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Distribution and differentiation of the laticifer system in *Chamaesyce thymifolia* (L.) Millsp. (Euphorbiaceae)

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SUMMARY

Leaves and stems of *Chamaesyce thymifolia* (L.) Millsp. (Euphorbiaceae) were analysed using light and electron microscopy with special emphasis on the distribution and differentiation of non-articulated laticifers. Stem laticifers were found among parenchyma cells of the cortex. Laticifers formed a discontinuous ring in the boundaries of primary phloem and branching into the cortex. In leaves, they formed a network in the mesophyll. In the vascular system, they appeared close to the phloem. Laticifer tubes were branched, forming a network in both organs. The development of *C. thymifolia* laticifers showed two distinct stages: undifferentiated and differentiated ones. The undifferentiated laticifers were characterized by electron-dense cytoplasm, a nucleus, abundant mitochondria, rough endoplasm reticulum and a considerable number of small vacuoles. During the laticifer tube development, a gradual degeneration of organelles was observed while a central vacuole with latex particles was formed. The differentiated laticifers presented intact cell membranes, cellular debris and an accumulation of latex particles inside the tubes. Plasmodesmata and primary pit fields could not be observed between the laticifer and the adjacent parenchyma cells. Developed laticifers presented peculiar cell walls that were thicker than the adjacent cell walls. Various morphological types of starch grains were observed in the *C. thymifolia* latex.

Key-words: cell wall, *Chamaesyce thymifolia*, latex, laticifer differentiation, non-articulated laticifer, starch grains.

INTRODUCTION

Laticifers are series of cells or long single cells containing latex (Fahn 1990). The latex is composed of water and isoprene units mixed with other compounds that vary

† In memoriam.

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depending upon the species and the environment (Hunter 1994). Laticifer tubes are present in several tissues and organs of the plant. Metcalfe (1967) pointed out that laticifers are found in as many as 900 genera in 20 families of dicots and monocots, and also in other groups, such as *Gnetum* (Gnetaceae) and *Regnellidium* (Marsileaceae). Frequently, laticifers are considered as a taxonomic character. French (1988) reported, for instance, the significance of laticifers in distinguishing subfamilies within the Araceae.

The function of the laticifer system in plants is not clear. It has been suggested that laticifers constitute a special type of storage or excretion system (Fahn 1979). A variety of functions have been attributed to these tubes, such as synthesis and accumulation of large amounts of substances which may best be described as secondary metabolites, including the suggestion that latex might serve to protect the plant against attacks from herbivorous animals (Bonner & Galston 1947; Compton 1987; Hunter 1994).

Laticifers are classified into articulated and non-articulated types on the basis of their origin (Esau 1965). The non-articulated laticifers develop from a single cell which greatly elongates during growth of the plant and sometimes forms branches. Articulated laticifers consist of simple or branched series of cells which are usually elongated. The end walls of such cells can become porous or can disappear completely (Fahn 1990). It has been suggested that non-articulated laticifers are coenocytes formed by apical intrusive growth (Mahlberg 1959, 1993; Cass 1985). However, Milanez & Monteiro-Neto (1956) believed that non-articulated laticifers are formed by fusion of cells during their development.

The origin and early anatomical development of non-articulated laticifers were studied in *Nerium oleander* by Mahlberg (1963) and in *Euphorbia marginata* by Mahlberg & Sabharwal (1968). Based on general studies on non-articulated laticifer differentiation it has been suggested that the laticifer fluid is formed by breakdown and degeneration of the vacuole and other cell organelles (Marty 1968, 1970; Fineran 1983; Inamdar *et al.* 1988; Roy & De 1992). In the present study, using light and electron microscopy, we analysed the distribution and differentiation of non-articulated branched laticifers of *C. thymifolia*.

