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Urtica membranacea pearl glands. I. Morpho-ontogenetic and histochemical aspects

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With 12 Figures

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Summary

CORSI G. & MAFFEI F. 1992 – *Urtica membranacea* pearl glands. I. Morpho-ontogenetic and histochemical aspects. – Phyton (Horn, Austria) 32 (2): 235–245, 12 figures. – English with German summary.

The data concern a morphological, ontogenetic and histochemical investigation of the pearl glands of *Urtica membranacea* Poir., which never have been studied before, with a comparison with similar studies concerning *U. dioica*. Light microscopy, and for the very first time E.S.M. as well as fluorescence microscopy were used in this study.

A specific characteristic of the pearl glands of *U. membranacea* is the presence of a pluricellular glandular trichome either on their top or at the point of origin on the epidermis.

The pearl glands of *U. membranacea* are always formed by the enlargement of the basal cell of the glandular trichome suggesting that the trichome itself, probably through hormone activity, takes part in the formation of the glands.

The function of these pearl glands appears to be connected with water metabolism but their mode of action is not yet clear.

Some probable roles are suggested for the associated glandular trichomes.

Zusammenfassung

CORSI G. & MAFFEI F. 1992. – Perldrüsen von *Urtica membranacea*. I. Morpho-ontogenetische und histochemische Aspekte. – Phyton (Horn, Austria) 32 (2): 235–245, 12 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Es wird über Untersuchungen berichtet, die Morphologie, Entwicklung und Histochemie von bisher noch nicht untersuchten Perldrüsen von *Urtica membranacea* Poir. zum Gegenstand haben. Sie werden mit ähnlichen Untersuchungen an *Urtica dioica* verglichen. Lichtmikroskopie und zum ersten Mal REM aber auch Fluoreszenzmikroskopie wurden für diese Arbeit eingesetzt.

Eine Besonderheit der Perldrüsen von *U. membranacea* ist das Vorhandensein mehrzelliger Drüsenhaare, entweder an ihrer Spitze oder an der Ansatzstelle an der Epidermis.

Die Perldrüsen von *U. membranacea* werden immer durch Vergrößerung der Basalzellen der Drüsenhaare gebildet, was bedeutet, daß das Haar selbst, möglicherweise aufgrund von Hormonaktivität, an der Bildung der Drüsen beteiligt ist. Die Funktion dieser Perldrüsen scheint mit dem Wasserhaushalt verknüpft zu sein, aber genaueres ist noch nicht bekannt.

Für die assoziierten Drüsenhaare werden einige mögliche Aufgaben diskutiert.

Introduction

Pearl glands vary greatly in their localization, form and number of cells. They have often been compared by hydathodes (UPHOF & HUMMEL 1962, MATHWIESER & al. 1987), and are thus considered to be connected with guttation. Nonetheless, their role is still obscure to some extent, which makes them all the more interesting. The pearl glands in a considerable number of *Angiosperms* have been described but they seem to be particularly frequent in the genus *Urtica* (MEYEN 1837, WALTER 1921, MATHWIESER & al. 1987).

The pearl glands of *Urtica dioica* are unicellular. Their distribution in the plant, their morphology, ultrastructure and the environmental conditions in which they develop, have been studied (MATHWIESER & al. 1987) as well as the nuclear DNA content (MATHWIESER & GUTTENBERGER 1987). Nothing is known about the pearl glands of *Urtica membranacea* Poiret which is, together with *U. dioica,* the most common of the European nettles. They seemed interesting also because of their comparison to those of *U. dioica.* Their difference in number, seen clearly on a macroscopic scale, under natural conditions (they being very frequent during the whole vegetative period in *U. membranacea* and extremely rare in *U. dioica* where they appear only if the plant is grown in greenhouse) (MATHWIESER & al. 1987 and personal observations) suggests others may be revealed by further investigations.

