Acta Bot. Neerl. 36(3–4), November 1987, p. 271–282

THE DEVELOPMENT OF THE STAMINAL FILAMENT OF GASTERIA VERRUCOSA

C. J. KEIJZER, I. H. S. HOEK and M. T. M. WILLEMSE

Department of Plant Cytology and Morphology, Agricultural University, Wageningen, The Netherlands

Keywords: anther, filament, pollen, stamen.

SUMMARY

The development of the lower part of the stamen of *Gasteria verrucosa* (Mill.) H. Duval, the filament, was investigated using interference contrast, transmission and scanning electron microscopy, and was related to the development of the anther.

The filament extends during its entire development, in the younger stages mainly in the basal region, in the older stages in the tip. Up to maturity the solidity is gradually improved by the progressive thickening of the tangential epidermal wall and the cuticle from the tip to the base of the filament and the increasing turgidity of the epidermis.

The amount of tracheary elements increases up to maturity as does the starch content of the epidermis and the parenchyma. Starting at the pollen mitosis stage, a progressive cytoplasmic degeneration can be observed from the tip to the base and from the central parenchyma to the epidermis. At anther dehiscence the filament tip shrivels and the starch has disappeared from the entire filament. Both the latter phenomenon and the cytoplasmic degeneration may be due to redistribution of substances to other floral parts. The presence of prominent intercellular spaces may be important for the supply of gas to the maturating locule.

1. INTRODUCTION

In contrast to the huge amount of work on the development of anther tissues, investigations concerning the development of the anther supporting organ, the filament, are scarce. SCHMID (1976) has written a prominent work on the systematics of filament anatomy, focussing on the different tissues. He has found some typical filament characteristics and has discussed the possible roles of the filament in anther dehiscence. BURCK (1906) has reported the retraction of water from the anthers through the filament in favour of anther dehiscence, but experiments of later works (see SCHMID & ALPERT 1977) contradict these results. A few works deal with the vascular tissue of filaments (IVANCICH 1906; LEINFELLNER 1956) or the anatomical background of filament movements (PFEFFER 1904; KNOLL 1914). In some cereals interrelationships have been found between a poorly developed vascular tissue and the occurrence of male sterility, although normal development of this tissue in male sterile plants has also been reported (SHIVANNA & JOHRI 1985). ANDERSON (1980) has reported to the growth of pollen tubes from the indehiscent anthers through the filament, the receptacle and the carpels to the ovules in the cleistogamous flowers of some Malpighiaceae spp.

In the present study we investigated the filament development of *Gasteria verrucosa* (Mill.) H. Duval from the premeiotic stage up to anther dehiscence. This development is related to different aspects of anther development in this species (KEIJZER 1983, 1987a, b, c; KEIJZER & CRESTI 1987; KEIJZER & WILLEMSE 1986).

2. MATERIALS AND METHODS

Stamens of *Gasteria verrucosa* (Mill.) H. Duval in different stages of development were fixed in 3% glutaraldehyde for 3–4 hours and 1% osmium tetroxide for 2–3 hours, both in 0.1 M cacodylate buffer (pH 7.2) at room temperature. After dehydration in ethanol they were embedded in the low viscosity resin of SPURR (1969). Ultrathin sections were stained with lead citrate and uranyl acetate (REYNOLDS 1963) and observed in a Philips EM 301 at 60 kV. For the detection of callose, grids with ultrathin sections were mounted in a mixture of aniline blue (JENSEN 1962) and a highly diluted aqueous solution of calcofluor white M2R (HUGHES & MACCULLY 1975) under a cover glass. When illuminated with light of 415 nm, the former dye stains the callose, the latter both callose and cellulose, but to a lesser extent than the former, due to the very low concentration.

For interference contrast microscopy 2 μm sections were cut from the same blocks and observed in water under a coverglass. For the same microscope intact filaments were mounted in the clearing solution of HERR (1971) and directly observed.

To observe the internal structure, using scanning electron microscopy, filaments were treated according to the procedure of CRESTI *et al.* (1986). They were observed in a Jeol SEM 35C at 15 kV. Also fresh filaments were observed in the SEM.

3. RESULTS

3.1. General development

The filaments of *G. verrucosa* consist of an epidermis, surrounding parenchyma with a central vascular bundle (*fig. 1*). During growth of the flower bud the filament stretches continuously, keeping the anther in the top of the bud. In the younger stages the extension in longitudinal direction occurs mainly near the base of the filament, later on it is gradually replaced to the tip and occurs much faster, keeping up with the extension of the tepals and the pistil. The anther itself only stretches slightly. The vascular bundle of the filament is continued in apical direction in the connective tissue of the anther. It consists of one strain of spirally thickened tracheary elements and two strains of sieve elements (*fig. 2*). Just under its ending the amount of tracheary elements in cross section increases up to twice the mean number in the rest of the filament, the so called

tracheid maximum (*fig. 3*).

