

Seasonal Variations in Epicuticular Wax Ultrastructures of *Quercus robur* Leaves

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Already the very young leaflets of oak (*Quercus robur* L.), newly emerging from their buds, are covered by a thin wax layer, but at this time it lacks any wax sculptures or crystalloids. Both in its amount and its composition this wax layer is quite different to that of mature leaves. After leaf unfolding a dynamic biosynthesis of wax lipids is started. Ten days after the leaf has emerged from its bud, wax crystalloids are seen for the first time, developing out of the wax layer in the form of platelets. These crystalloids increase in size and quantity within the next weeks and are present on both leaf surfaces. On the adaxial side a dense and uniform arrangement of fringed edged platelets is observed. Similar dense arrangements of platelets are found on the abaxial leaf side, but with an intensive concentration and orientation around the stomata. All platelets are composed of tetracosanol, the main wax component, accounting for more than 40% of wax.

Variations in surface wax ultrastructures are observed in July. Well formed platelets and melted away crystalloids are found close together in distinct parts of the same leaf, both on the adaxial and on the abaxial sides of the leaf. These observations pertain to preparations made from July to November with a trend toward recrystallization in October. High temperature and essential oils may be the reasons for the melting away or solution of the platelets.

Introduction

All aerial organs of higher plants are covered by a thin continuous wax layer. This layer represents the interface between land plants and their aerial environment and is a protective and a transpiration barrier of the epidermal plant cells. This layer is therefore responsible for the control of transpiration and gas exchange through the stomata [1–3].

On mature leaves the wax layers are often superimposed with wax sculptures or wax crystalloids, and show most different ultrastructures on the adaxial and the abaxial leaf surfaces, for example on broadleaf trees [4–8].

Epicuticular leaf waxes consist of homologous series of very long chained lipids and usually of triterpenoids, also. Yield and composition of these wax mixtures change during leaf development and growth as shown recently for *Fagus sylvatica* [9, 10], *Tilia tomentosa* [11], *Quercus robur* [12] and *Citrus aurantium* [13].

The very young oak (*Q. robur*) leaflets just emerging from buds contain a continuous wax layer quite different in yield and composition from that of the mature leaves. During May and June a dynamic biosynthesis of wax lipids is observed. Wax amount is doublet quantitatively with respect to leaf dry weight (0.57% to 1.10%) or leaf surface area (24.1 µg/cm² to 48.1 µg/cm²). Wax amount increased from 64 µg to 4574 µg per leaf in 1991. Wax of mature leaf consists of homologous series of hydrocarbons, wax esters, aldehydes, fatty acids and the triterpenols: taraxerol, β-amyrin, α-amyrin and lupeol, free and esterified with fatty acids [12].

The surface wax structures of *Q. robur* were studied continuously by scanning electron microscopy (SEM) in the present investigation during a vegetation period in 1990 and also 1991. These results are correlated with the chemical composition of the leaf wax, analyzed at the same stages, in order to study the variations of surface wax ultrastructures and their chemical implications as well as leaf developmental factors.

Materials and Methods

Leaves of the oak tree *Quercus robur* L. were harvested from an isolated tree, grown in the garden of the Botanical Institute, of the University of

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Cologne. Leaf samples were collected from April to November in 1990 and 1991. Leaves were harvested twice a week in April, May and June and then once a week for the rest of the season. Epicuticular waxes were extracted from leaves by immersing them in CHCl_3 . Wax components were isolated and analyzed as described by Gülz and Müller [12]. The patterns of the oak leaf wax components on 24th April, 4th May and in August 1990 in Fig. 7 are described in terms of peak area percentages of the gas chromatograms. For SEM fresh and air-dried leaves were prepared by sputtering with gold using an Emscope sputter coater and examined under a Hitachi S-405 A und Cambridge Stereoscan 100 scanning electron microscope at 15 and 25 kV, respectively. The SEM figures shown in this paper resulted primarily from preparations made in 1990.

