CHAPTER 4

TAXONOMIC SIGNIFICANCE OF CHARACTERS

4.1 Palynology

BREDENKAMP, C.L. & VAN WYK, A.E. 1996. Palynology of the genus *Passerina* (Thymelaeaceae): relationships form and function. *Grana* 35: 335–346.

CONTENTS

Grana: 35: 335–346, 1996

Palynology of the genus *Passerina* (Thymelaeaceae): relationships form and function

CHRISTINA L. BREDENKAMP and ABRAHAM E. VAN WYK

Bredenkamp, C. L. & Van WYK, A. E. 1996. Palynology of the genus *Passerina* (Thymelaeaceae): relationships form and function. – Grana 35: 335–346. ISSN 0017–3134.

Pollen of the genus *Passerina* L. differs markedly from that of other southern African members of the Thymelaeaceae. Grains of most members of the Thymelaeaceae are characterised by a typical croton pattern, comprising rings of more or less trihedral sexine units mounted on an underlying reticulum of circular muri. In *Passerina*, however, the supratectal subunits are fused completely to form a continuous reticulum, which replaces the underlying reticulum. The reticulum in *Passerina* is therefore secondary in origin and not homologous with the basal reticulum of typical crotonoid grains in the family. The croton pattern has often been used as indication of a possible relationship between the Euphorbiaceae and Thymelaeaceae. Pollen of *Passerina* is adapted to anemophily. Grain sculpturing clearly demonstrates secondary derivation of a reticulate pattern from the typical croton pattern, through reduction, aggregation and fusion. Pollen of *Passerina* represents a climax of a continuum of variation in the exine of pollen in the Thymelaeaceae. As *Passerina* is considered phylogenetically advanced in the subfamily Thymelaeoideae, the subtribe Passerininae is raised to tribal rank, namely tribe Passerineae.

Christina L. Bredenkamp, National Botanical Institute, Private Bag X101, Pretoria, 0001 Republic of South Africa; Abraham E. Van WYK, H.G.W.J. Schweickerdt Herbarium, Department of Botany, University of Pretoria, Pretoria, 0002 Republic of South Africa.

(Accepted February 20, 1997)

Established by Linnaeus (1737, 1753), the genus *Passerina* comprises about 17 species, all restricted to southern Africa (Thoday 1924, Bond & Goldblatt 1984). The palynological study of *Passerina* was undertaken as part of a monographic study of the genus. This study revealed that the pollen of the genus differs markedly from that of the other southern African genera of the Thymelaeaceae and indicated many similarities between the Thymelaeaceae and Euphorbiaceae (Nowicke 1994, Erdtman 1952, 1969).

Archangelsky (1966, 1971), using light microscopy (LM), recognised ten pollen groups in the Thymelaeaceae, of which two are represented by southern African Thymelaeaceae. The present LM, SEM and TEM study revealed new information, including a clear distinction between the pollen of *Gnidia* L. and *Passerina* L., which were both regarded as cryptostellate by Archangelsky (1966). Descriptions of the pollen of *Aetoxylon* (Airy Shaw) Airy Shaw, *Amyxa* Tiegh. and *Gonystylis* Teijsm. & Binn. by Nowicke et al. (1985) indicated that the phylogenetic relationships of the Thymelaeaceae were controversial issues.

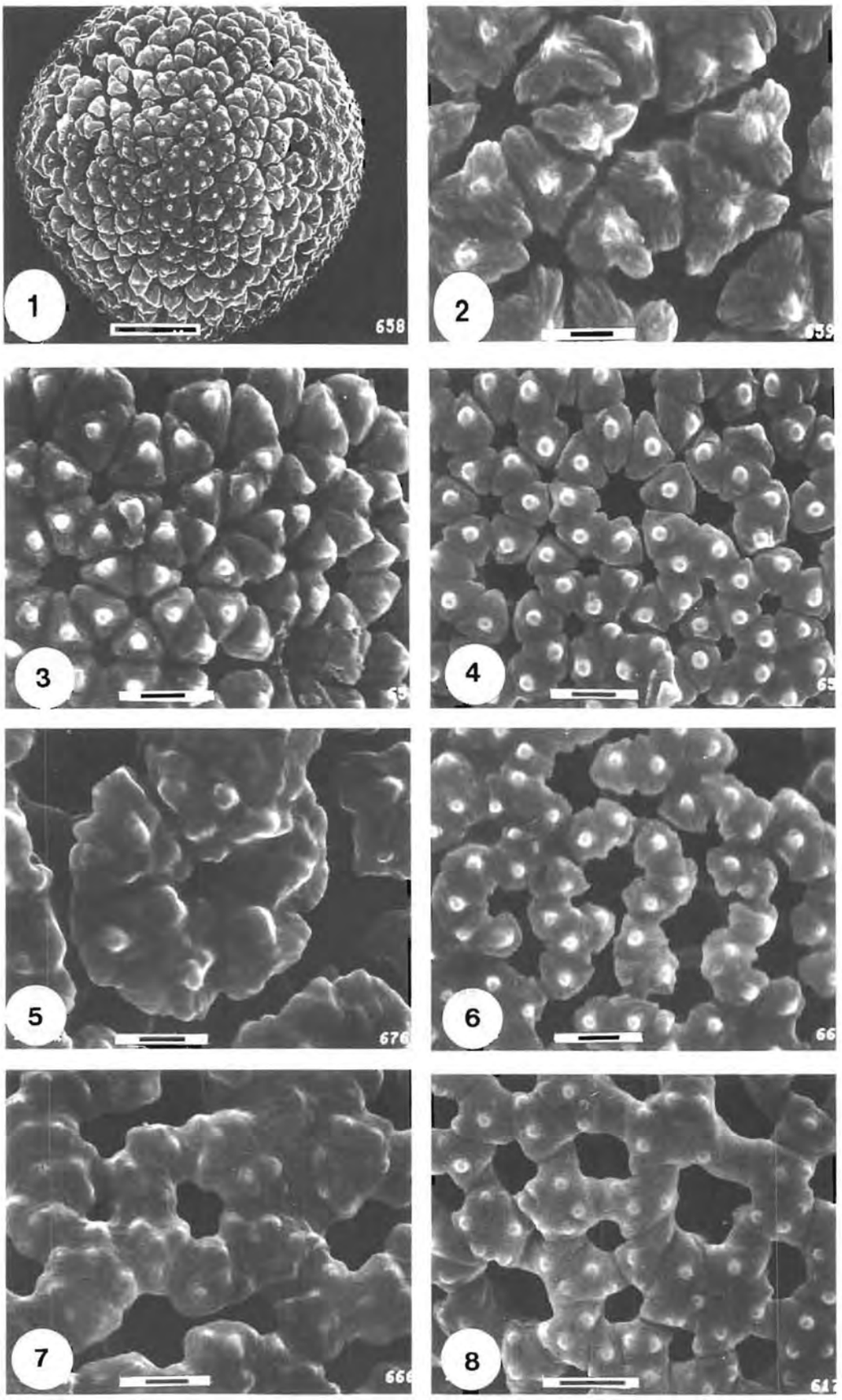
Pollen of Thymelaeaceae is remarkably uniform: spheroid in shape (Mohl 1835) and polyforate (Blaise 1959), panporate (Archangelsky 1966) or pantoporate (Punt et al. 1994), with a crotonoid tectum or a derivative thereof (Nowicke et al. 1985). The term "crotonoid pattern" was proposed by Erdtman (1952, 1966) who describes the sexine as follows: "regularly arranged excrescences (triangular or ±circular in cross section) supported (always?) by a baculate or baculoidate, or spongy layer" and arranged regularly in circles around foveoloid areas delimited by muroid ridges." Punt (1961) describes the *Croton* type of pollen for the Euphorbiaceae as having no apertures, but Nowicke (1994), using electron microscopy, disputes this and reports the presence of 3-colpate and pantoporate pollen in the Crotonoideae, thus concluding a close structural relationship between the pollen of the Crotonoideae and the Thymelaeaceae. Thanikaimoni et al. (1984) describe the omniaperturate pollen of *Croton matourensis* Aubl. and conclude that the "crotonoid pattern appears lax due to the well developed muri which delimit the lumina." Further, the ornamentation of the sculptural units is linked to the pollination mechanism. Nowicke et al. (1985) describe the subunits of *Croton* as triangular in surface view and forming a continuous array, but in the Thymelaeaceae these units appear to be attached to a basal ringlike network of muri (also described in literature as horizontal rods). An almost perfect continuum of variation in the distinction of the subunit has also been found for the Thymelaeaceae by Nowicke et al. (1985).

The present paper describes the pollen of *Passerina* and illustrates this continuum for southern African genera of the Thymelaeaceae. General trends in the evolution of palynological features in Thymelaeaceae and Gonystylaceae are proposed.

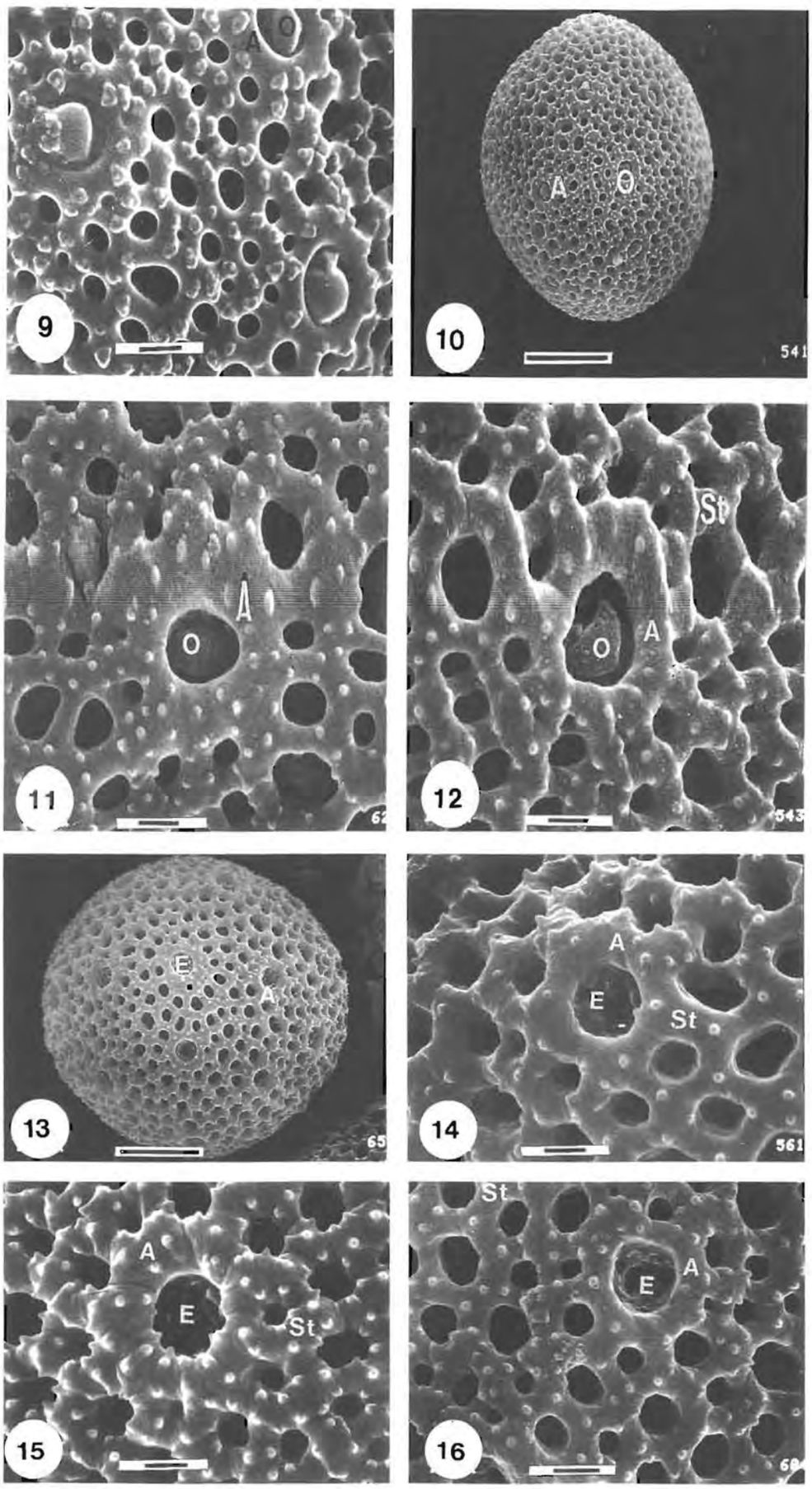
MATERIALS AND METHODS

During field work, aspects of pollination biology such as floral morphology, flower colour, presence or absence of nectar and scent, were recorded for the genus *Passerina*. Pollen of the 16 currently recognised species of *Passerina* in southern Africa (Arnold & De Wet 1993) as well as pollen of the genera *Cryptadenia* Meisn., *Dais* L., *Englerodaphne* Gilg, *Gnidia* L., *Lachnaea* L., *Peddiea* Harv., *Struthiola* L. and *Synaptolepis* Oliv. was studied by LM and electron

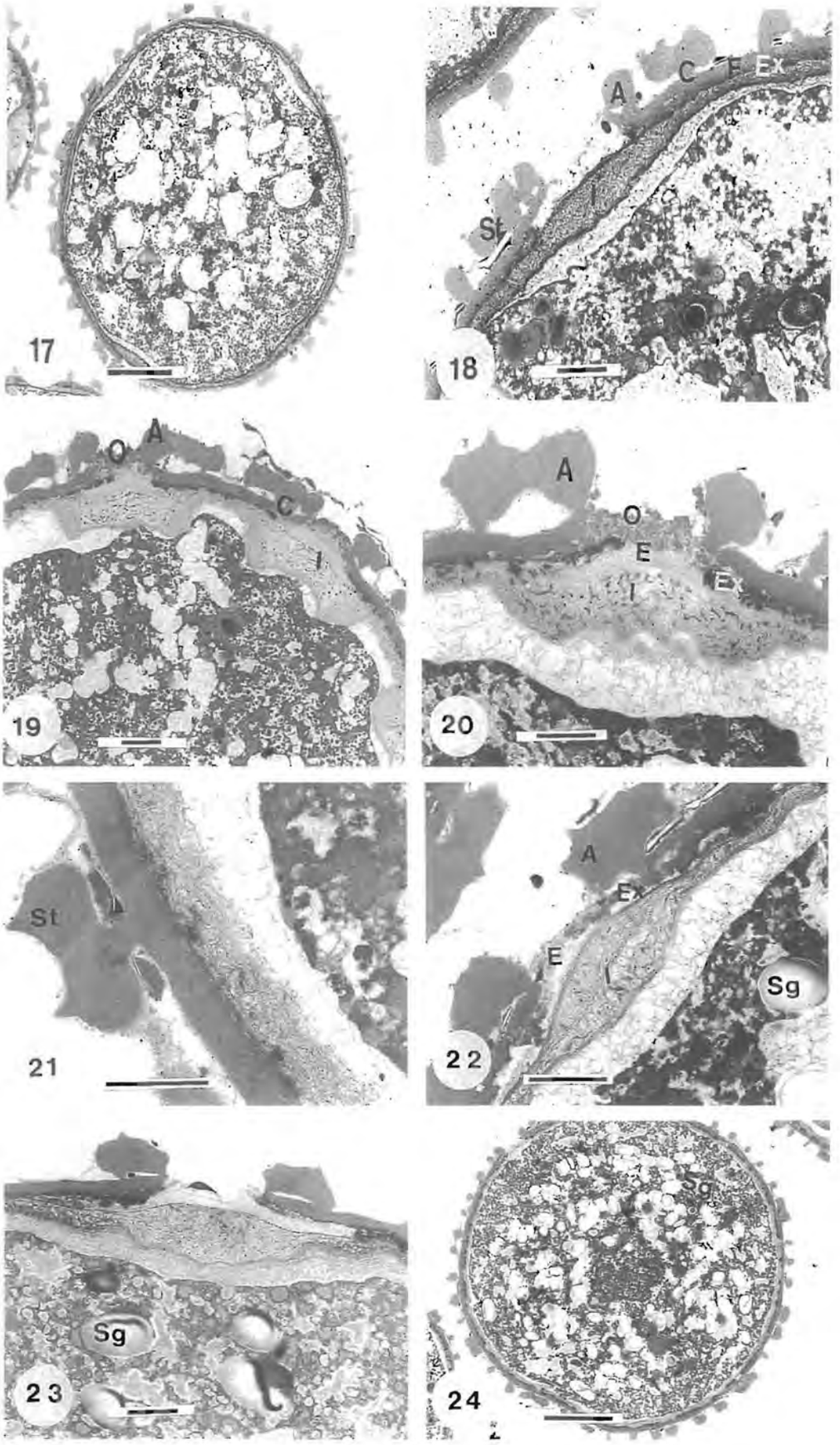
 ISSN 0017-3134



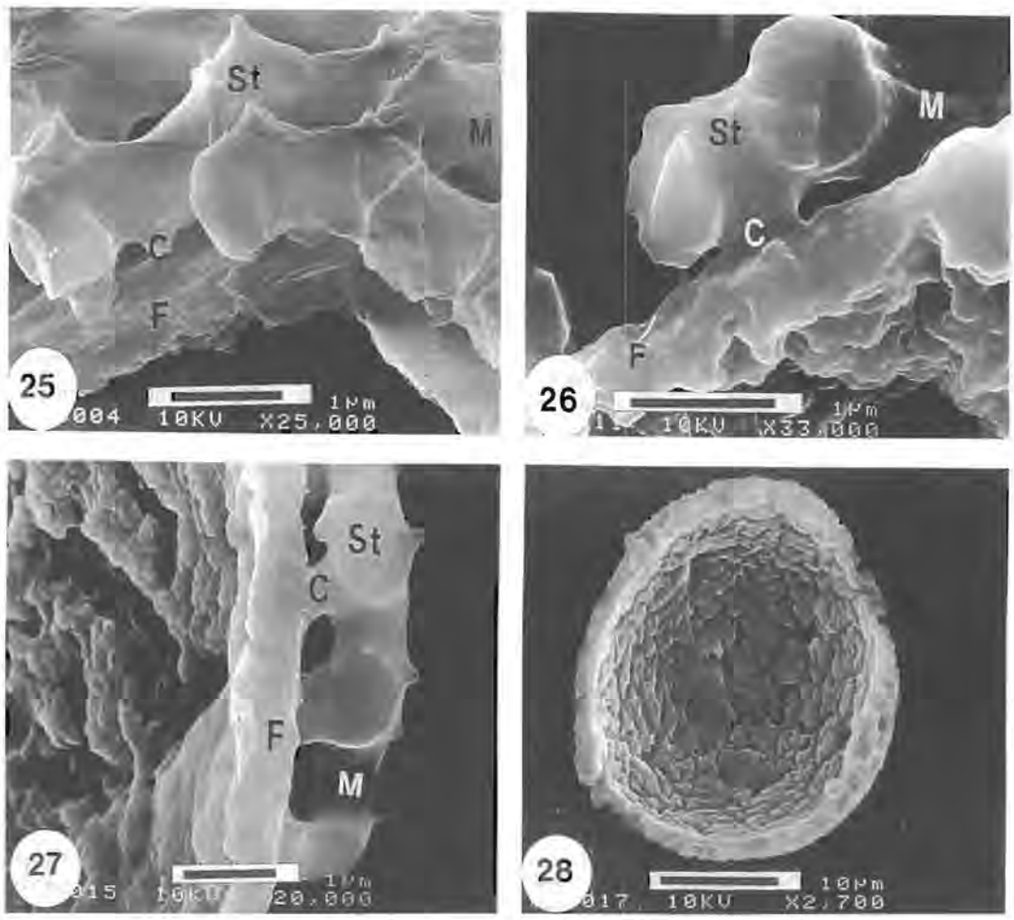
(For description see page 339).



(For description see page 339).



(For description see page 339).



Figs. 25–28. SEM micrographs of fractured pollen grains of *Passerina filiformis* (Killick 238), showing detail of the wall structure. (33–35) Transverse view of fractured wall, illustrating the secondary reticulum. (36) Fractured pollen grain showing surface of internal wall. C = columella, F = footlayer, M = murus and St = secondary reticulum. The black scale bar is 1 µm in Figs. 33–35 and 10 µm in Fig. 36.

microscopy. Anthers with pollen grains were removed from open flowers of herbarium specimens housed in the National Herbarium (PRE). For TEM, fresh flowers were collected, fixed and stored in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine. Species names and voucher specimens are supplied under "Specimens investigated".

SEM

Four methods were followed:

(1) Unacetolized pollen samples were air-dried on SEM stubs, sputter-coated with gold and viewed with an ISI-SX-25 SEM.

(2) Polliniferous samples were sonicated in 50% acetone for four minutes and then collected on 12 µm nuclepore filters locked in a multiple funnel manifold (Bredenkamp & Hamilton-Attwell 1988). Pollen was put through a graded ethanol series and the filters containing the pollen were air dried on SEM stubs, sputter-coated and viewed.

(3) Polliniferous samples were acetolyzed (Erdtman 1960) and thoroughly washed, first with distilled water and then ethanol. For LM, pollen was mounted in glycerine jelly and permanently sealed with entellan (Art. 7961, E. Merck, Darmstadt) (Frip 1983). Measurements of pollen grains were made with a Kontron image analyser and are based on at least 10 grains per sample. For SEM, the pollen-ethanol mixture was air-dried on SEM stubs, sputter-coated with gold and viewed.

Figs. 1–8. SEM micrographs of pollen grains of some southern African members of the Thymelaeaceae. (1) Lachnaea eriocephala (Richardson 15). 2–8 Portions of pollen wall showing detail of exine. (2) *Lachnaea eriocephala* (Richardson 15). (3) *Struthiola ciliata* (Bredenkamp 997). (4) *Gnidia capitata* (Van Rooyen 2178). (5) *Dais cotinifolia* (Germishuizen 5762). (6) *Englerodaphne pilosa* (Geldenhuys 1282); note presence of basal reticulum. (7) *Synaptolepis kirkii* (Ward 8473). (8) *Passerina paleacea* (Pillans 783). All specimens prepared by acetolysis technique (Erdtman 1960). The black scale bar is 10 µm in Fig. 1 and 1 µm in Figs. 2–8.

Figs. 9–16. SEM micrographs of selected species of Passerina showing whole grains as well as portions of pollen wall with detail of exine. (9) Unacetolyzed pollen of *P. ericoides* (Taylor 4042). (10) *P. obtusifolia* (Oliver 3679). (11) *P. paludosa* (Thoday 100). (12) *P. obtusifolia* (Oliver 3679). (13–14) *P. comosa* (Andreae 1288). (15) *P. galpinii* (Burgers 2259). (16) *P. vulgaris* (R.A. Dyer 180). A = annulus, E = endoaperture, O = operculum, and St = secondary reticulum. With exception of Fig. 13, which was prepared by the acetolysis technique, Figs. 10–16 were prepared by means of ultrasonic technique of Bredenkamp & Hamilton-Attwell (1986). The black scale bar is 1 µm in all figures, except for Figs. 10 and 13, where it is 10 µm.

Figs. 17–24. TEM micrographs of selected species of *Passerina* showing ultrathin sections of pollen grains. (17) cross section of whole grain and (18) enlargement showing detail of porus of *P. ericoides* (Bredenkamp 962). (19–21) *P. galpinii* (Bredenkamp 932), (19) part of cross section of whole grain, (20–21) enlargements showing detail of porus and structure of pollen wall. (22) *P. vulgaris* (Bredenkamp 924) showing detail of porus and intine. (23) *P. pendula* (Bredenkamp 908) and (24) *P. vulgaris* (Bredenkamp 924) showing starch granules. A = annulus, C = columella, E = endoaperture, Ex = endexine, F = footlayer, I = intine, O = operculum, St = secondary reticulum and Sg = starch grain. The black scale bar is 1 µm in all figures, except for Figs. 17 and 24, where it is 5 µm.

(4) Acetolyzed grains were crushed at liquid nitrogen temperatures, thawed and dusted onto double sided carbon adhesive tape. Coated with chromium and viewed with a Jeol 6 000 F field emission SEM.

TEM

TEM was used for the study of the wall structure in selected species. Anthers with pollen grains were removed from open flowers fixed in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine. Pollen was rinsed in 0.075 M phosphate buffer, pH 7.4–7.5, postfixed for one hour in 0.25% aqueous OsO_4, washed in three changes of water and dehydrated in a graded acetone series. Quetol 651 resin (Van der Merwe & Coetzee 1992) was used for embedding. Ultrathin sections were contrasted in 4% aqueous uranyl acetate for 10 minutes and rinsed in water three times. The sections were then contrasted with lead citrate (Reynolds 1963) and rinsed in water. A Phillips 301 TEM was used for examination of the sections. Descriptive terminology follows Erdtman (1969) and Punt et al. (1994).

RESULTS

General description of pollen grains of selected members of southern African Thymelaeaceae

Lachnaea eriocephala L. (Figs. 1–2)
Pollen grains monads, spheroid and pantoporate, mean diameter 49 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, attached to nexine by means of columellae which unite into tectum above. Tectum reticulate (basal reticulum) with supra-tectal triangular projections which are trihedral to shallowly trihedral with basal sides straight or emarginate, surfaces of lateral sides striate, with one single central spinule (see also Beyers 1992).
Cryptadenia uniflora Meisn.
Pollen grains monads, spheroid and pantoporate, mean diameter 53 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, sexine attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral, with basal sides straight, surface of lateral sides striate (see also Beyers 1992: 101).
Struthiola ciliata (L.) Lam. (Fig. 3)
Pollen grains monads, spheroid and pantoporate, mean diameter 38 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, sexine attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral, with basal sides straight, surface of lateral sides striate, with one single central spinule and units closely packed.
Gnidia capitata L. f. (Fig. 4)
Pollen grains monads, spheroid and pantoporate, mean diameter 33 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, sexine attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral, with basal sides straight and one single central spinule; some subunits tightly packed, possibly fused, others separated revealing horizontal rods (muri of basal reticulum).
Dais cotinifolia L. (Fig. 5)
Pollen grains monads, spheroid and pantoporate, mean diameter 66 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, sexine attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral; subunits with emarginate margins and fused into groups with exposure of muri of basal reticulum; spinules central and at bases of subunits.
Englerodaphne pilosa Burtt Davy (Fig. 6)
Pollen grains monads, spheroid and pantoporate, mean diameter 36 µm (Table I). Wall tectate and supra-ornate; sexine thicker than nexine, attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral, with one single central spinule, most subunits fused, forming half-circles or various patterns, muri of basal reticulum exposed.
Synaptolepis kirkii Oliv. (Fig. 7)
Pollen grains monads, spheroid and pantoporate, mean diameter 53 µm (Table I). Wall tectate and supra-ornate, sexine thicker than nexine, sexine attached to nexine by means of columellae which unite into tectum above. Tectum reticulate with supra-tectal triangular projections which are trihedral, spinules in groups of 4–6, most subunits almost completely fused, muri of basal reticulum exposed.

