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Laticifer Tissue Distribution and Alkaloid Location in *Vinca sardoa* (STEARN) PIGN. (*Apocynaceae*), an Endemic Plant of Sardinia (Italy)

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With 17 Figures

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Summary

SACCHETTI G., BALLERO M., SERAFINI M., ROMAGNOLI C., BRUNI A. & POLI F. 1999. Laticifer tissue distribution and alkaloid location in *Vinca sardoa* (STEARN) PIGN. (*Apocynaceae*), an endemic plant of Sardinia (Italy). – Phyton (Horn, Austria) 39 (2): 265–275, with 17 figures. – English with German summary.

Vinca sardoa (*Apocynaceae*) is an endemic plant of Sardinia (Italy) used in local folk medicine. Yet it has never been described in detail, particularly in regard to its secretory structures. The present research uses optical and electron microscopy to detect the laticifer cells in all plant organs, including the roots, where such cells had never previously been reported. Moreover, as opposed to what has been described in similar studies, these laticifer cells are articulated. In addition, histochemical tests

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were performed to detect the alkaloids in the latex and to quickly identify the same laticifer tissues in fresh samples. These tests always proved positive, indicating a close relationship between laticifer cells and the presence of alkaloids in the plant. TEM also showed that the laticifer cells have well-preserved cytoplasm and organelles which lead one to conclude that metabolic activity is still intact.

Zusammenfassung

SACCHETTI G., BALLERO M., SERAFINI M., ROMAGNOLI C., BRUNI A. & POLI F. 1999. Die Verteilung von Geweben mit Milchröhren und die Lokalisation von Alkaloiden in *Vinca sardoa* (STEARN) PIGN. (*Apocynaceae*), einer endemischen Pflanze Sardiniens (Italien). – Phyton (Horn, Austria) 39 (2): 265–275, 17 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Vinca sardoa (Apocynaceae) ist eine endemische Pflanze Sardiniens (Italien) und wird in der örtlichen Volksmedizin verwendet. Bisher wurde sie noch nie genau, insbesondere im Hinblick auf ihre sekretorischen Strukturen, beschrieben. Diese hier vorliegende Untersuchung setzt Licht- und Elektronenmikroskopie ein, um die Milchsaftzellen in allen Pflanzenorganen, einschließlich Wurzeln zu ermitteln; daß solche Zellen auch in Wurzeln vorkommen, wurde bisher noch nicht berichtet. Darüberhinaus, im Gegensatz zu dem was in ähnlichen Studien früher beschrieben wurde, sind diese Milchröhren gegliedert. Zusätzlich wurden histochemische Tests durchgeführt, um die Alkaloide im Milchsaft zu erkennen und um schnell die Milchröhren in frischen Proben nachzuweisen. Diese Tests fielen immer positiv aus und belegen damit den engen Zusammenhang von Milchröhren und dem Vorliegen von Alkaloiden in der Pflanze. TEM zeigte ebenfalls, daß die Milchsaftzellen ein gut erhaltenes Cytoplasma besitzen und Organellen, von denen man schließen kann, daß die Stoffwechselaktivität noch aufrecht ist.

Introduction

Vinca sardoa (Stearn) Pign. (*Apocynaceae*) is a species native to Sardinia which has never been subject to in depth study. In Sardinian folk medicine this plant is known for its hypotensive, astringent, hemostatic and depurative properties (ATZEI & PICCI 1975). The purpose of the present work has been to determine the distribution and location of laticifer vessels in the plant and histochemically detect the alkaloids present in the latex. Preliminary phytochemical studies had previously shown that various alkaloids were present in the above-ground portion of the plant (NICOLETTI & al. 1998). TEM was then used to characterize the ultrastructural features of the cells secreting and storing the latex.

Materials and Methods

Plant material

Vinca sardoa (Stearn) Pign. (*Apocynaceae*) plants were collected in Sardinia on the Marganai-Linas massif (Cagliari, Italy) at full flowering (March-May). The plants were identified following the keys reported in "Flora d'Italia" (PIGNATTI 1982) by M. BALLERO and a representative specimen of the species was deposited in the *Herbarium* of the Institute of Botany and Botanical Garden, University of Cagliari (CAG. no. 1301).

