| Phyton (Horn, Austria) | **Vol. 41** | Fasc. 1 | 35–48 | 29. 6. 2001 |
|---|---|---|---|---|

# Secretory Tissue Ultrastructure in *Tagetes patula* L. (*Asteraceae*) and Thiophene Localization through X-Ray Microanalysis

By

G. Sacchetti*), C. Romagnoli**), A. Bruni*) and F. Poli***)

With 11 Figures

Received July 10, 2000

Accepted August 5, 2000

Key words: *Tagetes patula* L., secretory tissue ultrastructure, thiophenes, X-ray microanalysis.

Summary

Sacchetti G., Romagnoli C., Bruni A. & Poli F. 2001. Secretory tissue ultrastructure in *Tagetes patula* L. (*Asteraceae*) and thiophene localization through X-ray microanalysis. – Phyton (Horn, Austria) 41 (1): 35–48, 11 figures. – English with German summary.

Transmission electron microscopy has been used to determine the ultrastructure of secretory tissues in seedlings (10 days old plants) and in flowering plants (50 days old plants) of *Tagetes patula*. All the secretory structures in seedling and in flowering plants showed a schizogenous origin. In particular, in *T. patula* seedlings canals were examined in roots and hypocotyl while secretory cavities were found in cotyledons. In flowering *T. patula* plants, canals in epicotyl and in flower corolla, as well as secretory cavities in leaves and flower bracts were examined. Plastids related to the synthesis and accumulation of lipid substances involved in the secretory process were then identified in some glandular structures. X-ray microanalysis showed the pre-

*) G. Sacchetti, A. Bruni, Department of Biology-Section of Botany, University of Ferrara, C.so Porta Mare 2, I-44100 Ferrara, Italy.

**) C. Romagnoli, Department of Animal Biology-Section of Botanical Garden, University of Modena and Reggio Emilia, Viale Caduti in Guerra 127, I-41100 Modena, Italy.

***) F. Poli, Department of Evolutionary and Experimental Biology, University of Bologna, Via Irnerio 42, I-40126 Bologna, Italy. For correspondence.

sence of sulfur in the secretory tissue cells and, above all, in the secretion itself. This supports the hypothesis of direct involvement of the *T. patula* glandular structures in the production of thiophene compounds.

## Zusammenfassung

SACCHETTI G., ROMAGNOLI C., BRUNI A. & POLI F. 2001. Die Ultrastruktur von Sekretionsgeweben in *Tagetes patula* L. (*Asteraceae*) und die Lokalisation von Thiophen mittels Röntgenstrukturanalyse. – Phyton (Horn, Austria) 41 (1): 35–48, 11 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Mit dem Transmissionselektronenmikroskop wurde die Ultrastruktur von Sekretionsgeweben in Sämlingen (10 Tage alte Pflanzen) und in blühenden Tagetes-Pflanzen (50 Tage alt) untersucht. Alle sekretorischen Strukturen in den Sämlingen und in den blühenden Pflanzen weisen eine schizogene Entstehung auf. Vor allem in *T. patula* Sämlingen wurden in Wurzeln und in Hypocotylen Gänge beobachtet, während einfache Hohlräume in den Cotyledonen gefunden wurden. In blühenden Pflanzen wurden sowohl Gänge im Epicotyl und in der Blumenkrone als auch Sekretionsbehälter in Blättern und in den Bracteen festgestellt. Plastiden, die mit der Synthese und Akkumulation von Lipiden im Zusammenhang stehen, wurden in einigen Drüsenstrukturen gefunden. Die Röntgenstrukturanalyse weist auf das Vorkommen von Schwefel in den Sekretionszellen und vor allem im Sekret selbst hin. Dies bestätigt die Hypothese einer direkten Beteiligung von Drüsenstrukturen bei der Produktion von Thiophenverbindungen.

## Introduction

*Tagetes patula* L. (*Asteraceae*) is a plant known for its property to produce and accumulate considerable amounts of thiophenes, polyacetylene derivatives which act as antibiotics, insecticides, fungicides and nematodicides (GOMMERS 1981, MARES & al. 1990, DOSDALL & al. 1992, HUDSON & al. 1993, ROMAGNOLI & al. 1994). Phytochemical studies have shown that some thiophenes are accumulated in several different organs of *T. patula* with specific distribution and with concentration varying throughout plant ontogenesis (TOSI & al. 1988). A study carried out by conventional and fluorescence microscopy on *T. patula* samples has located some histological elements responsible for the production and/or accumulation of thiophenes (POLI & al. 1995). In fact, these secretory tissues are part of a complex internal system of polyacetylene reservoirs (PAR) (LERSTEN & CURTIS 1989) present in the roots, hypocotyl, cotyledons, epicotyl, floral corolla, leaves and floral bracts. In *T. patula* both kinds of glandular structures could produce and/or accumulate thiophenes and essential oils (POLI & al. 1995).

The present work reports the results of an ultrastructural study using transmission electron microscopy (TEM) on the internal secretory tissue cells of seedlings (10 days old) and flowering plants (50 days old) of

*T. patula*. Then X-ray microanalysis was applied to identify the presence of sulfur in the cells and relate it to the accumulation of polyacetylene sulfates such as thiophenes.

