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RELEVANCE OF COMPARATIVE LEAF BLADE ANATOMY IN TAXONOMIC AND FUNCTIONAL RESEARCH ON THE SOUTH AFRICAN POACEAE

by

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1. INTRODUCTION

Synopsis: The large and economically important grass family is notoriously difficult to classify because the highly specialized and reduced flower necessitates the use of fine morphological distinctions for the separation of taxa. Fortunately leaf blade anatomy has proved to be an invaluable aid in the classification and identification of the Poaceae. This project was initiated primarily to increase our knowledge of the systematics of the grasses of southern Africa but with the secondary objective of serving as basic data for several applied biological disciplines. A brief outline of these objectives will be followed by a short explanation of the methodology adopted and of the layout and presentation of this thesis.

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1. INTRODUCTION

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1. INTRODUCTION

1.1 Background to agrostological research

In South Africa the Poaceae is an extremely important plant family due to the number of species occurring in the region and to the economic contribution grasses make to many land management practices. About 75 per cent of the surface area of South Africa is covered by natural grassland, either pure grassveld types or as an understorey to various bush and savanna veld types (Acocks, 1975). Most of this grassland is the primary food source of either domestic livestock or of wild herbivores, including many insects. Grasses are, therefore, a vital link in many complex and inter-related food chains affecting a wide spectrum of terrestrial vertebrates and invertebrates.

The conservation and sound management of this grass layer is critical for soil genesis and the disturbance and over-utilization of this component of the vegetation is the prime reason for increased soil erosion and karoo encroachment over large areas of South Africa. In addition many of the region's more important agronomic crops are grasses. Examples are maize, wheat and sugar.

The economic importance of grasses in the South African context cannot be questioned and an improved scientific understanding of the grass family is imperative to ensure the long-term survival of this sub-continent. This knowledge is essential for land-use practices such as agriculture, forestry and wildlife conservation. In related fields of study, such as desertification and fresh water production and conservation, a deeper knowledge of our grass flora could contribute to the future well-being of this region by stabilizing the traditional, pastoral subsistence of the rural populations.

There are 1 050 species and subspecific taxa of grasses presently recorded from South Africa (Gibbs Russell pers. comm., 1981). Members of this large family are extremely difficult to classify because of the degree of reduction and specialization that has occurred in the flower. This implies the use of extremely fine morphological distinctions for separating taxa, necessitating the constant use of the hand lens and the dissecting microscope. This results in difficulties in interpretation with the individual opinions of agrostologists sometimes differing.

Difficulties with identification and classification of grass taxa have negative effects on all related biological disciplines relying on accurate identifications and meaningful classifications. Sterile, vegetative grass material often proves extremely difficult or impossible to identify by morphological means. This fact also hampers research by limiting field work to the flowering season of the grass component of the vegetation because many of the studies involving grasses demand intensive, prolonged experimentation, or study, extending over all the seasons of the year. Reliable identification of grasses is thus essential, irrespective of growth stage. At present this is not feasible when conventional herbarium techniques and knowledge are used.

From the above it is obvious that a large body of basic and applied biologists employed in the agricultural and forestry industries and the various nature conservation authorities are, to varying degrees, reliant upon a knowledge of grasses in order to function effectively and efficiently. It is, therefore, clear that agrostological research in South Africa should receive a very high priority and that a modern up-to-date classification that reflects phylogenetic relationships as accurately as possible is required. Such a classification will enable identifications to be made with greater accuracy and should contribute greatly to the stabilization of the nomenclature of the Poaceae.

The present situation is confusing as, in the 25 years since the publication of Chippindall's "Guide to the Identification of Grasses" (1955), which included about 910 recognized names, there have been 230 name changes and 178 additional species have been recorded for southern Africa (Gibbs Russell & Smook, 1980). A modern classification, incorporating attributes from a wide spectrum of character sources, will also undoubtedly have increased predictive value and will benefit all concerned.

1.2 Objectives of this research project

The taxonomic importance of leaf anatomical characters has long been appreciated and has been conclusively demonstrated by many authors throughout the world. Duval-Jouve (1875) was the first to use grass leaf anatomy for taxonomy and Schwendener (1890) described details of the leaf in section. The important systems of grass classification published by Avdulov (1931) and Prat (1932, 1936) profoundly influenced the course of grass systematics and both relied heavily on anatomical details. Tateoka (1956, 1957), Stebbins (1956) and Brown (1958, 1961) added considerably to our knowledge of the types of anatomy present in the Poaceae. The first really comprehensive treatise on grass anatomy was published by Metcalfe (1960) and in the ensuing decades there has been considerable activity in the field of grass systematics following the lead given by these early comparative anatomists. Examples are the work of Jacques-Felix (1962) and Parodi (1961). This development explains why it has

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become generally accepted nowadays that anatomical details of the leaf blade are an essential ingredient of any satisfactory treatment of grass taxonomy.

Anatomical characters of the grass leaf blade, as viewed in transverse section and of the epidermis in surface view, have been shown to provide as useful taxonomic and phylogenetic clues as any other characters currently employed in grass taxonomy. Some leaf anatomical attributes are valuable criteria in their own right and are as reliable as any of those to be gained from other features of the plant, including spikelet morphology, and their value is enhanced if used in conjunction with as wide a spectrum of diagnostic characters as possible (Clifford & Watson, 1977).

The present project was undertaken with the above considerations in mind. The initial and prime objective of this study was, therefore, to provide additional comparative leaf anatomical information to assist in the construction of a modern classification of the South African representatives of the grass family. In addition to the classical characters of the inflorescence and spikelet it is hoped that this classification will incorporate characters from as many sources as possible, such as agronomic characters, cytogenetical information, embryo structure, phytogeographical data, seedling morphology and many more, including leaf blade anatomical attributes.

It is anticipated that the completion of this project will stimulate and facilitate taxonomic work on the South African representatives of the grass family, much of which is in urgent need of revision. This study should contribute substantially to the Poaceae volume of the Flora of Southern Africa, a work which should bring a high degree of stability to southern African grass names. The information obtained from a broadly-based survey of the leaf anatomy will also be vital for the preparation of the projected Genera of the South African Grasses.

Valuable clues to the relationships and identity of unknown grass specimens are found in the leaf anatomy and epidermis (Calderon & Soderstrom, 1973) and thus, a secondary objective of this study is the provision of a means of identifying sterile grass specimens or small fragments as a professional service to the scientific community. This facility will enable ecological, pasture management, land-use and environmental impact surveys to be conducted throughout the year and would greatly increase the productivity and efficiency of many research organizations. A comprehensive and representative reference slide collection, such as has been built up during the course of this project, should greatly facilitate such identifications and should permanently serve a useful function.

Identification of grass leaf blade cuticular fragments using anatomical criteria has been shown to be a useful technique for determining food preferences of grazing herbivores, both mammals and insects (Stewart, 1967; Cavender & Hansen, 1970). To date studies of this type in South Africa have been restricted geographically and are not generally applicable to the whole region. Studies of the variation in anatomical leaf characters, both temporal and spatial, are needed before the microscope technique can be generally accepted for determining herbivore diets. Studies on geographical variation and digestibility differences are currently being undertaken at the Botanical Research Institute and, together with the present study, should help eliminate the gaps which, in the past, have hampered the drawing of definite conclusions in the assessment of animal feeding preferences using epidermal information.

Identifications of grass leaf fragments are also needed for forensic and medicinal purposes as well as quality control of commercial stock feeds and hay as a precaution against adulteration. In pedological studies the examination of grass silica bodies, which decompose very slowly, have been used to determine the origins and development of soils (Smithson, 1956; 1958).

All these applied secondary objectives have been considered in the planning of this project and amenability to adaptation to these applications was an important consideration in the design of this project. To date this is the only broadly-based, comprehensive survey of the anatomy of the whole grass family to be undertaken in South Africa. It covers the full geographical ranges of all the species present in the subcontinent over a number of growing seasons. The aim of this study can, therefore, be summarized as being a detailed study of the leaf anatomy and epidermal histology of the southern African grass taxa for taxonomic and diagnostic purposes. In this thesis the relevance of these anatomical studies in taxonomic, as well as functional research, on the South African Poaceae will be evaluated by means of selected, detailed examples.

1.3 Methodology adopted in this study

In a wideranging but detailed study of a large number of taxa, it is absolutely essential that all observations made are directly comparable. To achieve this aim, uniform treatment must be accorded all material used and for this reason the choice of methods and techniques was critical.

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In order to render anatomical characters visible, sophisticated preparation techniques and good optical microscopes are required before structures can be observed, recorded and described. If all plant material used is prepared by using standardized techniques the resulting anatomical details observed can be directly compared. For this reason detailed consideration is given, in the following chapters, to the methods standardized upon in this study and of the techniques employed by other workers. The fact that the objectives of this study are multipurpose has also contributed to the need for careful evaluation and justification of the techniques chosen. The methods used, as well as those considered, are, therefore, discussed in some detail.

Similarly the description of anatomical structures requires a standardized terminology if ambiquity and misunderstanding is to be avoided. Considerable attention has, therefore, been given to describing and defining the anatomical attributes of the grass leaf blade. In addition these characters have been evaluated for possible taxonomic significance by critical consideration of inherent variation, both genotypic and phenotypic.

Once these fundamental aspects have been fully considered the significance of leaf anatomy in taxonomic studies of the South African Poaceae has been evaluated by citing examples illustrating the relevance of anatomical characters at different taxonomic levels as well as in unrelated taxonomic groups. The comparative anatomical work is still in progress and these initial examples are designed to illustrate the practicability and effectiveness of standardizing the technical approach as well as the choice of anatomical characters and the method of compiling the anatomical descriptions.

Finally, the basic information obtained in this study has been applied to functional studies of the grasses. Thus it is shown that this data has contributed considerably to our understanding of photosynthetic pathways in the Poaceae as well as the ecological significance of these photosynthetic types as deduced by their different geographical ranges in South Africa.

1.4 Presentation of this thesis

This thesis differs from the conventional presentation and layout and, therefore, it is felt that a brief introductory explanation should assist the reader considerably when consulting this work.

This research has been conducted over a ten year period and the results have been regularly published as separate papers. As a result thirteen articles have been included in this thesis, each with its own introduction, literature review, discussion and references. These papers have been published in several different journals and thus the format, layout, editorial policies and style are not consistent throughout. This approach has also resulted in information or deductions being repeated or stressed differently in different contexts. Finally, and most important, due to new information being gathered all the time, it has occasionally happened that original opinions and deductions have had to be altered when additional information became available. These apparent contradictions within a single work can only be appreciated if the time scale and method of publication are borne in mind.

The result of this procedure has been a somewhat inconsistent final presentation of this thesis, which in itself should be seen as an intermediate stage of an on-going research project. These separate, individual articles have been synthesized into a somewhat more coherent whole by inclusion of this introduction to the thesis as a whole, together with a detailed discussion of methods and a final discussion of the results gained from the study as a whole. The more important of these are enumerated in the final conclusions. Each chapter has been separately indexed, with references and a brief synopsis outlining the contents of the ensuing chapter. It is hoped that, with these additions, the reader will more easily be able to appreciate the composition and structure of this work.

The alternative approach, which seemed somewhat retrogressive and unproductive, was to re-write the published papers for incorporation in a more conventional thesis. As the published papers included here include 560 pages of double-spaced typed A4 manuscript, with 209 photomicrographs, a traditional thesis would have been a bulky, cumbersome work which would improve little, if at all, on the present presentation which is much more compact and manageable.

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2. MATERIALS AND METHODS

Synopsis: The collection, selection and standardization of the plant material examined in this study, is described and explained. The techniques adopted for fixation and preparation of leaf transverse sections and epidermides, as well as for illustrating and recording of the anatomical detail, are outlined. A brief review is given of the techniques for epidermis preparation employed in other, related studies and the technical results are evaluated according to the requirements of the present study. On this basis the choice of methods is justified. As this is essentially a multi-purpose project, a prime prerequisite was for the reference material to be suitable for several different applications. This requirement for flexibility was a major criterion on which the choice of techniques for preparation of the reference material was based.

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2. MATERIALS AND METHODS

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2. MATERIAL AND METHODS

This is primarily a comparative study of the leaf blade anatomy of the grass species occurring in southern Africa. It was, therefore, essential to standardize collecting methods, as well as the techniques for preparation of this material for microscopical examination, to ensure that all observations were comparable.

In this study classical botanical microtechnique procedures were employed wherever possible. Standard histological techniques were only modified as required by the specific characteristics of the grass leaf material used in this study, or where necessitated by such practical considerations as field work conditions and laboratory facilities available, or where a modification speeded up, simplified or increased the reliability of the technique. A further reason for standardizing on conventional microtechnical methods was to enable effective comparisons to be made with the bulk of previously published work. The final choice of the method of preparation of material was, therefore, dependent on the specific requirements of this project.

2.1 The Plant Material Studied

2.1.1 Collection of material

Healthy, fertile grass plants were collected randomly under natural conditions, in the field, throughout southern Africa, over a period of ten years. A sample of fully mature, green and undamaged leaf blade material was removed for fixation. The remainder of the plant was pressed and dried for use as a herbarium voucher specimen and for distribution to other herbaria as duplicates. To date 3 500 specimens have been collected for this purpose and all are lodged in the National Herbarium in Pretoria.

To facilitate the accurate identification of these voucher specimens particular care was taken to sample representative plants from the natural populations. Where considerable morphological variation was evident in the field, extremes of this variation were also collected. Accurate and detailed habitat and locality notes were taken at the time of collection. Furthermore, an effort was made to display the natural growth form of the plant on the herbarium specimen, as well as any noticeable vegetative and inflorescence characters of possible diagnostic use. The importance of the collection of voucher material cannot be overstressed as this was essential to provide a sound foundation on which later deductions could be made. It is felt that in this manner all species were adequately sampled over the whole of their geographical ranges at representative stages in their life cycle and in all their parts (Wheeler, 1970). If any of these criteria were not met it was always possible to use the collection in the National Herbarium for checking purposes.

These dried and mounted herbarium specimens were then identified by the author using keys, comparison with authenticated material and from personal experience. The specimens were also submitted for independent, objective diagnosis by the herbarium staff of the Botanical Research Institute. Cases of disagreement drew attention to differences in interpretation or unsatisfactory morphological diagnostic characters and particular attention was paid to these species on subsequent collecting expeditions. In addition special note was taken of the anatomy of these specimens to see if anatomical characters were present that could help resolve the diagnostic problem.

This method of randomly gathering material under natural conditions, over an extended time period, was chosen in an attempt to incorporate as wide a spectrum of phenotypic and genotypic variation as possible in the sample examined of any taxon. Collecting was planned so that there would be adequate representation of the ecological and geographical ranges, in the samples of any particular species. Therefore, any anatomical differences observed between the leaf specimens of any particular taxon, could be regarded as being of potential taxonomic significance. These structural differences could also be interpreted as reflecting functional differences as well.

2.1.2 Standardization of anatomical material sampled

To attain effective uniformity with terminology, definitions and descriptions in a comparative anatomical study, such as this, it is necessary for comparative material to be studied and to use standardized techniques for the preparation of this material for anatomical observation.

In the vegetative organs of the Poaceae the most important diagnostic characters appear in the leaf (Metcalfe, 1960) and in particular the leaf blade which provides more taxonomic characters than the sheath (Davies, 1959). For this reason, only leaf blade material was sampled for this study.

It must be noted, however, that minor anatomical variation occurs within a single leaf blade and that leaves at different levels on an individual plant also exhibit structural differences (Prat, 1932, 1948). In addition, leaves belonging to a single species grown in different habitats also vary structurally to a certain degree. This intraspecific variation is minor when

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compared with interspecific variation, usually being quantitative rather than qualitative.

Intraspecific anatomical variation of a particular leaf can thus be ascribed to three major causes: the age of the leaf, or its degree of maturity, the position of the leaf upon the plant, and the environment in which the plant is situated. Variation due to these factors – developmental, positional and environmental – must be fully considered before taxonomic use can be made of any differences observed.

A sampling procedure was, therefore, designed to minimize ontogenetic differences as much as possible but which would fully monitor the full range of phenotypic plasticity as a sound knowledge of ecological variation is essential for valid taxonomic conclusions, especially at the species level. Consequently, although plants were collected from widely separated localities under differing field conditions and at different times during different growing seasons, only leaves similarly situated and of similar age were sampled from the different plants collected.

In addition, it was necessary to select comparable parts of these particular leaf blades. This is because the greater part of an individual leaf lamina grows in length from a basal meristem and, as a result, along any given leaf there is a basipetal maturation gradient (Sangster, 1970). There is thus a gradual transition from a fully developed apex to a basal portion that has yet to complete ontogenetic development, with a corresponding gradation of mature to immature anatomical structures in a proximal direction. In addition, the shape of the lamina outline, width and number of vascular bundles can vary from the ligule to higher up the blade, especially in species with tapering apices (Arber, 1923; Burbidge, 1946).

In the light of the above all material sampled for anatomical investigation in this study was taken from leaf blades at a point about halfway between the blade apex and the ligule. Furthermore these leaves were healthy, green, mature leaves from as near the base of the plant as possible. Flag leaves on flowering culms were avoided at all times.

2.2 Microtechnique

2.2.1 Fixation of material

The fixative used throughout this study was Formalin-Aceto-Alcohol (Johansen, 1940), commonly known as FAA or Bles's fluid to zoologists (Gray, 1964). This solution was mixed in the following proportions:

50% ethanol . . . . . .85 mls
Formaldehyde 35–36% . . . . . .10 mls
Glacial Acetic Acid . . . . . 5 mls

The solution was made up in bulk in the laboratory and decanted into 10 ml pill vials fitted with plastic stoppers. These vials were individually arranged in wooden racks for transport in the field. Each vial contained an identification number, written in pencil on a cardboard tag. This number was the same as the unique collector's number on the herbarium voucher specimen and was subsequently used to identify a particular specimen throughout the various stages of preparation and examination.

Immediately upon removal of a basal leaf from the voucher plant, leaf segments 10–20 mm long were cut with sharp scissors from the central portion of the leaf blade. These segments were quickly placed in the fixative before any loss in turgidity occurred with corresponding inrolling or infolding.

It is essential that extreme care be taken to ensure that the leaves sampled actually belong to the flowering specimen collected as a voucher. Mixed tussocks are not uncommon, even on herbarium specimens, and this has led to several errors in the literature.

The critical factors in the decision to standardize on FAA as a fixative for this study were primarily practical considerations. As material may be stored almost indefinitely in FAA without exhibiting appreciable damage (Jensen, 1962) it is ideal for use on extended collecting trips. No changes of solution are required and, additionally, FAA does not require refrigeration. FAA is virtually the standard preservative of comparative anatomy, and has been extensively used in the past, and thus comparison with published results is often possible.

Generally the solution, mixed in the above proportions, gave satisfactory fixation for the purely anatomical requirements of this study. Only in certain of the delicate species of the Festucoideae and Danthonieae was distortion of the mesophyll caused. For these specimens a lower percentage of ethanol would probably have improved the fixation (Johansen, 1940) but regrettably this shortcoming was only detected after the commencement of the project.

Cytological detail was not well preserved and did not compare well with 3% gluteraldehyde solution at O°C (Feder & O'Brien, 1968). For practical reasons, mainly refrigeration problems, gluteraldehyde was not used in this study.

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2.2.2. Preparation of transverse sections

The following is a brief discussion of the techniques used for sectioning leaf blade material in this study.

2.2.2.1 *Desilicification*

After a thorough rinsing in running water to remove the fixative, the leaf segments were desilicified in 30% hydrofluoric acid for $1\frac{1}{2}$ hours (Breakwell, 1914). It was necessary to use a wax or polythene vessel for this purpose. The material was then rinsed again for at least 3 hours to remove all traces of the acid.

Unless the silica is removed by this means frequent tearing of the sections results. This can be attributed to the fact that the microtome knife strikes and dislodges silica bodies in the epidermis and drives them through the leaf tissue being sectioned (Parry & Smithson, 1958).

2.2.2.2 *Dehydration*

The method of Feder and O'Brien (1968) was employed. After washing, the fixed and desilicified specimens were transferred to methyl Cellosolve (synonyms: ethylene glycol monomethyl ether or 2-methoxyethanol) at about O°C. Two changes of this solvent were made in 4–24 hours. The specimens were subsequently transferred to 100% ethanol (O°C, 4–24 hrs), then to n-propanol (O°C, 4–24 hrs), and finally to n-butanol (O°C, 4–24 hrs). The time intervals are largely dependent on the size and permeability of the specimens but in this study 24 hrs were allowed for each solvent i.e. dehydration took four days.

This dehydration series has the advantage that the specimens may be stored for several months in the propanol or butanol in a deep freeze. As with all common solvents used for dehydration, improved results are obtained with delicate material when cold solvent (O°C) is used. Refrigeration during dehydration is thus preferable and the use of labour-saving automatic tissue processors are restricted somewhat. Fortunately, with the material used in this study, dehydration at room temperature appeared to have no detrimental effect.

2.2.2.3 *Infiltration*

After dehydration the leaf segments were infiltrated with Tissuemat (TissuePrep) which is a formulation of paraffin wax and synthetic polymer with a melting point of 56,5°C. This infiltration was done by placing each leaf segment on solidified Tissuemat in a small vial and covering the leaf segments with n-butanol. The unstoppered bottles were then placed in an embedding oven, preferably of the circulating-air type, at 56,5°C for 12–24 hours, or at least until the wax had melted and the plant material had sunk to the bottom. The wax then now poured out and replaced with fresh molten wax and left for a further 12 hours to complete the infiltration.

2.2.2.4 *Embedding*

An embedding mould, consisting of a brass base and frames, and large enough to hold the segments (about 30 mm x 15 mm), was filled with melted Tissuemat wax from a dispenser set at about 56,5°C. This mould was then placed on a warm platform and, before a film had formed on the block surface, the tissue to be embedded was removed from the vials with warmed forceps and positioned in the wax block. It was correctly orientated centrally and horizontally in the wax block using warm needles and forceps. An identification label was inserted into the wax. Once a film had formed over the surface, the mould and wax was plunged into ice-cold water and allowed to harden.

The transfer of the segments to the wax block must be done quickly, because, if a film forms on the wax surface, or if the leaf segments cool appreciably, then bubbles of air tend to become trapped around the specimen. This can lead to complications during sectioning.

2.2.2.5 *Sectioning*

The wax blocks were trimmed, particular care being taken to ensure that the opposite sides were parallel to one another, and then mounted on microtome object discs. These discs were then attached to a rotary microtome in such a way that the leaf segment was positioned at right angles to the blade, that the leaf was orientated with the adaxial surface upwards and that the parallel block sides were parallel with the microtome knife. A ribbon of 10 µm, serial sections was then cut.

The ribbons were cut into 75 mm lengths which were floated in a paraffin section mounting bath at about 50°C. This flattened the sections before the ribbons were floated on to microscope slides coated with an adhesive. The ribbons were arranged as desired and then dried on a hot plate set at a temperature slightly below the melting point of the wax. It is preferable to blot the sections (Gray, 1964), before drying, to ensure better adhesion. The slides and sections were stored at O°C until ready for staining.

The adhesive used for glueing the sections to the

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slides was made up by dissolving 5g of gelatin in 1 litre of warm distilled water and adding 0,5g of chrome alum to serve as a preservative (Jensen, 1962). Slides were merely dipped in this gelatin solution and allowed to dry before sections were affixed.

If the embedded material proved to be too hard and brittle, and continually fragmented or tore when sectioned, it was soaked in Mollifex for up to 3 weeks – depending on the degree of hardness.

2.2.2.6 *Staining*

The safranin and fast green staining combination (Johansen, 1940) was standardized upon throughout. The paraffin was removed from the sections with xylene and they were partially hydrated in an ethanol series of decreasing concentration to 50% before staining overnight in safranin (1% safranin in 95% alcohol diluted 50:50 with distilled water). After washing in water and destaining in acidified 70% alcohol they were dehydrated to absolute alcohol before counterstaining with fast green (0,5 %solution in 50% clove oil, 50% alcohol). A few seconds only was sufficient. The fast green was differentiated in a mixture of 50% clove oil, 25% absolute alcohol and 25% xylene before the final three changes in xylene. The sections were then mounted under coverslips with Permount mounting medium and dried on a hot plate at about 60°C for at least 24 hours.

This double-stain combination stained all the lignified cell walls, the cuticle and the nuclei red and the cytoplasm and cellulose cell walls stained green or blue.

Certain grass leaves, particularly permanently infolded leaves with copious hypodermal sclerenchyma development tended to curl excessively and become dislodged from the wax ribbon and, therefore, did not flatten completely. This resulted in these sections not adhering satisfactorily to the slide and they tended to become dislodged and lost during the long staining procedure. To overcome this problem the method of Christensen (pers. comm., 1973) was introduced. This method, whereby the slides, with the sections still in paraffin, are immersed into the stains and only deparaffinated immediately prior to mounting, proved successful in these cases.

2.2.3 Preparation of leaf epidermides

Leaf surface structure has long been used in studies of plant morphology and anatomy as well as in the classification of extant as well as fossil plant groups. Identification of plant material from the epidermis and the cuticle has therefore proved useful to taxonomists, palaeobotanists, animal nutritionists and toxicologists, the drug industry and police departments. Epidermal structure appears to be of particular importance in food preference studies of wild and domestic herbivores (including insects) by analysis of faecal or rumen samples.

In all these studies permanent anatomical reference material, backed by herbarium voucher specimens, should be prepared. Numerous techniques are available for the preparation of this reference material, and, depending on the objectives of the study in question, certain techniques are more suitable for specific applications than others. The choice of the method of preparation is, therefore, dependent on the particular requirements of the study being undertaken.

The present study is essentially a comparative anatomical study of grass leaf blades and a suitable technique for this type of study was chosen. This decision was taken after experimenting with several techniques on a wide range of leaf types and also after consideration of the techniques most often employed in comparable studies in the literature. Based on these considerations the manual scraping method (Metcalfe, 1960) proved most satisfactory for comparative anatomy of grasses and was standardized upon throughout this project. This decision was taken notwithstanding the fact that the manual scraping method was not found to be ideal for other purposes – for example blending techniques were found to be more suitable for faecal analysis (Wilson *et al.*, 1977).

A knowledge of the structure of the grass leaf epidermis is pertinent to several branches of science, for each of which a particular method of specimen preparation will prove more satisfactory. For this reason a brief review of most methods of epidermal preparation will be given. It must be noted that the technique chosen to prepare permanent mounts should be designed to meet the three requirements of speed, simplicity and reliability (Stace, 1965). This is of particular importance in taxonomic and diagnostic work. Experimental studies may demand the choice of a method not fulfilling these prerequisites.

2.2.3.1 *Direct methods*

These methods involve the removing of all mesophyll, fibrous and vascular tissue by mechanical or chemical means until the epidermal layer, or only the cuticular membrane, remains for examination.

Before epidermal mounts can be prepared, most of these techniques require some pre-treatment of the leaf. The most important of these are rehydration of herbarium material and the clearing and softening of

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leaves before preparation.

Hydration can usually be adequately accomplished by boiling herbarium material in water until it has been restored as nearly as possible to its original shape. This usually suffices for epidermal preparations but some authors advocate the use of a detergent solution for difficult material (Ghouse *et al.*, 1972). Hydration was only required for the herbarium material, that was sometimes used for checking purposes, in this study.

Decolourization and removal of chlorophyll is sometimes advocated in grass anatomical studies. Clearing was not used in this study but it would undoubtedly have been beneficial in rendering leaves containing large amounts of tannin in the epidermal cells, more visible. However the presence of these deposits in themselves are significant in a comparative study such as this. 70% alcohol, lactic acid (Clark, 1960), chloral hydrate, lactochloral, lactophenol and chloral phenol are clearing agents that have been used to clear grass epidermal preparations (Huynh, 1971). Commercial bleach solutions (sodium hypochlorite) have often been used for bleaching grass leaf material (Dilcher, 1974).

Pre-treatment procedures for making silicified structures more easily visible are often useful in studies using grass leaf material. Ammonia and hydrogen peroxide have been used for this purpose (Parry & Smithson, 1958) together with clearing in cedarwood oil. A mounting medium with the correct refractive index (± 1,51) is also necessary to make the silica more conspicuous.

2.2.3.1.1 *Mechanical maceration*

Although some of these methods appear unsophisticated, it is often more expedient to use this type of technique to isolate the whole epidermis, rather than the cuticular membrane alone. This applies particularly to the Gramineae (Stace, 1965) where the preparations are used for taxonomic purposes (Metcalfe, 1960).

The manual scraping method (Metcalfe, 1960) was the technique standardized upon for epidermal preparation in this study. This method essentially involved the physical removal of the opposite epidermis, the mesophyll, vascular and fibrous tissue by gentle and careful scraping with a razor or surgical scalpel blade. The fixed leaf segments to be scraped were placed on a hard surface (glass or porcelain tile), adaxial side uppermost and flooded with 70% alcohol. Cold, fibre-glass optic illumination is useful for this step as the alcohol evaporation rate is not increased. If the material has been cleared then generally the same clearing fluid is used for this scraping.

The leaf segments were scraped with a new, sharp blade held at an angle until a single-celled layer of epidermal cells remained. A dissecting microscope is essential and a black background helps considerably. Jeweller's forceps, flattened dissecting needles and fine camel-hair brushes are often necessary to successfully prepare epidermides of fragile or thin leaves.

These epidermal scrapes were double stained using a solution of methylene blue and alum for 60 sec., followed by ruthenium red for 60 mins. The leaf scrapes were placed in a container with distilled water to which a small quantity of the ruthenium red powder had been added. These aqueous solutions of this dye are unstable and it is vital that the solutions are prepared at the time of staining. This stain colours cellulose walls bright red – the intensity of the colour being proportional to the content of pectic substances. Sclerified walls appear blue and suberized walls green.

Manual peeling (Kaufman *et al.*, 1969) is a successful method for preparing dicotyledon cuticles but is considerably less suited to grass epidermides. In some species with thick, distinct cuticular membranes, this membrane alone can be stripped off, but peels usually include the whole epidermal layer, often with additional adhering mesophyll and fibrous tissue.

The method of blending leaf segments in a Wiley Mill or blender (Cavender & Hansen, 1970) has primarily been developed to prepare reference material for studies of herbivore diets. This method has been considerably modified in the present study (Wilson *et al.*, 1977) so that the same method is used in the preparation of the reference material as is used in the preparation of rumen and faecal samples for microscopic analysis.

Material to be prepared by this method can either be fixed in FAA or air dried – this applies equally to stomach, rumen, fistula, faecal or reference material. This material is then blended for 5 seconds (20,000 rpm) with a microblender and then washed through a 0,1 mm sieve. This reduces all material to a homogenous size of approximately 1,5 mm in length. Loss of fragments smaller than 0,1 mm is not critical as fragments smaller than this usually do not carry enough features to allow conclusive identifications (Martin, 1954). This material can be filtered and oven dried to constant weight. To make the cuticular fragments more visible the material can either be cleared in Hertwig's solution and finally mounted in Hoyer's solution (Anderson, 1954) or boiled in a reflux con-

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densor in a mixture of FAA and nitric acid (Wilson *et al.*, 1977).

2.2.3.1.2 *Chemical maceration*

Chemical maceration techniques are dependent upon two important structural features of the epidermis (Stace, 1965). The cuticle and the bulk of the cuticular layer are composed of cutin which differs chemically from cellulose – the main constituent of the cell wall. Furthermore, the cuticular membrance of most plants is separated from the cellulose wall by a thin layer of pectic material. The isolation of the cuticular membrane may thus be affected by use of two types of substance: macerating solutions of strong acids or enzymatic preparations. The acids dissolve away all polysaccharides leaving only the cutin unaffected and pectinase enzyme solutions digest the pectic layers, freeing the cuticular membrane.

The outer surface of these cuticular preparations is smooth or minutely striate or papillose. The lower surface, on the other hand, is protracted into flanges which represent the position of the epidermal cell walls. When viewed from above, or below, the cuticular membrane is thicker at the position of the flanges, which are consequently visible, especially if the cutin is stained, as a series of darker lines representing the shapes of the original epidermal cells. It is important to realize that these lines only demarcate the centre of each adjoining pair of cell walls and that original cell wall thickness cannot be inferred from this type of preparation.

Several of these acid and enzyme maceration techniques have been tested and it was found that they usually result in more detail being visible on the cuticle than in scraped preparations where mesophyll tissue and epidermal cell contents sometimes obscure detail. The disadvantages far outweigh this advantage, however. The methods are complicated and time-consuming and result in very brittle cuticular segments which are very easily damaged during staining and mounting. Best results are obtained with specimens that scrape easily and fragile, thin leaves are virtually impossible to prepare by these chemical means. Finally, because cell wall thickness cannot be determined using these techniques, quantitative measurements cannot be made and comparison with material prepared by mechanical means is not possible. It was, therefore, decided not to use any of these methods as standard procedure in the present study. However for restricted studies they may prove entirely satisfactory. Examples are desert perennials or grasses from saline habitats – environments with grasses characterized by thick cuticles.

A brief summary of chemical meceration techniques will be given to illustrate the extensive possibilities that exist. Jeffrey's solution (Stace, 1965, 1966; Kiger, 1971), Schulze's solution (Stace, 1965; Dilcher, 1974) and nitric acid (Pohl, 1967; Ghouse *et al.*, 1972) are the best known acid maceration techniques. Nitric acid has been extensively used for grass material, particularly for the preparation of reference slides in diet studies (Storr, 1961; McAllister & Bornman, 1972), and is a rapid method requiring only 4–5 mins. The main disadvantage is that the protoplasm of the epidermal cells is not entirely eliminated, a feature which may obscure observation of surface details (Huynh, 1971). Acetic acid and hydrogen peroxide (Boulos & Beakbane, 1971), cupric sulphate and hydrochloric acid (Mohan Ram & Nayyar, 1974) and acetolysis (Huynh, 1971) are other less well-known techniques that have been applied to removal of leaf cuticles. The sodium hydroxide maceration technique (Ghouse *et al.*, 1974) can be mentioned here as it is particularly useful for material containing resinous and tanniferous substances. It is both a clearing and macerating method that eliminates these coloured materials better than lactic acid treatment (Clark, 1960).

Pectic enzymes (Pectinase and Enzyme 19) can also be used for cuticle isolation (Hohl, 1948; Orgell, 1955) as can a solution of hemi-cellulose, cellulose and pectinase (Schieferstein & Loomis, 1956). Maceration in rumen fluid (Powell *et al.*, 1974) is a method that is of particular interest where the epidermal preparations are required as reference material for rumen or faecal analysis in food preference studies of mammalian herbivores. This method is presently being used in digestibility studies which form part of the applied aspects of this research project.

2.2.3.2 *Indirect methods*

These methods involve the making of replications of the epidermal patterns by using peelable casts. Staining is seldom used in these indirect methods and the detail seen of the imprints is as a result of optical phenomena – refraction and diffraction – and the method of mounting (North, 1956).

Indirect methods allow only for examination of surface patterns on the cuticle and histological details of epidermal cell arrangement and structure may not always be discernible. If this is required, manual scraping or certain chemical maceration techniques must be employed.

Imprinting methods make use of the fact that the outer surface of the cuticle usually follows the contours of the epidermal cells. These methods are useless for leaves with thick cuticles which do not show

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the outlines of the epidermal cells on the outer cuticular surface (Stace, 1965). This type of technique has not proved very successful for the study of grass epidermides. One of the main reasons probably is the common occurrence of ribs and furrows on grass leaf blades which results in the imprinted cast being of variable thickness with consequent focussing difficulties. Tearing is also a considerable problem in these cases with the region of the cast overlying the ribs being very fragile and thin. Imprinting is, nevertheless, a very important botanical technique because it is the only method suitable for studying fossil material.

Imprint methods employ synthetic plastics and natural fluids which are capable of making moulds. All these products are liquid at the time of application and solidify after exposure to air, resulting in transparent films which can be stripped off and examined microscopically. Either a single primary template is only made, or an additional, secondary, positive template may be produced as well.

Natural fluids used for producing primary templates are certain mucilages and latexes (Patel, 1968; Shah & Gopal, 1969) and a large range of synthetic plastics have also been used for this purpose. Examples are Archer's liquid plastic (Sinclair & Dunn, 1961), cellulose acetate film (Joy *et al.*, 1956) and solution (North, 1956), collodin (Long & Clements, 1934) and acrylic plastic (Hanna, 1972).

All these plastic compounds have the serious disadvantage of 'misting' when allowed to harden in a moist atmosphere. Thus, they cannot be used on specimens which become distorted as they dry or, like most biological material, have a naturally wet surface. Compounds have been found that overcome this difficulty. Duco cement (Williams, 1973), Rhoplex (Horanic & Gardner, 1967), silicone rubber (Sampson, 1961) and Formvar (Mueller *et al.*, 1954) are examples.

2.3 Microscopy

Anatomical observations were made with a Zeiss Standard RA or Reicherdt Univar microscope, both equipped with plano objectives. Photomicrographs were taken using the Univar and Kodak Panatomic-X (ASA 32) or Ilford FP4 (ASA 125) film. Bright field, phase contrast and interference contrast illumination were used.

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3. ANATOMICAL CHARACTERS OF THE LEAF BLADE

Synopsis: The anatomical characters of the grass leaf blade that are visible in transverse sections and abaxial epidermal scrapes, are defined, described, illustrated and discussed in an attempt to achieve the uniformity currently lacking in comparative anatomical studies of the Poaceae. Definitions of all the taxonomically useful attributes, together with explanatory diagrams, are tabulated in hierarchical 'keys'. This procedure is designed to facilitate the quick and consistent compilation of comparative descriptions as well as for easy conversion to various methods of data storage and processing. Detailed discussions on variation and the taxonomic usefulness of these characters are also included. By this means it is hoped to achieve uniformity with interpretation of the anatomical characters used and so to standardize their descriptive terminology.

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3. ANATOMICAL CHARACTERS OF THE LEAF BLADE

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Bothalia 12,1: 65–109 (1976)

A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section

R.P. ELLIS*

ABSTRACT

Descriptive "keys", including definitions and diagrams, for standardizing and simplifying the description of grass leaf structure as seen in transverse section are given. Over 500 characters are included with the possibility for expansion to 999. Notes on variation and taxonomic importance of the characters are also included.

Résumé

UN PROCEDE POUR STANDARDISER L'ANATOMIE COMPAREE DE LA FEUILLE DE POACEAE. I. LE LIMBE VU EN SECTION TRANSVERSALE

Des "clefs" descriptives incluant des définitions et des diagrammes pour standardiser et simplifier la description de la structure des feuilles de graminées vues en section transversale sont données. Plus de 500 caractères sont inclus avec la possibilité d'expansion jusqu'à 999. Des notes sur la variation et l'importance taxonomique des caractères sont également fournies.

INTRODUCTION

Anatomical investigations of the grass leaf-blade have long provided valuable taxonomic information. In fact, nowadays, it is generally accepted that anatomical details, especially of the leaf-blade and embryo, when used in conjunction with a wide spectrum of other diagnostic characters, are an essential ingredient of any satisfactory treatment of grass taxonomy. Furthermore, in the Poaceae (=Gramineae), with their highly specialized and reduced flowers, very fine morphological distinctions are often necessary to define differences between taxa. Anatomical data is, therefore, regarded as being of undoubted importance in the jigsaw of complete systematic evidence in this numerically large and important family.

The importance of anatomy in agrostological studies has resulted in the rapid accumulation of an extensive body of literature with attendant problems of lack of uniformity with definitions and descriptions. Valuable data is, therefore, commonly not applicable to the family as a whole and comparisons cannot be drawn with any degree of assurance. This problem was greatly ameliorated by the publication in 1960 of Anatomy of the Monocotyledons. 1. Gramineae by C. R. Metcalfe and the present paper is a further attempt to stabilize this terminology, and, at the same time to present a system whereby description and comparison of grass leaf anatomy will be simplified and standardized.

DESCRIPTIVE KEYS

In an attempt to achieve the necessary uniformity, descriptive "keys" have been compiled for use as a framework for anatomical descriptions of the grass leaf-blade as viewed in transverse section. The "keys", which incorporate both definitions and diagrams, have been designed to enable the user to standardize descriptions and the hierarchical layout has been chosen to facilitate the speed and ease at which complete, comparative descriptions can be compiled Anatomical characters, and all other information considered to be of diagnostic or taxonomic importance, and gathered from an extensive survey of the relevant literature, have been included. The keys should, therefore, prove adequate for all tribes of the Poaceae except for the Bambuseae where additional data on the fusoid cells must be included to satisfactorily distinguish between certain genera.

The hierarchical tabulation of the characters has been used to expedite their use but they do not in any other way conform to any acknowledged key format or design. However, if a statement, at any level of the hierarchy, does not apply to the specimen being examined, no subsequent statements or characters of a lower rank are relevant. The user, therefore, merely proceeds to the next character of equal rank or indentation, and, if applicable, works inwards noting all the relevant numbered end points before working back outwards until the same level or rank as that of the originally chosen statement is reached. Thus, the "keys" are not dichotomous or true indented keys, but by using this type of format all the possible characters are recorded in a constant descriptive sequence. Each character is assigned a constant number and the recording of these numbers effects a saving in time and space. Furthermore, this system enables easy conversion to edge punched and feature cards as well as for electronic data processing by computer. In addition, it is ensured that all possible structures are rapidly and routinely noted in a rational sequence which simplifies compilation of descriptions of the various taxa. By employing a standard sequence significant differences become more readily evident.

In order for the standardization of terminology and descriptions to be effective it is essential that comparative material be examined. Therefore, in this study, all descriptions refer to transverse sections taken at a point about halfway between the blade apex and the ligule of mature basal leaves. Flag leaves on flowering culms were avoided where possible. By standardizing on the material studied in this way intraspecific differences may be assessed.

*Botanical Research Institute, Department of Agricultural Technical Services, Private Bag X101, Pretoria.

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66 A PROCEDURE FOR STANDARDIZING COMPARATIVE LEAF ANATOMY IN THE POACEAE.
I. THE LEAF-BLADE AS VIEWED IN TRANSVERSE SECTION

1. OUTLINE OF THE LAMINA IN TRANSVERSE SECTION

Outline usually determined by the shape of the abaxial surface. Includes the entire transverse section of the leaf blade at a point about halfway between the leaf apex and the base of the lamina. Subdivided into leaves that can be or are relatively flattened and permanently infolded, acicular leaves.

OUTLINE OF THE LAMINA OF OPEN LEAVES:

Includes all leaves which are not permanently infolded to such a degree that the internal structure is altered. These leaves are normally open or expanded under favourable environmental conditions but exhibit various degrees of infolding or inrolling under conditions of water stress. Projecting and grooved midribs and keels must be considered in relation to the overall shape.

DESCRIPTION:

Expanded; commonly flattened; a line connecting both margins and the median bundle straight:
Nature of blade; ribs and furrows not considered:
Flat, even or straight 101*
Undulating gently 102*
Distinctly wavy or undulating:
Corrugated leaf i.e. waves rounded 103*
Pleated leaf i.e. waves pointed or angled 104*

V-shaped; two halves of the lamina folded toward each other on either side of the midrib i.e. a line connecting both margins with the median bundle angled at the median bundle; carinate:
Angle formed by the two arms of the lamina at the midrib:
Narrow; closed V i.e. less than 45° to each other 105*
Standard V i.e. between 45° and 90° to each other 106*
Broad, open V i.e. more than 90° to each other 107*
Wide, very open V i.e. almost 180°; presence of projecting keel gives appearance of V-shape 108*
Shape of arms of the lamina:
Straight 109*
Outwardly bowed; concave 110*
Outwardly curving; convex 111*
Outline heart-shaped 112*
Distinctly wavy or undulating:
Corrugated leaf i.e. waves rounded 113*
Pleated leaf i.e. waves pointed or angled; plicate 114*
Irregularly wavy or bent 115*
Symmetry of two halves of the lamina:
Symmetrical on either side of the median vascular bundle 116
Two halves assymmetrical on either side of the median vascular bundle 117*

U-shaped; two halves of the lamina curved upwards on either side of the midrib or with a prominent rounded keel i.e. no definite angle formed with the midrib; canaliculate:
Shape of the U formed:
Broad, wide i.e. horizontally elongated 118*
Tall, narrow i.e. vertically elongated 119*
Base and sides same length i.e. equidimensional 120*

Inrolled; lamina rolled inwards towards the adaxial surface:
Nature of inrolling:
Convolute; inrolled from ONE margin only; margins wrapped around each other 121*
Involute; inrolled from BOTH margins 122*
Degree of inrolling:
Tightly inrolled 123
Loosely inrolled 124

DESCRIPTION CONTINUED. Measurements taken by estimation utilizing the known diameter of the field of view of the objective where the thickness or width of the leaf almost fill this field of view. This standardisation is for the Zeiss Standard RA microscope fitted with X 10 Kpl W eyepieces.

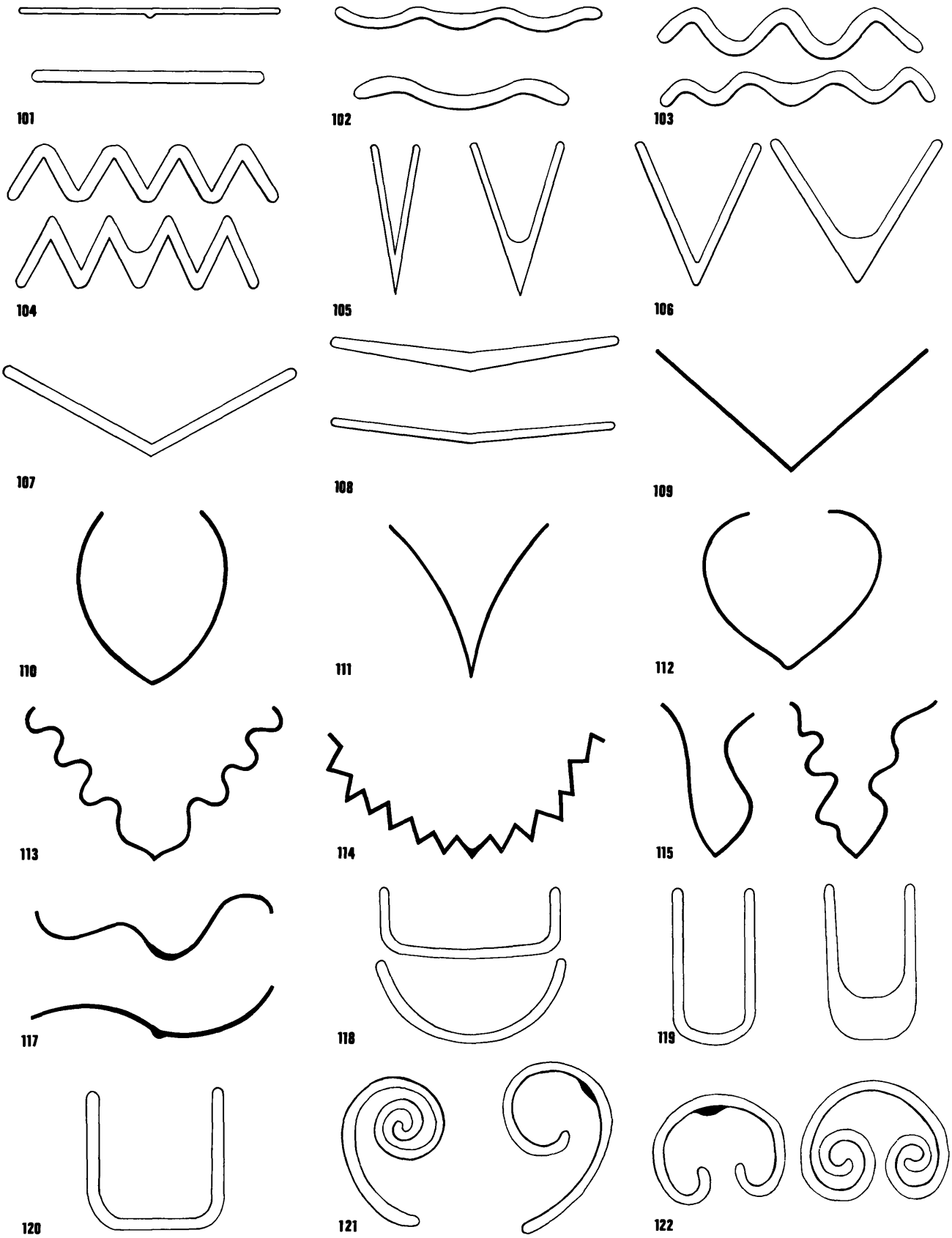
Width of one half of the lamina i.e. from blade margin to midrib or median vascular bundle if midrib indistinguishable:
X16 Objective:
Less than half the field of view at x 160 i.e. less than 0,56 mm wide 125
Between half and the full field of view at x160 i.e. from 0,56 mm – 1,125 mm wide 126
X2,5 Objective:
Less than half the field of view at x25 i.e. 1,125 mm – 3,6 mm wide 127
Between half and the full field of view at x25 i.e. from 3,6 mm – 7,2 mm wide 128
Up to twice the field of view at x25 i.e. from 7,2 mm – 14,4 mm wide 129
More than twice the field of view at x25 i.e. more than 14,4 mm wide 130

Thickness of lamina taken at a point about halfway between the median bundle and the margin. Depth of largest rib in central area of lamina measured:
X40 Objextive:
Less than one quarter of the field of view at x400 i.e. less than 0,112 mm thick 131
Between one quarter and half the field of view at x400 i.e. from 0,112 mm – 0,225 mm thick 132
Between half and three quarters of the field of view at x400 i.e. from 0,225 mm – 0,336 mm thick 133
Between three quarters and the full field of view at x400 i.e. from 0,366 mm – 0,450 mm thick 134
More than the full field of view at x400 i.e. more than 0,450 mm thick 135

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OUTLINE OF OPEN LEAVES



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1. OUTLINE OF THE LAMINA IN TRANSVERSE SECTION CONTINUED

OUTLINE OF THE LAMINA OF PERMANENTLY INFOLDED LEAVES:

Includes only leaves that are permanently infolded to the extent that the internal structure is altered. Often includes acicular, setaceous, filiform and junciform leaves. The entire section is considered under low magnification with the abaxial surface determining the outline.

DESCRIPTION:

Shape, outline; as formed by the abaxial surface:

Reduced V-shaped; two halves of the lamina folded toward each other on either side of the median bundle i.e. a definite angle formed:

Arms straight 150*
Arms angled; often hexagonal 151*

Reduced U-shaped; two halves of the lamina curved upwards on either side of the median bundle i.e. no definite angle formed:

Arms vertical or parallel to one another 152*
Arms rounded or outwardly bowed:
Elliptical; forming an incomplete oval or ellipse 153*
Rounded; forming an incomplete circle 154*

Triangular; base broad on either side of the median bundle 155*

Circular or *Round* in transverse section:

Hollow cylinder; margins not fused; adaxial surface not reduced; horseshoe-shaped 156*
Solid cylinder; margins fused or nearly so:
Adaxial surface reduced to a minute groove 157*
Adaxial surface not distinguishable; radially symmetrical; terete 158*

Number of **Vascular Bundles** present in section; includes median bundle or midrib if present:

3 vascular bundles in T/S 159
5 vascular bundles in T/S 160
7 vascular bundles in T/S 161
9 vascular bundles in T/S 162
11 vascular bundles in T/S 163
13 vascular bundles in T/S 164
15 vascular bundles in T/S 165
17 or more vascular bundles in T/S 166

Adaxial channel bounded by the arms of the lamina; may be open or closed at the leaf margin:

Vertical channel sides; parallel to each other:

Width of the channel; compared with the blade thickness at a point midway between median bundle and margin:
Wider than the leaf thickness 167*
Nearly the same as the leaf thickness 168*
Narrower than the leaf thickness 169*
Narrow cleft; less than half the leaf thickness 170*

Depth of the channel; compared with the blade thickness at the median vascular bundle:
Shallower than the leaf thickness 171*
Nearly the same as the leaf thickness 172*
Deeper than the leaf thickness 173*
Deep; more than twice the leaf thickness 174*

Non-vertical channel sides:

Rounded channel 175*
Triangular channel 176*
Channel reduced to small groove 177*
No channel present 178*

DESCRIPTION CONTINUED. Measurements taken by estimation utilizing the known diameter of the field of view of the objective where the thickness or width of the leaf almost fill this field of view. This standardisation is for the Zeiss Standard RA microscope fitted with X10 Kpl W eyepieces.

Width of one half of the lamina i.e. from blade margin to midrib or median vascular bundle if midrib indistinguishable:

X16 Objective

Less than half the field of view at x160 i.e. less than 0,56 mm wide 179
Between half and the full field of view at x160 i.e. from 0,56 mm – 1,125 mm wide 180

X2,5 Objective:

Less than half the field of view at x25 i.e. 1,125 mm – 3,6 mm wide 181
Between half and the full field of view at x25 i.e. from 3,6 mm – 7,2 mm wide 182
More than the full field of view at x25 i.e. more than 7,2 mm wide 183

Thickness of lamina taken at a point about halfway between the median bundle and the margin. Depth of largest rib in central area of lamina measured:

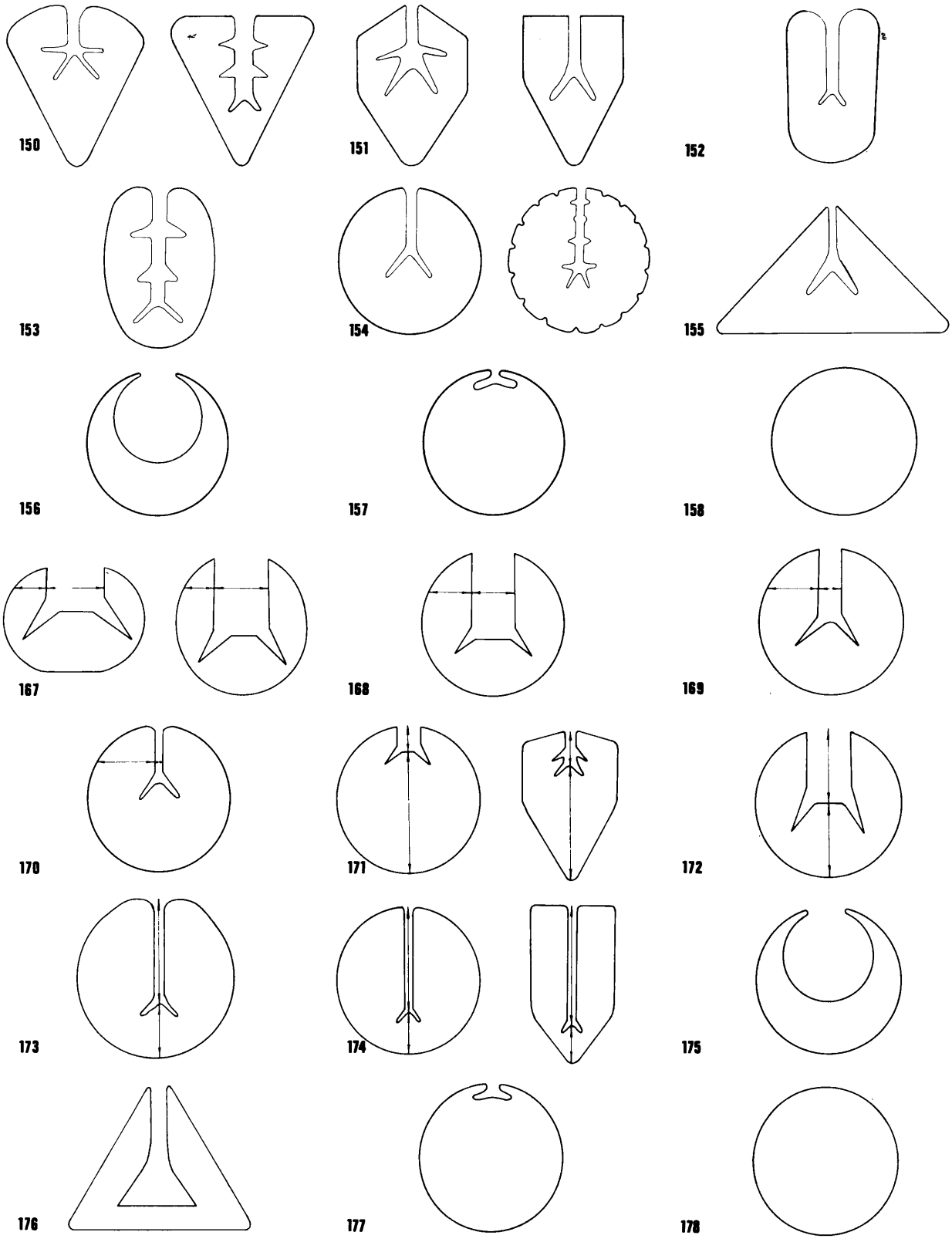
X40 Objective:

Less than one quarter of the field of view at x400 i.e. less than 0,112 mm thick 184
Between one quarter and half the field of view at x400 i.e. from 0,112 mm – 0,225 mm thick 185
Between half and three quarters of the field of view at x400 i.e. from 0,225 mm – 0,336 mm thick 186
Between three quarters and the full field of view at x400 i.e. from 0,336 mm – 0,450 mm thick 187
More than the full field of view at x400 i.e. more than 0,450 mm thick 188

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OUTLINE OF PERMANENTLY INFOLDED LEAVES



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2. ADAXIAL AND ABAXIAL LONGITUDINAL RIBS AND FURROWS

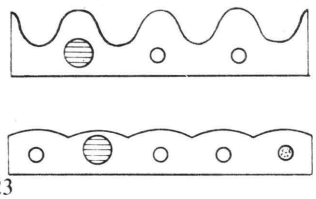
Includes all longitudinal ribs or ridges as well as the associated furrows or grooves commonly found on the adaxial leaf surface but sometimes on the abaxial surface as well. If ribs are present there must be corresponding furrows and vice versa. If furrows are not found between the ribs but in any other situation they are termed grooves, e.g. the grooves present on some ribs. Ribs and the associated vascular bundles form regular associations and patterns. The furrows and the adaxial channel of acicular leaves can be distinguished by the fact that the channel is the area bounded by the permanently infolded lamina and the furrows lie between the ribs which may also be in the channel.

DESCRIPTION:

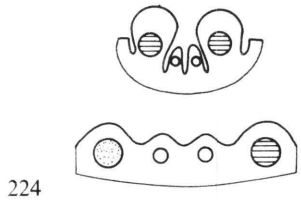
- No ribs or furrows present on either surface i.e. lamina surface straight or only slightly undulating with no regular pattern associated with the vascular bundles . . . 201*
- **Adaxial** ribs and furrows present:
  - *Depth* of adaxial furrows in comparison to the leaf thickness i.e. the depth of the larger ribs in the central region of the lamina between the margin and the median vascular bundle regarded as leaf thickness:
    - Slight, shallow furrows i.e. less than a quarter of the leaf thickness . . . 202*
    - Medium furrows i.e. a quarter to one half the leaf thickness . . . 203*
    - Deep furrows i.e. more than one half the leaf thickness . . . 204*
  - *Shape* of adaxial furrows:
    - Wide, open furrow i.e. obtuse angle formed by furrow sides at base . . . 205*
    - Narrow furrow i.e. sides of furrow almost vertical:
      - In form of cleft . . . 206*
      - Base fairly broad but sides steep . . . 207*
  - *Distribution* of adaxial furrows; important for acicular leaves:
    - Furrows between all vascular bundles . . . 208*
    - Furrows between 1st and 2nd order vascular bundles i.e. present over 3rd order vascular bundles . . . 209*
    - Furrows between 1st order vascular bundles i.e. present over 3rd and 2nd order vascular bundles . . . 210*
    - Furrow on either side of the median vascular bundle only . . . 211*
    - Two furrows on either side of median vascular bundle only . . . 212*
    - Deepest furrows on either side of the median vascular bundle . . . 213*
  - *Shape* of adaxial ribs as seen in T/S; all types present must be included:
    - Rounded, obtuse ribs i.e. apex rounded:
      - Situated over the vascular bundles . . . 214*
      - Situated between the vascular bundles (usually occupied by air spaces) . . . 215*
    - Flat-topped, square ribs i.e. apex flattened:
      - Sides rounded with flat top . . . 216*
      - Sides angled with flat top . . . 217*
    - Trangular ribs i.e. apex pointed:
      - One vascular bundle in each rib . . . 218*
      - Three vascular bundles in each rib . . . 219*
      - Grooved at apex . . . 220*
    - Massive ribs i.e. very large usually with bases narrow in relation to upper parts of rib:
      - Flattened apices . . . 221*
      - Rounded apices . . . 222*
  - *Distribution* of different ribs in association with the vascular bundles:
    - Ribs present over all vascular bundles:
      - Similar ribs over all vascular bundles . . . 223*
      - Ribs over 1st and 2nd order vascular bundles larger than those over the 3rd order vascular bundles . . . 224*
      - Ribs over 1st order vascular bundles with flattened tops and those over the 3rd order bundles triangular . . . 225*
    - Ribs not associated with all vascular bundles:
      - Ribs present only over 1st and 2nd order vascular bundles . . . 226*
      - Ribs present only over 1st order vascular bundles . . . 227*
      - Ribs present over midrib only . . . 228*
- **Abaxial** ribs and furrows present:
  - *No* abaxial rib development:
    - Surface smooth or with few undulations not regularly associated with the vascular bundles . . . 229*
    - Grooves present on abaxial surface:
      - Opposite larger vascular bundles . . . 230*
      - Between vascular bundles; often shallow . . . 231*
      - Groove on either side of midrib . . . 232*
  - *Ribs* present on abaxial surface:
    - Size of abaxial ribs:
      - Taller than the adaxial ribs . . . 233*
      - Same size as adaxial ribs i.e. section resembles a string of beads; moniliform . . . 234*
      - Smaller than adaxial ribs . . . 235*
      - Slight undulation associated with the vascular bundles . . . 236*
    - Distribution of abaxial ribs:
      - Present opposite 1st order vascular bundles only . . . 237*
      - Present opposite 1st and 2nd order vascular bundles only . . . 238*
      - Present opposite all vascular bundles . . . 239*
    - Composition of abaxial ribs:
      - Composed of sclerenchyma:
        - In form of rounded caps . . . 240*
        - In form of flat-topped caps . . . 241*
      - Composed of girder or strand of sclerenchyma in contact with the epidermis . . . 242*

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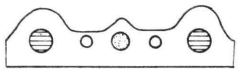
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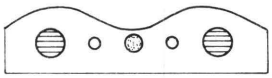
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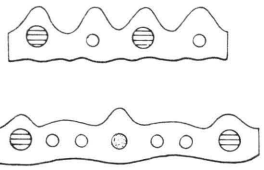
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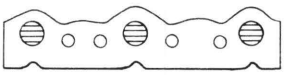
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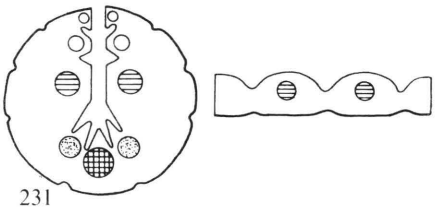
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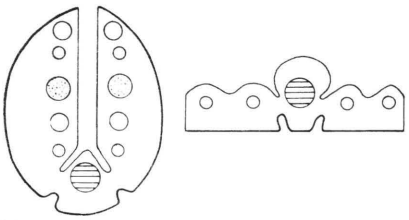
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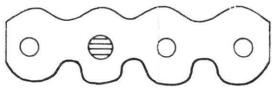
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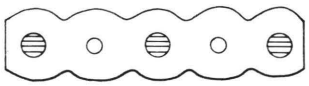
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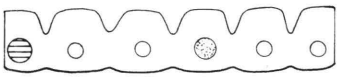
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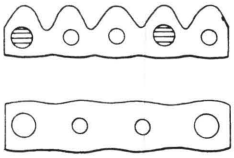
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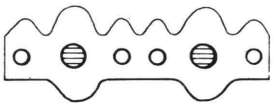
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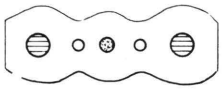
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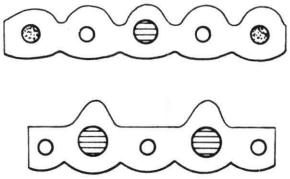
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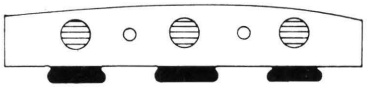
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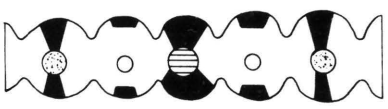
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72 A PROCEDURE FOR STANDARDIZING COMPARATIVE LEAF ANATOMY IN THE POACEAE. I. THE LEAF-BLADE AS VIEWED IN TRANSVERSE SECTION

3. MEDIAN VASCULAR BUNDLE, MIDRIB AND KEEL STRUCTURE

Keel and midrib distinguished by the presence or absence of parenchyma associated with the median vascular bundle. It is termed a midrib if the median bundle is solitary, structurally distinct and without associated parenchyma and a keel if parenchyma or bulliform cells are associated with the median bundle or bundles. Thus one or many vascular bundles may be incorporated in a keel. The keel does not necessarily project. In V-shaped leaves, only the median bundle is considered to comprise the keel (if parenchyma is present) unless there is a marked thickening in relation to the rest of the lamina. The number of vascular bundles in a keel is, in a general way, roughly proportional to the size of the keel.

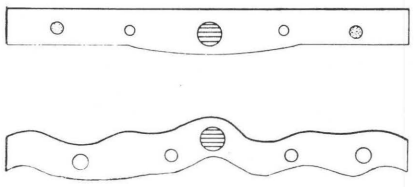
DESCRIPTION:

- Not possible to determine from preparation i.e. entire width of blade not sectioned 301
- **Median bundle** only present i.e. entire or more than half the blade width sectioned and no structurally distinct midrib is distinguishable 302*
- **Midrib** only present; distinguishable from other first order vascular bundles; no associated parenchyma developed i.e. keel inconspicuous:
  - *Projection* of midrib:
    - Projects abaxially:
      - Sclerenchyma causes projection 303*
      - Projection due to position or size of bundle 304*
    - Projection inconspicuous or marked; not projecting abaxially only:
      - Blade expanded or slightly inrolled 305*
      - Lamina V-shaped but no parenchyma at angle 306*
      - Acicular or terete leaves; parenchyma may be developed but no specifically in association with the midrib . . 307*
  - **Keel** developed; colourless parenchyma and/or bulliform cells associated with the median bundle; sclerenchyma development may also occur:
    - *Number* of vascular bundles comprising keel; in V-shaped leaves, if there is no thickening in the keel area the median bundle is considered to constitute the keel:
      - One vascular bundle comprising the keel:
        - Parenchyma of small, rounded cells surrounding or immediately adaxial to the median bundle 308*
        - Bulliform cells in adaxial epidermis above median bundle:
          - Leaf expanded, flat or slightly inrolled 309*
          - Leaf V-shaped:
            - Bulliform cells penetrate into leaf on either side of median bundle forming an inverted V 310*
            - Bulliform cells elongated and arranged in U above median bundle 311*
            - Elongated bulliform cells in U as well as layers of elongated parenchyma cells 312*
            - Bulliform cells and rounded parenchyma cells present 313*
      - Two vascular bundles present in keel; one being a superposed adaxial amphivasal 3rd order bundle 314*
      - Three vascular bundles present in keel:
        - Adaxial groove present 315*
        - No adaxial groove present 316*
      - Four vascular bundles present in keel; three abaxial and one adaxial amphivasal bundle 317*
      - Many vascular bundles present in keel:
        - All vascular bundles abaxially arranged:
          - Median bundle indistinguishable from other 1st order bundles:
            - One 1st order bundle and smaller bundles comprise keel 318*
            - Three 1st order bundles and other smaller bundles comprise keel 319*
            - Five or more 1st order bundles and other smaller bundles comprise keel. 320*
          - Median bundle structurally distinct (size, bundle sheath or sterome) from other 1st order bundles:
            - Large median bundle and other 3rd and 2nd order bundles only in keel 321*
            - Normal 1st order bundles present in addition to the median bundle 322*
        - Vascular bundles distributed adaxially, abaxially and in the interior of the keel; usually includes superposed and amphivasal bundles 323*
    - *Shape* of keel in transverse section:
      - Not really distinct from leaf outline 324*
      - V-shaped, pointed or inverted triangular keel:
        - Leaf V-shaped with V-shaped keel 325*
        - Leaf V-shaped with inverted triangle-shaped keel 326*
        - Leaf expanded with inverted triangular or pointed keel 327*
      - Rounded or semicircular keel; leaf not necessarily U-shaped:
        - U-shaped keel; slightly thicker than rest of lamina 328*
        - U-shaped keel; much thicker than rest of lamina 329*
        - Rounded or semicircular keel i.e. adaxial side of keel flat 330*
        - Rounded keel with raised, flattened adaxial side 331*
        - Rounded keel with single, central adaxial groove 332*
        - Rounded keel with distinct adaxial rib developed 333*
        - Rounded keel with numerous adaxial ribs and furrows 334*
      - Massive U-shaped keel; often with air-spaces 335*
    - *Sclerenchyma* associated with the keel:
      - Adaxial sclerenchyma:
        - Strands in subepidermal layers 336*
        - Strands fused forming a hypodermal band 337*
      - Abaxial sclerenchyma:
        - Most bundles with abaxial strands 338*
        - Most bundles with abaxial girders 339*
        - Central or solitary bundle with anchor-shaped girder 340*
    - *Air spaces* or *Lacunae* incorporated in the keel; definite lacunae present or enlarged parenchyma cells of keel breaking down:
      - One air-space in keel 341*
      - Two air-spaces in keel 342*
      - Four or more air-spaces in keel 343*

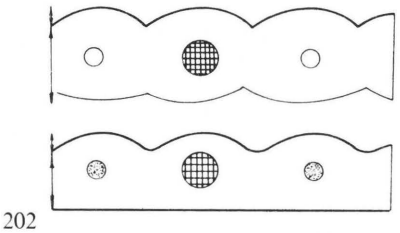
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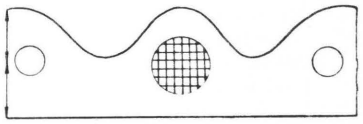
RIBS AND FURROWS



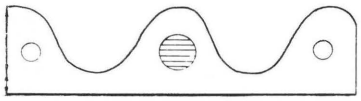
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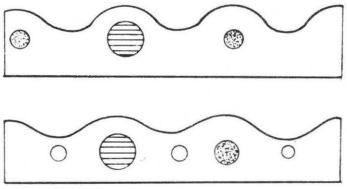
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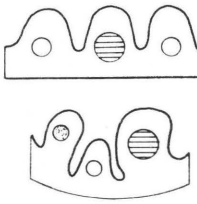
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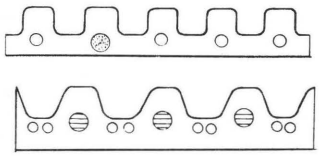
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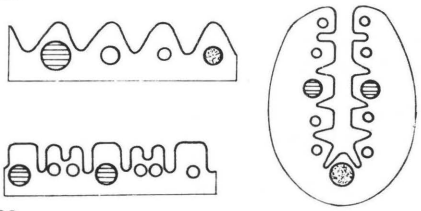
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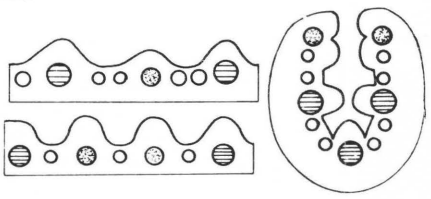
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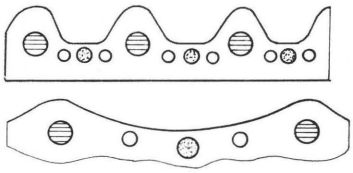
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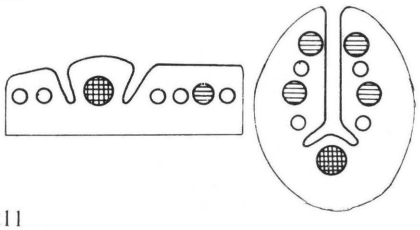
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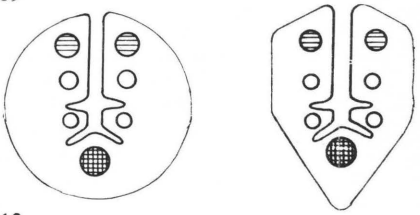
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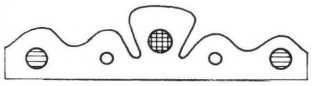
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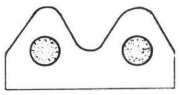
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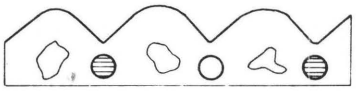
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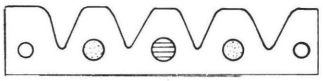
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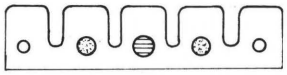
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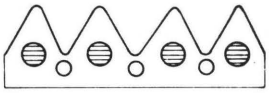
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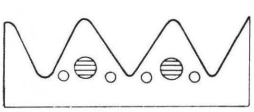
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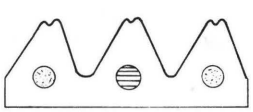
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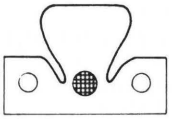
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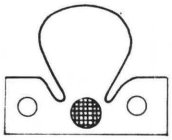
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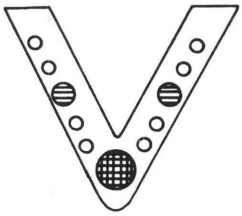


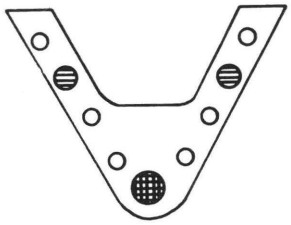
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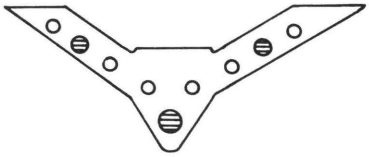
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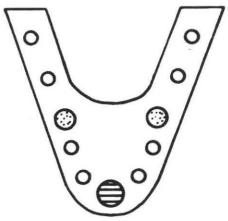

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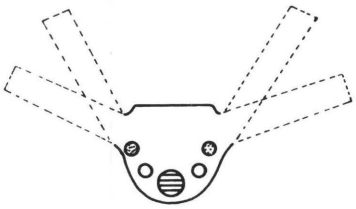

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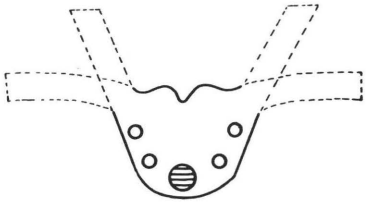

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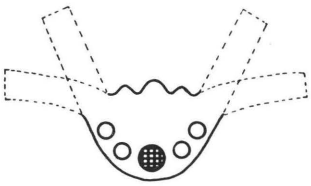

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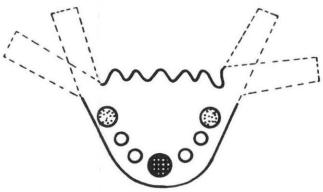

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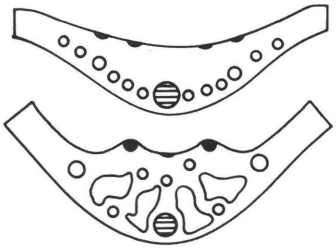

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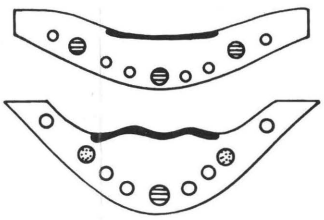

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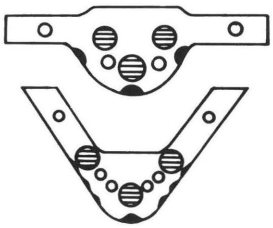

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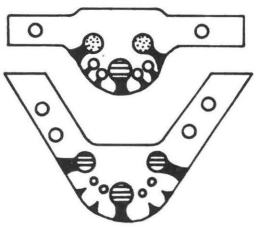

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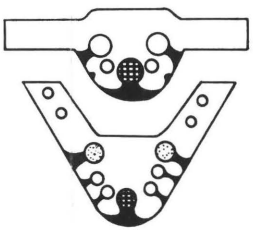

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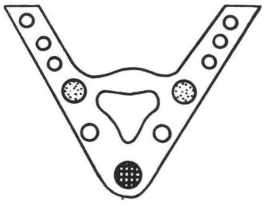

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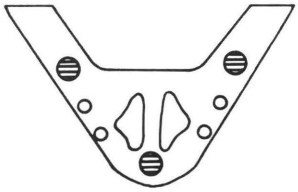

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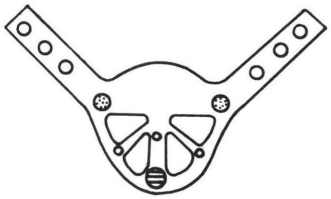

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74 A PROCEDURE FOR STANDARDIZING COMPARATIVE LEAF ANATOMY IN THE POACEAE. I. THE LEAF-BLADE AS VIEWED IN TRANSVERSE SECTION

4. VASCULAR BUNDLE ARRANGEMENT

Usually studied under low magnification. Only half the width of the lamina, from the margin to and including the median vascular bundle considered. When the section is incomplete and the median bundle is indistinguishable only those characters that are possible to determine are considered. In certain cases where the 2nd order vascular bundles are not clearly distinguishable from the 1st order bundles they are considered as 1st order bundles for the purposes of arrangement.

ARRANGEMENT:

Total number of 1st ORDER bundles in half the width of the lamina; includes the median vascular bundle:

- Not possible to determine; entire blade not sectioned 401
- 1 1st order vascular bundle in blade section i.e. median bundle 402
- 2 1st order vascular bundles in half lamina i.e. 3 in entire blade 403
- 3 1st order vascular bundles in half lamina i.e. 5 in entire blade 404
- 4 1st order vascular bundles in half lamina i.e. 7 in entire blade 405
- 5 1st order vascular bundles in half lamina i.e. 9 in entire blade 406
- 6 1st order vascular bundles in half lamina i.e. 11 in entire blade 407
- 7 1st order vascular bundles in half lamina i.e. 13 in entire blade 408
- 8 1st order vascular bundles in half lamina i.e. 15 in entire blade 409
- 9 1st order vascular bundles in half lamina i.e. 17 in entire blade 410
- 10 or more 1st order vascular bundles in half lamina 411

Arrangement and **Alternation** of different orders of vascular bundle along width of blade; central part of lamina between margin and midrib studied:

Variable from median bundle to margin; no regular pattern discernible:

- Progressively fewer 1st and more 3rd order bundles towards the margin 412
- Progressively more 1st and fewer 2nd and 3rd order bundles toward the margin 413
- Arrangement near margin differs from remainder 414

Regular arrangement from median bundle to margin:

Number of 3rd order bundles between consecutive 2nd or 1st order bundles at a position halfway to margin:

- No 3rd order bundles between consecutive larger bundles 415
- 1 3rd order bundle between consecutive larger bundles 416
- 2 3rd order bundles between consecutive larger bundles 417
- 3 3rd order bundles between consecutive larger bundles 418
- 4 3rd order bundles between consecutive larger bundles 419
- 5 3rd order bundles between consecutive larger bundles 420
- 6 3rd order bundles between consecutive larger bundles 421
- 7 3rd order bundles between consecutive larger bundles 422
- 8 3rd order bundles between consecutive larger bundles 423
- 9 3rd order bundles between consecutive larger bundles 424
- 10 or more 3rd order bundles between consecutive larger bundles 425

Number of 2nd order bundles between consecutive 1st order bundles:

- No 2nd order bundles between consecutive 1st order bundles 426
- 1 2nd order bundle between consecutive 1st order bundles 427
- 2 2nd order bundles between consecutive 1st order bundles 428
- 3 2nd order bundles between consecutive 1st order bundles 429
- 4 2nd order bundles between consecutive 1st order bundles 430
- 5 or more 2nd order bundles between consecutive 1st order bundles 431

Position of vascular bundles in blade; vertical situation in the section about halfway between median bundle and margin:

Same level of positioning for all bundles of different orders:

- All bundles situated in the centre of the blade 432*
- All bundles situated closer to the abaxial surface 433*
- All bundles situated closer to the adaxial surface 434*

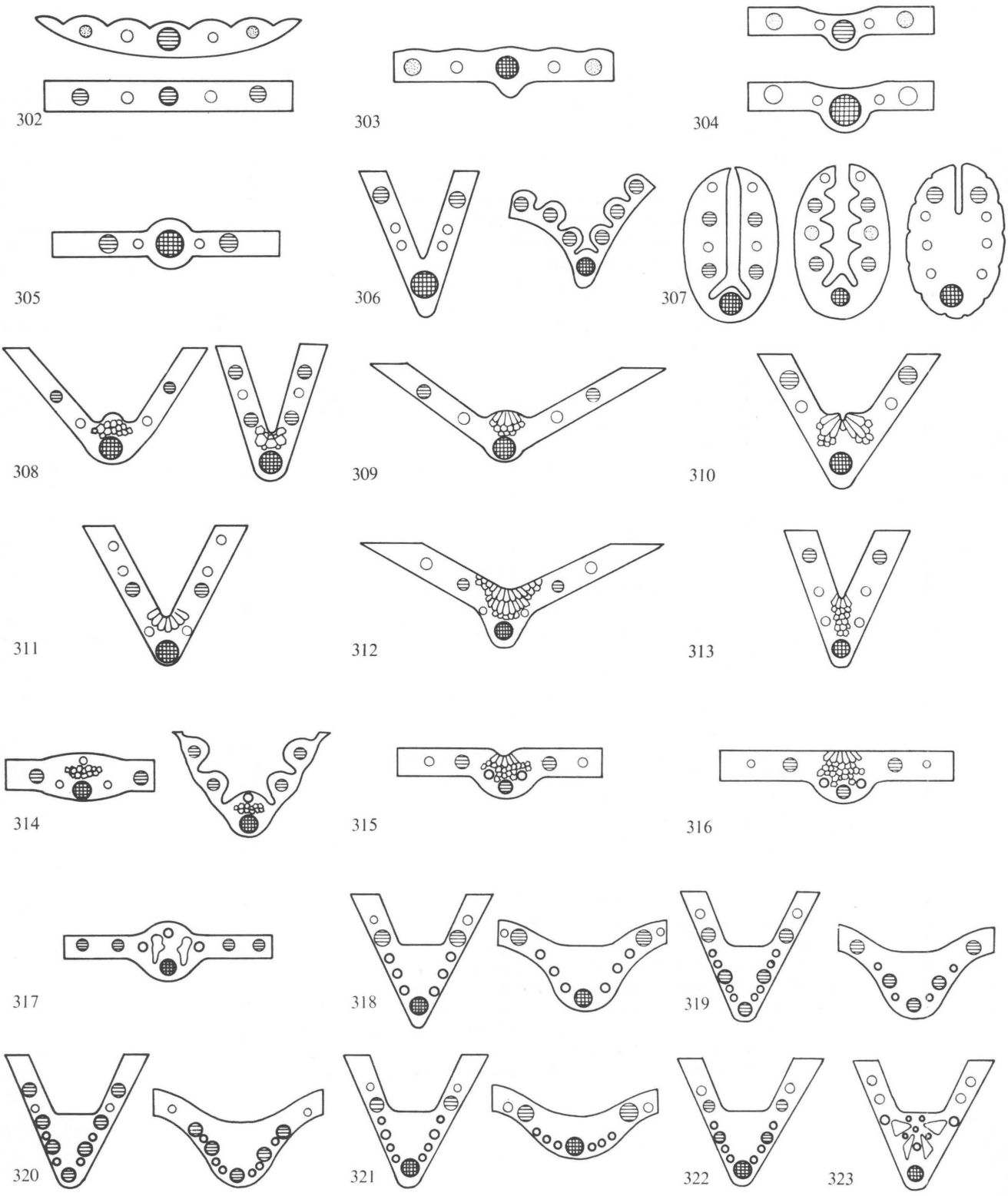
Different levels of positioning for bundles of different orders:

- 1st order bundles central and 3rd order bundles abaxial 435*
- 3rd order bundles central and 1st order bundles displaced adaxially in ribs 436*
- All bundles arranged irregularly and inconsistently 437*
- Bundles of all orders abaxial and an adaxial superposed, often amphivasal bundle in each rib 438*

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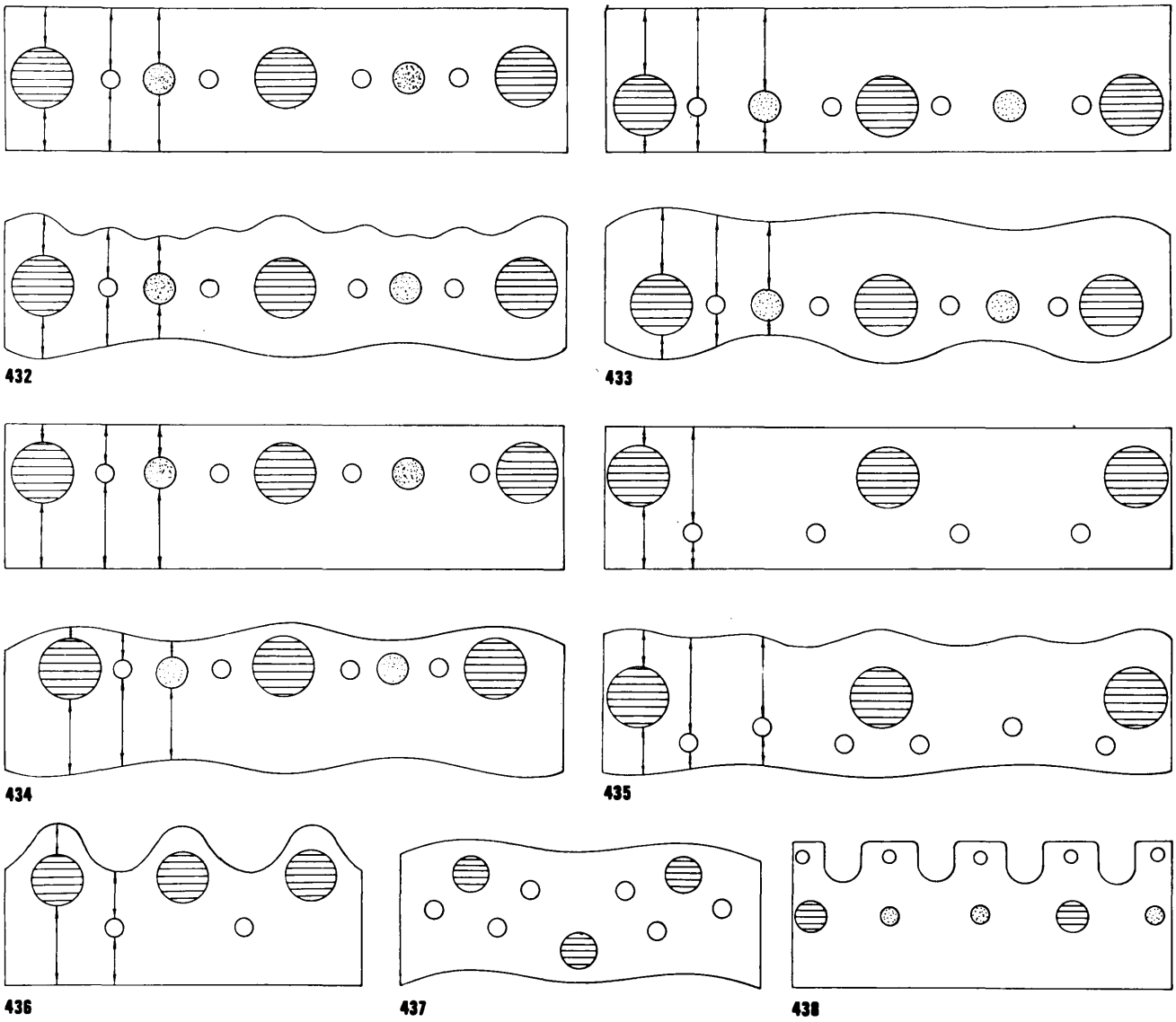
MIDRIBS AND KEELS



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VASCULAR BUNDLE ARRANGEMENT



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5. VASCULAR BUNDLE DESCRIPTION

Vascular bundles are considered to consist solely of the xylem and phloem tissue. For the purposes of describing the shape of the vascular bundles the bundle sheaths are not included as being part of the bundles. Thus, when a bundle is described as being circular or angular in outline, these terms refer only to the vascular tissue and exclude the inner or single bundle sheath.

DESCRIPTION:

Third order vascular bundles; usually very small bundles often with xylem and phloem indistinguishable and consisting of only a few lignified cells and a few phloem elements; when not obviously smaller than basic type bundles often distinguishable by the absence of sclerenchyma strands and/or the presence of bulliform cell groups adaxially:

- No third order bundles present in section 501
- *Shape* of third order bundles in section:
  - Rounded in outline; usually with many small parenchyma sheath cells:
    - Circular in outline 502*
    - Elliptical; vertically elongated 503*
  - Angular in outline:
    - Square-shape or pentagonal i.e. surrounded by a sheath of 4 or 5 large parenchyma cells 504*
    - Hexagonal or octagonal i.e. surrounded by 6 or more relatively small parenchyma cells 505*
    - Triangular in outline 506*
    - Vertically elongated; tall and narrow 507*
- *Nature* of vascular tissue of third order bundles:
  - Xylem and phloem groups distinguishable 508*
  - Vascular tissue consists of only a few vascular strands 509*

Second order vascular bundles; xylem and phloem easily distinguishable; bundles usually fairly large, often of similar size to the first order bundles; no conspicuously large metaxylem vessels or lysigenous cavities present; sclerenchyma arrangement usually the same as for first order bundles:

- No second order bundles present in section 510
- *Shape* of second order bundles in section:
  - Rounded in outline; usually with many small parenchyma sheath cells:
    - Circular in outline 511*
    - Elliptical; vertically elongated 512*
  - Angular in outline; parenchyma sheath of relatively few large cells; inner mestome sheath may also be present:
    - Square-shaped or pentagonal 513*
    - Hexagonal or octagonal 514*
    - Triangular in shape; often rather large 515*
    - Tall and narrow in outline; vertically elongated; bundles with straight vertical sides 516*

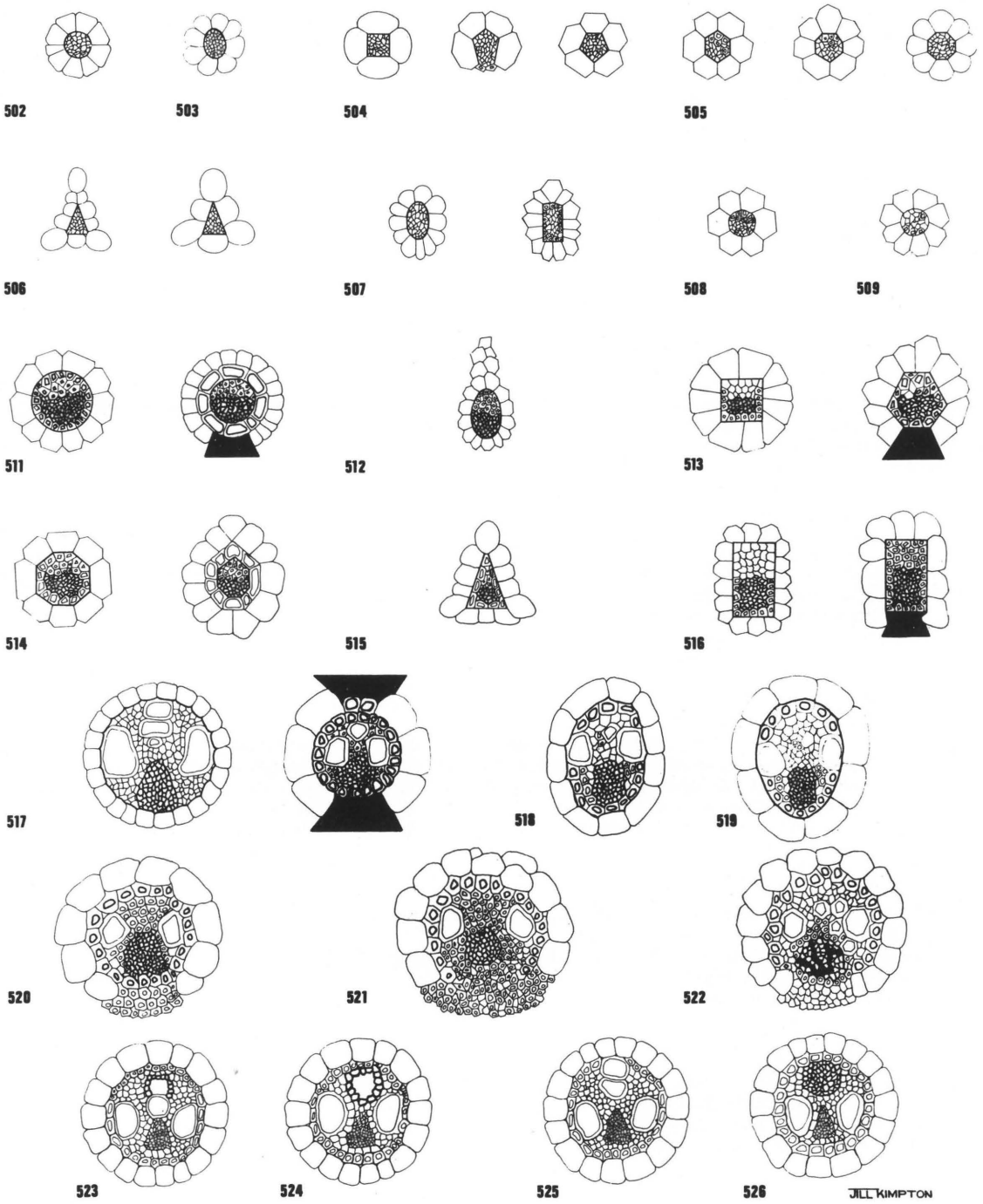
First order or **Basic type** vascular bundles; large metaxylem vessel present on either side of protoxylem elements; lysigenous cavity commonly present; associated with sclerenchyma girders or strands:

- *Shape* of first order bundles in section:
  - Circular or round in outline 517*
  - Elliptical; vertically elongated 518*
  - Egg-shaped; broadest side adaxial 519*
- *Relationship* of phloem to vascular fibres:
  - Phloem adjoins the inner or parenchyma sheath 520*
  - Phloem completely surrounded by thick-walled fibres 521*
  - Phloem divided by intrusion of small fibres resulting in sclerosed phloem 522*
- *Nature* of lysigenous cavity and protoxylem:
  - Lysigenous cavity and enlarged protoxylem vessel present 523*
  - Lysigenous cavity but no protoxylem vessel present 524*
  - Enlarged protoxylem vessel present but no lysigenous cavity 525*
  - No lysigenous cavity or protoxylem vessel present 526*
- *Size* of metaxylem vessels in relation to parenchyma sheath cells in T/S:
  - Narrow vessels i.e. parenchyma sheath cells wider than vessels 527
  - Wide vessels i.e. vessels with width equal to or slightly greater than parenchyma sheath cells 528
  - Very wide vessels i.e. width of vessels very much more than that of parenchyma sheath cells 529
- *Shape* of metaxylem vessels as seen in T/S:
  - Angular in T/S 530
  - Circular in T/S 531
- Thickening of walls of metaxylem vessels as seen in T/S:
  - Unthickened; secondary wall barely visible 532
  - Slightly thickened; intermediate between unthickened and distinctly thickened state 533
  - Distinctly thickened; inner secondary wall distinct 534

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VASCULAR BUNDLE DESCRIPTION



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6. VASCULAR BUNDLE SHEATHS

Includes the single or double sheath completely or partially surrounding each vascular bundle and adjoining the mesophyll. May also include adaxial or abaxial extensions of parenchyma cells which are considered to be part of the bundle sheath and not part of the system of colourless parenchyma. The different orders of vascular bundles may be surrounded by sheaths of different structure and thus the sheaths associated with the different orders of vascular bundles are considered individually.

ORDERS OF VASCULAR BUNDLE present in section; bundle sheaths of each order of vascular bundle present completely described before continuing with the descriptions of the sheaths of the other orders of vascular bundle present:

- Third order vascular bundle sheaths; describe fully: . . . 601
- Second order vascular bundle sheaths; describe fully: . . . 602
- First order vascular bundle sheaths; describe fully: . . . 603

DESCRIPTION. A complete description is given separately for each order of vascular bundle present:

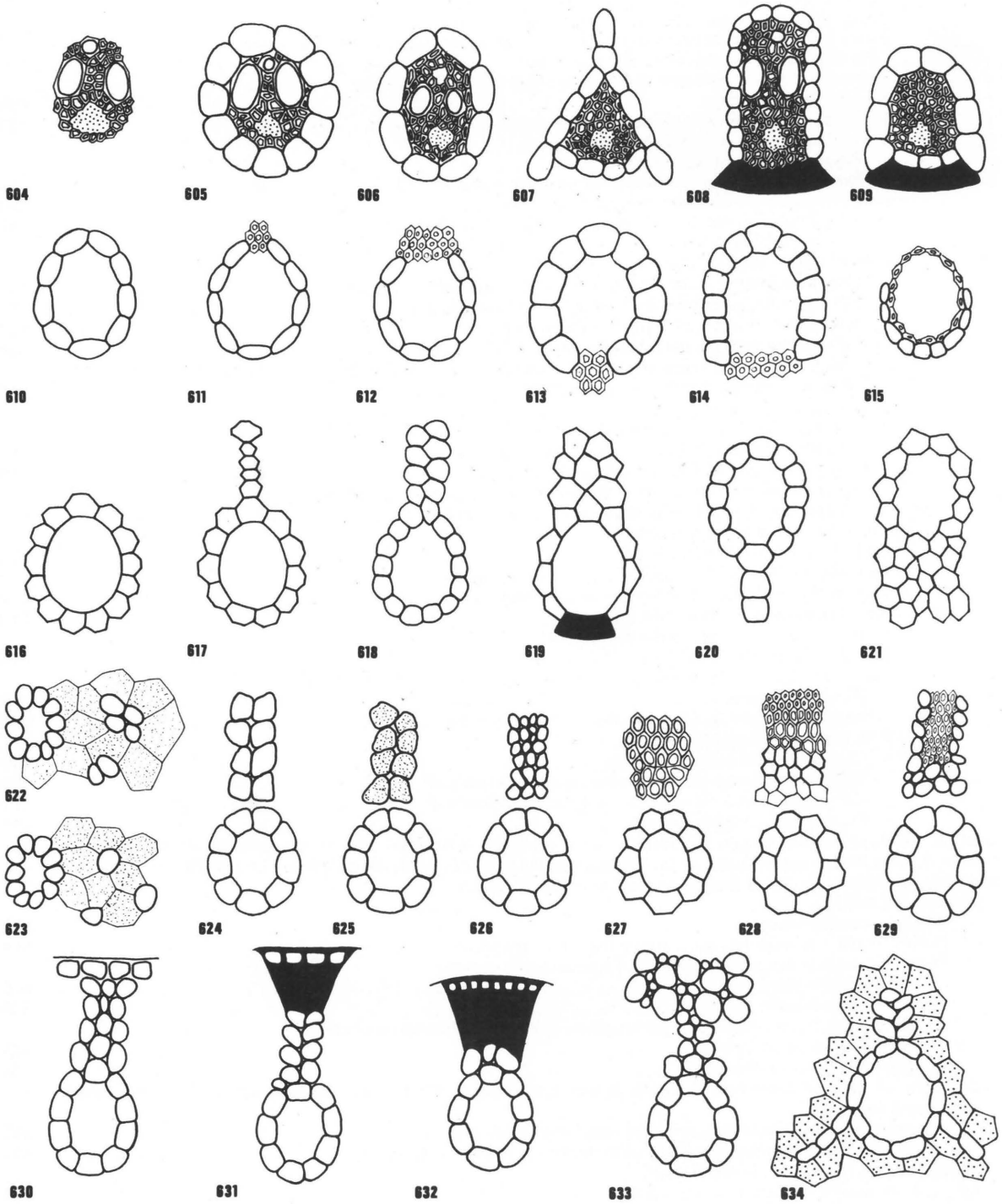
- **No** bundle sheaths surrounding the vascular bundles . . . 604*
- **Outer** or **Single** bundle sheath; parenchyma sheath; includes vascular bundles with single and those with double sheaths:
  - *Shape* of bundle sheath:
    - Sheath round, circular . . . 605*
    - Sheath elliptical, vertically elongated . . . 606*
    - Sheath triangular with adaxial apex . . . 607*
    - Sheath tall and narrow with vertical sides . . . 608*
    - Sheath horse-shoe shaped . . . 609*
  - *Extent* of bundle sheath:
    - Sheath complete; completely surrounding the bundle . . . 610*
    - Sheath incomplete due to interruption of sclerenchyma girders of various sizes:
      - Adaxial interruption:
        - Slight interruption caused by a narrow girder of 1 – 3 fibres wide . . . 611*
        - Wide interruption caused by broad girder of more than 3 fibres wide or by colourless parenchyma . . . 612*
      - Abaxial interruption:
        - Slight interruption caused by a narrow girder of 1 – 3 fibres wide . . . 613*
        - Wide interruption caused by broad girder of more than 3 fibres wide or by colourless parenchyma . . . 614*
    - Sheath reduced to two lateral strips at the sides of the phloem; adaxial interruption not due to sclerenchyma girders . . . 615*
  - *Extensions* of the bundle sheath; comprised of parenchyma cells associated with the sheaths and not part of the bulliform groups; extend to adjacent sclerenchyma girders or strands or to the epidermis:
    - No extensions of the bundle sheath present . . . 616*
    - Nature of the extensions:
      - Adaxial extension of the bundle sheath:
        - Narrow extension:
          - Uni-seriate; consisting of one column of cells . . . 617*
          - Bi-seriate; consisting of two columns of cells . . . 618*
        - Broad extension; tri- or multi-seriate . . . 619*
      - Abaxial extension of the bundle sheath:
        - Narrow extension; uni- or bi-seriate . . . 620*
        - Broad extension; tri- or multi-seriate . . . 621*
      - Distinctive cells similar to those of the parenchyma sheath found scattered in the mesophyll:
        - In groups of 2 – 6 with vascular tissue lacking . . . 622*
        - Solitary . . . 623*
    - Composition of the extensions:
      - Consist of large, thin-walled colourless cells; the same size or bigger than sheath cells . . . 624*
      - Consist of large, thin-walled cells with chloroplasts; the same size or bigger than sheath cells . . . 625*
      - Consist of relatively small, thin-walled colourless cells; smaller than the sheath cells . . . 626*
      - Consist of thickened parenchyma cells . . . 627*
      - Consist of colourless cells gradually decreasing in size as walls increase in thickness and eventually merge into the sclerenchyma strand . . . 628*
      - Consist of parenchyma cells on either side of sclerenchyma strands or girders . . . 629*
    - Extent of the extensions of the bundle sheath:
      - Extend to and in contact with the epidermis or an inconspicuous sclerenchyma strand (1 – 4 fibres) . . . 630*
      - Extend to conspicuous sclerenchyma strand . . . 631*
      - Extend to sclerenchyma strand almost extending to the bundle sheath . . . 632*
      - Extend to colourless subepidermal parenchyma . . . 633*
      - Extend into mesophyll tissue . . . 634*
    - Length of extension:
      - 1 – 2 cells long or deep . . . 635
      - 2 – 5 cells long or deep . . . 636
      - More than 5 cells long or deep . . . 637
  - *Number* of cells comprising the bundle sheath:
    - 4 parenchyma cells comprise the sheath . . . 638
    - 5 parenchyma cells comprise the sheath . . . 639
    - 6 parenchyma cells comprise the sheath . . . 640
    - 7 parenchyma cells comprise the sheath . . . 641
    - 8 parenchyma cells comprise the sheath . . . 642
    - 9 parenchyma cells comprise the sheath . . . 643
    - 10 parenchyma cells comprise the sheath . . . 644
    - 11 parenchyma cells comprise the sheath . . . 645
    - 12 parenchyma cells comprise the sheath . . . 646
    - 13 parenchyma cells comprise the sheath . . . 647
    - 14 parenchyma cells comprise the sheath . . . 648
    - 15 or more parenchyma cells comprise the sheath . . . 649

CONTINUE ON FOLLOWING PAGE AND THEN RETURN AND DESCRIBE SHEATHS OF ANY OTHER ORDERS OF VASCULAR BUNDLES THAT ARE PRESENT.

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VASCULAR BUNDLE SHEATHS



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6. VASCULAR BUNDLE SHEATHS CONTINUED

DESCRIPTION CONTINUED. A complete description is given separately for each order of vascular bundle present:

Structure of the parenchyma sheath cells; : cells of each order of vascular bundle considered separately and all cell types included:

- Not well differentiated from the chlorenchyma cells 650
- Distinct from chlorenchyma cells:
  - Shape of cells comprising sheath; include all shapes relevant for each order of vascular bundle:
    - Inflated, round, circular 651*
    - Radial walls straight; tangential walls inflated 652*
    - Radial and outer tangential walls straight; inner tangential wall inflated 653*
    - Radial and inner tangential walls straight; outer tangential wall inflated; fan-shaped 654*
    - Cells elliptical; elongated; often rather irregular 655*
  - Arrangement of cells of different shapes comprising the bundle sheath:
    - All cells similar in shape 656
    - Irregular; cells of different shapes comprise sheath 657
    - Two enlarged elongated cells abaxially situated:
      - Adjoining girder of sclerenchyma 658*
      - Normal parenchyma sheath cells between the elongated cells 659*
    - Cells at adaxial side of sheath elongated vertically; elliptical 660*
    - Sheaths of adjacent bundles continous with each other; *Triodia* type 661*
  - Size of cells comprising sheath:
    - Cells of sheath similar in size:
      - Large and inflated; generally larger than mesophyll cells; conspicuous 662*
      - Not markedly larger than the mesophyll cells; conspicuous 663*
      - Cells smaller than the mesophyll cells; inconspicuous 664*
      - Cells smaller than the inner bundle sheath cells 665*
      - Cells small; resemble cells of a mestome sheath i.e. thickened walls often especially the inner tangential walls; parenchyma sheath apparently absent 666*
    - Cells of sheath of various sizes:
      - Cells of different sizes comprise sheath; irregular 667*
      - Gradation in size with largest cells adaxially situated 668*
      - Gradation in size with largest cells abaxially situated 669*
      - Gradation with smallest cells in the centre; abaxial and adaxial cells larger 670*
      - Gradation with largest cells in the centre on each side 671*
  - Wall structure of bundle sheath cells:
    - Walls thin; no secondary thickening 672*
    - Walls slightly thickened; secondary cell wall not distinct 673*
    - Walls distinctly thickened; secondary wall distinct 674*
    - Inner tangential and radial walls thickened 675*
  - Chloroplast structure of the bundle sheath cells:
    - Translucent; sheath cells without chloroplasts 676
    - With small chloroplasts; not distinct from chloroplasts of the chlorenchyma:
      - Numbers of chloroplasts comparable to the mesophyll cells 677*
      - Numbers of chloroplasts fewer than in mesophyll cells 678*
    - With large, specialised chloroplasts:
      - Chloroplasts fill entire cell lumen 679*
      - Chloroplasts concentrated near the outer tangential wall 680*
      - Chloroplasts concentrated near the inner tangential wall 681*
      - Chloroplasts centrally situated 682*

Inner or **Mestome** bundle sheath; endodermis; only applicable when two sheaths are present; often difficult to distinguish from fibrous ground tissue of the vascular bundle; in contact with the metaxylem i.e. if metaxylem vessels in contact with parenchyma sheath then there is no inner bundle sheath:

Extent of sheath:

- No inner sheath present 683*
- Sheath complete; completely surrounding the xylem and phloem 684*
- Sheath incomplete due to interruptions of sclerenchyma girders:
  - Adaxial interruption 685*
  - Abaxial interruption 686*
- Sheath reduced; intermediate type; interruptions not due to sclerenchyma girders:
  - Abaxial; opposite phloem only 687*
  - Adaxial; opposite xylem only 688*

Structure of cells of inner bundle sheath in region adjacent to phloem; cells of each order of vascular bundle considered separately:

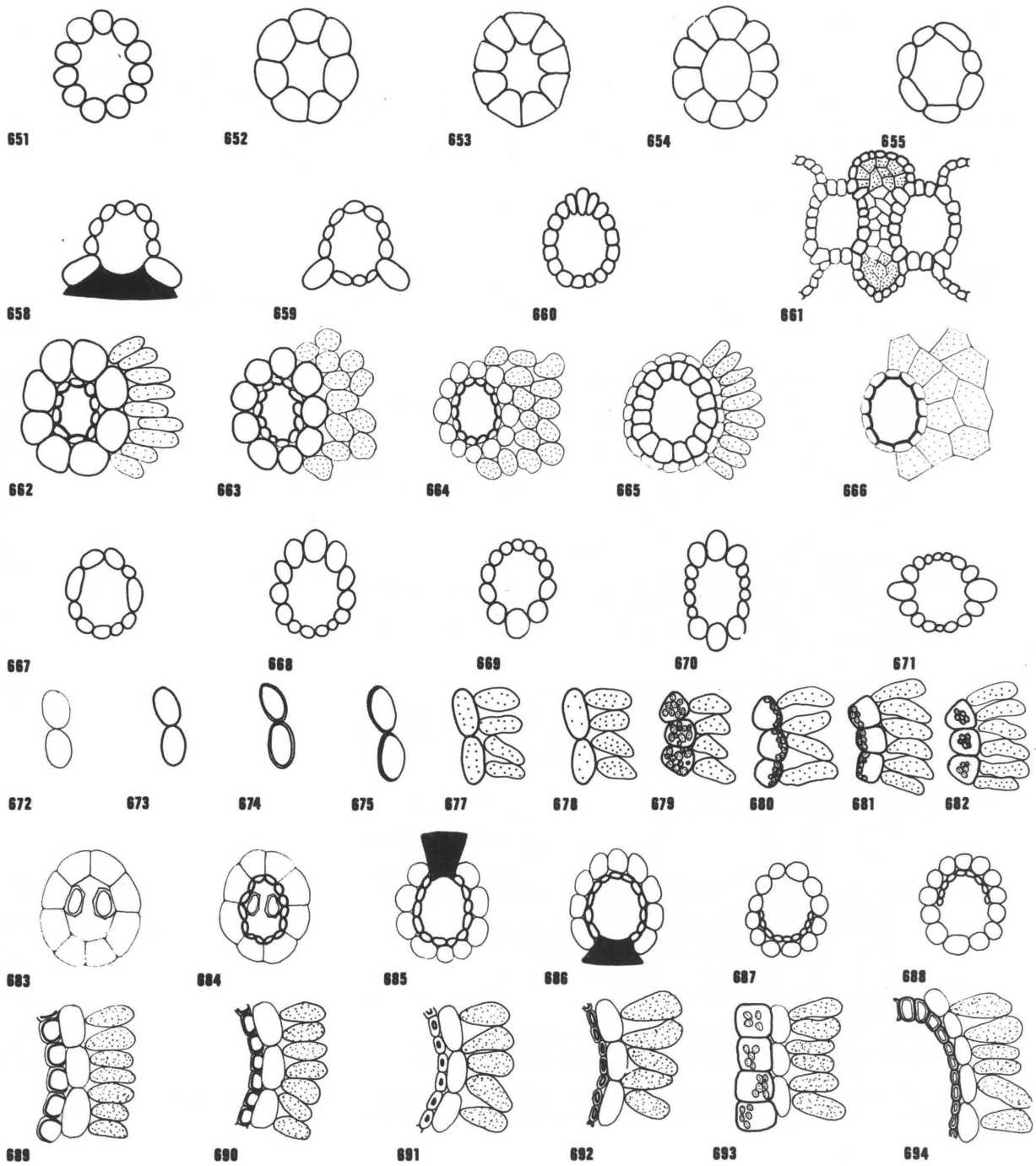
- Relatively large with inner tangential and radial walls thickened 689*
- Small, with inner tangential and radial walls thickened; U-shaped thickenings 690*
- Small, with uniformly thickened walls:
  - Walls heavily thickened; lumen small 691*
  - Walls not conspicuously thickened 692*
- Larger than outer bundle sheath; parenchymatous; with chloroplasts 693*
- Adaxially situated cells larger than lateral cells of the sheath 694*

N.B. SHEATH OF EACH ORDER OF VASCULAR BUNDLE PRESENT IN SECTION DESCRIBED COMPLETELY BEFORE CONTINUING WITH SCLERENCHYMA OF THE LEAF.

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VASCULAR BUNDLE SHEATHS



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7. SCLERENCHYMA OF LEAF

Includes all thickened fibrous tissue in the leaf, except that constituting part of the vascular tissue i.e. xylem and phloem fibres and thickened parenchyma. The sclerenchyma can be sub-divided into the circumvascular sclerenchyma surrounding, in contact with, or opposite the vascular bundles in the form of girders or strands, sclerenchyma found in the intercostal areas between successive bundles, sclerenchyma of the leaf margin and sclerenchyma of acicular leaves. Sclerenchyma associated with the midribs or keels included under midribs and keels.

SCLERENCHYMA STRANDS AND GIRDERS

Circumvascular sclerenchyma surrounding, in contact with, or opposite the bundles; refers only to longitudinal subepidermal sclerenchyma following the course above and below the vascular bundles. Complete description is given of the circumvascular sclerenchyma associated with each of the different orders of vascular bundle present in the leaf.

ORDERS OF VASCULAR BUNDLES present in leaf section; sclerenchyma associated with each order of vascular bundle present in section considered separately and each is completely described before commencing with other orders that might be present:

- Sclerenchyma associated with third order vascular bundles 701
- Sclerenchyma associated with second order vascular bundles 702
- Sclerenchyma associated with first order vascular bundles 703

DESCRIPTION

ADAXIAL sclerenchyma; sclerenchyma found above the vascular bundles; associated with the bundles:

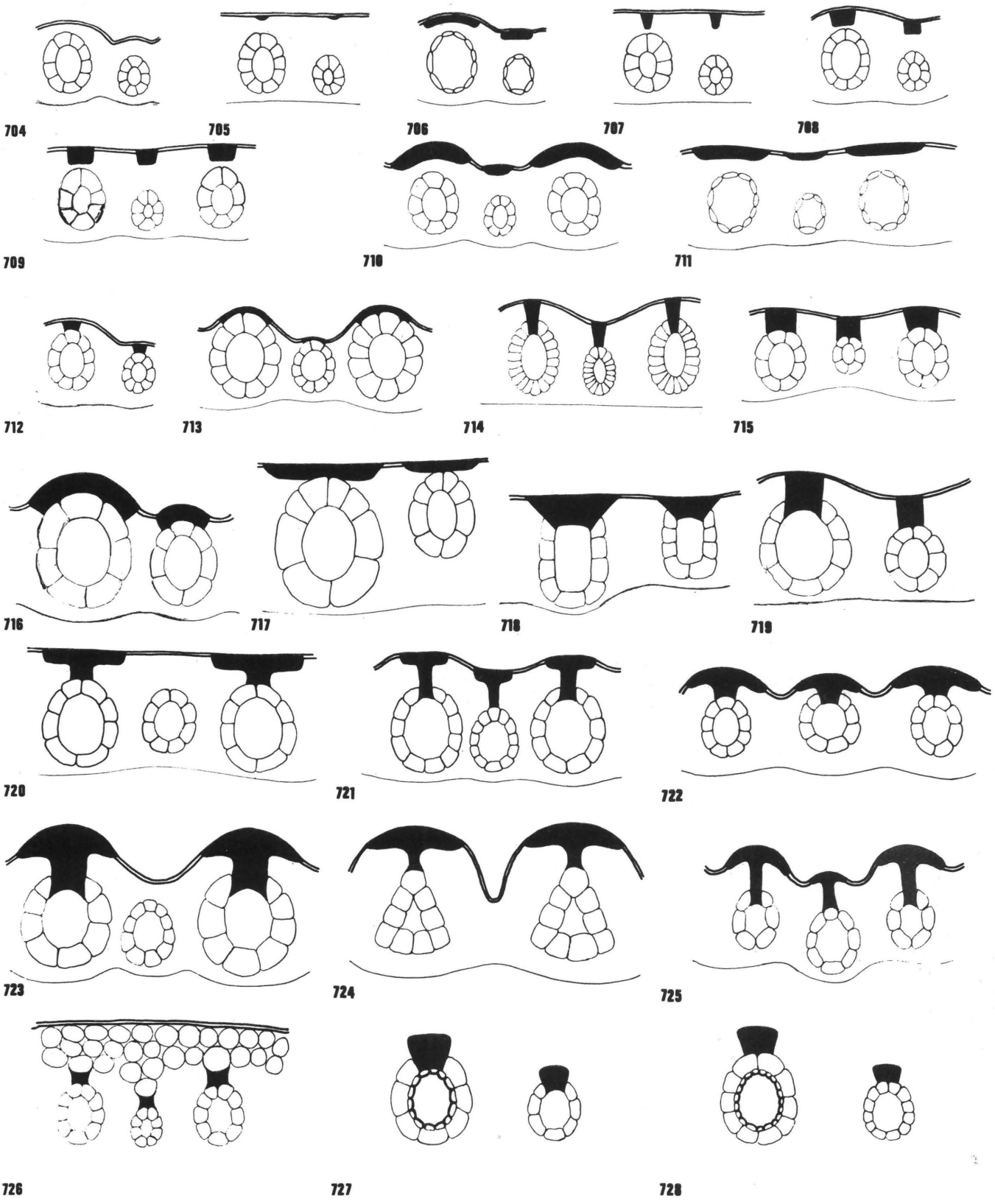
- No adaxial sclerenchyma present 704*
- *Strand* present; sclerenchymatous; not in contact with the bundle sheath; separated by parenchyma or mesophyll from sheath:
  - Minute strand consisting of only a few subepidermal fibres 705*
  - Small strand; inconspicuous; epidermal cells unaltered over the strand:
    - Shallow, forming a strip; only 2 – 4 fibres deep; subepidermal 706*
    - Narrow; only 2 – 4 fibres wide 707*
    - As deep as wide as seen in T/S 708*
  - Well-developed strand; conspicuous; epidermal cells over strand usually small and thick-walled:
    - As deep as wide as seen in T/S 709*
    - Wider than deep as seen in T/S; in form of band:
      - Arched; follows the shape of the adaxial rib 710*
      - Straight, horizontal band 711*
- *Girder* present; sclerenchymatous; in contact with, or interrupts the bundle sheath:
  - Very small girder; inconspicuous; epidermal cells usually unaltered over girder 712*
  - Small girder; epidermal cells over the girder usually small and thick-walled:
    - Small thin subepidermal strip 713*
    - Narrow; deeper than wide 714*
    - As deep as wide as seen in T/S; equidimensional 715*
  - Well-developed girder; conspicuous; epidermal cells over girder small and thickened:
    - Relatively wide and deep band; as wide or wider than the vascular bundle:
      - Arched; follows the shape of the adaxial rib 716*
      - Straight; horizontal band 717*
      - Narrowing towards bundle 718*
    - Relatively narrow and deep girder; narrower than the vascular bundle 719*
    - T-shaped; horizontal band connected to bundle or bundle sheath by a stem:
      - Stem short; shorter than horizontal cross-piece 720*
      - Stem relatively long; as long or longer than horizontal cross-piece 721*
    - Inversely anchor-shaped; arched band follows shape of adaxial rib:
      - Stem short and sturdy; more than tri-seriate 722*
      - Stem long or tall and sturdy; more than tri-seriate 723*
      - Stem short and thin or narrow; 1 – 3 seriate 724*
      - Stem long or tall and thin or narrow; 1 – 3 seriate 725*
- *Girder* extends from bundle sheath to bulliform or colourless cells; not to epidermis 726*
- *Girder* description continued:
  - Fibres interrupt the cells of the single or outer bundle sheath 727*
  - Fibres in contact with the cells of the single or outer bundle sheath 728*

CONTINUE ON FOLLOWING PAGE AND THEN RETURN AND DESCRIBE STRANDS AND GIRDERS OF ANY OTHER ORDERS OF VASCULAR BUNDLES THAT ARE PRESENT IN THE SECTION.

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ADAXIAL SCLERENCHYMA OF LEAF



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7. SCLERENCHYMA OF LEAF CONTINUED

Continuation of circumvascular sclerenchyma surrounding, in contact with, or opposite the bundles; refers only to longitudinal subepidermal sclerenchyma following the course above and below the vascular bundles. Complete description is given of the circumvascular sclerenchyma associated with each of the different orders of vascular bundle present in the leaf.

ABAXIAL sclerenchyma; sclerenchyma found below the vascular bundles:
- No abaxial sclerenchyma present . . . 729*
- *Strand* present; sclerenchymatous not in contact with the bundle sheath; separated by parenchyma or mesophyll from sheath:
  - Minute strand consisting of only a few subepidermal fibres . . . 730*
  - Small strand; inconspicuous; epidermal cells unaltered under the strand:
    - Shallow, forming a strip; only 2 – 4 fibres deep; subepidermal . . . 731*
    - Narrow but fairly deep; only 2 – 4 fibres wide . . . 732*
    - As deep as wide as seen in T/S . . . 733*
  - Well-developed strand; conspicuous; epidermal cells under strand usually small and thick-walled:
    - As deep as wide as seen in T/S . . . 734*
    - Wider than deep as seen in T/S; in form of a band:
      - Arched, follows the shape of the abaxial rib . . . 735*
      - Straight, horizontal band . . . 736*
- *Girder* present; sclerenchymatous in contact with, or interrupts the bundle sheath:
  - Very small girder; inconspicuous; epidermal cells usually unaltered under girder . . . 737*
  - Small girder; epidermal cells under the girder usually small and thick-walled:
    - Small, thin subepidermal strip . . . 738*
    - Narrow, deeper than wide . . . 739*
    - As deep as wide as seen in T/S; equidimensional . . . 740*
  - Well-developed girder; conspicuous; epidermal cells under the girder usually small and thickened:
    - Relatively wide and deep band; as wide or wider than the vascular bundle:
      - Arched, follow the shape of the abaxial rib . . . 741*
      - Straight, horizontal band . . . 742*
      - Narrowing towards bundle; triangular or trapezoidal . . . 743*
    - Relatively narrow and deep girder; narrower than the vascular bundle . . . 744*
    - Inverted T-shaped; horizontal band connected to bundle or bundle sheath by stem:
      - Stem short; shorter than horizontal cross-piece . . . 745*
      - Stem relatively long; as long or longer than horizontal cross-piece . . . 746*
    - Anchor-shaped; arched band follows shape of abaxial rib:
      - Stem short and sturdy; more than tri-seriate . . . 747*
      - Stem long or tall and sturdy; more than tri-seriate . . . 748*
      - Stem short and thin or narrow; 1 – 3 seriate . . . 749*
      - Stem long or tall and thin or narrow; 1 – 3 seriate . . . 750*
    - Continous or almost continuous abaxial hypodermal band . . . 751*
- *Girder* extends from bundle sheath to bulliform or colourless cells; not to the epidermis . . . 752*
- *Girder* description continued:
  - Fibres interrupt the cells of the single or outer bundle sheath . . . 753*
  - Fibres in contact with the cells of the single or outer bundle sheath . . . 754*

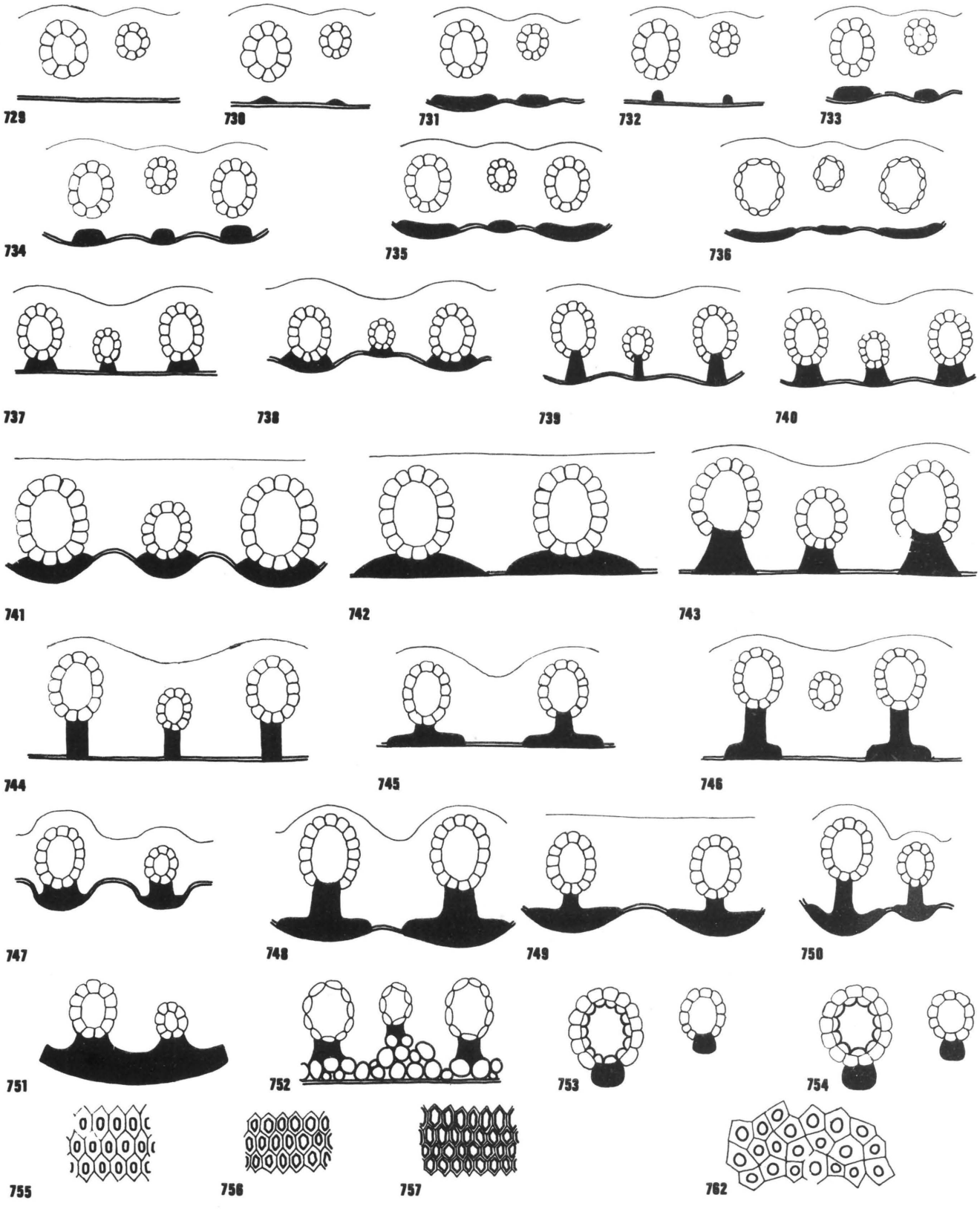
N.B. CIRCUMVASCULAR SCLERENCHYMA OF EACH ORDER OF VASCULAR BUNDLE PRESENT IN SECTION DESCRIBED SEPARATELY BEFORE COMMENCING WITH SCLERENCHYMA CELL STRUCTURE AND OTHER SCLERENCHYMA TISSUE OF THE LEAF.

SCLERENCHYMA CELL STRUCTURE:
- *Fibres,* circumvascular sclerenchyma comprised of thickened; elongated fibres:
  - Cell wall structure:
    - Very thick walled; lumen almost excluded . . . 755*
    - Cell walls of fibres of medium thickness; secondary wall distinct . . . 756*
    - Not markedly thickened; secondary wall not thick . . . 757*
  - Cell wall composition:
    - All cell walls lignified i.e. stain red with saffranin . . . 758
    - All cell walls of cellulose i.e. stain blue-green with fast green . . . 759
    - Some sclerenchyma cell walls lignified others with cellulose walls:
      - In individual girder or strand groups . . . 760
      - In different parts of the leaf section . . . 761
- *Collenchyma;* thickened parenchyma cells; wide lumens . . . 762*

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ABAXIAL SCLERENCHYMA OF LEAF



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7. SCLERENCHYMA OF LEAF CONTINUED

Continued from previous page and in contrast to the circumvascular sclerenchyma the types of sclerenchyma described below are not described in relation to the different orders of vascular bundles.

SCLERENCHYMA BETWEEN BUNDLES:
Supernumary strands not associated with the vascular bundles but situated intermediate between them, usually beneath the abaxial epidermis opposite the bulliform cell groups or furrows. Excludes sclerenchyma at the leaf margin and of the keel or midrib.
DESCRIPTION:

- *No* sclerenchyma present between the vascular bundles 763
- *Abaxial strands* of sclerenchyma fibres present:
  - Opposite adaxial furrows with or without bulliform cells 764*
  - Opposite bulliform cell groups not associated with furrows 765*
- *Hypodermal band;* sclerenchyma in form of more or less continuous abaxial band:
  - Extensions of girder or strand groups 766*
  - Hypodermal layer continuous 767*

SCLERENCHYMA IN LEAF MARGIN:
Includes all mechanical tissue situated in, or directly associated with the margin of the lamina. May incorporate or fuse with the ultimate, the penultimate or other lateral bundles.
DESCRIPTION:

- **No** sclerenchyma developed in association with the margin 768
- **Cap** of sclerenchyma at the margin; not in contact with the lateral bundle:
  - *Size* of cap:
    - Very small; consists of a couple fibres 769*
    - Relatively small; less than the width of a third order vascular bundle 770*
    - Well-developed; wider than third order vascular bundles 771*
  - *Shape* of cap:
    - Rounded cap 772*
    - Pointed cap 773*
    - Narrow, very pointed projection 774*
    - Crescent-shaped cap; sclerenchyma extends shortly along both abaxial and adaxial side of leaf 775*
    - Curved in shape with sclerenchyma extending along adaxial side of the leaf 776*
    - Curved in shape with sclerenchyma extending along abaxial side of the leaf 777*
  - *Epidermal* cells at margin:
    - Not fibrous; thin-walled or outer walls thickened; distinct from fibres 778
    - Small, fibrous; thickened on all walls; similar to fibres in T/S 779
  - *Angular* prickles of margin:
    - Enlarged bases present in margin 780
    - No prickle bases visible in margin 781
  - *Nature* of junction with mesophyll and remainder of lamina:
    - Adjoins normal mesophyll cells 782
    - Specialised enlarged parenchyma cells near cap or ultimate bundle 783
    - Lateral intercellular canal near margin; lysigenous duct not associated with the bundle 784
- **Hood** of sclerenchyma; hood-like structure formed by sclerenchyma extensions above or below ultimate and other lateral bundles; may or may not be in contact with bundles or bundle sheaths but must extend above or below or beyond ultimate lateral vascular bundle:
  - *Position* of fibrous hood:
    - Fibres extend along adaxial side of the leaf 785*
    - Fibres extend along abaxial side of the leaf 786*
  - *Extent* of fibrous hood:
    - Short hood; extends as far as or over ultimate lateral bundle 787
    - Well-developed hood; extends as far as or beyond penultimate bundle 788
- **Penultimate** bundle and associated sclerenchyma; may be no sclerenchyma development at the extreme margin but the penultimate lateral bundle may be intimately associated with specialized girders, strands or bands:
  - *Nature* of lateral fibrous groups:
    - Bundle sheath of penultimate bundle modified and cells are horizontally elongated and in contact with the normal sheath cells of the ultimate bundle 789*
    - Bundle sheath of penultimate bundle not modified but sclerenchyma continuous between the ultimate and penultimate bundles from adaxial to abaxial epidermis 790*
    - Well-developed adaxial girder or band developed above the penultimate bundle 791*
    - Extensive adaxial band developed above the lateral 3 or 4 bundles 792*

SCLERENCHYMA ARRANGEMENT IN ACICULAR LEAVES:

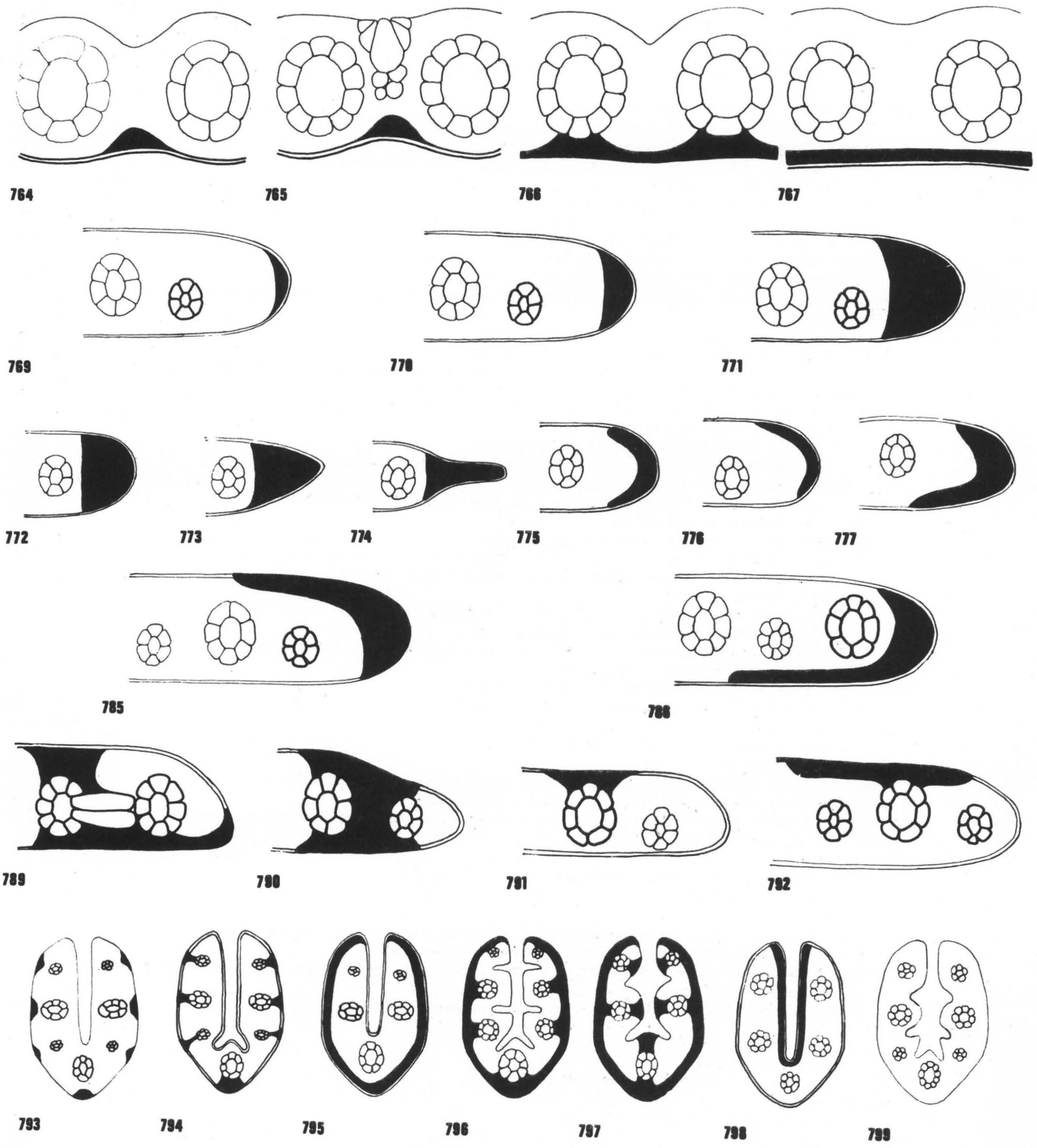
Refers to the sclerenchyma as seen in transverse section of permanently infolded leaves.
DESCRIPTION:

- Abaxial strands present opposite the vascular bundles 793*
- Abaxial girders present opposite the vascular bundles 794*
- Continuous, abaxial, subepidermal band; not connected to the vascular bundles 795*
- Continuous, abaxial, subepidermal band connected to bundles by girders 796*
- Continuous, abaxial, subepidermal band and girders extending to adaxial surface 797*
- Continuous, adaxial, subepidermal band not connected to the vascular bundles 798*
- No, or very poor sclerenchyma development 799*

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SCLERENCHYMA BETWEEN BUNDLES, AT MARGIN AND IN ACICULAR LEAVES



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8. MESOPHYLL OF LEAF

Applies to all the ground tissue between the adaxial and abaxial epidermides. It usually consists mainly of the assimilatory tissue or chlorenchyma but may be partly composed of translucent colourless cells. It does not include the bulliform cells (which are part of the epidermis) the sclerenchyma or the bundle sheaths or their extensions.

