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# Internal secretory structures and histochemistry in leaves of *Baccharis notosergila* Griseb. (Asteraceae, Astereae)

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**ABSTRACT**
*Baccharis notosergila* Griseb. (Asteraceae, Astereae) is an aggressive weed of natural grasslands but it also has medicinal properties. The aim of this investigation was to increase the knowledge of internal secretory structure types and identify the main substances accumulated in them. Plants were collected in Buenos Aires province, Argentina. Vouchers were deposited in LPAG herbarium. Leaves were conserved in alcohol 70°. Leaves cross sections were obtained, others were made diaphanous, and all were stained with dyes and with reagents for histochemical analysis. False ducts (formed by cavities) and cavities themselves were found. The cavity itself is a short space, lined by short epithelial cells. False duct is a long and extended space lined by very long epithelial cells, developed from a row of elongated cavities during leaf growth. False ducts run on abaxial side along the major veins (1st to 3rd orders). Cavities are isolated or two-three clustered and located on lateral-abaxial side from 1st to 5th order veins, at the end of veinlets (6th order) and in mesophyll. Both structures are enclosed by parenchyma sheath. The chemical compounds (resins, phenolic and lipophilic compounds) accumulated in cavities and false ducts can have multiple applications, either for their medicinal properties or might be used for the biocontrol of pathogens to improve sustainable agricultural systems.

**KEY WORDS**
Asteraceae,
Astereae,
cavities,
chemical substances,
false ducts.

**RÉSUMÉ**

*Structures sécrétrices internes et histochimie dans les feuilles de* Baccharis notosergila *Griseb. (Asteraceae, Astereae).*

*Baccharis notosergila* Griseb. (Asteraceae, Astereae) est une mauvaise herbe agressive des prairies naturelles, mais possède également des propriétés médicinales. L'objectif de cette recherche était d'améliorer les connaissances sur les types de structures sécrétoires internes et d'identifier les principales substances qui s'y accumulent. Les plantes ont été collectées dans la province de Buenos Aires, en Argentine. Les spécimens ont été déposés dans l'herbier du LPAG. Les feuilles ont été conservées dans de l'alcool à 70°. Les sections transversales obtenues ainsi que d'autres feuilles ont été éclaircies. Les préparations ont été colorées avec des colorants et des réactifs pour l'analyse histochimique. De faux conduits (formés par des cavités) et des cavités proprement dites ont été trouvés. La cavité proprement dite est un espace court, tapissé de cellules épithéliales. Le faux canal est un espace long et étendu, tapissé de longues cellules épithéliales, développé à partir d'une rangée de cavités allongées pendant la croissance de la feuille. Les faux conduits s'étendent sur la face abaxiale le long des nervures principales (du 1er au 3e ordre). Les cavités sont solitaires ou en groupes de deux-trois et situées sur le côté latéral-abaxial des veines de 1er à 5e ordre, à l'extrémité des veinules (6e ordre) et dans le mésophylle. Les deux structures sont entourées d'une gaine de parenchyme. Les composés chimiques (résines, composés phénoliques et lipophiles) contenus dans les cavités et les faux conduits peuvent avoir de multiples applications, soit pour leurs propriétés médicinales, soit pour le contrôle biologique des pathogènes afin d'améliorer les systèmes agricoles durables.

**MOTS CLÉS**
Asteraceae,
Astereae,
cavités,
substances chimiques,
faux conduits.

## INTRODUCTION

The genus *Baccharis* L. belongs to the family Asteraceae, subfamily Asteroideae and tribe Astereae. It has more than 400 species distributed in almost the entire American continent. This paper is about *Baccharis notosergila* Griseb. which is distributed in Argentina, Brasil, Paraguay and Uruguay. It is a perennial woody shrub with high adaptive capacity to different environmental conditions. In Argentina, it is a problematic weed that produces strong competition for water, nutrients and light with the species with forage value in areas with natural grassland, generating a process of degradation and reducing the livestock productivity (Sione *et al.* 2006). Many species of *Baccharis* have also been used in traditional medicine for treatment of diverse pathologies, such us gastrointestinal ailments, ulcers, fever, rheumatism, and infectious diseases. Alvarenga *et al.* (2018) evaluated the immunomodulatory activity of methanolic extracts from *Baccharis notosergila, B. punctulata* DC., and *B. trimera* (Less.) DC. The three species are a potential source of chemical compounds which had immunomodulatory activity on human mononuclear cells. In a comparative study of therapeutic activity of *Baccharis* genus Petroche Torres *et al.* (2022) expressed that some flavonoids would have anti-inflammatory and anti-oxidant activities. *Baccharis* exhibits an interesting secretory tissue. Today many papers referred specifically to secretory structures in Asteraceae, notably in the genus *Baccharis* (e.g., Tetley 1925; Ragonese 1988; Lersten & Curtis 1986, 1987, 1988, 1989; Werker *et al.* 1994; Poli *et al.* 1995; Castro *et al.* 1997; Simon *et al.* 2002; Melo-de-Pina & Menezes 2003; Łotocka & Geszprych 2004; Cury & Appezzato-da-Glória 2009). However, great variability exists among the species of Asteraceae coincidently as reported by Metcalfe & Chalk (1950). The most frequently internal secretory structures mentioned in Asteraceae are ducts and cavities (Castro *et al.* 1997; Budel *et al.* 2012, 2015, 2018; Bobek *et al.* 2015), whose location is different. Milan *et al.* (2006) cited secretory ducts on the xylem side in *Mikania glomerata* Spreng. (Eupatorieae). Plos *et al.* (2011) mentioned the presence of secretory cavities and ducts in the genus *Ophryosporus* Meyen (Eupatorieae) on the xylem and phloem sides. Delbón *et al.* (2012) in *Flourensia* Cambess. (Heliantheae) found ducts on the abaxial and adaxial sides of the vascular bundles, depending of the species. Similar encounter was reported by Hernández & Arambarri (2019) in leaves of *Pascalia glauca* Ortega (Heliantheae). In the leaf of *Baccharis obovata* Hook. et Arn., Molares *et al.* (2009) indicated presence of cavities on abaxial side, whereas Ornellas *et al.* (2019) in leaves of *B. stylosa* Gardner reported ducts on the abaxial and adaxial sides and mesophyll. There are still many doubts in the anatomical interpretation of the secretory structures that exert an important ecological role in plants (Sharifi-Rad *et al.* 2017). Further studies are needed to extend the knowledge of specialized secretory tissue as well as the chemical nature of secreted compounds which are useful for plant and humanity. Results would be useful in taxonomy, for medicine, the mechanism of defense against biotic and abiotic factors that each species possesses, and in management and conservation of species. The aims of this research were: 1) to deepen the knowledge of internal secretory structure types in leaves of *Baccharis notosergila* and 2) to check the main chemical substances secreted and/or accumulated in the secretory structures.

