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Structure and development of the secretory cavities of *Myrtus communis* leaves

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Abstract

The structure and development of *Myrtus communis* L. secretory cavities has been studied in young and expanded leaves, using light and scanning electron microscope. Secretory cavities are continuously formed during leaf development, but in mature leaves the rhythm of their appearance shows steep decrease. Each secretory cavity is developed from a single epidermal cell, which undergoes a periclinal division followed by anticlinal and several oblique cell divisions. The lumen of the secretory cavity is initiated by cell wall separation, *i.e.*, schizogenously. The secretory cells line the cavity, where the secreted material is collected. Secretory cavities are covered by modified epidermal cells, which do not seem to form any special aperture. Essential oils seem to be discharged after mechanical treatment of the leaf.

Additional key words: anticlinal and periclinal divisions, essential oils, myrtle, scanning electron microscope.

Introduction

Myrtus communis L. (*Myrtaceae*; common name: myrtle) is an evergreen sclerophyll perennial shrub native to the area of Mediterranean Basin. Essential oils from leaves, flowers and fruits of the plant are widely used in food, liqueur and cosmetic industries. Although the composition of the essential oils of this species has been repeatedly studied (*e.g.* Chalchat *et al*. 1998), the information concerning the structure and development of its glands seems to be scarce and incomplete.

Two of the known families with species secreting oil substances into similar intercellular spaces are *Rutaceae* and *Myrtaceae* (Fahn 1979). Very common, in both families, is the accumulation of the essential oil in secretory cavities, *i.e*., structures under the epidermis, which consist of a large intercellular space lined by an epithelium of secretory cells. Although the mature secretory cavities of *Myrtaceae* have identical appearance to those of *Rutaceae*, it seems that they have different ontogeny (Haberlandt 1896, Carr and Carr 1970). Detailed studies on the myrtaceous secretory cavities formation have been conducted with *Eucalyptus* species. According to Carr and Carr (1970) and Fahn (1979) the secretory cavities of this species are initiated from single epidermal cells (meristemoids). According to this model, from the first divisions of the epidermal meristemoid cell, an upper and a lower cellular strata are differentiated (Fahn 1979). Most of the gland cells, including the epithelial and most of the peripheral cells develop from the lower stratum. The upper cell stratum remains in the epidermis. List *et al*. (1995) support that the oil glands of *Melaleuca alternifolia* (Maiden & Betche) Cheel, another member of *Myrtaceae*, are formed either from an epidermal cell or a cell adjacent to the epidermis. Whether the model suggested by Carr and Carr (1970) and formulated by Fahn (1979) for *Eucalyptus* or the model of *Melaleuca alternifolia* (List *et al*. 1995) is applicable to *Myrtus communis* remains to be investigated.

Another question in the literature is the origin of the lumen of a secretory cavity, *i.e*., whether the lumen develops from cell wall separation (schizogenous origin) or from cell lysis (lysigenous origin). Views as to whether the lumen of a certain cavity develops schizogenously, lysigenously or schizo-lysigenously are contradictory (see review by Fahn 1988). Different aspects exist concerning the manner of the formation of the lumen in *Rutaceae* (see Fahn 1979). Concerning the secretory cavities of *Citrus deliciosa*, Bosabalidis and

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Tsekos (1982b) supported the lysigenous model of the cavity formation. The ontogenetic investigations carried out so far, showed that in the family of *Myrtaceae* the schizogenous formation of the cavity lumen is a rather common phenomenon (Carr and Carr 1970, Fahn 1979). By contrast, List *et al*. (1995), working with *Melaleuca alternifolia* support the schizogenous or schizo-lysigenous origin of the cavity lumen.

