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SECRETORY STRUCTURES IN *TAGETES MINUTA* (ASTERACEAE, HELENIEAE)

P. M. SIMON[1,3], L. KATINAS[1,2], and A. M. ARAMBARRI[2]

Summary: *Tagetes minuta* is an important worldwide resource due to its agrochemical and pharmacological properties. Despite of the great number of chemical studies on this species, the secretory structures are scarcely studied. An anatomical survey of all organs of *T. minuta* was carried out. Three types of secretory structures coexist in *T. minuta*: (1) cavities in foliar blade and involucral phyllaries; (2) ducts in root, stem, petioles, midvein, capitula peduncle, corollas, and styles; and (3) glandular trichomes on stem, leaves, phyllaries of the involucre, and corollas. Ducts have an uniseriate or biseriate epithelium, generally surrounded by a parenchyma sheath. Secretory cavities have a multilayered epithelium, and lack a parenchyma sheath. There is a lack of continuity between ducts and cavities.

Key words: Asteraceae, Helenieae, *Tagetes minuta*, secretory structures.

Resumen: Estructuras secretoras en *Tagetes minuta* (Asteraceae, Helenieae). *Tagetes minuta* constituye un importante recurso a nivel mundial debido a sus propiedades agroquímicas y farmacológicas. Si bien se han realizado numerosos trabajos químicos sobre esta especie, las estructuras secretoras han sido poco estudiadas. Un examen anatómico de todos los órganos de *T. minuta* demuestra que existen tres tipos de estructuras secretoras: (1) Cavidades en segmentos foliares y filarios del involucro; (2) conductos en raíz, tallo, pecíolo, vena media, pedúnculo del capítulo, corolas y estilos; y (3) tricomas glandulares en tallo, hojas, pedúnculos, filarios y corolas. Los conductos tienen epitelio uniseriado o biseriado y están generalmente rodeados de vaina parenquimática. Las cavidades tienen epitelio multiseriado y no poseen vaina parenquimática. No existe continuidad entre conductos y cavidades.

Palabras clave: Asteraceae, Helenieae, *Tagetes minuta*, estructuras secretoras.

INTRODUCTION

Secretory structures involve a wide variety of elements such as cavities, ducts, individual cells, and trichomes. A secretory cavity (gland or sac) is a more or less isodiametric internal space or lumen, surrounded by an epithelium of secretory cells (Fahn, 1979). When the spaces are elongated they constitute secretory canals (or ducts) (Esau, 1976). In other cases, the epidermal cells give rise to glandular trichomes of various degrees of complexity, which release the secretion between the cell wall and the cuticle. Lersten and Curtis (1989) concluded that there is no qualitative difference between ducts and cavities, and that these terms merely identify the two extremes of a range of reservoirs. In addition, the traditional classification of these structures into external and internal structures (Cutler, 1969; Esau, 1976) is confusing since individual structures sometimes transcend particular categories (Cutler, 1969). For example, some authors consider glands as internal structures (e.g., Cutler, 1969) whereas others consider them as external (e.g., Esau, 1976).

Lersten and Curtis are among modern authors who have contributed most to the knowledge of secretory structures in Asteraceae, e.g., in *Solidago* and *Conyza* (Lersten & Curtis, 1987, 1989), in *Eupatorium* (Curtis & Lersten, 1986; Lersten & Curtis, 1986), and in *Ambrosia* (Lersten & Curtis, 1988), belonging to the tribes Astereae, Eupatorieae and Heliantheae, respectively. On the other hand, secretory structures in genera of the tribe Heleniae have not been extensively studied.

The subtribe Pectidinae (Robinson, 1981) is one of the most easily recognized of the Helenieae, mainly by its conspicuous, large, secretory cavities in leaves and involucral phyllaries. The genus *Tagetes* L., with 55 species (Soulé, 1996), constitutes, together with *Pectis*, one of the largest genera of Pectidinae. It includes the popular *Tagetes minuta* L., commonly known as "chinchilla" and "Mexican marigold",

[1]División Plantas Vasculares, Museo de La Plata, Paseo del Bosque s/n, 1900 La Plata. E-mail: pmsimon@netverk.com.ar; katinas@museo.fcnym.unlp.edu.ar
[2]Morfología Vegetal, Facultad de Ciencias Agrarias y Forestales, Casilla de Correo 31, 1900 La Plata.E-mail: botgral@ceres.agro.unlp.edu.ar
[3]Corresponding author

distributed in the temperate grasslands and montane regions of southern South America, including Peru, Bolivia, Paraguay, Chile and Argentina. Its affinity for disturbed sites has allowed *T. minuta* to colonize many areas around the world such as Europe, Asia, Africa, Madagascar, India, Australia and Hawaii (Soulé, 1993).

