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# Glandular hairs and secretory ducts of *Matricaria chamomilla* (Asteraceae): morphology and histochemistry

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The aim of the present work is to characterize the glandular hairs and the secretory ducts of *Matricaria chamomilla* (Asteraceae) morphologically, anatomically and histochemically. The glandular hairs are multicellular and biseriate with two basal cells, two peduncle cells and a secretory head composed of six cells. The histochemical tests show that the glands are positive for lipids, essential oils, sesquiterpene lactones, and pectic-like substances. The secretory ducts show a lumen surrounded by a layer of specialized cells, with the exception in the root where the ducts do not show any secretory epithelium. The histochemical tests show that the ducts are always positive for lipids, while the tests for the presence of essential oils and sesquiterpene lactones are heterogeneous in the plant organs considered.

Key words: Asteraceae, glandular hairs, histochemistry, *Matricaria chamomilla*, morphology, secretory ducts

## Introduction

Many species belonging to Asteraceae are applied in therapy, because of their high content of pharmacologically active compounds produced by the secretory tissues. True chamomile, *Matricaria chamomilla* (= *Chamomilla recutita*), is one of the oldest pharmaceutical plants. Today, the plant is used in home-made remedies, mainly as a mild sedative and spasmolytic, and in pharmaceutical preparations as a stimulant, diaphoretic, anti-inflammatory, carminative, nervine, but also in perfumery and cosmetics (Hegnauer 1964, Uphof 1968, Ferri & Capresi 1979, Redaelli *et al*. 1981, Della Loggia *et al*. 1982, Schulz & Albroscheit 1988, Bruni 1999). The pharmacological activity of chamomile is well documented and mainly ascribed to flavonoids, coumarinic derivatives and essential oils (Hegnauer 1964, Schilcher 1987, Hausen 1992, Mares *et al*. 1993).

The active principles are extracted from the inflorescences, which are the most commonly used vegetal drug in Europe, but also from leaves and stems. Only dried capitula, or parts of them (tubular flowers, ligulate flowers and receptacle), are used in the preparation of home-made teas; different therapeutic activities are attributed to each commercial type of chamomile (Bruni 1999).

Little is known about the presence of secretory structures in chamomile. The presence of ducts and glandular hairs in leaves, stems and capitula has been recorded (Lassanyi *et al*. 1978, Tumino & Ragusa 1979, Halásová *et al*. 1980, Repčák *et al*. 1984, 1993), but all the information regarding their morphology and location is dated and incomplete. In this paper, the secretory structures of *M. chamomilla* both in the vegetative and reproductive organs were investigated with respect to morphology and location. A histochemical investigation was also performed on the main secretion compounds to identify any difference between the two types of secretory structures.

## Material and methods

The plant material examined for this study came from a population of *M. chamomilla* cultivated in the Botanical Gardens of Pisa. As a control, we also analyzed a natural population (in a private garden near Lucca, Italy). *Exsiccata* are in PI.

### Light microscopy (LM)

Sections of fresh material (root, stem, leaf, inflorescence peduncle and receptacle) were cut at 25 $\mu$m with a Leitz 1720 cryostat at –16 °C. Other sections were cut at 3 $\mu$m with a Leica 2055 microtome after fixing the material in FAA (10% formaldehyde, 5% acetic acid and 45% ethanol; Sass 1958) and embedding it in LR White acrylic resin (SIGMA). Parts of leaves, inflorescence peduncle, involucral bracts, receptacle, ligulate and tubulose flowers were also observed as a whole.

All materials underwent the following histochemical tests: Toluidine Blue (O'Brien & McCully 1981) as a generic stain; Alkanna tincture (Faure 1914), Nile blue (Cain 1947) and Sudan red 7B (Brundrett *et al*. 1991) for total lipids; Nadi reagent (David & Carde 1964), Sudan III and glacial acetic acid (Johansen 1940) for essential oils; concentrated sulphuric acid (Geissmann & Griffin 1971) for sesquiterpene lactones; Delafield hematoxylin (Faure 1914) for pectic-like substances. Standard control procedures were carried out at the same time.

