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Internal secretory spaces in thickened underground systems of Asteraceae species

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Abstract. Secretory structures are present in many vascular plants and have an important ecological role as a plant defence mechanism against herbivors and pathogens. Internal secretory spaces of lipid substances are widespread in the Asteraceae. However, information about the occurrence of these structures in thickened underground systems is sparse, compared with what we know about aerial systems. The main objective of the present paper was to investigate the occurrence, formation and chemical nature of the secretory structures in six Asteraceae species belonging to the following tribes: Eupatorieae (*Mikania cordifolia* and *M. sessilifolia*), Mutisiae (*Trixis nobilis*), Plucheeae (*Pterocaulon alopecuroides*) and Vernonieae (*Vernonia elegans* and *V. megapotamica*). The samples were collected in areas of Cerrado (tropical savanna) in the state of São Paulo, Brazil. The secretory structures found were cortical canals in roots (*T. nobilis*, *P. alopecuroides*, *V. elegans* and *V. megapotamica*), cortical cavities in roots (*M. cordifolia*, *M. sessilifolia* and *P. alopecuroides*), cavities in the secondary phloem of roots (*T. nobilis*), cortical cavities in the xylopodium (*M. cordifolia*, *M. sessilifolia*, *P. alopecuroides* and *V. megapotamica*) and in the underground stem (*T. nobilis*), and canals in the secondary xylem in the xylopodium (*M. cordifolia* and *M. sessilifolia*). Histochemical tests showed the presence of lipid substances in all structures.

Introduction

The occurrence of secretory structures in vascular plants has already been reported by various authors and, according to Fahn (1988), the main types are glands, nectaries, trichomes, oil cells, ducts and cavities. Some authors (Solereder 1908; Metcalfe and Chalk 1950, 1979; Robson 1977, 1981; Metcalfe 1983) have pointed out the importance of these structures in taxonomic studies, with the purpose of distinguishing orders, tribes, genera and even species, because different secretory structures may be found in different parts of the same plant or be confined to one of its organs. Consequently, a better understanding and definition of the function and the location of such structures are necessary. The secretory structures also play an environmental role. Increasing the capacity of plants to survive in their habitat by the production of substances is important for the interaction of plants with the biotic and abiotic environment (Harbone 1993), as well as a mechanism of plant defence against herbivores (Fahn 1979, 2002; Dussourd and Denno 1991).

Internal secretory spaces of lipid substances are widely distributed among the species of the Asteraceae (Fahn 1979), occurring both in aerial and underground organs. However, the role of these structures is not yet well understood. It is known that lipid compounds may act against herbivorous insects (Murphy 2001) and there is extensive literature on the aerial organs, whereas studies on underground structures are scarce (Appezzato-da-Glória *et al*. 2008*b*). The difficulties in sampling and processing underground material for analysis are probably the main limiting factors in the study of underground organs. However, studies of underground organs in Asteraceae are important, because such organs can be useful tools in taxonomic and even ecological studies.

Most studies on secretory structures have focussed on the occurrence of ducts in non-thickened roots (Grotta 1944; Williams 1947, 1954; Hoehne *et al*. 1952; Lersten and Curtis 1986, 1988; Joseph *et al*. 1988; Curtis and Lersten 1990; Poli *et al*. 1995; Luque *et al*. 1997; Sacchetti *et al*. 1997; Duarte and Estelita 1999; Pagni and Masini 1999; Melo-de-Pinna and Menezes 2002, 2003; Pagni *et al*. 2003; Lotocka and Geszprych 2004; Luque-Arias 2004; Machado *et al*. 2004; Fonseca *et al*. 2006; Vilhalva and Appezzato-da-Glória 2006; Appezzato-da-Glória *et al*. 2008*b*), with only few having studied the secretory structures in thickened underground systems (Hoehne *et al*. 1952; Panizza and Grotta 1965; Ragonese 1988; Curtis and Lersten 1990; Lotocka and Geszprych 2004; Machado *et al*. 2004; Vilhalva and Appezzato-da-Glória 2006; Appezzato-da-Glória *et al*. 2008*b*).

Because internal secretory spaces, which are common in Asteraceae, can be used as a tool in taxonomic studies, a more detailed analysis of such structures, as well as correct and clear terminology are needed. The presence of intermediary structures between well defined types (Meira 1991), e.g. between duct and cavity (Cutter 1978; Fahn 1979; Metcalfe 1983), can lead to dubious classification. For example, Curtis and Lersten (1990) pointed out these difficulties concerning terminology while studying rhizome of *Solidago canadensis* L. They observed

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that the so-called canals found in this species were actually cavities very close to each other whose septum broke during the process of tuberisation. Therefore, they proposed the term 'oil reservoir', which was also later adopted by Lotocka and Geszprych (rhizome of *Rhaponticum carthamoides* (Willd.) Iljin; 2004).

The present study aims to analyse the occurrence, formation and the chemical nature of the internal secretory spaces of six Asteraceae species belonging to the following tribes: Eupatorieae (*Mikania cordifolia* L.f. Willd. and *M. sessilifolia* DC), Mutisiae (*Trixis nobilis* (Vell.) Katinas), Plucheeae (*Pterocaulon alopecuroides* (Lam.) DC) and Vernonieae (*Vernonia elegans* Gardner and *V. megapotamica* Spreng.).

Materials and methods

Mikania cordifolia, *M. sessilifolia*, *Trixis nobilis*, *Pterocaulon alopecuroides*, *Vernonia elegans* and *V. megapotamica* were selected from the list of Almeida *et al*. (2005). The aim was to compare the location and the formation process of secretory structures in species of the same genera (in the case of *Mikania* and *Vernonia*) and among genera belonging to distant tribes (Eupatorieae, Mutisiae, Plucheeae and Vernonieae). Adult individuals were collected in natural populations in areas of Cerrado located in Botucatu (22°53′S, 8°29'W) and Itirapina (22°13′S, 47°54'W), São Paulo State, Brazil, where Asteraceae is well represented. The vouchers of the specimens (88 792, 88 791, 92 159, 88 790, 88 787 and 88 789, respectively) are deposited in the ESA Herbarium, Brazil.

