Chemistry and Morphology of Epicuticular Waxes from Leaves of Five *Euphorbia* Species

Herbert Hemmers and Paul-Gerhard Gülz

Botanisches Institut der Universität zu Köln, Gyrhofstraße 15, D-5000 Köln 41, Bundesrepublik Deutschland

Kurt Hangst

Museum für Ostasiatische Kunst, Universitätsstraße 100, D-5000 Köln 1, Bundesrepublik Deutschland

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The surface waxes of five *Euphorbia* species *(Euphorbiaceae)* were studied by chemical methods and by scanning electron microscopy. The yields of epicuticular waxes, expressed as percentages of the dry weights, differed from species to species. Qualitatively the five species showed the same wax composition but differences appeared in the amounts of single wax components and in their distribution patterns. The predominance of mainly saturated and long chained components as well as the high amounts of primary alcohols and triterpenols resulted in crystalline wax layers. The comparable chemical wax composition of all five species resulted in a quite similar morphological appearance of wax crystals.

Introduction

Aerial parts of higher plants are covered by a thin continuous waxy layer. These epicuticular waxes are composed of complex mixtures of lipophilic compounds in which certain constituent classes or single components usually dominate [1–4]. The epicuticular waxy layer acts as a barrier between the plant and its environment and therefore plays a fundamentally protective role. The most important functions are the reduction of transpiration and the control of gas exchange [5–7]. Further functions are the reduction of surface wettability [8–11], and protection against fungal pathogens, bacteria, virus and insect attacks [12–17]. The morphological appearance of surface waxes is very different. There are amorphous wax layers with more or less liquid consistency as well as strong structured crystalline wax coatings of varying forms [4, 18–20].

The different morphology of the wax structures is caused primarily by the chemical nature, composition and distribution patterns of the epicuticular waxes [18, 21–23]. The following study shows epicuticular wax structures of five *Euphorbia* species obtained by scanning electron microscopy in correlation with the chemical composition of these waxes. The species studied were *Euphorbia characias, E. cyparissias, E. lathyris, E. niccaensis* and *E. peplus,* all herbs or shrubs widespread in Europe.

Reprint requests to Dr. P.-G. Gülz.

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Materials and Methods

Plant material for this study was cultivated from seeds in the field of the Botany Institute in Cologne under the same environmental and horticultural conditions. Plants were extracted directly with chloroform by dipping the shoots into the solvent. The chloroform extract was decanted and concentrated by evaporation. The crude wax was fractionated by column chromatography on silica gel (Merck 60). The column was successively eluted with pentane for hydrocarbons, with 2-chloropropane for esters and aldehydes, and with methanol for free alcohols and free fatty acids. Yields and composition were described separately [24]. Pieces of fresh leaves of the five *Euphorbia* species were prepared for electron microscopy by sputtering with gold (about 800 A) and examinated in a Philips PSME 500 scanning electron microscope of 12 KV. Preservation of natural surfaces and individual wax platelets appeared excellent by this method.

Results and Discussion

The SEM micrographs show that the *Euphorbia* leaf surfaces consist of a dense continuous layer of wax which is superimposed by formations of wax crystals (Fig. 1 and 3). Fig. 2 shows that surface

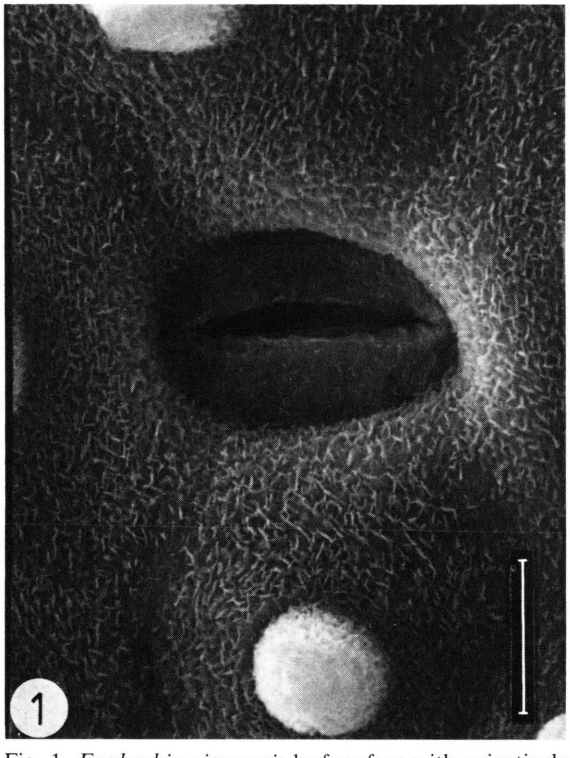

Fig. 1. *Euphorbia niccaensis* leaf surface with epicuticular wax layer, stoma and cuticular papillae. Bar = 10 μm.

