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GENERAL CONNECTIONS BETWEEN LATEX AND NECTAR SECRETIONAL SYSTEMS OF *ASCLEPIAS SYRIACA* L.

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Abstract

The intracellular latex secretion and the extracellular nectar secretion are anatomically connected to each other in *Asclepias syriaca* L. The whole gynostemium is interwoven by the non-articulated laticifers, and they can also be found in the tissues of the epimorph nectary. The latex system is divided into several branches in the gland parenchyma, and the epithelial glandular tissues of the nectary. The question as to whether there is also a functional connection as a consequence of the anatomical connection (or whether it is possible that certain materials of the latex are secreted into the nectar and are finally mixed into the honey) between the two secretional systems was answered by TLC and GC-MSD analyses of the latex and the honey.

By means of TLC analyses at 254 nm and reactions with general alkaloid reagents (Dragendorff reagent, Meyer reagent and 1% $Ce(SO_4)_2$ solution in 1 M H_2SO_4), several compounds were detected both in the latex and in the honey of *Asclepias*. Due to the lack of TLC standards, these compounds were not identified, and only the degree of compositional identity of the latex and the honey was established.

By means of GC-MSD (a gas chromatograph coupled with a mass selective detector) analyses, the following compounds which can also be found in the latex were identified in the honey of *Asclepias syriaca*: 2-propenoic acid 6-methylheptyl ester (retention time: 6.95 min), pentadecane (ret. time: 10.80 min), diethyl phthalate (ret. time: 11.43 min), hexadecane (ret. time: 12.05 min), heptadecane (ret. time: 13.20 min), 2,6,10,14-tetramethyl pentadecane (ret. time: 13.30 min), octadecane (ret. time: 14.32 min), 2-tetradecanoic acid methyl ethyl ester (ret. time: 14.45 min), phthalic acid butyl 2-methylpropyl ester (ret. time: 15.56 min), 1-eicosene (ret. time: 17.15 min), docosane (ret. time: 18.30 min) and *bis* (2-ethylhexyl) phthalate (ret. time: 20.94 min).

The functional connection between the two secretional systems consequent from their anatomical connection is strongly suggested by the partial compositional identity of the latex and the honey, and the direct connection of the latex-system with the glandular tissue of the nectary.

Key words: secretion, nectary, honey, *Asclepias*.

Introduction

In the past few decades, a number of data have been obtained regarding the nectar production of melliferous plants, the quantity of honey produced and its composition. The chemical composition of the nectar produced, and finally the honey, is an important medical question. In the case of plants that produce latex, it can be influenced consider-

ably by the connection of intracellular and extracellular secretion. This paper examines the anatomical and functional connection of the intracellular latex secretion and the extracellular nectar secretion of *Asclepias syriaca*, which is an important plant in bee-farming.

It is a well-known fact that the common milkweed is a plant that contains latex. The latex can be found in long, expanded, non-articulated laticifers as the product of intracellular secretion (METCALFE, 1985). In fact, the latex can be regarded as the cell fluid of laticifers (FREY-WYSSLING, 1933; ESAU, 1953), which may contain several biologically active compounds such as organic acids, terpenoids (ADAMS, 1987), alkaloids (SOKOLOV, 1952), saturated and aromatic hydrocarbons (CAMPBELL, 1983; SIMIONESCU, 1987), steroids and alcohols (BISBOAR, 1983), in the form of either solution or colloidal suspension. The chemical compounds of the *Asclepias* genus are also mentioned by HEGNAUER (1966).

In contradiction with the statements of SPRENGEL (1793), STADLER (1886), KNUTH (1909), FITTING (1930) and RENDLE (1953) regarding the nectar-secretional system of the common milkweed, GALLIL and ZERONI (1965) and KEVAN et al. (1989) provided information on the anatomy and tissue structure of the nectar-secretional system of *Asclepias* species, and the epithelial position of the glandular tissue, which is so important here.

