Chemoecology 10:033–040 (2000)
0937–7409/00/010033–08 $1.50 + 0.20

CHEMOECOLOGY

Chemical composition of the slippery epicuticular wax blooms on *Macaranga* (Euphorbiaceae) ant-plants

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Summary. The stems of many *Macaranga* ant-plants (Euphorbiaceae) are covered by epicuticular wax crystals rendering the surface very slippery for most insects. These wax blooms act as selective barriers protecting the symbiotic ant partners, which are specialized "wax-runners", against the competition of other ants. Glaucous stems occur almost exclusively among the ant-plants of the genus *Macaranga* (Federle *et al.* 1997). We analyzed the cuticular lipids of 16 *Macaranga* species by GC-MS and investigated the wax crystal morphology using SEM. Presence of crystalline wax blooms was strongly correlated with high concentrations (52%–88%) of triterpenoids. In contrast epicuticular waxes of glossy *Macaranga* surfaces contained only 0% to 36% of these dominant components. Therefore we conclude that triterpenoids are responsible for the formation of the thread-like *Macaranga* wax crystals. In all *Macaranga* ant-plants investigated, the principal components were epitaraxerol and taraxerone accompanied by smaller portions of taraxerol, β-amyrin and friedelin. Only in the case of the non-myrmecophytic *M. tanarius* did β-amyrin predominate. Moreover, we found that only in *M. tanarius*, the dense wax crystal lacework is torn into large mosaic-like pieces in the course of secondary stem diameter growth. Both chemical and macroscopic differences may contribute to a reduced slipperiness of *M. tanarius* stems and appear to be functionally important. The distribution of wax crystals and their composition amongst different sections of the genus suggests that glaucousness is a polyphyletic character within *Macaranga*.

Key words. epicuticular waxes – triterpenoids – fine structure – SEM – insect attachment – ant-plants – *Macaranga* – Euphorbiaceae

Introduction

In SE Asia, the pioneer tree genus *Macaranga* (Euphorbiaceae) comprises about 51 species, 24 of which are obligate ant-plants ("myrmecophytes"). The biology of ant-plant associations in this genus has been studied intensively in recent years (Fiala *et al.* 1989; Fiala & Maschwitz 1990, 1991, 1992a,b; Federle *et al.* 1998a,b; Heil *et al.* 1997, 1998). The ant partners of *Macaranga* (genera *Crematogaster* and *Camponotus*) nest inside the hollow twigs and predominantly feed on food bodies provided by their host plants.

The stems of some *Macaranga* myrmecophytes are glaucous, *i.e.* covered by epicuticular wax crystals. These wax blooms have been shown to be slippery for insects (Federle *et al.* 1997). Only the specialized ant partners of "waxy" host trees are capable of walking on the glaucous surfaces without any difficulty. The epicuticular wax crystals on vertical connecting stems thus act as selective barriers which protect the specialist ant partners against invasion of "foreign" generalist ants. Within the genus *Macaranga*, glaucous stems are found almost exclusively among the ant-plant species (50% of 26 ant-plant morphospecies; 6.7% of 30 non-myrmecophytic morphospecies; Federle *et al.* 1997).

The present study focuses on the chemical compounds that are responsible for the formation of the slippery *Macaranga* epicuticular wax crystals. Epicuticular wax crystals occur on various plant species and organs in specific shapes and arrangements (Barthlott *et al.* 1998). Corresponding surface wax mixtures were in many cases found to contain a dominant component (Baker 1982; Jetter & Riederer 1994). As suggested by recrystallization experiments (Jeffree *et al.* 1975; Jetter & Riederer 1994, 1995), these compounds are essential for the formation of the crystals and their identity, together with crystallization conditions, determines the crystal geometries.

Most extraction techniques remove epicuticular crystals together with intracuticular waxes. We therefore attempted a partially selective removal of epicuticular crystals by sampling from the outermost part of the cuticle. The composition of wax crystals could then be inferred from comparing glaucous versus non-glaucous *Macaranga* surfaces with regard to chemical composition and fine structure.

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Materials and methods

Plant material and sample preparation

Macaranga plants were collected from Peninsular Malaysia or Borneo and grown in the greenhouses of the Botanical Garden, University of Würzburg. 16 *Macaranga* species were selected to represent a wide range of character combinations including both ant-plants and species not associated with ants as well as glaucous and glossy species. *Manihot esculenta* (Euphorbiaceae) was included as a glaucous outgroup species. *Macaranga* species are named according to Whitmore (1975) and Davies (in press). As we collected samples from greenhouse plants of which we kept only one or a few individuals, no herbarium specimens were collected from our sampled trees. Nevertheless, voucher specimens of the investigated *Macaranga* species are in the collection of the second author.