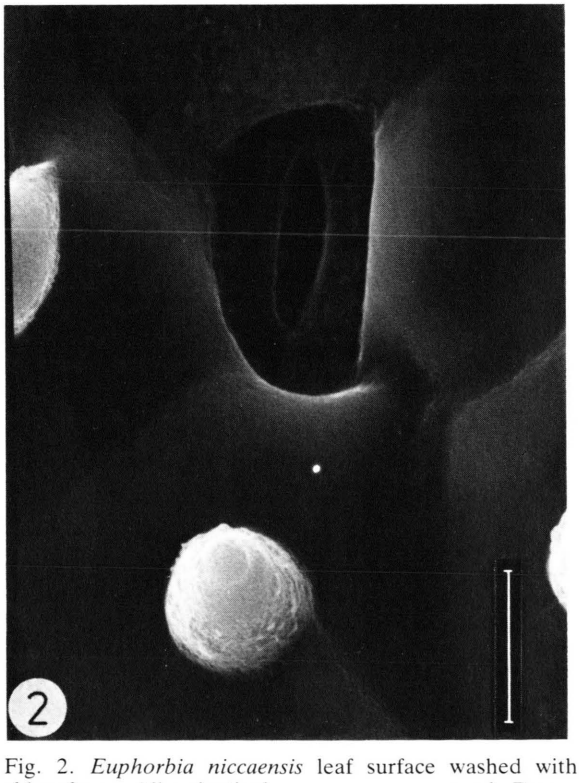

Fig. 2. *Euphorbia niccaensis* leaf surface washed with chloroform. All epicuticular waxes are removed. Bar = 10 μm.

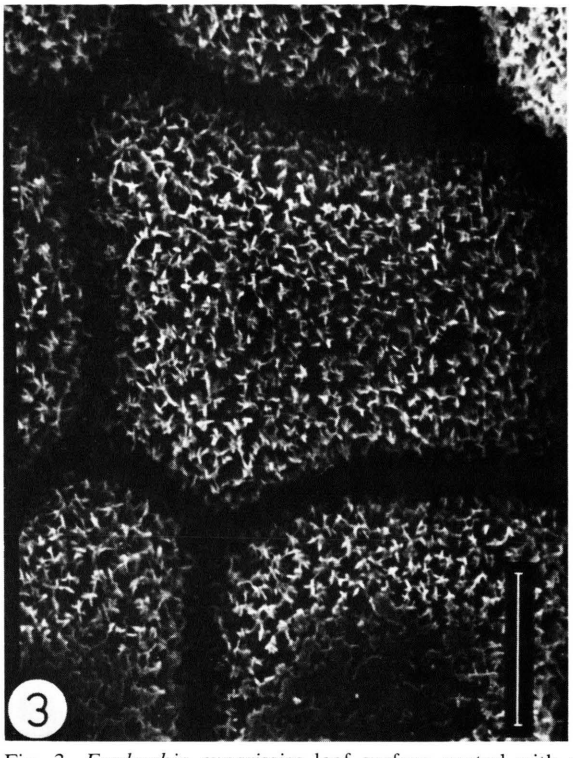

Fig. 3. *Euphorbia cyparissias* leaf surface coated with a dense layer of crystalline wax. Bar = 10 μm.

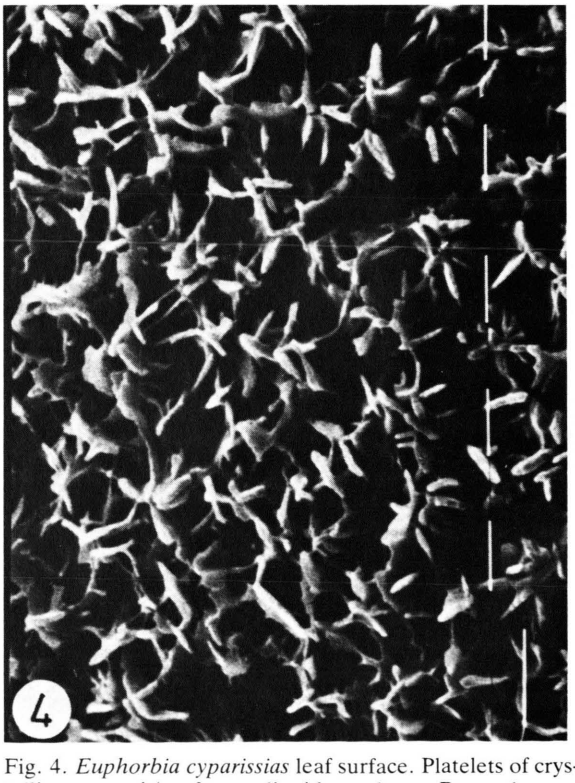

Fig. 4. *Euphorbia cyparissias* leaf surface. Platelets of crystalline wax arising from a liquid wax layer. Bar = 1 μm.

