"Epicuticular Waxes" from Exine Material of Pine Pollen

Christiane Niester*, Paul-Gerhard Gülz**, and Rolf Wiermann*

* Botanisches Institut der Universität Münster, Schloßgarten 3, D-4400 Münster, Bundesrepublik Deutschland
** Botanisches Institut der Universität Köln, Gyrhofstraße 15, D-5000 Köln 41, Bundesrepublik Deutschland

Z. Naturforsch. **42c**, 858–862 (1987); received April 29, 1987

Pinus mugo Turra, Pinaceae, Pollen, Wings, Localization of Epicuticular Waxes

"Epicuticular waxes" from pine pollen and from pollen wings have been investigated. Various hydrocarbons, aldehydes, wax esters, free fatty acids and primary alcohols in the form of homologous series were identified. A noteworthy observation was the presence of a large number of unsaturated fatty acids.

Although there were quantitative differences between the waxes from whole pollen and pollen wings, the distribution patterns of chain lengths of the individual compounds were very similar.

It is demonstrated unequivocally, through the use of pollen wing material, that the waxes are components of the exine. It is postulated that the pollen waxes provide an additional effective mechanism for protection against the effects of the physical and chemical environment.

Introduction

The epidermis, in particular the complex multiple-layered outer wall, is an important barrier between the plant and the environment. During evolution plants have aquired systems which provide effective protection for the outer cells from the various influences of the environment. The epicuticular waxes, made-up of a combination of different lipid classes [1], are one of these systems. This complex mixture of long-chained lipophilic substances which is supported by, and in places stored in, the cutinized wall layers, helps ensure that the cuticle is virtually permeable to water.

Plant cells such as pollen are particularly exposed to the influences of the environment. This holds especially for wind-borne pollen which may be dispersed to very high altitudes. The pollen, or male gametophyte, is protected by specially structured, resistant wall materials and their inclusions.

In this paper we demonstrate that pollen is capable of accumulating the components of epicuticular waxes. It is also shown unequivocally, through the use of wings isolated from *Pinus* pollen, that the extractable waxes are present in and/or on the exine.

Reprint requests to Prof. Dr. R. Wiermann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0700–0858 $ 01.30/0

Materials and Methods

Plant material

Pollen from *Pinus mugo* Turra was collected from South Tyrol (Italy; = Po. – 1984) and from the Botanical Garden, Münster (FRG; = Po. – 1986).

Isolation of wing material

150 mg of pollen were suspended in 10 ml of 0.2 M tris-HCl, pH 8.0, and sonicated for 10 min at 4 °C (Branson Sonifier, Microtip; 32 W/cm^2). The extract was immediately layered onto a 3-step, nonlinear glycerol-H_2O gradient containing, in order from the bottom layer, 10 ml of each of 83.3%, 75% and 50% glycerol. Following centrifugation for 10 min at $25 \times g$, the pollen wings collected at the interface between the top and the second glycerol layers [2].

The wing-enriched fraction was diluted with 5 ml H_2O and was centrifuged for 15 min at $9600 \times g$ and 4 °C. The supernatant was discarded and the pellet was washed thrice with 10 ml H_2O before being freeze-dried for 48 h.

The freeze-dried fraction was subjected to a microscopic examination for contamination by whole pollen and pollen fragments. For this, the wing: pollen-fragment ratio was estimated in 5 aliquots taken from 1 mg of the freeze-dried material suspended in 1 ml H_2O. If the fractions contained more than 1 pollen fragment per 300 wings, the fraction was resuspended in 10 ml of the tris-HCl buffer and repurified on a glycerol gradient.

Isolation and characterization of the epicuticular waxes from whole pollen and pollen wings

250 mg of whole pollen or wings were extracted over about 1–2 h with 100 ml chloroform through a 2 cm diameter filter (frit size: 4). The filtrate, designated the total epicuticular wax fraction was dried under vacuum and weighted.

The separation of the total wax fraction into individual lipid classes was accomplished using a 10 cm × 2 cm column of silica gel 60 (0.06–0.2 mm; Merck, Darmstadt, FRG) suspended in pentane. The column was eluated successively with 50 ml of each of pentane, 2-chloro-propane, methanol and chloroform [3]. Each solvent was freshly distilled prior to use. Each eluate was reduced to dryness and weighted before resuspension in 50 µl hexane. The lipid composition of each fraction was subjected to thin-layer and gas chromatographic analysis. The esterification of the free fatty acids in the methanol fraction and their subsequent separation from alcohols was performed according to Gülz [4].