Waxes were mechanically removed from stems (30 cm below the main shoot tip) and from young leaves by gentle brushing with a swab of dry glass wool held with forceps. This sampling method should provide samples enriched in epicuticular waxes. It consequently cannot yield quantitative information on area-related wax coverage. The collected samples were dissolved in $CHCl_3$. The resulting solutions of cuticular waxes were filtered and the solvent partially removed under reduced pressure. Prior to GC analysis hydroxyl- or carboxyl-containing compounds in all samples were transformed to the corresponding trimethylsilyl (TMSi) derivatives by reaction with bis-N,O-trimethylsilyltrifluoroacetamide (Macherey-Nagel, Düren, FRG) in pyridine (30 min at 70°C). For GC-analysis the sample volume was adjusted to concentrations of approximately 50 µg/ml for main components.

Chemical analysis

The composition of the mixtures was studied by capillary GC (Hewlett Packard 5890 II, Avondale, Pennsylvania, USA) with on-column-injection (30 m DB-1 WCOT i.d. 320 µm, J&W Scientific, Folsom, California, USA) and FI- or MS-detector (70eV, m/z 50–650, hp 5971). GC was carried out with temperature programmed injection at 50°C, oven 2 min at 50°C, 40°C min^{-1} to 200°C, 2 min at 200°C, 3°C min^{-1} to 300°C, 30 min at 300°C. The initial inlet pressure of the carrier gases was adjusted to 50 kPa hydrogen (FID) or 10 kPa helium (MSD). After 41 min the inlet pressure was raised by 10 kPa min^{-1} to a final value of 150 kPa (FID) or 110 kPa (MSD). By this method all common cuticular wax constituents including long-chain alkyl esters up to chain lengths of C_{46} could be detected. Individual compounds were identified by comparing their GC and MS characteristics with those of authentic standards and literature data. Standards for epimeric taraxerol isomers were obtained after reduction of taraxerone with $LiAlH_4$. A sample of taraxerol, which was provided by Robert B. Bates (structure confirmed by NMR; Bates *et al.* 1998), allowed an unambiguous assignment of epitaraxerol and taraxerol. The quantification of individual compounds was based on relative areas of integrated GC-FID peaks. FID response factors (based on mass units) of triterpenoids and aliphatic wax constituents can be considered as approximately equal.

SEM

Small pieces of *Macaranga* stem surfaces were cut out with a razor blade and dried over silica gel. Samples were glued with a double-sided tape onto SEM specimen holders, sputtered with gold for 2 minutes (25 mA) and investigated using a Zeiss DSM 962 scanning electron microscope (working voltage 15 kV).

Results

Wax composition

The waxes of all the glaucous *Macaranga* surfaces analyzed were found to contain high concentrations of triterpenoids (52%–88%; Table 1), while almost all the surfaces with glossy appearance yielded mixtures with only traces of these cyclic compounds (Table 1). In most of the non-glaucous species, waxes with only traces of triterpenoids were isolated. Glaucous and glossy surfaces are often present on different tissues or stages of development (e.g. leaves and stems of *M. hypoleuca*, young and adult stems of *M. hosei* and *M. pruinosa*). In these cases, glaucousness was again correlated with high triterpenoid portions in the waxes. Stems of *M. hullettii* and upper leaf surfaces of *M. hypoleuca* were the only non-glaucous surfaces that yielded waxes with considerable triterpenoid concentration (36% and 28%, respectively), but even these maximal figures were well below the minimum (52%) of all the glaucous samples investigated. The correlation between glaucous appearance and triterpenoid content of the wax was highly significant (U-test: $n = 21$; $U = 104$; $z = -3.79$; $P < 0.001$). The major triterpenoids occurring in waxes from glaucous *Macaranga* surfaces were identified as epitaraxerol, taraxerol, taraxerone, β-amyrin and friedelin. In most of these triterpenoid mixtures epitaraxerol and taraxerone prevailed (35%–73% of the total wax), both compounds occurring in ratios between 2:1 and 2:5 (Table 1). All the other triterpenoids were present in smaller portions amounting to a total of 6%–17%. Stems of *M. tanarius* were exceptional, because β-amyrin was the dominant surface lipid. It should be noted that in this species taraxerone and epitaraxerol could not be detected. Due to higher preconcentration of samples from glossy surfaces the detection limits for individual triterpenoids were even lower than for wax mixtures from glaucous surfaces. Nonetheless, the principal wax components could not be detected in most of the waxes from glossy *Macaranga* surfaces. Only the stem waxes of *M. puncticulata*, *M. winkleri*, and *M. hullettii* were found to contain small to intermediate percentages of triterpenoids. In all these samples taraxerol was identified as the major cyclic compound while taraxerone and epitaraxerol could not be detected. Somewhat higher concentrations of these principal components were only found on adaxial leaf surfaces of *M. hypoleuca*.