For anatomical analyses, whole underground systems of three adult plants from each species were fixed in formaldehyde : glacial acetic acid : 50% ethanol (1 : 1 : 18 , v/v, FAA 50) for 48 h (Johansen 1940) and dehydrated in a series of graded ethanol (50–100%) and embedded in plastic resin (Leica Historesin: Leica, Wetzler, Germany). Cross- and longitudinal serial sections, 5–10 µm thick, were cut with a rotary microtome, then mounted on glass slides and stained with toluidine blue (Sakai 1973). To determine the chemical nature of the substances found in the secretory structures, hand-cut sections of fixed material were submitted to the following histochemical tests: Sudan IV (Jensen 1962) for lipids, Nadi reagent (David and Carde 1964) for terpenoids, ruthenium red (Johansen 1940) for mucilage and ferric chloride (Johansen 1940) for phenolic compounds. The images were digitally captured with a Leica DMLB microscope by using a video camera plugged to a computer utilising the IM50 software for image analysis.

Results

All species studied showed thickened underground organs, a xylopodium (for definition see Alonso and Machado 2007 and Appezzato-da-Glória *et al*. 2008*a*) and a stem tuber (Table 1). The xylopodium of the stem structure and the stem tuber emitted adventitious roots whereas the xylopodium of the radicular structure emitted lateral roots. The presence and location of secretory structures in these organs are shown in Table 1.

The secretion had a natural yellowish-orange colour, which can be seen on the slides stained with toluidine blue (Figs 1*A*, *C*, *D*, *G*, *H*, 2*B, D–H*). The secretion stained positively only with Sudan IV (Fig. 3*A–C*, *E*, *G*), with the reddish colour indicating the presence of lipids. There was no reaction to the other stains and reagents used.

The xylopodium of *M. cordifolia*, although showing a secondary structure, had retained the cortex from the primary growth. These organs had cavities and canals. The cavities (150 µm diameter, 452 µm length) were observed in the cortex near the phloem (Fig. 1*A*) and their epithelium comprised cells of the endodermis with Casparian strips and cells of the cortical parenchyma opposite the endodermis (Fig. 1*B*). Figure 1*C* shows that this cavity originates from a schizogenesis process followed by cell lysis, with the dissolution and later division of epithelial cells to increase the lumen. The formation of a canal (100 µm diameter, 1.7 mm length) can be observed in the secondary xylem. It is originated by the separation of parenchyma cells of the vascular ray, forming its epithelium, which leads to an increase of the lumen after cell divisions (Fig. 1*D*). On the adventitious roots of the xylopodium, cavities (350 µm diameter, 1.3 mm length) occur on the cortex opposite the phloem. In cross-section, these cavities in the primary root structure show a diamond-shaped lumen circumscribed by four epithelial cells. Two of the epithelial cells are endodermic with visible Casparian strips and two are from the adjacent cortical parenchyma, which, as seen in a longitudinal section, suffers separation in different places, originating small intercellular spaces (Fig. 1*E*) in the secondary root structure. The union of these spaces, by complete cellular separation, gives rise to elongated structures (Fig. 1*F*). After the division and lysis of epithelial cells (Fig. 1*G*), the lumen of these cavities becomes roundish and bigger, as seen in the cross-section (Fig. 1*H*).

In the xylopodium of *M. sessilifolia* there are cortical cavities (100 µm diameter, 434 µm length), originating through the separation of cells from the endodermis with visible Casparian strips, and cells from the cortical layer opposite the endodermis.

Table 1. Occurrence and types of secretory structures in the underground systems of six Asteraceae species

Species	Occurrence and the type of the secretory structure	
Mikania cordifolia	Xylopodium (cortex and secondary xylem): cavities	Roots (cortex): cavities
Mikania sessilifolia	Xylopodium (cortex and secondary xylem): cavities	Roots (cortex): cavities
Trixis nobilis	Underground stem (cortex and secondary phloem): cavities	Roots: canals in the cortex and cavities in the secondary phloem
Pterocaulon alopecuroides	Xylopodium (cortex): cavities	Roots (cortex): canals and cavities
Vernonia elegans	Xylopodium: secretory endodermis	Roots (cortex): canals and secretory endodermis
Vernonia megapotamica	Xylopodium (cortex): cavities and secretory endodermis	Roots (cortex): idioblasts, canals and secretory endodermis