CHLORENCHYMA
All the tissue containing chloroplasts excepting the parenchyma bundle sheath which may or may not contain chloroplasts and is described under bundle sheaths. Does not include all the ground tissue between the adaxial and abaxial epidermides especially if any translucent cells without chloroplasts are present.

DESCRIPTION:

Radiate chlorenchyma; radially arranged around the vascular bundles which are often close together; panicoid; may be interrupted by sclerenchyma girders and thus divided into two groups on either side of the vascular bundle:
- *Number* of layers of chlorenchyma cells arranged around the bundles:
  - Single layer of cells:
    - Tabular 801*
    - Isodiametric 802*
  - Two layers of radiating cells 803*
  - Numerous layers of radiating cells; cells long and narrow; *Isachne* type 804*
- *Extent* of radiating cells around bundles:
  - Radiating cells completely surrounding bundles 805*
  - Interrupted above bundles by sclerenchyma or colourless parenchyma girders 806*
  - Interrupted below bundles by sclerenchyma or colourless parenchyma girders 807*
  - Reduced to two strips of chlorenchyma by large girders or colourless parenchyma 808*
- *Relationships* of successive radiating mesophyll groups to each other:
  - Separated by bulliform and colourless cell groups:
    - Completely divided; colourless cells continuous to abaxial epidermis 809*
    - Adaxially divided; colourless cells in adaxial half of leaf 810*
  - Separated by irregular chlorenchyma and intercellular air-spaces 811*
  - Radiating cells of successive bundles adjoin one another 812*

Indistinctly or **incompletely radiate** chlorenchyma; intermediate between radiate type and that not arranged in a definite pattern:
- Continuous between bundles 813*
- Chlorenchyma divided by groups of colourless cells; tending to be radiate 814*

Irregular chlorenchyma; not radiate or arranged in a definite pattern; vascular bundles usually widely spaced; festucoid:
- *Vertical* arrangement in the mesophyll between successive vascular bundles:
  - Occupying the major or entire area between the adaxial and abaxial epidermides:
    - All cells of the chlorenchyma similar; homogenous:
      - Regular small cells; isodiametric; tightly packed 815*
      - Irregular; cells of different size and shape; often with intercellular air-spaces 816*
    - Cells pallisade-like in the adaxial chlorenchyma 817*
    - Chlorenchyma cells adjoining the bundles larger than those further removed 818*
  - Occupying the lower abaxial half of the leaf thickness 819
  - Occupying the lower abaxial third of the leaf thickness 820
  - Confined to small patches surrounding abaxial and adaxial grooves which contain stomata 821*
- *Horizontal* arrangement in the mesophyll between successive vascular bundles:
  - Continuous between bundles:
    - Strap-shaped; horizontally elongated; bundles widely spaced 822*
    - Tall and narrow groups; bundles close together 823*
    - U-shaped; occupying the sides and bases of furrows or around bulliform cell groups 824*
  - Mesophyll groups divided by colourless cell groups 825*

Arm cells present or comprise chlorenchyma; walls invaginated:
- Invaginations extend almost to opposite wall; cells divided into compartments 826*
- Invaginations relatively short 827*

Fusoid cells present in mesophyll; translucent, elongated narrow collapsed cells; cavity between fusoid cells usually only visible in T/S; bamboo type:
- Successive fusoid cells separated by a vertical column of chlorenchyma one cell wide 828*
- Successive fusoid cells separated by numerous chlorenchyma cells 829*

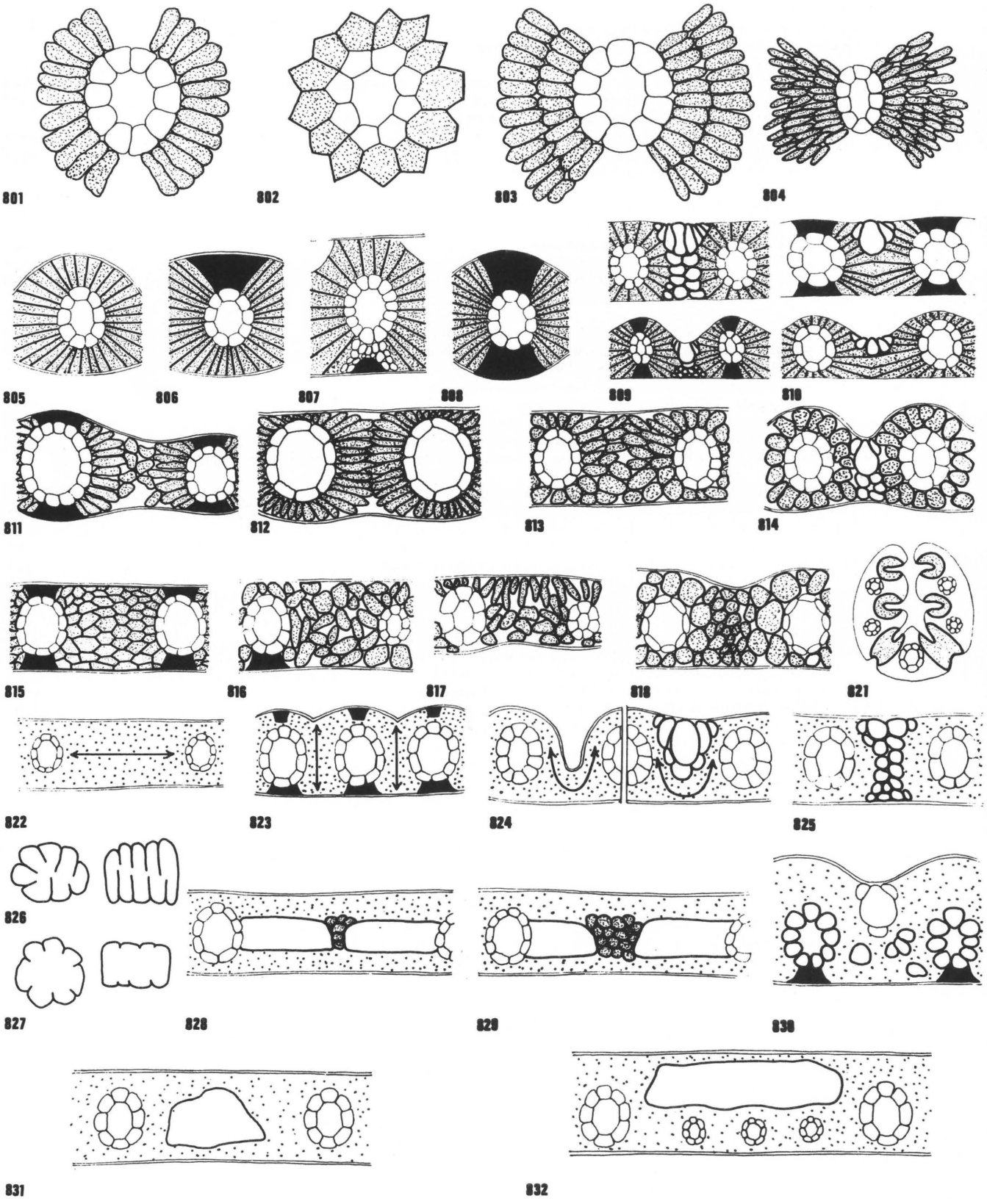
Distinctive cells, similar to parenchyma sheath cells but not associated with bundles present in mesophyll 830*

Lacunae or air spaces present in mesophyll:
- *Extent* of lacunae:
  - Between all or most adjacent vascular bundles 831*
  - Continuous air spaces above third order bundles 832*
- *Aerenchyma* associated with the lacunae:
  - Distinct lacunae bounded by compact mesophyll with no aerenchyma traversing the lacunae 833
  - Lacunae distinct but traversed by colourless aerenchyma cell chains 834
  - Irregularly defined air spaces in diffuse mesophyll with many intercellular spaces and chlorophyll-bearing aerenchyma; often subtending the stomata 835
  - Stellate cells representing a diaphragm interspersed by sclerotic strands present 836

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CHLORENCHYMA



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90 A PROCEDURE FOR STANDARDIZING COMPARATIVE LEAF ANATOMY IN THE POACEAE.
I. THE LEAF-BLADE AS VIEWED IN TRANSVERSE SECTION

8. MESOPHYLL OF LEAF CONTINUED

COLOURLESS CELLS

Translucent cells constituting the remaining cells in the zones between successive vascular bundles, excluding the chlorenchyma, the sclerenchyma and the bulliform cells. They vary in size and may be larger or smaller than the bulliform cells but are always without chlorenchyma and never part of the epidermis i.e. not in contact with the surface.

DESCRIPTION:

- **Absent**, no colourless cells present 837
- **Well defined** groups of colourless cells present:
  - *Closely* associated with inflated bulliform cells or groups:
    - Size of colourless cells:
      - Smaller than bulliform cells; uninflated; often same size as chlorencyma cells 838*
      - Similar in size or shape to bulliform cells; inflated 839*
    - Width of extension of bulliform cell group; composed of colourless cells:
      - Narrower than bulliform cell group 840*
      - Same width as bulliform cell group 841*
    - Number of extensions from each bulliform cell group:
      - 1 extension from each group 842*
      - 2 extensions from each group; one on either side of the vascular bundle 843*
    - Extent of intrusion into the mesophyll by colourless cells:
      - Girders extend to the opposite epidermis 844*
      - Strands not extending to the opposite epidermis 845*
      - Band occupying the adaxial half of the leaf composed of colourless cells 846*
  - *Not* closely associated with large bulliform cells:
    - Origin at the base of furrows:
      - Girder-like extension to the opposite epidermis between the bundles 847*
      - Girder-like extensions to the bundle sheath or towards the bundle sheath 848*
    - Origin on flat epidermis or with slight furrows:
      - Girder-like extension extends to the opposite epidermis:
        - Column of girder only uni- or bi-seriate 849*
        - Girder multi-seriate 850*
      - Girder of colourless cells does not extend to the opposite epidermis 851*
- **III defined** groups of smallish colourless cells; not associated with large or well defined bulliform groups; usually adaxial:
  - Adaxial half of leaf consisting entirely of colourless cells 852*
  - Abaxial half of leaf consisting mainly of colourless cells 853*
  - Present on adaxial side of small vascular bundles only 854*
  - Form arches over third order bundles and extend to the abaxial epidermis 855*
  - Abaxial irregular groups; may be associated with sclerenchyma strands 856*

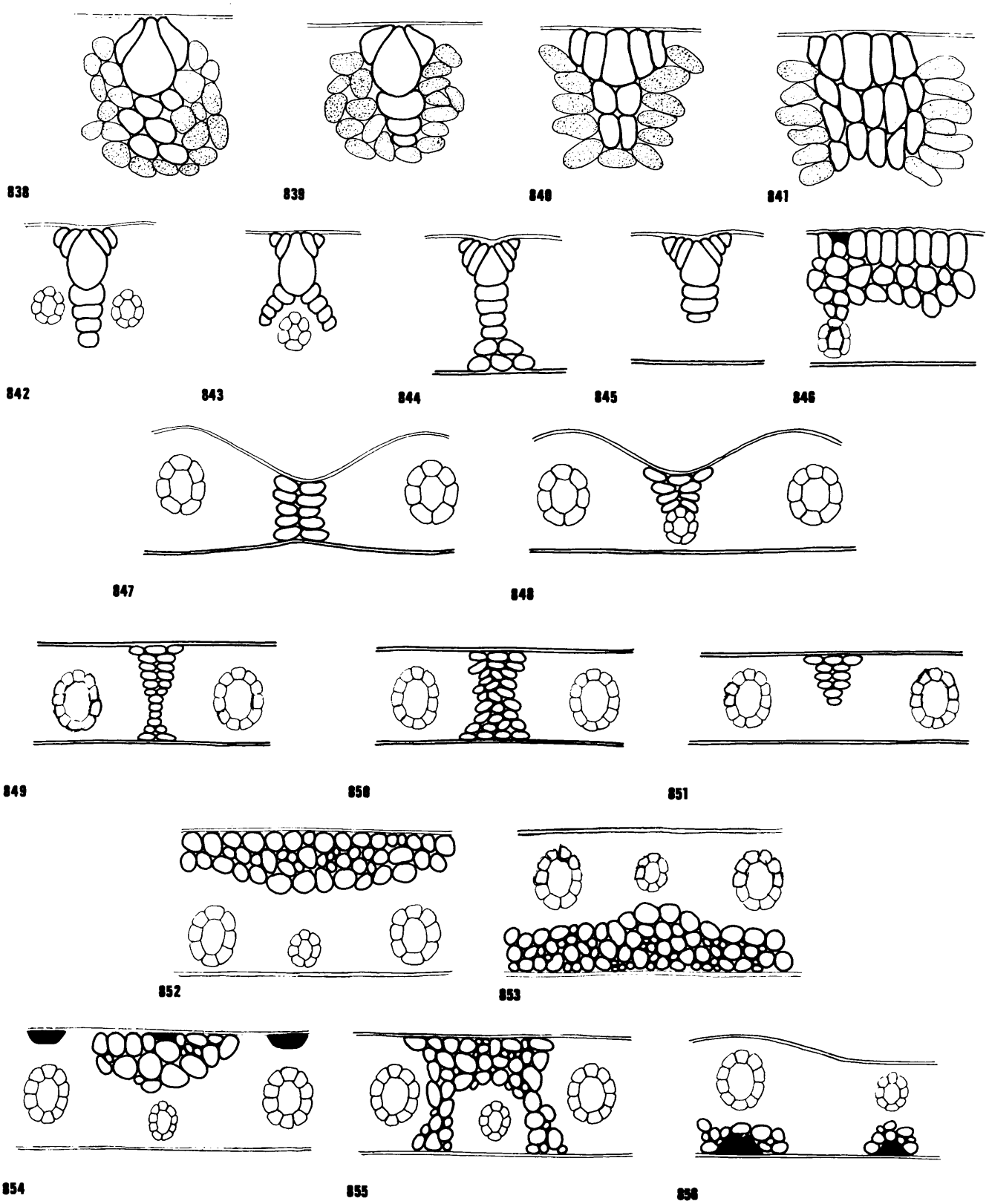
DESCRIPTION OF INDIVIDUAL CELLS:

- **Structure** of colourless cells:
  - Inflated, large parenchyma cells; resemble bulliform cells in size and shape 857
  - Smaller, thin-walled parenchyma cells; rounded in shape:
    - Cells regular in size and shape; tissue uniform in appearance 858
    - Cells irregular in size and shape; tissue irregular in appearance 859
- **Thick-walled** parenchyma or collenchyma cells 860

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COLOURLESS PARENCHYMA CELLS



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9. EPIDERMAL CELLS IN TRANSVERSE SECTION

Includes all cells and their appendages present in the epidermal layer. Can be subdivided into the bulliform cells and typical thickened epidermal cells.

ADAXIAL AND ABAXIAL EPIDERMIS of the leaf; cells of the upper and lower epidermis considered separately and the adaxial epidermis is fully described before commencing with the abaxial epidermis:

- Cells of adaxial epidermis 901
- Cells of abaxial epidermis 902

BULLIFORM CELLS

Colourless cells forming part of the epidermis but differing from the remaining epidermal cells by being markedly larger and inflated. Characteristically they are large and are restricted to groups often intimately associated with colourless cells but all gradations are found. Thus small solitary bulliform cells may be present or the epidermis may be comprised primarily of large, rounded and inflated cells. All these variations are included under bulliform cells for descriptive purposes. For bulliform cells of the keel see under midrib and keel.

DESCRIPTION: Adaxial and abaxial epidermides considered separately.

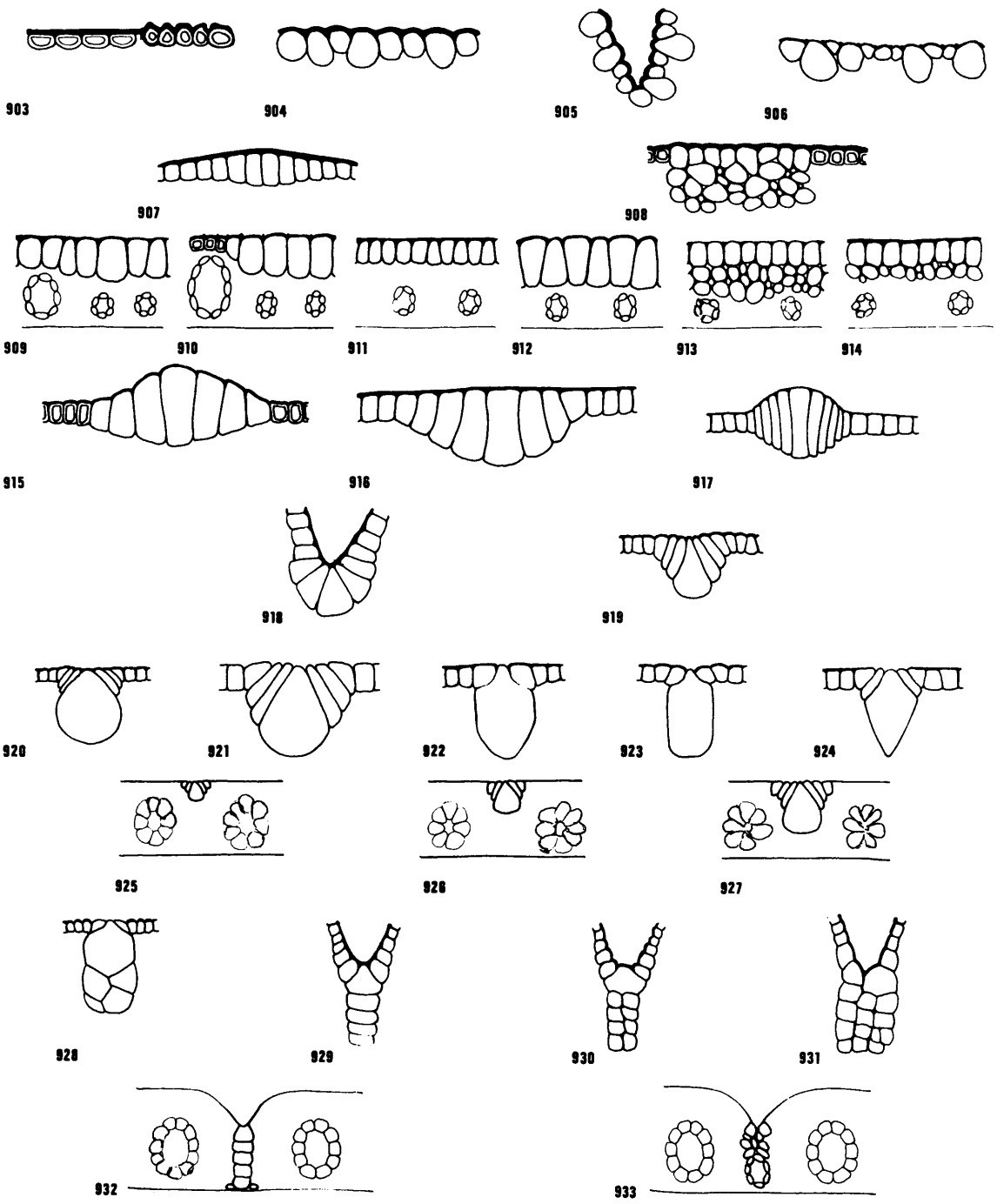
- **Absent,** no bulliform cells in epidermis being described; consists of small cells; often thickened 903*
- **Bulliform cells** only; not arranged in regular groups; epidermal cells rounded and inflated the same size or slightly larger than the cells of the bundle sheath:
  - All or most epidermal cells of this type 904*
  - Isolated cells larger; may comprise irregular small groups:
    - On sides of deep furrows 905*
    - Not in furrows; usually in leaves with flat surfaces 906*
- **Groups of bulliform cells** present in epidermis; well-defined and regular; distinct from normal epidermal cells:
  - *Small* bulliform cells; not conspicuously larger than the normal epidermal cells:
    - Not associated with colourless cells 907*
    - Closely associated with colourless cells; indistinct 908*
  - *Extensive* groups of large, inflated bulliform cells extending over one or many vascular bundles:
    - Not associated with colourless parenchyma:
      - Distribution of extensive bulliform groups:
        - Present throughout the epidermis; may be slightly reduced opposite the larger bundles 909*
        - Present in most of the epidermis but not present opposite the first order bundles and usually reduced over the second order bundles 910*
      - Size of constituent cells; taken in areas opposite the third order bundles:
        - Occupy less than $\frac{1}{4}$ of the leaf thickness 911*
        - Occupy more than $\frac{1}{4}$ of the leaf thickness 912*
    - Associated with colourless parenchyma cells together with which it forms a zone of colourless cells above or below the chlorenchyma; may be disrupted over the first order bundles:
      - Colourless zone comprises more than $\frac{1}{2}$ of the leaf thickness 913*
      - Colourless zone comprises less than $\frac{1}{2}$ of the leaf thickness 914*
  - *Restricted* groups of large, inflated bulliform cells; *Zea* type; parallel-sided cells i.e. inner tangential wall same length or only slightly shorter than the outer tangential wall:
    - Projecting above the level of the epidermis; cf. cushion cells of macro hairs 915*
    - Level with the general epidermal surface 916*
  - *Restricted* groups of tall and narrow bulliform cells; not inflated; parallel-sided cells or with outer tangential wall slightly shorter than the inner wall; lateral walls long and straight 917*
  - *Fan-shaped* groups; each cell of group with outer tangential wall shorter than inner tangential wall; median cell of group appreciably larger than the remainder:
    - Situated at bases of furrows 918*
    - Not found at bases of furrows or present in leaves without furrows; some cells parallel-sided 919*
  - *Resemble* fan-shaped groups due to central cell of group being larger and of a different shape than the rest which may be small by comparison; not necessarily with shorter inner tangential walls; *Sporobolus* type:
    - Shape of central cell of group which is always narrow at epidermis and often recurved or straight at area of contact with lateral bulliform cells of group:
      - Inflated, rounded; short area of contact with lateral cells 920*
      - Inflated, fan-shaped 921*
      - Narrower than deep; shield-shaped 922*
      - Elongated with parallel sides 923*
      - Elongated rather pointed base; diamond-shaped 924*
    - Size of central cell:
      - Relatively small; not much larger than bundle sheath parenchyma 925*
      - Occupy $\frac{1}{4} - \frac{1}{2}$ of the leaf thickness 926*
      - Occupy more than $\frac{1}{2}$ of the leaf thickness 927*
  - *Narrow* groups of bulliform cells and intimately associated large colourless parenchyma cells penetrating deep into the mesophyll; the contact with the adaxial epidermis made by the bulliform cell unclear; *Arundo* type 928*
  - *Bulliform* cells and closely associated colourless cells forming an extensive column or girder extending from the base of an adaxial furrow deep into leaf:
    - Nature of column or girder:
      - Column uni-seriate 929*
      - Column bi-seriate 930*
      - Column multi-seriate 931*
    - Position of column:
      - Extends from base of furrow to abaxial epidermis 932*
      - Extends from base of furrow towards a vascular bundle 933*

CONTINUE DESCRIPTION OF ADAXIAL EPIDERMIS OVERLEAF AND THEN RETURN AND DESCRIBE THE ABAXIAL EPIDERMIS.

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BULLIFORM CELLS



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9. EPIDERMAL CELLS IN TRANSVERSE SECTION CONTINUED

TYPICAL EPIDERMAL CELLS AND APPENDAGES

Includes all epidermal structures as seen in transverse section excluding the bulliform cells. Incorporates the cuticle, macro-hairs, prickles and papillae as seen in transverse section.

DESCRIPTION:

Cuticle and **Thickening** of epidermal cell walls:
- *Outer* tangential wall:
  - Outer walls not thickened and with a very thin cuticle 934*
  - Outer walls slightly thickened or with a thin cuticle 935*
  - Outer walls thickened and covered by a distinct, thick cuticle continuous over the epidermal cells:
    - Cuticle and cell wall equal to or greater than the depth of the average epidermal cells 936*
    - Cuticle and cell wall less than the depth of the average epidermal cells 937*
  - Outer tangential wall of each epidermal cell thickened individually:
    - Outer wall occupies half or more of the depth of the cells 938*
    - Outer wall occupies less than half of the depth of the cells 939*
    - Irregular; cells of different sizes comprise the epidermis 940*
- *Radial* and *Inner* tangential walls of epidermal cells:
  - All walls of epidermal cells thickened 941
  - Only outer wall thickened 942
  - No walls thickened 943

Macro-hairs as seen in transverse section; nature of hair bases described:
- *No* macro-hairs visible 944
- *Superficial bases;* not sunken into the leaf or embedded between inflated bulliform cells:
  - Epidermal cells not modified to form cushion cells associated with the base of the hair:
    - Swollen base not much larger than and situated between normal epidermal cells; included here are probably some elongated prickle hairs:
      - Hair short and thick 945*
      - Hair slender and elongated 946*
    - Constriction above bulbous base embedded in normal epidermal cells:
      - Hair very thin and slender 947*
      - Hair thickened and stiff 948*
  - Epidermal and other cells modified to form a cushion base:
    - Base surrounded by small thickened cells which may extend a short way up the hair:
      - Hair with enormous swollen base 949*
      - Base of hair not exceptionally large and swollen 950*
- *Sunken bases;* sunken in leaf or embedded between large, inflated epidermal cells or bulliform cells:
  - Constriction above bulbous base embedded between large epidermal cells:
    - Size and shape of hairs:
      - Hairs slender but relatively short 951*
      - Hairs thick and short 952*
      - Hairs long and very slender 953*
      - Hairs long and relatively thick 954*
    - Nature of bulliform or epidermal cells surrounding base:
      - Raised around hair forming a definite cushion 955*
      - Unraised bulliform cell groups 956*
  - Base bulbous and not constricted; embedded between bulliform cells 957*

Prickles and **Hooks** as seen in transverse section:
- *No* hooks or prickles visible 958
- *Small* hooks present in the epidermis; usually located in intercostal areas:
  - With short, curved barbs 959*
  - With straight, pointed and slender barbs:
    - Present between typical epidermal cells 960*
    - Present between inflated bulliform-like cells 961*
- *Thickened* prickles present in epidermis; usually located opposite the vascular bundles:
  - Pointed broad prickle; base not bulbous 962*
  - Small with short barb 963*
  - Bulbous base; barbed 964*
  - Bulbous base; no barb; asperite 965*

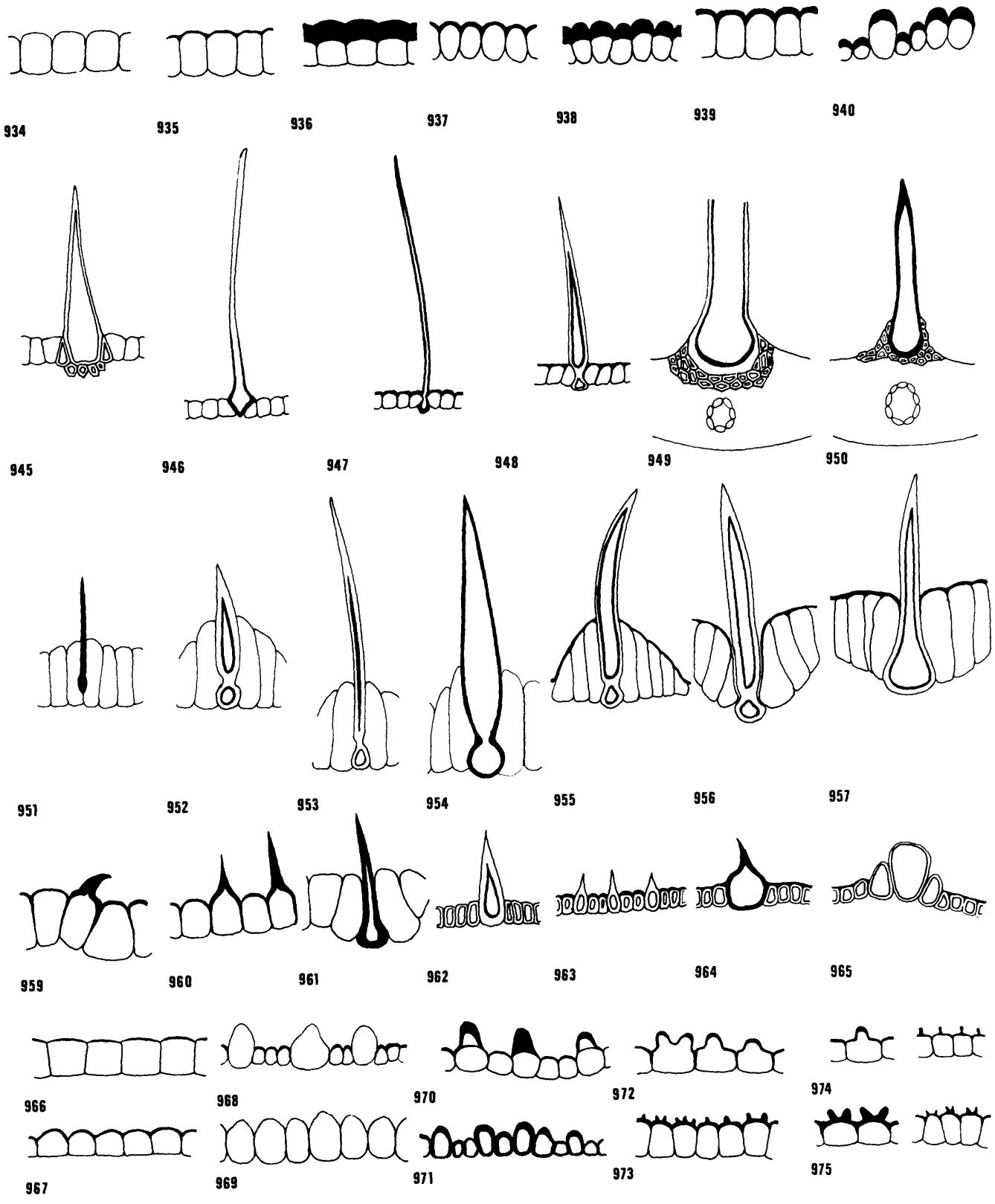
Papillae on the epidermal cells as seen in transverse section:
- *Shape* of outer tangential wall of the epidermal cells:
  - Outer walls of epidermal cells not arched to any marked degree; flattened 966*
  - Outer walls of epidermal cells arched but not papillose 967*
  - Arching of outer wall exaggerated; papillose; inflated papillae as wide as epidermal cells:
    - Thin-walled wide papillae scattered throughout the epidermis 968*
    - Entire or major part of epidermis composed of thin-walled wide papillae 969*
  - Papillae wide; as wide as or slightly narrower than epidermal cells; not inflated and thin-walled:
    - Distal, outer wall markedly thickened 970*
    - Papillus thickened and cuticular 971*
  - Papillae narrower than the epidermal cells:
    - Size of papillae:
      - Relatively broad but not much more than half the width of the epidermal cells 972*
      - Much less than half the width of the cell 973*
    - Number per cell as seen in transverse section:
      - One per cell 974*
      - Bifurcate; two per cell 975*

N.B. ADAXIAL AND ABAXIAL EPIDERMIDES DESCRIBED SEPARATELY.

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EPIDERMAL CELLS



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ANATOMICAL CHARACTERS

The following presents a brief conspectus of the nature of the various structures used in describing grass leaf anatomy, as well as their range of structural variation. Where relevant, an account is given of the function or development of these structures and the factors which contribute to their variability. Their importance as either taxonomic or diagnostic characters is stressed.

Outline of the lamina in transverse section

The foliage of the grass plant is comprised of the sheath, the ligule and the leaf-blade. In the following account, the leaf-blade, the expanded and free portion of the leaf as distinct from the sheath, is termed the lamina or the blade.

Outline of the lamina of open leaves

In many grasses with open leaf-blades the two halves of the lamina on either side of the median vascular bundle, midrib or keel, are relatively thin and wide, of equal width and usually symmetrically arranged about the median region. Infrequently, however, in certain species the portion of the lamina to one side of the midrib may not be symmetrical with that on the other half or perhaps the leaves may be relatively narrow and rather thick. These are exceptions to the general rule but are nevertheless useful for indentification purposes.

The lamina of most grass leaves in transverse section appears flattened and expanded and a straight line connects both margins and the median bundle or keel. However, expanded laminae may become variously altered by involution usually by becoming inrolled or infolded. Infolding results in the appearance becoming V- or U-shaped because the two lateral halves of the lamina tend to become folded adaxially towards each other on either side of the median vascular bundle which may, or may not, be adaxially grooved or incorporated into a keel. Grooves or keels serve to accentuate the V- or U-shape which characterizes many species. Inrolled leaves may appear variously U-shaped or may show either convolute inrolling from one margin only, or involute inrolling where the leaf-blade rolls inwards from both margins.

The degree of infolding or inrolling varies with the environmental conditions and thus is not of much value diagnostically (Metcalfe, 1960). However, leaves of grasses that are fully expanded under optimum conditions invariably exhibit a characteristic type of involution in response to environmental stress. Therefore, it is the nature, and not the degree, of the infolding or inrolling that is of importance taxonomically and diagnostically. Furthermore, it must be noted that some leaves remain flat on drying and in others the leaf folds inwards a little near the keel but the margins remain reflexed. Other responses are shown by the corrugations becoming more pronounced in species with plicate blades, and the production of filiform leaves in species which normally possess expanded blades. Further details of the involution process are included under bulliform and colourless cell function.

Expanded leaves of different species thus exhibit various, but constant, movements in response to adverse environmental change, the resultant shape being of specific or generic diagnostic value. Various members of the Andropogoneae (*Hyparrhenia, Cymbopogon* and *Dichanthium*) often show limited infolding near the keel with the margins remaining reflexed and several species of *Cymbopogon* produce both expanded and filiform leaves (Vickery, 1935). Some *Brachiaria* and *Oplismenus* species do not alter shape at all under conditions of water stress and remain flat and expanded. Certain grasses even show nyctinastic movements as described for *Leersia hexandra* by Goebel (Bor, 1960) in which the leaves are folded in dull weather but flatten in bright sunlight.

The variability exhibited by the leaf outline demonstrates the necessity for a sampling method which will adequately reveal the extremes of this variation. This should be achieved if adequate numbers of specimens of each species are collected, and fixed immediately, from different areas, over an extended period of time and from as many habitats as possible.

It appears reasonable to postulate that the type of folding adopted by the mature leaf in response to environmental conditions, as well as the symmetry of the leaf, may be correlated to the way in which the leaf is folded in the bud. Thus, Amedei (1932) has shown that *Chaetochloa palmifolia* (=*Panicum palmifolium*) is folded conduplicately in the bud and the mature leaf is more or less pleated depending on circumstances. The blades of grass leaves may be either folded, convolute or pleated in the bud (Bor, 1960). Therefore, the bud arrangements may simply be linked to the major types of involution exhibited in mature leaves except, perhaps, involute inrolling which is difficult to explain. Does it happen then, that a leaf which is convolute in the bud will exhibit definite V-shaped movements as a mature leaf instead of the expected convoluted inrolling? This possibility merits further investigation.

Apart from the characters of the shape of the outline, under optimum and adverse conditions, quantitative data of leaf width and thickness are also of value in describing and identifying leaves of different grasses. De Winter (1951) mentions the extreme thinness (about 0,05 mm) of the leaf of *Prosphytochloa prehensilis* (=*Potamophila prehensilis*) where the leaf is only about four cell layers thick. These quantitative characters are subject to considerable variation, and categories must be broadly defined to accommodate this variability. Detailed measurements have not been included in the keys and measuring is done by estimation of the leaf dimensions in relation to the known diameter of the field of view of the microscope used in the study. These broad categories are, nevertheless, useful for comparison with measurements given by other authors and can be easily modified as required.

Outline of permanently infolded leaves

In many grasses the lamina is very strongly and often permanently infolded or involuted. In such leaves the stomatal bands are invariably restricted to certain areas of the adaxial surface, usually on the sides of adaxial furrows within the channel. A thick cuticle and extensive sclerenchyma development is characteristic although this is not universally true as shown by Lewton-Brain (1904) for *Mibora minima* (=*Mibora verna*) which is entirely parenchymatous. However, these leaves can be distinguished by the fact that they are extremely narrow and permanently infolded to the extent that the internal structure is altered.

Numerous leaf-types defined on morphological criteria fall into this group. Examples are setaceous (wiry and bristle-like), filiform (thread-like), acicular (needle-like), junciform and cylindrical leaves. Anatomically these different types cannot be separated with any degree of certainty and a term inclusive of all permanently infolded leaves is required.

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Acicular leaves invariably fulfill the requirements of the definition of being permanently infolded to the extent that the internal structure is altered. In filiform leaves, often produced under adverse conditions by species which normally have open, expanded leaves, however, the blade is reduced to an enlarged midrib structure, with a mass of colourless parenchyma above the bundles and the lateral laminae being reduced or absent. Some species of *Cymbopogon* exhibit this reduction regularly. The extreme case of this specialization is seen in *Miscanthidium teretifolium* which is cylindrical in transverse section and the adaxial surface can be recognised only by the presence of a minute groove on the adaxial side of the cylinder (Metcalfe, 1960). In these cases, it must be stressed, the internal structural alteration of the leaf does not occur as a result of the leaf being permanently folded but rather as a result of loss of the lateral portions of the blade and a corresponding development of the midrib.

The cylindrical condition can probably arise by one of two different processes: the loss of the lateral regions and a concurrent development of the keel or by parenchyma development in a permanently infolded leaf excluding the adaxial channel. For convenience these two types of narrow leaf are included together under permanently infolded leaves. This further illustrates the need for a single term describing all reduced, permanently infolded and/or structurally altered grass leaves.

Transverse sections of these leaves often exhibit characters that are of taxonomic value because in assuming the permanently infolded, acicular form, the leaves of grasses of different affinities have not acquired precisely the same arrangement of vascular and mechanical tissue (Metcalfe, 1960). The outline and the shape of the adaxial channel and ribs and furrows may be distinctive as well. Burbidge (1946a) was able to separate ten species of *Triodia*, all possessing acicular leaves, using characters of the outline, the adaxial channel and adaxial and abaxial furrows. For descriptive purposes it is, therefore, necessary to describe the shape of the blade as outlined by the abaxial surface, to count the number of vascular bundles present in the section, to describe the adaxial channel and to include leaf width and thickness dimensions. Ribs and furrows and sclerenchyma are described in later "keys". With these descriptive criteria the differences present in permanently infolded leaves are of significant specific diagnostic value.

It is absolutely essential to standardize on the portion of the blade to be sectioned in permanently infolded leaves as the shape can vary from the base to higher up the leaf. Arber (1923, 1934) noticed that folded leaves may approach radial symmetry and become cylindrical in the apical regions. In *Triodia* (Burbidge, 1946a), towards the apex of the lamina the lateral veins disappear one by one, commencing with the marginal pair. There is a corresponding progressive reduction in the amount of mesophyll so that finally the pungent point is formed of the median vascular bundle surrounded by sclerenchyma. This progressive change, of many characters, along a single blade is equally important in open, tapering leaves and is the prime reason for standardizing on material from the central region of the lamina from ligule to tip.

In the cases of some narrow, folded leaves it is often debatable whether they in fact should be described under open or under permanently infolded leaves. In such cases it is perhaps safest to describe them

Longitudinal ribs and furrows

The adaxial and abaxial surfaces of the grass leaf blade may be either flat or longitudinally ribbed. The ribs are usually developed in association with and adjacent to the larger vascular bundles. They are generally characteristic of and more fully developed on the adaxial than the abaxial surface (Metcalfe, 1960). Ribs have also been termed ridges (Arber, 1934; Wilson, 1971) and if ribs are present there must be corresponding furrows between adjacent ribs. Furrow, in the context used here, is a term reserved exclusively for the depressed intercostal zone between adjacent ribs and which, with few exceptions, occur between the vascular bundles. If furrows are present in any other situation they are termed grooves to avoid possible confusion. Examples are the grooves present at the apex of the well-developed ribs of *Sporobolus artus* (Goossens, 1938) or those described immediately abaxial to the larger veins described by Sabnis (1921). In permanently infolded leaves the area bounded by the infolded lamina is characterized by being referred to as a channel.

In different species ribs and furrows may vary in height or depth, transverse shape, spacing and location. The ribs of a single grass blade can be of one or more distinct sizes or shapes, the ribs of each type being consistently associated with a specific order of vascular bundle. It is possible to measure the inclination of the ribs by measuring the angle formed by the sides of the two adjacent ribs at their base in the furrow (Wilson, 1971). Consequently, leaves with obtuse angles would have flatter surfaces than those with acute angles. The abaxial ribs may sometimes be taller than those of the adaxial surface. Where well-developed abaxial and adaxial ribs and furrows are similar and occur opposite each other the leaf section resembles a string of beads and is termed moniliform.

There are numerous examples of species of grasses possessing ribs or furrows that do not conform to the generalized definition outlined above. Lewton-Brain (1904) illustrates the enormous triangular ribs of *Aira caespitosa* where three vascular bundles are included in a single rib. *Glyceria fluitans*, another example illustrated by Lewton-Brain (1904), possesses low adaxial ribs situated between the vascular bundles which are situated beneath the 'furrows'. The ribs are occupied by copious air spaces. The flat-topped abaxial ribs of *Digitaria macroglossa* are exceptional because the ribs are in fact sclerenchyma caps and are not linked to the vascular bundles in any way.

Metcalfe (1960) comments on the correlation between prominent ribs and a marked capacity for involution, and states that this ability has been evolved in response to ecological conditions. Furthermore, this has occurred independently in various grasses and consequently leaves of this type are found in grasses between which there are no close taxonomic affinities. Rib development is nevertheless, often useful for specific diagnostic purposes and Gordon-Gray and Ward (1970) have contrasted the consistent flatness of the leaf surfaces of *Phragmites mauritianus* with the slight ribs developed on comparable surfaces of leaves of the closely related *P. australis*.

A paper by Wilson (1971) shows that the adaxial ribs of *Lolium perenne* appear to be under the control of a few genes as indicated by the rapid and immediate response to selection achieved in this study. Thus, in a species which appears to exhibit a wide range of phenotypic variation with respect to rib development, this variation may in actual fact be the direct manifestation of the genotype. This fact should be borne in mind when variation patterns of adaxial ribs are

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Median vascular bundles, midribs and keels

In order to eliminate any possibility of ambiguity arising from the use of these terms it is necessary to define them in the context that they are to be used here. The median vascular bundle is so termed only if the centrally situated vascular bundle of the leaf is structurally indistinguishable from the other basic or first order vascular bundles of the leaf, and if this median bundle is not associated with any parenchyma development or thickening of the leaf. On the other hand, a midrib is centrally positioned but structurally distinct from the other first order bundles with respect to size, bundle sheath, sclerenchyma girder or vascular structure and lacks associated parenchyma. When parenchyma or bulliform cells are developed in association with the median bundle or bundles, the whole structure, often incorporating many bundles, is termed a keel. The median bundle of a keeled leaf may be a midrib, and thus distinguishable from first order bundles, or a median vascular bundle, being indistinguishable from the other first order vascular bundles. A keel usually projects either abaxially, adaxially or both, but this is not necessarily so and in V-shaped leaves, unless there is a marked thickening in relation to the rest of the lamina, the median bundle alone is considered to comprise the keel, if it is accompanied by parenchyma.

This distinction of the three distinct types is considered necessary because in the past these terms have been interchanged by various workers. Metcalfe (1960) considers a projecting midrib, whether associated with parenchyma or not, to constitute a keel. Thus, no distinction is made between leaves with or without parenchyma in association with the median vascular bundle.

The size of the keel in an individual leaf varies, usually becoming progressively larger towards the base of the blade so that, in transverse section, the extent to which a keel is prominent depends on the level at which the section is taken. Therefore, the central part of the leaf must be used for comparative purposes.

Vickery (1935) states that, in addition to variation at different points along a single leaf, the size and degree of development of the keel varies tremendously in different leaves of the same species, so that the appearance in transverse section can only be of importance when taken in conjunction with the macroscopic appearance of the keels of a number of leaves. This is definitely not necessary in some groups and Fisher (1939) found the keel-size, amount of parenchyma and sclerenchyma and the bulliform cells—to be a valuable diagnostic character in separating four species of *Chloris*.

Keel structure, although subject to variation, is important diagnostically because the various types of keel that are recognised are easily distinguished except that the inconspicuous keel with one vascular bundle merges into that with three bundles. Thus, size may vary but shapes are constant and distinguisable. In addition there are numerous associated characters such as sclerenchyma, parenchyma and bulliform cells of the keel, air spaces and the nature of the adaxial side of the keel.

Bambusoid and oryzoid grasses display distinct keel structure with prominent lacunae, with or without diaphragms (Holm, 1896), and with a complex system of bundle arrangement. The keel is comprised of two or more vascular bundles distributed near both the abaxial and adaxial surfaces and sometimes in the interior as well. This characteristic structure has been discussed by various authors such as Holm (1892, 1895, 1896), Arber (1934), Jacques-Felix (1955), Schweickerdt & Marais (1956), Tateoka (1965) and Launert (1965). In addition to the Bambusae & Oryzeae, the Streptochaetae and *Prosphytochloa prehensilis* (=*Potamophila prehensilis*) in South Africa (de Winter, 1951) possess superposed adaxial bundles in the keel. Air spaces developing in the keels of older leaves are common in many other genera. *Sporobolus artus* var. *lysigenatus* and *S. pyramidalis* (Goossens, 1938) are examples.

Vascular bundle arrangement

The vascular bundles, or parallel longitudinal veins, are usually arranged in a single row embedded at various positions in the mesophyll as seen in section. Certain grasses, such as *Oryza coarctata* (Tateoka, 1963) and *Porteresia* (Tateoka, 1965a) have a normal abaxial vascular bundle in each rib, but in addition, a superposed, adaxial, amphivasal bundle is situated immediately anterior to each of the abaxial bundles. This type of arrangement is also found in the keels of some bambusoid and oryzoid grasses.

The arrangement most commonly found in grasses, is for all the bundles to be centrally positioned in the vertical plane of the blade. However, all bundles may be located closer to the abaxial or to the adaxial surface. Irregular arrangements, with bundles of different sizes or orders situated at different levels within the mesophyll are also found, In these instances, a regular pattern of arrangement of the different orders of bundles in the mesophyll is usually discernible. Cases where the bundles are located at irregular and inconsistent levels are rare, such as *Stipa tenacissima* (Metcalfe, 1960). This positioning of the vascular bundles in the blade appears to be a useful diagnostic character above the genus level that has been largely overlooked in the past.

The total number of vascular bundles in a section through a leaf blade taken halfway between the sheath and apex is inconsistent, varying with the width of the leaves. Little reliance can be placed on the total number of vascular bundles in a leaf as Vickery (1935) correctly stresses. She found that *Themeda avinacea* and *Cymbopogon exaltatus* may have flat expanded leaves 5–7 mm wide but under certain conditions filiform leaves consisting essentially of an enlarged keel are formed. This reduction in the width of the blade involves a reduction in the number of vascular bundles. Wider leaves do not necessarily have more vascular bundles than narrower leaves, however, as the bundles may be more or less crowded in different specimens of the same species. The total number of vascular bundles in a section is only considered to be useful in permanently infolded leaves, where there has been a reduction in the total number of vascular bundles.

The relative proportion and the alternation of the various sizes of vascular bundle along leaves of any one species of grass is remarkably constant. Slight variations in this respect may be found in leaves taken from different levels on a single plant, the variation being linked with differences in the width of the blades. For this reason basal leaves have been standardized upon for comparison. However, the proportion of first order bundles and smaller bundles in grasses belonging to different genera and species show marked differences.

The distribution patterns of bundles of different orders, and their variation, are important diagnostic characters. Thus, Holm (1891a) found a pattern of 1 first order bundle, 1 third order bundle, 1 second order bundle, 1 third order bundle, 1 first order

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bundle, etc., in *Uniola latifolia*. He notes that this formula is not strictly constant but that it gives the general features concerning the relative number and arrangement of bundles. For other species of *Uniola* he was unable to give a formula because of excessive variation, especially in leaves from different localities. Leigh (1960, 1961), in a study of a few *Eragrostis curvula* strains distinguished two groups both with a basic pattern of 3 or 4 second order bundles between successive first order bundles repeating itself either four or eight times along the width of the leaf.

The use of a formula to describe the pattern of arrangement of the various orders of vascular bundle is only suitable when a regular pattern from median bundle to margin is present. Often no pattern is obvious with progressively fewer first order vascular bundles and more third order vascular bundles nearer the margin or more first order vascular bundles and fewer second and third order vascular bundles laterally situated. For this reason the use of formulae to describe these patterns is not suitable. On the other hand, if a constant part of the blade is described, e.g. the central part of the lamina between the median bundle and the margin, these observations on bundle alternation will be comparable, even for leaves where the pattern changes from median bundle to margin.

Vascular bundle structure

Metcalfe (1960) visualizes the vascular bundle as consisting solely of the xylem and phloem elements, the bundle sheaths not being treated, for descriptive purposes, as if they constitute part of the bundle. For consistency and convenience this principle has been adopted in the present work and thus, when a bundle is described as being circular or angular in outline, these terms refer only to the outline of the xylem and phloem tissue.

The vascular bundles of the Poaceae are divided into three orders or ranks for descriptive purposes. These orders are not necessarily distinct but may intergrade and intermediate types are sometimes present. However, within a given leaf, three, and sometimes even four, classes of bundle are usually evident even although their structure may not conform exactly with that of the generalized definitions given below.

The three different orders in a single leaf are usually immediately evident because they differ in size. However, the relative diameters of the various orders cannot be used satisfactorily for the grass family as a whole, because equivalent orders of bundle differ in size and structure from one species to the next. Bowden (1964) classified bundles using the diameter as a criterion, but in species where all bundles are approximately the same size, but structurally different, this system is inadequate.

The first order bundles, or basic type (Metcalfe, 1960) are characterized by having a metaxylem vessel on either side of the protoxylem which may be non-functioning, and replaced by a lysigenous cavity or lacuna. A few well-developed protoxylem vessels may be present in addition to the lysigenous cavity, or both cavity and vessels may be absent. In the second order bundles the xylem and phloem are easily distinguishable, but the large metaxylem vessels are lacking as is the lysigenous cavity. The third order vascular bundles are often small bundles in which the xylem and phloem is reduced to a few elements or may even be indistinguishable using light microscopy.

In first order bundles the metaxylem vessels may appear to be paired, but these are merely the oblique overlapping ends of otherwise solitary vessel elements. Thus the number of metaxylem vessels in an individual bundle is unreliable as a diagnostic character because it varies with the level at which the sections are taken (Metcalfe, 1960). For this reason, only the size and shape of these vessels are mentioned in the descriptive keys.

The phloem of the first order vascular bundles may exhibit varying degrees of sclerosis. This is especially common in grasses from dry localities (Metcalfe, 1960). In *Triodia* (Burbidge, 1946a) and *Merxmuellera* (=*Danthonia*) (de Wet, 1960) the phloem may be divided into two or three groups by intrusion of small fibres. The phloem may adjoin the inner or parenchyma, bundle sheath or it may be completely surrounded by thick-walled fibres which isolate it from the xylem and the bundle sheath (Jefferies, 1916).

Bundles of the second order commonly resemble the first order bundles in shape and size and are only recognisable by the absence of the metaxylem vessels. Some authors have pointed out that second order bundles may show a tendency to develop into first order bundles (Vickery, 1935; Goossens, 1938).

In third order vascular bundles the metaxylem vessels are always lacking and in the smallest bundles of this order the xylem and phloem elements may be indistinguishable. These bundles, in fact, form a reduced conducting system and sometimes only a few lignified cells are present together with the phloem elements. Fisher (1939) notes that in these instances neither the protoxylem nor the metaxylem is present. In some genera the third order bundles are not conspicuously smaller than the basic type bundles, but are then distinguishable by the absence of sclerenchyma girders, or even strands, and/or the presence of bulliform cells adaxially.

The shape of the third order bundles may be classified as angular, inconspicuously angular, circular or elliptical in outline. They exhibit these shapes more clearly than do the larger bundles. This shape is taxonomically an important character, linked with the size and number of bundle sheath cells. Thus, small, angular bundles are, on the whole, characteristic of the panicoid, and non-angular bundles of the festucoid grasses.

Various classifications of the orders of vascular bundles employ the extent of development of the sclerenchyma strands and girders. Thus, Breakwell (1914, 1915) distinguishes primary bundles, in which the sclerenchyma is in direct contact with the bundle, and secondary bundles which possess an entire bundle sheath. Bowden (1964) also groups first and second order bundles as those possessing sclerenchyma girders and third and fourth order bundles as those with strands. As was pointed out above, the nature of the sclerenchyma developed in association with the various orders of vascular bundle is especially important in recognizing the larger types of third order bundle, but as bundles are defined here, bundle sheaths and sclerome are not included in vascular bundle descriptions.

Surprisingly few detailed studies have been conducted on the phloem of the Poaceae and these only recently. Thus, the mature phloem of *Avena* was studied by O'Brien & Thinman (1967), Buvat (1968) studied the sieve elements of *Hordeum* and Singh & Srivastava (1971) the phloem of *Zea*.

These studies have shown that the first-formed protophloem elements are located abaxially within a bundle and subsequent sieve elements differentiate successively in an adaxial direction to within a couple of cells of the differentiating xylem elements. Thus, in a mature bundle, all the major features of

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differentiation of the sieve elements and companion cells are visible. The sieve elements of the protophloem often lack companion cells and may differentiate without lateral expansion. The later-formed sieve elements, as a rule, are accompanied by one or more companion cells and extent laterally as a first step in differentiation. Furthermore, the protophloem is usually thin-walled and may become crushed in mature bundles. The metaphloem has thicker walls and may even be thick-walled as in *Distichlis stricta* (Roy, 1969).

The xylem consists of primary xylem only. In first order bundles it can be divided into the protoxylem, which is that area in which the tracheary elements have either annular or helical thickenings or both, and metaxylem, which is the remaining primary xylem. This metaxylem can be sub-divided into the early and the late metaxylem; the late metaxylem being the two large vessels so characteristic of these bundles.

The structure of these late metaxylem vessels is an indication of their specialization. Cheadle (1955, 1960) has shown that specialization in the Angiosperms proceeds from long vessel members, that are angular as seen in transverse section, and with long scalariform plates with many bars and perforations on oblique end walls, to short vessel members that are circular in section, with simple plates, with single perforations on transverse end walls. In the Poaceae no great variation occurs in the late metaxylem which is very specialized. Thus, simple plates are common and scalariform plates rare. A character that is variable, and revealed in section, is whether or not the vessel members are circular or angular.

Vascular bundle sheaths

In the Poaceae every vascular bundle is surrounded, either completely or partially, by one or two single layered bundle sheaths. These bundle sheaths are taxonomically very important, and as a general rule single sheaths are characteristic of the panicoid and double sheaths of the festucoid grasses. Structural variations of the bundle sheath are also important for diagnostic purposes, as well as constituting evidence for important physiological differences between the various groups of tribes of the Poaceae. As would be anticipated in a tissue undergoing extensive changes, even within genera, to almost every generalization there are exceptions. Notwithstanding, the bundle sheath is undoubtedly of the utmost importance from a comparative anatomical viewpoint and fortunately numerous ultrastructural, physiological and taxonomic studies of the structure and functions of the sheath have been undertaken.

Recent terminology, to avoid reference to physiological activities and anatomical structure, as well as to eliminate the concept of specific functions for the two layers (Lommasson, 1957), has been restricted to the terms double (comprised of an inner and outer) and single sheaths. Various other terms have been used in the past, but none of them are applicable to the grass family as a whole.

The single sheath, and usually the outer sheath, when there are two, commonly consists of a single layer of large, thin-walled or slightly thickened cells. In section they are generally inflated and conspicuous, being larger than the adjoining mesophyll cells. Panicoid grasses, in general, have more inflated single or outer sheath cells than those of the outer sheath of the festucoid species (Metcalfe, 1960). This sheath has often been referred to as the parenchyma bundle sheath but other terms include starch sheath, border parenchyma as well as mestome sheath. None of these terms are generally applicable, as for example *Aristida* species have an inner bundle sheath consisting of larger cells than the outer sheath, which may be composed of thickened cells.

The typical single or outer sheath cells are either translucent, without chloroplasts, or they may contain green pigment in plastids similar to, or differing from, the cholorplasts of the chlorenchyma of the mesophyll. Many early workers ignored the contents of these sheath cells and represented them as being empty (Lewton-Brain, 1904; Lohauss, 1905; Prat, 1936; Vickery, 1936). However, the nature of these contents, whether typical chloroplasts or specialized plastids is undoubtedly a character of systematic importance as Brown (1958) correctly stresses. In this connection, it is important that in older leaves of wheat few or no plastids are present in the bundle sheaths (Percival, 1929). Thus, age of leaf sampled must be standardized for this character to be reliable. The detailed structure and function of these bundle sheath cells will be elaborated on later.

The arrangement of the chloroplasts in the bundle sheath cells has been used for diagnostic purposes—whether they display a horse-shoe shaped arrangement against the inner or outer tangential wall, or an even distribution in the cells. However, Roth (1968), has shown in xerophytic species such as *Sporobolus virginicus* that the horse-shoe shaped arrangements occur under low-intensity illumination, such as in the mornings, afternoons, at night and in rolled up leaves, while on sunny days the chloroplasts are evenly arranged all over the walls. This is probably an adaptation to efficient light utilization, but cannot carry much weight as a diagnostic character.

The single or outer sheath may possess girder-like extensions which are of diagnostic importance. The extensions either consist of colourless cells resembling those of the bundle sheath or there may be a gradual transition to the sclerenchyma fibres of the adjacent strand. The extensions may be uni-seriate to very wide, and sometimes flank narrow sclerenchyma strands or girders as in the first order bundles of *Garnotia scoparia* (Tateoka, 1958). Burbidge (1946) and Decker (1964) discuss the unique outer bundle sheath of *Triodia* leaves where the sheath of one bundle is continuous with those of adjacent bundles.

The adjacent sclerenchyma girders may interrupt the single, or outer, sheath to a greater or lesser extent, either adaxially, abaxially or both. Thus, the outer or single bundle sheath may be entire, horse-shoe shaped or even reduced to two lateral arcs. In *Arundinella leptochloa* (Tateoka, 1958) this sheath is only developed adaxially adjacent to the xylem, not being replaced by a sclerenchyma girder abutting on the lateral parts of the phloem.

Schweickerdt (1941) has used the number of cells comprising this sheath to distinguish the closely related genera of *Monelytrum* and *Tragus*. This cell number appears to exhibit excessive variability in most instances, but it is relatively constant for the third order bundles of groups such as the Paniceae.

Various degrees of possible reduction of the vascular bundles, as perhaps evidenced by relic bundle sheath cells have been observed in *Arundinella* (Vickery, 1935) and in *Garnotia* (Tateoka, 1958). In these grasses the first order vascular bundles have well developed outer sheaths, *Garnotia* even having abaxial extensions. In smaller bundles these extensions are reduced, and only a few cells, resembling those of the bundle

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sheath, are irregularly present on the sclerenchyma girders or strands. The extreme situation is found between successive vascular bundles of some *Arundinella* and *Garnotia* species, where solitary, or groups of two to six cells, similar to those of the bundle sheaths are present, scattered in the mesophyll. Tateoka (1958) calls these groups of sheath-like cells, lacking associated vascular tissue, distinctive cells. Carolin, Jacobs & Vesk (1973) have shown that these distinctive cells are in fact isolated files of parenchyma sheath cells connecting with the bundle sheath proper. They consider this to be a specialization to the wide expanses of mesophyll between adjacent vascular bundles found in these species. These distinctive cells ensure that all mesophyll cells are ultimately in contact with the bundle sheath parenchyma cells.

The inner sheath normally consists of living, thick-walled, chloroplast-free cells which are elongated parallel to the vascular bundles. In section the cells of this sheath are nearly always smaller in diameter than the outer sheath and possess thickened walls, the thickening often being more conspicuous on the radial and inner tangential walls. Exceptions to these generalizations do occur as in *Aristida, Elytrophorus* (Schweickerdt, 1942) which has a thin-walled inner bundle sheath, and the Meliceae (Decker, 1964) where the inner sheath cells are uniformly thickened.

This sheath often resembles an endodermis and it has been shown to have similar physiological activity (van Fleet, 1942). It has been variously called the endodermis or mestome sheath in the past. Unfortunately, the term mestome sheath, has been used for parenchymatous sheaths as well and modern authors (Metcalfe, 1960; Barnard, 1964), therefore, refer to it merely as the inner sheath.

The inner sheath is sometimes difficult to recognise, especially in cases where the cells are not distinct from those of the fibres of the vascular bundle ground tissue. However, cells of this layer are always in contact with the metaxylem vessels (Vickery, 1935). Thus, if large inflated cells are immediately adjacent to the metaxylem vessels it can be assumed that no inner sheath is present.

In addition to sometimes not being sharply differentiated from the bundle ground tissue, in some smaller bundles the inner bundle sheath is sometimes only developed adjacent to the phloem (Fahn, 1967). For this reason Metcalfe (1960) proposed the introduction of a third bundle sheath category—intermediate—in addition to single and double sheaths.

The inner bundle sheath cells of *Aristida* are anomalous in two ways: the diameter of the inner bundle sheath cells is greater than those of the outer sheath, and they contain numerous chloroplasts. These larger inner sheath cells may also have uniformly thick walls. Holm (1901) was the first to record this double bundle sheath, Vickery (1935) interpreted this condition correctly and Lommasson (1957) considered it to be of taxonomic importance, so much so as to warrant the placing of *Aristida* in a separate tribe. Theron (1936) attached little taxonomic importance to the sheaths of *Aristida*, probably having misinterpreted the specialized bundle sheath.

Lommasson (1957) was further able to show that, in the leaf sheath, where both bundle sheaths lack chlorophyll, on the adaxial side against the culm, the cells of the outer bundle sheath were larger than those of the inner bundle sheath. The transition to the opposite condition, as in the leaf blade, occurs where chlorophyll is formed in the bundle sheath cells and mesophyll. He advanced the explanation that this anomalous condition represents the result of special activities occurring at the chlorophyll—vascular interface.

The structure and function of the parenchyma cells of the outer, or single sheath, and their dimorphic chloroplasts have been extensively studied. Zirkle (1929) recognised that the cells of the bundle sheath in *Zea* contained specialized chloroplasts concerned with starch storage. In 1944, Rhoades and Carvalho reported that in panicoid grasses, such as *Zea* and *Sorghum*, the single parenchyma sheath contained large specialized plastids, functioning in starch formation and storage. In the outer parenchyma sheath cells of the festucoid grasses, as represented by barley, wheat and oats, they noticed the plastids to be smaller than those in the mesophyll. In these species the plastids of the chlorenchyma, as well as those of the bundle sheath cells, form starch.

Rhoades and Carvalho further demonstrated that in festucoid grasses the mesophyll cells photosynthesise and accumulate starch during daylight. In these grasses the outer parenchyma sheath cells have numerous plastids which, however, are smaller than those of the mesophyll. Small amounts of starch, lying in the central region of the plastid were found in the sheath plastids as well as those of the mesophyll.

In *Zea* and *Sorghum* the chlorenchyma cells photosynthesise, but do not store starch at all. Thus, no trace of starch was found in the chloroplasts even if abundant starch was present in the bundle sheath plastids. The plastids of the single sheath appear to elaborate, and temporarily store starch, but have little photosynthetic activity. These workers showed, that the starch found in these plastids was derived from soluble carbohydrates made in the mesophyll plastids, and translocated to the sheath cells where starch synthesis occurred, and not from sugars synthesised in the plastids of the bundle sheath, which could be capable of photosynthesis since they contain a green pigment.

This transformation of soluble carbohydrates to starch in the bundle sheath plastids occurs only when the rate of movement of sugars into these cells is greater than the translocation from the bundle sheath cells into the vascular bundles. Starch, which accumulates throughout the day, is transformed back into soluble carbohydrates during the night, so that by morning the bundle sheath plastids are devoid of starch. Roth (1968) considers the possibility that these cells function in water storage as well, especially in xerophytic species.

Grasses with this specialized type of bundle sheath structure and function have been termed the eupanicoid subtype of the panicoid subfamily (Brown, 1961). The other subtype, the chloridoid grasses have a dark green parenchymatous sheath and a single layer of pale green radial chlorenchyma. Here the sheath cells photosynthesise in addition to accumulating starch. Brown (1961) postulates that the chlorenchyma may have assumed some unknown function to replace the activities of photosynthesis and starch storage which it has more or less lost. Otieno (1967) is of the opinion that the radiating mesophyll cell layer, of these chloridoid grasses, constitutes part of the bundle sheath which is then composed of three layers. This is not acceptable because all intermediate types between irregular and radiate chlorenchyma are found.

The starch-free bundle sheath plastid has numerous, small, colourless areas, resembling vacuoles, contained within a peripheral rim. It is in these vacuolar-like regions that starch is deposited. Thus, each plastid

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contains numerous simple starch grains embedded in, but protruding from, the surface of the plastid.

Ultrastructural studies of these dimorphic chloroplasts of the panicoid grasses, by Laetsch & Price (1969) on *Saccharum*, Downton & Pyliotis (1971) on *Sorghum bicolor* and Andersen *et al.* (1972) on *Zea* show, that the bundle sheath chloroplasts loose their grana during ontogeny. Young bundle sheath chloroplasts have well-developed grana, but mature plastids are agranal whereas mesophyll chloroplasts contain grana irrespective of their developmental stage. They conclude that the structure of the specialized chloroplasts in bundle sheath cells is a result of reduction and that this chloroplast dimorphism is a specialization of labour. There are various degrees of reduction in the sheath chloroplasts. *Zea*, *Coix* and various members of the Paniceae and Andropogoneae have rudimentary grana and Carolin, Jacobs & Vesk (1973) have shown that the Eragrostoideae in fact have well-developed grana. Johnson (1964) suggests the possibility that the degree of specialization of these chloroplasts has phylogenetic significance.

The chloroplast-containing sheath cells contain, in addition, a remarkably high concentration of additional cellular organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, but vacuoles are difficult to locate. The differences in the ultrastructural features of the cells of the bundle sheath and those of the chlorenchyma further suggest the existence of differences in the functions of these cells (Dobychina, 1970; Carolin, Jacobs & Vesk, 1973).

This detailed discussion of the chloroplasts and the bundle sheath has been considered necessary because in recent years this aspect has received considerable attention from the point of view of the Kranz syndrome. Thus, grasses with bundle sheaths containing specialized chloroplasts are typical of the high photosynthethic capacity grasses and those with no, or normal chloroplasts are characteristic of low photosynthetic capacity grasses (Black, 1971).

At present the advantages of highly developed sheath cells still remain unclear. Black (1971) infers that the anatomy of the high photosynthetic capacity plants results in more rapid rates of translocation, and perhaps higher concentrations of translocates, which may both prevent a feedback type of inhibition of photosynthesis by a product such as starch. Nutrients may, in addition, be supplied to non-photosynthetic parts of the plant such that high growth rates are facilitated.

The possibility of the inner bundle sheath, or the single sheath, when the inner sheath is lacking, having functions and characteristics similar to an endodermis has received considerable attention in the literature. In grasses with a single bundle sheath, the cells of this sheath do not have the anatomical characteristics associated with an endodermis, such as Casparian strips, thicker radial and inner tangential walls or suberised walls. This is applicable to a number of inner sheaths as well.

Van Fleet (1950) demonstrated, with a variety of histochemical reactions, however, that the cells of these sheaths share common substance reactions and may be induced to develop typical endodermal characteristics. Thus, normally the single sheath cells contain chlorplasts and do not exhibit characteristics of an endodermis. But in variegated and albino leaves, or in etiolated leaves, Van Fleet showed that those areas lacking chlorophyll show characters of a typical inner bundle sheath, i.e. endodermal characteristics. In *Pennisetum villosum* and *Oplismenus hirtellus* he further showed that casparian deposits are developed on the radial walls prior to the unilateral desposition characteristic of the mature inner sheath. Endodermal characteristics, therefore, appear at varying stages in the development of the leaf in at least some of the cells of an atypical inner sheath. Thus, in any sheath bounding the vascular tissue, some or all, attributes of a true endodermis can be detected. Van Fleet points out that the endodermal characteristics are always more pronounced opposite the phloem than opposite the xylem. Once again for comparative work it is essential to standardize upon leaves of equivalent developmental stages.

Schwendener (1890) stated that the walls of the parenchymatous sheath of *Zea* contained suberized lamellae, something Van Fleet (1950) was not able to show for normal maize leaves. O'Brien & Carr (1970) also described a suberized lamella, which is probably the site of suberin deposition, in the walls of the inner sheath of *Triticum* and *Avena* as well as in the parenchyma sheath of maize. The suberized lamellae modify the plasmodesmata of the numerous pit-fields connecting the cells of the inner sheath with those of the outer sheath and vascular parenchyma, or between cells of the single parenchyma sheath.

Using these ultrastructural details O'Brien & Carr (1970) propose a function for the inner bundle sheath and a further function, in addition to photosynthesis and starch storage, for the single sheath. These workers are of the opinoin that the suberized lamellae are relatively impermeable to water and thus there is the possibility that in grass leaves water loss is regulated at the vascular bundle as well as by the stomata. This control of water loss at the vascular bundles is probably essential for an adequate supply of solute to be maintained to the leaf tip, because, under conditions of water stress the lower part of the blade could conceivably transpire all the water available to the leaf. If the suberized lamellae restrict passive loss of water from the vascular bundles to the mesophyll, forcing the water to follow a symplastic route through the sheath cells, the water flow across the sheath could be regulated by the activity of the sheath cells.

Further circumstantial evidence for this possibility is that transfer cells, which are believed to help regulate solute exchange between tissue systems, are absent from the leaves of grasses. In dicotyledon leaves these transfer cells are thought to assist exchange between xylem and phloem, and between these and the adjacent mesophyll. O'Brien & Carr reason that if passive loss of water is restricted from the vascular bundles because of the suberized lamellae, and if the bulk of solute transfer must pass through the plasmodesmata, then transfer cells are unnecessary.

Sclerenchyma of the leaf

In the grass leaf blade, the sclerenchymatous tissue, or the sterome, includes all fibres as well as other thick-walled cells in certain instances. The sclerenchyma is commonly found in association with the vascular bundles, with the midrib or keel and in the margin.

Xylem and phloem fibres, and thickened vascular parenchyma, constitute part of the ground tissue of the vascular bundles. The mestome, or inner bundle sheath, is also fibrous, the fibres appearing, in some instances, ontogenetically the same as those of the hypodermal sclerenchyma, but differing in that they possess numerous pits which are lacking in the true

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sterome (Artswager, 1925). This tissue is included in the description of vascular bundle structure and will not be discussed further here.

The sclerenchyma associated with the vascular bundles is in the form of sub-epidermal longitudinal bands following the course of each vascular bundle. In transverse section this circumvascular sclerenchyma may surround, be in contact with, or may be situated above or below, but not connected to the bundle or it's sheath. This sclerenchyma tissue is termed a strand when it does not extend sufficiently deeply into the mesophyll to make contact with the bundle sheath cells (Metcalfe, 1960). These strands, therefore, appear in section as small hypodermal "islands" of thickened tissue situated above and below, or on one side only of each vascular bundle. When the inner face of a group of fibres is in contact with, disrupts, or envelopes the bundle sheath, it is termed a girder (Metcalfe, 1960). It resembles a girder in transverse section, extending from either or each epidermis to the bundle sheath. When girders are continuous from the vascular bundle to the epidermis on either side it is termed an I-beam construction (Gould, 1968).

All distribution patterns of the circumvascular sclerenchyma occur either as strands or girders. In addition, preliminary observations in the present study suggest that a further distinction between girders in contact with, and those disrupting the parenchymatous sheath, may be useful taxonomically.

In leaves possessing both strands and girders, the former are usually associated with the third order vascular bundles and the latter with larger bundles. Other leaves may have strands or girders only developed in association with all the vascular bundles of the leaf. The distribution and arrangement of the sclerenchyma associated with the bundles is, therefore, useful diagnostically. Furthermore, the amount of sclerenchyma present varies from species to species (Metcalfe, 1960). This can be misleading, however, because marked intraspecific variation will be found in specimens from different localitities or from different seasons as the work of Burduja & Toma (1970) on *Deschampsia flexuosa* and other species has shown. They found that on flowering tillers there was less sclerenchyma in the leaves than on non-flowering tillers where the fibre walls were also thicker with the lumen smaller. Taxonomically sclerenchyma distribution is seldom of more than specific diagnostic importance.

The distribution of sclerenchyma associated with the vascular bundles can be correlated with ecological factors as well. Grasses from arid areas are thus characterized by well developed sclerenchyma tissue while many tropical grasses often have a high proportion of the smaller bundles not accompanied by sclerenchyma.

The function of all the sclerenchyma present in the leaf is undoubtedly to provide mechanical support for the softer tissues. The development of sclerenchymatous tissues makes possible the withstanding of the physical stresses and strains imposed on an elongated, straplike leaf such as is predominant in the Poaceae. An additional function of the fibres is seen by their frequent silicification, especially in older leaves (Parry & Smithson, 1964) and they, therefore, can act as a depository for excessive silica. Silicified fibres result in elongated, pointed needles of silica.

The thickening of the fibre cell walls is usually by lignification, but in many specimens the staining reaction varies across a single sclerenchyma girder, or in girders from different parts of the lamina. In these cases, when stained with safranin and fast green, there is a gradation from typically red-staining fibres to structurally similar fibres which attract the blue or green dye. Hoefer (1941–42) also found variations in the reaction of fibres in different parts of the leaf of *Stipa tenacissima* when treated with lignin stains. In addition, lignin tests with phloroglucin and hydrochloric acid and tests with Maules's reagent did not give identical results. Hoefer's tests showed, in addition that there is zonation in the degree of lignification in different layers of the cell wall of individual fibres as seen with transverse section. It appears, therefore, that the degree of lignification varies, but in addition, lignins of various chemical constituents appear at present to be grouped under the general term "lignin".

In certain grasses sub-epidermal longitudinal strands are found between successive vascular bundles, either alone or in addition to the circumvascular strands or girders. These supernumary strands are usually abaxial and situated opposite the bulliform cell groups or furrows (Lewton–Brain, 1904). When sclerenchyma is found between the bundles in expanded leaves it is of special importance diagnostically.

Continuous abaxial hypodermal bands are found in certain species, especially those with acicular leaves. These bands may be a regular, narrow strip, 2–4 cells deep, located immediately beneath the epidermis, or may result from lateral extensions of the fibrous tissue comprising strands or girders.

Mechanical tissue of the leaf margin may occur in the form of a cap or hood. When the marginal sclerenchyma is not in contact with the lateral bundle, it is termed a cap, and a hood is formed when this sclerenchyma extends above or below the lateral bundles. The fibres of the hood may, or may not, be in contact with the bundle sheath of the ultimate bundle, but always extend inwards from the margin, as far as, or further than, this bundle. This lateral fusion with the sterome tissue associated with the lateral bundles forms the hood-like structure (Goossens, 1938). In some instances, there is no sclerenchyma development at the extreme margin but the ultimate and penultimate lateral bundles may be intimately associated with specialized sclerenchyma development. Immediately interior to the fibrous cap may be situated normal mesophyll cells, small groups of enlarged, colourless parenchyma cells or a lateral intercellular duct may be developed.

Sclerenchymatous tissue is also found developed in association with midribs and keels. This has been described together with the median vascular bundles, midribs and keels.

In reduced, permanently infolded leaves the distribution of the sclerenchyma follows that outlined above in basic pattern, but appears symmetrical and distinctive because of the nature of the leaf blade. In these leaves this arrangement of the mechanical tissue is of taxonomic importance because, in assuming the permanently infolded form, the leaves of different grasses have not acquired precisely the same sclerenchyma tissue arrangements (Metcalfe, 1960).

Mesophyll

In the Poaceae, the term mesophyll is generally applied to the ground tissue occupying all the space in the leaf not occupied by the vascular bundles, the bundle sheaths and the sclerenchyma. The mesophyll can be subdivided into the assimilatory chlorenchyma

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and the colourless parenchyma which consists of translucent cells often in close association with the bulliform cells. For this reason further details of colourless parenchyma will also be found under the discussion of bulliform cells.

Grass leaf blade chlorenchyma seldom exhibits a distinct differentiation into pallisade and spongy regions as seen in transverse section. In some species the adaxial chlorenchyma cells are more regularly and vertically arranged than the remainder. Metcalfe (1960) correctly stresses that this distinction is rare and at best unclear. Statements in the literature often imply that there is greater contrast between these zones than there actually is.

In dicotyledons, as Watson (1942) has shown with *Hedera helix*, the English ivy, the leaves respond to strong light by the production of pallisade tissue. On the same plant shade-grown leaves have no pallisade tissue at all. These cell differences are caused by factors affecting the processes of vacuolation and enlargement of the cells. Thus, the uppermost layer of mesophyll cells has an increased osmotic value due to a change in the starch-sugar ratio. This, according to Watson (1942), results in the absorbtion of water, increased vacuolation and expansion, with the resultant formation of pallisade tissue.

In the Poaceae, the above does not appear to apply and in certain instances the opposite may be the case. In the genus *Panicum*, the shade-loving forest species often have the upper layer of chlorenchymatous cells more or less vertically arranged in a pallisade manner in contrast to most other species of this genus where the assimilatory tissue is more of less radiate.

The arrangement of the chlorenchyma cells appears to be of fundamental taxonomic significance. Thus, in the festucoid type, the chlorenchymatous tissue is more or less homogenous without being arranged in any definite pattern in relation to the vascular bundles. In grasses of the panicoid type the assimilatory cells are arranged in a radiating manner around the vascular bundles as seen in transverse section. Each bundle is, therefore, situated in the centre of a regular circle, or partial circle of chlorenchyma. The former irregular arrangement is, in addition, generally associated with leaves in which the vascular bundles are widely spaced, whereas the radiate condition and closely placed vascular bundles are correlated (Vickery, 1935).

The division of the Poaceae into two sub-families with radiate or non radiate chlorenchyma is an oversimplification. Brown (1958) shows that within the panicoid grasses the Chlorideae and related tribes have the regularly radiate condition, whereas this radiate condition is less regular in other panicoid genera such as *Andropogon* and *Panicum*. Thus, the radiate condition is of more than one kind, and grasses with partially or incompletely radiate chlorenchyma also occur, as Metcalfe (1960) stresses. Nevertheless, the distinction between radiate and non-radiate chlorenchyma is important taxonomically provided that the limitations are recognized. For example, both conditions may occur in the same genus, as in *Sporobolus* where many species have regular, elongated chlorenchyma cells radiately arranged around the bundles, but in *S. panicoides* the cells are irregular in shape and arrangement (Goossens, 1938).

Early workers correlated the non-radiate condition with grasses from temperate regions, whereas radiate assimilatory tissue was linked with tropical grasses. The geographical pattern is not so distinct, however, with many exceptions and Metcalfe (1960) remarks that the mesophyll arrangement is of more fundamental significance taxonomically than geographically.

In transverse section the individual chlorenchyma cells of most panicoid grasses appear elongated, narrow and tabular in shape. Their long axes are at right angles to the bundles. In festucoid grasses the cells are often irregular in shape and size. In some species, such as *Poa annua,* the largest cells are found nearest the vascular bundles (Bobrov, 1955) or the chlorenchyma cells abutting on both epidermides may be somewhat elongated and regular as in *Triticum vulgare* (Hayward, 1948). The irregular condition may be characterized by small, isodiametric cells tightly packed together or by irregularly shaped cells with many intercellular air spaces between them. The largest intercellular air spaces are commonly seen subtending the stomata and projecting deeply into the mesophyll. Pool (1923) illustrates such distinct sub-stomatal chambers in *Andropogon furcatus.*

Slade (1970) in a study of sun and shade-grown leaves of *Poa alpina* has shown that in sun leaves the mesophyll cells are more or less isodiametric and not longitudinally elongated as in shade leaves. The intercellular spaces are relatively inconspicuous in sun leaves but extensive in shade leaves as seen in longitudinal section. Slade also demonstrated that in transverse section there was no noticeable change in the horizontal widths of the intercostal zones of chlorenchyma between adjacent vascular bundles, but that the depth or thickness of the mesophyll was greatest in sun-grown leaves. This is as a result of reduction in cell size and intercellular spaces in shade leaves seen in transverse section in contrast to the situation in longitudinal section above.

The overall effect of these anatomical changes in sun and shade leaves is to physically weaken the leaf blade. Slade (1970) has shown, in addition, that in low light intensity the cell walls are thinner and a well defined cuticle may be absent.

It appears, therefore, that the light intensity under which a leaf develops has a profound effect on the arrangement of the assimilatory cells into pallisade and spongy mesophyll, especially in dicotyledons (Watson, 1942), as well as the size and shape of the individual cells as shown by Slade (1970). The applicability of these findings to the chlorenchyma cell structure and arrangement in the Poaceae as a whole does not appear clear at present. Thus, if the radiate and non-radiate conditions are of fundamental phylogenetic significance it appears unlikely that major differences of tissue arrangement will occur under varying light intensities. Some generalizations do seem to apply and most forest dwelling species examined are characterized by very thin mesophyl tissue. *Prosphytochloa prehensilis* is an extreme example with the whole leaf being only four cell layers thick (de Winter, 1951).

Intercellular air spaces occur between the chlorenchyma cells of many grasses belonging to tribes of both the major subfamilies. These spaces may be very conspicuous in hygrophilous species (Arber, 1934; Vickery, 1935). In other aquatic species there are, in addition, distinct air cavities or lacunae present. All the species of *Elytrophorus* have these lacunae situated between adjacent vascular bundles (Schweickerdt, 1942) and in *Vetiveria* they occur over the smaller bundles (Kanmathy, 1969). The lacunae are traversed by colourless aerenchyma cells which are

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often stellate in shape and represent diaphragms in the air cavities. Kanmathy (1969) reports sclerotic strands interspersed in the stellate cells.

Cavities developing as a result of breakdown of parenchymatous tissue, especially in the region of the keel, and in the keel itself, are found in many grasses. These differ from lacunae in being indistinct with the breakdown of the cells continuing. This type of cavity has been used diagnostically for *Molinia caerula* (Lewton–Brain, 1904) and *Sporobolus artus* var. *lysigenatous* (Goossens, 1938).

A further modification of the mesophyll tissue is found in certain markedly infolded leaves where the chlorenchyma is confined to small bands adjacent to the adaxial and abaxial cleft-like furrows which contain the stomata. The tissue between the groups of assimilatory tissue is made up of colourless parenchyma. This unique type of anatomy is discussed by Burbidge (1946, 1946a) for *Triodia* species. Burbidge believes that this reduction of the chlorenchymatous tissue results in a corresponding narrowing of the width of the furrows and consequent protection of the stomata. De Wet (1956) found localized bands of chlorenchyma cells around the abaxial grooves in species of *Merxmuellera* (=*Danthonia*) and suggests that this may indicate relationships.

In some species, groups or isolated, bundle sheath-like cells with chloroplasts, but lacking associated vascular tissue are found scattered in the mesophyll. These are termed distinctive cells (Tateoka, 1958) and are described under vascular bundle sheaths.

Bamboos, and a few other grasses, are characterized by the presence of fusoid cells in the mesophyll of their leaves. These cells have been termed enlarged parenchyma cells (Page, 1947) and in transverse section have a fusiform or pyriform outline, being elongated transversely and alternating with chlorenchyma cells and the vascular bundles. Adjacent fusoid cells may be separated by a single vertical column of chlorenchyma or many chlorenchyma cells may separate them. The bulliform cells are located immediately adaxially to these chlorenchyma cells separating adjacent fusoid cells.

These conspicuous colourless cells have been incorrectly interpreted as intercellular spaces (Jacques-Felix, 1955). Their cellular nature was confirmed by Page (1947) and Metcalfe (1956).

The fusoid cells are, in fact, narrow and plate-like with the long axis of the cells lying at right angles to the long axis of the lamina. Longitudinal sections of the blade show these cells to have very narrow lumina in transverse section, the cells having collapsed in such a way that the tissue resembles a row of "I's". Between the dead, collapsed, mature cells there are large spaces which connect with the intercellular spaces of the chlorenchyma. In certain species the fusoid cells fail to collapse, but become rounded and separated from one another. The regularity and form of these collapsed cells seems to indicate that some force operates on the whole leaf at the same time (Page, 1947).

In addition, the Bambusae, as well as the Oryzeae, are characterized by having chlorenchyma comprised of what, in the grass anatomy literature, have become known as arm cells. These have also been called irregularly lobed or cleft cells (Page, 1947) or plicate mesophyll (Esau, 1960). These so-called arm cells possess inwardly directed projections or folds which may be continuous across the cell as seen in section and completely divide the cells into elongated compartments. The infoldings may end blindly in the lumina as well. These projections may be developed from the upper or the lower wall of the chlorenchyma cell, or from both the upper and lower, or from all walls (Brandis, 1907).

Chih-Ying Wu (1958) has shown that in some bamboo species the arm cells located above the fusoid cells have projections from the lower walls, whereas the cells below the fusoid cells and cavities have folds projecting from the upper walls. This phenomenon is mentioned by Arber (1934), but in the Oryzeae, even when fusoid cells are present, the arm cells illustrated by Tateoka (1963) have infoldings from all directions and of equal length.

It is not certain how these projections arise. They could be infoldings of the cell walls as an illustration of Carolin, Jacobs & Vesk (1973) indicates, but in some grasses their appearance suggests that they arise as fine partitions that at first traverse the cell completely, but subsequently become broken as the cells become enlarged (Metcalfe, 1960). Haberlandt (1884) called these "armpalisadezellen" and explained the significance of the infoldings as increasing the inner surface area of the cell, thereby creating space for more chloroplasts. Infoldings are common in many grass genera, and are termed arm cells or peg cells, and in the bamboos and their relatives it is possible that the pegs become "fused", eliminating the air spaces.

Fusoid and arm cells are important taxonomic characters. Thus, fusoid cells are especially characteristic of the Bambusae (Metcalfe, 1960), and when they occur in other genera, such as *Oryza* (Tateoka, 1963), affinities between these genera and the bamboos may be indicated. Arm cells, although characteristic of the Bambusae and Oryzeae, may also show variations in their occurrence. Thus, *Oryza tisseranti* is exceptional amongst the Oryzeae in not having arm cells (Jacques-Felix, 1958). Gordon-Gray & Ward (1971) noticed mesophyll cells with invaginated walls in *Phragmites* and suggest that this may reflect closer relationships to the Bambusae and Oryzeae than was previously thought possible. The extreme case is that of *Saccharum,* where Merida (1970) found cells with internal folds situated below the stomata in some varieties. This apparent occurrence of arm cells in a member of the Andropogoneae is certainly surprising.

Bambusoid and oryzoid grasses are very poorly represented in South Africa. For this reason, the accompanying descriptive key is not concerned with the details of arm and fusoid cells to any great degree. As presently constituted it cannot be expected to differentiate between the various bamboo genera.

The colourless parenchyma constituent of the mesophyll, especially that intimately associated with the bulliform cells is described, together with these bulliform cells, under the description of bulliform cells.

Bulliform and colourless cells

Bulliform cells are single, translucent cells, or groups of colourless cells, constituting part of, or the entire epidermis, but differing from other epidermal cells in being larger and more inflated. These cells occur most commonly, but not exclusively, at the bases of adaxial furrows (Metcalfe, 1960). Shields (1951) restricts the term bulliform cell to inflated epidermal cells present in the adaxial furrows. However, structurally similar cells may be present in the abaxial epidermis as well as, or instead of, the adaxial epidermis. Other terms used to describe these cells are hinge cells and motor cells, but the

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descriptive term bulliform, denoting their inflated appearance, is preferred to these terms referring to their disputed function in leaf involution.

Bulliform cells often form longitudinal, parallel, intercostal bands, and are commonly associated with underlying groups of structurally similar cells, which usually form vertically elongated units in the mesophyll when viewed in transverse section. Shields (1951) designated these associated cells, hinge cells. Here they are grouped with the colourless cells, a term incorporating all the translucent cells present in the mesophyll excluding bundle sheath cells devoid of chloroplasts, fusoid cells of bamboos and epidermal cells. The bulliform and associated colourless cells may appear in transverse section as deep girders, or may be restricted and superficial. The colourless cells associated with the bulliform cells are variable in shape and size, but all colourless cells, whether associated with the bulliform cells or not, are always without chloroplasts.

Bulliform cells have long been recognised as valuable taxonomic characters. As early as 1907, Brandis reviewed the distribution of bulliform cells in the Poaceae as a whole. The major character subdivisions he used were the presence of bulliform cells in the adaxial epidermis only, the presence of bulliform cells in both adaxial and abaxial epidermides, and the absence of distinct bulliform groups. The adaxial bulliform cell distribution was further subdivided into those grasses where bulliform cell groups alternated with all vascular bundles, those where the bulliform cells were present between successive first order bundles, but located over the third order bundles, those with bulliform cells on either side of the midrib and at the margins, those with bulliform cells only located on either side, or above the midrib. Using these distributional criteria of bulliform cells Brandis (1907) found uniform distribution in the tribes Bambuseae and Maydeae only. In all the other tribes studied various genera had different bulliform cell arrangement in some of their species.

The arrangement, frequency, distribution, as well as the relative size and shape of the bulliform cells and colourless cells are of taxonomic importance especially at the specific level. Thus, in *Elytrophorus*, Schweickerdt (1942) found the presence of conspicuous bulliform cells flanking the midribs only, to be the the oustanding diagnostic character of *E. africanus,* whereas in *E. interruptus* bulliform groups are present throughout the leaf width, but decrease in size towards the margin. Furthermore, in one species, the shape of the individual bulliform cells in transverse sections is rectangular, whereas in the other species they are trapezoidal with the outer wall being shorter than the inner wall. In *Leersia hexandra* there are groups of bulliform cells between all the vascular bundles and on both epidermides but other *Leersia* species have only two groups of bulliform cells on either side of the midrib (Holm, 1895). Goossens & Theron (1934) found the bulliform cells to be of significance in classifying the different varieties of *Themeda triandra.* The arrangement of the colourless cells in relation to the bulliform cells can be of specific diagnostic importance. In *Imperata cylindrica* (Vickery, 1935), where the bulliform cell groups occur over third order bundles, two rows of colourless cells are produced towards the abaxial surface, one on either side of each bundle.

The function of the bulliform cells has been the subject of much controversy in the literature and a totally satisfactory explanation of their function has, as yet, not been forthcoming. Duval-Jouve (1875) was the first to describe these cells and believed that the opening and inrolling of the blade resulted from changes in the turgor pressures of these cells. Some workers, such as Breakwell (1915), have carried this even further by postulating that rolling occurs only at times of water shortage and results in the leaf becoming tubular, thus protecting the greater number of adaxial stomata from excessive transpiration. This teleological viewpoint is not supported by the fact that many grasses, including drought resistant types which exhibit inrolling, have more stomata on the exposed abaxial surface than the protected adaxial surface (Vickery, 1935). Hayward (1948) mistakenly states that the outer surfaces of the bulliform cells are not cutinised, and thus readily lose water through diffusion. Metcalfe (1960) points out that in many grasses exhibiting involution the outer wall of the bulliform cells may be considerably thickened.

The conventional explanation of bulliform function thus accepts that the bulliform cells regulate the movement of the blade when it opens and closes. Goossens & Theron (1934) draw a comparison between bulliform cells and stomatal guard cells and the changing of cell shape and turgidity with turgor changes. However, whether the bulliform cells actually cause the rolling movements by collapse due to water loss, or whether their size and plasticity merely permit them to be compressed and so allow rolling to occur, has been the subject of some controversy.

Numerous facts point to caution in explaning the function of these cells. Brandis (1907) remarks on the fact that in mature leaves the bulliform cells may become entirely filled with solid silica making the leaf rigid. Bor (1960) quotes Goebel (1926) as describing nyctinastic movements in *Leersia hexandra* in which the leaves fold up in dull weather and flatten again in full sunlight. These movements obviously are not dependant on different water regimes within the bulliform cells. In addition, involution may occur in the absence of bulliform cells and often the thin lateral walls of bulliform cells are not collapsed or distorted in naturally rolled or wilted grass leaves (Shields, 1951).

Tschirch (1882) challenged this theory that movements depended only on turgor changes in the bulliform cells. While he agreed that rolling and unrolling of grass leaves was influenced by the loss and uptake of water, he thought that these movements were due, at least in some cases, to changes in the leaf fibres rather than the epidermal bulliform cells. He explained that the adaxial, subepidermal schlerenchyma fibres have a high capacity for imbibition and a marked tendency to shrink when dehydrated. This results in contraction of the adaxial ribs causing inrolling because the inflexible lower epidermis, reinforced by sclerenchyma gives the abaxial surface an unbroken rigidity. This contraction, coupled with cohesion among the chlorenchyma cells and among the bulliform cells may result in leaf involution. Thus, involution has also been defined as a cohesion and shrinkage phenomenon, the result of a differential contraction of the mesophyll and the rigid lower epidermis. As the mesophyll cells shorten in drying, they become stretched around the vascular bundles, causing the leaf to roll.

Shields (1951), in a detailed study of grass leaf involution, has shown that differential shrinkage of the adaxial and abaxial rib surfaces occurs. This shrinkage was more pronounced in all the species studied on the adaxial rib surface. This illustrates the lateral shrinkage in the adaxial sclerenchymatous

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fibres, where present, and in the mesophyll. In leaves lacking adaxial sclerenchyma girders or strands, large adaxial mesophyll cells collapse in the wilted leaf and the bulliform cells show varying degrees of buckling. Shields found that lateral and vertical contraction in the bulliform and associated colourless cells was no greater than in the adjacent mesophyll. In wilted leaves the abaxial ribs appear more prominent as a result of the smaller amount of shrinkage in the vascular tissue than in the surrounding cells. Shrinkage of the bulliform and colourless cells appears to play no part in involution except to facilitate the turning inward of the upper leaf surface.

The fact that the bulliform cell walls are hygroscopic and, therefore, may assist in movement was reported by Goossens & Theron (1934). This was supposedly shown by the fact that dead, detached leaf segments roll more tightly than in normal reversible involution and, conversely, increasing their water content leads to the partial unfolding of the dead leaf segment. Shields (1951) comments that this partial expansion of a dried leaf through adsorption by all the cell walls (which are also dead in the living blade) suggests that decreased hydration of the cell wall may play a passive part in reversible wilting. Since the bulliform cells are dead in this case, absorbtion of water by the protoplasm and large central vacuole cannot occur.

Involution does not result from plasmolysis. Plasmolysis, through vertical contraction of the mesophyll, may cause an increase in width, probably by relieving tissue strains through shrinkage of the protoplasts (Burstrom, 1942). As a result vascular bundles become more widely separated, and the thick-walled, stiff abaxial epidermis, which does not change in width, tends to curve backwards in plasmolysis so that the underside of the leaf becomes concave. Since plasmolysis involves turgor change the diametrically opposed characteristic of inrolling in wilted leaves implies that involution does not result from turgor changes.

Loss of water from the bulliform cells to the chlorenchyma in particular, may further contribute to the collapse of these cells. With a cuticle on their outer surface these epidermal cells are more resistant than the mesophyll cells to the drying effects of the atmosphere. However, Haberlandt (1928) showed that when an organ is transpiring rapidly, the epidermis loses water to photosynthetic tissue with its higher osmotic pressure.

Shields (1951) examined the bulliform cells in unwilted but tightly rolled leaf buds. They are small but turgid, rather than flaccid and collapsed, as in the wilted blades of mature leaves. Furthermore, unfolding from the developing bud involves general growth, particularly in the adaxial mesophyll, and does not result primarily from the enlargement of the bulliform cells, although these enlarge by stretching growth due to turgor changes. The rolling mechanism is, therefore, not related to the unfolding of the bud. Rolling involves cohesion and shrinkage in both living and non-living parts, and bud unfolding, turgor and stretching movements at a time when all cell walls are highly elastic.

To summarize, it has been shown that structural elements other than bulliform cells contribute to involution. 1. Rolling in drying is characterictic of certain grass leaves entirely lacking in bulliform cells, and if bulliform cells are present they may show no buckling or other distortion in the wilted leaf. 2. Since average measurable lateral shrinkage (which is negligible on the abaxial surface of wilted leaves) amounts to 7–12 per cent on the adaxial rib face (Shields, 1951) in naturally wilted leaves, subepidermal sclerenchyma and other adaxial elements of the mesophyll must contribute to involution. 3. Since dried leaves unfold partially when placed in water, involution must result in part from decreased water content in non-living cell walls which may or may not contain protoplasts. Rolling cannot result entirely from turgor movement, which, by definition, is a reversible change in the water content of living cells. Water loss by protoplasts alone is insufficient to cause involution in a leaf composed largely of non-living mechanical tissue. The form of the wilted leaf is determined by a number of elements in the mesophyll, the buckling of bulliform and colourless cells, where it occurs, being in part from passive compression.

Anatomical variations in the structure and distribution of bulliform cells in different genera exhibiting involutionary movements preclude the universal application of any one explanation of this phenomenon in wilting grass leaf blades. What is important from a descriptive point of view is the fact that the outline of the lamina can be markedly altered by this phenomenon. It appears to be constant within a species and, therefore, it is not the degree of infolding or unrolling that is important but rather the type of movement.

This is confirmed by the work of Dunlop (1913) on the curling of the leaves of different varieties of sugar cane. Involute curling, where the upper surface is protected, is characterized by the following anatomical structures: the bulliform cells are not prominent and the cells immediately interior to these cells are comparatively large and thin walled; the upper epidermis is not greatly lignified. Varieties of sugar cane exhibiting revolute curling, whereby the lower surface is protected, have large prominent bulliform cells attached to the vascular bundles by lignified cells. The vascular bundles are closer together and the upper epidermis is greatly lignified. Dunlop (1913) is of the opinion that this revolute curling is a permanent characteristic of the varieties showing it.

A further possible function of the bulliform and colourless cells has been advanced by Breakwell (1915). He states that the arrangement and distribution of the colourless cells allows light to penetrate to the chlorenchyma cells, even in the rolled up position of the leaf bud and thus aids in the development of the leaf. The rapid development of leaves with large bulliform groups and colourless parenchyma, such as *Astrebla pectinata,* is cited as circumstantial evidence for this possible function.

Epidermal cells in transverse section

Typical epidermal cells in transverse section normally do not exhibit any particularly important diagnostic characters, although examination of them in section can be of assistance in the interpretation of many epidermal structures as seen in surface view. Sections through the epidermis can aid in establishing the nature of the papillae, the attachment and structure of other epidermal appendages, the positioning of the stomata or the cuticle form and thickness.

Epidermal cells are usually square to rectangular in section, or the outer wall may be more or less arched or even papillate. The papillate appearance, as seen in surface view, may be due to a pronounced arching of the whole outer tangential wall, or more commonly (Vickery, 1935), due to the presence of a number of separate papillae arranged in a longitudinal row or rows. However, when these papillae are in a single row, and as wide as the epidermal cell, the outer wall

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also appears as strongly arched in section. Narrower papillae appear as distinct papillae on the outer tangential wall surface. Where more than one row of papillae are present on each cell they appear as bifurcate or multiple papillae. The papillae may be inflated and thin-walled, resemble conical warts or may be sharp-pointed expansions of the outer wall (Holm, 1891a) or teeth (Sabnis, 1921). It is also common for the distal ends of the papillae to be thickened.

The level of the stomata in different varieties of sugar cane has been correlated with differences in drought resistance by Merida (1970). They may be located at the same level as the epidermal cells or sunken below them as seen in transverse section. In certain grasses, such as *Spinifex hirsutus*, the stomata are present in depressions formed by the surrounding cells being enlarged and raised above the general level of the epidermal surface (Breakwell, 1915). The presence of stomata on both surfaces, or only the adaxial or abaxial surfaces can also be confirmed.

Micro-hairs are rarely seen in leaf sections but prickles and macro-hairs are commonly sectioned. The nature of the cushion cells surrounding the base of many macro-hairs, whether raised or not, can easily be determined. Interlocking prickles forming arches over the stomata can sometimes be seen as in certain *Danthonia* species (De Wet, 1960). Burbidge (1946a) found hairs consisting of a bulbous-based cell and a small apical cell imbedded in the epidermal cells at the base of grooves of leaves of all species of *Triodia*.

The size of the epidermal cells may vary over and between successive bundles as well as the cells of the adaxial and abaxial epidermides being of different sizes. In many grasses, especially members of the tribe Andropogoneae, the epidermal cells are exceptionally large, and can occupy up to half the leaf thickness. These are included under bulliform cells.

Cuticle thickness can be determined in leaf sections. Thus, it may be seen in many species that the lower epidermis is more strongly cuticularized than the upper. Slade (1970) has demonstrated the lack of a well defined cuticle in shade-grown leaves of plants which have distinct cuticles on the sun-grown leaves.

The thickness of this cuticle is of the utmost importance in faecal analysis studies, because during the digestion process all the cellulose cell-walls are dissolved away leaving the cutinized cuticular membrane behind. Thus, it is the relative thickness of the cuticular membrane, and not of the entire outer tangential wall, that is of importance in these studies of the diet of grazing animals. It should be stressed that the outer wall may be markedly thickened but lack a thick cuticle. The cuticle proper, therefore, consists of a layer of adcrusted cutin continuous over the entire leaf surface. Each cell does not have an individual cuticle but it may be individually thickened. This difference can readily be determined in transverse sections of these epidermal cells.

CONCLUSION

It is hoped that this attempt to introduce uniform standards to the description of grass leaf blades, as seen in transverse section, will stress the urgent need for standardization in these studies. If this can be achieved, anatomical descriptions will have a much wider applicability in the fields of comparative leaf anatomy and grass systematics in general.

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UITTREKSEL

Omskrywende sleutels, definisies en diagramme, vir die standaardisering, vereenvoudiging, en beskrywing van die grasblaar struktuur soos beskou in dwarssnit, word aangegee. Meer as 500 eienskappe is ingesluit met die moontlikheid vir uitbreiding tot 999. Aantekeninge van die variasie en die taksonomiese waarde van die eienskappe word ook verstrek.

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A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view

R. P. ELLIS*

ABSTRACT

Descriptive "keys", including definitions and explanatory diagrams, are given for the standardization and simplification of anatomical descriptions of the epidermides of grass leaf blades as seen in surface view. About 340 characters of the epidermis are included with ample room for expansion. Notes on variation and taxonomic importance of the characters are also included.

RÉSUMÉ

PROCÉDURE DE STANDARDISATION DE L'ANATOMIE COMPARÉE DE LA FEUILLE CHEZ LES POACÉES. II. L'ÉPIDERME VU DU DESSUS

On donne des "clés" descriptives, incluant des définitions et des diagrammes explicatifs, pour la standardisation et la simplification des descriptions anatomiques de l'épiderme du limbe foliaire des graminées, vu du dessus. Environ 340 caractéristiques de l'épiderme y sont incluses et il reste possible d'élargir considérablement ce nombre. On y joint également des notes sur la variation et l'importance taxonomique des caractères.

INTRODUCTION

The importance of anatomy in agrostological studies has resulted in the rapid accumulation of an extensive body of literature with attendant problems of lack of uniformity with definitions and descriptions. Valuable data are, therefore, commonly not applicable to the family as a whole and comparisons cannot be drawn with any degree of assurance. This problem was greatly ameliorated by the publication in 1960 of Anatomy of the Monocotyledons. 1. Gramineae by C. R. Metcalfe and the present paper is a further attempt to stabilize this terminology and, at the same time to present a system whereby description and comparison of grass leaf anatomy will be simplified and standardized. See Ellis (1976) for a more comprehensive introduction.

DESCRIPTIVE KEYS

In an attempt to achieve the necessary uniformity, descriptive "keys" have been compiled for use as a framework for anatomical descriptions of the epidermis of the grass leaf-blade as viewed in surface view. The "keys", which incorporate both definitions and diagrams, have been designed to enable the user to standardize descriptions and the hierarchical layout has been chosen to facilitate the speed and ease at which complete, comparative descriptions can be compiled. Anatomical characters, and all other information considered to be of diagnostic or taxonomic importance, and gathered from an extensive survey of the relevant literature, have been included. The keys should, therefore, prove adequate for all tribes of the Poaceae.

The hierarchical tabulation of the characters has been used to expedite their use but they do not in any other way conform to any acknowledged key format or design. However, if a statement, at any level of the hierarchy, does not apply to the specimen being examined, no subsequent statements or characters of a lower rank are relevant. The user, therefore, merely proceeds to the next character of equal rank or indentation, and, if applicable, works inwards noting all the relevant numbered end points before working back outwards until the same level or rank as that of the originally chosen statement is reached. Thus, the "keys" are not dichotomous or true indented keys, but by using this type of format all the possible characters are recorded in a constant descriptive sequence. Each character is assigned a constant number and the recording of these numbers effects a saving in time and space. Furthermore, this system enables easy conversion to edge punched and feature cards as well as for electronic data processing by computer. In addition, it is ensured that all possible structures are rapidly and routinely noted in a rational sequence which simplifies compilation of descriptions of the various taxa. By employing a standard sequence significant differences become more readily evident.

In order for the standardization of terminology and descriptions to be effective, it is essential that comparative material be examined. Therefore, in this study, all descriptions refer to material taken at a point about halfway between the blade apex and the ligule of mature basal leaves. Flag leaves on flowering culms were avoided where possible. By standardizing on the material studied in this way infraspecific differences may be assessed.

In describing epidermal structure, Metcalfe (1960) has been followed. Thus, for descriptive purposes, it is assumed that the long axis of the leaf is horizontal. Visualized like this, it is possible to refer to zones and files of cells that lie parallel to the long axis as being "horizontal". The direction at right angles to the long axis is vertical for descriptive purposes.

It is important to note that other workers have not necessarily adopted this descriptive viewpoint. In fact, most authors have neglected to explain how they have visualized the epidermis when compiling descriptions. This lack of a standard convention undoubtedly leaves room for misunderstanding and misinterpretation of descriptions. For example, Bobrov (1955) uses "elongated in the direction of the blade" for horizontal *cf.* Metcalfe (1960), whereas Martin (1955) visualizes the veins as being vertical.

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1. INTERCOSTAL LONG CELLS

Includes cells that are usually elongated horizontally and are relatively narrow vertically and are situated in the intercostal zones of the epidermis. Excludes the interstomatal long cells, stomata, intercostal short cells and costal long cells. Bulliform cells as seen in surface view fall under intercostal long cells but are difficult to identify with any degree of certainty and thus only cells which vary in appearance with focus are termed bulliform cells.

DESCRIPTION OF CELL SHAPE: All applicable differences present on specimen to be included.

Ratio of horizontal (length) and vertical (width) dimensions:
- *Elongated* cells; length 3x, or more than 3x longer than width . . . 101*
- *Shortened* cells; less than 3x longer than wide:
  - Length slightly greater than width . . . 102*
  - Length and width approximately equal . . . 103*
  - Length less than width; tall and narrow . . . 104*

Side walls; anticlinal horizontal long walls:
- *Parallel* to one another; cells rectangular, square or trapezoidal . . . 105*
- *Angled outwards;* cells hexagonal . . . 106*
- *Bowed outwards;* cells inflated . . . 107*

End walls; anticlinal vertical cross-members:
- *Vertical;* at right angles to the horizontal walls . . . 108*
- *Angled* or *Sloping* in relation to the horizontal walls . . . 109*
- *Rounded;* cells of the inflated type . . . 110*
- *Overlapping* one another; interlocking . . . 111*

Thickness of horizontal and vertical anticlinal walls of long cells; appearance probably dependant upon the extent of development of the cuticular flanges; in general this indicates cuticle thickness:
- *Unthickened;* thin-walled; no cuticular flanges present . . . 112*
- *Slightly* thickened; slight cuticular flanges probably developed . . . 113*
- *Moderately* thickened; cuticular flanges developed . . . 114*
- *Heavily* thickened; deep, well-developed cuticular flanges probably present . . . 115*
- *Pitted* or beaded; visible pits developed in thickened walls . . . 116*

Undulations of horizontal anticlinal walls of long cells; sinuosity; measured and described in terms of waves i.e. the variables frequency, amplitude and wave-lengh:
- *Straight* walls; not undulate . . . 117*
- *Irregular;* slightly undulating or wavy; wave-length long, amplitude shallow and frequency low . . . 118*
- *Slightly* undulating; faintly corrugated; wave-length short, amplitude shallow and frequency high . . . 119*
- *Moderately* undulating; often irregular; wave-length short, amplitude variable and frequency high . . . 120*
- *Deeply* undulating; strongly corrugated; wave-length short, amplitude relatively deep and frequency high:
  - Ω-shaped . . . 121*
  - U-shaped . . . 122*

VARIATION IN CELL SHAPE

Cell shape as determined by the relative lengths and shapes of the horizontal and vertical anticlinal walls:
- *Variable shape:*
  - Shape varies in single *files* of intercostal long cells . . . 123*
  - Shape varies in diffirent *areas* of the preparation e.g. near margin or midrib . . . 124*
  - Shape varies across a single intercostal *zone* e.g. hexagonal cells centrally and rectangular cells laterally . . . 125*
- *Constant* or relatively constant shape in intercostal zones throughout the preparation . . . 126

Cell size; usually cell length determines differences; ratio of length to width may vary but shape (rectangular cuboid or hexagonal) remains constant:
- *Variable* size:
  - Size varies in single *files* of intercostal long cells . . . 127*
  - Size varies in different *areas* of the preparation e.g. near margin or midrib . . . 128*
  - Size varies across a single intercostal *zone:*
    - Narrower cells in centre of intercostal zones . . . 129*
    - Wider cells in centre of intercostal zones . . . 130*
- *Constant* or relatively constant size in intercostal zones throughout the preparation . . . 131

DISTRIBUTION OF ASSOCIATED CELLS: All types of intercostal short cells and appendages present between the intercostal long cells of the files included.

No short cells between the adjacent long cells; long cells adjoin one another . . . 132*

Intercostal short cells separating adjacent long cells of the files; mentioned only if frequent i.e. between 50% or more of the long cells:
- *Single* short cells present between successive long cells . . . 133*
- *Pairs* of short cells present between successive long cells . . . 134*
- *Silico-suberose* couples or cork-silica cell pairs between successive long cells . . . 135*
- *Micro-hairs* present between successive long cells . . . 136*
- *Hooks* and/or prickles present between successive long cells . . . 137*

DESCRIPTION OF BULLIFORM CELLS: Usually grouped with intercostal long cells but it is often very difficult to distinguish them from inflated intercostal long cells; in surface view a bulliform cell typically has an outline or shape that varies with different focal levels; they are usually thin-walled and are often inflated.

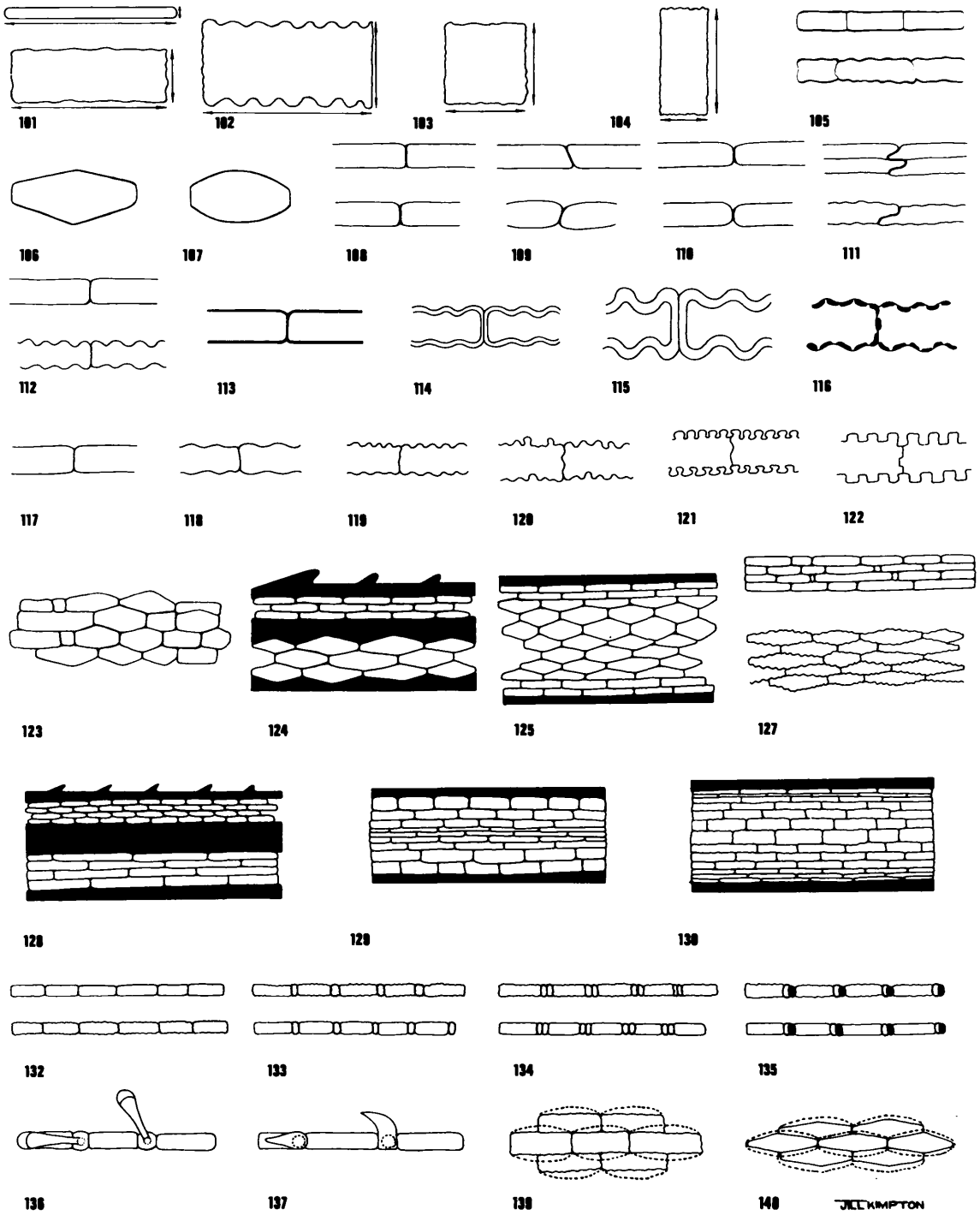
No bulliform cells present on surface of the preparation examined . . . 138*

Present; varying in appearance with focus; inflated, thin-walled:
- *Rectangular* in shape . . . 139*
- *Hexagonal* in shape . . . 140*

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INTERCOSTAL LONG CELLS



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2. STOMATA

The stomatal complexes as well as the interstomatal long cells are included in this section. They are both confined to the intercostal zones and are usually in well-defined bands.

STOMATAL COMPLEXES: The stomatal complex or stomatal apparatus consists of a pair of guard cells with their lumina enlarged at either end and more constricted in the middle as seen in surface view. These together with the pore between them form the stoma. Both the guard cells are accompanied on their outer, horizontal faces by a pair of subsidiary or accessory cells differing in shape and size from the other epidermal cells. The shape of the stomatal complex is determined by the outline of these subsidiary cells. This shape is often variable and then all different shapes that occur fairly frequently (about 25% of the stomata visible) must be mentioned.

DESCRIPTION: Determined by subsidiary cell shape in surface view. Intermediate types often present and all common types must be mentioned.

- **Absent;** no stomata visible on surface of preparation examined . . . 201
- **Types** of stomata present; determined by shape of subsidiary cells:
  - *Triangular;* panicoid types:
    - Low triangular; long and broadly angular subsidiary cells . . . 202*
    - High triangular; wide and markedly angular subsidiary cells:
      - Apex not evaginated; diamond-shaped stomatal complex . . . 203*
      - Apex drawn out into a point; evagination often containing the nucleus . . . 204*
  - *Dome-shaped;* subsidiary cells rounded:
    - Low dome-shaped; ovoid; vertical width of the subsidiary cells smaller in relation to the horizontal length . . . 205*
    - Tall dome-shaped; vertical width of the subsidiary cells greater in relation to the horizontal length . . . 206*
  - *Parallel-sided* subsidiary cells; rectangular in outline; stomatal complex long and narrow . . . 207*
  - *Flat-topped;* outer, horizontal walls of the subsidiary cells straight; often short and may be parallel:
    - Rounded with flattened top . . . 208*
    - Side walls and outer horizontal wall straight . . . 209*
- **Distribution** of the stomata in the intercostal zones:
  - *One* row of stomata in each intercostal zone most common:
    - Always one row of stomata in all intercostal zones . . . 210
    - Usually one row but sometimes two rows of stomata present . . . 211
    - Usually one row but sometimes two or three rows of stomata present . . . 212
  - *Two* rows of stomata in each intercostal zone most common:
    - Always two rows of stomata in all intercostal zones . . . 213
    - Usually two rows but sometimes one row of stomata present . . . 214
    - Usually two rows but sometimes three or more rows of stomata present . . . 215
  - *Three* rows of stomata in each intercostal zone most common:
    - Always three rows or stomata in all intercostal zones . . . 216
    - Usually three rows of stomata but sometimes more or less present . . . 217
  - *Four* rows of stomata in each intercostal zone most common . . . 218
  - *Five* rows of stomata in each intercostal zone most common . . . 219
  - *More* than five rows of stomata in each intercostal zone . . . 220
- **Arrangement** of the rows of stomata in the intercostal zones:
  - *Definite* rows of stomata present:
    - Rows separated by files consisting of intercostal long cells:
      - Separated by more than one file of intercostal long cells . . . 221*
      - Separated by only one file of long cells . . . 222*
    - Rows adjacent to one another; not separated by files of intercostal long cells . . . 223*
  - *Not* in definite rows but stomata present throughout the intercostal zone in any horizontal file of long cells; may be as many rows of stomata as there are intercostal files of long cells . . . 224
- **Position** of the rows of stomata in the intercostal zones:
  - *Centrally* situated rows in the centre of the intercostal zones . . . 225
  - *Restricted* stomatal rows; restricted to narrow zones adjoining the costal zones:
    - Double row adjoining the costal zones . . . 226
    - Single row adjoining the costal zones . . . 227
  - *Throughout* intercostal zones; stomatal rows not restricted to areas in the intercostal zones . . . 228

INTERSTOMATAL LONG CELLS: The interstomatal long cells lie in the same horizontal, longitudinal files as the stomata i.e. in the stomatal rows. They are only recognisable if the stomata are fairly frequent, regularly spaced and arranged in definite stomatal rows.

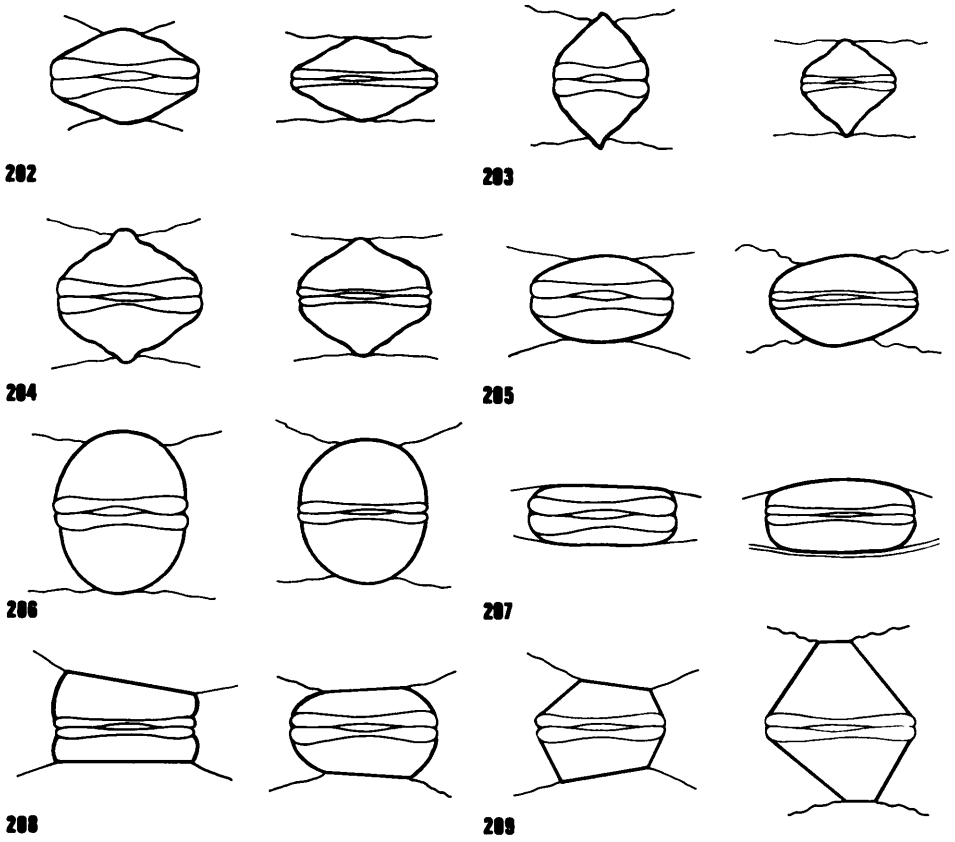
DESCRIPTION:

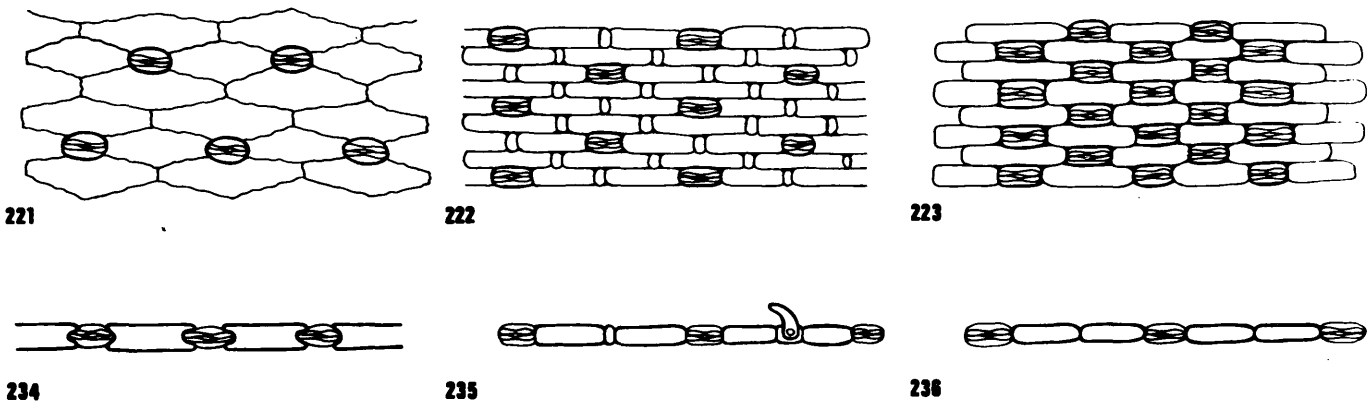
- **Absent;** no interstomatal long cells present:
  - *No* stomata present on surface examined . . . 229
  - *Infrequent* stomata; successive stomata of a row separated by more than three long cells . . . 230
- **Size** of intercostal long cells; greatest horizontal and vertical dimensions:
  - *Relatively long;* cells 3x or more longer than wide . . . 231
  - *Relatively short;* cells less than 3x longer than wide:
    - Length more than width . . . 232
    - Length and width approximately equal; square . . . 233
- **Arrangement** of files of interstomatal long cells in relation to successive stomata of a row:
  - *One* interstomatal cell between successive stomata; with concave ends . . . 234*
  - *Two* interstomatal cells between successive stomata:
    - Separated by short cells or hairs . . . 235*
    - Adjoin one another . . . 236*
  - *More* than two interstomatal cells between successive stomata of a row:
    - Separated by short cells or hairs . . . 237
    - Adjoin one another . . . 238

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STOMATA





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646 A PROCEDURE FOR STANDARDIZING COMPARATIVE LEAF ANATOMY IN THE POACEAE. II. THE EPIDERMIS AS SEEN IN SURFACE VIEW

3. INTERCOSTAL SHORT CELLS

Includes cells that are usually nearly equidimensional in shape but may be somewhat longer horizontally than they are vertically or their vertical width may exceed their horizontal length. They are, however, always smaller than the intercostal long cells with which they often alternate in horizontal rows. Intercostal short cells occur in pairs such as cork and silica cells or are solitary such as short intercostal cells and the cells from which micro-hairs, hooks and prickles arise. The silica bodies contained within typical silica cells of the cork-silica cell pairs are not included in this section but are described under Silica Bodies. Similarly, stomatal complexes and the cells of the cushion base of macro-hairs are excluded and described under the relevant sections.

DESCRIPTION OF SHORT CELLS: For variable types include all different types that occur on the preparation.

- **Absent;** no short cells in intercostal zones of surface examined 301
- **Solitary** short cells; includes cells from which hooks and micro-hairs arise:
  - *Unsilicified;* excludes only cells containing a distinct silica body; if cell walls only are silicified they are included here:
    - Tall and narrow in shape; vertical dimension greater than horizontal dimension:
      - Smooth outline 302*
      - Crenate outline 303*
    - Square or rectangular in shape:
      - Smooth walls 304*
      - Sinuous or undulating walls 305*
    - Round or elliptical in shape 306*
  - *Silicified* silica cells containing distinct silica body or phytolith; solitary; excludes silicified cork cells, hooks or prickles; silica body described under Silica Bodies:
    - Silica body and silica cell of same or similar shape 307*
    - Silica body and silica cell different in shape 308*
- **Paired short cells** situated between long cells:
  - *Silico-suberose couples;* intercostal cork-silica cell pairs:
    - Shape of cork cell; silica body described under Silica Bodies:
      - Tall and narrow:
        - With smooth outlines 309*
        - With crenate or irregular outlines 310*
      - Crescentic, enfolding the silica cell 311*
      - Square to rectangular in shape 312*
  - *Short cell pairs;* excludes cork and silica cells; included are cells from which hooks and micro-hairs arise:
    - Tall and narrow; both cells of the pair vertically elongated:
      - Smooth outline 313*
      - Irregular outline 314*
      - Crenate outline 315*
    - Square to rectangular; either one or both cells:
      - Straight walls 316*
      - Sinuous or undulating walls 317*

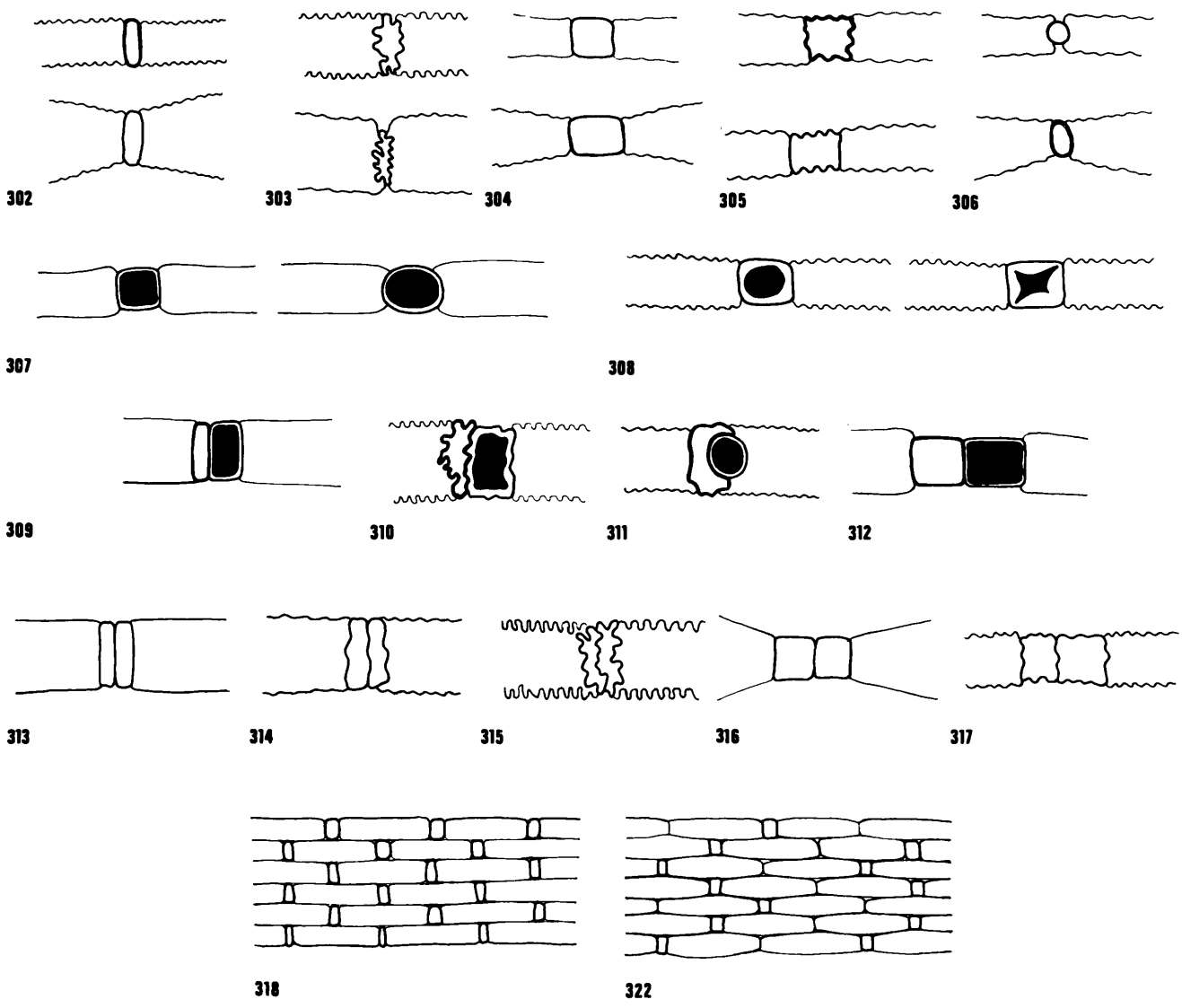
DESTRIBUTION OF SHORT CELLS:

- **Location** of short cells or cell pairs in relation to long cells:
  - *Between* each successive long cell in a file; present at both ends of each long cell:
    - Between all or most long cells i.e. 75% or more of the long cells with a short cell or cell pair between them 318*
    - Common between long cells only in the region flanking the costal zone 319
    - Irregular; presence varies for different areas of the preparation 320
    - Rare throughout the preparation 321
  - *Between* pairs of successive long cells in a file; present at only one end of each long cell:
    - Present between most or all alternative long cells i.e. 75% or more of the long cells 322*
    - Irregular; presence differs for different areas of the preparation 323
    - Rare throughout the preparation 324

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INTERCOSTAL SHORT CELLS



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4. PAPILLAE

Papillae are variously shaped protrusions from the outer walls of epidermal long and short cells particularly of the intercostal zones. When viewed in surface view they may appear as small conical, cutinised structures or may appear larger, inflated and usually thin-walled. A single papillus per cell is most commonly found but there may be numerous papillae, arranged in several rows or even of two different types, present on each individual long cell.

