Scanning electron microscopic studies on pollen from honey. IV. Surface pattern of pollen of *Sapium sebiferum* and *Euphorbia* spp (Euphorbiaceae) *

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Summary — Several species of *Euphorbia* and *Sapium* plants produce considerable amounts of nectar, which is collected by bees for honey production. In order to identify the nectar source species we used the usual method of melissopalynology (identification of pollen grains by light microscopy) as well as scanning electron microscopic (SEM) studies. Authenticated specimens of pollen were examined in a state of contraction as well as expansion. Four types of exine surface pattern could be distinguished by SEM. *E. Cærulescens*, *E. ledienii* and *E. tetragona* show the same 3 variations in exine structure (type A, B, C). It was therefore not possible to distinguish between these 3 *Euphorbia* species. However *Sapium sebiferum* showed a specific pattern and uniformity of exine structure (type D).

pollen / honey / Euphorbiaceae / light microscopy / scanning electron microscopy (SEM)

INTRODUCTION

The family Euphorbiaceae is one of the largest in the world, comprising > 300 genera (Heywood, 1982). Pollen of some genera gathered by honeybees are well-known in melissopalynology and enable the botanical origin of honey specimen to be identified. For example, it is possible to identify pollen of different species of Euphorbiaceae such as *Croton*, *Euphorbia*

or *Ricinus* in honeys from different countries (Zander, 1941). The existence of special honey sources with a large amount of nectar from *Euphorbia* species led us to investigate the possibility of identifying pollen of different species within the genus *Euphorbia*. These honeys are commonly known as 'noors-honeys', which can cause acute burning in the mouth and throat (Sosath *et al*, 1988). In addition we wished to determine pollen characters via

* These studies constitute part of a comprehensive research program led by E Hecker, Krebsforschungszentrum Heidelberg on several Euphorbiaceae.

examination of pollen grains that would distinguish *Euphorbia* honey from that of *Sapium sebiferum* (Euphorbiaceae). Microscopy (LM) as well as scanning electron microscopy (SEM) was therefore used. The melissopalynological study of the *Euphorbia* honeys was uninformative because of the naturally low content of *Euphorbia* pollen grains. Nevertheless these honeys contained high amounts of *Euphorbia* nectar, as confirmed by sensory test and bee botanical observations. Low pollen content is well-known for some other honey sources such as those of lime tree (Tiliaceae) and false acacia (Leguminosae) (Zander, 1935; Louveaux *et al*, 1970, 1978). Based on these facts, we examined authentic specimens of pollen gathered in South Africa (*Euphorbia*) and Louisiana, USA (*Sapium*). To compare complete the results of the LM and SEM examination we used Method I of Dustmann and Bote (1985) to obtain specimens from similar honey of expanded pollen grains for SEM study thus providing a possibility to transfer our results directly to melissopalynology.

MATERIALS AND METHODS

The Krebsforschungszentrum Heidelberg obtained the following authenticated pollen samples: 1) *Euphorbia coerulescens*: Jansenville / Caroo, South Africa, 1980; 2) *Euphorbia ledianii*: Kleinpoort / Caroo, South Africa, 1980; 3) *Euphorbia tetragona*: Keiskama Valley, South Africa, 1980; 4) *Sapium sebiferum*: Louisiana, USA, 1982.

After degreasing in ether, pollen grains were prepared for LM and SEM examination. The common palynological method of embedding in glycerine jelly (Erdtman, 1952) was used for LM. Pollen grains were prepared in 2 ways for SEM: 1) contracted pollen was directly dusted onto aluminium stubs provided with double-sided tape, then coated with a 20-nm gold layer; 2) for the expanded condition at onset of germination, *ie* the same condition and shape

found in the LM glycerine jelly preparations, pollen grains were transferred into filtered honey for 48 h at 35 °C. After using Method I of Dustmann and Bote (1985) – transfer to a wire screen, removal of 'pollenkitt' by ultrasonics, dehydration in acetone and a graded ethanol series, critical point drying (CPD) – pollen was dusted onto an aluminum stub provided with double-sided tape, and coated with a 20-nm gold layer. All SEM specimens were examined at an acceleration voltage of 10 kV.

The following equipment was used: Photo-Microscope III (Zeiss); SEM ISI-Super-II; Sputter-Coater ISI PS-2; Critical Point Drying Apparatus E 3000 (Polaron). Nomenclature for descriptions was according to Faegri and Iversen (1964).

RESULTS

LM examination

Expanded pollen grains of *Euphorbia coerulescens*, *E tetragona* and *Sapium sebiferum* are tricolporate and characterized by the same spherical shape (equatorial view). The ectexine includes the foot layer, columellae and a perforate tectum. The grains lack any supratectal sculpturing. Size varied from 32.5–42.5 µm within each species. In all pollen grains the characteristic contour of the living cell could be observed forming a yard around the pores which depended on intine thickness, and seen in polar view (fig 1).

