The Relations between the Amount of Carbohydrates in the Leaves of Armoedsvlakte Grasses and the Meteorological Factors.

By MARGUERITE HENRICI, Ph.D., Plant Physiologist, Veterinary Research Laboratory, Vryburg.

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PART I.

Within the last few years several authors (Molisch, 1921; Horn, 1922; Ahrns, 1924; Iljin, 1923) have pointed out that starch disappears from the leaves during wilting without the appearance of a corresponding amount of simple sugars. Experiments on cut leaves were carried out under constant laboratory conditions. Iljin (1923.2) tries to explain this discrepancy. In many, but not all of the investigated plants, he succeeded in proving that respiration is considerably increased during wilting, and in this manner a large amount of sugar disappears, also that the assimilation, i.e. the formation of starch, decreases at the same time. (Iljin 1923.1). Iljin, therefore, concludes that during the drying out of the plant the part of the amylase which hydrolyses the starch is not harmed at all, but that the synthetically active portion of the enzyme is destroyed.

It was found advisable to carry out these investigations under natural conditions and to determine in how far the results obtained in the laboratory agreed with those obtained in the veld. The conditions under which the experiments were carried out were, of course, different. Whole plants which had been exposed to changing meteorological conditions were taken from the veld and examined; whereas in Iljin's investigations single leaves were studied for a few hours only under constant laboratory conditions. Armoedsvlakte (Vryburg, Cape Province) proved an ideal place for this investigation, as, with the exception of the rainy season, wilting, or at least incipient drying, occurs daily. The moisture factor is, therefore, of paramount importance, and only in a few instances do light and temperature act as deciding factors.

No attempt was made to isolate every sugar, but merely to obtain by a simple method a general idea of the total amount of monosaccharides, disaccharides, starch, and the higher carbohydrates readily soluble in dilute acids. This separation appears to be sufficient for all practical purposes. The three first-mentioned carbohydrates were taken as direct assimilation products, whereas the carbohydrates soluble in dilute acid were determined separately with a view to the possible influence of the moisture-content, as it was thought that hemicellulose might be formed instead of cellulose during wilting.

I.—Method.

The method for the determination of reducing bodies was the "Citron" modification of the Fehling method, as described below.

For the determination of the water-soluble reducing bodies, chiefly glucose, ten grams of ground grass dried at 104° C. were usually taken. The grass was allowed to infuse with 200 c.c. of distilled water for about an hour with occasional stirring. After filtration and washing, the liquid was made up to 250 c.c. Reducing bodies were determined in this extract by indirect Fehling titration (see below). 100 c.c. of this solution were also boiled for one hour on a water bath with 10 c.c. of concentrated hydrochloric acid, cooled and neutralized with potassium hydrate using litmus as indicator. Generally a dark brown precipitate appeared, and this liquid was filtered, made up to a convenient amount (sol. 2), and titrated. This solution contained bodies soluble in water which reduce Fehling's solution after hydrolysis, chiefly disaccharides. The amount of glucose found in the first determination was of course deducted, both solutions being calculated on the same concentration. For the determination of the starch fresh grass had to be used, as it appears that in dried grass leaves the starch becomes horny and not hydrolysable by amylase. Five grams of fresh grass was generally used for the determination; 10 grams if very little starch was expected. The leaves were finely cut up with scissors, ground with sand, a little chalk, and about 20 c.c. of 10 per cent. alcohol, further extracted with more 10 per cent. alcohol, then with stronger alcohol, with ether, and finally with water. The residue was boiled with water for at least an hour on the water bath, after which time the infusion was cooled to 55° C., 0.2 grains diastase introduced, and the temperature kept at 55° C. for about an hour. It was then boiled up again, and examined with iodine under the microscope for starch. If starch was still present, the process was repeated until the microscopic examination was negative, as much as 0.4 grams of diastase being used if the starch was abundant. If less than 4 per cent. of starch was present, .2 gram of diastase was sufficient. After boiling, the infusion was filtered, the filtrate was made up to 250 c.c., 10 c.c. concentrated hydrochloric acid added and boiled in a Jena flask under a reflux condenser on a water bath for 2½ hours. After cooling, it was neutralized, filtered, and made up to a convenient volume, usually 500 c.c. This solution was determined with Fehling's solution as described below. The diastase used in these determinations itself contained reducing bodies, the amount of which had to be deducted from the result of the titration.

The residue after removal of the starch was rinsed into a Jena flask with 250 c.c. of water and 10 c.c. of concentrated hydrochloric acid, and boiled for 3 hours on a water bath under a reflux cooler. It was then neutralized and the reducing bodies present again determined. A certain proportion of the diastase used was soluble in acid, and was of a reducing nature, this amount, found to be 7.5 per cent., had to be deducted from the result of the titration.

In several preliminary experiments the solutions were cleared with lead acetate, but, in spite of careful precipitation of the lead, concordant results were not obtained, which indicates that there is some element in the grass leaves which inhibits the complete precipitation of the lead. The clarification was therefore omitted in all subsequent determinations.

The Titration: An approximately N/10 solution of sodium thiosulphate was made up and standardized with sublimed iodine or N/10 potassium bichromate (see Treadwell, 2, pages 544 and 547).

The Fehling's solution was Allihns modification, and consisted of two stock solutions, which were mixed in equal quantities for use. Solution A consisted of 34.639 grams of crystallized copper sulphate in half a litre of water, and solution B of 173 grams of Rochelle salts and 125 grams of potassium hydroxide in half a litre of water.