DESCRIPTION OF PAPILLAE: Include all diferent types present but continue with distribution and arrangement of each separate type before commencing with descriptions of other types of papillae that may be present on the specimen.

- **Absent;** no papillae present on the epidermal cells of the preparation 401
- **Circular** or **Rounded** papillae as seen in surface view; conical:
  - *Large;* diameter of the papillae more than $\frac{1}{2}$ the vertical width of the long cells; only a single horizontal row of large papillae can be accommodated on individual long cells:
    - Unthickened, dome-shaped, inflated 402*
    - Slightly thickened, inflated; apex often appears concave (interference contrast microscopy) 403*
    - Thick-walled, inflated; often fairly elongated; apex often appears concave (interference contrast microscopy) . . 404*
  - *Small;* diameter of the papillae usually less than $\frac{1}{2}$ the vertical width of the long cells; more than one horizontal row of small papillae can be accomodated side by side on individual long cells:
    - Thin-walled, inflated 405*
    - Thickened, cuticular, variously shaped 406*
- **Oblique** papillae; many papillae seen in side view; during specimen preparation the elongated and often hair-like papillae become folded over in many cases:
  - *Unthickened* papillae; walls not thickened:
    - Large; entire outer wall of long cell convex and inflated; often overarch neighbouring cells 407*
    - Distinct, not incorporating the entire outer wall; restricted to distinct part of the outer wall; may overarch neighbouring cells 408*
  - *Distally thickened;* only extremity of papillus thickened 409*
  - *Thickened* papillae; walls thickened 410*
  - *Globose,* inflated; often in chains in costal zones 411*

DISTRIBUTION OF PAPILLAE: Include all locations represented.

- **Intercostal zone** epidermal cells papillate.
  - *Bulliform cells* of intercostal zone with papillae:
    - Present on many or all bulliform cells; more than 50% of cells papillate 412
    - Present on relatively few bulliform cells; less than 50% of cells papillate 413
  - *Interstomatal* long cells with papillae:
    - Present on many or all interstomatal long cells; more than 50% of cells papillate 414
    - Present on relatively few interstomatal long cells; less than 50% of cells papillate 415
  - *Intercostal long cells* with papillae:
    - Present on many or all intercostal long cells; more than 50% of cells papillate 416
    - Present only on long cells at the edges of the intercostal zones; not in the central areas 417
    - Present on relatively few intercostal long cells; less than 50% of cells papillate 418
  - *Intercostal short cells* papillate; includes cork cells 419
- **Costal zone** epidermal cells papillate:
  - *Costal long cells* papillate:
    - Present on many or all costal long cells 420
    - Present on few costal long cells; infrequent 421
  - *Costal short cells* papillate 422

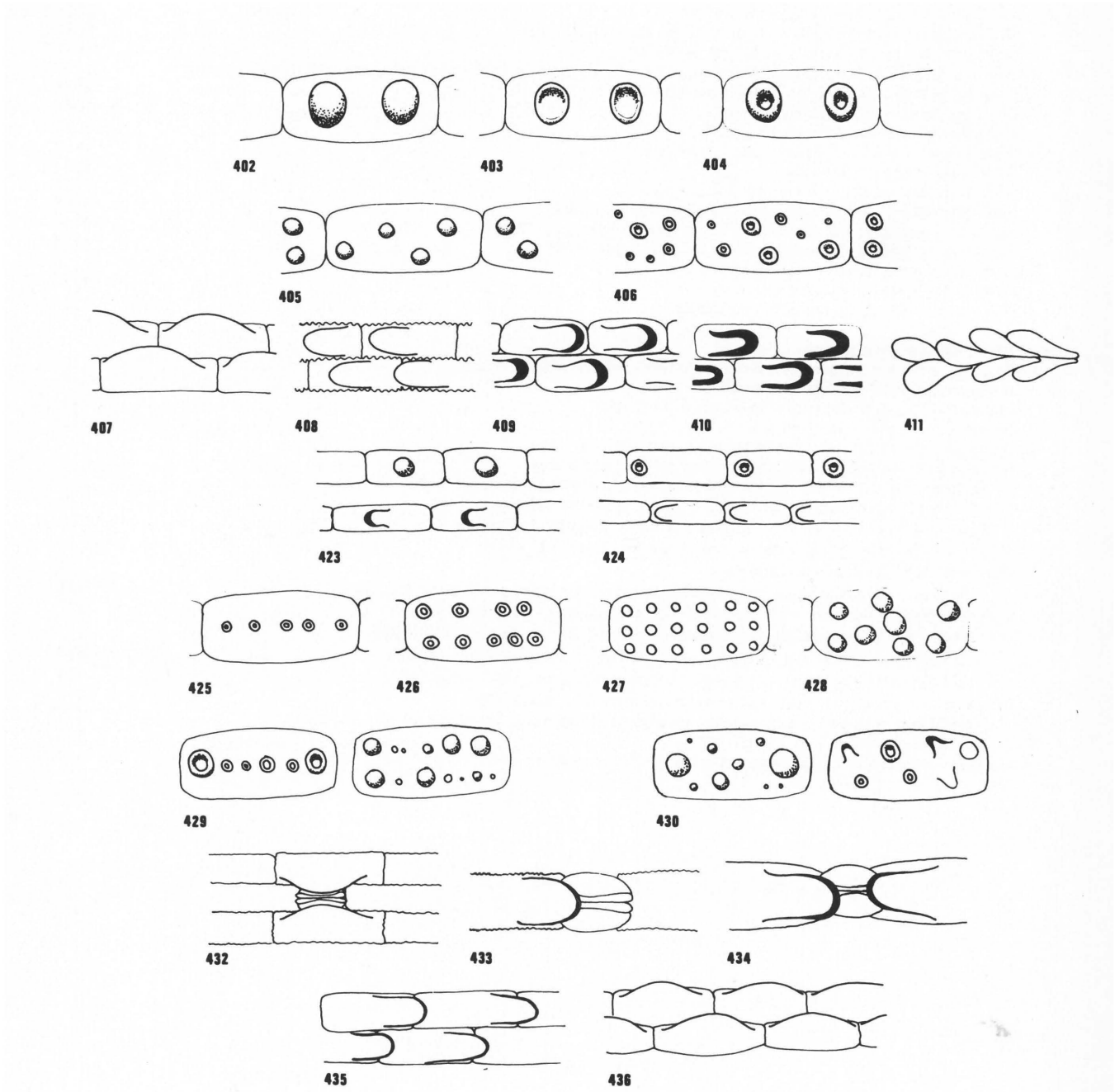
ARRANGEMENT OF PAPILLAE: Arrangement on individual cells and association with neighbouring cells.

- **One** papillus per cell:
  - *Centrally* positioned 423*
  - *Distally* situated on epidermal cells 424*
- **More** than one papillus per cell:
  - *Same type;* all papillae on individual cells of the same size and shape:
    - Arranged in a single horizontal row 425*
    - Arranged in two or more horizontal rows 426*
    - Arranged in vertical as well as horizontal rows 427*
    - Irregularly arranged 428*
  - *Different type;* papillae of different sizes or shapes present on individual cells:
    - Arranged in one or more horizontal rows 429*
    - Irregularly arranged 430*
- **Association** of papillae with neighbouring cells:
  - *No* overarching of neighbouring cells; papillae not oblique 431
  - *Overarch* adjacent cells in same or adjacent horizontal file of long cells:
    - Overarch the stomata:
      - From intercostal long cells 432*
      - From interstomatal long cells:
        - Overarch from only one adjacent interstomatal long cell in the same file 433*
        - Overarch from both adjacent interstomatal long cells of the same file 434*
    - Overarch neighbouring intercostal long cells:
      - From the same file of long cells 435*
      - From the adjacent file of long cells 436*

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PAPILLAE



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5. PRICKLE HAIRS

Robust, pointed structures with swollen bases arising directly from, and forming an integral part of the epidermis. Generally with thick, lignified walls. May conveniently be divided into two main types, prickles and hooks, on the basis of size. Intermediate types do occur, and also included are angular prickles and asperites. Descriptions refer to shape as seen in surface view but prickle hairs are often seen laterally and their lateral appearance is also included. Dimensions are gauged by the distance between parallel lines projected through the tip of the barb and the ends of the base furthest and nearest the barb tip.

PRICKLES:

Usually larger than hooks and with elongated, oval or elliptical bases. Excludes any prickles present on the leaf margin. Also known as aiguillons, asperites or teeth.

DESCRIPTION OF PRICKLES:

Absent; presumed missing i.e. no prickles observed on surface examined 501
Base size; estimated by comparison with the size (length) of the stomata of the same leaf:
Small prickles; base shorter than the stomata 502*
Medium prickles; base as long or slightly longer than the stomata 503*
Large prickles; base at least twice as long as the stomata 504*
Barb size; estimated by comparison with the size (length) of the base of the same prickle as seen in surface view:
Long barb; barb as long or longer than the base 505*
Short barb; barb shorter than the base 506*
Unbarbed or unpointed; incipient asperity 507*
Barb shape in relation to base; as seen in lateral view:
No prickles visible in side view 508
Barb developed basally from the apex of the base 509*
Barb developed from the apex of the base but slightly raised 510*
Barb not developed from the apex of the conical base; raised 511*
Barb with recurved point not developed from the apex of the base 512*
Barb orientation in relation to leaf blade:
Point to leaf apex; all in same direction 513
Point in both directions; apically and basally 514

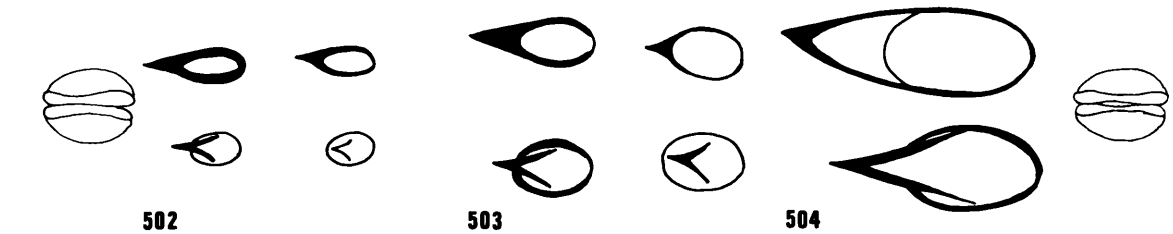
DISTRIBUTION OF PRICKLES:

Intercostal; present in intercostal zone; usually small and transitional prickles:
Interstomatal long cell files with prickles 515
Intercostal long cell files with micro hairs and prickles 516
Other intercostal files with prickles 517
Costal; present in costal zones overlying any vascular bundle order:
One-three files of cells comprise all or some of the costal zones:
Frequent; not more than 5 silica bodies between successive prickles of a file 518
Infrequent; more than 5 silica bodies between successive prickles of a file 519
Rare; present in only some 1–3 file costal zones; irregular distribution 520
More than three files of cells comprise all or some of the costal zones:
Single file of prickles per costal zone:
Frequent; not more than 5 silica bodies between successive prickles of a file 521
Infrequent; more than 5 silica bodies between successive prickles of a file 522
As many files of prickles present as there are files of silica bodies per costal zone:
Frequent; not more than 5 silica bodies between successive prickles of a file 523
Infrequent; more than 5 silica bodies between successive prickles of a file 524
Single row of prickles at margins of costal zone; often seen in side view:
Interlocking with prickles of adjacent costal zone; overlying stomatal groove 525*
Do not interlock 526
Irregular, scarce; often more common in costal zones nearer margin 527

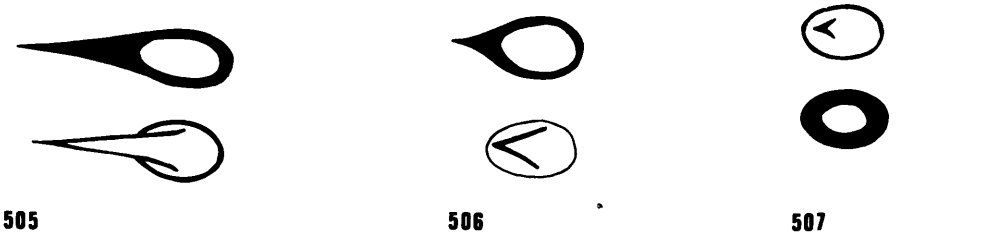
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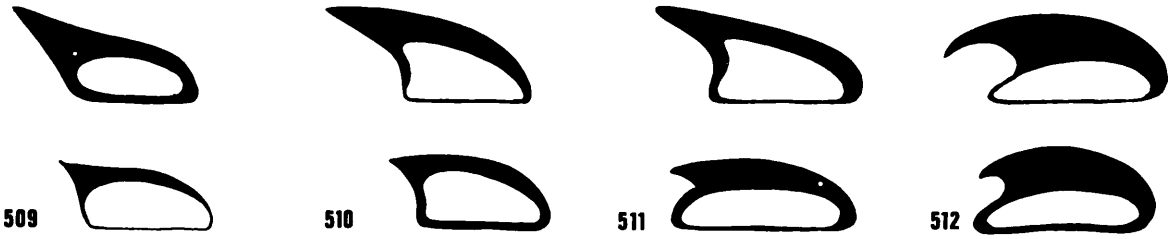
PRICKLES



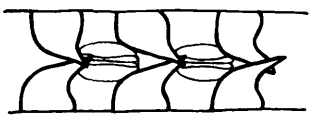
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525

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5. PRICKLE HAIRS CONTINUED

HOOKS:
Smaller than prickles usually with rounded bases. Generally present in intercostal zones. Also termed crochets or crown cells.

DISTRIBUTION OF HOOKS:

- **Absent;** presumed missing; not observed on surface of preparation examined 528
- **Costal;** present in costal zones of the leaf:
  - *Throughout* costal zones; not limited to any area of costal zones 529*
  - *On margins* of costal zones only 530*
- **Intercostal;** present in intercostal zones of the leaf:
  - *Adjacent* to costal zone; between intercostal long cells adjacent to costal zones 531*
  - *Centrally* located in intercostal zones; this type often in bulliform cell files 532*
  - *Interstomatal;* in same files as stomata 533*

ANGULAR PRICKLES OF MARGIN:
Includes only prickle hairs present on the leaf margin.

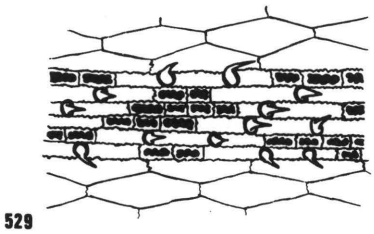
DESCRIPTION OF ANGULAR PRICKLES:

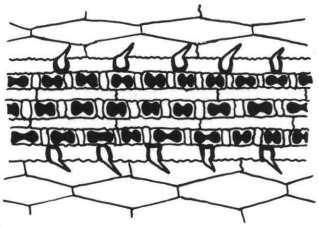
- No leaf margin present on preparation 534
- **Absent;** leaf margin present but no prickles present 535
- **Base size;** estimated by comparison with the length of the stomata of the same leaf:
  - *Small;* base shorter than the stomata 536*
  - *Medium;* base as long or slightly longer than the stomata 537*
  - *Large;* base much longer than the stomata; more than 2x 538*
- **Barb length** in relation to length of base:
  - *Short;* less than length of base 539*
  - *Medium;* as long as or slightly longer than base 540*
  - *Long;* much longer than the base, more than 2x as long 541*

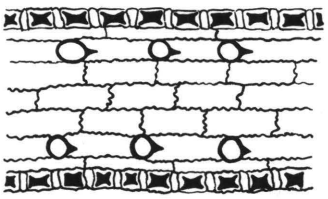
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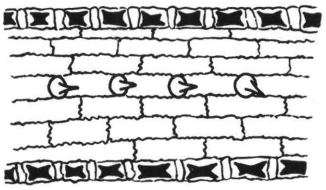
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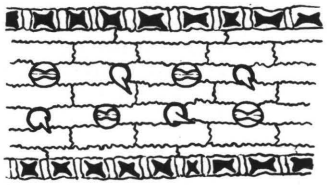
HOOKS AND ANGULAR PRICKLES


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6. MICRO-HAIRS

Micro-hairs are commonly termed bicellar trichomes although one-celled hairs do occur that are clearly homologous with the two-celled hairs. The distal cell is invariably very thin-walled and often is absent. Micro-hairs are valuable diagnostic characters and their presence alone is important. Thus preparations must be carefully examined as the hairs often occur infrequently and the mere presence of a solitary hair on a preparation may be taxonomically important.

DESCRIPTION: Continue further under Distribution for all types that occur.

- **Absent;** presumed missing i.e. none observed on surface of preparation examined . . . 601
- **Unicellular,** rounded to dome-shaped; **Sporobolus** type; associated with a cork cell . . . 602*
- **Bicellular,** two-celled; bicellar micro-hairs:
  - *Only basal cell* remains or is visible . . . 603
  - *Relative lengths* of basal and distal cells:
    - Approximately equal in length . . . 604*
    - Basal cell shorter than distal cell:
      - Basal cell less than $\frac{1}{2}$ the length of the distal cell . . . 605*
      - Basal cell only slightly shorter than the distal cell . . . 606*
    - Basal cell longer than distal cell:
      - Basal cell 2x, or more than 2x, longer than the distal cell . . . 607*
      - Basal cell slightly longer than the distal cell . . . 608*
  - *Relative shapes* of basal and distal cells:
    - Both cells approximately the same shape:
      - Cells of micro-hair narrow or thin:
        - Markedly elongated; micro-hair longer than the stomatal complex . . . 609*
        - Slender but short; micro-hair shorter than the stomatal complex . . . 610*
      - Cells of micro-hairs inflated and rounded . . . 611*
    - Basal cell less inflated than the distal cell; clavate hair . . . 612*
    - Basal cell more inflated than the distal cell . . . 613*
  - *Relative thickness* of basal and distal cell walls:
    - Walls of both cells approximately equal in thickness . . . 614*
    - Wall of distal cell thinner than wall of basal cell; often deciduous . . . 615*
  - *Shape* of distal cell:
    - Length equal to or less than width . . . 616*
    - Length slightly more than the width; length not more than 2x the width . . . 617*
    - Length much more than the width; length more than 2x the width . . . 618*
  - *Apex* of distal cell:
    - Broadly rounded to dome-shaped . . . 619*
    - Slightly tapered; tapering to a rounded apex . . . 620*
    - Tapered to a definite point . . . 621*
    - Sharply pointed . . . 622*
  - *Length* of basal cell:
    - Lenth approximately equal to width; not more than 2x the width . . . 623*
    - Length much greater than the width; more than 2x . . . 624*
  - *Shape* of base or attachment of basal cell:
    - Constricted base:
      - Inverted cone-shaped . . . 625*
      - Cupule-shaped . . . 626*
      - Parallel-sided; point of attachment small . . . 627*
    - Expanded base; not constricted:
      - Constriction above bulbous base . . . 628*
      - Parallel-sided . . . 629*
      - Bulbous i.e. broadly inflated . . . 630*
  - *Emergence* of base from short cell:
    - Turned at right angles to the short cell as seen in surface view . . . 631*
    - Emerges straight out of short cell; no angle at base . . . 632*

DISTRIBUTION: To be considered separately for each type of hair present.

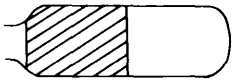
- **Intercostal;** occur in intercostal zone between long cells:
  - Between both intercostal long cells and interstomatal cells . . . 633
  - Between intercostal long cells only; not between interstomatal cells:
    - Near costal zone; between stomatal bands and costal zone . . . 634
    - In the centre of the intercostal zone . . . 635
  - Between interstomatal long cells only . . . 636

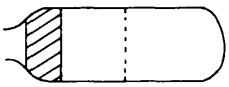
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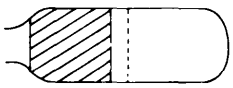
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MICRO-HAIRS

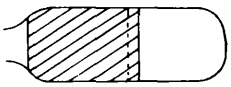

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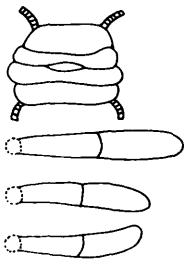

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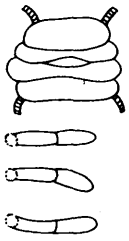

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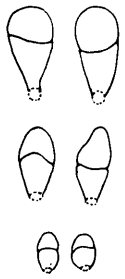

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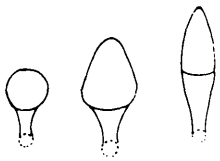

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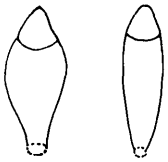

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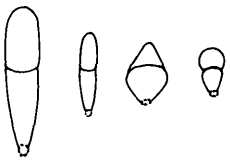

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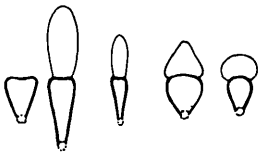

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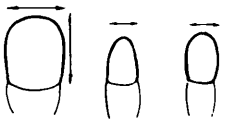

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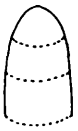

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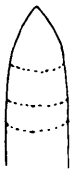

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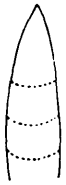

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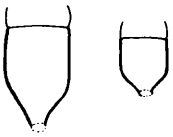

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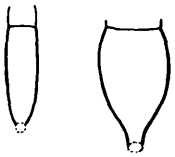

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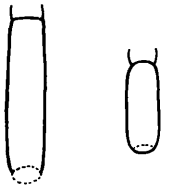

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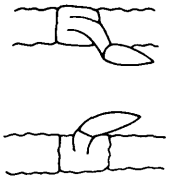

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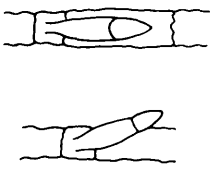

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7. MACRO-HAIRS

Includes all trichomes not included under micro-hairs or prickles. Invariably the macro-hairs are much longer than the micro-hairs and are generally unicellular although fine transverse partitions are sometimes present. The hairs vary in length, in flexibility, in the degree of cell-wall thickening and may possess superficial or sunken bases. Hairs with sunken bases may, or may not, be accompanied by specialized epidermal cells. Most uni-cellular macro-hairs are probably homologous with prickles and are often difficult to distinguish apart, especially in cases where prickles have long barbs or where macro-hairs are short and rigid. In such instances it is advisable to describe the hairs under both prickles and macro-hairs and to make a special note of this fact.

DESCRIPTION:

Absent: no macro-hairs seen and therefore presumed absent 701

Number of cells comprising the macro-hairs:

Unicellular macro-hairs present 702*

Bicellular macro-hairs present 703*

Multicellular macro-hairs present 704*

Flexibility of macro-hairs; correlated to wall thickness of hair cell/s:

Hard, stiff hairs usually with thickened walls 705*

Soft, thin-walled hairs often damaged during preparation; slender 706*

Epidermal cells associated with base of macro-hair:

One specialized hemispherical epidermal cell accompanying base of hair 707*

Two (sometimes one) specialized cells accompanying base of hair 708*

Many, usually smaller, specialized epidermal cells accompanying base of hair 709*

No specialized cells associated with base of macro-hair 710*

Nature of base of hair:

Swollen in relation to hair thickness 711*

Constricted in relation to hair thickness 712*

Undifferentiated in relation to hair thickness 713*

Association of the base of the macro-hairs with the epidermis; often difficult to ascertain from epidermal preparations:

Superficial; base constitutes part of the epidermis 714*

Sunken base embedded between and often below surrounding epidermal cells:

Sunken below general level of the epidermis 715*

Raised epidermal cells with sunken hair base surrounding hair; cushion hair 716*

Specialized types of macro-hairs; not included above:

Stalked, glandular bicellar hair; *Enneapogon* type 717*

Unicellular macro-hairs with swollen tips 718*

Crozier hairs 719*

Multicellular clavate hairs 720*

Length of macro-hairs; measurements taken by comparison with the known field of view of the microscope. These categories are standardized for the Zeiss Standard RA microscope fitted with x10 Kpl W eyepieces. If hairs of variable length are present include all relevant categories:

X16 Objective:

More than twice the field of view at x160 i.e. longer than 2,25 mm 721

Equal to or slightly more than the field of view at x160 i.e. between 1,125 mm – 2,25 m long 722

Less than the field of view at x160 i.e. less than 1,125 mm long 723

Frequency of occurrence of macro-hairs on surface of leaf examined:

Abundant; more than 10 hairs visible in field of view at x160 724

Frequent; between 3 and 10 hairs visible in field of view at x160 725

Scarce: 1 or 2 hairs visible in field of view at x160 726

Irregular; parts of leaf without hairs; present on other areas 727

Distribution of macro-hairs in the various zones of the leaf surface:

Intercostal exclusively 728

Costal exclusively 729

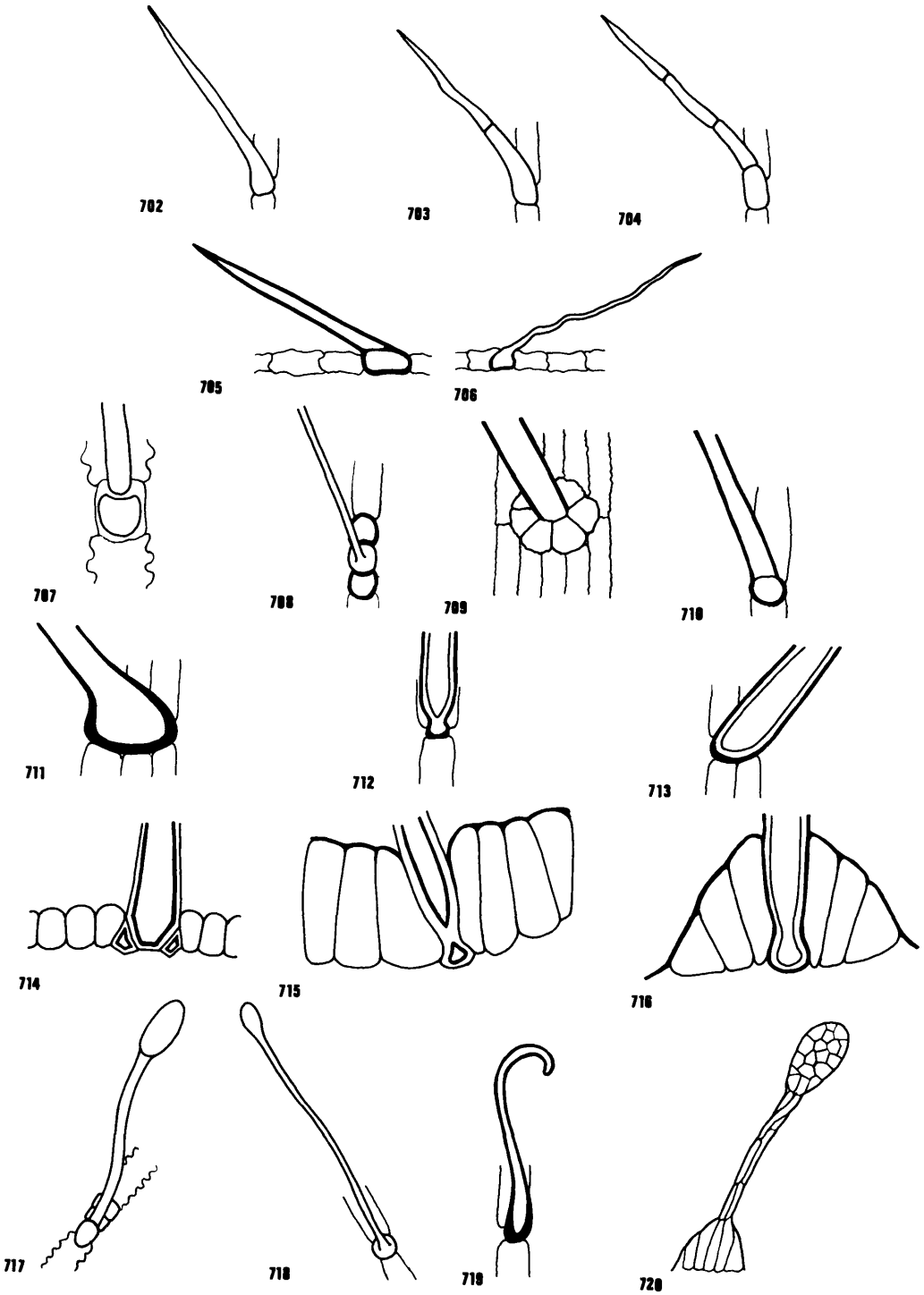
Both costal and intercostal in distribution 730

Leaf margins only or mainly 731

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MACRO-HAIRS



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8. SILICA BODIES

Silica cells are idioblasts present in both the costal and intercostal zones and containing silica bodies or opal phytoliths of characteristic shapes. The shapes of the silica cells are not necessarily the same as those of the silica bodies contained in them and it must be carefully noted that for descriptive purposes only the shape of the silica body is described. All applicable variations in shape present on the specimen must be included. Immediately after each different type of silica body has been described, continue with distribution and further descriptive details before commencing with any other types of silica bodies which may be present on the specimens.

DESCRIPTION: Based on outline of the silica bodies when viewed from above and when granules in focus.

- **Absent** i.e. no silica bodies observed on surface examined . . . 801
- **Vertically elongated,** tall; vertical dimensions greater than horizontal dimensions:
  - *Tall and narrow* (sometimes more square-shaped):
    - Smooth, regular outline . . . 802*
    - Irregular outline . . . 803*
    - Crenate or scalloped outline . . . 804*
  - *Crescent- or kidney*-shaped . . . 805*
  - *Elliptical* in shape . . . 806*
  - *Dumb-bell/cross*-shaped:
    - *Oryza*-type; vertically arranged dumb-bell shaped; vary with focus . . . 807*
    - Short, tall and perpendicular . . . 808*
    - Short, tall and tilted or angular . . . 809*
  - *Saddle*-shaped, tall . . . 810*
- **Equidimensional;** vertical and horizontal dimensions approximately equal:
  - *Small and crenate;* closely enveloped by accompanying short/cork cell . . . 811*
  - *Acutely angled,* sometimes resemble crystals; often intercostal . . . 812*
  - *Squarish,* angular, with irregular outlines . . . 813*
  - *Square,* cubical or slightly rectangular . . . 814*
  - *Cuboid,* more or less squarish with rounded corners; sub-circular . . . 815*
  - *Round,* circular in shape:
    - Fitting into a concavity of a cork cell . . . 816*
    - Not intimately associated with a cork cell . . . 817*
  - *Saddle*--shaped, rounded, shortened, double-axe shaped . . . 818*
  - *Cross*-shaped, with four apices not rounded cf. dumb-bell shaped:
    - Regular, perpendicular . . . 819*
    - Irregular, tilted . . . 820*
  - *Irregular dumb-bell* shaped; intermediate between cross- and dumb-bell shaped . . . 821*
  - *Dumb-bell* shaped, relatively short, not elongate:
    - Nature of central region of dumb-bell:
      - Wide middle/central portion . . . 822*
      - Constricted, narrow central portion . . . 823*
    - Nature of ends of dumb-bell shaped bodies:
      - Rounded . . . 824*
      - Flattened . . . 825*
      - Indented . . . 826*
- **Horizontally elongated;** horizontal dimensions greater than vertical dimensions:
  - *Oblong* in outline . . . 827*
  - *Saddle*-shaped; elongate . . . 828*
  - *Elongate* with rounded ends:
    - Smooth outline . . . 829*
    - Sinuous outline . . . 830*
    - Crenate outline . . . 831*
    - Nodular outline or shape . . . 832*
  - *Dumb-bell* shaped; elongated:
    - Nature of central region of dumb-bell:
      - Wide middle/central portion . . . 833*
      - Constricted, narrow central portion . . . 834*
    - Nature of ends of dumb-bell shaped bodies:
      - Rounded . . . 835*
      - Flattened . . . 836*
      - Indented . . . 837*
    - Length of central portion of dumb-bell shaped bodies:
      - Equal to one third of the total length of the body . . . 838*
      - Less than one third of the total length of the body . . . 839*
      - Greater than one third of the total length of the body . . . 840*

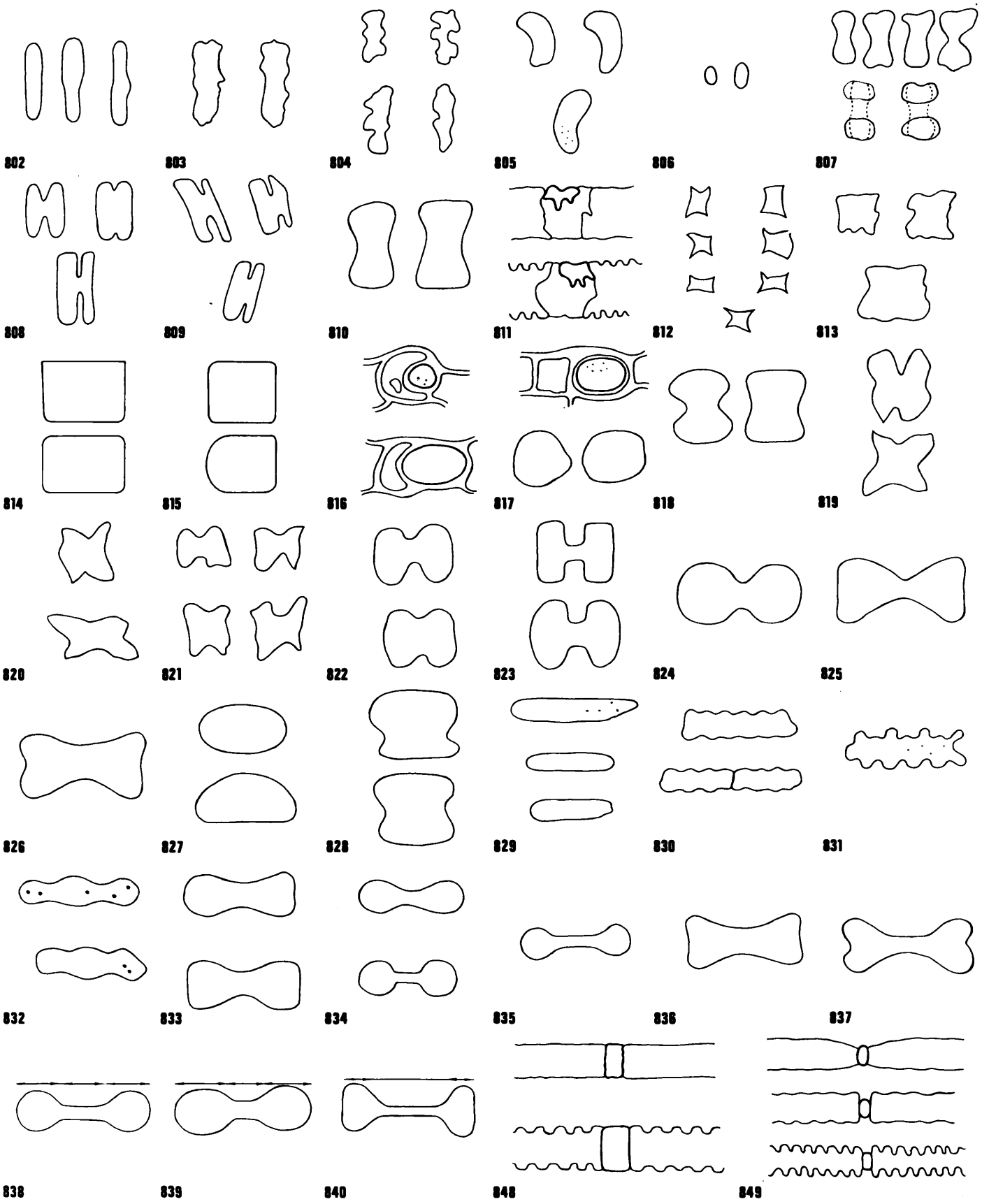
DISTRIBUTION AND DESCRIPTION CONTINUED: To be completed independently for each of the different types of silica body present on the specimen.

- **Distribution:**
  - *Costal zones:*
    - Present throughout the costal zones . . . 841
    - Present on outer edges of the costal zones only . . . 842
  - *Intercostal zones:*
    - Present throughout the intercostal zones . . . 843
    - Present only on outer edges of the intercostal zones . . . 844
- **Description** continued:
  - *Granules:*
    - Not seen in silica bodies . . . 845
    - Present in silica bodies . . . 846
    - Cracks in silica bodies visible . . . 847
  - *Width;* vertical dimensions:
    - Approximately the same as that of adjacent short or long cells . . . 848*
    - Much less than that of adjacent short or long cells . . . 849*

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SILICA BODIES



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9. COSTAL SHORT CELLS

All costal epidermal cells are incorporated in this section. Includes cork cells (suberised cells), silica cells (silicified short elements), prickles, ordinary equidimensional to rectangular costal short cells and costal long cells. Excludes only the bodies contained within the costal silica cells.

DESCRIPTION AND ARRANGEMENT: Include all applicable different types.

Horizontal arrangement; composition of all individual horizontal files, with or without silica bodies:

Costal short cells only; perhaps a few silica cells or costal long cells interspersed along files:

Short, square to slightly rectangular:

With sinuous walls 901*

With straight walls 902*

Rectangular cells but not more than 3x longer than wide:

Same width as intercostal long cells 903*

Narrower than intercostal long cells 904*

Costal long cells predominate; perhaps a few silica cells or costal short cells interspersed along files; includes only cells more than 3x longer than wide:

Width of long cells:

Same width as intercostal long cells 905*

Narrower than intercostal long cells; often extremely long 906*

Nature of other cells in files:

Files consist entirely of costal long cells 907*

Paired short cells between successive long cells 908*

Solitary short cells between successive long cells 909*

Silico-suberose couples; cork-silica cell pairs; closely associated cork and silica cells separated by normal costal short or long cells:

Frequency of occurrence in costal files of cells:

Common in files; separated by few (1–3) costal short cells:

Separated by short, or slightly rectangular cells 910*

Separated by rectangular cells 911*

Infrequent in files; separated by numerous short cells (or a few very long cells):

Separated by many short or slightly rectangular cells 912*

Separated by numerous rectangular costal short cells 913*

Separated by few very long costal long cells 914*

Relative shape of cork and silica cells comprising the pairs:

Cork cell same shape as silica body 915*

Cork cell tall and narrow; silica body not tall and narrow 916*

Cork cell crescentic and enfolding the silica body 917*

Intermediate between silico-suberose couples and alternating condition; a single cork cell may thus sometimes be associated with two adjoining silica cells and sometimes silica cells may be without cork cells:

Relative shape of cork and silica cells:

Cork cell same shape as silica body 918*

Cork cell tall and narrow; silica body not tall and narrow 919*

Cork cell crescentic and often enfolding the silica body 920*

Alternating silica cells and costal short cells; pairs not distinguishable as the sequence in a file is silica, short (cork), silica, short (cork), etc. and not silica, cork, short, silica, cork, short, silica etc.:

Regular arrangement along individual files:

Short to square short or cork cells 921*

Rectangular to long cork or short cells 922*

Irregular arrangement along individual files; short sections with 2–5 cork or short cells in place of alternating silica and short cells:

Short to square short or cork cells 923

Rectangular to long cork or short cells 924

Silica cells most common along individual files:

Silica cells in short rows (2–6) between 1–2 short cells 925*

Silica cells only; perhaps a few non-silicified short cells or prickles present 926*

Vertical arrangement of horizontal costal files adjacent to third and first order vascular bundles i.e. band with the least number of files and bands with the largest number of files per costal zone (if distinguishable):

Number of files per costal zone:

1 file of cells comprises costal zone 927

2 files of cells comprise costal zone 928

3 files of cells comprise costal zone 929

4 files of cells comprise costal zone 930

5 files of cells comprise costal zone 931

6 files of cells comprise costal zone 932

7 files of cells comprise costal zone 933

8 files of cells comprise costal zone 934

9 files of cells comprise costal zone 935

10 or more files of cells comprise costal zone 936

Composition of alternating files of cells comprising costal zone.

All files similar in composition 937

Composition of alternating files of costal zones differ:

For zones comprised of 3 files of cells:

1 file with silica cells and 2 files of costal short or long cells 938

2 files with silica cells and 1 file of costal short or long cells 939

For all zones comprised of more than 3 files of cells:

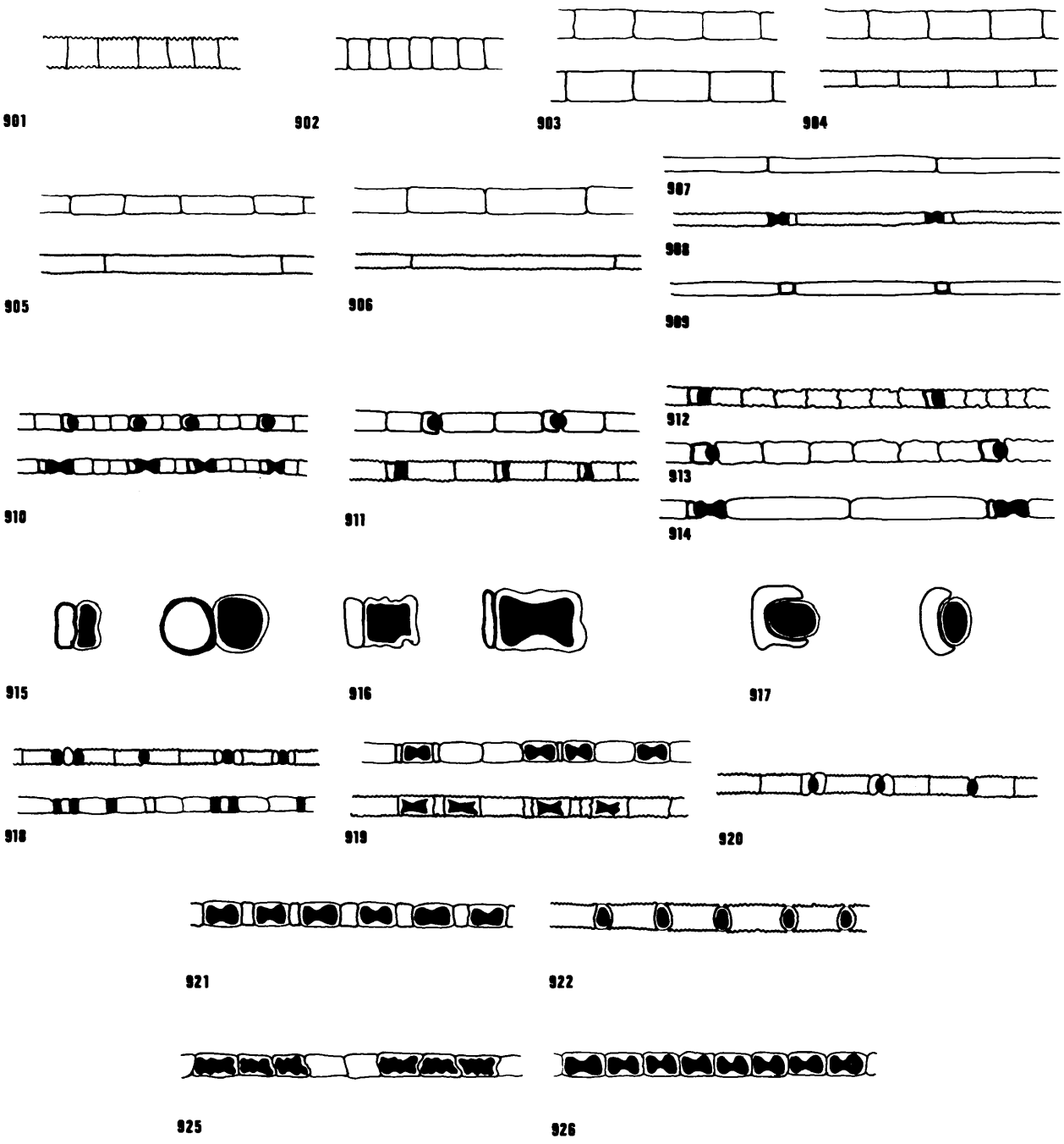
Files with silica cells alternate with a single file of costal short or long cells 940

Files with silica ceils separated by 2 or more files of costal short or long cells 941

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COSTAL SHORT CELLS



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ANATOMICAL CHARACTERS

The following presents a brief conspectus of the nature of the various structures used in describing grass leaf anatomy, as well as their range of structural variation. Where relevant, an account is given of the function or development of these structures and the factors which contribute to their variability. Their importance as either taxonomic or diagnostic characters is stressed.

Before commencing with a detailed description of the individual cell types, it is necessary to give a brief description of the plant cuticle as well as the arrangement of the individual cell types in the different zones of the leaf lamina. This background should result in a better appreciation of the relationship of the epidermal cells to one another and to the hypodermis and other internal tissues.

Epidermal preparations of grass leaf blades are either produced by macerating techniques, resulting in acellular preparations, or by scraping techniques by which the epidermal cell layer and attached cuticle is studied in surface view. It is important to have some understanding of the relationship between the epidermal cells and the cuticle to satisfactorily interpret the patterns and structure visible in these preparations.

Stace (1965) gives a lucid description of the cuticle of vascular plants. Separating any two cellulose cell walls of adjacent cells throughout the plant is a cellulose-free layer known as the middle lamella, consisting basically of calcium and magnesium pectates. The outer surface of the epidermal cells are adjacent to the environment, rather than a neighbouring cell, and it is on this surface that the cuticle is formed. Roelofsen (1952, 1959) ascertained the precise structure of this cuticle using polarising and electron microscopy.

External to the normal cellulose cell wall of the outer face of each epidermal cell, a thin layer of pectic material, which is presumably continuous with the middle lamellae of the underlying anticlinal walls of the epidermal cells, may be present. Exterior to this is usually a two-layered wall termed the cuticular membrane (Roelofsen, 1952, 1959). The inner portion of this, the cuticular layer, is composed primarily of a cellulosic framework between the micro-fibrils of which is encrusted large amounts of cutin by the process of cutinization (Esau, 1960). The outermost part of the cuticular membrane is usually the cuticle proper. This is a thin layer, lacking cellulose and composed mainly of cutin adcrusted to the cuticular layer by the process of cuticularization (Esau, 1960).

Rarely are all four layers as clearly defined as above, but generally there is an increase in cutin outwards and a corresponding increase in cellulose inwards. Very often the pectic layer is absent, the inner cutin-free cell wall gradually merging into the outer cellulose-free cutin layer.

Where the outer walls of adjacent epidermal cells meet, the cuticular layer usually projects downward between the cellulose cell walls, forming the three-dimensional network of cuticular flanges so evident on acellular epidermal preparations. The length of the flanges varies from their virtual absence to their reaching to the inner wall of the epidermis. The flanges are formed by the cuticular layer alone, the cuticle remaining a thin extra-cellular uninterrupted membrane continuous over the leaf surface, being interrupted only by the stomata.

From this description of cuticular membrane structure, it will be evident that in acellular preparations, whether the membrane is isolated by enzymatic maceration, usually a pectinase (Orgell, 1955), or acid maceration, the flanges will be present on the inner surface of the preparations. These represent the position of the epidermal cell walls and are clearly visible when viewed from above or below because the cuticular membrane is much thicker at the position of these flanges. The outer surface of such preparations will still retain any hairs, papillae or striations.

These flanges, however, are not synonomous with the cell walls which were present for some distance on either side of the flanges. Thus, in cuticular preparations cell wall thickness and, subsequently, epidermal cell size, cannot be determined. For this reason, some workers prefer the term 'cell outline' when referring to cuticular preparations (Stace, 1965).

In certain plants one or more of the sub-epidermal layers in addition may also be covered by a cuticular membrane. This inner cuticle on hypodermal and mesophyll cells, especially the cells lining the air chambers below the stomata, may sometimes be seen on cuticular preparations prepared by maceration. This inner cutin layer is continuous with the external cuticle through the stomata (Esau, 1960). Sitholey (1971) terms the entire structure, internal and external cuticle, the cuticular skeleton.

In scraped epidermal preparations, it will be seen that the outlines of the epidermal cells may either remain constant, or vary, with change in focus level. The former condition occurs when the cuticular flanges penetrate only between the upper portion of the anticlinal walls. Where the flanges extend to the whole depth of the epidermal cells, the cells may show differing patterns at their upper and lower surfaces. It is thus common for the outer wall to be distinctly sinuous whereas the inner wall is straight.

The mature thickness of the cuticular membrane is not attained until a fairly late stage in leaf development when cell enlargement is completed. The membrane is often thick enough to be isolated, and the flanges well enough developed to show cell outlines before the leaf is mature. Nevertheless absolutely mature leaves are essential for comparative taxonomic purposes.

Environmental conditions are known to affect the cuticular membrane and the cuticular flange depth. Thicker cuticles are correlated with sunshine, drier soil and air conditions, exposure to wind and higher altitude. This cuticular thickness is usually accompanied by a thickening of the outer epidermal cellulose cell wall.

The epidermal cells above and below the vascular bundles are usually variously modified. Depending on the extent and nature of this modification, the lamina of grass leaf blades is usually divided into longitudinal zones with the costal zones lying opposite the veins and the intercostal zones present between the veins (Metcalfe, 1960). This costal zone has variously been termed vascular bundle or nerve epidermis and the intercostal zone is also known as inter-vascular bundle or inter-nerve epidermis (Davies, 1959) and even longitudinal stomatal zone (Bobrov, 1955).

These two zones differ from one another most conspicuously in species where sclerenchyma is well developed in association with the vascular bundles

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only and where these sclerenchyma strands or girders are actually in direct contact with the epidermis (Jozwik, 1969). In these cases the costal epidermal cell walls are usually lignified and the cells are long and narrow. The intercostal cells are then much wider, unlignified and stomata are only present in this zone.

In many species the epidermis is more uniform and these two zones are then not so distinct. An example is where the hypodermal sclerenchyma occurs in the intercostal zones as well as the costal zones. In general, the more xeromorphic a leaf the less conspicuous are the veins on the cuticle (Stace, 1965) or the epidermis. The epidermis overlying vascular bundles with no sclerenchyma strands or girders need not necessarily be altered however and, in these instances, the costal zones are indistinguishable. This situation occurs frequently where bulliform cells overly the third order bundles. There is also variation in epidermal structure over vascular bundles of different orders and the lamina over the midrib, or from near the margins, can differ from the remainder (Metcalfe, 1960). Thus, in studying epidermal structure, comparison of epidermal preparations with transverse sections of the leaf are essential for accurate descriptions.

The presence or absence and frequency of commissural veins from one vascular bundle to another may also be taxonomically important (Slade, 1970). These cross veins are also known as transverse connections (Bobrov, 1955) or transverse anastomoses (Esau, 1960). These transverse vascular bundles may only contain a single file of tracheary elements and a single file of sieve-tube elements.

Intercostal long cells

The intercostal zone epidermis is generally made up of cells of two distinct sizes. The larger cells are elongated horizontally and are relatively narrow vertically. These are commonly referred to as long cells (Metcalfe, 1960; Gould, 1968) but are also termed undifferentiated (Davies, 1959) or fundamental (Prat, 1948) elements. These cells usually constitute the greater part of the epidermal surface area. Cells of the smaller category are normally much more nearly equidimensional in shape and are known as short cells (Metcalfe, 1960) or differentiated elements (Prat, 1948; Davies, 1959).

Both types of cell generally occur together in the same leaf. The shapes of long cells are fairly uniform and they exhibit inconspicuous differences such as variation in width and length, wall thickness and the extent to which walls are sinuate, papillate, or pitted. This contrasts markedly with the short cells which are often differentiated into such diverse structures as silica cells, cork cells, hooks, prickles or micro-hairs.

Gould (1968) is, nevertheless, of the opinion that significant differences in long cell structure exist among genera and even among species of the same genus. The basic types of long cells have proved very useful for broad surveys of the grasses (Metcalfe, 1960), but they are not adequate for all purposes since there are many intermediates and variants. For detailed studies of limited numbers of species, it may be necessary to introduce some additional types for descriptive purposes.

Although the shape, size and other characters of the long cells exhibit a wide range of genotypic variation, which has definite taxonomic applications, it must be remembered that these cells show an extreme degree of phenotypic variation which is elsewhere only paralleled by characters such as hairiness (Stace, 1965). Thus caution is needed in the taxonomic use of long cell characters.

Variation in epidermal cell size is great, especially that of the intercostal long cells. Thus, epidermal cells are larger on leaves from more humid or shady situations and smaller with drier air and soil and with greater altitude (Stace, 1965). Slade (1970) has shown that the mean length of long cells in shade leaves of *Poa alpina* was 51 per cent greater than in sun leaves. In general, these changes are proportional to a change in leaf area and cell length increase causes leaf length increases but the same number of cells are present in horizontal files in both sun and shade leaves.

Prat (1948) states that it is only the long cells that are sensitive to auxins. The length of a long cell in the meristematic base of the leaf can be suddenly increased by up to 200 times by hormone application. Short cells, on the other hand, are affected very little by hormones, probably on account of their precocious senescence and silicification (Blackman, 1969). Hence, their shape and size is more constant and reliable for taxonomic purposes.

Variability of epidermal cell size is not only dependent on environmental factors and genetic variation but is also due to leaf age, position of the leaf on the plant and the position of the epidermal cells in the leaf. Thus, in order for long cell size to be useful as a taxonomic character, care must be taken to use strictly comparable material and to ascertain the degree of variation present.

Long cell shape can be assessed quantitatively in two ways by finding the ratios of length: end width as well as the end width: median width (Borrill, 1961). These give a quantitative assessment of shape variation from square or rectangular to hexagonal.

The epidermal cells gain their characteristic adult shape gradually from the time that cell division ceases onwards, and in general this is not completed until the process of cell expansion has ended. It is for this reason that absolutely mature leaves must be examined to reliably determine cell shape.

The anticlinal walls of the epidermal cells are either straight or variously undulate or sinuous (Stace, 1965). These undulations are also known as folds (Wylie, 1943), corrugations (Martin, 1955) or waves (Watson, 1942).

The sinuosity of the cell wall can be expressed quantitatively by considering the undulations as waves and by measuring the variables frequency, amplitude and wave-length (Stace, 1956). These three variables amply express all the characters of the cell wall undulations except the shape of the waves. These may either be V-, U- or Ω-shaped.

The exact causes of the epidermal cell wall undulations are uncertain, but the two main theories suggest that it is either due to the tensions set up between the mesophyll and the epidermis or that it is caused by the method of hardening of the differentiating cuticular membrane (Watson, 1942). It has been observed that, whereas many cells have the anticlinal walls undulate throughout their height, some only have the outer part undulate, and thus the second hypothesis appears more likely.

Watson (1942) is of the opinion that the hardening of the cuticle extends gradually over the surface from the central area of the outer, free tangential wall.

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Further cell expansion will be limited at those points where this hardening first reaches the anticlinal walls, i.e. where the undulations extend most deeply into the cell lumen. In other places the cell wall is still expandable. Thus, the outward projecting undulations can further stretch until plasticity is limited by hardening of the cuticle. The final amplitude of the undulations is only reached when the cells attain full size and will depend to some extent on the rate of hardening of the cuticular membrane.

The relative advantages and disadvantages of undulate walls are unknown, but Wylie (1942) put forward the theory that the undulations of the anticlinal walls increase the area of lateral contact between adjacent epidermal cells. This fact, together with the pitting in the anticlinal epidermal cell walls is thought to favour lateral conduction of water through the epidermis which thus functions in water storage and transfer and not only as a protective covering for the leaf. However, the fact that straight walled long cells are commoner in xerophytes appears to cast some doubt on this proposed function (Stace, 1965). In shade conditions, where the cuticle hardens much less rapidly than in strong light, the undulations are much more pronounced and the lower ends of the anticlinal walls show an amplitude identical with that of the outer surface (Watson, 1942). In sunlight, on the other hand, the inner side usually remains straight although the outer side may be undulate because the plastic stage of the cell walls is much shorter. This explains this phenomenon, but does not satisfactorily ascribe a function to the undulations of the long cell anticlinal walls. However, the lateral interlocking and infolding of the epidermal cells probably adds rigidity to the leaf and prevents collapse when water is withdrawn (Fisher, 1939).

It is thus clear that, once again, mature leaves must be studied in order to ascertain the degree of undulation of the walls. Few other characters of the epidermis show as much variation as the cell wall undulations and, although this has been used taxonomically, its use requires the most extreme caution.

The size, shape and undulations of the intercostal long cells can vary vertically across a single intercostal zone. Thus the intercostal long cells adjoining the adjacent costal zone are often comparatively short and wide with markedly undulate walls whereas the centrally located long cells are longer and narrower with straighter walls (Kaufman *et al.*, 1969).

Silicification of long cells occurs erratically in many species (Parry & Smithson, 1964). In older leaves, either isolated long cells or large numbers of adjacent ones may become silicified. The opal of these cells is rather cloudy, contrasting strongly with the smooth, clear silica bodies which occupy silica cells. The shape of the long cell silica bodies conforms closely with the outline of the long cells themselves.

Stomata

Stomatal complexes have been the subject of extensive study throughout the plant kingdom by morphologists and anatomists as well as physiologists. Regrettably their terminology has become confusing with descriptive terms being used in differing senses by these different disciplines. The following terms (Van Cotthem, 1970) are necessary to describe the stomatal complexes of the Poaceae:

(a) stoma—the pair of guard cells and the pore or stomatal aperture (Stace, 1965) enclosed by them;

(b) stomatal complex—the stoma and surrounding cells participating in the mechanism of closing and opening of the pore. This is termed the stomatal apparatus by Stace (1965);

(c) subsidiary cells—epidermal cells surrounding the stoma which differ in shape or size from other epidermal cells. These are also known as accessory cells (Stace, 1965);

(d) neighbouring cells—epidermal cells surrounding the stoma which are indistinguishable from those of the remainder of the epidermis such as the interstomatal long cells.

Paliwal (1969) classifies the stomata of the Poaceae under the dwisahkoshik (biperigenous) category which is recognized by the presence of two subsidiary cells placed laterally on the guard cells. These two guard cells are characteristically shaped as seen in surface view. They are elongated and bone- or dumbbell shaped with expanded, thin-walled ends linked by narrow, thick-walled middle portions. The lumina and protoplast is consequently modified in shape, being an elliptical mass at either end connected by a thin protoplasmic strand through the thick-walled central canal (Brown & Johnson, 1962). The nuclei of the guard cells appear as two ellipses connected by a narrow thread (Fahn, 1967). The thickening of the walls of the central canal can ultimately isolate the opposite terminals of the guard cells resulting in the formation of two nuclei per guard cell (Flint & Moreland, 1946).

As a result of turgor increase in this type of guard cell, the thin-walled expanded tips swell so pushing apart the inflexible middle portions of the cells and thus opening the stomatal pore (Fahn, 1967). In young leaves, before the differentiation of the guard cell walls is complete, the stomata remain closed.

Each guard cell is physically and functionally (Fahn, 1967; Gould, 1968) closely associated with a subsidiary cell located laterally on the outer side, away from the stomatal pore. These are conspicuous, distinctively shaped cells and stand out in marked contrast with the remainder of the epidermal cells. These cells owe their origin to the adjacent protodermal cells (Paliwal, 1969). This is known as perigenous development of the stomatal complex in contrast to the mesogenous type where the subsidiary cells and the guard cells are formed from the same mother cell (Roth & Chausnitzer, 1969). Thus, formation of the stomatal complex involves three mitotic divisions. Two asymmetric divisions in the two lateral epidermal long cell rows give rise to the subsidiary cells and another asymmetrical division in the central row forms two unequal cells—the guard mother cell and the future interstomatal cell. This guard mother cell divides transversely to form the two guard cells (Zeiger, 1971). Further differentiation of the stomatal complex involves differential cell expansion and wall thickening (Kaufman *et al.*, 1971).

Grass stomatal complexes can be classified according to the shapes of the subsidiary cells as seen in surface view. Various types are recognized for descriptive purposes. The dome-shaped (Metcalfe, 1960) type is also known as ovoid (Prat, 1948) and the parallel sided type (Metcalfe, 1960) as rectangular (Reeder & Ellington, 1960) or lozengic (Prat, 1948). The triangular type has also been called diamond shaped or rhombic (De Lisle, 1963) and sometimes this type has a small lateral evagination in which the nucleus may be located at the triangle apex (Lersten & Pohl, 1969).

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These types are often sufficiently distinctive to be used for diagnostic and taxonomic purposes (Metcalfe, 1960). However, stomata of more than one type may occur together in a single leaf and the shape can be somewhat intermediate in certain species.

The stomata of the Poaceae are confined to the intercostal zones and each intercostal zone may include one or more stomatal bands depending on the species. Furthermore, each band may include one or more rows of stomata. The number of bands and rows of stomata in each intercostal zone varies not only from one species to another but also in different parts of a single leaf blade or in leaves taken from different levels of the same plant. De Winter (1951) has shown that the number of stomatal rows adjacent to vascular bundles of different orders differs in *Pseudobromus*.

The distribution and frequency of stomata are conspicuous characters and are often of considerable systematic and diagnostic value (Stace, 1965). However, they are often directly related to the ecology of the plant and are thus not likely to be of great phylogenetic value.

It has been shown that stomata become longer in shade, in moist air and in moist soil and Wilson (1971) has demonstrated considerable phenotypic variation both between and within populations in stomatal length. Other workers have found no difference in stomatal size between sun and shade leaves of a given species.

Stomatal frequency is a quantitative character that has been extensively used in the literature. However, the number of stomata formed on a given leaf is determined at the end of the period of cell division and long before the leaf reaches its full size. Thus, the stomatal frequency expressed as the number of stomata per unit area clearly decreases as the leaf expands and the degree of expansion is dependent on a number of environmental factors as well. Thus only mature leaves are acceptable for comparative measurements of stomatal frequency. Moreover the stomatal frequency often varies considerably on different leaves of the same plant or on different parts of the same leaf. Environmental factors can also regulate stomatal frequency. This is lowered by humidity, wet ground, shade, protection from wind and lower altitude but temperature variations affect frequency only minimally (Miskin & Rasmusson, 1970).

Fortunately the above variation can apparently be almost entirely cancelled by recording stomatal frequency in terms of the proportion of stomata to epidermal cells. Since the variation in stomatal frequency caused by environmental factors is apparently primarily due to the increase or decrease in epidermal cell size and not to cell number, this stomatal index is not affected. Unfortunately stomatal indices have not been used in the Poaceae, probably because the stomata exhibit such a regular pattern of zonation and are not randomly distributed as in dicotyledons for example.

There are numerous examples of variation in stomatal frequency and distribution on individual grass leaf blades. Although Miskin & Rasmusson (1970) found stomatal frequencies on the adaxial and abaxial surface of *Hordeum* to be the same, Bobrov (1955) found the stomata to be more abundant on the upper than on the lower surface of *Poa*. Stomata may even be absent on the abaxial surface altogether as on many permanently infolded leaves. However, in *Themeda triandra* the reverse occurs and there are fewer stomata on the upper surface (Mes & Aymer-Ainslee, 1935). At the base of the leaf there appear to be more stomata per unit area than elsewhere (Bobrov, 1955) and conversely stomata are more numerous nearer the leaf apex and nearer the margin (Mes & Aymer-Ainslee, 1935).

Variation in stomatal frequency is affected by the position of the leaf on the plant. Stomatal frequencies, therefore, appear to decrease progressively from the flag leaf to the basal leaves with the flag leaves having approximately twice as many stomata per unit area as the basal leaves (Miskin & Rasmusson, 1970).

This phenotypic variation can be taxonomically significant. Flint and Moreland (1946) found that the range in variation between varieties of sugar cane was less than that within a given variety. Factors such as position, rate of growth, turgidity of tissue and exposure to light resulted in greater variation in stomatal characteristics than that between varieties.

Certain structural adaptations of the stomata and associated structures are developed in response to environmental conditions. Grasses from dry localities often have stomata restricted to the sides and bases of furrows on the leaf surface. These leaves tend to become inrolled in dry conditions, the stomata thus being protected. In other xeromorphic species the stomata are protected by overlapping papillae, by interlocking prickles, the stomata may be sunken below the level of the epidermal cells (Merida, 1970) or the leaf may be permanently infolded with the abaxial surface being devoid of stomata.

Interstomatal long cells are cells that lie in the same horizontal files as the stomata and serve to separate the individual stomata in a file from one another. When the stomata in any given file are numerous, each successive stomatal complex is separated from its neighbour in the file by a single interstomatal long cell. Both end walls of these particular interstomatal cells will therefore be concave where they fit around the stomata.

Intercostal short cells

These cells are usually nearly equidimensional inl shape and are always shorter than the intercostal long cells with which they generally alternate in horizontar rows. The intercostal short cells are either solitary oe occur in pairs and are then known as silico-suberos. couples or cork-silica cell pairs (Kaufman *et al.*, 1970)

These intercostal short cells may be infrequent or absent in some species (Metcalfe, 1960). The frequency of occurrence of short cells and the contrasting of their presence in the costal and intercostal zones are worth considering for diagnostic purposes.

It is important to note that usually the costal short cells are not the same shape or arranged in the same manner as those of the intercostal zones. Thus, in comparative work, it is important to ensure that no confusion arises through comparing intercostal short cells with costal short cells (Metcalfe, 1960). Generally the morphology of the costal short cells appears to be taxonomically more important than the intercostal short cells but, nevertheless, both should be examined.

In short cell pairs, whether they be silico-suberose couples or not, the basal member of the couple is always the cork cell or suberous cell (Prat, 1948). It is thus possible to use these cell pairs for orientation purposes (Prat, 1948; Parry & Smithson, 1964; Sangster, 1970; Kaufman *et al.*, 1970). The silica cell or exodermic cell (micro-hair base) is always nearest

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the leaf-blade apex and the sequence from apex to ligule (or downwards on the culm and sheath) is long cell, silica cell, cork cell, long cell, etc.

The intercostal short cells, alternating with the long epidermal cells, are the products of assymmetric divisions of intercalary meristem cells (Kaufman *et al.*, 1970). Short cells produced by these divisions can give rise to silico-suberose couples, stomata or trichomes.

A brief description of the microscopic changes that characterize the primary stages of differentiation of cork-silica cell pairs is useful in that it gives a fuller appreciation of the development of the taxonomically very important silica bodies. A short epidermal cell, which is going to form a silico-suberose couple, undergoes a symmetrical, transverse division, giving rise to two daughter cells of equal size and similar morphology. The basal member of the pair then forms a cup-like depression along its upper side and the upper cell forms a basal protrusion conforming to the shape of the depression in the future cork cell. The potential silica cell also enlarges outwards resulting in this cell protruding from the epidermis somewhat. These changes involve only differential wall thickening and cell expansion.

The nucleus of the silica cell disintegrates, whereas the cork cell retains its nucleus. Later, after the complete breakdown of the nucleus, the upper cell begins to accumulate silica which is deposited as long chains in the lumen of the cell. The lower cell of each pair retains its nucleus at maturity and accumulates no silica. This is the cork cell and usually contains a solid deposit of some organic substance (Artschwager, 1930) and has suberized cell walls (Prat, 1948).

Papillae

The outer surface of epidermal cells may be flat or convex to various degrees or the cells may bear small conical processes. These are known as papillae and the epidermides or cells which bear papillae are termed papillate (Stace, 1965). These protrusions of the outer wall are variously shaped and all gradations from simple conical papillae to longer structures, better described as hairs, exist. These belong to the exodermic elements of Prat (1932) and in the context as used in the Poaceae cells with the entire surface of the outer wall inflated are also termed papillate. Thus, papillae are either distinct, highly cutinised (Metcalfe, 1960) protrusions or thin-walled, dome-shaped and inflated. Inflated papillae often serve to protect the stomata by overarching them, either from the interstomatal long cells or from the adjacent files of intercostal long cells.

Papillae occur mostly on the intercostal long cells but dome-shaped costal structures are characteristic of a few species (Metcalfe, 1960). There may be one to many papillae per cell, either in one or several rows. More than one type of papillus may be present on a given epidermis or cell. The papillus is generally centrally placed but may be distally located on the side of the cell nearest the apex of the leaf.

These structures occur sporadically throughout the Poaceae and they are particularly common in, and characteristic of, certain taxonomic groups such as the bamboos. Heavily cutinized papillae are particularly prevalent on grasses from dry localities (Metcalfe, 1960) or saline habitats.

Prickle hairs

These are tough, shortly pointed structures with swollen bases and short, sharp spines or barbs which arise directly from, and form an integral part of the epidermis. These structures generally have thick lignified walls (Metcalfe, 1960) and are the cause of the rough or scabrous feel of the leaf. As the prickle hairs are generally all reflexed in the same direction, the leaves only feel scabrous when rubbed in the opposite direction. Prickle hairs are often silicified (Parry and Smithson, 1964) resulting in the formation of hook-shaped opal phytoliths (Baker, 1960). Silicified prickle hairs appear to be composed of material of two different refractive indices probably opal of differing water contents as observed in *Avena sativa* by Baker (1960). These silicified prickle hairs are characterized by an inner core of translucent opal surmounted by an outer layered sheath of crystal clear opal, which is initially precipitated (Baker, 1960).

Prickle hairs may conveniently be divided into two main types distinguished mainly on the basis of differing size:

(a) Prickles which have elongated oval or elliptical bases and are usually larger than hooks. These structures are also known variously as aiguillons (Bowden, 1964), asperites (Davies, 1969), teeth (Leigh, 1961), or retrorse barbs (De Winter, 1951). The angular prickles of the leaf margin comprise a distinct group within this category and generally differ from the typical prickles occurring elsewhere on the same leaf. Structures that resemble, in size and shape, typical prickle bases but lack barbs are sometimes observed on grass leaf epidermides. This condition is known as incipient asperity (Davies, 1959) and these structures may be mature but unbarbed structures although Metcalfe (1960) points out that all prickles may pass through an unbarbed stage during their ontogenetic development. This possibility needs further investigation. In this context it is interesting to note that Vickery (1935) considers all prickle hairs to be reduced hairs.

(b) Hooks have rounded bases and are smaller than prickles. Hooks are generally located in the intercostal zones. These structures have also been termed crochets or crown cells (Prat, 1932).

Prickles and hooks may occur together or alone in the same grass leaf or they may both be absent. They are encountered in both zones of the leaf, but usually prickles are costal and hooks intercostal. The short barbs of these prickle hairs are usually directed towards the leaf apex (Metcalfe, 1960), as do the spines of the marginal angular prickles (Lersten & Pohl, 1969), and can thus be helpful in orientating epidermal preparations. There are exceptions where the prickle hairs point both apically and basally, however.

Prickle hairs are usually paired with short cells adjacent to them in the same files. It seems reasonable to assume that prickles and short cells are homologous (Metcalfe, 1960).

In some xerophytic grass species the prickles from adjacent costal zones overly the intervening intercostal zone. These interlocking prickles serve to protect the underlying stomatal groove. This type of situation is only found in leaves with narrow, relatively deep furrows as well as ribs especially on the abaxial surface.

Relative proportions of barb length to base size can be gauged by projecting parallel lines through the apex of the barb and the opposite ends of the prickle hair base. In this study base size is assessed by comparison with the length of the stomata on the same leaf.

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Micro-hairs

In the Poaceae the occurrence and form of micro-hairs are characters of considerable taxonomic significance. Many agrostologists (Reeder *et al.*, 1965) consider micro-hairs to be characters of fundamental systematic importance in this family where superficial morphological similarity does not necessarily represent close phylogenetic relationship.

Micro-hairs are typically less than 0,12 mm long (Johnston & Watson, 1977) and can be recognised by generally being much smaller than macro-hairs. They are usually two-celled and are often called bicellar hairs or trichomes (Lersten & Pohl, 1969) and consist of a basal cell connected to an intercostal short cell and a distal, apical cell. The bicellular nature of these hairs is not always immediately apparent because the distal cell, which is invariably very thin-walled, is easily damaged, and often missing, especially on herbarium material. Even when the distal cell remains intact it is often obscure because the very thin wall does not stain readily. The basal cell walls are sclerified and the distal cell wall is cellulosic (Prat, 1948). This difference in wall thickness is also taxonomically significant as it is in the panicoid grasses that the distal wall is exceptionally thin whereas in the chloridoid type of micro-hair the walls of both cells are practically the same thickness (Tateoka *et al.*, 1959).

It is suspected that micro-hairs function in the secretion of some undetermined substance, or substances (Metcalfe, 1960). It is possible that the distal cell is destroyed when the secreted material is discharged. This deciduous nature of the apical cell further restricts the use of the form of this cell for diagnostic purposes.

The term micro-hair is preferred to bicellar hair by Metcalfe (1960) because single-celled micro-hairs do occur in certain groups e.g. *Sporobolus*. These one-celled hairs are clearly homologous with bicellar micro-hairs and cannot be regarded as macro-hairs. Metcalfe (1960) is, therefore, of the opinion that distinctions in the size and functions of hairs are more fundamental than whether they are one- or two-celled.

Micro-hairs invariably occur in the intercostal zones of the leaf epidermis—either in the stomatal bands or between these bands and the costal zones. They arise from an intercostal short cell, or cell pair, situated between successive long cells in a file. Micro-hairs are present on either or both the adaxial and abaxial epidermides. Many species, especially those with permanently infolded leaves and lacking abaxial stomata, have no micro-hairs on the commonly examined abaxial surface, but they are present on the adaxial epidermis from which preparations are less easily made. This reason, and the fact that micro-hairs are easily rubbed off, leads Metcalfe and Clifford (1968) to advise caution in stating whether micro-hairs are absent and they suggest the use of the expression "none seen" as an alternative.

The absence or presence of micro-hairs is also possibly not as meaningful taxonomically as is generally accepted in the light of variation observed in *Cortaderia selloana* (Metcalfe & Clifford, 1968). Nevertheless, the presence or absence of micro-hairs appears to be constant for species, with only the above known exception, and both the presence and form of micro-hairs are very consistent at generic and major group levels (Johnston & Watson, 1977). They appear to be lacking in all festucoids, whereas all other groups universally possess them (Metcalfe, 1960; Watson & Clifford, 1976).

Two distinct types of micro-hair are easily recognized. The panicoid type has a relatively long and narrow or tapered distal cell whereas, in the chloridoid type, in contrast, the distal cell is markedly inflated or hemispherical. Numerous terms have been used to describe micro-hair shape but it is more satisfactory to express this as a ratio of total hair length to maximum hair width (Tateoka *et al.*, 1959). This ratio is, therefore, numerically larger in chloridoid than panicoid micro-hairs. Thus total length of the hairs, their dimensions and the relative lengths of the basal and distal cells appear to be the most satisfactory characteristics for taxonomic purposes. As the shape of the fragile distal cell can easily be distorted, caution must be exercised when relying on the shape of micro-hairs as a taxonomic character (Metcalfe, 1960).

Panicoid hairs have been described as rod-like (Tateoka *et al.*, 1959) but chloridoid hairs are variously known as club- or globe-shaped (Tateoka *et al.*, 1959), clavate (Decker, 1964) or "ice cream cone"-like (Reeder *et al.*, 1965).

Certain micro-hairs resemble neither of the above two types. *Neostapfia* and *Orcuttia* possess "mushroom-button" micro-hairs (Metcalfe, 1960; Stebbins & Crampton, 1961) and *Neostapfia* also has unique "crozier" hairs. Sunken micro-hairs occur in *Neeragrostis* (Nicora, 1962) and *Triodia* (Burbidge, 1946) where the hairs located in the adaxial groove consist of a bulbous-based basal cell embedded between the epidermal cells with a small apical cell. This description resembles papillae in section and may, in fact, represent mistaken papillae.

The hairs of the Pappophoreae have been discussed by numerous authors (Prat, 1936; Caceras, 1958; Jacques-Felix, 1962; Reeder, 1965). These are elongated bicellar hairs with a glandular, usually bulbous, apical cell and a slender, much elongated, basal cell. Stebbins and Crampton (1961) were of the opinion that the stalk was composed of two cells so that the hair is actually tricellar and De Wet (1958) states that the micro-hairs of *Schmidtia* and *Enneapogon* are typically eragrostoid. In the present context these hairs, together with crozier hairs, are considered with macro-hairs because of their larger size (greater than 0,12 mm long) and because they are associated with modified epidermal cells which characterize macro-hairs.

Silicification of the macro-hairs has been observed in some species (Parry & Smithson, 1964). Usually only the basal cell is observed in a silicified condition. However, the possibility of the distal cell becoming silicified but subsequently lost cannot be excluded.

Macro-hairs

Macro-hairs are much longer than micro-hairs. They are characteristically unicellular although sometimes fine, transverse partitions are seen (De Winter, 1951a; Metcalfe, 1960). Macro-hairs are often associated with one or more epidermal cells differing variously from the typical epidermal long cells. Intercostal macro-hairs generally conform to this definition but hairs frequently encountered on the costal zones and on the leaf margin are also referred to this type.

The distinction between macro-hairs and prickles is often not clear and Metcalfe (1960) considers these

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two hair types to be homologous. This is especially true when prickles possess exceptionally long barps as is common on leaf margins. In these cases, when it is difficult to decide where the distinction between a macro-hair and a prickle hair should be drawn, it is suggested that they be described under both hair types. However, if the hairs are relatively short and robust (as is characteristic of prickles) but are associated with specialized epidermal long cells then they can be regarded as macro-hairs. Prickles, therefore, arise from modified short cells whereas macro-hairs usually have modified long cells associated with their bases.

Macro-hairs vary considerably in length even on a single leaf, in flexibility, in wall thickness, and in the extent to which their bases are sunken between the surrounding epidermal cells. These hairs with sunken bases are often associated with epidermal cells that are larger, more inflated and raised above the general level of the leaf surface than are the typical long cells. A hemispherical protuberance is thus formed. This type of macro-hair is referred to as a cushion hair and is intercostal in occurrence. Bowden (1971) considers these thin-walled cushion cells to have high sugar concentrations and concludes that they are the sites of the leaf nectaries in *Andropogon gayanus*. Cushion hairs form the visible leaf pubescence which is visible with the naked eye or hand lens.

Macro-hairs are generally of no more than specific diagnostic value and variation in respect of length, frequency, cell wall thickness and the extent to which they are rigid and straight or flexible and bent have restricted taxonomic use. Metcalfe (1960) considers these differences to be useful only as confirmatory characters for the identification of species.

Hair frequency, in particular, is often unreliable for taxonomic purposes as the number of trichomes is determined when the leaf is very young and the density therefore drops as the leaf expands (Stace, 1965). Thus leaf age needs to be standardized but, in addition, macro-hair frequency is also affected by a variety of environmental conditions. Hairs are more abundant with more sunlight, greater wind exposure, drier air, drier soil and greater altitude. Glabrescence is another factor complicating macro-hair frequency (Stace, 1965). This involves the gradual loss of macro-hairs with age but fortunately in the case of cushion hairs the bases remain visible even when the hair is lost. Furthermore, the number of macro-hairs varies from leaf to leaf and on a single leaf the basal parts of the leaf blade are more pubescent than the distal parts (Liversidge, 1970).

The nature of the trichome base, particularly in the cushion type of macro-hair, frequently provides characters of importance (Stace, 1965). The hair base itself may be constricted or swollen in relation to the hair thickness. The nature of this base can often be more reliably determined from transverse sections of the leaf (Ellis, 1976). The extent that the base of the macro-hair is sunken below the level of the epidermal cells appears to be significant. Deeply sunken bases are generally characteristic of grasses from warmer climates and the more superficial bases are more common in temperate grasses (Metcalfe, 1960). The extent to which the hair bases are sunken or not is generally not easy to determine from epidermal preparations and, in addition, the sunken hairs easily become dislodged during preparation by the scraping method. It must be noted, however, that similar hair types occur in various genera between which there is not thought to be close affinity and thus macro-hair form is of only limited use above the species level.

Atypical macro-hairs such as crozier hairs and the stalked glandular hairs of the Pappophoreae (Stewart, 1964), on the other hand, are significant taxonomically at the generic level and above. These taxonomically important hairs are included under macro-hairs in the present context, although they are discussed more fully under micro-hairs, when they are often included by other authors.

A unique and distinctive type of multi-cellular clavate macro-hair has been described from several *Panicum* species (Kabuye & Wood, 1969). These hairs arise from conical bases in the intercostal zones. Above the base the hair consists of a multiseriate stem of elongated cells and widens to a head consisting of iso-diametric cells. In the mature condition these cells of the head contain a yellowish, sticky substance. Large cells on the distal side of the hair base hold the hair erect when they are turgid.

Silica bodies

Silica bodies are discrete deposits of hydrated silica present in the epidermal cells. These distinct solid opaline bodies are deposited in the lumens of specific and specialized silica cells or idioblasts. This deposition results in distinct structures, generally of constant shape and predictable location. Even although silica is virtually transparent and has a glassy appearance they are, nevertheless, easily visible microscopically because they have a distinct dark outline.

Silica bodies or Kieselkörper are also known as opal phytoliths (Parry & Smithson, 1964; Sangster, 1970). This term is distinct from the term silica body or spodogram (Edwards, 1935) which often includes both the typical bodies formed in the silica cells as well as atypical bodies sometimes present in other epidermal cells. This second type of intra-cellular silica deposit always conforms closely to the shape of the original cell whereas the shape of a phytolith may be independent of that of the idioblast in which it is laid down. This distinction is definitely justified but, as the term silica body has found such general acceptance in taxonomic and comparative anatomical literature, the term silica body has been retained.

Typical silica body deposition commonly occurs in costal idioblasts, which alternate with non-silica cells in parallel files throughout the costal zones. In addition, intercostal idioblasts occur at regular intervals between the intercostal long cells. Generally each apically orientated silica cell is contiguous with a basal cork cell and both together form a silico-suberose couple (Sangster, 1970) or cork-silica cell pair (Kaufman *et al.*, 1970).

Typical silica bodies, therefore, rarely occur in adjacent cells but are invariably located in alternate or more widely separated cells. This is a distinctive feature of these bodies and contrasts markedly with the characteristic silicification of adjacent cells in atypical silica deposition (Parry & Smithson, 1964).

The shapes of typical silica bodies or phytoliths are very important for taxonomic purposes (Metcalfe, 1960; Blackman & Parry, 1968). However, caution must be exercised as the shapes of these silica bodies are not necessarily the same as the outlines of the silica cells in which they are located. It is, therefore, essential to state unambiguously whether a silica cell or silica body is being referred to. In the past the lack of a clear distinction in this respect has been somewhat

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confusing and once again emphasises the need for standardizing terminology.

Silica cells become precociously filled with a gel of colloidal silica which becomes a solid transparent body as the protoplasm dies (Prat, 1948). In the centre of the mass of solid silica small spherical cavities, granules, vesicles or droplets are usually present (Fahn, 1967). These minute granules, which occur singly or in groups, have been regarded as the occluded nucleus (Prat, 1932), or remnants of the disintegrated cytoplasm (Blackman, 1969), which have been trapped during penetration of the silica. The presence of these granules suggests that the solidification of the silica gel occurs suddenly (Blackman & Parry, 1968), a fact which was demonstrated by Kaufman *et al.* (1969a). Sometimes the silica bodies are cracked or fragmented, but Blackman (1969) is of the opinion that this is an artefact resulting from stress during the preparation of leaf scrapes or strips.

For taxonomic purposes silica bodies are normally described as seen in surface view. Nevertheless an understanding of their three-dimensional appearance is useful. In transverse section silica bodies characteristically have a concave outer surface which may become exaggerated as a result of dehydration—either during the preparation of the material or naturally (Blackman, 1969). The degree of concavity is also dependent on where a particular section is taken as the bodies are generally more concave in their central parts. These trough-shaped silica bodies are typical of the costal zones and in the past these thin-walled concave costal epidermal cells have been mistaken for passage cells (Goossens & Theron, 1934) and Jeffries (1916) considered these "peculiar" cells to be plate cells.

Intercostal silica bodies, on the other hand, are often cone-shaped in three-dimensional view. The cone tapers toward the leaf interior as viewed in section (Parry & Smithson, 1964).

The preparation and interpretation of epidermal preparations showing silica bodies demands an appreciation of the properties of opal or solid hydrated silica (Parry & Smithson, 1958). This substance is chemically stable, is not destroyed by heat and is soluble in hydrofluoric acid. The refractive index of silica is low and consequently silica bodies have distinctly higher relief than the surrounding tissue when preparations are mounted in mounting media with higher refractive indices such as Canada balsam. It is interesting to note that this only applies to silica deposited in the silica cells and that the appearance of atypical silica is normally indistinct even in Canada balsam mounts (Blackman & Parry, 1968).

Various shapes of silica bodies have been recognized and these types are very important as they are characteristic of tribes or even sub-families of the Poaceae (Blackman & Parry, 1968). To avoid causing considerable confusion, however, it is essential that these shapes be described relative to their orientation on the leaf surface. This is because similar shaped silica bodies can be positioned at right angles in different species. Thus, the typical oryzoid silica body is characteristically dumb-bell shaped but in contrast to the panicoid type is orientated with the long axis vertically and not horizontally (Tateoka, 1963).

Shapes of silica bodies are normally described as seen in surface view. Round and oval silica bodies have also been termed hat-shaped (Parry & Smithson, 1964). Oblong, linear, rod-shaped or rectangular (Chih-Ying Wu, 1958) bodies belong to the category costal rods (Parry & Smithson, 1964). Crescent-shaped or half-moon (Prat, 1948) silica bodies have been described, as have saddle-shaped bodies. This type is also known as double-axe-shaped (Reeder & Ellington, 1960; Gould, 1968) or the battle-axe type (Prat, 1948). Dumb-bell silica bodies are bilobed structures with a trough-shaped outer surface and a narrow isthmus and the nodular or sinuous type has three or more expanded areas. The cross-shaped silica body is similar to the dumb-bell except that it has only a short narrow central part.

Silica body form and arrangement exhibit a limited degree of variation. Thus, costal silica bodies of a somewhat larger size are present on the abaxial surface of the leaf blade than on the same surface of the leaf sheath, but the sheath has many more intercostal bodies (Parry & Smithson, 1964), with the greatest number being concentrated at the base of the sheath (Blackman, 1968). The adaxial surface of the blade possesses fewer silica bodies with the intercostal bodies being absent and the costal bodies being less common but larger than those of the abaxial epidermis. Abaxial intercostal bodies are often more abundant towards the ligule with either a gradual, or an abrupt, change along the blade (Parry & Smithson, 1964). Alternatively in some species these intercostal bodies may be evenly distributed throughout the abaxial leaf epidermis.

Various environmental and ontogenetic factors may also affect the silica body pattern. pH of the soil and availability of silica can result in larger or smaller silica bodies (Sangster, 1970). On any given plant there may be variation depending on the position of the particular leaf on the plant (Parry & Smithson, 1964) with the number of silica bodies increasing from juvenile to adult leaves (Blackman, 1968), although the characteristic pattern and form of the bodies themselves is maintained. Intercostal silica bodies increase, and costal bodies decrease, from the juvenile to the adult leaves. Age of the leaf is also very important, as silica deposition follows the basipetal maturation gradient within the leaf (Sangster, 1970).

The mechanism by which silica accumulates in potential silica cells is unknown (Blackman & Parry, 1968). However, the consistent formation of regular bodies is indirect evidence for an active mechanism of silica deposition although no enzymes active in silica deposition are presently known.

A passive non-metabolic mechanism, whereby silica accumulates at the leaf surface due to transpiration, is also possible (Sangster & Parry, 1971). Prat (1948) suggests that developing silica cells undergo premature senescence, which is accompanied by a change in pH. Such a change could initiate silica precipitation. Alternatively, if the structure of the outer wall or cuticle allowed more rapid transpiration to occur, silica solutions would accumulate in these cells resulting in gel and opal formation (Blackman & Parry, 1968). The difficulty in explaining the regular pattern of typical silica body deposition casts some doubt on these passive theories of silica deposition as does the fact that silica is accumulated by silica cells whether still ensheathed or not (Kaufman *et al.*, 1969). This conflicts with Blackman (1968), who found that silica deposition only occurs when leaves are fully expanded. Nevertheless, exposure to sunlight and the atmosphere, and the corresponding increase in transpiration, is possibly not essential for the silicification process.

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In addition, different kinds of cells in the epidermal system may have different mechanisms whereby they accumulate silica (Kaufman *et al.*, 1969). Atypical silica is probably laid down not at the same time, nor by the same mechanism, as typical silica but rather as a result of environmental factors such as damage (Parry & Smithson, 1963) or high silica concentrations in the soil.

The uptake of silica by plants can be explained simply in terms of the concentration of silica in the soil solution and the amount of water transpired (Jones & Handreck, 1965). Silica is thus passively taken up in the transpiration stream but different plants take up different amounts of silica from a given soil. For example, in grasses the proportion of silica is ten to twenty times greater than in leguminous plants (Jones & Handreck, 1965). In addition, plants of the same species absorb different amounts of silica when grown on different soils.

The function of silica deposition is also not certain. It has been suggested that silicification represents the disposal of an unwanted substance. However, if this were the case then the atypical silicification of bulliform and long cells, which account for a much greater storage volume of silica than do the phytoliths (Parry & Smithson, 1963), would be a much more efficient system. On the other hand, deposition of silica bodies regularly and evenly over the leaf surface appears to distribute a given volume of silica over a greater area. If silica, therefore, functions as mechanical protection against insect attack (Ponnaiya, 1951; Yoshida *et al.*, 1959), this type of silicification could represent efficient use of this material and the greater deposition of silica on the abaxial surfaces of the leaf is in keeping with this proposed function (Parry & Smithson, 1964). Raeside (1970) is of the opinion that silica probably also helps to support plants when they are near their wilting points and this controlled petrification may be an economical means for grasses to attain rigidity with minimum expenditure of photosynthate (Drum, 1968).

A brief discussion of silica deposits other than the phytoliths proper, that are commonly present in the Poaceae, will probably help to make the distinction between these and the other types of silica deposits clear. Both intra- and extracellular deposits of silica occur in the grasses in addition to the typical silica bodies, sometimes in proportions which assume considerable physiological and anatomical significance (Sangster, 1970).

Two types of extracellular silicification occur. Membrane silicification results in thin films of silica bearing faint impressions of epidermal structures (Parry & Smithson, 1964). In addition, the intercellular air spaces between the sub-epidermal cells may become filled with silica.

Numerous types of atypical intracellular silicification are also found. These include long cell phytoliths (Sangster, 1968), which correspond to the original shape of the intercostal or costal long cells in which they are deposited and consequently have protuberances corresponding to the undulations of the cell wall. In other silicified long cells the silica may only be impregnated in the cell walls (Kaufman *et al.*, 1969). Pit phytoliths are minute opaline deposits in the simple pits of the outer periclinal walls of the epidermal long cells (Sangster, 1970a). Each aperture appears to be blocked and filled with silica. Intracellular silicification may also occur in the following epidermal cell types: bulliform cells, stomatal guard and subsidiary cells, cork cells, micro-hairs, macrohairs and very commonly, prickles (Parry & Smithson, 1964; Blackman & Parry, 1968; Kaufman *et al.* 1969). In addition, rectangular bodies may be formed in the cells of the sub-epidermal layers (Lanning *et al.*, 1958) and, especially in older leaves, silicification of sclerenchyma fibres and collenchyma occurs (Sangster, 1970a). Some of these atypical extra- and intracellular types of silica deposition can be expected to interfere with the mesophyll functions and could contribute to senescence. It is of interest to note that this type of silicification normally occurs much later than in the normal idioblasts (Sangster, 1970a).

CONCLUSION

It is hoped that this attempt to introduce uniform standards to the description of grass leaf blade epidermides will stress the need for standardization of terminology in these studies. If this can be achieved, anatomical descriptions will have a much wider application in the fields of comparative leaf anatomy and grass systematics in general.

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UITTREKSEL

Omskrywende sleutels, definisies en diagramme, vir die standaardisering, vereenvoudiging, en beskrywing van die epidermisstruktuur van grasblare, word aangegee. Ongeveer 340 *eienskappe is ingesluit met die moontlikheid vir uitbreiding tot* 999. *Aantekeninge van die variasie en die taksonomiese waarde van die eienskappe word ook verstrek.*

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4. LEAF ANATOMY AND GRASS TAXONOMY

Synopsis: The relevance of the standardization of the preparation procedures, terminology and definitions to comparative anatomical studies of the grasses will now be evaluated by means of a few selected examples. These anatomical descriptions have been compiled by recording all the structures and characters present in each representative specimen of any species by use of the relevant descriptive 'keys' and schedules. Thereafter computerized attribute lists for any particular taxon were produced from which the anatomical descriptions were compiled. These descriptions therefore incorporate the full range of variation exhibited by any given character in the sample studied and from this information the taxonomic value of the characters can be assessed. The relevance and significance of the anatomical studies of these selected examples to the taxonomy of these taxa is also discussed.

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4. LEAF ANATOMY AND GRASS TAXONOMY

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Bothalia 11,3: 273–275 (1974)

Anomalous vascular bundle sheath structure in *Alloteropsis semialata* leaf blades

R. P. ELLIS*

ABSTRACT

Alloteropsis semialata (R.Br.) Hitchc. of the Paniceae, would be expected to exhibit typical eupanicoid leaf anatomy with a single bundle sheath of large parenchymatous cells with specialized chloroplasts and radially-arranged chlorenchyma. Specimens from South Africa showed the following bundle-sheath and mesophyll deviations from the generalized panicoid model: 1. bundle sheath is double; 2. inner bundle sheath is composed of large cells with specialized chloroplasts; 3. outer sheath consists of many smaller cells with few, or without chloroplasts; 4. outline of the third-order bundles is tall and narrow; 5. chlorenchyma is not radially arranged. Other specimens of *A. semialata*, however, were found to have a large-celled outer bundle sheath devoid of chloroplasts and with indistinctly radiate chlorenchyma.

The genus *Alloteropsis* has been placed in the tribe Paniceae of the Poaceae. Species of this genus would, therefore, be expected to exhibit a typical panicoid leaf anatomy. Several specimens of *A. semialata* from South Africa have, however, been observed to exhibit fundamental differences in the structure and composition of the vascular bundle sheaths. The bundle-sheath number is considered to be a diagnositic character of importance in the basic classification of the Poaceae (Metcalfe 1954) and thus the anomalous condition observed in *A. semialata* warrants mention.

The panicoid grasses are generally characterized by the presence or absence of an inner bundle sheath (Avdulov 1931). The single or outer bundle sheath is composed of relatively few, large, inflated parenchymatous cells imparting upon the vascular bundle, *sensu* Metcalfe (1960), a distinctly angular outline. The chlorenchyma cells of panicoid grasses are more or less radially arranged.

Brown (1961) recognized three panicoid subtypes on leaf anatomy, the Paniceae falling under the eupanicoid subtype. This group of tribes is characterized by the inner bundle sheath usually being absent and with the chlorenchyma cells not radially arranged, as seen in transverse section. These cells are normaly tightly packed with few or no air spaces.

Within the panicoid grasses the main line of evolution appears to be from the typical eupanicoid structure towards the development of specialized plastids for starch conversion and storage in the parenchymatous sheath cells (Brown 1958). This is accompanied by a loss of the starch-synthesis function by the chloroplasts of the chlorenchyma, the cells of which show a corresponding, more regular, radial arrangement. Associated with this development is the complete loss of the inner bundle sheath.

Some species of *Panicum*, *Tricholaena*, *Oplismenus*, *Brachiaria* and *Eriochloa*, of the eupanicoid Paniceae, retain an endodermis-like inner bundle sheath (Brown 1958). Tateoka (1956) found, in some species of *Panicum* and other genera, that the cells of the parenchymatous sheath do not have the specialized starch plastids typical of the Paniceae. Thus there are exceptions to the generalized model for the eupanicoid subtype.

Metcalfe (1960) states that single bundle sheaths, radiate chlorenchyma and angular, third-order vascular bundles are diagnostic characters of the genus *Alloteropsis*. These characters are repeated in the descriptions given for *A. cimicina* (L.) Stapf and *Alloteropsis* sp. nov. Specimens of *A. semialata* from South Africa showed considerable differences from these generic characters and from the generalized eupanicoid anatomy.

The following bundle-sheath and mesophyll differences were observed in specimens of *A. semialata* (Figs. 1–4, 6, 7):

1. the bundle sheath of all orders of vascular bundle consists of two layers of parenchymatous cells.

2. the inner bundle sheath is composed of inflated, parenchymatous cells with a large, centrally-situated chloroplast. These cells are larger than those of the outer sheath. The inner bundle-sheath cells are unthickened except in first-order vascular bundles where the radial and inner tangential walls are slightly thickened.

3. the outer bundle sheath is comprised of numerous smaller, thin-walled cells without, or with a few normal chloroplasts.

4. the outline of the third-order vascular bundles is not angular, but tall and narrow.

5. the chlorenchyma cells are not tabular and radially arranged as seen in section, but are hexagonal to inflated in shape, with no definite pattern of arrangement. Furthermore, these cells are distinctly larger than the outer and inner bundle-sheath cells.

This anomalous structure was observed in the following specimens, the voucher specimens being deposited in the National Herbarium, Pretoria.

TRANSVAAL.—2528 (Pretoria): National Botanical Gardens, Brummeria (–CA) 5–11–1969 *R. P. Ellis 2*; 1–12–1972 *R. P. Ellis 1244*; Faerie Glen (–CD) 22–11–1972 *R. P. Ellis 721*. NATAL.—2729 (Volksrust): Clontarf Siding, Newcastle (–DB) 12–11–1969 *R. P. Ellis 36*.

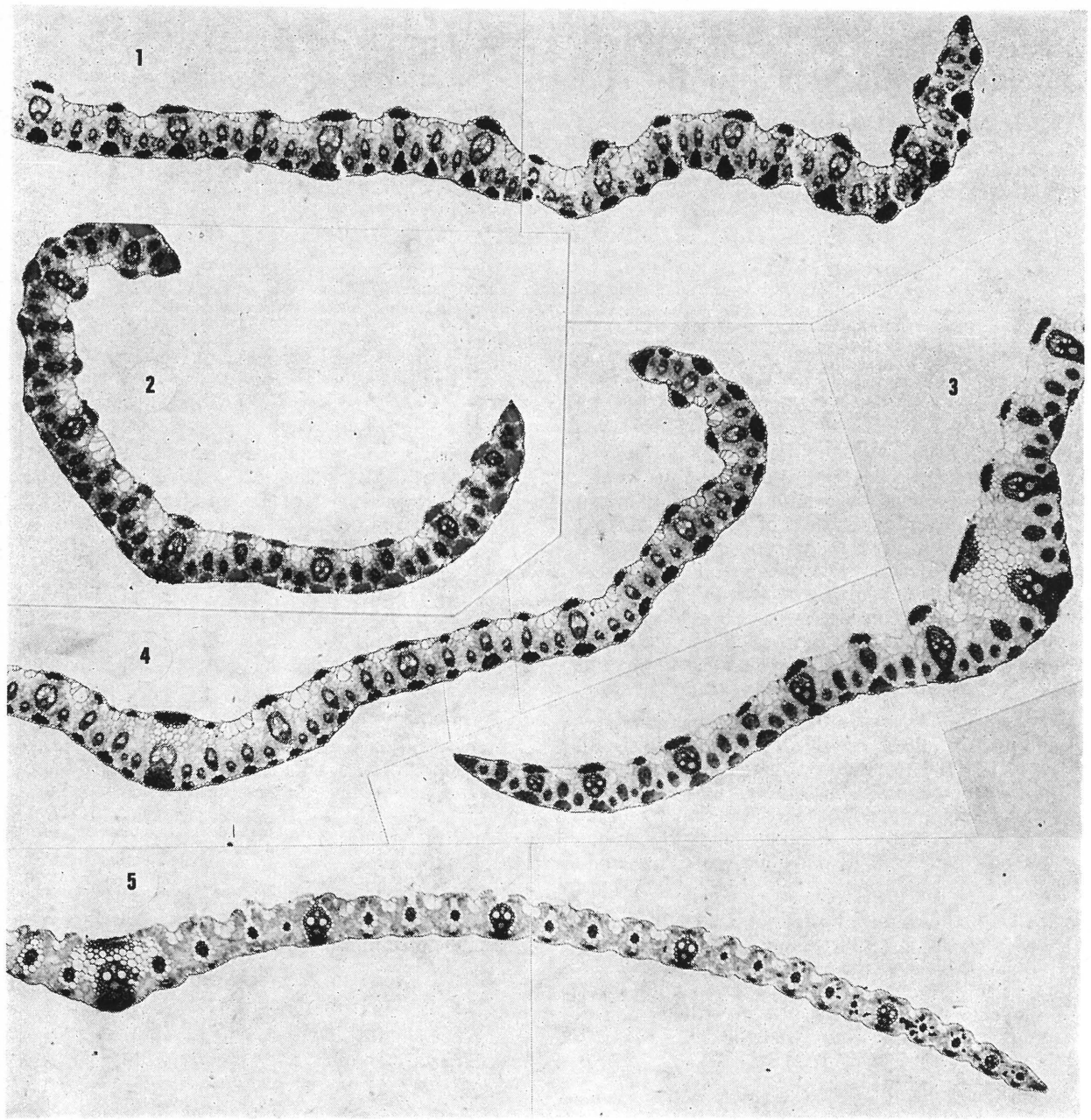
This structure bears a superficial resemblance to the bundle sheaths of the genus *Aristida* of the aristidoid subtype of the panicoid grasses (Brown 1958). Aristidoid grasses are characterized by two parenchyma sheaths and no endodermis-like inner sheath, but the chlorenchyma is distinctly radiate. In the sheaths of *Aristida* the larger inner-sheath cells also possess abundant chloroplasts, more than in the smaller, outer bundle-sheath cells (Lommasson 1957).

Other specimens of *A. semialata* were found to have a large-celled, outer bundle sheath devoid of chloroplasts, a typical mestome or inner bundle sheath around the larger bundles and the chlorenchyma indistinctly radiately arranged (Figs. 5, 8).

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FIGS. 1–5.—Leaf blade outline of **Alloteropsis semialata** in transverse section. A11 × 6,25, bright field. Fig. 1, *Ellis* 2. Fig. 2, *Ellis* 721. Fig. 3, *Ellis* 1244. Fig. 4, *Ellis* 36. Fig. 5, *Ellis* 733.

The specimens exhibiting this structure are: TRANSVAAL.—2530 (Lydenburg): Belfast (–CA) 6–2–1904 *Burtt-Davy 1323*. 2531 (Komatipoort): Ida Doyer Nature Reserve (–CC) 21–1–1971 *R. P. Ellis 378* and NATAL.—3030 (Port Shepstone): Eureka Farm, Izotsha (–CD) 20–12–1971 *R. P. Ellis* 733.

These specimens conform reasonably closely to the Metcalfe (1960) description for the genus except for the lack of chloroplasts in the sheath cells and the presence of a double bundle sheath.

The occurrence of two distinct types of anatomical structure within a single species is unexpected and further investigation is required. Chippindall (1955) notes that *A. semialata* is exceedingly variable in inflorescence size and form and it is not improbable that these anatomical differences are correlated with morphological ones.

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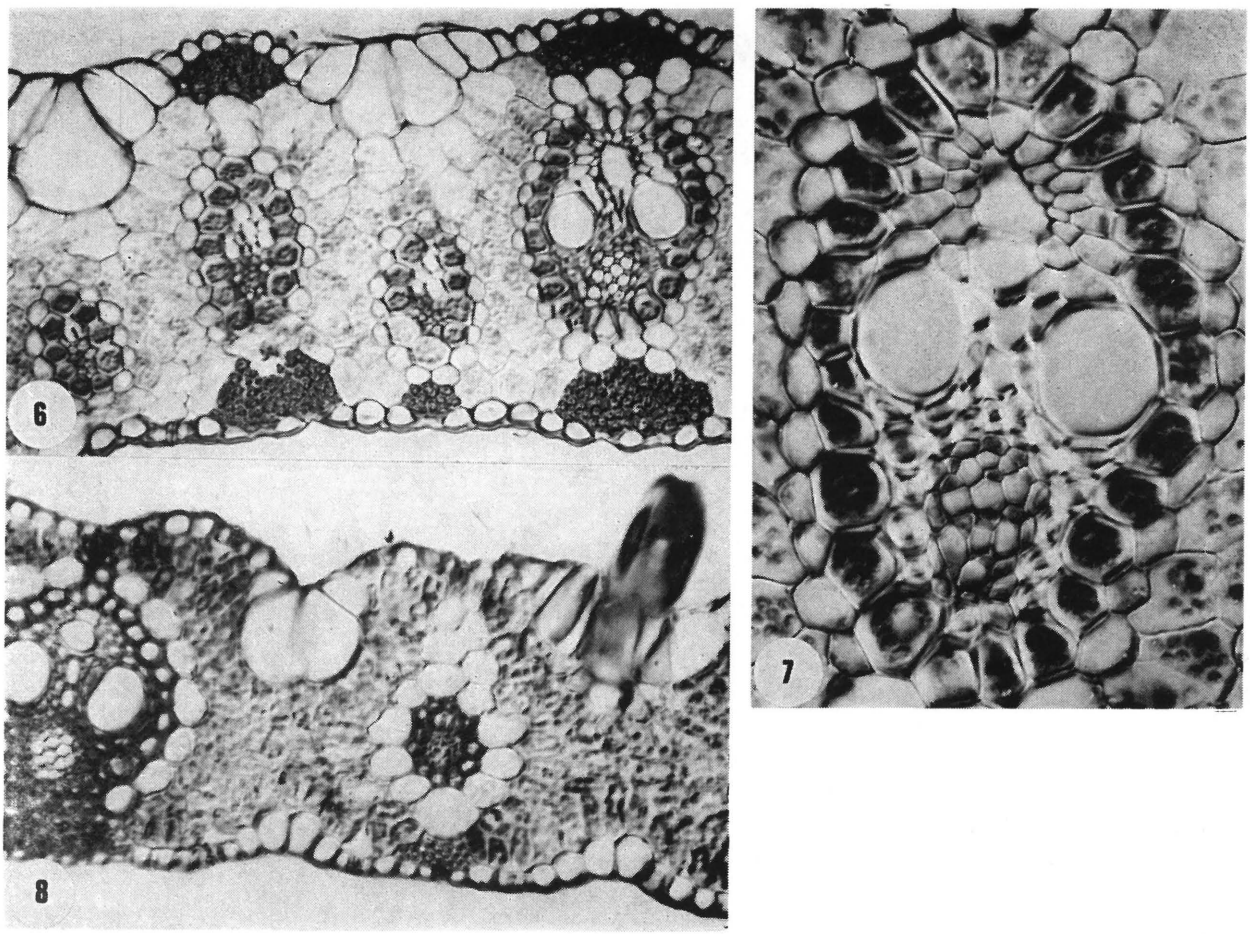
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FIGS. 6–8.—Transverse sections of the leaf blade of **Alloteropsis semialata.** Fig. 6, *Ellis* 36×40 interference contrast. Fig. 7, *Ellis* 36×100 interference contrast. Fig. 8, *Ellis* 733×40 interference contrast.

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Bothalia 11, 3: 235-241 (1974)

Comparative Leaf Anatomy of *Paspalum paspalodes* and *P. vaginatum*

R. P. ELLIS*

ABSTRACT

The leaf blade and epidermal anatomy of *Paspalum paspalodes* (Michx.) Scribn. (syn. *P. distichum* L.) and *P. vaginatum* Swartz is compared and discussed. Numerous anatomical differences are evident and the species can be separated on the basis of the distribution of sclerenchyma and the shape and thickness of the leaf margin. Another distinct diagnostic difference is the shape of the adaxial ribs and furrows and their associated papillae. *P. paspalodes* has two distinct types of abaxial epidermis: cuticular and papillate.

INTRODUCTION

The need for a detailed investigation of the relationship between *Paspalum paspalodes* (Michx.) Scribn. (syn. *P. distichum* L.) and *P. vaginatum* Swartz arose because of the conflicting opinions in the literature as to the nomenclature and status of these two related species. These historical, morphological and taxonomic aspects are discussed by Loxton (1974). The present anatomical investigation was conducted in conjunction with the above taxonomic study and here the leaf and epidermal anatomy are compared and differences and similarities discussed.

Leaf material was collected and fixed in FAA in the field from a number of localities in South Africa. Transverse sections of the leaf were prepared by use of wax embedding techniques, and double-stained in safranin and fast green. The method of Metcalfe (1960) was used for preparing epidermal material for microscopic study. To minimize the possibility of intraspecific variation and to facilitate and standardize comparison, tissue from a point midway between the apex and the ligule of the leaf blade was examined.

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TABLE 1.—The differences between *Paspalum paspalodes* and *P. vaginatum* as seen in transverse sections of the leaf blade.

Character	Paspalum paspalodes	Paspalum vaginatum
1. Width of blade at a point halfway between apex and ligule	1. More than 3,00 mm wide; usually wider than 7,20 mm	1. Less than 3,00 mm wide
2. Adaxial furrows	2a. Shallow furrows only; usually with distinct furrows less than $\frac{1}{4}$ of the leaf thickness deep, or leaf may be undulating with no regular furrows associated with the vascular bundles	2a. Furrows of medium depth; depth between $\frac{1}{4}$ and $\frac{1}{2}$ of the leaf thickness (Fig. 10)
	2b. Furrows always wide and open; obtuse angle formed by the furrow sides at the base	2b. Furrows in the form of a narrow cleft
3. Second-order vascular bundles	3. Tall and narrow with vertical, parallel sides; rarely some bundles may be elliptical in shape	3. Elliptical, vertically elongated in shape
4. First-order vascular bundle	4a. Lysigenous cavity and protoxylem vessel always present (Fig. 15, 16)	4a. Lysigenous cavity and protoxylem rarely present together; usually only a protoxylem vessel is present but both may be absent (Fig. 10)
	4b. Vessels wider than the cells of the parenchyma sheath and always angular in section	4b. Vessels not as wide as the cells of the parenchyma sheath and rounded or curcular in section
5. Parenchyma sheath of third-order vascular bundles	5. Sheath rounded or circular in shape	5. Sheath usually elliptical, vertically elongated; some bundles may possess round sheaths
6. Parenchyma sheath of first-order vascular bundles	6a. Commonly round or circular in shape; some bundles may have elliptical, vertically-elongated sheaths	6a. Sheaths elliptical or egg-shaped with broadest side adaxial
	6b. Parenchyma cells of sheath often rounded in shape; elongated, elliptical cells or cells with straight radial and inflated tangential walls uncommon	6b. Cells of sheath usually with straight radial and inflated tangential walls; rounded cells do occur infrequently
7. Sclerenchyma of leaf margin in transverse section	7. Relatively small to well-developed sclerenchyma caps present; rounded in shape, rarely narrow and pointed (Fig. 22, 23, 24)	7. Very small sclerenchyma cap consisting of a few fibres; pointed in shape (Fig. 20, 21)
8. Colourless cells	8 No colourless cells present.	8. Irregular abaxial groups of colourless cells associated with the sclerenchyma strands present (Fig. 10)
9. Adaxial epidermal cells	9 No macro-hair bases found	9. Sunken constricted bases of short, thick macro-hairs relatively common
10. Abaxial epidermal cells	10 Two distinct types of epidermis occur: (i) Epidermis may be composed of small, inflated, bulliform cells with a very thin cuticle and papillae (ii) Epidermis may be composed of relatively small cells with no papillae and the outer tangential wall with a distinct, continuous cuticle	10 Epidermis composed of relatively large inflated bulliform cells with a distinct thick cuticle; no papillae

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ANATOMY OF THE LEAF

Leaf Blade

Sections of the leaf blade of *P. paspalodes* and *P. vaginatum*, taken at a point midway between the leaf apex and the ligule, exhibit the following common anatomical features.

1. Adaxial ribs, wherever present, are rounded and present only over the first- and second-order vascular bundles. Adaxial furrows are situated over the third-order vascular bundles.

2. Abaxial ribs are absent in both species. Specimens of *P. paspalodes* with inflated, abaxial epidermal cells may possess an irregular, undulating, abaxial surface, but the undulations are not regularly associated with any order of vascular bundle to form ribs (Fig. 1).

3. One, rarely two, third-order vascular bundle is located between consecutive first- and second-order vascular bundles.

4. The first- and second-order vascular bundles are centrally positioned in the leaf section, whereas the third-order bundles are abaxially situated.

5. The bundle sheath of all vascular bundles is single, entire and without parenchymatous projections. The sheath cells contain specialized chloroplasts, which are larger than those of the chlorenchyma and are often grouped near the outer tangential walls of the sheath cells.

6. No adaxial or abaxial sclerenchyma strands or girders are associated with the third-order vascular bundles.

7. No sclerenchyma is developed between the bundles.

8. Adaxial papillae are developed on all, or most, epidermal cells in both species. They are always wider than half the width of the epidermal cells and are either thin-walled or have the distal wall thickened.

Notwithstanding the above structural similarities between the two species, they can, nevertheless, be readily separated and distinguished by the diagnostic characters of the leaf blade set out in Table 1.

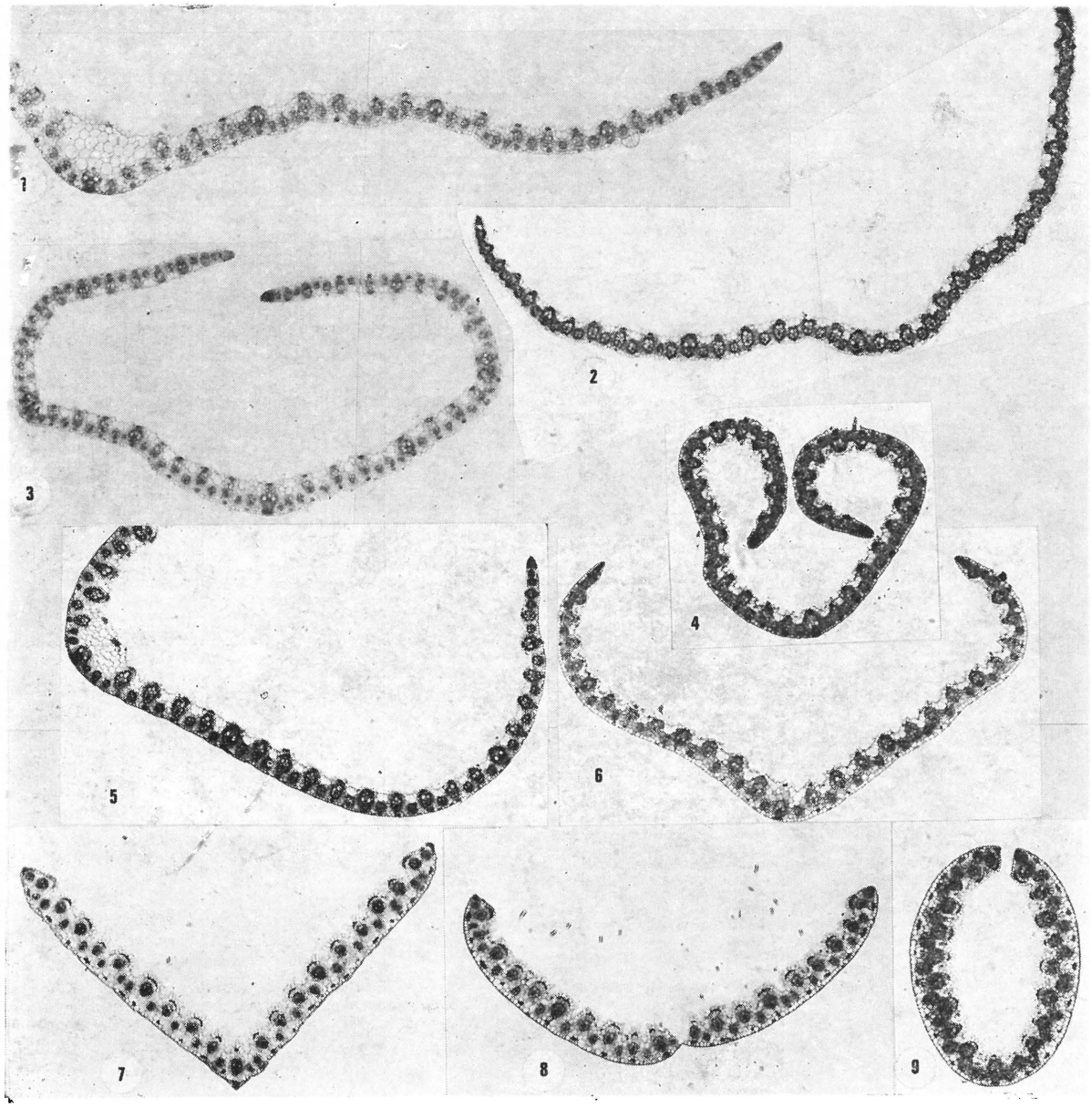


FIG. 1–9.—Leaf blade outline in transverse section. All ×6,25 bright field. Figs. 1–6, **Paspalum paspalodes.** Fig. 1, *Ellis* 1106. Fig. 2, *Ellis* 254. Fig. 3, *Ellis* 146. Fig. 4, *Ellis* 432. Fig. 5, *Ellis* 767. Fig. 6, *Ellis* 726. Figs. 7–9, **Paspalum vaginatum.** Fig. 7, *Ellis* 1114. Fig. 8, *Ellis* 276. Fig. 9, *Werger* 1622.

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Although these diagnostic characters exhibit variation, there are distinct discontinuities between the two species. Numerous other differences occur, but the variation shown by these characters is relatively great and may overlap with the extreme of the same character in the other species. These cannot be used alone as diagnostic characters but, when used in combination with other differences, are nevertheless important.

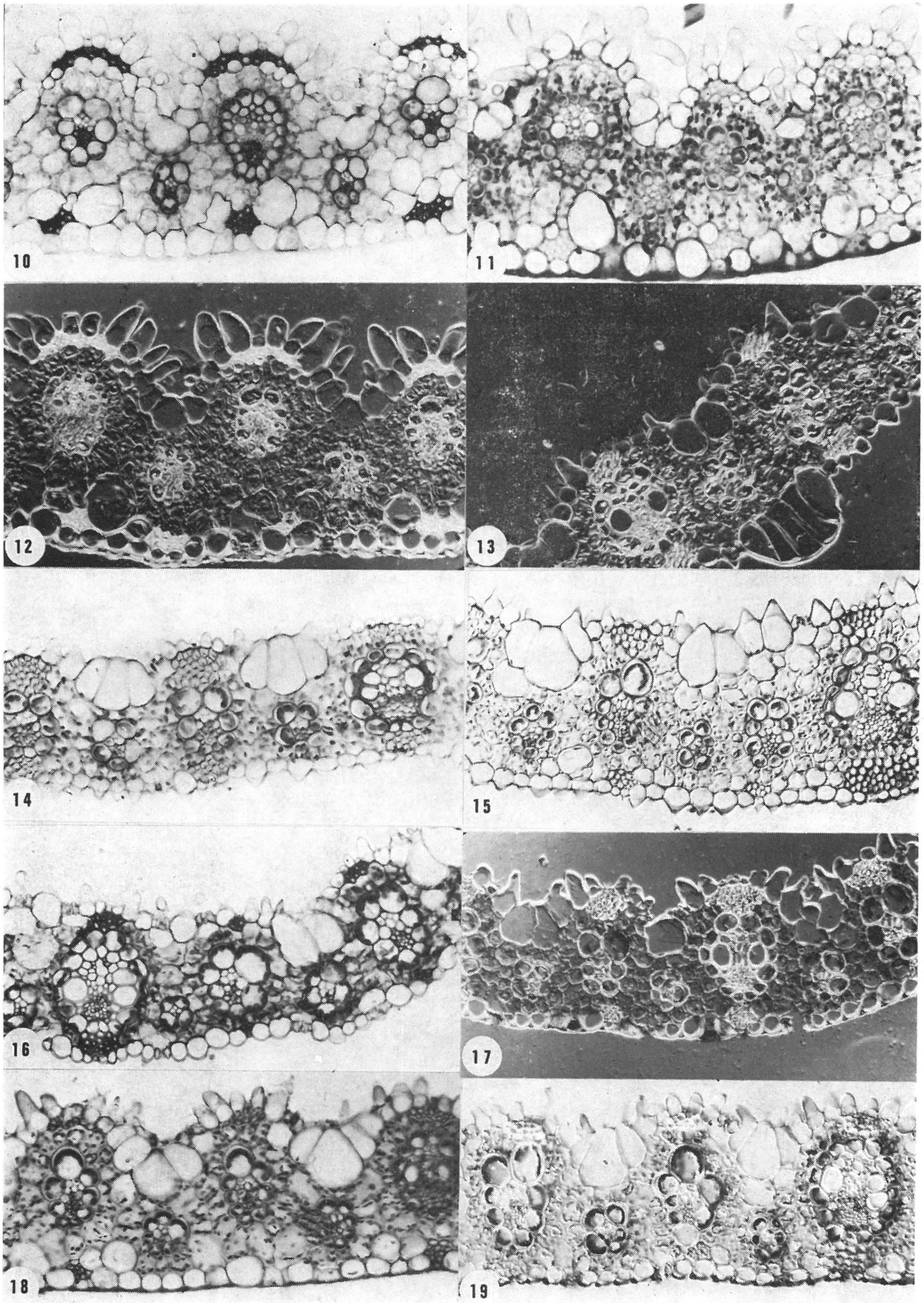


FIG. 10–19.—Transverse sections of leaf blade. All ×40. Figs. 10–12, **Paspalum vaginatum.** Fig. 10, *Ellis* 276 bright field. Fig. 11, *Werger* 1622 bright field. Fig. 12, *Ellis* 276 interference contrast. Figs. 13–19, **Paspalum paspalodes.** Fig. 13, *Ellis* 1106 interference contrast. Fig. 14, *Ellis* 146 lateral region of leaf, bright field. Fig. 15, *Ellis* 146 central region of leaf, interference contrast. Fig. 16, *Ellis* 254 bright field. Fig. 17, *Ellis* 432 interference contrast. Fig. 18, *Ellis* 762 interference contrast. Fig. 19, *Ellis* 767 interference contrast.

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The outline of the blade of *P. paspalodes*, as seen in transverse section, is expanded and gently undulating (Fig. 2) or broadly V-shaped with the arms of the "V" outwardly bowed (Fig. 5, 6) or irregularly wavy or bent (Fig. 2). Many leaves exhibit various degrees of inrolling from both margins (Fig. 3, 4, 6) and this loosely involute condition appears to be basic. Under adverse moisture conditions probably all leaves assume the involute shape of Fig. 4. Leaves of *P. vaginatum* are either V- (Fig. 7) or U-shaped (Fig. 8, 9), with the lamina arms either straight or concave, respectively. The angle formed by the arms of the lamina at the median vascular bundle is usually small, but may be 90° in certain cases.

In *P. vaginatum* the median vascular bundle is distinguishable only by the central position in the leaf section and is structurally identical to the other first-order bundles of the leaf. Some specimens of *P. paspalodes* possess only a median vascular bundle (Fig. 2, 3, 4, 6), but in others a keel comprising 7–9 bundles is developed (Fig. 1, 5). Only a single first-order bundle is present in the keel. Keel shape is often not distinct from the leaf outline, the keel merely being thicker than the rest of the lamina. Well-developed keels may be semicircular or rounded with a raised, flattened adaxial side.

The arrangement of the different orders of vascular bundle along the leaf differs in the two species. In *P. paspalodes* there are 3 or 4 second-order vascular bundles between successive first-order bundles. There is no regular arrangement in *P. vaginatum* and the number of second-order bundles between successive first-order bundles decreases rapidly from centre to margin. There is only one second-order bundle between the lateral pair of first-order bundles.

The bundle sheath of the first-order bundles of both species is composed of smaller parenchyma sheath cells than second- and third-order bundles. These sheath cells are normally thin-walled with large chloroplasts situated near the outer tangential wall. In a few specimens of *P. paspalodes*, however, the sheath cells are distinctly thickened on their inner tangential and radial walls thus resembling a mestome sheath. In these instances the chloroplasts are concentrated near this thicker, inner tangential wall (Fig. 14).

Sclerenchyma development associated with the first- and second-order vascular bundles in *P. vaginatum* is scant and in the form of a shallow, sub-epidermal strip adaxially and a small strand abaxially (Fig. 10, 11, 12). Girders were never found. In *P. paspalodes* both the adaxial and abaxial sclerenchyma is in the form of small strands (Fig. 18), but girders are sometimes developed when these strands are in contact with the bundle sheath (Fig. 15, 19).

The chlorenchyma of both species is irregular or sometimes indistinctly radiate with the adaxial cells tabular. It is continuous between the vascular bundles. The indistinctly radiate condition is more commonly found in *P. vaginatum*.

In both species the adaxial bulliform cells are either extensive groups present throughout the epidermis and only reduced opposite the first- and second-order vascular bundles, or they are in fan-shaped groups at the base of furrows.

The adaxial papillae of *P. vaginatum* are inflated, thin-walled and as wide as the epidermal cell on which the papillus is located (Fig. 12). They are longer than those of *P. paspalodes* and almost every cell is papillate. Those of *P. paspalodes* are narrower and often thickened distally (Fig. 13, 19).

Thus there are numerous anatomical differences between *P. paspalodes* and *P. vaginatum* and they can easily be separated on anatomical criteria of the leaf blade as seen in transverse section. The most outstanding and obvious difference is that of the sclerenchyma distribution and shape, as seen in cross-section, of the leaf margin. Figs. 20–24 illustrate this clearly, together with the distinct difference in leaf thickness at the margin. The shape of the adaxial ribs and furrows and their associated papillae is another distinct diagnostic difference between the two species.

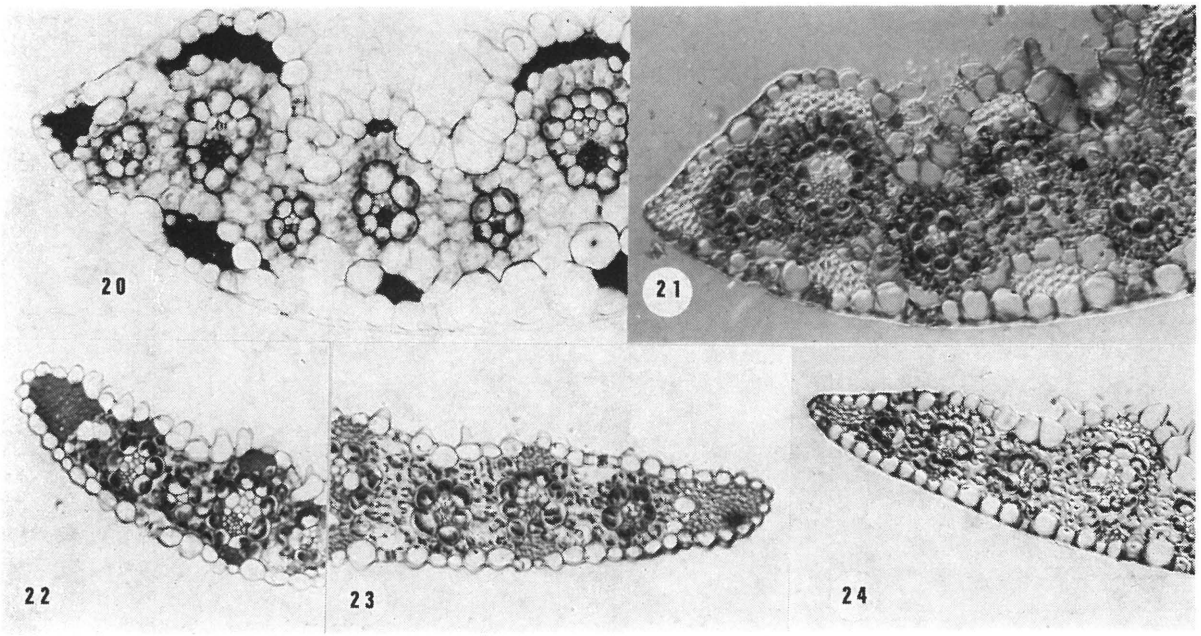


FIG. 20–24.—Transverse sections of the leaf margins. All ×40. Figs. 20–21. **Paspalum vaginatum.** Fig. 20, *Ellis* 276 bright field. Fig. 21, *Werger* 1622 interference contrast. Figs. 22–24. **Paspalum paspalodes.** Fig. 22, *Ellis* 254 bright field. Fig. 23, *Ellis* 1106 interference contrast. Fig. 24, *Ellis* 726 interference contrast.

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Epidermis

Epidermal scrapes of both the abaxial and adaxial epidermides were prepared for microscopical examination. The description of the epidermis of these two species is complicated by the fact that in *P. paspalodes* two distinct types of abaxial epidermis are present: those with a distinct, continuous cuticle and having no papillae (Fig. 27, 28), and those with a thin, indistinct cuticle and papillate epidermal cells (Fig. 29, 30).

Correlated with this difference are numerous others in the abaxial as well as the adaxial epidermides. For the sake of convenience these two forms of *P. paspalodes* will be termed cuticular and papillate respectively.

In *P. vaginatum* and cuticular *P. paspalodes* the abaxial intercostal long cells are elongated, with parallel side walls and vertical, anticlinal end walls. These walls are moderately undulating (Fig. 26, 28). In *P. vaginatum* cell shape and size are remarkably constant, but in cuticular *P. paspalodes*, cell-length is variable within a single file of long cells. Adjacent long cells are frequently separated by silico-suberose couples or solitary cork cells. In *P. vaginatum* and cuticular *P. paspalodes* there are no bulliform cells on the abaxial surface. In papillate *P. paspalodes* (Fig. 29, 30), however, the intercostal long cells are shortly rectangular or hexagonal in shape and resemble bulliform cells. They are not interspersed with intercostal short cells.

Two rows of stomata, separated by more than one file of intercostal long cells, are found in each intercostal zone in both species. Their shape, as determined by the shape of both subsidiary cells, is low triangular in *P. vaginatum* and cuticular *P. paspalodes*. In papillate *P. paspalodes* the stomata are low dome-shaped, with the subsidiary cells ovoid in shape.

The interstomatal long cells of both species are very similar in shape and size to the intercostal long cells. A single interstomatal cell is most commonly present between successive stomata in *P. vaginatum* and cuticular *P. paspalodes*, but in papillate *P. paspalodes* three or more interstomatal cells are found.

No prickle hairs were observed on the epidermal surface of either of the two species examined.

Macro-hairs were occasionally observed on the abaxial surface of papillate. *P. paspalodes*. These are typical cushion hairs with raised epidermal cells surrounding the sunken base of the hair (Fig. 13).

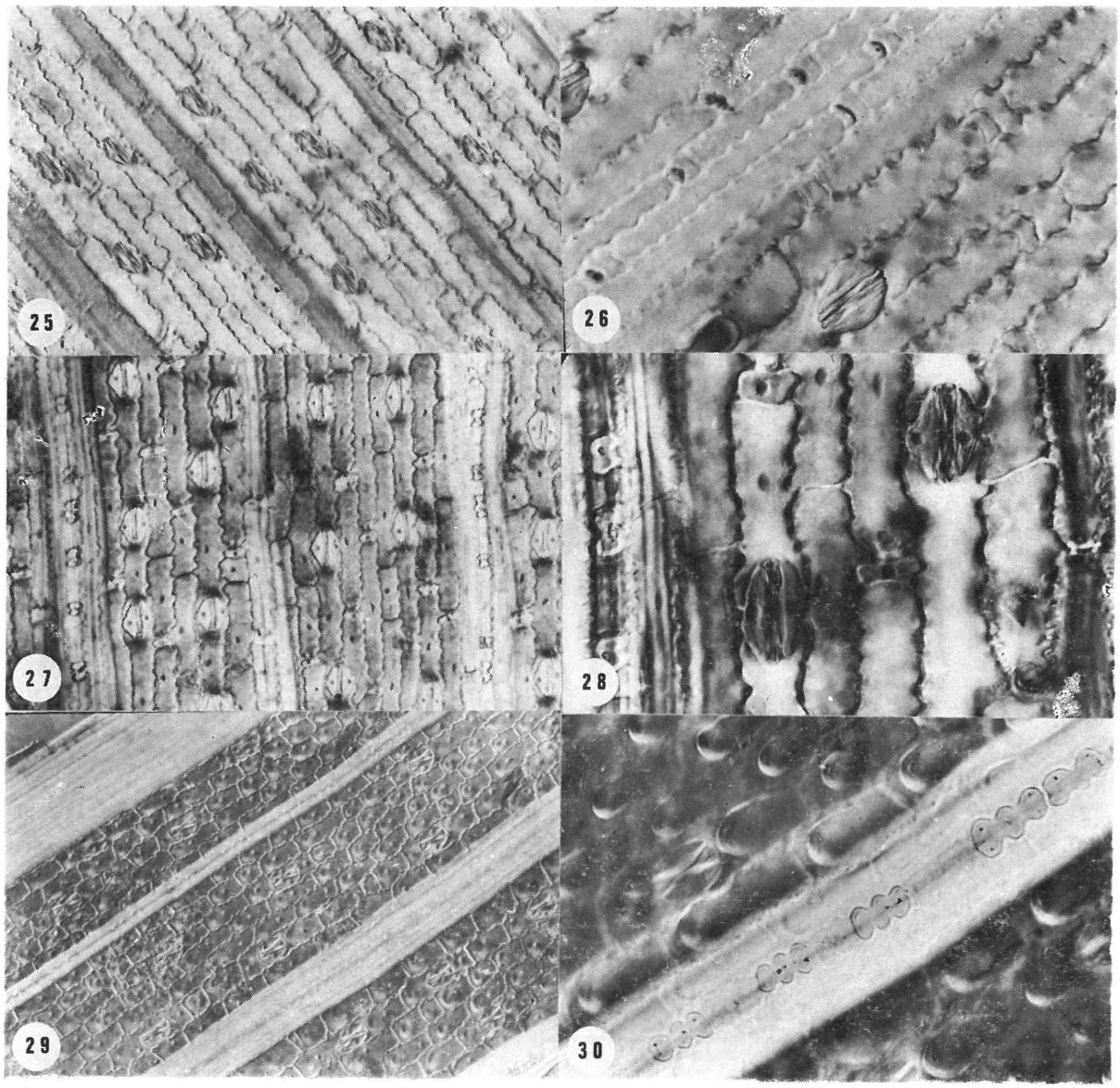


FIG. 25–30.—Abaxial epidermis, interference contrast. Figs. 25–26, **Paspalum vaginatum.** Fig. 25, *Ellis* 276 ×40. Fig. 26, *Ellis* 276 ×100. Figs. 27–30 **Paspalum paspalodes.** Fig. 27, *Ellis* 432 ×40. Fig. 28, *Ellis* 432 ×100. Fig. 29, *Ellis* 254 ×40. Fig. 30, *Ellis* 146 ×100.