SEM examination

Contracted pollen grains examined of the genera *Euphorbia* and *Sapium* were visible only in equatorial view depending on their prolate-oval shape. Differences in size and shape were non-significant. The characteristic margo along every colpus was hardly visible as the germination furrows were closed. Parts of the exine or single perfora-

Fig 1. LM, pollen grain of *Euphorbia* species. **a**, **b**: polar view, high focus and cross-section; note the characteristic contour of the living cell. **c**, **d**: equatorial view, high focus and cross-section (magnification $\times 787.5$).

tions (luminae) of the tectum were covered with 'pollen kitt'.

Expanded pollen grains of all specimens allowed a more detailed study in the polar as well as in the equatorial view. In *E. coerulescens*, *E. ledienii* and *E. tetragona* we found the same 3 variations in exine pattern, designated as type A, B and C. Only *Sapium sebiferum* showed uniformity in pollen exine pattern, which as it differed from type A, B and C was termed type D.

Description of pollen exine pattern in the different types

All pollen types are tricolporate; colpi are surrounded by a smooth, wide margo, flat-

tened straight to the colpus margin, the apertural endexine shows a verrucate pattern; the exine structure is tectate-perforate.

Type A

The exine pattern is regular tectate-perforate, the luminae are small and circular. The diameter is frequently smaller (up to $0.2 \mu\text{m}$) than the width of the muri (up to $0.7 \mu\text{m}$). The muri are plain or slightly vaulted (figs 2a, b; 3a, b)

Type B

The exine pattern is regular tectate-perforate. The luminae vary in size (up to $0.7 \mu\text{m}$) and shape (circular to oval). The muri are vaulted, with a width $\leq$ diameter of the luminae. The tectum pattern mainly at the intercolpium is partially striate-reticulate (figs 2c, d; 3d, c).

Type C

The exine pattern is irregular tectate-perforate. Most of the luminae are small ($\approx 0.2 \mu\text{m}$), with irregular sometimes elongated dimensions this possibly being determined by compressed and partially incomplete muri. The width of the high vaulted muri measures up to $0.7 \mu\text{m}$ (figs 2e, f; 3e, f).

Type D

The exine pattern is regular tectate-perforate characterized by small luminae (diameter $\approx 0.2 \mu\text{m}$). The muri are wide (up to $1.2 \mu\text{m}$) and generally plain (figs 2g,h; 3g,h).

Fig 2. SEM, structure pattern types of expanded pollen grains of *Euphorbia* species and *Sapium sebiferum* as seen in polar view. **a, b:** type A (*Euphorbia* sp); **c, d:** type B (*Euphorbia* sp); **e, f:** type C (*Euphorbia* sp); **g, h :** type D (*Sapium sebiferum*) (short marker = 5 μ m).

Fig 3. SEM, structure pattern types of expanded pollen grains of *Euphorbia* species and *Sapium sebiferum* as seen in equatorial view. **a, b:** type A (*Euphorbia* sp); **c, d:** type B (*Euphorbia* sp); **e, f:** type C (*Euphorbia* sp); **g, h:** type D (*Sapium sebiferum*) (short marker = 5 μ m).

DISCUSSION

Pollen grains of *Euphorbia coerulescens*, *E ledienii*, *E tetragona* and *Sapium sebiferum* exhibit similarities in size, overall shape and exine structural pattern. Using LM we were able to determine shape, construction of the exine and contour of the living cell. As volume of pollen grains changes in particular and possibly also shape and surface pattern hydration of the plasm (Blackmore and Barnes, 1985; Dustmann and Bote, 1987; von der Ohe and Dustmann, 1990) size cannot be a criterion in determining specific differences. The study of structural pattern of the ectexine was not satisfactory due to the limited depth of focus of the LM. SEM studies were carried out without acetolysis, a method already used in identifying ultra-structures in the pollen surface of apple varieties (Dustmann and Bote, 1987; von der Ohe, 1991).

Besides this, the acetolysis method (Erdmann, 1960) causes damage and loss of single pollen grains. This would not allow the SEM method to be used for Euphorbiaceae honey, which shows an extreme under-representation of pollen.

The differences in structural pattern formed by a perforate tectum observed via SEM do not allow differentiation of the 3 *Euphorbia* species. Although 3 structural pattern types (type A, B and C) were recognized, a reference to any species is not possible: all 3 types were found in each specimen of *Euphorbia coerulescens*, *E ledienii* and *E tetragona*. The pattern differs in shape and diameter of the luminae, which are sometimes elongated and formed by incomplete muri, as well as in the width and height of the muri. As all pollen grains were prepared in the same manner, we conclude that this variation is characteristic of these species of *Euphorbia*. In comparison, pollen of *Sapi-*

um sebiferum exhibited only 1 type of characteristic tectate pattern, type D, which was not found in any of the *Euphorbia* species.

The results of the present study show that there are no microscopic characters that distinguish pollen grains of the 3 species of *Euphorbia* via analysis of the exine structural pattern. However, it is quite possible to find specific identifying marks in different general of Euphorbiaceae via SEM.

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Résumé — Étude en microscopie électronique à balayage du pollen du miel.