## MATERIAL AND METHODS

Plants of *B. notosergila* having fully developed leaves were collected during the spring of 2018 and 2020 in Vieytes, Magdalena party (Pdo.), Buenos Aires province (Prov.), Argentina. The botanical material was identified and the vouchers were deposited in the Facultad de Agronomía, Universidad Nacional de La Plata (LPAG) herbarium.

For anatomy study, fresh mature leaves from basal, middle and apical stem regions were fixed in FAA (a solution of formaldehyde, glacial acetic acid, and 70% ethyl alcohol, Johansen 1940), then stored in 70% ethanol. To analyze the structures, freehand cross sections (CS) of the petiole and leaf-blade middle part were cut; the selected sections were bleached in (50%) sodium hypochlorite (NaClO), washed thrice with distilled water (DW), then were stained with an alcoholic solution (80%) safranin or (0.05%) Brilliant cresyl blue (Pérez & Tomasi 2002). For the paradermal view (PW), thirty leaves were made transparent using the method of Franklin (1945) modified, for that, they were boiled in ethyl alcohol 96º for 20 min, allowed to cool then washed and bleached in 50% (NaClO) for 2 h, then they were washed twice and submerged in a solution of (10 vol.) hydrogen peroxide + (5%) glacial acetic acid (1: 1 v/v) for 48 h, after that washed twice in DW and bleached again in (50%) NaClO. At the completion of the bleaching process, five washes were carried out to remove the NaClO, and samples were transferred into a solution of (5%) chloral hydrate for a minimum of 48 h. To complete the process, leaves were stained using different dyes and reagents, Safranin, Toluidine blue "O", and Oil red "O". The sections and diaphanous leaves were mounted in gelatin-glycerin on glass slides and sealed with nail polish. Secretory spaces dimensions in CS and PW of 20 samples were taken using ImageJ program (González 2018), the values were expressed in micrometer (µm).

The histochemical analysis was performed on leaves CS and PW. To analyze lipid compounds an alcoholic solution of Oil red "O" (ORO test) was used (Gurr 1971), the orange to red-color indicates positive test and essential oils were seen as droplets. Detection of tannins was performed using Ferric chloride ($FeCl_3$) test (Zarlavsky 2014), a green-blue color is a positive test. Resins were detected using a saturated solution of copper sulphate and heated gently (Cosa *et al.* 2014) a color emerald green indicates positive test. Toluidine blue "O" (TBO test) was used to contrast phenolic compounds a green turquoise color indicates positive test, and lilac-colored carbohydrates (Tapia-Torres *et al.* 2014).

Anatomical structures were examined and microphotographs were taken by using a stereoscopic microscope Bausch & Lomb, stereo zoom 5, and a digital camera, resolution 12MP; light microscopes Nikon E200 LED equipped with Micrometrics SE Premium software and a Lancet XSP-136D optical microscope equipped with digital camera solution disk.

For analyzing the secretory spaces we consider cavity and duct (= canal) according Metcalfe & Chalk (1989) who wrote: "Secretory cavities are intercellular spaces of various sizes and shapes in which chemical substances secreted from the surrounding cells are deposited. Secretory canals differ from cavities in being more elongated, and both are sometimes lined by epithelial cells from which secretion takes place. The space of ducts and cavities are commonly formed by the separation of cells that were previously in contact with each other and when formed in this way they are described as schizogenous". In our understanding the ducts from different plants and families are large intercellular spaces and have continuity with their main function to conduct substances from one place to another, whereas in cavities the intercellular space is shorter than duct and their main function is to store chemical compounds. These were named reservoirs by Lersten & Curtis (1989).

## RESULTS

Family Asteraceae Dumort.
Subfamily Asteroideae Lindl.
Tribe *Astereae* Cass.

*Baccharis* L.

*Baccharis notosergila* Griseb.

*Abhandlungen der Königlichen Gesellschaft der Wissenschaften zu Göttingen* 24: 183 (1879).