MATERIALS AND METHODS

Stems and leaves of the first, second and third internode of *C. thymifolia*, a herbaceous plant (syn. *E. thymifolia* Willd.), were collected from the Universidade Federal do Rio de Janeiro campus. For general light microscopy, the specimens were fixed in formalin–acetic acid–ethanol, dehydrated in ethanol and embedded in paraffin (58–60°C). Microtome sections of a thickness of 10–12 μm were cut and stained by astra blue–basic fuchsin. Serial paradermic sections of the leaf with the same thickness also were cut. Histochemical tests were carried out on freehand sections of the collected material: lignin was localized by 5% ethanolic phloroglucinol and 20% HCl (Johansen 1940); cellulose was localized by chlor–zinc–iodine (Jensen 1962); polyanions, especially pectin, were demonstrated with 0·2% aqueous ruthenium red (Jensen 1962); Lugoll's reagent (IKI) was applied to localize starch grains (Jensen 1962); and Sudan IV in 70% ethanol was used for the identification of lipids (Jensen 1962).

For transmission electron microscopy, fresh latex and stem fragments were fixed for 2 h at room temperature in a solution containing 2·5% glutaraldehyde, 2·0% paraformaldehyde in 0.075 M cacodylate buffer pH 7·4, while leaf fragments were fixed with 2·5% glutaraldehyde, 2·0% paraformaldehyde in 1·0% PIPES buffer pH 7·4.

Subsequently, the specimens were rinsed three times with the respective buffer, post-fixed for 1 h at room temperature with 1·0% osmium tetroxide, rinsed in distilled water, contrasted with 0·5% aqueous uranyl acetate at room temperature for 2 h, dehydrated in ethanol or acetone and embedded in Epon. Sections were stained with uranyl acetate followed by lead citrate and observed in a ZEISS 900 transmission electron microscope. In a cytochemical approach, 1% ruthenium red was used to detect negatively charged components in leaves and stem cell walls (Luft 1971). For scanning electron microscopy, after fixation the latex was dehydrated in ethanol, critical point dried in CO_2, covered with 20 nm gold and observed in a JEOL 25-5-II scanning electron microscope.