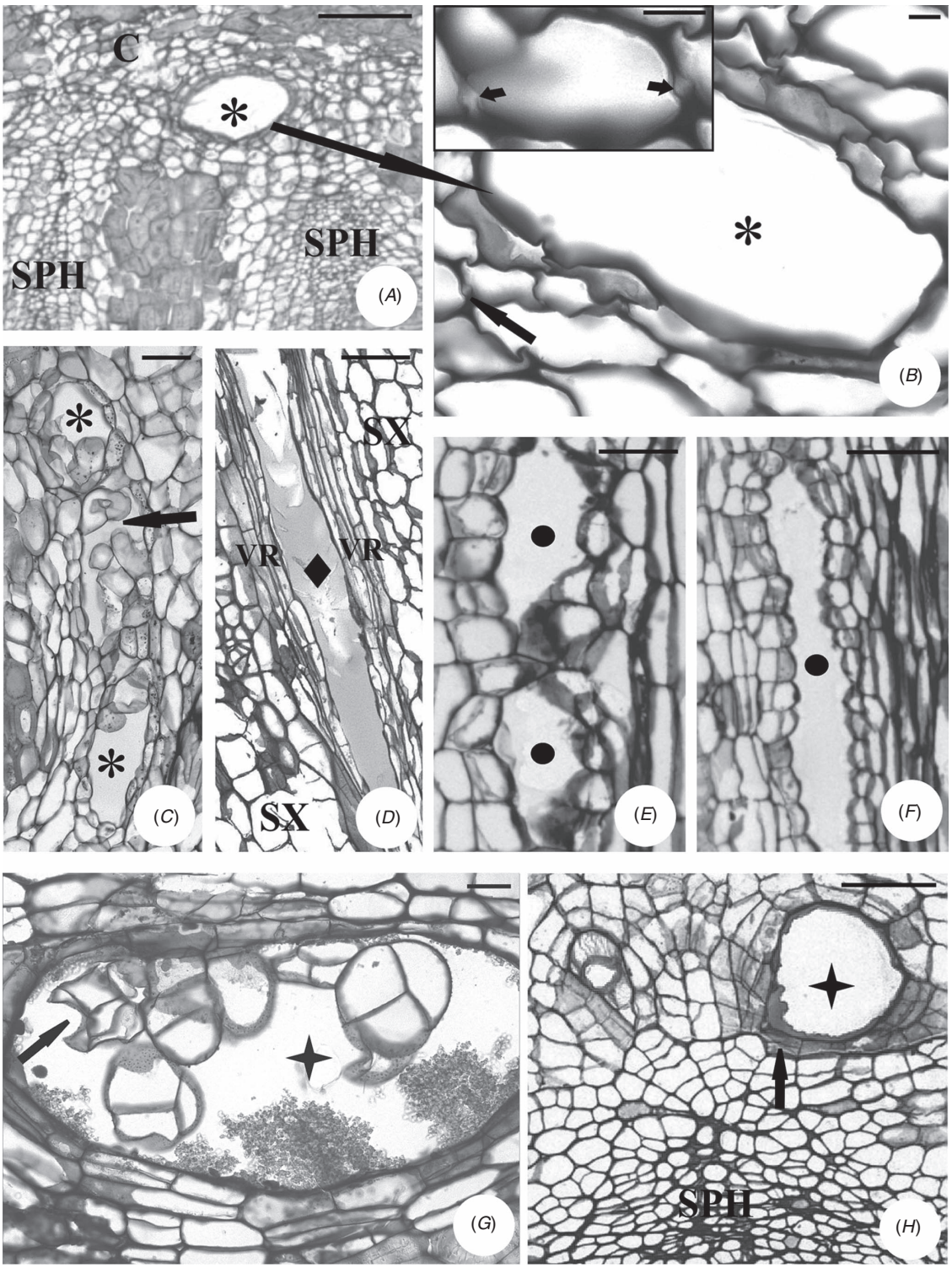

Fig. 1. *Mikania cordifolia*. (*A*, *B*, *D*) Cross-sections of the xylopodium. (*A*, *B*) Cortical cavity (✻). (*B*) Detail of cortical cavity, showing the Casparian strips on the endodermic cells (arrow). Inset shows a detail of an endodermic cell with Casparian strips (arrows). (*C*) Longitudinal section of the xylopodium, showing cortical cavity (✻) under cellular lysis (arrow). (*D*) Secondary xylem canal (◆). (*E*, *F*) Longitudinal section of an adventitious root, showing the formation of cortical cavity (●). (*G*, *H*) Cross-sections of adventitious roots, showing cortical cavities (✦); cellular lysis (arrow in *G*) and Casparian strips (arrow in *H*) are shown. C = cortex; SPH = secondary phloem; SX = secondary xylem; VR = vascular ray. Scale bars = 200 µm (*A*), 10 µm (*B*), 50 µm (*C*, *E*), 100 µm (*D*, *F*) and 30 µm (*G*, *H*).