The composition of the nectar produced by extracellular secretion, and finally the composition of the honey are extremely diversified. Besides the most important compounds, which are different types of carbohydrates (SOUTHWICK, 1981, 1983a,b), the nectar may contain vitamins (WEBER, 1942), other organic acids (MAURIZIO, 1960), antimicrobial compounds (KEVAN et al., 1989), etc. The toxic compounds of the nectar were submitted to detailed examinations by SCHULTZ-LANGER (1966, 1967) among others. In the world of plants there also occur honeys which have a harmful, toxic effect on the human organism. The honey of some tropical *Euphorbia* species has a strong, irritant effect (JURITZ, 1925). Compounds with the same physiological effect were also detected in the latex of these plants (UPADHYAY and HECKER, 1975; SOSATH, 1988).

The common origin of certain compounds of nectar and latex, and the connection between extracellular and intracellular secretion in the *Euphorbia* genus, have already been emphasized in previous publications (TÓTH-SOMA and GULYÁS, 1991; TÓTH-SOMA et al., 1993). In the present paper, the following questions are examined: is the latex system of *Asclepias syriaca* anatomically connected to its nectar-secreting system, and if it is, is there also a functional connection between them due to their anatomical connection? May certain compounds of the latex be secreted into the nectar, and finally into the honey?

Materials and methods

Anatomical examinations on the nectary.

The tissue structure of the nectaries in the stigmatic chambers of the gynostemium of *Asclepias syriaca* was examined; this is an excellent melliferous plant found all over Europe. To examine the gynostemium derived from its petals, we used the celloidin embedding method described by KISSER (1920) and ROmeis (1932), as modified by GULYÁS (1968). We made 20 to 30 µm thick cross-sections with sledge-microtome. For better examination of the gland, the cross-sections were stained with Erlich's haematoxylin and conserved in Canada balsam. The slides were examined under an NU-2 light microscope.

Chemical detections

The comprehensive analyses of milkweed latex and honey were performed by analytical techniques. By means of thin-layer chromatography, alkaloids and other compounds were detected which gave a positive reaction with alkaloid reagents. Other organic compounds were detected by the GC-MSD method.

From the latex and honey, a chloroform extract was made according to the method applied by SZÁSZ (1979) and TÓTH-SOMA et al. (1993). These extracts were examined on Kieselgel 60F 254 (MERCK®) thin-layer plates, by developing the chromatograms in an 85:15 (v/v) mixture of benzene and methanol. Preliminary examination of the plates was performed under 254 nm light. They were then developed with three general alkaloid reagents: a 1% solution of $Ce(SO_4)_2$ in 1 M H_2SO_4, the Meyer reagent and the Dragendorff reagent (HAIS and MACEK, 1961; MUNIER and MACHEBOEUF, 1949). The extracts were further examined with a Hewlett Packard GC-MSD system (an HP 5890A GC coupled to an HP 5970 MSD, 70 eV EI), using a 12 m × 0.2 mm × 0.5 µm HP-1 capillary column (OV-1 compatible), and He as carrier gas.

Results

During the anatomical examinations of the nectaries, their structure (already predicted in the literature) was observed. In the light microscopical pictures, the differentially stained gland-parenchyma and the epithelial glandular tissue could be clearly observed (Fig. 1, panel A). The whole gynostemium and both layers of the parenchyma of the epimorph nectary situated in the stigmatic chamber are interwoven by the inarticulate system of laticifers which is characteristic of the plant (Fig. 1, panels A, B, C). The nectar stored in the stigmatic chamber is responsible for the germination of the pollinium that comes from outside (Fig. 1, panel D). The laticifers closer to the epithelial glandular tissue are parallel with the secretional layer of the nectary (Fig. 1, panel E), and their final ramifications end on the border between the subepithelial parenchyma and the epithelial tissue, directly touching the secretional tissue (Fig. 1, panel F). It is clear, therefore, that the intracellular latex secretion and the extracellular nectar secretion are anatomically connected to each other.