## Materials and Methods

### Transmission electron microscopy

Ultrastructural analysis was performed on various organ samples of *Tagetes patula* L. (*Asteraceae*) plants, from the Botanical Garden of the University of Ferrara. Samples of roots, hypocotyl and cotyledons were taken from *T. patula* seedlings (10 days old plants); samples of epicotyl, leaves, flower corolla and flower bracts came from flowering *T. patula* plants (50 days old plants). All the samples were fixed following the method reported in TURNER & al. 1998 designated "osmium vapor fixation" by the authors. Following fixation, all specimens were dehydrated in an ethanol dehydration series at room temperature and embedded in an Araldite/Epon resin mixture as reported in POLI & al. 1989. The sections obtained with an LKB Ultratome III microtome were stained with uranyl acetate and lead citrate following the normal procedures. The grids were then examined and photographed with a transmission electron microscope (TEM, Zeiss EM 109 N).

### X Ray microanalysis

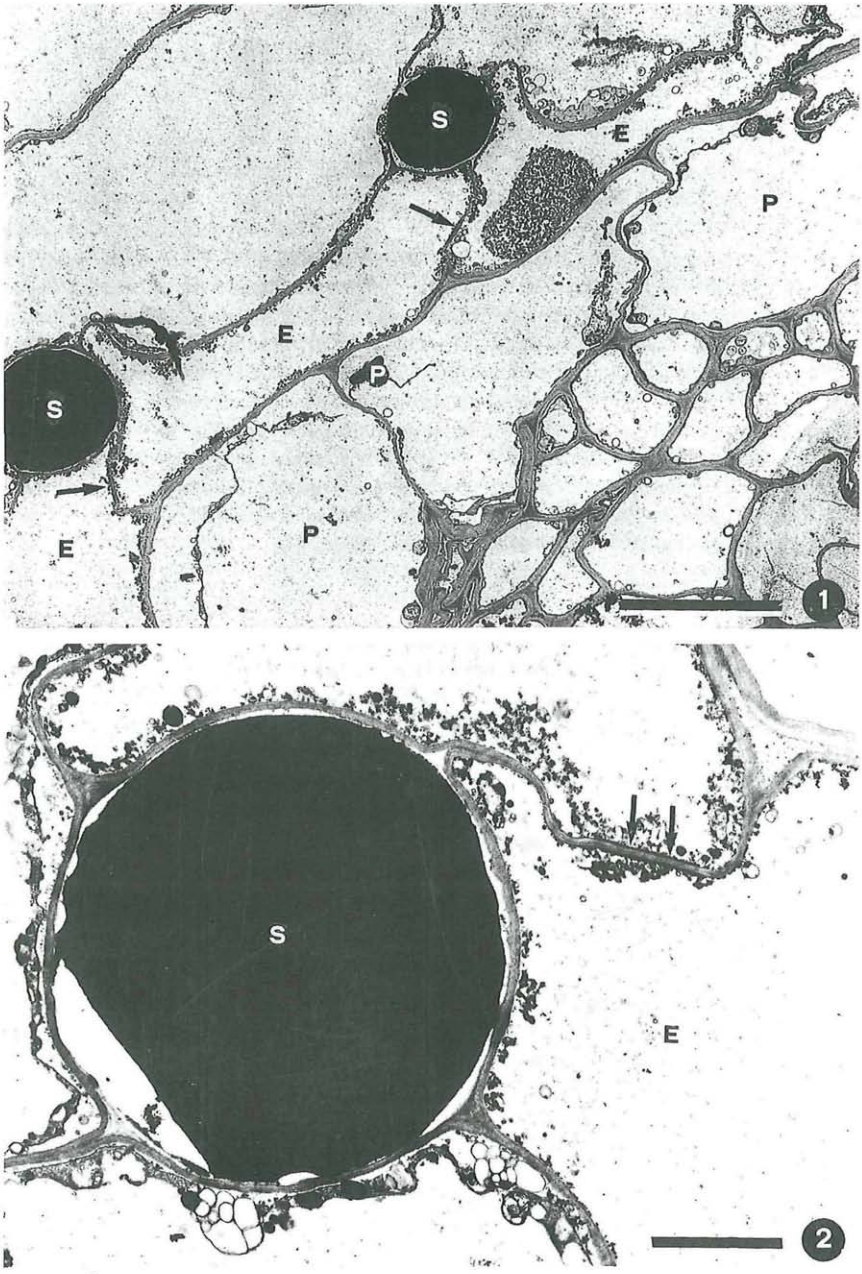
X Ray microanalysis was performed with a Link Analytical WDS spectrometer applied to a Stereoscan 360 electron microscope (Cambridge Microscope – Centro di Microscopia Elettronica, University of Ferrara). Analyses were performed on approximately 1000 Å thick unstained sections of samples fixed with $OsO_4$ for TEM, and placed on a suitable carbon support to prevent any interference between the support and the compounds to be identified. The instrument was configured to check the sulfur ($K_a$= 2,480 KeV) and was focused on the secretory cells and on the electron-dense deposits present in the reservoirs.