3.2. The pollen mother cell stage

In the pollen mother cell stage both the epidermis and the parenchyma are characterized by vacuolized thin-walled cells, surrounding some intercellular spaces (*fig. 1*). Near the tip of the filament some of these spaces are absent under the epidermis and cell wall corners are slightly thickened in these sites (*fig. 4*). Along the entire length of the filament the cuticle is thin (*fig. 5*). The epidermis and the periphere parenchyma layers contain some starch, the amount increasing towards the tip region. Lipid is scarce in all the tissues, except in the accompanying cells of the sieve elements. ER is scarce, dictyosome vesicles are present near the plasma membrane in both the epidermis and the parenchyma, especially near the base of the filament (*fig. 5*). The xylem contains 4–6 tracheary elements in cross section, each of the two phloem strains has three sieve elements.

3.3. The young microspore stage

In the epidermis the amount of starch is less than in the previous stage. In contrast, starch is also present in the central parenchyma layers (*fig. 6*), increasing towards the tip. Lipid droplets are now only found in the epidermis and the central parenchyma. Near the tip the number of subepidermal intercellular spaces is less than in the previous stage. The cuticle has the same structure as in the previous stage.

The walls of the sieve elements are thicker than in the previous stage and are visible after clearing the intact filament with HERR's (1971) solution (*fig. 7*). The amount of tracheary elements is higher than in the previous stage.

3.4. The young pollen grain after mitosis

Near the tip the epidermal cells are swollen in all directions if compared with the previous stage. Starch is still present in the same tissues as in the previous stage. In the lower half of the filament the amount of lipid in the central parenchyma is larger than in the previous stage and lipid is present now in the periphere parenchyma. Over the entire length of the filament signs of degeneration can be observed in some individual cells of the central parenchyma, their number increasing progressively from the base up to the tip. In these cells clusters of ribosomes, plasma and small vesicles are dispersed throughout the cell (*fig. 8*), the dictyosomes have all disappeared and the (small amounts of) plastids and mitochondria are floating in plasma-less areas. In the central parenchyma of the tip the number of intercellular spaces is small now (*fig. 9*). In the same region the outer tangential wall of the epidermis is thicker than in the previous stage and ridges are present on its outside (*fig. 10*) Accordingly, the surface of the cuticle is larger and also the thickness of the cuticle is larger than in the previous stage (*fig. 10*).

In the tip of the filament tracheary elements are still formed (*fig. 11*), raising their total number up to 10 in cross section, thus extending the tracheid maximum downward from the anther into the filament.

3.5. The mature anther stage

In this stage the development of the main part of the filament differs from the upper 50 μm. In the region of 50 to 500 μm under the tip starch is present in the epidermis. In the other tissues in this zone the amount is larger than in the previous stage (*fig. 12*), in contrast with the upper 50 μm. In the rest of the filament the amount of starch is equal to the previous stage. In the lower half (= 750 μ) of the filament lipid is scarce. In the upper 350 μm the amount of RER is less than in the previous stage, while large amounts of tubular SER are present now in both the epidermis and the parenchyma (*fig. 13*). In the upper 350 μm the intercellular spaces are absent under the epidermis and in the central parenchyma. In the lower parts the intercellular spaces are still present in all the tissues. The degeneration, in the previous stage only observable in some cells of the central parenchyma, can now also be found in the periphere parenchyma of the entire filament, although still more frequently near the tip. Over the entire length of the filament the amount of dictyosomes in both the epidermis and the parenchyma is larger than in the previous stage, their vesicles often close to the plasma membrane, comparable with *fig. 5*. The SER is still present in both tissues.

The outer tangential epidermal wall, its wall ridges and the cuticle are thicker than in the previous stage (*fig. 13*), showing gradual differences from the tip to the base (*fig. 14a, b, c, d*). In the former zone the cuticle sometimes dissociates from the wall (*fig. 15*). Also the cuticle, which is present in some of the intercellular spaces, shows such changes (*fig. 16*).

The amount of helically thickened tracheary elements is larger than in the previous stage, and both young tracheids and older stretched ones can be found in the mature filament (*fig. 17*). The pores between adjacent sieve elements and between sieve elements and accompanying cells are filled with callose, which

Fig. 1. The base of the filament in the pollen mother cell stage. Between the thin walled epidermis and parenchyma cells intercellular spaces are visible (arrows). X = xylem, P = phloem. (160 ×) (transverse section, interference contrast microscopy).

Fig. 2. The general composition of the vascular bundle. The hooked helical thickened tracheary elements (T) are situated opposite two separated strains of sieve elements (S), which are hooked as well. (1210 ×) (transverse section, interference contrast microscopy).

Fig. 3. In an intact anther cleared with HERR's solution, the ending of the vascular bundle in the connective tissue of the anther is visible. In basipetal direction part of the tracheid maximum (arrow) is visible. (310 ×) (light microscopy).

