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Development of mucilage cells of *Araucaria angustifolia* (Araucariaceae)

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Summary. The roles of mucilage cells were investigated through morphological and cytological analysis during leaf development in young *Araucaria angustifolia* plants. Differentiation began in leaf primordia in the shoot apex, when the young cells underwent a greater increase in volume in comparison with other mesophyll cells. The mucilage polysaccharides were synthesized by dictyosomes, from where they were taken by large vesicles and released into a cavity formed by detachment of the tonoplast, which was separated from the cytoplasm. At the end of differentiation, the cell was completely filled with mucilage, a gel consisting of a denser reticular structure surrounding less dense regions. The nucleus and cytoplasm were degenerated in mature cells. The *A. angustifolia* mucilage cells presented some cytological resemblances to the mucilage cells of members of some dicotyledonous families; however, differences in the dictyosomes and the secretion route were observed. Translocation and water storage of solutes was suggested by the use of the hydroxy pyrenetrisulfonic acid tri-sodium salt apoplastic tracer. The tonoplast detachment, dechromatinization, nuclear condensation, and general degeneration of the membrane systems observed during maturity indicated a programmed cell death process, one not yet described for angiosperm mucilage cells.

Keywords: *Araucaria angustifolia*; Araucariaceae; Development; Leaf; Mucilage cell; Programmed cell death.

Abbreviations: HTPS hydroxy pyrenetrisulfonic acid tri-sodium salt; PCD programmed cell death; LM light microscopy; TEM transmission electron microscopy.

Introduction

Mucilage cells are idioblasts that were first observed in the early 20th century, during studies of the leaf anatomy of members of genus *Araucaria* Juss by Barsali (1909), who described them as large intercellular spaces. Griffith (1950) and Vasiliyeva (1969) described them as cells with a great volume and lacking chloroplasts, while Monteiro et al. (1977), studying young *Araucaria angustifolia* plants (Bert.) O. Ktze (Brazilian pine), described them as another tissue in the mesophyll with a few small chloroplasts. Finally, Bamber et al. (1978) described the ultrastructure and observed that these unusual cells distributed in the mesophyll possessed pectic partitions subdividing the cell into numerous compartments. Therefore, the authors called them "compartmented cells". They only observed mature leaves of adult plants and characterized them as enucleated, lacking cytoplasm, vacuoles, and other cytoplasmic materials.

In preliminary studies (Mastroberti and Mariath 2003), we observed a few cytological characteristics of these cells during development and suggested that they were mucilage cells. We also described the leaf anatomy of young *A. angustifolia* plants and observed nucleated mucilage cells during leaf development. However, as was observed by Bamber et al. (1978), mature cells do not contain a nucleus or cytoplasm, which suggests that they might undergo programmed cell death (PCD). Even though Bamber et al. (1978) described relevant ultrastructural details, the ontogenesis and functions of these cells remained unknown.

Mucilage cells have been widely described and are present in members of many families, such as Lauraceae (Bakker and Gerritsen 1989, Bakker et al. 1991), Cactaceae (Trachtenberg and Fahn 1981; Trachtenberg and Mayer 1981a, b, 1982a, b), and Malvaceae (Bakker and Gerritsen 1992), and a few resemblances were found in the *A. angustifolia* mucilage cells (Mastroberti and Mariath 2003). However, when observed with the light microscope, the mucilage seems to be deposited in the protoplast, which is different from what occurs in di-

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cotyledons, in which the mucilage is deposited between the cell wall and the plasmalemma.

Araucaria angustifolia is a Brazilian native plant and little is known about its secretory structure. In Araucariaceae, the mucilage cells are present in members of genera *Araucaria* and *Wollemia* Jones, Hill & Allen and absent from members of genus *Agathis* Salisb. (Burrows and Bullock 1999).

Further data are required to complete the secretion pathway of mucilage cells, to describe their ontogeny in ultrastructural detail, and to provide evidence that these cells undergo PCD, a process not yet described for angiosperm mucilage cells. This work aims to describe the morphological and cytological aspects of *A. angustifolia* mucilage cells during development by light microscopy and transmission electronic microscopy and to investigate their function.

Material and methods

Experimental setting and sampling

Araucaria angustifolia (Araucariaceae) seeds were collected from the National Forest of São Francisco de Paula (Floresta Nacional de São Francisco de Paula, 29°23′S and 29°27′S, and 50°23′W and 50°25′W), Rio Grande do Sul, Brazil. Seeds were sown in a seedbed under full sun. Leaves were collected when young plants were about 4 months old (100–210 mm high). Six arbitrary stages were established according to the events which indicated that cytological changes had occurred in the mucilage cells during leaf development. The developmental sequence was based mainly on observations in the shoot apex. Immature leaves (3–15 mm in length) were collected from the shoot apex to observe stages 1–4. Recently expanded immature leaves (30–35 mm in length) were collected in order to observe stage 5. Mature or expanded leaves (30–45 mm in length) were collected from the middle portion of the stem to observe stage 6. Six samples were observed at each stage.

Transmission electron microscopy

The shoot apex and the leaf middle portion were fixed in a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) (Roland and Vian 1991). The material was postfixed in 2% OsO_4 plus 0.8% sodium phosphate-buffered $K_3Fe(CN)_6$ (Weber 1992), dehydrated in an acetone series, and embedded in a low-viscosity epoxy resin (Spurr 1969). Ultrathin sections were obtained with a Leica Ultracut UCT ultramicrotome with a diamond knife and stained with 2% aqueous uranyl acetate (Bozzola and Russel 1998) and 5% lead citrate (Hanaich et al. 1986). The sections were examined with a JEM 1200 ExII transmission electron microscope (Jeol) at the Electron Microscopy Center, Federal University of Rio Grande do Sul, Brazil.

Light microscopy

Some of the specimens were fixed with 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) (McDowell and Trump 1976), dehydrated in a graded ethanol series (Johansen 1940), and placed in a graded chloroform-ethanol series (Purvis et al. 1964). The material was then embedded in hydroxyethylmethacrylate (Gerrits and Smid 1983). Thin transverse and longitudinal sections were cut on a Microm HM 340 E rotary microtome with a glass knife. Sections were stained with 0.05% Toluidine Blue O (C.I. 52040), pH 4.4 (Feder and O'Brien 1968), and were observed in a bright field on a Leica DM R microscope. Some specimens processed for transmission electron microscopy (TEM) were semithin sectioned (350–500 nm) and stained with 1% Toluidine Blue O (C.I. 52040), pH 8–9 (Souza 1998).

Fluorescent apoplastic tracer

The cut ends of detached leaves were sealed with petrolatum drops to prevent dehydration of the detached area and to avoid entry of the dye. These fresh specimens were placed in a 0.02% aqueous solution of hydroxy pyrenetrisulfonic acid tri-sodium salt (HPTS) (Sigma) for 1 h (Oparka and Read 1994). Freehand sections of the specimens were carefully placed on a slide and mounted in glycerol to prevent a film of water forming between the surface of the section and the cover glass. Such a water film containing dye could terminate the whole section. The sections were observed by epifluorescence on a Leica DM R microscope with excitation filter for 350–390 nm and barrier filter for 400 nm.