Another stimulus for our research was the recently awakening of an interest for other structures in the *Urticaceae* connected with guttation (laminar hydathodes) especially in virtue of their systematic implications (LERSTEN & CURTIS 1991). This first study reports observations concerning pearl glands localization, morphology, ontogenesis and histochemistry. The last two aspects have not been fully investigated as yet even in the pearl glands of *U. dioica* (MATHWIESER & al. 1987). The histochemical tests used

were the same as those for the stinging hair (CORSI 1992), in order to reveal any relationship between the two structures. Light, scanning electron and fluorescence microscopy were used (the latter two for the first time in investigations concerning pearl glands). The interesting results concerning nuclear metabolism in pearl glands, from research already completed, will be reported in a note apart (CORSI in prep.).

Material and Methods

Material

Material for the present research was from the same spontaneous *Urtica membranacea* population growing in the Botanic Gardens of Pisa University, used for previous research on the stinging hair (CORSI & GARBARI 1990; CORSI 1992). Exsiccata in PI.

Light microscopy

The following material was used: leaf lamina and petiole strips; isolated pearl glands; 20 µm leaf sections obtained with 1720 digital Leitz Cryostat at –8° C; 3 µm semi – thin sections made with the Ultramicrotome LKB 2188 Ultrotome Nova after 12 hours fixation in FAA, dehydration in alcohol and embedding in glycol methacrylate. For the ontogenetic study, embryos and seedlings at various stages of development were used. The following stains were used throughout:

Sudan III (JOHANSEN 1940) Alkanna Tincture (FAURE 1914)	for lipids
Nile blue (CAIN 1947)	for neutral and acid lipids
Sudan III + glacial Acetic acid (JOHANSEN 1940)	for essential oils
Periodic Acid-Schiff's reagent (PAS) (O'BRIEN & MC CULLY 1981)	for polysaccharides
Ruthenium red (JENSEN 1962) Delafield's Haematoxylin (FAURE 1914)	for pectin-like substances
Iodine iodide tincture (FAURE 1914)	for starch
Potassium bichromate (FAURE 1914)	for tannins

Millon's reagent (FAURE 1914)	for proteins
Coomassie brilliant blue R (FAIRBANKS, STECK & WALLACH 1971)	for proteins
Lugol after Ringer's solution (FELDBERG 1950 in MARTY 1968)	for acetylcholine
p-dimethylamino-benzaldehyde in conc. HCl (REGULA & DEVIDÈ 1980)	for serotonin
Acrolein-Fucsin (PEARSE 1978)	for NH_2, NH, SH and imidazole groups in proteins
Sodium cobaltinitrite reagent (DAYANADAN & KAUFMAN 1975)	for potassium
Cobalt nitrate and Sodium nitrite (JOHANSEN 1940)	for potassium
Silver nitrate and U. V. light (RASCHKE & FELLOWS 1971)	for chlorides
Conc. Sulphuric acid (GEISSMAN & GRIFFIN 1971)	for sesquiterpene lactones
Picric acid (sol. 10%) (FAURE 1914)	for alkaloids

Controls were set up according to the methods suggested by the authors for each histochemical test.

Scanning electron microscopy

Leaf lamina and petiole strips for examination with the scanning electron microscope (SEM) was fixed in glutaraldehyde (2% with pH 7.4 phosphate buffer), dehydrated in increasing concentrations of alcohol and acetone mixtures then dried in a critical point drying apparatus before being sputter-conted with gold and examined at 15 KV in a Cambridge Stereoscan 90.

Fluorescence microscopy

Leaf lamina and petiole strips and isolated pearl glands were simply mounted in water or in glycerine and observed with a Wetzlar Orthoplan Leitz fitted with a high pressure Osram HBO 200 W mercury lamp. Leitz BG12 exciting filter and a K510 barrier filter were used, giving a mean excitation wavelength of 410 μm and a mean fluorescence wavelenght of 470 μm.

Results

Pearl glands are present in isolation (Figs. 1 and 2) and in groups (Fig. 5) on the under surface of leaves in correspondence with the ribs and in

particular near the petiole insertion point (where they are almost always found in groups); occasionally they are on the petiole itself.