Table I. *Pollen diameter in eight genera of the family Thymelaeaceae (measurements in* µm*).*

Taxon	Count (n)	Sum Σ	Mean $\bar{x}$	Standard deviation σ
Cryptadenia uniflora	39	2,060	53	7
Dais cotinifolia	37	2,450	66	3
Englerodaphne pilosa	50	1,800	36	3
Gnidia capitata	80	2,620	33	2
Lachnaea eriocephala	11	540	49	4
Peddiea africana	48	2,270	47	3
Struthiola ciliata	68	2,600	38	3
Synaptolepis kirkii	16	840	53	10

Description of pollen grains in the genus *Passerina* (Figs. 8–28)

Pollen grains monads, spheroid and pantoporate. Mean diameter of grains 32–44 µm (Table II). Pores composite (Thanikaimoni 1986: 120), endoapertures (ora) round or elongate, with uneven margins (Figs. 13–16). Pores 18–44, slightly protruding and larger than lumina of reticulation, distinguished by annuli (Figs. 10–11, 19–20 and 22)
04, 19–20 and 22). Opercula present in pores of unacetolyzed pollen grains (Figs. 9–12 and 19–20). "Supratectal" subunits, (as in typical croton pattern of most Thymelaeaceae), fused completely (Figs. 8, 19–21 and 25–28) to form a continuous secondary reticulum, often exhibiting faint indentations demarcating subunits (Fig. 15). Basal reticulum (rods) as in typical croton pattern no longer discernable (replaced by secondary reticulum derived from fused sexine subunits), and apparently lost through reduction, or fusion with the new reticulum. Spinules present on muri, average number of

Table II. *Pollen diameter in* Passerina *(measurements in* µm*).*

Taxon	Count (n)	Sum Σ	Mean $\bar{x}$	Standard deviation σ
P. burchellii	54	2,260	42	3
P. comosa	54	2,120	39	3
P. drakensbergensis	83	3,030	37	3
P. ericoides	53	2,020	38	10
P. falcifolia	53	2,340	44	4
P. filiformis	42	1,570	37	4
P. galpinii	56	231	42	3
P. glomerata	44	1,760	40	3
P. montana	57	2,050	36	3
P. obtusifolia	61	2,410	39	3
P. paleacea	57	1,870	33	3
P. paludosa	59	2,260	38	2
P. pendula	60	2,280	38	4
P. rigida	51	1,930	38	2
P. rubra	53	1,700	32	2
P. vulgaris	76	2,810	37	3

spinules surrounding a pore 9–28. Intine thickened at apertures, stratified, consisting of outer continuous polysaccharide layer and enzymatic inclusion (Thanikaimoni 1986) (Fig. 22). Horizontal network (muri) supported by columellae implanted on well developed footlayer (Figs. 18–22 and 25–28). Footlayer more strongly developed than endexine which displays granular electron-dense particles. Cytoplasm with many electron-dense particles, also containing large numbers of starch grains.

DISCUSSION

Form and function

In southern Africa *Passerina* is the only genus of the Thymelaeaceae that is wind-pollinated. The plants are ericoid shrublets, shrubs or small trees. They are resprouters, usually growing in sandy soil and even on primary sand dunes along the coast, always exposed to wind. These plants are often pioneers along roadsides or in disturbed places. Leaves are decussate, concave or closely involute, lined with woolly hairs, adapted to dry windy summers. Inflorescences and flowers are also adapted to dry, warm and windy summers as the relatively small flowers are arranged in terminal spikes or heads, subtended by bracts usually broader than the leaves. The calyx is 4-lobed, tube flask-shaped or subcylindric and lobes are spreading. Petals are absent and the flowers are without nectar and odour (Thoday 1924).

Adaptations of the flower to anemophily. – *Passerina* is largely endemic to the Fynbos Biome of the Cape and northern extensions of this biome on the eastern mountains of southern Africa. This biome is characterised by steep coastal mountains giving rise to "berg" winds. Extreme wind conditions appear all along the coastal plains. In the Eastern Cape Kopke (1988) reports persistent north-westerly winds as a feature of summer and winter, and high level winds from the north in summer. In view these wind patterns, the specialised wind pollination of *Passerina* seems very appropriate.

Flowering time is short, mostly between September and October, usually after the rainy season when wind velocities are high. Pollination takes place in the morning when flower colour is yellowish, orangy or pale pink. Calyx lobes are open and eight stamens exceed the calyx lobes in length, exposing anthers which are conspicuously yellow and bulging with pollen. Anthers are extrorse (unique for the Thymelaeaceae in southern Africa) and open explosively. All pollen is shed at once and towards the afternoon the anthers are empty, calyx lobes bend at right angles to the tube, and flower colour becomes a deeper red. The stigma is mop-like (penicillate) and dry.

Wind-pollinated plants are not obligately anemophilous, many may be facultatively entomophilous (Crane 1986). Non-sticky pollen grains could also be transported by insects with sticky or hirsute bodies, relating to amphiphily (Thanikaimoni 1986). The insect visual spectrum ranges from 300 to 700 nm and red tones would be invisible to bees, explaining why bees usually visit flowers which are yellow to blue (Richards 1986). Although the pollination of *Passerina* is mainly anemophilous, the yellow flowers are perceived by insects when the pollen is shed and they serve as secondary pollen vectors. However, birds are attracted to orange or red. This could explain the change in flower colour in *Passerina* from yellow, when the pollen is shed, to red later in the day. The persistent red calyx envelops the developing fruit and the dispersal of fruit by birds is a greater possibility.

Adaptations of pollen to anemophily. – Muller (1979) speculates that the functional significance of the crotonoid pattern is difficult to interpret. It can be considered a system of excrescences which upon volume reduction achieves efficient closure around porelike structures. Its apparently independent evolution in unrelated angiosperms suggests at least some adaptive advantage. A closed smooth surface appears to be a secondary modification in many cases, especially in wind-pollinated species. This trend was apparent in *Passerina* which portrayed a much smoother secondary reticulum.

Grain size and transport. – Effective wind transport obligates that the total structure of the pollen grain is influenced by aerodynamic considerations. Wind-dispersed pollen should be rather small, light, smooth and not sticky (lacking pollenkit) (Punt 1986). The diameter of the pollen of *Passerina* fell within the range of 32–44 µm. Muller (1979) and Crane (1986) agree that wind-dispersed grains fall within a spectrum of 20–30(–60) µm. For a typical wind-dispersed pollen grain (diameter 30 µm) in still air at 20 °C, the Reynolds number is around 0.1 at a terminal settling velocity of 5 cm s^{-1} (Crane 1986).

Wall structure. – The pollen wall of *Passerina* has a reticulum secondarily derived from the complete fusion of what was originally supratectal elements (still present in those extant Thymelaeaceae with crotonoid pollen). It clearly represents an evolutionary stage towards the development of a smoother exine sculpture. Smooth pollen can be advantageous in wind pollination as it serves to decrease pollen clumping and secondarily it allows reduction in effective radius without an overall thinning of the exine (Wodehouse

1935). The loss of ornamentation in anemophilous plants can be due to the energetic cost of sporopollenin Bolick (1990). One may speculate that, given enough time, grains of *Passerina* may loose their reticulate sculpture and become psilate. The present reticulate state may reflect a historical constraint introduced by the basal reticulum from which the current reduction has been derived.

Muller (1979) speculates that changes in pollen occur in response to selective pressure, any exine structure can be regarded as a compromise between the protective, harmomegathic and reservoir functions. The protective function of the pollen wall in *Passerina* was evident in the radial and tangential differentiation of exine layers which absorb the bending stresses during hydration and dehydration (Thanikaimoni 1986). The conspicuous homogeneous foot-layer (sole) also serves as a closed sealing layer.

Harmomegathic mechanisms involve the reaction of the complete pollen wall to the turgor pressure of the cytoplasm (Blackmore & Barnes 1986). Pantoporate grains of *Passerina* are well adapted to contraction upon dehydration and stretching during rehydration. The columellae in the pollen wall increase the possibility of bending and it has a well developed non-sporopollenous intine and aperture membranes (Figs. 18–20) which would be more capable of stretching and contracting than the exine. Intine is constrained by the exine, except at the apertures where it is distinctly thickened.

Reservoir function can be connected with characteristic cavities which hold tapetum-derived materials which play a significant role in producing an adhesive surface or as recognition substances. Thanikaimoni (1986) reports that pollen grains of anemophilous species have scanty electron-lucent heterogeneous pollenkit locked in the exine cavities and are less or not adhesive.

Apertures. – The pantoporate condition found in *Passerina* has obvious selective advantages. Protection of the cytoplasm and ease of germination are two factors with contradictory requirements. The pantoporate condition favours the reduction of large apertures for the protective function and compensates by optimising germination by increased number of apertures (Punt 1986). Increase in aperture number would obviously reduce the mechanical efficiency of the wall but this is taken care of by the compensative increase in exine thickness at the non-apertural region (Thanikaimoni 1986). The operculum is acquired to protect the water content of the pollen grain. Punt (1986) argues that smaller apertures inhibit desiccation. The xeromorphic adaptation of multi-aperturate operculate pollen grains with thick exine has a selective advantage in that it combines the mechanism against desiccation and that for quick germination to ensure rapid fertilisation (Thanikaimoni 1986). Multiple apertures also play an important role in the process of rehydration of the pollen grains as this process depends on the establishment of continuous water films between the apertural intine and the dry stigma surface. More apertures that are in contact with the stigma would obviously be activated and germination of pollen tubes would be more effective.

Dehydration. – TEM sections of the pollen grains of *Passerina* indicated that the grains varied considerably in their degree of hydration at the time of dispersal (Figs. 18–19). Starch granules were evident in grains of all members of the genus (Figs. 22–24). According to Heslop-Harrison (1979) the developing pollen lies immersed in the locular fluid during the first period of partial dehydration in the anther. Water will be withdrawn along water potential gradients occasioned by deficits developed elsewhere in the anther. The accumulation of starch at this time will presumably steepen the gradient by sequestering osmoticum and raising the water potential, thus enhancing the dehydration of the pollen grain. Baker & Baker (1979) claim a relationship between the presence of starch and wind pollination, this could possibly be due to the reduction in the mass of the pollen grain after dehydration. Further dehydration occurs in the air and desiccation attributable to the environment is most extreme in wind-dispersed pollen, demanding xeromorphic adaptations. Contrary to what one would expect, starch was also present in grains of all other southern African genera of Thymelaeaceae.

Settling on stigma. – In *Passerina* the stigma was mop-like (penicillate). According to Crane (1986) it is unlikely that plumose stigmas function as true sieves in angiosperms; it is more likely that inertial impaction is the means by which pollen becomes impacted on the stigma, but the possibility of electrostatic attraction should not be underestimated. However, this stigma type is normally associated with wind pollination.

Rehydration. – The source of water entering the pollen is the stigma. Stigmas without a free-flowing secretion pool are termed "dry" (Heslop-Harrison 1979). Our observation showed that the stigma in *Passerina* possibly represented the "dry" type. Heslop-Harrison (1979) states that dry stigmas offer rather difficult conditions for pollen hydration, and adjustment to these must require specialization of the exine and the apertural mechanisms. The evolution of porate exines seems to be such a specialization as porate exines are associated with dry stigmas. Apertures closest to the stigma form the first routes for the ingress of water. Enzymatic softening of the intine begins earlier at these sites.

Compatibility, pollen tube mechanism. – In *Passerina* the apertural intine was clearly stratified (Fig. 22). The enzymatic inclusion is sealed above and below by a continuous polysaccharide layer until hydration. The outer layer is then loosened and ultimately becomes disrupted with release of the underlying enzymes. The inner layer then becomes the precursor of the pollen tube. It conveys with it the poral intine with its enzyme load which degrades the cuticle of the stigmatic papilla (Thanikaimoni 1986). The functions of the enzymatic load of the apertural intine are still to be explained, but they play a role in: (a) softening of the intine at the germination site, as essential prelude to emergence of the tube tip, and (b) early interactions with the stigma, most probably during penetration of the cuticle and perhaps in the early nutrition of the pollen tube (Heslop-Harrison 1979).

Table III. *Summary of key pollen morphological characters in southern African Thymelaeaceae.*

CHARACTER	TAXON							
	Lachnaea eriocephala 49	*Cryptadenia uniflora* 53	*Struthiola ciliata* 38	*Gnidia capitata* 33	*Dais cotinifolia* 66	*Englerodaphne pilosa* 36	*Synaptolepis kirkii* 53	*Passerina* 32–44
Mean diameter µm								
Sexine triangular projections								
Shape	Trihedral						Indeterminate due to fusion	
Basal sides	Straight or emarginate		Straight		Emarginate	Indeterminate due to fusion		
Lateral sides	Striate			Smooth	Striate		Smooth	
Spinule	Single and central				Central and at base	Single and central	Groups of 4–6	Variable numbers
Arrangement	Separate		Separate, closely packed	Possibly fused, tightly packed	Fused into groups	Fused, forming patterns	Almost completely fused	Completely fused forming a secondary reticulum
Horizontal rods (basal reticulum)	Present							Absent

Taxonomic implications

In the present study a continuum of variation in the distinction of the triangular subunit of the croton pattern (Nowicke et al. 1985) for the southern African genera of Thymelaeaceae was illustrated (Figs. 1–8). In *Lachnaea eriocephala* (Figs. 1–2) the trihedral subunits had emarginate basal sides and the surfaces of the lateral sides were striate, with one single central spinule. In *Struthiola ciliata* and *Gnidia capitata* (Figs. 3–4) the subunits had straight basal sides, but in *Gnidia capitata* some subunits were tightly packed, possibly fused, while others were separated revealing mural rings. In *Dais cotinifolia* (Fig. 5) the subunits had emarginate margins, spinules were present at the bases of the subunits and fusion of the subunits into groups, with exposure of the mural rings, was clearly visible. In *Englerodaphne* (Fig. 6) most of the subunits were fused forming half-circles or an array of patterns, while fusion was almost complete in *Synaptolepis* (Fig. 7). The pollen of *Passerina paleacea* (Fig. 8) could be regarded as the climax of this continuum of variation, as all the subunits had fused completely to form a continuous secondary reticulum. The pollen wall of some members of *Passerina* still exhibited faint indentations demarcating the subunits (Figs. 8, 15), while the pollen wall was devoid of striation in many others.

Taxonomic position of the Thymelaeaceae

The Thymelaeaceae comprises about 500 species in 50 genera (Airy Shaw 1973, Cronquist 1981). A survey of the literature of the Thymelaeaceae reveals the confusion that exists with regard to the identity of the family and its taxonomical and phylogenetic relationships. Domke (1934) envisages a genetic relationship between the Thymelaeaceae, Malvaceae and Euphorbiaceae. Heinig (1951) discusses the relationships of the Thymelaeaceae with the Myrtales, Saxifragaceae, Lythraceae, Gonystylaceae and Malvales and comes to the conclusion that a polyphyletic origin of the Thymelaeaceae could be sought in both the Flacourtiaceae and Tiliaceae. Cronquist (1968) considers the Thymelaeaceae as completely at home in the Myrtales on account of the more primitive genera having an obviously compound pistil and he is convinced that the ancestry of the Myrtales lies in the Rosales. Takhtajan (1969) considers the Thymelaeales to have a common origin with the Euphorbiales and Malvales, all arising from a Flacourtiaceae-type ancestor. According to Archangelsky (1971) both the Euphorbiales and the Thymelaeales belong to the subclass Dilleniidae and originated from ancestral lines of the Dilleniales→Violales→Malvales. Hutchinson (1973) considers the phylogeny of the family as Magnoliales→Dilleniales→Bixales→Gonystylaceae→Thymelaeaceae.

Dahlgren (1975*a*, 1975*b*) places the Thymelaeales between the Euphorbiales and the Myrtales. In his treatment of the angiosperms (Dahlgren, 1980), the dicotyledons are divided into 24 superorders. Within the superorder Malviflorae a close affinity between the Malvales and Euphorbiales, as well as their affinity to the Urticales and the Thymelaeales, is recognised. In the classification diagram the relative positions approximated to the mutual similarity of attributes do not

reflect phylogenetical affinity between the Malviflorae and Myrtiflorae. The inclusion of the Thymelaeaceae in the Myrtales is overviewed by Dahlgren & Thorne (1984). Most members of the family possess Myrtalean characters of which some of the most important are intraxylary phloem, tough fibres permeating the phloem, 4-merous, perigynous flowers and an obturator descending from the base of the stylar canal to the ovules. On the other hand, the embryological and chemical evidence strongly argues against Myrtalean affinities. The very distinctive pollen of the Thymelaeaceae (which is also illustrated by this paper) is totally removed from that of any Myrtales and similar to that of most Euphorbiaceae. Mere sharing of the crotonoid pattern between taxa should never be interpreted as certain evidence of close evolutionary relationship. Note, however, that despite its very distinct appearance, the croton pattern has undoubtedly evolved convergently in many unrelated angiosperms (e.g. *Aragoa*-pollen (Scrophulariaceae), Nilsson & Hong1993) and is also present from an early stage in the fossil record (Muller 1979). Gertrud Dahlgren's (1989) diagram, illustrating a modified classification of the dicotyledons, sensu Dahlgren (1980), still maintains the close affinity between the Malvales, Euphorbiales, Urticales and Thymelaeales, included in the superorder Malvanae. In the latest revision of the classification of the Class Angiospermae, Thorne (1992) accepts the superorder Malvanae, but includes the Thymelaeaceae in the order Euphorbiales.

In spite of the views of Dahlgren & Thorne (1984), Cronquist (1988) argues that it is unnecessary to place the Thymelaeaceae in any other order than the Myrtales. He suggests the recognition of an order Thymelaeales, providing for this one family, but is of the opinion that this order would still stand alongside the Myrtales.

Relationships of taxa within the Thymelaeaceae

Archangelsky (1966) reviewed the pollen of 52 genera of the Thymelaeaceae and two of the Gonystylaceae. The reticulate pollen of *Octolepis* Oliv. is considered as the most primitive and crotonoid ("stellate" sensu Archangelsky) sculpturing is one of the complex variables of reticulate sculpturing. Typical crotonoid pollen are found in the genera *Dirca* L., *Ovidia* Meisn. and *Dicranolepis* Planch., while in the genera *Pimelia* Banks *et* Soland., *Gnidia* L., *Passerina* L., *Lethedon* Spreng., *Solmsia* Baill., *Daphnimorpha* Nakai, and Gonystylaceae the sculpture characteristic of the exine deviates from crotonoid to cryptocrotonoid ("cryptostellate"). Although Domke (1934) places the genera *Gonystylus* Teijsm. & Binn. and *Amyxa* Tiegh. in a subfamily Gonystyloideae of the Thymelaeaceae, the unique exine of the pollen of Gonystylaceae is so different that Archangelsky (1971) and Nowicke et al. (1985) distinguish the Gonystylaceae as a separate family. Archangelsky (1971) considers the Passerininae as a subtype, distinct from the pollen subgroups Dicranolepideae, Phalerieae, Daphneae, Thymelaeinae and Gnidiinae, with the pollen of *Thymelaea* as the common ancestral type.

Although the pollen of the genus *Passerina* is considered as "cryptostellate" (Archangelsky 1966, 1971), our study shows that the exine differs totally from that of the unique Gonystylaceae as well as the Thymelaeinae and Gnidiinae. The present study shows the continuum of variation from the separate triangular subunits on a basal reticulum (interconnecting tectal rods) of the croton pattern (Nowicke et al.1985) to the plain reticulum in *Passerina*. The reticulum in *Passerina* can be regarded as secondary, and derived through fusion of the supratectal trihedral subunits of an ancestral type depicting the typical croton pattern, accompanied by a loss of the basal reticulum.

Phylogeny

Most southern African genera of the Thymelaeaceae possess pollen with the crotonoid pattern which is known to have emerged early in the fossil record (Muller 1979). In the context of land plants, wind dispersal is widespread and probably a primitive condition. Wind pollination, however, is considered secondary and the initial shift to anemopily is thought to have occurred in the dry to seasonally dry tropics during the mid-Cretaceous (Crane 1986). Thanikaimoni (1986) discusses the phylogenetic value of apertural forms and concludes that the periporate pollen of *Buxus* L. can be derived from a tricolpate type by reduction of apertural area. The columellate wall in *Passerina* consists of an outer tectum and an infrastructure of cylindrical columns resting on a basal layer. This wall type is highly organised and occurs primarily in the angiosperms. Although the columellate pattern occurs in grains attributed to the earliest angiosperms, it is also present in a number of pre-Cretaceous pollen types, one from as early as the upper Carboniferous (Taylor & Zavada 1986). These authors suggest that plants with alveolar pollen walls might be more probable candidates as angiosperm ancestors than those with the homogeneous, unorganised pollen walls. In relation to the other southern African genera of the Thymelaeaceae, the pantoporate pollen grain of *Passerina* with its uniquely derived secondary reticulum can be regarded as phylogenetically advanced.

CONCLUSION

We agree with the ordinal placement of the Thymelaeaceae proposed by Dahlgren (1980). Concerning the classification within the Thymelaeaceae, the system of Domke (1934) has become outdated as Archangelsky (1971: Fig. 10) instated the new subfamilies Octolepidoideae, Microsemmatoideae and Synandrodaphnoideae as well as the family Gonystylaceae. Melchior (1964) has already stated that the tribe Gnidieae should be Thymelaeeae. However, if it is taken into consideration that the genus *Passerina* is anemophilous, lacks petals and petaloid scales, possesses a perigynous flower, extrorse anthers and pantoporate pollen with a secondary reticulum derived through fusion of sexine elements (and loss of typical crotonoid basal mural rings), it seems obvious that *Passerina* is phylogenetically more advanced than other genera in the subfamily Thymelaeoideae. Pollen of *Passerina* also represents the end result of an evolutionary specialization towards anemophily. Although it is recognised that the croton pattern (e.g. most Thymelaeaceae) is derived from the reticulate pattern (*Octolepis*), one must guard against equating all reticulate

sculpturing as primitive, as indicated by the secondary reticulate pattern of *Passerina*.

As Archangelsky (1971), on the basis of pollen morphology, suggested *Thymelaea* as the common ancestor of the Aquilariidae and the subfamily Thymelaeoideae, it seems appropriate not to form a new subfamily, but to raise the taxonomical position of the subtribe Passerininae (under the tribe Gnidieae) to the tribe Passerineae.

Tribus Passerineae (Domke) Bredenkamp & Van Wyk, stat. nov.

Subtribus Passerininae Domke in Untersuchungen über die systematische und geographische Gliederung der Thymalaeaceen: 108, 1934.

At the species level in *Passerina*, however, it is clear that although there are differences in the number of pores, the width of the annuli and the number of spinules, there does not seem to be clear discontinuity between species. An artificial classification at this level was therefore abandoned.

ACKNOWLEDGEMENTS

The authors wish to thank the National Botanical Institute under whose auspices this study was undertaken, Dr. S. Perold for assistance with the SEM, Mmes. H. du Plessis, A. Romanowski and C. Steyn for technical assistance, and Prof. J. Coetzee and Mr. C. van der Merwe, of the University of Pretoria, for assistance with the TEM.

SPECIMENS INVESTIGATED

Details of specimens examined. Fresh material collected for the TEM study of the wall structure is marked with an asterisk (*). All specimens are housed at PRE.

Cryptadenia uniflora Meisn. Western Cape; Letty 194
Dais cotinifolia L. Mpumalanga; Germishuizen 5762
Englerodaphne pilosa Burtt Davy. Western Cape; Geldenhuys 1282
Gnidia capitata L.f. Gauteng; Van Rooyen 2178
Lachnaea eriocephala L. Western Cape; Richardson 15
Passerina burchellii Thoday. Western Cape; Bolus 684
P. comosa C.H.Wr. Western Cape; Andreae 1288
P. drakensbergensis Hilliard & B.L.Burtt. KwaZulu-Natal; Edwards 974
P. ericoides L. Western Cape; Taylor 4042
**P. ericoides*. Western Cape; Bredenkamp 962
P. falcifolia C.H.Wr. Western Cape; Tysson 1449
P. filiformis L. KwaZulu-Natal; Killick 238
P. galpinii C.H.Wr. Western Cape; Burgers 2259
**P. galpinii*. Western Cape; Bredenkamp 932
P. glomerata Thunb. Western Cape; Taylor 6145
**P. glomerata*. Western Cape; Bredenkamp 973
P. montana Thoday. Mpumalanga; Giess 13136
P. obtusifolia. Thoday. Western Cape; Oliver 3679
**P. obtusifolia*. Western Cape; Bredenkamp 904
P. paleacea Wikstr. Western Cape; Pillans 783
**P. paleacea*. Western Cape; Bredenkamp 961.
P. paludosa Thoday. Western Cape; Thoday 100
P. pendula Eckl. & Zeyh. Eastern Cape; Fourcade 3043
**P. pendula*. Eastern Cape; Bredenkamp 908
P. rigida Wikstr. KwaZulu—Natal; Ward 7211
P. rubra Thoday. Eastern Cape; Acocks 22365
P. rubra. Western Cape; Bredenkamp 905
P. vulgaris Thoday. Western Cape; R.A. Dyer 180
**P. vulgaris*. Eastern Cape; Bredenkamp 924
Peddiea africana Harv. Western Cape; Haasbroek 1908
Struthiola ciliata (L.) Lam. Western Cape; Bredenkamp 997
Synaptolepis kirkii Oliv. KwaZulu-Natal; Ward 8473

REFERENCES

Airy Shaw, H. K. 1973. J. C. Willis's dictionary of the flowering plants and ferns. 8th ed. – Cambridge University Press, Cambridge.

Archangelsky, D. B. 1966. Pollen grains of the Thymelaeaceae and Gonystylaceae. (In Russian). – Botanical Journal, Moscow 51(4): 484–494 (In Russian).

Archangelsky, D. B. 1971. Palynotaxonomy of the Thymelaeaceae s.l. – In: Pollen morphology (ed. L. A. Kuprianova & M. S. Jakovlev), pp. 104–234. – Academy of Science, USSR, Komarov Botanical Institute, Leningrad (In Russian).

Arnold, T. H. & De Wet, B. C. 1993. Plants of southern Africa: names and distribution. – Memoirs of the Botanical Survey of South Africa 62: 515–519.