## Results

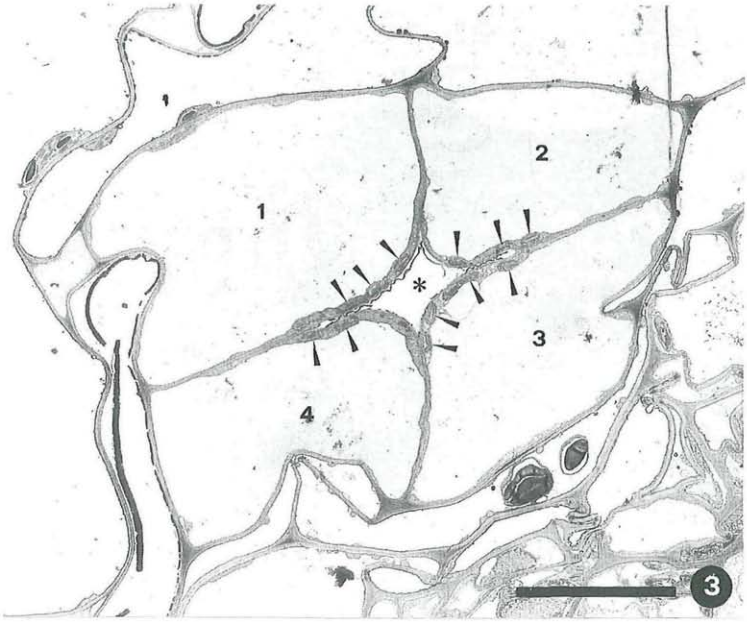
### *Tagetes patula* seedlings (10 days old plants)

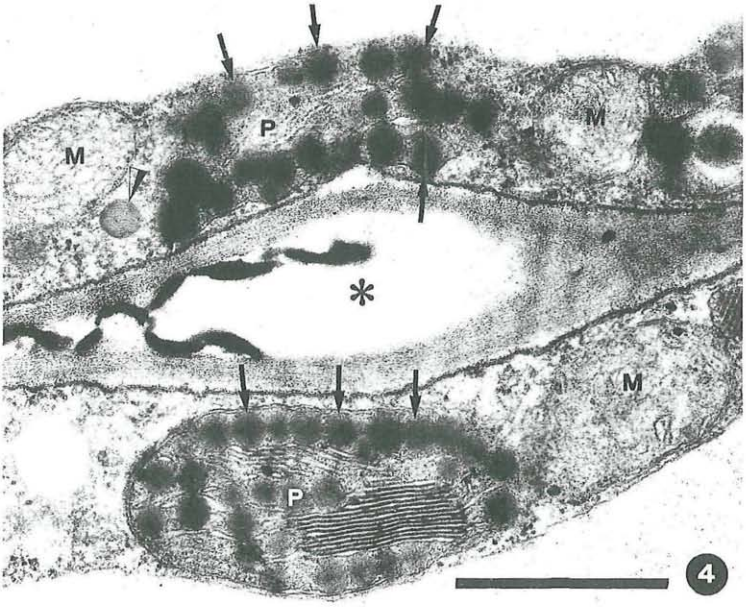
Ultrastructural analysis of the *T. patula* roots revealed some secretory canals in the area adjacent to the endodermis and an extremely electron-dense accumulation of secretion (Fig. 1). The endodermal cells appeared to mark the boundary of a schizogenous canal and presented numerous vesicles located against the wall, while it proved more difficult to identify the various cell organelles (Fig. 2). The presence of the Casparian strips confirm the endodermal location of the reservoirs (Figs. 1, 2).

In the cortical region of the hypocotyl, the epithelial cells delimited schizogenous canals (Fig. 3). At higher magnification the secretory cells showed a cytoplasm with vesicles and plastids close to the wall surrounding the secretory canal; these plastids had a particularly electron-dense stroma, numerous plastoglobules and some thylakoids (Fig. 4).




Figs. 1, 2. TEM Micrographs of root cross section of *Tagetes patula* seedlings (10 days old plants). Fig. 1. Note the secretory canals filled with secretion (S) and surrounded by glandular cells (E = endodermis; P = pericycle; arrows = Casparian strip). Bar = 10 μm. Fig. 2. At a higher magnification, the schizogenous nature of the secretory canal becomes more evident while it is difficult to identify the cell organelles in the adjacent glandular cells (E = endodermis; arrows = Casparian strip; S = secretion). Bar = 2 μm.




Figs. 3, 4. TEM Micrographs of hypocotyl cross section of *Tagetes patula* seedlings (10 days old plants). Fig. 3. The secretory schizogenous canal (asterisk) is lined by four epithelial cells (1–4). Note the particularly electron-dense cytoplasm of the epithelial cells beside the secretory cavity (arrowheads = plastids). Bar = 10 µm. Fig. 4. At a higher magnification, the cytoplasm beside the schizogenous space (asterisk) appears to have vesicles (arrowhead), plastids (P) with electron-dense stroma, few thylakoids and numerous plastoglobules (arrows; M = mitochondria). Bar = 1 µm.

The abaxial surface of the cotyledons, instead, presented secretory cavities. Also these secretory structures showed epithelial cells which bordered a schizogenous space (Fig. 5).

The cells had a particularly electron-dense cytoplasm and plastids with an electron-dense stroma, numerous plastoglobules and few thylakoids. Plastids of the neighbouring cells, instead, showed primary starch granules and a normal thylakoid system. Some deposits of electron-dense material had accumulated in the vacuoles (Fig. 6).