According to Haberlandt (1896), the content of the secretory cavity of *Rutaceae* is released into the atmosphere through a cap consisted of two to four specialized epidermal cells forming an opening resembling the stomatal pore. The same author observed that the secretory cavities of *Myrtus communis* release their content to the atmosphere after mechanical bending of the leaf and underlines that the epidermal cells through which the content of the secretory cavity is released to the atmosphere, should be different from that of *Rutaceae*. Carr and Carr (1970) working with *Eucalyptus* focused on the ontogeny and the origin of the lumen of the secretory cavity, but they did not make any special reference to the structure of the epidermal cells covering the mature secretory cavity. On the other hand, List *et al*. (1995) observed that the mature secretory cavities of *Melaleuca alternifolia* are capped by modified epidermal cells and they concluded that under a differential pressure the essential oil is able to pass through the epidermal lining of the cavity.

This work describes the ontogeny and the structure of *Myrtus communis* leaf secretory cavities as it has been revealed under the light and scanning electron microscope (SEM) and the structure of the epidermal cells above the secretory cavities as it has been revealed under SEM.

Materials and methods

Leaves were collected from wild growing individuals near Patras University Campus (38^o 14´ N, 21^o 44´ E, alt. 125 m). Leaves of three developmental stages (20 leaves from each developmental stage) were sampled: 2.5 - 3 cm long leaves, *i.e.*, fully expanded, 1.5 to 2 cm long leaves which are mature but not fully expanded, and young leaves <1 cm long. Samples from the blade from all three developmental stages of the leaf were carefully cut and fixed in 5 % glutaraldehyde in phosphate buffer at pH 7 at room temperature for 2 h. Tissue was then post-fixed in 1 % OsO_4 at 4 °C and dehydrated in a graded acetone series. For light microscopy, tissue samples were embedded in *Durcupan ACM* (*Fluka*, Buchs, Switzerland). Semi-thin sections (1 - 2 μm) of plastic-embedded tissue made on a *Reichert Om-U2* (Wien, Austria) microtome using glass knives, were stained with Toluidine Blue O. The sections were examined and digitally recorded with a *Zeiss Axioplan* microscope (*Zeiss*, Oberkochen, Germany) equipped with a video camera (*Sony, SSC-DC58AP*). For the determination of the number and density of the secretory cavities, 20 leaves from each developmental stage were observed and photographed using transmitted illumination under a stereomicroscope (*STEMI 2000-C*, *Zeiss*) equipped with a video camera as above. The digitally recorded micrographs were then used to determine dimensions, number and density of secretory cavities, using the *Image Tool 1.25* program (University of Texas Health Science Center, San Antonio, Texas, USA). For Scanning Electron Microscope (SEM) the dehydrated tissue samples were critical point dried, mounted with a double adhesive tape on stubs, sputter coated with gold and observed with a *JEOL 6300* (Japan) Scanning Electron Microscope (SEM). Non-fixed, uncoated leaves placed in the vacuum of the SEM were also observed. SEM pictures were digitally recorded.

Results

Numerous secretory cavities (400 - 1700 per leaf, Table 1) are located adjacent to the epidermis of *Myrtus communis* leaves, showing similar distribution to adaxial and abaxial surfaces. The diameter of mature secretory cavities ranges between 30 and 100 μm. Although secretory cavities appear very early during leaf development, comparing the total number of secretory cavities in three developmental stages of the leaf (Table 1), it is concluded that secretory cavities are continuously formed, but from the corresponding densities (number of secretory cavities per mm^2; Table 1) it is evident that the rhythm of secretory cavity

Table 1. Total number and density of secretory cavities of *Myrtus communis* leaves. Means ± SE, n = 20.

Leaves	Leaf length [cm]	Number of secretory cavities [leaf^{-1}]	Density of secretory cavities [mm^{-2}]
Young	<1	402 ± 33	23.65 ± 0.8
Non-fully expanded	1.5 - 2	1176 ± 49	13.71 ± 0.6
Fully expanded	2.5 - 3	1702 ± 47	9.53 ± 0.3

appearance decreases during leaf expansion.