### Fluorescence microscopy (FM)

For fluorescence microscopy, whole flowers and sections of leaves, stems and roots were used. Flavonoids were detected by fluorescence induction with fluorochrome aluminium chloride and ethanol (Guerin *et al*. 1971). A Leica DM LB fluorescence microscope with Group A filters (BP 340-380, dichroic mirror 450, LP 430 arrest filter) was used.

### Scanning electron microscopy (SEM)

Leaves, stems and flower heads were fixed in glutaraldehyde (2% with buffer solution at pH 7.4), dehydrated in an alcohol and acetone mixture, critical point dried, and sputter-coated with gold. Samples were examined at 15 KV with a Cambridge Stereoscan 90 scanning electron microscope.

## Results

### Glandular hairs

#### Morphology

The glandular hairs were multicellular and biseriate. Their morphology was the same in all organs of the plant. They consisted of two basal cells, two peduncle cells and a secretory head formed of six cells. The cells of the secretory head had a thin cuticle which lifted to form a large subcuticular chamber for the secretory material, which began to develop from the apical cells (Fig. 1A). SEM observations did not reveal any pore or crack, through which the secretory material could exude. The material was not released until the cuticle broke, either due to a mechanical event or at the end of the life of the gland (Fig. 1B). When glandular hairs degenerated, their cells collapsed; the degeneration began from the apical cells.

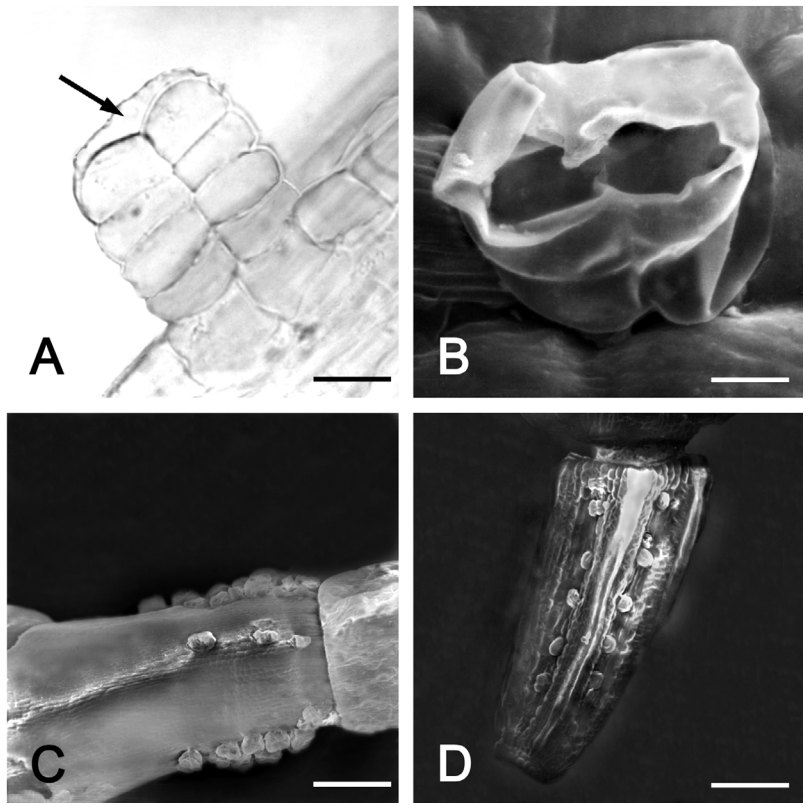


**Fig. 1.** Morphology and localization of the glandular hairs of *Matricaria chamomilla*. — **A**: Glandular hair of ligulate flower formed of two basal cells, two peduncle cells and a secretory head composed of six cells. The arrow shows the large subcuticular chamber of the secretory head; (scale bar = 0.9 µm). — **B**: Glandular hair of leaf showing a broken cuticle; (scale bar = 0.5 µm). — **C**: Tubulose flower showing glandular hairs mainly located at the base of the corolla; (scale bar = 8.3 µm). — **D**: Glandular hairs of ovary arranged in parallel rows on the external epidermis; (scale bar = 12.5 µm). **A** LM, **B**–**D** SEM.