*Tagetes patula* flowering plants (50 days old plants)

In the epicotyl and in all other parts of the cortical region of the stem, where numerous PAR were found, the secretory cells had the same ultrastructural features as found in the hypocotyl. In particular, the glandular epithelium was made up of cells which were smaller than the surrounding cortex cells and bordered a schizogenous canal (Fig. 7). Both the vacuoles and cytoplasm of these cells appeared particularly more electron-dense in comparison with those of the parenchymatic neighbouring cortex cells. The cytoplasm was most abundant next to the secretory cavity and contained numerous vesicles (Fig. 8).

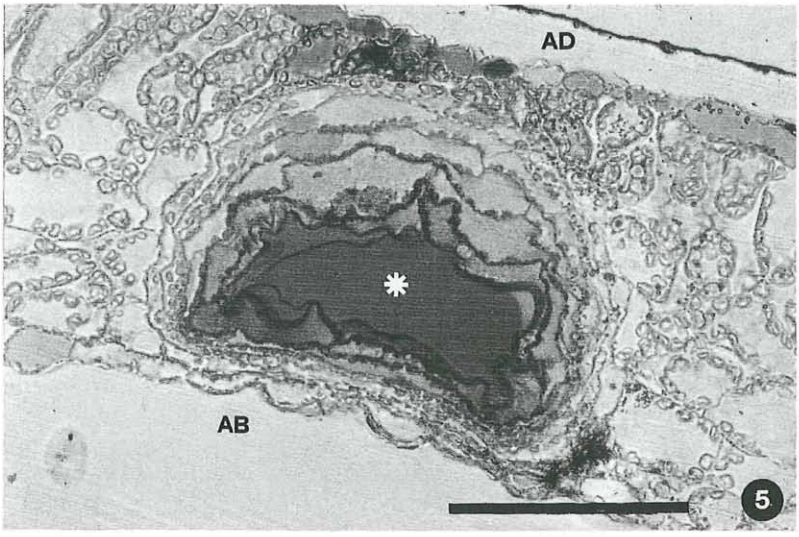
The abaxial surface of the leaves and flower bracts presented the same secretory cavities observed in cotyledons of *T. patula* seedlings.

In the flower corolla, schizogenous secretory canals, similar to those in the hypocotyl and epicotyl, were found. The secretory cells could be distinguished from the adjacent parenchymatic cells by the accumulation of vesicles next to the schizogenous canal (Fig. 9). In particular, these cells show highly electron-dense plastids and osmiophilic vesicles (Fig. 10).

To gain some indication as to the chemical composition of the secretion and of the secretory cells, X-ray microanalysis was performed on the same samples employed for ultrastructural study. Focusing the X-ray microanalysis ray on the secretion in the canals of the root, an appreciable amount of sulfur, osmium and chlorine were identified (Fig. 11, A). On the contrary, focusing the ray on the vacuoles of the cortical cell in the root, the concentration of sulfur was negligible, whereas the osmium and chlorine concentration was significant (Fig. 11, B). Similar results were obtained by applying microanalysis on the various cells of the secretory tissues in the various plant organs such as hypocotyl, epicotyl, leaf and flower; they all revealed different amounts of sulfur depending on the secretion concentration (Fig. 11, C-F).

## Discussion

Ultrastructural observation of the cells of the internal secretory system in *Tagetes patula* L. plants – 10 and 50 days old – confirms the hypothesis indicated in a previous study. The roots, hypocotyl, epicotyl and



