LATICIFER SYSTEMS IN *MANDEVILLA ILLUSTRIS* AND *M. VELUTINA* APOCYNACEAE

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ABSTRACT

Two Apocynaceae species from savanna (Cerrado) area of São Paulo State, Brazil were studied. In both *Mandevilla* species examined, the laticifer systems are of non-articulated branched type, characteristic of the family. In vegetative organs reported was the occurrence of a primary laticifer system of which the cells were differentiated in the embryo. However, additional laticifer cells were always produced during the growth of the shoot apex. A secondary laticiferous system produced by the cells of vascular cambium was identified in the tuberous root and in the stem. A proposed discussion on this apparently unique record in Apocynaceae was arisen to evaluate the main distinction between articulated and non-articulated laticifers.

KEY WORDS: Non-articulated laticifer, *Mandevilla*, Apocynaceae.

INTRODUCTION

The laticifers were reported in 22 angiosperm families, among them, the Apocynaceae (Metcalfe 1967). Laticifer classification is based on its initial number. So, non-articulated laticifers arise from single cells that divide coenocytically (without cytokinesis) to form multinucleated cells with growing tip that elongates (Fahn 1979). According to this concept, non-articulated laticifers generally originate only from primary tissues in the seedling; indeed this is one of the main distinctions between articulated and non-articulated laticifers (Rudall 1989). However, it was reported in Euphorbiaceae (Rudall 1989) and in Moraceae (Van Veenendall and Den Outer 1990) families that it is possible non-articulated laticifers to originate in the vascular cambium. There is no record of this occurrence in the Apocynaceae species. Besides, additional information on the latex composition in this family is necessary.

The present study deals with the organographic distribution, structure, ontogeny of laticifers and their composition in *Mandevilla illustris* and *M. velutina*, (Apocynaceae). These two species have been prescribed as folk medicine in certain regions of central-western Brazil where infusions or alcoholic extracts of tuberous roots were used for the treatment of venomous snake bites. Recently, Calixto et al. (1985), have been demonstrating scientifically their medicinal potential.

MATERIALS AND METHODS

Embryos of ripe seeds, shoot apices, leaves, stems, tuberous and non-tuberous roots of *Mandevilla illustris* (Vell.) Woodson and *M. velutina* (Mart. ex Stadelm.) Woodson were collected from savanna (Cerrado) area at the Experimental Station of Itirapina, São Paulo State, Brazil.

The material was fixed in Nawashin solution (Johansen 1940) for customary methods of embedding, sectioning and mounting of histological sections. Transverse and longitudinal sections, 8-10 μm thick, were stained with Heidenhain's hematoxylin and fast green (Milanez 1952).

For analysing thinner sections, the samples were fixed in 5% glutaraldehyde (2h) in 0,2 M potassium phosphate buffer, pH 7,2. After postfixing with 2% OsO4 (2h) and dehydrating in an ethanol series, the samples were embedded in Spurr (Spurr 1969). For light microscopy, sections, 1.0-1.5 μm thick, were stained with 1% toluidine blue in 1% sodium borate (Van Veenendaal and Den Outer 1990). For transmission electron microscopy (Zeiss EM 10) the sections were stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963).

For scanning electron microscopy transverse sections (120 μm thick) of the stems and xylopodium were cleared in 20% sodium hypoclorite solution, dehydrate in ethanol series. Finally, the sections were put between two slides and dehydrated by heating with a tungsten lamp (Exley et al. 1974). After that, they were mounted and sputter-coated with gold-paladium. SEM was used to investigate the tracheary element content and to obtain a better three-dimensional image of the cells.

For microchemistry tests, sections of the different organs were stained in ruthenium red for polysaccharides (Johansen 1940), Sudan black B for fatty substances (Jensen 1962) and aniline blue black for total protein (Fisher 1968).

RESULTS

In both *Mandevilla* species examined, the laticifer systems are of non-articulated branched type reported in the Apocynaceae family.