Results

Stage 1

Mucilage cell differentiation started in the primordial leaf. The differentiation of these cells occurred simultaneously with the leaf development (Fig. 1). Some mesophyll cells presented a larger volume increase, when compared with the other cells of this tissue, indicating that they could be immature mucilage cells (Fig. 2). This cellular type was abundant in the mesophyll.

The nucleus possessed denser chromatin in the young mucilage cells (Fig. 2); however, most ultrastructural characteristics were similar to those of the other mesophyll cells. Examples of this are the presence of chloroplasts containing large starch grains (Fig. 3) and mitochondria with undefined cristae (Fig. 4). Dictyosomes contained 6 to 8 cisternae and the *trans* face faced the plasmalemma, indicating that these organelles secreted polysaccharidic material to the cell wall (Fig. 4). This was also observed in the parenchymal cells of the mesophyll.

Stage 2

An increase in dictyosome activity and the presence of polysomes in the cytoplasm indicated a high cellular metabolic activity (Fig. 5). The general characteristics of the immature cells and the number of cisternae (6–8) of the dictyosomes were the same as in the previous stage (Fig. 5). However, the *trans* face of some dictyosomes was turned to the vacuole and the number of vesicles appeared to be smaller than in stage 1. These vesicles originated from groups formed by the dictyosome (Figs. 5 and 6). The tonoplast of the central vacuole had detached from the cytoplasm and formed a cavity in which small vesicles,

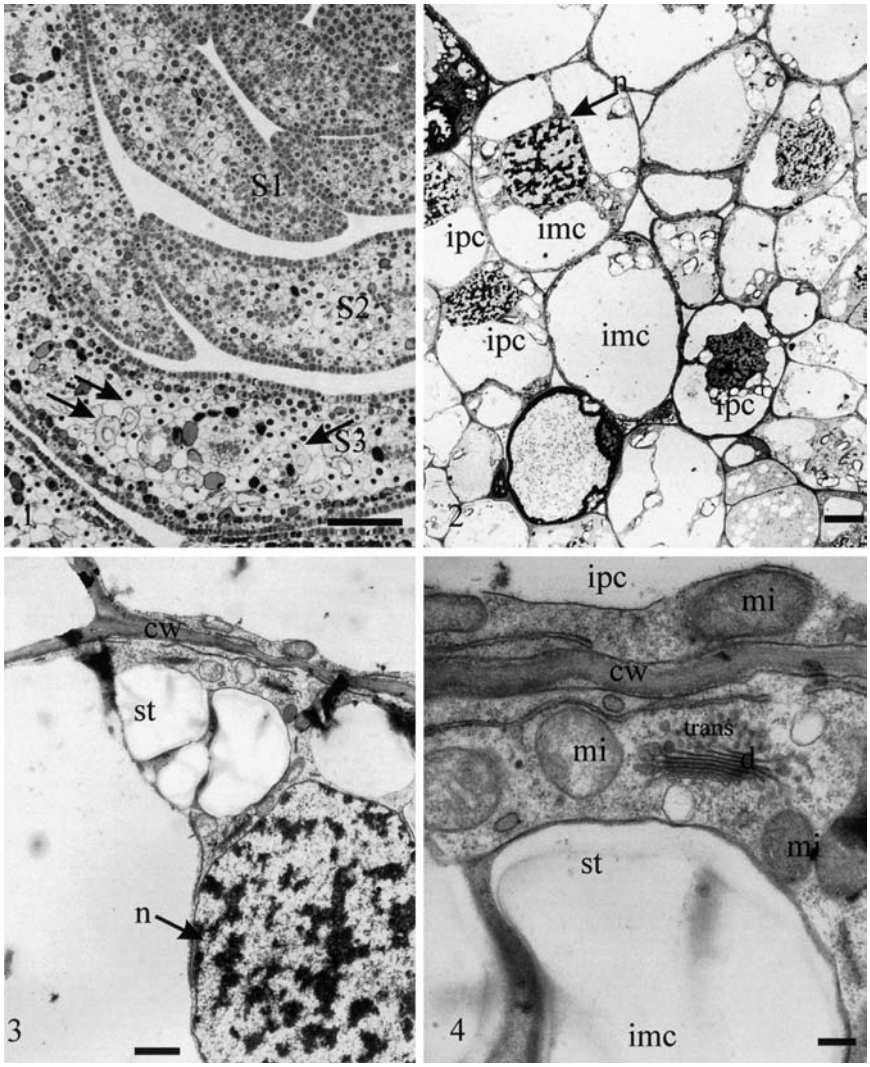


Fig. 1. Transverse section of shoot apex where immature leaves appeared at stages 1–3. LM. The mucilage cells also diverge during the immature stages of the leaves (arrows). *S1* Stage 1; *S2* stage 2; *S3* stage 3. Bar: 100 μm

Figs. 2–4. Transverse section of immature leaf at stage 1. TEM. *imc* Immature mucilage cell; *ipc* immature parenchymal cell; *cw* cell wall; *d* dictyosome; *mi* mitochondrion; *n* nucleus; *st* starch grain

Fig. 2. Immature mucilage cell with denser nuclear chromatin than the immature parenchymal cells. The greater volume of the future mucilage cell can be seen. Bar: 7 μm

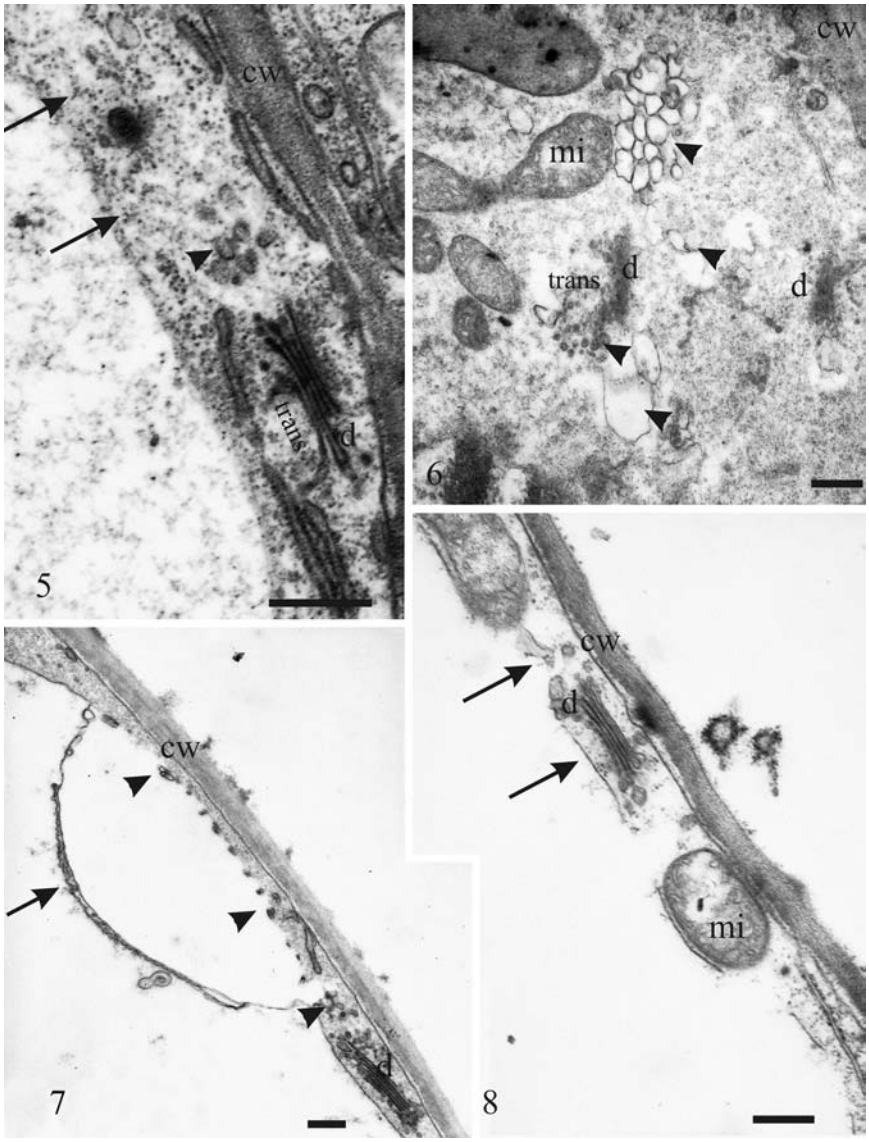
Fig. 3. Detail of immature mucilage cell showing large starch grains in the chloroplasts, as also observed in the immature parenchymal cells. Bar: 1 μm