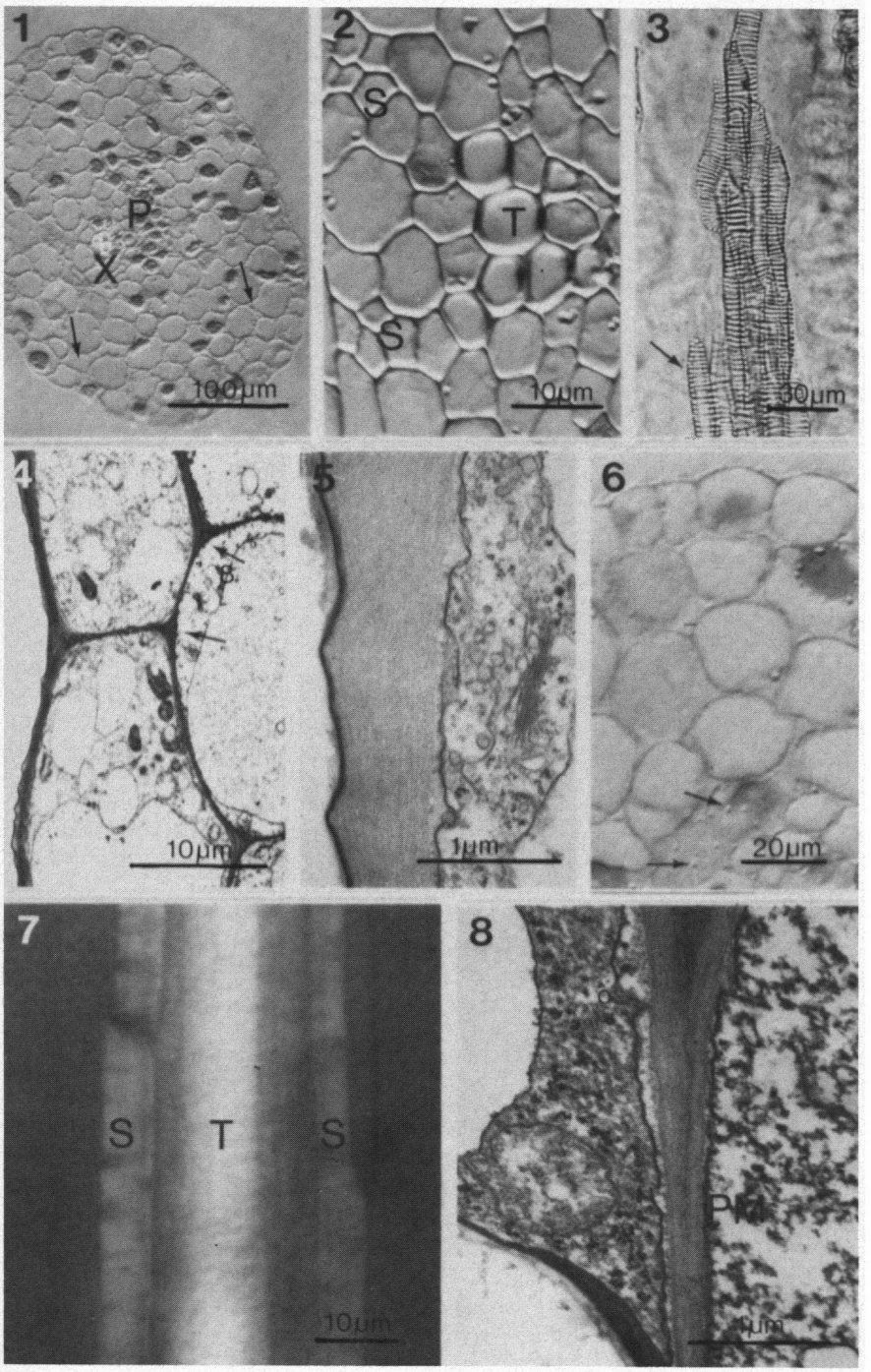
Fig. 4. In the pollen mother cell stage the cell walls of the epidermis near the base of the filament have thickened corners (arrows). (1825 ×) (transverse section, transmission electron microscopy).

Fig. 5. Detail of *fig. 4*. Dictyosome vesicles can be seen near the plasma membrane. (19300 ×).

Fig. 6. In the young microspore stage a small amount of starch can be seen in the epidermis whereas it is more abundant in the central parenchyma (arrows). (550 ×) (transverse section, interference contrast microscopy).

Fig. 7. In the young microspore stage the sieve elements (S) are visible after clearing intact fresh filaments with HERR's solution. In the centre tracheary elements (T) can be seen, although these are out of focus. (1100 ×) (longitudinal view, interference contrast microscopy).

Fig. 8. After microspore mitosis degenerating cells (right) can be found, bordering obviously vital ones. In the former clusters of ribosomes and plasma are visible, the plasma membrane (PM) is still present. (18500 ×) (transverse section, transmission electron microscopy).


1
P
X
100μm
2
S
T
S
10μm
3
30μm
4
10μm
5
1μm
6
20μm
7
S
T
S
10μm
8
1μm

could be histochemically detected with aniline blue and calcofluor white staining on the same grid as was used for the TEM-photograph (*figs. 18* and *19*).