They are unicellular, normally spheroid in shape (average diameter 170 μm), rarely oblong, narrower in the part inserted into the epidermis to form a short pedicel; the nucleus is considerably large. There is a glandular trichome, usually on the top (Fig. 1) but sometimes on the narrower part (Fig. 2); this trichome has a unicellular pedicel and a tetracellular head. The pearl glands emerge in correspondence of these trichomes: the basal cell nucleus begins to increase in size and the cell to swell to a much larger volume (Fig. 6). In the end it becomes a large roundish structure similar to a pearl in appearance.

When the swelling of the basal cell is symmetrical (Fig. 3) the glandular trichome is found at the top of the pearl gland; if the swelling is one-sided (Fig. 4) the glandular trichome is found on the narrow part, near the insertion into the epidermis. In groups of pearl glands, first the basal cell of one glandular trichome forms a pearl gland as described above; then, in the epidermal cells around the basal cell, the nuclei begin to increase in size (Fig. 7) and the cells begin to swell in turn, each one forming one of the pearl glands that make up the group.

On rare occasions, particularly on the ribs, when the pearl gland or a group of pearl glands has formed, the cells of the epidermis and just below, proliferate, forming a kind of pedestal supporting these structures.

Apart from water, histochemical tests indicate the presence of lipids (Fig. 8) and potassium (Fig. 9) (the latter in large quantities) in pearl glands. Other substances present are acetylcholine (Fig. 10) serotonin (Fig. 11) (mostly in the basal portion close to the insertion into the epidermis) chlorides and proteins.

Associated glandular trichomes were both morphologically and histochemically identical to those on the leaf epidermis as well as those on the pedestal of the stinging hairs (Corsi & Garbari 1990, Corsi 1992). They were rich in essential oils and proteins, but contained also potassium and polysaccharides (including pectin-like substances), tannins, chlorides, acetylcholine and serotonin.

By fluorescence microscopy pearl glands were not autofluorescent. Yellow autofluorescence was observed in the associated glandular trichomes (Fig. 12). In this feature too they were identical both to those on leaf epidermis and to those on the pedestal of the stinging structure (Corsi 1992).

No pore or aperture for expulsion of the secretion could be observed under the SEM on the surface of the pearl glands; neither under fluorescence preferential fracture lines as seen in some glandular trichomes (Modenesi, Serrato-Valenti & Bruni 1984) were observed. Under SEM pearl glands from which the secretion has escaped, do not appear to be broken but rather collapsed or deflated (Fig. 5).

Discussion

U. membranacea pearl glands show always a pluricellular glandular trichome either on their top or close to the insertion into the epidermis. The occasional presence of similar structures has been reported for *U. dioica* (MATHWIESER & al. 1987).

Several authors (STAHL 1920, WALTER 1921, ROUPPERT 1926, MATHWIESER & al., 1987) have suggested that these pearl glands originate when an increase insaline concentration is followed by water uptake.

If the high quantities of potassium and chlorides seen in *U. membranacea* may indicate a similar mechanism, the involvement, perhaps hormonal, of the glandular trichomes must not be excluded, since it is actually from their basal cells that the pearl glands originate. In *U. dioica,* where glandular trichomes are not always present, this kind of involvement has not been taken in account (MATHWIESER & al. 1987). The pearl glands in *U. dioica* should be seen as trichome hydathodes involved in guttation (MATHWIESER & al. 1987).

We are not as yet in a position to draw definite conclusions regarding the function of these structures in *U. membranacea.* They certainly seem to be involved somehow with water turnover but it is not yet clear whether they are really „water glands" (sensu TONZIG & MARRÉ 1971), i. e. structures by which the plant actively expels water and other substances to regulate its relations with the environment or whether they are more like the bulliform cells in *Gramineae* which do not expel water but alternately take it up and relinquish it to nearby cells varying their turgidity in the process thus changing the position of the leaves they are positioned on.