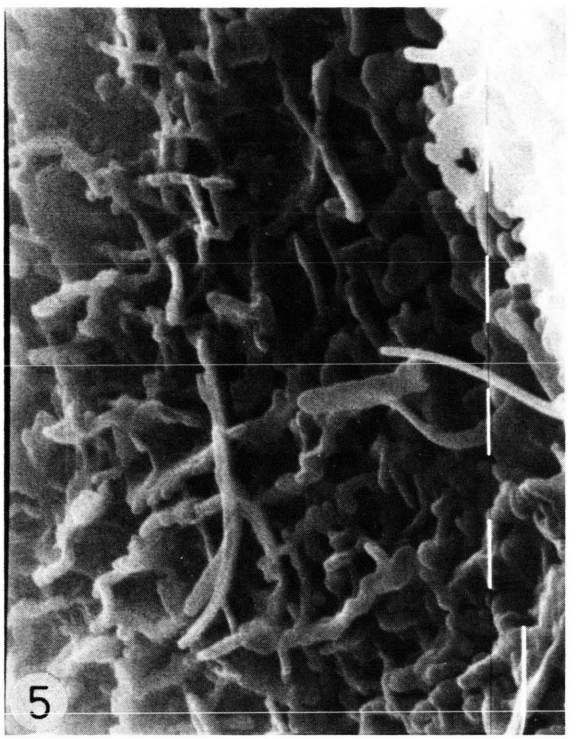

Fig. 5. *Euphorbia characias* leaf surface. Thick crystalline wax plates and occasional ribbons. Bar = 1 μm.

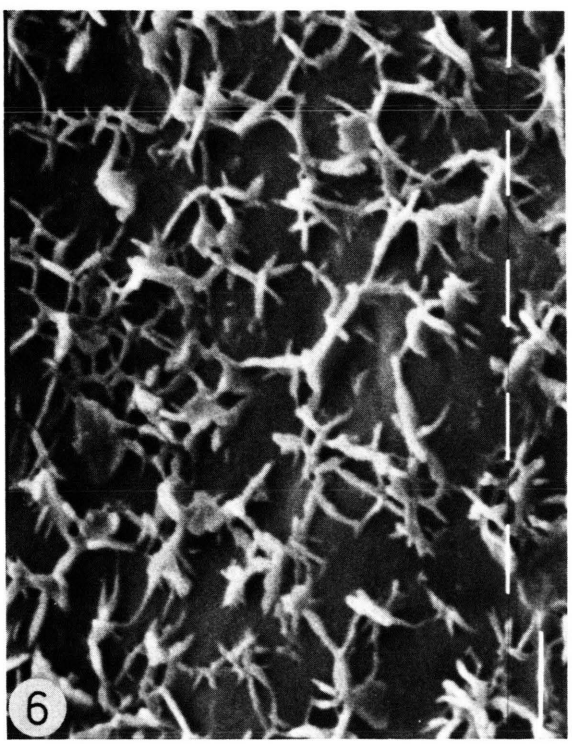

Fig. 6. *Euphorbia lathyris* leaf surface. Clusters of irregular shaped thin wax platelets with fringed edges. Bar = 1 μm.

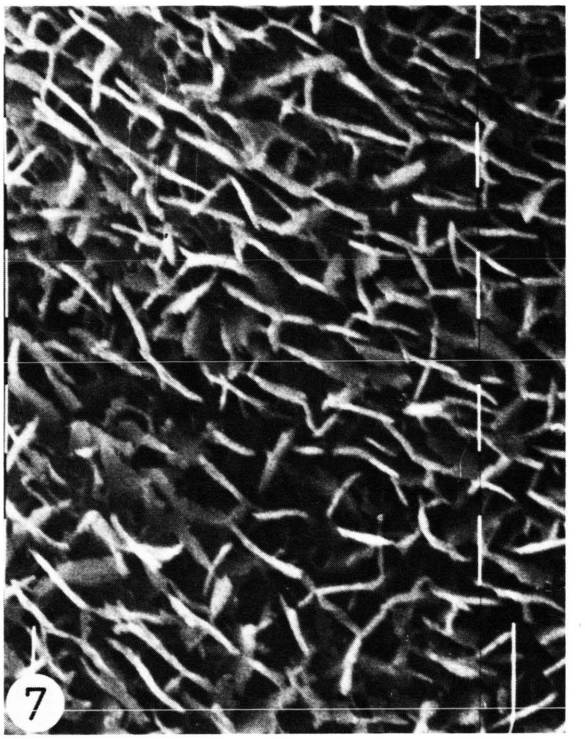

Fig. 7. *Euphorbia niccaensis* leaf surface with a dense layer of thin crystalline wax plates. Bar = 1 μm.