3.6. Twelve hours after anther dehiscence

At this stage the upper 500 μm of the filament is dehydrated and shriveled as is the entire dehisced anther (*fig. 20*). In the following 300 μm some of the protoplasts disconnect the walls and the cytoplasm appears disorganized in all the tissues (*fig. 21*). The lower parts of the filament are turgid, the cell shapes comparable with the previous stage, although the cytoplasm of some of them shows signs of degeneration (*fig. 22*). Apart from the characteristics described in 3.4, multi-membrane structures and electron-dense bodies can be observed in the latter cells. In the epidermis and parenchyma cells of the lower half (= 750 μm) of the filament dictyosome vesicles can be seen near both the plasma membrane and the tonoplast. Over the entire length the starch and most of the lipid is absent in these tissues.

The cuticle has the same structure as in the previous stage, apart from extreme undulations on the epidermis of the dehydrated zone (*fig. 23*). In the dehydrated tip the tracheary elements are open (*fig. 23*).

4. DISCUSSION

4.1. The vascular tissue

A vascular bundle with separated strains of sieve elements and relatively much phloem is rather common in filaments (SCHMID 1976). The presence of two bundles of sieve elements in both the filament and the connective tissue may have to do with the two-lobed structure of the anther. This is likely, since IVANCICH

Fig. 9. After the microspore mitosis, most of the intercellular spaces of the central parenchyma in the tip of the filament have disappeared (arrows). (1210 ×) (transverse section, interference contrast microscopy).

Fig. 10. After the microspore mitosis both the cuticle (C) and the outer tangential epidermal wall (W) are thicker than in the previous stage. Bordering the cuticle, ridges are present on the wall. (21600 ×) (transverse section, transmission electron microscopy).

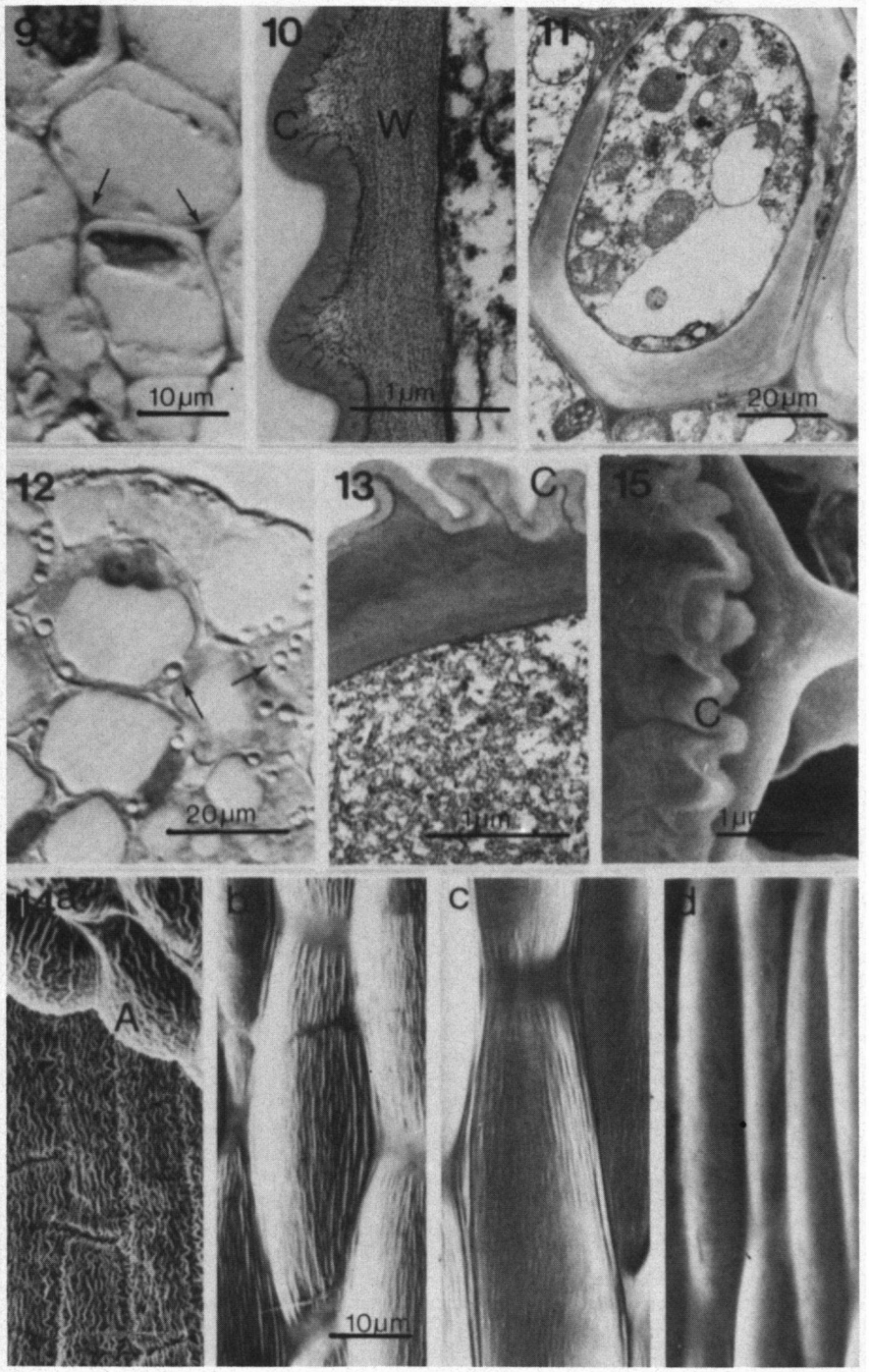
Fig. 11. After microspore mitosis newly formed tracheary elements can be found in the tip of the filament. In these elements the dictyosome activity is high. (6050 ×) (transmission electron microscopy).