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No micro-hairs were seen on *P. vaginatum*. On *P. paspalodes* (both cuticular and papillate forms) micro-hairs are infrequently present on isolated parts of the leaf. These have thickened distal cells tapering to a point. The basal cell is exceptionally short and very difficult to see. This is in agreement with Metcalfe (1960), but Türpe (1966) illustrates micro-hairs with the basal cell up to one third the total length of the micro-hair.

The silica bodies of papillate *P. paspalodes* are horizontally elongated and nodular in outline on both the abaxial (Fig. 30) and adaxial (Fig. 34) surfaces. *P. vaginatum* has horizontally elongated, but noticeably angular, silica bodies as well, but these are only present on the adaxial surface. Abaxial costal silica bodies are very rare (Fig. 25), but crescent-shaped intercostal silica bodies are common in *P. vaginatum* (Fig. 26). Cuticular *P. paspalodes* has rows of shortly dumb-bell-shaped silica bodies with wide central portions in the costal zones (Fig. 27, 28).

P. vaginatum and cuticular *P. paspalodes* bear no abaxial papillae. In papillate *P. paspalodes* the abaxial papillae are inflated, unthickened and dome-shaped, with a diameter greater than half the vertical width of the long cell on which it is located (Fig. 30). There are no papillae on the costal zones, but all intercostal and interstomatal long cells bear a single, centrally-positioned papillus. Adaxial papillae are found on *P. vaginatum* and both forms of *P. paspalodes*. They are extremely large, elongated, oblique and inflated on *P. vaginatum* (Fig. 31, 32). These papillae are the same width as the epidermal cell, which is thus protruded to form the papillus. Most epidermal cells on the adaxial ribs and furrows bear papillae. The adaxial bulliform cells of cuticular *P. paspalodes* bear papillae which taper to narrow, thickened distal ends (Fig. 33), whereas those of papillate *P. paspalodes* are more inflated and oblique, but are distally thickened as well (Fig. 34).

Specimens Examined

Paspalum paspalodes. TRANSVAAL.—2527 (Rustenburg): Schoemansville, Hartebeespoort Dam (–DD), *Ellis 432*. 2528 (Pretoria): National Botanical Gardens, Brummeria (–CA), *Ellis 767*. Constantia Park Extension (–CB), *Ellis 146*. O.F.S.–2926 (Bloemfontein): Botanical Gardens, Bloemfontein (–AA), *van Heerden 95*. NATAL.—3030 (Port Shepstone): Eureka Farm, Izothsa (–CB), *Ellis 726*. CAPE.—3028 (Matatiele): 16 Km S.W. of Matatiele (–BD), *Ellis 254*. 3228 (Butterworth): Fort Warden farm, Komga (–CC), *Ellis 1106*.

Paspalum vaginatum. TRANSVAAL.—2528 (Pretoria): Glasshouse in National Botanical Gardens, Brummeria (–CA), *Ellis 1235*. NATAL.—2930 (Pietermaritzburg): Isipingo beach mangrove fringes (–DD), *Werger 1622*. CAPE.—3228 (Butterworth): Mazeppa Bay (–BC), *Ellis 276*. Haga Haga (–CC), *Ellis 1114*. 3422 (Mossel Bay): Wilderness lagoon (–BB), *Ellis 1299*.

DISCUSSION

The material examined can be separated satisfactorily by a number of apparently unrelated diagnostic characters especially of the leaf blade as seen in transverse section. The most easily studied of these are the obvious differences of the leaf margin and of the shape and size of the adaxial ribs and furrows and their associated papillae. Distinct differences are not so readily evident in epidermal preparations, because of the complications caused by *P. paspalodes* having two distinct types of epidermis: papillate and cuticular.

Undoubtedly, when the abaxial epidermis is studied alone (Fig. 25–30), cuticular P. *paspalodes* resembles *P. vaginatum* more closely than it does papillate *P. paspalodes*. It can also be reasonably assumed that specimens of *P. paspalodes* will be found with shorter and relatively thicker and narrower leaves than typical *P. paspalodes*. Thus differences as seen in

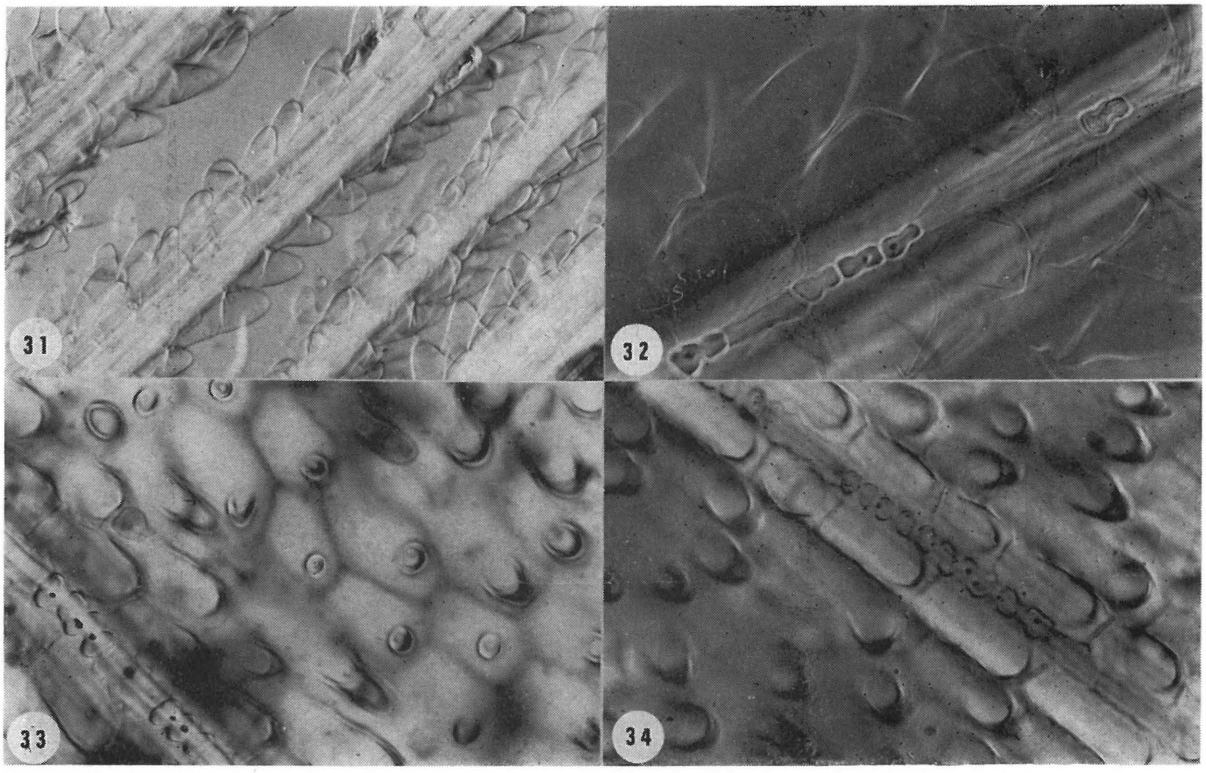


FIG. 31–34.—Adaxial epidermis, interference contrast. Figs. 31–32, **Paspalum vaginatum.** Fig. 31, *Ellis* 276 ×40. Fig. 32, *Ellis* 276 ×100. Fig. 33–34, **Paspalum paspalodes.** Fig. 33, *Ellis* 432 ×100. Fig. 34, *Ellis* 254 ×100.

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transverse section might not be so distinct and a complete range of intermediates may be found from papillate *P. paspalodes*, through narrower and thicker cuticular *P. paspalodes* to typical *P. vaginatum*. This development of an abaxial cuticle, and other associated differences, on *P. paspalodes* is not correlated with a saline environment as was expected. *P. vaginatum* is a maritime species and always grows in a saline habitat, but the cuticular specimens of *P. paspalodes* were collected at Hartebeespoort Dam (*Ellis* 432), a pond in the National Botanical Gardens, Pretoria (*Ellis* 146) and at Port Shepstone (*Ellis* 726). The reason for this cuticular development in moist habitats is obscure.

The capable technical assistance of Miss E. V. C. Taylor and Miss L. Breytenbach is acknowledged. For the photographic enlargements the author is grateful to Mr S. R. van Jaarsveld.

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Bothalia 12,2: 209—213 (1977)

Leaf anatomy of the South African Danthonieae (Poaceae). I. The genus *Dregeochloa*

R. P. ELLIS*

ABSTRACT

The anatomical structure, of the leaf blade as seen in transverse section, and the abaxial epidermis, of *Dregeochloa pumila* and *D. calviniensis* is described and illustrated. A generic description is included and the relationships of the genus are briefly discussed.

RESUME

ANATOMIE DE LA FEUILLE DES DANTHONIEAE (POACEAE) SUD-AFRICAINES. I. LE GENRE DREGEOCHLOA

La structure anatomique du limbe foliaire vu en coupe transversale, ainsi que l'épiderme abaxial de Dregeochloa pumila *et de* D.calviniensis *sont décrits et illustrés. On y joint une description du genre avec une brève discussion de ses relations.*

INTRODUCTION

The genus *Dregeochloa* Conert was described to accommodate the species *Danthonia pumila* Nees and a newly described species *Dregeochloa calviniensis* Conert (Conert, 1966). These two species have certain distinct characteristics of spikelet morphology, leaf anatomy and especially the structure of the mature caryopsis, which indicates that this genus occupies a unique and somewhat isolated position in the Danthonieae. Plants of the genus were previously assigned to the genus *Danthonia* DC. (Chippindall, 1955), and certain authors (De Wet, 1954, 1956; Jacques-Felix, 1962) have suggested their inclusion in *Asthenatherum* Nevski. However, they can readily be distinguished from both genera by the development of the leaves, the insertion of the central awn and the structure of the caryopsis (Conert, 1966). In addition, these species exhibit characteristic leaf anatomy which tends to confirm their placement together in a separate genus, but throws little light on the phylogenetic position of this genus. In the anatomical descriptions which follow, the following abbreviations will be used:

vb/s—vascular bundle/s
1′vb/s—first order vascular bundle/s
2′vb/s—second order vascular bundle/s
3′vb/s—third order vascular bundle/s
ibs—inner bundle sheath; mestome sheath
obs—outer bundle sheath; parenchyma sheath

ANATOMICAL DESCRIPTION OF THE GENUS *DREGEOCHLOA*

Leaf in transverse section

Leaf outline: U-shaped or canaliculate with two halves of lamina curved upwards to varying degrees on either side of median vascular bundle; forming no angle. Leaves narrow (between 1,60 mm and 3,50 mm wide), proportionately very thick (between 0,30 mm and 0,70 mm). *Ribs and furrows*: adaxial furrows present between all vbs; equally sized, rounded, adaxial ribs present over all vbs irrespective of order. Abaxial ribs and furrows well developed; furrows equal to or deeper than adaxial furrows; ribs in form of flat-topped sclerenchyma caps with horizontal prickles at edges interlocking over cleft-like furrow. *Median vascular bundle*: present; midrib or keel not developed. *Vascular bundle arrangement*: 5 1′vbs in blade; no 2′vbs present; all bundles located nearer abaxial surface. *Vascular bundle structure*: all bundles usually rounded in section although *D. pumila* exhibits a tendency for bundles to be vertically elongated and elliptical; phloem of 1′vbs adjoins inner, thickened sheath; no lysigenous cavity or protoxylem vessels developed; metaxylem vessels extremely narrow (diameter less than adaxial ibs cells), angular and only slightly thickened. *Vascular bundle sheaths*: round in shape in all 3′vbs and most 1′vbs, but in *D. pumila* 1′vb sheaths may be slightly elliptical. Obs always entire with no extensions; comprised of 11–15 cells around 3′vbs and 12–17 cells around 1′vbs; all cells similar in shape, thin-walled and conspicuous but not larger than mesophyll cells. *Sclerenchyma*: abaxial strands well developed in wide, straight, deep bands forming caps on flat abaxial ribs; no sclerenchyma between vbs; fibres thick-walled with lumen narrow; walls stain blue-green with safranin and fast green. *Margin*: sclerenchyma a well developed cap, somewhat pointed; crescent-shaped or fibres may extend abaxially. Sclerenchyma of margin never extends further than directly above and below ultimate vb. Margin without angular prickles. *Mesophyll*: indistinctly radiate; chlorenchyma of elongated, tabular cells relatively loosely and irregularly arranged; air spaces frequent. *Colourless cells*: entirely lacking except for bulliform cells. *Adaxial epidermis*: costal prickle hairs with bases not larger than, and situated between, normal epidermal cells; frequent; hairs slender and elongated, those of *D. pumila* (±0,10 mm) about twice the length of those of *D. calviniensis* (±0,05 mm); no papillae. *Abaxial epidermis:* outer tangential wall of cells flattened or slightly arched; not papillose.

Abaxial epidermis

Intercostal zones: cleft-like, intercostal, abaxial furrows make study and illustration of cells of this zone difficult. *Intercostal long cells:* length slightly greater than width; parallel-sided; moderate undulations of anticlinal walls. *Stomata:* appear low-dome shaped; 1–2 rows on each vertical wall of cleft-like furrow. *Papillae:* absent. *Prickle-hairs:* interlocking prickles present overlying stomatal furrow; single row of prickles on edge of costal zones. *Micro-hairs:* none observed. *Macro-hairs:* absent. *Silica-bodies:* present throughout costal zone; silica cells alternate with costal short cells.

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ANATOMICAL DESCRIPTIONS OF THE SPECIES

1. Dregeochloa pumila *(Nees) Conert*

Leaf in transverse section

Leaf outline: U-shaped leaves with base and sides about equal in length or more commonly less inrolled with wide, broad base to U. Width from 3,00 mm to 3,45 mm and thickness from 0,63 mm to 0,70 mm in freshly fixed leaves. Dried herbarium specimens narrower (2,00 mm wide) and thinner (0,30 mm thick). *Ribs and furrows:* adaxial furrows very shallow relative to leaf thickness; furrows narrow and cleft-like in sections from dried herbarium material but with broad bases with relatively steep sides in freshly fixed material. Abaxial ribs taller than adaxial ribs; interlocking prickles well-developed and common. *Vascular bundle arrangement:* 1′ vbs separated by single 3′vb except on either side of median vascular bundle where there are two 3′vbs. *Vascular bundle structure:* 3′vb diameter almost same as 1′vbs. *Vascular bundle sheaths:* obs cells rounded or with radial walls straight and tangential walls inflated; translucent, without chloroplasts or with few chloroplasts similar to those of mesophyll. Ibs not distinct around 3′vbs; probably absent; present around 1′vbs; entire, composed of small thickened cells; thickening may be on inner tangential and radial walls or equally on all walls when lumen may become more or less excluded. Adaxial cells of ibs larger than lateral cells; relatively thin walled. *Adaxial sclerenchyma:* small strands developed; shallow sub-epidermal strip or deeper groups of fibres. Strands similar in size over all vbs. *Mesophyll:* indistinctly radiate with chlorenchyma surrounding each individual bundle divided by abaxial furrows and large adaxial bulliform cells. Adaxial chlorenchyma groups narrow and composed of palisade-like cells between adjacent bulliform cell groups. *Adaxial epidermis:* bulliform cells resemble fan-shaped groups with exceptionally large central cell with straight sides occupying more than ½ the leaf thickness. Costal epidermal cells unthickened or with thin cuticle. *Abaxial epidermis:* bulliform cells small, in fan-shaped groups situated at base of furrows; largest bulliform cell not larger than obs cells. Costal epidermal cells with outer wall slightly thickened. Located on sides of adaxial ribs are horizontally projecting prickles with bases not larger than, and situated between normal epidermal cells; barbs thick and relatively very long (0,10 mm), interlocking over furrows.

Abaxial epidermis

Prickles: short, hook-like base; barb long (±0,10 mm); barb developed from apex of base; barbs lie at right-angles across abaxial furrows. *Silica bodies:* Round, circular to oblong in shape; sometimes shortly dumb-bell shaped with a wide middle portion (Fig. 8).

Specimens examined

S.W.A.—2615 (Luderitz): 1,6 km south of Luderitz lagoon (-CA), *Giess & van Vuuren 653*. 2715 (Bogenfels): Pomona (-AB), *Dinter 4018*.

CAPE.—2816 (Oranjemund): 2 km west of Beauvallon on road to Alexander Bay (-DA), *Ellis 2173*, *2174*: 9 km west of Beauvallon, *Tölken 5278*.

Discussion

D. pumila is a short (3–10 cm tall), tufted perennial, often forming small, dense, flat cushions up to 15 cm in diameter. It grows in crevices or in loose sand amongst shale-like, schist rocks in a region of extremely low winter rainfall (±50,0 mm), but where heavy sea mists occur frequently. It appears to be restricted to the coastal belt, less than 15 km from the sea, from just south of the Orange River mouth to the Luderitz area in South West Africa (Fig. 13).

This region undoubtedly is a harsh habitat with extremes of temperature, insolation and precipitation. The proximity to the sea has a moderating effect on seasonal temperature range and frequent mists probably offset the lack of rainfall somewhat, but an extremely large diurnal temperature range is to be anticipated on the dark rocks which this plant prefers. However, xerophytic adaptions are not well reflected in the anatomy of the leaf of *D. pumila*. The leaf is not permanently infolded and the usual copious sclerenchyma and thickened cuticle, often correlated with dry localities, is lacking (Figs. 1 and 2). The stomata are, however, restricted to abaxial furrows which are further protected by numerous interlocking prickles. This arrangement must limit excessive water loss to a certain degree. In addition the leaves are very short with a leathery, succulent texture and the plant forms a low, dense cushion. These growth characteristics are commonly found in xerophytes but are exceptional in the Poaceae.

No other members of the Danthonieae are found in the same habitat as *D. pumila* although *Asthenatherum glaucum* (Nees) Nevski and *Merxmuellera rangei* (Pilg) Conert are found in the adjoining sandy areas but they extend further into Namaqualand, the Namib Desert and even the Kalahari in the case of *A. glaucum*. Anatomically these species are easily seperated.

Sections taken from herbarium specimens have a somewhat more xerophytic appearance. The adaxial and abaxial furrows are more narrow and cleft-like and the bulliform cells appear reduced in size. In fact, these specimens superficially resemble *D. calviniensis* sections (of which only herbarium material was studied) more closely than fully turgid, freshly fixed specimens of *D. pumila*. This should serve as a caution when anatomical comparisons are made between sections of herbarium and fresh material. Thus, Dinter 4018 (Fig. 1) resembles the sections of *D. calviniensis* (Figs. 9 and 10) and it is only the sequence of the different orders of vascular bundle and the silica bodies and prickle hairs (Figs. 5 and 6) of the epidermis that reveal it to be a typical *D. pumila* specimen.

Remarkably little variation in leaf anatomy was evident in the specimens studied which may be expected from a species inhabiting a limited area with uniform climate, and occupying such a specialized niche. This species can readily be recognised by the elongated, deep bulliform cells associated with narrow columns of adaxial chlorenchyma cells, the thickness of the leaf and the abaxial furrows with interlocking prickles.

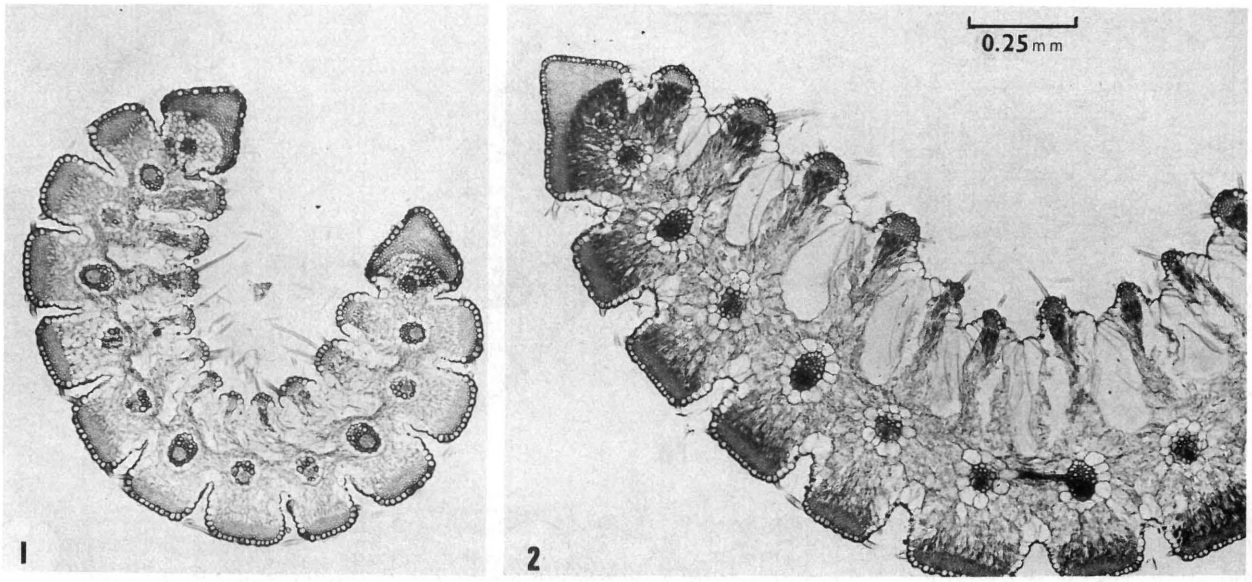
2. Dregeochloa calviniensis *Conert*

Leaf in transverse section

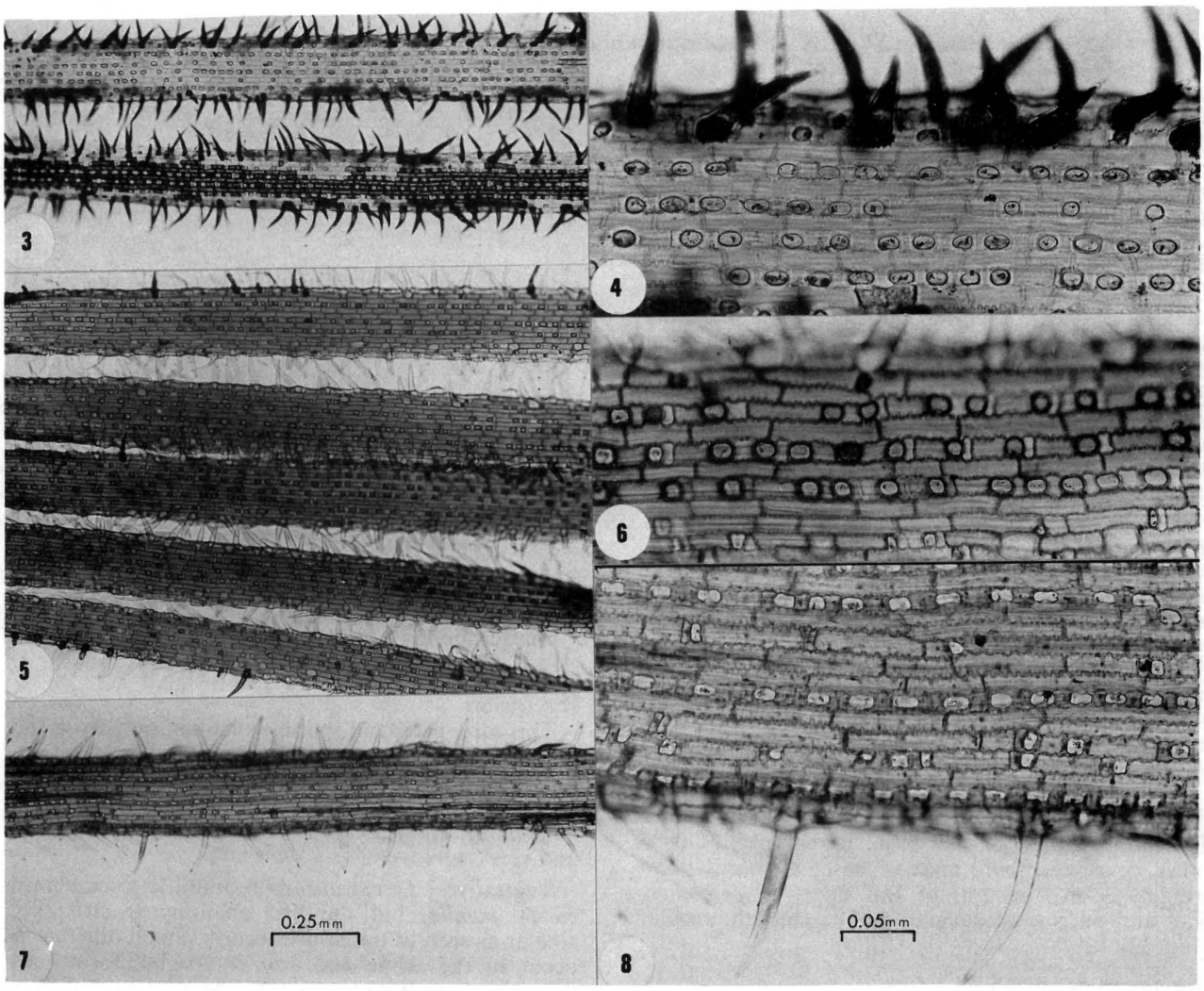
Leaf outline: U-shaped with base and sides equal in length to hollow cylindrical condition where margins almost meet. Generally more tightly inrolled than *D. pumila;* width from 1,60 mm to 2,30 mm in dried material. *Ribs and furrows:* adaxial furrows of medium depth and at least ¼ of leaf thickness. (This greater depth may be due to shrinkage in the herbarium material examined). Furrows narrow, cleft-like. Adaxial ribs same height as abaxial ribs although shaped differently; not moniliform; interlocking prickles small and infrequent. *Vascular bundle arrangement:* one 3′vb on either side of the median

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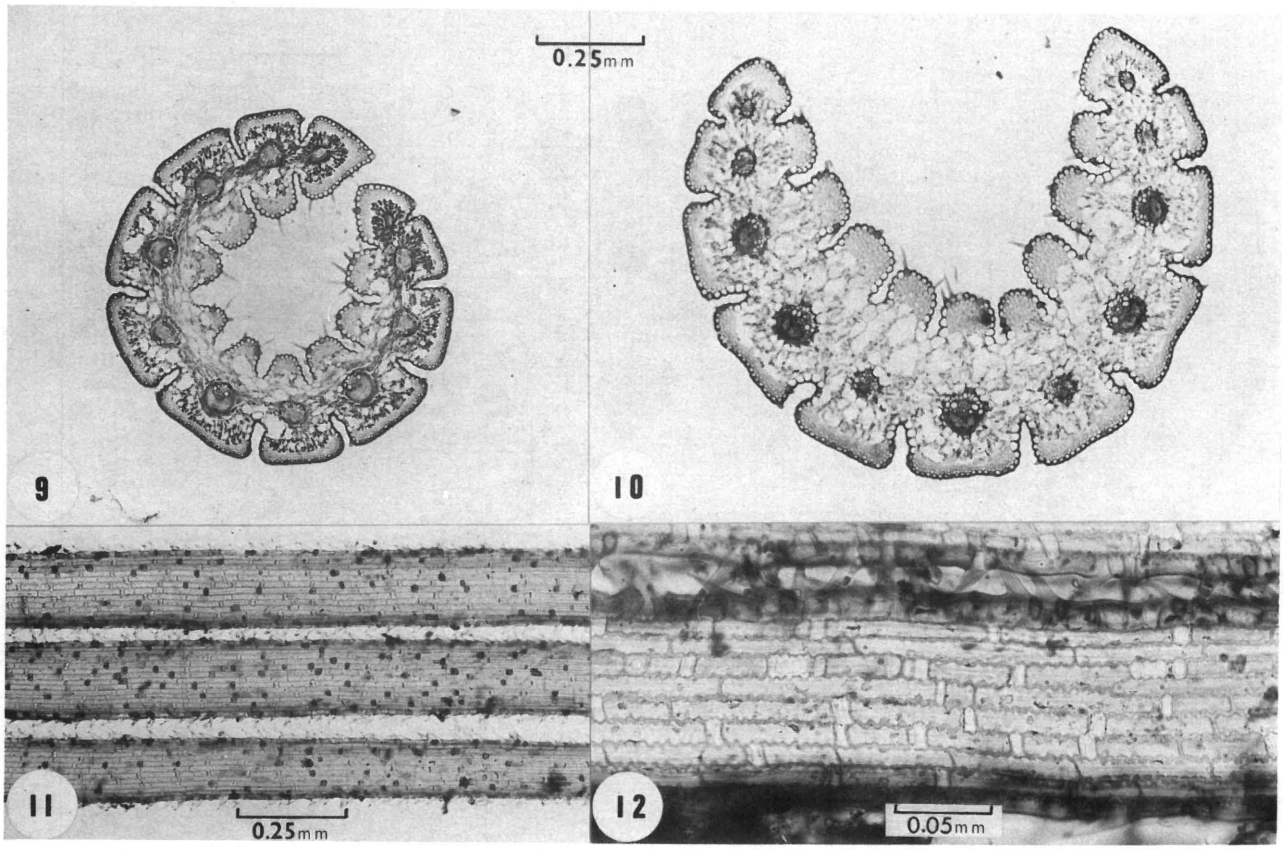
FIGS 1 & 2.—*Dregeochloa pumila*: transverse sections of the leaf blade; 1, *Dinter* 4018; 2, *Ellis* 2174.



FIGS 3–8.—*Dregeochloa pumila*: abaxial epidermis; 3 & 4, *Ellis* 2173; 5 & 6, *Dinter* 4018; 7 & 8, *Giess & Van Vuuren* 653.

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FIGS 9–12.—*Dregeochloa calviniensis*. 9 & 10, transverse sections of the leaf blade; 11 & 12, abaxial epidermis. 9, *Erasmus* s.n.; 10 11 & 12, *Dekkers* s.n.

vascular bundle; lateral two 1′vbs adjacent to one another with no 3′vbs present between them. *Vascular bundle sheath:* obs cells inflated and rounded; chloroplasts appear specialized and concentrated near inner tangential wall (Figs. 9 and 10). (This observation needs confirmation using freshly fixed material). Ibs well developed and distinct around both 1′ and 3′vbs, complete, composed of small thickened cells often with inner tangential and radial walls markedly thickened. Around 1′vbs the walls of cells adjacent to the phloem very thick with narrow lumen. Adaxial cells of ibs larger than lateral cells of sheath. *Adaxial sclerenchyma:* well developed strand; usually arched following shape of rib; similar over all vascular bundles. *Mesophyll:* indistinctly radiate with the chlorenchyma surrounding each individual bundle being divided by abaxial and adaxial furrows. *Adaxial epidermis:* bulliform cells appear absent, or small and present on sides and bases of furrows (Fig. 10). Costal epidermal cells all with outer wall slightly thickened. *Abaxial epidermis:* bulliform cells absent or only very small; situated on the sides and bases of furrows. Costal epidermal cells with distinct, thick cuticle on outer wall; thickness of cuticle and outer wall being equal to, or greater, than depth of epidermal cell. Interlocking prickles with very short barbs at sides of costal zones.

Abaxial epidermis

Prickles: short, hook-like base; barbs short (±0,05 mm), developed from apex of base; all barbs point irregularly in direction of leaf apex. *Silica-bodies:* Tall and narrow to square; outline smooth, regular (Fig. 12).

Specimens examined

CAPE.—3019 (Loriesfontein): Handelkraal, E.N.E. of Loriesfontein (-CD), *Dekkers s.n.* (PRE, iso). 3022 (Carnarvon): Brandewynskuil, between Vosburg and van Wyksvlei (-DB), *Erasmus s.n.*

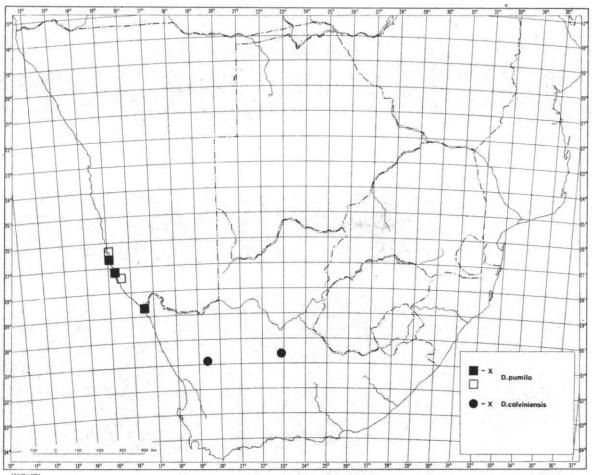


FIG. 13.—Distribution of *Dregeochloa pumila* and *D. calviniensis* compiled from specimens at the National Herbarium, Pretoria (PRE). X indicates localities of specimens studied anatomically.

Discussion

This rare, perennial species is known only from the above two localities and collections. At Handelkraal, on the margin of the Western Mountain Karroo, it is locally frequent on limestone. In contrast to *D. pumila*, this species forms small, dark green, dense and erect tufts from 15–25 cm high.

Vegetatively *D. calviniensis* bears little resemblance to *D. pumila*, but the leaf anatomy is strikingly similar as seen in transverse section. Small differences occur in the shape and size of the bulliform cells (this may have been accentuated by the fact that only herbarium specimens were studied), the size and thickening of the epidermal cells and the prickles and possibly the arrangement of the different orders of

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vascular bundles. The abaxial epidermis, however, shows important differences, especially in the shape of the silica bodies. Although *D. calviniensis* has tall and narrow silica bodies (Fig. 12) and *D. pumila* circular or oblong bodies (Figs. 4 and 6), both these shapes, and the fact that the silica cells and short cells are solitary or paired, and not in long rows, are typical festucoid characters (Metcalfe, 1960). This difference, although striking, can thus be expected even in two closely related taxa.

There appears to be a specialization and a centripetal concentration of chloroplasts in the parenchyma sheath cells of both *D. calviniensis* specimens examined (Figs. 9 and 10) and it is unfortunate that no fresh material was obtained to confirm this. In addition, the mesophyll, which is also poorly preserved, appears to consist of two layers of more or less radially arranged chlorenchyma cells. This configuration is typical of the Kranz syndrome but the $^{12}C/^{13}C$ ratios are typical non-Kranz (δ=–26, 32; Dekkers s.n.) as are those for *D. pumila* (δ=–25, 93; de Winter & Giess 6235). The possibility that *D. calviniensis* represents a very early stage in the aquisition (or late stage in the loss) of the Kranz syndrome still exists, and this warrants further investigation. If this is in fact the case then the relationship of *Dregeochloa* to *Asthenatherum*—a Kranz genus in the Danthonieae—becomes much clearer.

DISCUSSION AND CONCLUSIONS

In the past the leaf anatomy of this genus has, to a large extent, been misinterpreted. Thus, de Wet (1954) grouped *D. pumila* with the species now included in the genus *Asthenatherum*—all of which have "chloroplasts localized around the bundles." This, together with morphological criteria, he interpreted as indicating that *D. pumila* belonged to the genus *Asthenatherum*. Jacques-Felix (1962) stated that *D. pumila* had typical panicoid structure and also included this species with *Asthenatherum*. Conert (1962) noted that *D. pumila* did not have elongated radially arranged mesophyll cells. His observations led him to disagree with the above authors and he suggested that *D. pumila* occupied an isolated position in the Danthonieae near *Danthonia*.

In a later paper, de Wet (1960) grouped *D. pumila* with those species where the chlorophyll is not localised around the bundles but uniformly distributed throughout the mesophyll and which possess a thin-walled outer bundle sheath. These new observations led him to postulate that *D. pumila* formed a direct link between *Danthonia* and *Asthenatherum*. He was still of the opinion that in all other respects these two genera resembled one another very closely but Conert (1962) noted that the formation of the callus differed in addition to anatomical differences. This influenced Conert's (1962) proposal of an isolated position for *Dregeochloa* (=*D. pumila*).

Certain epidermal features have also been confused. De Wet (1954) described the silica bodies of *D. pumila* as being spherical and stated that micro-hairs were absent. In the 1960 publication de Wet described the silica bodies as dumbell-shaped and micro-hairs were observed. In the present study no micro-hairs were found but it must be stressed that, in this genus, the intercostal zones are difficult to preserve and study due to the deep adaxial furrows. On all preparations of *D. pumila* the silica bodies are horizontally elongated and oblong in shape although some silica bodies on Giess and van Vuuren 653 (Fig. 8) have indentations in the centre and could be described as being dumbell-shaped.

The observations of this study, based solely on leaf anatomy, confirm that these two species closely resemble one another and constitute a unique, distinct group. Their structure is unique amongst the Danthonieae and they show little anatomical resemblance to any other South African members of this tribe. This supports Conert's (1966; 1971) conclusions that this genus occupies an isolated position within the tribe. However, *Dregeochloa* has certain similarities in common with *Asthenatherum* (abaxial furrows and prickles, spikelet structure and distribution) which may indicate that these two genera had a common ancestor which gave rise to Kranz and non-Kranz groups. The region where these genera occur is a zone where Kranz genera (*Aristida*, *Stipagrostis*, *Eragrostis* etc.) and non-Kranz genera (*Schismus*, *Karoochloa*, *Chaetobromus* and *Merxmuellera*) are equally abundant. *Dregeochloa* shows no anatomical affinities with the genus *Merxmuellera* (=*Danthonia*) and its removal from *Danthonia* appears justified.

ACKNOWLEDGEMENTS

The author is indebted to Miss L. Breytenbach and Miss A. Vermeulen for capable technical assistance and to Dr J. Vogel and Mrs M. Greyling of the Natural Isotopes Division, N.P.R.L., C.S.I.R. for the $^{13}C/^{12}C$ determinations.

UITTREKSEL

Die anatomiese struktuur van die blaar in dwarssnee en die abaxiale epidermis van Dregeochloa pumila *en* D. calviniensis *word beskryf en geïllustreer. 'n Genus beskrywing is ingesluit en die verwantskappe van die genus word kortliks bespreek.*

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Bothalia 13,1 & 2: 185–189 (1980)

Leaf anatomy of the South African Danthonieae (Poaceae). II. *Merxmuellera disticha*

R. P. ELLIS*

ABSTRACT

The anatomical structure, of the leaf blade as seen in transverse section, and of the abaxial epidermis, of *Merxmuellera disticha* (Nees) Conert is described and illustrated. Three distinct anatomical "forms" are recognized viz. typical *M. disticha*, the Drakensberg form and the alpine bog form. These three anatomical groups also appear to have differing environmental requirements and probably warrant taxonomic status.

RÉSUMÉ

ANATOMIE FOLIAIRE DES DANTHONIEAE (POACEAE) D'AFRIQUE DU SUD. II. MERXMUELLERA DISTICHA

La structure anatomique du limbe foliaire en coupe transversale et celle de l'epiderme abaxial de Merxmuellera disticha *(Nees) Conert sont décrites et illustrées. On reconnaît trois "formes" anatomiques distinctes, soit la forme* M. disticha *typique, la forme du Drakensberg et la forme du marécage alpin. Ces trois groupes anatomiques semblent également avoir des exigences de milieu différentes et il est probable qu'elles méritent un statut taxonomique.*

INTRODUCTION

Merxmuellera disticha (Nees) Conert (1970) (= *Danthonia disticha* Nees) is probably the best known and most distinctive southern African representative of this genus. It is a wiry, tussock grass and is economically relatively important (Acocks, 1971) as it may become dominant, and completely usurp the position of better grazing grasses in certain areas. It occurs over extensive areas along the south coast and eastern mountain ranges and is an important constituent of the following veld types: *Themeda—Festuca* Alpine Veld, Stormberg Plateau Sweetveld and Karroid *Merxmuellera* Mountain Veld (Acocks, 1975).

This species is easily recognized by the inflorescence, which is an oblong, uninterrupted, distichous spike. It is the only species of this genus which can have 2-flowered spikelets and both the upper and lower glumes 3-nerved (Chippindal, 1955). *M. disticha* is thus distinct morphologically and only the plants with 2-flowered spikelets may be confused with *Pentaschistis basutorum* Stapf (Chippindal, 1955).

It was, therefore, most unexpected to discover that three distinct anatomical "forms" are present in this species. In addition, from the sample examined in this study, it appears that each of these three "forms" has different habitat requirements. For convenience, *M. disticha* sens. lat. has been sub-divided into three "forms" in the following descriptions and discussions: typical *M. disticha*, the Drakensberg form and the alpine bog form. Each of these forms exhibits charcteristic leaf anatomy and epidermal structure.

In the anatomical descriptions which follow, the following abbreviations will be used:

vb/s—vascular bundle/s
1'vb/s—first order vascular bundle/s
2'vb/s—second order vascular bundle/s
3'vb/s—third order vascular bundle/s
ibs—inner bundle sheath; mestome sheath
obs—outer bundle sheath; parenchyma sheath.

For definitions of terminology used see Ellis (1976, 1979).

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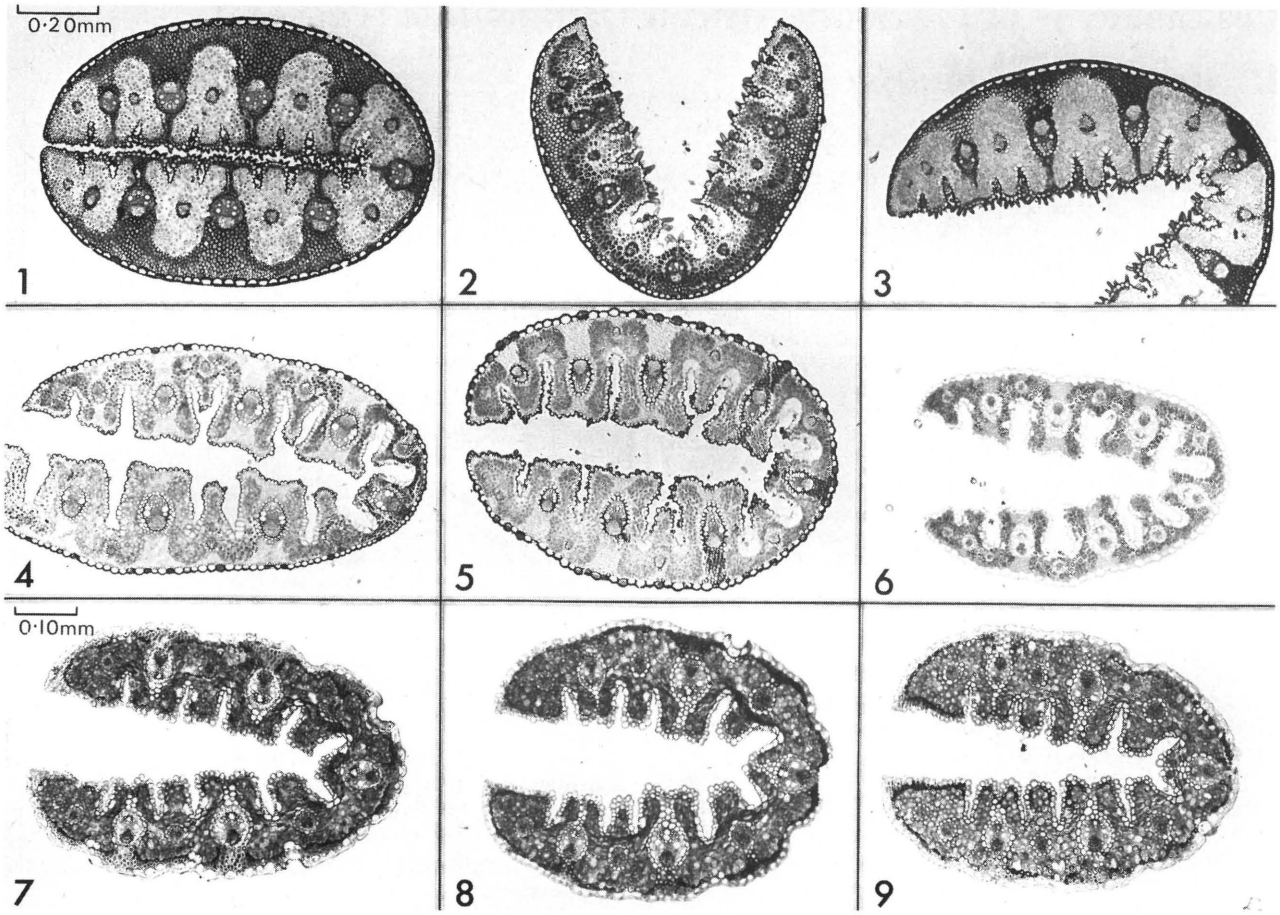
ANATOMICAL DESCRIPTION OF *MERXMUELLERA DISTICHA* SENS. LAT.

Leaf in transverse section

Leaf outline: infolded with an elliptical or U-shaped outline. Symmetry about the median bundle not perfect with the 1'vbs of opposite halves of the lamina alternating. This tendency particularly marked in the Drakensberg form (Fig. 4). Leaves narrow (<1,1 mm wide) when folded. Adaxial channel normally a deep, narrow cleft. *Ribs and furrows:* adaxial furrows narrow, cleft-like and located between all vbs except the penultimate 3'vb which lies at the base of a furrow. Adaxial ribs flat-topped and angular; 1'vb ribs larger than those overlying 3'vbs. Abaxial surface without ribs or furrows. *Median vascular bundle:* present; indistinguishable structurally from other 1'vbs. *Vascular bundle arrangement:* no 2'vbs present; one 3'vb between consecutive 1'vbs; 1'vbs decrease in size towards margin. *Vascular bundle structure:* vbs circular or elliptical in shape; phloem adjoins ibs; lysigenous cavity and protoxylem vessels present; metaxylem vessels circular, extremely narrow with thickening of the walls. *Vascular bundle sheaths:* obs of 3'vbs circular or with slight abaxial interruptions in the Drakensberg form (Fig. 5); no sheath extensions. Cells rounded or elliptical, inconspicuous and much smaller than the mesophyll cells; thin walled; chloroplasts few or absent. Ibs indistinct, complete and with uniformly thickened walls. Obs of 1'vbs elliptical or horse-shoe shaped with wide adaxial and abaxial interruptions; no extensions. Sheath cells inconspicuous; smaller than both the mesophyll and the ibs cells; rounded or, more often, elliptical; thin-walled or may resemble ibs cells with u-shaped wall thickenings in typical *M. disticha* only (Fig. 1). Few or no chloroplasts present. Ibs complete; inner radial and tangential walls thickened; often adaxial ibs cells larger than lateral cells. *Sclerenchyma:* small, shallow adaxial strands associated with 3'vbs. Inversely anchor- or T-shaped adaxial girders associated with 1'vbs; width and length of girder stem variable (Table 1); girder interrupts obs. Abaxial girders not associated with 3'vbs except in Drakensberg form (Figs 4 & 5). 1'vb girders usually trapezoidal narrowing toward the bundle; fibres interrupt the obs. Continuous abaxial hypodermal sclerenchyma well

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FIGS 1–9.—Leaf blade outline of *Merxmuellera disticha* sens lat. in transverse section. 1–3, typical *M. disticha* form. All × 160. (1, *Ellis* 2572; 2, *Ellis* 2564; 3, *Ellis* 669.) 4–6, Drakensberg form. All × 160. (4, *Du Toit* 675; 5, *Ellis* 1404; 6, *Ellis* 3152.) 7–9, Alpine bog form. All × 250. (7, *Ellis* 3315; 8, *Ellis* 3306; 9, *Ellis* 3316.)

developed in typical *M. stricta* (Fig. 1) but only a shallow interrupted hypodermal layer present in the alpine bog form (Figs 7–9) (Table 1). Fibres with both lignified and cellulose cell walls present in individual sections. *Margin:* relatively small, pointed, sclerenchyme cap developed. *Mesophyll:* not radiate; cells isodiametric, regular and tightly packed. Mesophyll tissue not continuous between all vbs; U-shaped chlorenchyma groups occupy sides and bases of furrows between consecutive 1'vbs. No colourless cells. *Adaxial epidermis:* bulliform cells poorly developed; basal cells of furrows may be slightly enlarged to form small, fan-shaped bulliform cell groups. Epidermal cells inflated with the outer wall slightly thickened. No macro-hairs, hooks or prickles developed. Cells variously papillate; one papillus per cell. *Abaxial epidermis:* no bulliform cells present. Cuticle and epidermal cell thickening differs in the three forms. Hooks, prickles and papillae absent; macro-hair bases present in alpine bog form (Figs 7 & 8).

Abaxial epidermis

Intercostal zones: not differentiated. *Stomata:* absent throughout abaxial epidermis. *Papillae:* absent except in some specimens of the Drakensberg form where long cells are inflated and tend towards obliqu e papillae (Fig. 15). *Prickles:* absent. *Hooks:* none observed. *Micro-hairs:* absent except in the alpine bog form where bicellar hairs with short basal, and elongated, tapering distal, cells occur (Fig. 16). *Macro-hairs:* absent except on all specimens of alpine bog form (Figs 16 & 17). Uni-cellular; 2–3 specialized epidermal cells associated with hair base; base swollen in relation to hair thickness; short >1,25 mm long. *Silica bodies:* variable (Table 1); rounded in typical *M. disticha* (Figs 10–12) to tall and narrow in the Drakensberg form (Figs 13–15). Present throughout abaxial epidermis; granules present; width approximately the same as that of adjacent costal long cells especially in typical and Drakensberg forms. *Costal cells:* silico-suberose couples alternate with single costal long cell throughout abaxial epidermis; cork cells crescentic and enfolding the silica body except for tall and narrow cork cells associated with tall and narrow silica bodies in the Drakensberg form; costal long cells rectangular with straight to slightly inflated walls; slightly undulated.

Specimens examined:

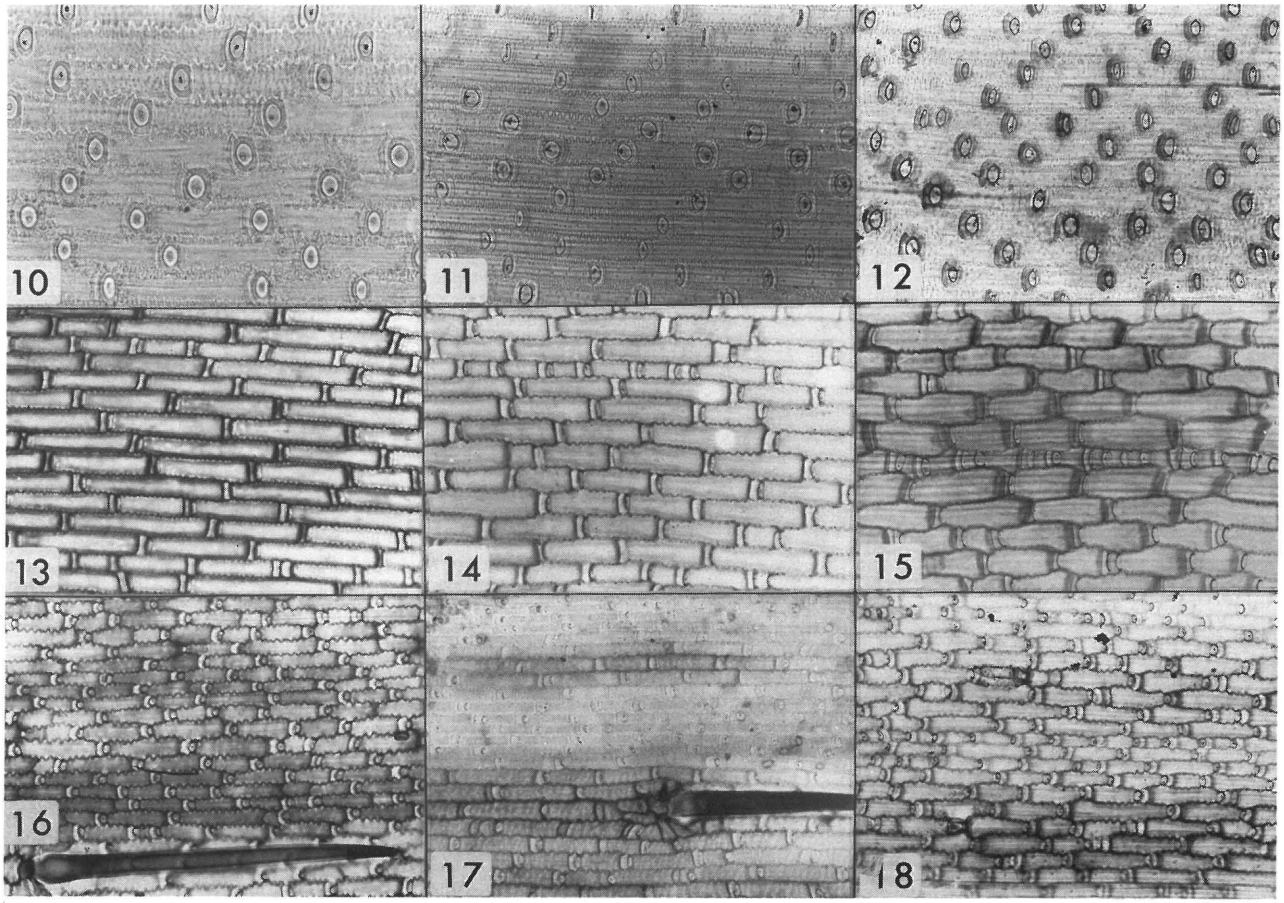
Typical M. disticha

O.F.S.—2828 (Bethlehem): Golden Gate National Park, Brandwag Peak (–DA), *Ellis 2391.*

CAPE.—3124 (Hanover): Lootsberg (–DC), *Theron 486.* 3126 (Queenstown): Jamestown (–BB), *Ellis 2603.* 3225 (Somerset East): Groot Riet Vlei (–AD), *Acocks 11961.* 3226 (Fort Beaufort): 40 km from Tarkastad on Adelaide road (–AD), *Ellis 2572.* 3325 (Port Elizabeth): Addo Elephant National Park (–BC), *Liebenberg 6656*, 7713; King Neptune Beach (–DC), *Ellis 2564.* 3420 (Bredasdorp): between Swellendam and Riviersonderend (–AA), *Ellis 1263*; Potberg (–AD), *Ellis 669*; Cape Agulhas (–CC), *Loxton 246.*

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FIGS 10–18.—Abaxial epidermis of *Merxmuellera disticha* sens. lat. All × 400. 10–12, *M. disticha* form. (10, *Liebenberg* 7713; 11, *Ellis* 1263; 12, *Ellis* 669.) 13–15, Drakensberg form. (13, *Ellis* 3157; 14, *Ellis* 3152; 15, *Ellis* 1404.) 16–18, Alpine bog form. (16, *Ellis* 3183; 17, *Ellis* 3192; 18, *Ellis* 3313.)

TABLE 1.—The anatomical differences between the different forms of *Merxmuellera disticha* as seen in transverse sections of the leaf blade and on epidermal preparations in surface view

Character	Typical Form	Drakensberg Form	Alpine bog Form
1. Number of 1'vbs in leaf section	1. 7 (sometimes 5)	1. 7 (sometimes 5)	1. 5 vbs
2. Outline of lamina	2. Permanently infolded but opening of 45° possible	2. Infolded but regular opening of 180° occurs	2. Permanently infolded
3. Depth of adaxial furrows	3a. Medium $< \frac{1}{2}$ leaf thickness 3b. All furrows of equal depth	3a. Deep $> \frac{1}{2}$ leaf thickness 3b. Furrows on either side of midribs deeper than rest	3a. Medium $< \frac{1}{2}$ leaf thickness 3b. All furrows of equal depth
4. Vertical position of vascular bundles in blade	4. All bundles centrally located	4. 1'vbs centrally and 3'vbs abaxially positioned	4. All bundles centrally located
5. Adaxial sclerenchyma girders —inversely T-shaped	5. T with long, narrow stem (1–3 seriate)	5. T with short, sturdy stem (> 3 seriate)	5. T with variable stem
6. Abaxial sclerenchyma	6. Continuous hypodermal layer with girders to 1'vbs only	6. Well developed tall or trapezoidal girders associated with both 3' and 1'vbs. Hypodermal layer sometimes developed	6. Thin hypodermal layer with trapezoidal girders associated with 1'vbs only
7. Bulliform cells	7. Poorly developed at base of furrows	7. Well developed on two furrows on either side of the median bundle	7. Absent
8. Adaxial papillae	8. Long, broad, hair-like distally thickened papillae on most epidermal cells	8. Outer walls of epidermal cells inflated with a few elongated, thickened papillae present	8. Outer walls of all epidermal cells inflated
9. Abaxial epidermal cells	9. Outer tangential wall flattened with smooth, continuous cuticle	9. Outer wall inflated and projecting; not conspicuously thickened	9. Outer walls irregular but with a markedly thickened cuticle
10. Abaxial micro-hairs	10. Absent	10. Absent	10. Present
11. Macro-hairs	11. Absent	11. Absent	11. Present
12. Silica bodies	12. Rounded or circular to elliptical in shape. Equidimensional to vertically elongated	12. Tall and narrow with smooth outlines to crescentic or kidney shaped. Vertically elongated	12. Crescent or kidney shaped to cuboid or rounded. Equidimensional
13. Cork cells	13. Crescentic, enfolding silica body	13. Tall and narrow adjacent to silica body	13. Crescentic, enfolding silica body

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Drakensberg form

O.F.S.—2828 (Bethlehem): Golden Gate National Park, Brandwag Peak (–DA), *Du Toit 675*. Wodehouse Peak, *Ellis 2382*; Witsieshoek—Mont-aux-Sources area (–DB), *Ellis 3133, 3152, 3157*.

NATAL.—2829 (Harrismith): Cathedral Peak, Organ Pipes Pass (–CC), *Ellis 1404, 3185, 3303, 3305*. 2929 (Underberg): Giants Castle Game Reserve (–AD), *McAllister 112*; Bannermans Pass, *Ellis 3312*.

LESOTHO.—2929 (Underberg): Mokhotlong (–AC), *Coetzee 834*.

Alpine bog form

NATAL.—2829 (Harrismith): Cathedral Peak, Organ Pipes Pass summit (–CC), *Ellis 3183, 3184, 3192, 3306, 3309*. 2929 (Underberg): Giants Castle, summit of Bannermans Pass (–AD), *Ellis 3313, 3315, 3316*; top of Sani Pass (–CB), *Du Toit 699*.

LESOTHO.—2929 (Underberg): above Sani Pass (–CA), *Du Toit 2207*.

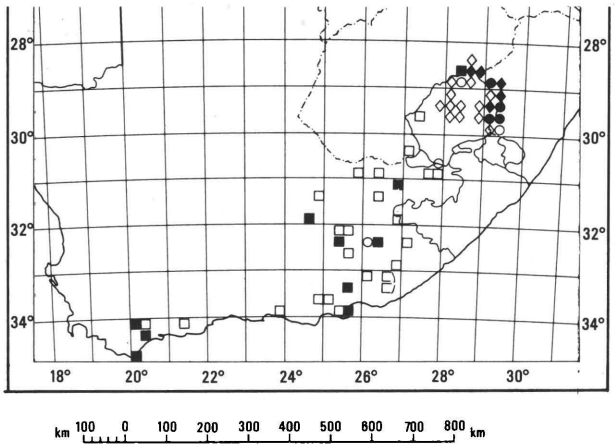


FIG. 19.—Distribution of *Merxmuellera disticha* in South Africa. □—typical *M. disticha* form; ◊—Drakensberg form; ○—Alpine bog form. Shaded symbols represent localities of specimens studied anatomically. Compiled from specimens at the National Herbarium, Pretoria (PRE).

DISCUSSION AND CONCLUSIONS

From the foregoing anatomical description of *M. disticha* sens. lat., and from the accompanying photomicrographs (Figs 1–18), it is evident that numerous anatomical differences exist between the three different forms of *M. disticha* recognized in this study (Table 1). These differences are of considerable magnitude and are much more obvious than are the anatomical differences among some of the other closely related *Merxmuellera* species e.g. *M. macowanii* (Stapf) Conert, *M. davyi* (C. E. Hubb.) Conert and *M. aureocephala* (J. G. Anders.) Conert or *M. drakensbergensis* (Schweick.) Conert and *M. stereophylla* (J. G. Anders.) Conert. In addition, the anatomical differences between *M. drakensbergensis* and *M. stereophylla*, for example, are merely a matter of degree and there is a tendency for the characters to grade from one species into the other. The differences among the three *M. disticha* forms, on the other hand, are distinctly disjunct with the characters being structurally different e.g. adaxial ribs, sclerenchyma girders, silica bodies etc. Furthermore, a number of correlated characters, from both the leaf blade in section and the epidermis, characterize each of the three forms. These diagnostic characters constantly occur in combination and with the detection of any single diagnostic character the remainder can safely be inferred. This evidence, based solely on leaf anatomical criteria suggests, therefore, that each of the forms of *M. disticha*, recognized in this study, warrants taxonomic status, possibly sub-specific rank.

In certain spikelet characters, differences are also exhibited among these three forms of *M. disticha*. From the sample examined, it appears that, in both the Drakensberg and alpine bog forms, only 2-flowered spikelets are found. In typical *M. disticha*, all specimens had three or more florets. In typical *M. disticha* and in the Drakensberg form the length of the upper and lower glumes is greater than 12 mm whereas, in the alpine bog form they are 11 mm or less in length. In both typical *M. disticha* and the Drakensberg form the lower glume was always distinctly 3-nerved, whereas in the alpine bog sample only a single prominent nerve was present with two poorly developed lateral nerves sometimes being evident. From the small sample examined, it would appear, that a detailed study of the spikelet morphology of *M. disticha* sens. lat. should confirm the anatomical groupings and assist in reaching a taxonomic decision

The three forms of *M. disticha* show distinct vegetative differences as well, and can be readily recognised in the field. The alpine bog form has very narrow, short, setaceous leaves between 100–200 mm long and forms fine, delicate, compact but low tussocks. The younger green leaves are normally more or less straight and erect and the older, dry leaves curl, thus forming an irregularly matted "cushion" out of which the needle-like green leaves project. Typical *M. disticha* plants have a similar structure, except on a much larger scale with the leaves being from 300–500 mm long. The setaceous leaves are thicker and much more rigid and fibrous, with a very high tensile strength. The old, dry leaves of the tussock form a dense curly mass. The Drakensberg form, on the other hand, has leaves which are often not setaceous, but are open and up to 3,5 mm wide. This is especially conspicuous under conditions of reduced radiation such as on misty, cloudy days. In this state, the Drakensberg form is unmistakeable and it is regrettable that in the preparation of herbarium vouchers the leaves become infolded and setaceous and the herbarium specimens resemble typical *M. disticha*. However, the green leaves are softer, more flexible and more easily torn and the old leaf blades are exceptionally curly.

Differences in habitat requirements between the three forms became evident while collecting material in the field for the anatomical investigation. Thus, the alpine bog and Drakensberg forms occur in the Drakensberg mountains but only in basaltic soils above the cave sandstone layers. On the summit of this escarpment they often occur in close proximity to one another. The alpine bog form is restricted to saturated, humic soils in shallow bogs or seepage areas and is often found in water about 100 mm deep. The Drakensberg form may be found on raised mounds in the selfsame seepage areas but obviously requires better drainage conditions. It is also more widespread being found down to altitudes of about 2 000 m, whereas the bog form is restricted to the summit at over 3 500 m.

Typical *M. disticha* has a wide distribution in the Cape Province (Fig. 19) being found at low altitudes along the southern coast and then throughout the eastern and north-eastern Cape mountains. Inexplicably it appears to be absent from the Transkei and Natal, but is found in Lesotho and the sandstone mountains of the eastern Orange Free State. Thus at Golden Gate typical *M. disticha* occurs on the slopes of Brandwag Peak (*Ellis* 2931), but higher up the same mountain, on the basalt cap known as Wode-

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house Peak, the Drakensberg form is found in black, peaty soils (*Ellis* 2382; *Du Toit* 675).

The type of intraspecific variation described here, has also been observed in *M. stricta* (Schrad.) Conert in similar habitats in the same mountains (Ellis, in prep.). Possibly the other closely related *Merxmuellera* species of the summer rainfall areas also represent the outcome of similar diversification and speciation e.g. *M. drakensbergensis* and *M. stereophylla* or *M. macowanii* and *M. aureocephala*. Within this genus, therefore, there appears to have been considerable adaptive radiation associated with altering environmental conditions. This applies particularly to altitude effects along this mountain range. These factors must be borne in mind when final taxonomic decisions are taken.

ACKNOWLEDGEMENTS

The author is grateful to the following organizations for permission to collect material on their property: the Department of Forestry, the National Parks Board and the Natal Parks, Game & Fish Preservation Board. The capable technical assistance of Miss R. Manders is gratefully acknowledged.

UITTREKSEL

Die anatomiese struktuur van die blaar in dwarsnee en die abaksiale epidermis van Merxmuellera disticha *word beskryf en geïllustreer. Drie afsonderlike anatomiese "vorme" word erken: tipiese* M. disticha, *die Drakensberg vorm en die alpienevlei vorm. Dit blyk ook dat hierdie drie anatomiese groepe ook verskillende omgewingsbenodigdhede het en waarskynlik taksonomiese status verdien.*

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Bothalia 13,1 & 2: 191–198 (1980)

Leaf anatomy of the South African Danthonieae (Poaceae). III. *Merxmuellera stricta*

R. P. ELLIS*

ABSTRACT

The anatomical structure, of the leaf blade as seen in transverse section, and of the abaxial epidermis, of *Merxmuellera stricta* (Schrad.) Conert is described and illustrated. In this variable species four distinct anatomical "forms" are recognized viz. the typical *M. stricta* form, the Cathedral Peak form, the Drakensberg form and the alpine form. The alpine and Cathedral Peak forms have recently been described as *M. guillarmodiae* Conert (1975). The degree of anatomical differentiation of these "forms" resembles the situation described in *M. disticha* (Nees) Conert (Ellis, 1980). Populations of both *M. stricta* and *M. disticha* from the Drakensberg mountains display extensive anatomical diversification which appears to be correlated with environmental factors. In addition, morphological differences are exhibited as well and the anatomical "forms" of *M. stricta* probably warrant taxonomic recognition.

RÉSUMÉ

ANATOMIE FOLIAIRE DES DANTHONIEAE (POACEAE) D'AFRIQUE DU SUD. III. MERXMUELLERA STRICTA

La structure anatomique du limbe foliaire en coupe transversale et celle de l'épiderme abaxial de Merxmuellera stricta *(Schrad.) Conert sont décrites et illustrées. Dans cette espèce variable on reconnaît quatre "formes" anatomiques distinctes, soit la forme* M. stricta *typique, la forme Cathedral Peak, la forme du Drakensberg et la forme alpine. Les formes alpine et Cathedral Peak ont récemment été décrites sous le nom de* M. guillarmodiae *Conert* (1975). *Le degré de différenciation anatomique de ces "formes" ressemble à la situation décrite chez* M. disticha *(Nees) Conert (Ellis,* 1980). *Dans les montagnes du Drakensberg des populations tant de* M. stricta *que de* M. disticha *montrent une large diversification anatomique qui est apparemment en corrélation avec des facteurs de milieu. En outre, des différences morphologiques sont également manifestes et il est probable que les "formes" anatomiques de* M. stricta *méritent d'être reconnues taxonomiquement.*

INTRODUCTION

Merxmuellera stricta (Schrad.) Conert (1970) [=*Danthonia stricta* (Nees) Schrad.] is widely distributed in the southern mountainous areas of South Africa. It occurs from Namaqualand in the north-west southwards to the south-western Cape, then eastwards to the north-eastern Cape mountains from where the distribution continues in a northerly direction along the Drakensberg mountains to Lesotho and the eastern Orange Free State. Together with *M. disticha* (Nees) Conert, *M. stricta* is an important constituent of the Karroid *Merxmuellera* Mountain Veld along all the higher mountains of the False Karoo and the Central Upper Karoo (Acocks, 1975). To the west of Beaufort West, *M. stricta* replaces *M. disticha* as the dominant grass of the Mountain Renosterbosveld (Acocks, 1975). *M. stricta* is also common in the fynbos communities of the south-western Cape.

M. stricta is a variable perennial, forming coarse, wiry tufts. Chippindall (1955) states that "There is considerable variation in the plants referred to *D. stricta*, and it is possible that they comprise more than one variety". In the north-west *M. stricta* may be confused with *M. dura* (Stapf) Conert, but *M. stricta* can be recognized by the glabrous condition of the lemma at the point of insertion of the central awn and is distinct anatomically (Ellis, in prep.). In the north-east, in the Drakensberg mountains, a situation exists, similar to that observed in *M. disticha* (Ellis, 1980), with three additional anatomical "forms" being present.

These anatomical "forms" appear to be correlated with morphological characters and habitat differences. Certain of the spikelet differences and other morphological characters must be of considerable magnitude as Conert (1975) has independently described a new species, *M. guillarmodiae* Conert, from *M. stricta* collections from the alpine region of the Drakensberg.

Unfortunately, the specimens cited by Conert (1975) as belonging to *M. guillarmodiae* fall into two of the anatomical categories recognized in the present study, while the remaining two "forms" fall in *M. stricta* as currently constituted. If the precedent created by the description of *M. guillarmodiae* is to be followed, it implies that a further two species require description. Similarly, by the same token, two new species, presently referred to *M. disticha*, also warrant description (Ellis, 1980).

In the present context *M. stricta* is viewed in its widest sense, and for convenience *M. stricta* sens. lat. has merely been sub-divided into four "forms" for descriptive purposes: the typical form (*M. stricta*), the Cathedral Peak form (*M. guillarmodiae*), the Drakensberg form (*M. stricta*) and the alpine form (*M. guillarmodiae*). Each of these "forms" exhibits characteristic leaf anatomy and epidermal structure which will be described and discussed according to the terminology of Ellis (1976; 1979).

In the anatomical descriptions which follow, the following abbreviations will be used:

vb/s—vascular bundle/s
l'vb/s—first order vascular bundle/s
2'vb/s—second order vascular bundle/s
3'vb/s—third order vascular bundle/s
ibs—inner bundle sheath; mestome sheath
obs—other bundle sheath; parenchyma sheath.

ANATOMICAL DESCRIPTION OF *MERXMUELLERA STRICTA* SENS. LAT.

Leaf in transverse section

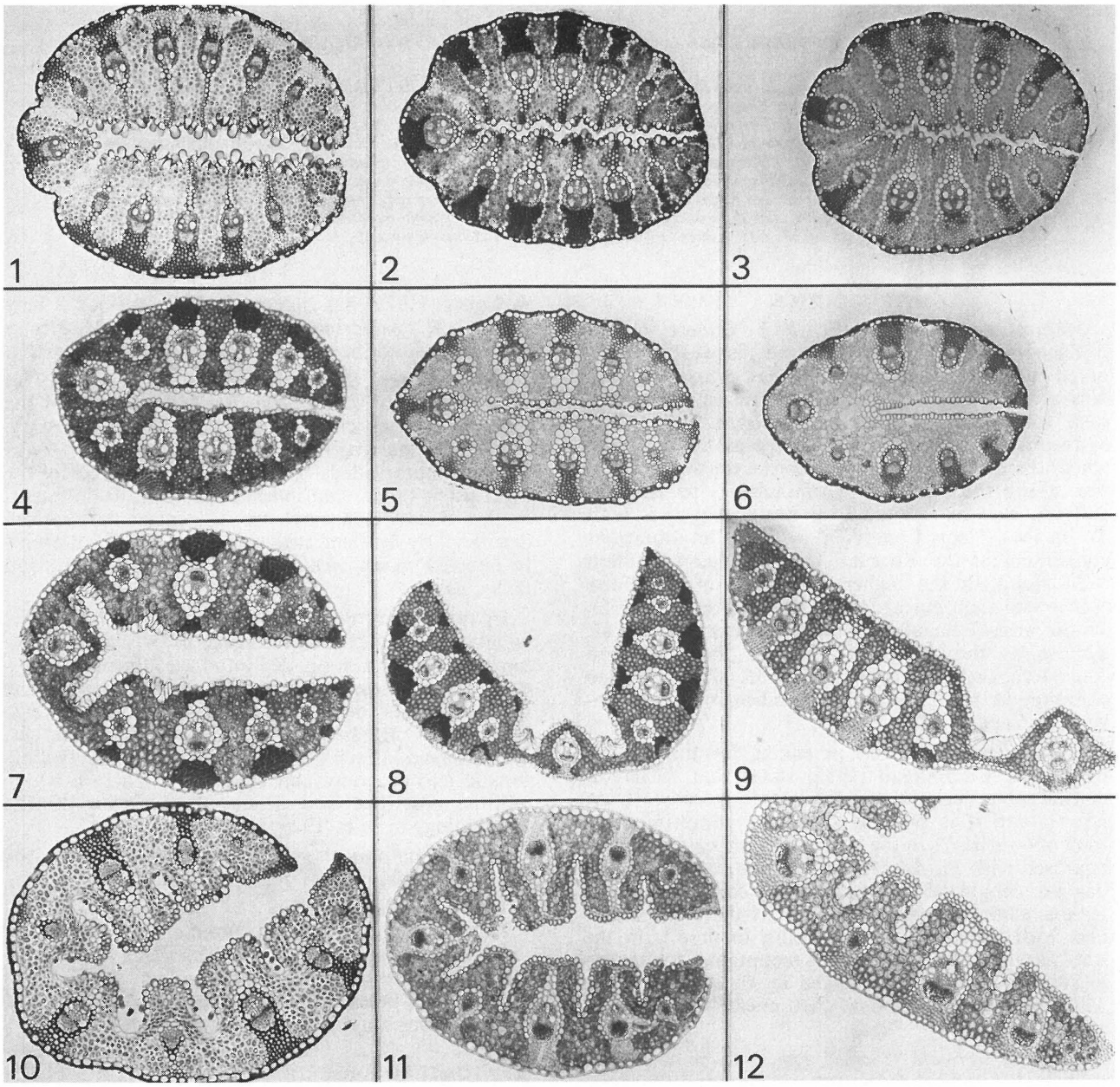
Leaf outline: infolded with an elliptical or U-shaped outline. Permanently infolded in typical and Cathedral Peak forms (Figs 1–6) but regular opening to 180°

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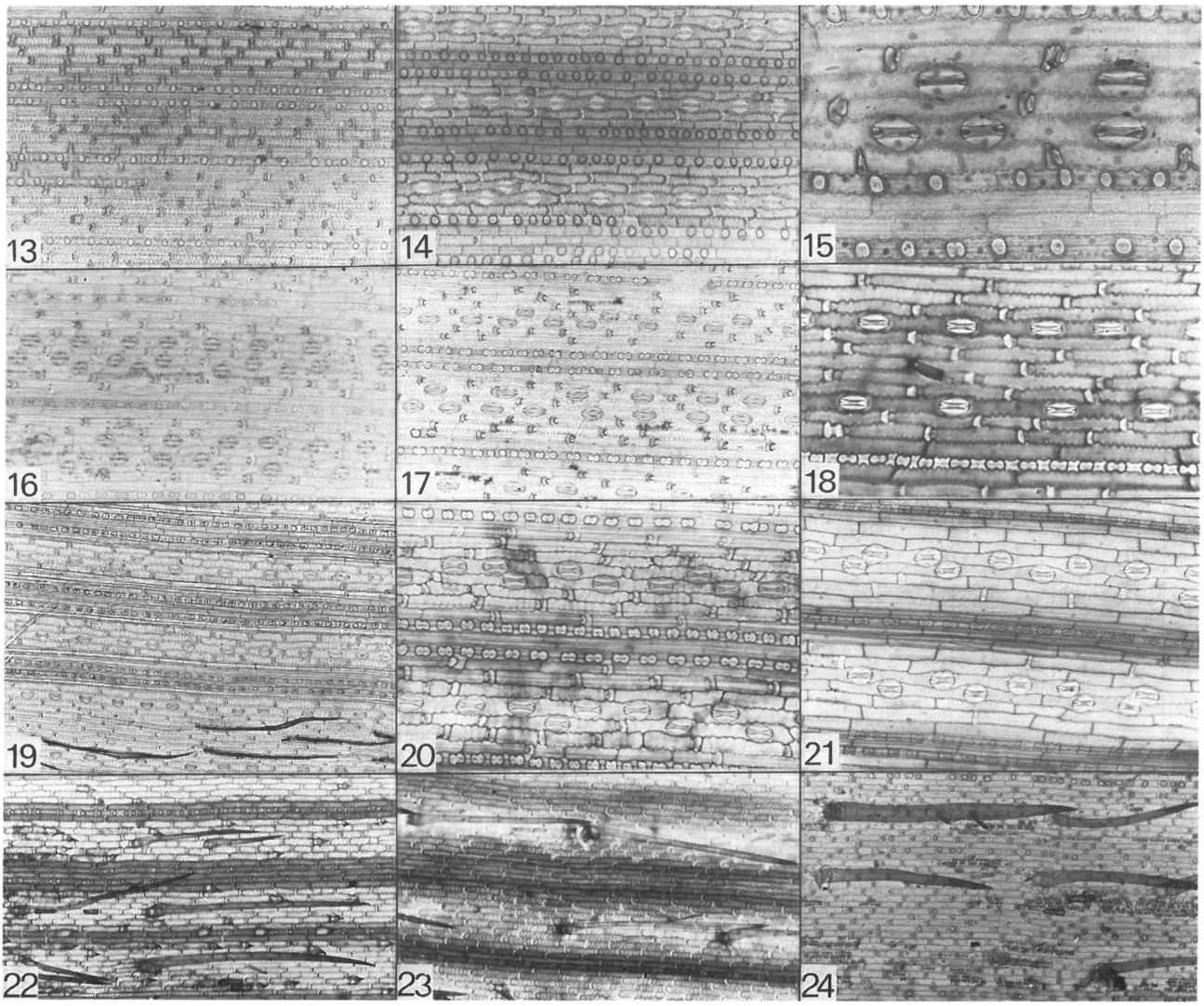
occurs in Drakensberg form (Fig. 9) and to 45° in the alpine form (Fig. 12). Lamina symmetrical about the median vb. 9–13 vbs present in leaf section (Table 1) with the alpine form always with 9 vbs (Figs 10–12) and typical *M. stricta* with 11 or 13 (Figs 1–3). Adaxial channel an extremely narrow and deep cleft in the typical and Cathedral Peak forms and is narrower than the lamina thickness in the Drakensberg and alpine forms when infolded. Leaves narrow (<1,1 mm wide) when folded. *Ribs and furrows:* adaxial furrows of variable depth but constant for each of the "forms" (Table 1); narrow, cleft-like. Similar ribs over all vbs when present; rounded or flat-topped with one vb per rib. Abaxial surface smooth except in typical *M. stricta* where grooves are present on either side of the median vb (Figs 1–3). *Median vascular bundle:* present; indistinguishable structurally from lateral l'vbs. *Vascular bundle arrangement:* no 2'vbs; 3'vbs absent between lateral l'vbs. 3 or 4 l'vbs in each half of the lamina in typical form but decreasing to two in alpine form. All bundles located in centre of blade. *Vascular bundle structure:* vbs circular or elliptical in shape; xylem and phloem distinguishable in all vbs; phloem adjoins ms; often divided by intrusion of fibres in typical *M. stricta* (Figs 2 & 3) and the Cathedral Peak form (Fig. 5). Lysigenous cavities present. Metaxylem vessels thickened, circular and very narrow. *Vascular bundle sheaths:* obs circular or elliptical but normally horseshoe shaped due to wide abaxial interruptions. Adaxial extensions present but cell composition differs in each of the "forms". Obs cell shape variable from round to elliptical but all cells in a given leaf similar in shape. Obs cells conspicuous but not larger than the



FIGS 1–12.—Leaf blade outline of *Merxmuellera stricta* sens. lat. as viewed in transverse section. 1–3, typical *M. stricta* form, all ×160. Note abaxial grooves on either side of median bundle only. (1, *Ellis* 2476; 2, *Ellis* 2445; 3, *Ellis* 2441.) 4–6, Cathedral Peak form, all ×160. Adaxial groove, but no furrows present. (4, *Ellis* 3295; 5, *Ellis* 2372; 6, *Trauseld* 833.) 7–9, Drakensberg form. Very deep adaxial furrow on either side of median vascular bundle. Leaf not permanently infolded. (7, *Ellis* 3318, ×250; 8, *Ellis* 3322, ×160; 9, *Ellis* 3321, ×250.) 10–12, alpine form, all ×250. Furrows developed between all vascular bundles. (10, *Ellis* 3181; 11, *Ellis* 3317; 12, *Ellis* 3308.)

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FIGS 13–24.—Abaxial epidermis of *Merxmuellera stricta* sens. lat. as seen in surface view. 13–15, typical *M. stricta* form. All with round silica bodies. (13, *Roberts* 2034, ×250, note absence of intercostal zones; 14, *Ellis* 1156, ×250, note stomatal files and intercostal zones; 15, *Ellis* 2317, ×640.) 16–18, Cathedral Peak form. Silica bodies dumb-bell shaped. (16, *Ellis* 2372, ×250; 17, *Galpin* 10357, ×250; 18, *Ellis* 3289, ×400.) 19–21, Drakensberg form. Dumb-bell shaped silica bodies. (19, *Acocks* 22069, ×160; 20, *Ellis* 1428, ×400; 21, *Ellis* 3290, ×400.) 22–24, Alpine bog form. Silica bodies crescent-shaped, round or irregularly dumb-bell shaped. Macro-hairs, micro-hairs and prickles common. All ×160. (22, *Du Toit* 2206; 23, *Ellis* 3181; 24, *Ellis* 1393.)

mesophyll cells; without chloroplasts (Fig. 26). Ibs complete; small cells with U-shaped thickenings; adaxial cells larger than lateral cells (Fig. 26). *Sclerenchyma:* Adaxial sclerenchyma variable from minute strands in the Cathedral Peak form (Figs 4–6) to well-developed, inversely anchor-shaped girders in typical *M. stricta* (Figs 1–3) or the alpine form (Figs 10–12) (Table 1). Abaxial girders well developed; either trapezoidal (Fig. 1 & 7) or narrower than the vb (Figs 2 & 5). In certain specimens of typical *M. stricta* individual girders fuse forming a continuous, abaxial, hypodermal band (Fig. 1). *Margin:* small, triangular, sclerenchyma caps developed. *Mesophyll:* not radiate; composed of regular, small, isodiametric, tightly packed cells (Fig. 26). Tall, narrow groups of chlorenchyma tissue between consecutive vbs separated by sclerenchyma girders. No colourless cells. *Adaxial epidermis:* bulliform cells poorly developed; basal cells of furrows may be slightly enlarged to form small, fan-shaped groups (Table 1). Epidermal cells inflated with outer wall slightly thickened. Macro-hairs and prickles absent; hooks occur irregularly. Cells variously papillate; one papillus per cell. Adaxial papillae best developed in typical *M. stricta* (Fig. 1). *Abaxial epidermis:* no bulliform cells developed. Hooks, prickles, macro-hairs and papillae lacking. Outer tangential wall flattened with smooth, continuous cuticle.

Abaxial epidermis in surface view

Intercostal zones: differentiated except in typical *M. stricta* specimens with continuous hypodermal sclerenchyma layer (Figs 1 & 13). Long cells medium to elongated with parallel, slight to moderately undulating, side walls. Silico-suberose couples between successive long cells. No bulliform cells. *Stomata:* absent in alpine form (Figs 22–24) and in those typical *M. stricta* specimens with hypodermal sclerenchyma layer (Fig. 13). Low dome-shaped, 1–2 files of stomata in centre of each intercostal zone; files adjacent to one another. One interstomatal cell between successive stomata. *Intercostal short cells:* silico-suberose couples; cork cell tall and narrow to crescentic; occur between most intercostal long cells. *Papillae:*

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TABLE 1.—The differences in leaf anatomy and spikelet morphology between the different forms of *Merxmuellera stricta*

Character	Typical *M. stricta* form	Cathedral Peak form	Drakensberg form	Alpine form
1. Outline of lamina	1. Permanently infolded	1. Permanently infolded	1. Infolded but regularly opens to 180°	1. Infolded but opening to 45° possible
2. Number of vbs in leaf section	2. 13 (sometimes 11)	2. 11 (sometimes 9)	2. 9 vbs	2. 9 vbs
3. Adaxial furrows	3a. Slight to medium $>\frac{1}{2}$ leaf thickness 3b. Present between all vbs	3a. Absent 3b. No furrows present	3a. Deep $>\frac{1}{2}$ leaf thickness 3b. A single furrow on either side of median vb only	3a. Medium $\frac{1}{2}$ leaf thickness 3b. Present between all vbs
4. Abaxial furrows	4. Single furrow on either side of median vb	4. Slight undulations associated with all vbs	4. Absent except when leaf open	4. Absent
5. Phloem	5. Usually divided into two groups by intrusive fibres	5. Sometimes exhibits sclerosed phloem	5. No intrusive fibres	5. No sclerosed phloem
6. Adaxial extensions of outer bundle sheath	6a. Parenchymatous grading gradually into fibres 6b. Long, narrow extensions of more than 5 cells	6a. Thin-walled colourless parenchyma 6b. Medium extensions of 4 or less cells	6a. Thickened parenchyma abruptly becoming sclerenchyma 6b. Short, wide extensions of 3 or 4 cells deep	6a. Thick-walled parenchyma decreasing in size to epidermis 6b. Long, wide or narrow extensions of more than 5 cells deep
7. Adaxial sclerenchyma	7. Inversely anchor-shaped with long, thin stem	7. Minute strand only; sometimes absent	7. Small strands narrowing towards obs extension	7. Inversely anchor-shaped girder with narrow or wide stem
8. Bulliform cells	8. Poorly developed only at base of furrow on either side of median vb	8. Absent	8. Well-developed fan shaped groups at base of furrow on either side of median vb	8. Poor to well developed fan shaped groups in the bases of all furrows
9. Epidermal hairs	9. Micro-hairs only; rare	9. Micro-hairs only	9. Micro - hairs but sometimes macro-hairs occur	9. Micro-hairs, macro-hairs and prickles common
10. Silica body shape	10. Rounded or elliptical	10. Dumb-bell shaped	10. Dumb-bell shaped but round in a few specimens	10. Rounded to elliptical but enfolded by crescentic cork cell
11. Lower glume length	11. (11–) 15,2 mm (–22)	11. (9–) 10,7 mm (–12)	11. (14–) 17,8 mm (–22)	11. (10–) 12,2 mm (–14)
12. Length of lower lemma awn	12. (9–) 12,5 mm (–16)	12. (8–) 9,8 mm (–11).	12. (8–) 12,0 mm (–14)	12. (5,0–) 5,5 mm (–6,5)
13. Hairs on back of lemma at point of insertion of awn	13. Glabrous; occasionally very sparse, scattered hairs present	13. Densely hairy; hairs 2–3,2 mm long	13. Always glabrous	13. Sparsely hairy; hairs up to 2 mm long

absent. *Prickle-hairs:* only present on alpine form (Fig. 22) (Table 1). Barbs short. *Micro-hairs:* present but very rare in typical *M. stricta* and then only in specimens with intercostal zones; common in other three "forms". Bicellular, elongated (especially in alpine form); basal cell slighly longer than distal cell; tapering distal cell thin-walled (Fig. 18). *Macro-hairs:* absent except on all specimens of alpine form; occur in groove on either side of median vb in one specimen of Drakensberg form (Fig. 19). Unicellular, inflexible; 2–3 specialized epidermal cells associated with base of hair; base somewhat swollen in relation to hair thickness. *Silica bodies:* differ in different "forms" (Table 1). Costal bodies rounded or elliptical in typical *M. stricta* (Figs 14 & 15) and in certain specimens of Drakensberg form; dumb-bell shaped in Drakensberg and Cathedral Peak form (Figs 18, 20, 21); small rounded to elliptical and associated with crescentic cork cell in alpine form (Figs 22 & 24). Granules present. Width same as adjacent costal long cells except in alpine form. *Costal cells:* silica cells alternate with costal short cells except in alpine form where silico-suberose couples alternate with costal short cells; files with silica cells alternate with files of costal long cells in all forms except alpine form.

Specimens examined.

Typical *M. stricta* form

CAPE.—3119 (Calvinia): Van Rhyns Pass (–AC), *Ellis 1139, 1140*; Kobe Mts (–CA), *Ellis 2445, 2447**. 3126 (Queenstown): Bushmanhoek Pass (–AD), *Ellis 2577**; Hangklip Mt (–DD), *Roberts 2034**. 3218 (Clanwilliam): Pakhuis Pass (–BB), *Ellis 1705**. 3219 (Wuppertal): Pakhuis Pass (–AA), *Ellis 1151, 1156*; Buffelberg Pass (–CA), *Ellis 1189*. 3225 (Somerset East): Grootfontein valley (–CB), *Van der Walt 184**. 3318 (Cape Town): Jonkershoek, Stellenbosch (–DD), *Ellis 2241, 2242, 2258*. 3319 (Worcester): Gydoberg (–AD), *Ellis 2476**; Karoopoort, Ceres (–BC), *Hafström & Acocks 100**. Franschoek Pass (–CD), *Ellis 687, 688*. 3320 (Montagu): 23 km from Barrydale on road to Montagu (–DC), *Ellis 643*. 3322 (Oudtshoorn): Swartberg (–AC), *Ellis 2579**; Robinsons Pass (–CC), *Ellis 2583**. 3323 (Willowmore): Uniondale (–CA), *Ellis 1641**. 3325 (Port Elizabeth): Gamtoos Valley (–CC), *Acocks 16112*. 3326 (Grahamstown): Peddie (–BC), *Fairall 240*. 3418 (Simonstown): Red Hill (–AB), *Ellis 2317, 2318*. 3419 (Caledon): Kleinmond (–AC), *Ellis 2514, Van Heerden 75*. 3420 (Bredasdorp): Bredasdorp (–AA), *Ellis 1265**.

Cathedral Peak form (*M. guillarmodiae*)

O.F.S.—2828 (Bethlehem): Golden Gate National Park, Brandwag Peak (–DA), *Ellis 2371, 2372*.

NATAL.—2829 (Harrismith): Royal Natal National Park (–CB), *Galpin 10357*; Cathedral Peak Forest Reserve (–CC), *Killick 1110†, 1576†, Ellis 3289, 3295, 3297*. 2929 (Underberg): Giants Castle Game Reserve (–AB), *Trauseld 833*; Nyiginye (–BA), *Du Toit* 2516; Highmoor Forest Reserve (–BC), *Du Toit 2500*.

Drakensberg form (*M. stricta*)

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 1428, 3290, 3299*. 2929 (Underberg): Giants Castle Game Reserve, Bannermans Pass (–AD), *Ellis 3318, 3321*; Loteni Game Reserve (–DA), *Ellis 3322*.

*Specimens with continuous abaxial hypodermal sclerenchyma layer and without stomata.

† Specimens cited by Conert (1975) as being *M. guillarmodiae*.

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LESOTHO.—2929 (Underberg): Sehlabathebe National Park (–CC), *Du Toit 2631, 2642.*

CAPE.—3028 (Matatiele): Quachas Nek (–BA), *Ellis 243**; Ramatselisonek (–BB), *Acocks 22069.* 3226 (Fort Beaufort): Great Winterberg (–AD), *Story 4522.**

Alpine form (*M. guillarmodiae*)

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve, summit above Windy Gap (–CC), *Ellis 1393, 3181, 3182, 3308.* 2929 (Underberg): Giants Castle Game Reserve, summit of Bannermans Pass (–AD), *Ellis 3317*; top of Sani Pass (–CA), *Du Toit 2206, 2242, 2286.*

LESOTHO.—2828 (Bethlehem): Butha Buthe (–CC), *Roberts 5844, Lubke 305*; Tsehlanyane valley (–CD), *Jacot-Guillarmod 3727†, 3734†.*

CAPE.—3027 (Lady Grey): Doodmans Kranz, Barkly East (–DC), *Galpin 6906†.* 3028 (Matatiele): Quachas Nek, Temrock Peak (–BA), *Liebenberg 5729.*

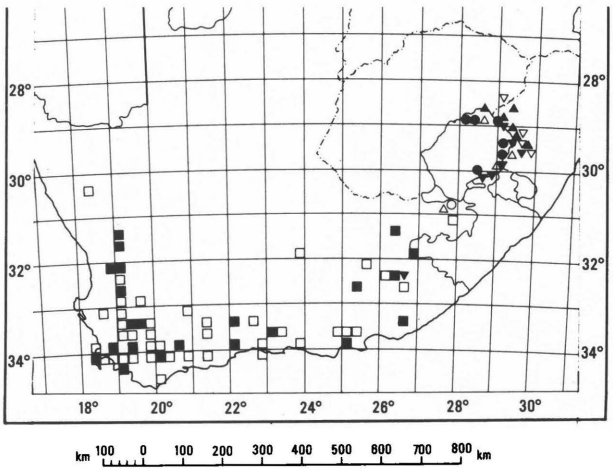


FIG. 33.—Distribution of *Merxmuellera stricta* in South Africa. □—typical M. stricta; △—Cathedral Peak form; ▽—Drakensberg form; ○—Alpine form. Shaded symbols represent localities of specimens studied anatomically. Compiled from specimens at the National Herbarium, Pretoria (PRE).

DISCUSSION AND CONCLUSIONS

All the specimens examined in this study have the lateral first order vascular bundles located adjacent to one another and lack the single third order bundle interspaced between them which is characteristic of *M. disticha* (Ellis, 1980). *M. dura*, considered to be closely allied to *M. stricta* (Chippindall, 1955; De Wet, 1960), does not share this characteristic and has, therefore, been excluded from the present considerations.

Anatomically the leaf structure of the typical *M. stricta* form is remarkably constant throughout its wide distributional area (Fig. 33). The only anatomical difference worthy of mention is the development of a continuous abaxial hypodermal sclerenchyma layer (Fig. 1) with the resultant exclusion of the intercostal zones and stomata (Fig. 13). This structure was present in 40% of the specimens examined in this study, and occurs in plants from widely scattered localities, throughout the range of this form, and does not appear to be correlated with any obvious environmental factor. Morphologically there is a definite tendency for these specimens to have shorter glumes [(11–) 12,1 mm (–15)] than the remainder of the typical *M. stricta* specimens [(15–) 17,5 mm (–22)]. These two characters, therefore, appear to be constantly linked and, on the basis of glume length, these shorter spikelets tend to resemble the Cathedral Peak form of *M. guillarmodiae* (Table 1). However, the *M. stricta* form has distinctly longer awns—averaging 11,1 mm as against 9,8 mm for the Cathedral Peak form. This form, however, does not have a continuous hypodermal sclerenchyma layer (Fig. 4) and has numerous stomatal files (Figs 16–18).

The anatomy of the Cathedral Peak form is unmistakeable, due to the shape of the adaxial groove which lacks ribs and furrows, (Figs 4–6) and due to the occurrence of dumb-bell shaped silica bodies (Figs 16–18). This type of anatomical structure appears to be strongly correlated with *M. guillarmodiae* spikelet characters, such as the presence of hairs on the back of the lemma up to the point of insertion of the central awn (Conert, 1975). However, as Conert (1975) points out the hair arrangement of the cited Cathedral Peak form specimens (*Killick* 1100, 1576) resembles that of *M. guillarmodiae,* but the hairs themselves, are longer. The typical *M. guillarmodiae* (alpine form) specimens examined have hairs up to 2,0 mm in length with the lemmas being sparsely hairy but the Cathedral Peak form specimens are densely hairy on the back of the lemmas with the hairs being from 2,0–3,2 mm long.

All morphological indications are, therefore, that this Cathedral Peak form is closely linked to the typical *M. guillarmodiae* or alpine form. However, one specimen, *Trauseld* 833, does not exhibit any of these *M. guillarmodiae* spikelet characteristics. Although the adaxial groove and silica bodies conform, it differs anatomically from the remainder of the Cathedral Peak form sample in that it lacks adaxial bundle sheath extensions and thus the mesophyll distribution is continuous adaxially and not restricted to separate tall and narrow groups (Fig. 6). This specimen was collected at Giants Castle and, although this area was revisited, no plants with Cathedral Peak form anatomy or morphology were located. The Drakensberg form, which *Trauseld* 833 resembles morphologically, is common in this area, but all specimens collected had the distinctive Drakensberg form anatomy. Specimens, conforming anatomically and morphologically with the Cathedral Peak form, do occur at Highmoor to the south of Giants Castle, e.g. *Du Toit* 2500.

Further collections from the southern Drakensberg are required to help clarify the relationships of this Cathedral Peak form. It has been grouped under *M. guillarmodiae* (Conert, 1975) together with the alpine form, which it closely resembles morphologically. Anatomically, however, it has most in common with the Drakensberg form. Both these latter "forms" have similar sclerenchyma girders and bundle sheath extensions but, most significantly, both have dumb-bell shaped silica bodies.

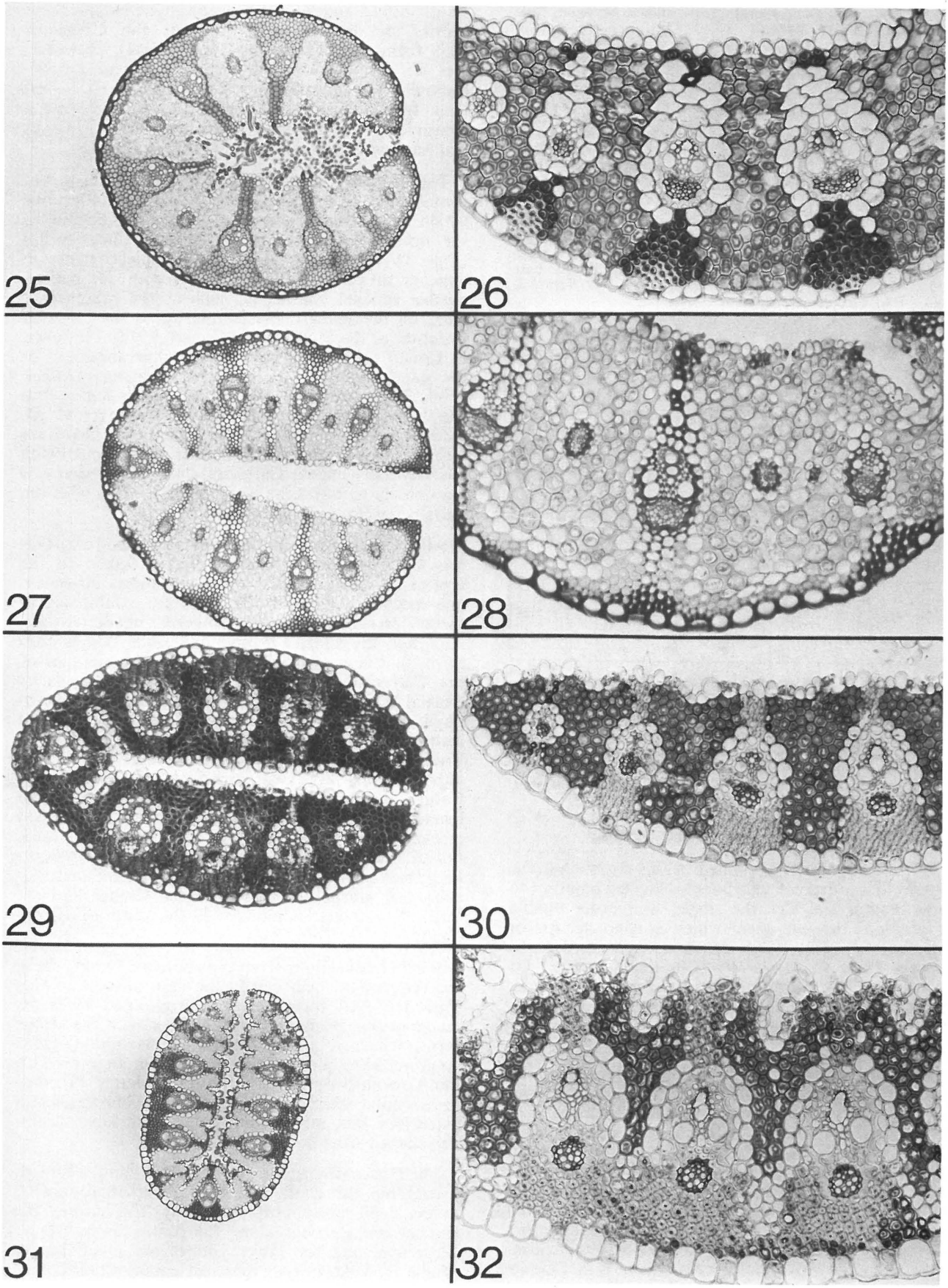
The Drakensberg form, on the other hand, although resembling the Cathedral Peak form anatomically, differs significantly morphologically. The back of the lemmas are glabrous along the centre, as in typical *M. stricta*, and the lower glumes are much longer (Table 1). Anatomically the most important difference is the two deep adaxial furrows on either side of the median vascular bundle (Fig. 7) and the laminae are capable of opening to 180° (Fig. 9) especially during conditions of low irradiance. This ability is undoubtedly functionally significant and was possibly partly responsible for the relative success of this Drakensberg form in the north-eastern mountains.

* Specimens with round or elliptical silica bodies.

† Specimens cited by Conert (1975) as being *M. guillarmodiae*.

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FIGS 25–32.—A comparison between the leaf anatomy of the setaceous-leaved, summer rainfall *Pentaschistis* species with the anatomy of the leaves of the *Merxmuellera stricta* group. 25, *Pentaschistis basutorum*, *Ellis* 2368, ×160. Note arrangement of large and small vascular bundles. 26, *Merxmuellera guillarmodiae*, *Ellis* 3289, ×400. Detail of mesophyll and bundle sheath structure typical of the *M. stricta* group. 27–28, *Pentaschistis fibrosa*. (27, *Du Toit* 646, ×160, note vascular bundle arrangement; 28, *Du Toit* 2301, ×400, outer bundle sheath cells thin-walled and inconspicuous.) 29–30, *Pentaschistis tysonii*. (29, *Ellis* 3302, ×250, third order bundles absent; 30, *Ellis* 3319, ×400, bundle sheath cells distinct.) 31–32, *Pentaschistis* sp. (31, *Ellis* 1409, ×100, no third order bundles between lateral first order bundles; 32, *Ellis* 3292, ×400.)

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This anatomical difference, although distinct and consistent, may, however, not be as significant as it initially appears. It can be simply explained by opposite developmental trends in respect of only a single character—a single adaxial furrow on either side of the median vascular bundle. The epidermis also closely resembles that of the Cathedral Peak form as both have dumb-bell shaped silica bodies (Figs 16–21).

The anatomical indications are, therefore, that the Drakensberg form resembles the Cathedral Peak form more closely than either of the other two forms. These similarities are not corroborated by spikelet structure, however, and in this respect the Drakensberg form displays typical *M. stricta* structure.

Some Drakensberg form specimens from the eastern Cape (*Ellis* 243; *Story* 4522), however, anatomically demonstrate this relationship with typical *M. stricta*. These specimens have round silica bodies, with characteristic *M. stricta* type epidermal structure but in section the anatomy is typical of the Drakensberg form. This observation, therefore, provides an important clue as to the affinities of this form and provides an anatomical link to support the morphological evidence. A degree of morphological and anatomical gradation appears to occur between these two forms in the areas of sympatry in the north-eastern Cape and once again further collecting is required to help confirm affinities between the Drakensberg, Cathedral Peak and typical *M. stricta* forms.

The anatomy of the alpine form specimens compares very favourably with that of the type specimen of *M. guillarmodiae* (*Jacot-Guillarmod* 3734). This form appears to be distinct, both anatomically and morphologically, from *M. stricta* (Conert, 1975) although the Cathedral Peak form is intermediate in most spikelet characters (Table 1). In addition it has specialized habitat requirements, being restricted to the higher alpine zone of the Drakensberg, often associated with boggy conditions. It is, therefore, not spatially associated with any of the other forms.

This alpine form of *M. guillarmodiae* exhibits striking resemblances, in habitat preferences, growth form and anatomy, with the alpine bog form described in *M. disticha* (Ellis, 1980). Anatomical similarities are rib and furrow distribution and form, mesophyll configuration and epidermal structure. The only difference is in the pattern of arrangement of the various orders of vascular bundle along the width of the lamina. This arrangement differs in the two "forms" but corresponds with the patterns found in either typical *M. stricta* or typical *M. disticha*. This difference is correlated with differences in inflorescence characters—a contracted panicle in *M. stricta* and a distichous spike in *M. disticha*—and appears to indicate the relationships of the alpine "forms". This seems to be an excellent example of convergent evolution in response to similar environmental conditions.

The most obvious morphological differences between the specimens of the four "forms" of *M. stricta,* recognized and examined in this study, are briefly summarized in Table 1. Typical *M. stricta* and the Drakensberg form have significantly longer lower glumes than do the alpine and Cathedral Peak forms. The longer glumes are associated with longer awns on the lower lemma except in the Cathedral Peak form which is intermediate between the alpine form and the other two. The Cathedral Peak and typical *M. stricta* forms are glabrous on the back of the lower lemma along the central vein up to the base of the central awn, although, occasionally, a few scattered hairs may be present in *M. stricta* type specimens. In both the alpine and Cathedral Peak forms the back of the lemma is hairy–sparsely hairy with hairs up to 2 mm long in typical *M. guillarmodiae* (the alpine form) but densely hairy with longer hairs (2,0–3,2 mm long) in the Cathedral Peak form.

Morphological characters, therefore, indicate two groups in *M. stricta* sens. lat.—the typical *M. stricta* and Drakensberg forms sharing certain characters whereas, the same characters differ considerably in the alpine and Cathedral Peak forms. This grouping is not confirmed by anatomical evidence which indicates close relationships between the Cathedral Peak and Drakensberg forms with typical *M. stricta* and the alpine forms being distinct. However, a few specimens with anomalous anatomy tend to break down the rigid anatomical divisions and, thereby, add substance to the morphological grouping.

The acceptance of the morphological groupings as reflecting affinities, implies that silica body structure is of no significance in this instance. This would normally be considered unlikely as silica bodies are usually of considerable value taxonomically (Metcalfe, 1960). Typical *M. stricta* specimens have classic rounded or elliptical festucoid-type silica bodies whereas, both the Drakensberg and Cathedral Peak forms have panicoid-type, dumb-bell shaped bodies (Clifford & Watson, 1977). All other indications are that typical *M. stricta* and the Drakensberg form are closely related except for silica body shape. In fact, these two "forms" of the same species possess silica bodies supposedly characteristic of different tribal groupings (Prat, 1932; 1936). De Wet (1954, 1956, 1960) notes the mixed character of the epidermis in *M. stricta* but actually refers to the association of micro-hairs (a panicoid character) with the festucoid elliptical silica bodies. Two panicoid epidermal characters may, therefore, occur in *M. stricta* sens. lat. but this is not supported by other anatomical evidence.

The recent description of *M. guillarmodiae* (Conert, 1975) as a separate species initially appears justified on the anatomical evidence presented in this paper. However, certain *M. guillarmodiae* specimens, included here in the Cathedral Peak form, differ dramatically from the type of *M. guillarmodiae* (the alpine form) and, applying the same criteria appear to merit specific status in their own right. The Drakensberg form would then also warrant specific status. This implies that a further two species require description, as is the case in *M. disticha* where an almost identical anatomical situation exists (Ellis, 1980). However, especially in *M. stricta*, these anatomical differences do not correlate very well with morphological differences.

This anatomical diversification within *Merxmuellera* populations in this restricted area of high altitudes and of climatic extremes also appears to be reflected by the description of *M. stereophylla*. (J. G. Anders.) Conert (Anderson, 1962) from *M. drakensbergensis* (Schweick.) Conert. The recognition of *M. stereophylla* was based on morphological and ecological grounds but, in this case, the anatomical differences are minimal in comparison with the differences between the anatomical "forms" of *M. stricta* and *M. disticha.*

In addition, two of the four setaceous-leaved *Pentaschistis* species from this same area, display remarkable anatomical similarities with *M. stricta*

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sens. lat. (Figs 25–32). *P. basutorum* Stapf (Fig. 25) and *P. fibrosa* Stapf (Figs 27 & 28) have typical *Pentaschistis* anatomy with thin-walled outer bundle sheath cells and somewhat diffuse mesophyll. The bundle sheath extensions and sclerenchyma associated with the third order bundles differ considerably from *M. stricta* sens. lat. (Fig. 26). P. *tysonii* Stapf (Figs 29 & 30) and *Pentaschistis* sp (Figs 31 & 32), on the other hand, display typical *Merxmuellera* type anatomy, and, on anatomical grounds only, appear to have more in common with *Merxmuellera* and especially the *M. stricta* group than they do with *Pentaschistis*.

The following *Pentaschistis* specimens were examined:

P. basutorum

O.F.S.—2828 (Bethlehem): Golden Gate National Park (–DA), *Ellis 2367, 2368, 2369, 2370, 2373*; *Liebenberg 7454*.

P. fibrosa

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 3300*; Sentinel (–DB), *Du Toit 646*. 2929 (Underberg): Highmoor Forest Reserve (–BC), *Ellis 3169*; Sani Pass (–CB), *Du Toit 2301*.

P. tysonii

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 3296, 3302*; *Killick 2280*. 2929 (Underberg): Giants Castle Game Reserve, Bannermans Pass (–AD), *Ellis 3314, 3319*.

Pentaschistis sp.

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 1409, 3291, 3292*. 2929 (Underberg): Giants Castle Game Reserve, Bannermans Pass (–AD), *Ellis 3320*.

For the above reasons the description of numerous new *Merxmuellera* species from this summer rainfall area is to be cautioned against for the time being. The indications are that this temperate region of high altitudes has only relatively recently been colonized by these typically winter rainfall grasses from the south. Adaptive radiation appears to be actively continuing and the taxonomic picture is not at all clear. Further studies, especially those of a biosystematic and autecological nature, are needed, within *Merxmuellera* and closely related genera in the Danthonieae, before reliable taxonomic conclusions can be reached. At present, the assigning of specific rank to any of these anatomical "forms" cannot be fully justified. However, for practical purposes, each of these anatomical "forms", described in both *M. stricta* and *M. disticha* (Ellis, 1980), deserve taxonomic recognition but infraspecific groupings are recommended until the status of this genus in this area is better understood.

In arriving at a final conclusion, it must be remembered, that, in both *M. stricta* and *M. disticha*, the anatomical differences between the "forms" are of considerable magnitude, disjunct and are correlated with other anatomical, morphological and ecological characteristics. In many instances these differences are, therefore, greater than are those between other *Merxmuellera* species and even between some of the genera of the Danthonieae.

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UITTREKSEL

Die anatomiese struktuur, van die blaar in dwarsnee en die abaksiale epidermis, van Merxmuellera stricta *(Schrad.) Conert word beskryf en geïllustreer. Vier afsonderlike anatomiese "vorme" word erken: die tipiese* M. stricta *vorm, die Cathedral Peak vorm, die Drakensberg vorm en die alpiene vorm. Die alpiene en Cathedral Peak vorme is onlangs beskryf as* M. guillarmodiae *Conert* (1975). *Die mate van anatomiese differensiasie van al hierdie "vorme" toon 'n ooreenkoms met die toestand wat in* M. disticha *beskryf is* (*Ellis,* 1980). *Populasies van, beide* M. stricta en M. disticha, *van die Drakensberge, vertoon anatomiese diversifikasie op groot skaal wat ook gekorreleer is met omgewingsfaktore. Boonop is morfologiese verskille ook sigbaar en hierdie anatomiese "vorme" van* M. stricta *verdien vermoedelik om taksonomiese erkenning te verkry.*

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Bothalia 13, 3 & 4: 487–491 (1981)

Leaf anatomy of the South African Danthonieae (Poaceae). IV. *Merxmuellera drakensbergensis* and *M. stereophylla*

R. P. ELLIS*

ABSTRACT

The leaf blade anatomy of *Merxmuellera drakensbergensis* (Schweick.) Conert and *M. stereophylla* (J. G. Anders.) Conert is described and illustrated. These two closely related species have virtually identical leaf anatomy—both the leaf in section and the abaxial epidermis. The close anatomical resemblance between these two species raises doubts about their specific status. This is especially significant when compared with the considerable differences observed between the anatomical 'forms' recognized in *M. disticha* (Nees) Conert (Ellis, 1980) and *M. stricta* (Schrad.) Conert (Ellis, 1980a).

RÉSUMÉ

L'ANATOMIE DE LA FEUILLE DU DANTHONIEAE (POACEAE) SUD AFRICAIN. IV. MERXMUELLERA DRAKENSBERGENSIS *ET* M. STEREOPHYLLA

L'anatomie de la feuille de Merxmuellera drakensbergensis *(Schweick.) Conert et* M. stereophylla *(J. G. Anders.) Conert est décrite et illustreé. Ces deux espéces étroitement apparentées ont une anatomie de la feuille virtuellement identique–tant la section de la feuille que l'epidermis abaxial. La ressemblance anatomique étroite entre ces deux espèces soulève des doutes au sujet de leur staut spécifique. Ceci est spécialement significatif quand on compare les différences considérables observées entre les "formes" anatomiques reconnues dans* M. disticha *(Nees) Conert (Ellis, 1980) et* M. stricta *(Schrad.) Conert (Ellis, 1980a).*

INTRODUCTION

Merxmuellera drakensbergensis (Schweick.) Conert (1970) (= *Danthonia drakensbergensis* Schweick.) and *M. stereophylla* (J. G. Anders.) Conert (1970) (= *D. stereophylla* J. G. Anders.) are wiry-leaved perennials forming rigid, erect tussocks. The unbranched culms grow vertically and the leaves are rigid and taper to a pungent apex. In both species these leaves are setaceous and tightly involute or canaliculate.

These two species are conspicuous components of the alpine vegetation of the Drakensberg mountains to which they are restricted. *M. drakensbergensis* occurs in the Barkly East and Maclear Districts of the north-eastern Cape, along the Drakensberg mountains of Natal and Lesotho and at Mariepskop in the Drakensberg of the north-eastern Transvaal. *M. stereophylla* has a more limited distribution, being found only in the Drakensberg areas of Natal and Lesotho at altitudes above 2 000 m. Although the distribution of these two species overlaps in the Natal and Lesotho alpine areas, they can, nevertheless, be distinguished both ecologically and morphologically.

M. drakensbergensis occupies mesic situations in the streambank and mud patch communities (Killick, 1963; Edwards, 1967) of the alpine belt along the summit of the high Drakensberg. The habitat of *M. stereophylla,* on the other hand, is essentially xeric and this species is common in the alpine grassland of the basalt cliffs as a crevice and ledge plant. In the *Danthonia* Tussock Grassland (Edwards, 1967) *M. drakensbergensis* is dominant around sponges and mud patches, but on rocky areas *M. stereophylla* is the principal grass. Although these two closely related species (Anderson, 1960) have long been confused, they are distinct ecologically and in the field can readily be distinguished by their differing habitat requirements.**

Vegetatively, these species can also be easily recognized. *M. stereophylla* has rigid, erect, grey-green leaves, whereas *M. drakensbergensis* has softer leaves which are olive-green in colour. *M. drakensbergensis* plants are up to 100 cm tall and *M. stereophylla* is a slightly smaller plant up to 80 cm high. A characteristic feature of *M. drakensbergensis,* which is not evident in *M. stereophylla,* concerns the behaviour of old leaf blades (Anderson, 1960). These normally break off above the ligule and the remaining portion of blade splits along the median nerve and the resultant halves recurve outwards in opposite directions. This useful field diagnostic character appears to be consistent and it is only in recently burnt plants that this character is not evident. However, as these species are badly injured by fire (Edwards, 1967) they are largely confined to fire-protected moist or rocky habitats. *M. macowanii* (Stapf) Conert also exhibits this vegetative characteristic and can, therefore, be confused with *M. drakensbergensis.* Both species are also streambank plants, but *M. macowanii* appears to be limited to the montane and sub-alpine belt below the summit of the Drakensberg.

The relatively recent description and recognition of *M. drakensbergensis* and *M. stereophylla* is somewhat surprising in view of these distinct ecological and vegetative differences. *M. drakensbergensis* was only described in 1938 (Schweikerdt, 1938), prior to which it was referred to *M. macowanii. M. stereophylla* received recognition as recently as 1960 (Anderson, 1960), although Chippindall (1955) mentioned an undescribed species from the high Drakensberg and was undoubtedly referring to this species. A probable reason for these species remaining undescribed for so long is the relative inaccessibility of the area in which they occur, as well as the fact that

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**According to Killick (1978) *M. drakensbergensis* is an ubiquitous species in the alpine belt of the Sani Pass area of the southern Drakensberg: it often occurs in flushes or along streambanks but is also found on rock outcrops and in Alpine Grassland, sometimes covering fairly large areas. *M. stereophylla,* on the other hand, appears to be restricted to dry outcrops at higher altitudes in this part of the Drakensberg. *M. drakensbergensis* is, therefore, not restricted to semi-aquatic communities in this area but displays a wider ecological tolerance. It, nevertheless, prefers deeper and moister soils than *M. stereophylla.*

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spikelet differences are slight and only a matter of degree. Thus the arrangement of the hairs on, and the length of, the lemmas (including lobes and awns) differ slightly (Anderson, 1960, 1962).

That these two species are very closely related (Anderson, 1960) is confirmed by the anatomy of their leaf blades which is almost identical. No consistent and measurable structural differences are evident and evidence from leaf anatomy, therefore, casts some doubt on the validity of granting these species specific status. This is especially significant when compared with the situation in *M. stricta* (Schrad.) Conert (Ellis, 1980a) and *M. disticha* (Nees) Conert (Ellis, 1980) where disjunct, relatively important anatomical differences were found to occur within each of these species. These anatomical differences were found to be consistently correlated with ecological and morphological differences and, therefore, these 'forms' of *M. stricta* and *M. disticha* appear to warrant similar taxonomic treatment to *M. drakensbergensis* and *M. stereophylla*. The granting of specific status to *M. drakensbergensis* and *M. stereophylla,* therefore, requires reassessment.

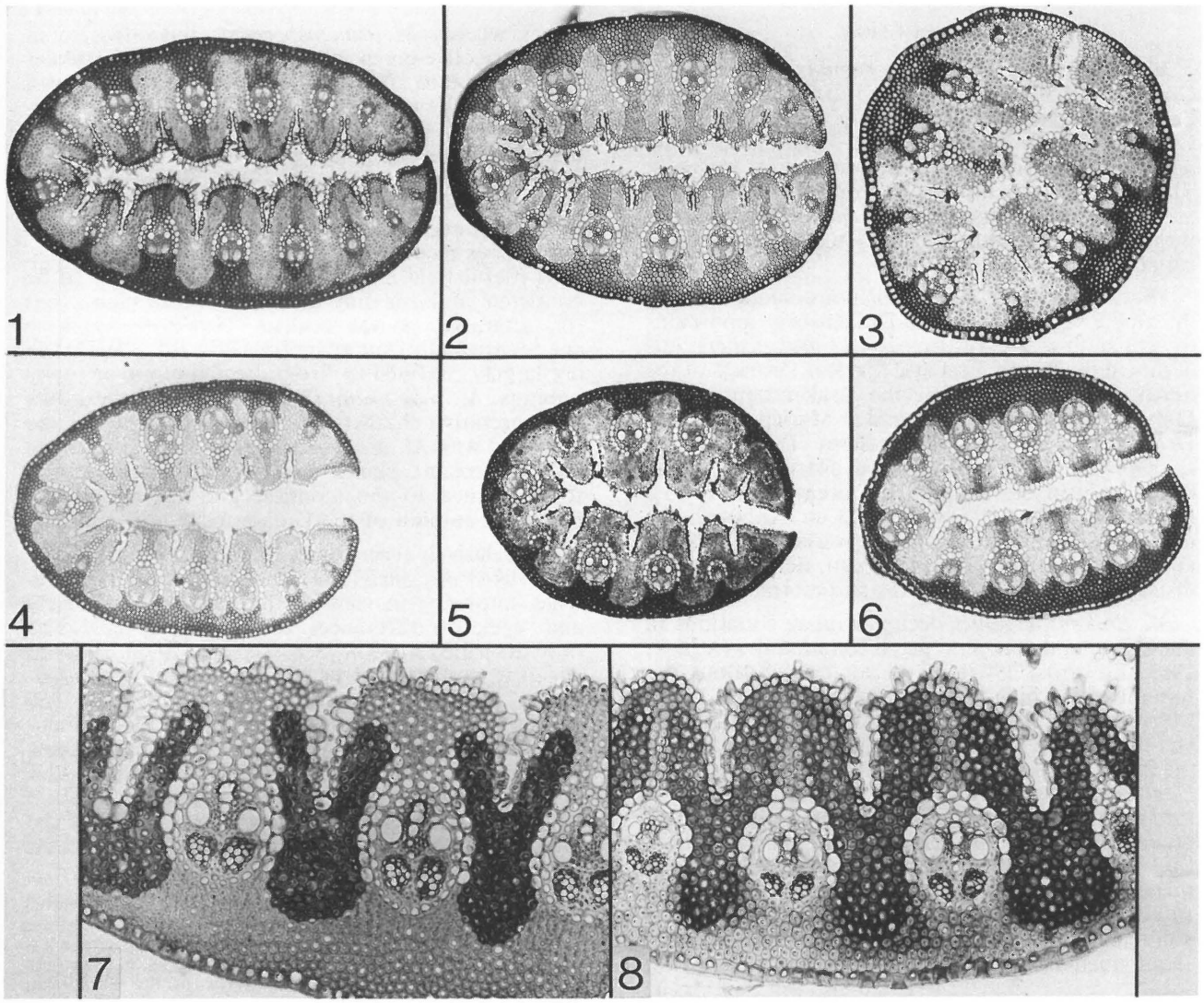
The only anatomical differences observed between these two species are slight differences in size and hence a combined description will suffice for both. Leaf anatomy and epidermal structure will be described following the terminology of Ellis (1976, 1979) and the following abbreviations will be used in the description:

vb/s	— vascular bundle/s
1'vb/s	— first order vascular bundle/s
2'vb/s	— second order vascular bundle/s
3'vb/s	— third order vascular bundle/s
ibs	— inner bundle sheath; mestome sheath
obs	— outer bundle sheath; parenchyma sheath

COMBINED ANATOMICAL DESCRIPTION OF *MERXMUELLERA DRAKENSBERGENSIS* AND *M. STEREOPHYLLA*

Leaf in transverse section

Leaf outline: permanently and tightly infolded with elliptical outline. Laminae slightly assymmetrical about the median vb such that adaxial furrows of one half of lamina align with adaxial ribs on other half of lamina. As a result margins overlap slightly. Adaxial channel always a deep, narrow cleft with a slight opening where margins overlap. *Leaf size:* setaceous; leaves narrow (0,56 mm–3,60 mm wide)



FIGS 1–8.—Leaf blade outline of *Merxmuellera drakensbergensis* and *M. stereophylla* in transverse section. 1–3, *M. drakensbergensis*, all ×160. (1, *Du Toit* 669; 2, *Liebenberg* 5707; 3, *Du Toit* 2313.) 4–6, *M. stereophylla*, all ×160. (4, *Jacot Guillarmod* 3733; 5, *Roberts* 3152; 6, *Edwards* 2284.) 7, *M. drakensbergensis*, ×400. (*Ellis* 3191.) 8, *M. stereophylla*, ×400. (*Ellis* 3139.)

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but tend to be thinner in *M. stereophylla* (0,56 mm–1,13 mm wide) than in *M. drakensbergensis*. This tendency reflected in number of vbs in leaf section. 11–15 vbs present with 11 vbs always in *M. stereophylla* (Figs 4–6) and *M. drakensbergensis* usually with 13 or 15 vbs (Figs 1 & 2) but sometimes only 11 vbs present (Fig. 3). *Ribs and furrows*: medium to deep cleft-like adaxial furrows between all vbs; rounded (Fig. 8) or slightly flat-topped (Fig. 7) ribs over all vbs; one vb per rib. Abaxial surface smooth or with very slight undulations associated with vbs (Fig. 3). *Median vascular bundle*: present but indistinguishable structurally from 1'vbs. *Vascular bundle arrangement*: no 2'vbs; 3'vbs absent between consecutive lateral 1'vbs. All bundles centrally located between upper and lower epidermides. *Vascular bundle structure*: vbs circular or elliptical in shape. Xylem and phloem distinguishable in all vbs; phloem adjoins ibs; phloem divided vertically into two equal groups by intrusion of fibres (Figs 7 & 8). Metaxylem vessel diameter narrow being only slightly greater than the diameter of the obs cells; slightly thickened. *Vascular bundle sheaths*: obs of all vbs horse-shoe shaped with wide abaxial interruptions. Adaxial interruptions usually fairly narrow (Fig. 8) but may be wide (Fig. 7) especially in *M. drakensbergensis*. No bundle sheath extensions present. Obs cells round or elliptical, sometimes with straight radial walls (Fig. 8); all obs cells similar in shape but small, being only slightly larger than the mesophyll cells in cross-sectional area; cell walls slightly but distinctly thickened; without chloroplasts. Inner sheath complete around all vbs; ibs cells similar in size to the obs cells but with considerably thicker walls, especially inner tangential wall (Fig. 8). *Sclerenchyma*: adaxial girders inversely anchor-shaped with narrow (Fig. 8) or sturdy (Fig. 7) stem; fibres interrupt obs. Abaxial sclerenchyma in form of continuous subepidermal band (especially well developed adjacent lateral 1'vbs) of varying thickness with large trapezoidal girders extending to, and interrupting, the obs; girders comprised of thick-walled fibres usually lignified although fibres near margin may be of cellulose (Fig. 1). *Leaf margin*: very small, pointed, poorly developed cap. *Mesophyll*: non-radiate; chlorenchyma of regular, small, isodiametric cells; tightly packed with air spaces not visible; in Y-shaped groups occupying sides and bases of adaxial furrows (Figs 7 & 8). No colourless cells present. *Adaxial epidermis*: poorly developed bulliform cells at bases of furrows. Macro-hairs absent. Pointed prickles with broad, but not bulbous bases; present throughout costal zones. Outer walls of epidermal cells arched and somewhat inflated and appear to be papillate. These may, however, represent sections, through varying planes, of the prickles. *Abaxial epidermis:* bulliform cells absent. Outer cell walls markedly thickened with a continuous, thick cuticle. No macro-hairs, prickles or papillae.

Abaxial epidermis in surface view

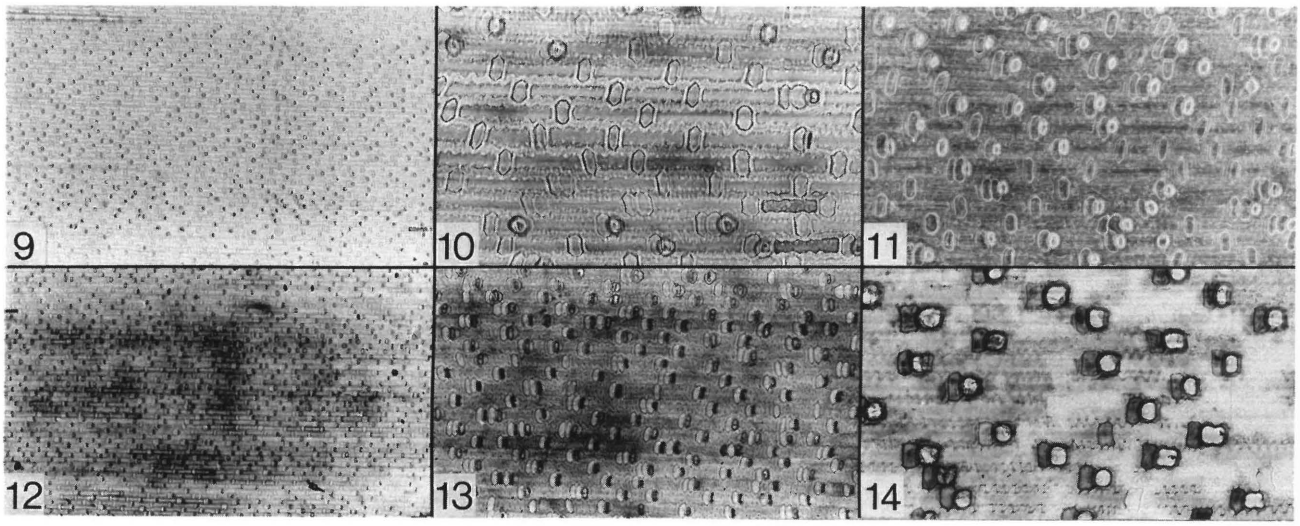
Intercostal zone: undifferentiated and entire abaxial epidermis similar in structure (Fig. 9 & 12) and essentially a costal zone due to development of continuous sub-epidermal fibrous layer. *Stomata*: absent from abaxial surface. *Prickle-hairs*: hooks and prickles not present. *Micro-hairs*: not seen on any of the specimens examined. *Macro-hairs*: absent. *Silica bodies*: equidimensional in surface view; either cuboid (Fig. 14), round (Fig. 10) or somewhat elliptical (Fig. 11); usually fitting into concavity in closely associated cork cell. Granules present; cracks sometimes present (Fig. 14). Width of silica bodies slightly narrower than costal long cells and cork cells. Similar silica bodies present throughout abaxial epidermis. *Costal cells*: silica cells and cork cells alternate with single costal long cells throughout abaxial epidermis; all files of similar cell arrangement. Silica may be relatively sparse (Fig. 10) with many silico-suberose couples actually consisting of a pair of cells (a cork and a silica cell) or even only a single cork or silica cell. Costal long cells elongated horizontally; at least 3× longer than wide; sides parallel; anticlinal walls heavily thickened and usually pitted (Fig. 10 & 13); undulations moderate to deep but difficult to distinguish clearly due to excessive cuticle thickening.

Specimens examined:

M. drakensbergensis

O.F.S.—2828 (Bethlehem): Witsieshoek-Mont-aux-Sources area (–DB), *Ellis 3137, 3138, 3154, 3155, 3156.*

NATAL.—2828 (Bethlehem): Sentinel (–DB), *Du Toit 669.* 2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 1398,*



FIGS 9–14.—Abaxial epidermis of *Merxmuellera drakensbergensis* and *M. stereophylla*. 9–11, *M. drakensbergensis*. (9, *Killick & Marais* 2183, × 160; 10, *Ellis* 1398, × 640, note number of cork cells associated with silica bodies; 11, *Staples* 242, × 160, phase contrast.) 12–14, *M. stereophylla*. (12, *Ellis* 3139, × 160; 13, *Ellis* 3139, × 400; 14, *Killick & Vahrmeijer* 4019, × 640.)