For the blank determination 20 c.c. of Fehling's solution were boiled with 25 c.c. of water for two minutes, the solution cooled under a tap, 20 c.c of 20 per cent. potassium iodide and 20 c.c. of 20 per cent. sulphuric acid added and immediately titrated to a colourless end-point, a few drops of starch solution being added as an indicator towards the end of the titration. 20 c.c. of Fehling's solution should require 26 to 30 c.c. N/10 thiosulphate.

For the determination of the sugar in the various solutions 25 c.c. of the extract was added to the boiling Fehling's solution, boiled for two minutes, and titrated as for the blank determination. The difference between the two results gave the reducing bodies as determined by Table 12.

II.—Results.

In consideration of the fact that total analyses of the grass material have not yet been made, this first report of the investigation is restricted to the direct products of assimilation.

A.—*Direct Products of Assimilation.*

The results are shown in the curves 1-10 and the tables 1-6. To aid in the interpretation of these curves, the rainfall and soil moisture during the years of these determinations are shown in curves 11 and 12. In curves 1-5 and in tables 1-5 the amount of glucose found in the starch determination is given, and in curves 6-10 and table 6 this value is multiplied by 0.9 to get the correct amount of starch.

The curves differ to some extent in shape, so that they cannot all be explained at the same time. They have, however, some points in common.

One special point must be emphasized. No grass shows wilting for a definite percentage of dry matter, and although wilting is often correlated with a high percentage of dry matter, there are so many exceptions in both directions that no general rule can be formulated. In young plants wilting often occurs at a very low percentage of dry matter, but, on the other hand, plants with a high percentage of dry matter are often not withered, although in this case they may be in the condition of incipient drying. According to results obtained by other authors, it might be surmised, *a priori*, that with an increasing percentage of dry matter the amount of direct-assimilation products would decrease, or at least that the starch would be hydrolysed and only sugars remain. From table 6 it can be seen that this view is only true to a limited extent, as high percentage of dry matter is not correlated with wilting.

In curve 7, *Eragrostis superba*, a uniform variation of the two factors, dry matter and assimilates, is shown except at the time when the plant was dying down. It is peculiar that before December the rise and fall of the curve of assimilation products is much steeper, but after this time gentler than the dry-matter curve. This point must be emphasized, as December is a critical period for all the grasses.

The curves of Digitaria (10), Themeda (6), and Chrysopogon (8) give a general impression of an inverse relation of dry matter and assimilates, especially during the period from August to December. The assimilates of *Aristida uniplumis* show no relation to the percentage of dry matter, which is very striking in consideration of the fact that *Aristida* scarcely alters in the percentage of its dry matter except in the spring. (Curve 9.)

Although there is often no relation between the amount of dry matter and the amount of assimilates, this does not mean that the latter is independent of the water-content and the condition (wilted or fresh) of the grass. This can be seen clearly in the curves 1-3 and 5 and tables 1-3 and 5.

Before the details of the curves and tables can be studied, the yearly curves of the assimilates must be characterized. A comparison of curves 1-5 shows that the different grasses differ somewhat, although they show some common points. There is no doubt that young leaves, but not the youngest, form the largest amounts of assimilation products. In two of the grasses, *Aristida uniplumis* and *Eragrostis superba*, the maximum amount of assimilates is produced before the rainy season.

In most grasses values for starch are rarely as high after New Year as before. The power of forming large amounts of starch is therefore not simply a characteristic of young leaves, for in the rainy season after New Year there is as much young grass as during the November rains, but the amount of starch present is usually insignificant in quantity. The young leaves are capable of forming a large amount of starch only at the one season. It has to be considered whether the smaller assimilation power in the second rainy period is real or only apparent. It does not seem impossible that the haulm formation which takes place in this period for *Digitaria*, *Chrysopogon*, and *Themeda* influences the amount of direct assimilates found in the leaves. *Eragrostis superba*, which produces haulms the whole year round, does not show a decrease of the assimilates in the second rainy period. It is likely that the sugars are formed in the leaves but immediately translocated to the root-crown, where they are needed for the haulm formation. As for *Eragrostis*, this process takes place the whole year round, a decrease of assimilates at any special time is not shown, whereas it occurs in the other grasses. Although this view is very likely, it does not abolish the fact of the pernicious influence of repeated wilting.

In comparing the amounts of carbohydrates before and after New Year, the possibility of temperature being an influencing factor must be considered. Temperatures in Spring are lower than after New Year, but on very hot days in November and December especially high values were found for *Digitaria*, so that high temperatures cannot entirely be responsible for the change after New Year. Differences in illumination cannot play a part, as the critical point falls in the time of strongest illumination.

It does not appear to be possible to explain the critical point as dependent entirely upon external factors, and the explanation must be sought in the internal conditions of the plant iself. It must be remembered that at this time the first continuous wilting occurs, although previously in September and October the grass wilted occasionally but never for long and only on odd days. The wilting also was not as pronounced as in midsummer, chiefly because the temperature was not so high and the light less strong, and therefore did not affect the metabolism much. When, however, the wilting continues day after day and lasts most of the time whilst the sun shines, then the metabolism is disturbed and probably is never completely restored.

This deduction is drawn from meteorological conditions of 1924 and 1925, not of 1923 and 1924, when the wilting in September was as bad as later on in the season. The results of the few determinations of that year, therefore, present slight differences, which will be discussed later.

Towards the end of the season before the grass leaves died down they contained but few direct assimilation products. In Europe it is a well known fact that with the cold, starch disappears from the leaves (Czapek, 1901, and Lidforss, 1907). It is therefore not necessary to insist on this point in South Africa.

After these general remarks the separate curves and tables may be interpreted.