Baker, H. G. & Baker, I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. – American Journal of Botany 66: 591–600.

Beyers, J. B. P. 1992. The generic delimitation of *Lachnaea* and *Cryptadenia* (Thymelaeaceae). – M.Sc. Thesis, University of Stellenbosch, Stellenbosch.

Blackmore, S. & Barnes, S. H. 1986. Harmomegathic mechanisms in pollen grains. – In: Pollen and spores: form and function (ed. S. Blackmore & I. K. Ferguson), pp. 137–149. – Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto.

Blaise, S. 1959. Contribution à l'étude caryologique et palynologique de quelques Thyméléacées. – Revue Generale de Botanique 66 (781): 109–161.

Bolick, M. R. 1990. The pollen surface in wind pollination with emphasis on the Compositae. – In: Plant systematics and evolution, Suppl. 5 (eds. M. Hesse & F. Ehrendorfer), pp. 39–51. – Springer, Vienna – New York.

Bond, P. & Goldblatt, P. 1984. Plants of the Cape Flora: a descriptive catalogue. – Journal of South African Botany 13: 429–434.

Bredenkamp, C. L. & Hamilton-Attwell, V. L. 1988. A filter technique for preparing pollen for scanning electron microscopy. – Pollen et Spores 30: 89–94.

Crane, P. R. 1986. Form and function in wind-dispersed pollen. – In: Pollen and spores: form and function (ed. S. Blackmore & I. K. Ferguson), pp. 179–202. – Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto.

Cronquist, A. 1968. The evolution and classification of flowering plants. – Houghton Mifflin Co., Boston.

Cronquist, A. 1981. An integrated system of classification of flowering plants. – Columbia University Press, New York.

Cronquist, A. 1988. The evolution and classification of flowering plants. – The New York Botanical Garden, Bronx, NY.

Dahlgren, G. 1989. The last Dahlgrenogram, system of classification of the dicotyledons. – In: The Davis & Hedge Festschrift, commemorating the seventieth birthday of Peter Hadland Davis and the sixtieth birthday of Ian Charleston Hedge: plant taxonomy, phytogeography, and related subjects (ed. Kit Tan), pp. 249–260. – University Press, New York.

Dahlgren, R. 1975*a*. A system of classification of the angiosperms to be used to demonstrate the distribution of characters. – Botaniska Notiser 128: 119–147.

Dahlgren, R. 1975*b*. The distribution of characters within an angio-

sperm system. I. Some embryological characters. – Botaniska Notiser 128: 181–197.

Dahlgren, R. M. T. 1980. A revised system of classification of the angiosperms. – Botanical Journal of the Linnean Society 80: 91–124.

Dahlgren, R. & Thorne, R. F. 1984. The order Myrtales: circumscription variation, and relationships. – Annals of the Missouri Botanical Garden 71: 633–699.

Domke, W. 1934. Untersuchungen über die systematische und geographische Gliederung der Thymelaeaceen. – E. Schweizerbart's Verlagsbuchhandlung (Erwin Nägele) G. M. B. H., Stuttgart.

Erdtman, G. 1952. Pollen morphology and plant taxonomy. Angiosperms. – Almqvist & Wiksell, Stockholm.

Erdtman, G. 1960. The acetolysis method. A revised description. – Svensk Botanisk Tidskrift 54: 561–564.

Erdtman, G. 1966. Pollen morphology and plant taxonomy. Angiosperms. – Almqvist & Wiksell, Stockholm.

Erdtman, G. 1969. Handbook of palynology. Morphology – taxonomy – ecology. – Munksgaard, Copenhagen.

Frip, P. J. 1983. A method of preserving glycerine jelly microscopic preparations. – South African Journal of Science 79: 228.

Heinig, K. H. 1951. Studies in the floral morphology of the Thymelaeaceae. – American Journal of Botany 38: 113–132.

Heslop-Harrison, J. 1979. Pollen walls as adaptive systems. Annals of the Missouri Botanical Garden 66: 813–829.

Hutchinson, J. 1973. The families of flowering plants. – Clarendon Press, Oxford.

Kopke, D. 1988. The climate of the eastern Cape. – In: Towards an environmental plan for the eastern Cape (ed. M. N. Burton), pp. 44–52. – Rhodes University, Grahamstown.

Linnaeus, C. 1737. Genera plantarum. – Conrad Wishoff, Stockholm.

Linnaeus, C. 1753. Species plantarum. – Laurentius Salvus, Stockholm.

Melchior, H. 1964. A. Engler's Syllabus der Pflanzenfamilien. Bd. 2. – Gebr. Borntraeger, Berlin – Nikolassee.

Mohl, H. 1835. Sur la structure et les formes des graines de pollen. – Annales des Sciences Naturelles 2,3: 148–180; 220–236; 304–346.

Muller, J. 1979. Form and function of angiosperm pollen. – Annals of the Missouri Botanical Garden 66: 593–632.

Nilsson, S. & De-yuan, H. 1993. The taxonomic significance of *Aragoa*-pollen (Scrophulariaceae). – Opera Botanica 121: 275–278.

Nowicke, J. W. 1994. A palynological study of Crotonoideae (Euphorbiaceae). – Annals of the Missouri Botanical Garden 81: 245–269.

Nowicke, J. W., Patel, V. & Skvarla, J. J. 1985. Pollen morphology and the relationships of *Aëtoxylon*, *Amyxa*, and *Gonystylus* to the Thymelaeaceae. – American Journal of Botany 72(7): 1106–1113.

Punt, W. 1961. Pollen morphology of the Euphorbiaceae with special reference to taxonomy. – Wentia 7: 1–116.

Punt, W. 1986. Functional factors influencing pollen form. – In: Pollen and spores: form and function (eds. S. Blackmore & I. K. Ferguson), pp. 97–101. – Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto.

Punt, W., Blackmore, S., Nilsson, S. & Le Thomas, A. 1994. Glossary of pollen and spore terminology. – LPP Contributions Series No. 1. LPP Foundation, Utrecht.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. – Journal of Cell Biology 17: 208–212.

Richards, A. J. 1986. Plant breeding systems. – George Allen & Unwin, London.

Takhtajan, A. 1969. Flowering plants: origin and dispersal. – Oliver & Boyd, Edinburgh.

Taylor, T. N. & Zavada, M. S. 1986. Developmental and functional aspects of fossil pollen. – In: Pollen and spores: form and function (ed. S. Blackmore & I. K. Ferguson), pp. 165–178. – Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto.

Thanikaimoni, G. 1986. Pollen apertures: form and function. – In: Pollen and spores: form and function (ed. S. Blackmore & I. K. Ferguson), pp. 119–136. – Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto.

Thanikaimoni, G., Caratini, C., Nilsson, S. & Grafström, E. 1984. Omniaperturate pollen with striate spines. – Bulletin du Jardin Botanique National de Belgique/Bulletin van de Nationale Plantentuin van België 54: 105–125.

Thoday, D. 1924. XVIII. – A revision of *Passerina*. – Kew Bulletin 4: 146–168.

Thorne, R. F. 1992. Classification and geography of the flowering plants. – The Botanical Review 58: 225–348.

Van der Merwe, C. F. & Coetzee, J. 1992. Quetol 651 for general use: a revised formulation. – Proceedings of the Electron Microscopy Society of Southern Africa 22: 31–32.

Wodehouse, R. P. 1935. Pollen grains: their structure, identification and significance in science and medicine. – McGraw-Hill Book Company, Inc., New York, London.

4.2 Mucilaginous epidermal cell walls

BREDENKAMP, C.L. & VAN WYK, A.E. 1999. Structure of mucilaginous epidermal cell walls in *Passerina* (Thymelaeaceae). *Botanical Journal of the Linnean Society* 129: 223–238.

CONTENTS

Botanical Journal of the Linnean Society (1999), *129*: 223–238. With 16 figures
Article ID: bojl.1998.0218, available online at http://www.idealibrary.com on IDEAL

Structure of mucilaginous epidermal cell walls in *Passerina* (Thymelaeaceae)

CHRISTINA L. BREDENKAMP*

National Botanical Institute, Private Bag X101, Pretoria, 0001 Republic of South Africa

ABRAHAM E. VAN WYK FLS

H.G.W.J. Schweickerdt Herbarium, Department of Botany, University of Pretoria, Pretoria, 0002 Republic of South Africa

Received June 1998; accepted for publication October 1998

Leaves of *Passerina* are inversely ericoid. Adaxial epidermal cells are relatively small; abaxial ones are large and tanniniferous. Mucilaginous epidermal cells are usually present in many Thymelacaceae, including *Passerina*, mainly in the abaxial epidermis. They are unequally divided by a periclinal wall-like septum into two separate compartments: (1) the outer, adjacent to the cuticle, containing mostly tanniniferous substances and (2) the inner, containing mucilage. This type of epidermis has often been incorrectly described as uni-, bi- or multiseriate. Transmission electron microscopy revealed mucilage, characterized by microfibrils, embedded between the innermost wall-like septum and outermost layers of the inner periclinal cell wall. As accumulation of mucilage increases, the innermost (adjacent to the cell contents) layer of the original periclinal cell wall is pressed against the cytoplasm, thus forming a clearly demarcated cellulose periclinal wall which divides the epidermis cell into two compartments, the inner with mucilage and the outer comprising the cell lumen. Existing controversy is critically discussed. Our observations confirm the authenticity of mucilagination in epidermal cell walls.

ADDITIONAL KEY WORDS:—cellulose – gelatinization – hydration – inner periclinal wall – slime – TEM.

CONTENTS

* Corresponding author. Email: clb@nbipre.nbi.ac.za

0024-4074/99/030223+16 $30.00/0

INTRODUCTION

Passerina L. comprises about 17 species, all confined to southern Africa (Thoday, 1924; Bond & Goldblatt, 1984). Members are perennial woody shrublets with ericoid leaves and reduced flowers, adapted to wind pollination. Most of the species are restricted to the Cape Floristic Region. The present paper emanates from a leaf-anatomical survey of the genus, undertaken as part of a monographic study of the group.

An outstanding feature of the leaf epidermis in many members of Thymelaeaceae is the presence of epidermal cells with so-called 'gelatinized' or mucilaginous inner periclinal cell walls. This type of epidermal cell has also been recorded in various other plant families (Solereder, 1908), and is particularly prevalent in taxa from regions with a Mediterranean climate. Following the gelatinization of the inner tangential cell wall, these epidermal cells appear distinctly two-celled, with an inner 'cell' filled with mucilage, and an outer one with a large vacuole, often containing tanniniferous substances. However, the structural interpretation of these cells in the plant-anatomical literature contains many inaccuracies. Hence, although the epidermal structure has been described in many ericoid-leaved members of the Cape and other Mediterranean floras, erroneous structural interpretations are common. For example, Christodoulakis, Tsimbani & Fasseas (1990) interpret the mucilaginous epidermis of *Sarcopoterium spinosum* (L.) Spach (Rosaceae) as "being composed of a flat mucilage-containing outer lumen with very thick outer wall and cuticle, a tannin-containing, mucilage-secreting cell in the middle, with thin walls around it, and a very large innermost lumen with mucilage." According to Gregory & Baas (1989) the tertiary wall has been described as a division wall, but its deposition is not preceded by a true cell and nuclear division. This concept of a multiseriate epidermis, encountered in many papers, is rejected by Gregory & Baas (1989) on the basis that ontogenetic studies are not available.

In spite of reports on the mucilaginous epidermis in leaves of various orders, families and genera by Solereder (1908), Haberlandt (1914), Frey-Wyssling & Mühlethaler (1965), Napp-Zinn (1973) and Frey-Wyssling (1976), Gregory & Baas (1989) doubt the classical interpretation that the inner mucilaginous portion of these cells originates from previously deposited cell wall material which is transformed into mucilage. They maintain that mucilage is deposited by the cytoplasm between the cell wall proper and the plasmalemma. Although this has been described in some taxa, e.g. in epidermal idioblasts of *Hibiscus schizopetalus* (Mast.) Hook.f. (Bakker & Gerritsen, 1992), the interpretation of the mucilaginous epidermis in *Passerina* needed clarification by means of an ontogenetic study. The present paper, based on results from light microscopy (LM) and transmission electron microscopy (TEM), describes the structure of mucilaginous epidermal cells in *Passerina* during maturation.

It also records the common occurrence of mucilaginous epidermal cells in various genera, families and orders and speculates on the ecological value of this adaptation to the Cape Mediterranean climate.

MATERIAL AND METHODS

Fresh leaf material of 16 species of *Passerina* (Appendix) was collected, fixed and stored in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine (a modified Karnovsky fixative, Karnovsky, 1965). Whenever possible, material from at least five different localities was studied for each species.

LM

LM was used for the study of the general leaf anatomy. The tenth leaf from the growing point of a twig was used for sectioning. A 1 mm wide segment of leaf material was cut from the centre of each leaf, thus including the main vein as well as both leaf margins in cross section. Samples were dehydrated, embedded in glycol methacrylate (GMA) and sectioned according to the methods of Feder & O'Brien (1968). Sections were stained in toluidine blue 'O', subjected to the periodic acid-Schiff's (PAS) reaction and mounted in Entellan (Art. 7961, E. Merck, Darmstadt).

TEM

TEM was used for the clarification of the structure of mucilaginous epidermal cell walls observed in the study of the general leaf anatomy. The second, fifth and tenth leaf, from the growing point of *Passerina falcifolia*, *P. paleacea* and *P. ericoides* were used in the TEM study of the wall structure. Leaf segments of $\pm$ 1 mm^2 were fixed in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine (a modified Karnovsky fixative, Karnovsky, 1965). The material was rinsed in 0.075 M phosphate buffer, pH 7.4–7.5, post-fixed for one hour in 0.25% aqueous OsO_4, washed in three changes of water and dehydrated in a graded acetone series. Quetol 651 resin (Van der Merwe & Coetzee, 1992) was used for embedding. Ultrathin sections were contrasted in 4% aqueous uranyl acetate for 10 minutes and rinsed in water three times. The sections were then contrasted with lead citrate (Reynolds, 1963) and rinsed in water. A Phillips 301 TEM was used for examination.

RESULTS

Generalized description of leaf epidermis

Observations are based on light microscopy of cross sections. LEAVES ericoid, adaxial surface concave, forming a groove which is more or less appressed to the stem; abaxial surface convex. ADAXIAL EPIDERMIS (Fig. 1) uniserial, with a thin cuticle. Epidermal cells relatively small, periclinal diameter (10–)15–25(–35) µm, anticlinal

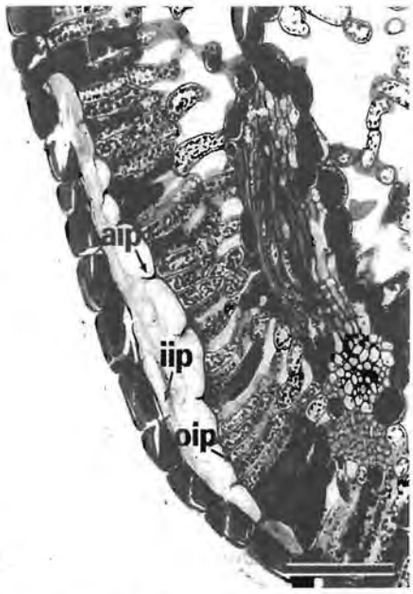
diameter 10–17(–20) µm; cell walls thin; vacuoles large, containing tanniniferous substances. Unicellular hairs and stomata present. MARGIN CELLS larger than adaxial epidermal cells, containing ample amounts of tannin; mucilage scanty. ABAXIAL EPIDERMIS uniserial with cuticle well developed, (10–)20–30(–60) µm thick, smooth or papillate. Trichomes absent, except for unicellular hairs in *P. comosa*. Stomata absent. Epidermal cells more or less oblong in outline; outer periclinal walls straight or convex, inner periclinal walls straight, convex or bulging towards the mesophyll; periclinal diameter of cells (20–)35–45(–60) µm, anticlinal diameter (20–)50–75(–105) µm. MUCILAGINATED CELL WALLS increase progressively from leaf margin to midrib, affecting mainly inner periclinal but also anticlinal cell walls. Mucilage with a layered appearance (also accounting for the misconcept of a multiseriate epidermis, Figs 4–6), occupying about two-thirds of the epidermal cell and separated from the cytoplasm by the innermost cellulose layer of the inner periclinal cell wall (Figs 1–6). CYTOPLASM compressed by mucilage, remaining as a thin layer appressed to the large, usually tanniniferous vacuole. ANTICLINAL LAYER of inner periclinal cell wall often plicate but gradually straightening and often diminishing as mucilage increases in cell walls, eventually breaking under pressure of accumulating mucilage to form a mucilage-filled cavity between the remains of the epidermal cells and large areas of mesophyll (Figs 1–3).

Ontogeny of mucilaginous epidermal cells

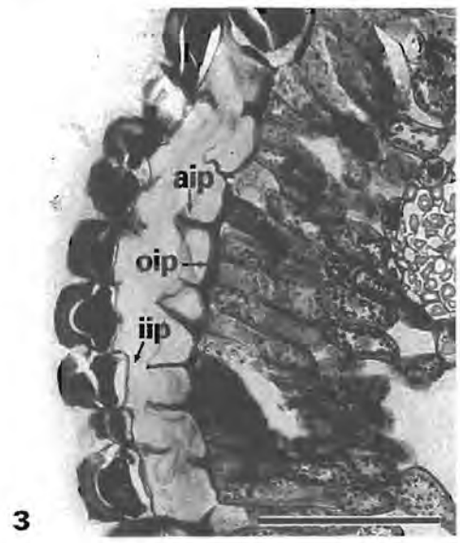
Formation of mucilage in epidermal cell walls is initiated in the second leaf below the growing point. Initially the inner periclinal cell wall thickens and becomes conspicuously striated (Fig. 7). Mucilage accumulates in the centre of the inner periclinal cell wall, resulting in the innermost (iip) and outermost cellulose layers (oip) of the cell wall being pushed apart. Microfibrils are visible in the mucilage and are orientated in the same direction as cellulose fibres of the inner periclinal cell wall (Figs 8 & 9). Both the inner periclinal and adjacent parts of the anticlinal walls are affected by this increasing mass of mucilage. The inner periclinal cell wall appears to be elastic and stretches to accommodate the increasing volume of

Figures 1–6. Light micrographs (LMs) of leaves of *Passerina* species in cross section, illustrating mucilagination of inner periclinal cell walls of epidermal cells. ab = abaxial epidermis, ad = adaxial epidermis, aw = anticlinal cell wall, c = cuticle, cy = cytoplasm, e = epidermal cell, ip = inner periclinal cell wall, aip = anticlinal layer of inner periclinal cell wall, iip = innermost layer of inner periclinal cell wall, oip = outer layer of inner periclinal cell wall, m = mucilage, mf = microfibrils, op = outer periclinal cell wall, p = palisade parenchyma, v = vacuole. Scale bar = 100 µm except in Fig. 4 where = 10 µm. Fig. 1. *P. falcifolia* (Bredenkamp 917), showing adaxial and abaxial epidermis with mucilage accumulating abaxially. Fig. 2. *P. falcifolia* (Bredenkamp 915), area of mucilage accumulation enlarged, mucilage separated from cytoplasm by innermost cellulose layers of inner periclinal cell walls (iip), rupture of anticlinal layers of inner periclinal cell walls (aip) and outer layers of inner periclinal cell walls (oip) forming a boundary between mucilage and palisade parenchyma. Fig. 3. *P. ericoides* (Bredenkamp 962), showing rupture of anticlinal layers of inner periclinal cell walls (aip) in epidermal cells and amorphous mucilage in mucilage cavity. Fig. 4. *P. ericoides* (Bredenkamp 956), illustrating layered appearance of mucilage. Fig. 5. *P. paleacea* (Bredenkamp 961), mucilage (m), occupying about two-thirds of epidermal cell. Fig. 6. *P. paleacea* (Bredenkamp 960), mucilaginous epidermal cells enlarged, illustrating striated appearance of mucilage.

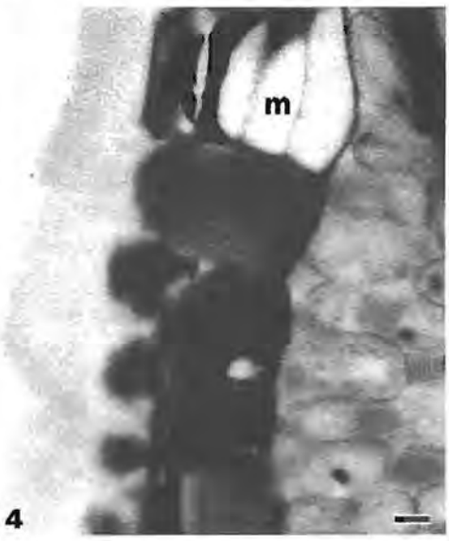
1



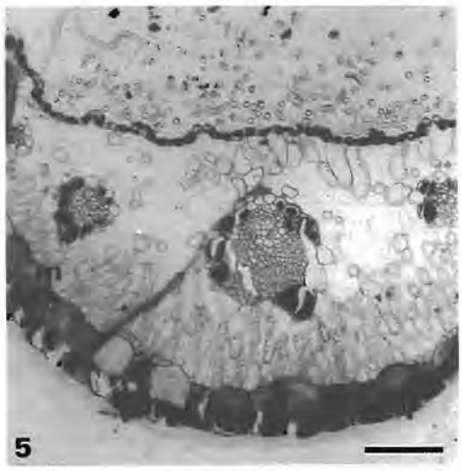
2



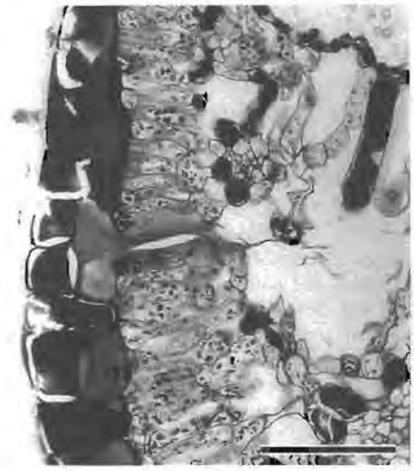
3



4



5



6

mucilage (Fig. 10, aip). The original anticlinal wall is pushed outwards and becomes pronouncedly plicate (Fig. 10, aw). However, anticlinal cell walls may also become mucilaginous, as illustrated by the orientation of the microfibrils of the mucilage accumulated between the anticlinal cell wall and the cytoplasm (Fig. 10, mf). Mucilaginated cell walls expand until the innermost cellulose layer of the inner periclinal wall presses almost against the vacuole, separated only by a very thin layer of cytoplasm (Fig. 10, cy).

Both early and intermediate stages of mucilagination are present in the fifth leaf below the growing tip (Figs 11 & 12). At this stage delineation of mucilage seemingly originating from the inner periclinal wall (aip) and that originating from the anticlinal wall (aw) (Fig. 12) is clearly demonstrated by orientation of microfibrils. The appressed cytoplasm and tannin-filled vacuole are wedged in between layers of mucilage (Fig. 12).

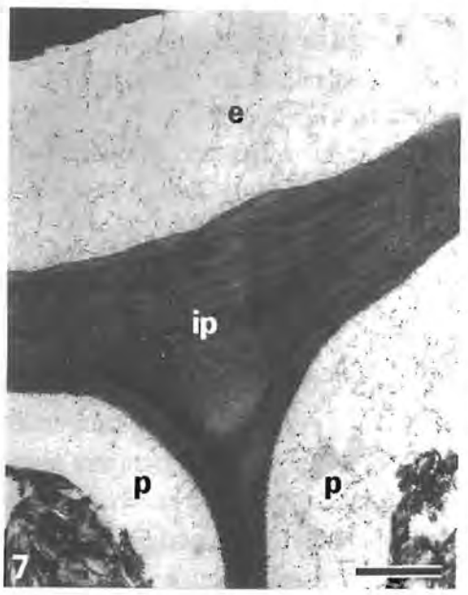
Mucilaginated cell walls stabilize towards the tenth leaf below the growing tip (Figs 13–16). Finally, the epidermal cell is characterized by a tannin-filled vacuole occupying most of the outer compartment of the cell and cytoplasm pressed against the vacuole by the surrounding mucilage (Fig. 13). The innermost cellulose layer of the inner periclinal cell wall 'is swollen' due to the increase of mucilage and becomes very conspicuous, superficially creating the impression of a second, mucilage-filled lumen (Figs 13–15). Mucilage, with microfibrils orientated in the same direction as cellulose of the inner periclinal wall, now occupies a large proportion of the inner portion of the cell. Finally, the anticlinal layers of the inner periclinal cell walls may rupture as a result of the pressure of the accumulating mucilage, and a larger intercellular mucilage-filled cavity is formed. At this stage the mucilage is completely hydrated and the identity of the microfibrils is lost (Fig. 16).

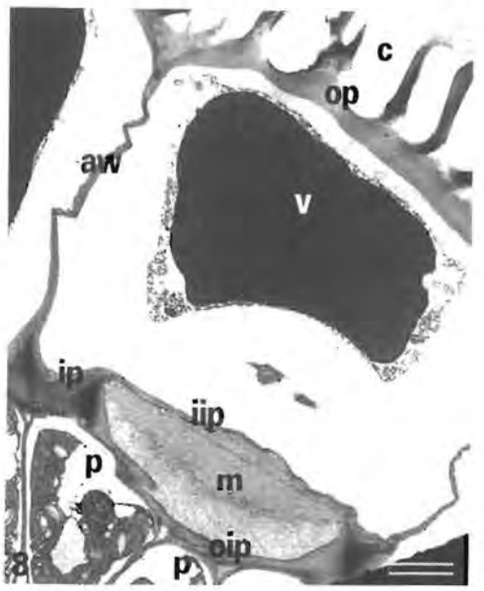
DISCUSSION

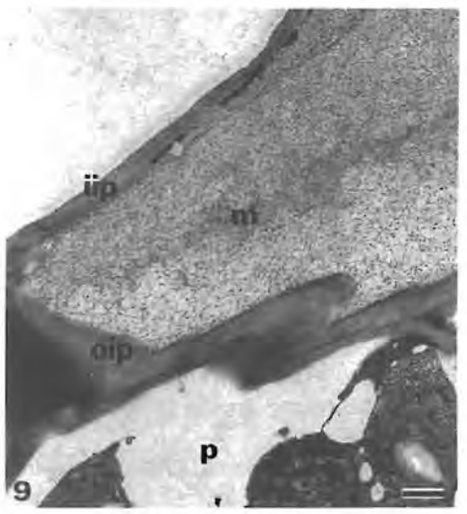
Climate and the distribution of Passerina

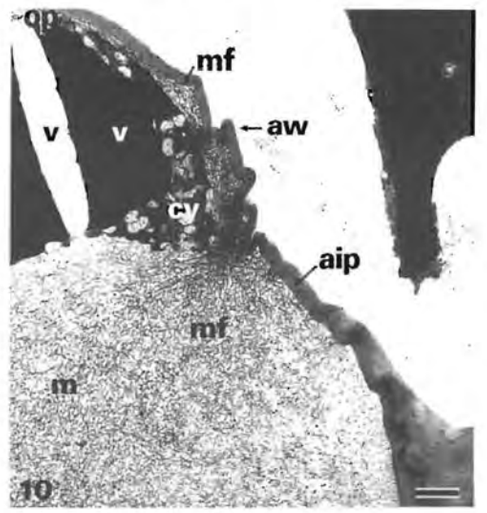
Passerina is confined largely to the Cape Floristic Region, its distribution extending easterly and northerly along the eastern mountains, coastline and escarpment of southern Africa. The climate is for the most part Mediterranean or semi-Mediterranean. In the west it rains in winter, except at high altitudes where moisture from fog and cloud condensation is provided by south-easterly winds in summer.