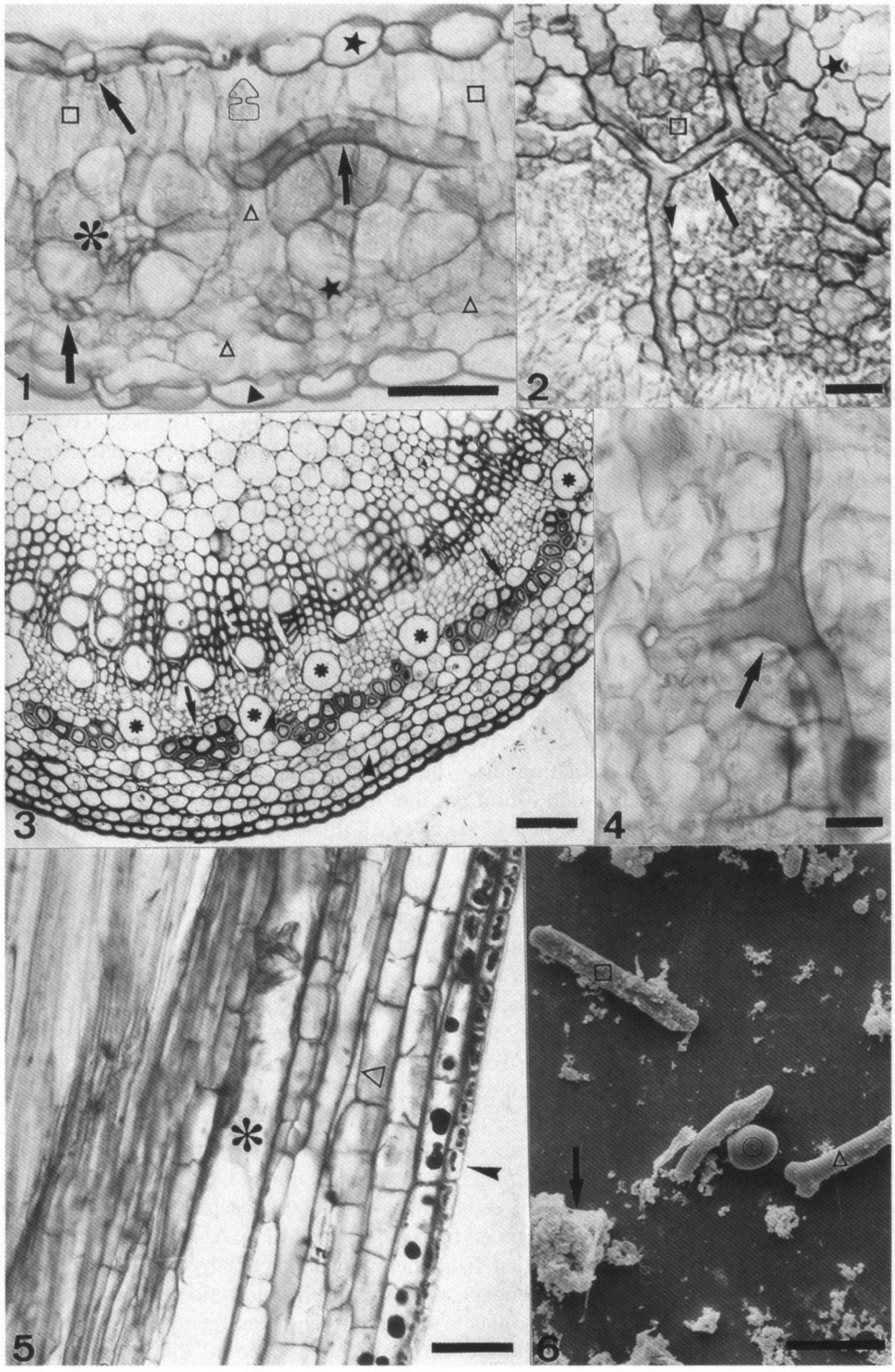
RESULTS

C. thymifolia presented branched non-articulated laticifer tubes. The leaf blades showed a compact uniseriate epidermis. The mesophyll consisted of a dorsiventral layer of palisade parenchyma and 5–6 layers of spongy parenchyma (Fig. 1). The laticifer system was found to form a network in the mesophyll (Figs 1, 2 and 4), characteristic for branched non-articulated laticifers. The bundle sheath presented C4 anatomy, while the vascular bundle showed a collateral arrangement (Fig. 1) and the laticifer tubes were often situated close to the phloem. Paradermic sections of the leaf blades showed that laticifers were either 'H' (Fig. 2) or 'Y' (Fig. 4)-shaped or they were found parallel to each other without connections. The laticifer cell wall was thicker and more intensely stained with astra blue–basic fuchsin than that of the adjacent parenchyma cells (Fig. 1).

The stem was bounded by a layer of epidermal cells. The cortex consisted of parenchyma cells (Fig. 3). Non-articulated branched laticifers occurred, forming a discontinuous ring intercalated by perivascular gelatinous fibres at the boundaries of the primary phloem and branching into the cortex (Figs 3 and 5). In longitudinal sections, laticifer tubes were often found parallel to the plant axis (Fig. 5). In the pith, laticifer tubes did not differ in diameter and cell wall thickness when compared to those found near the primary phloem. In the laticifer cell walls, cellulose was detected by chlor–zinc–iodine. Phloroglucinol tests revealed that the laticifer cell walls did not contain lignin; however, they showed pectic acids strongly labelled by ruthenium red in light and electron microscopy (data not shown).

The fresh latex was a milky white viscous fluid. When stained with Sudan IV, the contents of the laticifers appeared dark red. Lugoll's reagent revealed the presence of starch grains of unusual morphology as compared to those observed in other cells of the plant body. Using scanning electron microscopy, various morphological types of starch grains were observed in the latex which appeared as globular, rod, spindle and osteoid-shaped (Fig. 6).

