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The stinging hair of *Urtica membranacea* Poiret *(Urticaceae).* II. Histochemistry

By

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With 12 figures

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Summary

Corsi G. 1992. The stinging hair of *Urtica membranancea* Poiret *(Urticaceae).* II. Histochemistry. – Phyton (Horn, Austria) 32 (2): 247–253, 12 figures. – English with German summary.

This paper reports data concerning histochemical investigations on the stinging hairs of *Urtica membranacea* Poiret *(Urticaceae),* the cells of their pedestal and the small glandular trichomes found on the pedestal itself.

The results suggest that the pedestal cells are involved in the function of the stinging hairs. Several hypotheses are proposed concerning the rather more obscure role of the glandular trichomes.

Zusammenfassung

Corsi G. 1992. Das Brennhaar von *Urtica membranacea* Poiret *(Urticaceae).* II. Histochemie. – Phyton (Horn, Austria) 32 (2): 247–253, 12 Abbildungen. – Englisch mit deutscher Zusammenfassung. In dieser Arbeit wird über histochemische Untersuchungen an den Brennhaaren von *Urtica membranacea* Poiret *(Urticaceae),* den Sockenzellen sowie den kleinen Drüsenhaaren, welche an den Sockenzellen gefunden wurden, berichtet.

Die Ergebnisse weisen darauf hin, daß die Sockelzellen an der Funktion der Brennhaare mitbeteiligt sind. Weiters betreffen einige Hypothesen die eher unklare Rolle der Drüsenhaare.

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Introduction

The presence of histamine, acetylcholine and serotonin has been indicated for the stinging hairs of *Urtica (Urticaceae)* although it is doubtful whether these substances are actually those responsable for irritation (THURSTON & LERSTEN 1969).

Hardly anything is known in this respect about the stinging structures of *Urtica membranacea* which, moreover, have the rather singular characteristic of the pedestal possessing small glandular trichomes with a monocellular pedicel and a four-celled secreting head (CORSI & GARBARI 1990).

The aim of the present research was to discover, by means of specific histochemical tests, exactly which substances are present in the stinging liquid and in the secretion from the glandular trichomes, the latter in order to know more of the function of the glandular trichomes themselves, which is, as yet, only vaguely understood and is the object of several hypotheses (CORSI & GARBARI 1990). The response of the cells in the pedestal supporting the stinging hair to the histochemical tests was also recorded, to verify the hypothesis of their part in forming the irritating liquid (RAUTER 1872, HABERLANDT 1886, EMMELIN & FELDBERG 1947).

Material and Methods

The material for this research was taken from the spontaneous population of *U. membranacea* in the Botanic Garden of Pisa University. This population has already been used for previous research (CORSI and GARBARI, 1990). (Exsiccata in PI).

The parts used were: epidermal strips from leaves, petiole and stem; isolated stinging hairs; 20 µm-thick leaf and petiole sections cut by Leitz 1720 digital Cryostat at –8° C.

The following histochemical stains were used:

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)	for pectin-like substances
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 (DAYANANDAN & KAUFMAN 1978)	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. Preliminary observations were carried out by fluorescence microscopy. Leaf lamina or petiole strips and isolated stinging hairs were simply mounted in water or glycerine and observed under a Wetzlar Orthoplan Leitz with an Osram HBO 200 W high pressure mercury lamp. A Leitz BG 12 exciting filter and a K

510 barrier filter were used giving a mean excitation wavelength of 410 μm with a mean fluorescence wavelenght of 470 μm.

Results

The stinging hairs were positive to the histochemical tests for acetylcholine (Fig. 1) and serotonin (Fig. 3). These substances were found in the nucleus, which is lobed and contains vacuoles and parts of cytoplasm, as well as in the cytoplasm between the large vacuole areas towards the apex of the stinging hair itself.

The acrolein-fuchsin test, which among other things is specific for the imidazole group, typical of histamine and its precursors, was consistently negative. Substances other than water, found in small quantities, were lipids, pectin-like substances and proteins, as well as potassium (Fig. 4) and probably alkaloids (Fig. 2). The base of the stinging hair shows an intense yellow autofluorescence (Fig. 12).

Response to the histochemical tests seem to indicate that the pedestal cells are not involved in either the production of lipids or of pectin-like substances. They show a high serotonin content (Fig. 10) with potassium (Fig. 4) and chlorides also present.