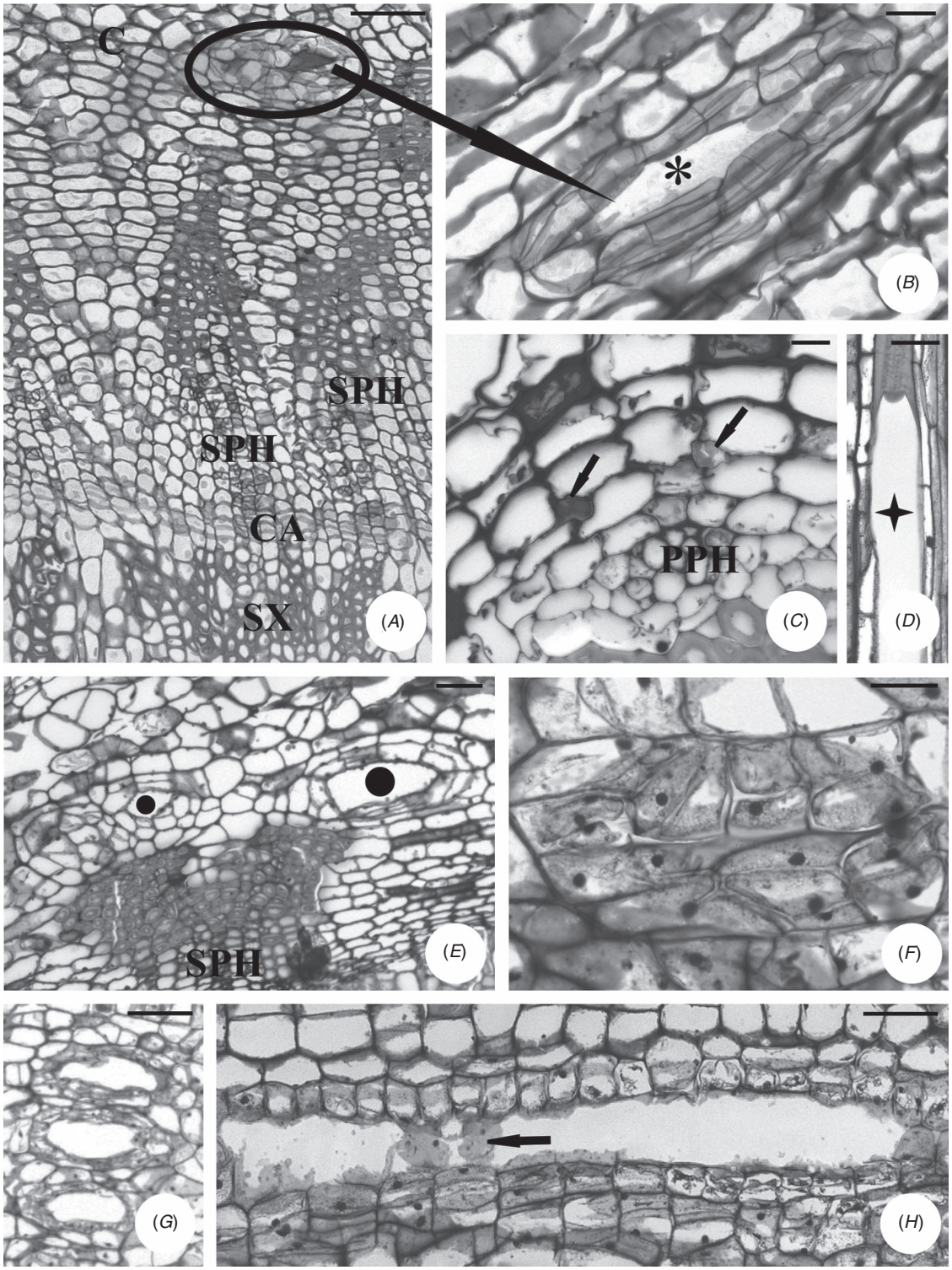

Fig. 2. *Pterocaulon alopecuroides*. (*A*) Cross-section of the xylopodium, showing cortical cavities. (*B*) Longitudinal section of the xylopodium, showing cortical cavities (✻). (*C*) Cross-section of the lateral root in primary growth, with cortical canals (arrows). (*D*) Longitudinal section of the lateral root in primary growth, showing the cortical canal (✦). (*E*, *F*) Cross-section of lateral roots in secondary growth, with cortical cavities (●). (*F*) The beginning of the cavity formation by separation of the cortical cells. (*G*, *H*) Longitudinal sections of lateral root. (*G*) Close cavities and (*H*) cavity formed by septum rupture. The arrow indicates cellular lysis. C = cortex; CA = cambium; PPH = primary phloem; SPH = secondary phloem; SX = secondary xylem. Scale bars = 100 µm (*A*, *G*), 30 µm (*B*, *D*, *F*), 10 µm (*C*) and 50 µm (*E*, *H*).

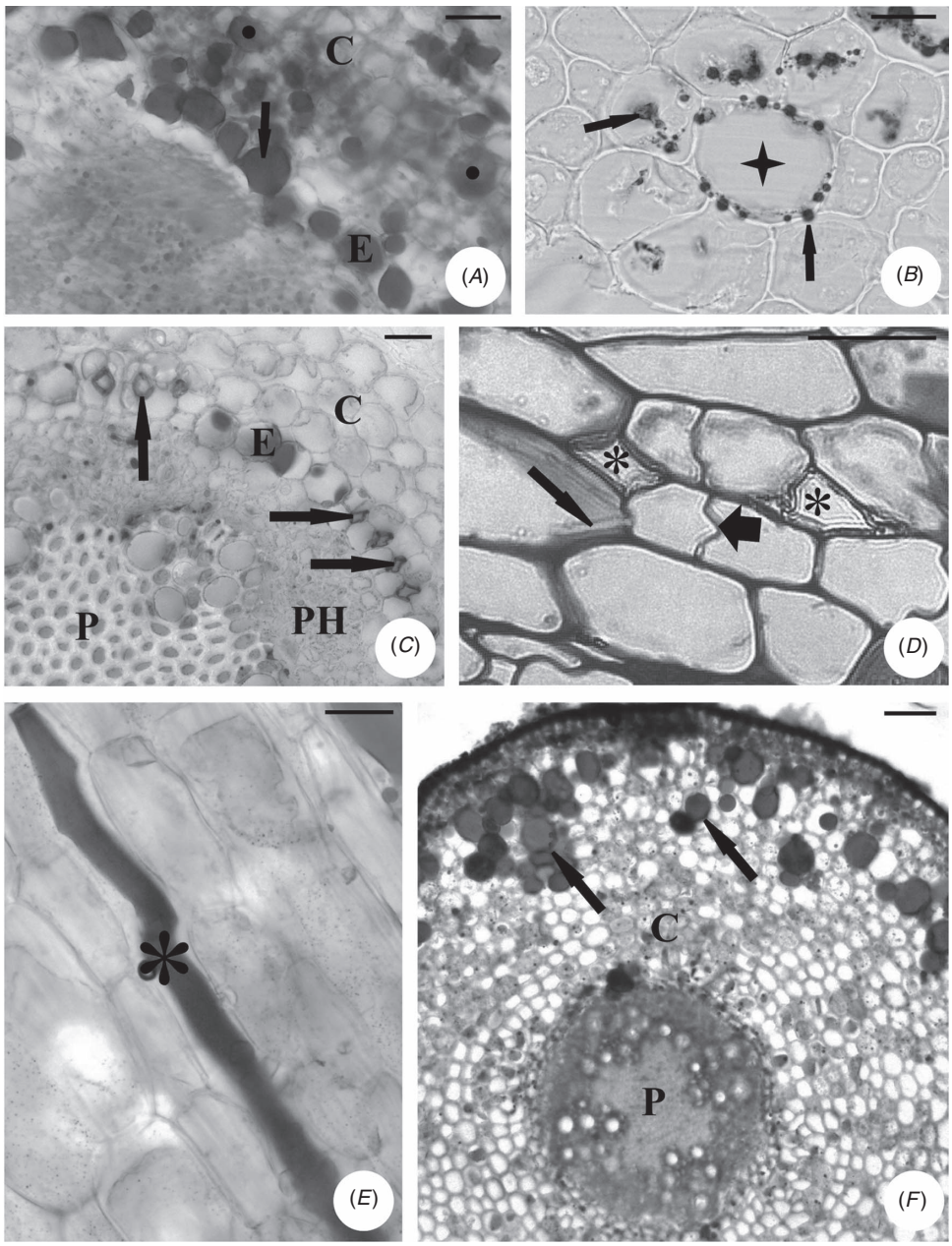

Fig. 3. Xylopodium of *Vernonia megapotamica*. (*A*) Cross-section of cortical (●) and endodermic cells (arrow) with lipid substances. (*B*) Longitudinal section, showing a cortical cavity (✦) and lipid drops in the epithelium (arrows). (*C*, *D*) Cross-sections of *V. elegans* adventitious roots. (*C*) Cortical canals (arrows) and endodermis with lipid substances secretion. (*D*) The cortical canals (✻) and the Casparian strips in endodermic cells (thin arrow), and an endodermic cell in division (wide arrow). (*E*, *G*) Adventitious roots of *V. megapotamica*. (*E*) Longitudinal section, showing the cortical canal (✻) and (*G*) cross-section with secretory idioblasts of lipid substance in the outer cortex (arrows). C = cortex; E = endodermis; P = pith. Scale bars = 30 µm (*A*–*E*) and 100 µm (*G*).