Themeda triandra (table 1 and curve 1): Table 1 leaves no doubt that with decreasing water-content of the leaves and the beginning of wilting the carbohydrate-content, especially that of starch, decreases. During the drought period of January, 1924, no starch was found, whereas in the rainy season of November, 1923, the amount of assimilation products was very high. In the following February there was practically no rain, but there was less evaporation, as the sky was often overcast, and some starch was found. In March heavy rains fell, the assimilation rose, and the starch-content increased considerably, never, however, reaching the November value in spite of an abundance of new leaves.

It is striking that no increase of carbohydrates occurred immediately after the rain (determination 16th March). This was also apparent in other grasses. In the case of this determination there were two reasons. On the day the grass was gathered the sky was overcast and the light therefore too weak to allow good assimilation, but more important still is the fact that the grasses had been exposed for some time to drought conditions and needed some time to recover their assimilation apparatus, a much longer period than that required to recover turgescence and normal suction force, longer even than to rebuild the destroyed chlorophyll (see "Henrici, 1926"). On the other hand, the assimilation did not fall as soon as the rain stopped. Provided that the soil was not too dry, high values were found as much as ten days after the rain at a time when the suction force was already considerably increased.

After the March rains, which produced many young leaves, April was dry. On the 8th, leaves with a very low percentage of dry matter, wilted, and therefore contained little starch, which condition held until the latter half of May, when after heavy rains the starch again increased. The leaves slowly died down, the chlorophyll was destroyed, the starch disappeared, and the amount of sugars diminished. Finally the brown leaves contained about 1 per cent. sugar and no starch at all.

In August, young leaves came up. They were very small, and contained large amounts of sugars but relatively little starch, which only increased after the elongation of the leaves. As the season progressed the carbohydrate-content depended on the condition of the leaves, but decreased towards Autumn. It is peculiar that the chlorophyll-content was much lower in November, 1924, than in November, 1923, which resulted in a lower assimilation and, therefore, a smaller amount of carbohydrates.

A few analyses for starch only were carried out from September to November, 1923. As mentioned before, the grass at this time contained much more chlorophyll than during the same period in 1924, and the starch value was very much higher, up to 12-15 per cent. when the leaves were fresh; but as soon as the leaves wilted, which often happened during the dry period of September and October, the amount went down to 1/4 to 1/6 of this value. In these experiments chlorophyll was certainly never a limiting factor.

Eragrostis superba (table 2 and curve 2): The yearly curve of *Eragrostis superba* differs from that of *Themeda*. Whereas *Themeda* has two distinct periods during which starch is abundant, *Eragrostis* shows three, the first occurring before the rains, and showing the absolute maximum; the second, after the drought of September and October, with a minimum of starch in the rainy period of November and December, followed by a decrease in January; and a third in February, with a maximum after the May rains. In June the carbohydrate-content falls. It is surprising to note that with the exception of the analyses made in Spring the starch maxima are always the same. It appears that the assimilation in *Eragrostis superba* is not influenced much by change of temperature or prolonged wilting, and that except in the Spring the age of the leaves has no effect at all. If starch disappears during the drought period, more sugar than usual appears, but this does not correspond to the amount of starch lost. The same sugar does not always predominate; in October the disaccharides are the chief sugars and later in the season the monosaccharides.

Aristida uniplumis (curve 3 and table 3): the curve of *Aristida uniplumis* is unique: there are no maxima during the rainy periods, but on the contrary the minimum is in December. Large amounts of starch are only found in young leaves in Spring and during the dry parts of February. After the end of March a sufficient number of green leaves for analysis could no longer be found, although the haulms and the leaves of other grasses were green well into the winter. The entirely different results of this and other grasses is the more striking because *Aristida* is a typical grass of the dry sand veld, but it is possible that it is just this unique behaviour which explains its habitat. An assimilation which though weak is constant and independent of rain is of great ecological importance. It would be interesting to repeat these determinations with a typical desert grass from the Kalahari.

Chrysopogon serrulatus (curve 4 and table 4): The curve of *Chrysopogon* shows an absolute maximum during the November rains and relative maxima in the rains of March and the beginning of February, although they do not reach half the value of the November maximum. The amount of disaccharides was small throughout the year, much smaller than the amount of glucose, but it is particularly striking that no disaccharides were found in March and April. At the end of May, when the grasses were drying out and the starch completely disappeared, disaccharides appeared in larger amounts. In all the other grass curves a high glucose-content corresponds to low disaccharide-content and vice versa.

In accordance with results obtained by Ahrns (1924), it does not seem impossible that *Chrysopogon* contains large amounts of maltose, but in these analyses it is calculated as "glucose," as no inversion with citric acid was resorted to. The ground grass always had a strong smell of malt.

Digitaria eriantha (curve 5 and table 5): The curve of *Digitaria* greatly resembles that of *Chrysopogon.* During the rainy season in November there is a large amount of starch, more than five times as much as under the most favourable conditions in March. The value for disaccharides is very low. It appears that, contrary to the case of *Eragrostis,* late rains did not restore the assimilatory system of *Digitaria* and *Chrysopogon,* and that the young leaves developed late in the season have a much lower assimilatory power than those which appeared with the first rains. For both grasses it is characteristic that there is no high starch content before the rain as in *Eragrostis.* The time of greatest development was during the rainy season, whereas *Eragrostis* formed haulms even before the rains. Of all the investigated grasses *Digitaria* is the one which wilts the quickest, so that it is not surprising that it was scarce before the rains and that it reached its assimilation maximum only after the rains.

B.—*Comparison of Carbohydrate Curves with those of Chlorophyll.*

When we compare the yearly carbohydrate curves (curves 6 to 10) with those of chlorophyll (Report 11 of the Director of Veterinary Education and Research, 1926), which are, however, of another year, we note a great similarity. In the curves of *Eragrostis superba* the absolute maxima of direct assimilation products and of chlorophyll are in August, long before the rains. Relative maxima occur in the rainy periods, while the intervening times of drought show minima of both chlorophyll and carbohydrates.