Embryos of ripe seeds presented laticifers along the hypocotyl-radicule axis and in the cotyledons (Figs 1-3). This primary system is developed during the seed germination. In adult plants, the early differentiation of this system was observed in the shoot apex, where the laticifer cells could be distinguished from neighbouring cells by their cytoplasmatic content, thicker walls and the absence of plasmodesmata. Soon after differentiation, the initials elongated vertically along the longitudinal axis of the plant body; their tapering apices grew intrusively and more rapidly among the surrounding cells; no fusions of laticifer cells with each other or adjoining parenchyma cells were observed. The laticifers were observed in all vegetative plant parts. The diameter of the lumen in laticifers varied in different parts of the plant body. Narrower laticifers were found in non-tuberous roots (Fig. 5). In the stem and petioles, the laticifers were noticed in cortex (Fig. 6), pith and around the vascular cylinder, close to the phloem (normal and intraaxillary ones). Laticifers were also observed in the colleters present of the base of the petiole. In the leaves, they always followed the vascular bundles (Figs 7-8) close to the phloem. They sent out branches through the intercellular spaces between the spongy parenchyma and the palisade tissue. Laticifers were observed just beneath the palisade tissue (Fig. 8) and sometimes they could reach the epidermis.

In addition to this primary laticiferous system, in *Mandevilla* a secondary laticiferous system was produced by the vascular cambium. The laticifers appear within the secondary phloem of the xylopodium and the tuberous root (Figs 9-11).

These laticiferous cells could be distinguished from the other cambium derived cells by their gray colour content resulted from the strong reaction with toluidine blue and from other characteristics mentioned above.

In the dissociated tissues of the underground organs, long laticifers were observed in the secondary phloem and sometimes their penetration into tracheary elements (Fig. 4).

The latex was milky in the aerial vegetative organs and yellow in the tuberous roots. The milky latex content showed positive reaction to total proteins, fatty substances and polysaccharides.

In the aerial stems, some tracheary elements exhibited a content that reacted to fatty substances as the laticifer content did (Fig. 12).

DISCUSSION

The classification of laticifers that is now most widely used (De Bary 1884 apud Rudall 1987) is based on structure and development. According to Milanez (1974), non-articulated laticifers arise, in the embryo, from cells the number of which is constant for each species. They grow intrusively into intercellular air spaces between neighbouring cells by wall elongation and successive mitoses without cytokinesis. So, all the system, even in the secondary structure, is produced by the autonomous apical growth of the embryonary cells and their branches. On the other hand, articulated laticifers consist of chains of cells the adjoining walls of which may sometimes break down, forming tubes or vessels. They can arise from primary and secondary meristems (Fahn 1979). Then, Rudall(1989) emphasizes the different origin between articulated and non-articulated laticifers, the former arising in both primary and secondary tissues.

Milanez (1974) contested the classification of the non-articulated laticifers, presenting arguments supporting the new laticifer initials formation in the secondary structure. This author mentioned papers where the researchers had difficulties in interpreting their results due to the theory that does not admit the formation of new laticifers from non-articulated ones. Schaffstein (1932) in Milanez (1974), presumed that in certain Asclepiadaceae, the presence of a secondary laticifer system, is similar to the articulated ones, but independent of origin, position and formation period, from those of continuous laticifers. Artschwager (1946), during his studies with *Cryptostegia grandifolia*, was not able to explain the presence of laticifers in the secondary structure; he admitted that some of them were so close to the vascular cambium that they appeared to be its products. Thus, the author prefered to suggest the presence of an intercalary growth of the laticifer cells in the cambium region. Milanez (1974) argued affirming that laticifer branches in the primary structure close to the cambium, probably would be a result of the secondary structure invasion by primary structure elements which generally are decadent due to the expansion of the derived cambium elements. Besides that, the same author questioned why the laticifer would change its growth from apical to intercalary.

However, Milanez (1966) admitted, for the first time, in *Cryptostegia grandifolia*, the origin of vertical laticifers in the secondary phloem due to longitudinal coalescence of the cambial derivates. Appezzato (1988) also observed the precocious differentiation of non-articulated laticifers from cambial zone of the tuberous root in *Mandevilla velutina*. Rudall (1989) and Van Veenendaal and Den Outer (1990) observed that secondary laticifers are produced by the vascular cambium in *Croton* spp. (Euphorbiaceae) and *Morus nigra* (Moraceae), respectively. These authors confirmed Metcalfes (1967) and Milanezs (1974) observations explaining why no secondary systems were reported in other studies. The most probable reason was the very young material used. Furthermore, up to now it was accepted that non-articulated laticifers generally arise only from primary tissues, distinctly from the articulated ones.