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3189, 3190, 3191, 3304. 2929 (Underberg): Sani Pass (–CB), *Du Toit 2313.*

LESOTHO.—2828 (Bethlehem): Maluti Mts (–CC), *Staples 242.* 2929 (Underberg): Pone Valley (–AC), *Coetzee 824;* Mokhotlong, *Liebenberg 5707;* Sani Pass summit (–CA), *Du Toit 2209.*

CAPE.—3028 (Matatiele): Naudes Nek (–CA), *Werdermann & Oberdieck 1139.*

M. stereophylla

O.F.S.—2828 (Bethlehem): Golden Gate National Park (–DA), *Roberts 3152;* Witsieshoek-Mont-aux-Sources area (–DB), *Ellis 3139.*

NATAL.—2828 (Bethlehem): Mont-aux-Sources (–DD), *Schelpe 1390.* 2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 1397, 1408, 3180, 3186, 3298, 3307, Killick 1317.* 2929 (Underberg): Giants Castle Game Reserve (–AB), *Killick & Vahrmeijer 4019, Edwards 2284.*

LESOTHO.—2828 (Bethlehem): Letseng-la-Terai (–CA), *Loxton & Ellis 997.* 2829 (Harrismith): Cleft Peak area (–CC), *Killick & Marais 2183.* 2929 (Underberg): Sani Pass summit (–CA), *Du Toit 2208;* Tschlanyane Valley (–CD), *Jacot Guillarmod 3733.*

DISCUSSION AND CONCLUSIONS

From the above anatomical description of the leaf blades of *M. drakensbergensis* and *M. stereophylla,* and from the accompanying photomicrographs (Figs 1–14), it is clearly evident that the structure of these two species is remarkably similar—both the leaf sections and the epidermis. The only difference detected in this study is a small variation in the cross-sectional area and width of the leaf sections. The magnifications of Figs. 1–6 are identical and a comparison of *M. drakensbergensis* leaf blades (Figs 1–3) with those of *M. stereophylla* (Figs 4–6) reveals that the leaves of *M. drakensbergensis* have a tendency to be larger. This difference is not consistent as numerous intermediates occur. Nevertheless this size difference is correlated with the number of vascular bundles present in the leaf blade. *M. stereophylla* has 11 vascular bundles, whereas *M. drakensbergensis* has 13 or 15. However, even this distinction is not consistent e.g. Fig. 4 which is intermediate with 12 vascular bundles and Fig. 3, *M. drakensbergensis,* with only 10 vascular bundles.

These slight quantitative differences are, nevertheless, characteristic and consistent for the majority of the specimens examined in this study. However, in this representative and extensive sample there were several specimens which proved difficult to identify satisfactorily using Anderson's (1962) key. For example, both *Ellis* 1397 and *Loxton & Ellis* 997 were initially determined as being *M. stricta* but, on checking, were identified as *M. drakensbergensis* and finally placed in *M. stereophylla*, mainly because they do not exhibit the outward recurving of the old leaf blades. In these specimens the arrangement of the hairs on the lemmas is apparently atypical. *Edwards* 2284 is another specimen which has caused problems with identification. Anderson and Conert both determined this specimen as being *M. aureocephala* (J. G. Anders.) Conert, but in this study it has been classified as being *M. stereophylla.*

In these morphologically atypical specimens the number of vascular bundels in the leaf section, and the corresponding width of the leaf blade, appear to be of no assistance in identification. Thus, *Ellis* 1397, collected in saturated, spongy ground is probably ecologically best placed in *M. drakensbergensis,* although it would be a very small, atypical specimen. It has only 11 vascular bundles in the leaf section, which is characteristic of *M. stereophylla.* The ecological, morphological and anatomical indications are, therefore, in conflict.

Ellis 1397 and 1408 are specimens collected near or at the summit of the Drakensberg escarpment at Cathedral Peak. The *Merxmuellera* populations at this particular locality were characterized by being extremely variable morphologically. Anatomical studies of the *M. stricta* and *M. disticha* (Ellis, 1980, 1980a) specimens from this locality revealed that the various specimens actually belonged to different 'forms' with distinct anatomical, morphological and ecological characteristics and that it was mere coincidence that they were found growing in such close proximity. The *M. drakensbergensis* and *M. stereophylla* specimens from these populations, on the other hand, show no correlation between morphological, anatomical and ecological characteristics and *Ellis* 1397 and 1408 in fact represent true intermediates.

This observation, together with the close resemblance of the leaf anatomy of these two species, indicates that *M. stereophylla* and *M. drakensbergensis* are very closely related, more so, in fact, than the anatomical forms of *M. stricta* and *M. disticha.* The specific status of *M. stereophylla* and *M. drakensbergensis* is, therefore, questioned and indications are that these two taxa warrant similar taxonomic treatment to the anatomical forms of *M. stricta* and *M. disticha.*

Both *M. drakensbergensis* and *M. stereophylla* exhibit the same type of arrangement of vascular bundles along the width of the lamina, there being no third order vascular bundles between the lateral first order bundles. This is essentially similar to the position described in *M. stricta* (Ellis, 1980a) and indicates the relationship of *M. stricta* to *M. stereophylla* and *M. drakensbergensis.* In addition, the anatomical evidence indicates that *M. stereophylla* and *M. drakensbergensis* exhibit a similar degree of divergence from the typical *M. stricta* anatomical form as do the other three anatomical forms of *M. stricta* (Ellis, 1980a). This implies that consideration should be given to the granting of equivalent taxonomic status to *M. drakensbergensis, M. stereophylla,* typical *M. stricta* and to the three other forms of *M. stricta.* The evidence gained in the present study indicates that subspecific rank is probably justified for each of the above entities of the *M. stricta* group.

M. drakensbergensis and *M. stereophylla* are thought to be related to three other species of *Merxmuellera,* all of which also occur in the Drakensberg mountains. These are *M. macowanii* (Stapf) Conert, *M. davyi* (C. E. Hubb.) Conert and *M. aureocephala* (J. G. Anders.) Conert and together these five species form a more or less distinct and closely related group within the genus (Anderson, 1962). This is not confirmed by anatomy, however, which indicates a relationship between *M. disticha* and *M. macowanii* and *M. davyi.* This is based on the alternating arrangement of lateral first order bundles with third order vascular bundles common to the latter three species (and probably to *M. aureocephala*) (Ellis, in press). A similar relationship exists between *M. disticha* and *M. macowanii* and *M. davyii* to that demonstrated between *M. stricta* and *M. drakensbergensis* and *M. stereophylla.* Indications are, once again, that similar patterns of adaptive radiation have occurred in the Drakensberg area from parental stock of both *M. stricta* and *M. disticha.* Thus, in both groups, cave sandstone and

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basalt, alpine bog, and streambank 'forms' appear to have evolved in response to the environmental conditions presently prevailing in the Drakensberg mountains. This unique situation will undoubtedly reward further population and cytogenetical studies.

ACKNOWLEDGEMENTS

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UITTREKSEL

Die anatomiese struktuur, van die blaar in dwarssnee en die abaksiale epidermis, van Merxmuellera drakensbergensis (*Schweick.*) *Conert en* M. stereophylla (*J. G. Anders.*) *Conert word beskryf en geïllustreer. Hierdie twee naverwante spesies is feitlik onuitkenbaar op anatomiese kenmerke alhoewel hulle 'n sekere mate van ekologiese en morfologiese skeiding toon. Die sterk anatomiese ooreenkoms tussen hierdie twee spesies bevraagteken hulle spesifieke status veral wanneer vergelykings getref word met die aansienlike anatomiese verskille wat in* M. stricta (*Schrad.*) *Conert* (*Ellis, 1980a*) *en* M. disticha (*Nees*) *Conert* (*Ellis, 1980*) *waargeneem is.*

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Bothalia 13, 3 & 4: 493–500 (1981)

Leaf anatomy of the South African Danthonieae (Poaceae). V. *Merxmuellera macowanii, M. davyi* and *M. aureocephala*

R. P. ELLIS*

ABSTRACT

Transverse sections and abaxial epidermal scrapes, of herbarium and freshly fixed leaf blade material, of *Merxmuellera macowanii* (Stapf) Conert, *M. davyi* (C. E. Hubb.) Conert and *M. aureocephala* (J. G. Anders.) Conert, were examined using light microscopy. The leaf anatomy of these three species is very similar in all respects with the exception of certain *M. aureocephala* specimens. In addition, the anatomy indicates a relationship between these three species and *M. disticha* (Nees) Conert. This group of species differs anatomically from *M. stricta* (Schrad.) Nees, and related species such as *M. drakensbergensis* (Schweick.) Conert and *M. stereophylla* (J. G. Anders.) Conert, in the sequence of vascular bundles along the width of the leaf blade and associated characters. However, the *M. aureocephala* specimens, not having the *M. disticha* type of vascular bundle arrangement, anatomically resemble the *M. stricta* group of species, and *M. aureocephala* appears to be intermediate between these two species groups.

RÉSUMÉ

L'ANATOMIE DE LA FEUILLE DU DANTHONIEAE (POACEAE) SUD AFRICAIN. V. MERXMUELLERA MACOWANII, M. DAVYI *ET* M. AUREOCEPHALA

Des sections transversales et des grattages epidermaux abaxiaux, d'herbarium et de matériel de feuille fraichement fixée, de Merxmuellera macowanii *(Stapf) Conert,* M. davyi *(C. E. Hubb) Conert et* M. aureocephala *(J. G. Anders.) Conert, ont été examinées en utilisant la microscopie lumineuse. L'anatomie de la feuille de ces trois espèces est très similaire dans tous les domaines à l'exception de certains specimens de* M. aureocephala. *De plus, l'anatomie indique une relation entre ces trois espèces et* M. disticha *(Nees) Conert. Ce groupe d'espèces diffère anatomiquement de* M. stricta *(Schrad.) Nees, et des espèces apparentées telles que* M. drakensbergensis *(Schweick.) Conert et* M. stereophylla *(J. G. Anders.) Conert, dans la succession des faisceaux vasculaires le long de la largeur de la feuille et des caractères associés. Cependant, les specimens de* M. aureocephala, *n'ayant pas le type d'arrangement de faisceaux vasculaires de* M. disticha *ressemblent anatomiquement au groupe d'espèces* M. disticha *et* M. aureocephala *apparait etre intermédiaire entre ces deux groupes d'espèces.*

INTRODUCTION

Merxmuellera macowanii (Stapf) Conert (= *Danthonia macowanii* Stapf), *M. davyi* (C. E. Hubb.) Conert (= *D. davyi* C. E. Hubb.), and *M. aureocephala* (J. G. Anders.) Conert (= *D. aureocephala* J. G. Anders.) (Conert, 1970) are all wiry, tufted, tussock-forming, perennial grasses. *M. macowanii,* in particular, forms large, lax tussocks up to 60 cm in diameter with leaves up to 100 cm long arching outwards from the tuft base. *M. macowanii* and *M. davyi* are summer-flowering, whereas *M. aureocephala* is a winter-flowing species.

These three species occur in mountain vegetation along the eastern escarpment of southern Africa. *M. davyi* is found at altitudes above 2 000 m on Mt Mlanje in Malawi, the Inyanga mountains of Zimbabwe and Mariepskop in the eastern Transvaal Drakensberg (Conert, 1975). *M. macowanii* occurs from the Transvaal Drakensberg southwards as far as the Witteberge, Stormberge and Amatole Mountains of the eastern Cape. It occurs between 1 500 and 3 000 m and is also found in the midlands of Natal. *M. aureocephala* appears to be localized and restricted to the high Drakensberg of Natal in the Cathedral and Cathkin Peak areas.

M. macowanii is frequently dominant along streambanks and in marshy areas of the montane and subalpine belts of the Drakensberg (Killick, 1963; Edwards, 1967) but is, nevertheless, a xeromorphic grass with sclerophyllous leaves. *M. davyi* and *M. aureocephala,* on the other hand, prefer more xeric habitats and occur on steep grassy slopes and in rocky situations in mountain grassveld (Anderson, 1962).

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These habitat preferences bear striking resemblances to the niches occupied by *M. drakensbergensis* (Schweick.) Conert and *M. stereophylla* (J. G. Anders.) Conert (Ellis, 1981). Furthermore, *M. macowanii* and *M. drakensbergensis,* both of which occupy mesic streambank and seepage habitats, display vegetative similarities in that the old leaf blades break off a short distance above the ligule, split along the mid-vein and then recurve outwards (Chippindall, 1955; Anderson, 1960). The above five species are considered to form a more or less closely related group within the genus (Anderson, 1962) and, therefore these ecological and morphological parallels are not unexpected.

The present study examined these relationships anatomically and indications are that two groups actually exist within these five species. *M. drakensbergensis* and *M. stereophylla,* therefore, display more anatomical similarities with each other than with either *M. macowanii* or *M. davyi.* It is significant that this anatomical sub-division separates species occupying similar niches and exhibiting similar old leaf blade behaviour. *M. aureocephala* specimens appear to be somewhat intermediate anatomically and possibly form a link between these two groups.

M. macowanii, M. davyi and *M. aureocephala* resemble one another anatomically, and, therefore, a combined description of their leaf blade anatomy will suffice. The terminology of Ellis (1976, 1979) will be used in the description with the following abbreviations:

vb/s	— vascular bundle/s
1'vb/s	— first order vascular bundle/s
2'vb/s	— second order vascular bundle/s
3'vb/s	— third order vascular bundle/s
ibs	— inner bundle sheath; mestome sheath
obs	— outer bundle sheath; parenchyma sheath

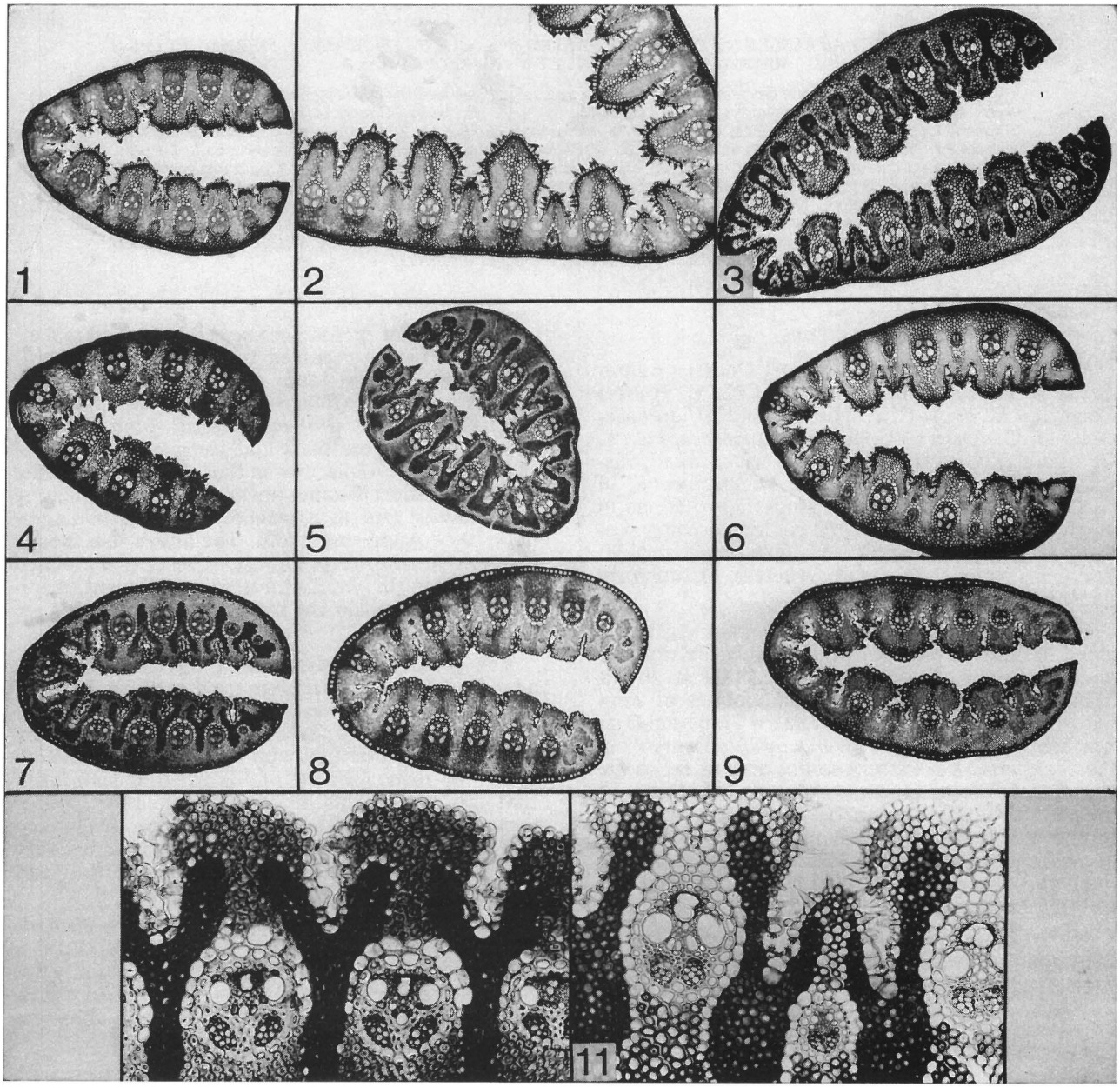
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COMBINED ANATOMICAL DESCRIPTION OF *MERXMUELLERA MACOWANII, M. DAVYI* AND *M. AUREOCEPHALA*

Leaf in transverse section

Leaf outline: infolded with reduced U- or V-shaped outline; opening to at least 45° possible (Fig. 2) except in certain *M. aureocephala* specimens which are permanently infolded with elliptical outlines (Figs 7 & 8). Adaxial channel deep and either cleft-like or variable depending on degree of infolding prevailing. Lamina always assymmetrical about the median vb; 1'vbs of opposite halves of lamina alternate and an extra 3'vb is usually present in one half e.g. four 3'vbs in the lower half and three in the upper half of Fig. 1. This assymmetry occurs in all specimens except typical *M. davyi* specimens (Figs 4 & 5). *Leaf size*: the total number of vbs in the leaf section varies from 13–17 in *M. aureocephala,* 15–17 in *M. davyi* and 17–27 in *M. macowanii*. Leaf thickness varies between 0,35–0,45 mm in *M. aureocephala* and *M. davyi* but up to 0,55 mm in *M. macowanii*. *Ribs* and *furrows*: massive adaxial ribs with rounded to triangular apices associated with 1'vbs and small triangular ribs with 3'vbs. Medium depth adaxial furrows between all vbs; cleft-like depending on degree of infolding of leaf; Y-shaped as massive ribs almost meet laterally and then furrow diverges on either side of rib over 3'vb (Fig. 11); found in all specimens except certain *M. aureocephala* specimens where lateral 1'vbs are not interspaced by 3'vbs (Figs 7, 8 & 10). Abaxial surface smooth. *Median vascular bundle*: present, characteristically smaller than lateral 1'vbs. *Vascular bundle arrangement*: no 2'vbs present; 1'vbs and 3'vbs alter-



FIGS 1–11.—Anatomy of the leaf blade in transverse section of *Merxmuellera macowanii, M. davyi* and *M. aureocephala*. 1–3, 6, *M. macowanii,* all × 100, considerable variation in leaf width evident. (1, *Story* 476; 2, *Ellis* 2394; 3, *Ellis* 3282; 6, *Codd & De Winter* 3239.) 4–5, *M. davyi,* ×100. (4, *Davidson & Mogg* 33315; 5, *Van der Schijff* 5832.) 7–9, *M. aureocephala,* all × 100, note sequence of vascular bundles. (7, *Killick* 3540; 8, *Ellis* 3179; 9, *Edwards* 843.) 10, *M. aureocephala,* × 400. (*Ellis* 3179.) 11, *M. macowanii,* × 400, phloem divided into two groups by intrusive fibres. (*Ellis* 3282.)

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nate along width of lamina except near margin where two or more consecutive 1'vbs may be present; at least two 3'vbs occur between the median vb and these successive 1'vbs near the margin (Table 1) except in certain specimens of *M. aureocephala* (Figs 7 & 8) where only a single 3'vb may be present on either side of the median vb followed by four or five 1'vbs. All vbs located in centre of blade. *Vascular bundle structure*: vbs elliptical (Fig. 11) or round (Fig. 10) in outline; xylem and phloem distinguishable in 3'vbs; phloem of 1'vbs divided into two similar groups by intrusion of fibres (Figs 10 & 11). Protoxylem vessel and lysigenous cavity present; metaxylem vessels circular, of slightly greater diameter than obs cells. *Vascular bundle sheaths*: obs elliptical or horseshoe-shaped with wide adaxial interruptions; interruption especially pronounced in some *M. aureocephala* specimens (Fig. 10) such that obs only present opposite xylem; these specimens without adaxial interruptions or extensions (Fig. 10). In all other specimens adaxial extensions present; of colourless cells gradually decreasing in size as walls increase in thickness until they merge into sclerenchyma strand (Fig. 11). Obs cells slightly larger in diameter than mesophyll cells; all similar in shape; rounded; without chloroplasts. Ibs entire with uniformly thickened walls (Fig. 11) or with inner tangential walls thickened (Fig. 10) in certain *M. aureocephala* specimens. *Sclerenchyma:* adaxial girders inversely anchor-shaped with long, wide stem on all bundles. Abaxial sclerenchyma continuous sub-epidermal band of varying thickness, with large trapezoidal girders extending to, and interrupting, the obs. Fibres either heavily lignified (Fig. 10) or resemble collenchyma in section (Fig. 11). Marginal sclerenchyma cap small and pointed. *Mesophyll:* arrangement non-radiate; cells uniform, small, isodiametric and tightly packed. Restricted to Y-shaped groups on sides and bases of furrows. Arms of Y uneven due to difference in size of adaxial ribs associated with 1'vbs and 3'vbs (Fig. 11) except in certain *M. aureocephala* specimens (Fig. 10). *Colourless cells*: absent. *Adaxial epidermis:* restricted groups of 3–4 bulliform cells present at base of furrows; better developed in *M. macowanii* (Fig. 11) than *M. aureocephala* (Fig. 10) and *M. davyi*. In *M. davyi* prickles with straight, broad barbs and without bulbous bases common and well-developed (Figs 4 & 5); present in *M. macowanii* to a slightly lesser degree (Figs 1–3) but absent in certain *M. aureocephala* specimens where adaxial epidermis consists of papillate cells. *Abaxial epidermis*: no bulliform cells; outer periclinal wall thickened and covered by continuous, thickened cuticle. No macrohairs, prickles or papillae occur.

Abaxial epidermis

Intercostal zone: undifferentiated; entire abaxial epidermis essentially costal in structure (Figs 14 & 16) due to hypodermal sclerenchyma development. *Stomata*: absent. *Prickle hairs*: not present. *Microhairs*: not developed on abaxial surface. *Macrohairs*: absent. *Silica bodies*: elliptical (Fig. 13) to tall and narrow (Fig. 15); outlines smooth. Closely associated with cork cell or pair of short cells. Width of silica bodies narrower than adjacent costal short and long cells (Fig. 13). Silica bodies sparsely developed or even absent (Fig. 17). *Costal cells*: silica cells and cork cells, either singly or in pairs, alternate with costal long cells throughout abaxial epidermis. Long cells elongated; at least 3× longer than wide; sides parallel; anticlinal walls heavily thickened and slightly undulating (Fig. 13) to strongly corrugated (Fig. 17).

Specimens examined:

M. macowanii

TRANSVAAL.—2530 (Lydenburg): Dullstroom (–AC), *Codd & De Winter 3239; De Winter & Codd 183.* 2730 (Vryheid): Wakkerstroom (–AD), *Devenish 1152.*

O.F.S.—2828 (Bethlehem): Golden Gate Highlands National Park (–DA), *Ellis 2394.*

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 1455, 3282; Killick 1090.* 2929 (Underberg): Estcourt (–BB), *Acocks 10659.* 2930 (Pietermaritzburg): Pietermaritzburg (–AC), *Edwards 2673;* Greytown (–BA), *Ellis 3372.*

CAPE.—3027 (Lady Grey): Barkly East (–DC), *Joubert s.n.* (Matatiele): Naudé's Nek (–CA), *Story 476.* 3126 (Queenstown): Buffelsfontein (–BC), *Stretton 182.*

M. davyi

TRANSVAAL.—2430 (Pilgrim's Rest): Mariepskop (–DB), *Van der Schijff 5832; Wedermann & Oberdieck 1908;* God's Window (–DD), *Davidson & Mogg 33315.*

M. aureocephala

NATAL.—2829 (Harrismith): Cathedral Peak Forest Reserve (–CC), *Ellis 3179, Killick 3450, 1727;* Mweni Pass, *Edwards 843.* 2929 (Underberg): Cathkin Peak area (–AB), *Edwards 2453.*

DISCUSSION AND CONCLUSIONS

The leaf anatomy of *M. macowanii* and *M. davyi* is remarkably similar—both the leaf in transverse section (Figs 1–6) and the abaxial epidermis (Figs 12–15). From the limited number of *M. davyi* specimens available for examination in this study (none of which was collected and fixed in the field), the only difference detected was a tendency for *M. davyi* leaves to be narrower with fewer vascular bundles per section. This is not a distinct difference, however, and several specimens overlap in this characteristic (Table 1). *M. macowanii* and *M. davyi* are considered to be closely allied (Anderson, 1962) and their leaf anatomy supports this close relationship. However, this anatomical evidence casts some doubt on the specific status accorded these two taxa and a closer comparison of these two species is necessary.

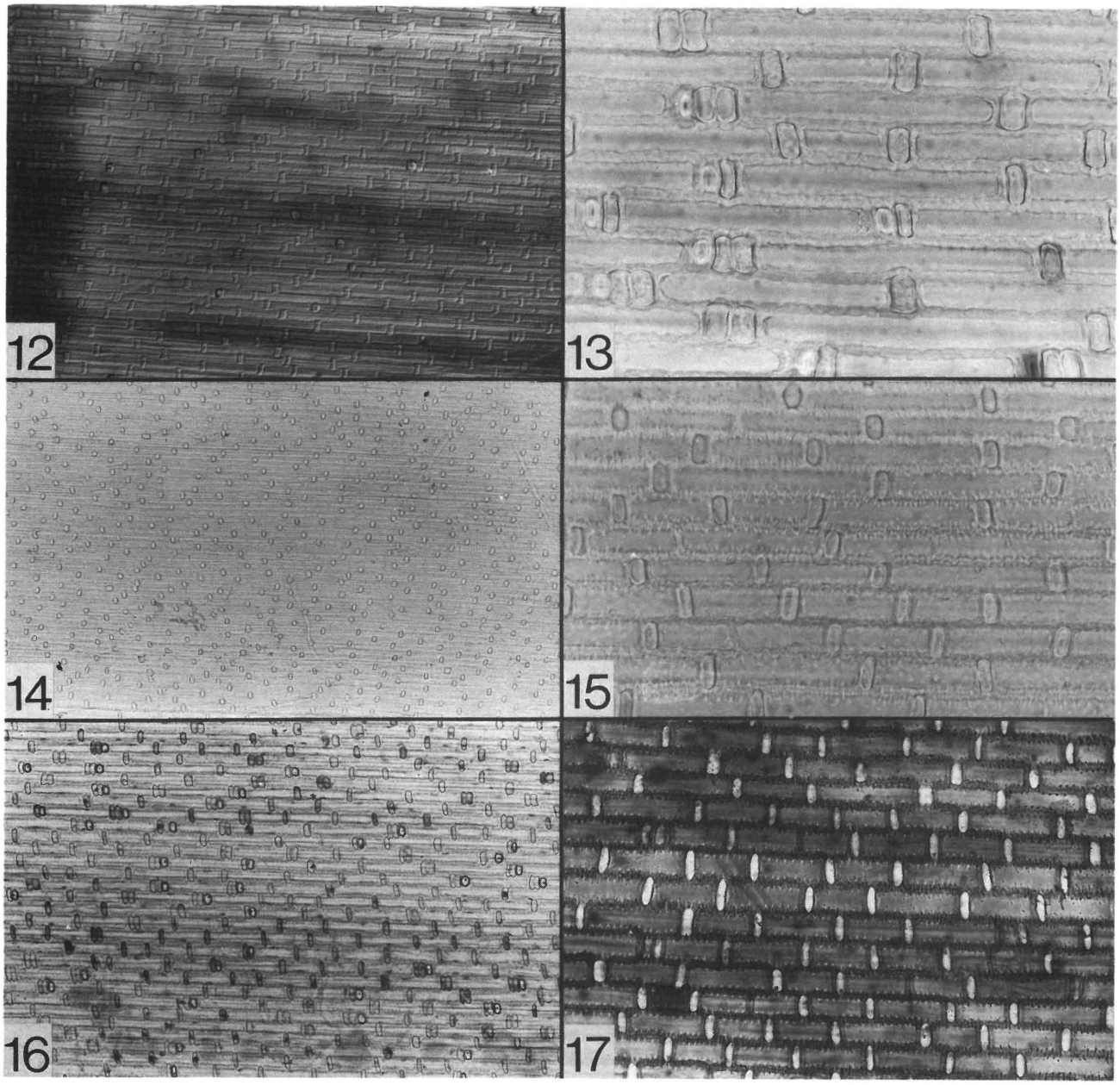
In contrast to the leaf anatomy, *M. macowanii* and *M. davyi* seem well separated ecologically and occupy different habitats—mesic streambank and seepage areas (Killick, 1963; Edwards, 1967) as opposed to drier rocky situations (Anderson, 1962). In addition, these two species are almost entirely separated geographically with only a small area of possible sympatry in the eastern Transvaal at Mariepskop and God's Window (Fig. 18). *M. davyi* extends northwards into central Africa along the eastern mountains, whereas *M. macowanii* occurs southwards as far as the north-eastern Cape.

Spikelet differences also appear to adequately differentiate these two species and the degree of fusion and the awned nature of the lemma lobes appear to be distinctive. In this respect *M. macowanii* and *M. davyi* apparently differ considerably and *M. davyi* actually bears a stronger resemblance to *M. aureocephala* than to *M. macowanii* which has characteristic adnate, awnless lemma lobes (Anderson, 1962).

Theoretically these ecological and morphological differences appear to be diagnostic yet in practice their application seems to have been inconsistent. Thus the specimens collected at God's Window and Mariepskop (*Davidson & Mogg* 33315 and *Van der Schijff* 5832) were initially identified as *M. maco-*

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FIGS 12–17.—The abaxial epidermis of *Merxmuellera macowanii, M. davyi* and *M. aureocephala* as seen in surface view. 12–13, *M. macowanii*. (12, *De Winter & Codd* 183, × 250, large number of single costal short cells without silica bodies; 13, *Stretton* 182, × 1 000, irregular short cell arrangement.) 14–15, *M. davyi*. (14, *Davison & Mogg* 33315, × 250, only single short cells occur; 15, *Wedermann & Oberdieck* 1908, × 1000.) 16–17, *M. aureocephala*. (16, *Killick* 3450, × 250; 17, *Ellis* 3179, × 640.)

wanii and have only recently been assigned to *M. davyi* (Conert, 1975)—notwithstanding the fact that a key was published in 1962 specifically to facilitate the identification of *M. davyi, M. macowanii* and other closely related species (Anderson, 1962). In addition, the specimen *Codd & De Winter* 3239 has similarly proved difficult to identify satisfactorily. In 1947 it was named *M. macowanii,* changed to *M. davyi* in 1975 but again placed in *M. macowanii* during the present study. Anatomical indications are that this specimen is better placed in *M. macowanii* (Fig. 6).

Therefore, although anatomical evidence appears to be in conflict with morphological and ecological indications, closer analysis shows that *M. macowanii* and *M. davyi* are, in fact, not consistently separable and are probably very closely related. In the light of the above evidence, a reassessment of their specific status, therefore, appears justified.

An additional consideration, which must be borne in mind when assessing the taxonomic status to be accorded these two taxa, is the almost identical situation observed in *M. drakensbergensis* and *M. stereophylla* (Ellis, 1981). These two species are also inseparable on anatomical grounds and a gradation in leaf size and vascular bundle number was also noted. Several other parallels exist between these two pairs of species. *M. drakensbergensis* and *M. macowanii* both occupy mesic, damp habitats and both display characteristic behaviour of the old leaf blades. *M. stereophylla* and *M. davyi* occur in drier, rocky situations and tend to have narrower leaves.

M. macowanii and *M. davyi* have purposely been considered separately from *M. drakensbergensis* and

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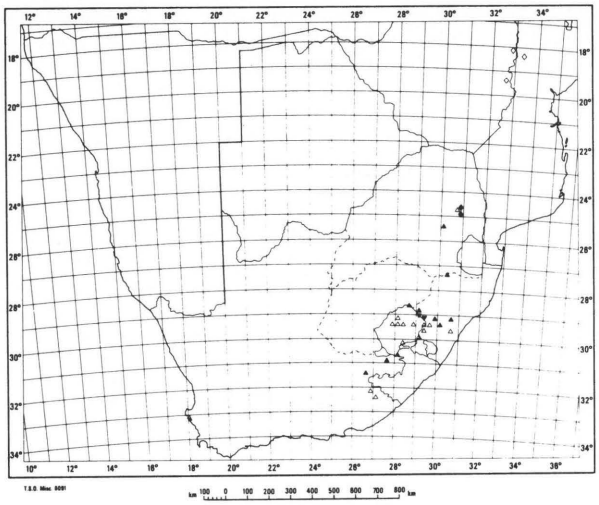


FIG. 18.—Distribution of *Merxmuellera macowanii* (△), *M. davyi* (◆) and *M. aureocephala* (▼) in southern Africa. Shaded symbols represent localities of specimens studied anatomically. Compiled from specimens at the National Herbarium, Pretoria (PRE).

M. stereophylla, even although they share so many common characteristics. This is because a distinct anatomical attribute consistently separates these two species pairs—the arrangement of the different orders of vascular bundles along the width of the lamina. In *M. macowanii* and *M. davyi* at least two alternating pairs of first and third order bundles, commencing with the median bundle, are present before consecutive lateral first order bundles are encountered (Table 1). In *M. drakensbergensis* and *M. stereophylla,* on the other hand, only a single third order bundle is present between the median bundle and successive lateral first order bundles. The alternating sequence of first and third order bundles is correlated with several other anatomical characters such as form of the adaxial furrows and shape of the mesophyll cell groups.

It is possible that position along the length of the leaf blade may affect this arrangement of the first and third order vascular bundles. However, it has been shown that, towards the apex of the lamina, the lateral veins disappear one by one, commencing with the marginal pair, until the median bundle remains to form the pungent tip (Burbidge, 1946). It is unlikely, therefore, that the bundle sequence, as noted here, will be affected by the position of the sections examined as all material was taken from the central third of the leaf blade between ligule and apex (Ellis, 1976) and, in addition, the relevant bundle sequence is not marginal but adjacent to the median bundle or midrib (Table 1).

Up to this point *M. aureocephala* has been excluded from this discussion because, in respect of this anatomical difference, it does not conform with either of the vascular bundle sequences described above but exhibits mixed and intermediate conditions. Fig. 7 illustrates the condition typical of *M. drakensbergensis* and *M. stereophylla* where a single third order bundle is followed by successive first order bundles. Fig. 9, on the other hand, shows the alternating pattern of first and third order bundles characteristic of *M. macowanii* and *M. davyi. M. aureocephala,* therefore, exhibits both types of bundle arrangement that consistently separate *M. macowanii* and *M. davyi* from *M. drakensbergensis* and *M. stereophylla.* In addition, similar differences are found in different leaves of the same plant and even in single leaves. Thus leaf samples were taken from duplicate specimens of *Killick* 3450 and one showed the *M. drakensbergensis/M. stereophylla* type of bundle sequence (Fig. 7) whereas the other showed the *M. macowanii/M. davyi* type (Table 1). An exceptional case is illustrated in Fig. 8 where each of these two types of vascular bundle arrangement occur within a single leaf—one type in each half of the lamina.

The presence of all these intermediates in *M. aureocephala* casts some doubt on the importance attached to this difference in bundle arrangement in the present study. *M. aureocephala,* therefore, appears to hold the key to the understanding of relationships within this group of closely related species. Unfortunately, however, the taxonomic status of *M. aureoce-*

TABLE 1.—The arrangement of first (1′) and third (3′) order vascular bundles along the leaf blade from the median vascular bundle (midrib) to the margin

Specimens examined	Midrib	Vascular bundle sequence											
Merxmuellera davyi													
Wedermann & Oberdieck 1908	1′	3′	1′	3′	1′	3′	3′						
Van der Schijff 5832	1′	3′	1′	3′	1′	1′	3′						
Davidson & Mogg 33315	1′	3′	1′	3′	1′	3′	1′	1′	3′				
Merxmuellera macowanii													
Killick 1090	1′	3′	1′	3′	1′	1′	3′						
Killick 1090 (another leaf)	1′	3′	1′	3′	1′	3′	1′	3′	3′				
Devenish 1152	1′	3′	1′	3′	1′	3′	1′	3′	3′				
Stretton 182	1′	3′	1′	3′	1′	3′	1′	1′	3′				
Ellis 1455	1′	3′	1′	3′	1′	(3′)	1′	1′	3′				
Story 476	1′	3′	1′	3′	1′	(3′)	1′	1′	(1′)	3′			
Acocks 10659	1′	3′	1′	3′	1′	3′	1′	1′	3′	(3′)			
Codd & De Winter 3239	1′	3′	1′	3′	1′	3′	1′	(3′)	1′	(1′)	3′		
De Winter & Codd 183	1′	3′	1′	3′	1′	3′	1′	(3′)	1′	(1′)	3′		
Joubert s.n.	1′	3′	1′	3′	1′	3′	1′	(3′)	1′	1′	3′		
Edwards 2673	1′	3′	1′	3′	1′	3′	1′	(3′)	1′	1′	3′	3′	
Ellis 2394	1′	3′	1′	3′	1′	3′	1′	3′	1′	1′	1′	3′	
Ellis 3282	1′	3′	1′	3′	1′	3′	1′	3′	1′	3′	1′	3′	(3′)
Merxmuellera aureocephala													
Edwards 843	1′	3′	1′	3′	1′	3′	1′	1′	3′	(3′)			
Killick 3450	1′	3′	1′	3′	1′	1′	1′	1′	3′				
Ellis 3179	1′	3′	1′	(3′)	1′	1′	1′	1′	3′				
Edwards 2453	1′	3′	1′	(3′)	1′	1′	3′	(3′)					
Killick 3450 (another leaf)	1′	3′	1′	1′	1′	1′	3′						

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phala itself appears somewhat tenuous. To date this species is only known from six collections, all from a restricted area, of less than 20 km in diameter, in the subalpine belt of the Cathedral and Cathkin Peak areas of the Drakensberg (Fig. 18). Morphologically it is very similar to *M. davyi* except that the spikelets are larger in all parts, and the glumes are lanceolate instead of narrowly lanceolate (Anderson, 1962). Its winter-flowering habit, in fact, is the single diagnostic character separating *M. aureocephala* from its four close relatives. However, the specimens assigned to *M. aureocephala* may actually represent examples of late or early flowering in the other *Merxmuellera* species, e.g. *Edwards* 2284 has been determined by Conert 1973 as being *M. aureocephala,* but it is now considered as being *M. stereophylla*. Winter visits to these inhospitable mountains, to study fertile field populations, seem essential to a better understanding of the taxonomic status of all the summer-rainfall area *Merxmuellera* species.

Until these field studies have been undertaken, the true significance of the different vascular bundle arrangement sequences cannot be assessed. However, assuming that these patterns are a phylogenetically important difference, the available evidence indicates that *M. aureocephala* occupies a basic systematic position in this group from which each of the two distinct types have been derived. This implies a close relationship for these five species as postulated by Anderson (1962) as well as an origin in the Drakensberg mountains and not in the temperate, winter-rainfall areas of the Cape.

If the distribution of these two types of bundle arrangement is examined in all the summer-rainfall *Merxmuellera* species, however, a different origin seems likely. *M. disticha* (Nees) Conert, including each of its anatomical forms, exhibits the alternating sequence of first and third order vascular bundles (Ellis, 1980). *M. disticha,* therefore, shares this character with *M. macowanii, M. davyi* and some *M. aureocephala* specimens (Table 2). All the four *M. stricta* (Schrad.) Conert anatomical forms (including *M. guillarmodiae* Conert), on the other hand, have similar bundle arrangement to *M. drakensbergensis* and *M. stereophylla* as well as other *M. aureocephala* specimens (Ellis, 1980a) (Table 2). Thus, within this group of 12 summer-rainfall *Merxmuellera* taxa (Table 3), *M. aureocephala* remains the only taxon intermediate for this anatomical character.

SPECIES	VASCULAR BUNDLE ARRANGEMENT
M.disticha	1° 3° 1° 3° 1° 3°(1° 3°)(1° 3°)
M.davyi	1° 3° 1° 3°(1° 3°) 1° 1° 3°
M.macowanii	1° 3° 1° 3° 1° 3°(1° 3°)(1° 3°)(1° 3°) 1° 1° 3°
M.aureocephala	1° 3° 1° 3° 1° 3° 1° 1° 3°
	1° 3° 1° 3° 1° 1° 1° 3°
	1° 3° 1° 1° 1° 1° (1°) 3°
M.drakensbergensis	1° 3° 1° 1° (1°)(1°) 3°
M.stereophylla	1° 3° 1° 1° (1°) 3°
M.guillarmodiae	1° 3° 1° 1° 3°
M.stricta	1° 3° 1° 1° (1°)(1°) 3°

TABLE 2.—The arrangement of first (1′) and third (3′) order vascular bundles along the leaf blade from median vascular bundle to margin in the summer-rainfall *Merxmuellera* species

Table 3 diagramatically illustrates each of these twelve *Merxmuellera* taxa arranged according to vascular bundle sequence and grouped into the various habitats occupied by these various taxa. It is immediately evident from Table 3, that in each of the niches occupied by *Merxmuellera* spp. a taxon displaying each of the bundle sequence types occurs. A representative of each type of bundle arrangement occurs in the cave sandstone, basaltic soils, alpine bogs, alpine xeric sites and mesic sites. In addition, morphological and anatomical similarities often exist between these pairs of taxa inhabiting similar niches e.g. the old leaf blade behaviour in *M. macowanii* and *M. drakensbergensis* and the mesophyll and epidermal structure in the alpine bog forms of *M. stricta* and *M. disticha*. Once again, *M. aureocephala* is the exception.

Typical *M. stricta* and *M. disticha* forms are both widespread in the Cape and extend to lower altitudes in the Drakensberg. Throughout this wide distributional range both species are very uniform in both morphology and anatomy. It is only at higher altitudes, above the cave sandstone, that anatomical and morphological diversification is prevalent. An alternative hypothesis is, therefore, that *M. stricta* and *M. disticha* have independently colonized the wide variety of microhabitats present at higher altitudes in the Drakensberg by evolving locally adapted ecotypes for each of the various niches. This adaptive radiation, in response to identical environmental conditions, has resulted in very similar phenotypic expressions by the ecotypic forms of each species. As *M. stricta* and *M. disticha* occur sympatrically throughout most of their ranges it must be assumed that they originally possessed distinct but diverse genotypes which somehow were capable of responding in similar ways to the different environmental conditions encountered in the Drakensberg. This appears to explain the unique morphological and anatomical convergence observed in all the habitats occupied. The bundle sequence may, therefore, reflect a basic genetic different between *M. stricta* and *M. disticha* ancestral forms that has been retained in all the ecotypic forms.

This hypothesis does not explain the position of *M. aureocephala*. If the origin of all these taxa is to be sought in putative ancestors of *M. stricta* and *M. disticha* then the only explanation for *M. aureocephala* lies in a hybrid origin. Once again population and cytogenetical studies seem necessary to elucidate this question.

From these antomical studies on the summer-rainfall *Merxmuellera* species (Ellis, 1980; 1980a; 1981) it is nevertheless clear that, at this stage, at least 12 entities can be recognized. A further two, presently placed in *Pentaschistis*, also merit consideration (Ellis, 1980a). All these taxa are undoubtedly interrelated and the most practical systematic treatment, at this stage, appears to be the upholding of only two basic species (*M. stricta* and *M. disticha*) with numerous infraspecific taxa, possibly of subspecific rank, included in each. *M. guillarmodiae, M. macowanii, M. davyi, M. drakensbergensis* and *M. stereophylla* should be reduced to subspecific rank, whereas, the anatomical forms of *M. stricta* and *M. disticha* justify taxonomic recognition with subspecific status as well. *M. aureocephala* is the one entity on which the present studies have shed very little light and a taxonomic recommendation at this stage would be unwise.

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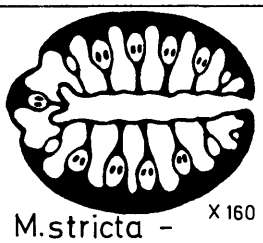
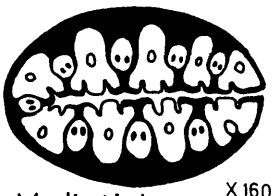
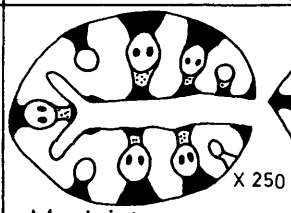
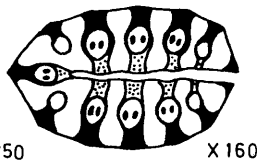
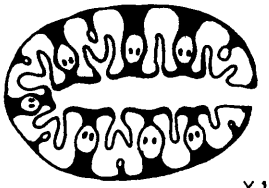
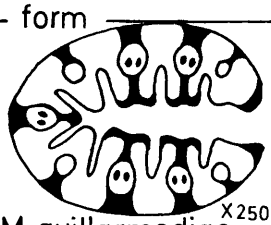
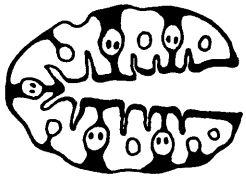
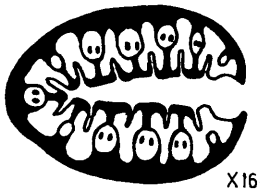
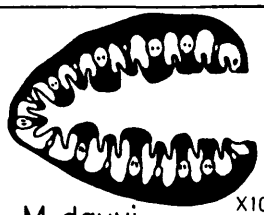
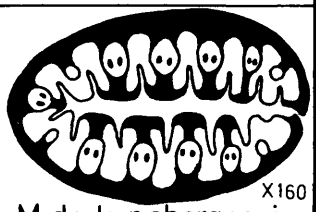
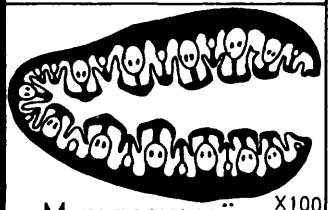
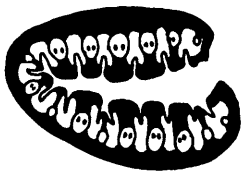
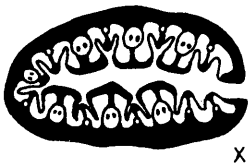
HABITAT	VASCULAR BUNDLE SEQUENCE	
	1°3°1°1° etc.	1°3°1°3° etc.
WIDESPREAD S.W.-N.E. Cape; O.F.S. Sandstone	 M. stricta - typical form X 160	 M. disticha - typical form X 160
DRAKENSBERG Subalpine belt Basalt	 M. stricta - drakensberg form X 250  M. guillarmodiae - Cathedral Peak form X 160	 M. disticha - drakensberg form X 160
DRAKENSBERG Alpine belt; summit Bogs + sponges	 M. guillarmodiae - alpine bog form X 250	 M. disticha - alpine bog form X 250
DRAKENSBERG Alpine; Tvl. northwards Xeric, rocky sites	 M. stereophylla X 160	 M. davyi X 100
DRAKENSBERG Alpine; E. Cape - Tvl. Mesic; streambanks	 M. drakensbergensis X 160	 M. macowanii X 100
DRAKENSBERG Subalpine belt Winter flowering	 M. aureocephala	 X 100

TABLE 3.—Diagrammatic representations of the leaf anatomy of the summer-rainfall *Merxmuellera* taxa according to habitat and vascular bundle arrangement

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UITTREKSEL

Dwarssnitte en abaksiale epidermale skrapings, van herbarium, asook vars gefikseerde blaarmateriaal, van Merxmuellera macowanii (*Stapf*) *Conert,* M. davyi (*C. E. Hubb.*) *Conert en* M. aureocephala (*J. G. Anders.*) *Conert is met behulp van 'n ligmikroskoop ondersoek. Die blaaranatomie van hierdie drie spesies is in alle opsigte dieselfde, met die uitsondering van sekere* M. aureocephala *eksemplare. Verder toon die anatomie 'n verwantskap tussen hierdie drie spesies en* M. disticha (*Nees*) *Conert. Hierdie spesiesgroep verskil anatomies van* M. stricta (*Schrad.*) *Conert, en verwante soorte soos* M. drakensbergensis (*Schweick.*) *Conert en* M. stereophylla (*J. G. Anders.*) *Conert, in die volgorde van die vaatbondels langs die blaarwydte en geassosiëerde kenmerke. Die* M. aureocephala *eksemplare wat nie die* M. disticha *tipe vaatbondelvolgorde toon nie, lyk anatomies na die* M. stricta *groep van spesies, en* M. aureocephala *is dus intermediêr tussen hierdie twee spesiegroepe.*

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5. LEAF ANATOMY AND FUNCTION IN THE POACEAE

Synopsis: The postulation that a correlation exists between certain anatomical characters of the leaf and the various photosynthetic pathways described for the Poaceae is confirmed and refined by examination of the anatomical reference material prepared during this study. It is demonstrated that these anatomical differences are not only functionally significant but that they are of taxonomic importance as they are not randomly distributed in the representative sub-families, tribes and genera that are surveyed. In addition the ecological implications of these structural and functional differences are also evaluated by examination of the distribution patterns of the grasses of southern Africa in conjunction with their particular photosynthetic pathways as inferred from leaf anatomy.

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5. LEAF ANATOMY AND FUNCTION IN THE POACEAE

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DISTRIBUTION OF THE KRANZ SYNDROME IN THE SOUTHERN AFRICAN ERAGROSTOIDEAE AND PANICOIDEAE ACCORDING TO BUNDLE SHEATH ANATOMY AND CYTOLOGY

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Abstract

Forty two genera and 133 species of the Eragrostoideae and 66 genera and 213 species of the Panicoideae collected in Southern Africa are classified into the four sub-divisions of the Kranz syndrome recognised in the Gramineae: C_3 and the NADP-ME, NAD-ME and PCK sub-types of the C_4 dicarboxylic acid pathway. This classification is based solely on anatomical criteria of the bundle sheath and mesophyll of the leaf blade as viewed in transverse sections with the light microscope. The resultant grouping of species and genera correlate very well with available information on their ultrastructure and physiology. The results support the contention that anatomy alone can confidently be used in predicting the specific photosynthetic CO_2 assimilation pathway operating in a given species. In addition, these pathways appear to be constant and specific for most species and genera and are therefore of considerable taxonomic importance.

Opsomming

DIE VERSPREIDING VAN DIE KRANZSINDROOM IN DIE ERAGROSTOIDEAE EN PANICOIDEAE VAN SUIDELIKE AFRIKA VOLGENS BONDELSKEDE-ANATOMIE EN SITOLOGIE

Twee en veertig genera en 133 *spesies van die Eragrostoideae en* 66 *genera en* 213 *spesies van die Panicoideae, wat in Suidelike Afrika versamel is, is in die vier onderafdelings van die Kranzsindroom wat in die Gramineae erken word ingedeel, te wete: die C_3-tipe en die NADP-ME, NAD-ME en PCK subtipes van die C_4-tipe. Hierdie indeling is uitsluitlik gebaseer op anatomiese kenmerke van die bondelskede en mesofil van die blaarlamina soos in dwarsdeursnee met die ligmikroskoop waargeneem kon word. Die gevolglike groepering van spesies en genusse korreleer baie goed met beskikbare inligting oor ultra-struktuur en fisiologie. Die resultate onderskraag die vermoede dat die anatomie alleen, met vertroue gebruik kan word om die spesifieke fotosintetiese CO_2-roete wat deur 'n bepaalde spesies gebruik word, te voorspel. Bo en behalwe dit, is hierdie fotosintetiese roete skynbaar konstant en spesifiek vir die meeste spesies en genera en is hulle gevolglik van groot taksonomiese waarde.*

Résumé

DISTRIBUTION DU SYNDROME DE KRANZ CHEZ LES ERAGROSTOIDEA ET PANICOIDEA D'AFRIQUE AUSTRALE, ÉTABLIE D'APRES L'ANATOMIE ET LA CYTOLOGIE DE L'ENVELOPPE DU FAISCEAU

42 genres et 133 *espèces d'Eragrostoidea,* 66 *genres et* 213 *espèces de Panicoidea récoltés en Afrique australe sont classées selon les quatre sous-divisions du syndrome de Kranz qui ont été reconnues dans les graminées: C_3 et les sous-types NADP-ME, NAD-ME et PCK de la voie en C_4 de l'acide dicarboxylique. Cette classification est basée uniquement sur les caractéres anatomiques de l'enveloppe du faisceau et du mésophylle du limbe foliaire examinés en coupe transversale au microscope optique. Le groupement en espèces et en genres qui en est résulté montre une excellente corrélation avec ce que l'on sait de leur ultra-structure et de leur physiologie. Ces résultats renforcent la théorie selon laquelle l'anatomie seule peut fournir une prédiction fiable de la voie spécifique d'assimilation photosynthétique du CO_2 qui se rencontre dans une espéce donnée. De plus, ces voies semblent être constantes et spécifiques pour la plupart des espèces et des genres et ont par conséquent une grande importance taxonomique.*

Introduction

Three different pathways of photosynthetic CO_2 assimilation have now been recognised among higher land plants. Corresponding to these pathways are the categories C_3, C_4 and crassulacean acid metabolism. Only the first two pathways occur in the Gramineae with the C_4 category being further sub-divisible into three variations depending on the reaction sequence for C_4 acid decarboxylation in the bundle sheath cells of leaf blades (Gutierrez, Gracen & Edwards, 1974; Gutierrez, Kanai, Huber, Ku & Edwards 1974a; Hatch, Kagawa & Craig, 1975). Each of these four categories is characterized by a distinctive syndrome of properties including differences in physiology, leaf anatomy and ultrastructure and ecological relationships.

The purpose of this paper is to further establish the correlations that have been demonstrated between the diagnostic anatomical features and various other characteristics of species assigned to each of these categories. Evidence will be presented to demonstrate that it is possible to assign any given segment of grass leaf blade to one of these four groups employing conventional methods of botanical microtechnique and light microscopy alone. Either herbarium material or preferably freshly fixed specimens can be used irrespective of collecting locality, habitat differences or position and age of the leaf chosen.

The coincidence between the anatomical classification and published information on the physiology and ultrastructure for each species will be compared to confirm the relevance of the anatomical criteria employed and to demonstrate the validity of predictions and extrapolations made from anatomical criteria alone. Thus, the detection of any one of a broad spectrum of distinguishing characters for each of the four categories should prove sufficient to infer the whole syndrome of related characters.

The Kranz syndrome is a remarkable example of structure related to function with the functional aspects of photosynthetic carbon fixation being correlated with the anatomy of the leaf. If these structural aspects of the syndrome correlate absolutely with the physiological and biochemical attributes it can reasonably be assumed that they are structurally essential to the particular photosynthetic fixation pathway with which they are associated.

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Thus, a detailed comparative anatomical survey should confirm and strengthen the predictive value of anatomy, aid in redefining aspects of Kranz anatomy and possibly expose new physiologically related characters. In view of the biological and probable economic significance of the C_4 pathway a broad, detailed anatomical survey is undoubtedly justified and of relevance to the various disciplines of plant science and agriculture.

Finally, the groupings obtained will be compared with the current classification of the Gramineae employed by S. African agrostologists to see whether accurate predictions of anatomy and physiology can be made from systematics alone. Whether the anatomical groupings are consistent within species and at higher hierarchical levels should prove indicative of either identificatory errors or the possible need for taxonomic adjustment. In addition a large-scale survey of this nature should pinpoint species where detailed study by experimentalists should prove rewarding.

Procedure

Segments taken from the middle third of grass leaf blades were fixed in the field in F.A.A. This material was desilicified, embedded in wax, sectioned on a rotary microtome and stained with Safranin and Fast Green. The leaf-blade transverse sections were then examined under a light microscope and assigned to one of the four categories according to a predefined set of criteria.

It is felt that a brief discussion of the physiology in addition to a full anatomical definition of each of the four groups will serve to give a better appreciation of the Kranz syndrome as a whole. A concise summary of the interrelated characteristics can in fact be found as the heading for Tables 1 and 2, but, as the characteristics are drawn from a wide field of disciplines, it is felt that a fuller background is necessary to gain a deeper appreciation of the Kranz syndrome by all the specialists who may be directly or indirectly affected by the full implications of this phenomenon—functional, evolutionary, applied and taxonomic.

The Kranz syndrome, as manifested in the Gramineae, can be classified as follows:

1. Non-Kranz or C_3 species
2. Kranz or C_4 species:
   - 2.1. Species with a Kranz mestome sheath
   - 2.2. Species with a Kranz parenchyma sheath:
     - 2.2.1. Species with centrifugal chloroplasts in the parenchyma sheath cells
     - 2.2.2. Species with centripetal chloroplasts in parenchyma sheath cells.

1. *Non-Kranz characteristics*

Species grouped in this category are variously known as C_3, non-Kranz or festucoid species and are abbreviated as n-K in this study.

Anatomically this category is characterized by a distinct, thick-walled mestome (or endodermal) sheath around all, or only the first order vascular bundles. Typically the cells of this inner sheath are markedly thickened on their radial and inner tangential walls and are chlorophyll-lacking. A parenchyma sheath is situated externally around the mestome sheath. This outer sheath is either without chloroplasts or the chloroplasts are few and of the same size as, or smaller than, the mesophyll chloroplasts (Fig. 46). In addition they are never more numerous than in mesophyll cells. The mesophyll is irregularly arranged with little differentiation between the bundles and an extensive leaf air space system is present (Byott, 1976).

These characteristics apply to all C_3 grasses but a further distinction can be made. The typical festucoid (Brown, 1958, 1961), or pooid (Carolin, Jacobs & Vesk, 1973) type is further distinguished by the indistinct nature of the parenchyma sheath cells, the walls of which are hardly thicker than the mesophyll cell walls. In addition the air spaces between the mesophyll cells are relatively large and numerous. Contrasting with this are the large, conspicuous parenchyma sheath cells with obviously thickened walls which are characteristic of the arundinoid and bambusoid types (Brown, 1961) as well as the C_3 species of the Paniceae (Fig. 46, 51) which, however, are free of chloroplasts. This type of sheath is often associated with closely packed, somewhat radially arranged chlorenchyma cells.

C_3 species fix CO_2 in photosynthesis by ribulose-1,5-diphosphate carboxylase into the 3-carbon molecule 3-phosphoglycerate by means of the Calvin pathway (Gutierrez, Edwards & Brown, 1976). This cycle occurs in the chlorenchyma cells and the bundle sheath cells (if they contain chloroplasts) and there is thus no division of functions and compartmentalization of reactions as is characteristic of C_4 species. In C_3 species starch is accumulated in the mesophyll cells during the day (Rhoades & Carvalho, 1944) whereas, in C_4 species it is only formed in the Kranz sheath cells and not the mesophyll cells.

2. *Kranz characteristics*

These are C_4, Kranz or panicoid species. The symbol K is used for this category in this study.

A single or a double bundle sheath may be present but this group is characterized by the presence of a Kranz or chlorenchymatous sheath of enlarged cells with walls thicker than the messophyll cell walls and containing specialized chloroplasts distinct from those of the mesophyll (Fig. 40, 41). These chloroplasts are in some way different from mesophyll chloroplasts, such as larger size, higher number per Kranz cell, and specific positions within the cell (Brown, 1975).

The chlorenchyma cells of the mesophyll are arranged more or less radially around each vascular bundle and each cell is in contact with or is separated from a Kranz sheath cell by not more than one other chlorenchymatous mesophyll cell (Hattersley & Watson, 1975). The mesophyll is markedly differentiated between successive bundles into radial mesophyll cells and colourless bulliform cells (Fig. 3) with the leaf air space system being restricted to certain parts of the leaf and individual air spaces are shorter as measured from the substomatal cavities (Byott, 1976).

The thickened Kranz sheath cell walls appear to be essential and are present in all C_4 grasses. The differentiation of biochemical activity between the mesophyll and the sheath cells appears to be the main functional difference from C_3 species where this division of labour is not found. The protoplasts of the sheath and mesophyll cells in C_4 species are, however, functionally closely associated with each other by means of numerous pits and plasmodesmata.

C_4 species fix CO_2 in the mesophyll cells by phosphoenolpyruvate carboxylase into the 4-carbon molecules oxaloacetate, malate and/ or aspartate (Gutier-

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rez *et al.*, 1974a: Gutierrez, Edwards & Brown, 1976). It may be that the initial C_4-dicarboxylic acid in all cases is oxaloacetate which is subsequently converted to either malate or aspartate (Huber, Kanai & Edwards, 1973). These C_4 acids are transferred to the Kranz sheath cells where decarboxylation occurs (Brown & Gracen, 1972; Gutierrez *et al.*, 1974a).

Depending on the relative quantities of aspartate and malate formed two sub-groups of the C_4 type are recognized. Furthermore three variations of C_4 photosynthesis are currently recognized depending on the reaction sequence for C_4 acid decarboxylation in the bundle sheath cells. Two are sub-divisions of the aspartate group and the third is equivalent to the malate formers. It must be emphasized that these distinctions cannot be used in an absolute sense as all C_4 plants appear to decarboxylate both C_4-acids, malate and aspartate (Downton, 1971), and to utilize the three decarboxylating enzymes (Gutierrez *et al.*, 1974, 1974a) to different degrees.

2.1. *Characteristics of species with a Kranz mestome sheath*

Species placed in this category are known variously as malate formers, or NADP-ME type species with eu-panicoid or andropogonoid anatomy. In this study they are referred to as MS or NADP-ME species.

The anatomy of this category is very distinctive, not being characterized by the presence of an inner, fibrous mestome sheath and an outer Kranz parenchyma sheath. Instead a single chlorenchymatous or Kranz sheath is present. Brown (1975) has suggested that this sheath is homologous with the mestome sheath and this condition applies when no cells are present between the metaxylem vessel elements and the laterally adjacent Kranz sheath cells of lateral primary or first order vascular bundles (Fig. 41), i.e. bundles with both metaxylem and protoxylem (Hattersley & Watson, 1976). Thus, in this single-sheathed group it is assumed that the Kranz sheaths have evolved from mestome sheaths.

Generally this single-sheathed condition is easily recognised but in a few species this condition is somewhat variable with cells sometimes being present between the metaxylem vessels and the Kranz cells (Fig. 61, 62). However, in these instances these cells may only be additional sclerenchymatous cells situated where mestome sheath cells are normally found, but it is unlikely that they will fulfil the ultimate criteria of mestome sheath cells—resistance to H_2SO_4 and the presence of Casparian strips (Brown, 1975). Fortunately the presence of these cells in the few species that possess them is very irregular. Thus, sometimes such cells may be located between one metaxylem vessel and the Kranz cells but not the other of a single bundle. In other cases these cells may only be associated with some of the first order bundles. Therefore, the MS condition can still be recognized in cross section and applies when metaxylem vessels are in direct contact with Kranz sheath cells in at least some first order vascular bundles. It is therefore necessary to study a few primary lateral vascular bundles before assigning a given specimen to either the malate or the aspartate category.

The Kranz sheath cells of this type are on the average smaller in transverse section than are the equivalent cells in aspartate forming types, i.e. species with double bundle sheaths. In addition, the Kranz sheath cells of the primary lateral bundles, but not necessarily others, are generally equal to or smaller than the adjacent mesophyll cells in transectional area (Hattersley & Watson 1975, 1976). Furthermore, the cells of the primary bundles are often smaller than those of the smaller bundles where the Kranz cells are often kidney-shaped as seen in transverse section (Fig. 44, 59). The mesophyll cells are normally indistinctly radially arranged and the individual cells are often square to isodiametric and not elongated as in the aspartate formers.

The chloroplasts of the Kranz cells are in the centrifugal position, i.e. towards the outer tangential wall (Fig. 40), and lack well-developed grana (Downton, 1970; Gutierrez *et al.*, 1974, 1974a). In some cases the chloroplasts may be located around the entire periphery of the sheath cells. The colour and texture of these chloroplasts when stained is variable but they usually are different shades of grey or brown. The individual chloroplasts in each individual sheath cell often vary in the intensity of pigmentation with the darkest chloroplasts being nearest the cell wall. The mitochondrial frequency is low with little internal membrane development (Hatch *et al.*, 1975).

Plants placed in this category are known as 'malate formers' because they form more of the C_4-dicarboxylic acid malate than aspartate, in the mesophyll cells as the first product of CO_2 fixation (Downton, 1970; Brown & Gracen, 1972) or subsequent to the formation of oxaloacetate which is converted to malate by malate dehydrogenase (Huber *et al.*, 1973). This malate is then transported to the bundle sheath cells where the dominant decarboxylating enzyme is NADP-malic enzyme which accounts for 70% or more of the total decarboxylating activity (Gutierrez *et al.*, 1976). This decarboxylation of malate gives rise to CO_2, $NADPH_2$ and pyruvate and it has been postulated (Downton, 1970) that this generation of $NADPH_2$ may eliminate the need for a photoreduction system in the Kranz cells and thus account for the absence or reduction of grana in the chloroplasts of these cells without serious consequence to Calvin cycle operation.

These species do not exhibit a post-illumination CO_2 burst (PIB) and the uptake of CO_2 is not appreciably affected by O_2 (Brown & Gracen, 1972). The correlation attempted by the above authors between the location of the chloroplasts and the presence or absence of the PIB does not always hold because no account was taken of the presence or absence of the parenchyma sheath.

2.2. *Characteristics of species with a Kranz parenchyma sheath*

These are the aspartate formers, with chloridoid or eragrostoid anatomy, under which the two subtypes, NAD-ME and PCK are grouped.

The anatomy of this category is typified by a well-developed inner or mestome sheath being present around the first order vascular bundles. Adjacent to this sheath externally is situated the specialized Kranz sheath which is, therefore, a modified parenchyma sheath (Brown, 1975). This condition can be recognized when cells are present between metaxylem vessel elements and the laterally adjacent Kranz sheath cells (Fig. 30, 35) in lateral first order vascular bundles (Hattersley & Watson, 1976). Note that the Kranz sheath must be present and this arrangement must be applicable to all lateral primary vascular bundles.

The Kranz cells of lateral first order bundles are generally larger in transectional area than the cells

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of the adjacent mesophyll (Hattersley & Watson, 1976). In addition these cells are wider radially than they are long as viewed in paradermal sections (Brown, 1974). In contrast the sheath cells of the malate formers are longer parallel to the vein than they are wide radially. This characteristic appears to be correlated with whether a species is an aspartate NAD or a malate or aspartate PCK former rather than with the sub-families Eragrostoideae and Panicoideae as suggested by Brown (1974).

The Kranz cell chloroplasts of the sub-types of this group vary in structure and position but all have well-developed grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a). It appears that, as these chloroplasts become filled with starch their volume increases and they tend to entirely fill the cells, thus obscuring the chloroplast positioning (Gutierrez *et al.*, 1974). This has an important bearing on light microscopy studies as the granal nature of these chloroplasts cannot be observed.

The Kranz sheath is surrounded by a single layer of radially arranged chlorenchyma cells (Fig. 3, 34) each of which is directly in contact with a Kranz sheath cell (Brown 1958, 1961).

The aspartate formers have higher frequencies of mitochondria in the Kranz sheath cells relative to the chloroplasts than do the malate formers. The aspartate former's mitochondria also have more extensive development of internal membranes (Hatch *et al.*, 1975).

The major initial photosynthetic product in this group is aspartate or aspartic acid. This amounts to 50% to 87% of the initial C_4-dicarboxylic acids (Downton, 1970). This aspartate is probably derived from the initially formed oxaloacetate by aspartate transaminase (Huber *et al.*, 1973). The decarboxylation of aspartate (or oxaloacetate) in the Kranz sheath cells does not generate reducing power (cf. $NADPH_2$ formed in malate decarboxylation) and these species therefore have well-developed granal sheath chloroplasts (Downton, 1970).

Aspartate formers exhibit a post-illumination CO_2 burst (PIB) which is oxygen-insensitive (Brown & Gracen, 1972).

2.2.1. *Characteristics of species with double bundle sheaths and having centrifugal chloroplasts.*

These are known as PCK-type or PEP-ck species This type is distinguished by the presence of a double bundle sheath with the specialized chloroplasts being centrifugally located, i.e. towards the outer cell wall, in the outer Kranz sheath cells (Gutierrez *et al.*, 1974, 1974a). These chloroplasts contain grana.

As seen under the light microscope these centrifugal chloroplasts appear as overlapping bodies, irregular in size and shape although they are often disc-like. In the stained condition they vary in colour from different shades of grey to black, with the darkest shade generally on the outer periphery (Fig. 1). These dark, dense chloroplasts do not appear to be granular and are best viewed in the sheath cells of third order bundles.

This typical condition may vary somewhat to the condition where the chloroplasts are arranged all around the periphery of the sheath cells or to the situation where the chloroplasts entirely fill the lumens of the sheath cells (Fig 7). This appears to be as a result of excessive starch accumulation (Gutierrez *et al.*, 1974, 1974a). In this condition the chloroplasts tend to be lightly and evenly pigmented and to have a fine, granular appearance with no striations (Fig. 7). This type of chloroplast is often consistently associated with a single, small, angular prismatic crystal centrally located in the chloroplast (Fig. 53).

The Kranz bundle sheath cells of species assigned to this category are rounded or elliptical with no straight radial walls. This condition is especially noticeable on the outer tangential surface of the cells resulting in the Kranz sheath as a whole having an irregular appearance (Fig. 53) in contrast to the compact, regular nature of NAD-ME type sheaths (Fig. 52). This condition is often most obvious around third order vascular bundles where the cell closest to the adaxial surface of the leaf and/or a lateral cell on either side of the bundle are often larger and more elongate than the remainder (Fig. 31). Kranz cell bundle sheath extensions are also relatively common in this category.

A single layer of radially arranged mesophyll cells surrounds each bundle, the individual cells of which are not as uniform in size and shape as are the chlorenchyma cells of NAD-ME type species (cf. Fig. 31 and 34).

PCK species have slightly fewer mitochondria per cell than do the NAD-ME species (Hatch *et al.*, 1975).

This category is characterized biochemically by the dominant decarboxylating enzyme being phosphoenolpyruvate carboxykinase, or PEP carboxykinase, which is responsible for 70% or more of the total decarboxylation activity (Gutierrez *et al.*, 1976). In these species the aspartate transported to the sheath cells is converted to oxaloacetate by aspartate transaminase and the oxaloacetate is converted to phosphoenopyruvate and CO_2 by PEP carboxykinase (Edwards, Kanai & Black, 1971).

2.2.2. *Characteristics of species with double bundle sheaths and having centripetal chloroplasts*

In this category are the NAD-ME type species. Chloroplasts in the Kranz sheath cells are centripetally positioned against the inner tangential cell wall and they contain grana (Gutierrez *et al.*, 1974, 1974a). These species also have a double sheath. It is these species which have strikingly short and wide parenchyma sheath cells cf. Brown, 1974.

The bundle sheath chloroplasts of this sub-type are generally very dense and stain darkly, often being dyed red with the safranin used in this study (Fig. 4). These chloroplasts are usually rather coarsely granular in nature especially when compared with PCK species (Fig. 8). Radial striations are often discernible particularly on the inner, centripetal face (Fig. 3, 6). This type of chloroplast very often almost entirely fills the Kranz cells. In these instances nuclei, if present are usually located adjacent to the outer tangential wall (Fig. 8).

The individual Kranz cells of this type can be distinguished from PCK type cells because they have straight radial walls and the outer tangential wall is less inflated. Each individual cell is thus more or less fan-shaped (Fig. 3). These cells are all similar in size and the sheath as a whole is thus conspicuous, compact and regular, either forming a complete ring or being horse-shoe shaped when sclerenchyma girders on the abaxial side of the leaf interrupt the parenchyma sheath (Fig. 5). The Kranz sheath is, thus, clearly distinguishable from the mesophyll tissue which consists of a single layer of radially

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TABLE 1 The Southern African genera of the Eragrostoideae grouped into the categories of the Kranz syndrome based on the anatomy of the leaf blade in transverse section. The number of species examined in each genus is given

TABEL 1 *Die Suid-Afrikaanse genera van die Eragrostoideae in klasse gegroepeer op grond van die anatomie van die blaar in dwarsdeursnit. Die aantal spesies wat in elke genus ondersoek is, word aangedui*

	n-K or/*of* C_3 NON-KRANZ	K or/*of* C_4 KRANZ		
	C_3 SPECIES No chlorenchymatous bundle sheath Kranz sheath absent No specialized chloroplasts High CO_2 compensation point—37–50 ppm. Low $^{13}C/^{12}C$ ratios i.e. δ = —23 to —32‰ *C_3 SPESIES* *Chlorenchiematiese bondelskede afwesig* *Kranzskede afwesig* *Geen gespesialiseerde chloroplaste* *Hoë CO_2-kompensasie punt—37–50 dpm.* *Lae $^{13}C/^{12}C$ verhouding d.i. δ = —23 tot —32‰*	C_4 SPECIES Chlorenchymatous bundle sheath present Kranz sheath present Specialized chloroplasts in bundle sheath cells Low CO_2 compensation concentration—less than 5 ppm High $^{13}C/^{12}C$ ratios i.e. δ = —9 to —16‰ *C_4 SPESIES* *Chlorenchiematiese bondelskede teenwoordig* *Kranzskede teenwoordig* *Gespesialiseerde chloroplaste teenwoordig in bondelskedeselle* *Lae CO_2-kompensasiepunt—minder as 5 dpm* *Hoë $^{13}C/^{12}C$ verhouding d.i. δ = —9 tot —16‰*		
		MS KRANZ MESTOME SHEATH Single bundle sheath (XYMS—) MALATE FORMERS No post illumination CO_2 burst—oxygen-insensitive Eupanicoid (Panicoid) anatomy *KRANZ-MESTOOMSKEDE* *Enkel bondelskede (XYMS—)* *APPELSUUR PRODUSEERDERS* *Geen na-beligting CO_2 vrystelling—suurstof onsensitief* *Eupanicoid (Panicoid) anatomie*	PS KRANZ PARENCHYMA SHEATH Double bundle sheath (XYMS+) around larger bundles ASPARTATE FORMERS Post illumination CO_2 burst (PIB)— oxygen-insensitive Chloridoid (Eragrostoid) anatomy *KRANZ-PARENCHIEMSKEDE* *Dubbel bondelskede (XYMS+) om groter bondels* *ASPARTIENSUUR PRODUSEERDERS* *Na-beligting CO_2 vrystelling (PIB)—suurstof onsensitief* *Chloridoid (Eragrostoid) anatomie*	
Double bundle sheath (XYMS+) Post illumination CO_2 burst—oxygen sensitive Festucoid (Pooid) anatomy *Dubbel bondelskede (XYMS+)* *Na beligting CO_2 vrystelling—suurstof sensitief* *Festucoid (Pooid) anatomie*		NADP CENTRIFUGAL CHLOROPLASTS NADP-ME type species Grana deficient or absent Mitochondria infrequent *SENTRIFUGALE CHLOROPLASTE* *NADP-ME tipe spesies* *Grana skaars of afwesig* *Mitochondria seldsaam*	PCK CENTRIFUGAL CHLOROPLASTS PCK type species PEP-ck species Grana developed Mitochondria frequent Mitochondria centrifugal *SENTRIFUGALE CHLOROPLASTE* *PCK tipe spesies* *PEP-ck spesies* *Grana ontwikkel* *Mitochondria volop* *Mitochondria centrifugaal*	NAD CENTRIPETAL CHLOROPLASTS NAD-ME SPECIES Grana developed Mitochondria frequent Mitochondria centripetal *SENTRIPETALE CHLOROPLASTE* *NAD-ME spesies* *Grana ontwikkel* *Mitochondria volop* *Mitochondria centripetaal*
Tribe/*Tribus* PAPPOPHOREAE			Enneapogon 6 spp Schmidtia 2 spp	
Tribe/*Tribus* ZOYSIEAE				Monelytrum 1 sp Mosdenia 1 sp Perotis 1 sp Tragus 3 spp
Tribe/*Tribus* CHLORIDEAE			Brachyachne 1 sp Catalepis 1 sp *Chloris* 3 spp Eustachys 1 sp *Microchloa caffra* Microchloa 2 spp Spartina 1 sp	*Chloris* 1 sp Craspedorachis 1 sp Ctenium 1 sp Cynodon 3 spp Enteropogon 1 sp Fingerhuthia 2 spp Harpochloa 1 sp *Microchloa caffra* Oropetium 1 sp Polevansia 1 sp Rendlia 1 sp Tetrapogon 1 sp
Tribe/*Tribus* ERAGROSTIDEAE			Dactyloctenium 3 spp Diandrochloa 2 spp Dinebra 1 sp *Eragrostis* 15 spp Stiburus 2 spp	Acrachne 1 sp Bewsia 1 sp Cypholepis 1 sp Diplachne 2 spp Eleusine 1 sp Entoplocamia 1 sp *Eragrostis* 39 spp Leptocarydion 1 sp Leptochloa 2 spp Lophachme 1 sp Odyssea 1 sp Pogonarthria 1 sp Tetrachne 1 sp Trichoneura 2 spp Tripogon 1 sp
Tribe/*Tribus* SPOROBOLEAE			*Sporobolus* 10 spp	*Sporobolus* 7 spp

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arranged chlorenchyma cells. The individual mesophyll cells are uniform in size and shape being elongated and narrow.

NAD-ME species appear to have the highest concentrations of mitochondria in the bundle sheath cells (Hatch *et al.*, 1975).

In this category high levels of NAD-malic enzyme account for 70% or more of the total decarboxylation activity in the Kranz sheath cells (Gutierrez *et al.*, 1976). Thus, in this type the aspartate is transported from the mesophyll cells to the bundle sheath where it is converted to oxaloacetate, the oxaloacetate to malate and the malate to pyruvate and CO_2 by aspartate transaminase, malate dehydrogenase and NAD- malic enzyme, respectively (Hatch & Kagawa, 1974).

Results and Discussion

Using the above anatomical criteria all specimens examined in this study were assigned to the relevant category solely on anatomical evidence. The following is a discussion of the material examined in this report. This anatomical classification is also compared and contrasted with relevant literature to assess the validity of using the anatomy of the leaf blade, as viewed in transverse section, as a marker for the whole Kranz syndrome.

Apart from published information, considerable use has been made of unpublished δ ^{13}C values which Dr J. C. Vogel of the Natural Isotopes Division of the Council for Scientific and Industrial Research Pretoria, and Prof. W. V. Brown of the University of Texas at Austin, U.S.A., have kindly made available to me.

The species are arranged alphabetically under genus in the same order as in Tables 1 and 2 which follow Loxton (1976) and serve to summarize the data and to display the taxonomic implications of the Kranz syndrome in these two sub-families. Each species is assigned to one of the four variations of photosynthetic CO_2 assimilation found in the Gramineae. This is denoted by the symbolic abbreviations given for each of the sub-divisions in Tables 1 and 2.

All voucher specimens of material examined are cited with collector's name and number together with quarter-degree collection locality according to the degree reference system of Edwards & Leistner (1971). These specimens are lodged in the National Herbarium, Pretoria, together with photomicrographs of most specimens examined. By including all the replicates of each species it is hoped to demonstrate that the anatomical criteria employed in this study are constant within species irrespective of locality of collection, sunlight, temperature and moisture conditions under which the leaf developed and prevailing at the time of collection. In addition broad generalizations may be possible regarding the geographical distribution patterns and concentrations of the various photosynthetic types in the grass component of different vegetation types.

Sub-family Eragrostoideae

All Southern African representatives of this subfamily examined were C_4 with markedly radiate mesophyll and are predicted to be aspartate formers as they have double bundle sheaths as reported by Brown (1975). The only variation is whether they are NAD-ME types with centripetal chloroplasts or PCK types with centrifugal chloroplasts. The statement (Brown, 1960) that in the Eragrostoideae the chloroplasts are attached to the centripetal wall is thus a generalization and does not always hold true. In addition the representatives of this sub-family with centrifugal chloroplasts should not possess parenchyma sheath cells that are short and wide, a characteristic which Brown (1974) attributed to this sub-family.

Tribe Pappophoreae

Both genera have a double bundle sheath with centrifugal chloroplasts and although no species have been biochemically typed it is predicted that they are PCK-type species (Fig. 1, 2).

ENNEAPOGON *Desv. ex Beauv.*

There is very little variation within this genus in the anatomical characters examined except that in the two species found in the N.W. Cape, *E. desvauxii* and especially *E. scaber*, the specialized chloroplasts entirely fill the bundle sheath cells in transectional view and it is only in older leaves that their centrifugal location becomes evident. However, the bundle sheath cells are typically rounded in transectional view and not with straight radial and/or tangential walls as is typical of species with centripetal chloroplasts.

E. cenchroides (*Licht. ex Roem. & Schult.*) *C. E. Hubb.* (Fig. 1.) **K-PS-PCK**

This agrees with Hattersley & Watson's (1976) grouping of this species as PS, i.e. with a double bundle sheath, using Metcalfe's (1960) data.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2031. Botswana.—2229–AA (Waterpoort), *Ellis* 1316, 1320. Transvaal.—2231–AC (Pafuri), *Ellis* 547. 2428–CD (Nylstroom), *Ellis* 197. 2430–BD (Pilgrims Rest), *Ellis* 1585. 2431–DD (Acornhoek), *Ellis* 104. 2528–CA (Pretoria), *Ellis* 409, 779. 2531–CB (Komatipoort), *Ellis* 1550. Cape.—2824–DC (Kimberley), *Ellis* 852.

E. desvauxii *Beauv.* **K-PS-PCK**

Typical PCK-type anatomy although the Kranz sheath cells are somewhat smaller than is typical of the other tribes possessing this sub-type. This applies to all the *Enneapogon* species.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2030, 2036. 2718–CB (Grünau), *Ellis* 897. Cape.—2816–BD (Oranjemund), *Ellis* 1812. 2824–DC (Kimberley), *Ellis* 842. 2921–BD (Kenhardt), *Ellis* 869. 2923–BC (Douglas), *Ellis* 856. 3123–DD (Victoria West), *van Heerden* 59.

E. pretoriensis *Stent* **K-PS-PCK**

Transvaal.—2527–DB (Rustenburg), *Ellis* 1228. 2528–CA (Pretoria), *Ellis* 835; –CB, *Ellis* 1788, 1796. 2529–AD (Witbank), *Ellis* 2062.

E. scaber *Lehm.* **K-PS-PCK**

A large proportion of the specimens of this species has the Kranz sheath cells almost entirely filled with specialized chloroplasts.

Cape.—2816–DB (Oranjemund), *Ellis* 1811. 2818–CC (Warmbad), *Ellis* 1740. 2820–DC (Kakamas), *Ellis* 875, 880. 2917–DB (Springbok), *Ellis* 2157. 2921–BD (Kenhardt), *Ellis* 861. 3323–BB (Willowmore), *Ellis* 1625.

E. scoparius *Stapf* **K-PS-PCK**

Transvaal.—2428–CD (Nylstroom), *Ellis* 195. 2429–AA (Zebediela), *Ellis* 1605. 2528–CC (Pretoria), *Ellis* 1502. 2429–AD (Witbank), *Ellis* 2060.

E. spathaceus *Goossens* **K-PS-PCK**

Transvaal.—2231–CB (Pafuri), *Ellis* 559, 1905, 1906.

SCHMIDTIA *Steud. ex Schmidt.*

Both species examined have rounded bundle sheath cells with centrifugal chloroplasts typical of PCK type species. The outer bundle sheath cells are

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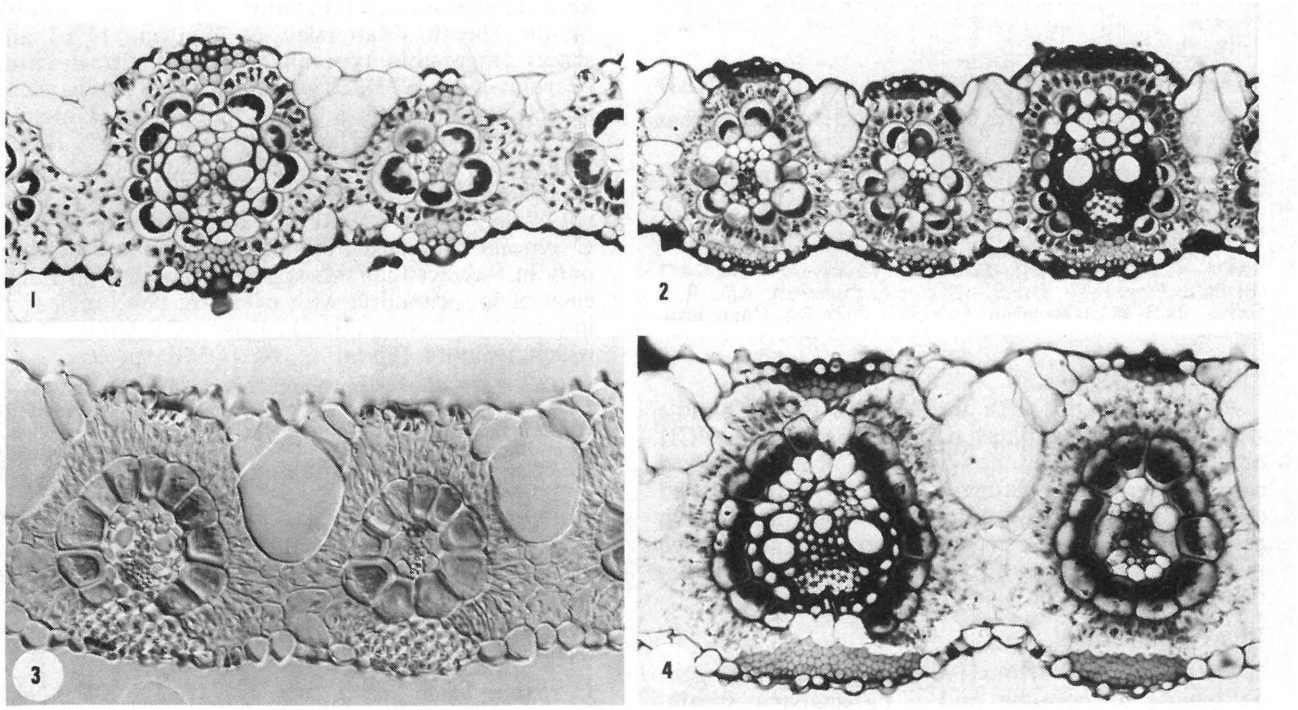


FIG. 1–2 The tribe Pappophoreae with double bundle sheaths and centrifugal chloroplasts. Fig. 1, *Enneapogon cenchroides*, K-PS-PCK, *Ellis* 409×640. Fig. 2, *Schmidtia pappophoroides*, K-PS-PCK, *Ellis* 929, ×400

FIG. 1–2 *Die tribus Pappophoreae met dubbel bondelskede en centrifugale chloroplaste. Fig.* 1, Enneapogon cenchroides, *K-PS-PCK*, Ellis 409×640. *Fig.* 2, Schmidtia pappophoroides, *K-PS-PCK*, Ellis 929, ×400

FIG. 3–4 The tribe Zoysieae with double bundle sheaths and centripetal chloroplasts. Fig. 3, *Tragus berteronianus*, K-PS-NAD, *Ellis* 294, ×640. Fig. 4, *Monelytrum luederitzianum*, K-PS-NAD, *Ellis* 928×400

FIG. 3–4 *Die tribus Zoysieae met dubbel bondelskede en centripetale chloroplaste. Fig.* 3, Tragus berteronianus, *K-PS-NAD*, Ellis 294, ×640. *Fig.* 4, Monelytrum luederitzianum, *K-PS-NAD*, Ellis 928, ×400

somewhat smaller in transectional area than is typical of PCK-type species.

S. kalihariensis *Stent* **K-PS-PCK**

CAPE.—2921–BD (Kenhardt), *Ellis* 862. 3018–CC (Kamiesberg), *Ellis* 2205.

S. pappophoroides *Steud.* (Fig. 2) **K-PS-PCK**

RHODESIA.—2031–AA (Bikta), *Ellis* 825. S.W.A.—1723–CB (Singalamwe), *Ellis* 1774. 1823–BC (Siambisso), *Ellis* 337. 2118–DB (Steinhausen), *Ellis* 2028. 2217–CA (Windhoek), *Ellis* 929. TRANSVAAL.—2230–CB (Messina), *Ellis* 1313. 2330–DC (Tzaneen), *Ellis* 1587. 2428–CD (Nylstroom), *Ellis* 196. 2431–DB (Acornhoek), *Ellis* 510; –DD, *Ellis* 97. 2528–AC (Pretoria), *Ellis* 1755. CAPE.—2824–DC (Kimberley), *Ellis* 837.

TRIBE ZOYSIEAE

The four S. African genera examined all have a double bundle sheath with the chloroplasts centripetally located in the outer sheath. The outer sheath cells form a conspicuous, compact and regular ring with straight radial wells, each bundle sheath cell being fan-shaped (Fig. 3,4). This is in agreement with the eragrostoid anatomy and ultrastructure (Carolin *et al.*, 1973) and PS sheath condition (Hattersley & Watson, 1976) of *Zoysia macrantha*. In addition *Z. japonica* has a $\delta^{13}C$ value of—14,7‰. (Smith & Brown, 1973).

Gutierrez *et al.* (1974, 1974a) report that *Z. japonica* is a PCK-type species and that it has centrifugal chloroplasts. It is predicted that the S. African genera of this tribe will all be NAD-ME types and this anomally warrants further study.

MONELYTRUM *Hack. ex Schinz*

M. luederitzianum *Hack.* (Fig. 4) **K-PS-NAD**

A double bundle sheath (Hattersley & Watson, 1976) and typical NAD-ME type anatomy.

S.W.A.—2217–CA (Windhoek), *Ellis* 928.

MOSDENIA *Stent*

M. leptostachys (*Fical. & Hiern*) Clayton **K-PS-NAD**

TRANSVAAL.—2329–DC (Pietersburg), *Ellis* 1601. 2529–AD (Witbank), *Ellis* 2071.

PEROTIS *Ait.*

Perotis indica, *P. rara* & *P. patens* are reported as being PS (Hattersley & Watson, 1976) from the data of Metcalfe (1960).

P. patens *Gand.* **K-PS-NAD**

RHODESIA.—1731–CC (Salisbury), *Ellis* 804. TRANSVAAL.—2230–DB (Messina), *Ellis* 1926. 2329–DC (Pietersburg), *Ellis* 1599. 2528–AC (Pretoria), *Ellis* 1326. 2530–BA (Lydenburg), *Ellis* 462.

TRAGUS *Haller*

Tragus biflorus is C_4 with a low CO_2 compensation point (Raghavendra & Das, 1976). *T. australianus* has PS bundle sheath structure (Hattersley & Watson, 1976) and eragrostoid anatomy and ultrastructure (Carolin *et al.*, 1973). It is expected that all species of this genus are NAD-ME type species.

T. berteronianus *Schult.* (Fig. 3) **K-PS-NAD**

This confirms the double bundle sheath report of Hattersley & Watson (1976) for this species.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2021. TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 100. 2528–CA (Pretoria), *Ellis*

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365. NATAL.—2830–CB (Dundee), *Ellis* 73. CAPE.—3123–DD (Victoria West), *Van Heerden* 62. 3227–DA (Stutterheim), *Ellis* 294.

T. koeleroides *Aschers.* **K-PS-NAD**

A $\delta^{13}C$ value of—12,0‰. (Vogel) confirms that this species is C_4.

CAPE.—2824–DC (Kimberley), *Ellis* 843. 3123–DD (Victoria West), *Van Heerden* 60.

T. racemosus (*L.*) *All.* **K-PS-NAD**

S.W.A.—2518–CA (Tses), *Ellis* 915. TRANSVAAL.—2629–AD (Bethal), *Ellis* 1837. O.F.S.—2827–DC (Senekal), *Ellis* 954. CAPE.—2923–BC (Douglas), *Ellis* 857. 3028–BA (Matatiele), *Ellis* 245.

TRIBE CHLORIDEAE

All species in this tribe are C_4 with a double bundle sheath and only vary as to whether they are PCK or NAD-ME type, i.e. whether the specialized chloroplasts are centrifugally or centripetally located (Fig. 5–12). These two groups are consistent within genera except for the genera *Chloris* and *Microchloa* in which both PCK and NAD-ME species or specimens were observed.

BRACHYACHNE *Stapf*

Hattersley & Watson (1976) record *B. convergens* as having a mestome and a parenchyma sheath.

B. patentiflora (*Stent & Rattray*) *C. E. Hubb.* (Fig. 9) **K-PS-PCK**

RHODESIA.—1832–CA (Rusape), *Ellis* 820. TRANSVAAL.—2528 –CC (Pretoria), *Ellis* 1211 (Cultivated).

CATALEPIS *Stapf & Stent*

C. gracilis *Stapf & Stent* (Fig. 7) **K-PS-PCK**

In some specimens examined the chloroplasts filled the entire parenchyma bundle sheath cells which are rounded and inflated (Fig. 7). Thus, on the basis of the parenchyma sheath cell-shape and not on the position of the chloroplasts, *C. gracilis* is assigned to this category. This grouping is somewhat tentative and resembles the situation in *Stiburus* of the Eragrostoideae. These genera may represent an intermediate condition and their biochemical typification should prove interesting.

TRANSVAAL.—2629–BC (Bethal), *Ellis* 1839, 1840, 1843. CAPE.—3028–BA (Matatiele), *Ellis* 234.

CHLORIS *Swartz*

Three S. African species are typically PCK-type (Fig. 10, 11) and a single species is distinctly NAD-ME type (Fig. 12) based on anatomical criteria. This observation is consistent with findings in the literature. e.g. *C. cucullata* is C_4 ($\delta^{13}C = -15,9‰$ Smith & Brown, 1973), has a double bundle sheath (Hattersley & Watson, 1976), has centrifugal chloroplasts and has been biochemically determined to be a PCK-type species (Gutierrez *et al.*, 1974, 1974a). This contrasts with *C. distichophylla* which is also an aspartate former as it shows a PIB (Brown & Gracen, 1972) and has both a mestome and a parenchyma bundle sheath (Hattersley & Watson, 1976) but has well developed grana (Brown & Gracen, 1972), centripetal chloroplasts and is a NAD-ME species (Gutierrez *et al.*, 1974, 1974a).

C. gayana *Kunth* (Fig. 10, 11) **K-PS-PCK**

This species has been extensively studied, all the results of which corroborate the anatomical findings of this study. It is a C_4 species with a $\delta^{13}C$ value of—13,1‰ (Vogel) and a low CO_2 compensation point (Downton & Tregunna, 1968). It has been shown to be an aspartate former (Downton, 1970), has a double bundle sheath (Hattersley & Watson, 1976) and shows eragrostoid type anatomy and ultrastructure (Carolin *et al.*, 1973). *C. gayana* has been biochemically determined to be a PCK-type species (Gutierrez *et al.*, 1974, 1974a; Hatch & Kagawa, 1974; Hatch, Kagawa & Craig, 1975) with well developed grana (Downton, 1970) and centrifugal chloroplasts (Gutierrez *et al.*, 1974, 1974a). In all the above respects *C. gayana* conforms with the predicted and deviates only in the fact that this species shows a high frequency of mitochondria with extensive development of internal membranes (Hatch, Kagawa & Craig, 1975), which is more typical of NAD-ME species.

S.W.A.—1823–BC (Siambisso), *Ellis* 331. TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1762. 2628–AB, (Johannesburg), *Ellis* 1385. 2630–AB (Carolina), *Ellis* 1534. NATAL.—3030–CB (Port Shepstone), *Ellis* 738. CAPE.—3227–DA (Stutterheim), *Ellis* 296; –DB, *Ellis* 288. 3228–BC (Butterworth), *Ellis* 275.

C. radiata (*L.*) *Swartz* **K-PS-PCK**

Very similar anatomically to *C. gayana* and *C. virgata* all of which have distinct crystals in bundle sheath cells.

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2003. 2528–CA (Pretoria), *Ellis* 761.

C. roxburghiana *Schult.* (Fig. 12) **K-PS-NAD**

This species has centripetally positioned chloroplasts with the parenchyma bundle sheath cells typical of the NAD-ME type species.

RHODESIA.—2130–DA (Nuanetsi), *Ellis* 1307. TRANSVAAL.—2231–CA (Pafuri), *Ellis* 1890; –CC, *Ellis* 1895. 2331–AB (Phalaborwa), *Ellis* 542.

C. virgata *Swartz* **K-PS-PCK**

S.W.A.—2118–DB (Steinhausen), *Ellis* 2033. 2518–CA (Tses), *Ellis* 920. TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1933. 2428–DB (Nylstroom), *Ellis* 2016. 2431–DD (Acornhoek), *Ellis* 103, 114. 2729–DB (Volksrust), *Ellis* 35. NATAL.—2830–CB (Dundee), *Ellis* 75. CAPE.—3028–BD (Matatiele), *Ellis* 249.

CRASPEDORACHIS *Benth.*

C. rhodesiana *Rendle* (Fig. 5) **K-PS-NAD**

Typical NAD-ME type anatomy.

RHODESIA.—1832–CA (Rusape), *Ellis* 817.

CTENIUM *Panz.*

C. concinnum *Nees* (Fig. 8) **K-PS-NAD**

Distinct NAD-ME type anatomy even although chloroplasts may fill cells. Distinct nuclei adjacent to outer tangential wall of bundle sheath cells as in Fig. 8.

TRANSVAAL.—2531–CC (Komatipoort), *Ellis* 380. 2628–AB (Johannesburg), *Ellis* 1833. 2630–CA (Carolina), *Ellis* 1849; –DA, *Ellis* 1858. CAPE.—3128–DB (Umtata), *Ellis* 257.

CYNODON *L. C. Rich.*

C. arcuatus has been examined biochemically and shown to be a NAD-ME species (Hatch & Kagawa 1974; Hatch, Kagawa & Craig, 1975).

C. dactylon (*L.*) *Pers.* **K-PS-NAD**

This has been shown to be a C_4 species: CO_2 compensation point 5 ppm. (Downton & Tregunna, 1968); $\delta^{13}C = -12,8‰$ (Smith & Brown, 1973), $-15,3‰$. (Bender, 1971). It has a double bundle sheath (Hattersley & Watson, 1976), PIB (Brown & Gracen, 1972) and eragrostoid anatomy and ultrastructure (Carolin *et al.*, 1973). In addition the chloroplasts are centripetal with well developed grana (Brown & Gracen, 1972). This is corroborated by the present observations although in some specimens

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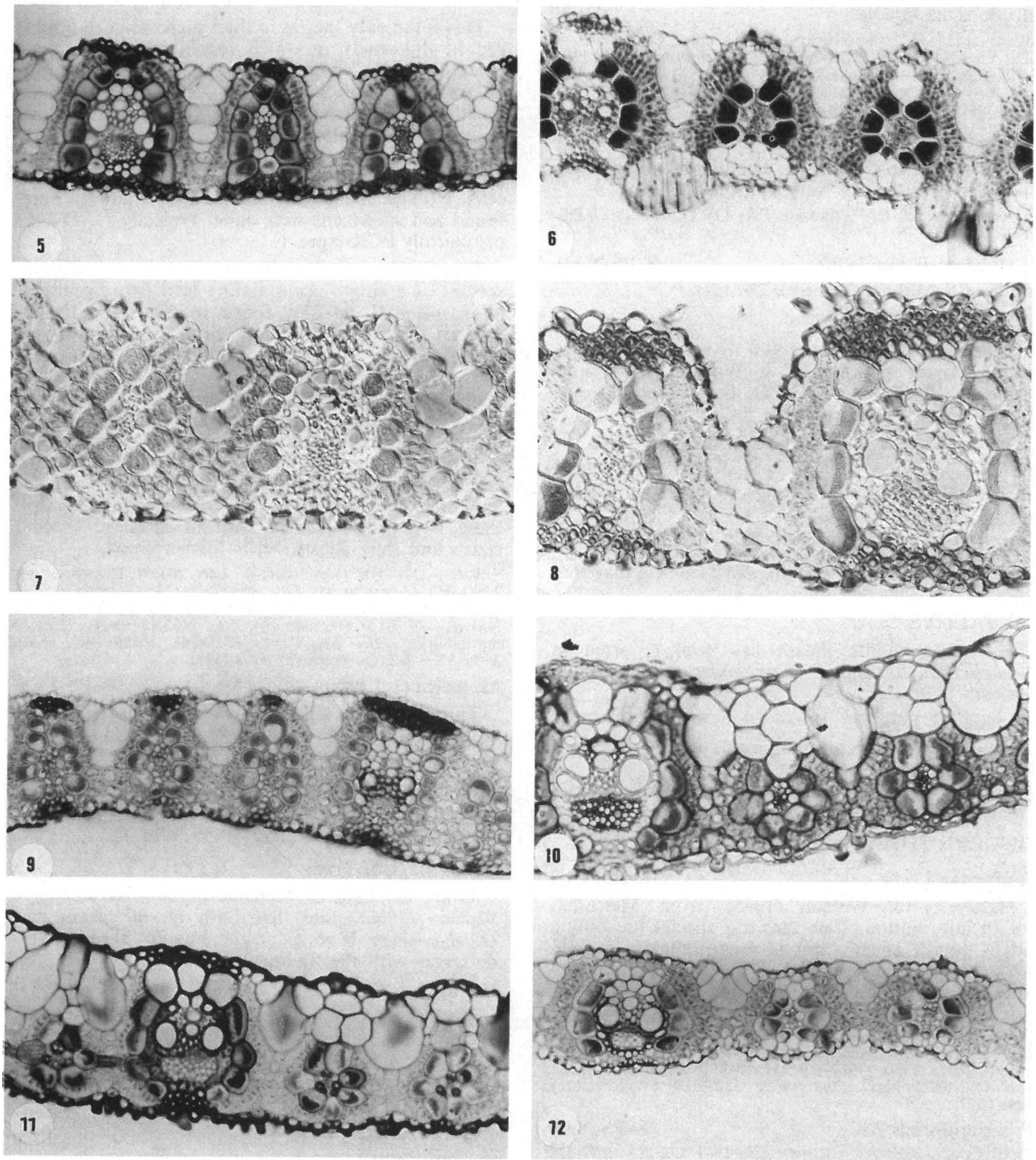


FIG. 5–12 The tribe Chlorideae showing the two anatomical sub-types of the aspartate forming type. Fig. 5, *Craspedorachis rhodesiana,* K-PS-NAD, *Ellis* 817, ×400. Fig. 6, *Cynodon hirsutus,* K-PS-NAD, *Ellis* 978×400. Fig. 7, *Catalepis gracilis,* somewhat atypical K-PS-PCK type as the chloroplasts almost fill inflated, rounded parenchyma sheath cells, *Ellis* 1843, ×640. Fig. 8, *Ctenium concinnum,* K-PS-NAD, *Ellis* 1858, ×400. Fig. 9, *Brachyachne patentiflora,* K-PS-PCK, *Ellis* 1211, ×400

FIG. 5–12 *Die tribus Chlorideae wat die twee sub-tipes van die aspartiensuurproduseerders toon. Fig.* 5, Craspedorachis rhodesiana, *K-PS-NAD,* Ellis 817, ×400. *Fig.* 6, Cynodon hirsutus, *K-PS-NAD,* Ellis 978, ×400. *Fig.* 7, Catalepis gracilis, *nie tipiese K-PS-PCK tipe nie omdat die chloroplaste amper die ronde parenchiemskedeselle vul,* Ellis 1843, ×640. *Fig.* 8, Ctenium concinnum, *K-PS-NAD,* Ellis 1858, ×400. *Fig.* 9, Brachyachne patentiflora, *K-PS-PCK,* Ellis 1211, ×400

FIG. 10–12 Variation within the genus *Chloris,* showing a species with centrifugal chloroplasts and a species with centripetal chloroplasts. Fig. 10–11, *Chloris gayana,* K-PS-PCK, *Ellis* 296, ×400. Fig. 12, *Chloris roxburghiana,* K-PS-NAD, *Ellis* 1895, ×400

FIG. 10–12 *Variasie in die genus* Chloris, *wat 'n spesies met centrifugale chloroplaste en 'n spesies met centripetale chloroplaste toon. Fig.* 10–11, Chloris gayana, *K-PS-PCK,* Ellis 296, ×400. *Fig.* 12, Chloris roxburghiana, *K-PS-NAD,* Ellis 1895, ×400

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the chloroplasts almost fill the Kranz cells. This species has colourless abaxial and adaxial sheath cells on most bundles.

S.W.A.—1823–BC (Siambisso), *Ellis* 332. TRANSVAAL.—2429–AA (Zebediela), *Ellis* 2053. 2528–CB (Pretoria), *Ellis* 138. O.F.S.—2827–DC (Senekal), *Ellis* 979. NATAL.—2729–DB (Volksrust), *Ellis* 34. CAPE.—3218–DD (Clanwilliam), *Ellis* 698. 3228–BC (Butterworth), *Ellis* 269.

C. hirsutus *Stent* (Fig. 6) **K-PS-NAD**

Very similar to *C. dactylon* but adaxial and abaxial colourless sheath cells very conspicuous.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 435. O.F.S.—2827–DC (Senekal), *Ellis* 978.

C. nlemfuensis *Vanderyst* **K-PS-NAD**

TRANSVAAL.—2330–CA (Tzaneen), *Ellis* 1594.

ENTEROPOGON *Nees*

E. acicularis has been shown to possess a double bundle sheath (Hattersley & Watson, 1976) and *E. monostachyos* has a low CO_2 compensation point (Raghavendra & Das, 1976).

E. macrostachyus (*Hochst. ex A. Rich.*) *Munro ex Benth.* **K-PS-NAD**

Classical structure of this grouping and predicted to be a NAD-ME type.

RHODESIA.—1932–CD (Melsetter), *Ellis* 826. TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1938, 1941. 2230–DB (Messina), *Ellis* 1931, 1932. 2231–AC (Pafuri), *Ellis* 545; –AD, *Ellis* 1912. 2529–AD (Witbank), *Ellis* 2078.

EUSTACHYS *Desv.*

A double bundle sheath has been reported in *E. distichophylla* and *E. paspaloides* (Hattersley & Watson, 1976).

E. mutica (*L.*) *Cufod.* **K-PS-PCK**

Centrifugal chloroplasts typical of this category.

TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 116. 2528–AC (Pretoria), *Ellis* 1754; –CA *Ellis* 1329. NATAL.—2930–CB (Pietermaritzburg), *Ellis* 64.

FINGERHUTHIA *Nees*

F. africana *Lehm.* **K-PS-NAD**

Hattersley & Watson (1976), from Metcalfe's (1960) information, state that this species has only a single bundle sheath and is thus a malate former. All the specimens examined in this study had two distinct sheaths around the first order vascular bundles.

S.W.A.—2718–CB (Grünau), *Ellis* 890. TRANSVAAL.—2429–AA (Zebediela), *Ellis* 1608. 2431–DD (Acornhoek), *Ellis* 117. 2531–BB (Komatipoort), *Ellis* 504. CAPE.—2816–DD (Oranjemund), *Ellis* 1810. 3017–BB (Hondeklip Bay), *Ellis* 2202. 3119–CA (Calvinia), *Ellis* 1723. 3323–BB (Willowmore), *Ellis* 1627.

F. sesleriiformis *Nees* **K-PS-NAD**

TRANSVAAL.—2629–AD (Bethal), *Ellis* 1841. O.F.S.—2827–DC (Senekal), *Ellis* 962.

HARPOCHLOA *Kunth*

H. falx (*L.f.*) *Kunth* **K-PS-NAD**

A typical species with centripetal chloroplasts although in most first order bundles the adaxial sheath cells are replaced by colourless cells.

TRANSVAAL.—2531–CC (Komatipoort), *Ellis* 382. 2628–AA (Johannesburg), *Ellis* 1376; –AB, *Ellis* 1372. NATAL.—2829–CC (Harrismith), *Ellis* 1410, 1422. 2929–DD (Underberg), *Ellis* 50. CAPE.—3028–BA (Matatiele), *Ellis* 238. 3227–AC (Stutterheim), *Ellis* 1484.

MICROCHLOA *R. Br.*

In this genus there is a certain degree of variation in the position of the chloroplasts in different specimens of *M. caffra*.

M. caffra *Nees* (Fig. 14, 17–20)

K-PS-PCK & K-PS-NAD

This is the only species in the Eragrostoideae observed in this study in which individuals of a single species varied between the anatomical categories as defined. All specimens possessed a double bundle sheath (cf. Hattersley & Watson, 1976) but in some individuals the chloroplasts were centripetal and in others centrifugal. This difference was accompanied by a difference in the shape of the bundle sheath cells. No specimens with intermediate anatomy were found and specimens were either typically NAD-ME or typically PCK-type.

This finding was totally unexpected, as different species of a single genus falling into two categories is exceptional and only found in *Chloris* in the S. African Chlorideae. However, this finding may be superficial and is probably due to indentificatory or classificatory inadequacies. Thus, in the somewhat inadequate sample examined, those specimens showing the PCK configuration (marked with an asterisk below) were collected on rock-mat communities (a pioneer community) whereas the others are from climax grassland communities with normal soil and moisture conditions. It is not improbable that these two ecotypes may warrant at least specific status and this species merits further study.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2035.* TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 95. 2528–CA (Pretoria), *Ellis* 1745*; –AC, *Ellis* 1757. 2530–BD (Lydenburg), *Ellis* 1558*. NATAL.—2829–CC (Harrismith), *Ellis* 1450. 2930–AC (Pietermaritzburg), *Ellis* 57. CAPE.—3028–BA (Matatiele), *Ellis* 240*b*. 3126–BB (Queenstown), *Ellis* 2599.

M. indica (*L.*) *Beauv.* (Fig. 15) **K-PS-PCK**

This species has a double bundle sheath (Hattersley & Watson, 1976) and centrifugal chloroplasts.

RHODESIA.—1731–CC (Salisbury), *Ellis* 812.

M. kunthii *Desv.* (Fig. 13, 16) **K-PS-PCK**

RHODESIA.—1731–CC (Salisbury), *Ellis* 810. Grid ref. unknown: Rhodesia, *Ellis* 1212 (=Gaff Y3).

OROPETIUM *Trin.*

Using the data of Metcalfe (1960), Hattersley & Watson (1976) state that both *O. africanum* and *O. thomaeum* have a single bundle sheath. This disagrees with the findings of this study.

O. capense *Stapf.* **K-PS-NAD**

Typical NAD-ME type anatomy although mestome sheath cells are thin-walled and inconspicuous.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2037. TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1934. 2231–AC (Pafuri), *Ellis* 543. 2330–DC (Tzaneen), *Ellis* 1588. 2527–DB (Rustenburg), *Ellis* 1230, 1231. 2528–CB (Pretoria), *Ellis* 1798. O.F.S.—2827–DD (Senekal), *Ellis* 944.

POLEVANSIA *de Wint.*

P. rigida *de Wint.* **K-PS-NAD**

CAPE.—3126–BC (Queenstown), *Stretton* 213.

RENDLIA *Chiov.*

R. altera (*Rendle*) *Chiov.* **K-PS-NAD**

Conspicuous Kranz sheath with centripetal chloroplasts which may be replaced adaxially by colourless parenchyma.

TRANSVAAL.—2329 –DD (Pietersburg), *Ellis* 1875. Natal.—2829–CC (Harrismith), *Ellis* 1433. 2929–BD (Underberg), *Ellis* 54.

SPARTINA *Schreb.*

A C_4 genus with $\delta^{13}C$ values of –13,1‰ for *S. alternifolia* (Smith & Brown, 1973), –14,4‰ for

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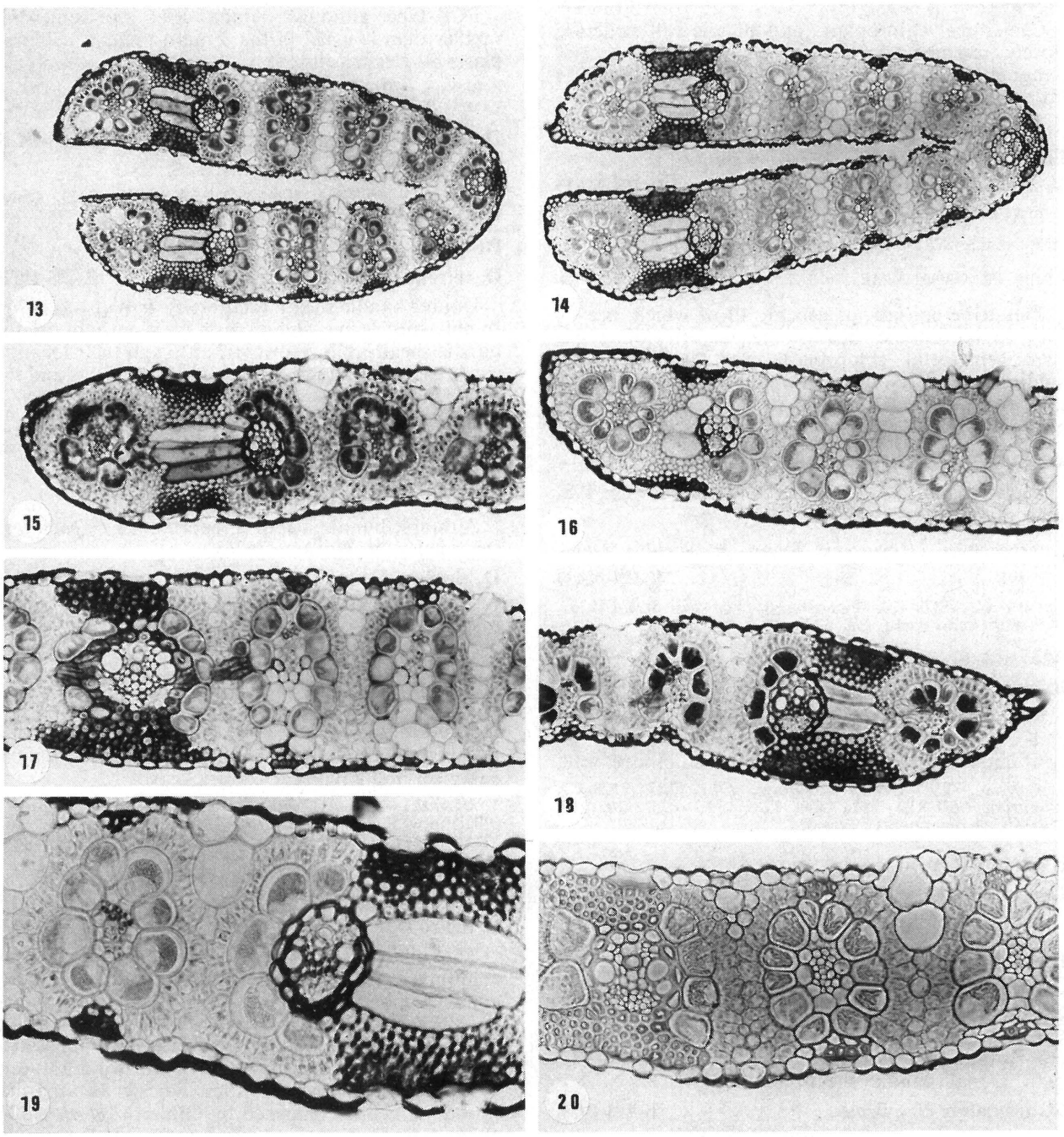


FIG. 13–20 The genus *Microchloa* and variation between PCK and NAD-ME types in *M. caffra*. Fig. 13, *Microchloa kunthii*. K-PS-PCK, *Ellis* 810, ×250. Fig. 14, *Microchloa caffra*, K-PS-PCK, *Ellis* 2035, ×250. Fig. 15, *Microchloa indica*. K-PS-PCK, *Ellis* 812, ×400. Fig. 16, *Microchloa kunthii*, K-PS-PCK, *Ellis* 1212, ×400. Fig. 17–20, *Microchloa caffra*, Fig. 17, K-PS-NAD, *Ellis* 1450, ×400. Fig. 18, K-PS-NAD, *Ellis* 1757, ×400. Fig. 19, K-PS-PCK, *Ellis* 1745, ×640, Fig. 20, K-PS-NAD, *Ellis* 57, ×640

FIG. 13–20 *Die genus* Microchloa *en variasie tussen PCK en NAD-ME tipes in* M. caffra. *Fig.* 13, Microchloa kunthii, K-PS-PCK, Ellis 810, ×250. *Fig.* 14, Microchloa caffra, *K-PS-PCK*, Ellis 2035, ×250. *Fig.* 15, Microchloa indica, *K-PS-PCK*, Ellis 812, ×400. *Fig.* 16, Microchloa kunthii, *K-PS-PCK*, Ellis 1212, ×400. *Fig.* 17–20, Microchloa caffra. *Fig.* 17, *K-PS-NAD*, Ellis 1450, ×400. *Fig.* 18, *K-PS-NAD*, Ellis 1757, ×400. *Fig.* 19, *K-PS-PCK*, Ellis 1745, ×640. *Fig.* 20, *K-PS-NAD*, Ellis 57, ×640

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S. cynosuroides and −13,4‰ for *S. pectinata* (Bender, 1971). In addition *S. maritima* and *S. townsendi*, both have a double bundle sheath (Hattersley & Watson, 1976).

S. capensis *Nees ex Trin.* **K-PS-PCK**

Centrifugal chloroplasts and bundle cell structure closely resemble the PCK type *Eragrostis* species although chloroplast size and concentration differ little from the mesophyll cells.

CAPE.—3423-AA (Knysna), *Ellis* 1300.

TETRAPOGON *Desf.*

T. tenellus (*Roxb.*) *Chiov.* **K-PS-NAD**

NAD-ME type anatomy.

TRANSVAAL.—2231-CB (Pafuri), *Ellis* 1909, 1910.

TRIBE ERAGROSTIDEAE

This tribe consists of genera, all of which, are C_4 with double bundle sheaths. The majority of genera have centripetal chloroplasts and are, therefore, predicted to be NAD-ME type. A few genera have centrifugal chloroplasts and in the genus *Eragrostis* both sub-types of aspartate formers are found.

ACRACHNE *R. Wright & Arnott ex Lindl.*

A. verticillata has a double bundle sheath i.e. P.S. anatomy (Hattersley & Watson, 1976.)

A. racemosa (*Heyne ex Roem. & Schult.*) *Ohwi.* **K-PS-NAD**

TRANSVAAL.—2329-BB (Pietersburg), *Van der Schijff* 5267. 2431-DB (Acornhoek), *Ellis* 522.

BEWSIA *Goossens*

B. biflora (*Hack.*) *Goossens* **K-PS-NAD**

Conspicuous Kranz sheath with centripetal chloroplasts. When chloroplasts are large and almost fill cell, nuclei are present adjacent to outer cell wall.

TRANSVAAL.—2527-DB (Rustenburg), *Ellis* 1225. 2528-CA (Pretoria), *Ellis* 419, 754; -CB, *Ellis* 139; -CC, *Ellis* 713. 2531-CC (Komatipoort), *Ellis* 381.

CYPHOLEPIS *Chiov.*

C. yemenica (*Schweinf.*) *Chiov.* **K-PS-NAD**

Grid ref. unknown, *de Wet* 1144.

DACTYLOCTENIUM *Willd.*

All species examined had centrifugal chloroplasts and anatomically resemble the genus *Chloris* of the Chlorideae very closely. *D. radulans* has typical eragrostoid ultrastructure (Carolin *et al.*, 1973) with granal bundle sheath chloroplasts.

D. aegyptium (*L.*) *Beauv.* **K-PS-PCK**

Hattersley & Watson (1976) concluded from the data of Metcalfe (1960) that this species has a double bundle sheath. It shows a C_4 $\delta^{13}C$ ratio of −12,2‰ (Smith & Brown, 1973).

S.W.A.—2118-DB (Steinhausen), *Ellis* 2022. 2518-CA (Tses), *Ellis* 919. TRANSVAAL.—2431-DB (Acorhhoek), *Ellis* 514. 2528-AC (Pretoria), *Ellis* 1759.

D. australe *Steud.* (Fig. 21) **K-PS-PCK**

NATAL.—3030-CD (Port Shepstone), *Ellis* 1463. CAPE.—3228-BC (Butterworth), *Ellis* 270; -CC, *Ellis* 1111.

D. giganteum *Fischer & Schweick.* **K-PS-PCK**

Double bundle sheath from data of Metcalfe (1960) (Hattersley & Watson, 1976). Characteristic PCK-type anatomy.

TRANSVAAL.—2231-CB (Pafuri), *Ellis* 1904. 2431-BA (Acornhoek), *Ellis* 529.

DIANDROCHLOA *de Wint.*

The anatomy of this genus is very similar to some of the PCK-type species of *Eragrostis.*

D. namaquensis (*Nees*) *de Wint.* **K-PS-PCK**

PCK-type although Kranz cells are somewhat smaller than is usual in the Eragrostoideae. Chloroplasts fill Kranz cells.

RHODESIA.—2028-BA (Bulawayo), *de Winter* 8331. TRANSVAAL.—2429-AA (Zebediela), *Ellis* 2092.

D. pusilla (*Hack.*) *de Wint.* **K-PS-PCK**

Typical PCK-type anatomy.

TRANSVAAL.—2528-CA (Pretoria), *Ellis* 2362, 2363. (cultivated).

DINEBRA *Jacq.*

D. retroflexa (*Vahl.*) *Panz.* **K-PS-PCK**

Double bundle sheath (Hattersley & Watson, 1976). In this species the chloroplasts may entirely fill the bundle sheath cells. However, although their location is not clear, the structure of these chloroplasts and the transectional shape of the bundle sheath cells is typical of grasses placed in this category.

TRANSVAAL.—2231-AC (Pafuri), *Ellis* 566. 2431-DD (Acornhoek), *Ellis* 520.

DIPLACHNE *Beauv.*

A double bundle sheath is reported for *D. parviflora* (Hattersley & Watson, 1976).

D. eleusine *Nees* **K-PS-NAD**

Typical NAD-ME type anatomy with Kranz sheath forming distinct, regular ring.

TRANSVAAL.—2529-AD (Witbank), *Ellis* 2057, 2058, 2059. NATAL.—2632-CC (Bella Vista), *Ellis* 1127. CAPE.—3328-CC (Butterworth), *Ellis* 2105, 2106.

D. fusca (*L.*) *Beauv. ex Stapf* **K-PS-NAD**

Adaxial Kranz cells of primary lateral bundles enlarged, and devoid of chloroplasts.

TRANSVAAL.—2231-AC (Pafuri), *Ellis* 558, 1908. 2331-AB (Phalaborwa), *Ellis* 532. CAPE.—3119-CC (Calvinia), *Ellis* 1715. 3228-CC (Butterworth), *Ellis* 2110. 3323-BB (Willowmore), *Ellis* 1630.

ELEUSINE *Gaertn.*

E. indica (*L.*) *Gaertn.* **K-PS-NAD**

The predictions of this study based purely on anatomy are substantiated by the various studies conducted on this species. Thus, it has been shown to have a double bundle sheath, (Hattersley & Watson, 1976), eragrostoid anatomy and ultrastructure (Carolin *et al.*, 1973) and it shows a PIB (Brown & Gracen, 1972). All these characteristics indicate an aspartate former, which is confirmed by Gutierrez *et al.* (1974, 1974a) who showed the major decarboxylating enzyme to be NAD-ME and that the chloroplasts are centripetally situated.

TRANSVAAL.—2231-AC (Pafuri), *Ellis* 550. 2531-CC (Komatipoort), *Ellis* 392. NATAL.—2831-CD (Nkandla), *Ellis* 81. 3030-CB (Port Shepstone), *Ellis* 728, 739.

ENTOPLOCAMINA *Stapf*

E. aristulata (*Hack & Rendle*) *Stapf* (Fig. 22) **K-PS-NAD**

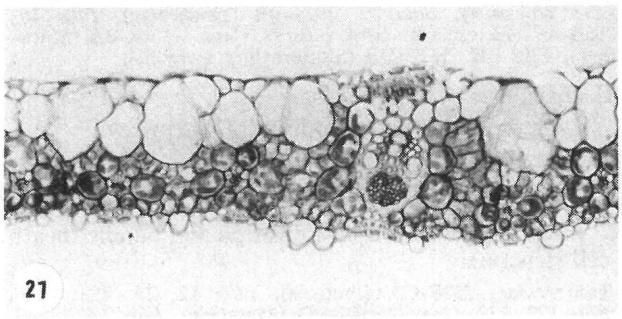
S.W.A.—2416-AB (Maltahöhe), *Ellis* 938.

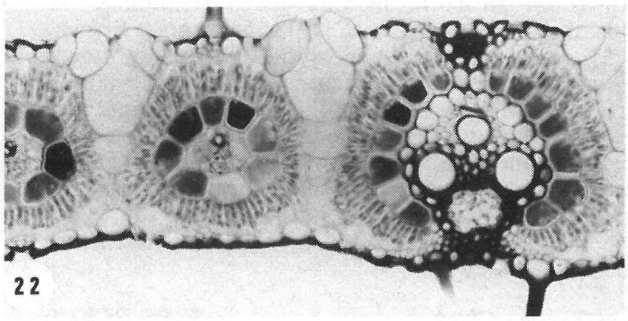
ERAGROSTIS *Wolf*

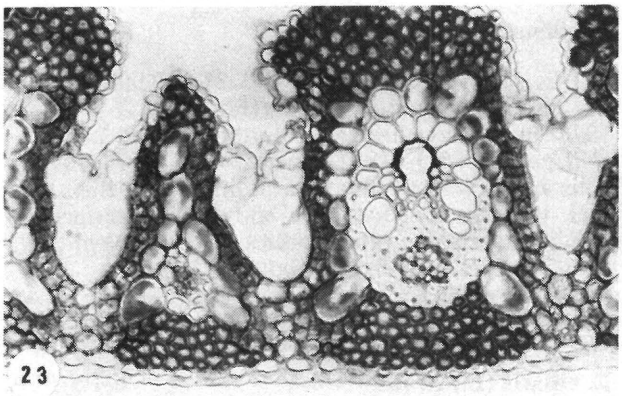
Anatomically 14 species of this genus can be classified as PCK-type species and 29 are NAD-ME-type species. This is the first report of centrifugal chloroplasts in this genus. Previous anatomical and ultra-

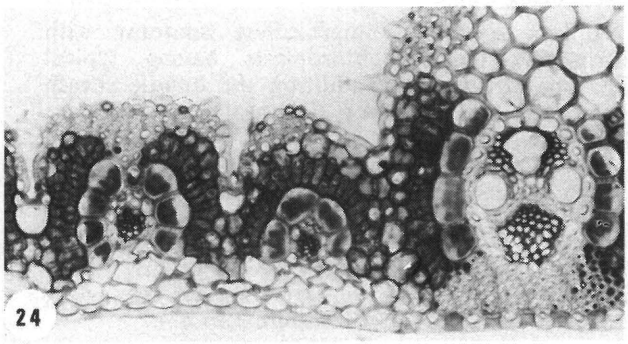
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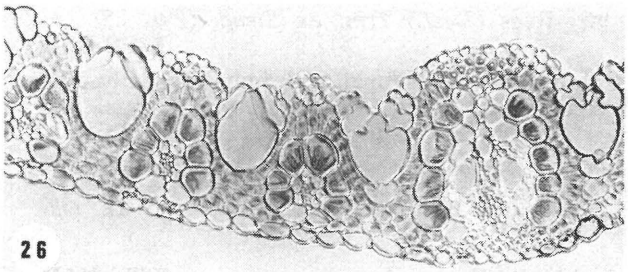
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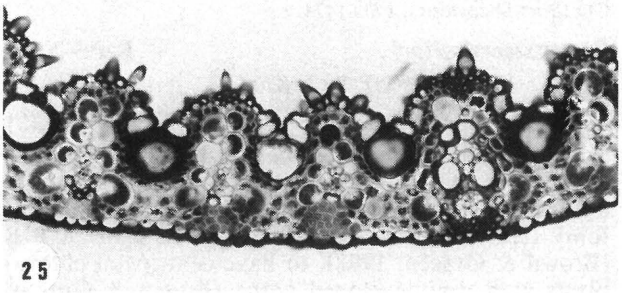


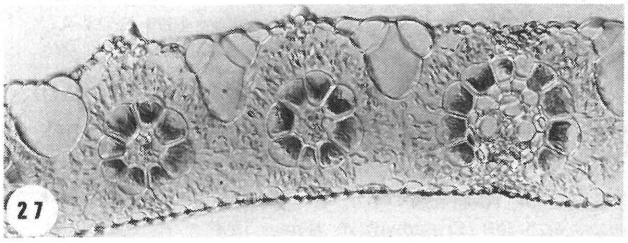


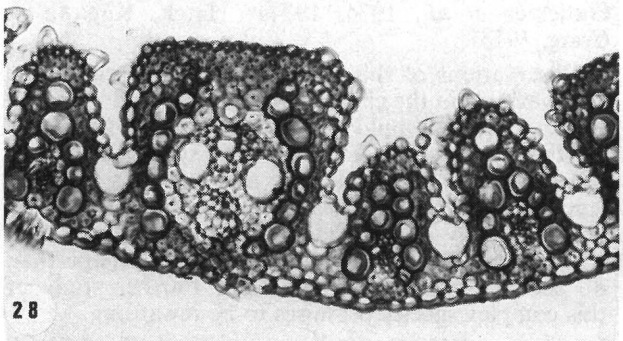












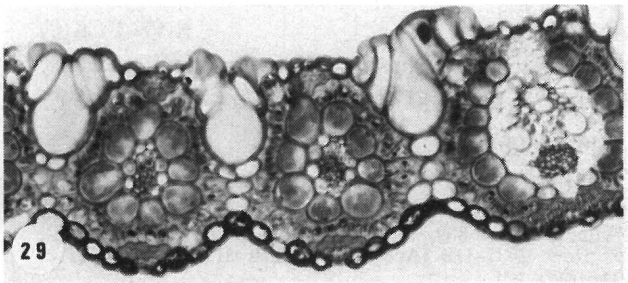


FIG. 21–29 The tribe Eragrostideae showing PCK and NAD-ME type anatomy. Fig. 21, *Dactyloctenium australe,* K-PS-PCK, *Ellis* 270×640. Fig. 22, *Entoplocamia aristulata,* K-PS-NAD, *Ellis* 938, ×400

FIG. 21–29 *Die tribus Eragrostideae wat PCK en NAD-ME tipe anatomie toon. Fig.* 21, Dactyloctenium australe, *K-PS-PCK, Ellis* 270, ×640. *Fig.* 22, Entoplocamia aristulata, *K-PS-NAD,* Ellis 938, ×400

FIG. 23–29 Variation within the genus *Eragrostis.* Fig. 23, *Eragrostis gummiflua,* K-PS-PCK, *Ellis* 262, ×640. Fig. 24, *Eragrostis plana,* K-PS-NAD, *Ellis* 277, ×640. Fig. 25, *Eragrostis atrovirens,* K-PS-PCK, *Ellis* 154, ×400. Fig. 26, *Eragrostis capensis,* K-PS-NAD, *Ellis* 27, ×400. Fig. 27, *Eragrostis barrelieri,* K-PS-NAD, *Ellis* 581, ×400. Fig. 28, *Eragrostis planiculmis,* K-PS-NAD but the Kranz cells are rounded and the chloroplasts are not typical of this type, *Ellis* 298, ×640. Fig. 29, *Eragrostis racemosa,* K-PS-PCK but somewhat intermediate in chloroplast structure, *Ellis* 231, ×640

FIG. 23–29 *Variasie in die genus* Eragrostis. *Fig.* 23, Eragrostis gummiflua, *K-PS-PCK,* Ellis 262, ×640. *Fig.* 24, Eragrostis plana, *K-PS-NAD,* Ellis 277, ×640. *Fig.* 25, Eragrostis atrovirens, *K-PS-PCK,* Ellis 154×400. *Fig.* 26, Eragrostis capensis, *K-PS-NAD,* Ellis 27, ×400. *Fig.* 27, Eragrostis barrelieri, *K-PS-NAD,* Ellis 581, ×400. *Fig.* 28, Eragrostis planiculmis, *K-PS-NAD maar die Kranzselle is rond en die chloroplaste is nie tipies van hierdie tipe nie,* Ellis 298×640. *Fig.* 29, Eragrostis racemosa, *K-PS-PCK maar effens intermediêr in chloroplaststruktuur,* Ellis 231, ×640

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structural studies have examined only chloridoid-eragrostoid structure in this genus (Hattersley & Watson, 1976; Carolin *et al.*, 1973; Johnson & Brown, 1973), with chloroplast position not being noted. However, in this genus alone in this sub-family there is variation between the NAD-ME-type and the PCK-type with intermediates between the two. This intermediate condition was observed in 13 species, nine of which resembled NAD-ME-type anatomy more closely (Fig. 28) and four the PCK-type (Fig. 29). This genus obviously merits further biochemical study.

E. aethiopica *Chiov.* **K-PS-PCK(?)**

Displays somewhat intermediate anatomy with centripetally located chloroplasts having typical PCK-type structure. In addition the bundle sheath cells are rounded and not typical of the Eragrostideae (cf. *Diandrochloa*).

TRANSVAAL.—2231-AC (Pafuri), *Ellis* 568. -CA, *Ellis* 1893. 2431-AB (Acornhoek), *Ellis* 534; -BA, *Ellis* 528.

E. annulata *Rendle ex S. Elliot* **K-PS-NAD**

Distinct NAD-type anatomy

S.W.A.—2518-CA (Tses), *Ellis* 921. 2818-AC (Warmbad), *Ellis* 896. CAPE.—2921-AC (Kenhardt), *Ellis* 874.

E. atrovirens (*Desf.*) *Trin. ex Steud.* (Fig. 25) **K-PS-PCK**

Chloroplasts centrifugal and bundle sheath cells large, differing in size and shape with adaxial and lower lateral cells being greatly elongated.