Themeda, on the other hand, has no absolute maximum of either chlorophyll or carbohydrates in the youngest leaves, but its maxima lie in the rainy and its minima in the drought periods.

Digitaria is very similar, but its maximum lies in the young spring leaves, although not in the youngest. The autumnal chlorophyll minimum corresponds to the carbohydrate minimum.

The similarity in the curves is not an accidental one, as the two processes are closely connected. If the chlorophyll-content becomes the limiting factor, the carbon assimilation will diminish and less sugars and starch will be formed. Deficiency of chlorophyll alone is seldom the limiting factor under the veld conditions of Bechuanaland, for it is generally associated with lack of water; only during the few days immediately following upon the rain is there sufficient water but insufficient chlorophyll to allow a maximum of assimilation. This also may account for the low values of assimilation products obtained immediately after the rains; the reformation of large amounts of chlorophyll takes several days.

C.—*Comparison of the Results with those obtained by other Authors.*

From these analyses it may be concluded that the carbohydrate-content of four of the investigated grasses depends more or less on their physiological condition, and, consequently, during the wilting, starch disappears without leaving a corresponding amount of sugar. In no case does the amount of carbohydrates depend on the percentage of dry matter or directly on the water-content of the grass. The external moisture factors, rain and soil humidity, have a much greater effect than the actual constitutional water-content. This is explained by the fact that wilting may occur at different proportions of water-content in the plants, and that it is the *wilting* itself and not the *low water-content* which leads to the decrease in starch.

The behaviour of the fifth of the investigated grasses, *Aristida uniplumis*, is quite different. This is very striking as this grass is typical of the dry sand veld of South Africa. It has its starch maxima during the dry periods and its minima during the rains.

It was not to be expected that results obtained with South African veld grasses would correspond exactly with those obtained by German authors (Ahrns 1924, Horn 1922). A larger variety of factors had to be considered; such as the age of the leaves, the direct influence of the season and the repeated drying out and recovery of the leaves. Even the few investigated grasses show differences. Repeated drying out of the leaves has no effect on the assimilation force of *Eragrostis superba*, whereas *Digitaria* under the most favourable conditions after drought is only capable of producing one-fifth to one-third as much starch as it produced before.

Thus analyses made in autumn cannot be directly compared with those made in spring, because the assimilation power is usually completely changed. This difference is not solely due to the age of the leaves, for after the late rains, there is an abundance of young leaves. The low values found for *Digitaria* at this time of the year are thus seen to be dependent upon the two factors, decreased assimilation power in the old leaves and an altogether smaller power in the new young leaves. In the case of *Eragrostis* both or at least one of these factors may be absent, probably the decreased assimilatory power of the old leaves. So that the assimilation activity of the old leaves may eventually make up for the small activity of the young leaves, which are also few in number as *Eragrostis* does not produce as many after the rains as *Digitaria* does.

In spite of these differences there are many points in common with the results obtained by Ahrns (1924) and Horn (1922). The investigations of Iljin (1923) are of special interest as he also worked on a steppe. He found that as long as the water loss was not too big the drought resistant plants showed a complete recovery of their assimilatory system. If the disturbances caused by the drought were too severe the plant lost its power of regulating this function (assimilation) in a normal way either temporarily or permanently; as anatomical disturbances may in this case have occurred. (Iljin 1922.) This observation may also be made for the grasses of Bechuanaland.

What happens to the disappearing carbohydrates in the grasses has not yet been experimentally established. It might be thought that a quicker migration of carbohydrates takes place during a drought than at ordinary times, but in the light of Iljin's (1923) observations this does not seem very probable. In the first place far less carbohydrates are formed, and it is quite probable that an increase of respiration really does take place as Iljin states. Direct experiments on respiration should support this hypothesis.

Résumé.

Under veld conditions in Bechuanaland the amount of direct assimilation products, especially starch, diminishes in *Eragrostis*

superba, *Digitaria eriantha*, *Themeda triandra* and *Chrysopogon serrulatus*, as soon as wilting or incipient drying occurs.

The maximum amount of assimilation products is found either in early spring or during the rainy season.

Aristida uniplumis, a typical grass of the dry grass veld, behaves differently, having its starch maxima in times of drought.

Wilting is not the only factor influencing the amount of carbohydrates. Age and season also play a big part, so that analyses made in autumn cannot be directly compared with those of spring.

Loss of starch and decrease of carbohydrates does not occur at a specific percentage of dry matter for a specific grass.

This is explained by the fact that wilting also does not occur at a definite water content.

PART 2.

To determine the total "carbohydrate" or "nitrogen-free extractives" complete analyses were made by the conventional methods of the agricultural chemist as given in T. B. Wood's "Course of Practical Work in Agricultural Chemistry for Senior Students." Moisture, ash, crude fibre, crude protein, and ether extract were determined. The sum of these subtracted from 100 was taken as "carbohydrates" or "N-free extractives." Data are calculated on "plant dry matter" (dried at 100°-104°). The water content of the fresh material is discussed elsewhere.

Results.

A glance at the tables 7-11 shows that the single species differ considerably in the total analyses. But, so far as possible, the common points may first be discussed.