Examined material. — **Argentina** • Prov. Buenos Aires, Pdo, Magdalena, Vieytes; 35°15'S, 57°37'W; 17.XII.2018; *F. Fernández & A. Carbone 1, 2, 3, 4*; LPAG • same location; 11.XI.2020; *F. Fernández & A. Carbone 6*, *7*, *8*; LPAG (in addition, specimens from Facultad de Ciencias Naturales y Museo Universidad Nacional de La Plata (LP) and Instituto de Botánica Darwinion (SI) were consulted) • Pdo, La Plata, Rufino de Elizalde; 22.III.1931; *Cabrera, A.L. 1683, 1684*; LP • same location; 05.IV.1932; *Cabrera, A.L. 2127*; LP • Pdo, Tigre, Las Conchas; 17.III.1927; *Molfino & Clos s/n*; LP • Pdo de la Costa, Punta Médanos; 22.XII.1981; *Troels Myndel Pedersen 13.194*; LP • Pdo, Ramallo, Reserva Municipal de Ramallo; 22.II.2002; *Torres Robles S. & Trevisan V. 806*; LP • Pdo, Berazategui, Hudson; 16.IV.1927; *Burkart 1302*; SI (image 15).

### Description

*Veins and vascular bundles in the leaf petiole and blade cross sections*

The petiole cross section shows three principal veins and two-four traces. Each vein of the petiole has one collateral vascular bundle with phloem and xylem and fiber caps on both sides surrounded by the parenchyma sheath. The primary and secondaries veins have one-three secretory structures situated next to and on the phloem side and enclosed by the parenchyma sheath (Fig. 1A, B). The leaf blade cross section of a young leaf (Fig. 1C), shows the primary vein formed by a collateral vascular bundle with fiber cap located only on the xylem side, and on the phloem side may be seen one to three secretory structures, all enclosed in the parenchyma sheath which has extensions to the adaxial and abaxial epidermis. In secondary veins the vascular bundle frequently shows scarce parenchyma sheath extensions to one epidermis and may has also one-three abaxial secretory structures, whereas in veins of 3rd order,

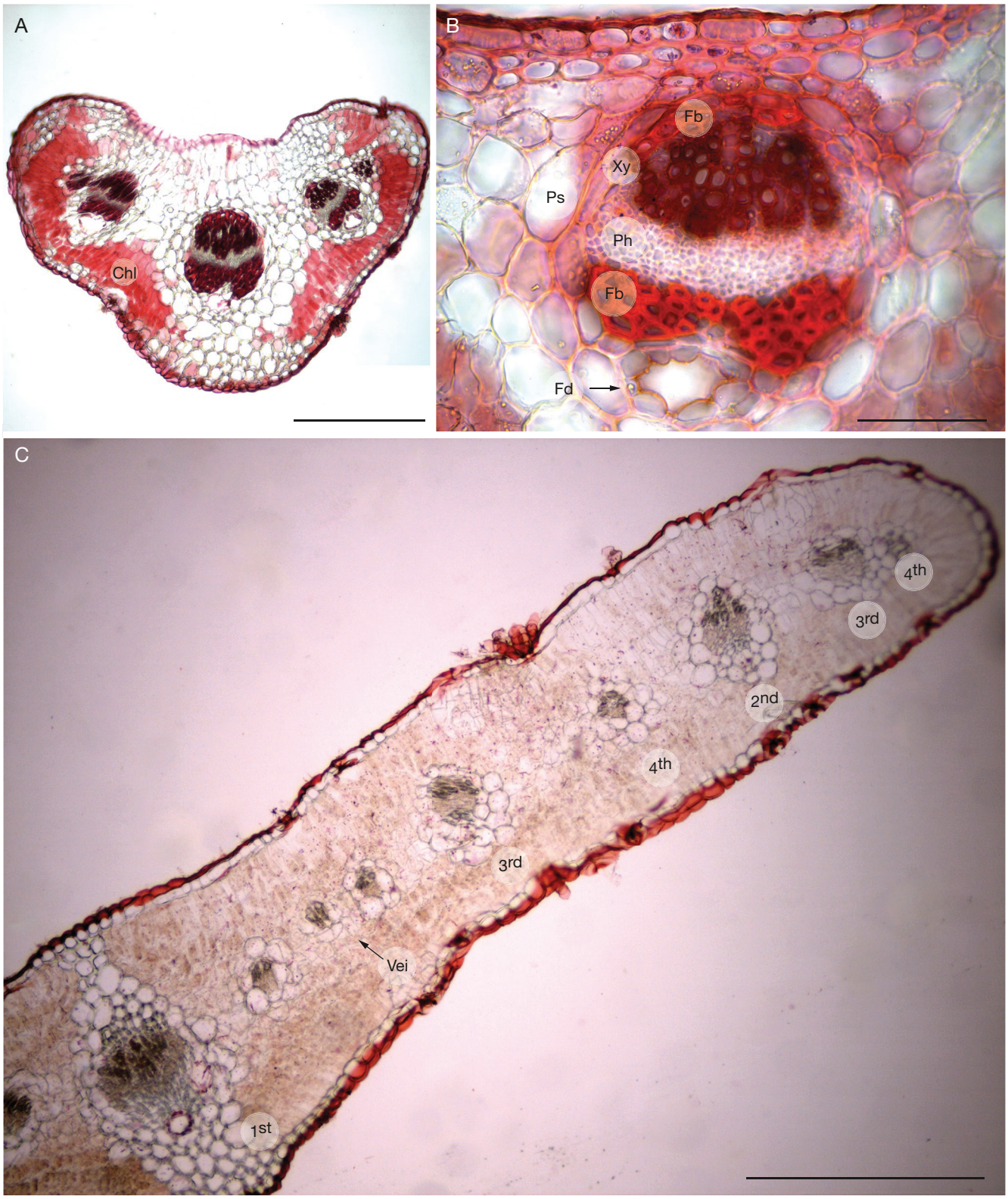


Fig. 1. — Leaf petiole and blade (CS): **A**, petiole showing three colateral vascular bundles and two traces; **B**, petiole, detail of a vascular bundle; **C**, leaf blade showing the order veins. Abbreviations: **Chl**, chlorenchyma; **Fb**, fibers; **Fd**, false duct; **1st to 4th**, first to fourth order veins; **Ph**, phloem; **Ps**, parenchyma sheath; **Vei**, veinlets; **Xy**, xylem. Scale bars: A, C, 300 µm; B, 100 µm.