RHODESIA.—2131-BC (Triangle), *Ellis* 1304. S.W.A.—1723-CD (Singalamwe), *Ellis* 313, 319. 1823-BC (Siambisso), *Ellis* 334, 335. TRANSVAAL.—2528-CA (Pretoria), *Ellis* 406; -CB, *Ellis* 154, 1790, 1793.

E. barbinodis *Hack.* **K-PS-NAD**

TRANSVAAL.—2430-DA (Pilgrim's Rest), *Ellis* 1574. 2528-AC (Pretoria), *Ellis* 1353; -CA, *Ellis* 1749; -CB, *Ellis* 491, 760.

E. barrelieri *Dav.* (Fig. 27) **K-PS-NAD**

TRANSVAAL.—2231-CA (Pafuri), *Ellis* 546. 2528-CA (Pretoria), *Ellis* 490, 581, 771.

E. bergiana (*Kunth*) *Trin.* **K-PS-NAD**

CAPE.—3023-DB (Britstown), *de Winter* 3595.

E. bicolor *Nees* **K-PS-PCK(?)**

Similar to *E. aethiopica* with intermediate anatomy.

TRANSVAAL.—2229-AA (Waterpoort), *Ellis* 1319.

E. biflora *Hack. ex Schinz.* **K-PS-PCK**

Characteristic PCK-type anatomy.

S.W.A.—2118-DB (Steinhausen), *Ellis* 2034. 2718-CB (Grünau), *Ellis* 889. TRANSVAAL.—2428-DA (Nylstroom), *Ellis* 1610. 2431-DB (Acornhoek), *Ellis* 517. 2528-CA (Pretoria), *Ellis* 429.

E. brizantha *Nees* **K-PS-NAD**

S.W.A.—2518-CA (Tses), *Ellis* 910.

E. caesia *Stapf* **K-PS-PCK (?)**

Somewhat atypical as chloroplasts not distinctly centrifugal in arrangement—may be peripheral or even centripetally concentrated.

TRANSVAAL.—2530-BA (Lydenburg), *Ellis* 441, 443. NATAL.—2829-CC (Harrismith), *Ellis* 1400.

E. capensis (*Thunb.*) *Trin.* (Fig. 26) **K-PS-NAD**

$\delta^{13}C = -12,2‰$. (Vogel, unpublished). Conspicuous bundle sheath with centripetal chloroplasts.

TRANSVAAL.—2528-CA (Pretoria), *Ellis* 11, 27, 372; -CB *Ellis* 155. 2531-BB (Komatipoort), *Ellis* 502. NATAL.—2729-BD (Volksrust), *Ellis* 29. 2929-BB (Underberg), *Ellis* 46. 2930-AC (Pietermaritzburg), *Ellis* 59. CAPE.—3028-BA (Matatiele), *Ellis* 242. 3227-DB (Stutterheim), *Ellis* 284.

E. chapellieri (*Kunth*) *Nees* **K-PS-PCK**

Typical PCK-type anatomy cf. *E. atrovirens.*

RHODESIA.—1832-CA (Rusape), *Ellis* 816.

E. chloromelas *Steud.* **K-PS-NAD (?)**

Intermediate chloroplast position and bundle sheath cell structure.

TRANSVAAL.—2528-CA (Pretoria), *Ellis* 12, 25, 752; -CB, *Ellis* 123, 137. CAPE.—3028-BD (Matatiele), *Ellis* 250.

E. cillianensis (*All.*) *Lutatt* **K-PS-NAD**

This is a C_4 species ($\delta^{13}C = -13,3‰$, Bender, 1971), has a double bundle sheath (Hattersley & Watson, 1976) and has centripetal chloroplasts and a predominance of NAD-ME (Guttierrez *et al.*, 1974, 1974a). This agrees with the anatomical findings of this study and this species does not show the intermediate condition of some other species cf. *E. aethiopica, E. caesia* and *E. chloromelas* above.

S.W.A.—2718-CB (Grünau), *Ellis* 888. TRANSVAAL.—2231-AC (Pafuri), *Ellis* 569; -CC, *Ellis* 1880. 2431-BA (Acornhoek), *Ellis* 527; -DD, *Ellis* 107. 2528-AC (Pretoria), *Ellis* 1765.

E. ciliaris (*L.*) *R. Br.* **K-PS-PCK**

Characteristic PCK-type structure.

RHODESIA.—1629-AA (Chirundu), *Ellis* 576. NATAL.—3030-CD (Port Shepstone), *Ellis* 1474.

E. crassinervis *Hack.* **K-PS-NAD**

TRANSVAAL.—2231-AC (Pafuri), *Ellis* 567.

E. curvula (*Schrad.*) *Nees* **K-PS-NAD (?)**

This species has been demonstrated to be C_4 ($\delta^{13}C = -13,4‰$, Vogel, unpublished), to have a double bundle sheath and chloridoid-eragrostoid anatomy (Hattersley & Watson, 1976), to show a PIB (Brown & Gracen, 1972), to have centripetal chloroplasts with well-developed grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a) and it is a NAD-ME-type species (Hatch & Kagawa, 1974; Gutierrez *et al.*, 1974, 1974a; Hatch, Kagawa & Craig, 1975).

Observations of this study show this species to be very variable in the characters examined and although some specimens would be grouped as NAD-ME-types on their chloroplast location and sheath structure, others definitely belong with those species grouped tentatively as PCK. In these specimens the chloroplasts are irregularly distributed in the bundle sheath cells which exhibit more variation in size and shape than do the NAD-ME-type specimens. Further study of this complex species promises to be rewarding.

TRANSVAAL.—2527-CA (Rustenburg), *Ellis* 350. 2528-CB (Pretoria), *Ellis* 1787; -CC, *Ellis* 722. 2530-AC (Lydenburg), *Ellis* 87; -BA, *Ellis* 447. O.F.S.—2827-DD (Senekal), *Ellis* 942, 943, 987. NATAL.—2729-BD (Volksrust), *Ellis* 33. 2829-CC (Harrismith), *Ellis* 1438. 2929-BB (Underberg), *Ellis* 45, 49. CAPE.—2921-BD (Kenhardt), *Ellis* 860. 3227-AD (Stutterheim), *Ellis* 307.

E. cylindriflora *Hochst.* **K-PS-NAD**

Characteristic NAD-ME-type structure.

RHODESIA.—1629-AA (Chirundu), *Ellis* 572. TRANSVAAL.—2231-AC (Pafuri), *Ellis* 548. 2431-DD (Acornhoek), *Ellis* 115.

E. cyperoides (*Thunb.*) *Beauv.* **K-PS-NAD**

$\delta^{13}C = -13,1‰$ (Vogel). Mestome sheath cells unusually large but Kranz cells typically NAD-ME-type.

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CAPE.—2816–DA (Oranjemund), *Ellis* 2172. 3017 (Hondeklip Bay), *Ellis* 2152. 3218–AB (Clanwilliam), *Ellis* 1168; –BB, *Ellis* 1690. 3318–AC (Cape Town), *Ellis* 1687.

E. echinochloidea *Stapf* **K-PS-NAD**

Chloroplasts distinctly centripetal but Kranz cells tend to be slightly more inflated than is usual.

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2010. 2429–AA (Zebediela), *Ellis* 1607. CAPE.—2921–BD (Kenhardt), *Ellis* 868. 2922–DC (Prieska), *Ellis* 858. 3018–CC (Kamiesberg), *Ellis* 2207.

E. elatior *Stapf* **K-PS-PCK**

NATAL.—3030–CD (Port Shepstone), *Ellis* 1473, 1475.

E. gummiflua *Nees* (Fig. 23) **K-PS-PCK**

Typical PCK-type structure of the bundle sheath cells.

TRANSVAAL.—2431–DB (Acornhoek), *Ellis* 515. 2528–CA (Pretoria), *Ellis* 1335; –CB, *Ellis* 156. 2628–AA (Johannesburg), *Ellis* 1378. O.F.S.—2827–DD (Senekal), *Ellis* 939. CAPE.—3028–CB (Matatiele), *Ellis* 262.

E. heteromera *Stapf* **K-PS-NAD (?)**

Somewhat intermediate structure with chloroplast position variable and bundle sheath cells distinctly inflated.

TRANSVAAL.—2231–CB (Pafuri), *Ellis* 1897. 2429–AA (Zebediela), *Ellis* 2087. 2527–DD (Rustenburg), *Ellis* 430. 2528–CA (Pretoria), *Ellis* 131. 2627–BB (Potchefstroom), *Ellis* 1499.

E. hispida *K. Shum.* **K-PS-PCK**

RHODESIA.—1832–CA (Rusape), *Ellis* 821.

E. homomalla *Nees* **K-PS-NAD**

Chloroplasts distinctly centripetal but bundle sheath cells slightly more rounded than is typical of NAD-ME-type anatomy.

S.W.A.—2416–AB (Maltahöhe), *Ellis* 936.

E. lappula *Nees* var. **divaricata** *Stapf* **K-PS-PCK**

Elongated adaxial and lateral bundle sheath cells with centrifugal chloroplasts. Typical of the Eragrostis PCK-type anatomy which characteristically has adaxial bundle sheath extensions of Kranz cells.

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2014. 2528–CA (Pretoria), *Ellis* 1340; –CD, *Ellis* 1366. 2628–AA (Johannesburg), *Ellis* 1377.

E. lappula *Nees* var. **lappula** **K-PS-PCK**

RHODESIA.—1832–CC (Rusape), *Ellis* 815. S.W.A.—1823–BC (Siambisso), *Ellis* 333. TRANSVAAL.—2529–AD (Witbank), *Ellis* 2069.

E. lehmanniana *Nees* **K-PS-NAD**

Definite NAD-ME type configuration of bundle sheath cells and chloroplasts. This contrasts with the variable condition in its close relative *E. curvula*.

TRANSVAAL.—2231–CB (Pafuri), *Ellis* 549. 2528–CA (Pretoria), *Ellis* 1328, 1747. O.F.S.—3026–BD (Aliwal North), *Ellis* 2094. CAPE.—2824–DC (Kimberley), *Ellis* 840. 2921–BD (Kenhardt), *Ellis* 867.

E. nindensis *Fical. & Hiern.* **K-PS-NAD**

Typical NAD-ME-type chloroplast location and structure although they may occupy the whole of the bundle sheath cell lumens.

RHODESIA.—1832–CA (Rusape), *Ellis* 818. 1731–CA (Salisbury), *Ellis* 797. S.W.A.—2718–CB (Grünau), *Ellis* 900, 901. TRANSVAAL.—2528–DA (Pretoria), *Ellis* 1805. 2530–BD (Lydenburg), *Ellis* 1559.

E. obtusa *Munro ex Fical. & Hiern.* **K-PS-NAD**

Characteristic NAD-ME-type structure.

TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 201. O.F.S.—2827–DC (Senekal), *Ellis* 982. 3026–BD (Aliwal North), *Ellis* 2093. CAPE.—3323–BB (Willowmore), *Ellis* 1626.

E. pallens *Hack.* **K-PS-NAD**

Structure typically NAD-ME-type.

TRANSVAAL.—2428–DA (Nylstroom), *Ellis* 1611. 2529–AD (Witbank) *Ellis* 2075.

E. paradoxa *Launert* **K-PS-NAD**

RHODESIA.—1731–CA (Salisbury), *Ellis* 809.

E. patens *Oliv.* **K-PS-PCK**

Characteristically NAD-ME-type.

RHODESIA.—1731–CA (Salisbury), *Ellis* 808.

E. patentissima *Hack.* **K-PS-PCK**

Centrifugal chloroplasts and inflated bundle sheath cells. Bundle sheath extensions present.

TRANSVAAL.—2530–BA (Lydenburg), *Ellis* 440. 2630–AC (Carolina), *Ellis* 1530; –CA, *Ellis* 1847.

E. pilosa (*L.*) *Beauv.* **K-PS-NAD (?)**

This species is a C_4 species as shown by a low CO_2 compensation point (Downton & Tregunna, 1968). Chloroplasts and bundle sheath cell structure somewhat intermediate.

TRANSVAAL.—2629–AD (Bethal), *Ellis* 1836; –BC, *Ellis* 1844.

E. plana *Nees* (Fig. 24) **K-PS-NAD**

Predicted to be a NAD-ME-type species.

TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1360; –CA, *Ellis* 775. CAPE.—2929–DD (Underberg), *Ellis* 224. 3028–BD (Matatiele), *Ellis* 248. 3228–BC (Butterworth), *Ellis* 277, 280.

E. planiculmis *Nees* (Fig. 28) **K-PS-NAD (?)**

Variable, similar to *E. curvula* in structure of bundle sheath cells and chloroplasts.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 153. CAPE.—3227–DA (Stutterheim), *Ellis* 298.

E. porosa *Nees* **K-PS-NAD**

S.W.A.—2217–CA (Windhoek), *Ellis* 923. 2818–AC (Warmbad), *Ellis* 894. CAPE.—2921–BD (Kenhardt), *Ellis* 871.

E, procumbens *Nees* **K-PS-NAD**

CAPE.—2921–BD (Kenhardt), *Ellis* 866.

E. pseudo-obtusa *de Wint.* **K-PS-NAD**

Typical NAD-ME-type bundle sheath and chloroplast position.

CAPE.—2824–DC (Kimberley), *Ellis* 838, 839.

E. pseudoscleranthe *Chiov.* **K-PS-NAD**

Distinctly NAD-ME type although chloroplasts may entirely fill bundle sheath cells.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1776. 2730–AB (Vryheid), *Ellis* 1517. NATAL.—2729–DB (Volksrust), *Ellis* 37.

E. racemosa (*Thunb.*) *Steud.* (Fig. 29) **K-PS-PCK (?)**

Although assigned to the PCK-type group this species does not resemble the other PCK-type *Eragrostis* in bundle sheath cell structure. In this respect this species resembles the variable, intermediate species such as *E. curvula*. The chloroplasts, however, are always definitely centrifugal.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 4, 26, 369; –CB *Ellis* 135, 1791; –DA, *Ellis* 1806. NATAL.—2729–BD (Volksrust), *Ellis* 31. CAPE.—3028–BA (Matatiele), *Ellis* 231. 3029–BA (Kokstad), *Ellis* 221. 3227–AD (Stutterheim), *Ellis* 306.

E. rigidior *Pilg.* **K-PS-NAD**

Typical structure of the NAD-ME-type.

RHODESIA.—1629–AA (Chirundu), *Ellis* 571. TRANSVAAL.—2230–DB (Messina), *Ellis* 1927. 2231–AC (Pafuri), *Ellis* 544. 2528–CA (Pretoria), *Ellis* 783; –CC, *Ellis* 1347.

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E. rotifer *Rendle* **K-PS-NAD (?)**

Intermediate type anatomy with PCK-type chloroplasts centripetally arranged in somewhat inflated bundle sheath cells.

RHODESIA.—2131–BC (Triangle), *Ellis* 1306. S.W.A.—2118–DB (Steinhausen), *Ellis* 2018. 2217–CA (Windhoek), *Ellis* 992. TRANSVAAL.—2531–BB (Komatipoort), *Ellis* 496.

E. sarmentosa (*Thunb.*) *Trin.* **K-PS-PCK**

PCK-type anatomy with two horizontally elongated bundle sheath cells abaxially situated.

S.W.A.—1723–CD (Singalamwe), *Ellis* 322. CAPE.—3320–DC (Montagu), *Ellis* 654. 3421–BB (Riversdale), *Ellis* 632.

E. sclerantha *Nees* **K-PS-NAD (?)**

A variable species. Some specimens with typical centripetal chloroplasts with radial striations, others with centripetal chloroplasts of the PCK-type and others with centrifugal chloroplasts. In bundle sheath cell structure this species closely resembles *E. racemosa*.

TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1864. 2527–DB (Rustenburg), *Ellis* 1224. 2528–AC (Pretoria), *Ellis* 1361. 2530–BA (Lydenburg), *Ellis* 445, 461. 2630–AC (Carolina), *Ellis* 1533.

E. spinosa (*L.f.*) *Trin.* **K-PS-NAD**

Distinctly NAD-ME-type in anatomical structure.

S.W.A.—2315–CA (Rostock), *Ellis* 1125. CAPE.—3119–CC (Calvinia), *Ellis* 1716.

E. stapfii *de Wint.* **K-PS-NAD**

NAD-ME type anatomy.

TRANSVAAL.—2527–CD (Rustenburg), *Ellis* 493. 2528–AC (Pretoria), *Ellis* 1352. 2630–AC (Carolina), *Ellis* 1531.

E. superba *Peyr.* **K-PS-NAD**

Probably the most characteristic NAD-ME anatomy of all the *Eragrostis* species examined.

S.W.A.—1823–BC (Siambisso), *Ellis* 339. 2118–DB (Steinhausen), *Ellis* 2020. TRANSVAAL.—2430–DA (Pilgrim's Rest), *Ellis* 1572. 2431–DD (Acornhoek), *Ellis* 96, 108, 112. 2528–BC (Pretoria), *Ellis* 1350. 2531–CB (Komatipoort), *Ellis* 1553. NATAL.—2832–AC (Mtubatuba), *Ellis* 82.

E. tef (*Zucc.*) *Trotter* **K-PS-NAD (?)**

The grouping of this species is uncertain as the chloroplasts tend to be peripherally arranged and the bundle sheath cells are inflated.

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2011. O.F.S.—2827–DC (Senekal), *Ellis* 961.

E. trichophora *Coss. & Dur.* **K-PS-NAD**

Typical NAD-ME-type anatomy.

TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1354, 1760; –CA, *Ellis* 405, 782. O.F.S.—2827–DD (Senekal), *Ellis* 945.

E. truncata *Hack.* **K-PS-NAD**

CAPE.—2923–BC (Douglas), *Ellis* 855.

E. uniglumis *Hack.* **K-PS-PCK**

Typical of the PCK-type *Eragrostis* species.

TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1356. 2529–AD (Witbank) *Ellis* 2073.

E. virescens *Presl.* **K-PS-NAD (?)**

Chloroplasts often entirely filling, or located on the periphery, of somewhat inflated bundle sheath cells cf. *E. curvula*.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 422, 758, 759, 772.

E. viscosa (*Retz.*) *Trin.* **K-PS-PCK**

PCK-type anatomy.

TRANSVAAL.—2431–BA (Acornhoek), *Ellis* 530.

LEPTOCARYDION *Hochst. ex Stapf*

L. vulpiastrum (*De Not.*) *Stapf* **K-PS-NAD**

Reported as having a single bundle sheath (Hattersley & Watson, 1976) from the data of Metcalfe (1960). This is not in agreement with the observations in the present study. Typical NAD-ME-type anatomy.

TRANSVAAL.—2430–BD (Pilgrims Rest), *Ellis* 1583. 2529–AD (Witbank), *Ellis* 2076, 2077.

LEPTOCHLOA *Beauv.*

Various species of this genus have been studied and all show expected NAD-ME-type characteristics cf. double bundle sheath (Hattersley & Watson, 1976), eragrostoid type anatomy and ultrastructure (Carolin *et al.*, 1973), PIB (Brown & Gracen, 1972), chloroplasts centripetal with well developed grana (Gutierrez *et al.*, 1974, 1974a; Brown & Gracen, 1972), and have been biochemically typed as NAD-ME species (Gutierrez *et al.*, 1974, 1974a).

L. panicea (*Retz.*) *Ohwi.* **K-PS-NAD**

Distinct NAD-ME-type anatomy although adaxial sheath cells of primary bundles are enlarged and colourless.

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 556, 1898, 1899. 2431–DB (Acornhoek), *Ellis* 519.

L. uniflora *Hochst. ex A. Rich.* **K-PS-NAD**

Using Metcalfe's (1960) data, Hattersley & Watson (1976) state that this species has a single sheath. This disagrees with the present observations. An extremely thin leaf in which only a single sheath cell on either side of the bundle possesses chloroplasts.

TRANSVAAL.—2230–DB (Messina), *Ellis* 1928. 2231–AC (Pafuri), *Ellis* 565.

LOPHACHME *Stapf*

L. digitata *Stapf* **K-PS-NAD**

TRANSVAAL.—2428–AC (Nylstroom), *Codd* 974. 2528–AC (Pretoria), *Codd* 3693; –DA, *Schweickerdt* 1828. 2529–CD (Witbank), *Codd* 10108.

ODYSSEA *Stapf*

O. paucinervis (*Nees*) *Stapf* **K-PS-NAD**

Typical NAD-ME-type structure.

S.W.A.—2214–DA (Swakopmund), *Ellis* 930. CAPE.—3218–AB (Clanwilliam), *Ellis* 1166, 1689.

POGONARTHRIA *Stapf*

P. squarrosa (*Licht.*) *Pilg.* **K-PS-NAD**

Possesses a double bundle sheath (Hattersley & Watson, 1976) and has distinct centripetal chloroplasts.

RHODESIA.—1731–CA (Salisbury), *Ellis* 801. TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 190. 2429–AA (Zebediela), *Ellis* 2042, 2043. 2431–DD (Acornhoek) *Ellis* 105. 2528–CA (Pretoria), *Ellis* 424; –CB, *Ellis* 1780. O.F.S.—2827–DC (Senekal), *Ellis* 955.

STIBURUS *Stapf*

The species of this genus have chloroplasts evenly distributed in the bundle sheath cells. These cells are rounded in transectional view. Although not typical PCK-type this genus has been referred to this category but further study is needed.

S. alopecuroides (*Hack.*) *Stapf* **K-PS-PCK**

The mestome sheath cells are extremely thickened and often two layers are present.

TRANSVAAL.—2430–DD (Pilgrim's Rest), *Ellis* 1569. 2529–CD (Witbank), *Ellis* 492. 2530–BA (Lydenburg), *Ellis* 437; –BD *Ellis* 1566.

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S. conrathii *Hack.* **K-PS-PCK**

TRANSVAAL.—2430-DC (Pilgrim's Rest), *de Winter* 9399.

TETRACHNE *Nees*

T. dregei *Nees* **K-PS-NAD**

Conspicuous, regular Kranz sheath with centripetal chloroplasts.

O.F.S.—2827-DD (Senekal), *Ellis* 947. 3026-AB (Aliwal North), *Ellis* 2604. CAPE.—3126-BB (Queenstown), *Ellis* 2602.

THICHONEURA *Anderss.*

T. eleusinoides (*Rendle*) *Ekman* **K-PS-NAD**

Typical NAD-ME-type anatomy.

TRANSVAAL.—2229-DD (Waterpoort), *Ellis* 1945, 1946, 1948.

T. grandiglumis (*Nees*) *Ekman* **K-PS-NAD**

Conspicuous Kranz sheath with centripetal chloroplasts.

TRANSVAAL.—2431-DD (Acornhoek), *Ellis* 101, 512. 2528-CA (Pretoria), *Ellis* 748; -CB, *Ellis* 1795. O.F.S.—2827-DC (Senekal), *Ellis* 953, 977.

TRIPOGON *Roem. & Schult.*

T. loliiformis has a double bundle sheath (Hattersley & Watson, 1976).

T. minimus (*A. Rich.*) *Hochst. ex Steud.* **K-PS-NAD**

RHODESIA.—1832-CA (Umtali), *Ellis* 819. 1930-DA (Umvuma), *Ellis* 792.

TRIBE SPOROBOLEAE

Sporobolus is the only S. African genus assigned to this tribe. All *Sporobolus* species mentioned in the literature are Kranz with typical C_4 ultrastructure (Johnson & Brown, 1973) and have a double bundle sheath (Hattersley & Watson, 1976) and are thus, most likely to be, aspartate formers. However, within this category there is variation, e.g. *S. airoides* and *S. cryptandrus*, have been shown to be NAD-ME-type species with centripetal chloroplasts and *S. poiretti* is a PCK-species with centrifugal chloroplasts. (Gutierrez *et al.*, 1974, 1974a). Brown & Gracen (1972) report no PIB for *S. poiretti* which is not as expected for an aspartate former.

The S. African *Sporobolus* species examined in this study can also be grouped as NAD-ME-type (7 species) or PCK species (10 species) based on anatomical criteria only.

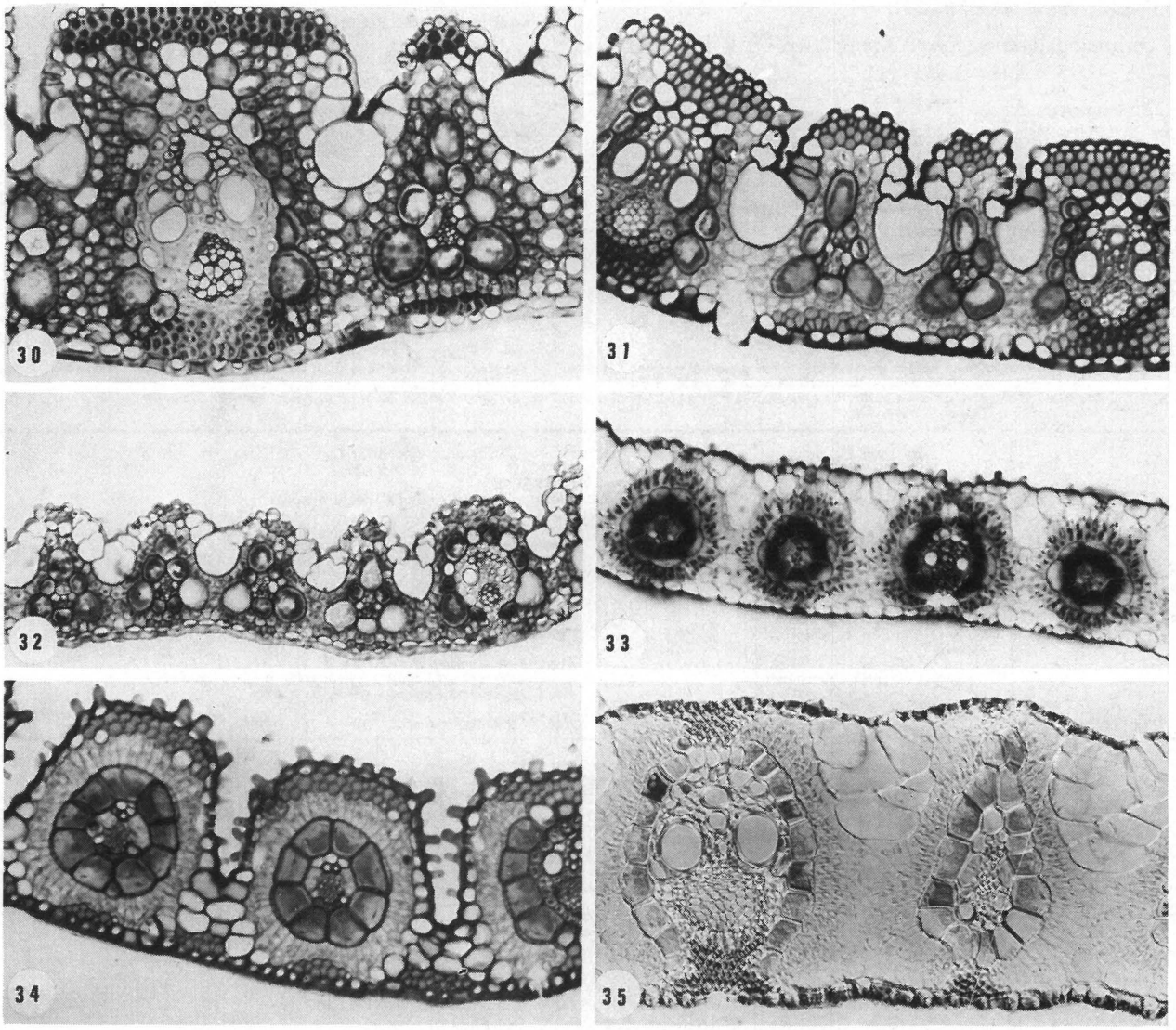


FIG. 30–35 The genus *Sporobolus* with chloroplasts either centrifugal or centripetal. Fig. 30–31, *Sporobolus africanus*, K-PS-PCK. Fig. 30, *Ellis* 278, ×640. Fig. 31, *Ellis* 263, ×640. Fig. 32, *Sporobolus pyramidalis*, K-PS-PCK, *Ellis* 289, ×640. Fig. 33, *Sporobolus coromandelianus*, K-PS-NAD, *Ellis* 912, ×400. Fig. 34, *Sporobolus virginicus*, K-PS-NAD, *Ellis* 265, ×640. Fig. 35, *Sporobolus consimilis*, K-PS-NAD, *Ellis* 531, ×400

FIG. 30–35 *Die genus* Sporobolus *met chloroplaste òf centrifugaal òf centripetaal. Fig.* 30–31, Sporobolus africanus, *K-PS-PCK. Fig.* 30, Ellis 278, ×640. *Fig.* 31, Ellis 263, ×640. *Fig.* 32, Sporobolus *p*yramidalis, *K-PS-PCK*, Ellis 289, ×640. *Fig.* 33, Sporobolus coromandelianus, *K-PS-NAD*, Ellis 912, ×400. *Fig.* 34, Sporobolus virginicus, *K-PS-NAD*, Ellis 265, ×640. *Fig.* 35, Sporobolus consimilis, *K-PS-NAD*, Ellis 531, ×400

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SPOROBOLUS *R.Br.*

S. africanus (*Poir.*) *Robyns & Tournay* (Fig. 30, 31) **K-PS-PCK**

Typical PCK-type structure with large inflated bundle sheath cells with centrifugal chloroplasts.

S.W.A.—1724–AD (Katima Mulilo), *Ellis* 345. TRANSVAAL.—2527–CA (Rustenburg), *Ellis* 354. 2531–CC (Komatipoort), *Ellis* 403. O.F.S.—2827–DC (Senekal), *Ellis* 983. NATAL.—2929–DD (Underberg), *Ellis* 209. CAPE.—3028–CB (Matatiele), *Ellis* 263. 3228–BC (Butterworth), *Ellis* 278.

S. centrifugus *Nees* **K-PS-PCK**

Typical PCK-type structure.

TRANSVAAL.—2530–BA (Lydenburg), *Ellis* 446. NATAL.—2829–CC (Harrismith), *Ellis* 1418, 1426. CAPE.—3227–CA (Stutterheim), *Ellis* 1491.

S. conrathii *Chiov.* **K-PS-PCK**

TRANSVAAL.—2528–CB (Pretoria), *Ellis* 1797.

S. consimilis *Fresen.* (Fig. 35) **K-PS-NAD**

Conspicuous bundle sheath with centripetal chloroplasts.

S.W.A.—2214–DA (Swakopmund), *Ellis* 934. TRANSVAAL.—2431–BA (Acornhoek), *Ellis* 531.

S. coromandeliamus (*Retz.*) *Kunth* (Fig.33) **K-PS-NAD**

S.W.A.—2518–CA (Tses), *Ellis* 912.

S. discosporus *Nees* **K-PS-NAD**

O.F.S.—2827–DC (Senekal), *Ellis* 981.

S. festivus *Hochst. ex A. Rich.* **K-PS-PCK**

PCK-type chloroplasts and with Kranz cell extensions of the bundle sheath.

RHODESIA.—1731–CC (Salisbury), *Ellis* 811. TRANSVAAL.—2528–AB (Pretoria), *van Heerden* 46.

S. fimbriatus *Nees* var. **fimbriatus** **K-PS-PCK**

This species has been biochemically typed as PCK-type (Hatch & Kagawa, 1974; Hatch, Kagawa & Craig, 1975) which confirms the predictions of this study.

TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 202.

S. fimbriatus *Nees* var. **latifolius** *Stent* **K-PS-PCK**

RHODESIA.—2030–DA (Fort Victoria), *Ellis* 794, 795.

S. lampranthus *Pilg.* **K-PS-PCK**

S.W.A.—2718–CB (Grünau), *Ellis* 902.

S. ludwigii *Hochst.* **K-PS-NAD**

CAPE.—2824–CD (Kimberley), *Ellis* 850

S. nitens *Stent* **K-PS-NAD**

Conspicuous, regular bundle sheaths with centripetal chloroplasts.

RHODESIA.—2030–DA (Fort Victoria), *Ellis* 827. TRANSVAAL.—2430–DA (Pilgrim's Rest), *Ellis* 1571. 2431–DA (Acornhoek), *Ellis* 516. 2527–DB (Rustenburg), *Ellis* 1222. 2528–CC (Pretoria), *Ellis* 1343. 2531–CB (Komatipoort), *Ellis* 1552.

S. panicoides *A. Rich.* **K-PS-PCK**

Bundle sheath cells, large rounded and varying in size with centrifugal chloroplasts.

RHODESIA.—1920–BA (Umtali), *Ellis* 822. TRANSVAAL.—2230–DB (Messina), *Ellis* 1929, 1930. 2428–CD (Nylstroom), *Ellis* 191.

S. pectinatus *Hack.* **K-PS-PCK**

Typical PCK-type anatomy.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1241, 1330; –DA *Ellis* 1804.

TABLE 2 The Southern African genera of the Panicoideae grouped into the categories of the Kranz syndrome based on the anatomy of the leaf blade in transverse section. The number of species examined in each genus is given

TABEL 2 *Die Suid-Afrikaanse genera van die Penicoideae in klasse gegroepeer op grond van die anatomie van die blaar in dwarsdeursnit. Die aantal spesies wat in elke genus ondersoek is, word aangedui*

n-K or/*of* C_3 NON-KRANZ	K or/*of* C_4 KRANZ	
C_3 SPECIES	C_4 SPECIES	
No chlorenchymatous bundle sheath	Chlorenchymatous bundle sheath present	
Kranz sheath absent	Kranz sheath present	
No specialized chloroplasts	Specialized chloroplasts in bundle sheath cells	
High CO_2 compensation point—37–50 ppm.	Low CO_2 compensation concentration—less than 5 ppm.	
Low $^{13}C/^{12}C$ ratios i.e. δ = —23 to —32‰	High $^{13}C/^{12}C$ ratios i.e. δ = —9 to —16‰	
C_3 SPESIES	*C_4 SPESIES*	
Chlorenchiematiese bondelskede afwesig	*Chlorenchiematiese bondelskede teenwoordig*	
Kranzskede afwesig	*Kranzskede teenwoordig*	
Geen gespesialiseerde chloroplaste	*Gespesialiseerde chloroplaste teenwoordig in bondelskedeselle*	
Hoë CO_2-kompensasie punt—37–50 dpm.	*Lae CO_2-kompensasiepunt—minder as 5 dpm*	
Lae $^{13}C/^{12}C$ verhouding d.i. δ = —23 tot —32‰	*Hoë $^{13}C/^{12}C$ verhouding d.i. δ = —9 tot —16‰*	
	MS	PS
	KRANZ MESTOME SHEATH	KRANZ PARENCHYMA SHEATH
Double bundle sheath (XYMS+)	Single bundle sheath (XYMS—)	Double bundle sheath (XYMS+) around larger bundles
	MALATE FORMERS	ASPARTATE FORMERS
Post illumination CO_2 burst—oxygen sensitive	No post illumination CO_2 burst—oxygen-insensitive	Post illumination CO_2 burst (PIB)— oxygen-insensitive
Festucoid (Pooid) anatomy	Eupanicoid (Panicoid) anatomy	Chloridoid (Eragrostoid) anatomy
Dubbel bondelskede (XYMS+)	*KRANZ-MESTOOMSKEDE*	*KRANZ-PARENCHIEMSKEDE*
	Enkel bondelskede (XYMS—)	*Dubbel bondelskede (XYMS+) om groter bondels*
	APPELSUUR PRODUSEERDERS	*ASPARTIENSUUR PRODUSEERDERS*
Na beligting CO_2 vrystelling—suurstof sensitief	*Geen na-beligting CO_2 vrystelling—suurstof onsensitief*	*Na-beligting CO_2 vrystelling (PIB)—suurstof onsensitief*
Festucoid (Pooid) anatomie	*Eupanicoid (Panicoid) anatomie*	*Chloridoid (Eragrostoid) anatomie*
	NADP CENTRIFUGAL CHLOROPLASTS	PCK CENTRIFUGAL CHLOROPLASTS / NAD CENTRIPETAL CHLOROPLASTS
	NADP-ME type species	PCK type species / NAD-ME SPECIES
		PEP-ck species
	Grana deficient or absent	Grana developed
	Mitochondria infrequent	Mitochondria frequent
		Mitochondria centrifugal / Mitochondria centripetal
	SENTRIFUGALE CHLOROPLASTE	*SENTRIFUGALE CHLOROPLASTE* / *SENTRIPETALE CHLOROPLASTE*
	NADP-ME tipe spesies	*PCK tipe spesies* / *NAD-ME spesies*
		PEP-ck spesies
	Grana skaars of afwesig	*Grana ontwikkel* / *Grana ontwikkel*
	Mitochondria seldsaam	*Mitochondria volop* / *Mitochondria volop*
		Mitochondria centrifugaal / *Mitochondria centripetaal*

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TABLE 2 (*continued*)/ TABEL 2 (*vervolg*)

Tribe/*Tribus*							
ARUNDINELLEAE			Arundinella	1 sp			
			Danthoniopsis	3 spp			
			Loudetia*	3 spp			
			Loudetiopsis	1 sp			
			Trichopteryx	1 sp			
			Tristachya	4 spp			
Tribe/*Tribus*	Acroceras	1 sp					
PANICEAE	*Alloteropsis semialata*		*Alloteropsis semialata*				
			Alloteropsis	2 spp	*Alloteropsis*	2 spp	
			Anthephora	2 spp	*Brachiaria*	11 sp	*Brachiaria* 1 sp
			Cenchrus	1 sp			
			Cymbosetaria	1 sp			
			Digitaria	30 spp	*Digitaria*	2 spp	
			Echinochloa	5 spp			
					Eriochloa	2 spp	
					Leucophrys	1 sp	
					Melinis	1 sp	
	Oplismenus	1 sp					
					Oryzidium	1 sp	
	Panicum	8 spp			*Panicum*	8 spp	*Panicum* 4 spp
			Paspalidium	1 sp			
			Paspalum	7 spp			
			Pennisetum	8 spp			
	Pseudechinolaena	1 sp					
					Pseudobrachiaria	1 sp	
					Psilochloa	1 sp	
					Rhynchelytrum	6 spp	
	Sacciolepis	1 sp					
			Setaria	13 spp			
			Stenotaphrum	1 sp			
			Stereochlaena	1 sp			
			Tarigidia	1 sp			
					Tricholaena	1 sp	
					Urochloa	6 spp	
Tribe/*Tribus*							
MAYDEAE			Coix*	1 sp			
			Zea*	1 sp			
Tribe/*Tribus*							
ANDROPOGONEAE			Andropogon	10 spp			
			Bothriochloa	3 spp			
			Chrysopogon	1 sp			
			Cymbopogon	4 spp			
			Dichanthium	2 spp			
			Diheteropogon	2 spp			
			Elionurus	1 sp			
			Eriochrysis	1 sp			
			Eulalia	1 sp			
			Hemarthria	1 sp			
			Heteropogon	2 spp			
			Hyparrhenia	10 spp			
			Hyperthelia	1 sp			
			Imperata*	1 sp			
			Ischaemum	3 spp			
			Microstegium	1 sp			
			Miscanthidium*	4 spp			
			Monocymbium	1 sp			
			Pogonatherum	1 sp			
			Rottboellia	1 sp			
			Saccharum*	1 sp			
			Schizachyrium	3 spp			
			Sehima	1 sp			
			Sorghastrum	1 sp			
			Sorghum	3 spp			
			Themeda	1 sp			
			Trachypogon	1 sp			
			Urelytrum	1 sp			
			Vetiveria	1 sp			
			Vossia	1 sp			

* Variable MS condition: double sheath sometimes present around larger vascular bundles
* *Veranderlike MS toestand: dubbel skede soms om die groter vaskulêre bondels teenwoordig*

S. pyramidalis *Beauv.* (Fig. 32) **K-PS-PCK**

PCK-type anatomy with bundle sheath extensions.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 790. 2531–CA (Komatipoort), *Ellis* 1539. O.F.S.—2827–DC (Senekal), *Ellis* 951. CAPE.—2824–DC (Kimberley), *Ellis* 846. 3227–DB (Stutterheim), *Ellis* 289.

S. smutsii *Stent* **K-PS-NAD**

S.W.A.—1823–BC (Siambisso), *Ellis* 330. TRANSVAAL.—2331–AB (Phalaborwa), *Ellis* 533. 2430–DA (Pilgrim's Rest), *Ellis* 1573.

S. stapfianus *Grand.* **K-PS-PCK**

Adaxial and lateral bundle sheath cells larger and elongated.

TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 109. 2528–CA (Pretoria), *Ellis* 9, 777, 1233. 2531–CC (Komatipoort), *Ellis* 395.

S. virginicus (*L.*) *Kunth* (Fig. 34) **K-PS-NAD**

Typical NAD-ME-type anatomy. Ultrastructure and anatomy eragrostoid (Carolin *et al.*, 1973) but with no prominent osmophilic layer in the cell walls (cf. *Cynodon dactylon*).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1232 (cultivated). CAPE.—3218–AB (Clanwilliam), *Ellis* 1167. 3228–CC (Butterworth), *Ellis* 264, 265, 1109, 1115.

SUB-FAMILY PANICOIDEAE

There is considerably more variation in this sub-family than in the Eragrostoideae where all genera are C_4 with double bundle sheaths. The variation in the Panicoideae is restricted to the tribe Paniceae where C_3 and malate as well as aspartate formers are found. In fact all four known different pathways of photosynthesis in the Gramineae occur in this tribe. The Arundinelleae, Maydeae and Andropogoneae are consistent in having representatives with a single bundle sheath only, i.e. malate formers or NADP-ME types. This sheath is a Kranz sheath and is homologous with the mestome sheath (Brown, 1975) but in the other Kranz Panicoideae the Kranz sheath is homologous with the parenchyma sheath. Thus, within this sub-family there have been two origins of the Kranz sheath (Brown, 1975).

TRIBE ARUNDINELLEAE

All South African genera in this tribe have a single Kranz bundle sheath and are thus expected to be malate formers and NADP-ME type species.

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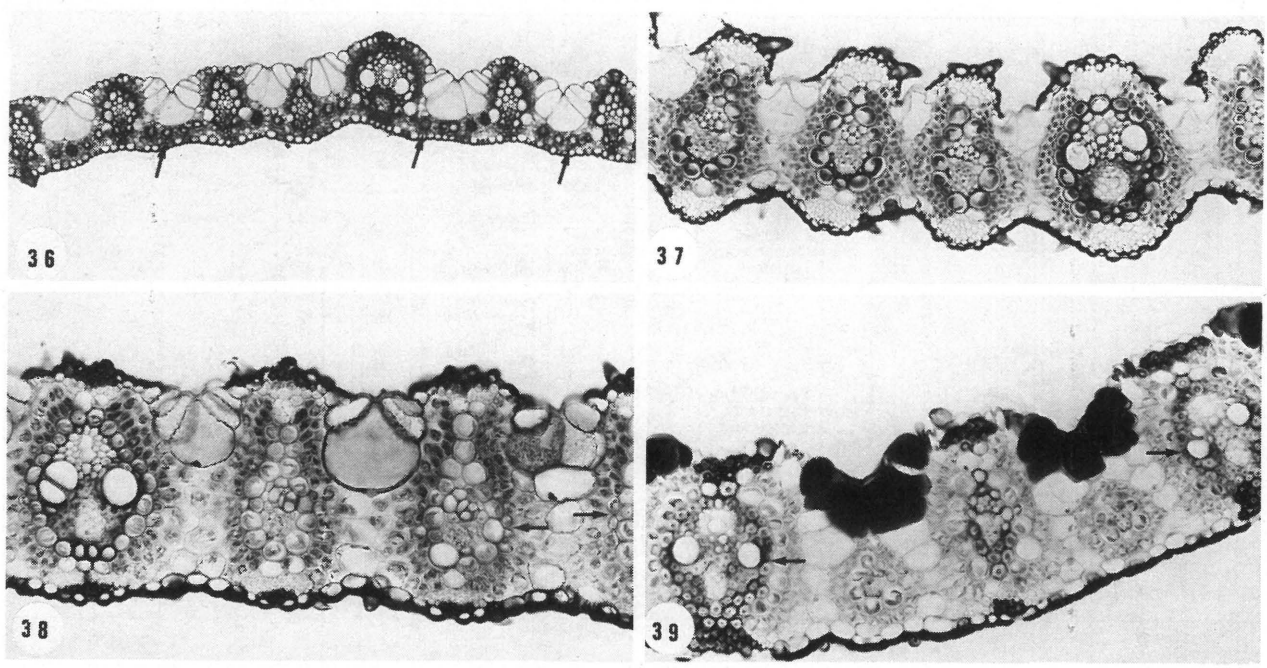


FIG. 36–39 The tribe Arundinelleae. Fig. 36, *Arundinella nepalensis,* K-MS-NADP with arrows indicating distinctive cells, *Ellis* 2116, ×160. Fig. 37, *Loudetiopsis glabrata,* K-MS-NADP, *Ellis* 344, ×400. Fig. 38, *Loudetia pedicellata,* K-MS-NADP, with small, rounded cells exterior to the Kranz mestome sheath cells, *Ellis* 2006, ×400. Fig. 39, *Loudetia simplex,* K-MS-NADP with arrows indicating an additional thickened cell layer interior to the mestome sheath, i.e. between the metaxylem vessels and the Kranz sheath, *Ellis* 1873, ×400

FIG. 36–39 *Die tribus Arundinelleae. Fig.* 36, Arundinella nepalensis, *K-MS-NADP en "distinctive" selle wat deur pyle aangedui word.* Ellis 2116, ×160. *Fig.* 37, Loudetiopsis glabrata, *K-NS-NADP,* Ellis 344, ×400. *Fig.* 38, Loudetia pedicellata, *K-NS-NADP met klein, ronde selle na die buitekant van die Kranz-mestoomskede,* Ellis 2006, ×400. *Fig.* 39, Loudetia simplex, *K-MS-NADP met pyle wat 'n ekstra verdikte sellaag aan die binnekant van die mestoomskede aandui, d.i. tussen die metaxileemvesel en die Kranzskede,* Ellis 1873, ×400

ARUNDINELLA *Raddi*

^{13}C values of two species of *Arundinella* (Smith & Brown, 1973) show this genus to be C_4 and the species examined by Hattersley & Watson (1976) had a single bundle sheath.

A. nepalensis *Trin.* (Fig. 36) **K-MS-NADP**

This species has a C_4 $\delta^{13}C$ ratio of $-12,1‰$ (Vogel) and has panicoid anatomy and ultrastructure (Johnson & Brown, 1973) with a single bundle sheath (Hattersley & Watson, 1976). This species is unique amongst the S. African grasses in having distinctive cells, which are Kranz cell extensions of the bundle sheath, scattered throughout the mesophyll.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1617; –CB, *Ellis* 1218. 2628–AB (Johannesburg), *Ellis* 1368. NATAL.—2730–AC (Vryheid), *Ellis* 479. 2829–CC (Harrismith), *Ellis* 1436. 3030–CB (Port Shepstone), *Ellis* 2116.

DANTHONIOPSIS *Stapf*

The species mentioned by Hattersley & Watson (1976) from Metcalfe's (1960) data are all typically NADP-ME type.

D. dinteri (*Pilg.*) *C. E. Hubb.* **K-MS-NADP**

A single, Kranz, bundle sheath with chloroplasts normally almost filling the Kranz cells.

TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1942, 1944, 1947, 2430–DA (Pilgrim's Rest), *Ellis* 1580.

D. parva (*Phipps*) *Clayton* **K-MS-NADP**

TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1936, 1937.

D. pruinosa *C. E. Hubb.* **K-MS-NADP**

Typical MS type anatomy with chloroplasts centrifugally positioned.

RHODESIA.—1731–CC (Salisbury), *Ellis* 802. TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1943. 2230–DB (Messina), *Ellis* 1924. 2231–CC (Pafuri), *Ellis* 1888, 1889. 2430–BD (Pilgrim's Rest), *Ellis* 1570.

LOUDETIA *Hochst. ex Steud.*

This genus has been categorized as a malate former although a certain degree of variation is exhibited. Hattersley & Watson (1976) report a double bundle sheath in *L. superba* and in this study a few specimens of *L. simplex* were noted to have two bundle sheaths.

L. flavida (*Stapf*) *C. E. Hubb.* **K-MS-NADP**

A single bundle sheath with rather small irregular sheath cells which are typical of NADP-ME type anatomy.

RHODESIA.—1731–CA (Salisbury), *Ellis* 798. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1334; –CB, *Ellis* 1786.

L. pedicellata (*Stent*) *L. Chipindal* (Fig. 38) **K-MS-NADP**

Cells resembling sheath cells, adjacent to the mestome sheath cells, are sometimes present. These are smaller than the sheath cells in transverse section, rounded and not radially arranged.

TRANSVAAL.—2428–DA (Nylstroom), *Ellis* 830; –DB, *Ellis* 2006, 2008.

L. simplex (*Nees*) *C. E. Hubb.* (Fig. 39) **K-MS-NADP**

Characteristic NADP-ME type anatomy but some specimens (R. Ellis 458, 1383, 1873) have a distinct double bundle sheath, the inner one of which is

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typical of a mestome sheath, being comprised of small, lignified thickened cells as seen in transectional view. In R. Ellis 1873 all first order bundles have this structure, thus it actually conforms structurally with the aspartate formers.

TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1873. 2528–CA (Pretoria), *Ellis* 427, 756; –CC, *Ellis* 1346. 2530–BD (Lydenburg), *Ellis* 1564; –BA, *Ellis* 438, 458. 2531CA (Komatipoort), *Ellis* 396; –CC, *Ellis* 383. 2628–AB (Johannesburg), *Ellis* 1383. NATAL.—2829–CC (Harrismith), *Ellis* 1452.

LOUDETIOPSIS *Conert*

Hattersley & Watson (1976) report both aspartate (double bundle sheath) and malate (single bundle sheath) formers within this genus. This situation warrants further study but unfortunately this genus is under represented in the present study.

L. glabrata (*K. Schum.*) *Conert* (Fig. 37) **K-MS-NADP**

This species has only a single bundle sheath.

S.W.A.—1724–AD (Katimo Mulilo), *Ellis* 344.

TRICHOPTERYX *Nees*

T. dregeana *Nees* **K-MS-NADP**

This species has a C_4 $\delta^{13}C$ ratio of $-13,3^0/_{00}$ (Brown) and a single bundle sheath.

TRANSVAAL.—2530–BA (Lydenburg), *Ellis* 454. 2531–CA (Komatipoort), *Ellis* 1543.

TRISTACHYA *Nees*

A genus well represented in this study with typical NADP-ME type anatomy throughout.

T. biseriata *Stapf* **K-MS-NADP**

Characteristic NADP-ME type bundle sheath arrangement and cell and chloroplast structure.

Transvaal.—2528–CA (Pretoria), *Ellis* 781, 1242, 1243, 1332, 1333; –CB, *Ellis* 1794.

T. eylesii *Stent and Rattray* **K-MS-NADP**

S.W.A.—1723–CD (Singalamwe), *Ellis* 315.

T. hispida (*L.f.*) *K. Shum.* **K-MS-NADP**

Kranz ($\delta^{13}C$=—$11,4^0/_{00}$, Brown, unpublished) with a single bundle sheath (Hattersley & Watson, 1976). This structure is consistent even in specimens with heavily sclerosed phloem.

TRANSVAAL.—2829–CC (Harrismith), *Ellis* 1430. 2929–BB (Underberg), *Ellis* 47. CAPE.—3227–AC (Stutterheim), *Ellis* 1483; –DA, *Ellis* 297. 3327–AA (Peddie), *Ellis* 588.

T. rehmannii *Hack.* **K-MS-NADP**

A single bundle sheath with centrifugal chloroplasts.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 725, 753, 1331; –DA, *Ellis* 1803. 2628–AB (Johannesburg), *Ellis* 1382.

TRIBE PANICEAE

This tribe exhibits more variation than any other similar grouping in the grass family, with C_3, NADP-ME, NAD-ME and PCK-type genera being represented. The genus *Panicum* includes species in all four categories and *Alloteropsis semialata* has C_3 and NADP-ME specimens. Tables 1 and 2 show clearly that all grass genera are consistently either C_3 or C_4 with the above two genera being the only two exceptions. These genera as presently constituted are, therefore, probably not meaningful in an evolutionary sense.

ACROCERAS *Stapf*

A. macrum *Stapf* (Fig. 46) **n-K**

This species has a C_3 $\delta^{13}C$ value ($-23,7^0/_{00}$; Brown, unpublished) as well as typical non-Kranz anatomy (Ellis, 1974c). Bundle sheath cells large and conspicuous usually devoid of chloroplasts but small, often colourless, plastids may be present.

TRANSVAAL.—2330–CA (Tzaneen), *Ellis* 1595. 2630–DA (Carolina), *Ellis* 1859, 1860. NATAL.—3030–CB (Port Shepstone), *Ellis* 2119. CAPE.—3228–BC (Butterworth), *Ellis* 266.

ALLOTEROPSIS *Presl.*

A very variable genus with two PCK-type species, two NADP-ME type species and a species with non-Kranz as well as Kranz specimens with a single bundle sheath.

A. angusta *Stapf* **K-MS-NADP**

This species has a C_4 δ^{13} value of $-12,2^0/_{00}$ (Brown) and NADP-ME type anatomy with a single bundle sheath. However, there are traces of a much reduced parenchyma sheath exterior to the Kranz mestome sheath.

ZAMBIA.—Mankoya Dist., *Drummond and Cookson* 6663. Mwinilunga Dist., *Milne-Redhead* 3711.

A. cimicina (*L.*) *Stapf* **K-PS-PCK**

This is a C_4 species ($\delta^{13}C$=$-13,4^0/_{00}$; Brown). Observations in this study show two definite sheaths which disagrees with the report of a single sheath (Hattersley & Watson, 1976). The chloroplast position could not be ascertained with any degree of certainty as no freshly fixed material was examined but it is expected that this will prove to be a PCK-type.

S.W.A.—1725–CC (Livingstone), *Killick and Leistner* 3327. MOZAMBIQUE.—2632–BC (Bela Vista), *de Lemos and Balsinhas* 244. NATAL.—2831 (Nkandla), *Ward* 3055. TANZANIA.—Ukirigura, *Greenway* 7384. INDIA.—Dehra Dun, *Parker* s.n.

A. gwebiensis *Stent and Rattray* **K-MS-NADP**

A C_4 species ($\delta^{13}C$=$-12,1^0/_{00}$; Brown) with a Kranz mestome sheath but retaining a residual parenchyma sheath.

ANGOLA.—Malange Dist., *Estes* 30. ZAMBIA.—Mwinilunga Dist., *Simon and Williamson* 1920; *Stent and Rattray s.n.*

A. paniculata (*Benth.*) *Stapf* **K-PS-?**

Kranz ($\delta^{13}C$–$13,0^0/_{00}$; Brown unpublished) with a double bundle sheath around primary vascular bundles. It was not possible to determine the chloroplast structure and locality in the specimen studied.

CONGO.—Isangai, *Germain* 4623.

A. semialata (*R.Br.*) *Hitch.* **n-K and K-MS-NADP**

A species in which are found two distinct anatomical and physiological forms (Ellis 1974a, b). Certain specimens show C_3 $\delta^{13}C$ values (Brown & Vogel) and typical non-Kranz anatomy. These specimens are marked with an asterisk below. Contrasting with these are specimens with C_4 $\delta^{13}C$ values and with a well-developed Kranz mestome sheath. Parenchyma sheath cells are present but they are small, thin-walled and without specialized chloroplasts. This is probably an intermediate stage in the evolution of the NADP-ME-type of anatomy.

TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1865*. 2428–DA (Nylstroom), *Barenbrug* 2. 2527–CB (Rustenburg), *Saayman* 15; –CD, *Saayman* 3*, 10, 16; –DA, *Saayman* 5; –DC, *Coetzee* 423*, *Repton* 4496*; –DD, *Coetzee* 127*, 496*, 497, *Saayman*, 1, 6, 12. 2528 –CA (Pretoria), *Ellis* 2, 1244, 1748; –CB, *Ellis* 1785, 1789; –CC, *Ellis* 721, *Saayman* 7, 9, 11, 21; –CD, *Saayman* 24, 25*. 2529–AD (Witbank), *Ellis* 2063. 2530–CA (Lydenburg), *Burtt-Davy* 1323*. 2531–AA (Komatipoort), *v.d. Schijff* 2035; –CC, *Ellis* 378*. 2627–BA (Potchefstroom), *Saayman* 8*, 17*, 19*, 20*; –BB, *Saayman* 13*, 14*. 2628–AB (Johannesburg), *Ellis* 1827*, 1828*, 1829*, 1830*. 2630–DA (Carolina), *Ellis* 1854*, 1861*, 1862*, O.F.S.—2727–BD (Kroonstad), *Brandmuller* 47. NATAL.—2729–DB (Volksrust), *Ellis* 36. 2832–AA (Mtubatuba), *Ward* 1585*, 1682; –AC, *Harrison* 339, *Strey* 5615. 3030–CB (Port Shepstone), *Ellis* 733*. CAPE.—3227–CB (Stutterheim), *Ellis* 2607*; –DB, *Ellis* 2606*. 3326–BC (Grahamstown), *Ellis* 2571.

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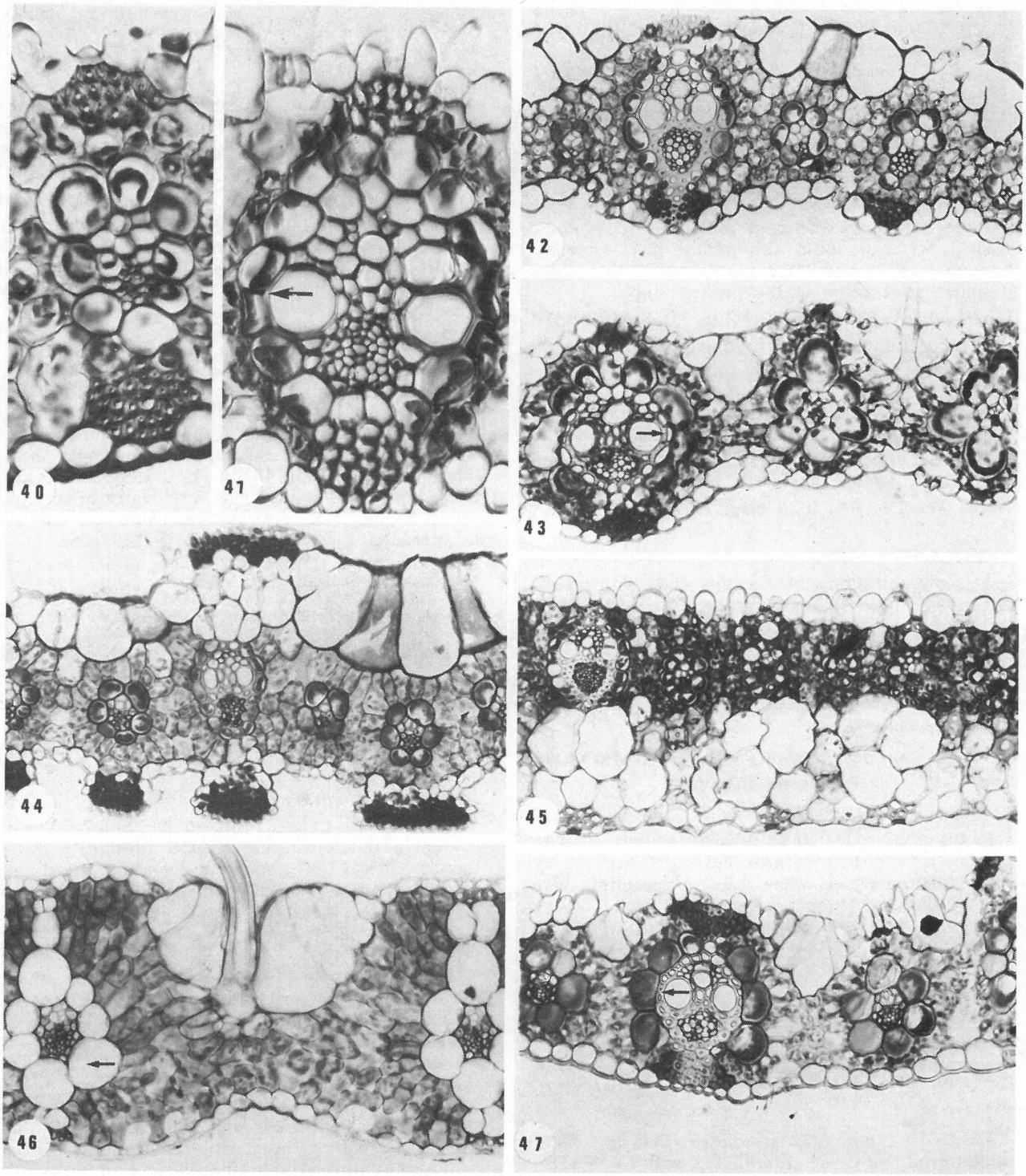


FIG. 40–47 Anatomical variation in the Paniceae. Fig. 40–41, *Paspalum paspalodes,* K-MS-NADP with Kranz mestome sheath adjacent to the metaxylem vessels (Fig. 41) and centrifugal chloroplasts (Fig. 40), *Ellis* 254, × 800. Fig. 42, *Paspalum dilatatum,* K-MS-NADP, *Ellis* 272, × 640. Fig. 43, *Urochloa panicoides,* K-PS-PCK with two bundle sheaths, *Ellis* 132, × 640. Fig. 44, *Digitaria macroglossa,* K-MS-NADP, *Ellis* 274, × 640. Fig. 45, *Stenotaphrum secundatum,* K-MS-NADP, *Ellis* 267, × 640. Fig. 46, *Acroceras macrum,* n-K with no specialized chloroplasts in the thickened parenchyma sheath cells, *Ellis* 266, × 640. Fig. 47, *Rhynchelytrum setifolium,* K-PS-PCK with a double bundle sheath and centrifugal chloroplasts, *Ellis* 302, × 640

FIG. 40–47 *Anatomiese variasie in die Paniceae. Fig.* 40–41, Paspalum paspalodes, *K-MS-NADP met Kranz-mestoomskede aangrensend aan die metaxileemvesels* (*Fig.* 41) *en centrifugale chloroplaste* (*Fig.* 40), Ellis 254, × 800. *Fig.* 42, Paspalum dilatatum, *K-MS-NADP,* Ellis 272, × 640. *Fig.* 43, Urochloa panicoides, *K-PS-PCK met twee bondelskede,* Ellis 132, × 640. *Fig.* 44, Digitaria macroglossa, *K-MS-NADP,* Ellis 274, × 640. *Fig.* 45, Stenotaphrum secundatum, *K-MS-NADP,* Ellis 267, × 640. *Fig.* 46, Acroceras macrum, *n-K met geen gespesialiseerde chloroplaste in die verdikte parenchiemskedeselle nie,* Ellis 266, × 640. *Fig.* 47, Rhynchelytrum setifolium, *K-PS-PCK met 'n dubbel bondelskede en centrifugale chloroplaste,* Ellis 302, × 640

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ANTHEPHORA *Schreb.*

The three species examined by Smith & Brown (1973) all have C_4 $\delta^{13}C$ values and Johnson & Brown (1973) report agranal chloroplasts centrifugally arranged in *A. cristata.*

A. argentea *Goossens* **K-MS-NADP**

Typical NADP-ME type anatomy with the somewhat larger bundle sheath cells of the third order bundles being distinctly kidney-shaped in section.

CAPE.—2820–DC (Kakamas), *Ellis* 877.

A. pubescens *Nees* **K-MS-NADP**

This species has a C_4 $\delta^{13}C$ value of $-12,5^0/_{00}$ (Smith & Brown, 1973) or $-11,0^0/_{00}$ (Vogel), has a single bundle sheath (Hattersley & Watson, 1976) and agranal chloroplasts (Johnson & Brown, 1973). Anatomically very similar to *A. argentea.*

S.W.A.—2217–CA (Windhoek), *Ellis* 927. TRANSVAAL.—2429–AA (Zebediela) *Ellis* 2041. 2528–AC (Pretoria), *Ellis* 1758; –BC, *Ellis* 1351. 2529–AD (Witbank), *Ellis* 2070.

AXONOPUS *Beauv.*

Available information indicates that this genus is C_4 as it has a low CO_2 compensation point (Downton & Tregunna, 1968) and C_4 $\delta^{13}C$ values (Smith & Brown, 1973). In addition a single bundle sheath is present (Hattersley & Watson, 1976) with reduced grana (Carolin *et al.,* 1973), i.e. NADP-ME-type anatomy.

A. compressus (*Swartz*) *Beauv.* **K-MS-NADP**

$\delta^{13}C = -12,6^0/_{00}$ (Vogel) with distinctive NADP-ME-type anatomy.

NATAL.—3030–CD (Port Shepstone), *Ellis* 1468.

BRACHIARIA (*Trin.*) *Griseb.*

All species studied appear to be Kranz from the isotopic discrimination values (Smith & Brown, 1973), to have a double bundle sheath (Hattersley & Watson, 1976) and the 11 species that have been biochemically typed are all PCK-types (Gutierrez *et al.,* 1976). A certain degree of variation has been reported. Thus *B. jubata* has a single bundle sheath (Hattersley & Watson, 1976) and *B. platyphylla* does not show a PIB (Brown & Gracen, 1972), both of which indicate a NADP-ME-type species. In this paper *B. glomerata* is reported as probably being of the NAD-ME-type.

B. advena *Vickery* **K-PS-PCK**

A double bundle sheath with an accentuated centrifugal positioning of the chloroplasts.

TRANSVAAL.—2729–BA (Volksrust), *Ellis* 1510.

B. brizantha (*Hochst. ex A. Rich.*) *Stapf* (Fig. 48) **K-PS-PCK**

This species has been shown to be a PCK-type (Gutierrez *et al.,* 1976) which agrees with the predictions based on the anatomy.

RHODESIA.—1731–CA (Salisbury), *Ellis* 814. S.W.A.—1723–CB (Singalamwe), *Ellis* 1775. TRANSVAAL.—2528–BC (Pretoria), *Ellis* 1349. –CB, *Ellis* 1123. 2530–DD (Lydenburg), *Ellis* 1535.

B. dura *Stapf* **K-PS-PCK**

PCK-type structure with adaxial and abaxial Kranz cell extensions of the bundle sheath.

S.W.A.—1723–CD (Singalamwe), *Ellis* 316. 1724–DB (Katima Mulilo), *Ellis* 341.

B. eruciformis (*Sibth. and J.E. Sm.*) *Griseb* **K-PS-PCK**

A confirmed PCK-type (Gutierrez *et al.,* 1976) exhibiting little variation.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2029. TRANSVAAL.—2431–BB (Acornhoek), *Ellis* 469. 2528–CA (Pretoria), *Ellis* 761. 2628–AB (Johannesburg), *Ellis* 1369. 2729–BB (Volksrust), *Ellis* 469. O.F.S.—2827–DC (Senekal), *Ellis* 971. CAPE.—3227–DB (Stutterheim), *Ellis* 1102.

B. filifolia *Stapf* (Fig. 50) **K-PS-PCK**

TRANSVAAL.—2630–DC (Carolina), *Ellis* 1521.

B. glomerata (*Hack.*) *A. Camus* **K-PS-NAD**

Although this species has been classified as a NAD-ME-type this has not been done with a great deal of confidence as only a single specimen was examined and the chloroplasts are not typically either PCK or NAD-type. However, in sheath cell structure this species resembles the NAD-ME-type and thus it has been tentatively grouped in this category.

S.W.A.—2416–AB (Maltahöhe), *Ellis* 937.

B. grossa *Stapf* **K-PS-PCK**

Large inflated parenchyma sheath cells with centrifugal chloroplasts.

RHODESIA.—2130–DA (Nuanetsi), *Ellis* 1310.

B. humidicola (*Rendle*) *Schweick.* **K-PS-PCK**

This species has been shown biochemically to be a PCK-type (Gutierrez *et al.,* 1976) which confirms the predictions of this study.

RHODESIA.—1731–CA (Salisbury), *Ellis* 806. S.W.A.—1724–DB (Katima Mulilo), *Ellis* 343.

B. nigropedata (*Munro ex Fical. and Hiern.*) *Stapf* (Fig. 49) **K-PS-PCK**

A PCK-type species (Gutierrez *et al.,* 1976) with classical anatomy of this type.

S.W.A.—2118–DB (Steinhausen), *Ellis* 2027. TRANSVAAL.—2329–DC (Pietersburg), *Ellis* 1602. 2527–DB (Rustenburg), *Ellis* 1229. 2528–AC (Pretoria), *Ellis* 1766; –CA, *Ellis* 778, 834.

B. serrata (*Thunb.*) *Stapf* var. **gossypina** (*A. Rich.*) *Stapf* **K-PS-PCK**

O.F.S.—2827–DC (Senekal), *Ellis* 972. CAPE.—3421–BA (Riversdale), *Ellis* 1650.

B. serrata (*Thunb.*) *Stapf* var. **serrata** **K-PS-PCK**

Large inflated Kranz sheath cells with typical PCK-type chloroplasts.

TRANSVAAL.—2527–DB (Rustenburg), *Ellis* 362. 2528–CA (Pretoria), *Ellis* 8, 128, 368. 2531–CC (Komatipoort), *Ellis* 404. O.F.S.—2827–DC (Senekal), *Ellis* 975. NATAL.—2930–AC (Howick), *Ellis* 62. CAPE.—3028–CB (Matatiele), *Ellis* 260. 3227–DA (Stutterheim), *Ellis* 293. 3421–BB (Riversdale), *Ellis* 635.

B. xantholeuca (*Hack.*) *Stapf* **K-PS-PCK**

Biochemically this species is PCK-type (Gutierrez *et al.,* 1976) which correlates with the anatomy. Crystals present in the bundle sheath cells.

TRANSVAAL.—2431–DB (Acornhoek), *Ellis* 111, 513.

CENCHRUS *L.*

$\delta^{13}C$ values for *C. incertus* (Smith & Brown, 1973) show this species to be C_4. *C. echinatus, C. pauciflorus* and *C. biflorus* all have a single, Kranz sheath (Hattersley & Watson, 1976) and *C. pauciflorus* has been shown to be a NADP-ME type species (Gutierrez *et al.,* 1974, 1974a) with centrifugal chloroplasts.

C. ciliaris *L.* **K-MS-NADP**

This study shows *C. ciliaris* to conform with the observations on other species of this genus, i.e. typical NADP-ME type anatomy.

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S.W.A.—2118–DB (Steinhausen), *Ellis* 2024. TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 198. 2528–AC (Pretoria), *Ellis* 1764; –CA, *Ellis* 1326. 2531–BB (Komatipoort), *Ellis* 495. NATAL.—2732–CA (Ubombo), *Ellis* 86. CAPE.—2824–CD (Kimberley), *Ellis* 853.

CYMBOSETARIA *Schweick.*

C. sagittifolia (*A. Rich.*) *Schweick.* **K-MS-NADP**

This species shows C_4 isotopic discrimination values of $-13{,}0‰$ (Vogel) or $-12{,}1‰$ (Brown) and exhibits NADP-ME type structure.

TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1939. 2230–AB (Messina), *Ellis* 2085. 2231–AD (Pafuri), *Ellis* 1911. 2431–DB (Acornhoek), *Ellis* 521.

DIGITARIA *Heist ex Fabric.*

A well represented genus in Southern Africa, most species of which are C_4, with a single bundle sheath and the species which have been examined ultrastructurally show typical panicoid structure with much reduced grana in the bundle sheath chloroplasts (Carolin *et al.*, 1973). This study has revealed two species with a mestome as well as a parenchyma sheath around the larger bundles. Further study of these species which are expected to be PCK-types, is needed.

D. abyssinica (*Hochst. ex A. Rich.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1237.

D. adscendens (*H.B.K.*) *Henr.* **K-MS-NADP**

A $\delta^{13}C$ value of $-11{,}6‰$ (Smith & Brown, 1973), has been reported for this species and it has the characteristic anatomy of a malate former.

TRANSVAAL.—2531–CC (Komatipoort), *Ellis* 393. 2630–AC (Carolina), *Ellis* 1529.

D. albomarginata *Stent* **K-MS-NADP**

A single bundle sheath with the cells of the third order bundles being larger than those of the first order bundles. This situation is characteristic of *Digitaria*.

NATAL.—2632–DD (Bela Vista), *Kok* 626.

D. apiculata *Stent* **K-MS-NADP**

Small rounded mesophyll cells sometimes present external to the mestome sheath. These cells could be remnants of the parenchyma sheath.

TRANSVAAL.—2330–CA (Tzaneen), *Kok* 554.

D. argyrograpta (*Nees*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2528–CB (Pretoria), *Ellis* 1345, 1784.

D. brazzae (*Franch.*) *Stapf* **K-MS-NADP**

This augments the deduction of Hattersley & Watson (1976) from the data of Metcalfe (1960), that this is a MS species.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1338, 1826.

D. debilis (*Desf.*) *Willd.* **K-MS-NADP**

S.W.A.—1723–CD (Singalamwe), *Ellis* 323. RHODESIA.—2131–BC (Triangle), *Ellis* 1303. TRANSVAAL.—2529–AD (Witbank), *Ellis* 2064. 2531–CA (Komatipoort), *Ellis* 1545.

D. decumbens *Stent* **K-MS-NADP**

This has been shown biochemically to be a NADP-ME type (Kanai & Black, 1972). This agrees with the anatomy.

TRANSVAAL.—2530–BD (Lydenburg), *Kok* 568.

D. diagonalis (*Nees*) *Stapf* **K-MS-NADP**

NADP-ME type anatomy with radially arranged mesophyll cells.

RHODESIA.—1731–CA (Salisbury), *Ellis* 803. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 791, 1802. 2531–CB (Komatipoort), *Ellis* 1549.

D. dinteri *Henr.* **K-MS-NADP**

S.W.A.—2718–CB (Grünau), *Ellis* 899.

D. eriantha *Steud.* **K-MS-NADP**

Typical NADP-ME type structure in all specimens examined.

TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 110. 2528–CA (Pretoria), *Ellis* 1327. O.F.S.—2827–DC (Senekal), *Ellis* 949, 950, 958, 959. CAPE.—2824–CD (Kimberley), *Ellis* 836.

D. eylesii *C. E. Hubb.* **K-MS-NADP**

TRANSVAAL.—2529–AD (Witbank), *Ellis* 2081.

D. flaccida *Stapf* **K-PS-PCK(?)**

Marginal, lateral primary bundles have a single bundle sheath but a second, and sometimes even a third sheath becomes progressively more developed toward the median bundle. This species may still be a malate former but this distinct anatomical difference has been accentuated by placing it with the aspartate formers. Bundle cell structure is not typical of the PCK type and further study is necessary.

TRANSVAAL.—2630–DA (Carolina), *Ellis* 1857. NATAL.—2829–CC (Harrismith), *Ellis* 1448.

D. gymnostachys *Pilg.* **K-MS-NADP**

NATAL.—2732–BC (Ubombo), *Kok* 482.

D. longiflora (*Retz.*) *Pers.* **K-MS-NADP**

TRANSVAAL.—2530–BA (Lydenburg), *Ellis* 456, 463. NATAL.—2830–CB (Dundee), *Ellis* 74.

D. macroglossa *Henr.* (Fig. 44) **K-MS-NADP**

NATAL.—3030–CB (Port Shepstone), *Ellis* 740. CAPE.— 3229–BC (Butterworth), *Ellis* 274.

D. milanjiana (*Rendle*) *Stapf* **K-MS-NADP**

S.W.A.—2118–DB (Steinhausen), *Ellis* 2032. TRANSVAAL.—2330–CA (Tzaneen), *Kok* 552.

D. monodactyla (*Nees*) *Stapf* **K-MS-NADP**

Bundle sheath structure is typically NADP-ME

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 418; –CB, *Ellis* 147; –CC, *Ellis* 714. 2628–AA (Johannesburg), *Ellis* 1375. NATAL.—2829–CC (Harrismith), *Ellis* 1451. CAPE.—3028–BA (Matatiele), *Ellis* 244.

D. nitens *Rendle* **K-PS-PCK(?**

Very similar to *D. flaccida* in anatomy. Probably not a PCK-type but anatomy not consistent with NADP-ME type structure.

RHODESIA.—2030–DA (Fort Victoria), *Ellis* 829.

D. pentzii *Stent* **K-MS-NADP**

RHODESIA.—2130–DA (Nuanetsi), *Ellis* 1311. TRANSVAAL.—2330–CC (Tzaneen), *Ellis* 1597. 2429–AA (Zebediela), *Ellis* 2048, 2050. 2430–DA (Pilgrims Rest), *Ellis* 1577.

D. perrottetii (*Kunth*) *Stapf* **K-MS-NADP**

NATAL.—2732–BC (Ubombo), *Kok* 482.

D. polyphylla *Henr.* **K-MS-NADP**

CAPE.—2823–AC (Grickwastad), *Kok* 508.

D. sanguinalis (*L.*) *Scop.* **K-MS-NADP**

This grass has been extensively examined, all the results of which are in agreement with the present study. This species is C_4: δ^{13} C-12,7‰ (Smith & Brown, 1973) and CO_2 compensation point is less than 5 ppm (Downton and Tregunna, 1968). It is a malate former as it has high malic enzyme (Huber *et al.*, 1973), no PIB (Brown & Gracen, 1972) and has a single Kranz sheath only (Hattersley & Watson,

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1976; Hatch & Kagawa, 1974). *D. sanguinalis* also has centrifugal chloroplasts with reduced grana (Brown & Gracen, 1972; Carolin *et al.*, 1973 and Gutierrez *et al.*, 1974, 1974a) and is a NADP-ME type (Gutierrez *et al.*, 1974, 1974a; Hatch & Kagawa, 1974 and Hatch, Kagawa & Craig, 1975).

RHODESIA.—1629–AA (Chirundu), *Ellis* 573. O.F.S.—2827–DC (Senekal), *Ellis* 960.

D. scalarum (*Schweinf.*) *Chiov.* **K-MS-NADP**

TRANSVAAL.—2630–DA (Carolina), *Ellis* 1855.

D. setifolia *Stapf* **K-MS-NADP**

Characteristic NADP-ME type anatomy.

NATAL.—2929–BB (Underberg), *Ellis* 43; –BD, *Ellis* 56. 2930–AC (Pietermaritzburg), *Ellis* 61.

D. setivalva *Stent* **K-MS-NADP**

TRANSVAAL.—2528–CB (Pretoria), *Ellis* 1122. O.F.S.—2827–DD (Senekal), *Ellis* 948.

D. smutsii *Stent* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 126.

D. swazilandensis *Stent* **K-MS-NADP**

This species has a Kranz $\delta^{13}C$ value of $-11,8^{0}/_{00}$ (Vogel). Although the leaf is very thin NADP-ME type structure is neverthless evident.

TRANSVAAL.—2231–CB (Pafuri), *Ellis* 1902, 1903. 2330–CA (Tzaneen), *Kok* 555. 2528–CA (Pretoria), *Ellis* 1240. NATAL.—3030–CB (Port Shepstone), *Ellis* 2121, 2122.

D. ternata (*A. Rich.*) *Stapf* **K-MS-NADP**

Anatomy is characteristically of the NADP-ME type.

TRANSVAAL.—2531–CC (Komatipoort), *Ellis* 388. 2729–BA (Volksrust), *Ellis* 1509. O.F.S.—2827–DD (Senekal). *Ellis* 941, 946. NATAL.—2729–DB (Volksrust), *Ellis* 39. 2929–DD (Underberg), *Ellis* 212, 215. 3030–CD (Port Shepstone), *Ellis* 1472. CAPE.—3128–DB (Umtata), *Ellis* 258.

D. tricholaenoides *Stapf* **K-MS-NADP**

This species is morphologically similar to *D. flaccida* but anatomically there is no evidence of a typical fibrous mestome sheath. Certain of the mesophyll cells adjoining the sheath cells are however, sheath-like in shape on some of the specimens examined.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 744, 1746; –CB, *Ellis* 1799. 2630–DC (Carolina), *Ellis* 1520. NATAL.—2829–CC (Harrismith), *Ellis* 1447.

D. valida *Stent* **K-MS-NADP**

TRANSVAAL.—2431–DB (Acornhoek), *Ellis* 511. 2531–DB (Komatipoort), *Ellis* 501.

D. zeyheri (*Nees*) *Henr.* **K-MS-NADP**

NADP-ME type anatomy distinct.

TRANSVAAL.—2330–CA (Tzaneen), *Kok* 551. 2428–CD (Nylstroom), *Ellis* 192. 2528–CA (Pretoria), *Ellis* 416. NATAL.—3030–CB (Port Shepstone), *Ellis* 734.

ECHINOCHLOA *Beauv.*

All species of this genus appear to be of the NADP-ME type.

E. colona (*L.*) *Link.* **K-MS-NADP**

A $\delta^{13}C$ value of $-13,1^{0}/_{00}$ (Bender, 1971), no PIB (Brown & Gracen, 1972) and a single bundle sheath (Hattersley & Watson, 1976). The chloroplasts are centrifugal with renduced grana and biochemically NADP-ME is the principle decarboxylating enzyme (Gutierrez *et al.* 1974, 1974a). The anatomy correlates with all the above information.

RHODESIA.—1731–CC (Salisbury), *Ellis* 807. 2131–BC (Triangle), *Ellis* 1305. TRANSVAAL.—2331–AB (Phalaborwa), *Ellis* 535. 2528–CA (Pretoria), *Ellis* 1615. 2729–BA (Volksrust), *Ellis* 1508. NATAL.—2831–CD (Nkandla), *Ellis* 80. 3030–CD (Port Shepstone), *Ellis* 1469.

E. crus-galli (*L.*) *Beauv.* **K-MS-NADP**

This is a C_4 species with a low CO_2 compensation point (Downton & Tregunna, 1968) and with C_4 $\delta^{13}C$ values (Bender, 1971; Smith & Brown, 1973). It is a malate former as it has high malic enzyme concentrations (Huber *et al.*, 1973), no PIB (Brown & Gracen, 1972) and a single bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts (Gutierrez *et al.*, 1974, 1974a) and with much reduced grana (Carolin *et al.*, 1973). This is almost the full syndrome of characters of a NADP-ME type species, which *E. crus-galli* has been shown to be (Gutierrez *et al.*, 1974, 1974a).

CAPE.—3028–BD (Matatiele), *Ellis* 253. 3227–AC (Stutterheim), *Ellis* 1485.

E. holubii (*Stapf*) *Stapf* **K-MS-NADP**

S.W.A.—2118–DB (Steinhausen), *Ellis* 2019. TRANSVAAL.—2231–AC (Pafuri), *Ellis* 557. 2428–DB (Nylstroom), *Ellis* 2015.

E. pyramidalis (*Lam.*) *Hitchc. and Chase* **K-MS-NADP**

TRANSVAAL.—2231–CA (Pafuri), *Ellis* 1892.

E. stagnina (*Retz.*) *Beauv.* **K-MS-NADP**

Typical NADP-ME type anatomy with the sheath cells around the third order bundles somewhat kidney-shaped in sectional view.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1615. CAPE.—3028–BD (Matatiele), *Ellis* 252.

ERIOCHLOA *H.B.K.*

Biochemical studies on this genus have shown that five species are PCK-type species (Gutierrez *et al.*, 1976). The work of Brown & Gracen (1972) on *E. gracilis* does not confirm the above as no PIB was found to be present, a condition associated with malate formers.

E. meyerana (*Nees*) *Pilg.* **K-PS-PCK**

A PCK-type species (Gutierrez *et al.*, 1976) which conforms with the double bundle sheath and centrifugal chloroplasts.

TRANSVAAL,—2230–DB (Messina), *Ellis* 1922. 2231–CC (Pafuri), *Ellis* 1882. 2331–AB (Phalaborwa), *Ellis* 536. 2531–BB (Komatipoort), *Ellis* 500. NATAL.—2632–CC (Bela Vista), *Ellis* 1126.

E. nubica (*Steud.*) *Hack. and Stapf ex Thell.* **K-PS-PCK**

Typical PCK-type anatomy with few, large, inflated bundle sheath cells with centrifugal chloroplasts.

RHODESIA.—2131–BC (Triangle), *Ellis* 1302. S.W.A.—2518–CA (Tses), *Ellis* 918. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 774. 2531–BB (Komatipoort), *Ellis* 499.

LEUCOPHRYS *Rendle*

L. mesocoma (*Nees*) *Rendle* **K-PS-PCK**

A PCK-type species belonging to the *Brachiaria—Eriochloa—Urochloa* group.

S.W.A.—2818–AC (Warmbad), *Ellis* 892.

MELINIS *Beauv.*

M. minutiflora is known to be a Kranz species ($\delta^{13}C = -12,7^{0}/_{00}$ Smith & Brown, 1973), with a double bundle sheath.

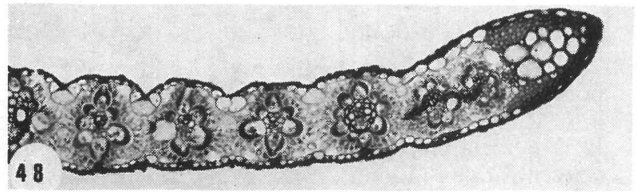
M. macrochaeta *Stapf and C. E. Hubb.* **K-PS-PCK**

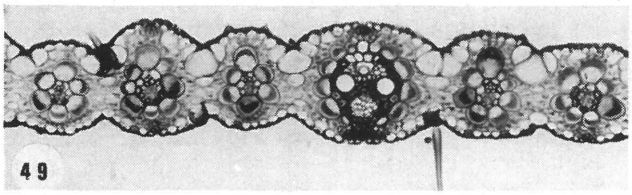
Characteristic centrifugal chloroplasts in outer bundle sheath cells.

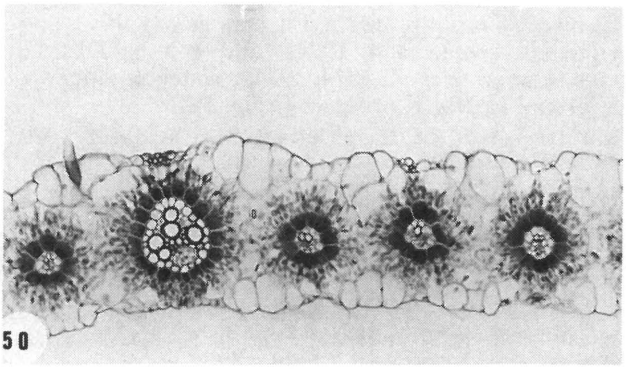
TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1877, 1878.

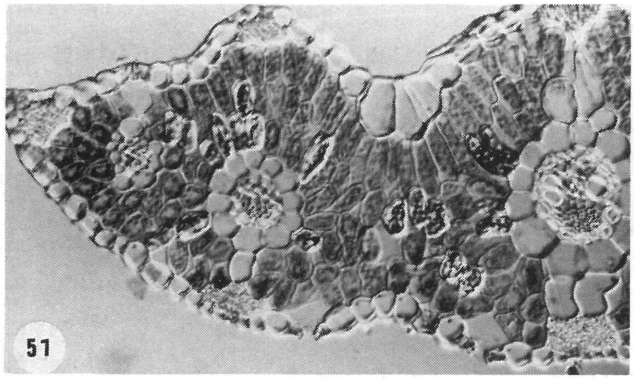
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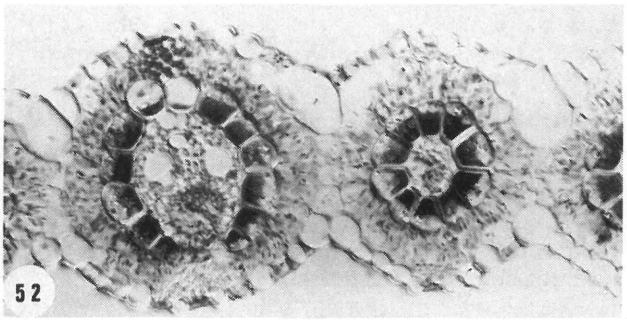
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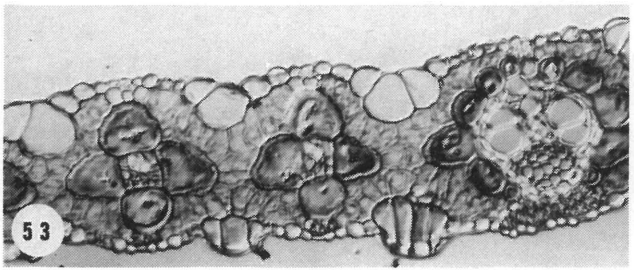












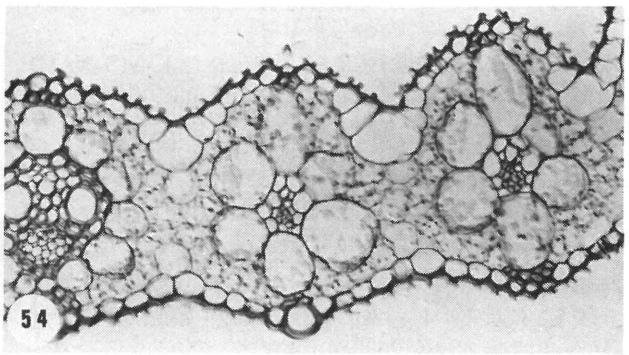


FIG. 48–50 Anatomical variation in the genus *Brachiaria*. Fig. 48, *Brachiaria brizantha*, K-PS-PCK, *Ellis* 1349, ×160. Fig. 49, *Brachiaria nigropedata*, K-PS-PCK, *Ellis* 834, ×160. Fig. 50, *Brachiaria glomerata*, K-PS-NAD with conspicuous, regular Kranz sheath with chloroplasts of the NAD type although they entirely fill the sheath cells, *Ellis* 937, ×250

FIG. 48–50 *Anatomiese variasie in die genus* Brachiaria. *Fig.* 48, Brachiaria brizantha, *K-PS-PCK*, Ellis 1349, ×160. *Fig.* 49, Brachiaria nigropedata, *K-PS-PCK*, Ellis 834, ×160. *Fig.* 50, Brachiaria glomerata, *K-PS-NAD met duidelike Kranzskede met chloroplaste van die NAD tipe alhoewel die skedeselle algeheel gevul word*, Ellis 937, ×250

FIG. 51–54 Variation within the genus *Panicum*. Fig. 51, *Panicum ecklonii*, n-K and lacking specialized chloroplasts, *Ellis* 30, ×640. Fig. 52, *Panicum arbusculum*, K-PS-NAD with centripetal chloroplasts, *Ellis* 898, ×400. Fig. 53, *Panicum maximum*, K-PS-PCK with centrifugal chloroplasts, *Ellis* 67, ×640. Fig. 54, *Panicum glabrescens*, K-PS-PCK, a senescing leaf but note inflated bundle sheath cells, *Ellis* 259, ×640

FIG. 51–54 *Variasie in die genus* Panicum. *Fig.* 51, Panicum ecklonii, *n-K en sonder gespesialiseerde chloroplaste*, Ellis 30, ×640. *Fig.* 52, Panicum arbusculum, *K-PS-NAD met centripetale chloroplaste*, Ellis 898, ×400. *Fig.* 53, Panicum maximum, *K-PS-PCK met centrifugale chloroplaste*, Ellis 67, ×640. *Fig.* 54, Panicum glabrescens, *K-PS-PCK, 'n verouderde blaar maar neem kennis van ronde bondelskedeselle*, Ellis 259, ×640

OPLISMENUS *Beauv.*

A non-Kranz genus with panicoid anatomy and having a few normal plastids in prominent parenchyma sheath cells (Carolin *et al.*, 1973).

O. hirtellus (*L.*) *Beauv.* **n-K**

A C_3 $\delta^{13}C$ value of $-31,0^0/_{00}$ (Smith & Brown, 1973) and with colourless bundle sheath cells devoid of specialized chloroplasts.

TRANSVAAL.– 2320–DD (Pietersburg), *Ellis* 1868. 2430–DA (Pilgrims Rest), *Ellis* 1579. 2527–CA, (Rustenburg), *Ellis* 310. 2531–CC (Komatipoort), *Ellis* 377, 391; –CD, *Ellis* 1544. NATAL.—2829–CC (Harrismith), *Ellis* 1460. 3030–CB (Port Shepstone), *Ellis* 2120. CAPE.—3228–AD (Butterworth), *Ellis* 282.

ORYZIDIUM *C. E. Hubb. and Schweick.*

O. barnardii *C. E. Hubb. and Schweick.* **K-PS-PCK**

TRANSVAAL.—2528–CA (Pretoria), *Schweickerdt* 2443, cultivated.

PANICUM *L.*

Panicum is the only grass genus with representatives in all three groups of C_4 plants as well as C_3 species. In this study no NADP-ME type species of *Panicum* were encountered and it is probable that there are no Southern African *Panicum* species of this type. Most *Panicum* species are of the NAD-ME type (Gutierrez *et al.*, 1976). In this study eight of the 12 C_4 species examined were of the PCK-type

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and may be more appropriately placed in the *Brachiaria* group (Gutierrez *et al.*, 1976). As this genus is definitely under-represented in this study further work on the S. African *Panicum* species is warranted. There is an extensive body of literature on this genus and selected examples have been taken to illustrate the characteristics of the four groups in *Panicum* and their correlation with the characteristics of these categories as defined in this study.

The non-Kranz *Panicum* species have typical C_3 $\delta^{13}C$ values, e.g. *P. ciliatum* $-26,3^0/_{00}$, *P. deppauperatum* $-25,2^0/_{00}$ (Smith & Brown, 1973), they show festucoid (pooid) anatomy with few plastids in the parenchyma sheath cells, e.g. *P. pygmaeum* (Carolin *et al.*, 1973) and exhibit a PIB, e.g. *P. miliodes* (Brown & Gracen, 1972).

P. antidotale is a typical NADP-ME species: $\delta^{13}C$ value of $-14,1^0/_{00}$ (Smith & Brown, 1973), a malate former (Downton, 1970), single bundle sheath and eupanicoid anatomy (Hattersley & Watson, 1976) with centrifugal chloroplasts and reduced grana (Gutierrez *et al.*, 1974, 1974a). Other biochemically determined NADP-ME species are *P. lanipes* and *P. macrophyllum*.

P. capillare is an extensively tested example of the NAD-ME type: $\delta^{13}C = -14,3^0/_{00}$ (Smith & Brown, 1973), CO_2 compensation point less than 5 ppm (Downton & Tregunna, 1968), it is an aspartate former (Downton, 1970) with low malic enzyme (Huber *et al.*, 1973) and the PIB is present (Brown & Gracen, 1972). A double bundle sheath is present (Hattersley & Watson, 1976) and chloroplasts are centripetal with grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a). Other determined NAD-ME *Panicum* species are: *P. decompositum*, *P. halli*, *P. makarikariense*, *P. miliaceum* and *P. turgidum* (Gutierrez *et al.*, 1974, 1974a; Hatch & Kagawa, 1974).

P. maximum, *P. fasculatum*, *P. molle* and *P. texanum* are verified PCK-type species (Gutierrez *et al.*, 1974, 1974a, 1976) with double bundle sheaths and centrifugal chloroplasts in which the grana are developed.

P. aequinerve *Nees* **n-K**

Non-Kranz $\delta^{13}C$ value $(-26,7^0/_{00}$, Brown) and normal C_3 anatomy (*Ellis* 1974*c*).

TRANSVAAL.—2531–CA (Komatipoort), *Ellis* 1541. NATAL.—2829–CC (Harrismith), *Ellis* 1453, 1457. 2929–DD (Underberg), *Ellis* 214. 3030–CD (Port Shepstone), *Ellis* 1470. CAPE.—3227–CA (Stutterheim), *Ellis* 1476; –DB, *Ellis* 283. 3228–CC (Butterworth), *Ellis* 2103, 2104.

P. aphanoneurum *Stapf* **K-PS-PCK**

PCK-type anatomy but in some cells chloroplasts entirely fill the cells and stain red.

S.W.A.—1723–CD (Singalamwe), *Ellis* 312.

P. arbusculum *Mez* (Fig. 52) **K-PS-NAD**

Chloroplasts distinctly centripetally arranged.

S.W.A.—2718–CB (Grünau), *Ellis* 898.

P. chusqueoides *Hack.* **K-PS-PCK**

A kranz $\delta^{13}C$ value of $-14,1^0/_{00}$ (Brown). PCK anatomy with very large bundle sheath cells especially around the third order bundles.

NATAL.—2732–DA (Ubombo), *Ellis* 1130. CAPE.—3228–CC (Butterworth), Ellis 1112, 2111, 2112.

P. coloratum *L.* **K-PS-NAD**

A species with a double bundle sheath (Hattersley A Watson, 1976) that has been determined to be a &ND-ME type (Gutierrez *et al.*, 1974, 1974a). The above authors state that the chloroplasts are evenly distributed in the bundle sheath cells. A similar situation was observed in the specimens examined in this study but all other indications are that this is a NAD-ME type species.

S.W.A.—1823–BC (Siambisso), *Ellis* 328. TRANSVAAL.—2528–CB (Pretoria), *Ellis* 1783. 2531–BB (Komatipoort), *Ellis* 508.

P. deustum *Thunb.* **K-PS-PCK**

A C_4 species ($\delta^{13}C = -11,9^0/_{00}$, Smith & Brown, 1973) with a double bundle sheath (Hattersley & Watson, 1976) and typical centrifugal chloroplasts.

TRANSVAAL.—2330–DC (Tzaneen), *Ellis* 1590. 2531–CB (Komatipoort), *Ellis* 1547; –CC, *Ellis* 389. NATAL.—2732–DA (Ubombo), *Ellis* 1129. CAPE.—3227–DB (Stutterheim), *Ellis* 1104.

P. dregeanum *Nees* **K-PS-PCK**

Kranz $\delta^{13}C$ value $(-12,3^0/_{00}$, Brown) and clear PCK anatomy.

TRANSVAAL.—2529–AD (Witbank), *Ellis* 2079.

P. ecklonii *Nees* (Fig. 51) **n-K**

Non-Kranz $\delta^{13}C$ value $(-24,4^0/_{00}$, Brown) and C_3 anatomy (Ellis, 1974c).

TRANSVAAL.—2630–DA (Carolina), *Ellis* 1856. NATAL.—2729–BD (Volksrust), *Ellis* 30. 2829–CC (Harrismith), *Ellis* 1421, 1449. 2929–CD (Underberg), *Ellis* 53.

P. glabrescens *Steud.* (Fig. 54) **K-PS-PCK**

Accentuated PCK type anatomy even in senescing leaves.

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 563. 2230–DB (Messina), *Ellis* 1925. NATAL.—3030–CD (Port Shepstone), *Ellis* 1467. CAPE.—3128–DB (Umtata), *Ellis* 259.

P. heterostachyum *Hack.* **n-K**

Non-Kranz $\delta^{13}C$ value $(-30,5^0/_{00}$, Brown).

TRANSVAAL.—2230–DB (Messina), *Ellis* 1923.

P. hymeniochilum *Nees* **n-K**

A C_3 species ($\delta^{13}C = -26,8^0/_{00}$, Brown) and non-Kranz anatomy (Ellis, 1974c) with colourless bundle sheath extensions.

TRANSVAAL.—2530–BD (Lydenburg), *Ellis* 1560, –DC, *Ellis* 465. 2630–DA (Carolina), *Ellis* 1523. NATAL.—3030–CB (Port Shepstone), *Ellis* 2125.

P. infestum *Anderss. ex Peters* **K-PS-PCK**

RHODESIA.—2130–DA (Nuanetsi), *Ellis* 1308.

P. laevifolium *Hack.* **K-PS-PCK**

A Kranz species ($\delta^{13}C = -12,1^0/_{00}$, Smith & Brown, 1973) with a double bundle sheath (Hattersley & Watson, 1976). Reported to be a malate former (Downton, 1970) but this appears to be incorrect. Anatomy is typically PCK-type.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1821. 2629–AD (Bethal), *Ellis* 1842. 2729–BA (Volksrust), *Ellis* 1507. NATAL.—2729–BD (Volksrust), *Ellis* 205. O.F.S.—2827–DD (Senekal) *Ellis* 940. CAPE.—3029–AD (Kokstad), *Ellis* 230.

P. laticomum *Nees* **n-K**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1919, cultivated. CAPE.—3227–DB (Stutterheim), *Ellis* 1101.

P. maximum *Jacq.* (Fig. 53) **K-PS-PCK**

An extensively studied species that is a Kranz ($\delta^{13}C = -13,3^0/_{00}$, Smith & Brown, 1973) aspartate former (Downton, 1970: Huber *et al.*, 1973) with a double bundle sheath (Kanai & Black, 1972; Hattersley & Watson, 1976) and PIB (Brown & Gracen, 1972). It has been determined as a PCK-type (Kanai & Black, 1972; Gutierrez *et al.*, 1974, 1974a, 1976)

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with centrifugal chloroplasts and well developed grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a). This species, together with the other PCK-type *Panicum* species possibly shows closer affinities with *Brachiaria* and its relatives than to the NAD, NADP and n-K *Panicum* species.

TRANSVAAL.—2330–CA (Tzaneen), *Ellis* 1592. 2528–CA (Pretoria), *Ellis* 125, 762; –CB, *Ellis* 1782. NATAL.—3030–CB (Port Shepstone), *Ellis* 67, 1466.

P. monticolum *Hook.f.* **n-K**

A C_3 isotopic discrimination value of $-31,1^0/_{00}$ (Brown) and non-Kranz anatomy.

TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1867, 1869.

P. natalense *Hochst.* **n-K**

A C_3 species ($\delta^{13}C = -23,8^0/_{00}$, Brown) with distinct non-Kranz anatomy (Ellis 1974c).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 417; –CB *Ellis* 140. 2530–BA (Lydenburg), *Ellis* 453, 460. 2531–CC (Komatipoort), *Ellis* 376. NATAL.—2829–CC (Harrismith), *Ellis* 1442. CAPE.—3029–AD (Kokstad), *Ellis* 223.

P. parvifolium *Lam.* **n-K**

Non-Kranz $\delta^{13}C$ value ($-31,2^0/_{00}$, Brown) with associated anatomical structure.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 412, 1816, cultivated; originally from Madagascar.

P. stapfianum *Fourc.* **K-PS-NAD**

An aspartate former (Downton, 1970), with a double bundle sheath (Hattersley & Watson, 1976) and possessing a compact conspicuous bundle sheath with centripetal chloroplasts.

O.F.S.—2827–DC (Senekal), *Ellis* 976.

P. volutans *J. G. Anders.* **K-PS-NAD**

TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 2002

PASPALIDIUM *Stapf*

Information in the literature points to this genus being C_4 with a single bundle sheath (Hattersley & Watson, 1976). Typical panicoid anatomy is accompanied by much reduced grana in the Kranz sheath cells (Carolin *et al.*, 1973).

P. obtusifolium (*Del.*) *Simpson* **K-MS-NADP**

A single bundle sheath with centrifugal chloroplasts.

TRANSVAAL.—2231–DB (Pafuri), *Ellis* 555, 1900, 1901.

PASPALUM *L.*

A typical eu-panicoid genus with a single bundle sheath (Fig. 41) in species previously examined (Ellis, 1974d; Hattersley & Watson, 1976).

P. dilatatum *Poir.* (Fig. 42) **K-MS-NADP**

This species has a low CO_2 compensation point (Downton & Tregunna, 1968) and a single Kranz bundle sheath (Hattersley & Watson, 1976) in which the grana are reduced (Carolin *et al.*, 1973). These characteristics are correlated with typical NADP-ME type anatomy.

TRANSVAAL.—2628–AB (Johannesburg), *Ellis* 1835. CAPE.—3228–BC (Butterworth), *Ellis* 272.

P. notatum *Fluegge* **K-MS-NADP**

This species exhibits the full syndrome of characteristics of the NADP-ME type: $\delta^{13}C = -13,2^0/_{00}$ (Smith & Brown, 1973); no PIB (Brown & Gracen, 1972); a single bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts with reduced grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a) and a high proportion of the total decarboxylating activity corresponds to NADP-malic enzyme (Gutierrez *et al.*, 1974, 1974a).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1753.

P. orbiculare *Forsk.* **K-MS-NADP**

A species with typical NADP-ME type anatomy.

S.W.A.—1723–CD (Singalamwe), *Ellis* 318, 327. TRANSVAAL.—2231–CB (Pafuri), *Ellis* 562. 2527–DD (Rustenburg), *Ellis* 433. 2528–CA (Pretoria), *Ellis* 1339, 1364, 1823. NATAL.—3030–CB (Port Shepstone), *Ellis* 729, 732.

P. paspalodes (*Michx.*) *Scribn.* (Fig. 40, 41) **K-MS-NADP**

This species is C_4 with a $\delta^{13}C$ value of $-10,3^0/_{00}$ (Brown) and a low CO_2 compensation point (Downton & Tregunna, 1968) and is most probably a malate producer as it has a single bundle sheath (Ellis, 1974d; Hattersley & Watson, 1976).

TRANSVAAL.—2527–DD (Rustenburg), *Ellis* 432. 2528–CA (Pretoria) *Ellis* 767, 1323; –CB, *Ellis* 146. NATAL.—3030–CB (Port Shepstone), *Ellis* 726. CAPE.—3028–BD (Matatiele), *Ellis* 254. 3227–DB (Stutterheim), *Ellis* 1106.

P. urvellei *Steud.* **K-MS-NADP**

A Kranz species ($\delta^{13}C = -11,3^0/_{00}$, Smith & Brown, 1973) with typical eu-panicoid structure.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 770; –CB, *Ellis* 152. NATAL.—3030–CB (Port Shepstone), *Ellis* 68, 730.

P. vaginatum *Swartz* **K-MS-NADP**

A single Kranz sheath surrounding the bundles with centrifugal chloroplasts (Ellis, 1974d).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1235, cultivated. CAPE.—3228–BC (Butterworth), *Ellis* 276; –CC, *Ellis* 1114. 3422–BB (Mossel Bay), *Ellis* 1299.

PENNISETUM *L. C. Rich. ex Pers.*

A typical C_4 eu-panicoid genus with a single bundle sheath with specialized chloroplasts usually with definite but reduced grana.

P. clandestinum *Hochst. ex Chiov.* **K-MS-NADP**

A C_4 $\delta^{13}C$ value of $-11,6^0/_{00}$ (Vogel) with typical malate former bundle sheath structure (Hattersley & Watson, 1976). This species is, therefore, predicted to be a NADP-ME type.

TRANSVAAL.—2528–CB (Pretoria), *Ellis* 583. CAPE.—3228–CC (Butterworth), *Ellis* 1113.

P. macrourum *Trin.* **K-MS-NADP**

Hattersley & Watson (1976) correctly infer from Metcalfe's (1960) data that this species has a single bundle sheath. Characteristic NADP-ME type anatomy in all respects.

TRANSVAAL.—2630–DC (Carolina), *Ellis* 1522. CAPE.—3219–AA (Wuppertal), *Ellis* 1161. 3227–AC (Stutterheim), *Ellis* 1482; –DA, *Ellis* 299. 3420–AB (Bredasdorp), *Ellis* 661.

P. natalense *Stapf* **K-MS-NADP**

NATAL.—2829–CC (Harrismith), *Ellis* 1441. 3030–CB (Port Shepstone), *Ellis* 2117.

P. purpureum *Schumach.* **K-MS-NADP**

A C_4 species with a single bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts. It has been biochemically determined to be a NADP-ME type (Gutierrez *et al.*, 1976) which is in agreement with the anatomical characteristics.

NATAL.—3030–CB (Port Shepstone), *Ellis* 741.

P. setaceum (*Forsk.*) *Chiov.* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 366.

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P. sphacelatum (*Nees*) *Dur. & Schinz.* **K-MS-NADP**

O.F.S.—2827–DC (Senekal), *Ellis* 952, 963.

P. thunbergii *Kunth* **K-MS-NADP**

Typical anatomy of this type.

TRANSVAAL.—2527–CA (Rustenburg), *Ellis* 348. 2528–CC (Pretoria), *Ellis* 717. 2530–AC (Lydenburg), *Ellis* 90. NATAL.—2730–AC (Vryheid), *Ellis* 480. CAPE.—3029–AD (Kokstad), *Ellis* 227. 3320–DC (Montagu), *Ellis* 659.

P. typhoides (*Burm.*) *Stapf & C.E. Hubb.* **K-MS-NADP**

A NADP-ME type (Kanai & Black, 1972; Hatch, Kagawa & Craig, 1975) with a single bundle sheath (Hattersley & Watson, 1976) and centrifugal chloroplasts.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 428.

PSEUDECHINOLAENA *Stapf*

P. polystachya (*H.B.K.*) *Stapf* **n-K**

Reported as a non-Kranz species from anatomical criteria (Ellis, 1974c).

NATAL.—3030–CB (Port Shepstone), *Ellis* 1465, 2123, 2124.

PSEUDOBRACHIARIA *Launert*

P. deflexa (*Schumach.*) *Launert* **K-PS-PCK**

A PCK type species with a double bundle sheath (Edwards & Gutierrez, unpublished). This species (= *Brachiaria deflexa*) has been erroneously grouped as a MS species, with a single bundle sheath by Hattersley & Watson (1976) using the data of Metcalfe (1960).

RHODESIA.—2030–DA (Fort Victoria), *Ellis* 796. TRANSVAAL.—2229–AA (Waterpoort), *Ellis* 1315. 2231–CC (Pafuri), *Ellis* 1879. 2330–DC (Tzaneen), *Ellis* 1589. 2430–BD (Pilgrims Rest), *Ellis* 1582. 2431–DB (Acornhoek), *Ellis* 523. 2528–AC (Pretoria), *Ellis* 1763, 1767. 2531–CC (Komatipoort), *Ellis* 394.

PSILOCHLOA *Launert*

P. pilgerana (*Schweick.*) *Launert* **K-PS-PCK (?)**

A Kranz species ($\delta^{13}C = -11,9‰$, Brown) with a double bundle sheath. Chloroplast position could not be accurately determined as no freshly fixed material was available for study.

S.W.A.—Grid ref. unknown, *Volk* 2917.

RHYNCHELYTRUM *Nees*

A C_4 genus with a double bundle sheath (Fig. 47). The outer bundle sheath cells have centrifugal chloroplasts but the sheath cells are not as large and inflated as is typical of PCK species. This applies to *Tricholaena* and *Melinis* as well, and these three genera form a recognizable group within the Paniceae.

R. bellispicatum (*Rendle*) *Stapf and C. E. Hubb* **K-PS-PCK**

RHODESIA.—1731–CA (Salisbury), *Ellis* 799.

R. brevipilum (*Hack.*) *Chiov.* **K-PS-PCK**

Distinctive PCK-type anatomy.

TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1940.

R. repens (*Willd.*) *C. E. Hubb.* **K-PS-PCK**

Has a double bundle sheath (Hattersley & Watson, 1976) with prominent grana in the outer sheath (Carolin *et al.*, 1973) and centrifugal chloroplasts. A typical mestome sheath is present and no chloroplasts were observed in this sheath cf. Brown (1975).

RHODESIA.—1731–CA (Salisbury), *Ellis* 805. S.W.A.—2217–CA (Windhoek), *Ellis* 926. TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 200. 2528–CA (Pretoria), *Ellis* 746. NATAL.—3030–CB (Port Shepstone), *Ellis* 731.

R. rhodesianum (*Rendle*) *Stapf and C. E. Hubb.* **K-PS-PCK**

TRANSVAAL.—2530–BD (Lydenburg), *Ellis* 1561, 1565. 2531–CC (Komatipoort), *Ellis* 397.

R. setifolium (*Stapf*) *Chiov.* (Fig. 47) **K-PS-PCK**

This species exhibits typical anatomy of the PCK type.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 122, 142; –CB, *Ellis* 1216; –CC, *Ellis* 723. 2628–AA (Johannesburg), *Ellis* 1379; –AB, *Ellis* 1384. 2531–CC (Komatipoort), *Ellis* 386. O.F.S.—2828–DA (Bethlehem), *Ellis* 2393. NATAL.—2930–AD (Pietermaritzburg), *Ellis* 58. CAPE.—3028–CB (Matatiele), *Ellis* 261. 3227–AD (Stutterheim), *Ellis* 302. 3320–DC (Montagu), *Ellis* 651.

R. villosum (*Parl. ex Hook.*) *Chiov.* **K-PS-PCK**

TRANSVAAL.—2329–DC (Pietersburg), *Ellis* 1598. 2527–CA (Rustenburg), *Ellis* 353. 2528–CA (Pretoria), *van Heerden* 26.

SACCIOLEPIS *Nash*

A non-Kranz genus with $\delta^{13}C$ values of $-27,1‰$ for *S. striata* (Smith and Brown, 1973) and $-26,3‰$ for *S. curvata* (Brown).

S. typhura (*Stapf*) *Stapf* **n-K**

Typical non-Kranz anatomy with double bundle sheath without specialized chloroplasts even in bundle sheath extensions.

TRANSVAAL.—2530–AC (Lydenburg), *Ellis* 92. 2630–CA (Carolina), *Ellis* 1527.

SETARIA *Beauv.*

A typical NADP-ME type genus with all species examined conforming to all the associated characteristics of this type: C_4 carbon isotopic ratios (Smith & Brown, 1973); low CO_2 compensation point (Downton & Tregunna, 1968); no PIB (Brown & Gracen, 1972); high malic enzyme (Huber *et al.*, 1973); a single bundle sheath (Hattersley & Watson, 1976); centrifugal chloroplasts (Gutierrez *et al.*, 1974, 1974a); reduced grana (Brown & Gracen, 1972; Carolin *et al.*, 1973) and four species have been biochemically determined as NADP-ME type (Gutierrez *et al.*, 1974, 1974a).

S. appendiculata (*Hack.*) *Stapf* **K-MS-NADP**

Single bundle sheath with sheath cells of third order bundles larger than those of first order bundles.

S.W.A.—2718–CB (Grünau), *Ellis* 905.

S. chevalieri *Stapf ex Stapf and C. E. Hubb.* **K-MS-NADP**

A pleated leaf with typical NADP-ME structure.

TRANSVAAL.—2527–CA (Rustenburg), *Ellis* 358. 2531–CA (Komatipoort), *Ellis* 1540. NATAL.—3030–CB (Port Shepstone), *Ellis* 736, 737.

S. flabellata *Stapf* **K-MS-NADP**

TRANSVAAL.—2527–CA (Rustenburg), *Ellis* 355. 2528–CC (Pretoria), *Ellis* 716. NATAL.—2929–BB (Underberg), *Ellis* 42. 2930–CB (Pietermaritzburg), *Ellis* 68. CAPE.—3327–AA (Peddie), *Ellis* 716. 3420–CA (Bredasdorp), *Ellis* 1271.

S. lindenbergiana (*Nees*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 724, 745, 1238, 1239. CAPE.—3325–BC (Grahamstown), *Ellis* 594.

S. nigrirostris (*Nees*) *Dur. and Schinz.* **K-MS-NADP**

Typical NADP-ME anatomy.

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 421, 787; –CB *Ellis* 1214. 2630–DC (Carolina), *Ellis* 1519.

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S. pallide-fusca (*Schumach.*) *Stapf and C. E. Hubb.* **K-MS-NADP**

O.F.S.—2827–DC (Senekal), *Ellis* 956, 973, 988. NATAL.—2730–AC (Vryheid), *Ellis* 470. 2929–DD (Underberg), *Ellis* 213. CAPE.—3028–BD (Matatiele), *Ellis* 251. 3029–AD (Kokstad), *Ellis* 226. 3227–CB (Stutterheim), *Ellis* 301.

S. perennis *Hack.* (Fig. 57) **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 3, 7, 367; –DD, *Ellis* 24. NATAL.—2929–BB (Underberg), *Ellis* 44.

S. sphacelata (*Schumach.*) *Stapf and C. E. Hubb. ex M. B. Moss* **K-MS-NADP**

A malate forming species showing no PIB (Brown & Gracen, 1972) and with characteristic NADP-ME anatomy.

S.W.A.—1723–CD (Singalamwe), *Ellis* 325. 1823–BC (Siambisso), *Ellis* 338. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 786, 1337; –CB, *Ellis* 1220. NATAL.—2832–AC (Mtubatuba), *Ellis* 83. CAPE.—3227–DB (Stutterheim), *Ellis* 290.

S. ustilata *De Wit* **K-MS-NADP**

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2004. 2528–CA (Pretoria), *Ellis* 1234. 2530–DD (Lydenburg), *Ellis* 1536.

S. verticillata, (*L.*) *Beauv.* **K-MS-NADP**

The anatomy of the specimens examined is in agreement with the findings of Hattersley & Watson (1976) viz. NADP-ME type with single bundle sheath.

TRANSVAAL.—2229–AA (Waterpoort), *Ellis* 1317. 2231–CC (Pafuri), *Ellis* 1883. 2528–CA (Pretoria), *Ellis* 764.

S. woodii *Hack.* **K-MS-NADP**

TRANSVAAL.—2431–DD (Acornhoek), *Ellis* 98.

STENOTAPHRUM *Trin.*

S. secundatum (*Walt.*) *Kuntze* (Fig. 45) **K-MS-NADP**

A C_4 carbon isotopic ratio of $-14,7^0/_{00}$ (Smith & Brown, 1973), single bundle sheath (Hattersley & Watson, 1976), no PIB and centrifugal chloroplasts (Brown & Gracen, 1972) with definite but reduced grana (Carolin *et al.*, 1973) characterize this species.

NATAL.—2831–CD (Nkandla), *Ellis* 79. CAPE.—3228–BC (Butterworth), *Ellis* 267; –CC, *Ellis* 1110. 3325–DC (Port Elizabeth), *Ellis* 602. 3419–DD (Caledon), *Ellis* 1268. 3423–AB (Knysna), *Ellis* 1644.

STEREOCHLAENA *Hack.*

S. cameronii (*Stapf*) *Pilg.* **K-MS-NADP**

RHODESIA.—2030–CB (Fort Victoria), *Ellis* 793.

TARIGIDIA *Stent*

T. aequiglumis (*Goossens*) *Stent* **K-MS-NADP**

A C_4 $\delta^{13}C$ value of $-11,3^0/_{00}$ (Brown) and typical malate former type anatomy.

CAPE.—2724–AA (Taung), *Kok* 602.

TRICHOLAENA *Schrad.*

T. monachne (*Trin.*) *Stapf and C. E. Hubb.* **K-PS-PCK**

This species is C_4 ($\delta^{13}C = -11,9^0/_{00}$, Brown) with a double bundle sheath and centrifugal chloroplasts.

S.W.A.—2118 (Steinhausen), *Ellis* 2025. TRANSVAAL.—2229–DD (Waterpoort), *Ellis* 1935. 2429–AA (Zebediela), *Ellis* 2054, 2055. 2430–DA (Pilgrims Rest), *Ellis* 1576. 2528–AC (Pretoria), *Ellis* 1358, 1761. 2529–AD (Witbank), *Ellis* 2065. 2531–CB (Komatipoort), *Ellis* 1551.

UROCHLOA *Beauv.*

Four species of this genus have been biochemically typed as PCK-type (Gutierrez *et al.*, 1976) and the anatomy of all species examined correlates with the biochemistry.

U. bolbodes (*Steud.*) *Stapf* **K-PS-PCK**

A PCK-type species (Gutierrez *et al.*, 1976) with characteristic anatomy.

TRANSVAAL.—2531–CB (Komatipoort), *Ellis* 1556.

U. brachyura (*Hack.*) *Stapf* **K-PS-PCK**

S.W.A.—2118–DB (Steinhausen), *Ellis* 2026. TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 199. 2528–CA (Pretoria), *Ellis* 407, 763.

U. mosambicensis (*Hack.*) *Dandy* **K-PS-PCK**

The dominant decarboxylase in this species is PEP carboxykinase (Gutierrez *et al.*, 1976) and it is, therefore, a PCK-type with expected anatomy.

TRANSVAAL.—2230–DB (Messina), *Ellis* 1916. 2431–DB (Acornhoek), *Ellis* 509. 2531–BB (Komatipoort), *Ellis* 503.

U. panicoides *Beauv.* (Fig. 43) **K-PS-PCK**

Possesses a double bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts and has been biochemically typed as a PCK species (Gutierrez *et al.*, 1976).

S.W.A.—2518–CA (Tses), *Ellis* 916. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 132, 408, 747, 765, 1781.

U. pullulans *Stapf* **K-PS-PCK**

Biochemically determined to be a PCK-type (Gutierrez *et al.*, 1976).

RHODESIA.—2130–DA (Nuanetsi), *Ellis* 1309.

U. trichopus (*Hochst.*) *Stapf* **K-PS-PCK**

RHODESIA.—1629–AA (Chirundu), *Ellis* 577.

TRIBE MAYDEAE

A small tribe in Southern Africa which exhibits the variable sheath condition of the NADP-ME type. Thus, around certain primary lateral vascular bundles a layer of cells is interpolated between the metaxylem vessels and the Kranz sheath. This cell layer is not necessarily present around all primary vascular bundles. Thus, in at least some first order lateral vascular bundles the metaxylem vessels are in direct contact with the Kranz cells and these species are then assigned to the NADP-ME category (Hattersley & Watson, 1976).

COIX *L.*

C. lachryma-jobi *L.* **K-MS-NADP**

In this species the mestome sheath is distinct with thickened walls and with specialized chloroplasts. In many of the primary bundles a few sheath-like cells are situated external to the mestome sheath. These cells have few, unspecialized chloroplasts. This is not the typical variable condition reported by Hattersley & Watson (1976) in which additional cells are located between the Kranz mestome sheath and the metaxylem vessels. This species has typical panicoid type anatomy and ultrastructure (Carolin *et al.*, 1973)

NATAL.—3030–CB (Port Shepstone), *Ellis* 2115, 2118.

ZEA *L.*

Z. mays *L.* **K-MS-NADP**

A species which has been extensively examined. It is C_4 with $\delta^{13}C = -13,6^0/_{00}$ (Smith & Brown, 1973 and with a low CO_2 compensation point (Downton) & Tregunna, 1968). It has been shown to be a malate former (Downton, 1970), with high malic enzyme (Huber *et al.*, 1973), no PIB (Brown & Gracen, 1972), a single bundle sheath (Hattersley & Watson, 1976), centrifugal chloroplasts with reduced grana (Gutierrez *et al.*, 1974, 1974a; Brown & Gracen, 1972) and

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characteristic panicoid anatomy and ultrastructure (Carolin *et al.*, 1973). This species has been biochemically shown to be a NADP-ME species (Hatch *et al.*, 1975). This study conforms to all the above except that a cell layer is sometimes positioned between the Kranz sheath and the metaxylem vessels.

TRANSVAAL.—2528–CA (Pretoria), *Ellis s.n.*, cultivated.

TRIBE ANDROPOGONEAE

A large tribe well represented in this study with uniform NADP-ME type anatomy throughout. Three genera, *Imperata, Miscanthidium* and *Saccharum* exhibit the variable MS condition where a double sheath is sometimes present around some of the larger vascular bundles.

ANDROPOGON *L.*

All species examined correlate perfectly with all the NADP-ME type characteristics: Kranz carbon isotypic values (Smith & Brown, 1973), no PIB (Brown & Gracen, 1972), a single bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts and reduced grana (Brown & Gracen, 1972; Gutierrez *et al.*, 1974, 1974a). *A. virginicus* (Kanai & Black, 1972), *A. gerrardii* and *A. scoparius* (Gutierrez, *et al.*, 1974, 1974a) have been biochemically determined as NADP-ME types.

A. abyssinicus *R.Br.* **K-MS-NADP**

NATAL.—2729–DC (Volksrust), *Edwards* 2819.

A. appendiculatus *Nees* **K-MS-NADP**

A single Kranz sheath with centrifugal chloroplasts.

TRANSVAAL.—2528–CC (Pretoria), *Ellis* 715; –DA, *Ellis* 1800 2530–AB (Lydenburg), *Ellis* 94. 2628–AB (Johannesburg), *Ellis* 1373. NATAL.—2729–BD (Volksrust), *Ellis* 38. 2829–CC (Harrismith), *Ellis* 1429. CAPE.—3227–CA (Stutterheim), *Ellis* 1479. 3320–DC (Montagu), *Ellis* 652.

A. eucomus *Nees* **K-MS-NADP**

TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1357. 2530–BA (Lydenburg), *Ellis* 457. 2628–AA (Johannesburg), *Ellis* 1381. 2630–DA (Carolina), *Ellis* 1526. O.F.S.—2828–CA (Bethlehem), *Ellis* 991.

A. festuciformis *Rendle* **K-MS-NADP**

NATAL.—2930–DA (Pietermaritzburg), *Killick* 284.

A. gayanus *Kunth* var. **squamulatus** (*Hochst.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2330–CA (Tzaneen), *Ellis* 1591. 2231–AC (Pafuri), *Ellis* 554.

A. huillensis *Rendle* **K-MS-NADP**

Typical NADP-ME type anatomy.

S.W.A.—1723–CD (Singalamwe), *Ellis* 321. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1341. 2525–DA (Carolina), *Ellis* 1525.

A. lacunosus *J.G. Anders.* **K-MS-NADP**

TRANSVAAL.—2630–DA (Carolina), *Ellis* 1851. NATAL.—2730–AC (Vryheid), *Ellis* 488.

A. ravus *J.G. Anders.* **K-MS-NADP**

TRANSVAAL.—2531–CC (Barberton), *Ellis* 399. NATAL.—2829–CC (Harrismith), *Ellis* 1424, 1425.

A. schinzii *Hack.* **K-MS-NADP**

TRANSVAAL.—2231–CB (Pafuri), *Ellis* 1907. 2529–AD (Witbank), *Ellis* 2061.

A. schirensis *Hochst.* **K-MS-NADP**

TRANSVAAL.—2428–DB (Nylstroom), *Ellis* 2007, 2009. 2528–CA (Pretoria), *Ellis* 423. 2530–BA (Lydenburg), *Ellis* 449, 452. O.F.S.—2827–DC (Senekal), *Ellis* 970. 2828–CA (Bethlehem), *Ellis* 993. NATAL.—2720–BD (Volksrust), *Ellis* 207.

BOTHRIOCHLOA *Kuntze*

A Kranz genus (Smith & Brown, 1973) with a single bundle sheath (Hattersley & Watson, 1976) and characteristic NADP-ME anatomy.

B. glabra (*Roxb.*) *A. Camus* **K-MS-NADP**

TRANSVAAL.—2230–DB (Messina), *Ellis* 1913, 1915. 2527–DD (Rustenburg), *Ellis* 434. 2531–CB (Komatipoort), *Ellis* 1555.

B. insculpta (*Hochst.*) *A. Camus* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 788; –CC, *Ellis* 1348. 2531–BB (Komatipoort), *Ellis* 497; –CB, *Ellis* 1554; –CC, *Ellis* 398.

B. radicans (*Lehm.*) *A. Camus* **K-MS-NADP**

TRANSVAAL.—2331–CC (Pafuri), *Ellis* 1896. 2431–BA (Acornhoek), *Ellis* 525.

CHRYSOPOGON *Trin.*

Species of this genus studied are C_4 (Smith & Brown, 1973), show no PIB (Brown & Gracen, 1972), and have a single bundle sheath (Hattersley & Watson, 1976). *C. fallax* is reported as showing the variable MS condition (Hattersley & Watson, 1976).

C. serrulatus *Trin.* **K-MS-NADP**

The single specimen examined showed no evidence of the variable sheath condition. In all primary vascular bundles a single sheath was present.

CAPE.—2824–DC (Kimberley), *Ellis* 847.

CYMBOPOGON *Spreng.*

A genus with C_4 $\delta^{13}C$ values (Smith & Brown, 1973), a single bundle sheath (Hattersley & Watson, 1976) and ill-defined grana in the Kranz sheath (Carolin *et al.*, 1973).

C. excavatus (*Hochst.*) *Stapf ex Burtt Davy* **K-MS-NADP**

TRANSVAAL.—2229–AA (Waterpoort), *Ellis* 1318. 2428–CD (Nylstroom), *Ellis* 194. 2429–AA (Zebediela), *Ellis* 2047. 2431–DD (Acornhoek), *Ellis* 99. 2528–CA (Pretoria), *Ellis* 750. 2531–CC (Komatipoort), *Ellis* 400, 402.

C. plurinodis (*Stapf*) *Stapf ex Burtt Davy* (Fig. 60) **K-MS-NADP**

Although collected from very different ecological areas and showing leaf outlines from open to terete, the characters examined in this study remain constant.

TRANSVAAL.—2528–CB (Pretoria), *Ellis* 157. O.F.S.—2827–DC (Senekal), *Ellis* 967. CAPE.—2824–DC (Kimberley), *Ellis* 848. 3017–BB (Hondeklip Bay), *Ellis* 1809; –BD, *Ellis* 1728. 3028–BA (Matatiele), *Ellis* 233. 3227–AD (Stutterheim), *Ellis* 304. 3420–AD (Bredasdorp), *Ellis* 670, 1661, 1683. 3421–BB (Riversdale), *Ellis* 637.

C. validus (*Stapf*) *Stapf ex Burtt Davy* **K-MS-NADP**

This species has a single bundle sheath (Hattersley & Watson, 1976).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1342. NATAL.—2829–CC (Harrismith), *Ellis* 1444. CAPE.—3219–AA (Wuppertal), *Ellis* 1150.

DICHANTHIUM *Willemei*

A genus possessing a single bundle sheath (Hattersley & Watson, 1976) and with grana absent or ill-defined (Carolin *et al.*, 1973).

D. aristatum (*Poir.*) *C.E. Hubb.* **K-MS-NADP**

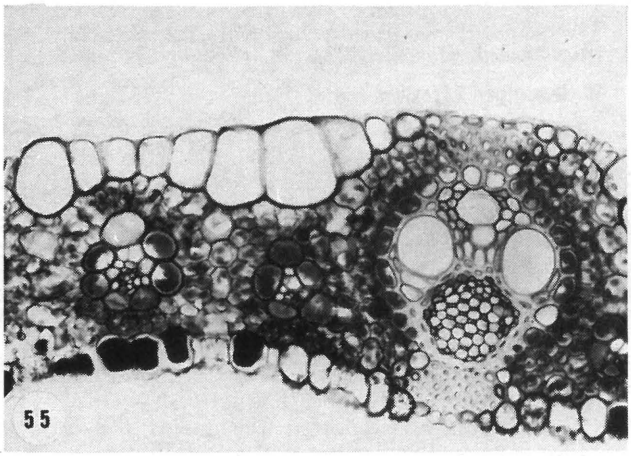
TRANSVAAL.—2528–CA (Pretoria), *Ellis* 130, 773.

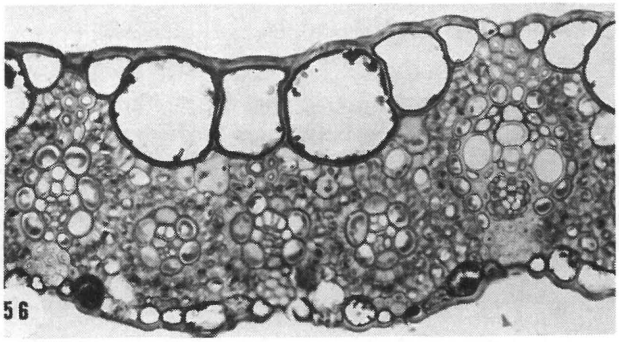
D. papillosum (*Hochst. ex A. Rich.*) *Stapf* **K-MS-NADP**

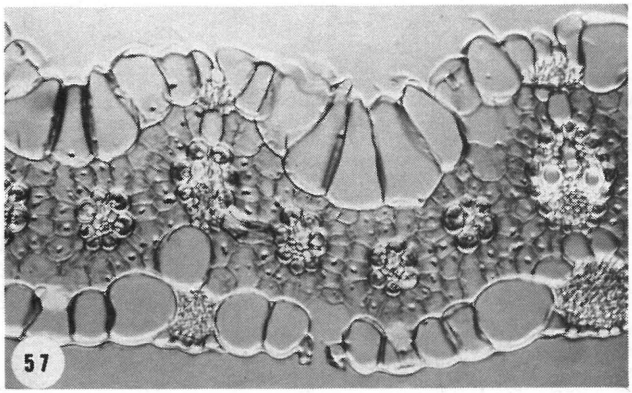
S.W.A.—2518–CA (Tses), *Ellis* 913. TRANSVAAL.—2231–AC (Pafuri), *Ellis* 564. 2428–CD (Nylstroom), *Ellis* 1614. 2431–DD (Acornhoek), *Ellis* 118. 2528–CA (Pretoria), *Ellis* 1750, 1820.