The number of plants examined and processed for conventional and electron microscopy was 50.

Conventional microscopy

Fresh cross and longitudinal sections of each plant organ were cut by hand using razor blades. These sections were stained for the identification of the laticifers alkaloid content and then examined under bright field. The following staining solutions were employed: Ellram reagent (FURR & MAHLBERG 1981), chloroauric acid ($HAuCl_4$), iodine-potassium iodide (IKI) (JOHANSSEN 1940), phosphotungstic acid (MORELLI 1981). All sections were stained for 24 h before observation. Negative controls were prepared by setting sections of each plant organ in a tartaric acid 10 % alcoholic solution for 24 h and then treating them with alkaloid indicators, following the suggestions given by the respective authors.

Samples of roots, stems, petioles, leaves and flowers were then fixed, embedded and sectioned following the procedures reported in POLI & al. 1995. Toluidine blue O (FEDER & O'BRIEN 1968) was used as general stain and Ninhydrin-Shiff's reaction (BRUNI & al. 1982) was used to detect cytoplasmic proteins. After staining, the sections were studied by a Zeiss Axiophot photomicroscope.

Transmission Electron Microscopy (TEM)

The same kinds of samples were fixed and processed for TEM following the standard procedures reported in POLI & al. 1989. A Zeiss electron microscope EM 109 N was used for observations.

Results

Histochemical and histological data

Conventional optical microscopy was used to observe sections of roots, stems, petioles, leaves and flowers of *Vinca sardoa*. This study revealed laticifer cells throughout the entire plant. The histochemical reactions indicated that alkaloids are stored in the laticifers of all the plant organs (Table 1).

Table 1.
Histochemical reactions, performed with different indicators, to check for alkaloids in various organs of *Vinca sardoa*. All tests were positive with different intensity.

	Ellram reagent color: yellow-brown	Chloroauric acid ($HAuCl_4$) color: purple-red	Phosphotungstic acid color: yellow-brown	Iodine-Potassium Iodide (IKI) color: brown-red
Roots	+	+++	+	+++
Stems	++	+++	++	+++
Petioles	++	+++	+	+++
Leaves	++	+++	+	+++
Flowers (corolla)	+	+++	+	++

+ weak reaction; ++ moderate reaction; +++ intense reaction.

There were numerous laticifer cells which appeared dark and were distributed throughout the cortical region of the roots (Figs. 1–2). Histochemical reaction with chloroauric acid solution ($HAuCl_4$) on longitudinal sections stained the latex a deep purple-red revealing numerous unbranched, articulated laticifers distributed throughout the entire cortical region (Fig. 2). The iodine-potassium iodide solution (IKI) stained the latex a deep brown-red evidencing the laticifers in the cortical area (Fig. 2a).

In the stem the laticifers were located in the cortical region, both close to the vascular bundles and below the epidermis. In the cross-sections, a comparison of the laticifer cells with the surrounding cells showed that the former were round and generally larger (Fig. 3). Alkaloid-specific reactions indicated that alkaloids were indeed present in the latex compounds (Fig. 4), while the same histochemical tests gave negative results on sections pretreated with tartaric acid (controls) (Fig. 5). The longitudinal stem sections showed unbranched and articulated laticifers with different alkaloid reactivity in the laticifer cells below the epidermis and close to the