The former hypothesis is supported by the considerable amounts of different substances that these glands contain, the latter by the lack of observations regarding liquid secretion or to find pores or any kind of aperture on their surface and by our always finding empty glands which were simply collapsed and not split nor burst, in contrast to those in *U. dioica* (MATHWIESER & al. 1987). The differences with respect to the pearl glands of *U. dioica* are of great interest. Apart from those already mentioned, there is the fact that in *U. membranacea* the number of pearl glands does not appear to be connected to the moisture content in the environment, as it is found in the other species (MATHWIESER & al. 1987); they are rarely seen on the pedestal of epidermic and subepidermic cells, whereas this is normal in

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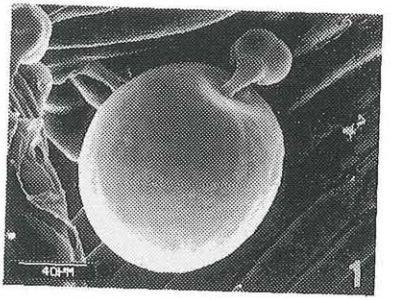
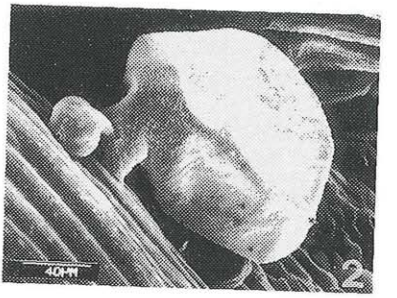
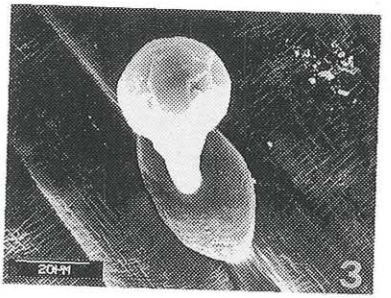
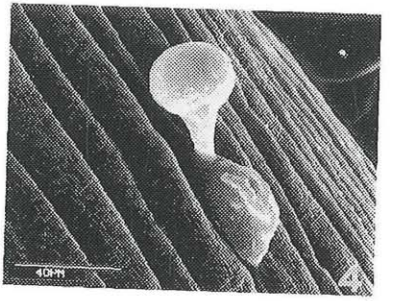
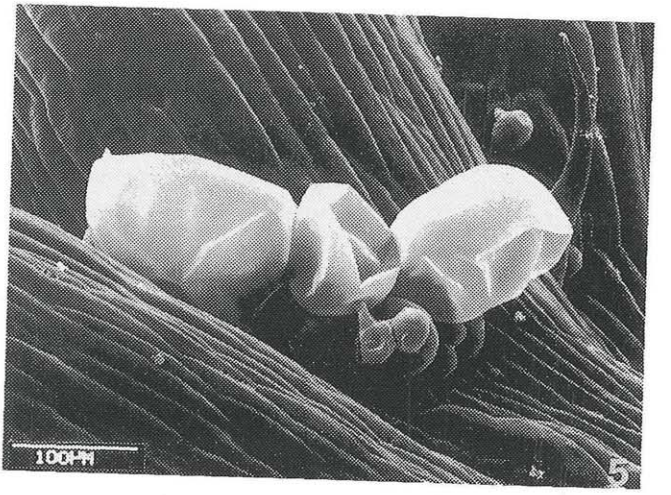
Fig. 1. SEM micrograph of a pearl gland, showing a glandular trichome on the top.
Fig. 2. SEM micrograph of a pearl gland, showing a glandular trichome on its narrow part.
Fig. 3. A glandular trichome basal cell with an all-round swelling (SEM micrograph).
Fig. 4. The glandular trichome basal cell with one-sided swelling (SEM micrograph).
Fig. 5. Group of deflated pearl glands from which the secretion has escaped (SEM micrograph).











U. dioica (MATHWIESER & al. 1987); lipid content of the secretion is far lower than in *U. dioica* (personal observations); even nuclear metabolism (the subject of a further paper) appears to be different from that of *U. dioica*.