Results

First observations of oak leaflets emerging from their buds were made on 24th April in 1990 and

6th May in 1991. In consequence of a spell of cold weather in April 1991 [14], the leaf unfolding was retarded in that year. The same coldness and late leaf emergence was found also for 1992. At the initial developmental stage, leaf area was found to be 1.3 cm^2 in both years. Fig. 1 shows an SEM of the adaxial (Fig. 1 A and 1 C) and abaxial (Fig. 1 B and 1 D) surfaces of very young already rolled leaflets. Epidermal cells are folded at this initial stage and are covered in a wax layer devoid of any wax sculptures or crystalloids. This wax layer was extracted by washing with chloroform and the folded epidermal cells with their intact cutin layer are now clearly visible. The wax extract was analyzed throughout the vegetation period as described by Gülz and Müller [12].

The adaxial leaf surface shows no trichomes, the abaxial bears numerous stomata and single glandular trichomes. The development of these organs is incomplete at that early stage. Stellate hairs are found on leaves of most *Quercus* species [15], but are absent on *Q. robur* leaves as well as on *Q. petraea* [16].

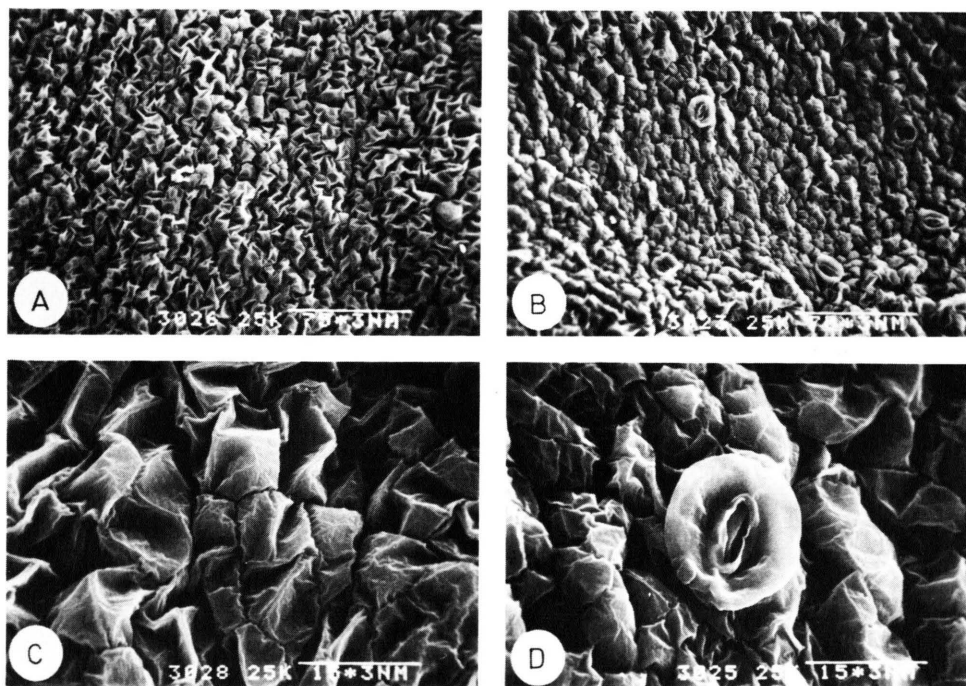


Fig. 1. *Q. robur* leaf surface structures of leaflets just after emerging from buds on 24th April 1990. A, adaxial epidermal cells still folded but covered with a wax layer. Bar = $75 \mu\text{m}$. B, abaxial epidermal cells still folded but covered with a wax layer, stomata and glandular trichomes. Bar = $75 \mu\text{m}$. C, adaxial epidermis. Bar = $15 \mu\text{m}$. D, abaxial epidermis. Bar = $15 \mu\text{m}$.