### Location

There were no glandular hairs on the inflorescence peduncle or on the receptacle (Table 1). They were few on involucral bracts, leaves and stems. The corolla showed glandular hairs only on the abaxial surface, mainly located at the base (Fig. 1C). Many hairs could be observed on the ovary, where they were arranged in parallel rows on the external epidermis (Fig. 1D).

### Histochemistry

The tests for lipids, essential oils, sesquiterpene lactones, and pectic-like substances were positive (a few examples are shown in Fig. 2; *see* also Table 2). The presence of flavonoids could not be shown because of the yellow autofluorescence that masked the action of the fluorochrome. No differences between tubular and ligulate flowers from a histochemical point of view were detected.

## Secretory ducts

### Morphology

The secretory ducts showed the same morphol-

**Table 1.** Localization of secretory structures in the floral and vegetative parts of *Matricaria chamomilla*. – absence, + presence.

| Secretory structures | Root | Stem | Leaf | Inflorescence peduncle | Receptacle | Bract | Corolla | Ovary | Stigma |
|---|---|---|---|---|---|---|---|---|---|
| Glandular hairs | – | + | + | – | – | + | + | + | – |
| Secretory ducts | + | + | + | + | + | + | – | – | + |

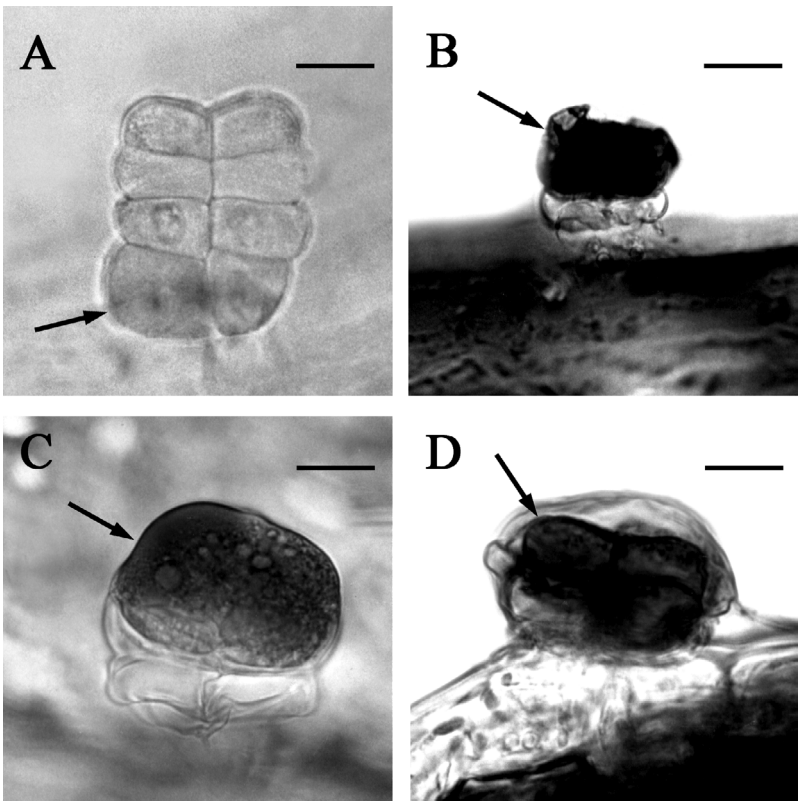


**Fig. 2.** Histochemistry of the glandular hairs of *Matricaria chamomilla*. — **A**: Hair of tubulose flower positive for total lipids (Alkanna tincture, scale bar = 2 µm). — **B**: Hair of tubulose flower positive for essential oils (Nadi reagent, scale bar = 2.8 µm). — **C**: Hair of tubulose flower positive for sesquiterpene lactones (concentrated sulphuric acid, scale bar = 1.6 µm). — **D**: Hair of ligulate flower positive for pectic-like substances (Delafield hematoxylin, scale bar = 1.6 µm). All LM. Arrows indicate the material stained.

ogy in all organs of the plant, except in the root, where the ducts did not show any secretory epithelium. In fact, they looked like intercellular spaces with a quadrangular shape, and they were located between the endodermis and the cortex (Fig. 3A). It seemed that the ducts originated in close relation with the endodermis. In a longitudinal view, they appeared elongated and unbranched. In the other organs, the lumen of the ducts was surrounded by a layer of specialized cells (Fig. 3B–E). The ducts were variable both in length and width. Anastomosis between neighboring secretory ducts was not observed.