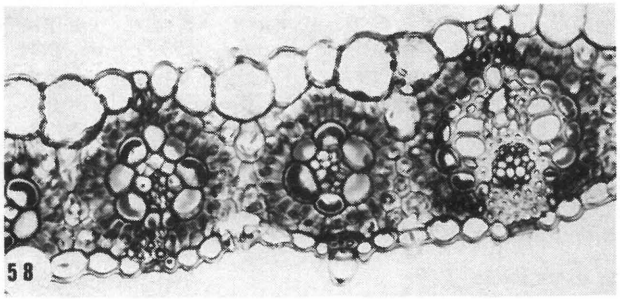
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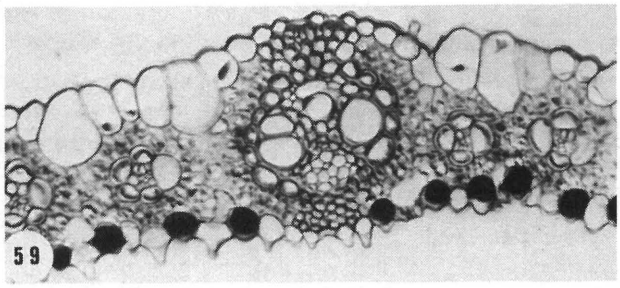
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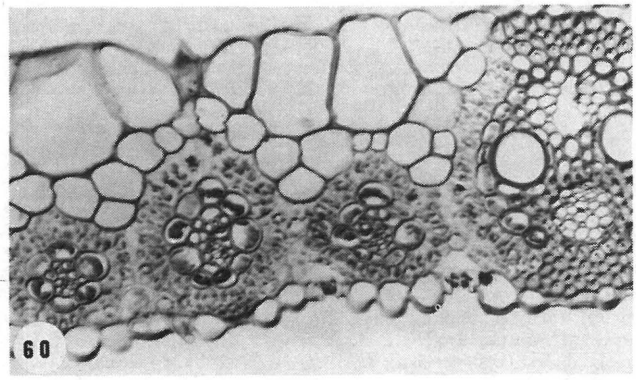

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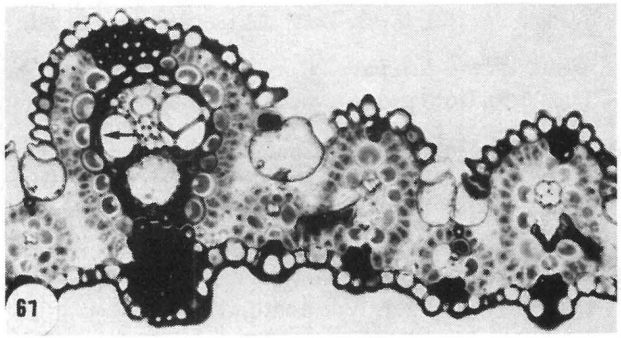

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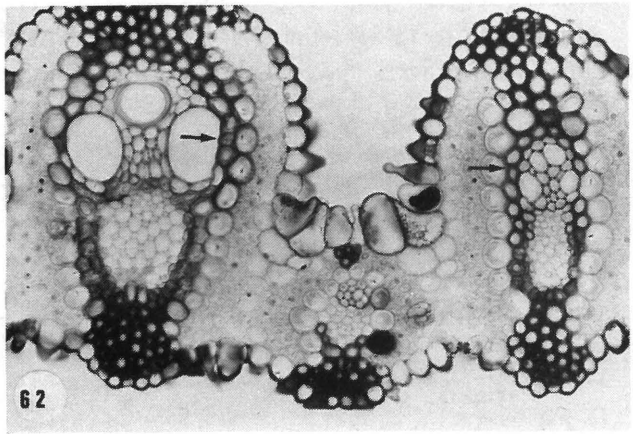

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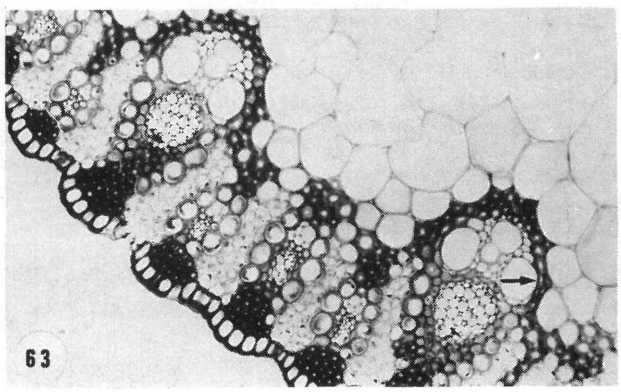

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DIHETEROPOGON (*Hack.*) *Stapf*

D. amplectens (*Nees*) *Clayton* **K-MS-NADP**

Using the data of Metcalfe (1960), Hattersley & Watson (1976) conclude that this species has a single bundle sheath. This agrees with the present observations.

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 553. 2527–CA (Rustenburg), *Ellis* 352; –DB, *Ellis* 1227. 2528–CA (Pretoria), *Ellis* 127, 751, 1777. 2531–CC (Komatipoort), *Ellis* 379. NATAL.—2730–AC (Vryheid), *Ellis* 481. 3030–CD (Port Shepstone), *Ellis* 1464.

D. filifolius (*Nees*) *Clayton* **K-MS-NADP**

NATAL.—2929–BD (Underberg), *Ellis* 55.

ELIONURUS *Humb. and Bonpl. ex Bonpl.*

A MS genus (Hattersley & Watson, 1976).

E. argenteus *Nees* **K-MS-NADP**

A C_4 species ($\delta^{13}C = -11,2^0/_{00}$, Vogel), with typical NADP-ME type anatomy.

TRANSVAAL.—2527–DB (Rustenburg), *Ellis* 359. 2528–CA (Pretoria), *Ellis* 10, 1744; –CB, *Ellis* 1215; –CC, *Ellis* 718. NATAL.—2929–BB (Underberg), *Ellis* 48, 51. CAPE.—3028–BA (Matatiele), *Ellis* 236.

ERIOCHRYSIS *Beauv.*

E. pallida *Munro* **K-MS-NADP**

TRANSVAAL.—2530–AC (Lydenburg), *Ellis* 93.

EULALIA *Kunth*

A single Kranz sheath (Hattersley & Watson, 1976) and grana ill-defined or absent (Carolin *et al.*, 1973) characterize this genus which is thus predicted to be of the NADP-ME type.

E. villosa (*Thunb.*) *Nees* **K-MS-NADP**

TRANSVAAL.—2531–CC (Komatipoort), *Ellis* 387. 2630–DA (Carolina), *Ellis* 1850. NATAL.—2730–AB (Vryheid), *Ellis* 1515. 2829–CC (Harrismith), *Ellis* 1443. CAPE.—3227–CA (Stutterheim), *Ellis* 1478.

HEMARTHRIA *R.Br.*

H. uncinata has a single bundle sheath (Hattersley & Watson, 1976), with panicoid anatomy with grana being absent or ill-defined (Carolin *et al.*, 1973).

H. altissima (*Poir.*) *Stapf and C.E. Hubb.*

K-MS-NADP

A species with characteristic NADP-ME anatomy.

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 560. 2331–BB (Phalaborwa), *Ellis* 540. 2527–DD (Rustenburg), *Ellis* 431. 2528–CB (Pretoria), *Ellis* 151. 2730–AC (Vryheid), *Ellis* 471. CAPE.—3320–DC (Montagu), *Ellis* 653.

HETEROPOGON *Pers.*

H. contortus (*L.*) *Beauv. ex Roem. and Schult.*

K-MS-NADP

A C_4 species ($\delta^{13}C = -11,8^0/_{00}$, Vogel), with no PIB (Brown & Gracen, 1972), with a single bundle sheath (Hattersley & Watson, 1976) and without grana (Carolin *et al.*, 1973). This species is thus confidently predicted to be a malate former and NADP-ME type.

TRANSVAAL.—2230–DB (Messina), *Ellis* 1918. 2429–AA (Zebediela), *Ellis* 2049. 2527–DB (Rustenburg), *Ellis* 363. 2528–CA (Pretoria), *Ellis* 6, 370; –CB, *Ellis* 141. 2530–AC (Lydenburg), *Ellis* 88. NATAL.—2929–BB (Underberg), *Ellis* 52. CAPE.—2824–CD (Kimberley), *Ellis* 849. 3227–DB (Stutterheim), *Ellis* 287.

H. melanocarpus (*Ell.*) *Benth.* **K-MS-NADP**

TRANSVAAL.—2231–CC (Pafuri), *Ellis* 1884, 1885, 1886.

HYPARRHENIA *Anderss. ex Stapf*

This genus is likely to be of the NADP-ME type but has been little studied. *H. variabilis* has been shown to have panicoid anatomy with grana either absent or ill-defined (Carolin *et al.*, 1973).

H. anamesa *Clayton* **K-MS-NADP**

CAPE.—3421–BA (Riversdale), *Ellis* 1649.

H. cymbaria (*L.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2330–CA (Tzaneen), *Ellis* 1593. NATAL.—2930–AC (Pietermaritzburg), *Ellis* 60.

H. dichroa (*Steud.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2530–DD (Lydenburg), *Ellis* 1538.

H. dregeana (*Nees*) *Stapf ex Stent* **K-MS-NADP**

TRANSVAAL.—2729–BA (Volksrust), *Ellis* 1506. NATAL.—2730–AC (Vryheid), *Ellis* 487. 2829–CC (Harrismith), *Ellis* 1445. O.F.S.—2827–DC (Senekal), *Ellis* 964.

H. filipendula (*Hochst.*) *Stapf* var. **pilosa** (*Hochst.*) *Stapf* **K-MS-NADP**

A MS species (Hattersley & Watson, 1976) with characteristic NADP-ME anatomy.

TRANSVAAL.—2230–DB (Messina), *Ellis* 1917, 1920. 2428–CD (Nylstroom), *Ellis* 189. 2431–DD (Acornhoek), *Ellis* 120. NATAL.—2729–DB (Volksrust), *Ellis* 40. 3030–CB (Port Shepstone), *Ellis* 66, 727. CAPE.—3421–BB (Riversdale), *Ellis* 630.

H. gazensis (*Rendle*) *Stapf* **K-MS-NADP**

RHODESIA.—1932–BD (Melsetter), *Ellis* 824.

H. hirta (*L.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2429–AA (Zebediela), *Ellis* 2052. 2527–CA (Rustenburg), *Ellis* 356; –DB, *Ellis* 361. 2528–CA (Pretoria), *Ellis* 134, 789, 1619; –CB, *Ellis* 158. 2530–BA (Lydenburg), *Ellis* 448. NATAL.—2720–BD (Volksrust), *Ellis* 206.

FIG. 55–63 Kranz structure in the tribe Andropogoneae. Fig. 55, *Ischaemum arcuatum*, K-MS-NADP with a single, Kranz bundle sheath, *Ellis* 273, ×640. Fig. 56, *Schizachyrium sanguineum*, K-MS-NADP, *Ellis* 136, ×640. Fig. 57, *Setaria perennis* (Paniceae), K-MS-NADP, *Ellis* 44, ×640. Fig. 58, *Themeda triandra*, K-MS-NADP, *Ellis* 295, ×640. Fig. 59, *Sorghum versicolor*, K-MS-NADP, *Ellis* 133, ×640. Fig. 60, *Cymbopogon plurinodis*, K-MS-NADP, *Ellis* 157, ×640

FIG. 55–63 *Kranz-struktuur in die tribus Andropogoneae. Fig.* 55, Ischaemum arcuatum, *K-MS-NADP met 'n enkel, Kranz-bondelskede*, Ellis 273, ×640. *Fig.* 56, Schizachyrium sanguineum, *K-MS-NADP*, Ellis 136, ×640. *Fig.* 57, Setaria perennis (*Paniceae*), *K-MS-NADP*, Ellis 44, ×640. *Fig.* 58, Themeda triandra, *K-MS-NADP*, Ellis 295, ×640. *Fig.* 59, Sorghum versicolor, *K-MS-NADP*, Ellis 133, ×640. *Fig.* 60, Cymbopogon plurinodis, *K-MS-NADP*, Ellis 157, ×640

FIG. 61–63 *Miscanthidium* species showing variable mestome sheath condition indicated by arrows. Fig. 61, *Miscanthidium junceum*, K-MS-NADP, *Ellis* 210, ×400. Fig. 62, *Miscanthidium capense*, K-MS-NADP, *Ellis* 1437, ×400. Fig. 63, *Miscanthidium teretifolium*, K-MS-NADP, *Ellis* 2080, ×400

FIG. 61–63 Miscanthidium-*spesies wat die verskillende mestoomskedekondisies aandui met pyle. Fig.* 61, Miscanthidium junceum, *K-MS-NADP*, Ellis 210×400. *Fig.* 62, Miscanthidium capense, *K-MS-NADP*, Ellis 1437, ×400. *Fig.* 63, Miscanthidium teretifolium, *K-MS-NADP*, Ellis 2080, ×400

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H. quarrei *Robyns* **K-MS-NADP**

TRANSVAAL.—2329–CD (Pietersburg), *Ellis* 1604.

H. tamba (*Hochst. ex Steud.*) *Andersss. ex Stapf* **K-MS-NADP**

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 552. 2528–CA (Pretoria), *Ellis* 784.

HYPERTHELIA *Clayton*

H. dissoluta (*Nees ex Steud.*) *Clayton* **K-MS-NADP**

Typical NADP-ME type anatomy with a single bundle sheath and radial chlorenchyma.

S.W.A.—1823–BC (Siambisso), *Ellis* 340. TRANSVAAL.—2231–AC (Pafuri), *Ellis* 551. 2429–AA (Zebediela), *Ellis* 2056. 2528–AC (Pretoria), *Ellis* 121. 2530–DD (Lydenburg), *Ellis* 1537. 2531–CC (Komatipoort), *Ellis* 401. NATAL.—2832–AC (Mtubatuba), *Ellis* 85.

IMPERATA *Cyrillo*

I. cylindrica (*L.*) *Beauv.* **K-MS-NADP**

Andropogonoid or panicoid type anatomy with a single bundle sheath (Hattersley & Watson, 1976) and without or with ill-defined grana (Carolin *et al.*, 1973). This species exhibits a variable MS condition (Hattersley & Watson, 1976) in some of the specimens examined.

S.W.A.—1723–CD (Singalamwe), *Ellis* 320. TRANSVAAL.—2528–CC (Pretoria), *Ellis* 720, 1336. 2730–AB (Vryheid), *Ellis* 1516. NATAL.—3030–CB (Port Shepstone), *Ellis* 69.

ISCHAEMUM *L.*

The species examined by Hattersley & Watson (1976) have a single bundle sheath.

I. afrum (*J.F. Gmel*) *Dandy* **K-MS-NADP**

TRANSVAAL.—2231–CC (Pafuri), *Ellis* 1881. 2428–CD (Nylstroom), *Ellis* 1613. 2431–DD (Acornhoek), *Ellis* 119. 2528–CA (Pretoria), *Ellis* 785, 1751; –CB, *Ellis* 1217. 2531–BB (Komatipoort), *Ellis* 505.

I. arcuatum (*Nees*) *Stapf* (Fig. 55) **K-MS-NADP**

TRANSVAAL.—2231–AC (Pafuri), *Ellis* 561. 2329–DD (Pietersburg), *Ellis* 1866. 2527–CA (Rustenburg), *Ellis* 309, 351. 2530–BB (Lydenburg), *Ellis* 1567. 2730–AC (Vryheid), *Ellis* 489. NATAL.—2829–CC (Harrismith), *Ellis* 1440. CAPE.—3228–BC (Butterworth), *Ellis* 268, 273.

I. franksae *J. M. Wood* **K-MS-NADP**

NATAL.—2829–CC (Harrismith), *Ellis* 1462.

MICROSTEGIUM *Nees*

A shade loving species with a very thin leaf and a single bundle sheath.

M. capense (*Hochst.*) *A. Camus* **K-MS-NADP**

TRANSVAAL.—2329–DD (Pietersburg), *Ellis* 1871. 2528–CA (Pretoria), *Ellis* 414, 712, cultivated.

MISCANTHIDIUM *Stapf*

Certain specimens of all the species of this genus examined showed a variable bundle sheath condition with additional cells being developed between the Kranz mestome sheath and the metaxylem vessels of first order bundles (Fig. 61–63).

M. capense (*Nees*) *Stapf* (Fig. 62) **K-MS-NADP**

O.F.S.—2827–DC (Senekal), *Ellis* 989. NATAL.—2829–CC (Harrismith), *Ellis* 1437.

M. erectum *Stent and C.E. Hubb.* **K-MS-NADP**

CAPE.—3028–BA (Matatiele), *Ellis* 247. 3227–DB (Stutterheim), *Ellis* 1110.

M. junceum (*Stapf*) *Stapf* (Fig. 61) **K-MS-NADP**

TRANSVAAL.—2428–CD (Nylstroom), *Ellis* 188. NATAL.—2929–DD (Underberg), *Ellis* 210. CAPE.—3228–BC (Butterworth), *Ellis* 281.

M. teretifolium (*Stapf*) *Stapf* (Fig. 63) **K-MS-NADP**

S.W.A.—1723–CD (Singalamwe), *Ellis* 317. 1725–CC (Livingstone), *Ellis* 1770. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1620. 2529–AD (Witbank), *Ellis* 2080.

MONOCYMBIUM *Stapf*

M. ceresiiforme (*Nees*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2530–BA (Lydenburg), *Ellis* 450, 459. 2630–DC (Carolina), *Ellis* 1518, 2629–DB (Bethal), *Ellis* 1528. NATAL.—2729–BD (Volksrust), *Ellis* 203, 1514. 2929–DD (Underberg), *Ellis* 208.

POGONATHERUM *Beauv.*

P. paniceum (*Lam.*) *Hack.* **K-MS-NADP**

This species has a single Kranz sheath (Hattersley & Watson, 1976).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 743, cultivated.

ROTTBOELLIA *L.f.*

R. exaltata *L.f.* **K-MS-NADP**

The observations in this study are in agreement with those of Hattersley & Watson (1976) and it is most probably a NADP-ME species.

NATAL.—3030–CB (Port Shepstone), *Ellis* 2113, 2114.

SACCHARUM *L.*

S. officinarum *L.* **K-MS-NADP**

A species which has been widely studied, all the aspects of which correlate with the predictions of this study. It is a C_4 plant with a $\delta^{13}C$ value of $-13,9‰$ (Smith & Brown, 1973) and a low CO_2 compensation point (Downton & Tregunna, 1968). It is a malate former (Downton, 1970) and it shows no PIB (Brown & Gracen, 1972), has a single bundle sheath (Hattersley & Watson, 1976) with centrifugal chloroplasts with reduced, or no grana (Brown & Gracen, 1972; Downton, 1970; Gutierrez *et al.*, 1974, 1974a). Sugar-cane has been biochemically typed as a NADP-ME species (Gutierrez *et al.*, 1974, 1974a). In this species cells between the Kranz cells and the metaxylem vessels are often present. It, thus, exhibits a variable MS condition (Hattersley & Watson, 1976).

NATAL.—3030–CB (Port Shepstone), *Ellis* 742.

SCHIZACHYRIUM *Nees*

S. scoparium has been shown to be a NADP-ME type with a single bundle sheath (Gutierrez *et al.*, 1974, 1974a) and the species examined in this study all show typically NADP-ME type anatomy.

S. jeffreysii (*Hack.*) *Stapf* **K-MS-NADP**

Possesses a single bundle sheath (Hattersley & Watson, 1976).

RHODESIA.—1731–CA (Salisbury), *Ellis* 803. 2131–BC (Triangle), *Ellis* 1301. TRANSVAAL.—2428–DA (Nylstroom), *Ellis* 832. 2429–AA (Zebediela), *Ellis* 2004, 2051. 2528–CB (Pretoria), *Ellis* 1120. 2529–AD (Witbank), *Ellis* 2066.

S. sanguineum (*Retz.*) *Alst.* (Fig. 56) **K-MS-NADP**

RHODESIA.—1932–BD (Melsetter), *Ellis* 823. TRANSVAAL.—2429–AA (Zebediela), *Ellis* 2045, 2046. 2528–CA (Pretoria), *Ellis* 136, 420. 2530–BA (Lydenburg), *Ellis* 464. 2531–CC (Komatipoort), *Ellis* 385.

S. ursulus *Stapf* **K-MS-NADP**

TRANSVAAL.—2528–AC (Pretoria), *Ellis* 1359; –CB, *Ellis* 1121.

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SEHIMA *Forsk*

S. galpinii *Stent* **K-MS-NADP**

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1752, 1817, 1819.

SORGHASTRUM *Nash*

S. nutana is a NADP-ME species with centrifugal chloroplasts with reduced grana (Gutierrez *et al.*, 1974, 1974a) and *S. pellitum* shows no PIB (Brown & Gracen, 1972) as is expected of a malate former.

S. friesii (*Pilg.*) *Pilg.* **K-MS-NADP**

TRANSVAAL.—2529–AD (Witbank), *Ellis* 2082, 2083, 2084.

SORGHUM *Moench.*

The following species of *Sorghum* have been widely studied: *S. bicolor, S. sudanense, S. halepense, S. leiocladum and S. vulgare.* All the results conform with the expected and to the results of this study. This genus has C_4 carbon isotopic values (Smith & Brown, 1973) and a low CO_2 compensation point (Downton & Tregunna, 1968). The species are malate formers (Downton, 1970) with high malic enzyme (Huber *et al.*, 1973), with no PIB (Brown & Gracen, 1972), a single bundle sheath (Hattersley & Watson, 1976; Hatch & Kagawa 1974) with centrifugal chloroplasts (Gutierrez *et al.*, 1974, 1974a) and reduced or ill-defined grana (Brown & Gracen, 1972; Downton, 1970; Gutierrez *et al.*, 1974, 1974a; Carolin *et al.*, 1973). *S. bicolor and S. sudanense* have been biochemically typed as NADP-ME species (Gutierrez *et al.*, 1974, 1974a; Hatch, Kagawa & Craig, 1975) which correlates with the above information.

S. almum *Parodi* **K-MS-NADP**

Exhibits a low CO_2 compensation point (Downton & Tregunna, 1968).

TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1822.

S. versicolor *Anderss.* (Fig. 59) **K-MS-NADP**

TRANSVAAL.—2231–CA (Pafuri), *Ellis* 1891. 2428–CD (Nylstroom), *Ellis* 1612. 2528–CA (Pretoria), *Ellis* 133, 776, 1818. 2531–BB (Komatipoort), *Ellis* 507.

S. verticilliflorum (*Steud.*) *Stapf* **K-MS-NADP**

TRANSVAAL.—2531–BB (Komatipoort), *Ellis* 506. NATAL.—3030–CB (Port Shepstone), *Ellis* 65.

THEMEDA *Forsk.*

A genus typical andropogonoid or eu-panicoid anatomy with a single bundle sheath with grana being absent or ill-defined (Hattersley & Watson, 1976; Carolin *et al.*, 1973).

T. triandra *Forsk.* (Fig. 58) **K-MS-NADP**

A C_4 $\delta^{13}C$ value of $-12{,}0‰$ (Smith & Brown, 1973) with a single bundle sheath (Hattersley & Watson, 1976).

S.W.A.—2118–DB (Steinhausen), *Ellis* 2023. TRANSVAAL.—2528–CA (Pretoria), *Ellis* 1, 371. O.F.S.—2827–DC (Senekal), *Ellis* 974. NATAL.—2832–AC (Mtubatuba), *Ellis* 84. CAPE—3227–DA (Stutterheim), *Ellis* 295. 3420–BC (Bredasdorp), *Ellis* 1670.

TRACHYPOGON *Nees*

T. spicatus (*L.f.*) *Kuntze* **K-MS-NADP**

TRANSVAAL.—2429–AA (Zebediela), *Ellis* 2091. 2528–CA (Pretoria), *Ellis* 426; –CB, *Ellis* 143. 2530–BC (Lydenburg), *Ellis* 451. 2630–CA (Carolina), *Ellis* 1846. NATAL.—2929–DD (Underberg), *Ellis* 211, 219. CAPE.—3029–BA (Kokstad), *Ellis* 222.

URELYTRUM *Hack.*

U. squarrosum *Hack.* **K-MS-NADP**

TRANSVAAL.—2527–DB (Rustenburg), *Ellis* 1226. 2528–CA (Pretoria), *Ellis* 145, 374, 425.

VETIVERIA *Bory*

Species of this genus have a single bundle sheath (Hattersley & Watson, 1976) with panicoid anatomy with grana being absent or ill-defined (Carolin *et al.*, 1973).

V. nigritana (*Benth.*) *Stapf* **K-MS-NADP**

RHODESIA.—1629–AA (Chirundu), *Ellis* 576. S.W.A.—1724–DB (Katima Mulilo), *Ellis* 342, 1771. TRANSVAAL.—2528–CB (Pretoria), *Ellis* 1131, cultivated.

VOSSIA *Wall. and Griff.*

V. cuspidata (*Roxb.*) *Griff.* **K-MS-NADP**

S.W.A.—1724–DC (Katima Mulilo), *Edwards* 4384.

CONCLUSIONS

The correlation between the anatomical criteria employed in this study and available physiological information from the literature is extensive (e.g. 57% of the eragrostoid genera and 80% of the panicoid genera mentioned have had previous relevant studies done) and almost perfect. This confirms and extends the hypothesis that the detection of any one aspect of the Kranz syndrome implies the whole syndrome—not only whether a plant is C_3 or C_4 but to what sub-type of the C_4 pathway it belongs. It can, therefore, be accepted that the anatomy of the bundle sheath and mesophyll is a valid marker and can be confidently used in predicting the physiology of any given species.

The only examples detected in this study where anatomical predictions have not been confirmed by physiological findings can be mentioned as these apparent anomalies require verification. *Sporobolus poiretti* (Gutierrez *et al.*, 1974, 1974a) and *Eriochloa gracilis* (Gutierrez *et al.*, 1976) are PCK-type species but Brown & Gracen (1972) report no PIB which is very unlikely in aspartate formers. *Panicum leavifolium,* an aspartate former probably of the PCK type, has been reported to be a malate former (Downton, 1970). These discrepancies could very easily result from the mis-identification of voucher or experimental material.

Some PCK species, e.g. *Chloris gayana* have high mitochondrial frequencies with extensive membrane development (Hatch, Kagawa & Craig, 1975) which is typical of NAD-ME species. Further study is warranted as high mitochondrial concentrations are possibly, therefore, not exclusive to, or essential only to the NAD-ME type.

In a few cases the anatomy of certain aspartate formers was such that specimens could not be placed in either sub-type with any degree of confidence. Biochemical typing is thus required of such species as *Catalepis gracilis* of the Chlorideae and *Stiburus* and the intermediate species of *Eragrostis* of the Eragrostideae. In these species the chloroplasts normally almost fill the Kranz cells which are in addition not characteristic of either the NAD-ME or PCK type. In addition the mesophyll cells do not show typical shape or arrangement.

Comparison of the results of this study with a taxonomic treatment of the Gramineae (Loxton, 1976) does not conform nearly as closely as does the

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coincidence between anatomical and physiological data. This is probably due to the lack of a modern, comprehensive classification of this family with a thorough taxonomic revision of generic and tribal limits. Thus, the anomalies between the Kranz characteristics and the taxonomic status and position of taxa may be the result of an imperfect systematic treatment rather than to the irrelevance of the Kranz syndrome to the taxonomy of the Gramineae.

Apart from *Alloteropsis* and *Panicum* of the Paniceae there are no other genera with both C_3 and C_4 species and it is likely that this distinction is at least a specific, if not a generic difference. Between malate and aspartate forming genera there is once again overlap only in the Paniceae within the genera *Panicum, Alloteropsis* and possibly *Digitaria*. A re-delimitation of generic limits within the Paniceae appears necessary which may result in the removal of these anomalies. For the present, however, malate and aspartate attributes do not appear to be significant above the species level although with further study there are indications that they are taxonomically as significant as the C_3 and C_4 differences. PCK and NAD-ME types do not show clear relationships at the specific and generic level although their presence in the various tribes definitely is not random. A thorough new treatment of the Gramineae combining both attributes of the Kranz syndrome and morphological criteria will undoubtedly lead to a more natural and evolutionary significant classification of the family. Comparative anatomical studies already confirm the advanced nature of the Kranz sub-families Eragrostoideae and Panicoideae.

The ecological implications of the Kranz syndrome are at this stage not at all clear although there is a definite pattern in their distribution which appears to be related to climatic factors (Vogel *et al.*, 1977) especially prevailing temperature during the growing season. Thus, C_3 species are prevalent in the winter rainfall areas and at higher altitudes along the higher mountain ranges in summer rainfall areas whereas C_4 species are dominant on the drier, hotter interior plateau and along the tropical eastern coastal belt. Malate and aspartate forming grasses also appear to display characteristic distribution patterns although this has not yet been examined in detail. However, 81% of the grasses listed by Leistner (1967) as occurring in the Southern Kalahari are aspartate formers but only 43% of the C_4 grasses in the Bankenveld of the Transvaal are aspartate forming species (Bredenkamp, 1975). Thus, aspartate formers appear to increase with a decrease in precipitation. Tew, Grossman, Fair & Cresswell (1974) have shown that the production of aspartic acid may be the system that allows grasses to exhibit low sensitivity to nitrogen such as found in primary grasses (as in the Kalahari) as compared to climax grasses of high nitrogen sensitivity where malic acid formation usually predominates. It may be, therefore, that aspartate formers are associated with soils of high nitrogen content. This has profound implications in the management of grassland communities for which a thorough knowledge of the Kranz syndrome has distinct economic implications.

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The Significance of the Occurrence of Both Kranz and non-Kranz Leaf Anatomy in the Grass species *Alloteropsis semialata*

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The occurrence of Kranz and non-Kranz leaf anatomy in a single plant species is recorded and the significance of this phenomenon is discussed.

Recent studies on the leaf anatomy of *Alloteropsis semialata* (R.Br.) Hitchc. have revealed the occurrence of two distinct types of anatomical structure within this single grass species[1]. Certain specimens of this grass display a double parenchyma sheath surrounding the vascular bundles as seen in transverse section (Fig. 1). The inner parenchyma sheath is composed of inflated cells with thickened walls and contain large chloroplasts almost entirely filling the cells. These cells are larger than the cells of the outer parenchyma sheath which are thin-walled and without, or with a few, chloroplasts similar in size to those of the mesophyll cells. The chlorenchyma cells are larger than the cells of either parenchyma sheath and are hexagonal to inflated in shape with small intercellular air spaces. Other specimens of *A. semialata* (Fig.2) have a typical mestome sheath of small thickened cells surrounded by a parenchyma sheath of large, thickened cells devoid of, or with few unspecialised chloroplasts. The chlorenchyma cells are tabular, indistinctly radiate, with larger intercellular air spaces.

Plants with large, specialised parenchyma bundle sheath cells with thickened walls and large chloroplasts are variously known as Kranz[2], high photosynthetic capacity[3], low CO_2–photorespiration[4] or compensation[5], C_4[6], or Hatch-Slack[7] plants. Non-Kranz, low photosynthetic capacity, high CO_2–photorespiration or compensation, C_3, or Calvin plants either do not possess a bundle sheath or, if present, it is thin-walled and without specialised chloroplasts. Correlated with these anatomical features are ultrastructural, cytological, physiological and biochemical characters constituting a syndrome of related functional and structural characters here referred to as the Kranz syndrome. Thus, within the species *A. semialata* we have both Kranz and non-Kranz plants. To illustrate the importance of this observation the differences between Kranz and non-Kranz plants will be briefly reviewed.

Cytological specialisations of Kranz plants include a high concentration of cellular organelles, such as chloroplasts, mitochondria and microbodies, and reduced vacuoles in the parenchyma sheath cells[3]. Ultrastructural studies of these organelles reveal the presence of dimorphic chloroplasts[8] in Kranz plants, the ontogeny of which has been studied by Laetsch and Price[9]. These parenchyma sheath plastids are often larger, contain more starch[10], with rudimentary, or no grana[6, 7, 11] and possess a peripheral reticulum[7] which is common to all C_4 plants[12]. These chloroplasts often exhibit a definite orientation within the parenchyma sheath cells[13]. This arrangement may be centrifugal or centripetal[14]. Johnson and Brown[6] consider this arrangement to be of taxonomic significance in certain groups (panicoid and eragrostoid) of the Poaceae. However, this has been invalidated by other investigations[13, 15]. The mitochondria in the parenchyma sheath cells of Kranz plants are larger than those of the mesophyll cells and their internal structure is similar to the peripheral reticulum of the chloroplasts[3, 7, 13]. Non-Kranz plants have a greater number of microbodies or peroxisomes per mesophyll cell than do Kranz plants where they are concentrated in the parenchyma sheath cells[4, 16]. Johnson and Brown[6] present a list of all the grass species of which the chloroplasts have been examined by electron microscopy.

Functionally these structural features of Kranz plants are concerned with the physiology and biochemistry of photosynthesis but it must be mentioned that photosynthetic capacity cannot be correlated with the following structures[15]: presence or absence of grana in the parenchyma sheath chloroplasts which may be lost during the ontogeny of the chloroplasts[17]; the presence or absence of starch grains in bundle sheath or mesophyll cell chloroplasts; the chloroplast size in sheath or mesophyll cells or the location of chloroplasts in the parenchyma sheath cells. The number and concentration of chloroplasts, mitochondria and microbodies in the parenchyma sheath cells, of mature leaves, are the most reliable cytological criteria correlated with Kranz plants[15] and with the taxonomy of the grasses[18].

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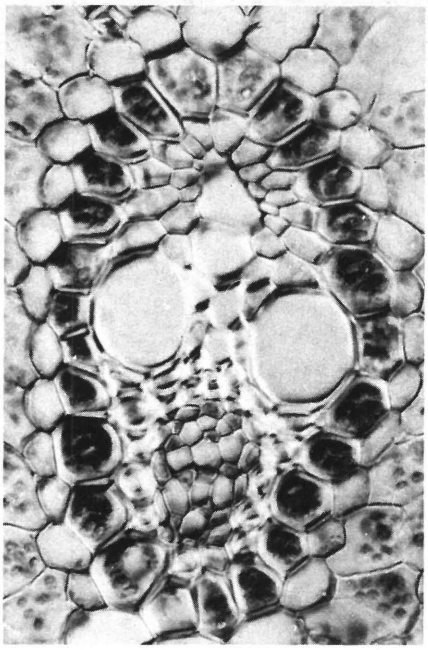


Fig. 1 A first order vascular bundle of the leaf of *Alloteropsis semialata* showing Kranz anatomy. The inner parenchyma sheath cells have specialised chloroplasts and thickened cell walls whereas the outer parenchyma sheath is thin-walled with normal chloroplasts.

Physiological characteristics

Kranz plants have the following physiological traits[3]: increase in the rate of photosynthesis with increasing light intensity and approaching a maximum value two to three times higher than in non-Kranz (low photosynthetic capacity) plants[19]; increased photosynthesis as the temperature rises above 15°–20°C with an optimum about 35°C[5]; below 16°C the chlorophyll is subject to photodestruction and developing leaves are chlorotic[20]; no inhibition of growth at normal oxygen concentration and less inhibition of growth by the oxygen produced by photosynthesis; less loss of reduced carbon, e.g. less glycolate oxidation; net fixation of CO_2 is less limited by low CO_2 concentration; the presence of two active photosynthetic carboxylases (phosphoenolpyruvate and ribulose–1,5 diphosphate) and their associated enzymes in the same leaf resulting in a more rapid uptake of CO_2 allowing the efficient uptake of atmospheric CO_2 and efficient carboxylation of any internal leaf CO_2 which may be released by respiration or photorespiration; low transpiration rate[21]; more rapid rates of translocation and the possible higher concentrations of translocates preventing a feedback type of inhibition of photosynthesis by products such as starch due to the highly developed parenchyma sheath cells. Starch thus accumulates in the bundle sheath when the rate of photosynthesis in the leaf exceeds the rate of translocation to the phloem[5,8].

This is the compartmentalization function for the thick-walled Kranz sheath[18,20,21] whereby the different enzymatic activities between the parenchyma sheath and mesophyll cells are segregated[6]. In fact, it is now recognised that the successful operation of the C_4 pathway is largely dependent upon the spatial separation of the two carboxylases involved in this pathway due to this compartmentation provided by the specialised Kranz-type anatomy[7,22].

Biochemically, plants with the Kranz syndrome synthesize C_4–dicarboxylic acids (oxaloacetic, malic and aspartic acids) as the initial products of CO_2 assimilation. This occurs in the mesophyll cells[23]. These acids are transported to the parenchyma sheath cells where, via the Calvin-Benson cycle, by carboxylation of the C_4 acids, the 3-carbon, phosphoglyceric acid is produced[23,24]. Non-Kranz plants produce phosphoglyceric acid as the first product of photosynthetic CO_2 fixation. Kranz plants utilise most of the available CO_2 producing a high $^{13}C/^{12}C$ ratio[25].

Kranz plants exhibit considerable physiological variability[26] and two biochemical types of C_4 plants have been proposed: the malate formers and the aspartate formers[20,22]. However, the type of non-structural storage polysaccharide, starch or fructosan, accumulated by grasses tends to generally conform with C_4 and C_3 photosynthesis[27,28] except for certain members of the non-Kranz tribes Arundineae and Stipeae which store starch instead of fructosan as in all other non-Kranz tribes.

The ecological implications of the concept of two distinct groups of plants with differing photosynthetic production capacities has been discussed by Black[3]. Briefly, Kranz plants, which fix CO_2 at higher rates[19] and with greater capacities, have an initial advantage when competing with non-Kranz plants. In certain stress environments, such as arid regions, where short periods of adequate water supply are followed by periods of high temperature and high light intensity, Kranz plants are favoured owing to their having evolved the ability to respond to increased light intensities and temperature and because they are about twice as efficient as non-Kranz plants in utilising water. These plants require only about half as much water as non-Kranz plants to produce one unit of dry matter[20]. This is perhaps linked to the observation[29] that in many xeric Kranz grass species the stomata do not appear to open appreciably at all. In addition, the arrangement of the chlorophyll tissue in Kranz leaves results in a very close association of the photosynthetic apparatus to the water columns of the conducting tissue[5]. This may have survival value for the plant under drought conditions especially if the suberin layer between the bundle sheath and mesophyll[30] acts as a barrier to water.

In agricultural environments, where many crops are non-Kranz, the plants which become pests are often high photosynthetic capacity plants. Black[3] postulates that photosynthetic capacity is the dominating factor in plant ecology and thus selective pressures for particular adaptations are supplied by environmental factors limiting photosynthesis[7]. This correlating of structural and functional aspects of photosynthesis with environmental selection pressures has important applications in agriculture. The situation in *Alloteropsis* probably presents a unique opportunity to study the

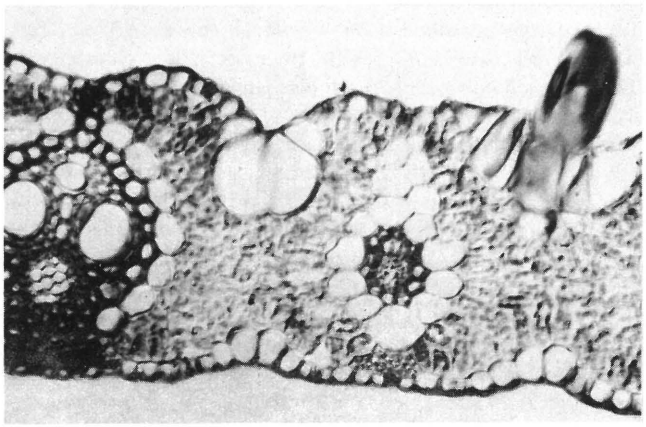


Fig. 2 Part of a section of a leaf of *Alloteropsis semialata* showing non-Kranz anatomy. The vascular bundles are surrounded by a typical inner mestome sheath and an outer sheath of large cells with few unspecialised chloroplasts.

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implications of this postulation, the results of which can provide information about the origin and evolution of plants and lead to a better understanding of the inherent genetic potential of the plant[7]. Breeding programmes could select components for an efficient photosynthetic system, such as greater carbon conservation[19], and attempt to improve them. This method of increasing dry matter production among temperate grass crops is particularly attractive. In addition, the introduction of temperate (non-Kranz) or tropical (Kranz) characters into species of the opposite type may serve to extend their range of cultivation[19].

A plant exhibiting any one of these Kranz characteristics can with a very high degree of confidence, be expected to exhibit any of the remaining characteristics[2–4,7,25]. This predictability of the whole syndrome upon the detection of any one aspect of it allows the generally accepted justification for regarding the leaf anatomy as a valid marker[7]. The Kranz syndrome is thus a remarkable example of form related to function with the functional aspects of photosynthetic carbon fixation being correlated with the anatomy of the leaf.

Distribution of plant types

From the above review of some aspects of the Kranz syndrome it will be realised that the anatomical differences observed in *Alloteropsis* imply important physiological, biochemical and ecological differences within this species, the taxonomic and phylogenetic implications of which are numerous and diverse. Kranz plants occur in the following families: Gramineae, Cyperaceae, Amaranthaceae, Chenopodiaceae, Portulacaceae, Euphorbiaceae, Nyctaginaceae, Aizoaceae, Compositae, and Zygophyllaceae[2,3,7,20]. These families and their known Kranz species[31] have a very high proportion of tropical species and it can generally be said that temperate plants are non-Kranz whereas the Kranz syndrome is largely a tropical phenomenon[7]. This, in conjunction with the fact that tropical environments are subject to the high temperatures and light intensities favouring Kranz plants, added weight to the idea that Kranz plants are plants of tropical origin. However, there are many typically tropical plants, and even grasses, such as the important bamboos and rices, which are non-Kranz. Laetsch[7], therefore, proposed that a combination of both aridity and high temperatures, as occur in some tropical environments, increases CO_2 diffusion resistance, thereby resulting in CO_2 levels limiting to photosynthesis. Kranz plants have probably evolved their efficient CO_2 fixation system in response to this selection pressure.

Apart from the Gramineae several other families exhibit both Kranz and non-Kranz genera: Amaranthaceae, Chenopodiaceae, Compositae, Saxifragaceae, Cyperaceae[2] and Euphorbiaceae[3]. Only in the genera *Atriplex*, *Bassia*, *Euphorbia* (which also has Crassulacean Acid Metabolism[20]), *Cyperus* and *Panicum* do differences between the species occur. *A. semialata* is the first known case where Kranz and non-Kranz have been reported for a single species[32].

Within the Poaceae the distribution of Kranz and non-Kranz species is very orderly[2]. All of the species in the sub-families Arundinoideae, Oryzoideae and Festucoideae are non-Kranz and all the species of the Eragrostoideae and Aristidoideae are Kranz. Only in the sub-family Panicoideae do both Kranz and non-Kranz types occur. In this sub-family the tribes Andropogoneae, Anthephoreae, Anthropogoneae, Arundinelleae Garnotieae, Maydeae, Tracheae and Melinideae are Kranz and the tribe Isachneae non-Kranz[2]. The tribe Paniceae alone posses both Kranz and non-Kranz genera. However, all genera are either Kranz or non-Kranz except for the genus *Panicum*[2,19] and the recently reported genus *Alloteropsis*[32].

The Southern African distribution of all the known non-Kranz members of the tribe Paniceae, including non-Kranz specimens of *A. semialata*, conform very closely. All these grasses are restricted to the higher areas of the Southern and Eastern Transvaal, especially along the Drakensberg escarpment and possibly extend northwards to the Eastern Highlands of Rhodesia. In Natal they occur in the midlands and not at higher altitudes in the Drakensberg, occurring only in the montane and sub-alpine belts[33]. The distribution reaches the coast near Pondoland and extends southwards to the East London area at lower altitudes. At higher altitudes the distribution probably extends along the Winterberg to the Bedford district.

This distribution follows closely that of the temperate and transitional forests as well as the inland tropical forests[34] in areas with rainfall of 750 mm or more. Indeed, it is in the shady, damp, ground layer of these forests that many of the non-Kranz members of the Paniceae occur. Examples are *Oplismenus hirtellus* (L.) Beauv., *Pseudechinolaena polystachya* (H.B.K.) Stapf and several (probably all) shade-loving species of *Panicum* such as *P. aequinerve* Nees, *P. hymeniochilum* Nees and *P. laticomum* Nees. The other non-Kranz species occur in open grassland of the following major veld types[34] – North-eastern Mountain Sourveld, North-eastern Sandy Sourveld, Turf Highveld and the Highveld Sourveld and Dohne Sourveld. These include *Sacciolepsis typhura* (Stapf) Stapf, and *Acroceras macrum* Stapf, which prefer damp habitats, and *Panicum ecklonii* Nees, *P. natalense* Hochst. and the non-Kranz specimens of *Alloteropsis semialata*, all three of which are important constituents of sourveld[39].

All the Kranz specimens of *A. semialata* have to date been collected from areas of lower altitude and rainfall (775 mm or less) surrounding the main distributional range of the non-Kranz species. These include the Pretoria and Middelburg areas in Bankenveld and Sourish Mixed Bushveld, the Tzaneen area in Lowveld Sour Bushveld and the Newcastle district in Natal Sour Sandveld.

This disjunct distribution of the Kranz specimens of *A. semialata*, circumscribing the higher, cooler and wetter areas, in which the non-Kranz specimens are found, appears to support the suggestions that the Kranz syndrome represents an adaptation to habitats with high temperatures and irradiance[2] as well as limited water supplies during the period of active growth[7,35]. However, it casts some doubt on the assumption that the syndrome arose under these conditions relatively early, and only two or three times in the evolution and radiation of the grass family[2,25]. The distribution of Kranz and non-Kranz *Alloteropsis* specimens in South Africa appears to indicate that the Kranz condition has evolved fairly recently in this taxon (as in *Panicum*[6]), and perhaps more than once, with a subsequent extension of distribution into warmer and drier areas.

Evolution

The full Kranz syndrome, with numerous associated differences, suggests a complex and integrated evolutionary change, the evolution of which would be unlikely to recur. When the syndrome was only known from tropical grasses this posed no problem, but the occurrence of the syndrome in several unrelated families suggested that the whole syndrome had arisen polyphyletically among these more advanced dicotyledon and monocotyledon families[36]. Initially this appeared difficult to explain. The distribution patterns of Kranz and non-Kranz plants (such as in *Alloteropsis*) together with the fact that the syndrome has been evolved independently in a number of unrelated taxa suggests, however, that the syndrome has evolved in response to similar selective forces operating in the environment. It also suggests that the non-Kranz condition, which is universally present in less advanced plants, is more

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primitive and appears to be the ancestral type. This possession of similar structural and functional features in unrelated taxa is a remarkable example of convergent evolution[7]. Many questions can be asked about the selective forces of the environment responsible for this adaptation as well as the structural changes necessary to bring about the Kranz condition. *Alloteropsis* would appear to be ideal material for studying the mechanisms of this change as well as the adaptive significance of the Kranz syndrome as a whole. If the syndrome has in fact been recently evolved, or is still evolving in *A. semialata*, examination of this species by electron microscopists, geneticists, biochemists and physiologists will be of the utmost importance to a fuller understanding of the functioning, evolution and taxonomic significance of this syndrome.

With regard to the changes required to acquire the Kranz syndrome, tissue culture experiments have shown that non-Kranz tissue of Kranz sugar cane can generate shoots with the C_4 photosynthetic pathway[7]. This demonstrates that both the mesophyll and parenchyma sheath cells have a genotype with the potential to form similar enzyme complements. Their compartmentalization into separate cell layers in Kranz plants results from the different phenotypic expression of this common genotype. In *Alloteropsis*, if Kranz and non-Kranz specimens have similar genotypes, the possibilities of this type of study are numerous.

If a non-Kranz chloroplast has this inherent potential to generate a Kranz-type chloroplast[20] then it is not surprising that the C_4 pathway often lacks taxonomic continuity. It may, therefore, be a highly mutable condition susceptible to being triggered off by a particular combination of environmental factors[20].

With the apparent exception of a single enzyme, all the C_4 enzymes also occur in C_3 plants[20]. This supports the idea that mesophyll and parenchyma sheath chloroplasts differ only quantitatively in their enzymology. The success of Kranz plants in fully utilising both carboxylation reactions can, therefore, once again be attributed to the compartmentation provided by the Kranz anatomy.

The above, together with the work of Björkman *et al.*[37] with *Atriplex* hybrids, suggest that the change from non-Kranz to Kranz is not as great as originally thought and that only a few genes may be involved. Hybrids between Kranz and non-Kranz parents were shown to be intermediate in leaf anatomy, ultrastructure and physiology by studies of segregation for structure, enzymes, CO_2 compensation and $^{13}C/^{12}C$ ratios. Crosses in other genera with C_4 and C_3 species, such as *Panicum* and *Euphorbia*[38] have been unsuccessful but *Alloteropsis* appears a likely subject to confirm and extend the valuable information gained from *Atriplex* hybrids.

Undoubtedly these genera represent taxa in which the evolutionary change from non-Kranz to Kranz has occurred. The distribution of Kranz and non-Kranz species in *Panicum* may indicate that this evolutionary change may be fairly recent or that it has occurred several times in the Paniceae. Johnson and Brown[6] are of the opinion that the Kranz syndrome evolved only once early in the phylogeny of the grass family and feel that a new taxonomic treatment of the Paniceae and *Panicum* could produce completely Kranz and non-Kranz groups from these taxa. The situation in *Alloteropsis* warrants mention. The Kranz and non-Kranz specimens of *A. semialata* can be distinguished on gross morphology and preliminary sorting of all the material in the National Herbarium in Pretoria does suggest that the Kranz and non-Kranz plants can perhaps be regarded as distinct species. Intermediates do seem to occur, however, and the question of the taxonomic status of the Kranz syndrome in *A. semialata* requires urgent attention.

Initially, when this anomalous vascular bundle sheath structure was detected in *A. semialata*, it was anticipated that specimens exhibiting intermediate types of anatomy would be found. It was hoped that intermediate anatomical types would supply evidence as to how the structural change from non-Kranz to Kranz occurs. However, to date, 19 specimens of *Alloteropsis*, from numerous localities, have been examined, but no variation between the two distinct types described above has been seen.

Specimens with intermediate anatomy would assist considerably in interpreting the anomalous panicoid bundle sheath structure in the Kranz plants of *A. semialata* (Fig. 1). It is suspected that the condition in Kranz *A. semialata* specimens represents a stage in the loss of the outer parenchyma sheath to give a typical single parenchyma sheath. No variation in the degree of development of the outer parenchyma sheath was observed in any of the specimens studied.

Of considerable interest is the size, shape and arrangement of the mesophyll cells surrounding the vascular bundles in Kranz specimens. They do not display the radial arrangement which ensures that most of the cells, if not all, are in contact with the parenchyma sheath cells as in most Kranz plants. It is interesting to speculate that the enlargement and elongation of the outer parenchyma sheath cells would result in the typical Kranz condition with the subsequent loss of chlorenchyma in the mesophyll cells which are no longer in contact with the bundle sheath cells. This implies the origin of the single layer of radiating mesophyll cells, found in many Kranz grasses, from the typical parenchyma bundle sheath of the non-Kranz festucoid grasses and appears unlikely. It is interesting to note the frequent description of Kranz anatomy in which the vascular bundles are described as being surrounded by two, concentric, chlorophyllous layers, an inner parenchyma and an outer mesophyll sheath[20,21].

If the above does occur, or if the outer parenchyma sheath becomes completely reduced, then the inner parenchyma sheath of the Kranz specimens of *A. semialata* probably represents a specialised mestome sheath. This is not impossible to conceive if one notes the thickened radial and inner tangential walls of these cells (Fig. 1). This differential thickening is common in mestome sheaths of the grasses. In addition Laetsch[7] describes the presence of a thin, electron opaque, lipid layer between the cell walls of the parenchyma sheath and mesophyll cells in Kranz plants. It has been demonstrated[18] that a similar osmophilic layer occurs in the mestome sheath of festucoid grasses. It is absent from the parenchyma sheath cells of these non-Kranz plants. Thus the inner parenchyma sheath in Kranz *Alloteropsis* plants, and the specialised parenchyma sheath of typical Kranz grasses may, in fact, be homologous with the inner mestome sheath of non-Kranz grasses. The situation is complicated, however, by the Eragrostoideae, where, in additon to a typical Kranz parenchyma sheath with an osmophilic layer, there is mestome sheath without a lipid layer[18]. In the Aristidoideae the osmophilic layer is lacking in both the parenchyma sheaths present in *Aristida*.

The above discussion must remain sheer speculation until ultrastructural, biochemical and physiological studies, hybridisation experiments and a taxonomic revision of the genus *Alloteropsis* are conducted. This interesting indigenous species, therefore, offers unique research opportunities to South African scientists of various disciplines.

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The Geographical Distribution of Kranz Grasses in South Africa

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A survey of the grasses in South Africa shows that C_3 and C_4 species are, to a marked degree, separated geographically. C_4 grass species are more abundant than C_3 grass species over most of the country. The only areas where C_3 grass species predominate are the winter rainfall region of the Western Cape and the summits of the Drakensberg and other mountain ranges in the Eastern Cape. This distribution pattern suggests that low temperatures during the growing season favour C_3 species and high temperatures C_4 species.

'n Ondersoek van die grassoorte in Suid-Afrika toon dat C_3 en C_4 spesies in 'n hoë mate geografies geskei is. Oor die grootste deel van die land is C_4 grassoorte meer volop as C_3 soorte. Die enigste gebiede waar C_3 grassoorte oorweeg is die winterreënstreek van die Weskaap en die kruine van die Drakensberg en ander bergkettings ten suide daarvan. Hier die verspreidingspatroon dui daarop dat lae temperature gedurende die groeiseisoen C_3 spesies bevoordeel en hoë temperature C_4 spesies.

Prior to 1965, all green plants were thought to assimilate carbon dioxide from the atmosphere via the Calvin-Benson photosynthetic pathway. At this time, however, an alternative method of carbon dioxide fixation was discovered in sugarcane, the Hatch-Slack pathway.[1,2] In the Calvin cycle the initial product of carbon dioxide fixation is the three-carbon substance phosphoglyceric acid (PGA), while a four-carbon molecule, oxaloacetic acid (subsequently transformed to malic and/or aspartic acid), constitutes the first carboxylation product in the Hatch-Slack mechanism.[3] Hence the designations C_3 and C_4, respectively, are now used to distinguish between plants utilizing the two different pathways.

It soon became evident that these two modes of photosynthesis are species specific and that C_4 plants differ in their leaf anatomy from C_3 species,[4] in particular the vascular bundles are surrounded by a distinct, thick-walled sheath ('Kranz') of cells containing specialized chloroplasts which differ in some way, such as size, shape or number, from the chloroplasts of the mesophyll.[4,5] The mesophyll cells are radially arranged and are either in direct contact with a Kranz cell or at the most one cell removed from one.[6] In the Kranz cells the malate and aspartate are decarboxylated and the resulting carbon dioxide converted to sugars and starch via the normal Calvin cycle.[7] C_3 plants have no such specialized sheath and

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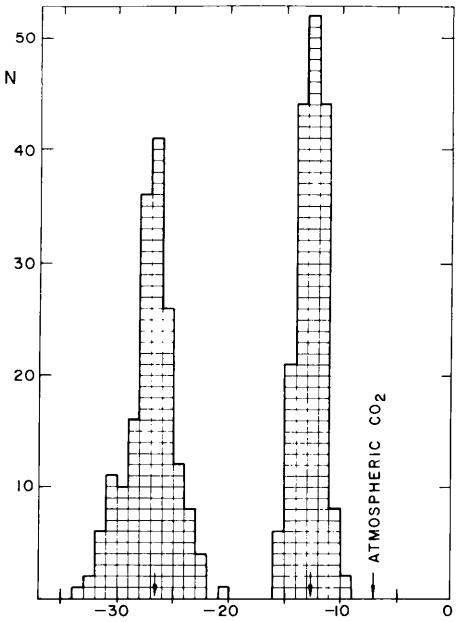


Fig. 1. Histogram of the relative ^{13}C content, δ, of 351 grass species showing the clear distinction between C_3 species ($\delta < -20‰$) and C_4 species ($\delta > -16‰$). Data from various sources.

the mesophyll cells show no regular pattern of arrangement.[4] The designation Kranz and non-Kranz is thus synonymous with C_4 and C_3.

Succulents, which exhibit Crassulacean acid metabolism (CAM), constitute a third category of plants, those capable of utilizing both the C_3 and the C_4 modes of carbon dioxide fixation.[7,8] This phenomenon is, however, of no further significance in the present context since it does not occur in the Gramineae.[9]

To date, several hundred Kranz plant species belonging to at least 13 different angiosperm families have been identified.[10] The syndrome is especially common in the grass family, but is also present among the Cyperaceae, Chenopodiaceae, Aizoaceae, Amaranthaceae and others. The known Kranz species have a marked tropical affinity, a fact that has given rise to the belief that the Kranz syndrome is an evolutionary adaptation in response to a tropical environment.[4] The ability of these plants to photosynthesize at high temperatures and high light intensities apparently substantiated this assumption.[4,11] Their high photosynthetic efficiency together with economy in water utilization, again, led to the proposal that aridity was an important environmental factor in determining the adaptive advantage of C_4 plants.[4,12]

Several field studies seem to support the contention that the C_4 photosynthetic pathway evolved in response to a hot, dry climate. Thus it has been found that the growing season of a non-Kranz species in a short-grass prairie ecosystem is confined to the cool moist months, while that of a Kranz species coincides with the hotter, dry months.[13] A strong correlation has also been established between the C_4 pathway and summer annuals in desert environments of the south-western United States and Mexico.[14] It has furthermore been observed that the non-Kranz form of *Alloteropsis semialata* is restricted to higher and cooler areas of South Africa, whereas the Kranz form of the species occurs mainly at lower, warmer altitudes.[15] The greater drought tolerance of the C_4 species of *Atriplex* as compared to that of the C_3 species of the same genus has moreover been demonstrated by experiment.[12]

On the other hand, certain growth-chamber and field experiments performed on C_3 and C_4 grasses at high light intensities and with both high and low water supply have failed to reveal differences in the overall growth rates.[16]

Furthermore, surveys of the vegetation in certain desert environments[17–19] have shown C_4 species to be rare in comparison with C_3 or CAM plants.

It thus remains to be established what the specific environmental conditions are that provide C_4 species with a competitive advantage over other plants and, by implication, what the natural habitats were that originally were responsible for the evolution of the C_4 pathway. It was considered that a possible approach to these problems was to investigate the distributional frequency of C_3 and C_4 species over a large region covering a wide range of climatic zones.

Since the majority of the known C_4 species belong to the Gramineae, and since the grasses constitute a significant proportion of most vegetational communities in South Africa, the survey was restricted to this family. The investigation was limited to South Africa, Lesotho and Swaziland, mainly because sufficient information on their flora is available and because the region as a whole comprises a wide variety of climatic types.

Methods

The C_3/C_4 status of most of the approximately one thousand grass species that occur in South Africa was ascertained and the frequency of occurrence calculated for each region. There are several methods available for distinguishing between C_4 (Kranz) and C_3 (non-Kranz) plants.[10] The criteria applied in this study were either the leaf anatomy or the ratio of the carbon isotopes $^{13}C/^{12}C$. The absence of a demonstrated case of CAM in the grass family permits the use of isotope ratios as a reliable diagnostic test.[9] Previously published lists of C_4 plants were also consulted.

In Fig. 1 the relative ^{13}C content of 351 different grass species is plotted, indicating the clear separation between C_3 and C_4 plants, since the samples with a relative ^{13}C content more negative than −20‰ are all C_3 plants, while those more positive than −16‰ are C_4. The relative isotope ratios of several species for which data have not been published elsewhere are given in Table 1. The main advantage of adopting the carbon isotopic composition as a criterion is that dried herbarium specimens can be used whereas other methods depend upon the availability of fresh leaf material.

For the anatomical survey a collection of 4 000 herbarium-vouchered, field-fixed anatomical preparations of grass leaf blade transverse sections was available. This comprised 680 grass species and, since the chloroplast structure is preserved, all could be classified as either C_3 or C_4.

The botanical survey of Acocks' *Veld Types of South Africa*[20] served as a basis for our investigation. The author defines a veld type as a 'unit of vegetation whose range of variation is small enough to permit the whole of it to have the same farming potentialities' and he divides the vegetational cover of South Africa into 70 different veld types which include 75 variations. The commonly occurring plant species are listed separately for each veld type or subdivision, so that it was possible to consider all vegetational units individually. In each case the grass species were classified as being either Kranz (C_4) or non-Kranz (C_3) and the percentages of C_4 species determined. On the basis of the frequencies established the various veld types were grouped into five categories, namely:

Group I: Exclusively C_4 areas: veld types in which 95% or more of the grass species are Kranz (C_4).

Group II: Predominantly C_4 areas: veld types in which 75% to 95% of the grass species are Kranz (C_4).

Group III: Intermediate areas: veld types in which 25% to 75% of the grass species are Kranz (C_4).

Group IV: Predominantly C_3 areas: veld types in which 5% to 25% of the grass species are Kranz (C_4).

Group V: Exclusively C_3 areas: veld types in which less than 5% of the grass species are Kranz (C_4).

Since Acocks does not list all the grass species which occur in

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Table 1. Relative ^{13}C content with respect to the SMOW standard, δ, (in parts per thousand, ‰) of some South African grass species.

Species	Collector & Specimen No.	Locality	Sa. No. M-	Anal. MC-	$\delta\ ^{13}C$ ‰
Alloteropsis semialata	Harrison 339	Mtubatuba	456	680	-11,5
" "	Ellis 423	Rustenburg	457	681	-26,7
Arundinella nepalensis	Ellis 1436	Cathedral peak	414	643	-12,1
Axonopus compressus	Ellis 1468	Port Shepstone	419	648	-12,6
Bromus catharticus	Van Zyl	Rietondale Pretoria	337	536	-24,2
Chloris gayana	Van Zyl	Rietondale Pretoria	232	479	-13,0
Cortaderia selloana	Van Zyl	Rietondale Pretoria	233	478	-24,4
Cymbosetaria sagittifolia	Ellis 2085	Limpoporivier	418	647	-13,9
Cynodon dactylon	Van Zyl	Rietondale Pretoria	338	537	-12,7
Cypholepis yeminica	Giess, Volk en Bleissner 6357	Grootfontein	684	977	-13,0
Digitaria decumbens	Van Zyl	Rietondale Pretoria	237	477	-12,9
" macroglosa	Strey and Huntley 5002	Ubombo Natal	659	953	-12,4
" swazilandensis	Ellis 2121	Port Shepstone	415	644	-11,8
Dregeochloa calviniensis	Dekkers s.n.	Loeriesfontein	429	660	-26,3
" pumila	De Winter and Giess 6235	Lüderitzbucht Lagoon	430	661	-25,9
Ehrharta calycina	Van Zyl	Rietondale Pretoria	332	521	-23,9
Elionurus argenteus	Acocks 382	Kuruman	489	706	-11,5
Elytrophoris globularis	Dinter 7390	Grootfontein	930	1264	-25,7
" spicatus	Schweichardt 2089	Grootfontein	931	1261	-26,2
Enneapogon brachystachus	Kinges 2250	Ausweich Lüderitz	648	928	-13,3
" cenchroides	Kinges 2451	Numis Lüderitz	649	927	-14,8
" scaber	Kinges 2694	Tschaukaib Hills Lüderitz	646	929	-13,0
" scoparius	Strey 2478	Kraaipoort Rostock	654	912	-14,2
Entoplocamia aristulata	Ellis 938	97 miles from Walvis Bay	644	933	-16,4
Eragrostis capensis	Bourquin 601	Zululand	472	698	-13,2
" curvula	Van Zyl	Rietondale Pretoria	247	475	-13,4
" cyperoides	Vogel	Spenserbaai	674	628	-13,1
" spinosa	Vogel	Namib	599	847	-14,9
" superba	Moll 4419	Tongoland	470	696	-13,1
Hemanthria altissima	Van Zyl	Rietondale Pretoria	249	476	-12,2
Heteropogon contortis	Ferrar 21	Barkley West	486	701	-11,4
Lasiochloa longifolia	Ellis 1259	Ceres	417	646	-27,6
Microstegium capense	Ellis 1872	Magoebaskloof	421	650	-12,5
Monelytrum luderitzianum	Ellis 928	Windhoek	643	934	-14,5
Odyssea paucinervis	Seydel 869	Swakopmund	652	915	-14,0
Panicum gilvum	De Winter and Giess 6911	Ondangua	933	1259	-11,0
" lanipes	Acocks 7595	Prieska	685	978	-12,6
" phragmitoides	Pentz 8084	Pretoria	932	1262	-12,7
Paspalum polystachyum	Van Zyl	Rietondale Pretoria	259	483	-12,1
" virgatum	Van Zyl	Rietondale Pretoria	262	482	-12,9
Phalaris arundinacea	Van Zyl	Rietondale Pretoria	329	522	-24,4
Phragmites communis	Vogel	Ndumo	322	490	-25,4
Poa binata	Van Zyl	Rietondale Pretoria	333	529	-23,3
Schmidtia kalahariensis	Kinges 2436	Weissenhorn Farm Lüderitz	653	952	-13,0
" pappophorroides	Louw 1887	Marko Aus	645	930	-12,3
Sporobolus fimbriatus	Van Zyl	Rietondale Pretoria	269	480	-13,2
Tragus koeleroides	Breuckner 516	Mafeking	474	700	-12,0
Triraphis fleckii	Ellis 895	Karasberg	658	955	-14,5
" pumilio	Giess 8780	Swakopmund	651	1398	-11,8

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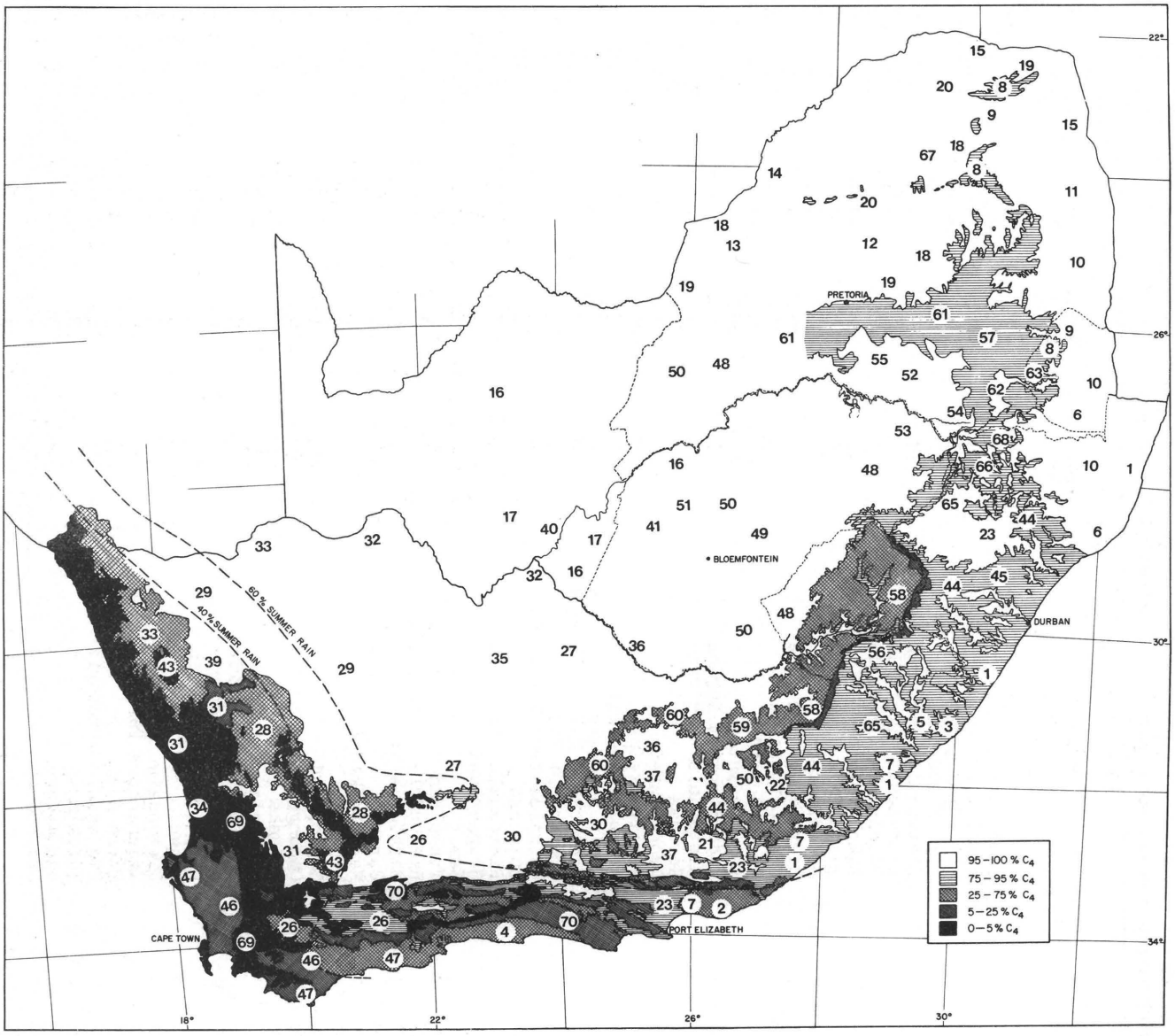


Fig. 2. Map showing the distribution of C_3 and C_4 grass species in South Africa. The numbers refer to the different veld types as listed by Acocks.[20]

any given veld type, the results based on his data were verified by using detailed plant check-lists available for specific regions.[21–42] It was found that the initial values obtained seldom differed significantly from the ratios derived by means of the extensive lists and that minor adjustments were necessary in but a few cases. This finding gives us confidence that the short lists of Acocks are sufficiently reliable for the classification outlined above and can thus be used whenever complete vegetational surveys do not exist or could not be consulted.

Results

The frequency distribution of C_3 and C_4 grass species for each of the veld types or variations delineated by Acocks is presented in Fig. 2.

The most striking result of the survey is that C_4 grass species predominate over most of South Africa (Groups I and II). The only regions where C_3 grass species are more abundant (Groups IV and V) are the highest parts of the Drakensberg mountains and the winter rainfall areas on the west and south coast of the Cape Province, with outliers on the mountains of the Cape Folded Belt.

The different vegetational units will be discussed briefly below. The numbers referred to in brackets are those of the different veld types used by Acocks in his publication.

Group I

The veld types in this group cover the greater part of South Africa, noticeably the northern and central interior and the Transvaal and Zululand Lowveld. Of the 39 veld types accommodated in this category, the most important are the Bushveld and False Bushveld types (9–22), a large portion of the Karoo and False Karoo types (excluding the southern mountainous False Karoo and western Karoo areas) (23a, 26a, 27, 29, 30, 31b, c, 32, 33b, c, 35–41) and the Pure and False Grassveld types which occupy the remainder of the central interior (48–56, 61a, 62, 65, 67, 68). The western variation of the Bankenveld (61a) is grouped here as it appears that the C_3 grass component decreases westwards.[29,30] There is a possibility, however, that some of the Pure Grassveld types in this category actually have a higher proportion of C_3 grasses than is to be concluded from Acocks and should thus be assigned to Group II.[25–27] Finally, the Zululand Palmveld (1b) and the Zululand Thornveld (6), together with the northern variation of the Valley Bushveld (23a) along the drier east coast river valleys, belong to Group I.

In all the areas of this category the very small proportion of C_3 grasses present are aquatics or shade-loving species, with the exception of *Schismus barbatus* in the Central Upper Karoo (27). This is a C_3 danthonoid species of southern affinity which

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probably makes use of the autumn rains of this region.[25]

Group II

These veld types are confined to the eastern parts of South Africa—east of the Drakensberg in the south and along the Transvaal Drakensberg in the north.

The majority of the Coastal Tropical Forest types (1a, c, d, 3, 5, 7a) are included in this category. Further inland the North-east Mountain Sourveld (8), several of the Pure and False Grassveld types (57, 61b, c, 63, 64, 66), and two of the Temperate and Transitional Forest and Scrub types (44a, 45) similarly belong to Group II, as do some of the Karoo veld types (24, 26b, c, 42) and notably the southern occurrences of the Valley Bushveld (23b–e).

At higher altitudes and latitudes all these veld types adjoin others with a higher proportion of C_3 species, whereas at lower altitudes and latitudes they border on exclusively C_4 areas.

The non-Kranz grasses of these veld types can be divided into forest-floor and margin species and those found in full sunlight in open grassland. The shade-tolerant forest species include *Oplismenus hirtellus, Panicum aequinerve, P. laticomum* and *Prosphytochloa prehensilis,* together with *Sacciolepis curvata* and *Leersia hexandra* from moist habitats. All of these have tropical affinities and belong to the tribes Oryzeae and Paniceae. *Ehrharta erecta, Stipa dregeana* and *Brachypodium flexum* are also forest-floor species but of southern affinity.

Non-Kranz grass species present in the open grassland are *Panicum ecklonii, P. natalense, Festuca costata, Poa binata* and C_3 specimens of *Alloteropsis semialata.* Thus, in both the forests and the grasslands of this class, temperate and tropical C_3 grass species occur in about equal proportions. However, the panicoid grassland C_3 species appear to be exclusively confined to the veld types of this category, whereas the festucoid and arundinoid C_3 species are also widely distributed further south, and the oryzoid species occur northwards in shady or aquatic habitats.

Some of the south-eastern and southern Karoo Veld types in this group have a few C_3 grass species listed for each area, such as the temperate species of the Arundinoideae: *Ehrharta erecta, E. calycina, Melica racemosa, Stipa dregeana, Merxmuellera stricta, M. disticha* and *Pentaschistis* spp. The areas in question either adjoin the exclusively C_4 Karoo types or are separated from them by mountain ranges with a higher percentage of C_3 grass species. All the non-Kranz grasses in these areas are southern elements and the C_3 members of the tropical Paniceae are not present.

Group III

The Coastal Tropical Forest areas south of the Great Fish River (2, 4, 7b) exhibit a large proportion of temperate C_3 grass species. Some Temperate and Transitional Forest and Scrub as well as Pure Grassveld types also show approximately equal numbers of C_3 and C_4 grass species, with the C_3 species increasing in abundance at higher altitudes. This holds true for the western extensions of the Dohne Sourveld (44b), the Stormberg Plateau Sweetveld (59), the Karroid Merxmuellera Mountain Veld (60), and the Themeda-Festuca Alpine Veld (58). In addition to the arundinoid genera enumerated in Group II, members of the Festuceae become important in these veld types. Such genera as *Bromus, Festuca, Helictotrichon* and *Koeleria* are listed.

Along the southern Cape coast the areas of Coastal Rhenosterbosveld (46) and Coastal Macchia (47) east of Swellendam fall into this category. A transition to higher percentages of C_3 species in the west seems to be suggested by the check-list for the Riversdale area.[36] In addition to the typical temperate festucoid and arundinoid grasses, species from the tropical tribes Eragrosteae, Sporoboleae, Paniceae, Andropogoneae and Aristideae are also found here.

The western Karoo areas have a greater proportion of C_3 grass species than those of the central Karoo. The Western Mountain Karoo (28) and the typical Namaqualand Broken Veld (33a) have thus been regarded as belonging to this group as has the Spekboomveld (25) in the south.

Group IV

The areas of the Coastal Rhenosterbosveld (46) and Macchia (47) on the west coast contain only a small grass component in comparison with the eastern regions of these veld types. C_4 species probably do not occur here but as little information is available the grouping must be regarded as somewhat tentative. In addition, the Coastal Rhenosterbosveld (46) and Coastal Macchia (47) areas to the west of Swellendam are placed in this category.

Acocks does not list grasses in the False Macchia (70), but since at least the mountainous areas of this veld type harbour a very high C_3 grass constituent, it is classified as belonging to Group IV. Here, too, the C_4 grass species apparently become more abundant towards the east.[36]

The proportion of C_3 grass species increases with altitude in the Alpine belt of the Drakensberg (58), so that above about 2 800 m the only C_4 species found amongst 25 common non-Kranz species are *Harpochloa falx, Eragrostis caesia* and *Stiburus alopecuroides.*[35,36,42] For this reason a small area on the top of the mountains is somewhat arbitrarily placed into Group IV. The exact delineation must, however, await a detailed investigation of the region.

Group V

Three of the western Karoo veld types—the Strandveld (34b), the Namaqualand Coast-belt (31a) and the Mountain Rhenosterveld (43) can be regarded as being exclusively C_3 in grass composition, although grass species are not numerous. However, this is probably due to human interference and, if protected, arundinoid genera such as *Ehrharta, Chaetobromus, Lasiochloa, Schismus,* and *Merxmuellera* would become plentiful. Another interesting effect of human activity in the Namaqualand coastal area is the increasing importance of the C_4 species *Eragrostis spinosa* and *E. cyperoides,* both of which are migrants down the west coast from the Namib desert.

The Macchia (69) of the south-western Cape Province has also been assigned to Group V because Acocks mentions only C_3 genera for this veld type. However, it is unlikely that no C_4 species occur in the fynbos. The check-list for Jonkershoek[39] shows less than 5% C_4 species, but the check-lists for the Cape Peninsula[40,41] and the Cape Hangklip area[37,38] both have larger proportions of C_4 grasses. The latter two areas should thus be classified under Groups III or IV. However, these areas include coastal habitats where C_4 species occur in saline environments and this fact probably accounts for the misleadingly high number of C_4 species in the check-lists. The coastal strip of the fynbos should probably be included in the Coastal Macchia (47) veld type, in which case the remaining fynbos (69) would then undoubtedly belong to Group V.

Discussion

The unexpectedly distinct separation between C_3 and C_4 grass species in South Africa enables us to draw some conclusions as regards the environment preferred by the two types of grass.

1) According to the *climatic* maps[43,44] of the region, only a very small portion of the country has a tropical (megathermal) climate, namely the extreme northern part of Zululand, parts of the Transvaal lowveld and the Limpopo valley in the north-east. Enclosing this area and extending southwards along the Zululand coast is a mesothermal zone classed as subtropical (B'_4 in Thornthwaite's classification).[44] In both the tropical and subtropical zones all veld types belong to Group I with exclusively C_4 grass species. But since most of the rest of the country where the climate is warm temperate or cool temperate

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(B'_4 to B'_3 after Thornthwaite) also has exclusively C_4 grasses in the vegetational cover, it must be concluded that climatic zones other than the tropics constitute a favourable environment for these plants.

2) A comparison of Fig. 2 with the *rainfall* map of Southern Africa[45] reveals that precipitation figures for both exclusively C_4 areas and predominantly C_3 areas extend over the complete range registered for the region. Thus the exclusively C_3 area (31) on the west coast south of the Orange River receives less than 100 mm rain annually, while on the top of the Drakensberg range, a predominantly C_3 area, the annual precipitation exceeds 1 250 mm. Similarly, the exclusively C_4 Arid Karoo (29) south of Goodhouse averages less than 100 mm rain per year, while along the Zululand Coast (1b)—also exclusively C_4—the rainfall exceeds 1 000 mm per year. Differences in precipitation can, therefore, not account for the distribution of C_3 and C_4 grass species. The same variation is encountered when the moisture index according to the Thornthwaite classification[43,44] is considered: C_3 as well as C_4 areas range from Arid (type E) over Semi-Arid, Sub-Humid to Humid (type B2). The frequent claim that the C_4 photosynthetic pathway is a specific adaptation to arid climates, therefore, does not sufficiently explain the current distribution of the C_3 and C_4 grasses in the region.

3) The only climatic factor that seems to account for the geographic distribution of the C_3 and C_4 grasses is the *temperature* during the growing season.[46] In the winter rainfall area of the western Cape Province, the grass grows during the winter months when temperatures are relatively low. Thus the mean daily maximum and minimum temperatures in July at Jonkershoek, Klaver and Port Nolloth are 16.8°, 19.9°, 16.9°C and 6.9°, 8.1°, 7.0°C respectively.[47] The one region in the summer rainfall area with predominantly C_3 grass species—on the summit of the Drakensberg in Lesotho—shows January temperatures which are lower than elsewhere in the country. The only weather station in this area is Oxbow, at an altitude of 2 951 m, and there the mean daily maximum and minimum temperatures for January are 22°C and 2°C. In this highest Alpine zone, up to 90% of the grass species are C_3.[42] In the uniform rainfall area along the Cape Folded Belt relatively high percentages of C_3 grass species mostly occur on the cooler mountain ranges and not so much in the warm valleys.

It thus seems that the most important determinant for the absence of C_4 grasses in a specific region is the occurrence of low temperatures during the growth period: below a mean daily maximum of about 25°C during the rainy season, grasses with the C_3 pathway become more successful than C_4 species. This may be related to the experimental observation that some C_4 grass species exhibit reduced growth or other pathological symptoms after exposure to temperatures below about 10°C.[48] If this is a general property of C_4 grasses, it would adequately explain their scarcity in areas where average night temperatures during the growing season fall below approximately 8°C. It would then be necessary to ascribe the presence of the few C_4 grasses encountered on the top of the Drakensberg range to favourable micro-climatic conditions. A further exception would be the presence of C_4 grass species in saline habitats in the south-west Cape Province.

It must, however, be pointed out that the linear correlation between the percentage of C_4 grass species and normal July (summer) minimum temperatures established for several localities in North America[49] does not hold at all for Southern Africa. For instance, this relationship would predict only 25% C_4 species in the grass population at Bethlehem, Orange Free State ($\overline{Tn} = 13.1$°C), while actually over 95% of the Gramineae are of the C_4 types at this locality.

It is of interest to note that the C_3 species in the predominantly C_3 areas (Groups IV and V) chiefly belong to the temperate sub-families Festucoideae and Arundinoideae (especially the tribe Danthonieae), while the tropical panicoid and bambusoid-oryzoid C_3 grasses are mainly absent. The equatorial bambusoid and oryzoid grasses (C_3) form only an insignificant element in the South African flora, being limited to moist habitats in subtropical Zululand. An exception is the indigenous bamboo, *Arundinaria tessellata,* which grows on stream banks and forest margins between 1 600 and 2 700 m on the eastern escarpment. The panicoid C_3 species, on the other hand, are common, with distribution patterns practically coinciding with the Group II category. In fact, these grasses are almost exclusively responsible for the C_3 component of all these veld types. At high altitudes they are increasingly replaced by the southern temperate types.

This may also be due to a temperature effect since some tropical C_3 species have been found to respond unfavourably to low temperatures in a manner similar to that mentioned above for some C_4 taxa.[50]

Anatomically these tropical and temperate C_3 species also show differences: the festucoids are characterized as having either an indistinct outer bundle sheath with irregular, diffuse mesophyll or with compact mesophyll of isodiametric cells and a relatively more conspicuous outer bundle sheath. In contrast, the bambusoid-oryzoid grasses have large, thick-walled outer bundle sheath cells and mesophyll comprised of arm-cells, while the panicoid C_3 species have similar bundle sheaths, but the mesophyll has no arm-cells. It is possible that the latter type is also physiologically an intermediate between festucoid (C_3) and classical C_4 types as has been claimed for *Panicum milioides*.[51] This would be in keeping with the fact that their distributional range lies between the summer rainfall C_3 and C_4 areas.

The high efficiency of carbon dioxide assimilation typical of the C_4 photosynthetic pathway constitutes a definite advantage for Kranz plants in that it enables them successfully to overcome increased stomatal resistances caused by high temperatures in conjunction with water stress. As has been pointed out already, this adaptive advantage does not, at first sight, appear to explain the predominance of C_4 species in environments ranging from the central arid desert over the cool temperate highveld to the humid subtropical east coast. A possible explanation lies in the predominantly orographic nature of the precipitation over the whole summer rainfall area. This results in the frequent occurrence of dry spells, which the vegetation has to contend with, even in regions of high precipitation.

It is noteworthy that differences do exist between the C_4 grass species present in the different climatic zones. Thus 86% of the species in the hot dry desert environment of the southern Kalahari[24] belong to the sub-type of Kranz plants that form aspartate as the initial photosynthetic product, whereas only 14% are malate formers. In contrast, only 40% of the grasses in the Central Bankenveld are aspartate formers.

Scrutiny of the worldwide distribution maps[52,53] of some tribes and sub-families of the Gramineae seems to support the observation that the aspartate formers, such as the sub-family Eragrostoideae, are concentrated in arid regions while malate formers, such as the tribe Andropogoneae, thrive in hot, more humid climates.

It appears from the foregoing discussion that the recording of distributional patterns on a regional scale reveals quite clearly the environmental conditions which determine the relative success of C_3 and C_4 grass species. However, the limiting factors need not necessarily be connected with the C_4 pathway as such but may involve other physiological processes. Certain tendencies identified within the two major groups may, in fact, point in this direction. It is noteworthy that the widespread occurrence of C_4 taxa in the Gramineae does not seem to be accompanied by a similar predominance of C_4 species in other plant families. Thus it has been found that while all the grasses of the Central Namib Desert are of the C_4 type, only 20% of the other plants investigated are Kranz or CAM, and most of these are succulent CAM species.[19]

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Nevertheless, the distribution map resulting from the present study does seem to suggest heat and aridity as the most likely nomers which provided the selective pressure in the development of the C_4 pathway and not so much a tropical environment as such which is normally associated with humidity.

Applications

Apart from the possible application of these findings to studies in plant geography, ecology and physiology, they may also be put to use in more practical fields such as pasture, wildlife management and crop selection. They can, furthermore, be extended to determine the actual frequency of C_3 and C_4 plants in the grass cover of the veld. Since C_3 grasses in predominantly C_4 areas are often localized and scarce, and vice versa, the distribution map of the grass *cover* is separated even more clearly into C_3 and C_4 areas than the species distribution map presented here.[54] Because the C_4 pathway is relatively rare amongst the other plant families and $^{13}C/^{12}C$ isotope ratios of the two types of plant are distinctly different, measurements of the carbon isotope ratio in animal bone from a C_4 grass area can reveal the browsing/grazing ratio in the diet of different herbivores.[53]

It is to be expected that major climatic changes in the past would have affected the geographical distribution of the C_3 and C_4 grasses as well as the vegetation cover in general. Such changes would be revealed by the carbon isotope ratio in fossil bones of suitably selected animal species. Isotope ratio measurements could thus also provide information on climatic fluctuations during the Pleistocene.

The technique can furthermore be employed to investigate the diet of prehistoric man as regards, for instance, the spread and utilization of the C_4 food plants maize and millet.[55]

Thus an avenue of research which has, until recently, been purely basic now appears to have several interesting applications in very diverse fields of study.

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Photosynthetic Pathways and the Geographical Distribution of Grasses in South West Africa/Namibia

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Analysis of floristic lists for South West Africa/Namibia shows that, throughout the territory, more than 95% of the grass species occurring in any given area display the C_4 photosynthetic pathway. Exceptions are areas in the north-east and south-west where between 5% and 18% of the grass species are of the C_3 type. The south-western district of Lüderitz falls within the winter rainfall area and it is only here that temperate C_3 genera are found. The C_3 species in the north-east belong to tropical groups. Most of the South West African C_3 grasses grow in specialized habitats and are either hydrophytes or sciophytes. Subdivision of the C_4 grasses into the three subtypes of the C_4 pathway reveals distinctive distributional trends. Malate formers or NADP-me species clearly become more abundant with increasing rainfall, whereas the aspartate formers show the opposite tendency. However, within the aspartate forming group the results show that it is specifically the NAD-me type of species which dominate in areas of very low precipitation, notably in the Namib and pre-Namib areas where rainfall is less than 200 mm/yr. The PEP-ck species form a group intermediate between the malate formers and the NAD-me grasses, especially as far as their water requirements are concerned.

'n Ondersoek van plantlyste van Suidwes-Afrika toon dat minstens 95 persent van die grasspesies in enige gegewe gebied die C_4-fotosinteseweg volg. Die noordoostelike en suidwestelike gebiede is uitsonderings waar tussen 5 persent en 18 persent van die grasspesies van die C_3-soort is. Die suidwestelike gebied van Lüderitz is 'n winterreënvalgebied en slegs hier word die gematigde C_3-species gevind. Die C_3-spesies van die noordoostelike gebied behoort tot die tropiese groep. Die meeste van Suidwes-Afrika se C_3-grasse groei in gespesialiseerde habitats en is òf hidrofiete òf skiofiete. Onderverdeling van die C_3-grasse in die drie subtipes vertoon opvallende verspreidingspatrone. Die groep wat hoofsaaklik appelsuur produseer, en ook NADP-me-spesies genoem word, neem toe met toenemende reënval, terwyl die groep wat hoofsaaklik aspartiensuur produseer volopper word met afnemende reënval. Binne die aspartaatgroep is dit egter die NAD-me-tipe wat domineer in gebiede met 'n baie lae reënval, veral in die Namib-woestyn en in die voor-Namib met 'n reënval van minder as 200 mm/jaar. Die PEP-ck-grassoorte is 'n groep tussen die malaatproduseerders en die NAD-me-spesies wat hul waterbehoeftes betref.

It has been shown previously that South African grasses exhibiting either the C_3 or the C_4 photosynthetic pathway are, to a marked degree, geographically separated.[1] This large-scale distribution pattern indicates that C_4 grasses predominate over most of South Africa, and it is only in the winter rainfall region of the western Cape, and along the summits of the eastern Cape and Drakensberg mountains, that C_3 species increase in abundance. This distribution pattern suggests that low minimum temperatures during the growing season favour species with the C_3 pathway and that C_4 grasses were more successful when temperatures are higher. Similar conclusions are arrived at independently in an analysis of the North American grass flora.[2] Large-scale patterns of grass distributions, therefore, indicate that minimum temperature during the growing season is the environmental parameter having the strongest correlation with the relative abundance of C_4 grasses in a particular region.

Floristic surveys in Costa Rica[3] and Kenya[4] reveal that the highest proportion of C_4 grasses is found at low altitudes with high temperatures, high insolation and with a low incidence of available soil moisture. At moister, higher altitudes with no water stress, C_3 species replace the C_4 grasses. The data from these and other studies,[5,6] as well as observational and experimental information on a few C_4 species,[7-10] thus support the hypothesis that the C_4 pathway is adapted to arid areas.[11-17] These deductions are apparently contradicted by our observation that C_3 grasses dominate in the most arid regions along the west coast of South Africa.[1] For this reason we suggested that the prevailing temperature during the growing season was the most significant environmental factor limiting the relative success of the C_3 or C_4 pathway. The presumed dependence of C_4 plants on arid environments is further questioned by the observation that C_3 plants predominate in the non-grass component of the flora of the central Namib Desert.[18] We are of the opinion, therefore, that large-scale distribution patterns do not corroborate extrapolations from limited studies of particular species or local habitats and that the role of water stress in the success of the C_4 pathway has not been adequately demonstrated. In order to investigate this apparent discrepancy further and to obtain more information on the optimum environmental requirements of the C_3 and C_4 pathways, we have undertaken a survey of the distribution of grass species in South West Africa/Namibia.

South West Africa has specific advantages in this respect. It covers a large area, extending from 17° to 29° south latitude. Temperatures throughout the territory are relatively uniform and fairly high with average summer maximum temperatures of about 30°C experienced throughout the region except for a narrow strip along the coast where maximum summer temperatures of about 20°C prevail.[19-23] The principal climatic variable is precipitation, which increases from less than 50 mm/yr in the south-west and

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along the Atlantic coast to over 500 mm/yr in the extreme north-east.[24] The vegetation ranges from true desert communities to tree savanna and woodland in the north-east.[25]

The climate of South West Africa is less complex than that of South Africa, which includes a wide range of climatic zones and experiences abrupt climatic changes over relatively short distances.[24] It was therefore considered that the gradual climatic changes in South West Africa, reflected mainly by a shallow moisture gradient, would be ideal for investigating the ecological requirements of the C_3 and C_4 pathways in the grasses. In addition, the distribution patterns of the three subtypes of the C_4 pathway that are now generally recognized, could also be examined and related to geographic patterns in precipitation.

Plants with the C_4 pathway, or Kranz syndrome, possess a wide range of physiological, biochemical, anatomical and ultrastructural characters that have been extensively studied and shown to be consistently related.[26,27] It has therefore been possible to demonstrate that the detection of any one of a wide spectrum of characteristics is sufficient to identify the entire syndrome. The C_3 and C_4 photosynthetic pathways are also related to the taxonomy of the grasses and are not randomly distributed in the Poaceae, but are restricted, with few exceptions, to certain subfamilies and tribes.[26-32] The relationship of the C_3 and C_4 pathways to grass systematics is, therefore, well appreciated and the classification of the Poaceae can be helpful in predicting photosynthetic and other physiological characteristics.[33,34]

This association of functional and structural features also applies to the three subtypes of the C_4 pathway.[31,32] Since the diagnostic criteria of these groups are not widely appreciated, a brief discussion of their distinguishing characteristics is given below.

All C_4 grasses initially fix CO_2 in the mesophyll cells, which results in the formation of oxaloacetate which is subsequently converted into either malate and/or aspartate.[35-37] Depending on the relative quantities of malate and aspartate formed, two distinct subgroups of C_4 plants are recognized: malate and aspartate formers.

This biochemical distinction is associated with structural differences reflected in the leaf anatomy as illustrated in Fig. 1. Malate formers are characterized by a single chlorenchymatous, or Kranz, sheath, with centrifugal chloroplasts present around the larger vascular bundles (Fig. 1,1 – 3).[26,27,31,32] This condition is readily recognized as no cells are present between the metaxylem vessel elements and the laterally adjacent Kranz sheath cells of the primary, or first order, vascular bundles. In aspartate formers (Fig. 1,4 – 9) a mestome, or inner bundle, sheath is situated between the metaxylem elements and the Kranz sheath.[31,32] Ultrastructurally, the malate formers lack well-developed grana in the chloroplasts[35,38,39] and have a low mitochondrial frequency.[40] In addition, malate formers do not exhibit a post-illumination CO_2 burst[36] as the aspartate formers do.

The malic and aspartic acids, formed by the first carboxylation reaction of C_4 photosynthesis, are transferred to the Kranz sheath cells where decarboxylation occurs, by the NADP-malic enzyme in the case of the malate formers.[41] In the aspartate formers it has been shown that 70% or more of the decarboxylation activity is due to one or other of two enzymes, either PEP-carboxykinase or NAD-malic enzyme.[41,42] Two subtypes within the aspartate formers can, therefore, be recognized. For convenience these are termed PEP-ck type and NAD-me type species, respectively. The predominance of either of these decarboxylating enzymes is also associated with anatomical differences in the plants.[32] PEP-ck species have chloroplasts located centrifugally against the outer wall of the Kranz sheath cells (Fig. 1,4 – 6), whereas the chloroplasts are centripetally located in NAD-me species (Fig. 1,7 – 9). In addition the mesophyll of NAD-me plants consists of a single layer of distinct, radially arranged, narrow tabular cells (Fig. 1,7 – 9), whereas in

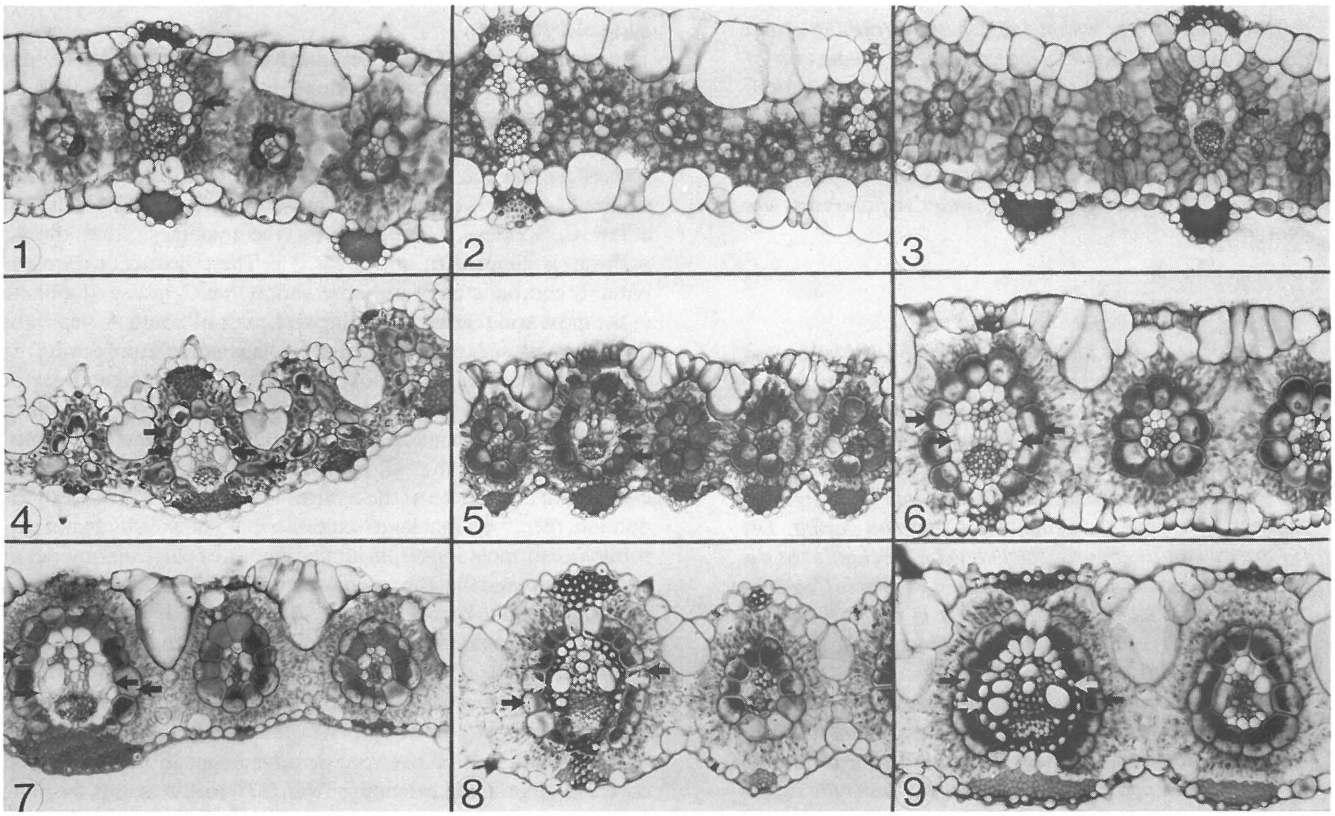


Fig. 1. C_4 leaf anatomy of selected South West African grasses. Note that all have a Kranz bundle sheath and a single layer of radially arranged mesophyll cells. 1 – 3, Malate formers with single, Kranz bundle sheath (arrowed). 1, *Anthephora pubescens*; 2, *Setaria sphacelata*; 3, *Digitaria eriantha*. 4 – 9, Aspartate formers with inner mestome sheath and outer Kranz sheath, i.e. double bundle sheath (indicted by arrows). 4 – 6, PEP-ck type species with specialized chloroplasts in centrifugal position: 4, *Eragrostis viscosa*; 5, *Rhynchelytrum repens*; 6, *Leucophrys mesocoma*. 7 – 9, NAD-me type species with centripetal chloroplasts in Kranz sheath cells. 7, *Eragrostis porosa*; 8, *Panicum arbusculum*; 9, *Monelytrum lüderitzianum*. Magnification × 150.

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Table 1. Relative ^{13}C content with respect to the SMOW standard, δ, of some South West African grass species.

Species	Collector and specimen no.	Locality	Sample no. (M -)	Analysis no. (MC -)	$\delta^{13}C$ (‰)
Panicum brevifolium	O. H. Volk 1870	Okavango R, SWA	1060	1642	−27.2
P. impeditum	M. G. L. Mills 74	Kalahari Gemsbok Park, Cape	1055	1643	−15.2
P. lanipes	W. Giess & M. Müller 13993	Okaukuejo, SWA	1059	1641	−12.1
P. pansum	H. Merxmüller & W. Giess 1875	Grootfontein, N. SWA	1058	1639	−11.2
P. pearsonii	K. L. Tinley 1391	Tsumeb, SWA	1057	1640	−12.1
P. schinzii	W. Giess 8435	Karibib, SWA	1061	1645	−12.1
P. trichonode	W. Giess 14954	Tsumeb, SWA	1056	1644	−12.3
Acrachne racemosa	W. Giess 3187	Kaokoveld, SWA	1062	1646	−12.9

PEP-ck species the radial arrangement is less regular and the chlorenchyma cells are larger and less elongated (Fig. 1,4 – 6).

Our knowledge of the structural and functional features of these subdivisions of the C_4 group of plants is considerable but little or no information is available on the possible ecological implications of these differences in the mechanism of photosynthesis. No attempt has been made to examine the environmental significance of these structural and functional differences, although it is relatively simple to classify grasses into the three categories on anatomical grounds alone. We have made certain taxonomic deductions from published reports. Thus, from Tieszen *et al.*'s[4] data it can be inferred[32] that malate formers (all the Andropogoneae) require higher soil moisture than the aspartate formers (the Chlorideae, Eragrosteae and Sporoboleae). However, taxonomic criteria cannot be used to distinguish the subtypes of the aspartate group and anatomical or biochemical distinctions are required. This is because many genera include NAD-me as well as PEP-ck species.[32]

Methods

The most recent floristic description of the grasses of South West Africa, in the *Prodromus einer Flora von Südwestafrika,*[43] was used as a basis for the present study. For each of the 347 grass species listed as occurring in the territory, the magisterial districts in which they were collected are given according to the Farm Map of 1950.[43] The lists of grasses compiled for each of the magisterial districts in this way were augmented with detailed checklists for specific areas.[44-48] Collectors' lists of collections from Etosha Pan submitted for identification to the Botanical Research Institute, Pretoria,were also consulted because the *Prodromus* includes very few grasses from this district. The Lüderitz-North district (LUN) is a restricted diamond-mining area in which very little plant collecting has been undertaken, and consequently the species lists from here are incomplete. The Caprivi Strip was ignored as it falls within the region covered by the Flora Zambesiaca and was not included in the *Prodromus.*

Classification of the South West African grasses as being either C_3 or C_4 was based on either the leaf anatomy or the ratio of the carbon isotopes, $^{13}C/^{12}C$. The relative isotope ratios of *Acrachne racemosa* and several *Panicum* species, for which data have not been published previously, are given in Table 1. The percentage of C_4 species in the total number of grasses was thus calculated for each of the magisterial districts (Fig. 2).

The C_4 grasses were further classified as being either malate or aspartate formers, and, if the latter, as either PEP-ck or NAD-me types. This subdivision was based solely on the anatomy and cytology of the bundle sheath and mesophyll cells[26,27,31,32] of freshly fixed leaf-blade material or on published biochemical analyses.[37-40,42,49,50] The division into malate and aspartate formers therefore, was made on the basis of the presence of a single or a double bundle sheath, respectively. All the C_4 species could be classified as being either malate or aspartate formers even if anatomical material was not available in every case. This was because all grass genera indigenous to South West Africa comprise only aspartate or only malate forming species. The genera which appear to include both malate and aspartate species (*Alloteropsis, Digitaria* and *Panicum*)[32] are represented in the territory by species of one C_4 subtype only. The frequency of occurrence of species representing the malate and aspartate C_4 photosynthetic subtypes is expressed as a percentage of the total of C_4 grasses for the various districts and presented graphically in Fig. 3.

In this study the assignment of the aspartate formers to the PEP-ck and NAD-me subtypes was based on anatomical criteria such as Kranz chloroplast position, Kranz cell shape and mesophyll cell structure and arrangement,[32] as well as on published biochemical findings.[38-40,42,50] Taxonomically, these aspartate subtypes are not separated as clearly as are the malate and aspartate forming species and, therefore, extrapolation based on the classification of the grasses cannot be used to determine whether a given aspartate forming species is a PEP-ck or NAD-me type or not.[32] Thus, *Eragrostis, Sporobolus* and *Panicum,* genera with many South West African representatives, have both PEP-ck and NAD-me representatives. Species for which no anatomical material was available could not therefore be classified beyond the aspartate

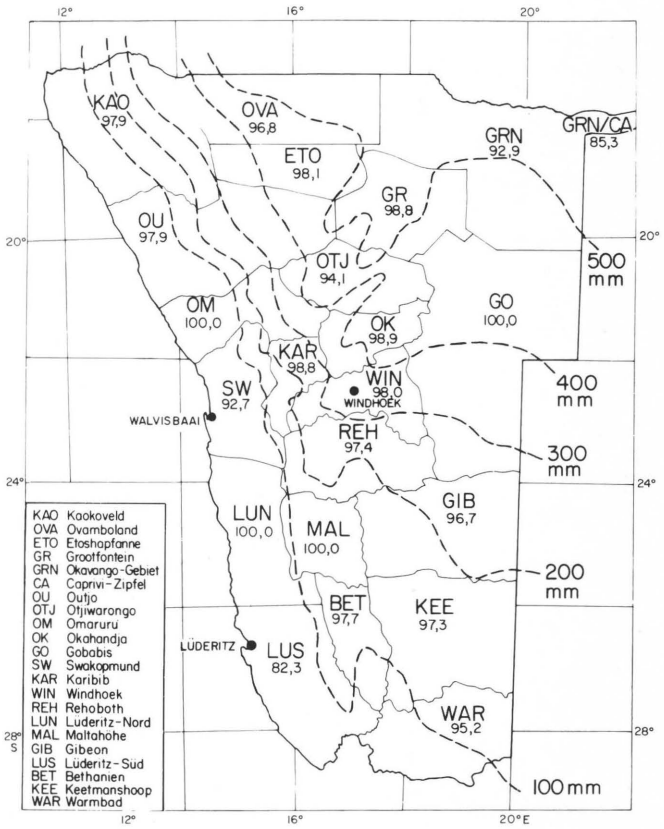


Fig. 2. Isohyets and magisterial districts of South West Africa. The numbers on the map refer to the percentage of C_4 grass species recorded for each magisterial district.

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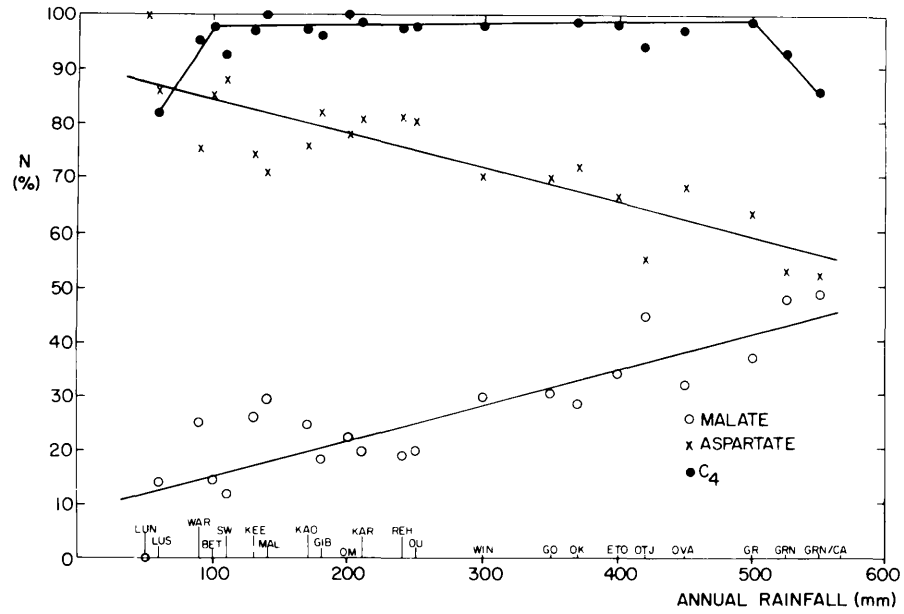


Fig. 3. The frequency (*N*) of C_4 grasses (●) expressed as a percentage of the total number of grass species, and the frequency (*N*) of malate (o) and aspartate (x) forming species expressed as a percentage of the total of C_4 grass species and plotted as a function of the estimated annual rainfall for each magisterial district of South West Africa. For abbreviations used refer to Fig. 2.

group. These species comprised 19% of the total of aspartate-forming grasses; the bars in Fig. 4 represent this uncertainty.

The average rainfall figures given for the magisterial districts in Figs 3 and 4 are of necessity only approximate, expecially where a steep precipitation gradient is present. They nevertheless reveal the general rainfall pattern with sufficient accuracy for the present purpose.

Results and discussion

Distribution of C_3 grasses

Of the 347 grass species listed for South West Africa,[43] only 31 (8.9%) are of the C_3 type. As is evident from Figs 2 and 3 these C_3 species are concentrated in two widely separated areas—the extreme south-west and the extreme north-east—where they constitute between 5% and 18% of the grass flora. In the remainder of the territory less than 5% of the grass species are of the C_3 type.

The annual rainfall in the south-western Lüderitz-South (LUS) district is less than 100 mm, while that in the north-east exceeds 500 mm.[24] The rainfall, therefore, does not appear to influence the occurrence of C_3 grasses in the country. This observation corroborates our previous conclusions that precipitation is not an important factor in determining the relative success of C_3 grasses.[1] A more detailed examination seems warranted.

The C_3 grasses found in South West Africa are taxonomically extremely diverse. They are classified into 21 genera, eight tribes and five of the six subfamilies of the Poaceae—the sixth, the Eragrostoideae, being the only subfamily without any C_3 representatives. Three tribes, the Danthonieae, Paniceae and Aristideae, and one genus, *Panicum,* include both C_3 and C_4 species, as shown in Table 2.

This broad taxonomic spread of C_3 species is exceptional, as normally their representatives in any local grass flora reflect either a strong temperate or a tropical bias.[1] Thus, either tropical panicoids, aristidoids, bambusoids and oryzoids or the more temperate festucoids and danthonoids usually predominate. In South West Africa both temperate and tropical C_3 grasses are found (Table 2).

Our analysis shows, however, that these temperate and tropical species are separated geographically—the temperate grasses grow in the Lüderitz-South district, while the tropical C_3 species are present in the north-eastern districts of Okavango (GRN) and Ovamboland (OVA).

The extremely arid south-western district falls almost entirely within a winter rainfall region. The growing season therefore corresponds with the cooler months of the year, with an average daily maximum temperature of 17.5°C at Lüderitz in July.[19-23] These temperatures are below the optima for most C_4 plants[3] and the winter minimum temperatures (Lüderitz: July average daily minimum 10.1°C) are probably sufficient to produce pathological

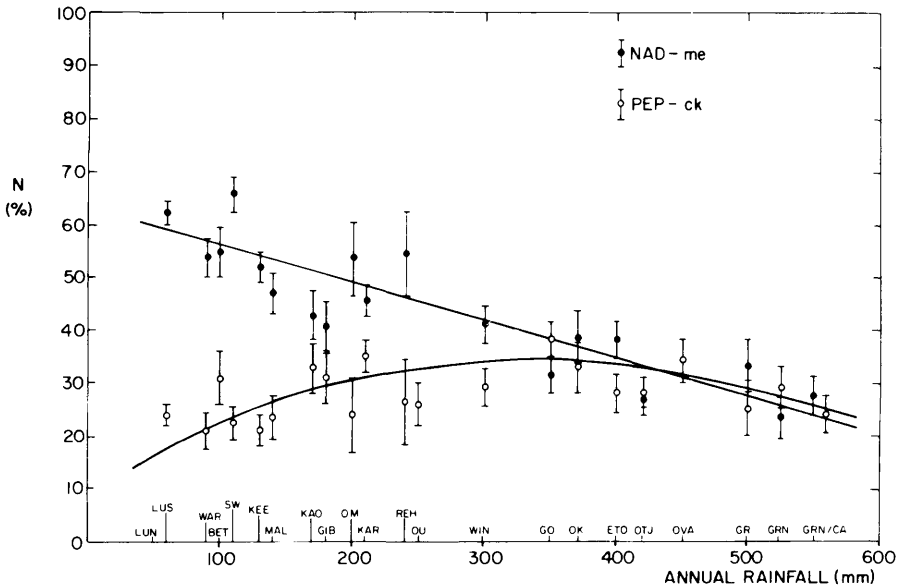


Fig. 4. The frequency (*N*) of NAD-me (♦) and PEP-ck (◊) species expressed as a percentage of the total of C_4 grass species and plotted as a function of the estimated annual rainfall for each magisterial district of South West Africa. For abbreviations used refer to Fig. 2.

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Table 2. Taxonomy and ecology of the C_3 grasses of South West Africa/Namibia

Species	Tribe	Sub-family	Ecological notes	
Dregeochloa pumila *Merxmuellera rangei* *Pentaschistis patula* *Chaetobromus involucratus* *Schismus barbatus* *Karroochloa schismoides*	Danthonieae	Arundinoideae	TEMPERATE	Drought-tolerant species
Ehrharta delicatula *E. pusilla* *E. triandra* *E. virgata* *E. longifolia*	Ehrharteae		Restricted to the winter rainfall area in the south-west. Temperatures during growing season relatively low.	
Polypogon monspeliensis *P. semiverticillatus* *P. minutiflorus* *Agrostis lachnantha*	Agrosteae	Festucoideae		HYDROPHYTES Annuals with short life-cycles and restricted to ephemeral but moist localities or perennials from wet habitats
Puccinellia angusta *Poa heterogama*	Festuceae			
Elytrophorus globularis *E. spicatus*	Danthonieae(?)	Arundinoideae		
Phragmites australis *P. mauritianus* *Arundo donax*	Arundineae		TROPICAL Summer growing season with high temperatures. Primarily in the north-eastern districts	Hygrophilous species of open water situations. Either emergent or rooted with floating leaves.
Oryza longistaminata *Leersia hexandra*	Oryzeae	Oryzoideae		
Acroceras macrum *Sacciolepis typhura* *S. africana* *S. auriculata*	Paniceae	Panicoideae		
Oplismenus burmannii *Panicum brevifolium*				Shade-loving species
Sartidia angolensis	Aristideae	Aristidoideae		Grassland species

symptoms in C_4 species.[2] It is therefore not surprising that several perennial C_3 species are found in exposed positions in this district but nowhere else in the country. These are drought-tolerant plants and many exhibit specialized growth forms and leaf anatomy, as does *Dregeochloa pumila*, for instance.[51] Such adaptations may enable these species to make use of moisture condensed from the frequent fogs which occur in the region.

All the C_3 grasses of this south-western winter rainfall area belong to temperate tribes and represent northerly extensions of the flora of the Cape Province where representatives of the Danthonieae, Ehrharteae, Agrosteae and Festuceae are common. Apart from a few members of the Danthonieae, which are perennials, the remainder are annuals with short life cycles. All the representatives of the Festuceae, Agrosteae and Ehrharteae belong in this category and, in addition, appear to avoid the harsh climate by occupying protected, often shady positions where moisture conditions are more favourable. This is so evident that all these species have been classified as hydrophytes, as they all may possess lacunae in the mesophyll of their leaves. If these plants are not found in association with open water, they appear to grow wherever sufficient water accumulates, when it rains, to enable them to complete their life cycles before the water has evaporated. Most C_3 species therefore occupy sites with mesic microclimates in this otherwise arid region. Whether the increase in soil moisture or the lower temperatures of the growing season favour the occurrence of these C_3 grasses is not clear from the South West African evidence. Distribution data on temperate C_3 plants from South Africa[1] and elsewhere[52,53] indicate that it is indeed the minimum temperatures which are the major selective factor in the success or otherwise of the C_3 pathway in grasses.

In the north-east, in the Okavango and Ovamboland districts, the C_3 species comprise more than 5% of the grasses (Figs 2 and 3). The C_3 grasses in this hot, summer rainfall area occupy specialized habitats and are either hydrophytes or obligative sciophytes (Table 2). The hygrophilous species are either perennials associated with permanent water bodies or ephemerals which grow in seasonally inundated sites. The perennials belong to the tropical tribes Oryzeae and Paniceae as well as the Arundineae. The classification of the annual, *Elytrophorus*, is uncertain. This genus has been placed in the Danthonieae,[54] although some authors consider it to be typically chloridoid.[33] If the genus were to be placed in the Eragrostoideae, then it would be the only one of C_3 type in an otherwise completely C_4 subfamily.[27,32]

The restricted and specialized habitats occupied by these tropical C_3 grasses help provide insight into the environmental conditions which limit the distribution of C_3 grasses, or, conversely, in

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response to which the C_4 pathway has evolved. In a hot tropical region the habitats occupied by C_3 grasses have three factors in common: relatively high moisture regimes, reduced solar radiation and low leaf temperatures. In some of the aquatic communities where C_3 grass species predominate, for example *Phragmites*, this diminished irradiance may be due to self-shading effects.[4]

The oryzoid, bambusoid and tropical arundinoid C_3 grasses are characterized anatomically by the presence of arm cells in the mesophyll. They also have an outer bundle sheath of large, thick-walled cells, a feature shared with the C_3 panicoid species. This structure differs considerably from that of the temperate festucoid C_3 grasses, which have diffuse, irregularly arranged mesophyll and thin-walled parenchyma sheath cells, and may imply physiological and ecological differences between the two kinds of grasses. This hypothesis appears to be supported by the spatial separation of the tropical and temperate C_3 species into areas of different climate in South West Africa. Caution is needed when extrapolating data on temperate species to tropical C_3 plants such as the bamboos and rices.

The above discussion shows that, although the average annual precipitation does not constitute the limiting factor for the success or otherwise of C_3 grasses, they are nevertheless apparently restricted to environments where water stress is not severe. This is especially noticeable in an arid territory like South West Africa but, where moisture availability is not a restriction, low minimum temperatures during the growing season undoubtedly provide temperate C_3 grasses with a competitive advantage. Water availability combined with reduced radiation and leaf temperatures appear to be strongly associated with the relative abundance of tropical C_3 species in regions with hot, tropical climates.

Distribution of C_4 grasses

C_4 species dominate the grass flora throughout South West Africa (Fig. 2) and comprise more than 80% of the grass species present in any district. It is only in the north-eastern Okavango and in the south-western Lüderitz-South districts, that the abundance of C_4 species drops below 95% (Fig. 3). It appears, therefore, that C_4 grasses have considerable advantages over their C_3 counterparts throughout the territory and the prevailing conditions are undoubtedly more favourable for the C_4 photosynthetic pathway everywhere except in certain habitats of deep shade and abundant water supply as discussed above. The uniformly high summer temperatures in the territory are considered to be in the optimum range for C_4 photosynthesis (30° – 45°C)[55] and the high proportion of C_4 grasses found throughout the region (Figs 2 and 3) appears to confirm this hypothesis. Their abundance further suggests that C_4 grasses exhibit a wide tolerance of moisture conditions, from extremely arid (less than 100 mm/yr) to reasonably mesic (about 550 mm/yr)[24] in the woodland communities. This negligible effect of rainfall can be illustrated further by the high percentage of C_4 species along the Natal Coastal Belt,[1] an area experiencing precipitation in excess of 1000 mm/yr.

Restricted water availability in hot, tropical climates has been proposed as the primary environmental factor determining the relative success of plants possessing the C_4 photosynthetic pathway in these particular circumstances. Our results indicate, however, that C_4 grasses as a whole are common in areas with widely differing precipitation regimes. In order to examine more critically the role of moisture in determining the relative success of C_4 grasses, we have studied the distribution patterns of the subtypes constituting the C_4 pathway.

Distribution of malate and aspartate forming species

It is evident from Fig. 3 that the malate forming C_4 grasses are uncommon in the districts experiencing the least rainfall but steadily grow in number with increasing precipitation. The north-eastern districts, which receive the highest rainfall in South West Africa, have the greatest proportion of malate forming species—45% of the total of C_4 grass species. Extrapolating from Fig. 3, a continuation of this trend can be predicted. Hence, to the north and east of the Okavango district, where the rainfall exceeds 750 mm/yr, malate forming grasses should be dominant. Floristic lists for the Lengwe National Park in Malawi[56] appear to confirm this prediction: 55% of the grass species in this area of 850 mm/yr rainfall are malate formers.

It has been demonstrated that the Andropogoneae in Kenya attain their highest development at intermediate levels of soil moisture.[4] We assume that all andropogonoid grasses are malate formers.[32] In Kenya it has been shown that they are replaced by C_3 species at higher moisture levels[4] which, in this instance, are associated with high altitudes and thus lower temperatures. Malate forming C_4 grasses, therefore, appear to be most successful in conditions of minimal water stress but with high temperatures and irradiance. In South West Africa, where the temperature lowering effects of increasing altitude are not present, this is illustrated by the relatively large number of aquatic malate forming grasses in open water conditions in the north-eastern districts. Thus, 16 aquatic malate forming species are found there, belonging to the following genera: *Paspalidium, Paspalum, Echinochloa, Eriochloa, Miscanthidium, Vossia* and *Vetiveria*. These are all emergent hydrophytes in contrast to seasonally inundated soak-zone species[57] and occur in addition to 12 C_3 grass species and only a single aspartate former.

It appears that, given suitably high temperatures, malate forming grasses generally attain their greatest abundance in conditions of reduced water stress. The malate subtype of C_4 plants is undoubtedly not well represented in arid and semi-arid tropical regions—an environmental requirement generally associated with C_4 plants.

Aspartate forming C_4 grasses attain their maximum frequency in areas of low rainfall and decrease in abundance with increasing moisture availability (Fig. 3). This trend is also evident in Kenya, since the Chlorideae, Eragrosteae and Sporoboleae, all species which we can predict to be aspartate formers,[32] are most abundant in conditions of low soil moisture availability.[4] It is interesting to note that, where aspartate forming plants are found in aquatic habitats in South West Africa, they are invariably associated with high levels of salinity, for example *Odyssea, Oryzidium, Sporobolus* and *Diplachne*.[57]

Distribution of NAD-me and PEP-ck type species

As the aspartate subtype of C_4 plants can be subdivided into NAD-me and PEP-ck categories, it is necessary to examine the distribution of each of these before generalizations can be made for the aspartate formers as such. Figure 4 illustrates the geographical distribution of PEP-ck and NAD-me grasses in South West Africa.

There are significant differences between the distribution patterns of NAD-me and PEP-ck species which are not evident in that for the combined aspartate formers. The frequency of NAD-me grasses increases from 25% of the total of C_4 species where rainfall is 550 mm/yr to 55% where the rainfall is less than 100 mm/yr. NAD-me grasses dominate in arid areas with low and unpredictable rainfall and high temperatures and radiation. It is, therefore, specifically this subtype which substantiates the belief in the adaptation to aridity of the C_4 pathway.

The PEP-ck subtype appears to be intermediate between the NAD-me type and the malate forming NADP-me type (Figs 3 and 4) with respect to moisture requirements. In South West Africa they achieve their maximum abundance in areas receiving rainfall of 350 mm/yr and decline when the precipitation departs from this level. Where rainfall is less than 350 mm/yr, PEP-ck species are generally found in relatively moist habitats such as along drainage lines or amongst boulder outcrops. In areas with rainfall in excess of 350 mm/yr, PEP-ck grasses once again are found primarily in more specialized habitats. *Panicum maximum* is a good example of a common PEP-ck species found in the north-eastern savannas or woodlands.[43] In these districts, this grass is almost exclusively restricted to growing beneath the canopies of the savanna trees.[58]

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From the results presented in this paper, the implications of this observation are that such semi-shade habitats must be more xeric than the open grassland. The reason, however, may be due to a factor completely unrelated to moisture.

In this study it was not possible to examine the undoubtedly important effects of nutrients on the distribution of grasses. Thus, nutrient drop from the tree canopy may also account for the relative abundance of aspartate forming PEP-ck species beneath the trees in a grass cover otherwise dominated by malate forming grasses.

It is of interest to note that the major anatomical difference between the PEP-ck and NAD-me aspartate forming grasses is the location of the specialized chloroplasts in the Kranz sheath cells. They are centripetal in NAD-me species and centrifugal in the PEP-ck group (Fig. 1).[32] In this respect the PEP-ck species resemble the malate formers more closely than the NAD-me kind. Ultrastructurally, however, these centrifugal chloroplasts differ in that they are agranal in the malate forming grasses but contain grana in the aspartate formers.[38,39] Mesophyll cell arrangement and structure in PEP-ck species bear strong similarities to the structure of the mesophyll in malate formers (Fig. 1). Except for the presence of a single or double bundle sheath, malate and PEP-ck grasses exhibit many anatomical similarities, so it is not surprising that these two subtypes initially become more abundant with increasing rainfall.

Summary and conclusions

Summer temperatures throughout most of South West Africa are remarkably uniform except for the narrow Atlantic coastal belt. These temperatures are relatively high and well within the optimum range for the C_4 photosynthetic pathway. Rainfall, on the other hand, exhibits a shallow gradient and increases in a northerly and easterly direction from less than 100 mm/yr to over 500 mm/yr.

Throughout South West Africa, C_3 species constitute a minor component of the grass flora. In the south-west, with winter rainfall and consequently relatively low temperatures during the growing season, temperate C_3 plants comprise up to 18% of the grass species present. In the north-east, with high temperatures during the growing season, the C_3 type once again makes up about 15% of the grass species. In the latter districts, however, tropical C_3 grasses are present, so that there is a distinct spatial separation of the temperate and tropical C_3 grasses in the territory. Growing season temperatures are, therefore, important in determining whether C_3 grasses with tropical or temperate characteristics are present in a particular area. In addition, high temperatures favour the presence of C_4 grasses.

C_3 grasses reach their maximum relative abundance in the most arid, south-western district of Lüderitz-South and, hence, it appears as if precipitation has no influence on the presence of these C_3 plants. However, closer examination of the C_3 grasses of this district showed that they favour relatively moist microenvironments. In the north-east, where C_3 grasses once again increase in abundance, the same high moisture requirements were observed for the tropical C_3 species. Thus it appears that grasses exhibiting the C_3 photosynthetic pathway are limited, to a large extent, to habitats with minimal water stress.

C_4 grasses are dominant throughout the territory, whether the climate is extremely arid or relatively mesic. In an environment of consistently high temperatures and irradiance during the growing season, the amount of rainfall has very little effect on the presence of C_4 species as a whole. However, the three subtypes of C_4 grasses show quite different distribution patterns which appear to depend on the rainfall. Malate formers are found to increase in abundance with increasing precipitation, whereas NAD-me species decrease. PEP-ck aspartate forming species are intermediate in their moisture requirements. In hot tropical climates, therefore, it can be predicted that NAD-me species will be more successful in truly arid areas, whereas malate forming NADP-me grasses will be more common in areas with reduced water stress.

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6. GENERAL DISCUSSION

Synopsis: The preceding chapters have described the microtechnique used in this study and defined and tabulated the anatomical attributes present in the grass leaf blade as seen in transverse section and of the epidermis in surface view. Selected examples are also given of the application of these techniques and anatomical characters to taxonomic and functional studies. The applicability of the methods chosen for specimen preparation and for the recording and synthesis of the anatomical details will now be assessed and evaluated. The examples chosen to illustrate the relevance of this approach in taxonomic and functional studies will be discussed in more detail to demonstrate their applicability to the Poaceae as a whole.

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6. GENERAL DISCUSSION

6.1 Introduction

This discussion will attempt to explain and justify the methods of preparation of the basic anatomical material, and the extraction and synthesis of the descriptive information, as outlined in the foregoing chapters, in terms of the objectives of this study. In addition, to further justify the approach adopted, and to motivate for the continuation of this project, selected examples of results obtained to date have been described to demonstrate the relevance and applicability of the methods used in taxonomic and functional studies in the Poaceae. An evaluation of the current and future relevance of this project can, therefore, be based on the potential significance of the information assembled during the course of this research, as inferred and extrapolated from the selection of results given in Chapters 4 and 5.

The examples included in the foregoing chapters by no means represent the total results to date but are only a sample chosen to indicate the significance, relevance and applicability of the information forthcoming from this study. For example, a total of only 17 taxa have been dealt with in Chapter 4. This represents a small proportion of the 650 species which are ready to be written up, having been collected, prepared and photographed. This backlog in the writing up of the anatomical descriptions is partly attributable to the fact that specific descriptions are undoubtedly more meaningful when dealt with in the generic and tribal context but, unfortunately key species have sometimes proved difficult to collect in the field. Consequently the final comparative descriptions and discussions have been delayed until material of these rarer species has been acquired – either freshly fixed or, as an alternative, herbarium material will be used.

Similarly the taxonomic distribution of the Kranz syndrome has only been documented in the Eragrostoideae and Panicoideae (Chapter 5, pages 140–181) but sufficient data exists to analyse all the C_3 subfamilies as well. Indications are that interesting correlations exist between the panicoid, festucoid and bambusoid/oryzoid types of C_3 anatomy and the ecological requirements of grasses possessing these different anatomical characteristics (Chapter 5, pages 186 and 192–194). This is the first mention of a possible physiological sub-division of the C_3 photosynthetic pathway in grasses and undoubtedly warrants detailed publication. Finally, the application of grass anatomical studies in feeding preference studies of grazing herbivores has also been examined as part of this study (Wilson *et al.*, 1977) using the present data as a basis for comparison and identification. Judging from these examples it appears reasonable to conclude that this project has made, and will continue to make, a significant scientific contribution to a wide range of botanical and allied disciplines.

Interpretation and synthesis of the basic anatomical information, prepared during the course of this study is, to a large extent, dependent on current taxonomic opinions and physiological and biochemical knowledge. In the foregoing it has been shown that this project has contributed significantly to, or has the potential to contribute to, our understanding of grass classification and photosynthesis based on present knowledge and understanding. With the general acceptance and synthesis of the implications of this new information in taxonomic and functional knowledge, further hypotheses are likely in the future for which the same basic anatomical data gathered during the present study will hopefully be relevant and usable. One of the basic prerequisites of this research project is, therefore, future applicability. This consideration has strongly influenced the approach to this project, particularly the methods adopted for the preparation, storage and recording of the anatomical data.

In this discussion it will be demonstrated that the standard anatomical procedures adopted, provided certain essential structures are accentuated, are adequate to infer a considerable amount concerning the taxonomic relationships and functioning of grasses. An additional proviso is that strictly comparative material must be examined, processed and described in a consistent standardized manner. In addition to the above applications, material prepared in this manner can also contribute significantly in several applied fields such as herbivore dietary studies, soil studies, weed research and forensic botany. These applications, not discussed in the preceding chapters, were also taken into consideration when planning and designing this research project.

All the above considerations have influenced this presentation to varying degrees and, consequently, it differs in many respects from the usual thesis where a logical well rounded-off write-up of a completed study on a restricted topic is the norm. It is felt that submission of this thesis at the present stage is justified due to the long-term and co-operative nature of the project and because significant contributions have already been made to our understanding of the classification and functioning of the grasses. This discussion will also, where necessary, examine in detail the extent of the scientific contribution outlined in the

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results presented in the preceding chapters.

One of the advantages of a broadly based, large-scale, but standardized, anatomical survey of this nature has been the comparability of various structures in many specimens or species. Nevertheless, this rigidity in technique has at the same time proved to be somewhat restricting in certain instances. Thus a large comparative collection of leaf blade transverse sections has been assembled from which much information can be gained about the taxonomic distribution of any character visible in transverse section. At the same time very little additional information has been gathered about the three-dimensional structure of the characters themselves. For instance longitudinal paradermal sections would reveal the very important structural differences between the Kranz mestome and parenchyma sheath cells and their relationships with the mesophyll cells (Brown, 1974). Maceration techniques are required to reveal the three-dimensional structure of silica bodies, so important in pedological studies (Smithson, 1956, 1958). In addition controlled sampling, which is impossible under natural, field conditions, would have helped considerably in understanding the development and differentiation of the leaf cells and tissues. These shortcomings are inevitable in a primarily taxonomically orientated study such as this but, nevertheless, this project has contributed significantly to our appreciation of the relevance of the anatomical structures as taxonomic characters.

6.2 Techniques and Methods

The techniques used for specimen preparation in most of the taxonomically orientated published works on grass leaf anatomy differ considerably from the methods adopted in this study and outlined in Chapter 2. The use of different techniques for the preparation of leaf material for anatomical study is, to a certain degree regrettable, as it implies that precise comparisons, extrapolations and deductions cannot always be confidently made between the results of different studies. This is an important consideration as the literature on grass leaf anatomy is surprisingly extensive as the following incomplete sample of local South African studies illustrates: Christopher (1942), Clarkson (1949), De Wet (1960a, b), De Winter (1951a, 1951b, 1965), Engelbrecht (1956), Fisher (1938, 1939, 1940), Goossens (1938), Goossens & Theron (1934, 1938), Louw (1943), Nixon (1953), Robbertse (1959), Schweickerdt (1941, 1942) and Schweickerdt & Marais (1956).

Due to the extent of the local and international literature, comparison with the results of previous studies was an important prerequisite for this study and, consequently, considerable attention was paid to incorporating this requirement into the anatomical preparations prepared for this project. In the light of modern technology and knowledge, however, the material examined by most earlier authors suffers from certain drawbacks. In order to overcome the limitations imposed on these studies by the techniques employed for section preparation in particular, certain improvements and deviations from standard practice have been incorporated into the methods used in this study.

A compromise was therefore reached, which of necessity, was not ideal from either point of view. Nevertheless, it should be obvious from the photomicrographs included in Chapters 4 and 5, that the sectioned material prepared during the course of the present study is a definite improvement on previous anatomical preparations although the high standards of fixation, impregnation, sectioning and staining expected of purely anatomical studies were not always attained.

6.2.1 Transverse sections

The methods of preparation and illustration of transverse sections of grass leaf blades used by virtually all earlier workers differed in three important respects from the techniques employed in the present study:

(1) the plant material utilized for anatomical examination normally had been removed from dried herbarium specimens and not freshly fixed in the field. Rehydration of the leaf tissues, either by gently boiling (Metcalfe, 1960) or by soaking in a glycerine/alcohol mixture (De Winter, 1965), was invariably imperfect with the result that the mesophyll and colourless cells, in particular, were often not restored to their original shape. Description and illustration of these structures has consequently, not always been accurate in the past. It must be stressed, however, that characters that cannot be easily studied in dried material are of limited use for identification purposes (Clifford & Watson, 1977) but, nevertheless, may prove enlightening for purposes of classification.