Fig. 12. In the mature anther stage both the amount and size of the starch grains (arrows) in the filament tip are less than in the previous stage. This photograph was made 200 μm under the anther attachment site. (820 ×) (transverse section, interference contrast microscopy).

Fig. 13. In the mature anther stage large amounts of SER are present in the epidermis of the upper 350 μm of the filament. The wall ridges under the cuticle (C) are higher than in *fig. 10*. (18500 ×) (transverse section, transmission electron microscopy).

Fig. 14. In the mature anther stage the undulating cuticle with underlaying wall ridges appears progressively from the base to the tip of the filament. Fig. 14a shows the tip with a part of the anther (A), fig. 14d shows the base. The figs. 14b and 14c represent the intermediate stages. the longitudinal cell size increases gradually from the tip to the base, i.e. from fig. 14a to fig. 14d. (950 ×) (longitudinal view, scanning electron microscopy).

Fig. 15. In the mature anther stage the undulating cuticle (C) near the tip sometimes dissociates from the epidermal wall. (13500 ×) (transverse section, scanning electron microscopy).


9
10μm
10
C
W
1μm
11
20μm
12
20μm
13
C
1μm
15
C
14a
A
b
10μm
c
d

(1906) reported the tangential splitting of both phloem and xylem in the filaments of some Amentaceae.

SCHMID (1976) related the emphasis on phloem to the considerable nutritional needs of the developing pollen. In *G. verrucosa*, the development of both the pollen and the locule wall tissues (KEIJZER & WILLEMSE 1986) justify such a hypothesis. Moreover, the stamens develop inside the closed flower bud in which the evaporation is low (KEIJZER 1987a). MAGENDANS (1983) demonstrated that the amount of tracheary elements in vein endings of *Hedera canariensis* Willd. leaves, grown in a relative humidity of 97%, is half as much as in 70%, thus decreasing the xylem-to-phloem ratio in humid conditions. This might be another reason for the emphasis on phloem. The experimental evidence for this hypothesis is very interesting for anther development studies, but is is difficult to obtain since decreasing the relative humidity inside the flower bud appeared to be lethal for the stamen (KEIJZER 1983).

The exclusively helical wall thickenings in the tracheary elements, often found in filaments (SCHMID 1976), remain open during the continuous extension (GROOTAARTS 1978) of the filament of *G. verrucosa*. Important water sinks in the anther are the swelling epidermis and endothecium cells near maturity (KEIJZER 1987a) and the pollen grains (WILLEMSE 1972; KEIJZER & WILLEMSE 1986). The new xylem elements which are continuously deposited until maturity, may serve as a compensation for the decreasing capacity of the older extending ones.

The increase of tracheary elements near the ending of the vascular bundle (tracheid maximum) resembles the storage tracheids ('Speichertracheiden') near

Fig. 16. In the filament tip the intercellular cuticles show similar changes as the epidermal cuticle, being undulations and in some sites dissociation from the wall. (9200 ×) (transverse section, transmission electron microscopy).

Fig. 17. In the mature anther stage both young and older stretched, helically thickened tracheary elements can be found in the vascular bundle of the filament after clearing with HERR's solution. (1000 ×) (longitudinal view, interference contrast microscopy).

Fig. 18. In the mature anther stage callose deposits (C) are visible in the pores between two bordering sieve elements. (27000 ×) (transverse section, transmission electron microscopy).