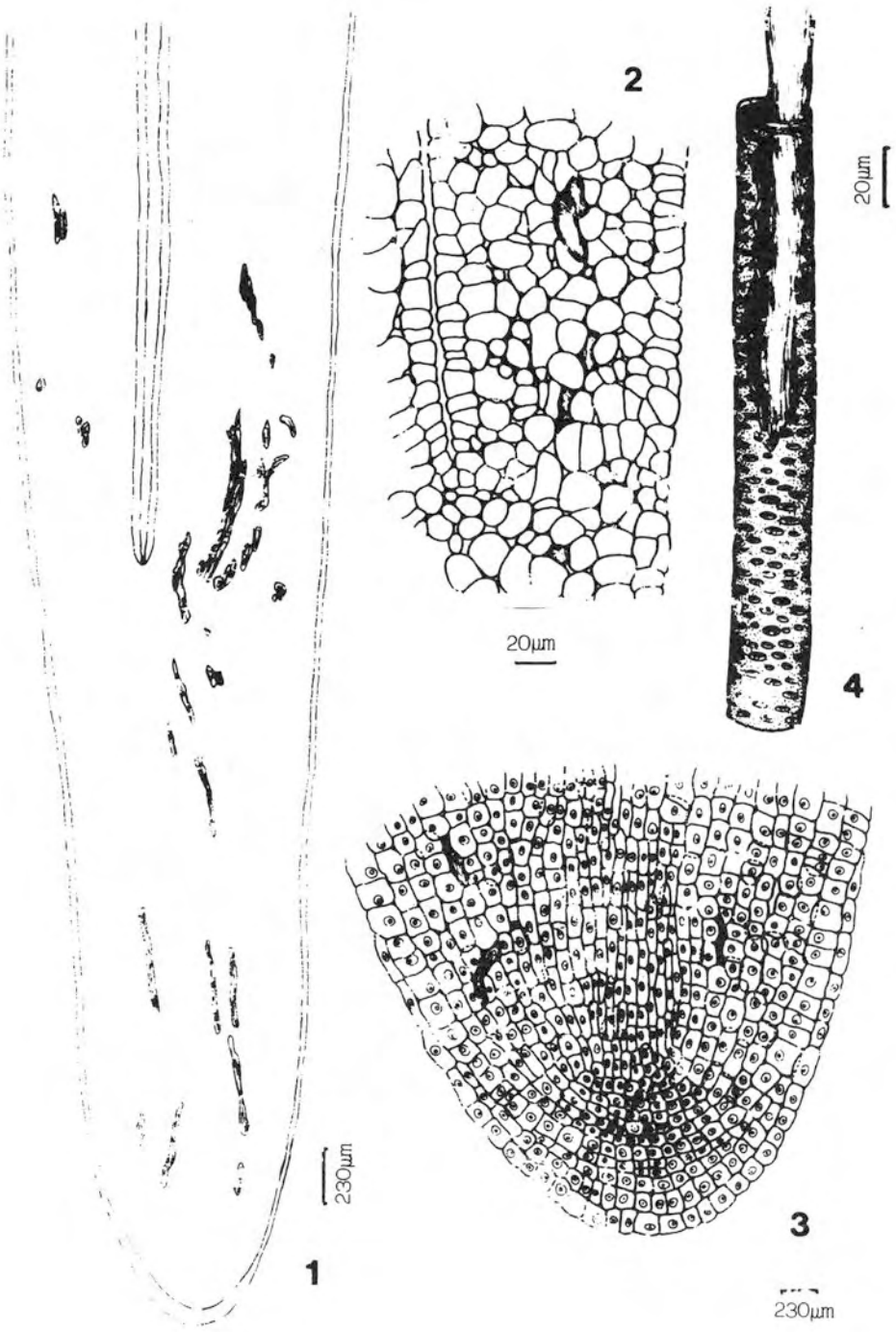
According to Van Veenendaal and Den Outer (1990), the main criteria by which laticifer initials of the vascular cambium could be distinguished were: strong reaction to toluidine blue, cell-wall thickness and cell size.

The mentioned authors still demonstrated that the presence of long, undivided (complete) and unbranched laticifers in macerations of young secondary phloem could be an evidence of their independence of those present in the primary structure. This state was also observed in the present study.

In the majority of the Apocynaceae species, the laticifer cells are already present in the embryo and they arise from the cotyledonary node (Solereder 1908).