(2) leaf sections have, in the past, been either cut by hand (De Winter, 1965) or with a sledge microtome (Metcalfe, 1960) while held between pieces of pith in the microtome clamp. Several earlier workers concluded that microtome sections were not entirely satisfactory and actually recommended the use of freehand sections e.g. Burr & Turner (1933), Vickery (1935), Goossens (1938) and Fisher (1940). Another argument in favour of hand sections is that

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they are simple and information which is difficult to obtain has little practical value for identification purposes (Clifford & Watson, 1977). Embedding has been considered too time-consuming and unsatisfactory due to the hardness of the leaf blades of many grass species (De Winter, 1965). In this study it has been found that once these drawbacks were overcome by rapid dehydration (Feder & O'Brien, 1968), mass production using a tissue processor and by softening in Mollifex, sections of embedded material proved to be far superior to material sectioned by hand or with the sledge microtome. This is because consistent, controllable section thickness was possible, several duplicate sections were routinely cut and staining and mounting were much easier because an adhesive (Jensen, 1962) was used to glue the wax ribbons to the slides. Hand cut and sledge microtome sections of most grass leaves are often incomplete and seldom are of equal thickness throughout. They are, therefore, not routinely suited to photographic illustration and, as a result, most earlier workers used camera lucida drawings to illustrate their work.

(3) to date the soaking of all material to be sectioned, in commercial sodium hypochlorite solutions (Javel) has been standard practice in most studies. The hypochlorite solution has the effect of bleaching the cell contents and stretching the hand cut sections (De Winter, 1965). Examination of these sections is therefore, limited to the study of tissue distribution and cell wall characteristics – cytological detail being impossible to ascertain. This is a serious limitation in the light of the current interest in chloroplast detail and its significance in relation to the Kranz syndrome. Certain authors purposely omitted the bleaching step in selected preparations in order to determine the distribution of the chlorenchyma (De Winter, 1965) but, nevertheless, chloroplast detail was not normally included in the camera lucida illustrations.

At the time of commencement of this project in 1970, renewed interest in grass leaf anatomy was centred around chloroplast structure and distribution, and mesophyll cell arrangement, as a result of the discovery of the C_4 dicarboxylic acid pathway of photosynthesis in 1965 (Kortschak *et al.*, 1965; Hatch & Slack, 1966) and its correlation with Kranz leaf anatomy (Brown 1975, 1977). For this reason it was considered essential to plan this project in the light of these new developments and consequently methods of section preparation were chosen which would enhance the study of chloroplast, bundle sheath and mesophyll cell structure. Bleaching was accordingly discontinued and a concerted effort was initiated to sample and fix as many grass species in the field as possible. This material was wax embedded and sectioned with the rotary microtome to enable photomicrographic recording of the anatomical structure on a routine basis.

6.2.2 Photomicrography

The use of photomicrography as a standard practice is a somewhat controversial deviation in comparative studies of grass leaf anatomy. Good photomicrographs are unquestionably an improvement on camera lucida drawings as all detail is illustrated and not just structures considered relevant to the study being undertaken. A wider application of published illustrations is, consequently, assured. In addition, the degree of detail and accuracy of photomicrographs does not depend on the experience, training and interpretation of the microscopist in deciding what must, and must not, be included in the line drawing. As the microtechnique procedures used in this study routinely resulted in sections of good quality and contrast, photomicrographs of acceptable standard were virtually always possible and usually of a higher standard than in other studies where photomicrographs have been published e.g. Clifford & Watson, 1977. The epidermal scrapes, on the other hand, were not so uniform and often residual mesophyll and fibrous tissue made photography difficult.

With modern photolithographic printing techniques, the publication of photomicrographs is no longer more expensive than line drawings and their use should be encouraged except where schematic, diagrammatic illustrations are required. In the present study the anatomical structure of every specimen collected has been recorded photographically – both the leaf in section and the abaxial epidermal structure. This includes 3 000 collections representing 650 species for which visual records are available. Thus inter- and intraspecific anatomical variation is documented which can conveniently be compared and contrasted with the external morphology of the herbarium voucher specimens. By this means anatomical detail is made available to the taxonomist in a convenient manner – a practical impossibility using individual drawings.

These photomicrographs of freshly fixed, wax embedded leaf material can easily be compared with the line drawings of earlier authors as both methods of preparation clearly show cell wall structure and tissue distribution – especially cells with thickened walls such as sclerenchyma, collenchyma and fibres. Caution must be exercised however, as cytoplasmic

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detail and the limited focal depth of photomicrographs of freshly fixed material can be somewhat confusing when compared with the usually somewhat stylized drawings compiled from sections made from herbarium material. Mesophyll cell structure and spatial arrangement, especially as regards the air-space system, and other thin-walled structures, such as the parenchyma sheath cells of the festucoid grasses, are without doubt more clearly resolved and interpreted in wax embedded sections prepared from freshly fixed material. The methods adopted for section preparation and illustration in this study have, therefore, proved successful and represent a definite advancement in comparative anatomical studies of the Poaceae.

6.2.3 Epidermal preparations

The choice of technique for the preparation of transverse sections was relatively simple and only influenced by two main considerations: the use of the preparations in comparative, taxonomic studies and the need to resolve structures relevant to the Kranz syndrome. Epidermal preparation techniques, on the other hand, proved much more difficult and a satisfactory, multi-purpose technique was not found. Instead comparative anatomical considerations were given precedence, and the standard scrape method adopted (Metcalfe, 1960). The various applications of epidermal studies had to receive a lower priority. The epidermal reference material prepared for this study will, consequently, not prove ideal for dietary studies of grazing herbivores using faecal or rumen analysis for instance. In my experience (Wilson *et al.*, 1977) more specialized techniques are needed for these specific applications and should be adopted in such instances.

Maceration has been used by many previous workers to prepare reference epidermal material for dietary studies (Baumgartner & Martin, 1939; Dusi, 1949; Martin, 1955, 1964; Storr, 1961; Croker, 1959, Hercus, 1960; McAllister, 1967 and McAllister & Bornman, 1972). Leaf surface imprinting techniques have also been used to a lesser extent for similar purposes (North, 1956; Sampson, 1961; Sinclair & Dunn, 1961 and Dilcher, 1974). Nevertheless, the majority of workers employing the faecal and rumen analysis technique have used the scrape method for preparing leaf fragments for identification (Dusi, 1949; Stewart, 1965, 1967; Kok, 1968; Liversidge, 1970; Field, 1972; Nolte, 1973; Kok & Van der Schijff, 1973 and Lensing, 1977, 1979). The results of these studies are, therefore, directly comparable with the present study and can readily be incorporated into the data base. In actual fact the scraping technique adopted in all these studies is almost identical to the method used in this study. The method differs slightly from normal practice in taxonomically orientated studies where bleaching is routinely used (Metcalfe, 1960) to soften the tissues and to serve as a lubricant. Glycerine has been substituted as a lubricant in many of the above applied studies (Lensing, 1977).

6.3 Anatomical characters

Systematic anatomical studies of plants have tended to concentrate mainly on vegetative structures, such as leaves and wood and, to a lesser extent, fruits and seeds. Plant taxonomists, on the other hand, have stressed reproductive structures as sources of taxonomic criteria. The history of grass taxonomy and anatomy closely follows this general pattern. Initial classifications relied heavily on spikelet morphology but gradually vegetative anatomical criteria, especially of the leaf, began to receive consideration. Thus, for the grasses, there is considerable anatomical information on the leaf but very little on stems, a fair amount on fruit structure (probably due to the economic importance of cereals) and remarkably little on inflorescences and flowers.

It is for precisely this reason that the leaf blade was specifically singled out for study in this project. The grass leaf has been shown to possess a multitude of interesting anatomical characters and the grasses are exceptional in that their leaf anatomy has been relatively thoroughly and extensively studied as reviewed in Chapter 3. What now remains is a detailed assessment of the taxonomic significance of the anatomical characters of the grass leaf blade and this is the prime purpose of the present study.

Due to the significant early work on grass leaf anatomy, notably that of Prat (1932) and Metcalfe (1960), it is now relatively easy to draw up long lists of well-defined attributes of the leaf, for which there is extensive, comparative data. In Chapter 3 these characters are defined, their terminology standardized and they are systematically grouped for convenient reference and use – an essential precondition for their evaluation as taxonomic criteria.

Defining taxonomic characters, whether for purposes of classification or identification, is of necessity arbitrary. There is always scope for disagreement over definitions and there is always room for improvement. Invariably, viewed with hindsight, and after practical testing, improvements become evident. This inevitably also applies to the long list of characters defined in Chapter 3 but it is only in practice that the relative merits of the various characters

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will be fully assessed.

Notwithstanding the above, the majority of the characteristics mentioned appear to be clearly and unambiguously defined and are individually applicable to most grass leaves. Several researchers were given the descriptive keys for the leaf blade as seen in transverse section (Chapter 3, pages 19–50) and the epidermis in surface view (Chapter 3, pages 65–84) to describe given specimens. These tests showed that the descriptions so obtained were remarkably consistent, implying that observation and detection of the characters was reliable and that the same meaning was given to definitions by the various workers. In addition, by classifying the characters and arranging them hierarchically and sequentially all specimens are checked for the same array of characters and, consequently, the absence of characters also becomes meaningful. Furthermore, although the list of attributes may be considered excessive, the pictorial presentation and pseudo-key format used, allow rapid characterization of any particular anatomical preparation.

In compiling the attribute lists presented in Chapter 3, all the anatomical characteristics of the leaf blade, that were located in the literature, and that have been used taxonomically, were incorporated. Many of these characters are well defined and widely recorded and thus generally accepted as being good taxonomic characters but the majority have only been used in restricted grass groups by specialist workers and may be considered to be of limited use. Grass anatomists, and taxonomists in particular, have usually subjectively chosen those characteristics they consider to meet their requirements for suitable taxonomic characters (e.g. Clifford & Watson, 1977) but, nevertheless, today many anatomical characteristics, such as micro-hairs (Johnson & Watson, 1977) and silica bodies, are acknowledged to be significant and reliable characters. Some of these anatomical attributes are valuable generic criteria that are as near perfect as are any of those to be gained from other aspects of the plant, including spikelet morphology.

The arbitrary restriction of the spectrum of potentially useful taxonomic attributes by choice appears to be a practical necessity in most taxonomic studies where a wide range of characteristics, other than leaf structure, are also considered. However, in the present study only anatomical criteria were considered and, therefore, as many attributes as possible could be evaluated. This was indeed fortunate because, in the morphologically rather uniform grass family, the richness of anatomical diversity exhibited by the leaf blade is truly phenomenal and a list of useful characteristics could probably be extended indefinitely. In fact leaf anatomy has become so important that professional agrostologists cannot now hope to progress without the use of a compound microscope in addition to the standard tool, the dissecting microscope.

This project will also attempt to identify and evaluate further taxonomically important characters which may help in producing an improved classification of the Poaceae. This has already been done with respect to some of the anatomical characteristics of the Kranz syndrome (Chapter 5, pages 140–176). This concern for future applicability influenced the decision to include all possible characteristics – even those presently considered insignificant.

That the leaf blade alone was singled out for anatomical study appears to be justified by historical precedent judging from the significance that has been attributed to this organ in the literature on grass anatomy (Metcalfe, 1960). Notwithstanding this emphasis on the leaf blade, it is certain that other organs will also reveal taxonomically useful features but extensive and intensive study is still required. Leaf sheaths, for instance, often differ anatomically from the blades and, when data becomes available for them, many new diagnostic features will no doubt be forthcoming. In addition, recent studies on the stem of certain Eragrosteae (Sanchez, 1979) stresses the potential significance of studies on other organs.

When the large number of attributes dealt with in Chapter 3 is considered, together with the detailed resolution required to detect many of them, it was initially expected that many of these characters would not prove functional and practical. Surprisingly however, in practice, most of the characteristics described for the lamina in transverse section could consistently and repeatably be observed even by several independent workers examining the same specimen. This applies, in particular, to specimens prepared using the techniques outlined in Chapter 2 and applied throughout this study. Material prepared from herbarium specimens often proved confusing with certain structures being impossible to resolve to the level required. This difficulty in interpretation applied particularly to mesophyll and colourless cell structure, as discussed in the description of *Dregeochloa* (Chapter 4, page 108) and if freshly fixed material was not available for confirmation then the detail demanded proved too fine for confident observation and description.

This suitability of preparations and attributes applies to at least 90% of the transverse sections pre-

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pared to date – the only exceptions being the soft, fragile leaves of some festucoid and danthonoid grasses for which the alcohol concentration of the fixative proved to be too harsh (Chapter 2, page 10). The same does not apply to the epidermal scrapes prepared during this study, however. Certain structures, notably thin-walled papillae, micro-hairs and silica bodies, are sometimes almost impossible to distinguish and may tax the microscopists' ability to resolve them, requiring excessive closure of the aperture iris diaphragm or even interference contrast illumination (Chapter 4, page 105, figs 31–34), for their resolution. This shortcoming is directly attributable to the method as different scrapes of the same leaf are often inconsistent, being differently stained (dependent on pH) and of varying thickness and clarity. An improvement in the method of preparing grass epidermides for anatomical study by means of transmitted light is necessary but it is doubtful if any method exists whereby repeatably good peels, scrapes or imprints can be obtained from members of the entire family. Leaves with abaxial ribs prohibit the successful application of most of these techniques throughout the family. Thus in *Dregeochloa* (Chapter 4, page 109, figs 3–8) it was not possible to adequately describe intercostal detail.

A more detailed evaluation of the merits of the individual attributes presented in Chapter 3 now follows. Comments on the detection, circumscription and taxonomic usage of these characters, in this and other research, will also be included.

6.3.1 The lamina in transverse section.

The range of available comparative information is less extensive for the leaf blade anatomy in transverse section than for the epidermis (Clifford & Watson, 1977) and the information that exists is often less satisfactory. It was partly to help overcome these shortcomings in our knowledge, that the present study was planned according to the format described. Emphasis was placed particularly on methods designed to improve our state of knowledge of the range of variation revealed by transverse sections of grass leaves.

In addition to obvious taxonomic relevance, some of the anatomical structure seen in sectional view is functionally associated with different photosynthetic pathways (Chapter 5), while much still remains unexplained. It is nevertheless clear, that some of the attributes recordable in transverse section, such as the arrangement of the chlorenchyma and the number of vascular bundle sheaths, have proved to be highly informative taxonomically (Brown, 1977; Ellis, 1977a; Chapter 5) and rank in importance with any other characters from different aspects of the plant's form. No doubt many other characteristics mentioned and defined in Chapter 3 will also prove to be useful indicators of the identity and relationships of grasses. Aspects of the schlerenchyma distribution and arrangement, leaf section outline and vascular bundle distribution and arrangement have thus been demonstrated to be significant in the genus *Merxmuellera* as described in Chapter 4.

6.3.1.1 *Leaf outline.*

Characters of the leaf outline, as seen under low magnification have received very little attention from previous workers. For instance, Clifford and Watson (1977) mention only midrib outline and vascularization, and rib size and distribution in their list of anatomical attributes. The type of folding and symmetry present in mature leaves is seldom mentioned but this detail may, in actual fact, reflect the situation in the bud (Amedei, 1932). Rolled versus folded leaves in the bud is considered to be taxonomically significant (Clifford & Watson, 1977) and indications from mature leaves, consequently warrants examination.

Leaf blade width is used by some authors and greater, or less, than 10 mm wide appears to be useful taxonomically as well as practically. It seems as if the categories arbitrarily decided upon in this study are too refined and tend to stress size differences for narrower leaves but further subdivisions are needed for leaves wider than 10 mm. However, in wide-leaved grasses it is seldom possible to obtain a complete section and, in any event, the voucher specimen is always available for measurement of these criteria. In these cases the possible difference between the width of dry leaves on the voucher and the width of turgid leaves of the fixed segments may vary considerably and should be accommodated for. Most measurements given in grass revisions refer to measurements of dried material and thus, at the present time, caution is called for in the use of these attributes and their full taxonomic implications are not fully comprehended.

The attention accorded the permanently infolded leaf type in this study (Chapter 3, pages 22 & 23) appears fully justified on the basis of the results, described in Chapter 4, of the study of *Merxmuellera,* a genus almost exclusively represented by species with setaceous leaves (Ellis, 1981a; Pages 132–137). Many of the anatomical characteristics typical of setaceous leaves, such as sclerenchyma distribution and vascular bundle number, are directly related to

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the degree of reduction associated with the infolding and appear to be of specific, or even sub-specific, importance and reflect ecotypical variation (Ellis 1980; 1980a; Pages 112–124). These characteristics are useful diagnostic aids.

6.3.1.2 *Ribs and furrows.*

The characters of the longitudinal ribs and furrows, both adaxial and abaxial, appear to be satisfactory as set out in the relevant descriptive 'key' in Chapter 3 (Pages 24 & 25). Their oversimplification may obscure several important attributes, such as the distinctive massive ribs, or the ribs of differing size or shape often consistently associated with different orders of bundle. These differences are easily and unambiguously recorded and observed. From the studies to date it appears as if more detailed attention to the abaxial rib description would have been justified as when they occur they are distinctive and diagnostic (Ellis, 1977; Pages 107–111). In species possessing these abaxial ribs, such as *Dregeochloa* and *Asthenatherum*, the preparation of acceptable epidermal scrapes is exceedingly difficult and very little detail of intercostal structure is evident. In good transverse sections, however, a lot can be inferred and, in this respect, the inclusion of the descriptive 'keys' for epidermal detail as evidenced from the sections (Pages 48 & 49) has proved most useful.

6.3.1.3 *Midribs and keels.*

Midrib and keel structure and vascularization, characters used by Clifford and Watson (1977), appear to be adequately handled by the relevant attribute list of Chapter 3. It must be noted, however, that, in the case of these characters, the position along the leaf lamina length, from which the section is taken, is critical. In this respect the standardization on the mid-laminar region of mature, basal leaves in this project, has reaped dividends although with respect to keel development, anatomical development is probably better accentuated nearer the ligule. Nevertheless, essential features such as basic shape and vascular arrangement are evident in the middle of the blade and the comparative nature of the sections allows for a good assessment of the degree of keel development to be made. Keel development appears to be an inconsistent characteristic of little taxonomic significance in many groups such as *Paspalum* (Page 101, figs 1–6). Keel development is apparently strongly influenced by the amount, and degree of aeration of, the water in the environment as evidenced by the *Leersia* sample assembled during this study.

6.3.1.4 *Vascular bundles.*

Metcalfe (1960) has demonstrated that structural details of the vascular bundles of the blade offer many opportunities for defining taxonomic characters but Clifford and Watson (1977) are of the opinion that only bundle sheath number has, as yet, been recorded sufficiently widely for use in classification. Bundle sheath structure and number, cell and chloroplast size and shape are very significant characters with undoubted application in grass systematics and functional studies (Ellis, 1977a; Pages 140–176; Ellis *et al.*, 1980; Page 190). Considerable attention has recently been paid to the anatomy and ultrastructure of the bundle sheath, and associated structures, constituting the Kranz syndrome (Brown, 1975, 1977; Hattersley & Watson, 1976; Laetsch, 1968; Carolin *et al.*, 1973; Johnson & Brown, 1973). The identificatory and classificatory value of the presence or absence of bundles with double sheaths (and whether these sheaths are Kranz or not) has not yet been fully evaluated. However, these features are not always in accord with the bulk of other systematic evidence as in *Panicum,* where both double and single, as well as Kranz and non-Kranz, sheaths are present (Ellis, 1974c, 1977a; Pages 165–167). These examples of characters that do not totally corroborate present taxonomic groupings should receive attention as they serve to illustrate the worth of these criteria and may help with making divisions within the major groups of genera which are consistently grouped by most of the conventional characters currently used. Bundle sheath characteristics have already been used in sub-dividing the genus *Panicum* (Gould & Clarke, 1978) and also appear to be meaningful in *Alloteropsis* (Pages 97–99 and 177–181).

In future bundle sheath detail will undoubtedly increase in taxonomic and functional importance. Fortunately the structures are always present, even on sterile specimens, and can usually be discerned even on dried material. Bundle sheath structure is, therefore, applicable to as wide a range of grass identificatory problems as are most spikelet characters and wider use of these attributes is justified.

Other characters of the vascular bundles, apart from bundle number, arrangement and position, which have been shown to be very important for distinguishing relationships within the summer-rainfall *Merxmuellera* species (Chapter 4, pages 112–137), are not as easy to observe, define and use as are the bundle sheath attributes. Detail of the vascular bundle itself does not appear to be significant except possibly the degree of sclerosis of the phloem, as in the *Merxmuellera* species already mentioned. Shape and size of the metaxylem vessels as defined in the

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relevant descriptive schedule (Pages 30 & 31) have proved unsatisfactory in practice and present difficulties with interpretation. Details of the protoxylem and lysigenous cavity also appear to be worthless and depend largely on the position from which the section was taken. Nevertheless, it seems worthwhile persevering with these attributes as they are easily visible even in sections prepared from herbarium material.

Bundle sheaths, as already described, are taxonomically most useful, especially their number and cytological detail. Bundle sheath extensions, on the other hand, are very variable and difficult to use and vary even within a given leaf as in *Merxmuellera macowanii* and *M. aureocephala* (Page 131, figs 1–11). This characteristic, therefore, presents difficulties with recording and description. In addition, the structural detail required of the bundle sheath cells in the descriptive key (Chapter 3, pages 32 & 33) is probably somewhat detailed and excessive as it is sometimes difficult to assign the cells of a given leaf to the relevant categories with a high degree of confidence. In some specimens these characters may, therefore, not be sufficiently distinguishable for reliable taxonomic use.

6.3.1.5 *Sclerenchyma.*

Most workers have been unable to make satisfactory use of the sclerenchyma patterns exhibited by grass leaves and Metcalfe (1960) felt that these characters are 'seldom of more than specific diagnostic value'. Although they made very little use of sclerenchyma characters, Clifford and Watson (1977) feel that a concerted effort aimed at defining sclerenchymatous attributes, followed by a comprehensive recording of comparative data, would have rewarding results. In the present study differences in girder structure have been shown to be important in distinguishing between the anatomical forms of *Alloteropsis* (Ellis, 1974; Page 97) and between the setaceous-leaved *Merxmuellera* species (Ellis, 1981a; Pages 132–136). In the *Paspalum* species studied (Ellis, 1974a; Pages 100–103), absence of sclerenchyma associated with the third order bundles proved significant. These observations confirm Metcalfe's (1960) views but further comparative information is required before an assessment can be made of the value of sclerenchyma characters at the generic, and higher levels.

Initial indications are that, the presence or absence of sclerenchyma in association with all, or only some, orders of vascular bundle, the presence of girders or strands, adaxial and/or abaxial sclerenchyma development and sclerenchyma found between the veins are some attributes of the sclerenchyma that may prove taxonomically significant. It has been shown in *Aristida,* for example, that all species produce intercostal sclerenchyma (De Winter, 1965) but this type of sclerenchyma is lacking in many other genera. This shows that, irrespective of possible ecological interpretations, this character is probably taxonomically useful, at least at the generic level.

6.3.1.6 *Mesophyll.*

The mesophyll of the leaf, including the chlorenchyma as well as the colourless and bulliform cells, exhibit many characteristics that have long been recognized as being taxonomically useful. However, examination of dried herbarium material often results in these attributes being exceedingly difficult to distinguish. One of the prime reasons for deciding to collect field-fixed material for this study was to ensure that these important characters would be clearly visible and unambiguously recordable.

The arrangement of the chlorenchymatous cells in the mesophyll is an anatomical feature bestowing a characteristic appearance on grass leaf sections. Chlorenchyma characters are also taxonomically most significant, distinguishing the various tribes of the Poaceae (Brown, 1958; 1960). In addition they are functionally associated with the type of photosynthetic pathway present (Brown, 1975, 1977). Thus in the C_3 festucoid grasses, the chlorenchyma cells are homogenous, with no obvious differentiation of palisade cells. In the C_4 andropogonoid, panicoid and chloridoid tribes these chlorenchyma cells are orientated so as to radiate from the vascular bundles. However, examples occur, especially in the arundinoids and danthonoids, such as *Cortaderia* and *Dregeochloa* (Ellis, 1977; Page 111), where there is a tendency to radiateness and it is difficult to decide between the radiate and non-radiate condition.

Without reference to very good sections, definite repeatable recordings of these very important differences is not always possible especially in veiw of the fact that herbarium material has largely been used in the study of grass leaf anatomy in the past. This difficulty has led Hattersley and Watson (1975) to propose the 'lateral cell count' and the 'maximum cells distant count' as a more precise indicator of photosynthetic pathway than 'radiateness', especially in distorted herbarium material. As these counts are dependent on chlorenchymatous mesophyll cells, all intervening colourless cells being ignored, they are only possible to determine in unbleached material. These measurements cannot, therefore, be made on the vast majority of grass leaf material prepared in the past.

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In this study it is possible to infer these 'lateral cell counts' from the relevant descriptive schedule on leaf chlorenchyma (Chapter 3, pages 42 & 43). Unfortunately the pertinent details are grouped under two major subdivisions of the chlorenchyma – radiate and irregularly arranged chlorenchyma and thus only half of the information will be recorded for any given specimen. In addition the character detail necessitates examining sections made from well-preserved material and thus the advantage of using the 'lateral cell count' (Hattersley & Watson, 1975), instead of deciding whether the mesophyll is radiate or not, is lost.

Details of the mesophyll cells themselves, when possible to record accurately, appear to be very significant in distinguishing anatomically between the subtypes of the C4 photosynthetic pathway (Chapter 5, pages 140–143). This serves, once again, to accentuate the advantages to be gained from well-preserved, embedded material, as prepared during the course of this study. The taxonomic significance of these mesophyll characters distinguishing the C4 subtypes is, as yet, not absolutely clear, but regular, routine recording of the relevant chlorenchyma detail will enable comparative data to be rapidly assembled, facilitating an early decision on the taxonomic importance of these anatomical and functional differences. It has recently also become apparent that functional differences, once again reflected in the structure of the mesophyll and bundle sheath cells, exist between the C3 grasses (Ellis *et al.*, 1980; Pages 192–194). This is the first mention of this possibility and will probably prove to be one of the most significant observations forthcoming from this study. This phenomenon of different C3 anatomical types definitely warrants detailed recording and analysis which will happen as a matter of course due to the design of this project.

It has already been openly acknowledged that a definite shortcoming of the descriptive schedules as constituted is their possible inadequacy as regards the mesophyll of the Bambuseae, where additional details of the fusoid cells (Chih-Ying Wu, 1958) probably are required to adequately distinguish between certain genera (Ellis, 1976; Page 59). In the South African context this inadequacy is insignificant as only two indigenous bamboo species occur in this area. Nevertheless, additional information is urgently required regarding taxonomically useful anatomical characters as this sub-family is also not adequately dealt with in Metcalfe's (1960) standard work. Fortunately scope for future expansion and elaboration has been allowed for in the design of the descriptive schedules and this detail can accordingly be incorporated where necessary.

Arm cells are considered to be characteristic structures of the mesophyll of the Bambuseae (Metcalfe, 1960). However, they appear to be more widespread among grasses than is generally supposed. Thus they have often been overlooked in *Phragmites* (Gordon-Gray & Ward, 1971) and have been observed in several *Ehrharta* species in this study. These cells justify detailed ultrastructural investigation, since they may represent large transfer cells (Clifford & Watson, 1977). Arm cells are easily visible in material prepared for this project and consequently their taxonomic distribution will be easy to document.

6.3.1.7 *Bulliform cells.*

Bulliform cells, and their associated colourless cells, are a conspicuous feature of grass leaf blades when viewed in transverse section but, unfortunately, they present the greatest difficulties in categorization and definition for diagnostic purposes. They undoubtedly offer potentially useful diagnostic attributes but before they can be gainfully used in practice, unambiguous description of the differences and variation are essential. The sub-division of the bulliform cell characteristics, as tabulated in Chapter 3 (Pages 46 & 47), is probably as refined as is possible without causing confusion and losing clarity.

Bulliform cell group structure is often variable along the width of a given blade, from the midrib to the margin (Ellis, 1974a; Pages 98, figs 1–5), and this is the major reason for these structures being difficult to define for taxonomic purposes. It is also necessary to ignore the two zones on either side of the midrib or median bundle as these frequently constitute 'midrib hinges' even in leaves without bulliform cells midway between midrib and margin. Some of the anatomical forms of *Merxmuellera stricta* (Chapter 4, page 118, figs 1, 7–9) are good examples of this phenomenon. In addition, their true nature and detailed structure, as well as their functioning, need further investigating before they can be successfully used for taxonomic purposes. In the meantime, description only of bulliform cells midway between the median bundle and the margin, as advocated in this study (Ellis, 1976; Chapter 3, pages 46–47), appears advisable to prevent confusion until such time as our knowledge of these interesting structures is considerably increased.

Metcalfe (1960) defined several types of bulliform cell which have been adopted, with little refinement, in the relevant descriptive schedule in Chapter 3 (Ellis, 1976). Clifford and Watson (1977) found that, in practice, it was necessary to lump several of Metcal-

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fe's (1960) categories, especially the subdivisions of the simple fan-shaped type. This demonstrates the caution that is needed before a final decision can be made regarding the use of these structures in taxonomic studies.

The inclusion of the descriptive 'key' on epidermal cells and appendages in the descriptions of the leaf in transverse section was a somewhat radical deviation from standard practice. Nevertheless this decision appears fully justified even although the structures may not necessarily be present and visible on every section. Using the embedding method, however, serial sections are obtained, increasing the chances of all structures being located on every slide. The observation and recording of these structures nevertheless assist in interpreting epidermal characteristics as seen in surface view. For example, whether macro-hairs have raised or sunken bases can be easily determined. In addition the epidermal details are often difficult to resolve satisfactorily due to, for instance, excessive ribbing. This was the situation with the adaxial epidermis of *Paspalum vaginatum* (Ellis, 1974a; Page 105, figs 31 & 32) and the abaxial epidermis of *Dregeochloa pumila* (Ellis, 1977; Page 109, figs 3–8) and the detail observed in transverse section contributed considerably to a better understanding of the epidermal structure of these species.

6.3.2 The abaxial epidermis.

Epidermal criteria, when carefully defined, are useful and reliable guides to taxonomic affinities and leaf epidermal preparations provide a wealth of taxonomically significant information in conveniently accessible form. Indeed Clifford & Watson (1977) put forward several reasons why grass leaf epidermal features, if sufficiently widely recorded, should not in practice be more informative for identification purposes than are the conventional aspects relating to floral or inflorescence structure. Most plant material that requires identification can be expected to possess leaves, or consist largely of leaves or leaf fragments, from which epidermal preparations can be obtained. Flowering structures, by contrast, are mainly ephemeral and are commonly absent. In addition, epidermal cuticle is relatively resistant to the processes of digestion (Stewart, 1970) and decay so that it is actually possible to observe leaf epidermal structure from animal rumen contents (Wilson *et al*., 1977) and faeces (Stewart, 1971; Stewart & Stewart, 1970), as well as from fossiliferous deposits (Palmer, 1976). It should be remembered, however, that leaf cuticles do not always survive maceration and digestion to appear in the faeces and for this reason the analysis of rumen and stomach contents seems worthy of more detailed appraisal especially in conjunction with the use of a rumenotomy trocar (Wilson *et al* ., 1977).

Consideration of the leaf epidermis of grasses is traditionally limited to the abaxial surface of the lamina (Metcalfe, 1960) and this trend has been largely followed in this study. Adaxial surfaces can, undoubtedly, provide copious data of taxonomic value if surveyed sufficiently extensively. This has been demonstrated in the Arundinoideae (Renvoize, pers. comm.) where micro-hairs were often present on the adaxial surface although absent on the abaxial surface of species with permanently infolded leaves. Prominent ribbing and irregularities in cell size caused by the presence of bulliform cells commonly combine to render the preparation of scrapes of this adaxial surface most difficult and time consuming. The abaxial epidermis presents few problems by comparison and there is generally no difficulty in obtaining good preparations except where abaxial ribs are well developed as in *Dregeochloa* (Ellis, 1977; Chapter 4, pages 107–111). The inclusion of adaxial, and abaxial, epidermal cell detail, as viewed in transverse section, in the descriptive schedules (Ellis, 1976), to some extent compensates for these shortcomings. Nevertheless, in the present study, adaxial scrapes were prepared whenever practical and the structural detail can be recorded for both surfaces using the relevant epidermal descriptive keys (Ellis, 1979; Chapter 3, pages 64–84) as has been done for *Paspalum vaginatum* and *P. paspalodes* (Ellis, 1974a; Chapter 4, pages 104 & 105, figs 25–34).

Metcalfe's (1960) convention for orientation of epidermal preparations for descriptive purposes has been adhered to (Ellis, 1979; Chapter 3). This requires that the epidermis be viewed through the microscope as though the long axis of the leaf is arranged horizontally; that is, with the veins running across the field of view. 'Vertical' thus indicates 'at right angles to the long axis of the leaf'. This standardization is essential to simplify the description of cellular structure particularly with reference to the proper understanding of the shapes of epidermal structures which cannot be accurately described unless orientation with respect to the leaf surface is clearly understood. In this respect the diagonally orientated photomicrographs of *Paspalum* (Ellis, 1974a; Chapter 4, pages 104 & 105, figs 25–34) can be validly criticised for being confusing and this practice has subsequently been discontinued.

An assumption that has generally been made in describing grass leaf epidermal structure is that the parallel venation of the leaf is reflected in the abax-

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ial epidermis as a marked difference in appearance between the costal zones, overlying the veins, and the intercostal zones between them. This banding exhibited in the epidermis is attributable to the adherence beneath the costal zones of sclerenchyma, which occurs sub-epidermally in association with the veins. However, comparison with transverse sections is essential to confirm, whether, or not, certain vascular bundles are not associated with sclerenchyma. They may, therefore, not be reflected as costal zones in surface view. This situation can clearly be seen in leaf sections of *Paspalam* where the minor third order bundles are not accompanied by abaxial sclerenchyma strands or girders (Ellis, 1974a; Chapter 4, page 102, figs 10–19). This example stresses the need for transverse sections to be studied in conjunction with epidermal preparations for complete understanding of the spatial relationships between the various tissues.

Cell sizes can also exhibit variation from the midrib to the margin as in the case of the silica bodies in the typical form of *Merxmuellera disticha* (Ellis, 1980; Page 114, figs 10–18). Transverse sections, once again, clearly illustrate this type of variation. Bearing this variation in mind, it seems advisable to restrict epidermal descriptions to the central part of the blade between midrib and margin. This consideration has unfortunately been omitted from the descriptive schedules for the epidermis (Ellis, 1979; Chapter 3, pages 64–84) although it was repeatedly stressed in the schedules for the leaf in transverse section (Ellis, 1976; Pages 19–63). This shortcoming is, to some extent, understandable as it is very often not possible to obtain scrapes inclusive of the median bundle and the margin and thus precise postioning is not always possible to ascertain.

6.3.2.1 *Long and short cells.*

The distinction made in this study between costal and intercostal long and short cells should help ensure precise descriptions in the future and prevent possible confusion as to exactly what cells are being referred to. The recognition of these two categories of cells, of distinct orders of size, has long been standard practice in grass histology studies and is usually clear cut and obvious. As in all biological material a few exceptions occur where this distinction tends to break down, such as in the adaxial epidermis of *Paspalum paspalodes* (Ellis, 1974a; Page 105, figs 33 & 34) because here all the intercostal cells are more or less isodiametric. These exceptions, nevertheless, do not seriously detract from the practical value of the distinction made between long and short cells, nor from the diagnostic worth of attributes which are dependent on this distinction.

Costal short cells are almost invariably present but intercostal short cells may be absent. The short cell patterns of occurrence may characterize an epidermis by their presence individually or in combination, but it is important to distinguish between costal and intercostal short cells as their configuration often differs in the same leaf. The separation of costal and intercostal short cells into separate descriptive schedules in Chapter 3 (Ellis, 1979) ensures that this distinction is unambiguously made. Costal short cell patterns of arrangement appear to be more important diagnostically than intercostal short cell distribution patterns and Clifford and Watson (1977) merely recorded intercostal short cell presence or absence. The inclusion of data on intercostal short cell patterns will undoubtedly significantly enhance the value of epidermal descriptions but at present these are difficult to define satisfactorily and more systematic details are needed.

The interpretation of short and long cell patterns of arrangement for diagnostic purposes is of necessity subjective. Metcalfe's (1960) arbitrary distinction between short cells mainly in files of 5 or more, short cells mainly paired and solitary short cells are reasonably satisfactory, being unambiguously applicable to most abaxial grass epidermides. Further details of vertical arrangement have been included in this study but it remains to be seen whether these will prove taxonomically useful in any groups although in *Merxmuellera* (Ellis, 1980a; Chapter 4, pages 112–137) indications are that this is not the case.

The appearance of the intercostal long cells is a distinctive characteristic of grass leaf epidermides. Whether their walls are straight or not, the degree of thickening of the anticlinal walls and whether the outer walls are pitted or not appear to be meaningful. Quantitative measurements of cell size, on the other hand, do not appear as useful because considerable variation is exhibited although ratios of length to breadth appear to be more consistent. The pits often present in the outer epidermal cell walls, such as in *Merxmuellera drakensbergensis* (Page 127, figs 9–11) and *M. aureocephala* (Page 133, figs 16 & 17), deserve detailed study. These structures may actually be ectodesmata (Clifford & Watson, 1977) and may be physiologically significant.

6.3.2.2 *Silica bodies.*

Silica bodies, so typical of mature grass leaves, occur in a diversity of shapes which, from a taxonomic point of view, provide a wide range of reliable, readily discernible data. There is, as yet, incomplete accord in the literature as to exactly what differences in sili-

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ca body shape should receive recognition. Metcalfe (1980) recognized twenty categories but Clifford and Watson (1977) found it desirable to lump these into seven far more inclusive groups in order to reduce the number of intermediates between the categories. Thus Metcalfe's (1960) cross-shaped, dumb-bell shaped, nodular and elliptical forms frequently occur in combination in the same genus or even the same leaf as shown in *Paspalum* (Page 104, figs 25–34) and *Merxmuellera stricta* (Page 119, figs 13–24). Further systematic application of these characteristics should lead to a better appreciation of the most satisfactory categorization from a taxonomic point of view.

Silica bodies are undoubtedly taxonomically useful and even appear to be characteristic of tribal groupings. Thus cross-shaped and dumb-bell shaped forms are largely confined to the panicoid and andropogonoid grasses whereas saddle-shaped bodies typify the chloridoid grasses (Prat, 1959). The horizontally elongated sinuous or crenate type appears to be limited to the festucoid grasses and the oryzoid type is consistently recognizable. Clifford and Watson (1977) feel that horizontally elongated, or more or less spherical, smooth silica bodies appear to be significant and the present studies in the South African Danthonieae (Chapter 4, pages 107–137) appear to confirm this except that some of the anatomical forms recognized also have dumb-bell shaped bodies.

6.3.2.3 *Stomata.*

The subsidiary cells of the stomata exhibit conspicuous variation which constitutes valuable identificatory criteria. Their shape ranges from the markedly triangular appearance of the subsidiary cells of many panicoid, andropogonoid and chloridoid grasses, through various dome-shaped intermediates to the parallel-sided forms typically found in the festucoid grasses. Although these shapes appear to be taxonomically significant, the different types often occur in combination on the same leaf. The parallel-sided type is often exclusive to a given epidermis but mixtures of domes with either triangular or parallel-sided types are often found such as in *Paspalum* (Ellis, 1974a; Chapter 4, pages 104, figs 25–30). It is very unusual to find examples where triangular subsidiaries occur in conjunction with those of the parallel-sided type (Clifford & Watson, 1977).

Stomatal subsidiary cell shape has long been considered to be of taxonomic significance (Prat 1932, 1936). In addition the mere absence of stomata from the abaxial leaf surface may be meaningful as has been demonstrated in some *Merxmuellera* species (Ellis 1980, 1980a, 1981, 1981a; Chapter 4, pages 112–137). Occurrence of species lacking abaxial stomata may, in some cases, constitute a useful generic diagnostic criterion although in other genera, such as *Merxmuellera,* there is variation from species to species in this respect. The recording of the presence or absence of abaxial stomata from a sufficiently large sample is needed before the significance of this character can be fully appreciated.

Recent studies have revealed that there is taxonomic variation in stomatal insertion among grass leaves (Watson & Clifford, 1978). Thus in most festucoids the guard-cells are clearly overlapped by the adjoining interstomatal cells whereas in nearly all the non-festucoid grasses the stomata are not sunken below the general level of the surrounding epidermis and their guard-cells are flush with the interstomatals or slightly overlap them. This distinction is clearly visible in epidermal preparations using different levels of focus as well as in transverse sections of the leaf. Unfortunately this potentially important distinction has not been incorporated into either of the appropriate descriptive schedules presented in Chapter 3 (Ellis, 1976, 1979). Fortunately allowance was made for later incorporation of characters into these 'keys' and this distinction most definitely merits inclusion as a character for routine, standard description. It is unambiguous and easy to record, even from herbarium material, and does not require sectioning. It is only in instances where overarching interstomatal papillae occur that interpretative difficulties may arise. A good example are the *Paspalum paspalodes* specimens possessing papillate abaxial epidermides (Ellis, 1974a; Page 104, fig 30).

6.3.2.4 *Epidermal appendages.*

The possession of epidermal papillae is a constant feature of many grass species and a number of genera. Examples have been cited in Chapter 4 of *Paspalum* (Ellis, 1974a) and *Merxmuellera* (Ellis 1980, 1980a, 1981, 1981a) where adaxial papillae are present on all species examined. Abaxial papillae are particularly common in the andropogonoid grasses.

Papillae, when present, are readily recognizable except sometimes when thin-walled papillae overlie unremoved mesophyll tissue. Papillae may also go undetected if the focal plane is not on the upper, outer surface of the cells. Interference contrast illumination is particularly useful in enhancing papillus detail in photomicrographs as illustrated in *Paspalum* (Page 105, figs 31–34).

The various types of hairs encountered on grass leaves present difficulties with clear definition. In most instances the major hair types generally recog-

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nized are clearly separable but sometimes intermediate forms occur especially between prickles and macro-hairs. This difficulty is not crucial as these hair types are of restricted taxonomic significance. This is so evident, in fact, that Clifford and Watson (1977) actually ignored macro-hairs and prickles because they feel these structures are too variable at generic level to warrant the effort needed for their comprehensive recording. Notwithstanding, these structures can undoubtedly be of assistance with the identification of species or even ecotypes as was the case with the macro-hairs described in the alpine bog forms of *Merxmuellera disticha* (Ellis, 1980; Page 112–116) and *M. stricta* (Ellis, 1980a; Page 117–124). Interlocking prickles overlying abaxial stomatal grooves appear to be characteristic of certain genera such as *Dregeochloa* (Ellis, 1977; Page 109, figs 3–8) and *Asthenatherum.* The continued recording of the details of these hair types, even at the generic level, therefore, seems justified.

Micro-hairs, on the other hand, are widely acknowledged as being exceptionally significant taxonomically (Tateoka *et al.*, 1959). Their detection, however, calls for careful examination of the epidermis at high magnification and they are easily overlooked by all but trained observers. Furthermore, the distal cell is frequently missing altogether and otherwise is thin-walled, collapsed, poorly stained and exceedingly hard to detect. In spite of these frustrating and time-consuming drawbacks, micro-hair detail is vital taxonomically and diagnostically and time spent on their elucidation is time well spent.

The most important distinction is that recognized between micro-hairs possessing inflated, more or less hemispherical, distal cells and those with elongated, parallel-sided tapering distal cells. The presence of these two types of micro-hairs are characters of considerable systematic significance. In addition the mere presence or absence of micro-hairs is also meaningful. Micro-hairs are totally absent from genuine festucoid grasses but are nearly universally present in panicoids, andropogonoids, chloridoids and arundinoid-danthonoids. The presence or absence of micro-hairs thus constitutes a valuable criterion for identification as this information immediately eliminates about half the genera of herbaceous grasses.

Cortaderia selloana is the only known species in which some specimens possess and others lack micro-hairs (Metcalfe & Clifford, 1968). In *Merxmuellera* a similar situation has been recorded in both *M disticha* (Ellis, 1980; Pages 112–116) and *M. stricta* (Ellis, 1980a; Pages 117–124) but in this case different anatomical ecotypes can be recognized which correlate with presence or absence of micro-hairs. This observation is significant and tends to lend weight to the suggestion that taxonomic recognition be given to the anatomical forms distinguishable in the summer rainfall *Merxmuellera* species (Ellis, 1981a; Pages 132–136) as the occurrence of micro-hairs is generally accepted to be under genetic control (Metcalfe & Clifford, 1968).

6.4 Anatomy and grass taxonomy.

Prior to 1875 the classification of the Poaceae was based on morphological (mostly spikelet) characters but during the past hundred years other non-morphological characters have been discovered and extensively applied to grass systematics. A brief historical summary of the more noteworthy anatomical studies will serve to place the present study in perspective and will emphasize the importance of anatomy in grass systematics. It is now recognized that probably the best clue to the relationships of an unknown grass are to be found in its leaf anatomy and epidermal structure (Calderon & Soderstrom, 1973) – a statement which clearly stresses the significance of the present project.

In 1875 Duval-Jouve reported that there are two basic types of leaf anatomy in the grass family. Schwendener (1890) examined the mestome sheath of numerous taxa. Pée-Laby (1898) noticed chloroplast concentrations in certain bundle sheaths. Holm (1901) examined the leaf anatomy of many species and was the first to record the unique bundle sheath condition in *Aristida.* Lohauss (1905) noted that the classical Festuceae contained both anatomical types recognized by Duval-Jouve (1895). Avdulov (1931) was the first to utilize basic leaf anatomy ('festucoid' and 'panicoid') to construct a classification of the family in addition to other non-morphological criteria. Hubbard (1948), Potztal (1953), De Wet (1956), Stebbins (1956) and Brown (1958) continued the study of leaf anatomy in relation to the systematics of the Poaceae. Stebbins (1956) proposed four different anatomical types and Brown (1958) recognized six.

Epidermal structure, especially micro-hairs and silica bodies, was utilized by Prat (1932, 1936) in an attempt at taxonomic clarification in conjunction with the non-morphological characters employed by Avdulov (1931). Tateoka, Inoue & Kawano (1959) quantified micro-hair characteristics and these are now recognized as being significant characters in grass systematics.

In the following discussion the examples of the application of anatomical criteria in the systematics and

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taxonomy of the southern African Poaceae, presented in Chapter 4, will be evaluated and elaborated on where necessary. In several instances the implications of the anatomical observations have already been adequately discussed in Chapter 4 and these will be excluded from the present discussion. It will be noted that anatomical characters of the leaf blade are useful at various taxonomic levels – from sub-specific to sub-family rank – and, in addition, they correlate with morphological and other non-morphological criteria as shown by Jacques-Felix (1962) and Auquier (1963). These include basic chromosome number, chromosome size, starch grain type, embryo characteristics, characters of lodicules, first seedling leaf, root hairs and persistence of nucleoli.

6.4.1 *Alloteropsis semialata.*

6.4.1.1 *Introduction.*

The observation (Ellis, 1974; Chapter 4, pages 97–99) that two distinct anatomical forms exist within *Alloteropsis semialata* (R. Br.) Hitchc. is, without doubt, the single most important finding of this study to date – both from a taxonomic and functional point of view. *A. simialata* remains the only known angiosperm species known to contain both Kranz and non-Kranz elements (Brown, 1977) as suggested by Ellis (1974b; Pages 177–181). The significance of these reports can be gauged by the considerable mention they have received in the recent literature. In physiological, ecological and taxonomic papers the exceptional situation noted in *A. semialata* has been extensively cited and is considered to be crucial to the better appreciation of the systematic and functional implications of the Kranz syndrome. These references to the unique position of *A. semialata,* as inferred from its anatomy, will be mentioned where pertinent in the following discussion.

The functional and ecological significance of the situation observed in *A. semialata* has been discussed and evaluated in Chapter 5 (Pages 177–181). The present discussion will concentrate on assessing the taxonomic implications of the observation with the objective of gaining a better understanding of the relevance of the Kranz syndrome to grass taxonomy and to obtain a more natural classification of the genus *Alloteropsis.* Understandably the complete separation of the taxonomic and physiological aspects is impossible and the Kranz syndrome, with its important physiological implications, will, of necessity, be referred to where relevant. The consequences of the unusual degree of anatomical variation observed in the leaf blades of grass specimens, presently assigned to the single species, *A. semialata,* can, therefore, only be appreciated if the full implications of the C_3 and C_4 photosynthetic pathways are borne in mind. Some specimens of *A. semialata* have Kranz anatomy and the C_4 photosynthetic pathway, whereas others exhibit non-Kranz anatomy and the C_3 pathway of CO_2 assimilation (Ellis, 1974b; Pages 177–181; 1977; Page 160). This fact has been confirmed by several authors including Brown (1975, 1977), Cresswell (1980), Frean *et al.* (1980a, b), Hattersley & Watson (1976), Hattersley *et al.* (1977), Winter *et al.* (1976) and Apel & Peisker (1979). These two photosynthetic types will be termed the C_3 and C_4 forms of *A. semialata* for the purposes of this discussion.

Since 1974, when the initial reports of the anatomical and functional differences present in *A. semialata* were published (Ellis 1974, 1974b), work on this species, and the genus as a whole, has been continued by the present author in co-operation with several other interested scientists, both local and overseas. Table 1 (Page 213) is a summary of results obtained to date. Eighty-five specimens, from a wide range of localities, have been examined anatomically – the leaf in transverse section as well as epidermal structure. The anatomical studies have been the prime concern of this study but the late Prof. W.V. Brown of the University of Texas confirmed many of the diagnoses. Fifty of the specimens have had their carbon isotope ratios determined, either by Dr. B.N. Smith of the University of Texas or by Dr. J.C. Vogel of the C.S.I.R., Pretoria. Cytogenetical studies have been initiated and are being undertaken by Mrs. L. Saayman through the Departement of Genetics at the University of Pretoria. Finally, Dr. S. Jacobs of the Royal Botanic Gardens, Sydney, is co-operating with a comparison of the anatomy and external morphology of South African and Australian representatives of this genus. A brief evaluation of preliminary indications from this basic data will be given.

It is clearly evident from Table 1 that all the data collected to date fully substantiate the initial impressions of Ellis (1974, 1974b) that two distinct entities are presently classified within the single species, *A. semialata.* The original inferences were based solely on anatomical criteria of the leaf blade in section but have now been corroborated by a wide range of attributes such as epidermal structure, carbon isotope ratios and chromosome numbers. No specimen, from the considerably enlarged sample, has been found to deviate from the predicted characteristics of the two distinct forms originally described. Indications from each of the attribute types listed in Table 1 (Page 213) will be discussed individually.

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SPECIES NAME	COLLECTION LOCALITY	HERBARIUM VOUCHER COLLECTOR NO.	LEAF ANATOMY IN SECTION: NON-KRANZ	KRANZ: MS	KRANZ: PS	EPIDERMAL MACROHAIRS: PRESENT	EPIDERMAL MACROHAIRS: ABSENT	δ ^{13}C ‰: -22 to -32‰ C_3	δ ^{13}C ‰: -9 to -16‰ C_4	CHROMOSOME NUMBER	SOIL pH
	ZAMBIA										
	Mwinilunga District	Milne-Redhead 7021	1			1		-20,0 (3)			
	TRANSVAAL										
	2??? - DD, Macobaskloof	Ellis 1865	1, 2			1		-25,5 (3)			
	2470 - AD, Pense	Ellis 2812, 2813	1			1				D	
	- AD, Trichardtsdal	Ellis 2830	1			1					
	2?27 - CD, Poeiernek	Saayman ?	1			1				I	5,25
A. serialata non-Kranz, C_3 form	- DC, Uitkomst	Coetzee 423	1			1		-26,7 (4)			
	- DC, Hekpoort	Repton 4496	1, 2			1		-26,1 (3)		P	
	- DD, Uitkomst	Coetzee 127	1			1		-26,7 (4)			
	- DD, Uitkomst	Coetzee 496	1			1		-26,1 (4)		L	
	2528 - CD, Waterkloof	Saayman 25a, b, c	1			1		-25,5 (4)			5,45
	- CD, Waterkloof	Vogel s.n.	1			1				O	5,45
	2529 - CD, Middelburg	Ellis 2836	1			1					
	2530 - AA, Die Berg	Ellis 2854	1			1				I	
	- CA, Belfast	Burtt-Davy 1323	1			1					
	2531 - CC, Ida Doyer Nat. Res.	Ellis 378	1, 2			1		-26,2 (4)		D	
	2627 - BA, Sterkfontein	Saayman 8	1			1		-27,1 (4)			5,25
	- BA, Krugersdorp Nat. Res.	Saayman 17a, b	1			1					
	- BA, Tarlton	Saayman 19	1			1		-25,2 (4)			
	- BA, Tarlton	Saayman 20	1			1		-25,5 (4)		2n=18	
	- BB, N. of Krugersdorp	Saayman 13a	1			1					5,65
	- BB, near Krugersdorp	Saayman 14	1			1					6,55
	2628 - AB, Bapsfontein	Ellis 1827, 1829	1, 2			1					
	- AB, Bapsfontein	Ellis 1828	1, 2			1		-26,7 (3)			
	- AB, Bapsfontein	Ellis 1830	1, 2			1		-26,4 (3)			
	2630 - DA, Athole Res. Farm	Ellis 1854, 1862	1, 2			1					
	- DA, Athole Res. Farm	Ellis 1861	1, 2			1		-25,4 (3)			
	2730 - AD, Oshoek	Smook 1202	1			1					
	NATAL										
	2731 - CD, Ngome Forest	Ellis 3348	1			1					
	2832 - AA, Hluhluwe Game Res.	Ward 1585	1			1		-26,0 (3)			
	2929 - AD, Cathedral Peak	Ellis 3271	1			1					
	3030 - CB, Izotsha	Ellis 733	1, 2			1		-26,8 (3)			
	CAPE										
	3029 - DA, Kokstad	Ellis 2979	1			1					
	3227 - CB, Komga	Ellis 2607	1			1					
	- DB, Komga	Ellis 2606	1			1		-26,7 (4)			
	3326 - BC, Grahamstown	Ellis 2571	1			1		-28,5 (4)			
	AUSTRALIA										
	Alligator Riv., Northern Territory	Jacobs 1786		1					-13,8 (4)	2n=18 (2)	
	Oenpelli, Northern Territory	Specht 1247		2					-11,4 (3)		
	Corinda, Queensland	Jacobs 1490		2						2n=18 (2)	
	Gilbert Riv., Queensland	White 1470		2					-11,3 (3)		
	Blackwater, Queensland	Henderson 646		2					-12,8 (3)		
	TAIWAN										
A. semialata Kranz, C_4 form	Okamato, Formosa									2n=18 (5)	
	AFRICA										
	Madagascar	Humbert 7108							-11,6 (3)		
	Zimbabwe	Moffet & Hurcombe								2n=54	
	TRANSVAAL										
	2429 - DA, Maboomspruit	Barenbrug 2		2			1		-11,6 (3)		
	2430 - AB, Ofcalaco	Ellis 2809		1			1			P	
	2527 - CB, Hartebeespoort Dam	Saayman 15		1			1				
	- CD, Hennops River	Saayman 10		1			1			O	
	- CD, Hartebeespoort	Saayman 16		1		1					6,55
	- DA, Brits	Saayman 5		1			1			L	
	- DD, Pelindaba	Saayman 1		1			1		-11,2 (4)		6,20
	- DD, Santa Barbara	Saayman 6b, c		1			1			Y	6,35
	- DD, Doornraantjie	Saayman 12		1			1				
	- DD, Uitkomst	Coetzee 497	1, 2				1		-12,3 (4)	P	
	2528 - CA, Brummeria	Ellis 2	1, 2				1				
	- CA, Brummeria	Ellis 1244	1, 2				1		-11,5 (3)	L	
	- CA, Brummeria	Manders 9	1				1				
	- CA, Sinoville	Ellis 1748	1, 2				1		-11,4 (3)	O	
	- CB, Cullinan	Ellis 1789	1, 2				1		-10,9 (3)		
	- CB, Roodeplaat Dam	Ellis 1785	1, 2				1		-12,2 (3)	I	
	- CC, Faerie Glen	Ellis 721	1, 2				1				
	- CD, Waterkloof	Saayman 24	1				1		-10,1 (4)	D	5,45
	- CD, Waterkloof	Vogel s.n.	1				1				5,45
	2529 - AD, Loskop Dam	Ellis 2063	1				1				
	- CD, Middelburg	Ellis 2832, 2833	1				1			2n=36 or 2n=54	
	2531 - AA, Numbi Dist.	Van der Schijff	2				1		-10,6 (3)		6,15
	2627 - BB, Muldersdrift	Saayman 7a	1				1				6,35
	- BB, Krugersdorp	Saayman 9	1				1				
	- DB, near Santa Barbara	Saayman 21	1				1		-13,3 (4)		
	- DB, near Doornraantjie	Saayman 11	1				1				7,35
	2628 - AD, Suikerbosrand	Bredenkamp 886	2				1		-12,6 (3)		
	O.F.S.										
	2727 - BD, Heilbron	Brandmuller 47		2			1		-12,3 (3)		
	2828 - BD, Witsieshoek	Ellis 3160		1			1				
	NATAL										
	2729 - DB, Newcastle	Ellis 36		1,2			1		-11,7 (3)		
	2730 - CB, Utrecht	Edwards 1052		2			1		-11,8 (3)		
	2832 - AA, Hlabisa	Ward 1682		2			1		- 9,3 (3)		
	- AC, Mtubatuba	Harrison 339		1			1		-11,5 (4)		
	- AC, Mtubatuba	Strey 5615		1			1		-11,2 (4)		
A. angusta	ZAMBIA										
	Mankoya Dist.	Drummond & Cookson 6663		1, 2		1 (few)			-12,1 (3)		
	Mwinilunga Dist.	Milne-Redhead 3711		1, 2			1		-12,4 (3)		
	INDIA										
	Dehra Dun	Parker s.n.			1				-11,6 (3)	N	
	Madras	-							-11,0 (3)	O	
A. cimicina	TANZANIA									T	
	Lake Province	Greenway 7384			1, 2	1			-13,1 (3)		
	S.W.A./NAMIBIA									K	
	E. Caprivi	Killick & Leistner 3327			1, 2	1			-12,9 (3)	N	
	MOZAMBIQUE										
A. papillosa	2632 - BC, Maputo	de Lemos & Balsinhas 244			1, 2	1			-13,4 (3)	O	
	NATAL										
	2831 - BB, Hlabisa	Ward 3055			1, 2	1			-12,4 (3)	W	
A. paniculata	CONGO									N	
	Isangai	Germain 4623			2	1			-13,0		

1. R.Ellis; 2. W. V. Brown; 3. B. N. Smith; 4. J. Vogel; 5. C. Hsu, 1965

TABLE 1. – Tabulated summary of co-operative observations in the study of the genus *Alloteropsis*.

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6.4.1.2 *Distribution and Ecology.*

The specimens examined to date constitute a representative sample collected from throughout the distributional range of *A. semialata* in South Africa (Page 215, fig. 1). The localities of both the C3 and C4 specimens of *A. semialata,* examined in this study, include the complete spectrum of habitats occupied by this species except for a somewhat under represented sample in central Natal and the Transkei. It can safely be assumed, from such a representative sample, that all possible variation in the attributes examined should have been incorporated. Very little variation was actually observed, however, and the C3 and C4 forms of *A. semialata* were consistent for their specific characteristics and no intermediates were detected.

A large collection of *A. semialata* herbarium voucher specimens has been assembled during the course of this study and most of these specimens have been further verified as being either the C3 or the C4 form by examination of leaf anatomy and $^{13}C/^{12}C$ ratios. It has therefore been possible to document the morphological characters correlated with the structural and functional differences between these two forms. These differences will be elaborated on later but, at this stage, it will suffice to state that the C3 and C4 forms from South Africa are consistently recognizable on vegetative features alone. By this means all the *A. semialata* herbarium material at the National Herbarium, Pretoria and at the Royal Botanic Gardens, Kew, has been assigned to either the C3 or the C4 form by the author. This data has been used in compiling the accompanying distribution maps of South Africa (Page 215, fig. 1), Africa (Page 216, fig. 2) and the worldwide distribution of this species (Page 217, fig. 3) and the other species of *Alloteropsis.*

The C3 form of *A. semialata* is found in the Eastern Cape, Transkei, Natal and then extends northwards and westwards into the highveld of the Transvaal (Page 215, fig. 1). Further to the north localities have been recorded along the Transvaal Drakensberg, the Zoutpansberg and the Chimanimani Mountains of Zimbabwe. Isolated records are known from Malawi and the Mwinilunga District of Zambia but the C3 form has not been collected further northwards in Africa (Page 216, fig. 2), or in Asia or Australia. This is in contrast to the C4 form which is widely distributed in Africa, India, South-east Asia, the East Indies and eastern Australia (Page 217, fig. 3).

Morphological variation of the C3 *A. semialata* plants increases in a northward direction and several specimens from central Africa are atypical and have consequently not been assigned to either form (Page 216, fig. 2). These plants are less robust, lack conspicuous pubescence of the leaves and, unfortunately, are represented by incomplete herbarium specimens from which it is difficult to make reliable diagnoses. A similar trend appears to be detectable along the sub-tropical Natal coastal belt. This is significant because the remainder of the South African and Zimbabwean material, all from higher altitudes and, consequently, with cooler climates, form a consistently uniform morphological entity. It appears, therefore, that with increasing temperatures, the typical and distinctive C3 form (= var. *eckloniana* Nees) exhibits increasing modification and apparently ultimately intergrades morphologically with the C4 form. For this reason the distinctive var. *eckloniana* segregate has not been considered to be of taxonomic significance (Clayton, in press).

This variation stands in marked contrast with the situation in the higher inland plateau of South Africa where, even where their distributions overlap, the C3 and C4 forms are easily distinguishable on the basis of a number of vegetative characteristics. In this region taxonomic separation of these two forms appears fully justified and is practically possible. The intermediate forms, especially those from Central Africa, appear to hold the key to the question of the relationships of the C3 and C4 forms and further study of these populations should also shed additional light on aspects of the evolution of the Kranz syndrome. Milne-Redhead 3021 is an example of a specimen from Zambia with unique intermediate carbon isotope characteristics (Table 1, Page 213).

In South Africa the C4 form of *A. semialata* occurs in isolated pockets along the periphery of the northern half of the distributional range of the C3 form (Page 215, fig. 1). To the east and north-east all the localities of the C4 form are at lower altitudes even although they often occur in the same quarter-degree grid squares (Edwards & Leistner, 1971) as the C3 form. This disjunct distribution of the C4 form, often in close proximity to the C3 form, could be interpreted as indicating that the C4 form has evolved independently from the C3 form on a number of separate occasions.

The occurrence of the C4 form in the eastern orange Free State, the Vaal Basin and northern Natal is worthy of comment. Some of these collections were made at relatively high altitudes, such as Ellis 3160 from Witsieshoek at 2 000 m. This does not conform with the generally accepted view that the C4 pathway is adaptive in hot, arid climates. The only

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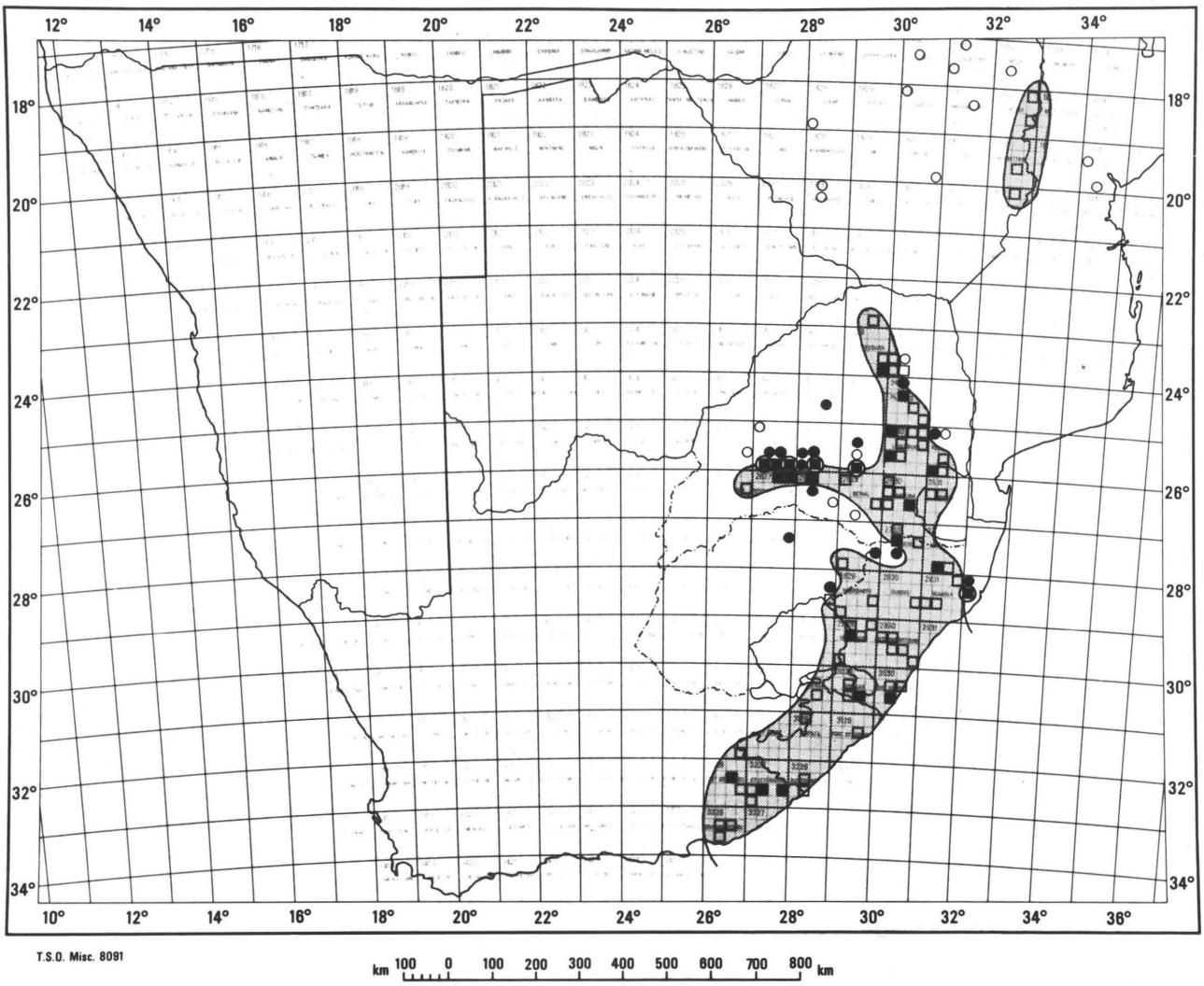


FIG. 1. – Distribution of the C3 (□) and the C4 (○) form of *Alloteropsis semialata* in Southern Africa. Shaded symbols represent localities of specimens studied anatomically. Compiled from specimens at the National Herbarium, Pretoria (PRE).

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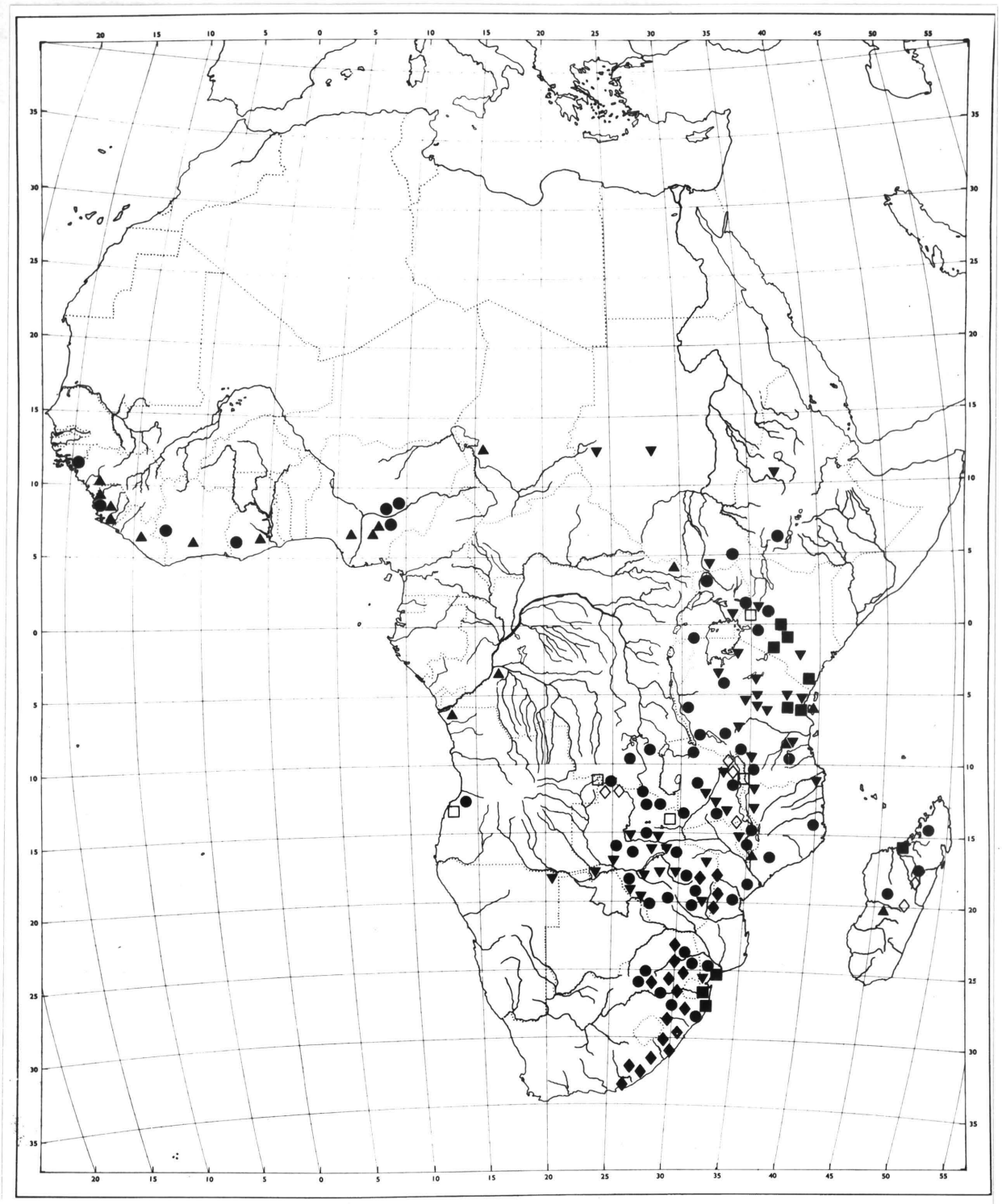


FIG. 2. – Distribution of the species of *Alloteropsis* in Africa: C4 form of *A. semialata* (●); C3 form of *A. semialata* (⧫); atypical C3 form of *A. semialata* (◊); *A. angusta* (□); *A. papillosa* (■); *A. cimicina* (▼) and *A. paniculata* (▲). Compiled from specimens at the National Herbarium, Pretoria (PRE) and the Royal Botanic Gardens, Kew (K).

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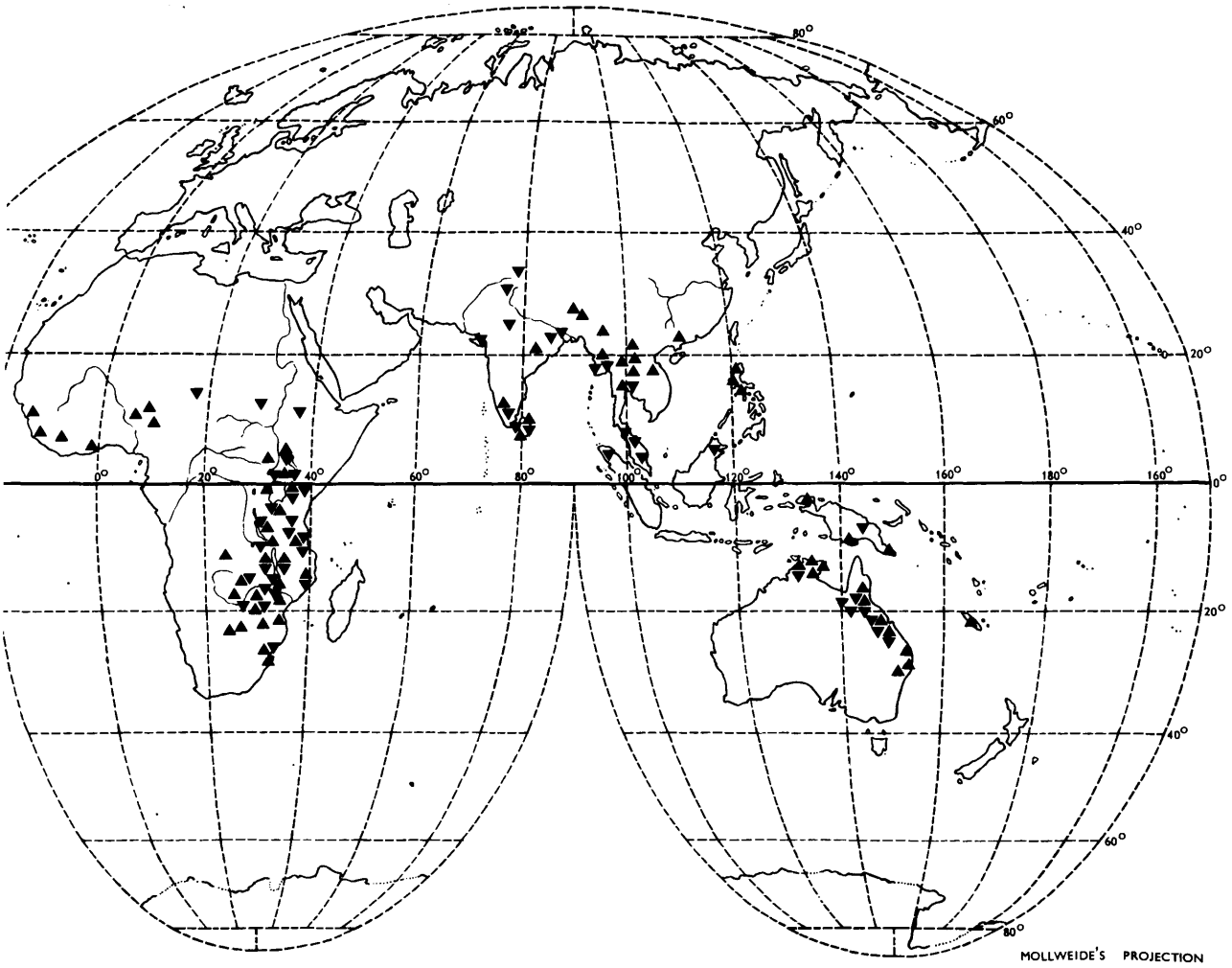


FIG. 3. – World-wide distribution of the C4 form of *A. semialata* (▲) with a Kranz mestome sheath and C4 *A. cimicina* (▼) with a Kranz parenchyma sheath. Compiled from the collection of the Royal Botanic Gardens, Kew (K).

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factor common to all these highland grassveld localities appears to be the poor, sour soils of these veld types (Acocks, 1975).

There are only two localities where the C_3 and C_4 forms of *A. semialata* are known to occur in close proximity in the same plant community – Waterkloof Heights in Pretoria and Sandton near Johannesburg (Frean *et al.*, 1980). In both instances the C_3 and C_4 forms are morphologically distinct and no intermediates are evident. At the Pretoria site the vegetation is a *Protea caffra* savanna on moderately acid soils (pH 5,45). The C_3 *A. semialata* form is most common on the upper slopes in the *Protea* community and the C_4 form occurs in a disturbed grassland below this slope. The zone of overlap between the C_3 and C_4 forms is clearly transitional between the *Protea* savanna and the grassveld. The *A. semialata* plants from this locality are clearly differentiated into the C_3 and C_4 forms on the basis of anatomy and $^{13}C/^{12}C$ ratios (Table 1) in addition to morphology. The CO_2 compensation points of 50 ppm and 5 ppm respectively (Vogel, unpublished) also confirm this photosynthetic difference.

Soil pH's from this region of sympatry of the C_3 and C_4 forms in the bankenveld area of the southern Transvaal have been analysed. Results indicate that the C_3 form prefers strongly to moderately acid soils whereas the C_4 form occurs on soils that are only slightly acid (Table 1, Page 213). Soil pH may therefore account for the continued separation of these two forms even when they occur in close proximity to one another.

The C_4 form is never abundant and conspicuous under natural conditions. This is in marked contrast to the C_3 form which, especially at higher altitudes, can become very common on certain south-facing slopes particularly in early summer when the large, bright green tufts are conspicuous. The C_4 form, on the other hand, seldom projects above the general grass canopy and only is visible when flowering. Both forms respond to winter fires which appear to stimulate early growth and flowering.

The C_4 form of *A. semialata* is widely distributed throughout sub-Saharan Africa except for the equatorial region of the Congo Basin and the more arid regions of Somaliland and the Kalahari (Page 216, fig. 2). Similar patterns are also evident in the distributions of the other C_4 *Alloteropsis* species – both the malate and aspartate forming types. On a worldwide scale this trend is also evident as a comparison of the distributions of the malate forming C_4 form of *A. semialata* and the aspartate forming *A. cimicina* shows (Page 217, fig. 3).

6.4.1.3 *Leaf Anatomy.*

All of the 41 specimens of the C_3 form of *A. semialata*, that have been examined, have similar non-Kranz leaf anatomy. They possess a double bundle sheath with the outer parenchyma sheath consisting of conspicuous, large, somewhat thick-walled cells without, or with few, chloroplasts (Page 99, fig. 8; Page 178, fig. 2). The inner sheath is a typical fibrous mestome sheath except that the cells often contain bodies which resemble chloroplasts. Ontogenetic studies by Cresswell (1980) and Frean *et al.* (1980) have confirmed ultrastructurally that these are vestigial chloroplasts which degenerate leaving the inner sheath almost devoid of chloroplasts. Only a single chloroplast type is present and it appears unlikely that these chloroplasts represent an early stage in the evolution of a Kranz sheath. In this C_3 form the arrangement of the mesophyll cells resembles the radiate condition – particularly along the adaxial half of the blade.

This type of panicoid C_3 anatomy differs considerably from the classical, festucoid C_3 type which is characterized by inconspicuous, thin-walled outer parenchyma sheath cells and irregularly arranged mesophyll. Several other South African panicoid C_3 grasses have virtually identical anatomy to the C_3 form of *A. semialata* (Ellis, 1974c), except that the mestome sheath is without chloroplats. *Acroceras macrum, Panicum ecklonii, P. natalense* and *Sacciolepis typhura* are examples.

In America, *Panicum milioides* and *P. hians* have been shown to possess photosynthetic characteristics intermediate between the C_3 and C_4 pathways (Brown & Brown, 1975). It is interesting to note that the anatomy of these two American species appears to resemble the South African panicoid C_3 grasses. *P. milioides* and *P. hians*, together with three other *Panicum* species, have recently been transferred to the genus *Steinchisma* (Brown, 1977) which is characterized by reduced photorespiration and other intermediate photosynthetic characteristics.

The possibility that the South African C_3 panicoid grasses possess intermediate photosynthetic characteristics as well, cannot be overlooked. This possibility is further strengthened by their geographical distribution which is intermediate between that of the tropical oryzoid and bambusoid C_3 grasses and the temperate festucoid and danthonoid species (Vogel *et al.*, 1978; Page 186). Furthermore these C_3 panicoid grasses (including the species placed in *Steinchisma*) grow in full sunlight in con-

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trast to the other C3 members of the Paniceae which are forest species restricted to shady habitats. These forest species also differ in bundle sheath and mesophyll anatomy.

Panicum milioides has a typical C3 $\delta\ ^{13}C$ ratio but has an intermediate CO_2 compensation point (15–20 ppm)(Brown & Brown, 1975). The CO_2 compensation point of C3 *A. semialata* has been determined to be about 50 ppm (Vogel, unpublished), which is typically C3, and *A. semialata* does not appear to be similar to the *Steinchisma* group in this respect. Physiological and ultrastructural aspects of the C3 and C4 forms of *A. semialata* are being further investigated (Cresswell, pers. comm.) and the results forthcoming should be carefully compared with published results for *P. milioides* and *P. hians* (Brown & Brown, 1975). This physiological work on *A. semialata* should help considerably in gaining a better appreciation of the relationships of the C3 and C4 forms of *A. semialata* both with respect to each other and to the Paniceae.

In addition to those anatomical attributes correlated with the Kranz syndrome, the C3 and C4 forms of *A. semialata* differ considerably. Examples are in the sequence, pattern and positioning of the vascular bundles of different orders along the width of the blade, the shape of the adaxial ribs and furrows and the form and shape of the sclerenchyma girders (Ellis, 1974; Pages 98 & 99, figs 1–8). These differences, irrespective of the other anatomical differences associated with the Kranz syndrome, are of considerable magnitude and appear to be taxonomically significant. In no other species of the Poaceae has such a degree of anatomical variation been observed and, therefore, on this basis alone *A. semialata* warrants taxonomic revaluation. The fact that no intermediates have been observed in a large and representative sample of 85 specimens adds weight to this opinion.

The leaf anatomy of the C4 form of *A. semialata* has been described in detail (Ellis, 1974; Pages 97–99; 1974b; Pages 177–181) and has been found to be consistent in a sample of 36 specimens from throughout the distribution range of the C4 form in South Africa (Page 213, table 1; Page 215, fig. 1). The bundle sheath and mesophyll structure remains remarkably consistent and the non-photosynthetic characters of the bundles and girders are also uniform throughout this representative sample.

No freshly fixed Australian or Asian material has as yet been examined to ascertain whether the non-photosynthetic characters mentioned above are similar to the condition described in the C4 form of *A. semialata* from South Africa. Correspondence with Prof. W.V. Brown and Dr S. Jacobs indicates that this is indeed so but I have yet to personally confirm this fact. This comparison is vital as the African and Australian representatives appear to differ in basic chromosome number (Page 213, table 1) and may possibly not represent a single species.

The Australian C4 form definitely has a Kranz mestome sheath and an outer sheath devoid of chloroplasts (Hattersley *et al.*, 1977) and in this respect compares favourably with the South African C4 form. However, from the photomicrograph published by Hattersley *et al.* (1977) it is not possible to ascertain any detail of the non-photosynthetic characteristics. This is unfortunate because the possibility that the C4 form needs to be definitely established and vital clues may be forthcoming from the diploid Australian populations.

It appears reasonable to postulate that the vestigial chloroplasts, present in the mestome sheath cells of present-day C3 representatives of *A. semialata*, are remnants of cells that gave rise to the Kranz chloroplasts of the mestome sheath cells of the C4 form with a concurrent degeneration of the outer parenchyma sheath cells. Vascular bundle sequence and sclerenchyma girder structure of the present C4 form however do not appear to confirm this hypothesis. This is in contrast to the situation found in the *Alloteropsis* species with Kranz parenchyma sheaths – the *Coridochloa* group of *A. cimicina, A. papillosa* and *A. paniculata*. These species closely resemble the C3 form of *A. semialata* as regards bundle distribution, ribs and furrows, sclerenchyma and epidermal macrohairs (Table 1). The derivation of these 'aspartate' forming types from the C3 form is easily visualized and requires only the development of specialized chloroplasts in the outer sheath cells. This is not so with the C4 form of *A. semialata* (and *A. angusta*) where the complicated, yet consistently repeated, sequence of vascular bundles of different sizes and their association with the sclerenchyma girders, makes an origin from a C3-like ancestor appear unlikely. Further study of the Australian C4 form, and of the *Coridochloa* group, is essential before a better understanding of relationships within this genus can be expected.

The outer bundle sheath cells in the C4 form of *A. semialata* (Ellis, 1974; Page 99, fig. 7; 1974b) deserve special mention. In this form the mestome sheath is Kranz and, therefore, it has been classed as a malate former (Ellis, 1977; Page 160) but it was noted as being exceptional in that outer bundle sheath cells are present. It is noteworthy that these

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parenchyma sheath cells are small, thin-walled, lack chloroplasts and are often collapsed and difficult to distinguish. This unique condition may represent an intermediate stage in the evolution of the NADP-ME type of anatomy (Page 160). Brown (1975) considers that this structure demonstrates that a Kranz sheath can be derived from a typical mestome sheath. The clearly distinguishable inner tangential and radial differential thickening of the Kranz cells of the C4 form clearly seems to lend support to this postulated derivation from the mestome sheath (Ellis, 1974b; Page 180) and the condition of the outer sheath may represent an intermediate stage in the degeneration of this sheath. Ultrastructural ontogenetical studies by Frean *et al.* (1980) confirm the derivation of the specialized photosynthetic tissue of this single Kranz sheath from the mestome or inner sheath. This observation in *A. semialata* has led Brown (1975) to propose that all single-sheathed Kranz grasses may have their Kranz sheaths homologous with the mestome sheath – a scheme with obvious taxonomic implications, especially in the Panicoideae (Ellis, 1977; Pages 157–158, table 2) where it is implied that there have been two origins of the Kranz sheath.

6.4.1.4 *Epidermal structure.*

Epidermal scrapes have been prepared of all the *A. semialata* material examined anatomically. Table 1 clearly illustrates a further intraspecific difference between the C3 and C4 forms of *A. semialata*: namely that all the C3 specimens possess abaxial epidermal macrohairs whereas the C4 form is glabrous and lacks hairs of this type. The presence or absence of macrohairs is, therefore, an additional difference between the two forms of *A. semialata*; a difference which correlates with the Kranz and non-Kranz anatomical attributes characterizing each of these forms.

A single C4 specimen (*Saayman* 16) is exceptional in that it possesses elongated, unicellular hairs which, according to definition (Ellis, 1979; Chapter 3, pages 79–80), should be called macrohairs. These particular hairs resemble the angular prickles of the margin, however, and appear to be essentially prickles with exceptionally elongated barbs. Both these hairs, and the marginal prickles, are associated with cushion cells at their bases and, in this respect, resemble typical macrohairs. In this specific instance the hairs appear to be intermediate between prickles and macrohairs and clearly illustrate the difficulty in precisely defining biological structures. Nevertheless, the fact that the hairs of this C4 specimen differ from the C3 macrohairs, appears to confirm that considerable differences exist between the two forms of *A. semialata*.

The difference between the pubescent C3 form and the glabrous C4 form has been confirmed by Frean & Cresswell (1979) and Frean, Barrett & Cresswell (1980) using scaning electron microscopy. These authors have shown that the area covered by the macrohairs is 8,0% on the upper surface and 10,0% on the lower surface. They are of the opinion that these hairs constitute a boundary layer at the leaf surface which could be physiologically important with regard to gas exchange and transpiration. Notwithstanding this development of hairs, the transpiration rates of the C3 form are noticeably higher when compared to C4 plants (Frean, Barrett & Cresswell, 1980).

In addition to macrohairs, the epidermides of these two forms differ in several other respects such as width of costal zones, size of silica bodies and stomata and the shape of the intercostal silica bodies. Frean & Cresswell (1979) have shown that the adaxial surface of the C4 form has conspicuous wax crystals which are lacking on the C3 form. The convolutions of the adaxial surface of the C3 form are also distinctive and the stomata vary in number and distribution between the two types. The stomatal index, stomatal frequency and stomatal size also differ (Frean *et al.*, 1980). The stomatal frequency is considerably higher in the C3 form but variation in the stomatal index is not so significant. The differences in stomatal frequency could influence physiological patterns of water utilization efficiency. The stomata and the epidermal cells of the C4 form were found to be larger in area than those of the C3 form. This may be an example of stomatal length being an indicator of ploidy level (De Wet, 1954) as the C4 form appears to be a polyploid in contrast to the diploid C3 form (Page 213, table 1).

6.4.1.5 *Carbon Isotope Ratios.*

In C4 plant species, CO_2 incorporation by phosphoenolpyruvate carboxylase does not discriminate against the ^{13}C atoms of CO_2 as does that by ribulose diphosphate carboxylase in C3 species (Whelan, Sackett & Benedict, 1973). As a result, the organic matter of C4 plants has a $^{13}C/^{12}C$ ratio not much different from that of CO_2 in the air. The organic matter of C3 species is, on the other hand, much lower in ^{13}C. C4 species thus have high negative ratios (between –9 and –18‰), whereas C3 plants have low ratios (between –22 and –38‰) (Bender, 1968, 1971; Smith & Brown, 1973; Troughton, *et al.*, 1974). Therefore, there is a gap of at least 4‰ between high and low ranges in grasses, which makes the carbon istotope ratio a very reliable

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method of characterizing a specimen as C_3 or C_4 (Smith & Epstein, 1971). In fact, these ratios are considered to be the best evidence obtainable for indicating whether an angiospermous plant is Kranz or non-Kranz provided it is neither aquatic or succulent (Brown, 1977).

The carbon isotope ratios of fifty of the *Alloteropsis* specimens examined anatomically in this study have also been determined (Table 1) in order to verify the anatomical indications as to whether they are Kranz or non-Kranz. All the specimens diagnosed as Kranz on anatomical criteria have $^{13}C/^{12}C$ ratios between –9,3 and –13,8‰, whereas the non-Kranz specimens are between –25,4 and 28,5‰. The *Alloteropsis* sample is, therefore, clearly divisible into C_3 and C_4 components which correlate perfectly with anatomical indications. Specimens with non-Kranz anatomy have C_3 photosynthesis and those with Kranz structure, the C_4 photosynthetic pathway.

The following *Alloteropsis* species are C_4: *A. paniculata, A. papillosa, A. cimicina, A. angusta* and the C_4 form of *A. semialata.* The C_3 form of *A. semialata* is the only exception in this respect. All grass genera are either entirely non-Kranz or entirely Kranz – the only known exceptions being *Panicum* and *Alloteropsis* (Brown, 1977). For *Panicum* it can be proposed that the genus is artificial and the Kranz and non-Kranz species should be separated. This has recently been done and many American C_3 *Panicum* species have been transferred to *Dicanthelium* (Brown & Smith, 1975; Gould & Clark, 1978). *Alloteropsis semialata* remains the only known angiosperm species containing both C_3 and C_4 elements and the taxonomic status of these forms needs reappraisal.

A specimen examined during the course of this study (Milne-Redhead 3021) is unique in having a $^{13}C/^{12}C$ ratio intermediate between the C_3 and C_4 ratios. The ratio of this plant was determined to be –20,0‰ and on rechecking –18,5‰. This is the only known example of a grass being intermediate for this characteristic and further studies of this exceptional plant should prove rewarding.Unfortunately its collection locality in north-western Zambia means that further material from this population will be difficult to come by.

This intermediate $^{13}C/^{12}C$ ratio is the reason for several *A. semialata* specimens not being classified as either C_3 or C_4 in Fig. 2. These unclassified specimens resemble Milne-Redhead 3021 morphologically and could possibly also represent specimens with intermediate carbon isotope ratios.

6.4.1.6 *Cytogenetics.*

This critically important aspect of this entire investigation has unfortunately made unsatisfactory progress and, at this stage, only a very brief preliminary report can be given. It is hoped to personally undertake this study during the growing season of 1981/82 so that future planning of research can be placed on a firmer basis.

Initial indications are that the C_3 form of *A. semialata* in South Africa is diploid (2n=18), whereas the C_4 form is polyploid, either tetraploid (2n=36) or hexaploid (2n=54) (Table 1). It is of particular interest to note that C_4 Australian and Asian representatives of *A. semialata* seem to be diploid (Brown, pers. comm.) in contrast to Africa where a high incidence of polyploidy is present.

This observation appears to cast some doubt on the proposal that the Kranz syndrome has evolved in the South African non-Kranz populations, from where it has spread to the East (Brown, 1977). It also negates the other possibility that reverse evolution has occurred and that the non-Kranz form has evolved from the Kranz form. This leaves the third possibility, that the two forms are not closely related but that they reflect convergent evolution, as the most plausible explanation of the situation in *A. semialata*.

Preliminary karyotype studies indicate that the chromosomes of the C_4 form differ structurally from those of the C_3 form (Saayman, pers. comm.) and, therefore, a true polyploid series may not exist in *A. semialata*. The tetraploid and the hexaploid C_4 specimens do not show multivalent pairing but have normal bivalents only. This normal bivalent pairing suggests that the C_3 and C_4 forms each represent a good species. If this is indeed so, then it implies that the C_3 and C_4 forms are not as closely related as morphological criteria indicate and tends to support the possibility of convergent evolution.

The most feasible explanation of the situation as presently understood is that a diploid, non-Kranz ancestor of the C_4 form originally existed in South Africa. Later a diploid Kranz taxon evolved here from which the tetraploid and hexaploid C_4 forms evolved. Apparently the diploid Kranz population in South Africa has died out but diploids have persisted in Australia. A knowledge of the breeding system in operation in both forms of *A. semialata* is vital, both for taxonomic decisions to be made and before hybridization experiments can be begun. If it proves possible to hybridize these two forms with different photosynthetic pathways, valuable information re-

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garding the stages in the evolution of the Kranz syndrome will be forthcoming. It is imperative that this cytogenetical study be continued as it is basic to further progress in all other disciplines.

6.4.1.7 *Morphology.*

From the foregoing it should be obvious that the C3 and C4 forms of *A. semialata* differ in many respects and it now remains to be seen whether they can be distinguished morphologically. No difficulty was experienced in sorting the 84 specimens of *A. semialata*, examined during the course of this study, into two distinct groups which agreed entirely with the Kranz/non-Kranz difference. The following are detailed descriptions of the C3 and C4 forms based on the specimens verified as being C3 or C4 by either anatomy or carbon isotope ratios. Diagnostic differences are in italics.

Alloteropsis semialata – C3 form

Caespitose perennial with persistent *stiff tomentose sheath bases or their remains*. Culms erect, simple *300–700 mm high*, 2- to 3-noded with the uppermost internode long, exserted. Nodes pubescent or silky-pubescent. Sheaths tight around the culm, strongly striate, the basal ones crowded, *very firm and more or less tomentose. Cilate margins.* Ligule reduced to a ciliate rim. Blades 100–300 mm long, 3–9 mm wide, linear from a more-or-less narrowed base, tapering to an acute point, flat or *folded*, firm; *hairy*, hairs ± 1,25 mm long. Racemes *2–6* (mostly 3–5, $\bar{x}$ = 3,73 ± 0,09) digitate from a silky pubescent node, suberect or obliquely spreading, usually stiff, *ca. 40–120 mm long*, secund or subsecund, *dense* and often interrupted in the lower part; rhachis slender, straight or slightly flexuose, triquetrous, scabrid on the angles and *usually with hairs longer than 1,5 mm*; pedicels mostly 2-nate, angular, the longer 2,5 mm long, the apices cupular. Spikelets ovate-lanceolate to ovate-oblong, shortly acuminate, *dorsally compressed*, length (excluding awn) *4,5–7 mm*, ($\bar{x}$ = 5,06 ± 0,06); *awn 1–3 mm long*, greenish or tinged with brown or purple. Lower glume ovate, acutely acuminate, mucronulate or very shortly awned, *3,5–4,0 mm long*, membranous *3–5-nerved*, glabrous or minutely pubescent. Upper glume firmer, similar to the spikelet in shape and size, 5-nerved, densely ciliate along the marginal nerves with appressed or ultimately spreading cilia about 1 mm long, either without wings or sometimes with narrow or broad fimbriate wings from the marginal nerves and then ciliate from the edges of the wings. Lower lemma about as long as the spikelet, glabrous or delicately pubescent with an acute or minutely truncate tip, sometimes mucronate or produced into a point, distinctly chartaceous except for a triangular hyaline portion at the base, sometimes mottled with brown. Palea about 2,0 mm long, deeply 2-fid, with obtuse ovate auricles extending to above the middle. Upper lemma almost as long as the lower, lanceolate, acuminate, chartaceous 5-nerved delicately ciliolate, produced into a mucro or fine bristle occasionally up to 3 mm long (usually 2 mm) its palea with ovate acute auricles. Anthers about 2,5–3.0 mm long, *orange coloured. Non-Kranz anatomy.*

Alloteropsis semialata – C4 form

Caespitose perennial with persistent *inflated silky-tomentose sheath-bases or their remains.* Culms erect, simple *300–1,110 mm high*, 2- to 3-noded with the uppermost internode long, exserted. Nodes pubescent or silky pubescent. Sheaths tight around the culm, strongly striate, *glabrous at the top*, the basal ones crowded, inflated and silky tomentose. *Tomentose margins.* Ligule reduced to a ciliate rim. Blades 120–350 mm long, 3–6 mm wide, linear from a more-or-less narrowed base, tapering to an acute point, flat or *convolute*, very firm, usually *glabrous* very occasionally with loose hairs, 1,0–1,5 mm long. Racemes 2–4 (mostly 2–3, $\bar{x}$ = 2,34 ± 0,06) digitate from a silky pubescent node, suberect or obliquely spreading, usually stiff, *ca. 60–150 mm long*, secund or subsecund, *dense to rather loose* and often interrupted in the lower part, rhachis slender, straight or slightly flexuose, triquetrous, scabrid on the angles or with *hairs 1,1–5,0 mm long*; pedicels mostly 2-nate, angular, hairy, 1–5 mm long, the apices cupular. Spikelets ovate-lanceolate to ovate-oblong, shortly acuminate, *slightly dorsally compressed*, length (excluding awn) *5–7 mm*, ($\bar{x}$ = 6,18 ± 0,05); *awn length 2,5–4,5 mm*, greenish or variously tinged with brown or purple. Lower glume ovate, acutely acuminate, mucronulate or very shortly awned, *3–5 mm long, membranous 3-nerved*, glabrous or very minutely pubescent. Upper glume firmer similar to spikelet in shape and size, 5-nerved, densely ciliate along the marginal nerves with appressed or ultimately spreading cilia about 1 mm long, either without wings or sometimes with narrow or broad fimbriate wings from the marginal nerves and then ciliate from the edges of the wings. Lower lemma about as long as the spikelet, glabrous or delicately pubescent with an acute tip (often mucronate or produced into a point), distinctly chartaceous except for a triangular hyaline portion at the base. Palea 2,0–2,5 mm long, deeply 2-fid, with obtuse ovate auricles extending to above the middle. Upper (fertile) lemma almost as long as the lower, lanceolate, acuminate, chartaceous, 5-nerved delicately ciliolate, produced into a

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mucro or fine awn 2,5–4,5 mm long, its palea with ovate acute auricles. Anthers about 2,5 mm long, *yellow to dark orange coloured. Kranz anatomy.*

The specimens examined thus sort into two distinct groups on the basis of their sheath bases, leaf hairiness, spikelet size and number of racemes, in addition to the non-Kranz–Kranz difference. Statistical tests indicate that there is a correlation between spikelet length and number of racemes. 't' tests between spikelet length and raceme number give:

Raceme number, t = 1,83, p = 0,08 (n = 193)

Spikelet length, t = 1,39, p = 0,13 (n = 372).

This is not significantly different but is a useful distinction for these types of characters, and, when combined with leaf hairiness and differences in sheath bases, as well as Kranz and non-Kranz, two quite distinct species can be distinguished. This applies to all the *A. semialata* material from South Africa and Zimbabwe in the National Herbarium, Pretoria.

Clayton (in press) maintains *A. semialata* as a single polymorphic species exhibiting a wide range of variation. He places *A. gwebiensis* and *A. homblei* in synonomy in *A. semialata* and maintains *A. angusta* as distinct but regards it merely as an impoverished form of *A. semialata.* Clayton, therefore, considers all the *Alloteropsis* species having a Kranz mestome sheath, and with similar vascular bundle and sclerenchyma girder arrangement to be very closely related, probably within a single species. This conclusion is in complete agreement with the anatomical observations of this study with the exception of the C_3 form of *A. semialata.* Although he mentions var. *eckloniana* as being a distinctive segregate he notes that it merges completely with the rest of *A. semialata* and, therefore, regards it as being of limited taxonomic significance. This decision is taken with due regard to the unusual degree of variation in the leaf anatomy reported in this study (Ellis, 1974, 1974b).

Although the C_3 and C_4 specimens verified during this study appear to be morphologically distinct at the species level, the C_3 specimens from Malawi and Zambia appear to intergrade with the C_4 form and, at this stage, it seems difficult to justify raising the C_3 form to specific rank. Further examination of material from these areas is required before a final decision can be taken on the taxonomic status to be accorded this C_3 form.

A. cimicina and *A. paniculata*, together with a recently described species, *A. papillosa* (Clayton, 1978), are maintained as separate species (Clayton, in press). These species have a double bundle sheath with the outer sheath being Kranz (Table 1). Anatomically this group is, therefore, uniform and in addition, they have been placed in the same subgenus, *Coridochloa,* on morphological criteria (Butzin, 1968). *A. cimicina*, an annual, and *A. papillosa*, a perennial, both have a papillose palea of the upper floret and constitute the section *Coridochloa. A. paniculata*, an annual with a glabrous palea, is placed in the section *Mezochloa* (Butzin, 1968). The anatomical distinction between those species with a Kranz mestome sheath and those species with a Kranz parenchyma sheath, therefore, is reflected in the classification and it only remains to decide what taxonomic rank this difference warrants. At the moment it appears as if the mestome sheath/parechyma sheath difference should be regarded as a sub-generic character as Brown (1977) does for *Panicum*. Attempts at sorting all the material examined in this study into possible generic groupings on morphological grounds has resulted in groupings which do not correspond with this bundle sheath difference. There is no question of establishing new genera which are heterogenous for this character and thus this sub-generic grouping of the parenchyma sheath and mestome sheath *Alloteropsis* species appears to be the most satisfactory at present.

In conclusion it can be stated that, although considerable information on the genus *Alloteropsis* has been assembled, final taxonomic synthesis awaits further cytogenetical data. Significant progress has been made with regard to the classification of the genus but the cytogenetical information is required to allocate taxonomic rankings to the groupings. *Alloteropsis* has proved to be an excellent example of where anatomical indications have proved highly significant and of relevance in both taxonomy and functional studies.

6.4.2 *Paspalum paspalodes* and *P. vaginatum*.

At the time at which this study (Ellis, 1974a; Chapter 4, pages 100–106) was undertaken in 1973, a certain degree of uncertainty existed as to the taxonomic status to be accorded these two taxa. This had resulted because these taxa are so similar morphologically, grow in the same type of habitat and have sympatric distributions – coastal salt marches in tropical to sub-tropical regions worldwide (Renvoize & Clayton, 1980). Most authors considered them to be separate species (Chippindall, 1955; Bor, 1960; Gordon Gray, 1972), but, sometimes *P. paspalodes* and *P. vaginatum* are recognized only as sub-species in one and the same species (Jovet & Guedes, 1972). A comparative anatomical study of these two taxa was con-

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sequently undertaken in an attempt to help resolve this uncertainty. The results are presented in Chapter 4 (Pages 100–106).

The South African material examined in this study revealed numerous, consistently present, anatomical differences. They can be separated on the basis of the distribution of the sclerenchyma, the form and the thickness of the margin, the shape of the adaxial ribs and furrows and the papillae. These differences, in conjunction with spikelet characters, distributional data and habitat differences led Loxton (1974) to uphold them as two distinct species in this region.

A firm, convincing decision as to the taxonomic status to be accorded these two entities was also required because, there existed, at that time, confusion as to the correct nomenclatural treatment for these taxa (Loxton, 1974). This disagreement over the correct names to be applied to these two species has, as yet, not been finally resolved (Renvoize & Clayton, 1980). The decision to examine their leaf anatomy was pertinent as unnecessary confusion and argument may have been avoided if they had not proved to be distinct species. However, the anatomical and morphological studies (Ellis, 1974a; Loxton, 1974) confirmed their specific status and now a stable decision on their correct names is required.

A brief discussion of this nomenclatural problem is not only of interest but clearly illustrates the absolute necessity for the closest possible co-operation between anatomists and competent taxonomists in any comparative anatomical study. It also confirms the importance of basing such anatomical studies on reliably authenticated herbarium voucher specimens lodged in internationally recognized herbaria (Dunn, 1960). Such an approach has been adopted in the present project and, therefore, the anatomical data of this specific study remain relevant and can easily be updated inrespective of nomenclatural changes that may subsequently be necessary to resolve the question of the correct names to be applied to *P. paspalodes* and *P. vaginatum*.

The confusion regarding the correct names for the two entities, regarded as being *P. paspalodes* and *P. vaginatum* in this study has arisen because, recently, it has become evident that both species are actually represented on the type sheet of *Paspalum distichum* L. On this sheet there are three specimens, one of which has been faintly annotated 'Br' by Linnaeus (Fosberg, 1977). The type of *P. distichum* was not indicated by Linnaeus (1759) in his original description but he did specifically acknowledge that his description was based on a Patrick Browne specimen from Jamaica. Thus the Browne specimen must be the true type (Vickery, 1975). Unfortunately, in the microfiche edition of the Linnean Herbarium this faded incription 'Br', indicating the true type of *P. distichum* is not visible. This fact has further complicated this complex nomenclatural question.

The third specimen from the left, bearing the inscription 'Br', on sheet number 79/9 in the Linnean Herbarium is regarded as being the holotype of *P. distichum* (Renvoize & Clayton, 1980). This specimen has glabrous upper glumes whereas the other two specimens on this same herbarium sheet have pubescent upper glumes (Vickery, 1975).

All the authors who have concerned themselves with the typification of *P. distichum* (Vickery, 1975; Guedes, 1976; Fosberg, 1977; Renvoize & Clayton, 1980) specifically mention only three specimens on the Linnean type sheet. Examination of the IDC microfiche edition of the Linnean Herbarium, as well as a print of a 35 mm microfilm of specimen 79/9, clearly reveals, however, that four separate plants are actually represented on this sheet – three fertile culms and a large vegetative specimen folded so as to enfold the 'third specimen from the left'. The above authors have apparently chosen to ignore this sterile specimen, without mentioning this fact, and thus, strictly speaking, the holotype – the third specimen from the left – is in fact this sterile specimen. This imprecision is most unscientific and confusing for anyone attempting to logically unravel this typification problem. Fosberg (1977) mentions, for instance, that, of the three specimens, the third from the left is the type, but, in a subsequent paragraph, refers to the center specimen as the type! He is possibly referring to its central location on the sheet but, nevertheless, these inaccuracies cannot be condoned. In selecting a holotype it has been assumed, therefore, that the above authors were actually referring to the specimen bearing the third *inflorescence* from the left. This definition thus selects a definite specimen, with precise spatial location on the type sheet, as the holotype.

Because these two species are so similar morphologically and because of this typification difficulty there has long been confusion over their true identity. This has led to the name *P. distichum* being persistently misapplied to specimens with appressed pubescent upper glumes as in Chippendall (1955) and Bor (1960) for instance. This concept unquestionably represents the same species as the two unmarked fertile specimens on the Linnean type sheet (Guedes, 1976) and Bor (1968) contends that these specimens actually should be called *P. paspalodes*

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(Michx.) Scribn. This has been done in the present study (Ellis, 1974a). Specimens that match the holotype of *P. distichum*, and have glabrous upper glumes, are generally called *P. vaginatum* Sw. as in the present study.

Subsequent to the unearthing of this typification error (Bor, 1968) some authors have begun to use *P. distichum* in the true sense and have placed *P. vaginatum* in synonomy under *P. distichum* (Launert, 1970; Simon, 1968). This, according to Fosberg (1977), is the correct procedure following the International Code's guide for determination of types (Stafleu *et al*, 1972) where the choice made by the original author is regarded as final. In Fosberg's (1977) opinion, and strictly according to nomenclatural rules, the Code leaves no alternative but to accept the specimen marked 'Br' as the type and to call the plant so indicated as *P. distichum* L. This Vickery (1975) has felt obliged to do in a supplement to the Flora of New South Wales where the name *P. distichum* has been substituted for the species previously regarded as *P. vaginatum* and another name has been found for the plants originally called *P. distichum*; namely *P. paspalodes*. As we shall see, this correction, although strictly following the Code of 1972, is not in agreement with general opinion and will probably require yet further adjustment if the alternative proposal, outlined below, prevails.

Another possible solution for this confusing issue is to reject the name *P. distichum* as a *nomen confusum* under Article 69 of the International Code (Stafleu *et al*, 1972) as the name has been used in different senses. *P. distichum* has constantly been misapplied and has become a persistant source of error (Clayton, 1972; Loxton, 1974; de Winter & Vorster, 1974). For the acceptance of this proposal a new name is required for the species erroneously regarded as *P. distichum* by most authors. The earliest available name is *P. paspalodes* (Bor, 1968) which has consequently been adopted by the above authors. In addition, and by implication, true *P. distichum* also needs a new name for which the generally used, but mistakenly applied *P. vaginatum* has proved to be the earliest alternative valid name. These were the names adopted in this study (Ellis, 1974a), and which agreed with expert opinion at that time.

Before this proposal to reject *P. distichum* as a *nomen confusum* had gained general acceptance the Leningrad Code of 1975 was published (Stafleu *et al*, 1978). New procedures for rejecting names under Article 69 were laid down and the Article itself was altered so that a name must be rejected if it has been widely and persistently used for a taxon not including its type. As this is precisely the situation with *P. distichum*, a formal proposal for the rejection of the name has been published (Renvoize & Clayton, 1980) as required by the new Article 69. This proposal will be submitted to the General Nomenclature Committee for referral to the relevant committee for the Spermatophyta. A final and binding vote will only be taken at the Nomenclature Section of the International Botanical Congress in Sydney in 1981. Only then will a final decision regarding this proposal to reject the name *P. distichum* be forthcoming.

Guédès (1976) has submitted an alternative case for the retention of the name *P. distichum* in the old sense for the species recognised as *P. paspalodes* in this study. Without actually seeing the type but only from examination of photographs (on which the 'Br' is invisible) Guédès selects as a type of *P. distichum* the second specimen from the left. This proposal is extremely practical as it has the advantage of maintaining nomenclatural stability and does away with the need for any name changes. *P. distichum* would continue to be applied to specimens with pubescent glumes and *P. vaginatum* for glabrous specimens as in Chippendall (1955), Bor (1960) and Gordon-Gray (1972).

Unfortunately the arguments of Jovet & Guédès (1972) for the retention of the name *P. distichum* are based on incomplete evidence and must, therefore, be disregarded. Neither the annotation 'Br', nor the hairs on the upper glume of the two left hand specimens are visible on the IDC photographs of the type and, consequently, these authors base their argument on the shape of the inflorescence and spikelets. In rejecting the third specimen from the left as the type of *P. distichum*, Guédès (1976) argued that it apparently only had a single spike and, as a result, does not really agree with Linnaeus' description and that Linnaeus probably had all three specimens in front of him, when writing his description. Examination of the actual type specimen reveals that there are two racemes (Renvoize & Clayton, 1980) and, therefore, Guédès choice of a lectotype is rejected.

On consideration of all the evidence it does appear as if the proposal to reject *P. distichum* as a *nomen confusum* is the most agreeable compromise possible in this particular ambiguous nomenclatural problem. However, there still appears to be one obvious weakness in this argument – the question of the inscription 'Br' on the type specimen. If Linnaeus actually wrote these letters then there is little doubt as to what specimen is the type and rejection can follow as a mere formality. However, if the fact that Linnaeus

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wrote the 'Br' is queried then a simple solution on the grounds of rejection may not be possible. Strangely no attempt has been made to qualify the contention that Linnaeus was responsible for the inscription and all authors avoid the issue except Fosberg (1977) who specifically states that the sheet was marked 'Br' by Linnaeus. As Savage (1945) makes no mention of this very significant inscription, it would appear as if the onus lies with proposers of nomenclatural change to obtain additional handwriting and other evidence to support their contention that Linnaeus actually designated the type specimen on the sheet. If this is not done the possibility will always exist that the 'Br' was added after Linnaeus, or even after 1945 for that matter. This possibility would have profound implications for the proposal to reject the name *P. distichum* and thus warrants further qualification.

This digression into the field of nomenclature, although not strictly relevant to anatomical studies as such, illustrates very clearly the need for mutual understanding between experts in the various disciplines of botany. No single aspect of botany can stand alone in isolation and all are interdependant upon one another. Comparative anatomy, in particular, is almost entirely reliant on taxonomic decisions, taken by morphologists and other co-operating workers, to serve as a basis for comparison with anatomical criteria. A mutual understanding of techniques, methods and approaches is, therefore, essential.

6.4.3 The Danthonieae.

The implications of the anatomical studies on the leaves of selected examples from this tribe, have been discussed fully in Chapter 4. It will be superfluous to repeat these discussions here and the reader is referred to the relevant sections of Chapter 4 (Pages 107–137).

6.4.3.1 *The genus Dregeochloa.*

This genus, is easily distinguished on anatomical criteria and numerous characters are common to the two species comprising the genus (Ellis, 1977; Pages 107–111). Anatomical observations confirm the decision to remove these two species from *Danthonia* and to place them in a distinct genus (Conert, 1966). This stresses the value of anatomical attributes at the generic level and emphasizes the importance of the current project to the systematics of the South African grasses.

6.4.3.2 *The summer rainfall Merxmuellera species.*

The leaf anatomy of this complex of eight species has proved extremely helpful in obtaining a better understanding of relationships within this group. The anatomy of each of the species included has been individually described (Ellis, 1980, 1980a, 1981, 1981a; Pages 112–137) and considerable intraspecific variation was noted which could be related to the ecological niches occupied by each of the anatomical forms recognized. Indications, based on the results of this study, are that only two of the eight species should be maintained and 12 sub-species recognized within these two species. Anatomy can,therefore,contribute much even at intraspecific levels and ecotypic variation is readily manifested in the anatomical structure of the leaves.

Leaf anatomy in the grass family is relevant from the subfamily level to subspecies and varieties. Results fortcoming from this study should contribute at all levels to an improvement of the taxonomy of the South African grasses.

6.5 Anatomy and functional studies.

Anatomy has a considerable contribution to make towards a better appreciation of the physiological functioning, particularly of photosynthesis, of the grass plant and consequently of ecological requirements and distribution patterns. This has been described in detail in Chapter 5 (Ellis, 1977a, 1974b; Ellis *et al.*, 1980, Vogel *et al.*, 1978) and the implications of anatomical criteria in this type of research are undoubtedly of considerable importance. The taxonomic significance of the photosynthetic differences in grasses is not yet fully appreciated and has been further elaborated on in Chapter 6 (Pages 218–221).

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7. FINAL CONCLUSIONS

Synopsis: The significance of the scientific contribution made by this study will be accentuated in this resumé of the major conclusions forthcoming to date. These highlights will emphasize the taxonomic and functional importance of this project to agrostological studies. The contribution made to date, and discussed fully in the preceding chapters, will enable an evaluation of the future potential of this study to be made and should justify the continuation, and even expansion, of this project. Although basically a simple, fundamental approach has been adopted in the project design and approach, microscopic preparations of a high standard have been produced resulting in the conclusions being relevant and pertinent to biological disciplines other than pure comparative anatomy. This study has consequently made important contributions to agronomic and pasture management practices over and above the taxonomic input it was planned to contribute.

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7. FINAL CONCLUSIONS

(1) The routine fixing of fresh grass leaf-blade material in the field under natural conditions has proved to be a significant advancement over previous comparative anatomical studies. This approach has enabled transverse sections of much better quality and resolution to be attained and, coupled with microtechnique procedures designed to elucidate chloroplast structure and distribution, has been particularly valuable in documenting details of the Kranz syndrome in the South African grasses. In addition, the large, representative collection of leaf transverse sections built up during the course of this study appears to be the most comprehensive in the world and will undoubtedly contribute considerably to our understanding and appreciation of the relevance of attributes of the leaf-blade in section to grass systematics.

(2) The collection of authenticated herbarium voucher material has provided a sound taxonomic basis for comparison with the anatomical indications and will assist considerably in arriving at a more natural classification of the South African grasses. The collection of replicate material from different climatic areas has enabled the anatomical variation inherent in any given taxon to be monitored and described as well as the structural effects of environmental differences to be ascertained. This is the first study in which geographically representative samples of the individual taxa have been studied anatomically and, as a result, the taxonomic significance of anatomical characters from infraspecific to sub-family level have been determined as has the functional and ecological implications of these structures.

(3) The use of photomicrography to illustrate anatomical structure is a new innovation in comparative anatomical studies of the grasses that has been implemented in this study. The results fully justify this approach as the quality of the photomicrographs is far superior to other published works and, in addition, accurately and objectively portray the actual condition pertaining in the plants themselves. A further benefit of this approach is that the anatomical results are made available to agrostologists for easy and meaningful comparison with the herbarium vouhcer material, both now and in the future, and should assist considerably in arriving at more balanced taxonomic decisions.

(4) The standardization of the terminology used to describe the grass leaf-blade as seen in transverse section and of the epidermis in surface view fulfilled a very real shortcoming in comparative studies of the grasses. The definition of the characters and their organization and arrangement into logical sequences has facilitated the rapid and consistent description of the leaf anatomy irrespective of subjective considerations. In addition the design and format of the 'keys' allows easy conversion to modern methods of data storage and processing – either by computer or edge-punched cards.

(5) The observation that two consistently and distinctly different anatomical forms exist within the species *Alloteropsis semialata*, is undoubtedly the single most important contribution of this study to date. The detailed anatomical study of this species, incorporating its world-wide distribution range, has verified the original finding as no gradation or intermediates have been found in the large sample studied. The unique situation described in *A. semialata* is tremendously important as regards the taxonomic significance of the Kranz syndrome and its phylogenetic implications. Cytogenetical studies of the two forms of this species are urgently required and should be accorded a high priority. This should assist in determining the actual relationships of the C_3 and C_4 forms of *A. semialata* and should provide a better understanding of the other grass genera that are heterogeneous for this character.

(6) The anatomical studies of the selected group of summer-rainfall *Merxmuellera* species have clearly demonstrated that taxonomic adjustment is required to ensure a more meaningful and natural classification of these taxa. These studies have also contributed to a clearer picture of the relationships of these species as well as their evolutionary history. Anatomical studies, such as this, can contribute substantially to an improved classification of the Poaceae with significant benefits to basic and applied biological science.

(7) The studies of the *Merxmuellera* species have also clearly demonstrated the adaptive radiation that has occurred in this mountainous region. The various niches occupied by the representatives of this genus are all occupied by distinct anatomical forms and possible instances of convergence are described. This well-documented example can obviously serve as a basis for a better appreciation of the relationships of closely related grass species in many other areas where a high incidence of speciation has occurred.

(8) The design and scope of this project has enabled a detailed taxonomic survey of the Kranz syndrome in the South African Poaceae to be under-

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taken. This has put South Africa in the forefront of recent advances in photosynthetic research and the anomalies detected during this study have created opportunities for meaningful physiological studies. Thus the report of the exceptional situation observed in *Alloteropsis semialata* in particular, has enabled local physiologists to gain a better understanding of the biochemistry of the C_4 photosynthetic pathway.

(9) The basic anatomical data assembled in this study has made possible a survey of the geographical distribution patterns of grasses with differing photosynthetic pathways. The results have shown that basic comparative anatomical research can play a key role in applied biological research. This survey has also clearly illustrated the interaction between environmental factors and function and structure in the grass family.

(10) The correlation of anatomical structure with the three recently described subtypes of the C_4 photosynthetic pathway has been a very important, original contribution resulting from this study. This information has further enhanced the understanding of the ecological requirements of C_4 grasses and should aid considerably in a better appreciation of the functioning of the Kranz syndrome.

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SUMMARY

RELEVANCE OF COMPARATIVE LEAF BLADE ANATOMY IN TAXONOMIC AND FUNCTIONAL RESEARCH ON THE SOUTH AFRICAN POACEAE

by

ROGER PEARSON ELLIS

Promoter: Prof. Dr. P.J. Robbertse
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The anatomy of the grass leaf blade, viewed both in transverse section and in surface view, has been extensively studied, both locally and internationally, and is generally considered to be of exceptional taxonomic importance in the Poaceae. Leaf anatomy thus provides a wealth of diagnostic characters for classification or identification purposes and a comparative anatomical study of grass leaves is consequently relevant in grass systematics as well as in applied fields such as pedology, palaeobotany, forensic botany and food preference studies of grazing herbivores. More recently the relevance of leaf anatomy in functional studies of the Poaceae has become evident, particularly with regard to the differing photosynthetic pathways operational in this family. For these reasons the prime objective of this study has been to gather basic, standardized and comparative leaf anatomical data on the grasses occurring in South Africa for synthesis in systematic and functional research.

The microtechnique procedures standardized upon for the preparation of transverse sections and epidermal scrapes are given in detail together with a brief review of alternative methods for epidermal specimen preparation. This review is included to enable techniques better suited to the applied aspects of epidermal identification to be utilized where necessary because the overriding factor in deciding to standardize upon the scrape method in this study was its suitability in comparative studies and to enable comparison with published works on this subject.

A detailed review of the anatomical characteristics of the leaves of grasses is given in order to standardize the terminology. Definitions and diagrammatic illustrations are included together with brief discussions of the variation and taxonomic significance of these attributes. A hierarchical 'pseudo-key' format has been used to facilitate the compilation of anatomical descriptions and to capitalize on modern methods of data handling such as information-retrieval and key-generating techniques.

Selected examples of the application of these anatomical criteria to taxonomic problems are given. The leaf anatomy of *Alloteropsis semialata, Paspalum paspalodes* and *P. vaginatum*, the genus *Dregeochloa* and the summer-rainfall *Merxmuellera* species is described in detail and the taxonomic indications discussed. This clearly demonstrates that comparative anatomy has a significant role to play in the classification of the Poaceae – from infraspecific to generic and even tribal levels. In addition, the results of ecotypic variation are clearly demonstrated and serve as a caution in suggesting taxonomic changes based on observations from insufficiently representative samples.

The anatomical data assembled during the course of this study was also used for a survey of the Kranz syndrome in the Eragrostoideae and Panicoideae subfamilies. In addition to the anatomical characteristics associated with the C_3 and C_4 photosynthetic pathways it was conclusively demonstrated that certain anatomical differences are also correlated with the sub-divisions of the C_4 pathway currently recognized on biochemical evidence. Thus malate and aspartate formers can be distinguished either by the mestome or the parenchyma sheath being Kranz and the aspartate formers are further divisible into the NAD-me and the PEP-ck sub-types on the basis of Kranz chloroplast positioning, Kranz cell shape and mesophyll arrangement. With this knowledge of the photosynthetic status of all the grasses occurring in South Africa it proved possible to examine the geographical distribution of these pathways in the grass flora of this region as well as South West Africa. This revealed interesting relationships between the occurrence of these pathways and climate and contributed significantly to an understanding of the adaptive significance of these important photosynthetic differences.

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OPSOMMING

TOEPASLIKHEID VAN VERGELYKENDE BLAARANATOMIE IN TAKSONOMIESE EN FUNKSIONELE NAVORSING OOR DIE SUID-AFRIKAANSE POACEAE

deur

ROGER PEARSON ELLIS

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Die anatomie van die grasblaarlamina, gebaseer op dwarsneë en oppervlakstruktuur van die epidermis, is reeds plaaslik sowel as internasionaal deeglik bestudeer en word as van besondere taksonomiese waarde in die Poaceae beskou. Blaaranatomie voorsien dus 'n groot aantal diagnostiese kenmerke vir gebruik in die identifisering en klassifisering van grasse en het gevolglik 'n praktiese toepassing in grastaksonomie, sowel as in ander toegepaste rigtings soos bodemkunde, fossiele plantkunde, geregtelike plantkunde en voedselvoorkeurstudies by weidende herbivore. Onlangs het die toepaslikheid van blaaranatomie ook in funksionele studies van die Poaceae duidelik na vore gekom veral met betrekking tot verskillende fotosintetiese weë wat by die familie voorkom. Om genoemde redes is die hoofdoel van hierdie ondersoek die inwin van gestandaardiseerde en vergelykbare basiese gegewens oor die blaaranatomie van die Suid-Afrikaanse grasse met die oog op die samevoeging van hierdie kenmerke in taksonomiese en funksionele navorsing.

Die gestandaardiseerde mikrotegniek-metodes vir die voorbereiding van dwarsneë en epidermale skrapings, word breedvoerig uiteengesit saam met 'n kort oorsig van alternatiewe metodes vir die voorbereiding van epidermis-preparate. Hierdie oorsig word ingesluit om lesers in staat te stel om tegnieke wat beter geskik is vir toegepaste aspekte van identifisering van epidermisse te gebruik waar dit benodig word. Die oorheersende faktor in die besluit om op die skraapmetode in hierdie studie te standaardiseer was die geskiktheid daarvan vir vergelykende studies en om vergelyking met reeds gepubliseerde navorsing oor hierdie onderwerp te kon tref.

'n Breedvoerige samevatting van die anatomiese kenmerke van grasblare word gegee om die terminologie te standaardiseer. Definisies en skematiese illustrasies word ingesluit saam met bondige besprekings van die variasie en taksonomiese waarde van hierdie kenmerke. 'n Hiërargiese stelsel in 'pseudosleutelformaat' is gebruik om die samestelling van anatomiese beskrywings te bespoedig en om op moderne metodes van datahantering soos inligtingonttrekking en sleutelontwikkeling te kapitaliseer.

Uitgesoekte voorbeelde van toepassings van hierdie anatomiese kenmerke op taksonomiese probleme word gegee. Die blaaranatomie van *Alloteropsis semialata, Paspalum paspalodes* en *P. vaginatum*, die genus *Dregeochloa* en die somerreënval *Merxmuellera* spesies word breedvoerig beskryf en die taksonomiese neigings word bespreek. Hierdie beskrywings toon duidelik aan dat vergelykende anatomie 'n betekenisvolle rol in die klassifisering van die Poaceae kan speel – van infraspesifiek tot generiese en selfs tribus vlak. Boonop word die resultate van ekotipiese variasie duidelik aangedui en dien as 'n waarskuwing om nie taksonomiese veranderinge voor te stel wat gebaseer is op waarnemings van onvoldoende verteenwoordigende monsters nie.

Die anatomiese inligting wat bymekaar gemaak is gedurende die loop van hierdie projek is ook gebruik vir 'n opname van die Kranssindroom in die Eragrostoideae en Panicoideae subfamilies. Bykomend tot die anatomiese kenmerke vereenselwig met die C_3 en C_4 fotosintetiese weë was dit oortuigend bewys dat sekere anatomiese verskille ook gekorreleer kan word met die onderverdelings van die C_4-tipe wat tans op grond van biochemiese gegewens erken word. Die malaat- en aspartaatvormers kan herken word weens die feit dat óf die schlerenchimatiese óf die parenchimatiese bondelskede van die Krans-tipe is. Die aspartaatvormers is ook verder onderverdeelbaar in die NAD-me en die PEP-ck subtipes op grond van die posisie van die Krans chloroplaste, Krans selvorm en mesofil rangskikking. Met hierdie kennis van die fotosintetiese status van al die grasse wat in Suid-Afrika voorkom was dit moontlik om die geografiese verspreiding van hierdie fotosintetiese bane in die grasflora van hierdie gebied, asook Suidwes-Afrika, te ondersoek. Hierdie ondersoek het interessante verwantskappe tussen die voorkoms van die verkillende fotosintetiese tipes en die klimaat getoon en het 'n betekenisvolle bydrae gelewer tot die kennis van die aanpassingsbelang van hierdie belangrike fotosintetiese verskille.

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CURRICULUM VITAE

Roger Pearson Ellis was born on the 19th December, 1944 in Johannesburg. He attended the Port Shepstone High School in Natal and passed the Natal Senior Certificate Examination in 1962. He attended the University of Natal in Pietermaritzburg from 1963–1969 and was awarded a B.Sc. degree with Botany and Zoology as major subjects in 1967, B.Sc. (Hons) in Botany in 1968 and an M.Sc. in 1970. This degree was awarded on submission of a thesis entitled 'Anatomy of *in vitro* grown *Nicotiana tabacum* cultures'.

In 1968 he was appointed as Professional Officer at the Botanical Research Institute (Department of Agricultural Technical Services) in Pretoria. He still holds this post and was promoted to Chief Professional Officer in 1977 and heads the Anatomy-Cytogenetics Section of this Institute. During this period of service he has been engaged in systematic anatomical research and has concentrated on the leaf anatomy of the South African grasses – a subject on which he has published several papers.

Mr Ellis is a member of several scientific societies such as the South African Association of Botanists, the Grassland Society of Southern Africa, the Southern African Weed Science Society and the Southern African Wildlife Management Association. He has been an active member of these societies, presenting papers at congresses and serving on the committee of the Northern Transvaal branch of the South African Association of Botanists. In 1979 he was elected chairman of this committee.

The following is a list of published papers:

(1) Ellis, R.P. & Bornman, C.H., 1971. Anatomical aspects of growth proliferation in *Nicotiana tabacum* tissue cultured *in vitro*. *Jl S. Afr. Bot.* 37: 109–126.

(2) Ellis, R.P. & Bornman, C.H., 1971. Aspects of cellular growth and development in *Nicotiana tabacum* tissue cultured *in vitro*. *Jl S. Afr. Bot.* 37: 269–280.

(3) Bornman, C.H. & Ellis, R.P., 1971. Differentiation of pseudothallial tracheary elements in *Nicotiana tabacum* tissue cultured *in vitro*. *Jl S. Afr. Bot.* 37: 281–290.

(4) Ellis, R.P., 1974. Comparative leaf anatomy of *Paspalum paspalodes* and *P. vaginatum*. *Bothalia* 11: 235–241.

(5) Ellis, R.P., 1974. Anomalous vascular bundle sheath structure in *Alloteropsis semialata* leaf blades. *Bothalia* 11: 273–275.

(6) Ellis, R.P., 1974. The significance of the occurrence of both Kranz and non-Kranz leaf anatomy in the grass species *Alloteropsis semialata*. *S. Afr. J. Sci.* 70: 169–173.

(7) Ellis, R.P., 1974. non-Kranz (C_3) leaf anatomy in the panicoid grasses: additional records from South Africa. *Bothalia* 11: 286–287.

(8) Ellis, R.P., 1974. *Smodingium* dermatitis: the intercellular secretory canals of the aerial axis and their relationship to this toxicity. *Bothalia* 11: 259–262.

(9) Findlay, G.H., Whiting, D.A., Eggers, S.H. & Ellis, R.P., 1974. *Smodingium* (African 'poison ivy') dermatitis. *Brit. J. Dermatology* 99: 535–541.

(10) Gaff, D.F. & Ellis, R.P., 1974. Southern African grasses with foliage that revives after dehydration. *Bothalia* 11: 305–308.

(11) Ellis, R.P., 1976. A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia* 12: 65–109.

(12) Ellis, R.P., 1977. Leaf anatomy of the South African Danthonieae (Poaceae). I. The genus *Dregeochloa*. *Bothalia* 12: 209–213.

(13) Ellis, R.P., 1977. Distribution of the Kranz syndrome in the Southern African Eragrostoideae and Panicoideae according to bundle sheath anatomy and cytology. *Agroplantae* 9: 73–110.

(14) Wilson, D.E., Hirst, S.M. & Ellis, R.P., 1977. Determination of feeding preferences in wild ruminants from trocar samples. *J. Wildl. Mgmt.* 41: 70–77.

(15) Vogel, J.C., Fuls, A. & Ellis, R.P., 1978. The geographical distribution of Kranz grasses in South Africa. *S. Afr. J. Sci.* 74: 209–215.

(16) Ellis, R.P., 1979. A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. *Bothalia* 12: 641–671.

(17) Ellis, R.P., Manders, R. & Obermeyer, A.A., 1979. Anatomical observations on the peduncle of *Xyris capensis*. *Bothalia* 12: 637–639.

(18) Ellis, R.P., 1980. Leaf anatomy of the South African Danthonieae (Poaceae). II. *Merxmuellera disticha*. *Bothalia* 13: 185–189.

(19) Ellis, R.P., 1980. Leaf anatomy of the South African Danthonieae (Poaceae). III. *Merxmuellera stricta*. *Bothalia* 13: 191–198.

(20) Ellis, R.P., Vogel, J.C. & Fuls, A., 1980. Photosynthetic pathways and the geographical distribution of grasses in South West Africa/Namibia. *S. Afr. J. Sci.* 76: 307–314.

(21) Ellis, R.P., 1981. Leaf anatomy of the South African Danthonieae (Poaceae). IV. *Merxmuellera*

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drakensbergensis and *M. stereophylla. Bothalia* 13: 487–491.

(22) Ellis, R.P., 1981. Leaf anatomy of the South African Danthonieae (Poaceae). V. *Merxmuellera macowanii, M. davyi* and *M. aureocephala. Bothalia* 13: 493–500.

(23) Werger, M.J.A. & Ellis, R.P., 1981. Distribution of photosynthetic pathways in the arid parts of South Africa. *Flora* 171: 64–75.

(24) Ellis, R.P., 1982. Leaf anatomy of the South African Danthonieae (Poaceae). VI. *Merxmuellera arundinacea* and *M. cincta. Bothalia* 14: in press.

(25) Ellis, R.P., 1982. Leaf anatomy of the South African Danthonieae (Poaceae). VII. *Merxmuellera dura* and *M. rangei. Bothalia* 14: in press.

(26) Ellis, R.P. & Soderstrom, T.R., 1982. *Dichaetaria wightii*, a first record of a C_3 species in the Chloridoideae (Poaceae). *Bothalia* 14: in press.

(27) Soderstrom, T.R. & Ellis, R.P., 1982. Taxonomic status of the endemic South African bamboo, *Arundinaria tessellata. Bothalia* 14: in press.

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