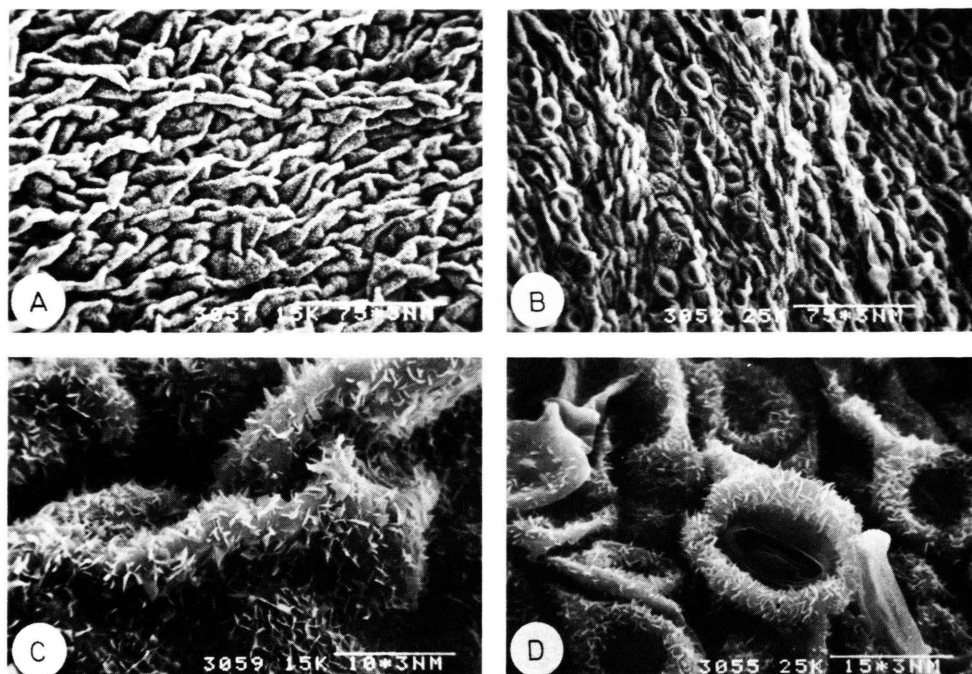


Fig. 2. *Q. robur* leaf surface structures of unfolding leaves 10 days later, on 4th May 1990. A, adaxial epidermal cells still folded, but showing the first development of wax crystalloids. Bar = 75 μ m. B, abaxial epidermal cells still folded, but showing the first development of wax crystalloids. Bar = 75 μ m. C, adaxial cells with wax crystalloids. Bar = 10 μ m. D, abaxial cells with wax crystalloids. Bar = 15 μ m.

Ten days after leaf unfolding dynamic variations in surface ultrastructures are observed. Wax crystalloids are found to crystallize out of the wax layers on both adaxial and abaxial leaf surfaces, forming fringed, edged platelets (Fig. 2). In 1990 this observation was first made on 4th May and in 1991 on 16th May. Leaf surface area at this stage was found to be 2.8 cm² and 3.9 cm² respectively. After this stage, a dynamic process of leaf growth starts. During May and June, leaf surface areas increase up to 30.9 cm² in 1990 and 47.5 cm² in 1991 as mean values for the remainder of the season. The amount of wax was doubled during this period [12].

In May epidermal cells, stomata and trichomes developed to their final size. Fig. 3 shows the growth process of adaxial epidermal cells during April to June. In this period leaf growth can primarily be observed with reference to different rates of cell enlargement for single cells. It would appear, that all cells found in the mature leaf are present at the initial stage of leaf unfolding. Wash-

ing the leaves with chloroform allowed epidermal cells and walls to be seen clearly in SEM figures. This leaf treatment does not destroy the cutin layer. However the functions of the cuticle (cutin + wax) as a barrier to the exchange of external and internal substances is destroyed. Leaves treated with chloroform lost their water very quickly. At room temperature these leaves reach their dry weight within 90 min (Fig. 4). The transpiration barrier is no longer effective. Therefore the function of the cuticle is exclusively conditioned by its wax layer.

During May, wax crystalloids increase quantitatively. The adaxial and abaxial leaf surfaces have nearly the same density of crystalloids in the shape of platelets. Around the stomata a greater concentration and characteristic orientation of these platelets is observed. The base of glandular trichomes is already covered with the platelets (Fig. 5).