Fig. 4. Detail of immature mucilage cell showing dictyosomes with 8 cisternae and mitochondrion with undefined cristae. The dictyosomes of the immature mucilage cells secreted vesicles towards the cell wall. All these characteristics were also observed in the parenchymal cells. Bar: 200 nm

also originating from the dictyosome, lined this structure forming a new membrane (Fig. 7). This membrane limited the recently formed cavity, establishing the mucilage storage area. In this stage, the mitochondrial cristae were more evident (Fig. 8). The tonoplast became further detached with the participation of dictyosome vesicles that were also involved in the cavity growth (Figs. 9 and 10). In the chloroplasts, the starch grains were reduced and osmiophilic bodies were present in the stroma, like in the parenchymal mesophyll cells. In light microscopy (LM), polysaccharide was observed in the cell lumen and attributed to the beginning of the secretion process (Fig. 11).

Stage 3

The volume increase of the mucilage cells achieved its peak and it was possible to observe that they took up a large part of the mesophyll. In addition, the mucilage presented a granular structure when observed in LM (Fig. 12). With TEM, the mucilage cell nuclei appeared denser (Fig. 13), and in a few cells, the mucilage formed thicker networks, parallel to the cytoplasm, unlike that seen in LM. The cytoplasm of the studied cells also became denser (Fig. 14) and the mitochondria (cristae and membrane) diverged like in the parenchymal cells of the mesophyll. The chloroplasts contained very small starch grains (if any), underdeveloped thylakoids and grana, and many osmiophilic bodies in the stroma in comparison with those in the parenchymal cells of the mesophyll (Fig. 15). Secretory vesicles of the dictyosomes increased in volume, adding their contents to the mucilage-filled cavity (Fig. 16). Some vesicles seemed to have originated from dilation of the dictyosome cisternae, resulting in the reduction of 2 to 3 cisternae (Fig. 16). This might indicate dictyosome degeneration, the end of the secretory activity of these cells and the metabolic activity as a whole, since polysomes were also no longer observed.



Figs. 5–8. Transverse section of immature leaf at stage 2. Beginning of secretory cavity formation. *cw* Cell wall; *d* dictyosome; *mi* mitochondrion

Fig. 5. Presence of polysomes in the cytoplasm indicating high cell metabolic activity (arrows). The characteristics of the organelles are similar to stage 1; however, a few vesicles originating from dictyosomes are not turned towards the cell wall (arrowhead). Bar: 500 nm

Fig. 6. Vesicle groups originating from the abundant dictyosomes in the cytoplasm (arrowheads). Bar: 400 nm

Fig. 7. Tonoplast initiates separation of cytoplasm (arrow), with cavity formation. The vesicles originating from the dictyosomes line the cavity to form a new membrane (arrowheads). Bar: 400 nm

Fig. 8. Membrane (arrows) surrounds the cavity recently formed by the dictyosomes. Mitochondrial cristae are more evident. Bar: 400 nm

Stage 4

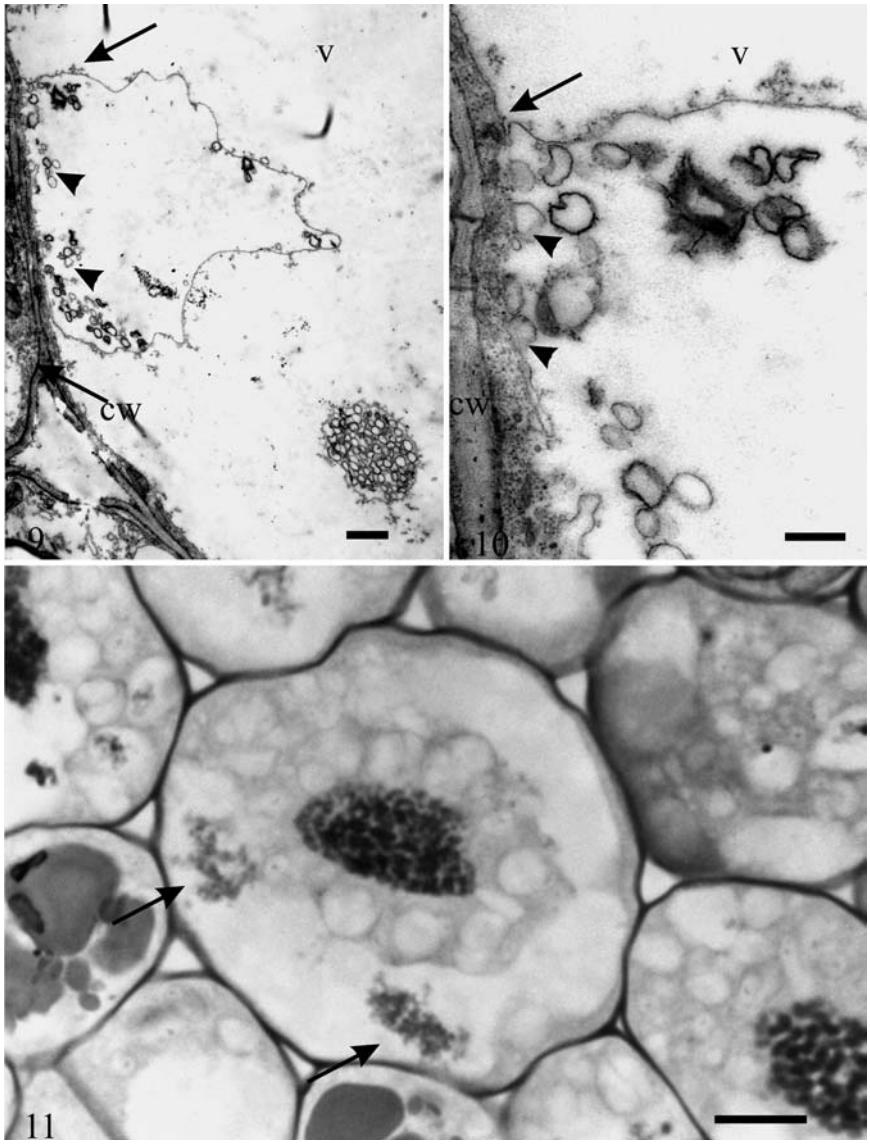
Stage 4 was characterized by the gradual degeneration of the protoplasm and changes in the mucilage structure. Few mucilage cells with nuclei were observed. The cytoplasmic content was reduced and the secretion, predominant in the entire cell, presented denser mucilage rays, now evident with LM (Fig. 17). Some mucilage cells possessed a central zone lacking this secretion, which was surrounded by a larger mass of the mucilage (Fig. 17). Two cells were observed by TEM (Fig. 18). In one of them, a membrane was observed close to this mucilage mass surrounding the region with no secretion. This could indicate the presence of the central vacuole, which was reduced by the continuous mucilage deposition (Fig. 18).