Such cells form the epithelium after separation. The growth of the lumen is followed by divisions in the epithelial cells (Fig. 4*A*). It is possible to observe canals (200 µm diameter, 2.5 mm length) in the vascular ray (Fig. 4*B*) in the secondary structure of the xylopodium of *M. sessilifolia*. Such canals result from the separation of vascular parenchyma cells along its extension (Fig. 4*C*–*F*), thus forming an elongated structure. The division of epithelial cells increases the lumen (Fig. 4*B*). The adventitious roots of this xylopodium, in the primary structure, show cortical cavities (100 µm diameter, 1 mm length) opposite the phloem, with a roundish lumen and an epithelium formed by a layer of cells, namely two cells from the endodermis and two from the cortical parenchyma opposite the endodermis. In the secondary structure, the epithelium shows cellular division, followed by an increase in the lumen. The presence of these cavities, as seen in the longitudinal plane,

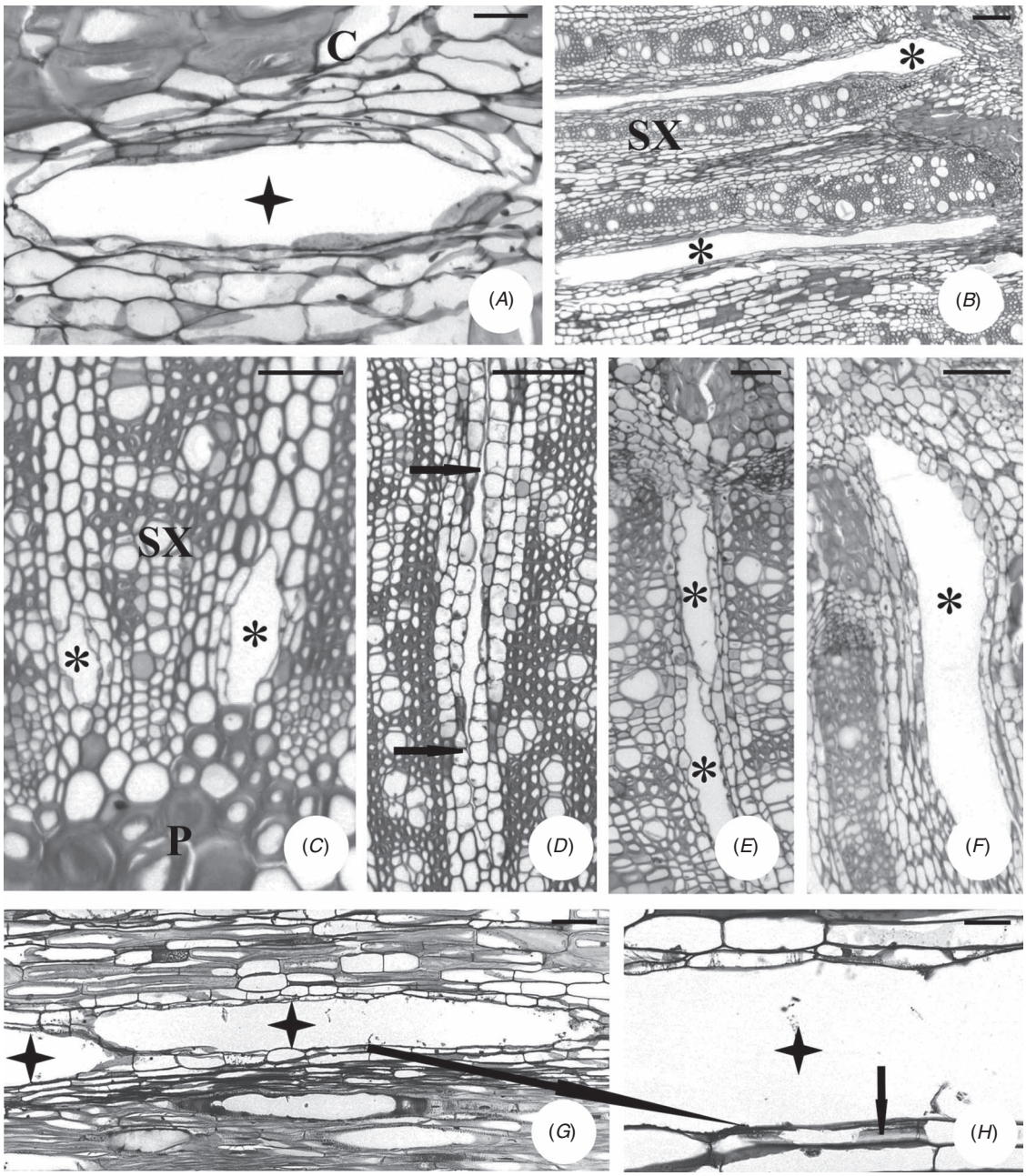

Fig. 4. *Mikania sessilifolia*. (*A–F*) Cross-sections of the xylopodium. (*A*) Cortical cavity (✦). (*B*) Secondary xylem canal (✻). (*C–F*) Formation of the secondary xylem canal (✻) by cellular separation of the vascular ray (*C*) near the pith, (*D*) average portion of secondary xylem (arrows), (*E*) near the vascular cambium and (*F*) on the cortex. (*G*, *H*) Longitudinal sections of adventitious roots, showing cortical cavities (✦). (*H*) Detail of an endodermic cell with Casparian strips (arrow). C = cortex; P = pith; SX = secondary xylem. Scale bars = 50 µm (*A*), 200 µm (*B*), 100 µm (*C–G*) and 30 µm (*H*).

results in a sequential separation of the endodermic cells with Casparian strips and the cortical parenchyma cells opposite the endodermis. In a more advanced stage of development, as seen in the longitudinal section, the cavities join in lines without anastomosis between then, maintaining their length (Fig. 4*G*, *H*).