Light and scanning electron microscope examination of fixed plant material revealed that secretory cavities of *M. communis* leaves develop from single epidermal cells (meristemoids). The epidermal initial cell undergoes a periclinal division to give rise to two cells (Fig. 1*A*), which in turn undergo anticlinal divisions (Fig. 1*B*,*C*). Further periclinal (Fig. 1*D*,*E*) and anticlinal (Fig. 1*F*) divisions form a group of cells with dense cytoplasm. Further divisions give rise to both the future secretory cells as well as to the cells surrounding the cavity (Fig. 1*G*). The walls of the central cells separate, forming the lumen of the secretory cavity (Fig. 1*H*, 2*A*-*C*). The secretory cells, forming the secretory epithelium, line the cavity retaining intact cell walls (Fig. 1*I*, 2*B*-*D*). The essential oil is collected in the cavity (Fig. 2*E*). When a leaf was observed under the vacuum of the SEM, droplets of the secreted substance were observed on leaf surface (Fig. 2*F*).

SEM examination of fixed and critical point dried plant material revealed that each secretory cavity is covered by modified epidermal cells. As seen in surface view, the number of these modified epidermal cells ranges from one to four (Fig. 3*A*-*D*). According to our observations, these "cap" cells do not seem to form any special opening.

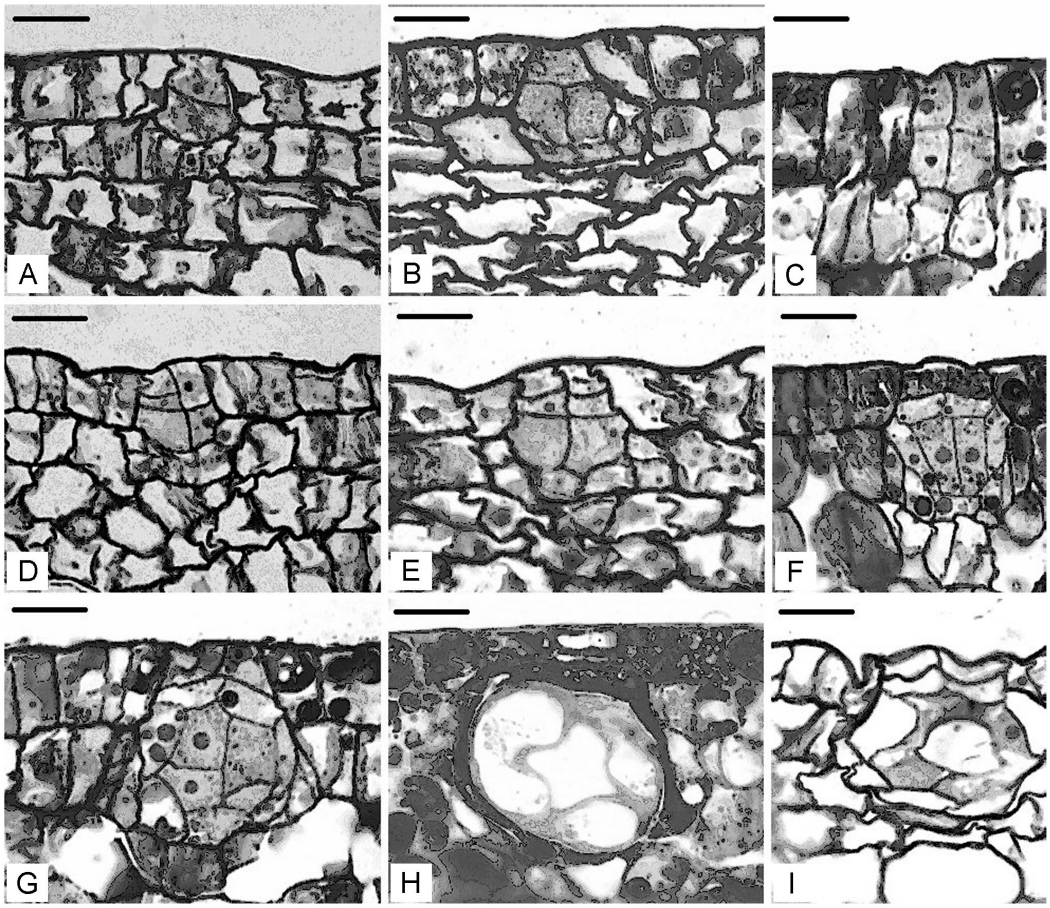

Fig. 1. Light microscope micrographs of cross-sectioned *Myrtus communis* leaves, showing the development of a secretory cavity. A periclinal division of a meristemoid cell initiates the formation of a secretory cavity (*A*); each derivative cell undergoes