Stage 5

At stage 5, nuclear degeneration began. The cytoplasmic matrix and the organelles became disorganized and dispersed compared with those of the parenchymal cells (Fig. 19). Both membrane system degeneration and nucleus chromatin loss were observed (Fig. 20). Some zones with a dilated nuclear membrane indicated nucleus degeneration (Fig. 20).

Stage 6

Stage 6 is characterized by full protoplasm degeneration. Longitudinal sections showed the loss of cell adhesion between the mature mucilage cells, culminating in the alter-



Figs. 9–11. Transverse section of immature leaf at stage 2. Secretory cavity formation. *cw* Cell wall; *v* vacuole

Fig. 9. Tonoplast detachment becomes more evident (arrow) with the liberation of many vesicles (arrowheads). Bar: 1 μm

Fig. 10. Detail of detached tonoplast (larger cell) and vesicles liberated into the recently formed cavity (arrowheads). Bar: 250 nm

Fig. 11. Mucilage cell during differentiation. LM. Note the polysaccharidic content in the mucilage cell (arrows). Bar: 10 μm

ation of their original outline (Fig. 21). The mucilage cell outline became amorphous with sinuous cell walls. The nucleus and cytoplasm could no longer be observed and the cell was completely filled with structured mucilage forming dense regions (Figs. 22 and 23). These denser regions of mucilage contained less dense reticular regions (Fig. 24). Adjacent to the plasmalemma, layers of varying electron density were attributed to protoplasmic fragments (Fig. 24).

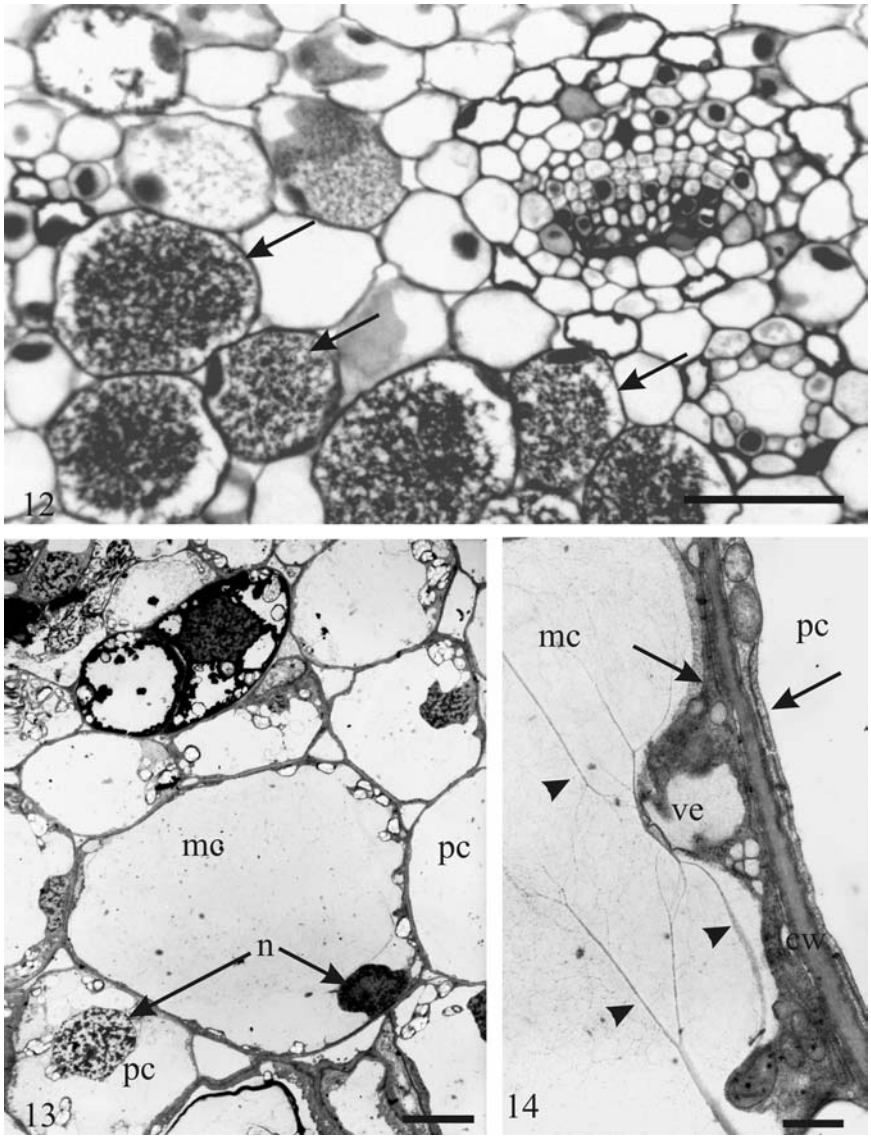
Fluorescent apoplastic tracer HPTS

HPTS is a fluorescent compound that has been used to trace apoplastic transport pathways in plants. Dipping mature leaves in HPTS showed that water is stored in mucilage cells via the mucilage. The green fluorescence emitted by HPTS through the UV filter (340–380 nm) demonstrated that the water present in air humidity was absorbed by the epidermis (Fig. 25) and then moved through an apoplastic pathway to the mesophyll, where mucilage cells absorbed high amounts of dye (Fig. 26), perhaps as a result of the biochemical breakdown of the plasmalemma once the cell underwent cell death.