Very-long-chain ($\geq$ C20) aliphatic compounds typical for cuticular waxes as fatty acids, n-alkyl acetates, n-alkanols, n-alkanes, and n-alkanals were exclusively detected in wax mixtures of glossy *Macaranga* surfaces (Table 1).

Fine structure of Macaranga *surfaces*

Macaranga stems either had a very dense web of thread-like epicuticular wax crystals on their cuticles, or they carried only few or no crystals (Table 1; *M. aetheadenia*, *M. hullettii*, *M. hypoleuca*, *M. lamellata*, *M. pruinosa*, *M. tanarius*, and *Manihot esculenta* investigated by SEM). Only on surfaces that looked macroscopically glaucous, were crystals abundant (Fig. 1A, B, E, F; Fig. 2C, D, G). In contrast, glossy *Macaranga* stem surfaces carried no wax crystals at all (*M. triloba* and *M. hullettii* investigated by SEM; Fig. 1C and D).

Table 1 Chemical composition (peak area%) of epicuticular waxes from *Macaranga* surfaces

	Organ	Glaucous	Myrmecophytic	Epitaraxerol [%]	Taraxerone [%]	Taraxerol [%]	β-Amyrin [%]	Friedelin [%]	Other triterpenoids detected*	Sum of triterpenoids [%]	aliphatic compounds^{+}	Sum of identified compounds [%]
***Pachystemon* sensu stricto**												
M. aetheadenia	stems	+	+	40	33	8	4	3	D(tr.)	88	n.i.	88
M. beccariana	stems	+	+	36	30	2	3	2	A(tr.), B(1%)	74	n.i.	74
M. glandibracteolata	stems	+	+	35	26	2	2	5	D(1%)	71	n.i.	71
M. havilandii	stems	+	+	33	26	3	1	6	A(3%)	72	n.i.	72
M. hullettii	stems	−	+	n.i.	n.i.	23	3	5	D(3%), E(2%)	36	n.i.	36
M. hypoleuca	stems	+	+	40	31	2	4	6	B(tr.)	83	n.i.	83
	leaf ad.	−		9	10	2	2	5	D(tr.)	28	ol(21%)[a)], ac(13%)[b)], fa(7%)[c)]	69
	leaf ab.	+		32	25	2	4	2	C(2%), D(3%)	70	n.i.	70
M. lamellata	stems	+	+	22	35	2	5	2	D(1%)	67	n.i.	67
M. motleyana ssp. griffithiana	stems	+	+	25	36	3	5	4	C(1%), D(1%)	75	n.i.	75
M. motleyana ssp. motleyana	stems	+	+	24	26	1	1	4	n.i.	56	n.i.	56
M. triloba	stems	−	+	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	fa(6%)[d)], ol(4%)[e)], al(3%)[f)]	13
***Pachystemon* sensu lato**												
M. puncticulata	stems	−	+	n.i.	n.i.	tr.	n.i.	n.i.	n.i.	tr.	al(6%)[g)], ol(4%)[g)]	10
***M. pruinosa* group**												
M. hosei, adult	stems	+	+	27	32	3	3	2	C(tr.), D(2%), E(1%)	70	n.i.	70
M. hosei, juvenile	stems	−	−	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	fa(4%)[h)]	4
M. pruinosa, adult	stems	+	+	22	13	12	2	2	A(1%), C(tr.), E(tr.)	52	n.i.	52
M. pruinosa, juvenile	stems	−	−	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	fa(5%)[h)]	5
Winklerianae												
M. winkleri	stems	−	+	n.i.	n.i.	1	1	n.i.	n.i.	2	an(14)[i)]	16
Individually distinctive species												
M. gigantea	stems	−	−	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	−	ac(4%)[j)], ol(tr.)[j)]	4
M. tanarius	stems	+	−	n.i.	n.i.	n.i.	65	5	A(3%)	73	n.i.	73
Reference species												
Manihot esculenta	stems	+	−	12	29	8	3	n.i.	A(3%)	55	an(5%)[k)]	60

tr. = traces (< 1%), n.i. = not identified, ad. = adaxial, ab. = abaxial

* A = β-Amyrinon, B = Epitaraxeryl-acetate, C = Lupenon, D = α-Amyrin, E = Lupeol