## Location

The secretory canals were present in the root, stem, leaf, peduncle of the inflorescence, receptacle, involucral bracts, and stigma of ligulate and tubular flowers (Table 1). In the root, the ducts were located out of the endodermis, in few discrete arrays just outside the primary phloem; each array consisted of a few ducts (Fig. 3A). In the stem, there were many secretory ducts distributed in the cortical parenchyma, near the cribro-vascular bundles (Fig. 3B). Many secretory ducts, with large lumen, were located in the receptacle

**Table 2.** Histochemistry of the glandular hairs of *Matricaria chamomilla*. + positive, ++ strongly positive.

| Compounds tested | Stem | Leaf | Bract | Corolla | Ovary |
|---|---|---|---|---|---|
| Lipids | + | + | ++ | ++ | ++ |
| Essential oils | + | + | + | + | + |
| Sesquiterpene lactones | + | + | + | + | + |
| Pectic-like substances | + | + | + | + | + |

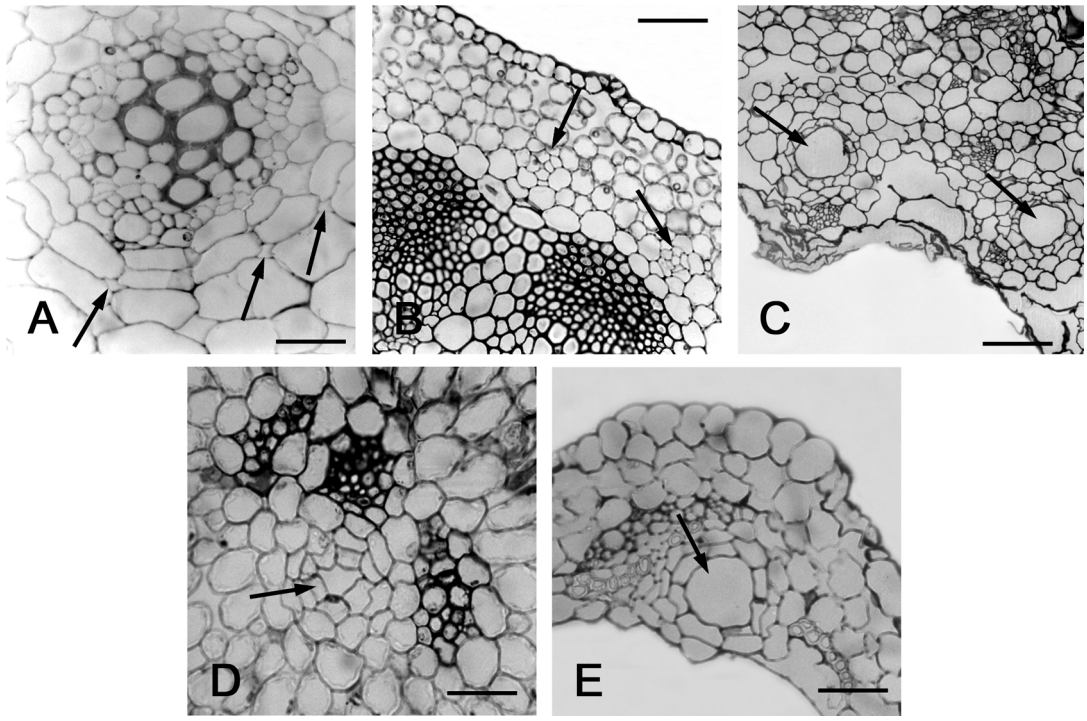


**Fig. 3.** Morphology and localization of the secretory ducts of *Matricaria chamomilla*. — **A**: Secretory ducts (arrows) in the root. They are quadrangular-shaped intercellular spaces located between the endodermis and the cortex; (scale bar = 3.3 µm). — **B**: Secretory ducts (arrows) in the stem, located in the cortex near the bundles; (scale bar = 5.5 µm). — **C**: Secretory ducts (arrows) in the receptacle showing a large lumen; (scale bar = 6.6 µm). — **D**: Secretory duct in the leaf (arrow) localized near the veins showing a small lumen; (scale bar = 2.8 µm). — **E**: Secretory duct (arrow) in the bract with a large lumen located in a median position, parallel to the vein; (scale bar = 5 µm). All LM and stained with Toluidine Blue (TBO).