an anticlinal division (*B*,*C*). Periclinal, anticlinal and oblique divisions of the derivatives give rise to all cells of the secretory cavity (*D*-*G*). Separation of secretory cell walls forms the lumen of the secretory cavity (*H*); inner walls of secretory cells remain intact (*I*). *Bars* represent 20 μm.

Discussion

Citrus deliciosa (*Rutaceae*) secretory cavities develop from a pair of initial cells, an epidermal and another meristematic cell of the sub-epidermal cell layer (Bosabalidis and Tsekos 1982a). On the other hand, according to Carr and Carr (1970), *Eucalyptus* (*Myrtaceae*) secretory cavities develop from single

epidermal cells. Data reported here show that the formation of the secretory cavities of *M. communis* leaves follows the model of *Eucalyptus*, as it has been described by Carr and Carr (1970) and further formulated by Fahn (1979). It is tempting to hypothesize that the secretory cavity ontogeny from a single meristemoid cell observed both in *Eucalyptus* species and in *Myrtus communis*, may be common in the family of *Myrtaceae*.

Contradictory views have been suggested concerning the origin of the intercellular space (lacuna or lumen) of the secretoty cavity (reviewed by Fahn 1979). It has been supported that in several species of *Rutaceae* (*Citrus medica, Citrus sinensis, Poncirus* sp.) the lumen is lysigenously formed. This has been reported also for *Citrus deliciosa* (Bosabalidis and Tsekos 1982b). On the contrary, the schizogenous or/and the schizo-lysigenous model has been supported for *Ruta graveolens* (see review by Fahn 1979). By contrast, Carr and Carr (1970)