These differences, with others that may emerge from further research by electron microscopy, which is already planned, do not seem to be connected with differences in habitat as the two entities share the same habitat. Research in progress will tell, we hope, whether these differences have to do with the type of life form of the two species (*U. membranacea* is an annual therophyte, *U. dioica* a perennial chamephyte) or to other metabolic and/or ecological diversities. Neither we can exclude that the observed differences are part of that great variability seen in pearl glands (UPHOF & HUMMEL 1962) nor that the term "pearl glands" really indicates vastly different structures.

To conclude, the presence of glandular trichomes on pearl glands in *U. dioica* has not been given much importance (MATHWIESER & al. 1987) and no hypothesis has been put forward to explain their role. In *U. membranacea,* apart from the part they play in the formation of pearl glands, which we have already discussed here, the fact that they contain many substances of precise and complex biological activity seems to point to yet another role, that is in regulating plant-environment interaction and/or perhaps in the functioning of the pearl gland, which will be the subject of further research.

Acknowledgements

Thanks are due to Mr. Antonio Masini for his helpful technical advise.

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Fig. 6. The glandular trichome basal cell beginning to swell to a much larger volume (phosphomolibdic acid) ×400.

Fig. 7. A pearl gland with a very large nucleus (arrow). The epidermal cells (double arrows), around the associated glandular trichome basal cell, are beginning to increase in size their nucleus, a step towards the formation of other pearl glands (Feulgen) ×150.

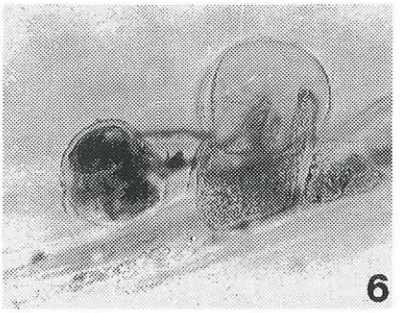
Fig. 8. Pearl gland positive for the histochemical test for liphophilic substances (Alkanna tincture) ×150.

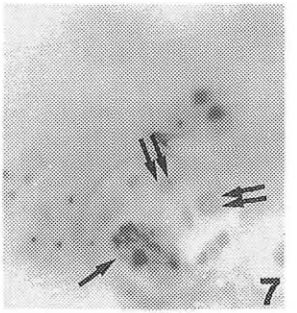
Fig. 9. Pearl gland positive for the histochemical test for potassium (sodium cobaltinitrite reagent) ×150.

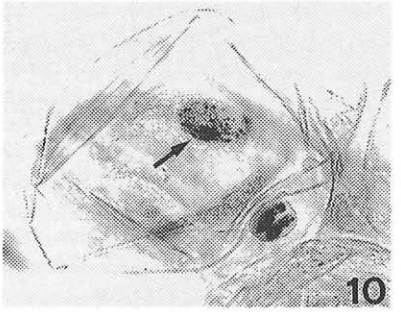
Fig. 10. Pearl gland positive for histochemical test for acetylcholine (arrow) (Lugol after Ringer's solution) ×200.

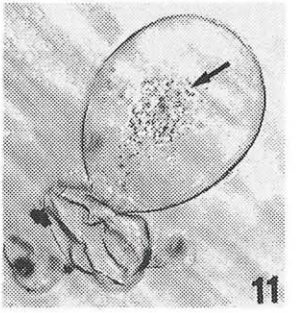
Fig. 11. Pearl gland positive for histochemical test for serotonin (arrow) (p-dimethylamino-benzaldehyde in conc. HCl) ×200.