$^{+}$ ac = n-alkyl-acetate, al = n-alkanal, an = n-alkane, fa = fatty acid, ol = n-alkanol

The distribution of homologues is characterised by (Range of chain lengths; *major homologue*)

[a)] (C26–C34; *C28*) [b)] (C24–C32; *C28*) [c)] (C24–C32; *C26*) [d)] (C20–C36; *C34*) [e)] (C26–C34; *C32*) [f)] (C30–C34; *C32*) [g)] (C26–*C28*) [h)] (*C20*–C30) [i)] (C27–C33; *31*) [j)] (C24–*C26*) [k)] (C29–*C31*)

C16 and C18 fatty acids were excluded from quantification due to possible interference by contamination of the glasswool used for wax sampling

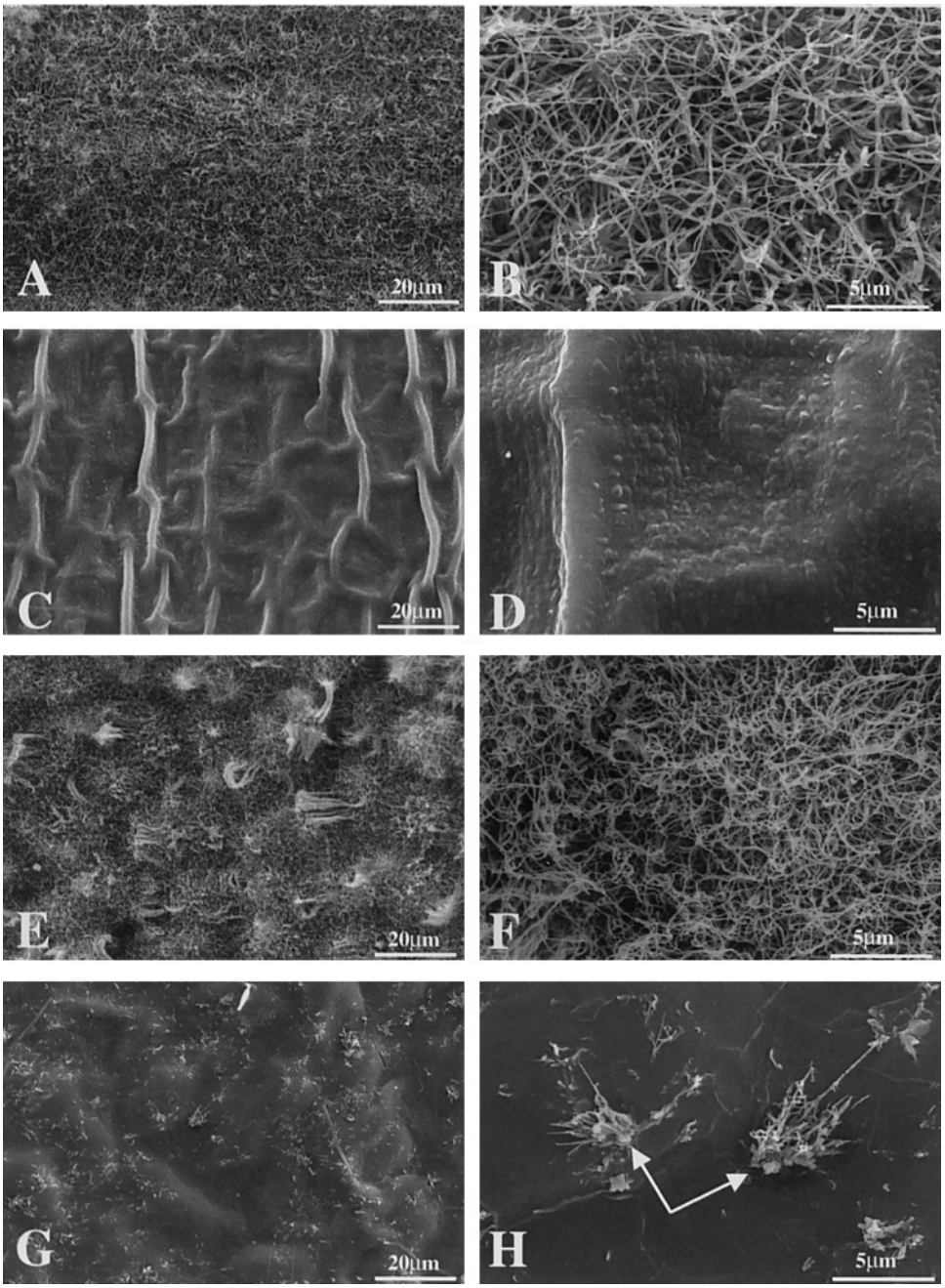

Fig. 1 Fine structure of glaucous and glossy *Macaranga* surfaces. A: *Macaranga lamellata* stem surface (1000 ×). B: *M. lamellata* stem surface (5000 ×). C: *M. triloba* stem surface (1000 ×). D: *M. triloba* stem surface (5000 ×). E: *M. hypoleuca* abaxial leaf surface (1000 ×). F: *M. hypoleuca* abaxial leaf surface (5000 ×). G: *M. hypoleuca* adaxial leaf surface (1000 ×). H: *M. hypoleuca* adaxial leaf surface (5000 ×) crystal patches (arrows)

Only in the case of the glossy adaxial leaf surfaces of *M. hypoleuca*, did we find small patches of crystal threads scattered over a smooth cuticle (Fig. 1G and H). It should be noted that this exceptional, intermediate morphology coincides with the results of the chemical analysis, in which we found intermediate concentrations (19%) of the crystal-forming components epitaraxerol and taraxerone (see Table 1).

Macaranga crystal threads were thin and long (probably more than 10 µm, see Fig. 1B, F) and had few ramifications. There was considerable variation of diameters between different threads (30–420 nm; x = 115 nm, s.d. = 65 nm, n = 120 crystals measured). High magnification images showed that individual strings can be composed of two ''sub-threads'' which form a double helix (Fig. 2A). Thick crystal strings (> 100 nm diameter) were often not cylindrical (like the thin threads) but appeared to be flat ''belts'' (Fig. 2B) possibly composed of parallel threads. Large crystal strings may thus represent aggregates of smaller units.

The basic crystal geometry of all the *Macaranga* species investigated appears to be identical, but some variation occurs with respect to thread diameter and degree of ramification. This pattern was present even in *M. tanarius*, in which β-amyrin was the dominant component. On a larger scale, however, the glaucous *M. tanarius* samples had a strikingly different surface structure. Its crystal threads did not form a homoge-