According to Mahlberg (1961) in *Nerium oleander* the laticifers arise from approximately 28 cells in the embryo, immediately below the shoot apex; no new laticifer cell is produced during the growth of the shoot apex. These results were different from the present ones. On the other hand, in *Plumeria alba* and *Vallaris solanacea* the laticifer cells differentiate only in the shoot apex and not in the embryo (Murungan and Inamdar 1987a, b). Indeed, they observed that new laticifer cells are continuously produced by the apical meristem as it was reported in the present study.

According to Fay et al. (1989), an absence of plasmodesmata in the laticifer walls of *Hevea* sp. could be related to the

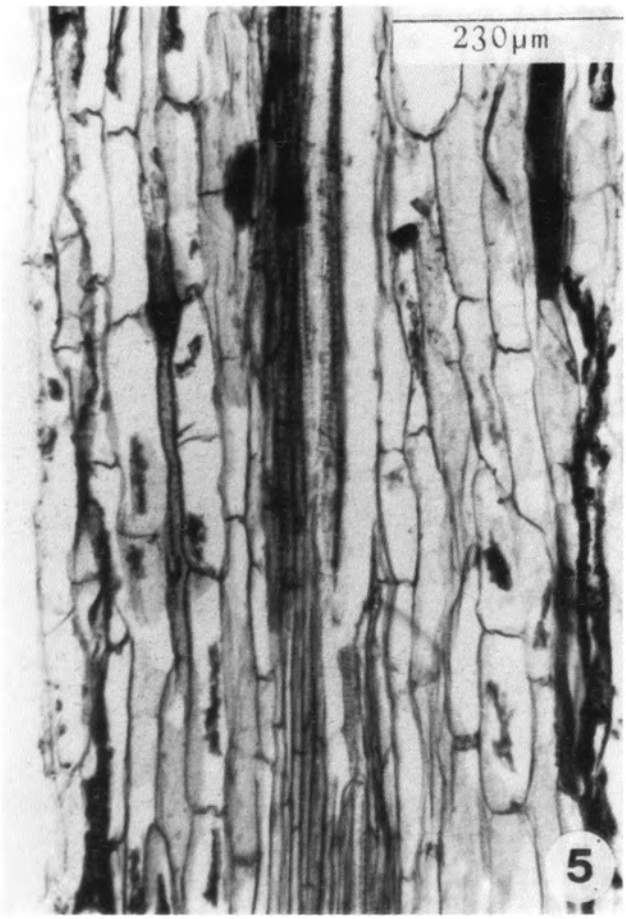


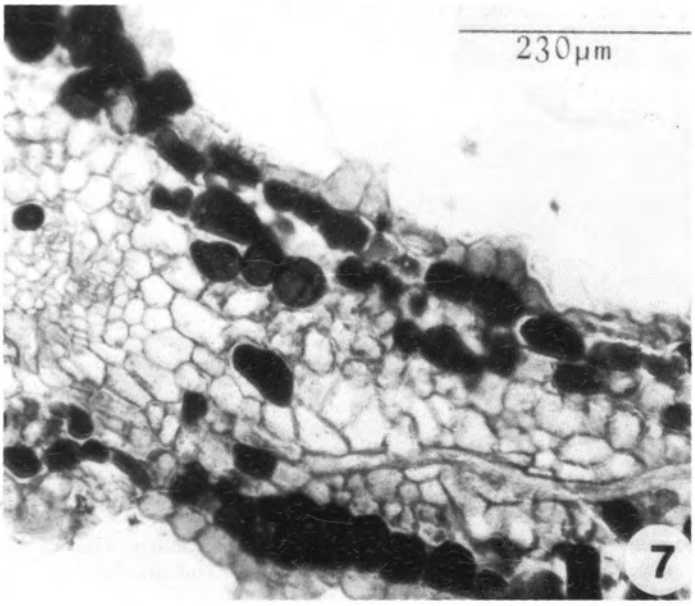
Figs 1-3. *Mandevilla* spp. Drawings of longitudinal sections of the embryo in ripe seed. Dark areas represent laticifers. (1) General view of the embryo and its initials laticifers distribution; Cotyledon (2) and radicule (3), showing the presence of the laticifer initials and laticifers.
Fig. 4. Drawing of the laticifer penetrating into tracheary element.