Tagetes minuta is an economically important worldwide resource in many countries due to its oil with agrochemical and pharmacological properties. The insecticidal activity of floral, foliar, and root extracts of *T. minuta* against crops pests such as weevils (Sriharan *et al.*, 1994; Weaver *et al.*, 1994), mosquitoes (Perich *et al.*, 1994, 1995; Maradufu *et al.*, 1978), nematodes (Kumar *et al.*, 1998; Zumarán *et al.*, 1994), fungi (Bii *et al.*, 2000), bacteria (Hethelyi *et al.*, 1986), and viruses (Hudson, 1990) are widely recognized. Various medicinal properties and use as a condiment and in the essential oil industry (as "Tagetes oil") are fully demonstrated and exploited (Neher, 1968; Martijena *et al.*, 1998; Craveiro *et al.*, 1988).

Due to the economic value of *T. minuta* many chemical studies have been performed (e.g., Kaplan, 1960; Filipov, 1997; Israilev & Seeligmann, 1983, 1985; Maradufu *et al.*, 1978; Font Quer, 1981; Russin *et al.*, 1988). The species is rich in many secondary compounds, including monoterpenes, sesquiterpenes, flavonoids, thiophenes, and aromatics. Differences in the oil activity between organs of *T. minuta*, such as between leaves and florets, have been documented (Bii *et al.*, 2000).

Secretory cavities, ducts and secretory cells have been reported in the genus *Tagetes* (Van Tieghem, 1872; Thouvenin, 1884; Leblois, 1887; Col, 1903; Metcalfe & Chalk, 1950), focusing on *Tagetes erecta* (Thouvenin, 1884) and *T. patula* (Van Tieghem, 1872). Until now, secretory structures have been scarcely analyzed in *T. minuta*. A developmental study in *T. minuta* by Del Fueyo (1986) identified glands (= secretory cavities) in leaves and phyllaries, and secretory ducts in petiole, and midvein, interpreting them as schizogenous. The presence of these structures in roots, capitula peduncle, florets, and fruits, on the other hand, has never been investigated.

In this paper we examine the ocurrence, structure and distribution of secretory structures in root, stem, leaves, capitula peduncle, involucre, florets, and fruits of *T. minuta*.

Material And Methods

Roots, stems, leaves, capitula peduncle, phyllaries of the involucre, florets and fruits were obtained from herbarium (see Appendix 1) and living specimens.

Samples from herbarium specimens were reconstituted in boiling water. Whole foliar segments and phyllaries were cleared according to Strittmatter (1973). The midregion of plant organs were isolated and free hand cut transversely. Transverse serial sections in whole organs such as florets and fruits were also made. Some sections were stained with Safranin while the rest were left unstained. Material was also fixed in formalin-acetic acid-alcohol and processed by the usual techniques of paraffin infiltration. Transverse serial sections were cut 10-15 µm thick and stained with Cresyl violet.

Light microscope observations and drawings were carried out on a Leitz SM Lux microscope equipped with a camera lucida. Selected light microscope images were transferred electronically from the microscope to the computer using the Photo Express version 1.0 software, and then sized, processed and organized into plates with Corel Draw 9, Corel Photo-Paint 10 and Microsoft PowerPoint computer programs. Leaf and phyllary tissues were prepared for scanning electron microscopy by sputter-coating with gold/paladium, and examined and photographed with a Jeol JSM-T100 Scanning Electron Microscope.

We follow Fahn (1979) in the use of terms, i.e., "ducts" for the elongated internal secretory structures, and "secretory cavities" for the more or less isodiametrical secretory structures. Both structures are defined as including the epithelium and lumen. All secretory structures are described in transection, except when indicated.

Results

Root. Ducts are distributed in the cortex and in the vascular cylinder (Fig. 1A, 2A). *Cortex ducts*: Four to six, ranging from 10 µm to 25 µm diam., situated among the endodermis and the parenchyma cells of the cortex in roots with primary growth, or in the periderm in roots with secondary growth. Uniseriate epithelium, with 5-6 epithelial cells. Parenchyma sheath is absent. *Vascular cylinder ducts*: Ten to 12, 15 µm to 40 µm diam., situated in the phloem. Uniseriate epithelium, with 5-7 epithelial cells. Parenchyma sheath is absent.