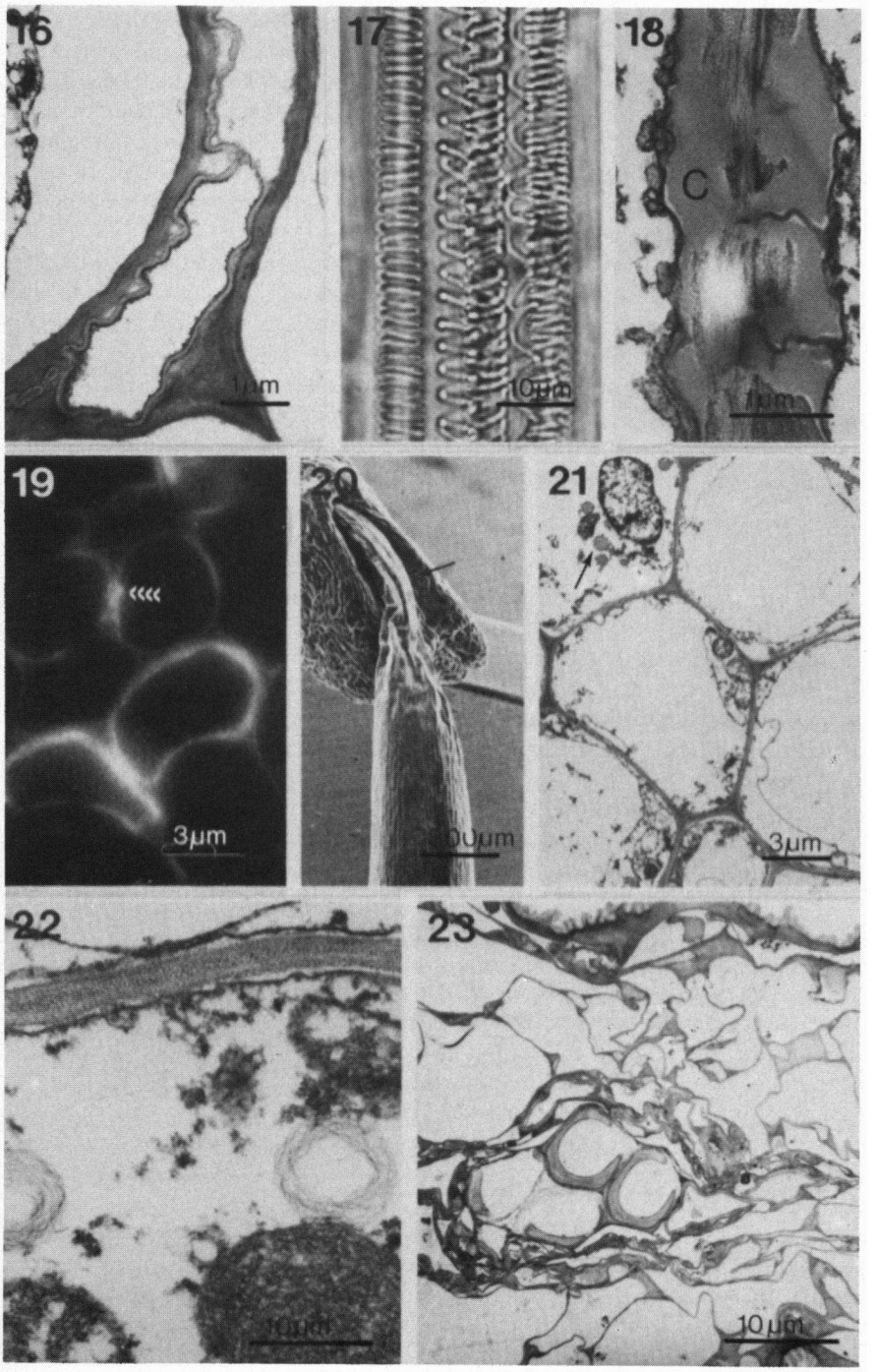
Fig. 19. UV-microscopical view on the same grid as in *fig. 18* after staining with aniline blue and calcofluor white. The callose site (arrow), which is yellow due to aniline blue, can be distinguished as a white spot on this picture. The fainty (blue) calcofluor white staining of the rest of the cell walls serves as an orientation of the tissue in this ultrathin section. (3600 ×).

Fig. 20. The dehydrated and shriveled anther and filament tip (arrow) after anther dehiscence. (32 ×) (longitudinal view, scanning electron microscopy).

Fig. 21. After anther dehiscence the cytoplasm in the tissues of the filament tip has degenerated. Apart from the characteristics shown in *fig. 8*, organelles in plasma-less areas can be observed (arrow) (3050 ×) (transverse section, transmission electron microscopy)

Fig. 22. The degenerating cytoplasm in part of the parenchyma cells halfway the filament after anther dehiscence. The plasma membrane is situated against the cell wall. Apart from the characteristics shown in *figs. 8* and *21*, multi-membrane like structures and electron-dense bodies can be found. (1620 ×) (transverse section, transmission electron microscopy).

Fig. 23. In the dehydrated filament tip the cuticular undulations lay close to each other due to the shrinkage of the epidermal wall. In contrast with the shrinkage of the other tissues, the tracheary elements remain open. (1500 ×) (transverse section, transmission electron microscopy).


16
1µm
17
10µm
18
C
1µm
19
3µm
20
21
3µm
22
10µm
23
10µm

the tip of the vascular bundle of other plant organs (HEINRICHER 1885; HABERLAND 1924). SCHMID's (1976) suggestion that these tracheids might store water to prevent premature anther dehiscence seems unlikely, given the large amount of water in the epidermis and endothecium cells (KEIJZER 1987a) in comparison with the low capacity of this growing tracheid complex. Prevention of premature dehiscence, if necessary, will presumably need a considerable supply of water from or through the filament (KEIJZER 1987a), despite the extension of the tracheid maximum into the filament tip.