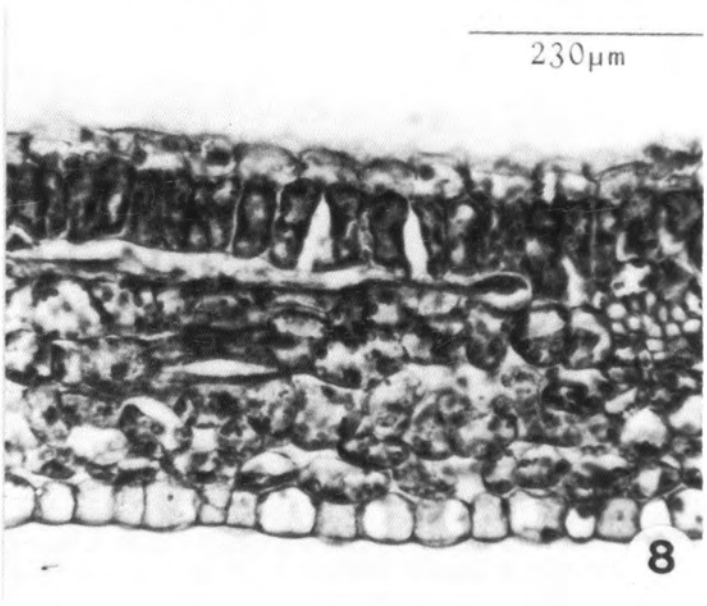
fact that they need to maintain a high turgor pressure and require a high concentration of osmoticum, which is inconsistent with possible leaks through the plasmodesmata. The absence of those communications in mature laticifers imply that the metabolits translocate apoplastically through the laticifer wall.

Among the studies concerning laticifers the information about their occurrence in roots is rare (Fahn 1979). This occurrence is probably, due to their lesser diameter as compared to the ones present in other organs as it was verified in *Mandevilla spp*. This diameter difference makes the laticifer identification difficult. They were also observed in *Apocynum cannabinum* and *Thevetia neriifolia* (Metcalfe and Chalk 1950). Their records in colleters are rare, too. Murugan and Inamdar (1987) reported them in *Plumeria alba*.

In *Mandevilla* spp. some tracheary elements of the aerial stems exhibit a content that reacted to fatty substances tests as the laticifers did. This characteristic was reported by Ravelo (1981) in *Mandevilla benthamii* and by Gonalvez (1962/65) in *Ralwolfia grandifolia*. According to this author the content could be a result of the protoplasm degeneration during va-