Discussion

"Compartmented cells" are mucilage cells with an unusual secretion pattern

In stage 1, mucilage cells increased significantly in volume. This was the first difference that could be observed



Figs. 12–14. Transverse section of immature leaf at stage 3. The granular aspect of the mucilage in LM appears as a denser net. *cw* Cell wall; *mc* mucilage cell; *n* nucleus; *pc* parenchymal cells; *ve* vesicle

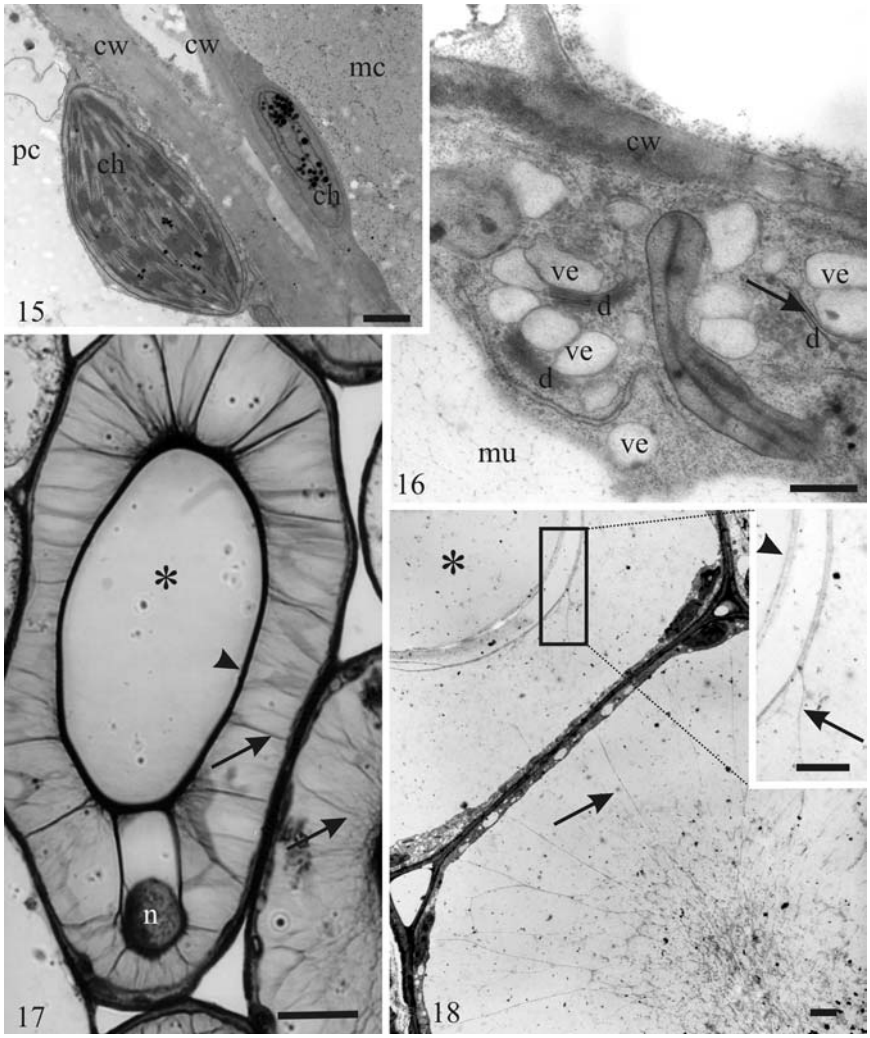
Fig. 12. Mucilage cells reach their maximum size, filling a large portion of the mesophyll (arrows). Bar: 50 μm

Fig. 13. Mucilage cell nucleus is denser than that of the neighboring parenchymal cell. Bar: 10 μm

Fig. 14. Detail comparing the cytoplasm of a mucilage cell and a parenchymal cell (arrows), showing a more compact cytoplasm in the mucilage cells. Note the large vesicle with polysaccharidic content and the net structure of the mucilage (arrowheads). Bar: 1 μm

between mesophyll parenchymal cells, because no cytological differentiation was observed in the cytoplasm or organelles. Moreover, almost all cell development was shown to occur in the shoot apex region, following leaf development. In general, the mucilage cells of members some angiosperm families also show these same characteristics in the initial stages (Bakker and Gerritsen 1992). However, Trachtenberg and Fahn (1981) and Bakker et al. (1991) observed that immature mucilage cells from *Opuntia ficus-indica* (L.) Mill. and *Cinnamomum* L. species occurred together with mature cells in the shoot apex. As for *Opuntia polyacantha* Haw., Mauseth (1980) described a nonsequential development of mucilage cells. A sequential development following leaf maturity was observed in the mucilage cells of *A. angustifolia*, *Aloe arborescens* Mill. (Trachtenberg 1984), and *Hibiscus schizopetalus* (Bakker and Gerritsen 1992).

In stage 2, *A. angustifolia* mucilage cells contained chloroplasts with a morphology different from that of chloroplasts of parenchymal cells. In immature leaves, these organelles were insignificant in mucilage cells, with reduced thylakoids and grana. The same occurs with all the mucilage cells of the species mentioned above. Moreover, Trachtenberg (1984) proposed that the substrate for mucilage synthesis in *Aloe arborescens* mucilage cells is the starch grains present in the chloroplasts. The main role of these idioblasts is not photosynthetic, as in other mesophyll cells (Dickison 2000). In *Opuntia* species, oil drops are rare in mucilage cell chloroplasts (Mauseth 1980, Trachtenberg and Mayer 1981a), but in *A. angustifolia*,



Figs. 15 and 16. Transverse sections of immature leaf at stage 3. *ch* Chloroplasts; *cw* cell wall; *d* dictyosomes; *mc* mucilage cell; *mu* mucilage; *pc* parenchymal cell; *ve* secretory vesicle

Fig. 15. Comparison of the chloroplasts of a mucilage cell and a parenchymal cell. Those of the mucilage cell show reduced thylakoids, with no grana and many osmiophilic bodies. Bar: 1 μm

Fig. 16. Volume of secretory vesicles originating from the dictyosomes has increased. Some cisternae seem to form vesicles directly, indicating dictyosome degeneration and the end of secretory activity (arrow). Bar: 500 nm

Figs. 17 and 18. Transverse sections of immature leaf at stage 4. Gradual degeneration of the protoplasm and changes in the mucilage structure. *n* Nucleus

Fig. 17. LM. Nucleated mucilage cell with reduced cytoplasmic content and denser mucilage rays (arrows). Some cells possess a central region without secretion (asterisk), surrounded with a denser region (arrowhead). Bar: 10 μm