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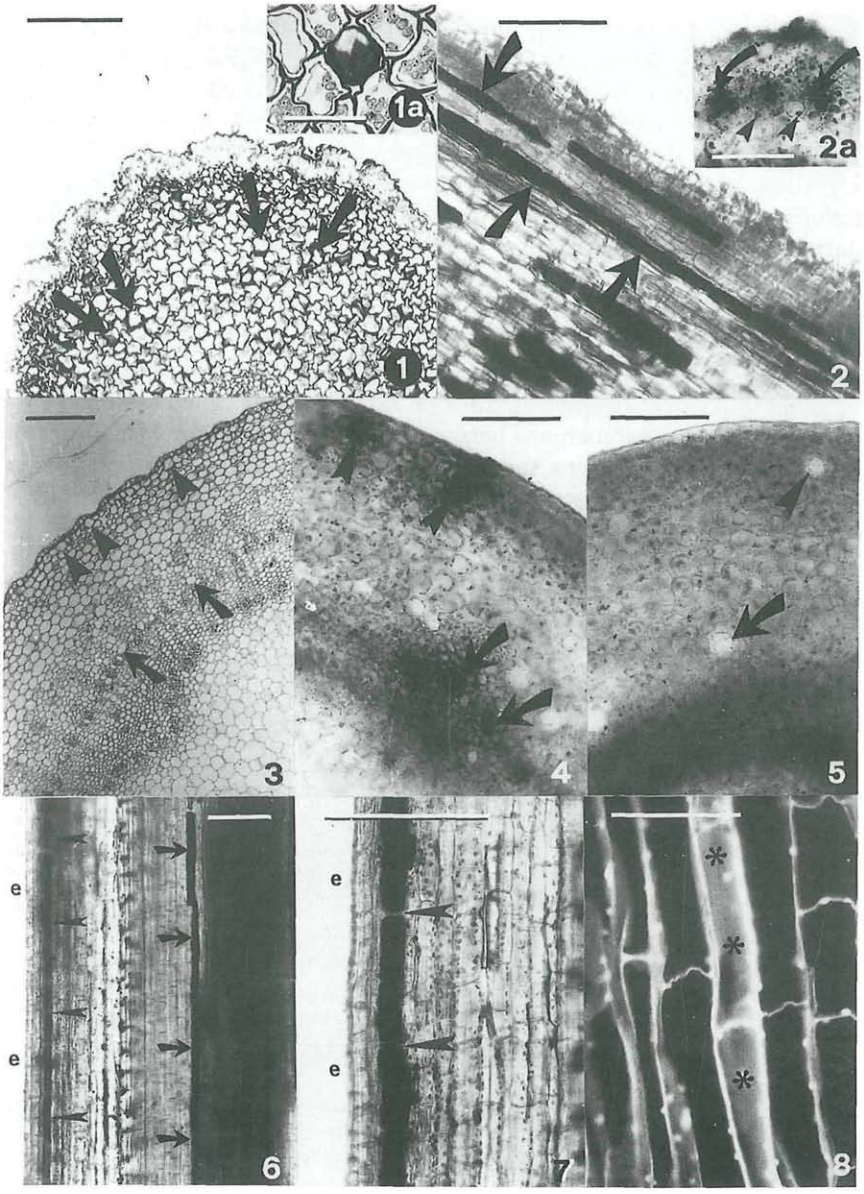
Figs. 1–2. Laticifers in the root of *V. sardoa.* Fig. 1. Resin-embedded cross-section stained with toluidine blue O (TBO). Note the numerous laticifers (arrows) distributed throughout the cortical portion. Scale bar = 100 μm. Fig. 1a. Detail of Fig. 1 showing the star-shaped laticifer cell surrounded by round parenchymal cells. Scale bar = 25 μm. Fig. 2. Hand-cut longitudinal section stained with chloroauric acid solution ($HAuCl_4$); the alkaloid indicator stains the latex an intense purple-red. The laticifers, distributed throughout the cortical area, are clearly unbranched and articulated (arrows). Scale bar = 100 μm. Fig. 2a. Hand-cut cross-section treated with iodine-potassium iodide solution (IKI). The latex in the laticifers stains an intense brown-red (arrows: laticifers; arrowheads: starch grains). Scale bar = 100 μm.