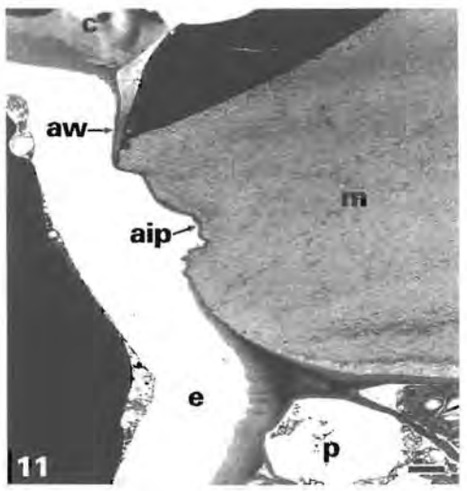
Figures 7–12. Transmission electron micrographs (TEMs) of abaxial epidermal cells in leaves of *P. falcifolia* (Bredenkamp 917) in cross section, showing initiated and progressed mucilagination of inner periclinal and anticlinal epidermal cell walls. Figs 7–10. Representing second leaf and Figs 11 & 12, fifth leaf below growing tip. Scale bar = 1 µm except Fig. 8 where = 5 µm. Fig. 7. Striated inner periclinal cell wall during initiation of mucilagination. Fig. 8. Mucilage accumulated between the innermost and outermost cellulose layers of the inner periclinal cell wall (iip and oip respectively). Fig. 9. Higher magnification of accumulated mucilage in inner periclinal cell wall in Figure 8. Fig. 10. 'Elastic' anticlinal layer of inner periclinal cell wall (aip) and plicate anticlinal cell wall (aw). Fig. 11. Delineation of mucilage originating from inner periclinal wall and that originating from anticlinal wall. Fig. 12. Higher magnification of mucilage in Figure 11, showing orientation of microfibrils correlating with origin from inner periclinal and anticlinal walls.

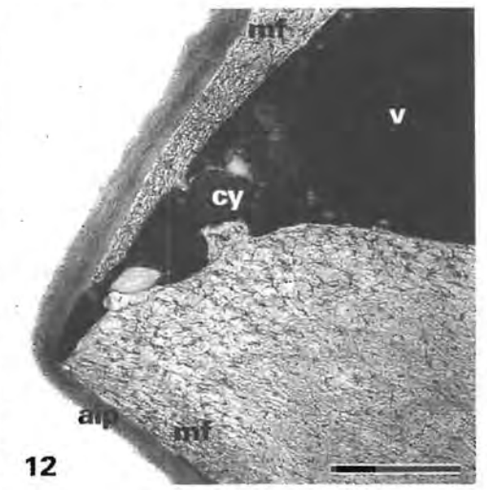












Along the south coast, winter rainfall is complemented by some summer rain which increases eastwards. The western Karoo and Namaqualand are characterized by winter precipitation and summer drought (Campbell, 1985; Cowling *et al.*, 1995). KwaZulu-Natal and the eastern mountains of southern Africa are predominantly summer-rainfall areas.

General morphology of Passerina

The plants are perennial shrublets, shrubs or small trees. They are resprouters, usually growing in sandy soil on plains, mountains and even on primary sand dunes along the coast, always exposed to wind. Some species are often pioneers along roadsides or in disturbed places. Leaves are decussate, concave or closely involute, ericoid and lined with woolly hairs, the latter apparently an adaptation to the dry windy summers of the Mediterranean or semi-Mediterranean climate.

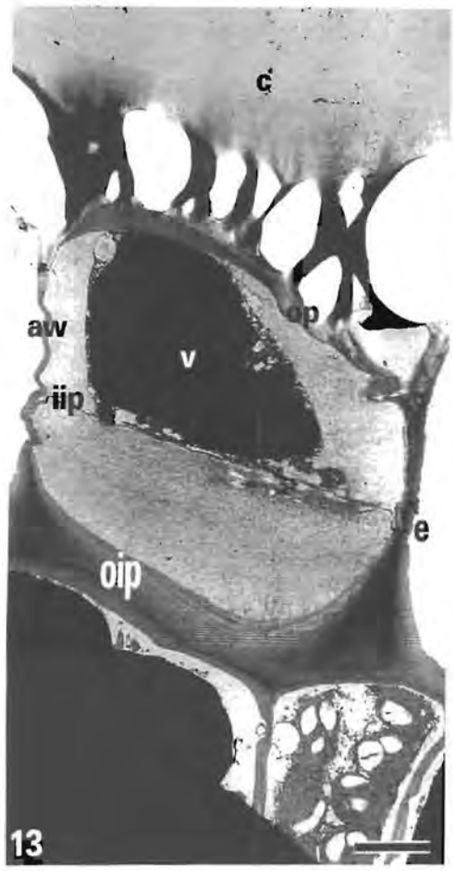
Mucilaginous cell walls in epidermal cells

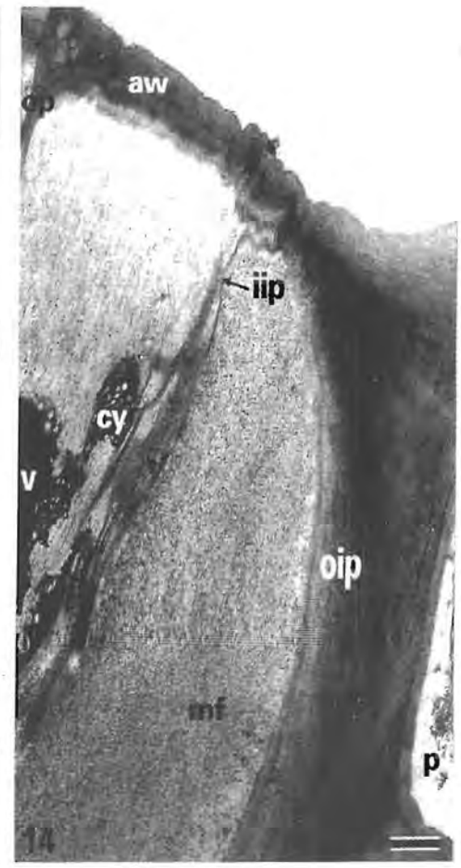
Historical review

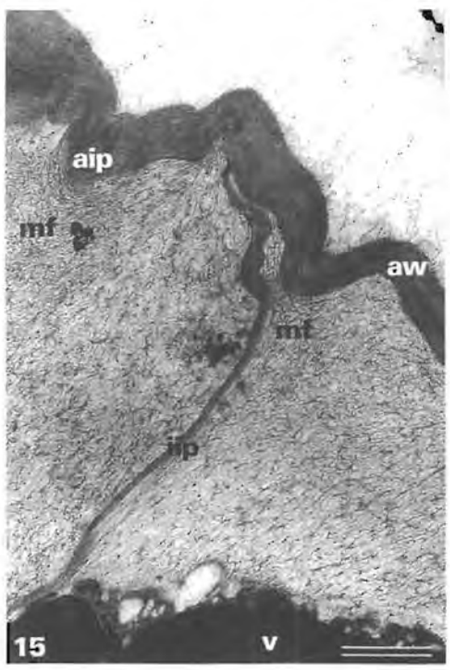
Solereder (1908) documented the 'gelatinization' of the epidermis of the leaf in various orders, families and genera of dicotyledons, including the Thymelaeaceae. Based on the origin of mucilage, he distinguished between two types of mucilaginous epidermal cells, namely those with mucilage derived from the gelatinization of portions of the cell wall, and special mucilage-secreting cells. Haberlandt (1914) described the 'mucilaginous inner walls' commonly present in certain Sapindaceae and Rutaceae, and Napp-Zinn (1973) used the term 'Verschleimung (Gelifikation)' to describe this transformation of the cell wall in reporting on the presence of the phenomenon in many taxa.

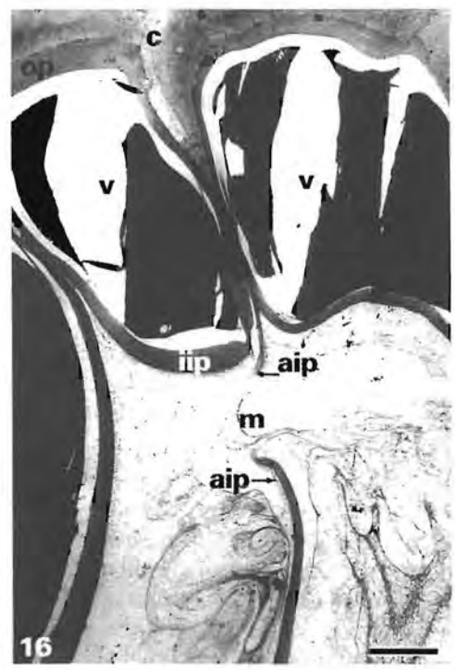
More recently Baas & Gregory (1985) and Gregory & Baas (1989) concluded that, in most cases, mucilage production in cells results from the Golgi apparatus producing numerous vesicles filled with polysaccharides which are deposited between the plasmalemma and the cell wall by reverse pinocytosis. These authors refuted the following interpretations: assimilating function of the cell wall; mucilagination or gelification; previously deposited wall material transformed into mucilage; deposition of cellulose layers on top of unilateral slime deposits in epidermal cells; a tertiary division wall not preceded by true nuclear and cell division; multiseriate

Figures 13–16. **TEM**s of abaxial epidermal cells in leaves of *P. falcifolia* (Bredenkamp 917) in cross section, showing progressed mucilagination of inner periclinal and anticlinal cell walls. Representing tenth leaf below growing tip. Scale bar = 1 μm except in Figures 13 and 16 where = 5 μm. Figs 13–15. Innermost cellulose layer of the inner periclinal cell wall (iip) clearly demarcated. Fig. 14. Larger magnification of right side of epidermal cell in Fig. 13, showing innermost layer of inner periclinal cell wall (iip) separated from outer layer of inner periclinal cell wall (oip) by mucilage containing microfibrils (mf). Fig. 15. Larger magnification of left side of epidermal cell in Figure 13, showing innermost layer of inner periclinal cell wall (iip), separating mucilage originating from inner periclinal wall and that originating from anticlinal wall (aw). Fig. 16. Ultimate rupture of anticlinal layer of inner periclinal cell wall (aip), initiating formation of mucilaginous cavity.









mucilaginous epidermis. Trachtenberg & Fahn (1981) supported the secretory function of the Golgi apparatus in mucilage production, although Fahn (1988) added that mucilage may accumulate either inside the cell wall or in the space between it and the retreating protoplast.

Bakker & Gerritsen (1992) discussed the structure of mucilage cells in the shoot apex and mesophyll as well as the leaf epidermis (single cells or idioblasts) of *Hibiscus schizopetalus*. However, mucilage secretion in these cells should not be confused with mucilagination of epidermal cell walls as indicated for *Passerina* in the present study. Their study reported the involvement of the Golgi apparatus, the rough endoplasmic reticulum as well as the presence of plastids with starch granules in the deposition of mucilage between the plasmalemma and the cell wall. No 'tertiary wall' is formed.

Although many authors agree on the Golgi apparatus as the source of mucilage, Kristen, Liebezeit & Biedermann (1982) found a direct release of polysaccharides and proteins by the tubular components of the endoplasmic reticulum in *Isoetes lacustris* L. Likewise, Trachtenberg (1984) studying mucilage secretion in *Aloe arborescens* Mill., found polysaccharides and structural changes mainly in the plastids of young leaves. In mature leaves secretory evidence appears to be centred in the plasma membrane.

In addition to the above mentioned controversy on the origin of mucilage, many authors have misinterpreted mucilaginous epidermal cells. Yakovleva (1988), reporting on the ultrastructure of slime cells of the leaf epidermis in 35 dicotyledonous plant species, described three types of slime cells based on the location of the slime in relation to the cell wall. In the first type the slime remains within the cell lumen, separated from the cytoplasm by the cell wall. The second type is characterized by the release of slime outside the cell wall, and the third type by the presence of two layers of slime. Yakovleva's erroneous interpretation of the 'first type' (*sic*) is frequent in the literature. What Yakovleva (1988) interpreted as a slime-filled lumen is in reality the central gelatinized part of the inner periclinal cell wall. According to our view, all three types of slime cells fit perfectly into the concept of mucilaginous epidermal cell walls. The interpretation by Christodoulakis *et al.* (1990) on the mucilaginous epidermal cells of *Sarcopoterium spinosum* (Rosaceae), closely agrees with that of Yakovleva (1988). Similar erroneous interpretations have been made by Carlquist (1990) who interpreted the mucilaginous epidermal cell walls in leaves of *Geissoloma* Lindl. ex Kunth as a multiseriate epidermis. Lersten & Curtis (1992) also interpreted epidermal cells with mucilaginous inner periclinal cell walls in certain species of *Polygonum* L. as a biseriate epidermis. In their paper on anatomical adaptations in the leaves of selected fynbos species, Van der Merwe, Van der Walt & Marais (1994) also incorrectly described epidermises with mucilaginous cell walls as multiseriate.

Process of mucilagination of epidermal cell walls in Passerina

Mucilaginous epidermal cells in *Passerina* are the result of cell wall transformation, thus agreeing with the classic interpretation of cell wall 'gelatinization'. Early stages of mucilagination of the periclinal as well as anticlinal cell walls are reflected by conspicuous striations of the affected walls (Fig. 7). The cell walls become progressively more mucilaginous as hydration of the matrix progresses (Frey-Wyssling, 1976). Microfibrils in this cellulose mucilage are orientated in the same direction as the original cellulose wall. As mucilagination continues, faintly marked layer boundaries become discernible. The undulated anticlinal cell wall, as well as the anticlinal layers of the inner periclinal cell wall, stretches to accommodate the increasing volume of

TABLE 1. Comparison of the process of mucilagination in the cell walls of *Passerina* with mucilage formation in mucilaginous cells and idioblasts

Mucilagination of cell walls	Mucilage formation in cells and idioblasts
1. Mostly in epidermal cells of leaves and seed coats. In *Passerina*, most epidermal cells of the abaxial leaf epidermis are affected.	1. In the cortex and mesophyll of stems and leaves, more rarely in the epidermis.
2. Periclinal and anticlinal epidermal cell walls mucilaginous. Inner periclinal wall most active and forming most of the mucilage.	2. Cell walls do not become mucilaginous.
3. Polyuronans of cell wall matrix hydrate and swell to a soluble colloidal mucilage.	3. Mucilage secreted mainly by Golgi apparatus and endoplasmic reticulum, accumulating between cell membrane and cell wall
4. Cellulose mucilage (Frey-Wyssling, 1976) interspersed by ultrastructural microfibrils.	4. Pectin mucilage (Frey-Wyssling, 1976) without ultrastructural microfibrils.
5. Innermost layer of inner periclinal cell wall (erroneous 'tertiary wall') separates mucilage from cytoplasm; appressed to vacuole.	5. No cell wall or part of cell wall (erroneous 'tertiary wall') present. Mucilage accumulates between plasmalemma and original cell wall.

mucilage. The cytoplasm remains clearly delineated from the mucilage by the innermost layer of the inner periclinal cell wall and is appressed to the vacuole containing tanniniferous substances. With further hydration, the layered boundaries of the mucilage become less obvious, the microfibrils disappear and an amorphous jelly remains. At this stage the anticlinal layers of the inner periclinal cell walls between adjacent epidermal cells often disintegrate, causing a cavity bordered by the innermost layers of the inner periclinal cell walls and the original inner periclinal cell walls of a group of epidermal cells (Figs 1–3). This cavity, filled with an amorphous mucilage, forms a lining for the adjacent palisade parenchyma cells.

Mucilagination as described for *Passerina* is a genetically induced attribute of the cell wall and should not be confused with the process of mucilage formation in mucilage idioblasts, mucilage cells and cells bordering mucilage cavities. In these latter cases the mucilage is secreted mainly by the Golgi apparatus and the endoplasmic reticulum and accumulates between the cell membrane and the cell wall. The importance of mucilage formation by the Golgi apparatus should not be underestimated as there are many diverse and important mucilage producing cells present in plants. The two processes are compared in Table 1.

Chemical composition of epidermal cell walls

For the interpretation of cell wall architecture we mainly follow Brett & Waldron (1996). The earliest formed layer of the cell wall is the middle lamella, the next layer deposited by daughter cells is the primary cell wall, which continues to be deposited while the cell is growing in surface area. When cell differentiation takes place, a further secondary wall is laid down. All the wall layers consist of two phases: a microfibrillar phase and a matrix phase. Components of the matrix phase include pectins as well as hemicelluloses. The microfibrillar phase is distinguishable from the matrix phase by its high degree of crystalinity and its relatively homogeneous chemical composition. It is composed of extremely long thin microfibrils consisting of cellulose molecules aligned parallel to the long axis of the microfibril.

Matrix polysaccharides are formed in the endoplasmic reticulum and Golgi apparatus (Ray, Eisinger & Robinson, 1976; Brett & Waldron, 1996), while cellulose

is formed by enzymes in the plasmamembrane (Brett & Waldron, 1996). Wall hemicelluloses are characterized mostly by polyuronans. These substances have a considerable hydration capacity. In contact with water they swell to a soluble colloidal gel with a very high water content and are consequently responsible for the formation of mucilage in cell walls (Frey-Wyssling, 1976).

Classical histologists distinguish between cellulose- and pectin-slimes (Frey-Wyssling, 1976). On a chemical basis this classification seems questionable, but ultrastructurally cellulose-slimes appear to be reinforced by fibrils which are absent in pectin-slimes. Ultrastructural fibres are conspicuous in the mucilaginous cell walls of *Passerina* (Figs 8–15), thus agreeing with the concept of a 'cellulose slime'.

According to Frey-Wyssling (1976) cellulose-slimes comprise cell walls with a matrix swollen to such an extent that their ultrastructural cellulose fibrils become separated from each other. The fibrils can slide along one another, with loss of the original elasticity of the cell wall. In spite of fundamental change in mechanical properties, a gel (mucilage) reinforced by fibrils is still present. This is a rehydration process only, no mucilage is added from the cytoplasm. Frey-Wyssling (1976) reasoned that although cellulase is available in higher plants, it is not used for recycling the glucose components of cellulose. The process of senescence should therefore not be confused with mucilagination.

Possible functions of mucilage

During this century many authors have speculated on the functions of mucilage in plants. Solereder (1908) suggested that gelatinization of the epidermis of the leaf serves for the storage of water. For Haberlandt (1914) the greatly thickened mucilaginous inner walls of epidermal cells probably represent a water storing device, a function which in the typical epidermis would be assigned to the cell sap of the vacuole. In a review of speculation on functions of mucilage cells in vegetative organs, Gregory & Baas (1989) concluded that no ecological preference can be deduced because of a lack of data on habitat and life form, and they emphasized the need for experimental data.

Mucilaginous epidermal cell walls are particularly common in plants from regions with a Mediterranean climate (Van der Merwe *et al.*, 1994). In the seasonally dimorphic subshrub *Sarcopoterium spinosum*, Christodoulakis *et al.* (1990) describe a 'large mucilage lumen' in each of the affected epidermal cells. Although we disagree with their interpretation of the mucilaginous epidermis, the analogy of the epidermal structure between *S. spinosum* and *Passerina* is striking, especially as both are adapted to Mediterranean conditions. They speculate that the 'mucilage lumen' has a similar role to that of the hydatenchyma of plants from arid or salty areas. Water is absorbed during the relatively humid season of spring and conserved for the vital activities of the leaves during the long arid summer. Mucilage may also act as a light density filter protecting the palisade tissue from excessive radiation.

Phenolics in epidermal tissue

Phenolics in some plants from the Cape Floristic Region were studied by Glyphis & Puttick (1988). They concluded that mean seasonal values for all assays increase from lowest concentrations in winter to highest concentrations in autumn. Total

phenols for *Passerina vulgaris* range from 5.2% of dry weight in leaf material in winter to 9.8% in autumn. This increase in phenols from winter to autumn correlates well with the dry warm summers of the Mediterranean climate of the Cape. Ormrod, Landry & Conklin (1995), working on *Arabidopsis thaliana* (L.) Heynh., showed that the presence of UV-absorptive substances in the epidermal cells of leaves protects mesophyll tissue from the harmful effects of UV-B radiation. Hence it is speculated that in *Passerina* the large quantities of phenols in the vacuoles of the epidermal cells (Figs 1–16) may well be a response to UV-B radiation which is high during the dry warm summers of the Cape (Musil & Bhagwandin, 1992).

Speculations on functions of mucilaginous epidermis in Passerina

A four-fold mechanism for the protection of the mesophyll tissue in *Passerina*, a response to the Mediterranean climate of the Cape, is proposed. The hydrophobic cuticle protects the leaves against desiccation. The convex outer periclinal epidermal cell wall focuses light rays onto the mesophyll. Large vacuoles filled with phenols and the mucilage formed by the cellulose-slimes (inner periclinal walls) protect the mesophyll from UV-B radiation. The mucilaginous inner periclinal wall forms excessive quantities of mucilage, resulting in a gelatinous layer or 'slime cushion' with the rupture of adjacent anticlinal layers of inner epidermal periclinal cell walls. A primary function of this mucilage is probably to serve as a regulator of hydration within the leaf, protecting the leaf against water loss at certain times and also to serve as a water-accumulating environment for the development and function of the leaf in times of drought.

Ecology

In *Passerina*, formation of the hydrophilic mucilaginous epidermis is apparently genetically determined and it is, to a greater or lesser extent, present in all species. The modification of the abaxial epidermis, whether strongly mucilaginous or mostly tanniniferous with a few mucilaginous cells, may depend on the humidity of the environment. We suggest that the hydrophilic mucilaginous epidermis allows the accumulation of water, when available, for later use by the plant. This may account for the observation that in *Passerina* increased mucilagination occurs in species of which the plants grow at the sea shore (*P. ericoides*, *P. paleacea*), in high rainfall areas (*P. filiformis*, *P. galpinii*, *P. pendula* and *P. rubra*), on mountain slopes (*P. falcifolia*) and at high altitudes on the Drakensberg Mountains (*P. drakensbergensis*). On the other hand, in species occurring in the arid western Karoo and Namaqualand (*P. glomerata* and *P. comosa*), the abaxial epidermis has cells with large vacuoles almost completely filled with tannin and few cells containing mucilage.

Systematic value

In *Passerina* mucilaginous epidermal cell walls cannot be used as a taxonomic character at species level as the character is present to varying degrees in all species, irrespective of their environment. At the family level, mucilaginous cell walls are regarded as common in the Thymelaeaceae (Solereder, 1908; Metcalfe & Chalk, 1979). It is therefore concluded that mucilagination, as a taxonomic character, is useful at the family level only. In Thymelaeaceae mucilaginous epidermal cell walls

have already been reported in species of the genera *Arthrosolen* C.A. Mey., *Chymococca* Meisn., *Cryptadenia* Meisn., *Daphne* L., *Diarthron* Turcz., *Dicranolepis* Planch., *Edgeworthia* Meisn., *Gnidia* L., *Lachnaea* L., *Lagetta* Juss., *Lasiadenia* Benth., *Lasiosiphon* Fresen., *Linodendron* Griseb., *Linostoma* Endl., *Lophostoma* Meisn., *Ovidia* Meisn., *Passerina* L., *Peddiea* Harv., *Phaleria* Jack, *Pimelea* Gaertn., *Struthiola* L., *Synaptolepis* Oliv., *Thymelaea* Endl. and *Wikstroemia* Endl. (Solereder, 1908; Beyers, 1992).

CONCLUSIONS

Although mucilagination (also referred to as 'gelatinization') of cell walls has been reported by many authors, up to now it has been grossly confused with the process of mucilage formation in specialized mucilage-secreting cells. Moreover, epidermal cells with mucilaginous inner tangential walls have frequently been interpreted erroneously as a biserial epidermis. Our study has shown that the periclinal as well as anticlinal walls of epidermal cells in *Passerina* are conspicuously mucilaginous, positively confirming the authenticity of mucilaginous cell walls, especially in epidermal tissue. The development of mucilagination of epidermal cell walls is probably an advanced state, especially, in *Passerina* where this phenomenon is considered an adaptation to survive the dry warm summers typical of the Mediterranean climate of the Cape Floristic Region. The character is present in all species of *Passerina*, irrespective of their environment. Mucilaginous epidermal cell walls are also well known in other families and genera in the Cape Floristic Region and in the Mediterranean flora.

ACKNOWLEDGEMENTS

The authors wish to thank Mmes H. du Plessis, A. Romanowski and C. Steyn for technical assistance, and Prof. J. Coetzee and Mr C. van der Merwe, of the University of Pretoria, for assistance with the TEM.

REFERENCES

Baas P, Gregory M. 1985. A survey of oil cells in the dicotyledons with comments on their replacement by and joint occurrence with mucilage cells. *Israel Journal of Botany* **34:** 167–186.

Bakker ME, Gerritsen AF. 1992. The development of mucilage cells in *Hibiscus schizopetalus*. *Acta Botanica Neerlandica* **41,1:** 31–42.

Beyers JBP. 1992. The generic delimitation of *Lachnaea* and *Cryptadenia* (Thymelaeaceae). Unpublished M. Sc. Thesis, University of Stellenbosch, Stellenbosch.

Bond P, Goldblatt P. 1984. Plants of the Cape flora: a descriptive catalogue. *Journal of South African Botany* **13:** 1–455.

Brett CT, Waldron KW. 1996. *Physiology and biochemistry of plant cell walls*. 2nd edn. London: Chapman & Hall.