Thin-layer chromatography

Thin-layer silica gel plates (20 cm × 20 cm; 0.2 mm silica-gel thickness; silica gel 60, Merck, Darmstadt, FRG) were developed in benzene. Bromothymol blue (0.04 g/100 ml 0.01 N NaOH; Merck) was used for staining. Unsaturated bonds were identified on similar plates impregnated with $AgNO_3$ [5].

Gas chromatography

Between 0.3 and 0.5 µl of each fraction was analysed using a Hewlett Packard 5710A gas chromatograph with FID, integrator and a 12 m glass capillary column packed with OV-101 (Macherey and Nagel, Düren, FRG). The carrier gas was N_2. The temperature programme consisted of 2 min at 160 °C followed by an increase up to 340 °C at a rate of 4 °C/min. Individual compounds and compound-classes were identified by comparison with standard mixtures, and were quantified using measurements of peak areas.

Results

"Epicuticular waxes" of pollen and pollen wings

"Epicuticular waxes" comprised between 1.3% (Po. – 1984) and 1.6% (Po. – 1986) of the dry weight of *Pinus* pollen. The wings, which are derived exclusively from the exine [6–8], contained 3.5% waxes.

The waxes of pollen and their wings contained hydrocarbons, aldehydes, wax esters, fatty acids and primary alcohols (Fig. 1). As expected, these substances were present as homologous series. Although thin-layer chromatography of the fractions obtained following column fractionation indicated the presence of ketones, their presence could not be confirmed by gas chromatography.

Characterization of the individual lipid classes

Hydrocarbons were present in such small quantities that a detailed characterization of these substances was not possible. The distribution pattern of the other components is shown in Fig. 2. It should be noted that although low concentrations of odd-numbered wax esters were observed, odd-numbered

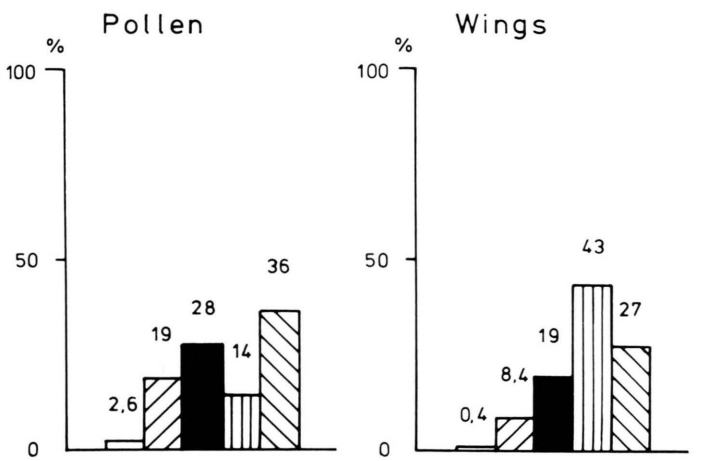

Fig. 1. Composition of the "epicuticular waxes" from pine pollen and wing material (□: hydrocarbons; ▨: aldehydes; ■: wax esters; ▥: free fatty acids; ▧: primary alcohols).