and inflorescence peduncle, in the parenchymal tissue around the cribro-vascular bundles (Fig. 3C). Also in the leaf, the secretory ducts were arranged all along the veins. Their number varied from one to four and they had a small lumen (Fig. 3D). In the bracts, there was only one secretory duct with a large lumen, located in a median position, parallel to the vein (Fig. 3E). The stigma showed two secretory ducts, one in each lobe.

## Histochemistry

The secretory ducts were always negative for the presence of pectic-like substances (Table 3). The tests for the presence of lipids were always positive (Fig. 4). The tests for the presence of essential oils and sesquiterpene lactones gave different results depending on the plant organs considered (Fig. 4 and Table 3). Also in the

**Table 3.** Histochemistry of the glandular ducts of *Matricaria chamomilla*. – negative, + positive, ++ strongly positive.

| Compounds tested | Root | Stem | Leaf | Inflorescence peduncle | Receptacle | Bract | Stigma |
|---|---|---|---|---|---|---|---|
| Lipids | + | + | + | ++ | ++ | ++ | ++ |
| Essential oils | – | + | + | ++ | ++ | ++ | ++ |
| Sesquiterpene lactones | – | + | + | + | + | + | + |
| Pectic-like substances | – | – | – | – | – | – | – |

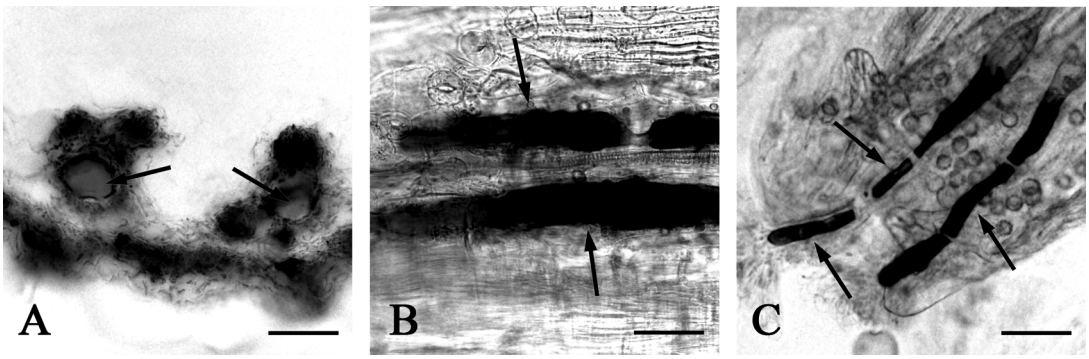


**Fig. 4.** Histochemistry of the secretory ducts of *Matricaria chamomilla*. — **A**: Ducts of receptacle positive for total lipids (trasversal section, Sudan Red 7B, scale bar = 8.3 µm). **— B**: Duct of bract positive for essential oils (longitudinal view, Nadi reagent, scale bar = 4 µm). — **C**: Ducts of stigma positive for sesquiterpene lactones (longitudinal view, concentrated sulphuric acid, scale bar = 4 µm). All LM. Arrows indicate the material stained.

secretory ducts, the presence of flavonoids could not be detected because of their intense yellow autofluorescence.

## Discussion

Two different types of secretory structures coexist in *Matricaria chamomilla*. The multicellular biseriate glandular hairs are similar to those commonly found in the family, which have been described for many other species (Vermeer & Peterson 1979, Pagni 1995, Pagni & Masini 1999, Corsi & Nencioni 1995, Duke & Paul 1993, Pagni *et al*. 2003, Ciccarelli *et al*. 2007).