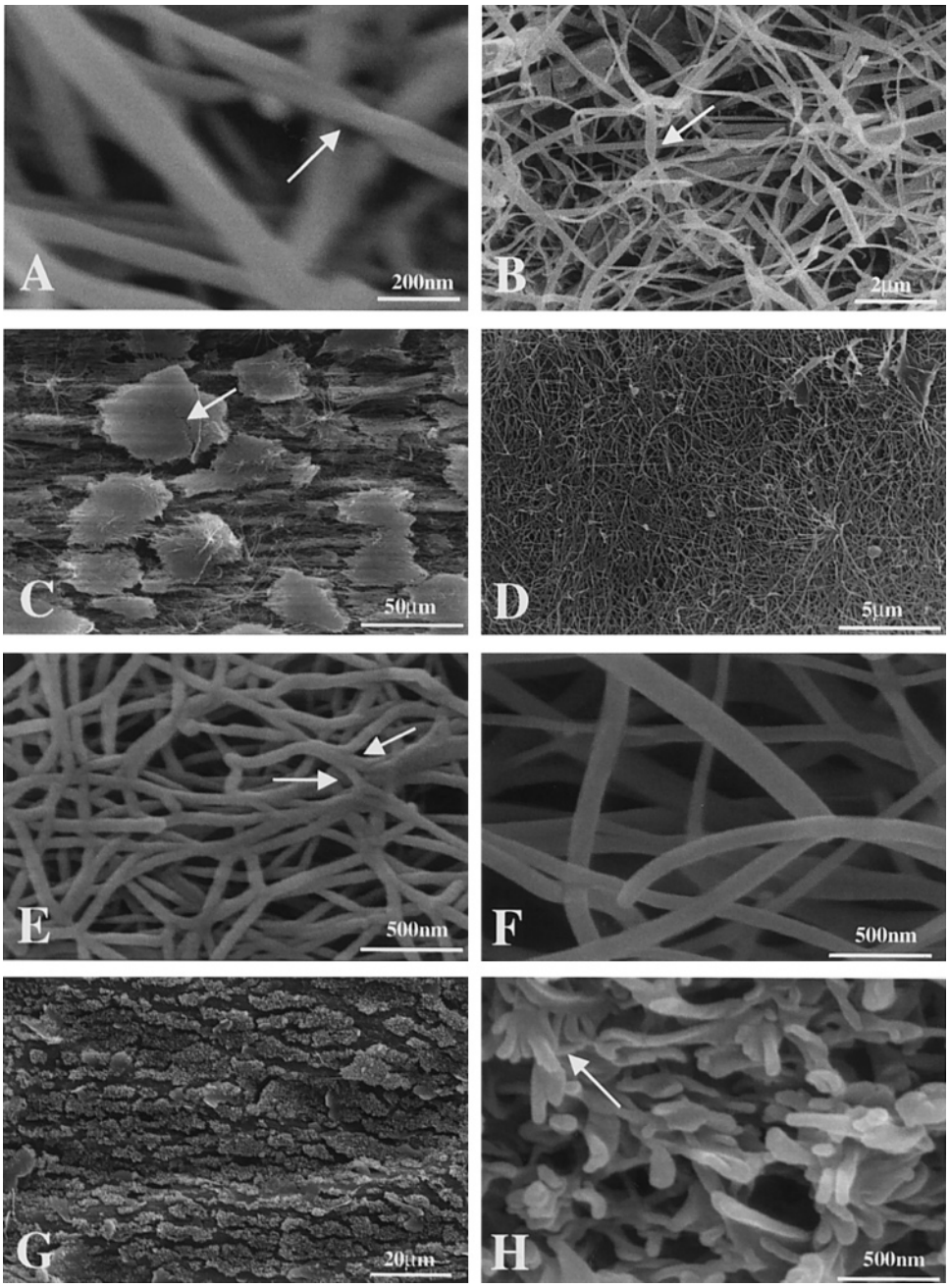

Fig. 2 Structural details of epicuticular wax crystals on surfaces of *Macaranga* spp. and *Manihot esculenta*. A: *M. lamellata* stem surface (100000 ×) double helix (arrow). B: *M. hypoleuca* stem surface (10000 ×) crystal ribbons (arrow). C: *M. tanarius* stem surface (500 ×) crystal layer pieces, note the beginning fracture (arrow). D: *M. tanarius* stem surface (5000 ×) outermost wax crystal layer (on a mosaic piece). E: *M. tanarius* stem surface (50000 ×) outermost wax crystal layer (on a mosaic piece), note the ramifications (arrows). F: *M. tanarius* stem surface (50000 ×) inner wax crystal layer (between the mosaic pieces). G: *Manihot esculenta* stem surface (1000 ×). H: *Manihot esculenta* stem surface (50000 ×); note the finger-like ramifications (arrow). All magnifications indicated refer to the instrument parameter of the SEM

nous surface as observed in all the glaucous *Macaranga* ant-plants. The outermost layer of the wax crystal mesh in *M. tanarius* rather appeared to be torn into flat pieces (10–100 µm in diameter, Fig. 2C). This effect was also visible at low magnifications and we proved its presence in fresh surface samples of 5 haphazardly chosen *M. tanarius* plants (collected 30 cm below the shoot tip). We thus assume that the fracture of the wax crystal web is not a drying artifact, but a process probably caused by secondary growth of diameter of stems. Crystals on mosaic pieces (Fig. 2D and E) had similar shape and dimensions as epitaraxerol/taraxerone crystals on other *Macaranga* surfaces. Areas between mosaic pieces (Fig. 2F) obviously had a reduced crystal density.

We found similar "mosaic" surfaces in *Manihot esculenta* stems (Fig. 2G). Here, the crystals were again threads with diameters in the same order of magnitude as on *Macaranga*. However, they were shorter and much more ramified than in *Macaranga*. The large number of visible wax crystal tips were conspicuously swollen (Fig. 2H).