The glandular trichomes on the pedestal, appear to produce a mixed lipophilous and hydrophilous secretion, since they contain an essential oil (Fig. 6) and pectin-like substances (Fig. 5). Phenol-like substances (probably tannins) (Fig. 7) are also present together with proteic substances in masses of considerable size (Fig. 8) with potassium (Fig. 4) chlorides and, above all, acetylcholine (Fig. 9) and serotonin (Fig. 10). Tests for NH_2, NH, SH and imidazole groups in the proteins were also positive (Fig. 11). Under the observation conditions used here, the glandular trichomes show an intense yellow autofluorescence (Fig. 12).

Discussion

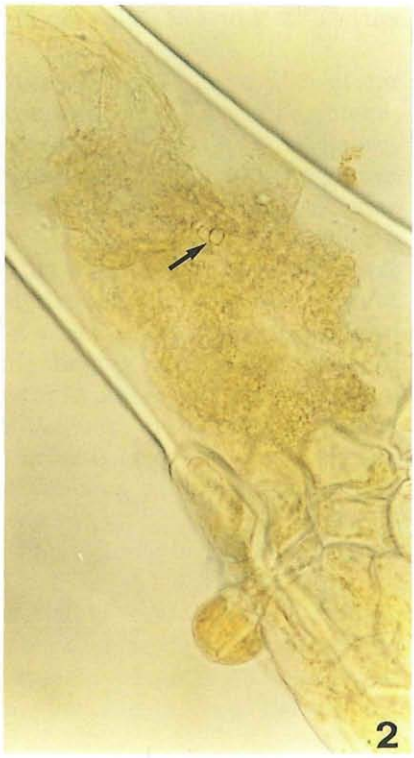
Two of the pharmacologically active substances already reported as present in the stinging hairs of other species of *Urticaceae* (THURSTON & LERSTEN 1969) i. e. acetylcholine and serotonin were found in the stinging hairs of *U. membranacea*.

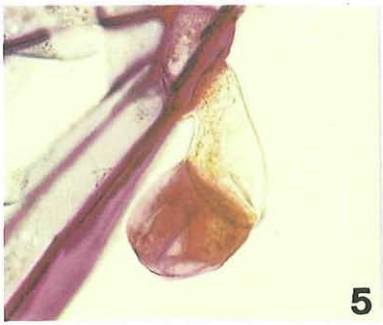
Fig. 1. A stinging hair positive in the histochemical test for acetylcholine in corrispondence of the nucleus (arrow) as well as in the citoplasmic strands traversing the highly vacuolated region (double arrows) (Lugol after Ringer's solution) ×100.

Fig. 2. A stinging hair with cristals of probable alkaloids (arrow) (Picric acid) ×250.

Fig. 3. A stinging hair positive in the histochemical test for serotonin (arrows) (p-dimethylamino-benzaldehyde in HCl) ×250.

Fig. 4. A stinging hair positive in the histochemical test for potassium (sodium cobaltinitrite) x 100. Also the glandular trichomes on the pedestal are positive (arrow).

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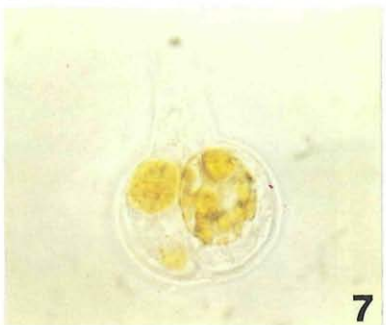
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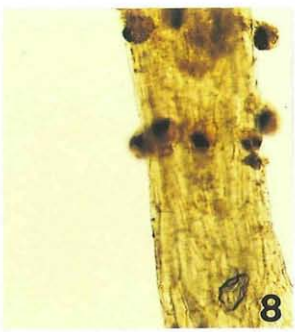
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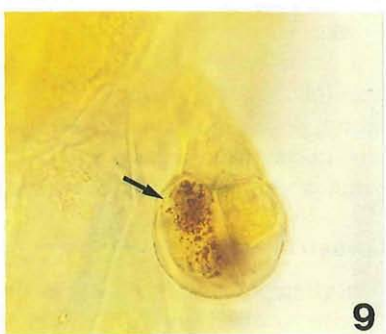
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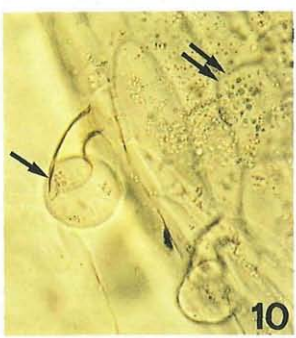
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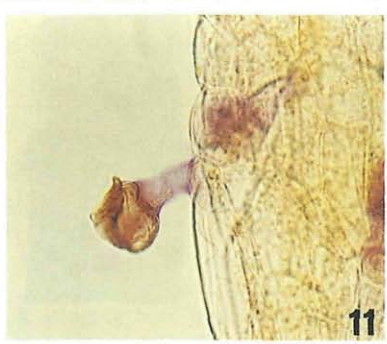
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Serotonin had already been identified as present in the stinging hairs of *U. membranacea* by paper – and thin – layer chromatography as well as by spectro-photometry and spectro-photo-fluorometry (REGULA & DEVIDÉ 1980).