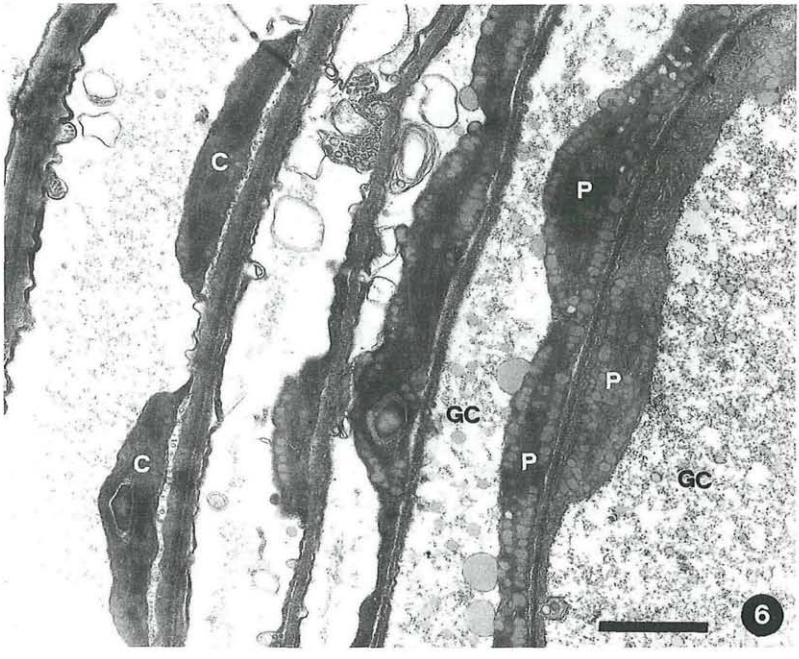
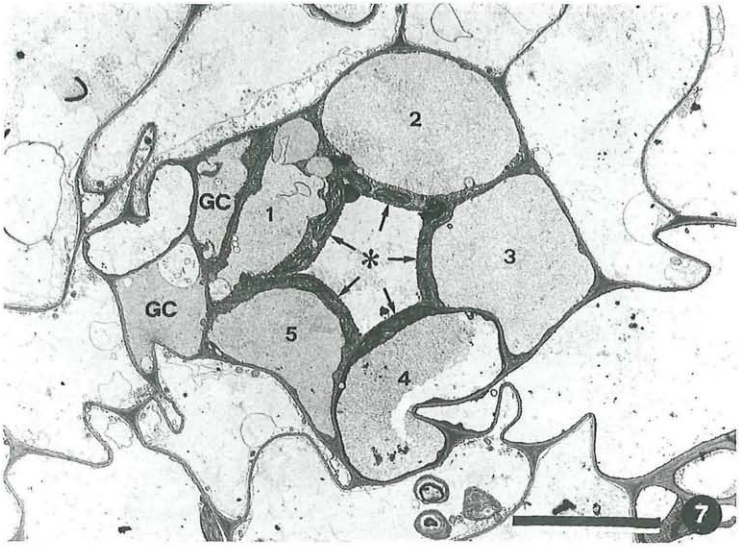
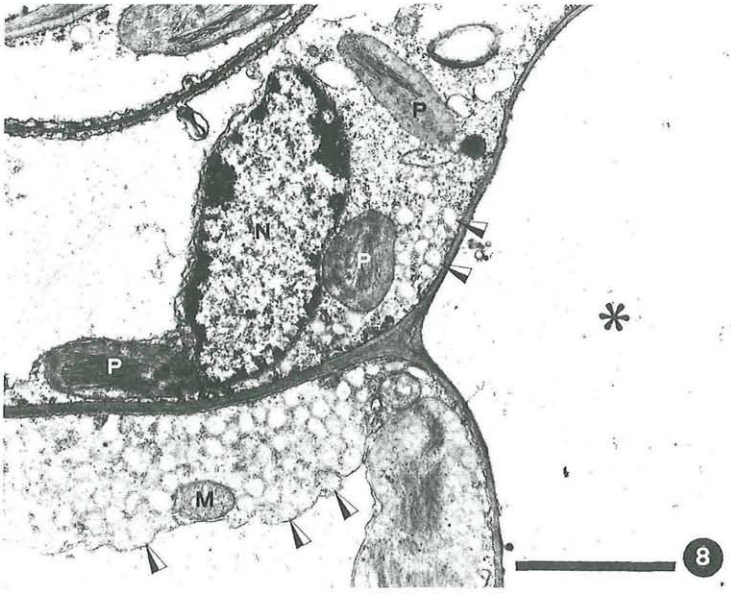