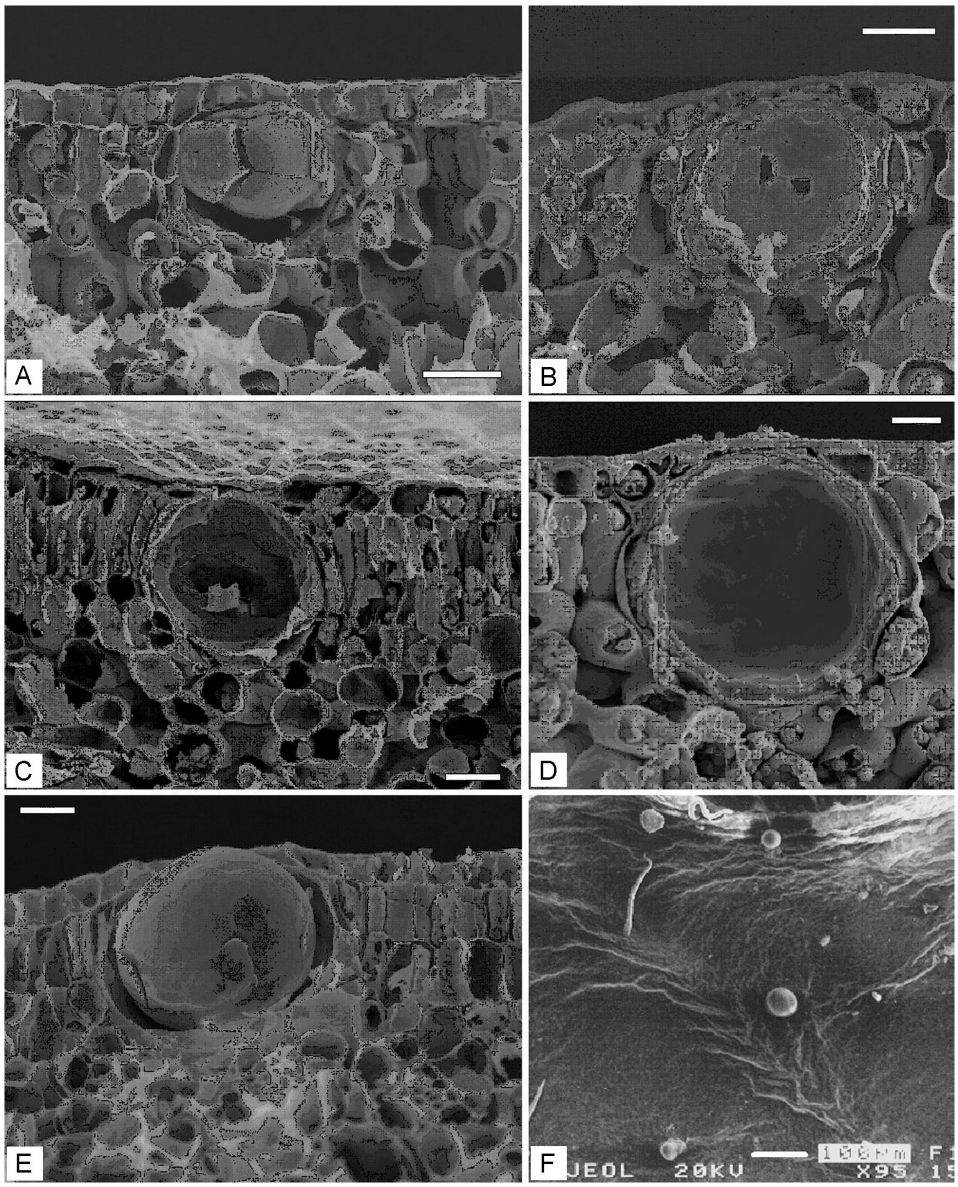

Fig. 2. SEM micrographs of leaf secretory cavities in *Myrtus communis*: cross-sectioned leaves (*A-E*), surface view of a leaf (*F*). *A-C* show intact secretory cells and the initiation of the lumen; *D* - mature secretory cavity; *E* - the content of a secretory cavity. *F* - droplets of oil appeared above secretory cavities when a fresh leaf was observed under the vacuum of the SEM. *Bar* in *A*, *C*, *D*, *E* represents 20 μm, in *B* 50 μm and in *F* 100 μm.