Campbell BM. 1985. A classification of the mountain vegetation of the Fynbos Biome. *Memoirs of the Botanical Survey of South Africa* **50:** 1–121.

Carlquist S. 1990. Leaf anatomy of Geissolomataceae and Myrothamnaceae as a possible indicator of relationship to Bruniaceae. *Bulletin of the Torrey Botanical Club* **117:** 420–428.

Christodoulakis NS, Tsimbani H, Fasseas C. 1990. Leaf structural peculiarities in *Sarcopterium spinosum*, a seasonally dimorphic subshrub. *Annals of Botany* **65:** 291–269.

Cowling R, Richardson D, Paterson-Jones C. 1995. *Fynbos: South Africa's unique floral kingdom*. Cape Town: Fernwood Press.
Fahn A. 1988. Secretory tissues in vascular plants. *Tansley Review* **14:** 229–257.
Feder N, O'Brien TP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* **55:** 123–142.
Frey-Wyssling A. 1976. *The plant cell wall*. Berlin: Gebrüder Borntraeger.
Frey-Wyssling A, Mühlethaler K. 1965. *Ultrastructural plant cytology*. Amsterdam: Elsevier.
Glyphis JP, Puttick GM. 1988. Phenolics in some southern African Mediterranean shrubland plants. *Phytochemistry* **27:** 743–751.
Gregory M, Baas P. 1989. A survey of mucilage cells in vegetative organs of the dicotyledons. *Israel Journal of Botany* **38:** 125–174.
Haberlandt G. 1914. *Physiological plant anatomy*. Reprint edn 1965. New Delhi-5; Today & Tomorrow's Book Agency.
Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27:** 137a.
Kristen U, Liebezeit G, Biedermann M. 1982. The ligule of *Isoetes lacustris*: ultrastructure, mucilage composition, and a possible pathway of secretion. *Annals of Botany* **49:** 570–584.
Lersten NR, Curtis JD. 1992. Foliar anatomy of *Polygonum* (Polygonaceae): survey of epidermal and selected internal structures. *Plant Systematics and Evolution* **182:** 71–106.
Metcalfe CR, Chalk L. 1979. *Anatomy of the dicotyledons*. Vol. 1, 2nd edn. Oxford: Clarendon Press.
Musil CF, Bhagwandin N. 1992. The SUN program for computation of solar ultraviolet spectral irradiances: solar exposure limits in South Africa. *South African Journal of Science* **88:** 406–410.
Napp-Zinn K. 1973. Anatomy of the leaves – II. Leaf anatomy of the angiosperms. In: Zimmermann W, Carlquist S, Ozenda P, Wulff HD, eds. *Encyclopedia of plant anatomy*. Vol. 8, part 2A. Berlin: Gebrüder Borntraeger, 184–189.
Ormrod DP, Landry LG, Conklin PL. 1995. Short term UV-B radiation and ozone exposure effects on aromatic secondary metabolite accumulation and shoot growth of flavonoid-deficient *Arabidopsis* mutants. *Physiologia Plantarum* **93:** 602–610.
Ray PM, Eisinger WR, Robinson DG. 1976. Organelles involved in cell wall polysaccharide formation and transport in pea cells. *Berichte der Deutschen Botanischen Gesellschaft* **89:** 121–146.
Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17:** 208–212.
Solereder H. 1908. *Systematic anatomy of the dicotyledons*. Vol. 2. Oxford: Clarendon Press.
Thoday D. 1924. XVIII. A revision of *Passerina*. *Kew Bulletin* **4:** 146–168.
Trachtenberg S. 1984. Cytochemical and morphological evidence for the involvement of the plasma membrane and plastids in mucilage secretion in *Aloe arborescens*. *Annals of Botany* **53:** 227–236.
Trachtenberg S, Fahn A. 1981. The mucilage cells of *Opuntia ficus-indica* (L) Mill. – development, ultrastructure, and mucilage secretion. *Botanical Gazette* **142:** 206–213.
Van der Merwe AM, Van der Walt JJA, Marais EM. 1994. Anatomical adaptations in the leaves of selected fynbos species. *South African Journal of Botany* **60:** 99–107.
Van der Merwe CF, Coetzee J. 1992. Quetol 651 for general use: a revised formulation. *Proceedings of the Electron Microscopy Society of Southern Africa* **22:** 31–32.
Yakovleva OV. 1988. Slime cells of the leaf epidermis in the dicotyledonous plants (electron microscope data). *Botanical Journal USSR* **73:** 977–987.

APPENDIX: SPECIMENS EXAMINED AND VOUCHER SPECIMENS CITED

Fresh material collected for the TEM study of the wall structure is marked with an asterisk (*). All specimens are housed at PRE.

Passerina burchellii Thoday, *Bolus* 684, Baviaanskloof, Genadendal, Western Cape.
P. comosa C.H.Wright, *Andreae 1288*, Seweweekspoort, Western Cape.
P. comosa C.H. Wright, *Bredenkamp 1034*, Seweweekspoort, Western Cape.
P. drakensbergensis Hilliard & B.L.Burtt, *Edwards 974*, Royal Natal National Park, KwaZulu-Natal.
P. drakensbergensis Hilliard & B.L. Burtt, *Bredenkamp 1018*, Ndedema Gorge, Cathedral Peak Forest Reserve, KwaZulu-Natal.
P. drakensbergensis Hilliard & B.L.Burtt, *Bredenkamp 1019*, Ndedema Gorge, Cathedral Peak Forest Reserve, KwaZulu-Natal.

P. drakensbergensis Hilliard & B.L.Burtt, *Bredenkamp 1020*, Ndedema Gorge, Cathedral Peak Forest Reserve, KwaZulu-Natal.
P. ericoides L., *Taylor 4042*, Pearly Beach, Western Cape.
**P. ericoides* L., *Bredenkamp 956*, Milnerton, Cape Town, Western Cape.
**P. ericoides* L., *Bredenkamp 962*, Cape Maclear, Cape Town, Western Cape.
P. falcifolia C.H. Wright, *Tysson 1449*, Knysna, Western Cape.
**P. falcifolia* C.H. Wright, *Bredenkamp 915*, Tsitsikamma, Western Cape.
**P. falcifolia* C.H. Wright, *Bredenkamp 917*, Gouna, Western Cape.
P. filiformis L., *Killick 238*, Table Mountain, Pietermaritzburg, KwaZulu-Natal.
P. filiformis L., *Bredenkamp 1016*, Oribi Gorge, KwaZulu-Natal.
P. filiformis L., *Bredenkamp 896*, Kiwane, Eastern Cape.
P. filiformis L., *Bredenkamp 1036*, Steenbras River Mouth, Western Cape.
P. filiformis L., *Van Wyk & Bredenkamp 1*, Umtamvuna River Bridge, KwaZulu-Natal.
P. galpinii C.H. Wright, *Galpin 4491*, Melkhoutfontein, Western Cape.
P. galpinii C.H. Wright, *Bredenkamp 923*, Mossel Bay, Western Cape.
P. galpinii C.H. Wright, *Bredenkamp 932*, Riethuiskraal, Western Cape.
P. galpinii C.H. Wright, *Bredenkamp 933*, Still Bay, Western Cape.
P. galpinii C.H. Wright, *Bredenkamp 946*, De Hoop Nature Reserve, Western Cape.
P. glomerata Thunb., *Bredenkamp 973*, Tulbagh, Western Cape.
P. glomerata Thunb., *Bredenkamp 977*, Groenfontein, Western Cape.
P. glomerata Thunb., *Bredenkamp 984*, Citrusdal, Western Cape.
P. glomerata Thunb., *Bredenkamp 994*, Cedarberg Mountains, Western Cape.
P. glomerata Thunb., *Bredenkamp 1002*, Cedarberg Mountains, Western Cape.
P. montana Thoday, *W. Gies 13136*, Auas Mountains, Namibia.
P. montana Thoday, *Bredenkamp 1028*, Marikele Nature Reserve, Northern Province.
P. montana Thoday, *Bredenkamp 889*, Golden Gate, National Park, Free State.
P. montana Thoday, *Bredenkamp 890*, Golden Gate, National Park, Free State.
P. montana Thoday, *Bredenkamp 893*, Ladybrand, Free State.
P. sp. nov., *Bredenkamp 1044*, Waboomberg, Ceres, Western Cape.
P. obtusifolia Thoday, *Bredenkamp 919*, Perdepoort, Oudtshoorn, Western Cape.
P. obtusifolia Thoday, *Bredenkamp 929*, Rooiberg, Western Cape.
P. obtusifolia Thoday, *Bredenkamp 967*, Jonaskop, Western Cape.
P. obtusifolia Thoday, *Bredenkamp 971*, Karoo National Botanical Garden, Western Cape.
P. obtusifolia Thoday, *Bredenkamp 1033*, Seweweekspoort, Western Cape.
P. paleacea Wikstr., *Bredenkamp 940*, Puntjie, Western Cape.
P. paleacea Wikstr., *Bredenkamp 950*, De Hoop Nature Reserve, Western Cape.
P. paleacea Wikstr., *Bredenkamp 952*, Harold Porter National Botanical Garden, Western Cape.
**P. paleacea* Wikstr., *Bredenkamp 960*, Kommetjie, Cape Town, Western Cape.
**P. paleacea* Wikstr., *Bredenkamp 961*, Cape Maclear, Cape Town, Western Cape.
P. paludosa Thoday, *Thoday 100*, Riet Valley, Cape Flats, Western Cape.
P. paludosa Thoday, *Bredenkamp 1035*, Rondevlei Nature Reserve, Western Cape.
P. pendula Eckl. & Zeyh., *Fourcade 3040*, Zuur Anys, Eastern Cape.
P. pendula Eckl. & Zeyh., *Bredenkamp 908*, Groendal Nature Reserve, Eastern Cape.
P. pendula Eckl. & Zeyh., *Bredenkamp 909*, Groendal Nature Reserve, Eastern Cape.
P. rigida Wikstr., *Bredenkamp 1013*, Umtamvuna River Mouth, KwaZulu-Natal.
P. rigida Wikstr., *Bredenkamp 897*, Kleinmond West, Eastern Cape.
P. rigida Wikstr., *Bredenkamp 898*, Port Alfred Eastern, Cape.
P. rigida Wikstr., *Bredenkamp 899*, Kenton-on-Sea, Eastern Cape.
P. rigida Wikstr., *Bredenkamp 911*, Jeffrey's Bay, Eastern Cape.
P. rubra C.H. Wright, *Bredenkamp 900*, Grahamstown, Eastern Cape.
P. rubra C.H. Wright, *Bredenkamp 914*, Kareedouw, Eastern Cape.
P. vulgaris Thoday, *Bredenkamp 901*, Grahamstown, Eastern Cape.
P. vulgaris Thoday, *Bredenkamp 907*, Groendal Nature Reserve, Eastern Cape.
P. vulgaris Thoday, *Bredenkamp 924*, Kleinbrak, Western Cape.
P. vulgaris Thoday, *Bredenkamp 926*, Riversdale, Western Cape.
P. vulgaris Thoday, *Bredenkamp 943*, Bontebok National Park, Western Cape.

4.3 Epidermis

BREDENKAMP, C.L. & VAN WYK, A.E. 2000. The epidermis in *Passerina* (Thymelaeaceae): structure, function and taxonomic significance. *Bothalia* 30: 69–86.

CONTENTS

Bothalia 30,1: 69–86 (2000)

The epidermis in *Passerina* (Thymelaeaceae): structure, function and taxonomic significance

C.L. BREDENKAMP* and A.E. VAN WYK**

Keywords: anatomy, cuticle, epicuticular waxes, epidermis, *Passerina*, southern Africa, stomata, taxonomy, Thymelaeaceae

ABSTRACT

Epidermal features were studied in all 17 species of *Passerina*, a genus endemic to southern Africa. Leaves in *Passerina* are inversely ericoid, the adaxial surface concave and the abaxial surface convex. Leaves are inversely dorsiventral and epistomatic. The adaxial epidermis is villous, with unicellular, uniseriate trichomes and relatively small thin-walled cells, promoting flexibility of leaf margins owing to turgor changes. In common with many other Thymelaeaceae, abaxial epidermal cells are large and tanniniferous with mucilaginous cell walls. The cuticle is adaxially thin, but abaxially well developed, probably enabling the leaf to restrict water loss and to tolerate high light intensity and UV-B radiation. Epicuticular waxes, present in all species, comprise both soft and plate waxes. Epidermal structure proves to be taxonomically important at family, genus and species levels. Interspecific differences include arrangement of stomata and presence or absence of abaxial epidermal hair. Other diagnostic characters of the abaxial epidermal cells are arrangement, size and shape, cuticular ornamentation and presence or absence of wax platelets. Two groups of species on the basis of abaxial epidermal cell orientation are recognised. Many leaf epidermal features in *Passerina* are interpreted as structural adaptations to the Mediterranean climate of the Cape.

CONTENTS

INTRODUCTION

The genus *Passerina* L. comprises about 17 species, all endemic to southern Africa (Thoday 1924; Bond & Goldblatt 1984). Despite the now outdated revision by Thoday (1924), taxonomic boundaries in *Passerina* remain a problem, mainly owing to the apparent lack of marked morphological differences between the species. The present paper emanates from a comparative leaf-anatomical survey of the genus, undertaken as part of a monographic study of the group. This survey highlighted the importance of the epidermis as a source of taxonomic evidence.

The combined distribution of all the *Passerina* species is shown in Figure 1. Most species of *Passerina* are

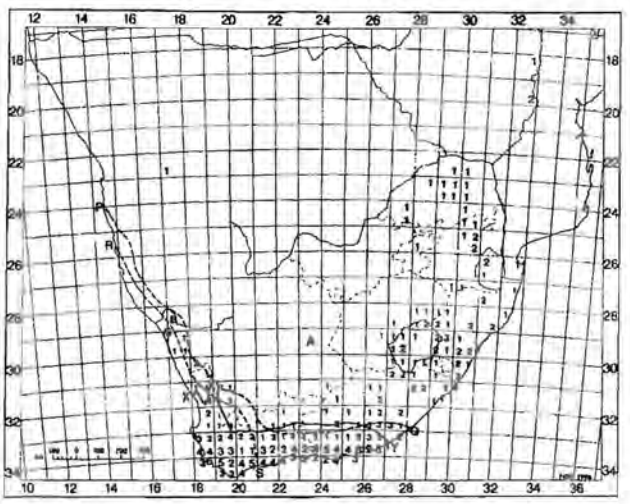


FIGURE 1—Number of species per grid in the distribution of *Passerina*. Lines PQ and RS: boundaries between summer (A), intermediate (B) and winter (C) rainfall areas. Line XY shows northern boundary of Cape Supergroup rock outcrops.

* National Botanical Institute, Private Bag X101, 0001 Pretoria.
** H.G.W.J. Schweickerdt Herbarium, Department of Botany, University of Pretoria, 0002 Pretoria.
MS. received: 1999-06-07.

TABLE 1.—*Passerina* specimens examined and housed at PRE

Species	Collector	Locality
burchellii Thoday	*Bredenkamp 1545*	WESTERN CAPE.—3319 (Worcester): Jonaskop, (–DC).
	*Bolus *687; Stokoe *2542*	WESTERN CAPE.—3419 (Caledon): Baviaanskloof, Genadendal, (–BA).
comosa C.H.Wright	*Andreae *1288; MacDonald *2125*	WESTERN CAPE.—3321 (Ladismith): Seweweekspoort, (–AD).
drakensbergensis Hilliard & B.L.Burtt	*Edwards 974*	KWAZULU-NATAL.—2828 (Bethlehem): Royal Natal National Park, (–DB).
	*Bredenkamp *1018, 1019, *1020*	KWAZULU-NATAL.—2829 (Harrismith): Ndedema Gorge, Cathedral Peak Forest Reserve, (–CD).
ericoides L.	*Bredenkamp *962*	WESTERN CAPE.—3418 (Simonstown): Cape Maclear, (–AD).
	*Bredenkamp *+956*	WESTERN CAPE.—3318 (Cape Town): Milnerton, (–CD).
	Taylor 4042	WESTERN CAPE.—3419 (Caledon): Pearly Beach, (–CB).
falcifolia C.H.Wright	*Bredenkamp *+917*	WESTERN CAPE.—3323 (Willowmore): Gouna, (–CC).
	*Bredenkamp *915*	WESTERN CAPE.—3324 (Steytlerville): opposite Tsitsikamma Lodge, (–CD).
	Tyson 1449	WESTERN CAPE.—3423 (Knysna): Knysna, (–AA).
filiformis L.	*Killick 238*	KWAZULU-NATAL.—2930 (Pietermaritzburg): Table Mountain, (–CB).
	*Bredenkamp *1016, *1017*	KWAZULU-NATAL.—3030 (Port Shepstone): Oribi Gorge, (–CB).
	*Bredenkamp *1012; Van Wyk & Bredenkamp 1*	KWAZULU-NATAL.—3130 (Port Edward): Umtamvuna River Bridge, (–AA).
	Bredenkamp 896	EASTERN CAPE.—3327 (Peddie): Kiwane, (–BA).
	Bredenkamp 1036	WESTERN CAPE.—3418 (Simonstown): Steenbras River Mouth, (–BB).
galpinii C.H.Wright	*Bredenkamp *946*	WESTERN CAPE.—3420 (Bredasdorp): De Hoop Nature Reserve, (–AD).
	Galpin 4491	WESTERN CAPE.—3421 (Riversdale): Melkhoutfontein, (–AD).
	*Bredenkamp *932*	WESTERN CAPE.—3421 (Riversdale): Riethuiskraal, (–AD).
	Bredenkamp 933	WESTERN CAPE.—3421 (Riversdale): Still Bay, (–AD).
	Bredenkamp 923	WESTERN CAPE.—3422 (Mossel Bay): Mossel Bay, (–AA).
glomerata Thunb.	*Bredenkamp *988, 994, 1002*	WESTERN CAPE.—3219 (Wuppertal): Cederberg Mountains, near Algeria, (–AC).
	Bredenkamp 984	WESTERN CAPE.—3219 (Wuppertal): Citrusdal, (–CA).
	Bredenkamp 977	WESTERN CAPE.—3219 (Wuppertal): Ceres, Karoo, Farm Groenfontein, (–DC).
	*Bredenkamp *973*	WESTERN CAPE.—3319 (Worcester): Tulbagh, (–AC).
montana Thoday	*Giess 13136*	NAMIBIA.—2217 (Windhoek): Auas Mountains, (–CA).
	Bredenkamp 1028	NORTHERN PROVINCE.—2427 (Thabazimbi): Marikele Nature Reserve, (–BC).
	*Bredenkamp *1024*	MPUMALANGA.—2430 (Pilgrim's Rest): World's View, (–DD).
	*Bredenkamp *1025*	MPUMALANGA.—2430 (Pilgrim's Rest): God's Window, (–DD).
	*Bredenkamp 889, *890*	FREE STATE.—2828 (Bethlehem): Golden Gate National Park, (–DA).
	*Bredenkamp *893*	FREE STATE.—2927 (Maseru): Ladybrand, (–AB).
obtusifolia Thoday	*Bredenkamp 971*	WESTERN CAPE.—3319 (Worcester): Karoo National Botanical Garden, (–CB).
	Bredenkamp 967	WESTERN CAPE.—3319 (Worcester): Jonaskop, (–CD).
	*Bredenkamp 1033, *1034*	WESTERN CAPE.—3321 (Ladismith): Seweweekspoort, (–AD).
	*Bredenkamp *929*	WESTERN CAPE.—3321 (Ladismith): Rooiberg, (–CB).
	*Bredenkamp *919*	WESTERN CAPE.—3322 (Oudtshoorn): Perdepoort, (–CD).
paleacea Wikstr.	*Bredenkamp 960*	WESTERN CAPE.—3418 (Simonstown): Kommetjie, (–AB).
	*Bredenkamp *+961*	WESTERN CAPE.—3418 (Simonstown): Cape Maclear, (–AD).
	Bredenkamp 952	WESTERN CAPE.—3418 (Simonstown): Harold Porter National Botanical Garden, (–BD).
	Bredenkamp 950	WESTERN CAPE.—3420 (Bredasdorp): De Hoop Nature Reserve, (–AD).
	*Bredenkamp *949*	WESTERN CAPE.—3420 (Bredasdorp): Waenhuiskrans, (–CA).
	Bredenkamp 940	WESTERN CAPE.—3421 (Riversdale): Puntjie, (–AC).
paludosa Thoday	*Bredenkamp *1035; Jungle *156*	WESTERN CAPE.—3418 (Simonstown): Rondevlei Nature Reserve, (–BA).
	Thoday 100	WESTERN CAPE.—3418 (Simonstown): Riet Valley, Cape Flats, (–BA).
pendula Eckl. & Zeyh.	*Fourcade 3040*	EASTERN CAPE.—3324 (Steytlerville): Suuranys, (–CB).
	*Bredenkamp *908, *909*	EASTERN CAPE.—3325 (Port Elizabeth): Groendal Nature Reserve, (–CB).
rigida Wikstr.	*Ward 7211*	KWAZULU-NATAL.—2832 (Mtubatuba): St. Lucia Park, (–AD).
	*Bredenkamp *1013*	KWAZULU-NATAL.—3130 (Port Edward): Umtamvuna River Mouth, (–AA).
	*Bredenkamp *899*	EASTERN CAPE.—3326 (Grahamstown): Kenton-on-Sea, (–DA).
	Bredenkamp 898	EASTERN CAPE.—3326 (Grahamstown): Port Alfred, (–DB).
	Bredenkamp 897	EASTERN CAPE.—3327 (Peddie): Kleinmond West, (–CA).
	Bredenkamp 911	EASTERN CAPE.—3424 (Humansdorp): Jeffreys Bay, (–BB).
rubra C.H.Wright	*Bredenkamp 914*	EASTERN CAPE.—3324 (Humansdorp): Kareedouw, (–CD).
	*Bredenkamp *905*	EASTERN CAPE.—3325 (Port Elizabeth): Colchester, (–DB).
	*Bredenkamp *900*	EASTERN CAPE.—3326 (Grahamstown): Grahamstown, (–AD).
vulgaris Thoday	*Bredenkamp *926*	WESTERN CAPE.—3321 (Ladismith): foot of Gysberg Pass, (–CC).
	Bredenkamp 907	EASTERN CAPE.—3325 (Port Elizabeth): Groendal Nature Reserve, (–CB).
	Bredenkamp 901	EASTERN CAPE.—3326 (Grahamstown): Grahamstown, (–AD).
	Bredenkamp 943	WESTERN CAPE.—3420 (Bredasdorp): Bontebok National Park, (–AB).
	*Bredenkamp *924*	WESTERN CAPE.—3422 (Mosselbaai): Kleinbrak, (–AA).
sp. nov. 1	*Bredenkamp *1044, *1046, *1047*	WESTERN CAPE.—3319 (Worcester): Waboomberg, (–AD).
sp. nov. 2	*Esterhuysen *12189, *26859*	WESTERN CAPE.—3218 (Clanwilliam): northern Cederberg Mountains, (–BB).
sp. nov. 3	*Stokoe *9302*	EASTERN CAPE.—3322 (Oudtshoorn): Swartberg Pass, Prince Albert area, (–AC).
	*Esterhuysen *28006*	EASTERN CAPE.—3324 (Steytlerville): Cockscomb, (–BD).
sp. nov. 4	*Schlechter *9302*	EASTERN CAPE.—3322 (Oudtshoorn): Montagu Pass, (–AC).
	*Esterhuysen *10734*	EASTERN CAPE.—3323 (Willowmore): Kouga Mountains, (–DA).

* Material used for the SEM study of the ad- and abaxial epidermises. +Fresh material collected for the TEM study.

endemic to the Cape Floristic Region. From here the distribution of *P. filiformis* and *P. montana* extends east and north along the eastern mountains and Great Escarpment of southern Africa. In the Cape the climate is for the most part Mediterranean or semi-Mediterranean. In the west, it rains in winter; along the south coast, winter rainfall is complemented by some summer rain which increases eastwards. The western Karoo and Namaqualand (Succulent Karoo Biome) are characterised by winter precipitation and summer drought. KwaZulu-Natal and the eastern mountains of southern Africa are predominantly summer rainfall areas. Distribution of the species of *Passerina* coincides with the geography and climate along the whole distribution area. *P. ericoides*, *P. paleacea*, *P. paludosa*, *P. galpinii* and *P. burchellii* are endemic to Western Cape. The first three species are found along beaches and salt marshes only, *P. galpinii* grows mainly on calcrete in the Agulhas Plain area (Goldblatt & Manning in press) and *P. burchellii* is found on the high mountains at Genadendal and Villiersdorp. *P. comosa* grows on mountain slopes and summits in the Kamiesberg, Great Winterhoek and Klein Swartberg Ranges. *P. glomerata* is found from Worcester to Tulbagh, in the Clanwilliam area and extends to the Witteberg south of Matjiesfontein. *P. obtusifolia* is ubiquitous in the Cape, distributed from Worcester in Western Cape to Alice in Eastern Cape and on some of the mountain ranges in and around the Little Karoo. A new species, of which the plants are often buried under snow during winter, grows at high altitudes in the Ceres Karoo. *P. vulgaris* is a pioneer with a wide distribution from Western Cape to East London in Eastern Cape. *P. falcifolia* is found on mountain ranges between George and Uitenhage and *P. pendula* is endemic to the KwaZunga Catchment Basin and the Zwartkops River area of Eastern Cape. *P. rubra* is common in the Port Elizabeth to Uitenhage area, with outliers in the Swellendam and Bredasdorp Districts. *P. drakensbergensis* is endemic to the high Drakensberg in the Bergville District of KwaZulu-Natal and *P. rigida* is distributed all along the coast, from northern KwaZulu-Natal to the Cape Peninsula. *Passerina* sp. nov. 2 is found on the northern Cederberg Mountains, *P.* sp. nov. 3 at mountain tops in the Uitenhage area and the Swartberg Pass and *P.* sp. nov. 4 on the Kouga Mountains and the Montagu Pass.

The most important studies applying the 'anatomical method' for the delimitation of the Thymelaeaceae were published by Van Tieghem (1893) and Supprian (1894). The presence of mucilaginous epidermal cells in *P. ericoides* (= *Chymococca empetroides* Meisn.) as opposed to the total lack thereof in the other species, was also mentioned by Supprian (1894). Subsequently, Gilg (1894) critically discussed the 'anatomical method' as applied by Van Tieghem (1893) and Supprian (1894) for the delimitation of the Thymelaeaceae and concluded that certain characters would not uphold criticism. He regarded former systems based on floral morphology as more suitable for a taxonomic grouping of the Thymelaeaceae.