Figs. 3–8. Laticifers in the stem of *V. sardoa.* Fig. 3. Resin-embedded cross section of the stem stained with TBO. The arrowheads indicate the laticifers below the epidermis and the arrows those near the vascular bundles. Scale bar = 100 μm. Fig. 4. Hand-cut cross section stained with IKI solution. The laticifer cells both below the epidermis (arrowheads) and near the vascular bundles (arrows) stain an intense brown-red. Scale bar = 100 μm. Fig. 5. Hand-cut control cross-section pretreated with tartaric acid. This section, subsequently treated with a IKI solution, is clearly negative both for the laticifer cells below epidermis (arrowhead) and for those near the vascular bundles (arrow). Scale bar = 100 μm. Fig. 6. Hand-cut longitudinal section stained with $HAuCl_4$ solution showing the laticifers below epidermis (e: epidermis; arrowheads: laticifers) and those near the vascular bundles (arrows). Scale bar = 100 μm. Fig. 7. Particular of Fig. 6. The latex in the laticifers below the epidermis (e) is intensely stained. It is clear that these laticifer tissues are unbranched and articulated (arrowheads indicate the wall between the laticifer cells). Scale bar = 100 μm. Fig. 8. Resin-embedded longitudinal section of the stem stained with Ninhydrin-Schiff's reaction to determine the presence of cytoplasmic proteins. The laticifer cell cytoplasm (asterisks) appears intensely positive when compared to the parenchymal cell cytoplasm. Scale bar = 50 μm.



vascular bundles (Figs. 6, 7). In this part of the plant, the Ninhydrin-Schiff's protein reaction indicated a greater accumulation of proteins in the laticifer cell cytoplasm than in the surrounding cells (Fig. 8).

Cross-sections of resin-embedded petiole specimens showed numerous laticifer cells both below the epidermis and in the phloem parenchyma (Fig. 9). In fresh samples, the histochemical reactions clearly detected the presence of alkaloids in laticifer cells (Fig. 10). $HAuCl_4$ produced a particularly intense stain of the laticifers below the epidermis (Fig. 10a).

Cross-sections of fresh and resin-embedded leaf specimens revealed laticifer cells, particularly close to the abaxial surface just below the epidermis (Fig. 11). No particular interaction between laticifer vessels and vascular bundles were found here. In the fresh sections the alkaloid indicators reacted with the latex producing an intense stain (Fig. 11a).

In the flower, the laticifer vessels were present in the corolla while they were absent in both the androecium and the gynoecium. Cross-sections of resin-embedded samples revealed laticifer cells below the epidermis and in the parenchyma between the vascular bundles (Figs. 12, 13). Fresh longitudinal sections, treated with chloroauric acid solution, showed a strongly purple-red stain (Fig. 14).

Ultrastructural data

The ultrastructural characteristics of the mature laticifer cells were similar in all plant organs. The present data refer to the mature laticifer cells in the stem as these were best preserved by the fixing and embedding

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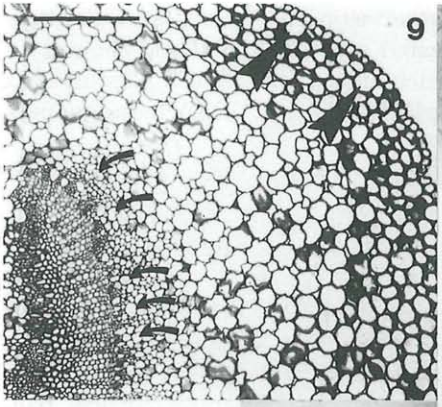
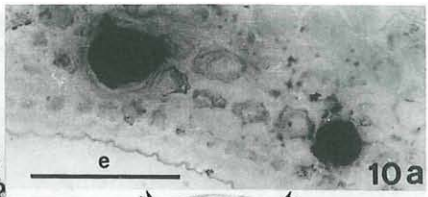
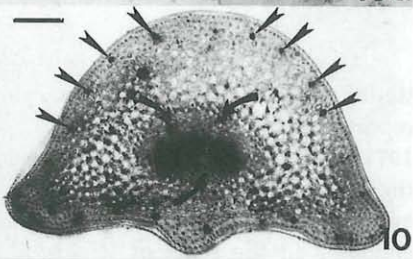
Figs. 9–10. Laticifers in petioles of *V. sardoa*. Fig. 9. Resin-embedded cross-section stained with TBO. Arrowheads indicate the laticifer structures below the epidermis and the arrows indicate those in the phloem parenchyma. Scale bar = 100 μm. Fig. 10. Hand-cut cross-section treated with $HAuCl_4$ solution. Note the laticifer structures (arrows and arrowheads) in both petiole areas where the latex is strongly stained. Scale bar = 100 μm. Fig. 10a. Detail of Fig. 10 where the $HAuCl_4$ solution has intensely stained the laticifer latex below the epidermis (e). Scale bar = 50 μm.