Ash-content: Little can be said about the ash-content, as it varies considerably, is influenced by adhering dust, and does not increase continuously with the advancing season. *Aristida uniplumis* has the lowest ash-content, of 5.9-6.9 per cent.; it shows also the smallest variations, probably because dust cannot so well adhere to a roll-leaf (see Theiler, Green and du Toit, 1924, p. 29). *Eragrostis superba* follows next with a much wider range of values, 5.7-10.7 per cent., the lowest value being in young grass and the highest in brown leaves and a general tendency of higher values with the advancing season. *Themeda triandra* follows with values of 6.8–10.1 per cent., which cannot be arranged according to the season; *Themeda* is the grass which is often entirely buried in sand and dust. *Chrysopogon* shows decidedly higher values, 8.0–11.7 per cent., the latter value being found in very old leaves, but for the others nothing conclusive can be said. *Digitaria eriantha* has the highest ash-content with a range of 9.9–14.4 per cent., the higher values occurring before the rains. It may be mentioned that the grass with the lowest ash-content shows also the lowest phosphorus-content, on the other hand *Digitaria* and *Chrysopogon* show also a higher phosphorus content than the two grasses with medium ash-content (Henrici, 1927).

Crude protein: According to the protein-content, two different types of grasses can be distinguished. *Chrysopogon*, *Eragrostis superba*, *Themeda triandra* and *Digitaria* distinctly show two maximal values in protein-content, the first occurring between September and November, the second in March. The lowest values of protein are found in old grasses, that is to say in green and brown leaves in winter and in green leaves during the drought of January. It is peculiar that not all the aforementioned grasses have the absolute maximal values at the same time. For *Digitaria* and *Chrysopogon* they range from September to October. *Eragrostis superba* has the highest value only in the second rainy period and *Themeda* shows the same values in spring as in the second rainy period.

Aristida uniplumis represents the second type. It only shows one maximum in spring and small fluctuation from January to the end of March.

The absolute values of crude protein vary widely for the species. *Aristida uniplumis* takes a special place as green leaves in the spring and summer were analysed, no leaves being available at any other time of the year. Its values vary between 7-9.8 per cent. The minimum value is not low at all, but it must be remembered that it is not a winter value. Amongst the other four grasses, *Themeda* has the lowest maximal value and the lowest minimal value for old green leaves. The respective figures are 11.3 per cent. and 5.5 per cent. for green leaves, and 4.5 per cent. in brown leaves. *Chrysopogon* shows only 3.2 per cent. in very leached brown leaves, but 7.5 per cent. in brown leaves which have recently died, and the maximal value in green leaves going up to 13.3 per cent. The minimal values for *Eragrostis* are similar, 3.0 per cent. for very leached, 4.6 per cent for brown leaves which have not yet been leached; the maximal value being 15.4 per cent. The drought value is only 5.1 per cent. *Digitaria eriantha* has the highest values all the year round. Even in very old brown leaves it is still 5.4 per cent., in October it is as high as 20 per cent., and with the exception of the drought period it never goes lower than 11 per cent.

The Ether-extract: The minimal values for the ether-extract are found in very young and in leached old leaves. Some of the grasses show also a low percentage of ether-extract during the drought period in January. Only *Eragrostis superba* has the tendency of increasing the ether-extract with advancing season; this may be due to a peculiar process in the chloroplasts of this grass. It was observed that in weekly sections made through its leaves an oily substance occurred in the granules, the substance increased with the advancing seasons. A qualitative or quantitative study of the nature of this substance was not done.

Crude Fibre: Generally the fibre-content of the young leaves is low, although for the different species the percentage varies considerably. It is not always the first spring leaves which have the lowest fibre-content, but the leaves which are developed in the time of the first rains may have the lowest fibre-content (*Eragrostis superba* and *Themeda triandra*). During drought, not all the grasses behave alike. *Chrysopogon* scarcely shows any change, *Themeda* and *Eragrostis* increase their fibre-content during drought, *Aristida* on the other hand decreases it. With progressing season the fibre-content increases, but for *Chrysopogon* only towards autumn and winter, after having been nearly constant from October to March. Leaves which have just died down show a considerably higher fibre-content than old leached leaves (*Eragrostis* and *Digitaria*). It is considered that this phenomenon is due to bacteriological activities, depolymerising the cellulose to carbohydrates which are soluble in dilute acid.

With the exclusion of very old leaves the percentage of fibre may vary little or much. The range for—

Aristida uniplumis is	38.3-53.9 per cent.
Digitaria	26.7-33.5 per cent.
Themeda	25.6-31.4 per cent.
Chrysopogon	24.2-29.9 per cent.
Eragrostis	21.6-31.1 per cent.

This would mean that from the point of view of palatability *Eragrostis* and *Chrysopogon* are the best, *Aristida uniplumis* the worst of the investigated grasses, if only the chemical analysis had to decide. *Bews* (1918, p. 51ff.), however, pointed out that not only the chemical analysis but the transverse leaf-section has to be consulted, and for this purpose indeed the result differs very much from the chemical analysis. The sections of the five investigated grasses are given as an example.

Aristida is doubtless the worst of the grasses, but *Digitaria* changes places with *Eragrostis*. *Digitaria* has in all stages of growth very soft leaves which are easily cut in freehand sections. *Eragrostis* has very hard nerves. *Aristida uniplumis* has a higher fibre-content in the young leaves than during drought. It is thought possible that a depolymerisation takes place during drought, as *Aristida* has a maximum of reducing carbohydrates at that time. The process will be discussed in the paragraph on relation of fibre and carbohydrates.