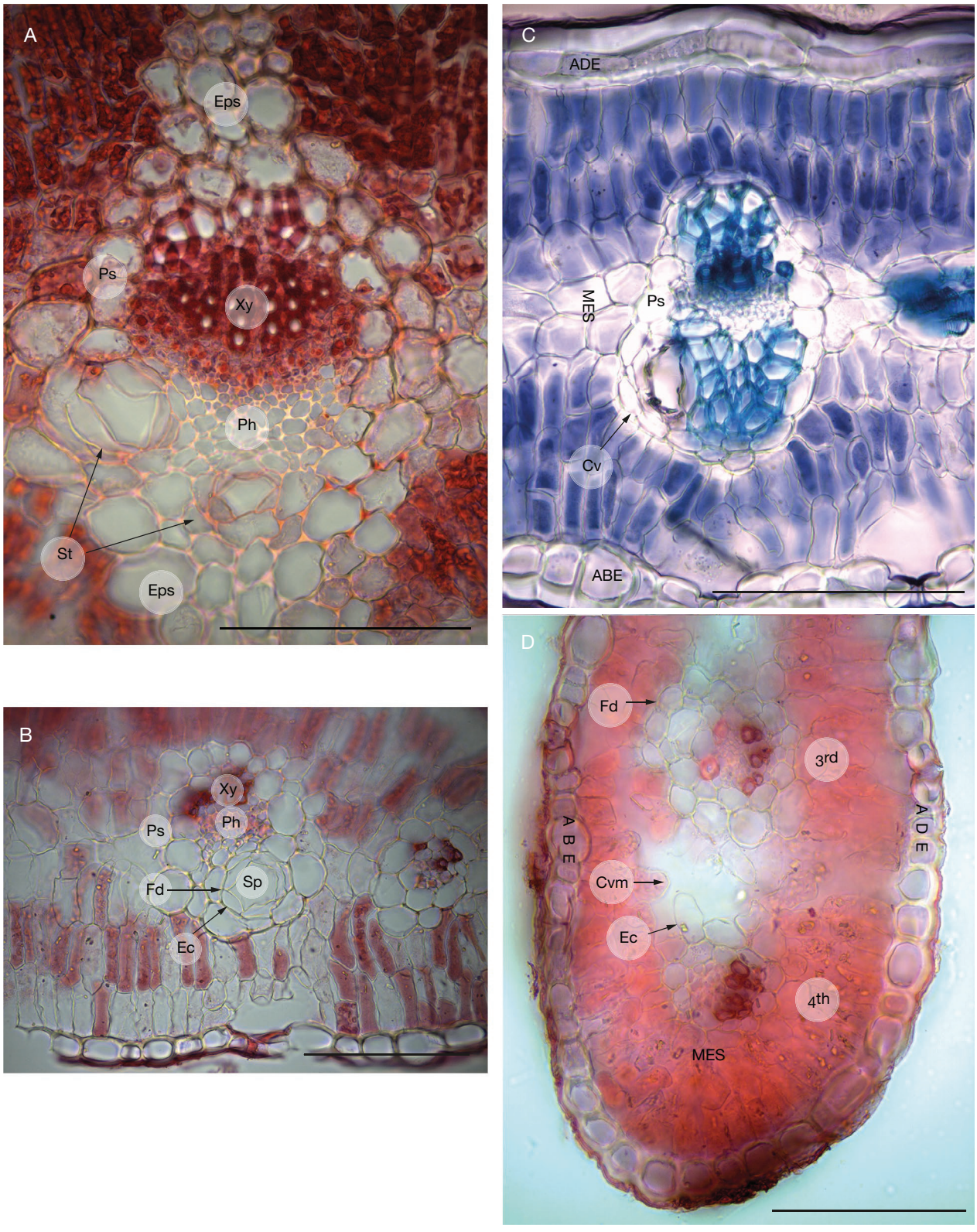


FIG. 2. — Order veins, secretory structures (CS): **A**, primary vein, parenchyma sheath extensions with two secretory structures; **B**, third order vein, one false duct; **C**, vascular bundle and lateral-abaxial cavity enclosed in the parenchyma sheath; **D**, cavity in the mesophyll. Abbreviations: **Cv**, cavity; **Cvm**, cavity in mesophyll; **ABE**, abaxial epidermis; **ADE**, adaxial epidermis; **Ec**, epithelial cells; **Eps**, extension of parenchyma sheath; **Fd**, false duct; **MES**, mesophyll; **3rd, 4th**, order veins; **Ph**, phloem; **Ps**, parenchyma sheath; **Sp**, space; **St**, secretory structures; **Xy**, xylem. Scale bars: 100 µm.