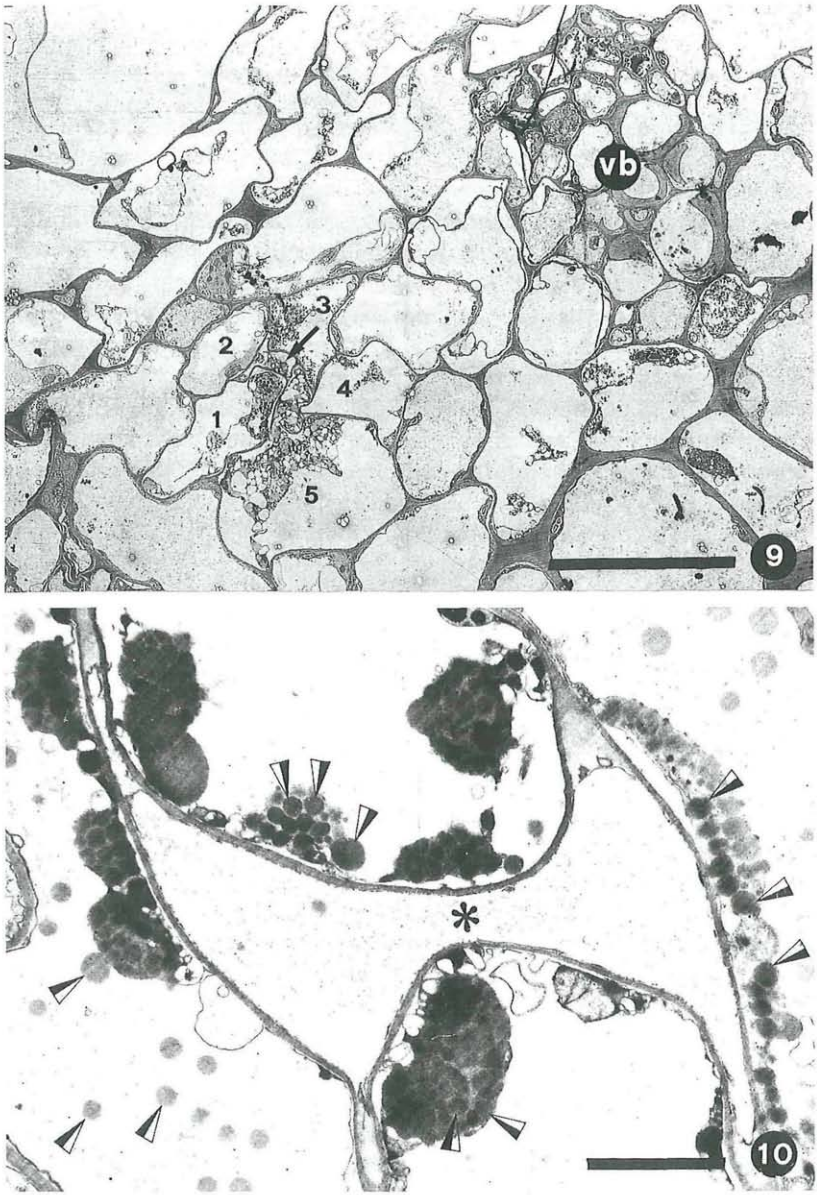

Fig. 5. Semithin cross section of cotyledon of *T. patula* (10 days old seedlings). Note the schizogenous secretory cavity bordered by glandular cells (AB = abaxial surface; AD = adaxial surface; asterisk = schizogenous space). Bar = 100 µm.

Fig. 6. Stronger magnification of glandular cells (GC) of the secretory cavity showing electron-dense cytoplasm, vacuole filled with electron-dense material, plastids (P) with few thylakoids and numerous plastoglobules. Note the chloroplast (C) of the neighbouring cells with a normal thylakoid system and starch granules. Bar = 1 µm.




Figs. 7, 8. TEM micrographs of epicotyl cross section of flowering *Tagetes patula* (50 days old plants). Fig. 7. The secretory schizogenous canal (asterisk) is lined by five cells (1-5). Two glandular cells (GC) are not in contact with the secretory cavity. Note that the secretory cells are smaller than the surrounding cortical ones and the vacuoles are more electron-dense. Again here the electron-dense cytoplasm (arrows) appears next to the schizogenous canal. Bar = 10 μm. Fig. 8. At a higher magnification, the cytoplasm of the glandular cells appears rich in vesicles (arrowheads), with numerous plastids (P) (M = mitochondria; N = nucleus; asterisk = secretory canal). Bar = 2 μm.




Figs. 9, 10. TEM micrographs of floral corolla samples of *Tagetes patula*. Fig. 9. Note the secretory canal (arrow) which can be identified by the presence of glandular cells (1–5) with vesicle-rich cytoplasm (vb = vascular bundle). Bar = 20 μm. Fig. 10. The secretory canal (asterisk) appears schizogenous in nature. Note the plastids with an highly electron-dense stroma and numerous vesicles (arrowheads) with different reactivity to osmium. Bar = 2 μm.

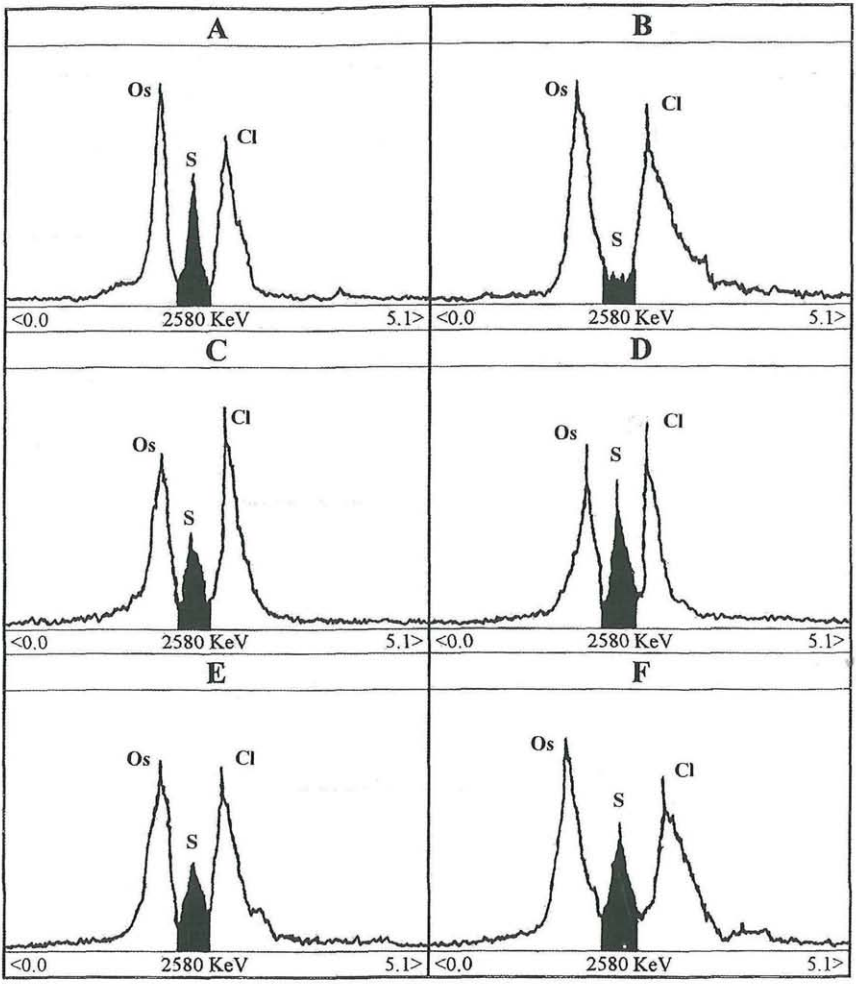



Fig. 11. Energy spectra in X-ray microanalysis configured for sulfur obtained after 100 s of analysis at 20 KeV. Spectrum A: ray focused on the secretion in the root glandular canal. Spectrum B: ray focused on the cortical cell vacuole in the root. Spectrum C: ray focused on the glandular cells of the secretory canals in the hypocotyl. Spectrum D: ray focused on the glandular cells of the secretory canals in the epicotyl. Spectrum E: ray focused on the cellular structures of the secretory cavity in the leaf. Spectrum F: ray focused on the glandular cells of the secretory canals in the flower corolla.