Stem. *Ducts*: Twelve to 20, ca. 30 µm diam., alternating with the phloem and associated with

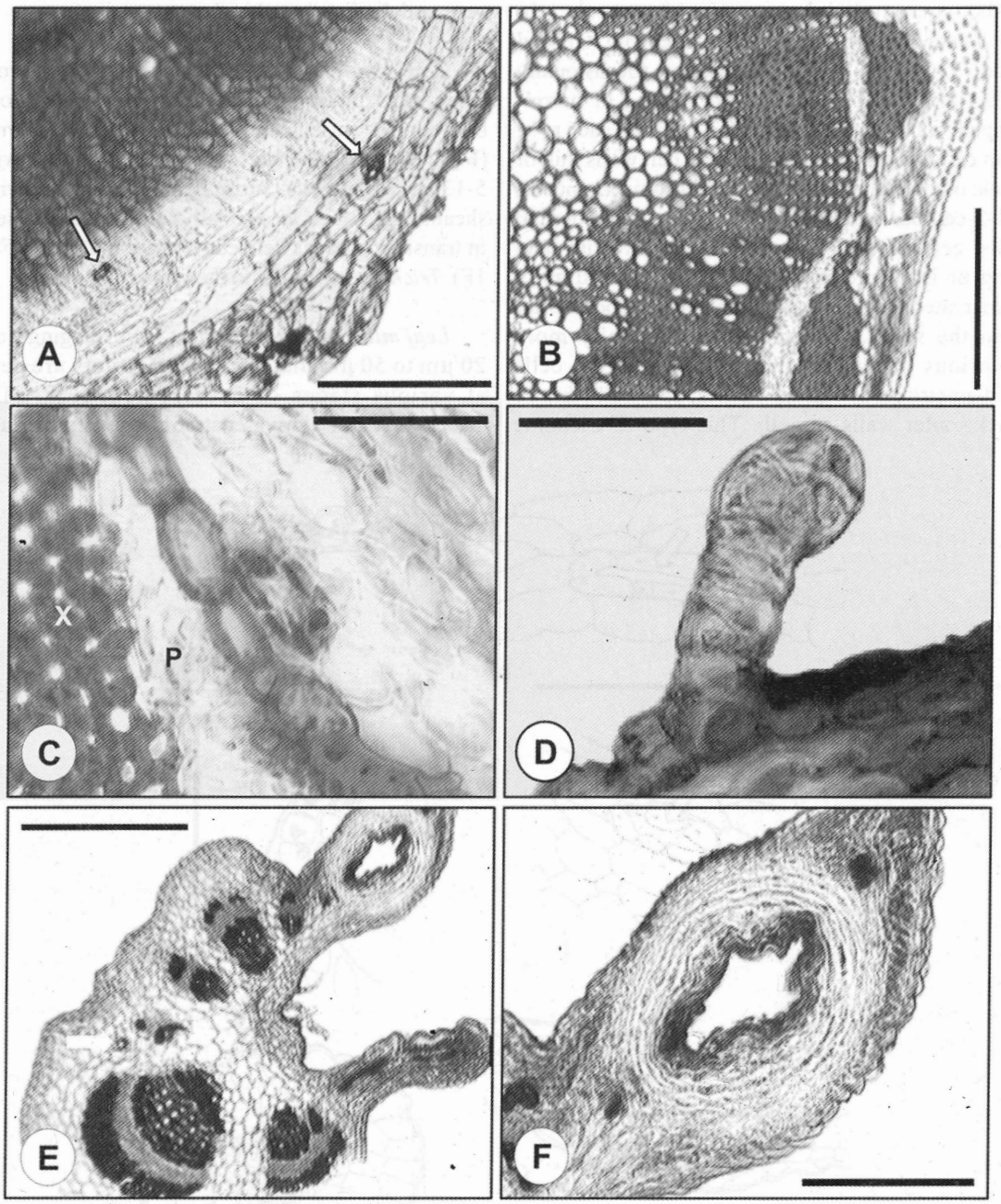

Fig. 1. Light micrographs of cross section in organs of *Tagetes minuta* showing the secretory structures (arrows). **A**: root showing two secretory ducts, one in the periderm and one in the phloem; **B**: enlarged view of stem showing the cortex where the secretory ducts are found; **C**: secretory duct in stem cortex, associated with sclerenchyma tissue; **D**: biseriate capitate glandular trichome in stem. **E**: transition area between the foliar blade and the petiole showing ducts (arrow) and one cavity; **F**: enlarged view of the foliar secretory cavity. **A**, **E**, **F**: Simon 1078 (LP); **B** - **D**: Simon 20 (LP). Scale bars: 200 µm on **A**, **B**, **F**: 50 µm on **C**, **D**: 0.5 mm on **E**. X = xylem; P = phloem.

schlerenchyma tissue, in the cortical parenchyma (Fig. 1B). Uniseriate epithelium (Fig. 1C), with 6-10 epithelial cells, surrounded by a parenchyma sheath. *Trichomes* (Fig. 1D, 2B): Differentiated into stalk and head, 70-200 µm long. Stalk biseriate (occasionally uniseriate); uniform in breadth; oblong or barrel-shaped; contents translucent; 4-7-celled in each row; with cells subopposite or opposite; cell walls thin or thick; outer walls straight or slightly convex, smooth; a 1-2-cellular base imbedded among the epidermal cells; cells of the basal tiers usually broader than long or isodiametrical. Head sharply or slightly demarcated from the stalk; rounded in shape; shorter than the stalk; contents dense, generally more notorious in the terminal tier; 2-4-tiered, cells isodiametrical, of various shapes; cell walls thin or thick; outer walls smooth. This type of trichome corresponds to the capitate hairs or "biseriate capitate glandular hair" of Ramayya (1962), and are very widely distributed in Asteraceae.