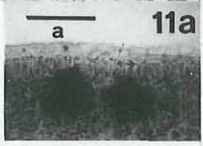
Fig. 11. Laticifers in the leaves of *V. sardoa*. Resin-embedded cross-section of the leaf stained with TBO. The arrow indicates a laticifer cell (a: abaxial surface). Scale bar = 100 μm. Fig. 11a. Hand-cut cross-section of the leaf stained with IKI solution. The latex in the laticifers is strongly stained a brown-red (a: abaxial surface). Scale bar = 50 μm.

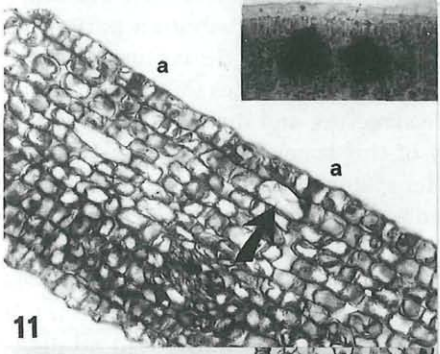
Figs. 12–14. Laticifers in the flower corolla of *V. sardoa*. Fig. 12, Resin-embedded cross-section of flower corolla stained with TBO. The arrowheads indicate the laticifers below the epidermis and the arrows those in the parenchyma, between the vascular bundles. Scale bar = 200 μm. Fig. 13. Detail of the previous figure. Note the laticifers between the vascular bundles (arrows) and below the epidermis (arrowheads). Scale bar = 100 μm. Fig. 14. Hand-cut longitudinal section of the flower corolla stained with $HAuCl_4$ solution (arrows: laticifers). Scale bar = 50 μm.