The fine structure of laticifers from stem and leaf blade was similar. The laticifers markedly differed from the neighbouring cells by their elongated shape and thicker cell walls. Two clearly defined stages of differentiation of laticifer tubes were observed: undifferentiated (Fig. 7) and differentiated ones (Figs 11 and 12). At the undifferentiated stage, laticifers could be distinguished from neighbouring cells by the presence of a dense cytoplasm, abundant mitochondria, rough endoplasmic reticulum, dictyosomes and many small vacuoles. The cytoplasm was relatively homogeneous possessing a large number of free ribosomes. Short cisternae, with occasional dilatation areas of rough endoplasmic reticulum, were observed. Mitochondria showed an elongated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profile, with developed cristae and a moderate electron-opaque matrix. Small vesicles were formed by rough endoplasmic reticulum (Fig. 7) and in some images it appeared that small vacuoles were formed by the fusion of these vesicles. Plasmodesmata and primary pit fields were not observed. The undifferentiated tubes presented amyloplasts with starch grains, nuclei, and peroxisomes similar to those found in other mesophyll and cortex cells.

During development, the laticifers of *C. thymifolia* presented a cytoplasmic organization similar to that described above but with only one large central vacuole surrounded by an intact tonoplast. Globular latex particles were always present inside the central vacuole (Fig. 8). In a subsequent step, a partial disorganization of the central vacuole and extensive degeneration of organelles was seen. The number of mitochondria was reduced, showing only few cristae and an empty matrix (Fig. 9). Subsequently, a partial degeneration of the nucleus was observed and fusion of small vesicles with the central vacuole could be seen.

In a later developmental stage, a large number of membrane systems could be observed. Simultaneously, the tonoplast was broken down completely and cellular debris could be observed among the latex particles. After this moment, laticifer tubes were formed by only one compartment (Fig. 10). Differentiated laticifers were characterized by total or partial protoplast degeneration while the cell membrane remained intact, accumulating latex particles inside the tubes. The latex particles were cross-linked by an amorphous material (Fig. 11).

Undifferentiated laticifers presented compact cell walls in which cellulose microfibrils were very difficult to identify (Fig. 8). Subsequently, there was an increase of thickness and alteration of microfibril disposition (Figs 10 and 11). Cell walls of developed laticifers presented a clear wavy disposition of cellulose microfibrils close to the cell membrane (Fig. 12). Lignification of the laticifer cell walls was not revealed by phloroglucinol using light microscopy.

DISCUSSION

The laticifers of *C. thymifolia* are non-articulated and branched. The genera *Euphorbia* and *Chamaesyce* are characterized by the presence of non-articulated laticifers in embryonic and adult tissues (Biesboer & Mahlberg 1981; Fineran 1983; Mahlberg 1993). In paradermic sections of the leaves a laticifer network is demonstrated easily. The laticifer system presents its tubes close to the vascular bundles, branching throughout

Fig. 1. Transverse section of a *C. thymifolia* leaf showing the distribution of laticifer tubes (arrows) in the leaf blade. Palisade parenchyma (open square); spongy parenchyma (open triangle); vascular bundle (asterisk); adaxial epidermis (star); abaxial epidermis (black triangle); stomata (open arrow). Light microscopy. Bar = 50 μm.