Leaf petiole. *Ducts*: Three to five, ranging from 35 µm to 50 µm diam., situated abaxially in the petiole, between the vascular bundles, in the parenchyma (Fig. 1E, 3A). Uniseriate to biseriate epithelium, with 5-12 epithelial cells, surrounded by a parenchyma sheath (Fig. 3B). Occasionally, cavities are present in transitional areas between petiole and blade (Fig. 1F). *Trichomes*: Similar to the stem trichomes.

Leaf midvein. *Ducts*: Three to five, ranging from 20 µm to 50 µm diam. (Fig. 3C). Ducts are seen at various stages of development (Fig. 3D). Developed ducts with uniseriate to biseriate epithelium,

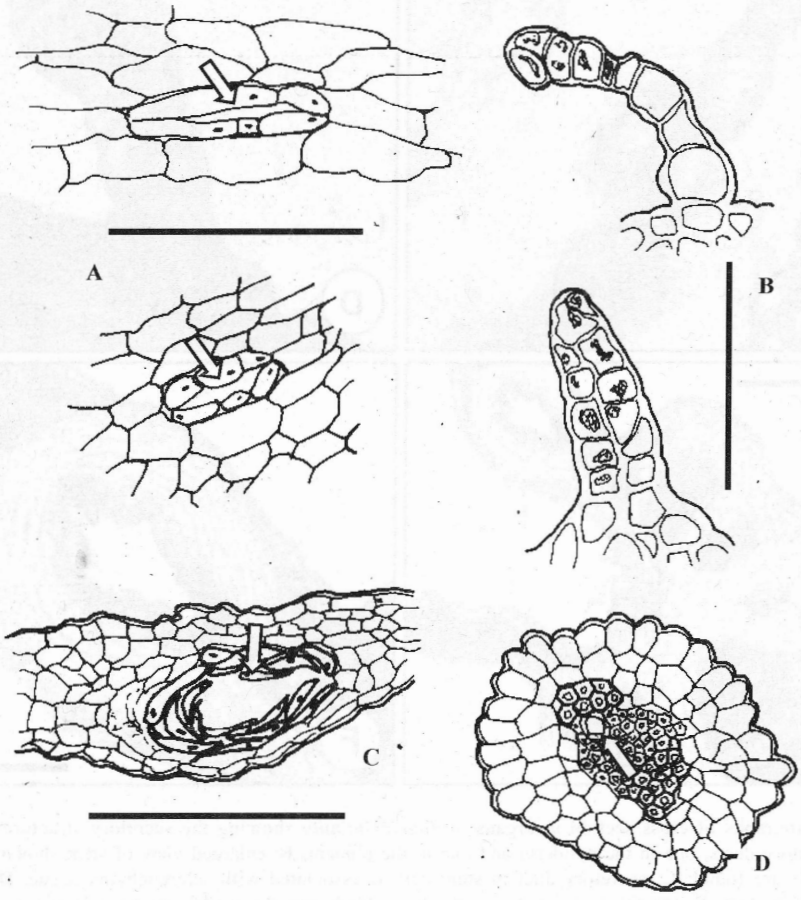

Fig. 2. Secretory structures in *Tagetes minuta* (arrows). **A**: secretory ducts in the root cortex (above) and phloem (below) showing the uniseriate epithelium; **B**: biseriate capitate glandular hairs in different views; **C**: secretory cavity in an involucral phyllary showing the multiseriate epithelium; **D**, secretory duct in the style in contact with peripheric vascular and central stigmatoid tissue. **A**: Simon 1078 (LP); **B**: Simon 20 (LP); **C**: Ringuelet 185 (LP); **D**: Mangieri 11 (LP). Scale bars: 100 µm.

with 3-5 epithelial cells, surrounded by a parenchyma sheath (Fig. 3E). *Trichomes*: Three-5-celled, oblong, similar to the stem trichomes.

Leaf blade. *Cavities.* Surface view: Leaves in *T. minuta* are pinnatisect, constituted by 8-20 segments; the secretory cavities are conspicuous,