Carbohydrates: The amount of carbohydrates differs considerably for the different species and for the same species it varies with the season. The meteorological conditions do not act alike on the different species. *Themeda triandra* does not change much, its carbohydrate-content varies from 46.8-53.5 per cent. *Digitaria* on the contrary shows a wide range of variation from 33.8-54.9 per cent., the lowest value being in October. *Chrysopogon serrulatus* with a range of variation from 44.8-50.4 per cent. has high values in spring and February, and low values in very old leaves. *Eragrostis superba* on the whole shows a higher carbohydrate-content, 45.2-68.5 per cent., the latter figure in very old leaves which show a low fibre-content. Except for the brown leaves the higher values are rather found before the New Year. Rain shortly before the sampling of the grass does scarcely alter the percentage of carbohydrates (see analyses of the 28th and 30th October, 1924). *Aristida uniplumis* has the lowest content of carbohydrates, with a variation of 28.5-46.9 per cent. Its highest value is in time of drought, the lowest value is found in old green leaves simultaneously with the highest percentage of fibre.

In the preceding paragraph several times allusion was made to an interrelation of fibre and carbohydrate content. The question arises whether always a connection between these two figures exists. It is true that for *Chrysopogon* and *Aristida* the sum of the percentage of fibre and carbohydrate varies but little, but for the other species the differences are up to 10 per cent., so that a general correlation cannot be thought of. One point may be recapitulated: *Aristida uniplumis* shows during drought a low fibre and a high carbohydrate content, whilst *Eragrostis superba* and *Digitaria* have just the opposite, high fibre and low carbohydrate content.

In the first part of this investigation, the direct assimilation products (sugars and starch) have been determined. A further figure has been got by boiling the starch-free plant material with 1.4 per cent. hydrochloric acid for three hours under the reflux cooler (König, p. 282). According to König this value includes starch, pentosans and hexosans of the hemicelluloses. As in this investigation the starch was determined by hydrolysis with diastase (official and tentative methods of the Association of Agricultural Chemists, 1920, pp. 95-96), the reducing value of the acid solution was only due to pentosans and some hexosans of the hemicelluloses. Hemicelluloses are found in grass leaves (Czapek I, 1913, p. 489) as structural framework and possibly also as reserve-substance. According to Pringsheim (1923, p. 183ff.), the reserve-substances occur earlier than the "Gerüstsubstanz." Reserve-substances are generally hexosans, and structural units often pentosans. A young plant has therefore less pentosans than an adult plant. In the leaves of the grasses under investigation the sclerenchymatic and collenchymatic tissue contains plenty of hemicellulose. It is likely to be pentosan, but it is not excluded that also some hexosans occur. Before explaining in detail the obtained figures, a discrepancy has to be pointed out. If the percentage of the direct assimilates and the percentage of these "hemicelluloses" are added, the figure arrived at does not correspond to the figure for "carbohydrates" of the total analyses, the carbohydrates being 10-15 per cent. more.

Pringsheim (1923, p. 93) also speaks of a difference in adding up the constituents of the total analyses in which pentosans and lignin were determined separately, in straw of cereals the discrepancy being 1.0-8.4 per cent. In general, however, this author thinks that the difference from 100 per cent. after subtraction of ash, protein, cellulose, pentosans, should give the lignin in substances like wood and straw. If the lignin is determined directly and a difference in the two figures occurs, it would be chiefly due to hexosans, that is to say, polysaccharides which are hydrolysed easier than cellulose (page 93), like starch, which does not depolymerise into pentosans and is, therefore, not determined as furfurol. The difference observed in straw is 1.0-14 per cent.; Pringsheim, however, points out that these substances have no important rôle in the material analysed by him.

In this investigation the lignin was not determined separately, but according to Pringsheim's deduction the observed difference is likely to be lignin, as the starch and sugars have been determined directly. The substance of this "difference" is characterised by its solubility in 1.25 per cent. sulphuric acid and 1.25 per cent. potassium hydroxide, but its insolubility in 1.4 per cent. hydrochloric acid. According to Rosenthaler (1923, p. 82) lignin is not soluble in dilute alkali, or dilute acid, or, at least, soluble to a very small degree only. According to this separation of the carbohydrates it may be an oxycellulose which is not soluble in water and alcohol, in alkali partly soluble, but soluble in dilute acid and reducing boiling alkaline copper solution (p. 95). It seems, therefore, likely that not the whole difference is lignin, but rather a mixture of some carbohydrates showing the specific solubility mentioned. It seems also probable with regard to the fact that the difference observed in this investigation is bigger than Pringsheim's.

The difference found in this investigation varies considerably, but is sometimes of the same order of size as Pringsheim (1923, p. 96) stated it to be in the straw of cereals and other plants.

The variations for the 5 grasses are:—

Chrysopogon	23–37	per cent.
Aristida	14–30	,,
Eragrostis...	20–35	,,
Digitaria	0–27	,,
Themeda	1–36	,,

The minima and maxima of this "difference" do not coincide for the various species. *Chrysopogon*, *Aristida*, and *Themeda* show a minimum in old leaves, *Eragrostis* and *Digitaria* in one of the rainy periods. *Chrysopogon* and *Eragrostis superba* have their maximum in drought whilst *Digitaria* has its maximum at the end of the season, and *Themeda* in the rainy season.

Considering this different behaviour in every respect it is hardly possible to draw any conclusion which holds good for all the grasses except that there is certainly an interrelation between the amount of hemicellulose and the size of this "difference." If the one is high the other is low and vice versa. For *Digitaria, Themeda,* and *Chrysopogon* an interrelation between assimilates and difference can be detected. If only a small amount of assimilates is present the "difference" is high and vice versa, without, however, the lowest percentage of assimilates corresponding always to the highest of the difference. The correlation may be due to two facts. Either the meteorological conditions influence directly each of the compounds, increasing the one and decreasing the other; or, in decreasing the assimilates, more of the compounds included in the "difference" are formed.

In this connexion the investigation of Cannon (1924, p. 121) may be remembered; he points out that under arid conditions plants may convert the polysaccharides largely into anhydrides or wall material, or under less arid conditions, into pentosans or mucilages (succulents).