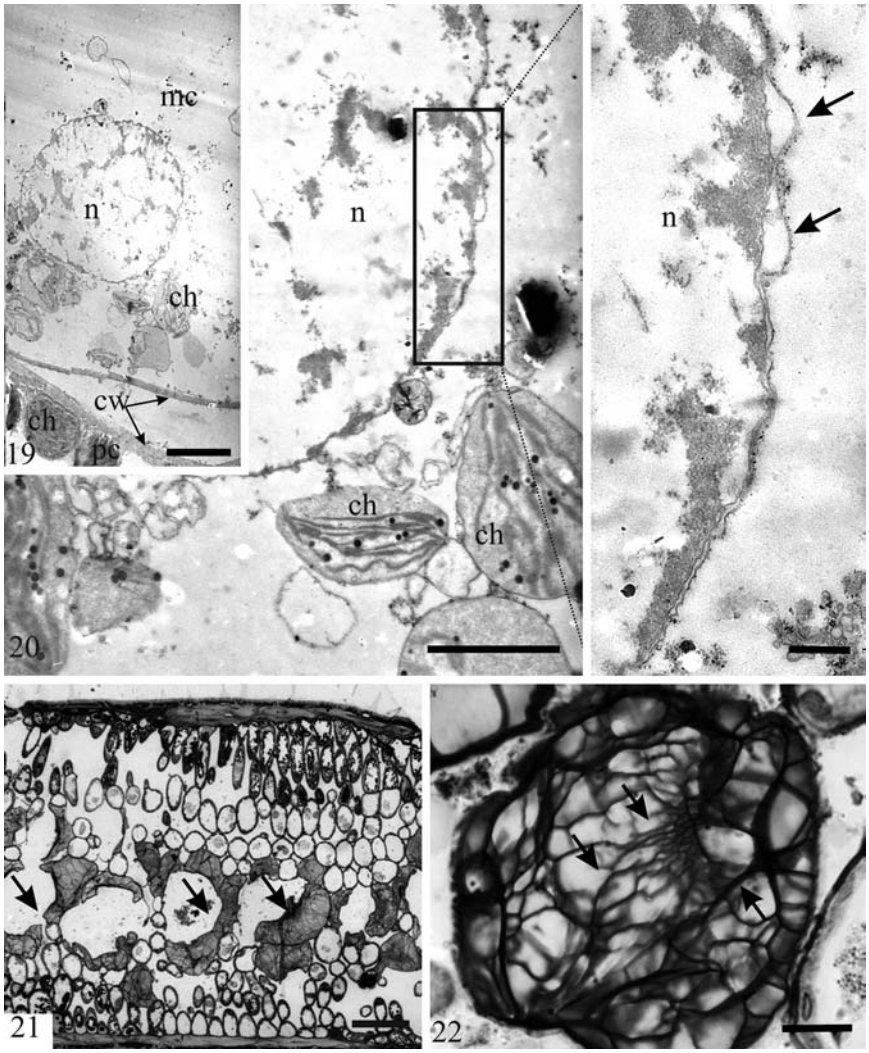
Fig. 18. TEM. In one of the two mucilage cells observed, denser mucilage can be seen surrounding the central secretory region of the cell (asterisk), which appeared to be the central vacuole. **Inset** One more electron-dense membrane can be seen, which appears to be the tonoplast (arrowhead). Note the presence of the mucilage rays (arrows). Bars: 2 μm

the number of oil drops increases with cell development. The presence of a high number of oil drops in plastids can be associated with nonphotosynthetic chromoplasts (Clowes and Juniper 1968). With the degeneration of organelles, these oil drops could be another energy source for the secretion process, since the chloroplasts of mucilage cells persist until very close to the time of cell death.

The smooth and rough endoplasmic reticulum in *A. angustifolia* mucilage cells seemed to have characteristics similar to those of mucilage cells of members of other families. The participation of the smooth endoplasmic reticulum, in addition to the dictyosome, in the early secretory process of *A. angustifolia*, as has been observed for *Opuntia ficus-indica* (Trachtenberg and Fahn 1981), was not supported. Horner and Lersten (1968) also described the participation of the endoplasmic reticulum for *Psychotria bacteriophila* trichomes, and Meyberg (1988) observed the same in *Nymphoides peltata* (Gmel.) Kuntze. However, the stereological analyses carried out by Trachtenberg and Mayer (1981b) demonstrated that the apparent increase in endoplasmic reticulum was not significant in *Opuntia ficus-indica*.

In the mucilage cells of many members of the studied families (Trachtenberg and Fahn 1981, Bakker et al. 1991, Bakker and Gerritsen 1992), the number of dictyosome cisternae ranges from 6 to 8 on average, but may reach 16 or 18 (hypertrophic) due to the high activity during the secretion phase. The dictyosomes in *A. angustifolia* mucilage cells were not hypertrophic, but the released vesicles showed a large increase in size when compared with neighboring parenchymal cells.

In most of the mucilage cells studied so far (Mollenhauer 1967; Bouchet 1971, 1973; Trachtenberg and Fahn 1981; Trachtenberg 1984; Bakker et al. 1991; Bakker and Gerritsen 1992), the secretion accumulates between the cell wall and the plasmalemma, pressing the



Figs. 19 and 20. TEM. Transverse sections of immature leaf at stage 5. Beginning of nuclear degeneration. *ch* Chloroplast; *cw* cell wall; *mc* mucilage cell; *n* nucleus; *pc* parenchymal cell

Fig. 19. Protoplast is disorganized compared with the parenchymal cells of the mesophyll. Bar: 4 μm

Fig. 20. The organelle membrane system is unstructured and chromatin loss can be seen in the nucleus. In the detail (boxed area), areas with dilated membranes are visible in the nucleus, indicating nuclear degeneration (arrows). Bar: 2 μm

Figs. 21 and 22. LM. Transverse sections of mature leaf at stage 6. Protoplasm and nucleus are degenerated

Fig. 21. Cell adhesion between the mucilage cells is lost, leading to the formation of large intercellular spaces (arrows). Bar: 50 μm

Fig. 22. Detail of the mucilage cell with sinuous cell walls. The nucleus and cytoplasm are no longer visible and the cell lumen is completely filled with dense regions of the secretion forming a netlike structure (arrows). Bar: 10 μm

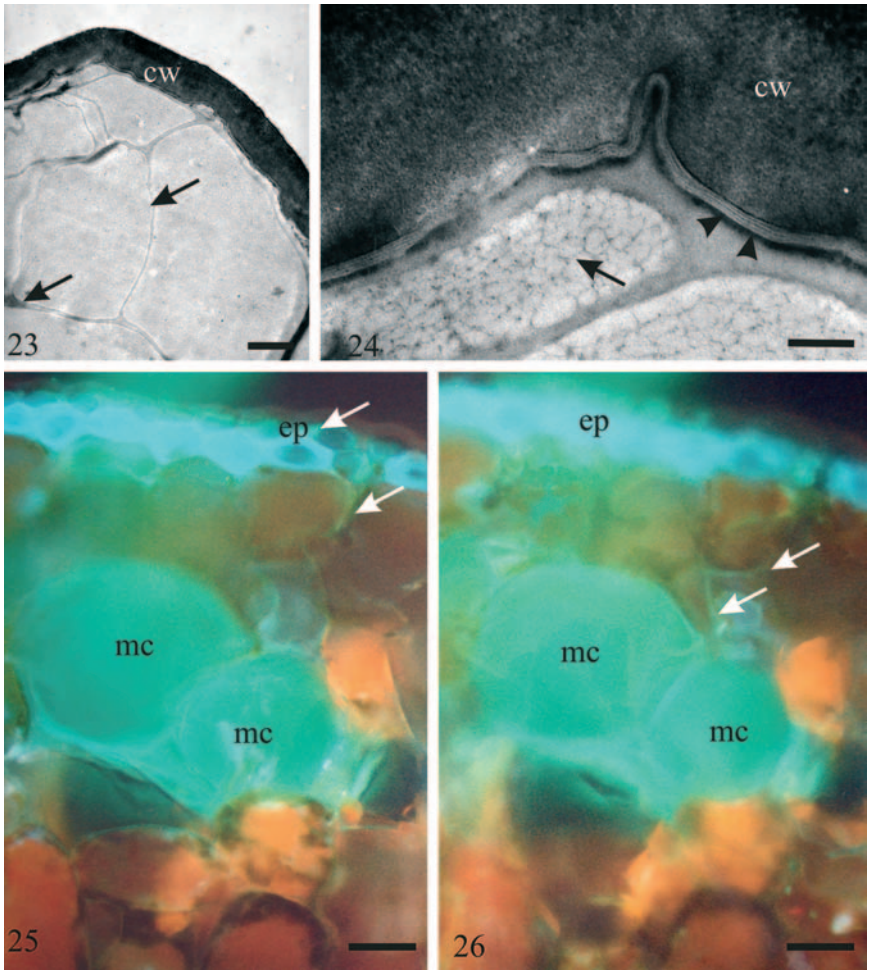
protoplast away from the cell wall and reducing the vacuole volume. The protoplast then degenerates, appearing as an electron-dense residual mass. In contrast, in *A. angustifolia* mucilage cells the secretion was deposited into a specialized vacuole or cavity between the central vacuole and the cytoplasm, and the cytoplasm was compressed against the cell wall (Fig. 27). The central vacuole then disappeared.