The accumulation of secretion in a subcuticular chamber and its release following the breaking of the cuticle is a common feature of many glandular hairs (Schnepf 1974, Dell & McComb 1974, Hammond & Mahlberg 1977, Ascensao & Pais 1982, Duke & Paul 1993, Pagni 1995, Pagni & Masini 1999, Pagni *et al*. 2003, Ciccarelli *et al*. 2007).

In chamomile, hairs can be found on the vegetative organs, but primarily on the capitulum, where they are in large amounts, especially on the ovary, where they are heterogeneously distributed. Tumino and Ragusa (1979) showed the presence of glandular hairs on involucral bracts, corolla and ovary. Halásová *et al*. (1980) and Repčák *et al*. (1984) reported the presence of hairs on the vegetative organs and parts of the capitulum, but not on the ovary. Ciccarelli *et al*. (2007) described the usefulness of the presence, morphology and distribution of glandular hairs of the ovary for Asteroideae taxonomy. For example, hairs of *M. chamomilla*, like other Anthemideae species, show a characteristic intercostal distribution on the ovary.

Because of their abundance, the presence of secretory hairs in the ovary is significant. Their secretion, beyond carrying out ecological actions, such as providing floral rewards to pollinators and chemical defence, could probably play an important role in the maturation process of fruits and seeds. The presence of secretory tissues in the ovary is very frequent. Structures of different types, such as secretory ducts and isolated cells, can be found in many species in other families (Robson 1981, Pagni *et al*. 1986).

The secretory ducts of *M. chamomilla* are typical schizogenic ducts; their morphology is similar to that reported for other species of Asteraceae (Ascensao & Pais 1988, Corsi & Nencioni 1995, Pagni 1995, Pagni & Masini 1999, Pagni *et al*. 2003). The root ducts lacking a specialized epithelium are observed in the family (Tetley 1925, Sacchetti *et al*. 1997, Pagni & Masini 1999, Pagni *et al*. 2003). According to Tetley (1925) and Williams (1954), since these ducts are so close to the phloem, they probably aid the sieve tubes in the transfer of organic material. As for glandular hairs, the secretory ducts can be found in the vegetative organs, but primarily in the capitula. Our findings on their location are in agreement with Repčák (1984)

and Halásová (1980), though not completely, since those authors did not report the presence of ducts in the stigma of the ligulate flowers. We found ducts in the stigma of both ligulate and tubulose flowers, which are characterized by a very large lumen.

Comparing the distribution of secretory ducts with that of glandular hairs in the capitulum (*see* Table 1), we noted that, with the exception of involucral bracts, they are always located in different floral parts. This may suggest that the two types of secretory structures play a partially different ecological role.

The histochemistry showed slight differences in secretion: the hairs have a hydrophilic and lypophilic secretion, the ducts do not show any pectic-like substances. The hairs, because of their partially mucilaginous secretion and early development, may also have a lubrificant role to facilitate the process of distension of the organ in which they are located. From a phytotherapeutic point of view, the mucilaginous compounds secreted by glandular hairs are responsible of the mild sedative and anti-inflammatory actions of chamomile (Bruni 1999). Nevertheless, our histochemical results showed that a large number of substances, with important ecological effects (Harborne 1993), exists in both secretions. Some of them (i.e. lipids, essential oils, sesquiterpene lactones) are well-known secondary metabolites in chamomile (Hegnauer 1964).

The secretory ducts of the root differ from those of other plant organs, not only in their structure, but also in secretion. They did not show a presence of essential oils, sesquiterpene lactones or pectic-like substances. One explanation could be the absence of a specialized epithelium. These ducts, in fact, may aid the sieve tubes, with which they are in close contact, in the transfer of organic material. Alternatively, the different material present in the secretory ducts of the root could be linked to the soil environment, which is very different from the aerial one (for example pathogens, ecological conditions, etc.).

Since the capitula are richer in secretory structures, they are obviously to be preferred to the vegetative organs for the extraction of active substances. Moreover, our study shows that each part of the capitulum has a different type and/or different frequency of secretory structures, whose secretion can slightly vary depending on the floral part considered. This is in accordance with the phytochemical analysis of flowerhead fractions, that show different concentrations of secondary metabolites (Redaelli *et al*. 1980, 1982).

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