The functional connection resulting from the anatomical connection was verified by TLC examinations. These examinations revealed that several compounds that are present in the latex are also present in the honey (Fig. 2). On the chromatograms examined under 254 nm light, four compounds were found which were present in the extracts of both honey and latex (Fig. 2, spots a-d, plate I). On the plate developed with Meyer reagent, there were five compounds (Fig. 2, spots a-e, plate II), the plate

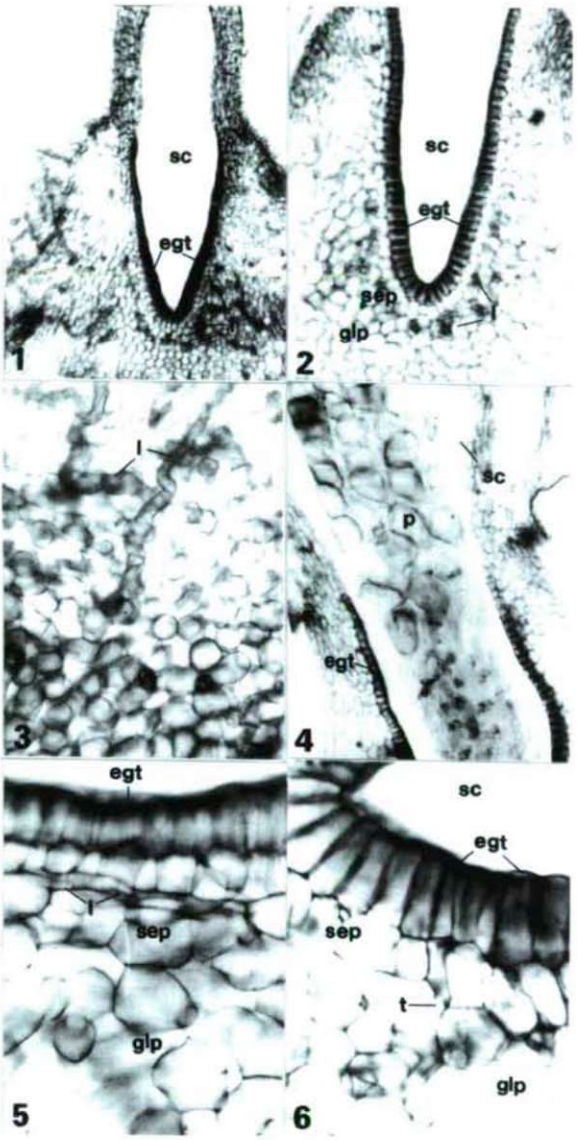

Fig. 1. Cross-section of the floral nectaries of *Asclepias syriaca* L. Enlargement: panel A 80×, B 125×, C 265×, D 125×, E 500×, F 530×, epithelial glandular tissue (egt), subepithelial parenchyma (sep), gland-parenchyma (glp), laticifers (l), stigmatic chamber (sc), pollinary (p). See explanation in text.

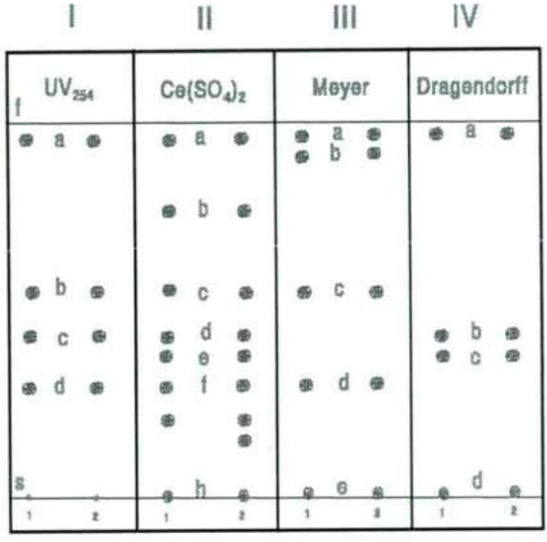

Fig. 2. TLC of latex (1) and honey (2) extracts of *Asclepias syriaca* L. The distances of the spots from the front from a to h. 1-2: numbers of samples, f: front, s: start.

developed with $Ce(SO_4)_2$ revealed eight compounds (Fig. 2, spots a-h, plate III), and on the plate developed with Dragendorff reagent, there were four compounds (Fig. 2, spots a-d, plate IV) that could be found in both extracts. Due to the lack of TLC standards, we did not investigate these compounds qualitatively, but the compositional identity of the latex and the honey was thus clearly demonstrated.