Fig. 8. *Euphorbia peplus* leaf surface with small crystalline wax platelets which have fringed edges. Bar = 1 μm.

waxes can be completely removed with chloroform. These extracted waxes consist of homologous series of *n*-alkanes, wax esters, aldehydes, alcohols, fatty acids and furthermore of free and esterified triterpenols.

Their composition and distribution patterns are shown in Fig. 9. These waxes contained qualitatively the same constituents but differed from species to species in the relative quantity of single components and additionally in their distribution patterns. As can be seen waxes are complex mixtures of numerous substances with different functional groups and most of them form homologous series. This leads to a melting point depression of the single components. The complex mixtures form primarily congealed liquid layers similar to the liquid phases in GLC. These liquid layers be designated as mother liquor in which single wax components dissolve each other. From this mixture the main components may crystallize. In general all *Euphorbia* leaf surfaces are cov-

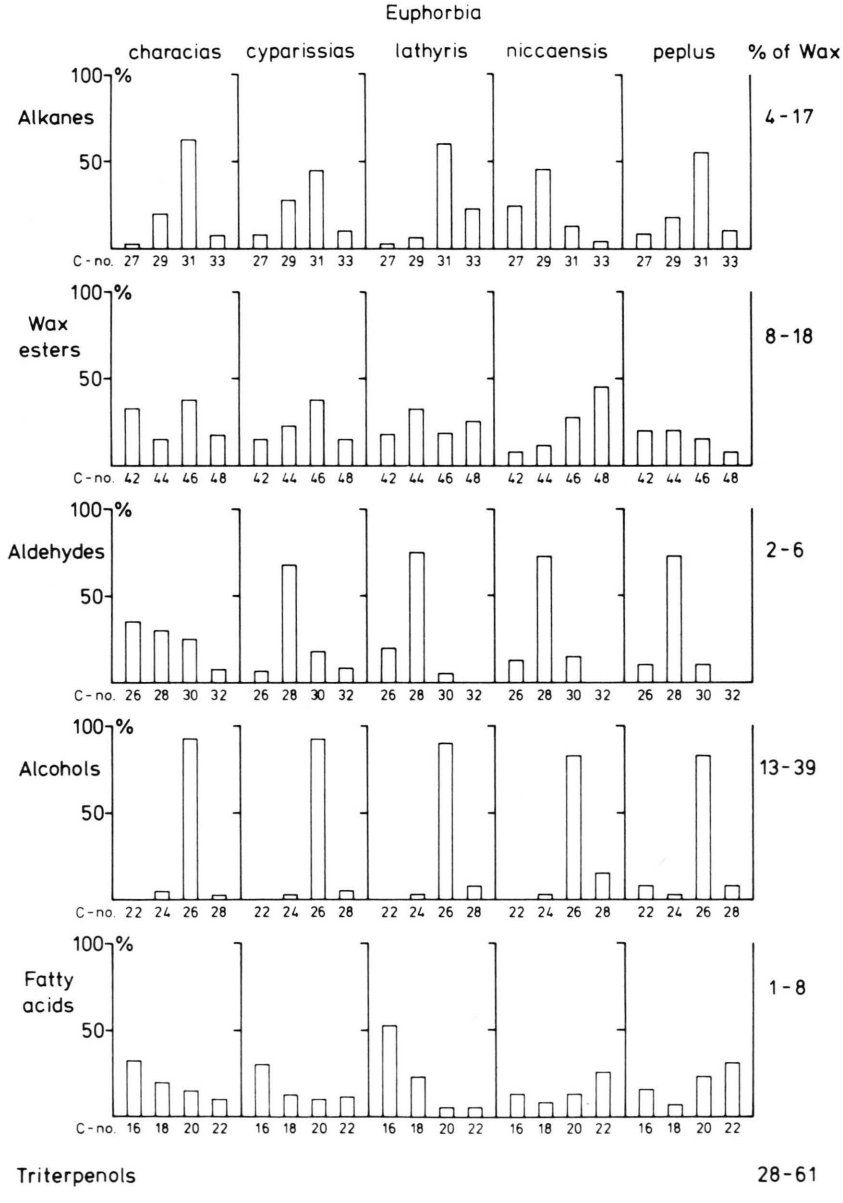

Fig. 9. Composition and distribution patterns of epicuticular waxes from leaves of five *Euphorbia* species. All waxes consist of alkanes, wax esters, aldehydes, primary alcohols, fatty acids, and triterpenols (horizontal bars). The patterns of each *Euphorbia* species (vertical bars) were described in peak area percent of the gas chromatograms.