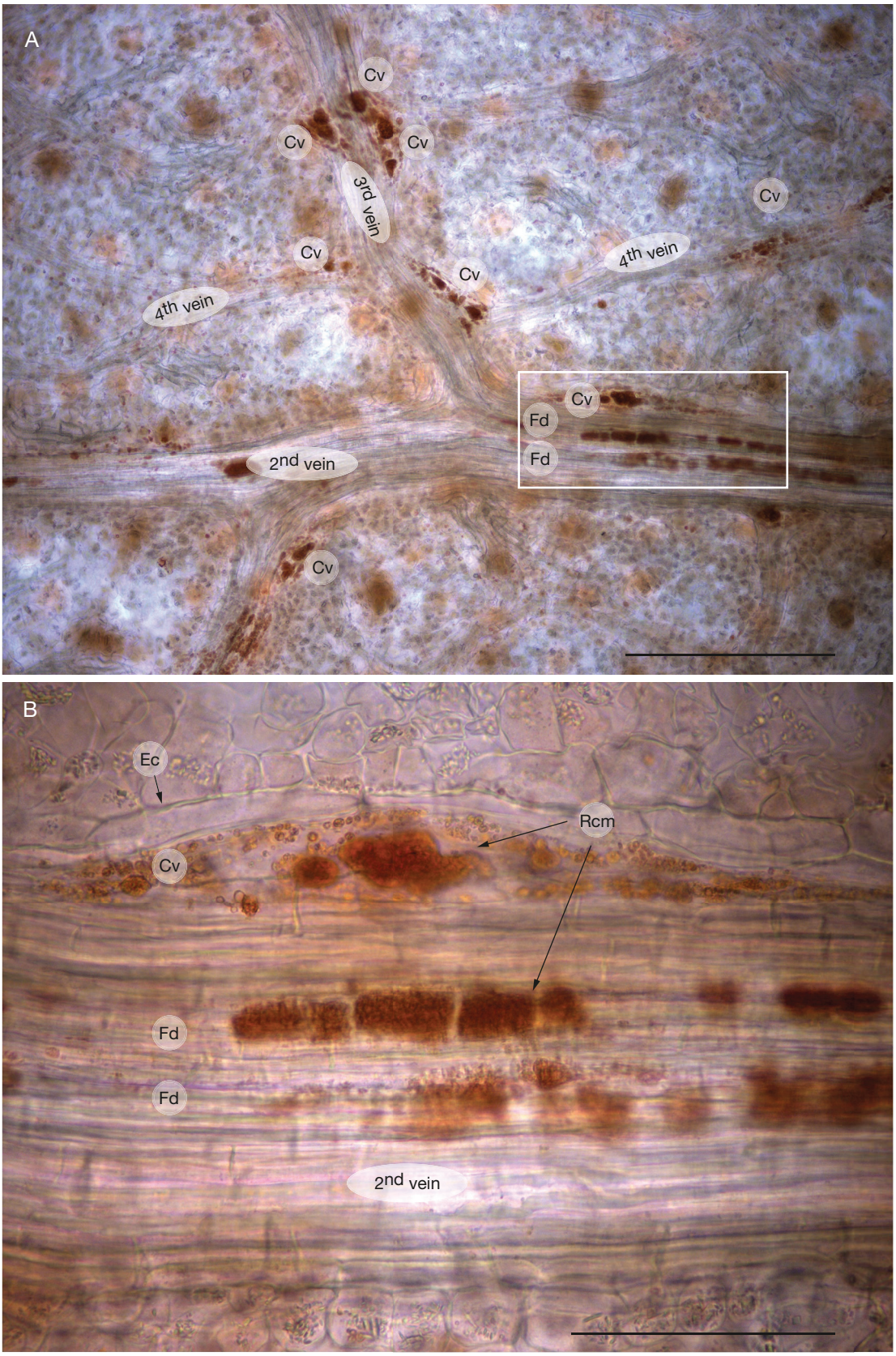


FIG. 3. — Secretory structures (PW): **A**, two false ducts and a cavity on abaxial side of 2nd order vein and cavities on 3rd and 4th order veins; **B**, detail of photograph showing the two false ducts on abaxial side of secondary vein and the cavity located on lateral-abaxial side. Abbreviations: **Cv**, cavities; **Fd**, false ducts; **Ec**, epithelial cells; **2nd, 3rd, 4th** order veins; **Rcm**, resinous complex mixture. Scale bars: A, 300 µm; B, 100 µm.