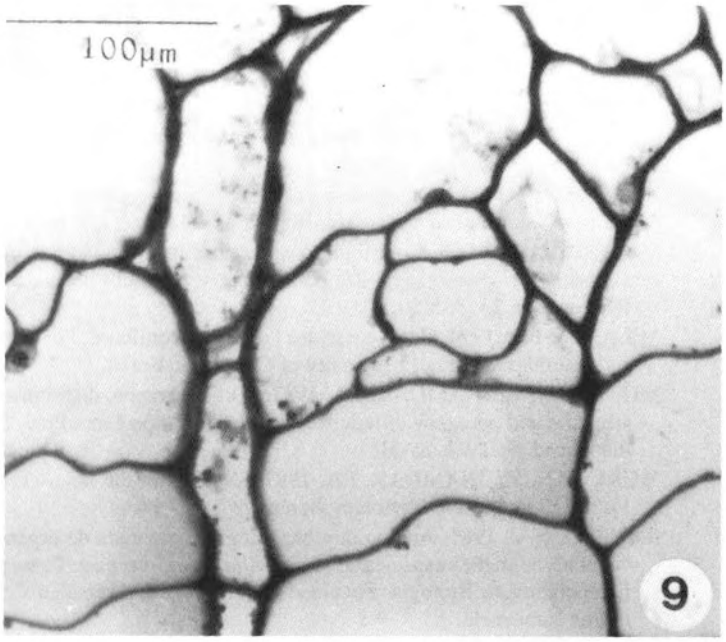


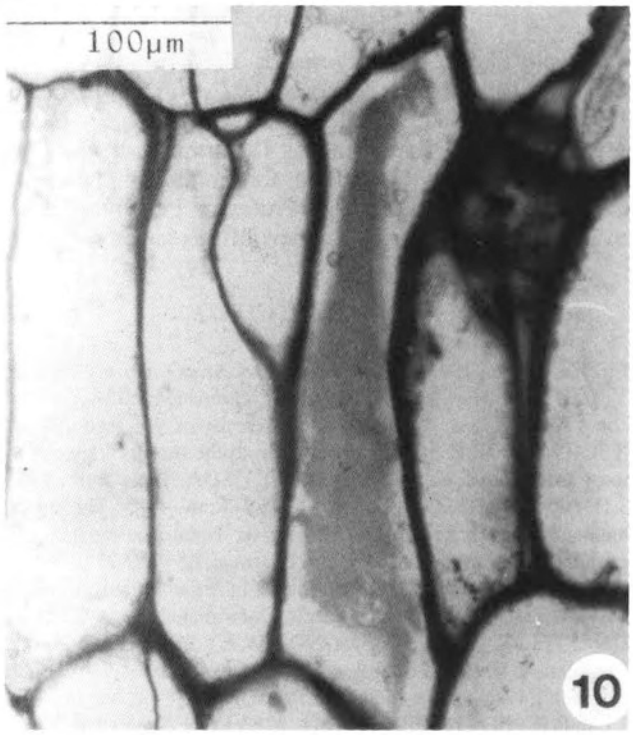
Figs 5-8. *Mandevilla* spp. Longisection of non-tuberous root showing a narrow laticifer (5). Longisection of stem showing laticifer in the ground tissue branching towards the epidermis (6). Transection of leaf showing laticifer near the vascular bundle (7) and below the palisade tissue (8).

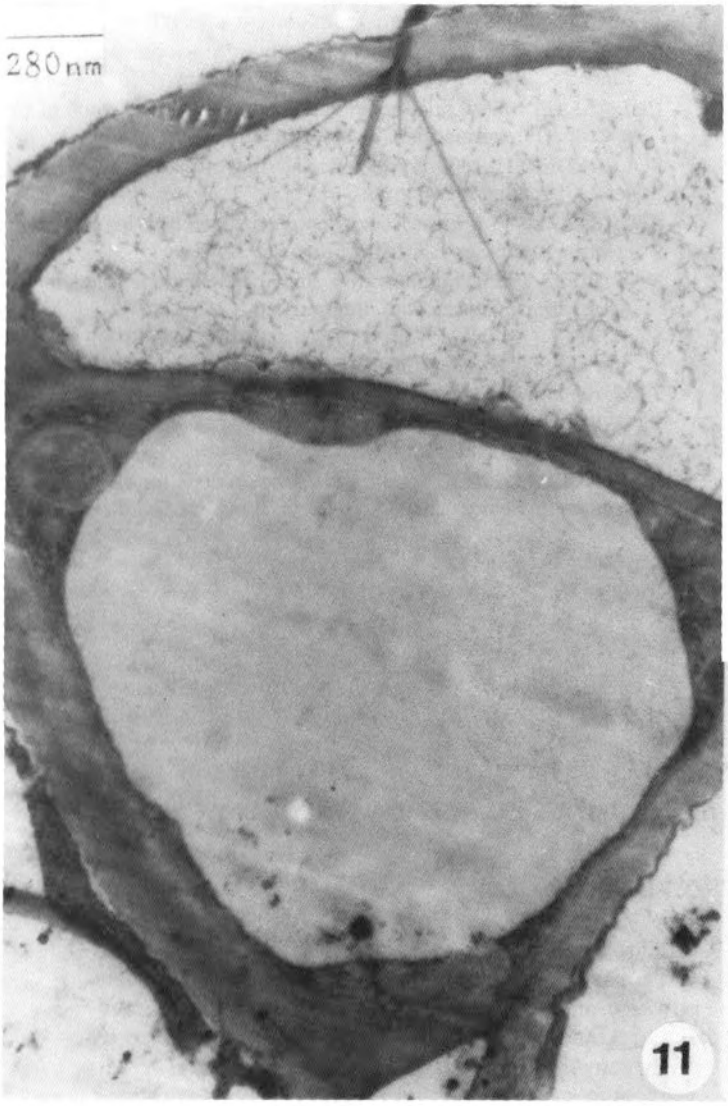
scular differentiation. But, De Castells et al. (1984) reported non-articulated laticifers in *Jatropha gossypium* penetrating into tracheary elements of the petiole and stem. And, Cortella (1989) observed in *Ipomoea purpurea* the presence of non-functional vessels filled by latex.