In the GC-MSD examinations, several identical compounds could be observed (Fig. 3). The mass spectra (with the help of the NBS mass spectrum library) permitted identification of the following compounds in the honey extract (Fig. 3, B): 2-propenoic acid 6-methylheptyl ester (ret. time: 6.95 min), pentadecane (ret. time: 10.80 min), diethyl phthalate (ret. time: 11.43 min), hexadecane (ret. time: 12.05 min), heptadecane (ret. time: 13.20 min), 2,6,10,14-tetramethyl pentadecane (ret. time: 13.30 min), octadecane (ret. time: 14.32 min), 2-tetradecanoic acid methyl-ethyl ester (ret. time: 14.45 min), phthalic acid butyl 2-methylpropyl ester (ret. time: 15.56 min), 1-eicosene (ret. time: 17.15 min), docosane (ret. time: 18.30 min) and *bis* (2-ethylhexyl) phthalate (ret. time: 20.94 min). The peaks of these compounds can also be seen on the gas chromatogram of the latex extract (Fig. 3, A). As these peaks appeared at the same retention times with the same mass spectra for both extracts, they are considered to relate to identical compounds.

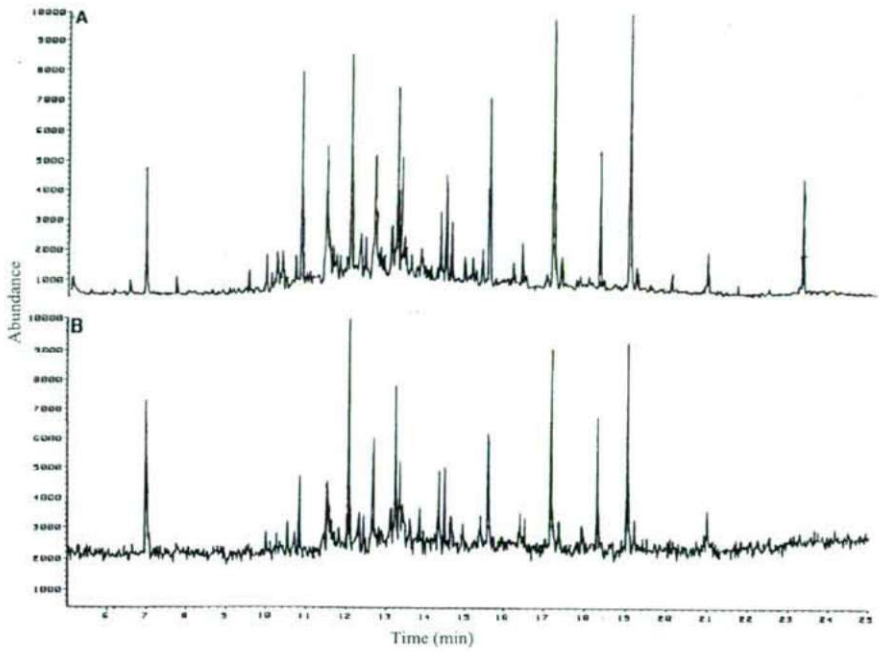

Fig. 3. Gas chromatograms of latex (**A**) and honey (**B**) extracts of *Asclepias syriaca* L.

Discussion

Our anatomical examinations of the gynostemium and the nectary verify that the intracellular latex-secretional and the extracellular nectar-secretional systems of *Asclepias syriaca* L. are anatomically connected to one another. The parenchyma layer of the nectary is richly interwoven by inarticulated laticifers which are also present in the gynostemium and are characteristic of the whole plant. After several ramifications, the laticifers end on the border between the epithelial glandular tissue and the gland-parenchyma of the nectary. Therefore, a functional connection between the two secretional system is anatomically possible. This assumption was strongly indicated by the TLC and GC-MSD analyses of the honey and the latex. Our results harmonize with the anatomical observations made so far on the nectary (GALID and ZERONI, 1965; KEVAN et al. 1989) supplemented by the position of the laticifers in the gynostemium compared to their glandular tissue. Our results provide new data concerning the observation of the connection between the two secretional systems mentioned above.

Our results suggest that the *Asclepias* honey sold commercially should be examined medically and toxicologically.

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