The twentieth century yielded very little anatomical work on the Thymelaeaceae. Standard works were those of Solereder (1908) and Metcalfe & Chalk (1950, 1979). Thoday (1921) described the structure and behaviour in drought of the ericoid leaves of *P. filiformis* and *P.* cf. *falcifolia*; he also supplied some notes on their anatomy. In a discussion of inversely dorsiventral leaves, Kugler (1928) included a description of the leaves of *P. filiformis* (= *P. pectinata* Hort.). More recently, leaf anatomy of the genera *Lachnaea* L. and *Cryptadenia* Meisn. was treated by Beyers (1992) and leaf and involucral bract characters of systematic use in *Gnidia* L. were studied by Beaumont *et al.* (1994). The scanty information on leaf anatomy in Thymelaeaceae calls for further research in this field, especially in the genus *Passerina*.

Previous leaf anatomical studies identified mucilagination of the epidermal cells as being of possible taxonomic importance. Recently Bredenkamp & Van Wyk (1999) clarified the structure of the epidermal cells and origin of the mucilage, concluding that mucilagination of epidermal cells is of taxonomic importance mainly at the family level.

The wide distribution of *Passerina* in the Cape Floristic Region, along the southern and eastern coastline and along the Great Escarpment of southern Africa as far north as Zimbabwe, illustrates the adaptation of these plants to a wide range of habitats, including Mediterranean and summer rainfall regimes. Decreasing rainfall from the eastern Escarpment to the northwestern Cape is reflected by adaptive changes in the leaf structure of the group. The present paper provides a description of epidermal characters in *Passerina* as well as an assessment of their taxonomic significance. It also speculates on the possible adaptive value of the observed structural features of the leaf.

MATERIAL AND METHODS

Fresh leaf material of 17 species of *Passerina* (Table 1) was collected, fixed and stored in a 0.1 M phosphate-buffered solution at pH 7.4, containing 2.5% formaldehyde, 0.1% glutaraldehyde and 0.5% caffeine [modified Karnovsky fixative; Karnovsky (1965)]. Whenever possible, material from at least five different localities was collected, fixed and air-dried for each species and herbarium specimens were made.

Light microscope (LM) studies

The LM was used for general leaf anatomy as well as epidermal studies. Unless stated otherwise, the tenth leaf from the growing point of a twig was used in all comparative studies. To prepare transverse sections of the main vein as well as both leaf margins, a 1 mm wide segment of leaf material was cut from the centre of each leaf. Samples were dehydrated, embedded in glycol methacrylate (GMA) and sectioned according to the methods of Feder & O'Brien (1968). Sections were stained with the periodic acid/Schiff's reaction and in toluidine blue 'O', then mounted in Entellan (Art. 7961, E. Merck, Darmstadt).

The following three methods were followed in the study of the cuticles:

1. GMA transverse sections of leaves were stained for 10 minutes in 1% Sudan Black B dissolved in 70% ethanol. Sections were rinsed twice in 70% ethanol for a few seconds and mounted in glycerine jelly.

2. Cuticular mounts were obtained by maceration according to the method of Kiger (1971). Specimens were slightly over-stained in a 1% acqueous safranin solution, dehydrated in methyl cellusolve and mounted in Entellan.

3. Epidermal mounts were obtained by removing small pieces of ad- and abaxial epidermis manually and by paradermal hand sections. Epidermises were stained in 1% safranin dissolved in 50% ethanol, dehydrated in a graded ethanol series and mounted in Entellan.

Scanning electron microscope (SEM) studies

The SEM was used to study the epidermal surface features (including epicuticular waxes), as well as to verify the structure of the cuticle. Leaves from air-dried material were used for all species. Whole leaves were used as they are small and ericoid, but trichomes were manually removed adaxially to reveal the stomata. Leaves were mounted onto aluminium stubs with silver paint, exposing the ad- and abaxial surfaces separately and sputter-coated with gold. For the purpose of studying epicuticular waxes, the sputter-coating process was modified to prevent high temperatures from changing the wax surfaces. Specimens were sputter-coated for 30 seconds and left to retain their normal temperature for one minute. This was repeated five times after which the specimens were viewed with a Jeol 840 SEM.

For the verification of the authenticity of epicuticular wax droplets and small round protrusions observed in certain species of *Passerina*, leaves were washed in chloroform for one minute, before they were pasted onto aluminium stubs. The procedure described above was used for sputter-coating and viewing.

Transmission electron microscope (TEM) studies

The TEM was used for the study of the structure of mucilaginous epidermal cell walls in *Passerina*. The second, fifth and tenth leaf from the growing points of *P. ericoides*, *P. falcifolia* and *P. paleacea* were used to study the structure of the cell wall. Leaf segments of ± 1 mm² were fixed in a modified Karnovsky fixative (Karnovsky 1965). Fixed material was rinsed in 0.075 M phosphate buffer, pH 7.4–7.5, post-fixed for one hour in 0.25% aqueous OsO_4, washed in three changes of water, dehydrated in a graded acetone series and embedded in Quetol 651 resin (Van der Merwe & Coetzee 1992). Ultrathin sections were contrasted in 4% aqueous uranyl acetate for 10 minutes and rapidly rinsed in water three times. The sections were then contrasted with lead citrate (Reynolds 1963), rinsed in water and examined with a Phillips 301 TEM.

For the verification of wettability and possible absorption of water by laminar epidermal hairs, we follow Alvin (1987). He proposed a mechanism through which water is absorbed by the specialised abaxial epidermal trichomes of *Androstachys johnsonii* Prain. This process involves the wettability of the hairs which he investigated by spraying the glabrous adaxial surfaces of the leaves with water. Water seeped round the leaf margins to the abaxial surface, wetting approximately 50% of the abaxial surface within 5 minutes. In the present study, the glabrous abaxial surfaces of five cymbiform leaves (from dried herbarium specimens) were pasted onto a sticky surface, exposing the villous concave adaxial surface. A drop of water was placed in the adaxial groove at the base of each leaf (average leaf size 2.5 × 4.0 mm) and left overnight. This experiment was repeated using 0.5% aqueous safranin, followed after 20 minutes by a rinse with water.

Terminology

Trichome structure

We have followed the terminology of Stace (1965) and Theobald *et al.* (1979).

Cuticle

Although the interpretation proposed by Martin & Juniper (1970) for the cuticle of plants has been widely followed by many workers, Holloway (1982) reviewed the historical perspective of the plant cuticle and attempted to adopt the most workable interpretation of the cuticular membrane (CM) in practice. In response, we follow Jeffree (1986), whose uncomplicated and pragmatic interpretation distinguishes three main zones, namely the cuticle proper, the cuticular layer and the cell wall. The cuticular membrane comprises the cuticle proper plus the cuticular layer and is bonded to the outer periclinal walls of the epidermal cells by a pectin-rich layer, which is equivalent to the continuous middle lamella. A layer of epicuticular wax generally coats the cuticle proper.

Cuticular ornamentation (LM and SEM)

We follow Wilkinson (1979) in our choice of terminology to describe cuticular ornamentation.

Epicuticular wax

The recognition of soft waxes in the present study is based on the criteria proposed by Amelunxen *et al.* (1967), Wilkinson (1979) and Barthlott *et al.* (1998).

RESULTS

Macromorphology of the leaf

Leaf arrangement decussate, sometimes imbricate, closely adherent to stem or spreading at angle of 5°–20°(–60°); spreading of leaves often prominent in juvenile plants. *Lamina* inversely ericoid; adaxial surface concave, often forming a groove facing stem and lined with woolly hairs; abaxial surface convex, orientated more or less acroscopically, thus exposing a large surface area to the environment; cuticle often amber-coloured (in herbarium material) and outline of epidermal cells often macroscopically visible. *Leaf shape* cymbiform (boat-shaped), falcate or cigar-shaped; plane shape linear, oblong, lanceolate, ovate or trullate. *Leaf base* sessile or cuneate. *Leaf apex* truncate and hump-backed, obtuse, rounded, acumi-

nate or acute to almost spine-tipped. *Margins* sometimes ciliate. *Size* (1.5–)2.5–4.0(–8.0) × (0.8–)1.2–2.0(–3.0) mm.

Anatomy of the leaf

Transverse section (LM): *leaves* epistomatic. *Adaxial epidermis* concave, villous, with unicellular, uniseriate trichomes; cuticle relatively thin, 2–5 µm; epidermal cells uniserial, relatively small (10–)15–25(–35) × 10–17(–20) µm; vacuoles large with tanniniferous substances, cell walls thin; stomata present, with guard cells at same level, sunken below, or raised above adjacent epidermal cells. *Abaxial epidermis* convex, glabrous or sparsely hairy; cuticle relatively thick (10–)20–40(–70) µm; epidermal cells relatively large, periclinal diam. of cells (20–)30–60(–65) µm, anticlinal diam. (25–)30–75 (–105) µm (Table 2), tanniniferous, often with mucilaginous cell walls. *Mesophyll* inversely dorsiventral (Kugler 1928); spongy parenchyma situated adaxially and palisade parenchyma abaxially. *Main vascular bundle* collateral, surrounded by parenchymatous bundle sheath with ample amounts of tanniniferous substances; bundle sheath adaxially irregularly biseriate, abaxially strengthened by sclerenchyma. *Secondary vascular bundles* ± 6; bundle sheaths irregular, parenchymatous and tanniniferous. Figure 2A, B.

Adaxial (dorsal) epidermis

Cuticle

Transverse section (LM): cuticular membrane 2–5 µm thick, smooth, ridged along boundaries of guard cells (Figure 2G), gradually thickening close to leaf margins, equalling abaxial cuticle in thickness and sculpturing at margins.

Surface view (LM and SEM): smooth (Figure 2C), except in *Passerina* sp. nov. 1, where markings on epicuticular wax are most probably caused by snow (Figure 3D, E).

Epidermal cells

Transverse section (LM): cells uniserial, irregularly shaped, relatively small with periclinal diam. (10–)15–25(–35) µm, anticlinal diam. 10–17(–20) µm; cell walls thin, outer periclinal wall convex; vacuoles large, containing tanniniferous substances (Figure 2A, F–H). Margin formed by a few rows of conically shaped or anticlinally elongated cells.

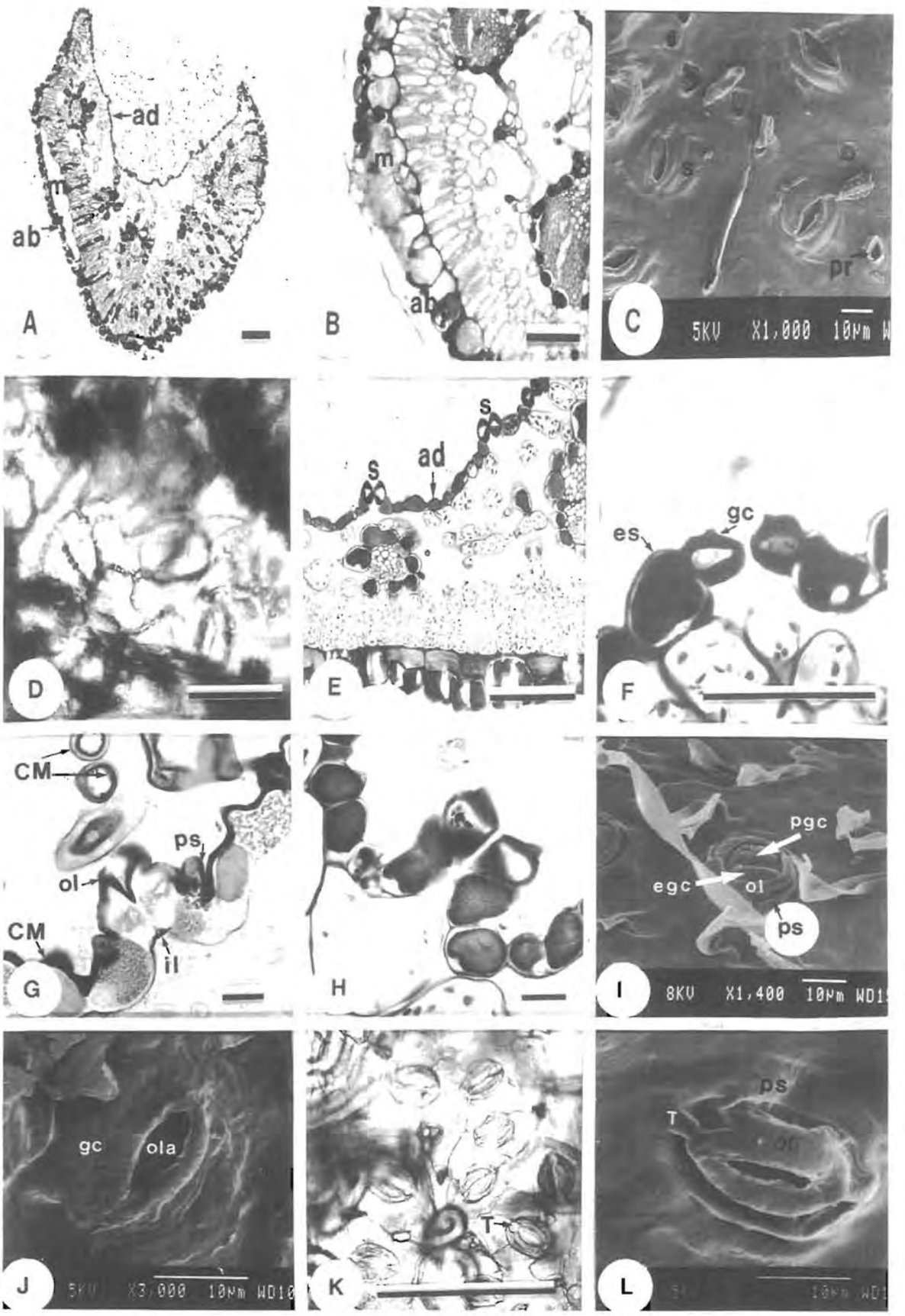
Surface view (LM and SEM): cells polygonal, 4–many-sided, walls usually undulate with loose, wide u-shaped curves of shallow amplitude (Figures 2D, K; 3C), arranged in rows and straight-walled in *Passerina* sp. nov. 1 (Figure 3D, E). Nodular walls observed in *P. falcifolia* (Figure 2D). Vacuoles with ample tanniniferous substances.

Stomatal complex

Transverse section (LM): *lamina* epistomatic; stomata dispersed randomly over adaxial surface, but absent from edges of leaf margin; raised or at same level as other epidermal cells (Figure 2E–H); dispersed in two columns in adaxial epidermal folds, with ± 3–5 rows of epidermal cells in between; raised, sunken or arranged in stomatal crypts in *Passerina* sp. nov. 1 (Figure 3F). *Guard cell* outline in all species varying between widely obtrullate, very widely obtrullate or widely depressed obtrullate, with angles slightly rounded; cell walls thick-

TABLE 2.—Dimensions of abaxial epidermal cells and cuticular membrane (CM) in *Passerina*. Measurements in µm in cross section and surface view

Name	Width of CM	Periclinal diam.	Anticlinal diam.	Length × width	Shape of cell
comosa (Figure 4B, C)	10–40	30–60	70–75	45–55 × 35–40	slightly oblong
glomerata	(20–)30–40(–50)	(20–)30–35(–40)	(25–)30–55(–60)	30–40 × 30–35	isodiametric
ericoides (Figure 4D–F)	20–32	35–60	40–60	35–50 × 40–50	± isodiametric
obtusifolia (Figure 4G–I)	20–30	40–55	55–75(–105)	(30–)40–55(–60) × (45–)50–70(–95)	transversely oblong
burchellii (Figure 4J–L)	60(–70)	45	75	(65–)85(–125) × 45–50	angles rounded, oblong
drakensbergensis (Figure 5A–C)	20	30–35	50–55	50–65 × 30–40	oblong
montana	30–35	30–45	45–65	40–60 × 35–40	isodiametric to slightly oblong
sp. nov. 1 (Figure 5D–F)	20	35	40	45–55 × 35–40	oblong
vulgaris	(10–)20–30	30–45	35–45(–70)	35 × 30–40	transversely oblong
filiformis	20–35	35(–65)	55–75(–90)	35–55 × 25–30	oblong
falcifolia	20	40	40	60–75 × 35–50	oblong
pendula	30	45–55	60–65	50–65 × 30–40	oblong
rigida (Figure 5G–I)	20–30	35–50	35–55	35–45 × 35–40	isodiametric to slightly oblong
galpinii	40–50	30–35	(40–)55–60(–70)	30–40 × 30	isodiametric to slightly oblong
rubra	20–30	30–50	45	(30–)35(–55) × 35–40	isodiametric to slightly oblong
paleacea	20(–40)	35(–65)	50–60	45–50 × 30–35	slightly oblong
paludosa (Figure 5J–L)	20	35–45	60–70	95–100 × 45–50(–95)	oblong



ened (Figure 2F, H); periclinal and anticlinal dimensions for individual guard cells 10.0–12.5(–15.0) × (10.0–) 12.5–15.0(–20.0) µm. *Cuticular membrane* (Figures 2G; 3B) covering outer periclinal walls of epidermal and guard cells, as well as poral epidermal walls of guard cells, smooth or slightly crenate when lining the pore (Figure 3B), contracted into a pair of ± continuous outer stomatal ledges above guard cells, thus forming an entire outer cavity (not divided into compartments); inner stomatal ledges and inner cavity present. *Epidermal cells* surrounding guard cells not different from other epidermal cells in size, shape or staining properties (Figure 2F). *Peristomatal cuticular rims* conspicuous on epidermal cells bordering guard cells (Figure 2G).

Surface view (LM and SEM): *stomata* anomocytic; outline elliptic to circular, dimensions (20–)26–30(–35) × (15–)24–30(–35) µm, circular in *Passerina* sp. nov. 1, dimensions 27.5 × 27.5 µm. *Epidermal cells surrounding guard cells* 3–5(6), irregularly shaped with sinuate walls and long axis parallel to guard cells, corresponding in orientation, size, shape and staining properties to other ± elongated epidermal cells (Figures 2D, K; 3C); pentagonal to heptagonal epidermal cells in *Passerina* sp. nov. 1, with walls slightly sinuate to straight, possibly nodular (Figure 3E). *Stomata* raised above or at same level as other epidermal cells in all species (Figure 2I, J, L); dispersed in two columns in adaxial epidermal folds, with ± 3–5 rows of epidermal cells in between, sunken or arranged in stomatal crypts in *Passerina* sp. nov. 1 (Figure 3D). *Guard cells* often conspicuously raised (Figure 2I, J). *Peristomatal cuticular rims* conspicuous on epidermal cells bordering guard cells (Figures 2I, L; 3A), rims also visible as 1–4 small semilunar protrusions bordering guard cells in cuticular preparations and epidermal peels (Figure 3C) (rims should not be confused with small subsidiary cells, an interpretation which could result in stomata being erroneously classified as paracytic or cyclocytic). *Outer stomatal ledges* ± continuous, present above guard cells (Figures 2I–L; 3A, C). *Stomatal poles* (where guard cells meet) retuse; T-pieces (cuticular thickenings of common walls between guard cells) well developed (Figures 2I, J, L; 3C).

Trichomes

LM and SEM: *adaxial surface* of leaf villous. *Trichomes* nonglandular, unbranched, devoid of surface features or constrictions, mostly strongly spiralled (Figure 3G, H), terete, with central lumen, covered by cuticle (Figures 2G; 3I). *Hair bases* with pore, poral rim somewhat thickened (Figures 2C; 3C, G); hair base cells mostly 4–6 and slightly radially elongated (Figure 3C, G). *Trichomes bordering leaf margin* conspicuous in *P. burchellii*, *P. paludosa* and *P. pendula* (Figure 3I, J). *Trichome foot* scarcely modified, inserted between epidermal cells (Figure 3I), usually straight, but with individual trichomes strongly spiralled (Figure 3J) in *P. pendula* (brown in dried material).

Wettability and the possible absorption of water by the laminar epidermal hairs in *Passerina* were assessed by means of laboratory tests. We found that water had formed a film over the felty layer of hair at the leaf base, whereas the adaxial surface had remained dry. A treatment with 0.5% aqueous safranin revealed that only the exposed parts of the spiralised hairs in the felty indumentum at the leaf bases stained pinkish. Although the longer hairs at the leaf margins were stained, those on the rest of the adaxial surface remained unstained.

Abaxial (ventral) epidermis

Trichomes

Abaxial surfaces of bracts and young leaves in *P. comosa*, *P.* sp. nov. 3 and *P.* sp. nov. 4 tomentose to sparsely hairy (Figure 4B), older leaves often glabrous. Description of trichomes as described under adaxial epidermis.

Epidermal cells

Transverse section (LM and TEM) (Figures 2A, B, E; 3K–L): epidermis uniserial. *Stomata* absent. *Epidermal cells* more or less oblong in outline; outer periclinal walls straight or convex, inner periclinal walls straight, convex or bulging towards mesophyll, often mucilaginous and then superficially resembling a multiple epidermis; periclinal diam. of cells (20–)30–60(–65) µm, anticlinal diam. (25–)30–75(–105) µm (Table 2). *Mucilaginous cell walls* increasing progressively from leaf margin to midrib (Figure 2B), affecting mainly inner periclinal but also anticlinal cell walls (Figure 3K, L); mucilage with a layered appearance (Figures 2E; 3K), eventually occupying about two-thirds of epidermal cell and separated from cytoplasm by innermost cellulose layer of inner periclinal cell wall (Figure 3L). *Cytoplasm* compressed by mucilage, remaining as a thin layer appressed to large, usually tanniniferous vacuole. *Anticlinal layer* of inner periclinal cell wall often plicate but gradually straightening and often disappearing as mucilagination increases, eventually breaking under pressure of accumulating

FIGURE 2.—LM photographs and SEM micrographs of epidermis of inversely ericoid leaf in *Passerina*. A, *P. falcifolia*, *Bredenkamp 917*, ad- and abaxial epidermis with mucilage accumulating abaxially; B, *P. galpinii*, *Bredenkamp 946*, mucilaginous abaxial epidermal cells; C, *P. filiformis*, *Bredenkamp 1016*, smooth adaxial cuticle, stomata and poral rims of hair bases; D, *P. falcifolia*, *Bredenkamp 915*, adaxial epidermal walls undulate, nodular; E, *P. ericoides*, *Taylor 4042*, stomata at different levels in relation to adaxial epidermis; F, *P. comosa*, *Bredenkamp 1034*, PAS staining of guard cell walls and surrounding epidermal cells, showing width; G, *P. pendula*, *Bredenkamp 909*, t/s adaxial epidermis stained with Sudan Black B, showing cuticular membrane; H, *P. pendula*, *Bredenkamp 909*, raised stomata stained with toluidine blue; I, *P. paleacea*, *Bredenkamp 961*, with peristomatal rim, raised epidermal and poral walls of guard cells, conspicuous outer stomatal ledges; J, *P. galpinii*, *Bredenkamp 946*, with distinct outer stomatal ledge aperture; K, *P. filiformis*, *Bredenkamp 1016*, and L, *P. pendula*, *Bredenkamp 909*, with T-pieces at stomatal poles. Abbreviations: ad, adaxial epidermis; ab, abaxial epidermis; CM, cuticular membrane; e, epidermal cell; egc, epidermal wall of guard cell; es, epidermal cell surrounding guard cell; gc, guard cell; ic, inner cavity; il, inner stomatal ledge; l, lumen of trichome; m, mucilage; oc, outer stomatal cavity; ol, outer stomatal ledge; ola, outer stomatal ledge aperture; p, pore; pgc, poral wall of guard cell; pr, trichome poral rim; ps, peristomatal rim; s, stomata; sc, stomatal crypt; t, trichome; T, T-piece at stomatal pole. Scale bars: A, B, D, E, F, K, 100 µm; C, G, H, I, J, L, 10 µm.

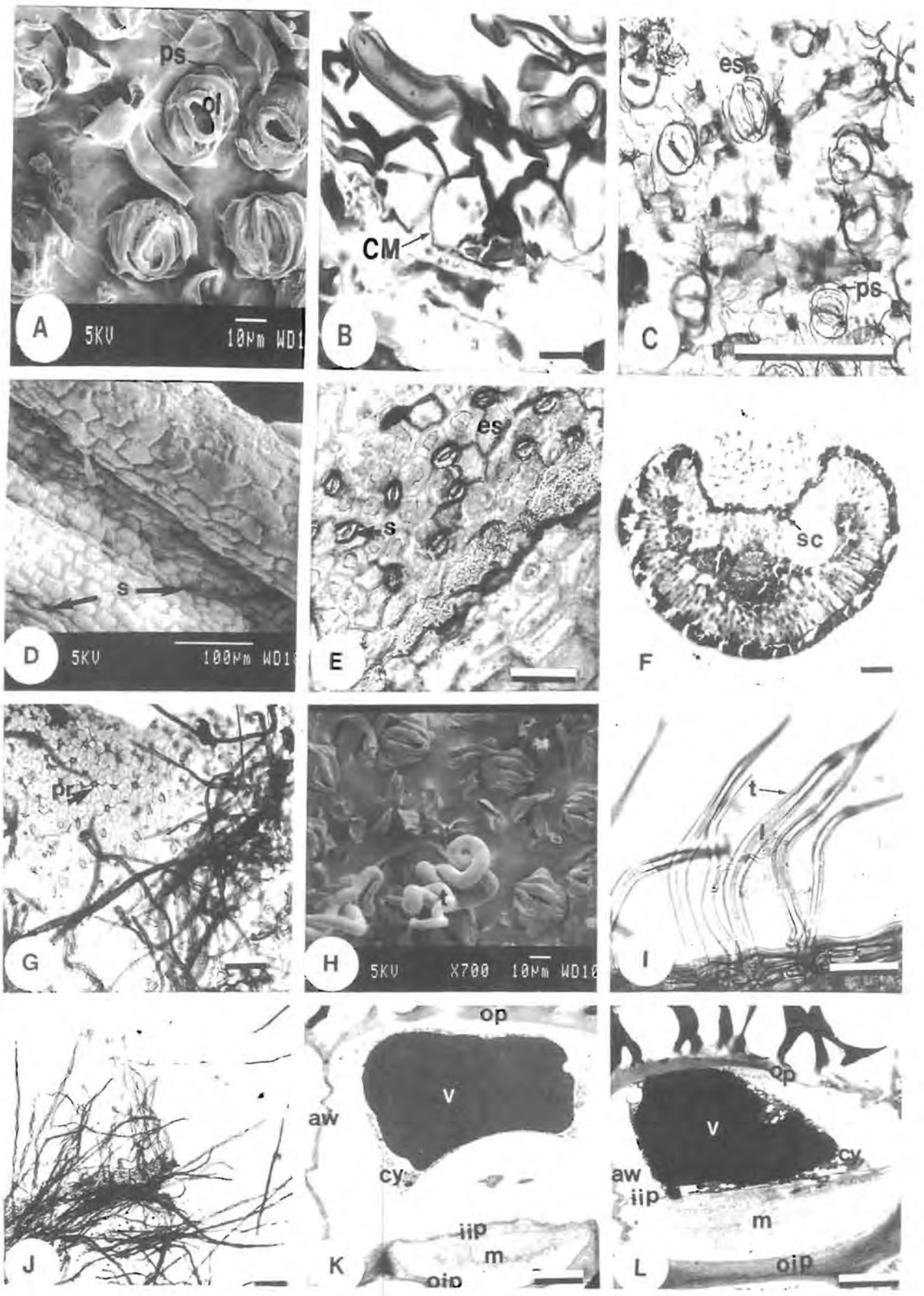


TABLE 3.—Abaxial epidermal characters in *Passerina*

Name	Epidermal cells		Abaxial hair present	Ornamentation of CM						Epicuticular wax		
					Papillate							
	random	in rows		Smooth	Molar-like crown	One dome per cell	Several domes per cell	Several globular papillae per cell	Striate	Soft wax	Platelets	Plates
Group A												
P. glomerata (Figure 6C)	X			X								X
P. ericoides (Figures 4D–F; 6D)	X			X						X		
P. obtusifolia (Figures 4G–I; 6E)	X					X				X		
P. burchellii (Figures 4J–L; 6F)	X						X			X		X
Intermediate												
P. comosa (Figures 4B, C; 6A,B)		X	X		X						X	X
P. sp. nov. 3		X	X				X					X
P. sp. nov. 4		X	X	X				X			X	
P. drakensbergensis (Figures 5A–C; 6G)		X						X				X
P. montana		X		X				X				X
Group B												
P. sp. nov. 1 (Figure 5D–F)		X					X					X
P. sp. nov. 2		X					X				X	
P. vulgaris		X							X			X
P. filiformis (Figure 6H)		X							X			X
P. falcifolia		X							X			X
P. pendula (Figure 6I)		X							X		X	X
P. rigida (Figures 5G–I; 6J)		X							X		X	X
P. galpinii		X							X	X		
P. rubra		X							X	X		
P. paleacea (Figure 6K, L)		X							X	X		
P. paludosa (Figure 5J–L)		X							X	X		

mucilage, resulting in a mucilage-filled cavity between remains of epidermal cells and adjacent mesophyll (Figure 2A) (Bredenkamp & Van Wyk 1999).