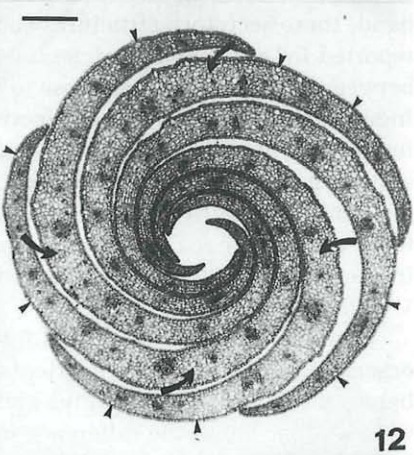


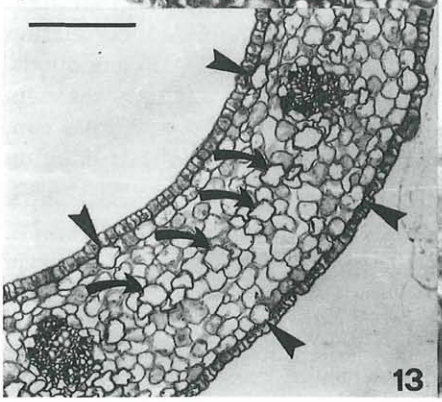


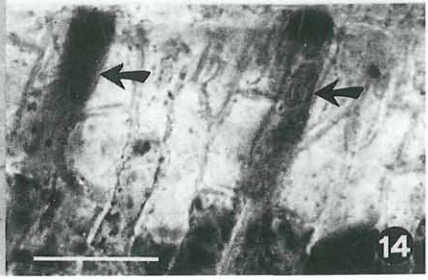












method. The mature laticifer cell has a large central vacuole full of latex accumulated in a large osmiophilic droplet. Part of the latex, in the form of smaller droplets, also adhered to the tonoplast (Fig. 15). The vacuole was surrounded by a thin layer of well-preserved parietal cytoplasm. Plastids, mitochondria and RER profiles appeared well-structured and showed no signs of degradation (Fig. 16). In particular, the laticifer cell plastids held numerous osmiophilic droplets, while the plastids in the surrounding parenchymal cells contained only starch (Fig. 17).

Discussion

All the organs in *Vinca sardoa* presented unbranched, articulated laticifer tissues while unbranched, non articulated laticifer tissues have been reported for the genus *Vinca*, as for all *Apocynaceae* (METCALFE 1967, FAHN 1979). In particular, FAHN 1979 asserts that laticifer cells are not present in the roots of plants belonging to the genus *Vinca*. The present results, however, show numerous laticifer cells throughout the entire cortical parenchyma in the roots of *V. sardoa*. In the stem and leaves, on the other hand, these secretory structures showed the same distribution pattern as reported for other *Vinca* species, i.e. mainly located in the cortical portion, between the epidermis and close to the vascular bundles (METCALFE 1967). Indeed, in our opinion, the distinctive structure and distribution of laticifer cells emphasizes the particularity of this species as it differs *V. minor* and the genus as a whole in the laticifer system distribution and structure. This unique data can be further added to the morphological-systemic features which have led taxonomists to suggest a new species (PIGNATTI 1982). In fact, *V. sardoa* was, until recently, considered a variety in the species *V. difformis* (ATZEI & PICCI 1975).

The histochemical reactions for alkaloids proved positive in all plant organs although to different extents. This indicates an evident correlation between the laticifer structures and the presence of alkaloid compounds. On the other hand, the difference in alkaloid reactivity found in the stem laticifer cells below the epidermis and close to the vascular bundles can, most likely, be explained by the different degree of alkaloid accumulation