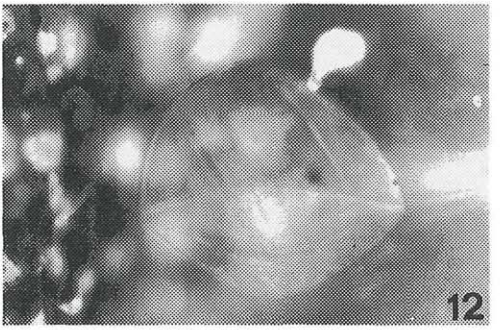
Fig. 12. Yellow autofluorescence in the glandular trichome on the top of the non fluorescent pearl gland, ×150.











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Recensio

BELL Adrian D. 1991. Plant Form. An Illustrated Guide to Flowering Plant Morphology. With line drawings by Alan BRYAN. – Kl. 8°, XIV + 341 Seiten, 196 Farbphotos und 156 Strichzeichnungen; kart. – Oxford University Press, Oxford, New York, Tokyo. – £ 25,– (Paperback). – ISBN 0-19-854219-4.

Der Autor versucht in erster Linie an Hand der Abbildungen die Grundzüge der Angiospermen-Morphologie darzustellen. Das Buch besticht durch die hervorragenden Farbphotos und die meist präzisen Strichzeichnungen (z. T. Photo und zugehörige Strichzeichnung kombiniert). Die Auswahl der Beispiele ist erfreulich originell, indem auch weniger häufig abgebildete, nicht in allen Lehrbüchern enthaltene Arten und insbesondere tropische Vertreter zum Zuge kommen [z. B. gleich als Titelbild ein ausgezeichnetes Photo des Blütenstandes von *Norantea guyanensis (Markgraviaceae),* zu dem noch zwei Photos früherer Entwicklungsstadien kommen (p. 88), um die Entwicklung der Kannenblätter zu illustrieren].

Der Text ist einfach formuliert und weist meist lexikalische Kürze auf.

Die meist 2 oder 4 Seiten umfassenden Kapitel sind zu zwei Teilen gruppiert. Teil 1 „Morphological description" umfaßt eine Einführung in die Darstellungsweise morphologischer Fakten (*Philodendron pedatum* vegetativ, als ausführlich dargestelltes Beispiel), Blatt-, Wurzel- und Sproßachsen-Morphologie, Morphologie der Fortpflanzungsorgane, Sämlings-Morphologie, vegetative Vermehrung, Gras-, Seggen-, Orchideen- und Kakteenmorphologie, Domatien und schließlich Sonderfälle *(Gesneriaceae* z. T., *Podostemaceae* und *Tristichiaceae, Lemnaceae).* Teil 2 „Constructional organization" (Seiten 215–315) befaßt sich mit der Entwicklungsmorphologie, der „Architektur" des Pflanzenkörpers. Es geht in erster Linie um Fragen der Aktivität von Apikalmeristemen bzw. Knospen (Andauer der Aktivität, Hemmung, Förderung, Rhythmik) und deren Auswirkungen auf Habitus, Wuchsform, Verzweigung, Symmetrie usw. Zum Beispiel werden Blattstellung, Konkauleszenz und Rekauleszenz (allerdings ohne daß diese beiden Termini gebraucht werden), Beiknospen, Kauliflorie, Akrotonie/Basitonie, monopodiales/sympodiales Wachstum, Lang- und Kurztriebe, Dichotomie, Teratologien, Chimären, Gallen u. a. sowie zuletzt die Typen der Sproßkonstruktion an Hand der Modelle nach HALLÉ, OLDEMAN und TOMLINSON behandelt.

Durch die breite Stoffauswahl, die auch Kapitel umfaßt, die üblicherweise in Lehrbüchern (noch) nicht dargestellt sind, durch vielfach dynamische (Veränderungen während des Wachstums der Pflanzen einschließende) Betrachtungsweise, durch Berücksichtigung neuer Interpretationen und die dargestellten Beispiele ist das Buch lehrreich und in vielen Bereichen überaus anregend. Am meisten gilt dies für die Kapitel, in denen ein Problemkreis im Detail dargestellt ist (z. B. *Philodendron-* und Grasmorphologie; Teil 2), weniger natürlich für diejenigen, in denen viele Fakten bzw. Termini erläutert werden.