ered with wax crystals of various density. Clusters of irregular shaped wax plates surrounded by zones of fluid wax coatings could be observed in the micrographs of *E. cyparissias* (Fig. 4) and *E. lathyris* (Fig. 6) whereas *E. niccaensis* (Fig. 7) and *E. peplus* (Fig. 8) showed thin plates with fringed edges, randomly distributed. *Euphorbia niccaensis* (Fig. 7) and *E. characias* (Fig. 5) showed more compact crystal structures than the other plants. The plates of *E. niccaensis* appeared larger than those of *E. peplus*. The latter are intersperced by smooth wax coatings. The wax formations of *E. characias* (Fig. 5) were thick well rounded plates with occasional ribbons. The similar morphology of the wax structures, occurring always as platelets of varying forms on the surface of the leaves, are primarily a function of the chemical nature, the composition and distribution patterns of these surface waxes. All species examined had generally the same wax composition with a predominance of very long chained and primarily saturated substances. There were especially high amounts of triterpenols and free primary alcohols with strongly defined main components. This results in fine crystalline platelets as shown in the Figs. 1 and 3 to 8.

[1] G. Eglinton and R. J. Hamilton, Science **156,** 1322 (1967).
[2] P. E. Kolattukudy, in: Chemistry and Biochemistry of Natural Waxes (P. E. Kolattukudy, ed.), p. 290, Elsevier, Amsterdam 1976.
[3] A. P. Tulloch, in: Chemistry and Biochemistry of Natural Waxes (P. E. Kolattukudy, ed.), p. 235, Elsevier, Amsterdam 1976.
[4] E. A. Baker, in: The Plant Cuticle (D. F. Cutler *et al.*, eds.), p. 139, Academic Press, New York 1982.
[5] M. Grncarevic and F. Radler, Planta (Berl.) **75,** 23 (1967).
[6] D. A. Reicosky and J. W. Hanover, Am. J. Bot. **63,** 449 (1976).
[7] E. Sutter and R. W. Langhans, Can. J. Bot. **12,** 2896 (1982).
[8] P. J. Holloway, Pestic. Sci. **1,** 156 (1970).
[9] A. G. Netting and P. v. Wettstein-Knowles, Planta (Berl.) **114,** 289 (1973).
[10] T. J. Swiecki, A. G. Endress, and O. C. Taylor, Can. J. Bot. **60,** 316 (1982).
[11] B. E. Juniper and C. E. Jeffree, Plant Surfaces (E. Arnold, ed.), London 1983.
[12] P. Schütt, Eur. J. For. Path. **1,** 32 (1971).
[13] P. Schütt, Eur. J. For. Path. **2,** 43 (1972).
[14] L. L. Jackson, L. Dobbs, A. Hildebrand, and R. A. Yokiel, Phytochemistry **12,** 2233 (1973).
[15] T. J. Swain, Ann. Rev. Plant Physiol. **28,** 479 (1977).
[16] S. Woodhead, C. G. Galetti, and G. B. Marini Bettolo, Phytochemistry **21,** 455 (1982).
[17] D. S. J. Atkin and R. J. Hamilton, J. Nat. Prod. **45,** 694 (1982).
[18] P.-G. Gülz and K. Hangst, Z. Naturforsch. **38c,** 683 (1983).
[19] C. E. Jeffree, E. A. Baker, and P. J. Holloway, in: Microbiology of Aerial Plant Surfaces (C. H. Dickinson and T. T. Preece, eds.), p. 119, Academic Press, New York 1976.
[20] W. Barthlott und E. Wollenweber, Tropische und Subtropische Pflanzenwelt **32,** 1, Akad. Wiss. Lit., Mainz 1981.
[21] D. M. Hallam, Planta (Berl.) **93,** 257 (1970).
[22] C. E. Jeffree, New Phytol. **75,** 539 (1975).
[23] C. E. Jeffree and A. P. Sandford, New Phytol. **91,** 549 (1982).
[24] H. Hemmers and P.-G. Gülz, Phytochemistry (1986), in press.