In splitting up the carbohydrates in direct assimilates, and substances which after removal of starch are soluble in dilute acid or in dilute sulphuric acid and dilute alkali, it was thought possible to trace some relation between the starch disappearing in the drought and the other carbohydrates. Unfortunately there was not enough material to make total analyses of all the plants of which the direct assimilates were determined. But the limited number of total analyses gives a fairly good idea of the seasonal changes of the carbohydrates.

First may be considered the polysaccharides soluble in 1.4 % hydrochloric acid if boiled for 3 hours under the reflux cooler (see König, p. 282), which probably contain pentosans and some hexosans of the hemicellulose type, since in this investigation, the starch was previously separated by amylase.

With the exception of *Themeda*, in no grass do these hemicellusoses fall below 9 per cent. The upper limit varies with the species; for *Chrysopogon*, *Aristida uniplumis*, and *Themeda triandra* it is about 20 per cent., *Eragrostis superba* goes up to 27 per cent., *Digitaria* even to 34 per cent. Drought does not affect all the grasses in the same way. Whilst *Chrysopogon* and *Eragrostis superba* have their lowest content of these hemicelluloses in January, *Digitaria* and *Aristida uniplumis* show a maximum at the same period. High values of hemicellulose in *Chrysopogon* and *Themeda* and *Eragrostis* are found in old brown leaves, for *Eragrostis superba* also in young leaves. Low values for *Digitaria* are found during the second rainy period, for *Aristida uniplumis* in very old and in young leaves. As can be seen from these data, the different grasses behave specifically.

It has to be considered if in time of minima of direct assimilates maxima of another carbohydrate or of fibre occur. Attention must be drawn to the fact that the minimum of starch and sugars of four of the investigated grasses occurs in a time of a minimum of phosphorus (Henrici, 1927). Reeds and Koch (1907) have pointed out that phosphorus deficiency leads to a disturbance in the carbon dioxide metabolism, that in place of starch erythrodextrin and cellulose are formed. If in the investigated grasses erythrodextrin would be formed, it would nevertheless be found in the analysis amongst the direct assimilates, as the diastase would convert it into reducing sugar. As generally the assimilates are low in the time of drought it is not likely that erythrodextrin is formed in any larger amount, although no direct test was made except with iodine. Neither the solutions nor the tissues gave any red colour. If any changes occur at all they must be with the other carbohydrates. It is peculiar that phosphorus deficiency and drought act similarly for an increase in wall substances and a decrease of starch.

As has been shown previously, in time of drought, that is to say in time of a phosphorus-minimum, *Eragrostis* and *Digitaria* increase their fibre-content, *Themeda, Digitaria, and Aristida* their percentage of hemicellulose, *Chrysopogon, Themeda*, and *Eragrostis* the proportion of substances determined by difference. This means that in all of the investigated grasses one or two classes of carbohydrates are increased which are less soluble than starch; which class of carbohydrate is formed depends entirely on the species. The conditions of *Eragrostis* and *Digitaria* correspond closest to the observations of Reeds and Koch (1907) who found an increase of cellulose. Cannon's result (1924) for different degrees of aridity are confirmed in this investigation for different species in the same degree of aridity. As a rule it may be said that during drought accompanied by a simultaneous minimum of phosphorus a carbohydrate less soluble than starch is present in a larger amount. Whether it is more the influence of the deficiency of phosphorus or of the drought is difficult to decide as both factors have the same effect on the carbon metabolism.

If in the grasses a minimum of phosphorus leads to a minimum in assimilates and to an increase in less soluble carbohydrates, the question arises whether a maximum of phosphorus tends to a maximum in direct assimilates and a decrease in less soluble polysaccharides. With regard to direct assimilates, the answer is in the affirmative, especially in the first rainy season, only it has to be remembered that favourable moisture conditions may act on both processes, assimilation and intake of phosphorus, independently, and that from the increase of both it must not be concluded that the one process is dependent on the other. The observation that young leaves in the early spring show a high phosphorus content and a high percentage of assimilates supports rather the idea that phosphorus content and assimilates show a direct interrelation although at this time soil and air are very dry.

It is more difficult to demonstrate a coincidence of the minimum of the less soluble carbohydrates with a phosphorus maximum. For *Digitaria* and *Chrysopogon* it exists in young leaves and in the rainy periods. Also for *Themeda, Eragrostis,* and *Aristida* it can be detected, but the differences are not so obvious and may therefore be more accidental in the sense that favourable moisture conditions influence independently both processes. In the whole question interpretation is obscured by the fact that in the second rainy period the amount of direct assimilates is never so large (with the exception of *Eragrostis*) as in the first, regardless of favourable moisture conditions and high phosphorus content, so that *a priori* more insoluble polysaccharides are found in this period. In this connexion a point may be raised which has not been under discussion. The smaller amount of direct assimilates in the second rainy period has not found a satisfactory explanation. In the interval experiments have been done which show that the intake of CO_2 is by no means small at this time (although not so big as in spring time) and it is thought that for some reason the grass does not form starch, but translocates sugar quicker; the reason is in all probability the haulm formation which takes place on the highest scale in this period. It is probable that the sugars migrate directly to the place of their consumption, to the rootcrown, and that they are not condensed to starch but transformed to cellulose and hemicellulose as structural material for the haulms. This process cannot be shown by an analysis of leaves only, although the amount of less soluble carbohydrates in the second rainy period is greater than in the first. It is characteristic that *Eragrostis* does not show a decrease in assimilates as it forms haulms the whole year round and never shows a very high amount of direct assimilates.