Histamine, if present, does not appear in quantities sufficient for histochemical detection. Many other substances, such als lipids, proteins, pectin-like substances, potassium and perhaps alkaloid-like substances were found. Except for alkaloids, already reported for the stinging hairs of other species of the genus *Urtica* (THURSTON & LERSTEN 1969), none of these substances have been reported previously.

This is probably because, until now, the stinging apparatus has never been subjected to histochemical analysis and all chemical and/or pharmacological investigations have been aimed at substances considered to be toxic and in some way involved in the stinging action. The presence of potassium is interesting, since it supports the hypothesis (ROUPPERT 1915 in UPHOF & HUMMEL 1962) that the stinging hair, with the obvious function of repelling animals, may also function in the active excretion of water and because WINTERITZ (1907) suggested potassium as an active agent of the *Urtica* sting.

The pedestal cells are highly positive for serotonin in the histochemical test.

The work on *U. dioica* left some uncertainty regarding the site of the formation of the substances contained in the stinging liquid. Some authors (RAUTER 1872; HABERLANDT 1886; EMMELIN & FELDBERG 1947) suggest that these toxic substances are formed in the surrounding tissues and subsequently transported into the stinging hair. Others (THURSTON 1974) consider that they are formed in the hair itself.

Fig. 5. Pectin-like substances in a glandular trichome on the stinging hair pedestal (Ruthenium red) ×400.

Fig. 6. Essential oil in a glandular trichome on the stinging hair pedestal (Sudan III + glacial acetic acid) ×700.

Fig. 7. Phenol-like substances (probably tannins) in a glandular trichome on the stinging hair pedestal (Potassium bichromate) ×800.

Fig. 8. Proteic substances in a glandular trichome on the stinging hair pedestal (Millon's reagent) ×800.

Fig. 9. Acetylcholine (arrow) in a glandular trichome on the stinging hair pedestal (Lugol after Ringer's solution) ×400.

Fig. 10. Serotonin in a glandular trichome (arrow) on the stinging hair pedestal and in the pedestal cells (double arrow) (p-dimethylamino-benzaldehyde in HCl) ×220.

Fig. 11. Positivity for NH, NH_2 and imidazole groups in a glandular trichome on the stinging hair pedestal (Acrolein – fucsin) ×220.

Fig. 12. Autofluorescence in the basal part (arrow) of the stinging hair and in the glandular trichomes on the stinging hair pedestal (double arrow) ×160.

The massive quantities of serotonin present in the pedestal cells and the large pits on their walls in contact with the stinging hair (CORSI & GARBARI 1990), seem to suggest that the former of the two groups of authors cited is correct, at least concerning this substance in *U. membranacea*. The pedestal cells are also positive in the tests for chlorides and potassium, which might indicate that they are playing a part in the guttation seen in stinging hairs. The presence of serotonin which, as well as affording protection against predators, has an auxin-like activity (GROSSE 1982), could also be seen in this light.

The glandular trichomes on the pedestal appear to be particularly rich in substances. The positive results of specific tests for acetylcholine, serotonin and NH_2, NH, SH and imidazole groups indicate that they too, like the cells of the pedestal, may participate in the formation of toxins which may then be transported into the stinging hair. Neither does the presence in these structures of potassium, chlorides and pectin-like substances, exclude the possibility of their being involved in some way in water balance. However, their high content in phenols (most probably tannins) and essential oils with, perhaps, their intense autofluorescence, may indicate that they are in some way involved in regulating plant-animal relationships. At present none of these possible functions can be excluded for the glandular trichomes.

Research, still in progress, will perhaps clarify the function of these structures which, by their presence, make the stinging hair of *U. membranacea* particularly interesting.

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Recensio

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