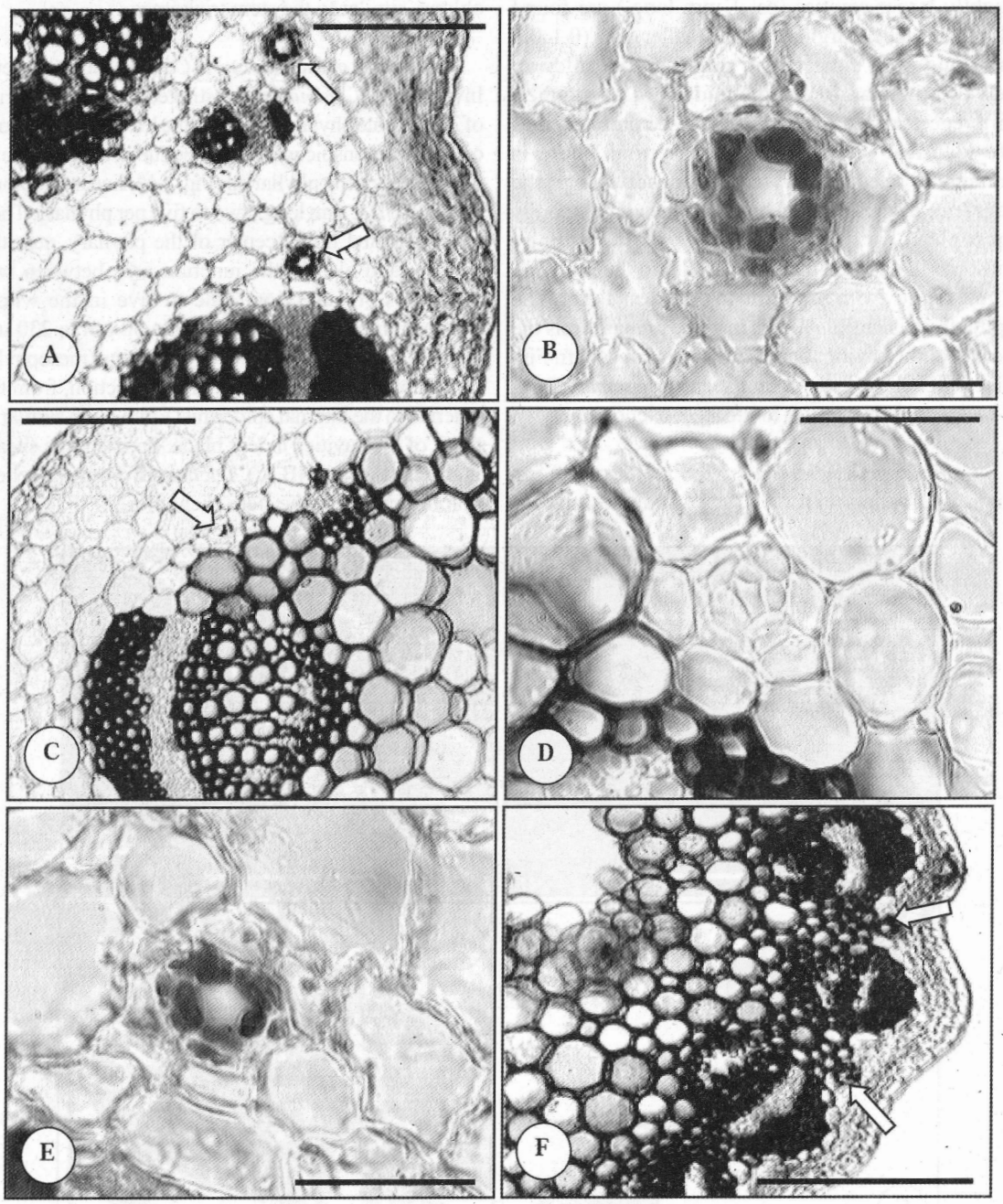

Fig. 3. Light micrographs of cross section in organs of *Tagetes minuta* showing the secretory structures (arrows). **A**: enlarged view of the petiole showing two secretory ducts at both sides of one small vascular bundle; **B**: detail of one petiole secretory duct showing the biseriate epithelium and the surrounding parenchyma sheath; **C**: enlarged view of the midvein showing the area between the vascular bundles where the secretory ducts at different stages of development are found; **D**: secretory duct of the midvein in development stage; **E**: mature secretory duct of the midvein showing biseriate epithelium and the surrounding parenchyma sheath. **F**: enlarged view of the capitula peduncle showing the area between the vascular bundles where the secretory ducts are found. **A**: Simon 1078 (LP); **B** - **F**: Simon 20 (LP). Scale bars: 200 μm on **A**, **C**, **F**: 50 μm on **B**, **D**, **E**.

large, pellucid, bulging above the surface of the segments, rounded-elliptic to linear in shape, ten to 15 per segment. They occur in two regions, the slightly larger cavities (ca. 1 mm long.) are found along the blade margin, and the smaller ones (0.1-0.3 mm long.) are clustered in groups about midway between the margin and the midvein. Despite their differences in position and size, the secretory cavities are identical in structure. Transection: There is commonly one secretory cavity in each half of the segment, at the level of both, palisade and spongy mesophyll, or just at the level of the spongy mesophyll, near the margin, parallel to the midvein. They range from 170 µm to 350 µm long. The epithelium is multilayered (in some preparations the border of the cavities appears disintegrated) (cfr. Fig. 1F). Parenchyma sheath not seen. *Trichomes*: Two-3-celled, sparse, similar to the stem trichomes.