The underground stem of *T. nobilis*, although showing a secondary structure, retained the structure from primary growth. This stem has cavities (62 µm diameter, 400 µm length) in the internal cortex, opposite the phloem (Fig. 5*A*), originating by separation of common cortical cells, which then form the epithelium. The epithelium later suffers cellular divisions to increase the lumen. There are also cavities (230 µm diameter, 600 µm length) present in the phloematic ray (Fig. 5*A*), which, as seen in the cross-section, are originated by a separation and later lysis of cells, with a septum rupture between the spaces, leading to lumen expansion (Fig. 5*B–D*). In the longitudinal plane, cavities can be seen as little elongated spaces with a determined lumen. In the

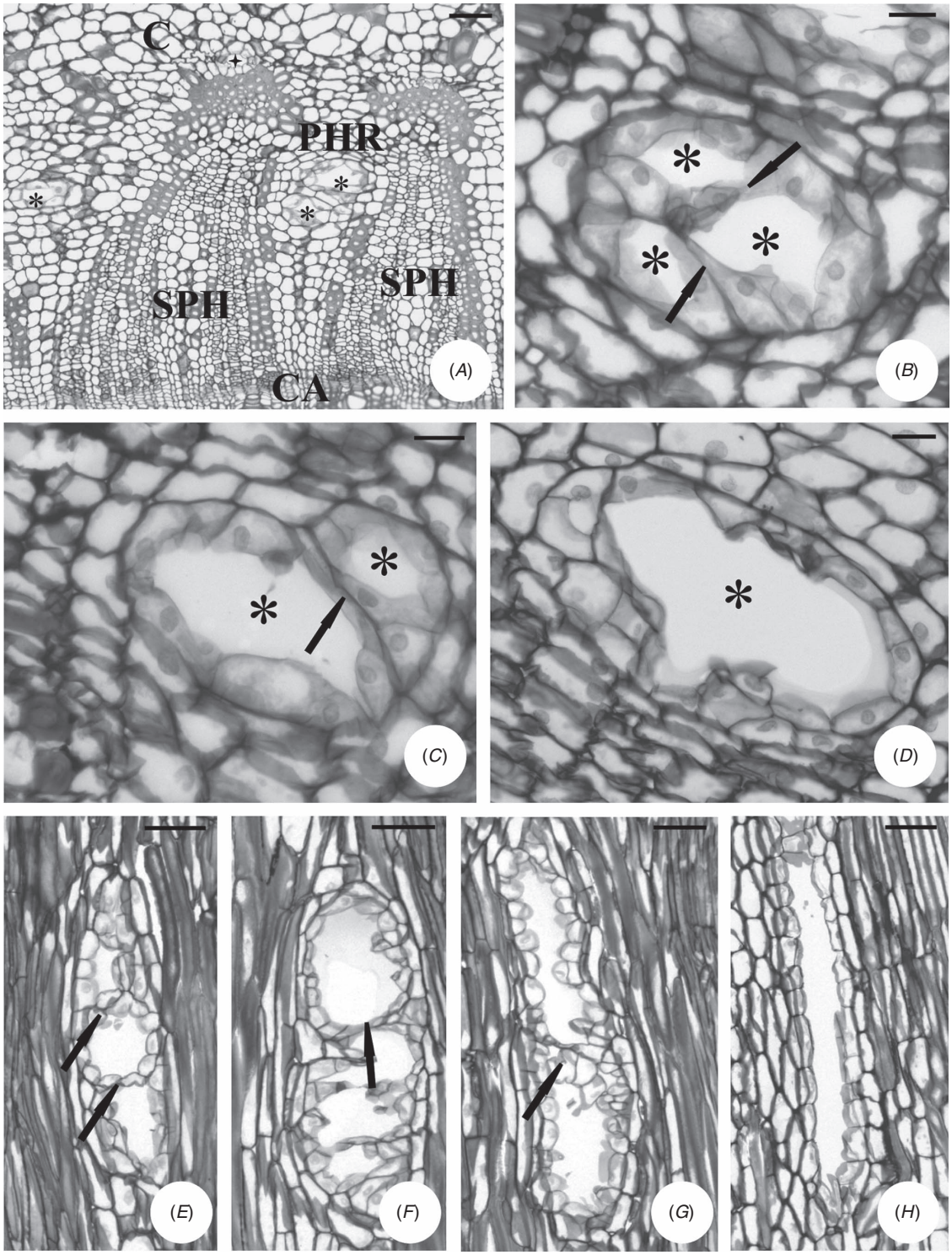

Fig. 5. *Trixis nobilis*. (*A–D*) Cross-sections of the thickened underground stem, showing cortical cavities (✦) and the cavities in the phloem ray (✻). (*B–D*) Formation of the cavities in the phloem ray (✻) by septum rupture (arrows). (*E–H*) Longitudinal sections of the adventitious roots, showing the formation of the cavities in the phloematic ray by septum rupture (arrows). C = cortex; CA = cambium; PHR = phloem ray; SPH = secondary phloem. Scale bars = 100 µm (*A*, *E–H*) and 30 µm (*B–D*).

adventitious roots of *T. nobilis*, in the primary structure, there are cortical canals (50 µm diameter, 625 µm length) opposite the phloem. These canals show a diamond-shaped lumen, resulting from the separation of cells of the endodermis and the cortical cells of the parenchyma, opposite the endodermis. These cells form the epithelium. In the secondary structure, the epithelial

cells divide and the lumen canal becomes bigger and roundish. In the phloematic ray, small cavities are formed as a result of the separation of cells from the parenchyma. In the longitudinal section, it can be observed that the isolated cavities after septum rupture result in the formation of elongated structures (70 µm diameter, 700 µm length) (Fig. 5*E–H*).