The persisting intercellular spaces, a general feature in filaments (STRAW 1956; SCHMID 1976), may also serve as a transport system. They may have to do with the appearance of gas in the locular cavity, previously related to transport through stomata and intercellular spaces of the anther (KEIJZER & CRESTI 1987). The cuticle, present in some of the intercellular spaces, makes the transport of gas rather plausible. In contrast, PFEFFER (1904) and KNOLL (1914) have reported the transfer of water from the cells to the intercellular spaces, causing voluminar changes on behalf of filamental movements.

4.2. The extension and rigidity of the filament

The dictyosome vesicles near the plasma membrane in the extending parts of the filament probably have a function in the deposition of wall materials (CHRISPEELS 1980). Furthermore, the presence of tubular SER in the filament base near maturity is remarkable and might promote membrane formation in rapidly growing tissues (CRESTI & KEIJZER 1985).

The gradual thickening of both the outer tangential epidermal wall and the cuticle may promote the rigidity of the filament. We related similar phenomena in the anther to the improved ability to evaporate in favour of dehiscence (KEIJZER 1983; KEIJZER & WILLEMSE 1986), although the thickening of the cuticle contradicts such an explanation (KEIJZER & WILLEMSE 1986). However, since the cuticular undulations on the filament can also be found in the region that remains turgid after dehiscence, a direct relationship with evaporation is less likely. Moreover, the gradual change from the thin cuticle at the base to the thick undulated configuration at the tip (comparable with the cuticular types 6 and 2 respectively from the classification of HOLLOWAY 1982) contradicts the rather sharp border between the shriveled and the turgid part of the filament. In contrast, this gradual thickening is accompanied by the proceeding cytoplasmic degeneration from the tip to the base of the filament, suggesting a rigidity function during a decreasing turgidity. These ideas remain speculative, so the cuticular changes will be subject of further study, since we observed them currently in other species and on other reproductive organs like pistils (WILLEMSE & FRANSSEN-VERHEYEN 1986).

A second rigid structure may be the swollen epidermis. Since the increasing vacuolation is preceded by starch breakdown, osmotical water uptake is likely, comparable with the anther epidermis (WOYCICKI 1924; KEIJZER 1987a).

SCHMID (1976) wondered whether the turgidity in the filament after anther dehiscence is real turgidity, thus emphasizing the importance of other rigidity

factors like cell walls. The degeneration and plasma membrane retraction that we find in the final developmental stages of the filament support this idea.

Despite the development of different mechanisms to keep the anther rigid after the cytoplasmic degeneration, the question remains as to why this degeneration occurs relatively long before anthesis. Breakdown products of the degenerating cells may be redistributed to other floral parts. LINSKENS (1974) found a redistribution of amino acids and proteins from the stamen to the style after fertilization in *Petunia hybrida*. Since this shift occurs rather quickly, transport through the sieve elements is likely. In this light the callose deposition in the sieve pores shortly before maturity is remarkable. However, since we could not find such deposits between all the sieve elements, this transport system may remain (partly?) intact. Apart from breakdown products of degenerating cells, also the sudden disappearance of the starch during anther dehiscence may be a comparable mobilization of reserve substances for redistribution. Moreover, this turn of starch into sugars may cause an osmotical retraction of water from neighbouring tissues and promote dehydration of the dehiscing anther and the filament tip, apart from the role of evaporation in this process (KEIJZER 1983, 1987a). BURCK (1906) proposed water retraction to the nectaries, but his experiments could not be confirmed in later studies (see SCHMID & ALPERT 1977).

ACKNOWLEDGMENTS

The authors thank mr. S. Massalt, mr. A. B. Haasdijk and mr. P. A. van Snippenburg for the preparation of the photographs.

REFERENCES

ANDERSON, W. R. (1980): Cryptic self fertilization in the Malpighiaceae. *Science* **207**: 892–893.

BURCK, W. (1906): On the influence of the nectaries and other sugar containing tissues in the flower on the opening of the anthers. *Recueil des Trav. Bot. Neerl.* **3**: 163–172.

CHRISPEELS, M. J. (1980): The endoplasmatic reticulum. In: P. K. STUMPF & E. E. CONN (eds.): *Biochemistry of Plants: a comprehensive treatise*, Vol. 1: *The Plant Cell* (Ed. N. E. TOLBERT). pp. 390–412. Academic Press, New York.

CRESTI, M. & C. J. KEIJZER (1985): The structure of ER in pollen grains and pollen tubes after osmium tetroxide potassium ferricyanide staining. *J. Submicr. Cytol.* **17**: 615–620.

–, –, A. TIEZZI, F. CIAMPOLINI & S. FOCARDI (1986): The stigma of Nicotiana: ultrastructural and biochemical studies. *Am. J. bot.* **73**: 1713–1722.