Capitula peduncle. *Ducts*: Ten to 15, ca. 30 µm diam. alternating with the vascular bundles, next to the phloem, in the parenchyma (Fig. 3F). Uniseriate epithelium, with 6-8 epithelial cells, surrounded by a parenchyma sheath. *Trichomes*: Eight-10-celled, oblong, similar to the stem trichomes.

Phyllaries of the involucre. *Cavities.* Surface view: Involucre in *T. minuta* is constituted by a single series of 3-4 connate phyllaries. Like in the leaves, the secretory cavities are conspicuous, large, pellucid, bulging above the surface of the phyllaries (Fig. 4A), linear to oblong in shape, 0.2-2 mm long., two to five per phyllary. They are distributed in the center of the phyllary, near the margin, and also in the junction area between two phyllaries. Transection: One to five in the whole involucre transection, ranging from 50 µm to 230 µm long., at the level of the parenchyma tissue (mesophyll), or occupying the whole phyllary transection, from the external to the internal epidermis. Epithelium similar to those of the cavities in leaf blade. Parenchyma sheath is absent (Fig. 2C, 4B, C). *Trichomes*: Four-10-celled, oblong, similar to the stem trichomes.

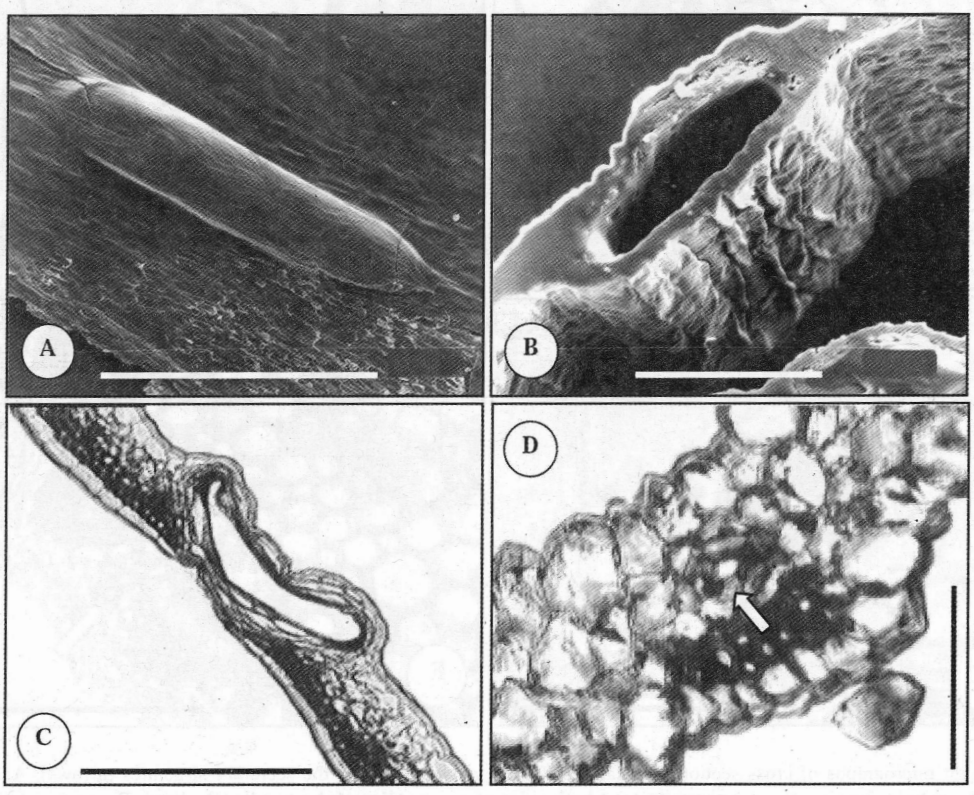

Fig. 4. **A-B**: SEM micrographs of secretory cavities in *Tagetes minuta*. **A**: secretory cavity in the external surface of a phyllary of the involucre; **B**: cross transection of a secretory cavity in a phyllary of the involucre. **C-D**: light micrographs of cross section in organs of *Tagetes minuta* showing the secretory structures (arrows). **C**: phyllary of the involucre showing one secretory cavity bulging above the external surface; **D**: corolla with a secretory duct adaxial to the vascular bundle, **A**, **B**: Ringuelet 185 (LPAG); **C**, **D**: Mangieri 11 (LP). Scale bars: 100 µm on **A**, **B**: 200 µm on **C**: 50 µm on **D**.