The xylopodium of *P. alopecuroides* has cortical cavities (30 µm diameter, 220 µm length) near the phloem (Fig. 2*A*), originating through separation of cortex cells, which then form the epithelium. Cellular divisions of the epithelium results in the increase of the lumen (Fig. 2*B*). The lateral roots of the xylopodium, in the primary structure, have cortical canals (86 µm diameter, 1 mm length) with origin and location similar to those described for *T. nobilis* (Fig. 2*C*, *D*). However, their shape is irregular and the epithelium does not divide. In the secondary structure, canals are not visible. Nevertheless, the separation of the cortical cells of the parenchyma (Fig. 2*F*) leads to the formation of new secretory cavities (Fig. 2*E*). In the longitudinal section, we observed that these small cavities are very close to each other (Fig. 2*G*). With the development of the organ, cells between the cavities, which form septa separation, suffer lysis, resulting in the formation of elongated structures (50 µm diameter, 450 µm length) whose epithelium divides itself following the growth of the lumen (Fig. 2*H*).

The xylopodium of *V. elegans* and *V. megapotamica* has a secretory endodermis of lipid substances. Cells from the cortical parenchyma in *V. megapotamica* show lipid content (Fig. 3*A*). In *V. megapotamica*, cortical cavities (30 µm diameter, 50 µm length) can be observed, with the lumen being formed by the separation of cortical cells. These cells constitute the epithelium of the cavity (Fig. 3*B*). The adventitious roots of these two species have a lipid substance-secreting endodermis (Fig. 3*C*) and, in the primary growth, cortical canals are observed (15–50 µm diameter, 900 µm length). Their origin, location and shape are similar to those described for *T. nobilis* (Fig. 3*C–E*). However, cellular divisions in the epithelium were not observed. . In *V. elegans*, an anticlinal division of a single cell in endodermis with visible Casparian strips, results in two cells which are part of the epithelium of two adjacent canals (Fig. 3*D*). In the outer cortex of adventitious roots *V. megapotamica*, idioblasts containing lipid substances can be observed (Fig. 3*G*).

Discussion

Col (1903) determined the difference between canals and cavities for the first time, with cavities being shorter and wider than canals. In the present study, when analysing the thickened underground systems and their adventitious and laterals roots, canals and cavities of different shapes, origins and location inside the organs were observed.

These structures originated from cellular separation only (schizogenous), cellular dissolution (lysigenous), or had a mixed origin, i.e. first cellular separation, followed by lysis of epithelial cells, leading to the increase of lumen in these spaces (Evert 2006). In the present study, cavities formed during a schizolysigen process were observed on the xylopodium as well as on the adventitious roots of *M. cordifolia*, on the lateral roots of *P. alopecuroides* (cortical cavities) and on the underground stem and adventitious roots of *T. nobilis* (cavities in the phloematic ray). All other secretory structures originated from the schizogenous process alone.

The presence of cortical canals was verified in the adventitious roots of *T. nobilis*, *V. elegans* and *V. megapotamica* and on the lateral roots of *P. alopecuroides*. They originated from a schizogenous process and are located opposite the primary phloem poles in which the endodermis participated on the formation of cortical canals. These are types of secretory structures, which are quite common in Asteraceae roots (Tetley 1925; Williams 1947, 1954; Metcalfe and Chalk 1950; Lersten and Curtis 1986; Luque *et al*. 1997; Luque 2001; Melo-de-Pinna and Menezes 2002, 2003; Lotocka and Geszprych 2004; Appezzato-da-Glória *et al*. 2008*b*). The cortical canals are elongated longitudinally and therefore different from the cavities observed in the study species.

It is important note the occurrence of canals in the roots of *T. nobilis*, according to Melo-de-Pinna and Menezes (2003), because the only references to the presence of canals in the Mutisieae tribe have been for the genera *Ianthopappus* (Melo-de-Pinna and Menezes 2002) and *Richterago* (Melo-de-Pinna and Menezes 2003). The occurrence of canals in *T. nobilis* not only complements the information about the tribe, but also verifies the occurrence of the same structure as in the roots of Mutisieae.

In the adventitious roots of *M. cordifolia* and *M. sessilifolia*, cortical cavities were elongated structures, visibly shorter than the canals, and not constituting elongated structures in the whole extension of the root. Lersten and Curtis (1986) observed these kinds of cavities in *Eupatorium rugosum* Houtt root, also belonging to the Eupatorieae tribe, and called them structures ‘tubular cavities’. These authors noticed that, in their case, all the tubular cavities were schizogenous as in the *Mikania* species studied here.

The occurrence of canals and cavities was also observed in the adventitious roots of *T. nobilis* (Mutisieae tribe). The cavities originated by a process similar to that described by Curtis and Lersten (1990) for the rhizome of *S. canadensis* (Astereae tribe). Curtis and Lersten (1990) called these structures ‘oil reservoir’ and verified that at the beginning of the formation of these reservoirs, schizogeny occurred, as described in the present study for *T. nobilis*.

The occurrence of secretory structures in thickened underground organs could be verified for five of the study species. All of them showed cortical cavities originating from cellular separation and, with the exception of *V. megapotamica*, multiplication of epithelial cells for lumen growth was observed. Multiplication of epithelial cells followed by cellular lysis was verified only for *M. cordifolia*. In *T. nobilis*, in addition to the cortical cavities, the underground stem also had cavities in the secondary phloem due to the fusion of the smaller cavities. This fusion may be occurring, so that these structures can follow the growth of phloematic ray, as observed by Luque-Arias (2004) in *Coespeletia* roots. For *M. cordifolia* and *M. sessilifolia*, the presence of canals in the secondary xylem, originated by cell separation in the vascular ray after multiplication of epithelial cells, as well as cortical cavities, was reported. The presence of secretory canals in the secondary xylem

of the xylopodium in Asteraceae has been reported only for *Isostigma megapotamicum* (Speng) Sherff (tribe Heliantheae) by Vilhalva and Appezzato-da-Glória (2006). However, in their study the canal was originated from cells derived from the vascular cambium.