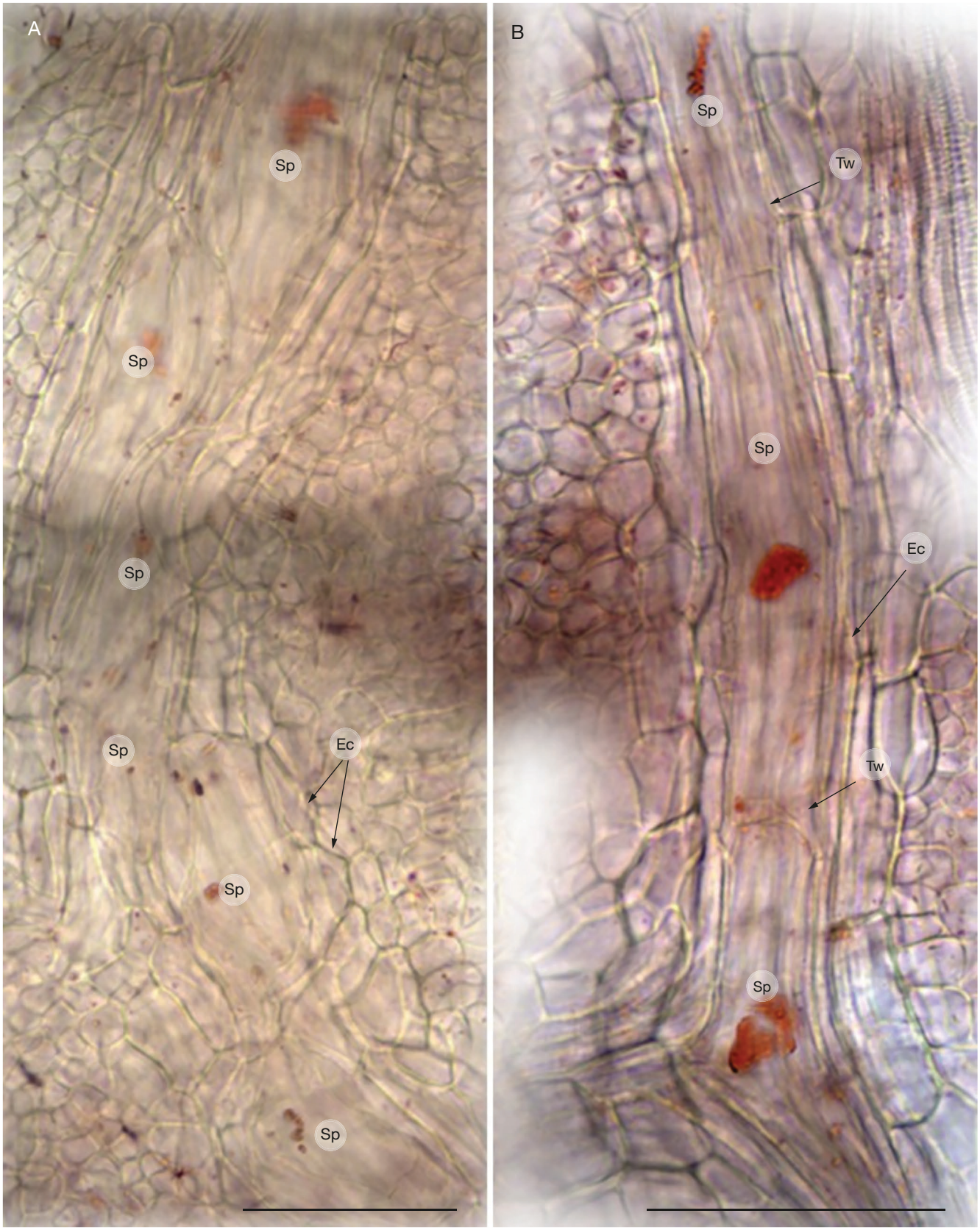


Fig. 4. — Cavities in a row going to form a false duct: **A**, short cavities in a row having short space and short epithelial cells; **B**, elongated cavities in a row having large space and elongated epithelial cells; these cavities still exhibit thin walls that contact each other. Abbreviations: **Ec**, epithelial cells; **Sp**, space; **Tw**, thin wall. Scale bars: 100 µm.