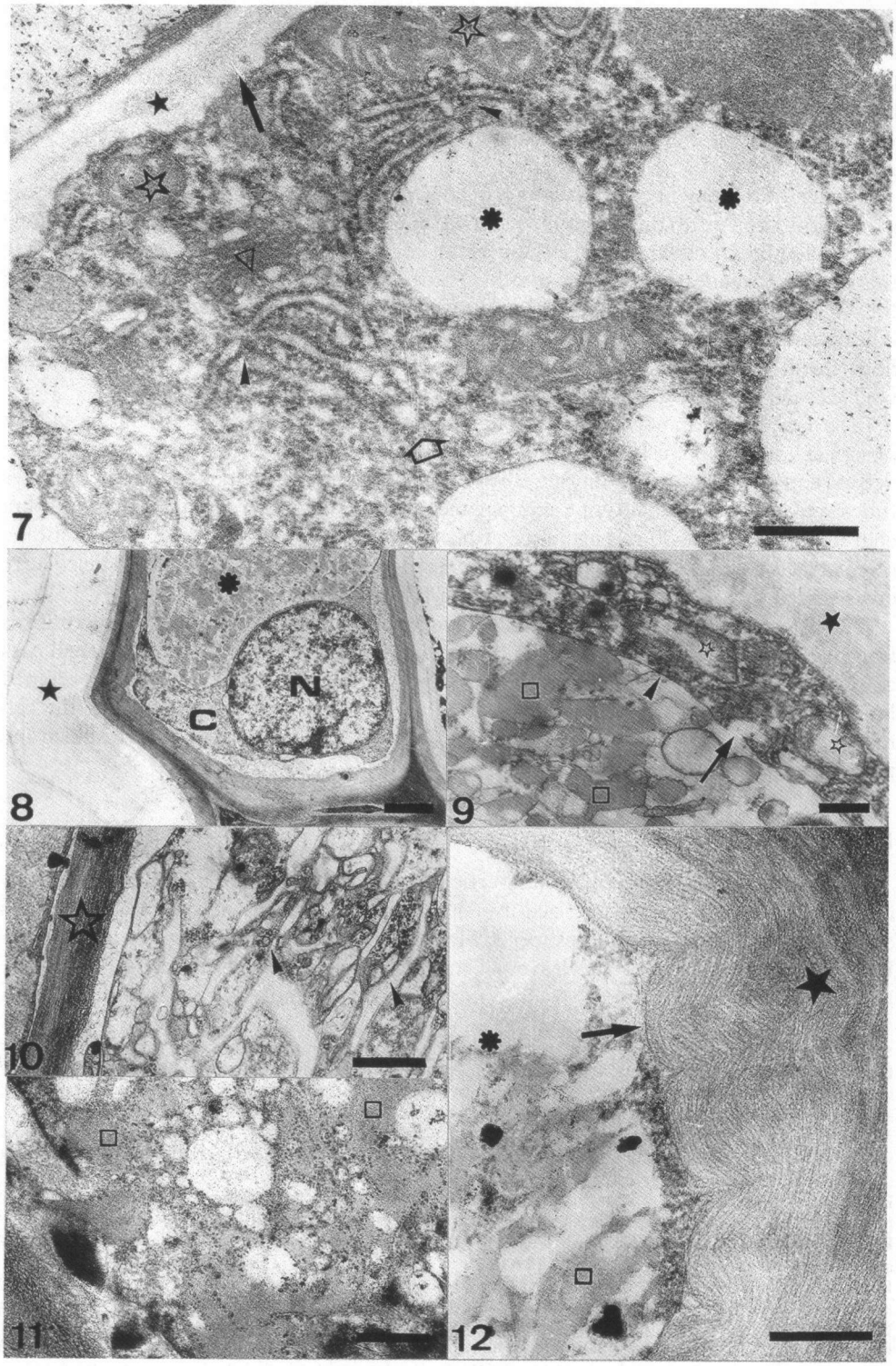
Fig. 2. Paradermic section of a leaf blade showing a laticifer tube forming an 'H' profile (arrow). Epidermis (star); palisade parenchyma (open square). Light microscopy. Bar = 10 μm.

Fig. 3. Transverse section of a stem showing the distribution of laticifer tubes (asterisks) close to the primary phloem (arrowheads). Gelatinous fibres (arrow). Light microscopy. Bar = 100 μm.

Fig. 4. Transverse section of a leaf showing a fork or 'Y' shape in a laticifer tube (arrow). Note the strongly stained cell wall. Light microscopy. Bar = 10 μm.

Fig. 5. Longitudinal section of a stem. Observe a laticifer tube with its content near to the phloem (asterisk) and another immersed into the cortex (star). Epidermis (arrowhead); parenchyma cells of cortex (open triangle). Light microscopy. Bar = 100 μm.