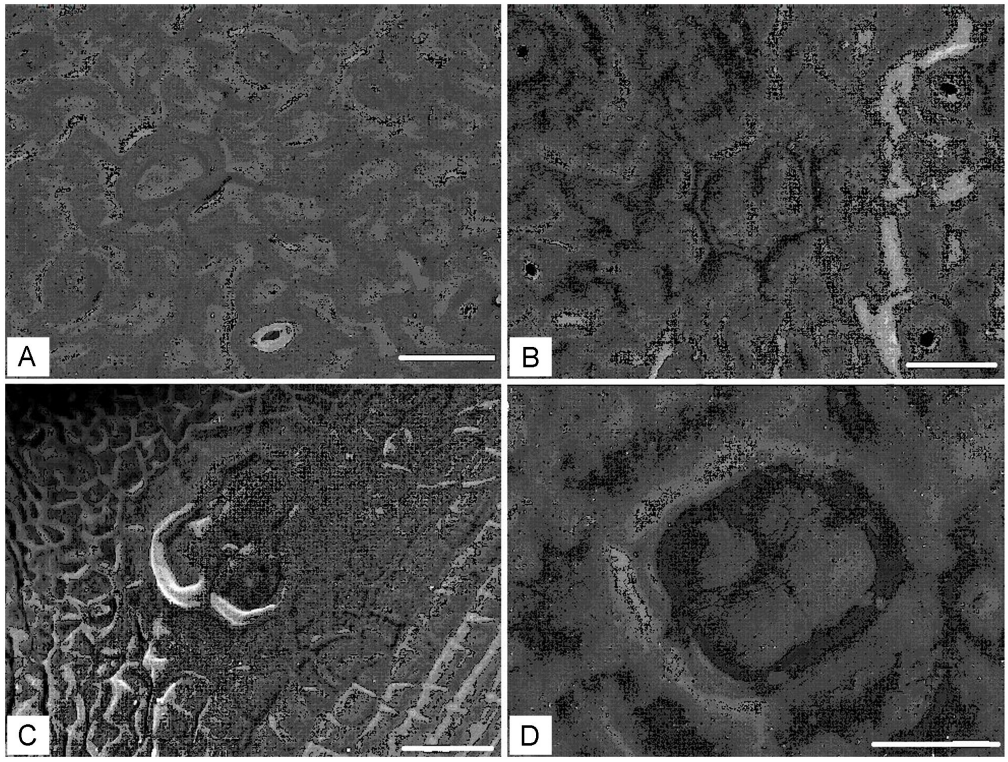

Fig. 3. Surface view of *Myrtus communis* leaves under SEM. Micrographs show the modified epidermal cells above secretory cavities. The "cap" of the cavity consists of one (*A*) to four (*D*) cells. *Bars* represent 20 μm.

and Fahn (1979) supported that the lumen of the secretory cavity of *Eucalyptus* species is schizogenously formed.

Yet, List *et al*. (1995), working with the secretory cavities of *Melaleuca alternifolia,* another member of *Myrtaceae*, suggested a schizogenous or a schizo-lysigenous formation of the cavity. Our data on *Myrtus communis* secretory cavities support also the schizogenous formation of the lumen. It seems that the schizogenous formation of the lumen of the secretory cavity is common in the family of *Myrtaceae*.

According to Haberlandt (1896), the secretory cavity of *Ruta graveolens* is covered by modified epidermal cells, which form an aperture like stomatal pore. He hypothesized also that *Myrtus communis* should have a different covering structure from *Ruta*. Carr and Carr (1970) in their detailed work with *Eucalyptus* species did not make any special reference concerning the epidermal cells above the secretory cavity. List *et al.* (1995) reported that glands of *Melaleuca alternifolia* leaves are covered by modified epidermal cells and that under a differential pressure the secreted oil was able to pass the epidermal lining. SEM examination of *M. communis* leaves revealed that its secretory cavities are covered by one to four modified epidermal cells, without any opening, in agreement with Haberlandt' s hypothesis. Under the vacuum of the SEM column, the secreted material passes through the cap cells covering the secretory cavity; this is also in agreement with the case of *Melaleuca alternifolia* (List *et al*. 1995). These data are also in agreement with Haberlandt's statement that in the case of *M. communis* the essential oil is discharged after mechanical bending of the leaf.

References

Bosabalidis, A., Tsekos, I.: Ultrastructural studies on the secretory cavities of *Citrus deliciosa* Ten. I. Early stages of the gland cells differentiation. - Protoplasma **112**: 55-62, 1982a.

Bosabalidis, A., Tsekos, I.: Ultrastructural studies on the secretory cavities of *Citrus deliciosa* Ten. II. Development of the essential oil-accumulating central space of the gland and process of active secretion. - Protoplasma **112**: 63-70, 1982b.

Carr, D.J., Carr S.G.M.: Oil glands and ducts in *Eucalyptus* l'Herit. II. Development and structure of oil glands in the embryo. - Aust. J. Bot. **18**: 191-212, 1970.

Chalchat, J.C., Garry, R.P., Michet, A.: Essential oil of myrtle (*Myrtus communis* L.) of the Mediterranean Littoral. - J. Essential Oil Res. **10**: 613-617, 1998.

Fahn, A.: Secretory Tissues in Plants. - Academic Press,

London 1979.
Fahn, A.: Secretory tissues in vascular plants. - New Phytol. **108**: 229-257, 1988.
Haberlandt, G.: Physiologische Pflanzenanatomie. 2nd Ed. - W. Engelmann Verlag, Leipzig 1896.
List, S., Brown, P.H., Walsh, K.B.: Functional anatomy of the oil glands of *Malaleuca alternifolia*. - Aust. J. Bot. **43**: 629-641, 1995.