IV. Sculpture de l'exine des pollens de diverses espèces d'Euphorbiacées. De nombreuses plantes de la famille des Euphorbiacées produisent des quantités considérables de nectar, que les abeilles utilisent pour le miel. L'existence des «miels de noors» ayant un fort pourcentage de nectar d'*Euphorbia* et de *Sapium* nous a permis de rechercher des moyens d'identification de ces pollens, afin de déterminer sans ambiguïté l'origine botanique des miels. Pour ce faire, des échantillons de pollen d'*Euphorbia coerulescens*, *E ledienii*, *E tetragona* et *Sapium sebiferum* ont été observés en microscopie optique et en microscopie électronique à balayage (MEB). L'analyse en microscopie optique n'a permis aucune différenciation : les pollens d'*Euphorbia* aussi bien que de *Sapium* sont tricolporés, avec une marge, et montrent en vue polaire le contour typique du cytoplasme (fig 1). Il n'est pas possible de mettre en évidence des différences

dans la structure de l'exine en raison de la faible profondeur de champ du microscope optique. Pour les études au MEB, on a fait passer des pollens en état de contraction à l'état d'expansion, en les mettant dans du miel filtré. Après les avoir isolés, on les a transférés sur un crible métallique (Dustmann et Bote, 1985).

Tous les pollens étudiés ont montré une structure de l'exine tecto-perforée. Quatre types de structures ont été trouvés : type A (fig 2a, b; 3a, b), type B (fig 2c, d; 3c, d), type C (fig 2e, f; 3e, f) et type D (fig 2g, h; 3g, h). Les sculptures se différencient par la disposition et la taille des perforations et par l'architecture des muri. Les types A, B et C se rencontrent chez toutes les espèces d'*Euphorbia* et ne permettent donc pas de différencier les 3 espèces *coerulescens*, *ledienii* et *tetragona*. Par contre *Sapium sebiferum* présente une sculpture spécifique et une uniformité de la structure de l'exine (type D) qui, par ses muri extrêmement larges et plats, se distingue de tous les autres types. Il est donc possible de différencier les miels d'*Euphorbia* de ceux de *Sapium sebiferum*.

pollen / miel / Euphorbiaceae / microscopie optique / microscopie électronique à balayage

Zusammenfassung — Raster-elektronen-mikroskopische Studien an Pollen aus Bienenhonig. IV. Oberflächenmuster von Pollen aus verschiedenen Euphorbiaceae Arten. Zahlreiche Pflanzen aus der Familie der *Euphorbiaceae* produzieren beträchtliche Mengen an Nektar, der von Honigbienen eingetragen wird. Das Auftreten sog "Noors-Honige" mit hohen Anteilen an *Euphorbia*- und *Sapium*-Nektar veranlaßte uns, eine Möglichkeit zu finden, diese Pollen zu identifizieren, um die botanische Herkunft der Honige sicherzustellen.

Hierzu wurden authentische Pollenproben von *Euphorbia coerulescens*, *E ledienii*, *E tetragona* und *Sapium sebiferum* sowohl mit dem Lichtmikroskop als auch mit dem Raster-Elektronenmikroskop (REM) untersucht. Lichtmikroskopische Analysen ermöglichten keine Differenzierung: Sowohl *Euphorbia*- als auch *Sapium*-Pollen sind tricolporat, marginat und zeigen in der Pollage die typische Kontur des Plasmas (Abb 1). Unterscheidungen der Exine-Struktur waren aufgrund der geringen Tiefenschärfe des Lichtmikroskopes nicht möglich. Für die REM-Untersuchungen wurden kontrahierte Pollen zur Expansion in pollenfreien Honig überführt und nach Isolierung in ein Edeldahlnetz präpariert (Dustmann und Bote, 1985). Alle untersuchten Pollen zeigen eine tectat-perforate Exine-Struktur. Insgesamt wurden 4 verschiedene Struktur-Typen gefunden: Typ A (Abb 2a, b; 3a, b), Typ B (Abb 2c, d; 3c, d), Typ C (Abb 2e, f; 3e, f) und Typ D (Abb 2g, h; 3g, h). Die Oberflächenmuster unterscheiden sich in Anordnung und Größe der Perforationen sowie im Aufbau der Muri. Typ A, B und C treten bei allen *Euphorbia*-Arten auf und lassen somit eine Differenzierung von *E coerulescens*, *E ledienii*, und *E tetragona* nicht zu.

Sapium sebiferum zeigt jedoch ein spezifisches Muster und eine einheitliche Exine-Struktur (Typ D), die sich aufgrund der extrem breiten, flachen Muri von allen anderen Typen absetzt.

Die Ergebnisse zeigen, daß mit Hilfe des Raster-Elektronenmikroskopes die hier untersuchten Pollen der Gattung *Euphorbia* nicht untereinander aber von *Sapium sebiferum* unterschieden werden können und somit eine Differenzierung der entsprechenden Honige möglich ist.

Pollen / Honig / Euphorbiaceae / Lichtmikroskopie / Raster-Elektronenmikroskopie

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