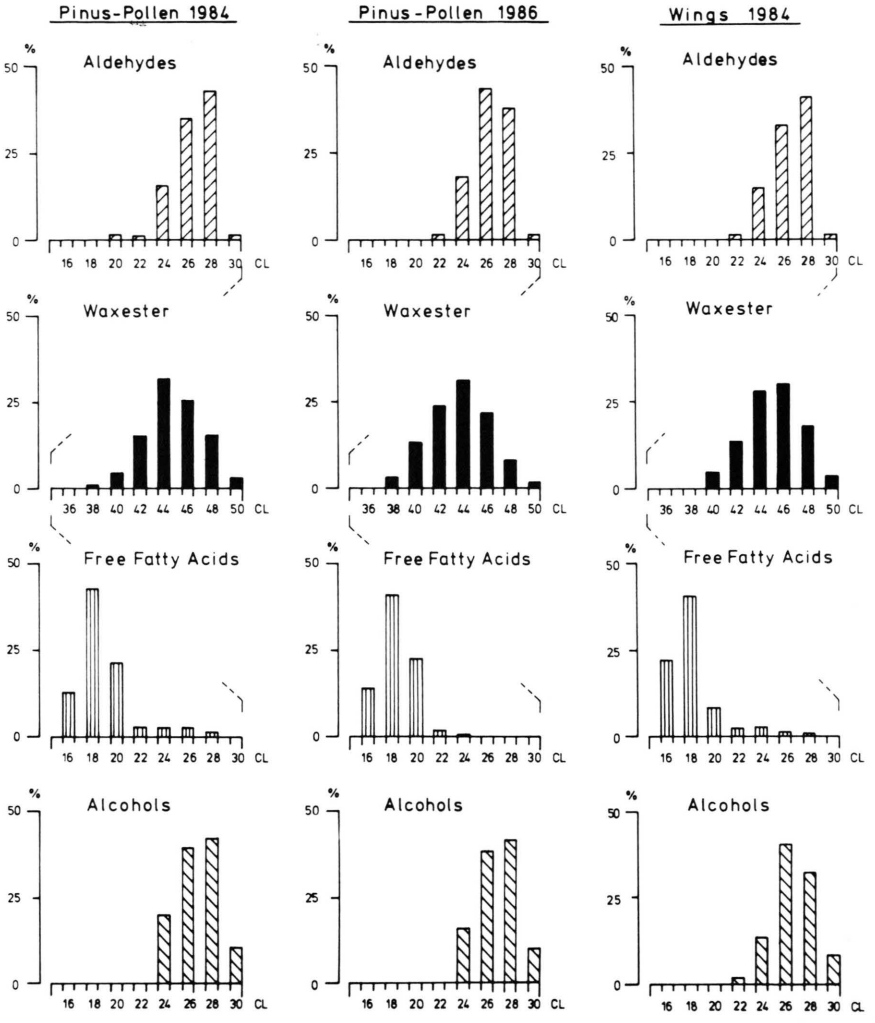

Fig. 2. Distribution patterns of epicuticular waxes from pollen and wing material. (The values are percentages of the total weight of each lipid class.)

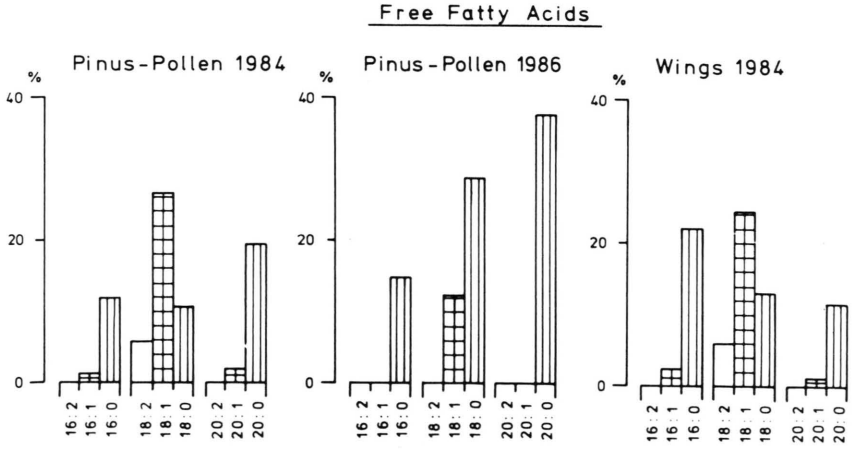

Fig. 3. Distribution patterns of free fatty acids of waxes from pollen and wing material.
□: 2 unsaturated bonds.
▦: 1 unsaturated bond.
▥: saturated.

aldehydes, fatty acids and alcohols were either not detected or were present only in trace amounts.

The presence of a large number of unsaturated (16:1, 18:1, 18:2, 20:1, 20:2) fatty acids is noteworthy (Fig. 3). Low concentrations of unsaturated wax esters were also identified. The presence of these unsaturated substances was confirmed by thin-layer chromatography on $AgNO_3$-impregnated silica-gel plates [5].

A comparison of the composition of the waxes in pollen and in pollen wings shows that although differences in the quantitative composition may occur, the distribution patterns of chain lengths are, in general, similar between the two fractions.

Discussion

The outer pollen wall, the exine, is a compartment where various substances may be localized. The accumulation of carotenoids in and/or on the exine structure has long been recognized. Recently, it has been shown that flavonoids and very complex phenylpropanes, such as *E*-caffeoyl-(*E*)-feruloyl- and di-(*E*)-feruloylspermidine, are also present [9–11]. This paper describes for the first time the role of the exine as a carrier of "epicuticular waxes". The evidence was obtained from exine material isolated in the form of *Pinus* pollen wings.