flower corolla have secretory canals, whereas cotyledons, young leaves and flower bracts have glandular cavities (POLI & al. 1995).

The ultrastructural characteristics of the secretory cells described in the various organs are basically similar. In particular, the epithelial cells of the schizogenous reservoirs presented an electron dense cytoplasm rich in vesicles and plastids with an electron-dense stroma, numerous plastoglobules and few thylakoids. These ultrastructural features, also described in numerous other species with secreting structures, are typical of cells which produce lipid substances (FIGUEIREDO & PAIS 1994). In particular, the presence of plastids such as those described, may be related to the production of terpene compounds (CHENICLET & CARDE 1985) which, in the secretory structures of *T. patula* are stored with the thiophenes (BICCHI & al. 1985, POLI & al. 1995). These ultrastructural findings are also supported by previous histochemical and phytochemical studies at UV microscope showing that all glandular structures, PAR and secretory cavities lipid secretion (essential oils) contain thiophene compounds emitting a light blue fluorescence (POLI & al. 1995). Moreover, the similarities pointed out in POLI & al. 1995 between the internal secretory structures of *T. patula* and the PAR described in *Ambrosia trifida* (LERSTEN & CURTIS 1989) could also reflect a relationship between the presence and/or accumulation of thiophene compounds in both these *Asteraceae* and a significance of their localization. In fact, while the presence of thiophenes and polyacetylenes in the roots of *T. patula* has been associated with the strong nematodicide activity of these plants (GOMMERS 1981), on the other hand, the similarly strong influence of the ragweed *A. trifida* on plant-parasitic nematodes, due to chemical compounds exuded from the roots (WANG & al. 1998), leads to enforce the analogy between the reservoirs of the two *Asteraceae* also by a morpho-functional and allelopathic point of view. Besides, VAN FLEET 1971 associated the morphology and enzymatic arrangement of the endodermis in the genus *Tagetes* with the production of conjugated, unsaturated sulfate compounds such as thiophenes. Later, MAKJANIC & al. 1988, employing an experimental technique generally used in biomedicine, identified an accumulation of sulfur in the endodermal cells of the root. In the present work, the use of X-ray microanalysis confirmed the presence of sulfur both in the endodermal cells and in the secretion accumulated in the schizogenous spaces in the root. This accumulation was also found in all secretory tissues in *T. patula* and this could be related to previous phytochemical studies which showed that significant amounts of thiophene compounds are accumulated in *T. patula*, both in the roots and in the other organs studied here (TOSI & al. 1988). In fact, the sulfur identified by the X-ray microanalysis can most likely be attributed to the thiophenes 2,2':5',2"-tertienyl (α-T), 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH), 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) and 5-(3-buten-1-ynyl)-

2,2'-bithienyl (BBT) that have 3, 2, 2 and 2 atoms of sulfur in the molecule respectively and have been identified as having the highest concentrations in *T. patula* (LAM & al. 1988).

The presence of osmium and chlorine in all the samples can be attributed to the process used in preparing the specimens for electron microscopy. The localization of these polyacetylene derivatives also in the epigeous organs of *T. patula* could be associated to the strong biological activity that these metabolites show against fungi (MARES & al. 1990, ROMAGNOLI & al. 1994) or to a possible role that these compounds may play against herbivores as repellant agents.

## Acknowledgements

Investigations supported by grants from Consiglio Nazionale delle Ricerche (CNR), Ministero dell'Università e della Ricerca Scientifica (MURST) of Italy. Thanks go to the Centro di Microscopia Elettronica of the University of Ferrara for the technical support provided.

## References

BICCHI C., DÁMATO A., FRATTINI C., NANO G. M., CAPPELLETTI E. & CANIATO R. 1985. Analysis of essential oils by direct sampling from plant secretory structures and capillary gas chromatography. – J. High Res. Chrom. Commun. 8: 431–435.

CHENICLET C. & CARDE J.-P. 1985. Presence of leucoplasts in secretory cells and of monoterpenes in the essential oil: A correlative study. – Israel J. Bot. 34: 219–238.

DOSDALL L. M., GALLOWAY M. M. & ARNASON J. T. 1992. Toxicity and residual action of the photoactivated compound, cyano-alpha-terthienyl, and its efficacy for reducing pre-imaginal populations of mosquitoes. – J. Am. Mosquitoes Control Assoc. 8: 166–172.

FIGUEIREDO A. C. & PAIS M. S. S. 1994. Ultrastructural aspects of the glandular cells from the secretory trichomes and from the cell suspension cultures of *Achillea millefolium* ssp. *Millefolium*. – Ann. Bot. 74: 179–190.