The structural aspect of the mucilage has been studied by Bamber et al. (1978) and Mastroberti and Mariath (2003). The secretion initially appears granular and later as a denser net, reflecting the biochemical alterations that the mucilage appears to undergo due to the developmental stage of the cell. Immunocytochemical studies are being performed to analyze the pectic epitopes and arabinogalactan proteins, with the objective of observing such changes throughout the leaf development.

Table 1 shows the differences and similarities between *A. angustifolia* mucilage cells and some members of angiosperm families.

Mucilage cells undergo a PCD

PCD in plants has been studied by Greenberg (1996), Jones and Dangl (1996), and Pennel and Lamb (1997), who described the important role this process plays in xylogenesis, reproduction, senescence, and defense against pathogens. *Araucaria angustifolia* mucilage cells underwent an apoptotic process without cell destruction. The ultrastructural evidence of PCD or apoptosis was the condensation of the chromatin (also called heterochromatin by Buzek et al. [1998]) and its movement to the edges of the nucleus, cytoplasm degeneration (membrane systems), and DNA fragmentation.



Figs. 23 and 24. TEM. Transverse sections of mature leaf at stage 6. *cw* Cell wall

Fig. 23. Detail of the mucilage structure in which denser zones (arrows) surround less dense secretion regions. Bar: 1 μm

Fig. 24. Less dense regions appear as a thin network (arrow). Adjacent to the plasmalemma, layers of varying electron density can be seen which may represent protoplasmic fragments (arrowheads). Bar: 100 nm

Figs. 25 and 26. HPTS apoplastic tracer test in fresh leaf tissue. Transverse section. Epifluorescence. *ep* Epidermis; *mc* mucilage cells. Two focal planes showing mucilage cell water storage and translocation through epidermal water absorption (Fig. 25) following an apoplastic pathway (arrows) and filling the mucilage cells (green fluorescence) (Fig. 26). Bars: 50 μm

Membrane degeneration in apoptotic cells was observed by Obara et al. (2001) and Yu et al. (2002), who described the tonoplast as the first membrane to be ruptured, releasing enzymes that favor the depolarization of other membrane systems, including rapid nucleus degeneration. In *A. angustifolia* mucilage cells, the tonoplast was also affected by the involution of the cytoplasm, from which it became detached during the secretion phase. A cell cavity was formed.

Sineonova et al. (2000) observed other apoptotic events in chloroplasts of *Nicotiana tabacum* L., which showed reduction or degeneration of thylakoids during leaf senescence. In the mucilage cells, chloroplasts underwent great alterations, such as grana and thylakoid reduction.

Chloroplast degeneration and detachment of the tonoplast from the cytoplasm have also been observed in the cell death of tracheal elements (Buvat 1989). The xylogenesis mentioned previously is considered a form of PCD characterized by events similar to those that occur in the mucilage cells, in addition to the cell permanence. Therefore, both tracheal elements and mucilage cells remain functional after their death.

During stage 5, the nucleus presented a stage of dechromatinization and nuclear membrane detachment, suggesting cell death. Some elements have been studied that allow us to understand the biochemistry of these events and, in particular, the PCD regulators. Greenberg (1996) and Matile (1997) described the participation of the vacuole in PCD. The latter suggested that certain proteins in the cytoplasm degrade tonoplast phospholipids, releasing endonucleases to fragment the nuclear DNA, as well as toxic compounds and hydrolases.

The detachment of the tonoplast, the thylakoid reduction in the chloroplasts, the dechromatinization of the chromatin, and the detachment of the nuclear membrane in mucilage cells can be attributed to the same apoptotic model described by the authors quoted above.

What is the function of mucilage cells?

Many functions are attributed to mucilage, such as adhesive in seed dispersal, regulation of germination and cap-

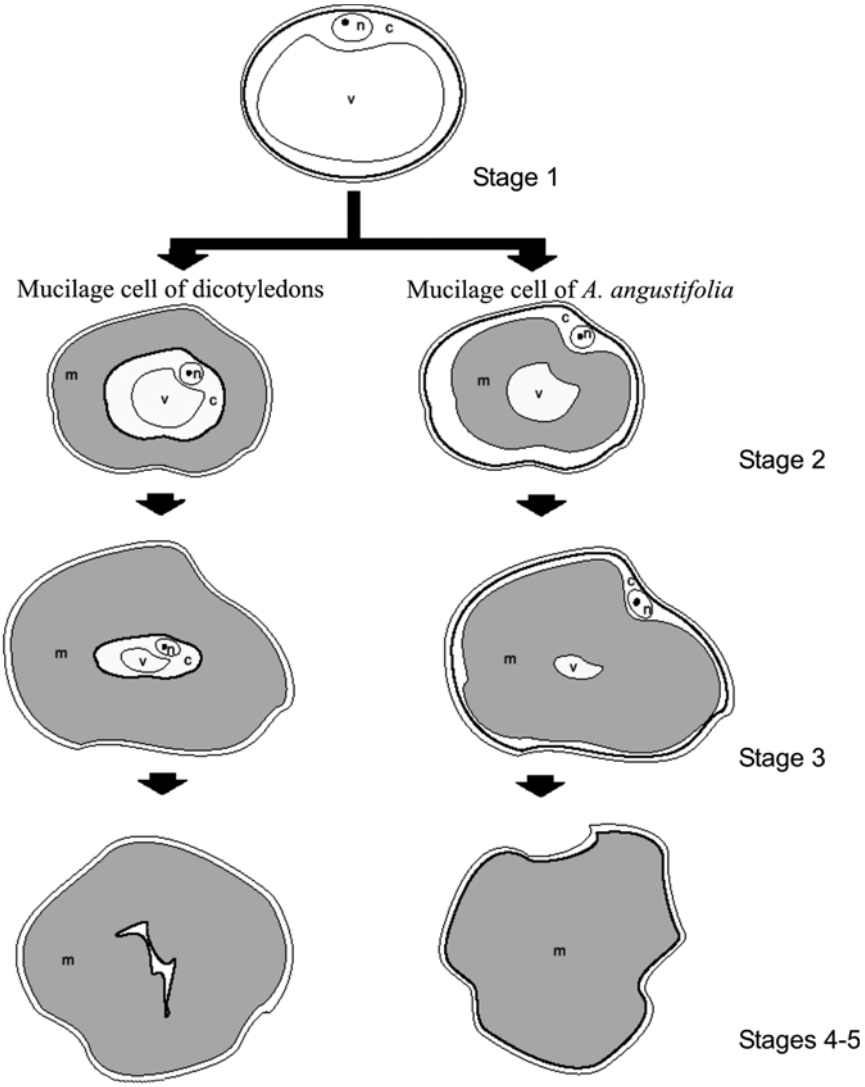


Fig. 27. Comparison of mucilage cell development in dicotyledons and *A. angustifolia*. *n* Nucleus; *c* cytoplasm; *v* vacuole; *m* mucilage; thick line, plasmalemma. Mucilage cell secretion in dicotyledons occurs between the plasmalemma and the cell wall. In *A. angustifolia*, secretion occurs between the cytoplasm and central vacuole into a specialized cavity