Discussion

Many plants carry epicuticular wax crystals on their leaves and/or primary stems. These wax crystals show a considerable ultrastructural and chemical diversity and they can be of systematic significance (Barthlott *et al.*

1998). In most cases the formation of special crystal habits is correlated with the presence of a particular compound class in the wax mixture formed by individual constituents occurring at high relative amounts (Baker 1982; Jetter & Riederer 1994). For few crystal types this assumption has been corroborated by recrystallization experiments with epicuticular wax mixtures and with pure compounds (Jeffree *et al.* 1975; Jetter & Riederer 1994, 1995). Consequently, Crystal formation is a spontaneous process occurring when single compounds in a mixture are concentrated above threshold values. Gülz *et al.* (1992) stated that wax crystals can usually be observed if one lipid class is present in concentrations of at least 40% of the total wax.

Our data strongly suggest that the epicuticular wax crystals of *Macaranga* trees are formed by triterpenoids. In all the glaucous *Macaranga* species investigated, triterpenoids made up more than 50% (in some cases $>80\%$) of the total wax. A high content of the two compounds epitaraxerol and taraxerone is probably responsible for crystal formation. Only in *M. tanarius* did the wax crystals consist mainly of β-amyrin.

Several references show a clear correlation between triterpenoid content of epicuticular waxes and glaucousness of plant surfaces. Although only limited information is available on the fine structure of triterpenoid crystals, several sources of data from the literature suggest a great morphological diversity. In agreement with the results of our study, the glaucous appearance of the leaves of two *Dudleya* species (Crassulaceae) was related to high contents of taraxerone (39%–42%) in *D. farinosa* and of β-amyrenyl acetate (40%–45%) in *D. brittonii* (Manheim & Mulroy 1978). Similar to the epitaraxerol/taraxerone and β-amyrin crystals on *Macaranga* surfaces, the wax crystals of *D. brittonii* were characterized as primary threads (20 μm length, 2 μm thickness) with secondary threads (2 μm length, 0.1 μm thickness, Barthlott & Wollenweber 1981). Thread-like wax crystals of the fern *Polypodium aureum* are mainly composed of fernene (Barthlott & Wollenweber 1981), a triterpenoid with a different carbon ring system lacking oxygen-containing functional groups. Apart from triterpenoids, also flavonoids and (dihydro-) chalcones have been reported to form threads (Barthlott & Wollenweber 1981).

In contrast to the thread-like crystals on *Dudleya brittonii*, crystals in the shape of "quadrangular rodlets" were reported from the upper surface of *Tilia tomentosa* leaves, where β-amyrenyl acetate was also the dominant triterpenoid (49%, Gülz 1994). In the wax gourd *Benincasa hispida* four isomeric triterpenol acetates ($\sim 50\%$) and three triterpenol isomers ($\sim 17\%$, Meusel *et al.* 1994) were found to form epicuticular wax crystals, classified as "terete rodlets" (Barthlott *et al.* 1998). From a principal component analysis of chemical wax composition and ultrastructural examinations of 55 varieties of seven *Sedum* species, Stevens *et al.* (1994) concluded that triterpenoids (4%–62% of total wax) are primarily responsible for the glaucousness of leaf surfaces, even when they are not the main constituents of epicuticular wax. Germanicyl formate, fern-8-en-3-yl formate, taraxerone and taraxeryl acetate were identified as principal triterpenoid wax constituents. The crystals were described as "irregularly shaped platelets" without consideration of individual triterpenoids.

No ultrastructural information is available about the epicuticular crystals of epitaraxerol, taraxerone and β-amyrin. Thread-like crystals like the ones found in our study have also been reported from other plant families and may be formed by different triterpenoid components. We cannot yet decide whether the combination of both or only one of the principal components (epitaraxerol/taraxerone; β-amyrin) determines the shape of the crystals on *Macaranga* surfaces. A possible modulating influence of minor wax constituents should also be considered. These questions can only be answered by recrystallization experiments with pure compounds.

In all the glossy *Macaranga* surfaces triterpenoids occurred at much lower concentrations or could not be detected at all. The fact that the *M. hullettii* stem cuticle lacks crystals, even though it contained 23% taraxerol, may be explained by the relatively low total triterpenoid concentration. It seems plausible that according to Gülz *et al.* (1992) the total triterpenoid concentration in this case (36%) is still below a critical threshold range for the formation of crystals. An intermediate form between glossy and glaucous surfaces with small patches of crystal threads was found on the adaxial side of *M. hypoleuca* leaves. This indicates that the crystallization process may start at much lower concentrations (19%) of the crystal-forming components epitaraxerol and taraxerone.