Fig. 6. Starch grain types found in latex. Globular (open circle); rod (open square), spindle (black triangle) and osteoid shaped (open triangle). Latex particles (arrow). Scanning electron microscopy. Bar = 100 μm.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the mesophyll. In the stem, these tubes appear on the primary phloem boundaries, branching into the cortex. The *C. thymifolia* stem has laticifer tubes associated with the primary phloem and they appear dispersed in the pith. This suggests that they can cross the stele from the pith and that they originate from the same initial cell. It has been reported in other plants that branched non-articulated laticifers are formed only in primary tissues and can cross the stele from the pith (Fahn 1979). A similar distribution occurs in *A. syriaca* (Wilson & Malhberg 1978), *A. curassavica* (Giordani 1977), *E. characias* (Marty 1968), *E. pulcherrima* (Fineran 1983), *Ficus carica* (Rachmilevitz & Fahn 1982) and *Jatropha dioica* (Cass 1985). In other species, such as *Nelumbo nucifera*, laticifer tubes occur only in the vascular bundle (Esau & Kosakai 1975). They may also occur as isolated ducts or as a linear compact mass, as described in *Calotropis gigantea* (Roy & De 1992). Such a distribution was not found in *C. thymifolia*.

It was possible to identify *C. thymifolia* laticifers by the thickness and staining of the cell walls. In this plant, the laticifer cell wall was strongly stained by ruthenium red, suggesting a higher quantity of pectic substances. The walls are thicker than those of the adjacent parenchyma cells. However, it has been reported that laticifer cell walls may have the same thickness as adjacent cells or are thicker. In that case, they are highly hydrated and contain a large portion of pectic substances and hemicelluloses (Fahn 1979). The laticifer forms a soft, plastic primary cell wall during its early development (Sperlich 1939). However, the thickness of the laticifer cell wall is quite variable. This is more evident in those plants in which the laticifer wall is thicker than that of the adjacent cells (Fahn 1979). The irregularity in cell wall thickness appears to be the result of plasticity of the wall (Malhberg 1993). It has been reported that the cell wall of laticifers is relatively rich in pectic material which is responsible for its hygroscopic character (Frey-Wyssling 1932, 1942). The laticifer cell wall of *C. thymifolia* shows a wavy arrangement of cellulose microfibrils, suggesting a rearrangement of the cellulose microfibrils in later stages of development.

During the early stages of laticifer differentiation many small vesicles are formed in the cytoplasm (Stockstill & Nessler 1986). These vesicles were reported to result directly

Fig. 7. Initial stage of laticifer differentiation characterized by cytoplasm rich in organelles. Mitochondria (open stars) and rough endoplasmic reticulum (arrowhead) are the most abundant organelles. Dilatation of rough endoplasmic reticulum is frequent (open arrow). Note the cell membrane (arrow) close to the compact cell wall. Small vacuoles (asterisks); Golgi apparatus (open triangle). Transmission electron microscopy. Bar = 0·5 μm.

Fig. 8. Intermediary stage of laticifer differentiation, showing accumulation of latex particles in the central vacuole with an intact tonoplast (asterisk). Nucleus (N); cytoplasm (C); Cell wall of the gelatinous fibre (star). Transmission electron microscopy. Bar = 1·0 μm.

Fig. 9. Laticifer differentiation presenting accumulation of latex particles (open square) in the central vacuole with an intact tonoplast (arrowhead). Note a fusion of a small vesicle with the tonoplast (arrow). Mitochondria (open stars) were present in partially degenerated cytoplasm. Cell wall (star). Transmission electron microscopy. Bar = 0·5 μm.

Fig. 10. Final stage of differentiation of a laticifer. Latex particles together with a fluid phase of latex. Some membrane systems can be seen (arrowheads). Note the electron density of the cell wall (open star) and the arrangement of the microfibrils. Transmission electron microscopy. Bar = 1·0 μm.

Fig. 11. Differentiated laticifer showing latex (open squares) and electrondense cellular debris. Transmission electron microscopy. Bar = 1·0 μm.