GROOTAARTS, H. (1978): *Generatieve en vegetatieve ontwikkeling van de bloem van Gasteria verrucosa.* Internal report Department of Plant Cytology and Morphology, Agricultural University, Wageningen.

HABERLANDT, G. (1924): *Physiologische Pflanzenanatomie*, 2 Auflage, Engelman, Leipzig.

HEINRICHER, E. (1885): Über einige im Laub dikotyler Pflanzen trockenen Standortes auftretende Einrichtungen, welche mutmasslich eine ausreichende Wasserversorgung des Blattmesophylls bezwecken. *Bot. Zentralbl.* **23**: 25–33, 56–61.

HERR, J. M. JR. (1971): A new clearing squash technique for the study of ovule development in angiosperms. *Am. J. Bot.* **58**: 785–790.

HOLLOWAY, P. J. (1982): Structure and histochemistry of plant cuticular membranes: an overview. In: D. F. CUTLER, K. L. ALVIN & C. E. PRICE (eds.): *The Plant Cuticle*. Academic Press, London, New York.

HUGHES, J. & M. E. MACCULLY (1975): The use of an optical brightener in the study of plant structure. *Stain Technol.* **50**: 319–329.

IVANCICH, A. (1906): Der Bau der Filamente der Amentaceen. *Österr. Bot. Z.* **8**: 385–394.

JENSEN, W. A. (1962): *Botanical Histochemistry*. Freeman & Co., San Francisco and London.

KEIJZER, C. J. (1983): Hydration changes during anther development. In: D. L. MULCAHY & E. OTTAVIANO (eds.): *Pollen, biology and implications for plant breeding*. pp. 197–201. Elsevier biomedical, New York.

– (1987a): The processes of anther dehiscence and pollen dispersal. 1. The opening mechanism of longitudinally dehiscing anthers. *The New Phytol.* **105**: 487–498.

– (1987b): The processes of anther dehiscence and pollen dispersal. 2. The formation and the transfer mechanism of pollenkitt, cell wall development in the loculus tissues and a function of the orbicules in pollen dispersal. *The New Phytol.* **105**: 499–507.

– (1978c): The processes of anther dehiscence and pollen dispersal. 3. The dehydration of the filament tip and the anther in three monocotyledons species. *The New Phytol.* **106**: 281–287

– & M. CRESTI (1987): A comparison of the anther development in male sterile Aloe vera and male fertile Aloe ciliaris. *Ann. Bot.* **59**: 533–542.

– & M. T. M. WILLEMSE (1986): Functional morphological relationships in the developing locule of Gasteria verrucosa. In: C. J. KEIJZER: *Stamen Development*. Thesis, Agric. Univ. Wageningen, The Netherlands. pp. 63–88.

KNOLL, F. (1914): Zur Ökologie und Reizphysiologie des Andröceums von Cistus salvifolius L. *Jahrb. wiss. Bot.* **54**: 498–527.

LEINFELLNER, W. (1956): Die Gefassbündelversorgung des Lilium Staubblattes. *Österr. Bot. Z.* **103**: 346–352.

LINSKENS, H. F. (1974): Translocation phenomena in the Petunia flower after cross- and self-pollination. In: H. F. LINSKENS (ed.): *Fertilization in higher plants*. pp. 285–292. North-Holland Publishing Company, Amsterdam, Oxford.

MAGENDANS, J. F. C. (1983): Anatomy of vein endings in Hedera leaves; influence of wet and dry conditions. *Med. Landbouwhogeschool Wageningen* **83**–6.

PFEFFER, W. (1904): *Pflanzenphysiologie*. 2 Auflage, engelmann, Leipzig.

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

SHIVANNA, K. R. & B. M. JOHRI (1985): *The angiosperm pollen, structure and function*. Wiley Eastern Ltd., New Delhi.

SCHMID, R. (1976): Filament histology and anther dehiscence. *Bot. J. Linn. Soc.* **73**: 303–315.

– & P. H. ALPERT (1977): A test of Burck's hypothesis relating anther dehiscence to nectar secretion. *The New Phytol.* **78**: 487–498.

SPURR, A. R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastr. Res.* **71**: 173–184.

STRAW, R. M. (1956): Adaptive morphology of the Penstemon flower. *Phytomorph.* **6**: 112–119.

WILLEMSE, M. T. M: (1972): Morphological and quantitative changes in the population of cell organelles during microsporogenesis of Gasteria verrucosa. *Acta Bot. Neerl.* **21**: 17–31.

– & M. A. W. FRANSSEN-VERHEIJEN (1986): Stylar development in the open flower of Gasteria verucosa (Mill.) H. Duval. *Acta Bot. Neerl.* **35** (3): 297–309.

WOYCICKI, Z. (1924): Recherches sur la déhiscence des anthères et le rôle du stomium. 2. La formation de la fente dans les anthères et les phenomènes qui l'accompagnent. *Rev. Gén. Bot.* **36**: 253–268.