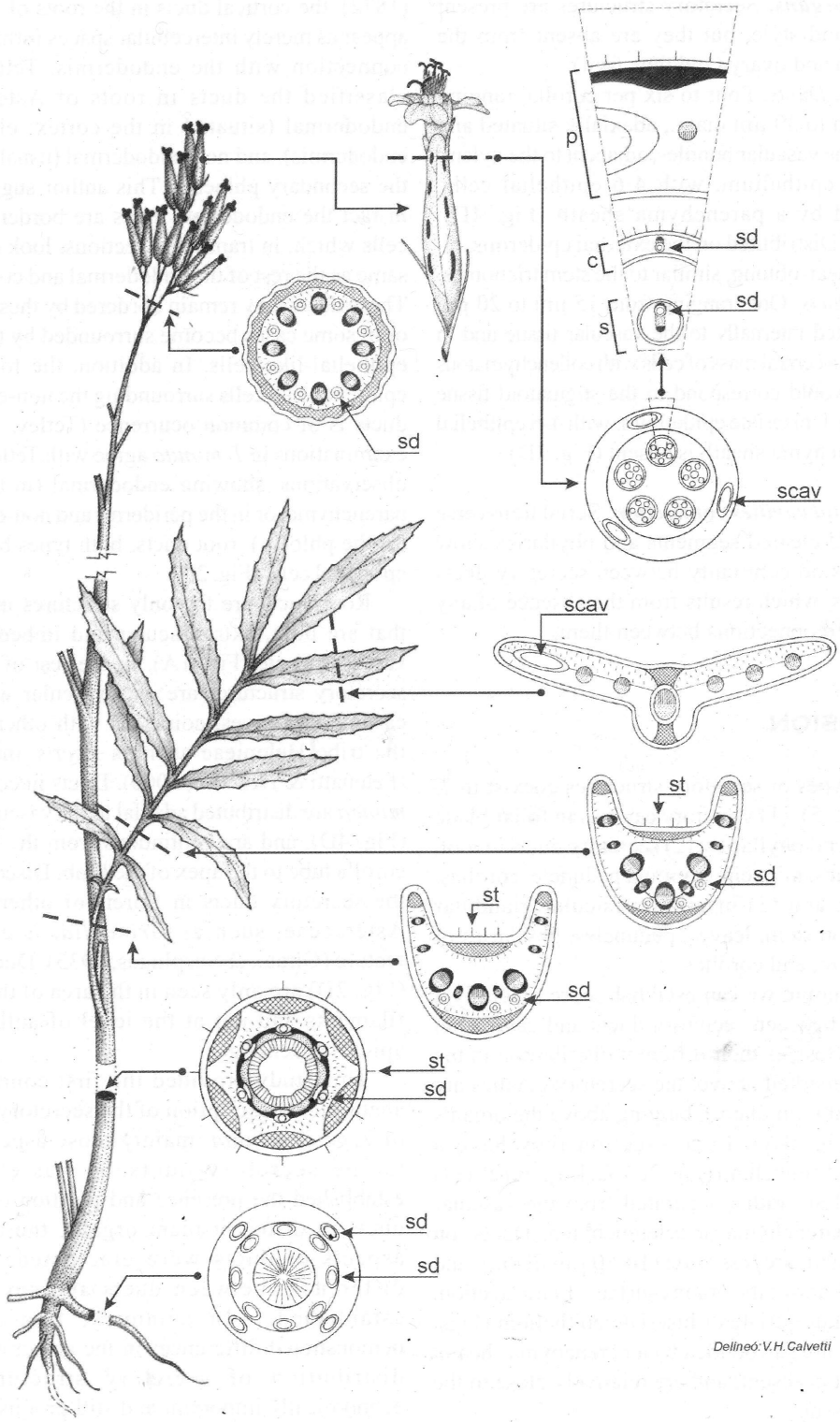

Fig. 5. Diagrammatic overview of *Tagetes minuta* with the organs in transection, showing the ocurrence and position of the secretory structures. From bottom to top: root, stem, petiole, midvein, blade, capitula peduncle, capitulum. c = corolla; p = phyllary; s = style; s cav = secretory cavity; sd = secretory duct; st = secretory trichome.

Floral organs. Secretory structures are present in corolla and style, but they are absent from the androecium and ovary (and thus fruit).

Corolla. *Ducts*: Four to six per corolla, ranging from 20 µm to 30 µm diam., adaxially situated and aligned to the vascular bundles, adjacent to the xylem. Uniseriate epithelium, with 4-6 epithelial cells, surrounded by a parenchyma sheath (Fig. 4D). *Trichomes*: Distributed on the external epidermis, 5-9-celled, linear-oblong, similar to the stem trichomes.

Style. *Ducts*: One, ranging from 15 µm to 20 µm diam., situated internally to the vascular tissue and in contact with a central mass of cells with collenchymatous walls that would correspond to the stigmatoid tissue (Esau, 1976). Uniseriate epithelium, with 4-6 epithelial cells. Parenchyma sheath is absent (Fig. 2D).

Ducts and cavities connection. Serial transverse sections and cleared segments and phyllaries show that there is no continuity between secretory ducts and cavities, which results from the absence of any type of interconnections between them.