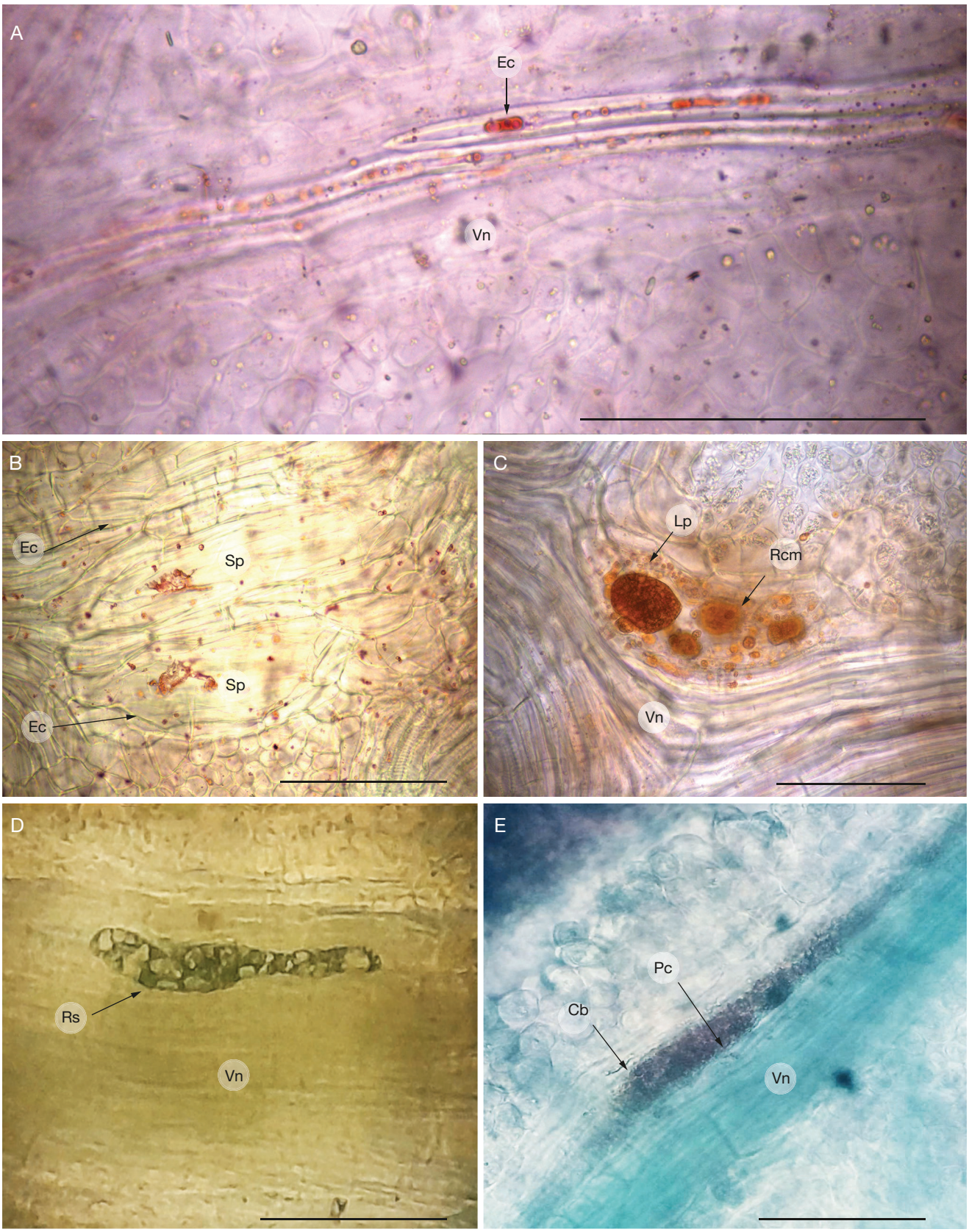


FIG. 5. — Epithelial cells, cavities shapes and chemical compounds (PW): **A**, Detail of large epithelial cells of a false duct with resins and oils (ORO test); **B**, a couple of oblong initial cavities in the apical region of a young leaf; **C**, isolated curved cavity adapted the angle of branching veins, storing resins and lipids (ORO test); **D**, space of cavity filled with resins, emerald green color (solution of copper sulphate test); **E**, space of isolated oblong-elongated cavity showing carbohydrates (lilac color) and phenolic compounds (turquease) (TBO test). Abbreviations: **Cb**, carbohydrates; **Ec**, epithelial cells; **Lp**, lipids; **Pc**, phenolic compounds; **Rcm**, resinous complex mixture; **Rs**, resins; **Sp**, space; **Vn**, vein. Scale bars: 100 µm.

the vascular bundle does not have sheath extensions and on the phloem side there is only one duct. The 4th to 6th order veins do not present ducts (Fig. 1C).

*Cavities and duct, characteristics in cross section*
Cavities and ducts in cross sections are rounded in outline, and the space size of both structures is similar having a mean diameter value of 41.7 µm. They are lined by uniseriate epithelium formed by 4-6 (-8) cells (Fig. 2A, B). Some secretory structures are located on abaxial side and others on abaxial-lateral side (Fig. 2A). The last are cavities (Fig. 2C). Cavities also are found in the mesophyll (Fig. 2D). The epithelial cells frequently contain spheroid and red colored droplets of essential oils when treated with ORO, the space may be empty or filled with a resinous substance in which were identified resins, lipids and phenolic compounds (Fig. 2B; Fig. 5C-E).

*Cavities and duct locations*
As was expressed above, the ducts are on abaxial (phloem) side of 1st to 3rd order veins. Cavities are isolated or in small clusters on lateral abaxial side of the 1st to 5th order veins, at the end of 6th order veins (veinlets), and rarely in the mesophyll (Fig. 3 A, B).

*False ducts, origin and characteristics in paradermal view*
In paradermal view of *B. notosergila* leaf, we observed in the apical region of the leaf blade a row of initial cavities contacting one another (Fig. 4A). Then, we found a row of elongated cavities contacting their ends (Fig. 4B). These observations illustrate that with the leaf growth the cavities suffer elongation, and this row of several cavities keep the walls in contact each other forming a false duct. It is possible, later the walls in contact each other disappear and they merge forming a true duct. The false duct in paradermal view exhibit elongated and narrow epithelial cells (length from 152.9 to 644.7 µm) with slightly thick cellulosic walls. They would be a derived state after cavities elongation. The large epithelial cells come into contact with each other by touching their ends or overlapping. Inside were identified predominantly essential oils and resins (Fig. 5A).

*Cavities, origin and characteristics in paradermal view*
Initial cavities may be in a row (Fig. 4A) or they may appear as an individual structure or forming small clusters of two-three cavities, whose shape is variable, oblong, oblong-elongated, oblong rounded, triangular or rounded. They have a length from 83.9 to 508.8 µm; their epithelial cells are short (length 21.7-97.9 µm) and with narrow walls (Fig. 5B). These cavities accumulate and reserve a resinous complex mixture. Lipids (essential oils), resins, phenolic compounds (tannins), and carbohydrates were identified (Fig. 5C-E).

## Discussion

*Cavities and ducts, origin and development*
The research of the secretory tissue of *Baccharis notosergila* leaves has permitted to get more information and illustrates better the existence of cavities themselves and cavities arranged in a row that will form the false ducts with the leaf growth. This result contributes with a new species in which its secretory structures correlate with conclusions reached by Lersten & Curtis (1987, 1989) for *Conyza canadensis* (L.) Cronquist and *Solidago canadensis* L., and by Arambarri & Hernández (2024) for *Conyza bonariensis* (L.) Cronquist and *Solidago chilensis* Meyen. The false ducts in analyzed species of *Baccharis*, *Conyza* Less., and *Solidago* L. are formed by cavities. Arambarri & Hernández (2024) observed in false ducts of *C. bonariensis* and *S. chilensis* a differentiation of parenchyma sheath cells enveloping the cavities named by the authors "attached parenchyma cells" (Apc). These Apc were not observed in *B. notosergila* however, where the false ducts are surrounded by parenchyma cells.

*Secretory structures, presence and dimensions in cross section*
The presence of secretory structures on vascular bundle phloem side in genus *Baccharis* was previously documented by Budel *et al.* (2018) and Ornellas *et al.* (2019). However, the secretory structure dimension has received little attention, but Molares *et al.* (2009) reported dimensions for *Baccharis obovata*, and we found similar values.