In maceration of the underground organ tissues of *Mandevilla* spp. was verified that laticifers can penetrate into tracheary elements. This event could also occur in the aerial stems.

Many Apocynaceae laticifers have rubber particles insoluble in ethanol (Van Die 1955). But they were not reported in the present study. Proteins and lipids were localized in the *Mandevilla* spp. laticifers, however, they lack starch grains. The same characteristics were reported in *Vallaris* laticifers by Murugan and Inamdar (1987b).







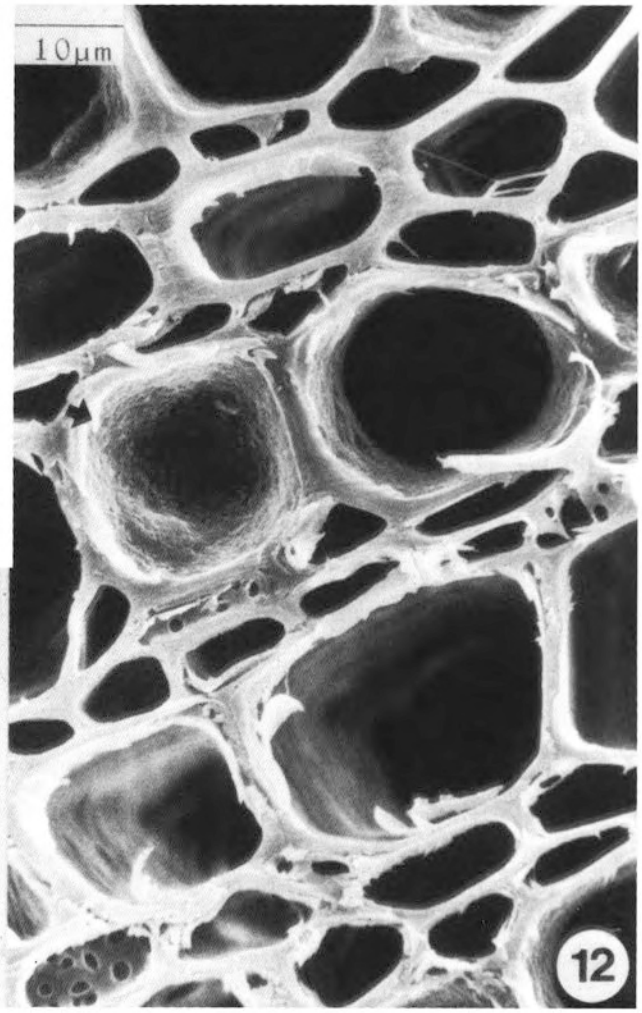


Fig. 9 and 10. *Mandevilla* spp. Transection (9) and longitudinal section (10) of the cambial region from tuberous root. The laticifer cells content reacts with toluidine blue.
Fig. 11. Ultrastructure of the laticifer cell showing its thicker walls devoided of plasmodesmata.
Fig. 12. SEM of a transection of stem showing the secondary xylem. The content of the tracheary cell (arrow) exhibits the same reaction to fatty substances as the laticifers.

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SYSTEMY RUREK MLECZNYCH U *MANDEVILLA ILLUSTRIS* I *M. VELUTINA* (APOCYNACEAE)

STRESZCZENIE

Zbadano dwa gatunki Apocynaceae z sawanny (Cerrado) w obszarze Sao Paulo, Brazylia. Systemy rur mlecznych u obu gatunków *Mandevilli* należą do typu nieczłonowanego, charakterystycznego dla tej rodziny. W narządach wegetatywnych stwierdzono występowanie pierwotnego systemu rur mlecznych, którego komórki były zróżnicowane w zarodku. Jednak dodatkowe komórki mleczne produkowane były zawsze w czasie wzrostu wierzchołka pędu. Wtórny system rurek mlecznych wytworzony przez komórki miazgi został rozpoznany w bulwiastym korzeniu oraz w łodydze. Proponuje się dyskusję nad tym unikatowym spostrzeżeniem u Apocynaceae w celu ustalenia różnicy między członowanymi i nieczłonowanymi rurkami mlecznymi.

SŁOWA KLUCZOWE: nieczłonowane rurki mleczne, *Mandevilla*, Apocynaceae.