Within the genus *Macaranga*, glaucousness shows a remarkable distribution. Glaucous stems occur almost exclusively among the ant-plants of the genus (Federle *et al.* 1997). This distribution pattern suggests an adaptive significance of glaucousness in *Macaranga* ant-plants. Since the associated ant partners of these plants are perfectly capable of running on the slippery stems, the wax crystals protect these ants against other insects, especially predators and competitors. Selection and protection of highly adapted plant-ants by forming a wax crystal barrier is probably beneficial for a host plant, because these ants provide a more efficient control of herbivores and vine overgrowth than do opportunistic species (Federle *et al.* 1997). Federle *et al.* (1997) had evaluated "glaucousness" by the macroscopic appearance of *Macaranga* trees only. Our data show that this glaucous appearance is explained by the presence of thread-like triterpenoid crystals.

It is still largely unknown how chemical composition and structure of wax crystals are related to slipperiness for insects. Many plant species form wax blooms that are slippery for insects comprising a variety of different crystal structures (e.g. Knoll 1914; Stork 1980b; Eigenbrode 1996). The fact that insects have difficulties attaching to wax crystals can be explained by several hypothetical mechanisms. First, surface roughness strongly decreases the surface energy of a substrate and thus its wettability by liquids. Insects

using adhesive liquids (see e.g. Walker *et al*. 1985; Ishii 1987; Lees & Hardie 1988; Gorb 1998; but see Stork 1980a) would have a poorer attachment due to wet adhesion. Second, air gaps between the wax crystals would prevent the action of a suction cup (even though the use of suction has never been proved in non-aquatic insects). Third, wax crystals could simply break off the surface and detach with an insect's leg. The best-known case of slippery wax blooms are the trapping zones of *Nepenthes* pitchers. Juniper (1986) hypothesized that *Nepenthes* wax scales are easily detached, because they are suspended only on thin stalks from the epidermal surface. No such structure is present in *Macaranga*, where wax crystal threads form a dense three-dimensional lacework. Nevertheless, it seems possible that the thin crystal threads break. The particular chemical composition and specific mosaic structure of the wax blooms on *M. tanarius* could be interpreted in this context. *M. tanarius* is one of the few non-myrmecophytic *Macaranga* species with glaucous stems (Federle *et al*. 1997). Even though they are not permanently ant-inhabited, non-myrmecophytic *Macaranga* species strongly depend on non-specific, foraging ants, which also provide efficient biotic defense against herbivory (Fiala *et al*. 1994). If the stems of such trees were slippery, beneficial ants would be excluded from the tree. However, the glaucous twig surfaces of *M. tanarius* are interspersed with some velvety hairs, which appear to reduce the barrier effect on insects (Federle *et al*. 1997). Moreover, the macroscopic mosaic structure of the wax bloom could cause a reduced slipperiness. However, we do not assume that the different chemical composition of the wax crystals on *M. tanarius* has any functional significance.

In *Macaranga*, genus sections have been established by Whitmore (1975) and Davies (in press), which are well confirmed by molecular phylogenetic data (Frank Blattner, unpubl. res.). Glaucousness occurs in four sections of the genus, which all contain also non-glaucous species. In *Macaranga*, glaucousness even varies between closely related subspecies (subspp. of *M. pruinosa* and *M. kingii*, Federle *et al*. 1997). Glaucousness appears to be an evolutionarily labile plant feature (Jeffree 1986) and may have been acquired and/or lost several times during the phylogeny of *Macaranga*.

However, the chemical composition of the wax crystals appears to be less variable. Epitaraxerol/taraxerone crystals occur not only in at least two *Macaranga* sections (Section *Pachystemon sensu stricto* and *M. pruinosa* group; Table 1), but also in the distantly related Euphorbiacean outgroup species *Manihot esculenta*. Thus, the capacity to produce these particular crystals may be a basic trait in the Euphorbiaceae. The *M. tanarius* wax crystals formed by a different component apparently represent an independent development.

Acknowledgements

The authors are indebted to Bianka Pink and Daniela Seifert for skillful technical assistance, Fritz Thiele from the Botanical Garden Würzburg for great efforts in cultivating many *Macaranga* species and Robert B. Bates (University of Arizona) for providing a taraxerol standard. This work was financially supported by the German Research Council (DFG, SFB-251) and the Fonds der Chemischen Industrie.

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Received 7 October 1999; accepted 3 December 1999.

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