*Secretory structures and taxonomy*
The genera *Baccharis*, *Conyza* and *Solidago* belong to Astereae tribe. In the genus *Baccharis*, Smiljanic (2005) reported for *B. platypoda* DC. and *B. stylosa* secretory spaces located on adaxial and abaxial vascular bundle sides. Molares *et al.* (2009) for *B. obovata* indicated ducts located on the abaxial side. Ornellas *et al.* (2019) for *B. stylosa* reported ducts associated to the vascular bundles on the abaxial side but also indicated their presence on adaxial side and in the mesophyll. In this paper for *B. notosergila,* secretory spaces were found located on the abaxial side (included lateral-abaxial), terminal in veinlets, and in the mesophyll. In the genus *Conyza,* Lersten & Curtis (1987) reported for *C. canadensis* secretory structures located predominantly on abaxial side. Pérez & Apóstolo (2022) studied *C. bonariensis* (L.) Cronquist var. *bonariensis* and *C. b*. var. *angustifolia* (Cabrera) Cabrera and they reported the secretory structures located predominantly on abaxial vascular bundle side, rare on adaxial and on both vascular bundle sides. Arambarri & Hernández (2024) in *C. bonariensis* consigned false ducts and cavities located on abaxial side from 1st to 3rd order veins, and adaxial in 4th order veins. In the genus *Solidago,* Lersten & Curtis (1989) found in *S. canadensis* secretory spaces predominantly located on the abaxial side (1st to 3rd order veins), in 4th order veins on both vascular bundle sides; in veinlets on the xylem (adaxial) side and in the mesophyll. Similar findings were reported in *S. chilensis* by Pérez *et al.* (2018) and Arambarri & Hernández (2024). Evidently, there is a predominance of secretory structures on the abaxial side in the species of *Baccharis*, *Conyza* and *Solidago*. It accords well with Castro *et al.* (1997) who reported that the tribe Astereae has predominantly secretory structures on abaxial (phloem) side, whereas Eupatorieae and Heliantheae frequently have secretory spaces on the adaxial (xylem) side. Several authors found this location studying different species of Eupatorieae and Heliantheae tribes (Milan *et al.* 2006; Plos *et al.* 2011; Delbón *et al.* 2012; Hernández & Arambarri 2019).

*Secretory structures and evolution*
The chemical compounds that are secreted by glandular trichomes, and transported and/or stored in false ducts and cavities (reservoirs, according Lersten & Curtis 1989) allow to *B. notosergila* competitive and survival in adverse conditions. According Fahn (2002) and Liesenfeld *et al.* (2019) this acquired capacity would be result of its evolutionary history.

*Chemical compounds*
Ariza Espinar (1973) in *Baccharis* spp., had indicated that the secretory structures release other chemical compounds besides essential oils. Espírito-Santo *et al.* (1999) determined tannins in *B. dracunculifolia* DC. Budel *et al.* (2012) and Jasinski *et al.* (2014) established the volatile oils are the principal substances secreted in *Baccharis*. Tosoratto *et al.* (2016) analyzing several species of *Baccharis* identified pectates, lipids and tannins. In the present research were identified lipids (essential oils) in the epithelial cells and predominantly abundant in young leaves; a complex mixture of resins, lipids, phenolic compounds, carbohydrates and other substances having a natural dark color was found filling the internal secretory spaces predominantly in old leaves. The most notable chemical compounds found in leaves are the essential oils (volatile terpenoids). In the genus *Baccharis* monoterpenes and sesquiterpenes are the most frequent (Budel *et al.* 2018; Minteguiaga 2019). These essential oils are complex chemical compounds that have multiple functions: reduction of abiotic stress, allelopathy, inter-plant signaling, defense against herbivores and microbial pathogens, attract benefactors such as parasitoids (predators of the herbivores) and pollinators, and seed dispersal. By other way, preclinical studies have documented their medicinal antimicrobial, antioxidant, anti-inflammatory and anticancer activities (Glas *et al.* 2012; Sharifi-Rad *et al.* 2017; Minteguiaga 2019). The resins are a combination of nonvolatile terpenoids, and may be mixed with gums and other substances. Resins are able to protect the plants from biotic (pathogenic) and abiotic factors. They have an account of the ecological roles as the co-evolution occurring between plants and insects. Resins have been used since ancient times in medicine. The list of uses is seemingly endless (Barnett 2004). The phenolic compounds (flavonoids, phenols, tannins) have been found forming complex mixtures with other compounds in secretory structures of vegetative and reproductive organs in different species and families of plants. Among the phenolic compounds, the tannins and flavonoids provide defense against damage by UV-B radiation. Tannins play a role in the defense of the plant against herbivores by reducing the digestibility of nutrients (Castro & Demarco 2008). It was determined that phenolic compounds content in *Baccharis uncinella* DC., may have allelopathic effects on germination and seedling growth, and might be used as biological herbicide (Dias *et al.* 2017).

## CONCLUSION

In the leaf of *Baccharis notosergila* false ducts formed by cavities and cavities themselves were found. False duct is a long and extended space lined by very long epithelial cells, developed from a row of elongated cavities during the leaf growth. They are located on the abaxial vascular bundle side from 1st to 3rd order veins. The cavity itself is a short intercellular space lined by short epithelial cells. Cavities are isolated or in small clusters. They are located on lateral-abaxial side and in the angles of 1st to 5th order veins, at the end of veinlets, and in the mesophyll. False ducts and cavities contain a complex mixture of lipids, resins, phenolic compounds, carbohydrates and other substances. Extracts of these substances may be a new source of medicinal compounds or useful in organic control of pathogens or as herbicide to improve sustainable agriculture systems.

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