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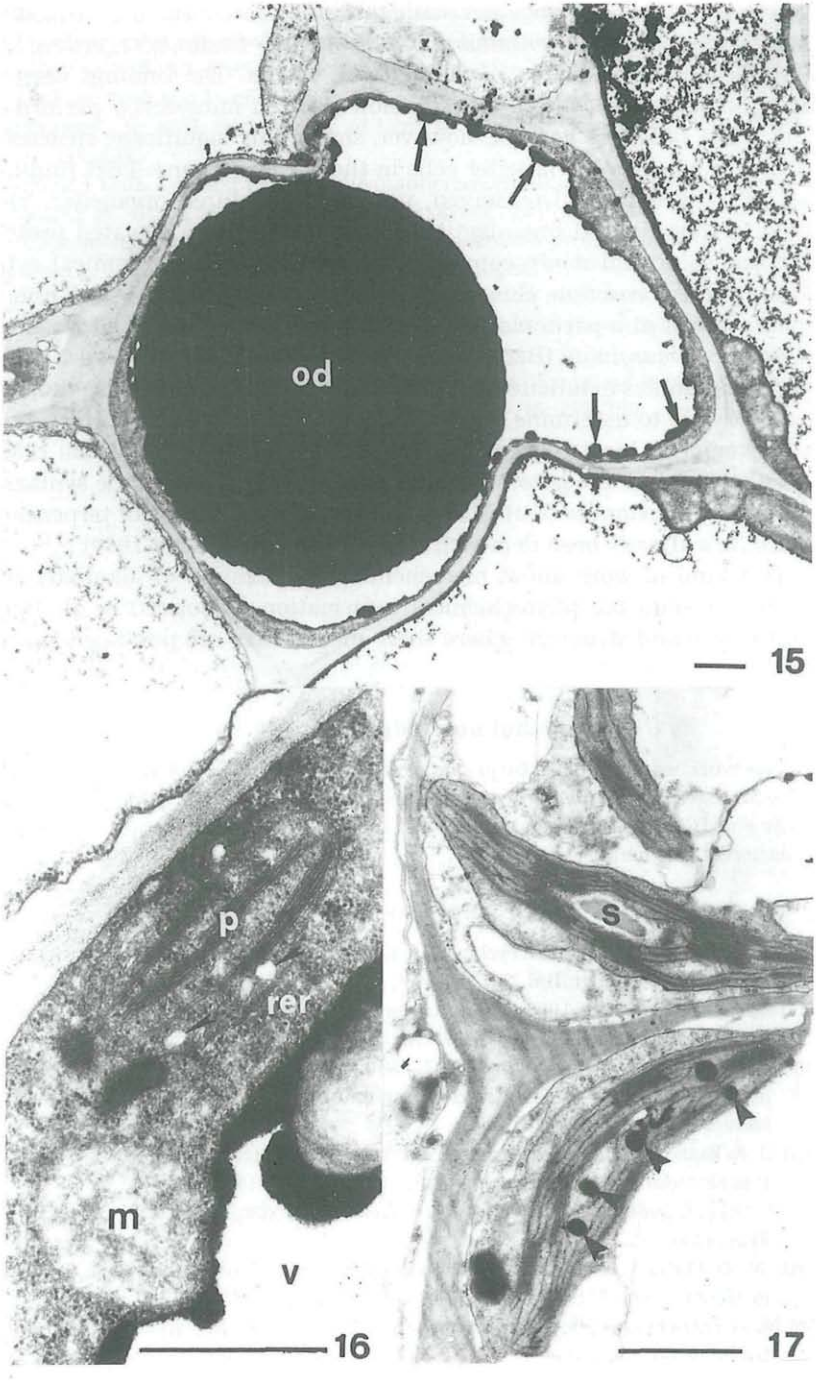
Figs. 15–17. TEM micrographs of mature laticifers in the stem of *V. sardoa*. Fig. 15. The mature laticifer cell presents a large vacuole filled with latex gathered in a large osmiophilic droplet (od). The arrows indicate the latex adherent to the tonoplast. Scale bar = 1 μm. Fig. 16. The vacuole (v) appears surrounded by a thin layer of well-preserved parietal cytoplasm containing RER (rer), plastids (p) and mitochondria (m). The arrowheads indicate trace of osmiophilic droplets in the plastid lost during TEM procedures. Scale bar = 1 μm. Fig. 17. The parenchymal cell adjacent to the laticifer (above) reveals well-structured plastids containing droplets of starch (s). The plastid in the young laticifer cell (below) contains osmiophilic droplets (arrowheads). Scale bar = 1 μm.



in the latex. Our data, together with those of similar research (CORSI & BIASCI 1998), support preliminary phytochemical findings regarding the isolation and identification of alkaloids in plants. The findings derived from optical, and above all, transmission electron microscopy performed on all plant laticifers has not, however, shown any significant structural differences between the laticifer cells in the various organs. TEM findings revealed cells with well-preserved, normally structured organelles. This type of ultrastructural organization appears to indicate elevated protein synthesis, as optical microscopy had revealed in the histochemical cytoplasmic protein reaction. Similar findings regarding protein synthesis in laticifer tissues at a particular stage of differentiation have been observed in *Euphorbia marginata* (BRUNI & al. 1982). As regards the presence of osmiophilic granules in laticifer cell plastids, in light of the above findings, it is not possible to determine whether these are latex precursors or simply the plastoglobuli located in many plant species (BACKHAUS & WALSH 1983). On the other hand, the possibility that plastids play a role in the synthesis of lipophilic substances (FAHN 1979) and in the biosynthesis of terpenes in general, has already been demonstrated (GRUMBACH & FORN 1980).

This kind of work about histochemical localization of alkaloids will help to correlate the phytochemical information (NICOLETTI & al. 1998) with the site and structure where these metabolites are produced and/or stored.

Acknowledgements

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