GOMMERS F. J. 1981. Biochemical interactions between nematodes and plants and their relevance to control. – Helminth. Abs. Ser. B, Plant Nematol. 50: 9–24.

HUDSON J. B., GRAHAM E. A., ROSSI R., CARPITA A., NERI D. & TOWERS G. H. 1993. Biological activities of terthiophenes and polyenes from the *Asteraceae*. – Planta Med. 59: 447–450.

LAM J., BRETELER H., ARNASON T. & HANSEN L. (Eds.) 1988. Chemistry and biology of naturally occurring acetylenes and related compounds (NOARC). Vol. 7. Bioactive molecules. – Elsevier, Amsterdam, Oxford, New York, Tokyo.

LERSTEN N. R. & CURTIS J. D. 1989. Polyacetylene reservoir (duct) development in *Ambrosia trifida* (*Asteraceae*) staminate flowers. – Am. J. Bot. 76: 1000–1005.

MAKJANIC J., VIS R. D., GRONEMAN A. F., GOMMERS F. J. & HENSTRA S. 1988. Investigation of P and S distributions in the roots of *Tagetes patula* L. using micro-PIXE. – J. Exp. Bot. 39: 1523–1528.

MARES D., FASULO M. P. & BRUNI A. 1990. Ultraviolet-mediated antimycotic activity of α-terthienyl on *Microsporum cookei*. – J. Med. Vet. Mycol. 28: 469–477.

Poli F., Sacchetti G. & Bruni A. 1995. Distribution of internal secretory structures in *Tagetes patula* (*Asteraceae*). – Nord. J. Bot. 15: 197–205.

— , Bonora A., Vannini G. L., Bruni A. & Fasulo M. P. 1989. Callus formation, cell suspension culture and plant regeneration in *Ranunculus serbicus* Vis. – J. Plant Physiol. 135: 637–639.

Romagnoli C., Mares D., Fasulo M. P. & Bruni A. 1994. Antifungal effects of α-tethienyl from *Tagetes patula* on five dermatophytes. – Phytother. Res. 8: 332–336.

Tosi B., Lodi G., Dondi F. & Bruni A. 1988. Thiophene distribution during the ontogenesis of *Tagetes patula*. – In: Lam J., Breteler H., Arnason T. & Hansen L. (Eds.), Chemistry and biology of naturally occurring acetylenes and related compounds (NOARC), Vol. 7. Bioactive molecules, pp. 139–150. – Elsevier, Amsterdam, Oxford, New York, Tokyo.

Turner G. W., Berry A. M. & Gifford E. M. 1998. Schizogenous secretory cavities of *Citrus limon* (L.) Burm. F. and a revaluation of the lysigenous gland concept. – Int. J. Plant Sci. 159: 75–88.

Van Fleet D. S. 1971. Enzyme localization and the genetics of polyenes and polyacetylenes in the endodermis. – Adv. Front. Plant Sci. 26: 109–143.

Wang W., Zhu X. R. & Liu W. Z. 1998. Influence of ragweed (*Ambrosia trifida*) on plant-parasitic nematodes. – J. Chem. Ecol. 24: 1707–1714.

Phyton (Horn, Austria) 41 (1): 47–48 (2001)

# Recensiones

**Klemun Marianne (Ed.) 1998. Werkstatt Natur – Pioniere der Forschung in Kärnten.** Katalog zur Ausstellung anläßlich des 150jährigen Bestehens des Naturwissenschaftlichen Vereines für Kärnten. – Gr. 8°, 300 Seiten, zahlr. Abb.; kart. – Carinthia II, 56. Sonderheft. – ATS 120,–. – ISBN 3-85328-013-7.

Der erste Abschnitt betrifft die Gründungsgeschichte (1848) des Naturwissenschaftlichen Vereines für Kärnten und des Museums, die Vereinsgeschichte inkl. Satzungen und die Aufgabenstellung der beiden Institutionen. Dabei werden Vorgeschichte, zeitgenössisches Umfeld, Museumswesen im allgemeinen, andere Museen als Vorbilder etc. behandelt. P. 129–158, als Bildteil deklariert, zeigen Abbildungen von Pflanzen und Tieren (auf p. 154 ein Photo des größten Käfers der Welt, Titanus giganteus, Cerambycidae), Sammelgeräten, Mikroskopen, Karten etc. Der „Objektteil" (p. 169–299) ist in neun Kapitel gegliedert. Die ersten beiden gelten Museen im allgemeinen und Gründung und Beschreibung des Kärntner Landesmuseums. Das nächste ist dem ersten Kustos Friedrich Simony (1813–1896) gewidmet (p. 170–176). Kapitel 5 behandelt Exkursionen, Sammeln und Präparieren. Kapitel 8 (p. 251–286) umfaßt Botanik und behandelt neben dem Botanischen Garten die Botaniker Rainer Graf (1811–1872), Friedrich Welwitsch (1806–1872), David Pacher (1816–1902), Gustav Adolf Zwanzinger (1837–1893), Markus Jabornegg-Gamsenegg (1837–1910), Hans Sabidussi (1864–1941), Anton Wallnöfer (1856–1926), Robert Benz-Albkron (1863–1921), Erwin Aichinger (1894–1985).