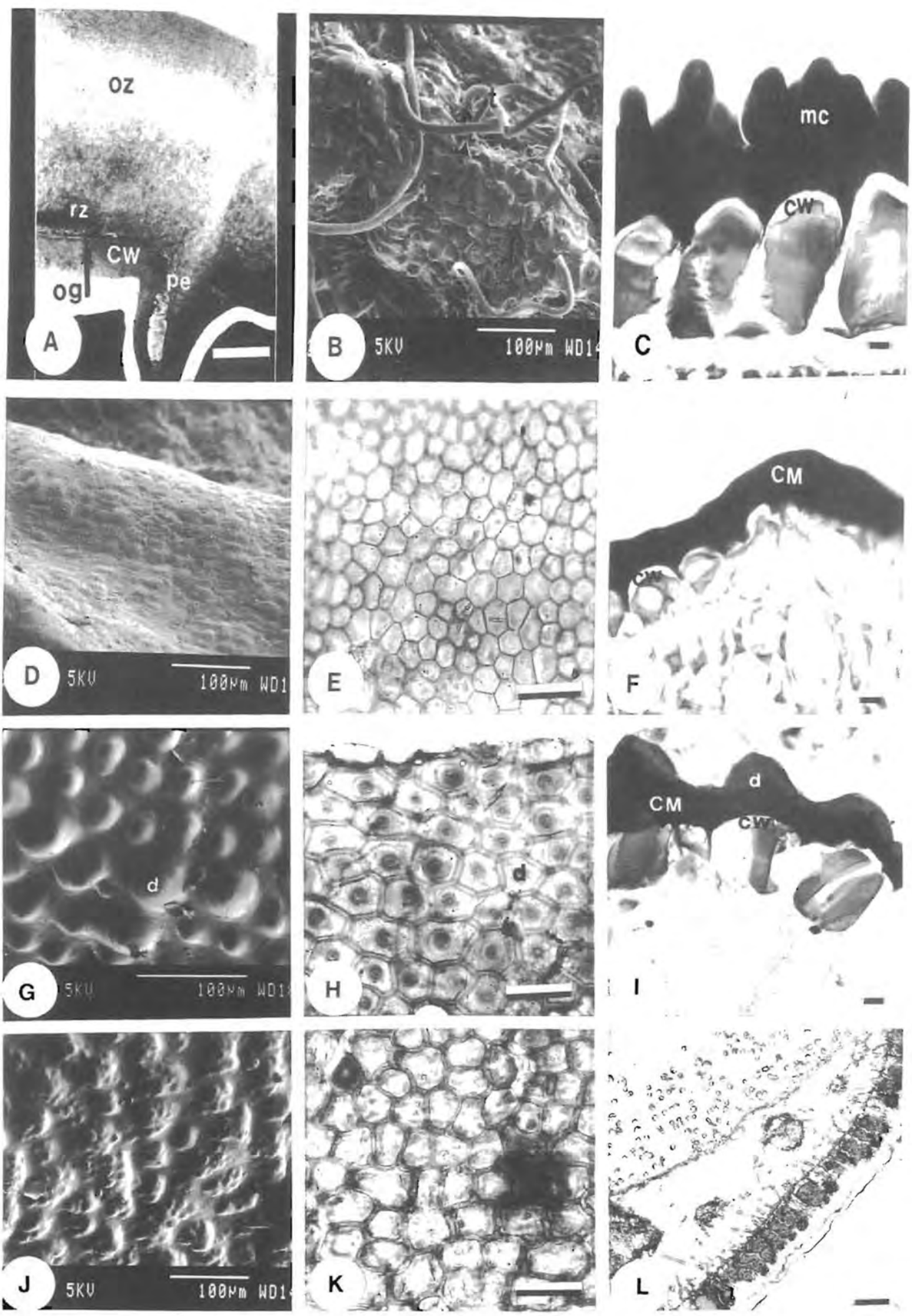
Surface view (SEM micrographs and cuticular preparations): *shape* pentagonal to heptagonal, cells mostly isodiametric or transversely oblong in *P. glomerata*, *P. ericoides* (Figure 4D, E) and *P. obtusifolia* (Figure 4G, H), but oblong in *P. burchellii* (Figure 4J, K); cells mostly slightly oblong or oblong in all other species of *Passerina* (Figure 5; Table 2). *Arrangement* random in *P. glomerata*, *P. ericoides*, *P. obtusifolia* and *P. burchellii* (Figure 4D–K), in rows in all other species of *Passerina* (Figure 5; Table 3).

Cuticle

Transverse section (LM): *epicuticular wax* absent owing to chemical treatment during fixation, embedding and staining. *Cuticular membrane* (CM) well developed, (10–)20–30(–70) µm thick (Table 2); cuticle proper delineated by narrow, lightly stained outer zone and cuticular layer by wider, darker stained zone; cuticular pegs present, formed by layer projecting into grooves between anticlinal walls of adjacent epidermal cells. *Outer periclinal cell walls* not staining with Sudan Black B (Figures 4I; 5C, I, L).

TEM: cuticle structure corresponding to the cuticular structural type 3, described by Holloway (1982). Cuticle proper and CM not distinguishable. *Cuticular membrane* (Figure 4A) comprising a wide, mainly amorphous outer zone and narrow faintly reticulate inner zone; osmiophilic granules aligned on border of clearly defined cell wall; cuticular pegs with unknown (possibly pectinaceous) substance (stained light grey) between cell wall and peg, forming part of middle lamella.

FIGURE 3.—LM photographs and SEM micrographs in *Passerina*. A–F, structure of stomatal complex. A–C, *P. rigida*, *Bredenkamp 1013*, *Ward 7211*: A, surface view of stomata showing peristomatal rims, raised guard cells and pronounced outer stomatal ledges; B, t/s adaxial epidermis stained with Sudan Black B, with crenate surface of cuticular membrane lining poral walls of guard cells; C, epidermal maceration stained with safranin, showing structure of epidermal cells surrounding guard cells, peristomatal rims. D–F, *Passerina* sp. nov. 1, *Bredenkamp1046*: D, sunken stomata in cavity of cymbiform leaf; E, epidermal maceration stained with safranin, with structure of epidermal cells and sunken stomata; F, t/s leaf, with raised stomata as well as stomatal crypts. G–J, structure of trichomes. G, *P. rubra*, *Bredenkamp 905*, with poral rims in relation to adaxial epidermal cells. H, *P. falcifolia*, *Bredenkamp 915*, with unicellular, long, spiralised, pointed trichomes; I, *P. paludosa*, *Bredenkamp 1035*, with trichome foot and conspicuous lumen; J, *P. pendula*, *Bredenkamp 909*, trichomes strongly spiralised. K, L, TEM micrographs of abaxial leaf epidermal cells of *P. falcifolia*, *Bredenkamp 917*, in cross section: K, mucilage accumulated between innermost and outermost cellulose layers of inner periclinal cell wall; L, innermost cellulose layer of inner periclinal cell wall. Abbreviations: aw, anticlinal cell wall; cy, cytoplasm; iip, innermost layer of inner periclinal cell wall; oip, outer layer of inner periclinal cell wall; m, mucilage; op, outer periclinal cell wall; v, vacuole. Scale bars: K, L 5 µm; A, B, H, 10 µm; C–F, G, I, J, 100 µm.



Cuticular ornamentation

In transections and surface view of leaves, LM and SEM studies showed that two groups of species, henceforth called Groups A, Intermediate and B (Table 3), can be distinguished on the basis of the arrangement and shape of epidermal cells as well as cuticular ornamentation.

Group A

Epidermal cells mostly isodiametric or transversely oblong in surface view; arranged randomly; cuticle mostly papillate; *outer periclinal walls* of cells convex in all species. *Cuticular membrane* (CM) smooth in *P. ericoides* and *P. glomerata* (Figures 4D–F; 6C); papillate in *P. obtusifolia*, with one dome per cell, situated ± centrally on outer periclinal wall of pentagonal or heptagonal cells (Figures 4G–I; 6E); with several domes per cell in *P. burchellii* (Figures 4J–L; 6F).

Group B

Epidermal cells mostly oblong in surface view, arranged in rows; concavities (depressions in centre region of cell) and convexities (roundish cells forming a low dome) more or less alternating (Figure 5G, J); cuticle with ridges at junction of epidermal cell walls mostly conspicuously raised, exhibiting a definite striate pattern (Figure 5D, G, J), otherwise ± plane.

Cuticular membrane pronounced at junctions of epidermal cell walls and grooved between anticlinal walls of adjacent cells (Figure 5I), more or less smooth in *P. vulgaris*, *P. filiformis*, *P. falcifolia*, *P. pendula*, *P. rigida*, and *P. galpinii*, except in *Passerina* sp. nov. 1, in which the presence of snow, at the time of collecting, seemed to have caused markings on the cuticular wax (Figure 5D, E). Small globular papillae visible between cuticular ridges in *Passerina* sp. nov. 1 (Figure 5D–E), *P. rubra*, *P. paleacea* and *P. paludosa* (Figure 5J–L).

Intermediate

Epidermal cells arranged in rows but CM less pronounced at junctions of epidermal cell walls and cuticular ridges less conspicuous, were recorded in *P. comosa* (Figures 4B; 6A, B), *P. drakensbergensis* (Figure 5A, B), *P. montana*, *P.* sp. nov. 3 and *P.* sp. nov. 4. CM smooth or with small globular papillae in *P. montana* and *P.* sp. nov. 4; domed with a 'molar'-like crown in *P. comosa* (Figure 4B, C), with several domes per cell in *P.* sp. nov. 3 and with 9 or 10 globular papillae per cell in *P. drakensbergensis* (Figures 5A–C; 6G).

Epicuticular waxes

Soft waxes present, coating entire abaxial surface: wax protruding through amorphous layer of CM in a variety of configurations: droplets conspicuous in *P. comosa*, *P. ericoides* and *P. burchellii* (Figure 6A, D, F); droplets and small round protrusions forming flat, shapeless lumps in *P. paleacea* (Figure 6L). *Crystalloids*: wax platelets and plates present or absent (Table 3); thin wax platelets, with margins entire or non-entire, flaking from wax surface in *P. comosa* and *P. rigida* (Figure 6A, J) and changing to plates as margins become distinctly edged. Upright plates separating from surrounding wax in *P. filiformis* (Figure 6H). Platelets and plates varying from sparse to abundant; platelets ± square to irregularly shaped, plates ± square to oblong and usually arranged perpendicular to cell rows.

The authenticity of epicuticular wax droplets and small round protrusions, observed in *P. ericoides*, *P. obtusifolia* and *P. paleacea* (Figure 7), was verified by washing leaves in chloroform for one minute and comparing them to unwashed specimens under SEM. Epicuticular wax droplets were clearly discernible in unwashed *P. paleacea* (Figure 7A), while small pores appeared in the cleaned, de-waxed cuticle after washing (Figure 7B–E). Similar pores were also present in *P. ericoides* (Figure 7F). No pores were present in the papillate CM of *P. obtusifolia*, but the corroded apices of the papillae clearly showed an accumulation of epicuticular waxes at these points (Figure 7G–I).

DISCUSSION

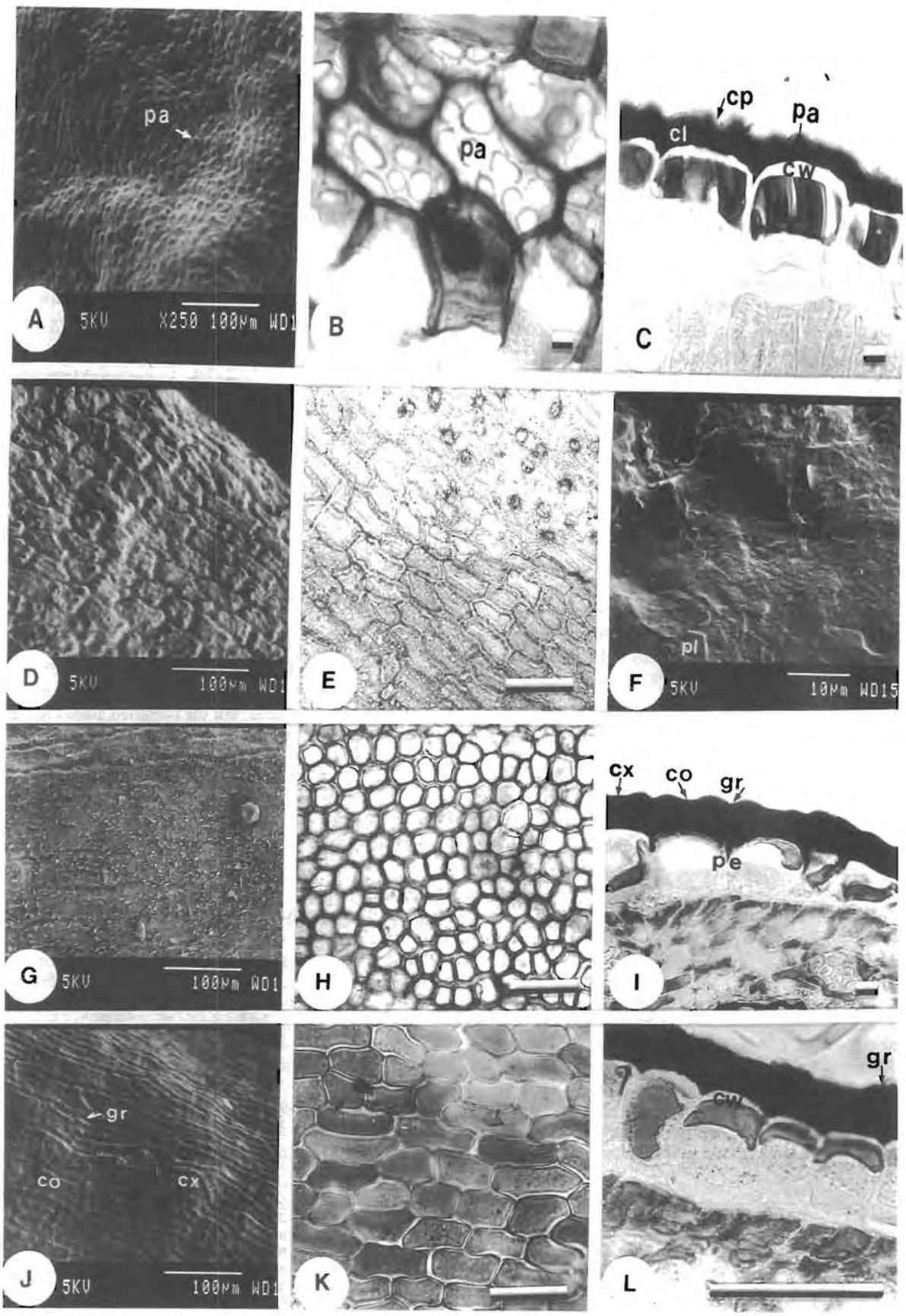
Adaxial epidermis

Plants of high mountains in the tropics usually have straight to curved anticlinal epidermal cell walls, the percentage of species with undulated walls increasing as altitude decreases (Wilkinson 1979). The straight-walled arrangement of the cells in *Passerina* sp. nov. 1 (Figure 3D, E), a high-altitude montane species, seems to comply with this pattern.

Stomatal complex

In all but one species of *Passerina* the stomata are usually raised or at the same level as other epidermal cells (Figure 2E, G, H), indicating that this character is of lim-

FIGURE 4.—A, TEM micrograph of cuticular membrane in *Passerina paleacea*, *Bredenkamp 961*, with wide, amorphous outside zone, narrow faintly reticulate inner zone, osmiophilic granules at border of cell wall and cuticular peg. B–L, LM photographs and SEM micrographs of abaxial leaf epidermis in *Passerina*. Epidermal macerations stained with safranin and t/s of epidermis stained with Sudan Black B. B, C, *P. comosa*, *MacDonald 2125*, *Andraea 1288*: B, trichomes present; C, CM domed, with 'molar-like' crown to each dome. D–F, *P. ericoides*, *Bredenkamp 956*, *962*, *Taylor 4042*: D, CM smooth, epidermal cells randomly arranged, ± isodiametric, outer periclinal cell walls convex; E, cells randomly arranged, ± isodiametric; F, convex outer periclinal walls and smooth CM. G–I, *P. obtusifolia*, *Bredenkamp 1034*: G, CM with one dome per cell; H, epidermal cells randomly arranged, transversely oblong with one dome per cell; I, convex outer periclinal cell wall and CM with one dome per cell. J–L, *P. burchellii*, *Bolus 687*, *Bredenkamp 1545*; J, CM with several domes per cell; K, randomly arranged cells, transversely oblong with rounded angles, several domes per cell; L, t/s epidermis in polarised light showing CM with several domes per cell. Abbreviations: CM, cuticular membrane; cw, outer periclinal cell wall; d, dome; mc, molar-like crown; og, osmiophilic granules; oz, amorphous outside zone; pe, cuticular peg; rz, narrow faintly reticulate inner zone; t, trichome. Scale bars: A, 5 µm; C, F, I, 10 µm; B, D, E, G, H, J–L, 100 µm.



ited taxonomic value at species level, except in *Passerina* sp. nov. 1, which has stomatal crypts or sunken stomata. Classification of the stomatal complex into stomatal types is often a problem owing to the subtle distinction of subsidiary cells (Wilkinson 1979; Van Wyk *et al.* 1982).

Patel (1978) considers subsidiary cells as morphologically and physiologically different from other epidermal cells and proposes a number of criteria to distinguish subsidiary cells in mature epidermis. Of these criteria we used the following in the distinction of subsidiary cells: size, shape, contents and position of cells. We found that the cells adjacent to the guard cells did not differ from other epidermal cells, except that they might be raised or sunken (Figures 2K; 3C). Furthermore, when stained with PAS, periclinal walls of subsidiary cells should be lightly stained compared with other epidermal cells, owing to less carbohydrates in these cell walls according to Patel (1978). In *Passerina* the periclinal walls of the cells adjacent to the guard cells stained homogenously with other cells in the stomatal complex (Figure 2F) and the anticlinal walls are not comparatively thinner than those of other epidermal cells, thus the cells adjacent to the guard cells cannot be considered subsidiary cells (Figure 2F, H). Stained with Sudan Black B, the contents of the cells surrounding the guard cells do not differ from those of other epidermal cells and no lipid bodies are present (Figure 2G).

We therefore conclude that the epidermal cells surrounding the guard cells in *Passerina* are not differentiated as subsidiary cells and we classify the stomatal apparatus in *Passerina* as anomocytic. This corresponds to the prevailing state in the Thymelaeaceae (Solereder 1908; Metcalfe & Chalk 1979). However, although we prefer to regard the epidermal cells surrounding the guard cells as similar to other epidermal cells, the presence of conspicuous peristomatal cuticular rims on the outer periclinal cell walls of epidermal cells around the guard cells may be used in support of a view that these cells are subsidiary cells. The stomatal apparatus could then be classified as staurocytic (Wilkinson 1979) or anomotetracytic (Dilcher 1974). As the number of epidermal cells surrounding the guard cells varies from 3–5(6), it would seem appropriate to classify the stomatal apparatus as anomostaurocytic (Van Wyk *et al.* 1982).

Trichomes

Passerina leaves are often cymbiform with spiralised trichomes densely arranged in the concave ventral space. This indumentum is likely to play an important role in the water relations of the plant. Water droplets precipitating from the atmosphere, or running down from leaves directly above, would accumulate in the concave leaf space. Droplets would be repelled by the hydrophobic cuticle of the trichomes and owing to cohesion forces cause a moisture layer in the upper part of the dense trichomes. One may speculate that water vapour escaping through the stomata would not be drawn outwards by capillary forces because of the water-repelling nature of the cuticle surrounding the trichomes, thus retaining a high concentration of moisture in the vicinty of the stomata. The overall high concentration of water vapour over the adaxial surface of the leaf is likely to decrease the transpiration rate. Laboratory tests to assess the wettability and the possible absorption of water by the laminar epidermal hairs in *Passerina*, suggest that the wettability of the spiralised hairs is quite low and that absorption of water by these trichomes is highly improbable. However, our suggestion of an overall high concentration of water in the adaxial cavity of the leaf, which serves to decrease the transpiration rate, is supported by these tests.

Cuticular ornamentation

Cuticular thickness may be affected by light, temperature, soil, atmospheric moisture and altitude (Wilkinson 1979). In *Passerina*, with many species adapted to the Cape Mediterranean climate, all members have a relatively thick cuticle, but it was the thickest in *P. comosa*, *P. glomerata*, *P. burchellii*, *P. galpinii* and *P. paleacea* (Table 2). The first two species grow in the northwestern parts of the Western Cape and on the mountains in and around the Little Karoo (= Karoo Mountain Centre *sensu* Weimarck 1941), areas with high light intensity, high temperature and low atmospheric moisture. *P. burchellii*, growing on high mountains at Villiersdorp and Genadendal, is exposed to high light intensity as well as high and low critical temperatures. *P. galpinii* grows on calcrete and *P. paleacea* is exposed to salt spray and wind. In *P. drakensbergensis*, *P. falcifolia*, *P. paludosa* and *P.* sp. nov. 1, the thickness of the CM is ± 20 µm. Of these species, *P. falcifolia*, from the mountains between George and Uitenhage, and *P. drakensbergensis*, from high altitudes in the Bergville District of KwaZulu-Natal, are exposed to relatively high atmospheric moisture. However, it is difficult to speculate on the functional significance of the relatively thin cuticles in *P. paludosa*, from salt marshes in the Cape Peninsula, and *P.* sp. nov. 1, a species from Waboomberg, one of the highest points in the Western Cape and often covered by snow in winter.

FIGURE 5.—Abaxial leaf epidermis and structure of CM in *Passerina*. Epidermal macerations stained with safranin and t/s of epidermis stained with Sudan Black B. A–C, *P. drakensbergensis*, *Bredenkamp 1018*, *1019*: A, cells arranged in rows with 9 or 10 globular papillae per cell; B, inner surface facing upwards, cells oblong in shape with 9 or 10 papillae per cell; C, CM layered, with cuticular layer and cuticle proper, also globular papillae. D–F, *Passerina* sp. nov. 1, *Bredenkamp 1044*, *1046*: D, several domes per cell, CM irregularly marked by ice crystals; E, cells arranged in rows, oblong in shape with CM irregularly marked by ice crystals; F, geometrical plates, flat or slightly raised. G–I, *P. rigida*, *Bredenkamp 1013*, *Ward 7211*: G, cells arranged in rows, plates abundant; H, cells arranged in rows, isodiametric to slightly oblong; I, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls, concavities and convexities not conspicuous. J–L, *P. paludosa*, *Bredenkamp 1035*, *Thoday 100*: J, cells arranged in rows, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls, concavities and convexities conspicuous; K, cells arranged in rows, cells oblong; L, CM pronounced at junctions of epidermal cell walls, grooved in midline of joining walls. Abbreviations: cl, cuticular layer; co, concavity; cp, cuticle proper; cw, outer periclinal cell wall; cx, convexity; gr, groove in CM; pa, papillae; pe, cuticular peg; pl, plates. Scale bars: A, D, E, G, H, J–L, 100 µm; B, C, F, I, 10 µm.