ture of insects by carnivorous plants, and lubricants for the growing root tip, and water storage (Fahn 1979). Mucilage can also contribute to damage protection when plants are exposed to low or freezing temperatures, as suggested by Clarke et al. (1979).

According to Esau (1965) and Fahn (1979), polysaccharides contribute to drought resistance. Bamber et al. (1978) and Mastroberti and Mariath (2003) observed through histochemical tests that pectin is abundant in *A. angustifolia* mucilage cells. It is well known that pectin has a hydrophilic nature and is present in mucilage.

Araucaria angustifolia is a xeromorphic species that exists in regions with a temperate rainy climate, where the average maximum and minimum temperatures of the coldest month are 18 °C and −3 °C, and the average maximum temperature of the hottest month is 27 °C (Mattos 1994). In these cells, mucilage probably plays a protective role against low-temperature stress, as suggested by Clarke et al. (1979).

An apoplastic tracer was utilized in this work. Apoplastic tracers are nontoxic and able to move as freely as water through cell walls. Canny (1990, 1993) stated that tracers are sufficiently dissociated at apoplastic pH and do not cross the plasmalemma and stain cell contents. Canny (1993) demonstrated that the fluorochrome does not pass through the plasmalemma in live cells, although water does. The ions K^+, Ca^+, P^-, and Cl^- cross this membrane more rapidly. However, biochemical studies of the membrane potential of xylem parenchymal cells (ray cells) and tracheal elements have shown that there are changes in the regulation of ions entering and leaving through the plasmalemma, via alteration of the ion channels by K^+ and Ca^+. This allows the entry of solutes and water into the tracheal elements that have al-

Table 1. Comparison of mucilage cells of angiosperms and *A. angustifolia*[a]

Characteristic	Estimation for species:					
	Opuntia ficus-indica (Cactaceae)	*Opuntia polyacantha* (Cactaceae)	*Cinnamomum burmanni* (Lauraceae)	*Hibiscus schizopetalus* (Malvaceae)	*Aloe arborescens* (Liliaceae)	*Araucaria angustifolia* (Araucariaceae)
Mucilage cells						
General characteristics	numerous; ↑ volume	numerous; ↑ volume	numerous; ↑ volume (21 μm); large vacuole	↑ volume (90 μm); large vacuole	numerous; ↑ volume (300 μm); large vacuole	numerous; ↑ volume (180 μm); large vacuole
Distribution	random	random	random	row or pairs in mesophyll and in shoot apex; singly in epidermis	–	rows
Development	nonsequential; majority of stages occur in shoot apex	nonsequential; majority of stages occur in shoot apex	nonsequential; majority of stages occur in shoot apex	sequential; majority of stages occur in shoot apex	sequential; follow maturity of leaf	sequential; majority of stages occur in shoot apex; follow maturity of leaf
Mucilage						
Structure	lamellate	fibrillar (?)	fibrillar	granular (mesophyll), lamellar (epidermis)	granular	densest regions (lamellate and reticulate)
Basic compound	polysaccharides, no proteins	polysaccharides	polysaccharides	–	polysaccharides (starch)	polysaccharides (pectins, proteins)
Synthesis location	Golgi	Golgi	Golgi	Golgi	chloroplasts, vesicle from plasmalemma	Golgi and rough endoplasmic reticulum
Function	calcium control, water storage	–	–	–	water storage	water storage
Suberin layer	absent	absent	present	absent	absent	absent
Chloroplasts	↓ thylakoids, ↓ oil drops and grana	↓ thylakoids, ↓ oil drops	↓ thylakoids, starch grains persistent	↓ starch grains, ↓ thylakoids	↑ volume of starch grains, ↓ thylakoids	↑ oil drops, ↓ tylakoids and grana
Golgi	hypertrophic; ↑ activity	hypertrophic; ↑ activity; formation of large vesicles	hypertrophic; ↑ activity; numerous	hypertrophic; ↑ activity; numerous	↓ number	↑ activity; formation of large vesicles

[a] ↑, Increase; ↓, decrease; –, no information

ready undergone cell death (De Boer and Wegner 1997). If this biochemical alteration also occurs in the plasmalemma of mucilage cells, this would allow the entry of HTPS fluorochrome connected to these solutes via the symplast into the cell lumen. The water absorbed by the epidermis would reach the mucilage cells faster than the parenchyma of the mesophyll, because, as in tracheal elements (De Boer and Wegner 1997), the plasmalemma of the mucilage cells would allow the water to enter more easily, to be stored by the mucilage, just as it allowed the HTPS to enter. However, the necessary biochemical studies have not been performed. The action of the apoplastic tracer HPTS could indicate that these cells play an important physiological role in water and/or solute storage and translocation throughout the apoplast–symplast solute pathway.

These cells could act as water regulators during unfavorable periods, such as in cold weather or during seedling establishment.

Conclusions

The secretion pathways in angiosperms and *A. angustifolia* (gymnosperm) are different. In angiosperms, the vesicle membranes fuse with the plasmalemma and the vesicles containing secreted substance are eliminated by invaginations of the plasmalemma (granulocrine secretion) or by an active molecular or ionic process (eccrine secretion) (Fahn 1979). In contrast, in *A. angustifolia* mucilage cells the vesicles containing the secretory material are eliminated from the cytoplasm by tonoplast detachment. The secretion is deposited between the central vacuole and the cytoplasm, and the cytoplasm is compressed against the cell wall. A specialized cavity is formed where the mucilage is deposited.

The PCD process in mucilage cells was observed as vacuole reduction and protoplasm degeneration, thylakoid reduction in the chloroplasts, dechromatinization of the chromatin, and detachment of the nuclear membrane. This can be attributed to the same apoptotic model described by Matile (1997), Sineonova et al. (2000), and Obara et al. (2001).

The *A. angustifolia* mucilage cells were dead at maturity, but they remained physiologically functional. The action of the apoplastic tracer HPTS could indicate that these cells have a role in water storage and translocation throughout the apoplast solute pathway; however, more studies must be performed to confirm this.

In addition, immunocytochemical studies are being performed to characterize the pectic epitopes and mucilage proteins from the plasmalemma and cell wall of these cells, in order to verify the biochemical alterations during their development.

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