Few studies of secretory structures in thickened underground organs of the Asteraceae refer to the occurrence of cortical secretory canals, e.g. in rhizomes of *Calea pinnatifida* Banks (tribe Heliantheae) (Hoehne *et al*. 1952), *Solidago microglossa* DC. (tribe Anthemideae) (Panizza and Grotta 1965) and *Eupatorium inulaefolium* H.B.K. (tribe Eupatorieae) (Ragonese 1988), rhizophores of *Smallanthus sonchifolius* (Poepp & Endl.) H.Robinson (tribe Heliantheae) (Machado *et al*. 2004) and the xylopodium of *Pterocaulon angustifolium* DC. (tribe Plucheeae) (Appezzato-da-Glória *et al*. 2008*b*). The presence of cortical secretory reservoirs was reported for the rhizomes of *Solidago canadensis* (Curtis and Lersten 1990) and *R. carthamoides* (Lotocka and Geszprych 2004) and secretory canals in the secondary phloem were reported for the xylopodium of *Calea verticillata* (Klatt) Pruski (tribe Heliantheae) (Vilhalva and Appezzato-da-Glória 2006). However, only in the case of *E. inulaefolium* and *R. carthamoides*, was the origin of these structures noted, being a result of separation and multiplication of the epithelium cells, as occurred for five of the species studied here.

Within the Vernonieae, Vilhalva (2004) suggested that the general lack of secretory canals on the leaves may be transferred to underground systems, giving this observation a taxonomic connotation. Actually, the species of the tribe presented herein, also lack canals on the xylopodium, a fact that may contribute to their identification. In the present study, it was observed that underground organs in the species of Vernonieae had common characteristics, which can be used as a common trait for this tribe. The xylopodium of *V. elegans* and *V. megapotamica* have a lipidic secretory endodermis. This characteristic has already been described for rhizophores of *Vernonia herbacea* (Vell.) Rusby and *V. platensis* (Spreng.) Less. (Hayashi and Appezzato-da-Glória 2005) and for tuberous roots of *V. brevifolia* Less. and *V. grandiflora* Less. (Hayashi and Appezzato-da-Glória 2007). Lipids were observed in the cortical parenchyma of the xylopodium of *V. megapotamica*, as has also been observed for the rhizophores of *V. herbacea*, *V. plantensis* (Hayashi and Appezzato-da-Glória 2005) and tuberous roots of *V. brevifolia* (Hayashi and Appezzato-da-Glória 2007). Secretory idioblasts in the radicular cortex of *V. megapotamica* have also been reported for *Chresta sphaerocephala* DC. by Appezzato-da-Glória *et al*. (2008*b*).

Among the study species, *V. elegans* and *V. megapotamica*, which belong to the Vernonieae, had secretory cells not only in the canals and cavities but also in the endodermis, confirming the observations made by our research team in other studies on Asteraceae (Hayashi and Appezzato-da-Glória 2005, 2007; Appezzato-da-Glória *et al*. 2008*b*). Tetley (1925) and Williams (1954) suggested that the secretion in the endodermis is related with that the secretory structures, considering that the canals complement the phloem in the transportation of organic matter and the endodermis constitutes a passage for phloem substances to the canal.

Although there is no literature explaining the functional differences between canals and cavities, Lersten and Curtis (1988) justified the use of the term reservoir because this structure has preferably a storage function rather than a transporting function. Moreover, in *P. alopecuroides* (Plucheeae), the formation of cavities on the lateral roots similar to the oil reservoir described by Lersten and Curtis (1988) was verified. It is interesting that these cavities seem to merge into the cortical canals formed during the primary growth of the root, because they are not found in the secondary growth. Lotocka and Geszprych (2004) affirmed that, in underground organs, some reservoirs stop working and disappear with time, possibly being obliterated, which could explain why the cortical canals of the primary structure were not observed in the secondary growth of the lateral roots of *P. alopecuroides*.

The location of the cavities and canals opposite or near the root phloem and in the thickened underground organs of the species analysed in the present study, as well as the lipid nature of the secretions, are probably related to defence processes against herbivores as discussed by Appezzato-da-Glória *et al*. (2008*b*). In roots of *Santolina leucantha* Bertol. (Asteraceae, Anthemideae), the intense secretory activity of secondary products into the canals might have an important environmental role in preventing herbivores (Pagni and Masini 1999). Lersten and Curtis (1989) suggested that in leaves of *S. canadensis*, the location of the secretory reservoirs near the larger veins may provide protection to the phloem against herbivory. Franceschi *et al*. (2005) reported the occurrence of reservoirs of chemical substances (phenols, terpenoids and alkaloids) distributed along various tissues of the bark, and defined them as constitutive chemical defences in conifers, protecting the phloem nutrients against the use by organisms such as herbivores. Williams (1954) pointed out the importance of secretory canals near the phloem, with the function in transportation of photo-assimilates.

It is important to emphasise that there is a lack of literature on the formation and occurrence of secretory structures in thickened underground organs in other families of vascular plants, with the exception of laticiferous structures, as e.g. in rhizome of *Cyclanthus bipartitus* Poit. (Cyclanthaceae) (Wilder and Harris 1982) and in tuberous roots of *Mandevilla illustris* (Vell.) Woodson and *M. velutina* (Mart. ex Stadelm.) (Apocynaceae) (Appezzato-da-Glória and Estelita 1997). Laticiferous structures also play a role in the protection against herbivory (Fahn 2002).

Neither the function of these structures, nor the nature of secreted compounds are well defined, including the gum-secreting canals of the secondary xylem of the tubers of *Vochysia thyrsoidea* Pohl (Vochysiaceae) (Jeronymo and Paviani 1992) and secretory cavities of lysigenous origin in bulbs of *Oxalis latifolia* H.B.K. (Oxalidaceae) (Estelita-Teixeira 1982). This is probably due to the fact that the Asteraceae constitutes the largest group of angiosperms (Bremer 1994) and within their different growth forms, many species show thickened underground organs (Tertuliano and Figueiredo-Ribeiro 1993). Therefore, more information on secretory structures is of crucial importance. These structures may have great economical potential (Cutter 1978), being

possible new sources of chemical compounds important for medicinal purposes (Wagner 1977).

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