Fig. 12. Differentiated laticifer showing a wavy arrangement of cellulose microfibrils (arrow) in the cell wall (star) close to cell membrane. Note mature laticifer tube without internal compartmentalization (asterisk). Latex (open square). Transmission electron microscopy. Bar = 1·0 μm.

from the dilatation of the endoplasmic reticulum in *F. carica* (Rachmilevitz & Fahn 1982), *A. syriaca* (Wilson & Mahlberg 1978) and *Cannabis sativa* (Mesquita & Santos Dias 1984). This also occurs in *C. thymifolia* where these dilatation areas form bands within the cytoplasm that will degenerate. Stockstill & Nessler (1986) observed that the large vacuoles are the fusion products of many small vacuoles in *N. oleander*. Dictyosomes are another possible source of vesicle formation, according to Rachmilevitz & Fahn (1982) and Wilson & Mahlberg (1980), who suggested that vesicles may bud off from the cisternae of dictyosomes.

The differentiation of laticifers in *C. thymifolia* involves the development of small vacuoles which, after fusion, form a central vacuole. This central vacuole disintegrates in mature laticifers. This also occurs in *A. syriaca* (Wilson & Mahlberg 1978), *Nelumbo nucifera* (Esau & Kosakai 1975), *Papaver bracteatum* (Nessler & Mahlberg 1978) and *F. carica* (Rachmilevitz & Fahn 1982). However, Inamdar *et al.* (1988) reported that one of the most important features observed in the mature non-articulated branched laticifers of *Allamanda violacea* was the presence of a large vacuole. Similar observations were reported in the laticifers of other species, e.g. *A. syriaca* (Wilson & Malhberg 1980), *N. oleander* (Stockstill & Nessler 1986), and *F. carica* (Rachmilevitz & Fahn 1982).

Gradual degeneration of the laticifer cytoplasm was observed in *C. thymifolia* after the central large vacuole was formed. When the laticifers were completely differentiated, the tonoplast disappeared. The mitochondria, dictyosomes, endoplasmic reticulum and nuclei also degenerated during laticifer development. Similar observations were reported in *A. violacea* (Inamdar *et al.* 1988). In contrast, it has been described that a few intact organelles may persist in the cytoplasm in *E. pulcherrima* (Fineran 1983). Cellular autophagy is the main cause of central vacuole formation in non-articulated laticifers (Stockstill & Nessler 1986; Inamdar *et al.* 1988; Roy & Deepesh 1992). Marty (1970) reported the presence of numerous vacuoles in undifferentiated non-articulated laticifers, with isolated areas of the cytoplasm. The vacuoles were considered autophagic and were interpreted to digest the included areas of cytoplasm to form the large central vacuole of the cell.

Latex is an emulsion or suspension of many solid particles in a liquid with variable refractive indexes, producing characteristic plant products (Biesboer & Mahlberg 1981). The colour of latex varies in different plant species and may be white and milky in *Euphorbia* and *Asclepias*. *C. thymifolia* presents a milky latex which contains different shapes of starch grains, such as globular, rod, spindle and osteoid-shaped. Morphological types of starch grains formed in the genera *Euphorbia* and *Chamaesyce* may be considered as a taxonomic character (Rao & Prasad 1986). The rod, spindle and osteoid starch grain shapes are the most frequent types in non-articulated laticifers and are predominant in *C. thymifolia*. It has also been reported that rod-shaped grains can be observed in the latex of herbaceous taxa and the other types in xerophytic, succulent taxa (Biesboer & Mahlberg 1981).

The functional role played by the laticifer system is not well established. For some investigators, it represents a special type of inner secretory tissue found in plants (Esau 1965; Fahn 1979; Mahlberg 1993). It might store several types of substances, which are not reused in the metabolism of the plant and probably do not act as cellular energy reserves (Bonner & Galston 1947; Biesboer & Mahlberg 1978). Another possible role played by laticifers is the participation in plant defence mechanisms. Latex of *C. papaya* might provide antifungal action (Giordani *et al.* 1991). In contrast, laticifers of

C. thymifolia are infected by protozoa of the genera *Phytomonas* and bacteria. However, these micro-organisms are not observed in other plant tissues (Da Cunha 1993). Primary pit-fields, plasmodesmata and lignification of the laticifer cell wall of *C. thymifolia* were not observed. It is possible that the laticifer system in this species acts as a closed compartment inside the plant that supports chemical and physical impediments against herbivory.

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