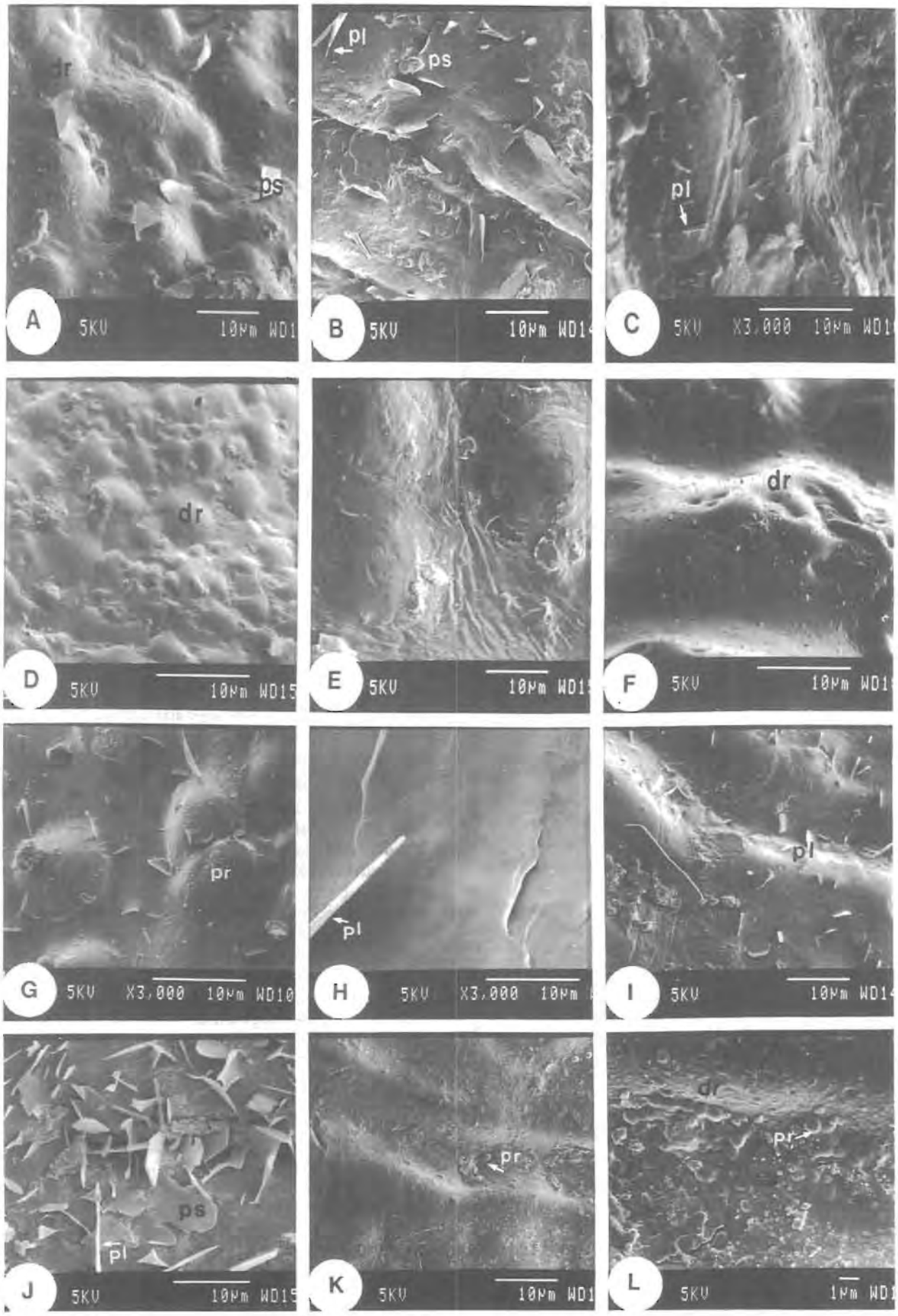


FIGURE 6.—SEM micrographs of abaxial leaf surfaces, the CM and epicuticular waxes in *Passerina*. A, B, *P. comosa*, *MacDonald 2125*: A, droplets present in epicuticular wax, platelets flaking from smooth wax coating; B, wax platelets flaking from smooth wax coating, plates present. C, *P. glomerata*, *Bredenkamp 973*, outer periclinal wall convex, plates scarce, square to oblong, raised 30°–90°; D, *P. ericoides*, *Bredenkamp 956*, droplets present in epicuticular wax; E, *P. obtusifolia*, *Bredenkamp 929*, smooth wax coating also covering domes. F, G, *P. burchellii*, *Stokoe 2542*: F, droplets at apices of domes; G, small round protrusions at apices of papillae. H, *P. filiformis*, *Bredenkamp 1016*, upright plates separate from surrounding wax, orientated at an angle to cell rows; I, *P. pendula*, *Bredenkamp 908*, plates frequent, perpendicular to cell rows, square to oblong, flat or raised; J, *P. rigida*, *Bredenkamp 1013*, platelets and plates ; K, L, *P. paleacea*, *Bredenkamp 961*, wax droplets, protrusions and flat shapeless lumps contributing towards soft wax coating or smooth layer. Abbreviations: dr, droplets in epicuticular wax; pl, plates; pr, small round protrusions of epicuticular wax; ps, platelets. Scale bars: A–K, 10 µm; L, 1 µm.

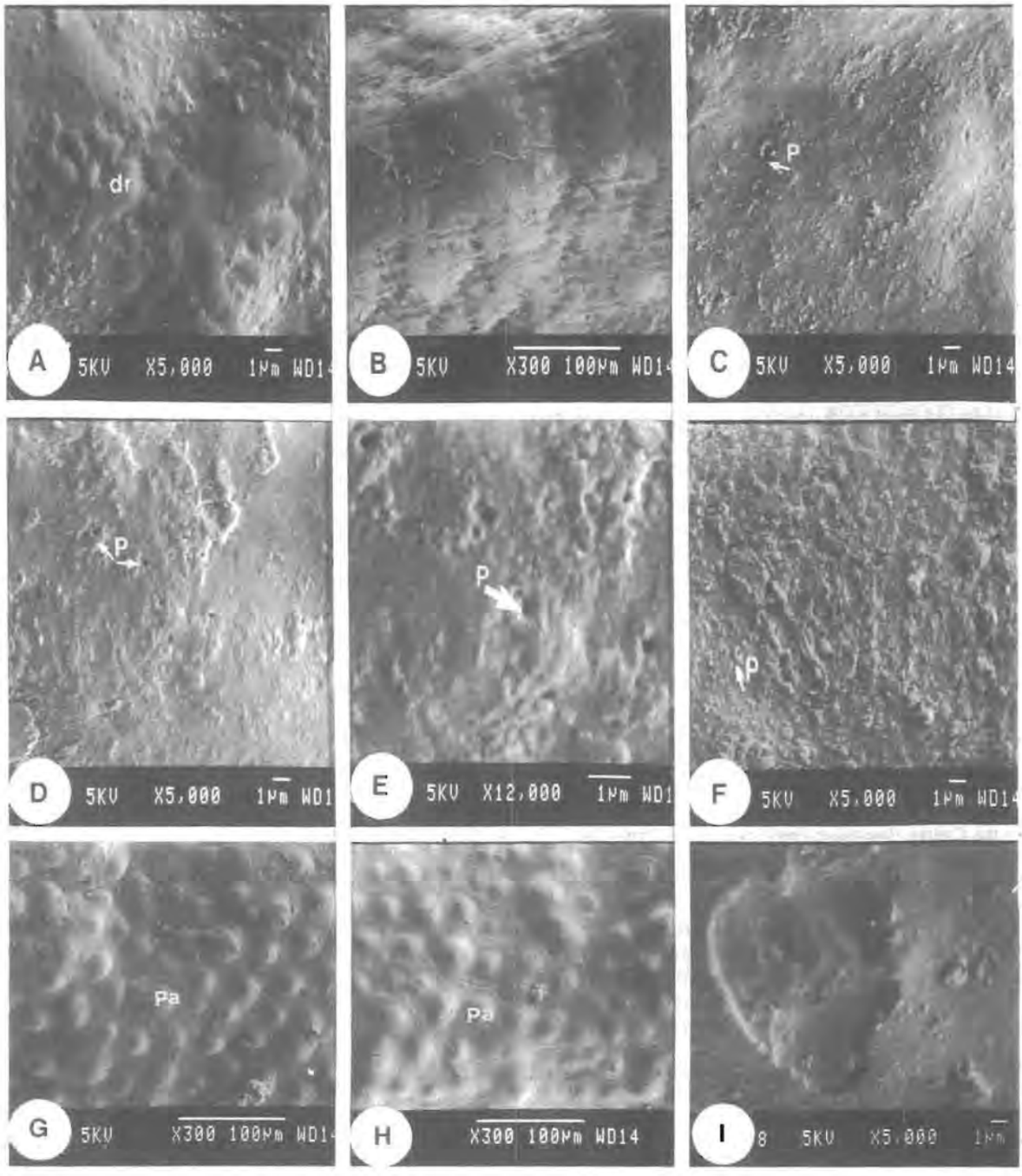


FIGURE 7.—SEM micrographs of abaxial leaf surfaces of *Passerina* washed in chloroform for one minute, compared to unwashed specimens. A–E, *P. paleacea*, *Bredenkamp 961*: A, unwashed leaf showing droplets in smooth wax coating; B, low magnification of washed leaf, showing CM devoid of epicuticular wax; C–E, higher magnifications showing pores in CM. F, *P. ericoides*, *Bredenkamp 956*, washed specimen showing pores in CM. G–I, *P. obtusifolia*, *Bredenkamp 929*: G, unwashed specimen; H, I, washed specimens showing corroded apices of papillae. Abbreviations: dr, droplets in epicuticular wax; p, pore; pa, papillae. Scale bars: A, C–F, I, 1 µm; B, G, H, 100 µm.

Haberlandt (1914), following a study of plants in tropical rain forests, considered the function of papillose epidermal cells as concentrating limited light by acting as lenses. Bredenkamp & Van Wyk (1999) speculate that, in *Passerina*, the convex outer periclinal epidermal cell wall may well focus light rays onto the mesophyll, whereas large vacuoles filled with phenols and the mucilage formed by the cellulose slimes (inner periclinal walls) protect the mesophyll from potentially dangerous UV-B radiation. According to Wilkinson (1979) the presence and prominence of papillae are diagnostically unreliable because they vary with the climate or distribution of the species; only morphologically distinct types can be used for diagnostic purposes. However, distinct epidermal cell papillae characterise *P. comosa*, *P. obtusifolia* *P. burchellii*, *P. drakensbergensis* and *P.* sp. nov. 2 (Figures 4B–C, G–L; 5A–C). The presence of these papillae could have been induced by the high light intensity of the areas in which these plants grow.

Epicuticular waxes

In their study of the epicuticular waxes in the families of the Dilleniidae and Rosidae, Ditsch & Barthlott (1997) documented the numbers of genera, species and hybrids in which different wax types occur, without identifying the various taxa. The epicuticular waxes of 12 genera, 31 species and two hybrids were studied in the Thymelaeaceae. Of these, nine genera and 26 species have wax flakes, one species has angled platelets and four genera and five species have no crystalloids. Our

observations indicate that the simple plate-type waxes found in *Passerina* correspond well to those described by Ditsch & Barthlott (1997) in the Thymelaeaceae. Of the 17 species in *Passerina*, two have wax flakes, eight have platelets or angled plates and seven are devoid of crystalloids (Figure 6, Table 3).

The mechanism of wax extrusion through the cuticle is highly controversial (Baker 1974; Jeffree *et al.* 1975; Hallam 1982). Baker (1982) discusses the extrusion of wax by means of 'pores and channels, the liquid extrusion theory, polymerization theory and the crystallization theory'. Hallam (1982) proposes that wax or wax precursors in their protein or glycoprotein 'shells' move through the cuticle and burst on the surface, liberating the wax from the 'package'; on crystallization, the protein coats stick to the surface as the wax crystals develop.

Our results indicate small pores in the cleaned, dewaxed cuticle of *P. paleacea* and *P. ericoides* (Figure 7B–F), after washing leaves in chloroform. Both Baker (1982) and Hallam (1982) are convinced that detailed investigations by many investigators have failed to confirm the presence of pores or microchannels in certain plant cuticles and that pores have not been shown to connect with the plasmalemma of the epidermal cytoplasm below. Although the presence of pores has been confirmed by our study, further research on the ultrastructure of the CM in *Passerina* could be most informative.

Freeman *et al.* (1979), working on *Citrus*, found amorphous wax layers on immature leaves and fruit, with small protrusions and isolated regions of upright platelets developing, eventually followed by cracks and irregular plates. Similarly in *Passerina*, wax droplets, protrusions and flat, shapeless lumps contribute towards a soft wax coating or a smooth layer. Species of *Passerina* with soft wax coatings, without platelets or plates, are summarised in Table 3. In *P. comosa*, *P. filiformis* and *P. rigida* (Figure 6B, H, J) platelets and plates are formed as a result of cracks developing on the outer wax surface, crystallising into irregularly shaped flakes, which gradually become square or oblong with 'entire' or 'non-entire' margins, often becoming distinctly edged. In *P. filiformis* (Figure 6H) upright plates separate from the surrounding wax, orientating themselves at an angle to the cell rows, eventually resulting in most plates being arranged more or less perpendicularly to the cell rows. Wax type, as well as the presence or absence of plates and platelets, is apparently genetically determined (Baker 1982). For example, *P. ericoides*, *P. rigida* and *P. paleacea* (Figure 6D, J, K) all grow along the sea shore, where they are subjected to wind, salt spray and high light intensity, and yet, *P. ericoides* and *P. paleacea* have coverings of soft waxes only, whereas platelets and plates are abundantly present in *P. rigida*. However, in plate waxes the number of platelets and plates, size, configuration and distribution of the surface wax structures can be considered as environmentally induced (Baker 1974, 1982).

Functions of epicuticular waxes

Possible functions of epicuticular waxes are discussed by Jeffree (1986). In *Passerina*, large areas of the abaxial epidermis are exposed to the atmosphere because the inverse-ericoid leaves are usually closely appressed to the stem. In response to the warm, dry summers of the Mediterranean climate of the Cape, it is proposed that the CM, including the abaxial epicuticular waxes, has a water-proofing function, protecting the leaves against desiccation and limiting transpiration to the adaxial epidermis only. As the leaves are decussately arranged, the water-repelling function of the waxes would cause droplets of water to run off the abaxial epidermis, into the concave, hairy adaxial surface of the lower leaf, resulting in a decreased transpiration rate owing to the higher adaxial water concentration. According to Jeffree (1986) the wettability of the plant surface is determined by its microroughness. The presence of crystalloid platelets and plates, and especially their arrangement perpendicular to cell rows, may facilitate the retention of moisture.

Systematic value

Epicuticular waxes have been proven taxonomically valuable, among others in the study of the Centrospermae (Engel & Barthlott 1988), Dilleniidae and Rosidae, including the Thymelaeaceae (Ditsch & Barthlott 1997), at sectional level in *Eucalyptus* L'Hér. (Hallam & Chambers 1970) and at species level in *Hordeum* L. (Baum *et al.* 1989). In *Passerina* the presence or absence of crystalloid platelets or plates combined with characteristics of the CM and the outer periclinal cell walls of the abaxial epidermis, makes it possible to distinguish between two groups in the genus. This distinction is species-specific for most of the 17 species examined (Table 3).

Ecological aspects of leaf epidermis

The structure and function of the epidermis should be considered in context with gross leaf morphology and arrangement. Leaf arrangement is of vital importance to the physiology of the plant. The epidermis serves as an envelope, physically protecting the mesophyll, the largest part of the abaxial epidermis forming a multifunctional barrier to the environment. The thin adaxial epidermis is concealed in the groove of the cymbiform leaf in most cases; it is almost covered by dense, long, spiralised uniserial trichomes and contains the stomata, which are often raised. This arrangement is likely to reduce the rate of transpiration, especially if moisture can be retained by the indumentum. The abaxial epidermis is probably multifunctional. The whole of the CM has a waterproofing function and the epicuticular waxes also have a water-repelling function. At the same time the CM may play a major part in focusing light rays onto the palisade parenchyma. Large tanniniferous vacuoles may play a role in the possible absorption of UV-B radiation, and mucilage formed by the cellulose slimes (inner periclinal walls) possibly protects the mesophyll from desiccation (Bredenkamp & Van Wyk 1999).

The expansion and inrolling of the leaf margins in *Passerina*, as a result of changing turgor pressure in the epidermal cells, were described by Thoday (1921). He regards the main mechanism involved as the co-ordina-

tion between the turgor pressure and the difference in size and thickness of cell walls of the ad- and abaxial epidermis, whereas the plicate anticlinal cell walls of the abaxial epidermis protect the cells against bending stress. Stomata (or at least the indumentum) are exposed when the leaf margins expand and are protected in a villous groove when the leaf margins are rolled inwards, thus regulating the rate of transpiration.

CONCLUSIONS

Leaf shape and structure in Thymelaeaceae exhibit a transformation series from mainly dorsiventral, the prevailing family feature, to isobilateral or centric in *Diarthron* Turcz., *Pimelea* Banks & Soland. and *Thymelaea* Juss. (Metcalfe & Chalk 1950). All the mentioned states are present in *Lachnaea* and *Cryptadenia* (Beyers 1992) and, as the most advanced state, inversely dorsiventral leaves in *Passerina*. A transformation series can also be illustrated by the presence of amphistomatic, hypostomatic and epistomatic leaves in the Thymelaeaceae (Metcalfe & Chalk 1950), the epistomatic state in *Passerina* considered to be the most advanced (the collateral vascular bundles of the leaves, with xylem arranged adaxially and phloem abaxially, rule out the possibility of resupination of the leaves).

The most pronounced epidermal characters of the Thymelaeaceae are anomocytic stomata (Metcalfe & Chalk 1950), unicellular trichomes and mucilagination of epidermal cells. In the present study the presence or absence, distribution of or changes in the above-mentioned structures, were used as distinguishing characters at both generic and species levels. Mucilagination of epidermal cells is often found both ad- and abaxially in the leaves of Thymelaeaceae. In *Passerina*, mucilagination takes place in the abaxial epidermis only. At species level the sunken stomata and stomatal crypts of *Passerina* sp. nov. 1 are used in the delineation of the new taxon and *P. comosa* is distinguished by the presence of unicellular trichomes on the abaxial surface of the leaves.

On the basis of abaxial cuticular characters, it has been possible to distinguish two groups of species in the genus. Group A comprises *P. burchellii* Thoday, *P. comosa* C.H.Wright, *P. ericoides* L., *P. glomerata* Thunb. and *P. obtusifolia* Thoday. Group B comprises *P. drakensbergensis* Hilliard & B.L.Burtt, *P. falcifolia* C.H.Wright, *P. filiformis* L., *P. galpinii* C.H.Wright, *P. montana* Thoday, *P. paleacea* Wikstr., *P. paludosa* Thoday, *P. pendula* Eckl. & Zeyh., *P. rigida* Wikstr., *P. rubra* C.H.Wright, *P. vulgaris* Thoday, *P.* sp. nov. 1, *P.* sp. nov. 2, *P.* sp. nov. 3 and *P.* sp. nov. 4. Certain species in each of the two groups seem to be naturally allied. Distribution patterns of *P. obtusifolia* and *P. glomerata* coincide at Worcester and transitional types can be clearly distinguished. Transitional types are similarly present in *P. filiformis* and *P. vulgaris* in the Cape Peninsula and in *P. filiformis* and *P. falcifolia* near Knysna.

Hence it can be concluded that the conspicuous differences as well as the concise characters of the ad- and abaxial epidermis, critically described and discussed in this paper, can be used as taxonomic tools at the family, genus and species levels. Furthermore, the leaf epidermis in *Passerina* is probably most valuable to the plant in terms of ecological adaptation, considering the wide distribution of the genus in southern Africa as well as the accompanying geographical and climatic variation. The gross leaf morphology and the ad- and abaxial epidermal characters have been most useful in the interpretation of the possible functioning of the leaves and are of vital importance in the survival strategies of the plant.

ACKNOWLEDGEMENTS

The authors wish to thank Mmes H. du Plessis and C. Steyn and Dr E. Steyn for assistance with the LM, Mrs A. Romanowski for developing and printing many excellent photographs and Prof. J. Coetzee and Mr C. van der Merwe, both of the University of Pretoria, for assistance with the SEM and TEM.

REFERENCES

ALVIN, K.L. 1987. Leaf anatomy of *Androstachys johnsonii* Prain and its functional significance. *Annals of Botany* 59: 579–591.

AMELUNXEN, F., MORGENROTH, K. & PICKSAK, T. 1967. Untersuchungen an der Epidermis mit dem Stereoscan-Elektronenmikroskop. *Zeitschrift für Pflanzenphysiologie* 57: 79–95.

BAKER, E.A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. *New Phytologist* 73: 955–966.

BAKER, E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In D.F. Cutler, K.L. Alvin & C.E. Price, *The plant cuticle*: 139–165. Academic Press, London.

BARTHLOTT, W., NEINHUIS, C., CUTLER, D., DITSCH, F., MEUSEL, I., THEISEN, I. & WILHELMI, H. 1998. Classification and terminology of plant epicuticular waxes. *Botanical Journal of the Linnean Society* 126: 237–260.

BAUM, B.R., TULLOCH, A.P. & BAILEY, G.L. 1989. Epicuticular waxes of the genus *Hordeum*: a survey of their chemical composition and ultrastructure. *Canadian Journal of Botany* 67: 3219–3226.

BEAUMONT, A.J., EDWARDS, T.J. & VAN STADEN, J. 1994. Leaf and involucral bract characters of systematic use in *Gnidia* (Thymelaeaceae). *Proceedings of the Electron Microscopy Society of South Africa* 24: 47.

BEYERS, J.B.P. 1992. *The generic delimitation of* Lachnaea *and* Cryptadenia *(Thymelaeaceae)*. M.Sc. thesis, University of Stellenbosch, Stellenbosch.

BOND, P. & GOLDBLATT, P. 1984. Plants of the Cape flora: a descriptive catalogue. *Journal of South African Botany* 13: 1–455.

BREDENKAMP, C.L. & VAN WYK, A.E. 1999. Structure of mucilaginous epidermal cell walls in *Passerina* (Thymelaeaceae). *Botanical Journal of the Linnean Society* 129: 223–238.

DILCHER, D.L. 1974. Approaches to the identificaton of angiosperm leaf remains. *Botanical Review* 40: 1–157.

DITSCH, F. & BARTHLOTT, W. 1997. *Mikromorphologie der Epicuticularwachse und das System der Dilleniidae und Rosidae*. Akademie der Wissenschaften und der Literatur, Mainz.

ENGEL, T.E. & BARTHLOTT, W. 1988. Micromorphology of epicuticular waxes in Centrosperms. *Plant Systematics and Evolution* 161: 71–85.

FEDER, N. & O'BRIEN, T.P. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* 55: 123–142.

FREEMAN, B., ALBRIGO, L.G. & BIGGS, R.H. 1979. Ultrastructure and chemistry of cuticular waxes of developing *Citrus* leaves and fruits. *Journal of the American Society for Horticultural Science* 104: 801–808.

GILG, E. 1894. Studien über die Verwandtschaftsverhältnisse der Thymelaeales und über die 'anatomische Methode'. *Botanische Jahrbücher* 18: 489–574.

GOLDBLATT, P. & MANNING, J.C. in press. Cape plants: conspectus of the Cape flora of South Africa. *Strelitzia*.

HABERLANDT, G. 1914. *Physiological plant anatomy*. Reprint edn 1965. Today & Tomorrow's Book Agency, New Delhi.

HALLAM, N.D. 1982. Fine structure of the leaf cuticle and the origin of leaf waxes. In D.F. Cutler, K.L. Alvin & C.E. Price, *The plant cuticle*: 197–214. Academic Press, London.

HALLAM, N.D. & CHAMBERS, T.C. 1970. The leaf waxes of the genus *Eucalyptus* L'Héritier. *Australian Journal of Botany* 18: 335–386.

HOLLOWAY, P.J. 1982. Structure and histochemistry of plant cuticular membranes: an overview. In D.F. Cutler, K.L. Alvin & C.E. Price, *The plant cuticle*: 1–32. Academic Press, London.

JEFFREE, C.E. 1986. The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In B. Juniper & R. Southwood, *Insects and the plant surface*: 23–64. Edward Arnold, London.

JEFFREE, C.E., BAKER, E.A. & HOLLOWAY, P.J. 1975. Ultrastructure and recrystallization of plant epicuticular waxes. *New Phytologist* 75: 539–549.

KARNOVSKY, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* 27: 137a.

KIGER, R.W. 1971. Epidermal and cuticular mounts of plant material obtained by maceration. *Stain Technology* 46: 71–75.

KUGLER, H. 1928. Über inverse-dorsiventrale Blätter. *Planta* 5: 89–134.

MARTIN, J.T. & JUNIPER, B.E. 1970. *The cuticles of plants*. Edward Arnold, London.

METCALFE, C.R. & CHALK, L. 1950. *Anatomy of the dicotyledons* 2. Clarendon Press, Oxford.

METCALFE, C.R. & CHALK, L. 1979. *Anatomy of the dicotyledons* 1, edn 2. Clarendon Press, Oxford.

PATEL, J.D. 1978. How should we interpret and distinguish subsidiary cells? *Botanical Journal of the Linnean Society* 77: 65–72.

REYNOLDS, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17: 208–212.

SOLEREDER, H. 1908. *Systematic anatomy of the dicotyledons* 2. Clarendon Press, Oxford.

STACE, C.A. 1965. Cuticular studies as an aid to plant taxonomy. *The Bulletin of the British Museum (Natural History), Botany*, ser. 4: 1–78.

SUPPRIAN, K. 1894. Beiträge zur Kenntnis der Thymelaeaceae und Penaeaceae. *Botanische Jahrbücher* 18: 306–353.

THEOBALD, W.L., KRAHULIK, J.L. & ROLLINS, R.C. 1979. Trichome description and classification. In C.R. Metcalfe & L. Chalk, *Anatomy of the dicotyledons* 1: 40–53.

THODAY, D. 1921. On the behaviour during drought of leaves of two Cape species of *Passerina*, with some notes on their anatomy. *Annals of Botany* 35: 585–601.

THODAY, D. 1924. XVIII. A revision of *Passerina*. *Kew Bulletin* 4: 146–168.

VAN DER MERWE, C.F. & COETZEE, J. 1992. Quetol 651 for general use: a revised formulation. *Proceedings of the Electron Microscopy Society of Southern Africa* 22: 31, 32.

VAN TIEGHEM, PH. 1893. Recherches sur la structure et les affinités des Thyméléacées et des Pénéacées. *Annales des Sciences Naturelles Botanique*, 7,17: 185–294.

VAN WYK, A.E., ROBBERTSE, P.J. & KOK, P.D.F. 1982. The genus *Eugenia* L. (Myrtaceae) in southern Africa: the structure and taxonomic value of stomata. *Botanical Journal of the Linnean Society* 84: 41–56.

WEIMARCK, H. 1941. *Phytogeographical groups, centres and intervals within the Cape Flora*. Lunds Universitets Årsskrift. N.F. Avd. 2, Bd. 37, Nr. 5. Otto Harrassowitz, Leipzig.

WILKINSON, H.P. 1979. The plant surface. In C.R. Metcalfe & L. Chalk, *Anatomy of the dicotyledons*, edn 2, 1: 97–165. Clarendon Press, Oxford.