DISCUSSION

Three types of secretory structures coexist in *T. minuta* (Fig. 5): (1) secretory cavities in foliar blade and involucral phyllaries; (2) secretory ducts in root, stem, petioles, midvein, capitula peduncle, corollas, and styles; and (3) biseriate vesicular glandular trichomes on stem, leaves, peduncles, phyllaries of the involucre, and corollas.

At this point, we can establish more precisely a distinction between secretory ducts and cavities in *T. minuta*. Besides their different distribution in the plant, as remarked above, the secretory cavities are wide (90-300 µm diam.), bulging above the organ's surface (Fig. 4A). In transection they have a multilayered epithelium (Fig. 2C), lack a parenchyma sheath, and are rather separated from the vascular tissue by parenchyma or sclerenchyma. Ducts, on the other hand, are less wide (10-50 µm diam.), and never bulge above the organs surface. In transection, they have a uniseriate or biseriate epithelium (Figs. 1C, 3B, E), are surrounded by a parenchyma sheath, or the sheat is absent, and are relatively close to the vascular tissue.

Some additional remarks can be made with respect to the duct characteristics in roots and the location of secretory structures. According to Van Tieghem (1872), the cortical ducts in the roots of Asteraceae appear as merely intercellular spaces formed in close connection with the endodermis. Tetley (1925) classified the ducts in roots of Asteraceae as endodermal (situated in the cortex, close to the endodermis), and non-endodermal (usually found in the secondary phloem). This author suggested that in fact the endodermal ducts are bordered by four cells which, in transverse sections, look exactly the same as the rest of the endodermal and cortical cells. These ducts may remain bordered by these four cells or in some cases become surrounded by thin-walled epithelial-like cells. In addition, the formation of epithelial-like cells surrounding the non-endodermal ducts is of common ocurrence (Tetley, 1925). Our examinations in *T. minuta* agree with Tetley's (1925) observations, showing endodermal (in the cortical parenchyma, or in the periderm) and non-endodermal (in the phloem) root ducts, both types bordered by epithelial cells (Fig. 2A).

Root ducts are the only structures in *T. minuta* that are both, extravascular and imbedded in the vascular tissue (Fig. 1A). In the rest of the organs secretory structures are extravascular wich, in the case of the leaves, coincides with other genera of the tribe Helenieae such as *Pectis* and *Flaveria* (Petenatti & Del Vito, 2000). Ducts in corollas of *T. minuta* are distributed adaxial to the vascular bundles (Fig. 4D), and are continuous from the base of the corolla tube to the apex of the limb. Discontinuity of the secretory ducts in florets of other genera of Asteraceae, such as *Grindelia*, is an unusual feature (Giraux & Susplugas, 1935). Ducts in styles (Fig. 2D) are only seen in the area of the staminal filaments and not at the level of anthers or the apical appendages.

This study provided the first comprehensive anatomical description of the secretory structures of *Tagetes minuta*, mainly those aspects related to the secretory ducts. It was clarified or established the presence and position of secretory ducts in different plant organs, the anatomical aspects of ducts were elucidated, and clear differences between ducts and cavities were established. In summary, this study has demonstrated differences in the anatomy, size, and distribution of secretory structures of an economically important and still promising species of Asteraceae that can help to anchor the great amount of chemical information about *Tagetes minuta* to sites and structures.

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Appendix 1. Collection data (country, province, locality, date of collection, collector and number, and herbarium abbreviation) of the anatomically examined specimens analyzed of *Tagetes minuta*.

ARGENTINA. *Prov. Buenos Aires*. La Plata, 1995, Ringuelet 185 (LPAG), V-1995, Arambarri 156 (LPAG), 22-IV-1931, Finastera s.n. (LP); Punta Lara, 6-IV-1966, Rossi s.n. (LPAG); Berisso, balneario Bagliardi, 1996, Bayón 318 (LPAG). *Prov. Chaco. Dpto. Nueve de Julio*, Las Breñas, 16-XI-1998, Simon 20 (LP). *Prov. Misiones*. Caá-Guazú, IX-1940, Mangieri 11 (LP).

BOLIVIA. *Dpto. Tarija*, Cainguá, 15 km N de Villa Montes, 4-VI-1971, Krapovickas et al. s.n. (LP); *Dpto. Santa Cruz*, vicinity of abandoned old Jardín Botánico, along Río Pirai and roadsides on W side of Santa Cruz, 17° 47' S, 63° 13' W, 8-VII-1987, Nee 35074 (LP).

PARAGUAY. *Dpto. Cordillera*, Altos Colonia Bernal Cué, 18-VI-1973, Schinini s.n. (LP).

PERÚ. *Dpto. Cusco*, desvío km 82, Estancia Pisacucho, 20-V-1958, Cabrera and Fabris 13457 (LP).