Various hydrocarbons, aldehydes, wax esters, free fatty acids and primary alcohols were identified in the wax from the exine. The hydrocarbons were only present in small quantities. The dry weight of the pollen waxes constituted between 1.3 and 1.6% of the total pollen dry weight. These values are below the 2.1 to 2.5% reported for 1-year-old needles from *Pinus cembra* [12].

An overall similarity was observed in the major constituents and their distribution patterns of waxes isolated from pollen collected from different sources and different years (Fig. 2).

Although waxes from whole pollen wings exhibited quantitative differences, the distribution patterns of chain lengths of the individual components were similar. Differences were observed, however, in the amounts of fatty acids. It is possible that the exine is more accessible to the extraction solvents after the removal of the wings from the pollen corpus. The extra steps necessary for the purification of the wings may also be involved.

The "epicuticular waxes" from gymnosperms have been extensively studied [13–17]. A comparison of our observations with those of Günthardt [18] and Günthardt and Wanner [12] on *Picea* and *Pinus* needles is of relevance. Pollen waxes contain no secondary alcohols, such as non-acosan-10-ol, which are typical for almost all gymnosperm needle waxes [14–16]. An unexpected observation was the high content of unsaturated fatty acids with chain lengths of C_{16}–C_{20}.

There is clear evidence in *Pinus*, as in a number of other plant systems, for organ-specific composition of cuticular waxes. In comparison to needle waxes, pollen waxes can be identified by the following characteristics: the levels of hydrocarbons are low, they contain considerable amounts of characteristic aldehydes not previously identified in needle waxes, and they are deficient in the secondary alcohols and ω-hydroxy-fatty acids typical of needle waxes. Through the accumulation of waxes in and/or on the exine, pollen has aquired an additional and effective protective mechanism against the effects of the environment. According to Schönherr [19], such waxes may possibly provide a substantial increase in the barrier against water diffusion.

Acknowledgements

This work was financially supported by a grant from the Deutsche Forschungsgemeinschaft to R. Wiermann. We thank Rosemary and Joe Holtum, Münster, for help in preparing the manuscript.

[1] P. E. Kolattukudy, in: The Biochemistry of Plants, **Vol. 4** (P. K. Stumpf and E. E. Conn, eds.), pp. 571–645, Academic Press, New York, London, Toronto, Sydney, San Francisco 1980.
[2] K. Schulze Osthoff and R. Wiermann, J. Plant Physiol., in press (1987).
[3] P. G. Gülz, Ber. Dtsch. Bot. Ges. **99,** 89–97 (1986).
[4] P. G. Gülz, Z. Naturforsch. **37c,** 1053–1056 (1982).
[5] L. J. Morris, J. Lipid Res. **7,** 717–731 (1966).
[6] P. Martens and L. Waterkeyn, La Cellule **62,** 171–222 (1962).
[7] G. Shaw and A. Yeadon, Grana Palynologica **5:2,** 247–252 (1964).
[8] J. R. Rowley and B. Walles, in: Proceedings of the 8th International Symposium on Sexual Reproduction in Seed Plants, Ferns and Mosses (M. T. M. Willemse and J. C. Went, eds.), pp. 56–57, Pudoc, Wageningen 1985.
[9] R. Wiermann and K. Vieth, Protoplasma **118,** 230–233 (1983).
[10] B. Meurer, V. Wray, L. Grothjahn, R. Wiermann, and D. Strack, Phytochemistry **25,** 433–435 (1986).
[11] S. Gubatz, S. Herminghaus, B. Meurer, D. Strack, and R. Wiermann, Pollen et Spores **XXVIII,** 347–354 (1986).
[12] M. S. Günthardt and H. Wanner, Flora **172,** 125–137 (1982).
[13] J. Borges Del Castillo, C. J. W. Brooks, R. Cambie, G. Eglinton, R. J. Hamilton, and P. Pellitt, Phytochemistry **6,** 391–398 (1967).
[14] D. Corrigan, R. F. Timoney, and D. M. X. Donnelly, Phytochemistry **17,** 907–910 (1978).
[15] R. A. Franich, L. G. Wells, and P. T. Holland, Phytochemistry **17,** 1617–1623 (1978).
[16] G. Herbin and P. A. Robins, Phytochemistry **7,** 1325–1337 (1968).
[17] P. Schütt und H.-J. Schuck, Flora **162,** 206–214 (1973).
[18] M. S. Günthardt, Epicuticular Wax of *Picea abies* needles. 6. International Symposium on the Structure, Function and Metabolism of Plant Lipids, Neuchatel, Switzerland, L-802 (1984).
[19] J. Schönherr, Planta **131,** 159–164 (1976).