It may, however, be stated that a coincidence of a minimum of phosphorus and assimilates occurs in the drought-period combined with an increase of less soluble carbohydrates. Although it cannot be asserted that the maximum of phosphorus and assimilates are always combined with a minimum of less soluble carbohydrates, the broad correlation certainly exists.

Comparison of the Data with other Analyses.

Comparison can be offered with analyses on mixed grass samples of Armoedsvlakte from November, 1919, to June, 1920 (Theiler, A., Green, H. H., and du Toit, P. J, 1924, p. 29).

On the mixed sample the content of proteins is highest in November and still high in January and decreases rapidly from February to June. The percentage of ether-extract decreases continuously, the nitrogen-free extractives and the fibre increasing steadily. On the whole the order of size of the data obtained in this investigation agrees very well with those of 1919-20, except for the very old leaves which were not analysed separately in the previous years and although in this investigation the increase of fibre and carbohydrates (N-free extract) is not so steady. The analytical data are dependent on the rainfall, and differences in the data may be due to its different distribution.

The protein-content in 1924-25 decreases much earlier but shows a second increase: this may be due to two factors. The grass of 1924-25 was already older in December (having come up in August), and the second rainy period brought up a lot of young green grass with a high percentage of protein. In the mixed sample there was naturally at this time of the year a lot of dry brown grass leaves with little protein, which decreases the average value.

The second rise in protein causes by itself some fluctuations in the gradual increase of fibre and carbohydrates.

Comparison with European material may be made. Unfortunately in plant physiological literature there are scarcely any data, in view of the fact that every determination of the total analysis includes different compounds and cannot be directly used for physiological guidance (see Benecke and Jost, 1924, p. 6). In spite of this drawback it may be said that on the whole Armoedsvlakte grass is at any time of the year richer in fibre and much poorer in protein, except in spring, and most of the time richer in carbohydrates than European meadow grass.

Further analytical data on South African and European grasses are found in papers by Marchand and Smit (1919) and Vipond (1914). According to Vipond's (p. 15) table the Armoedsvlakte grass compares very well as to the protein content with other South African grass, the percentage of which seldom exceeds 8.0 per cent.; the values in Vipond's investigation are not recalculated for "material dried at 100°," which would increase the values about 10 per cent. Old Armoedsvlakte grass is poor in protein and corresponds to Vipond's "coarse veld hay." The content of carbohydrate of Armoedsvlakte grass shows the same order of size or is rather higher, the fibre with the exception of *Aristida uniplumis* is rather lower than in grasses analysed by Vipond. Ash and ether-extract are about the same. A *Digitaria* analysis by Marchand is of special interest (Analysis 3777, p. 8), as in Armoedsvlakte also a *Digitaria* was analysed. Marchand's species is poorer in protein and much richer in fibre than the species of Bechuanaland, ash and carbohydrates being about the same.

In conclusion it can be said that from the point of view of protein and carbohydrates the Armoedsvlakte grasses are not poorer than other South African grasses in spite of many adverse meteorological and edaphic conditions. Nothing need be said in this place of the mineral content.

Résumé of the Second Part.

1. Total analyses were done on single species of Armoedsvlakte grasses (Vryburg District, Bechuanaland) in the course of their development and the data were compared as far as possible in the light of meteorological factors.

2. During the drought the protein content decreases, the percentage of fibre and carbohydrates increases. The opposite takes place in the second rainy period.

3. Special attention is given to the content of fibre and carbohydrates. The carbohydrates are divided into direct assimilates, polysaccharides soluble in 1.4 per cent. HCl, polysaccharides soluble in 1.25 per cent. H_2SO_4 and 1.25 per cent. KOH, but not soluble in 1.4 per cent. HCl. The first group chiefly contains pentosans of the hemicelluloses, the second group is likely to be a mixture of lignin and perhaps also oxycelluloses.

4. It was found that in the drought period a less soluble carbohydrate occurs in the place of starch, at the same time as a minimum of phosphorus in the grass.

5. In general there is a coincidence of a maximum in the percentage of phosphorus, a maximum of direct assimilates and a decrease of less soluble carbohydrates; but this process is more difficult to trace and is not without exception.

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EXPLANATION OF TABLES.

TABLE 1.—Carbohydrates, Themeda triandra.

Tables 1 to 5 are arranged in the same manner. After the date (column 1), the condition of the plant (column 2), then the dry matter of the leaves in percentages (column 3) are marked; columns 4, 5, 6, give the contents of the water soluble reducing bodies, chiefly glucose, of water soluble bodies reducing Fehling after hydrolysis, chiefly disaccharides, and of starch, all three in percentages of dry matter; the column 7 shows the reducing bodies soluble in dilute acid, chiefly hemicellulose, also in percentage of dry matter.

TABLE 2.—Carbohydrates, Eragrostis superba.

TABLE 3.—Carbohydrates, Aristida uniplumis.

TABLE 4.—Carbohydrates, Chrysopogon serrulatus.

TABLE 5.—Carbohydrates, Digitaria eriantha.

TABLE 6.—Dry matter and total amount of assimilation products of the investigated grasses.

In this table for all investigated grasses the total amount of assimilates (with the corrected starch value) is given, to prove that there is no direct relation between them and the amount of dry matter.

TABLE 7.—Total analyses of Themeda triandra in percentage of stove dry material.

TABLE 8.—Total analyses of Eragrostis superba in percentage of stove dry material.

TABLE 9.—Total analyses of Aristida uniplumis in percentage of stove dry material.

TABLE 10.—Total analyses of Chrysopogon serrulatus in percentage of stove dry material.

TABLE 11.—Total analyses of Digitaria eriantha in percentage of stove dry material.

TABLE 12.—Sugar values of the 1/10 N Thiosulphate solution.