The process of leaf growth finishes in June. At that stage a dense arrangement of fringed, edged

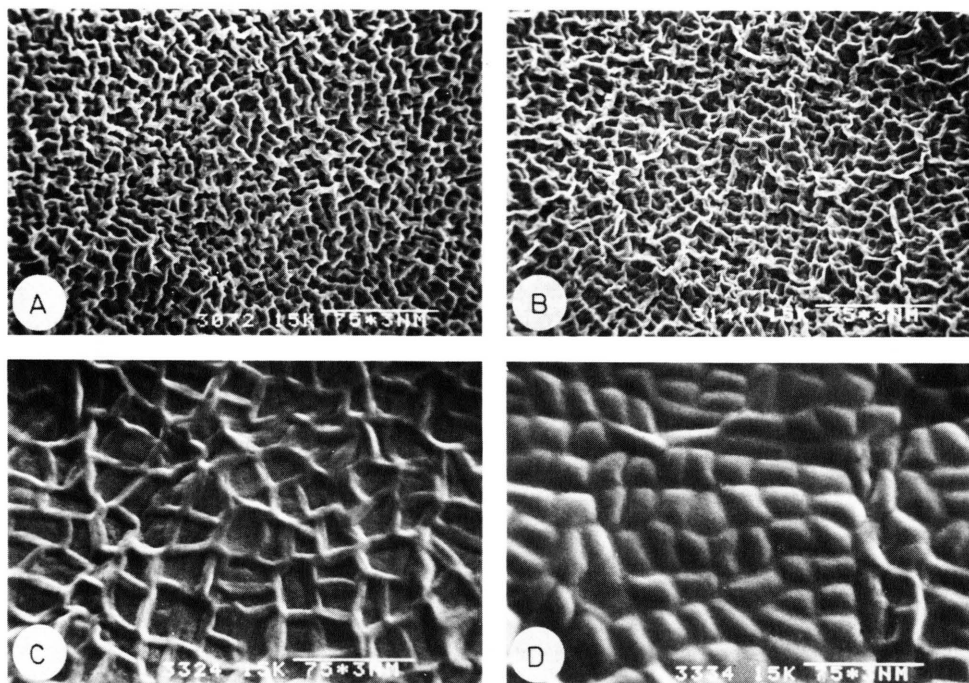


Fig. 3. *Q. robur* adaxial epidermis after washing with chloroform. Bar = 75 µm. A, epidermal cells on 30th April 1990; B, epidermal cells on 4th May 1990; C, epidermal cells on 22th May 1990; D, epidermal cells on 26th June 1990.

platelets is found on the adaxial and abaxial leaf surfaces. The shape of these wax crystalloids is identical on both leaf sides (Fig. 5C and 5D).

The epidermal cell surfaces are not even, stomata and trichomes are clearly recognizable and rise above level of the other cells in mature leaves (Fig. 5A and 5B).

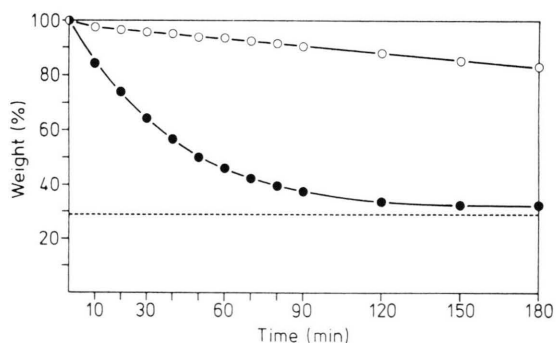


Fig. 4. *Q. robur*, decreasing of leaf weight due to water loss at room temperature. —○—, untreated leaves; —●—, leaves washed with chloroform.

Similar surface wax structures are found also for *Q. petraea* [16].

All developmental stages and ultrastructural observations in 1990 are reproducible in 1991.

Variations in the shape of wax crystalloids are seen within July. The sharp edged platelets melt away on both leaf surfaces. Mostly, the elevated parts of the stomata and also the adaxial cells show this melting first. In a distinct area of the leaf, shaped crystalloids and parts which have melted away are seen close together (Fig. 6). When the contents of the glandular trichomes empties over the wax layer (Fig. 6H), wax crystalloids are practically dissolved. During the following months both kinds of wax structures are seen with several intermediate forms. The melting process can go so far that no more crystalloids are seen. The wax melts together to form lumps and seals. From October to November no difference could be seen in surface structures of green and yellow leaves. From October to November it appeared that the melted parts became less frequent and recrystallization was observed (Fig. 6D).

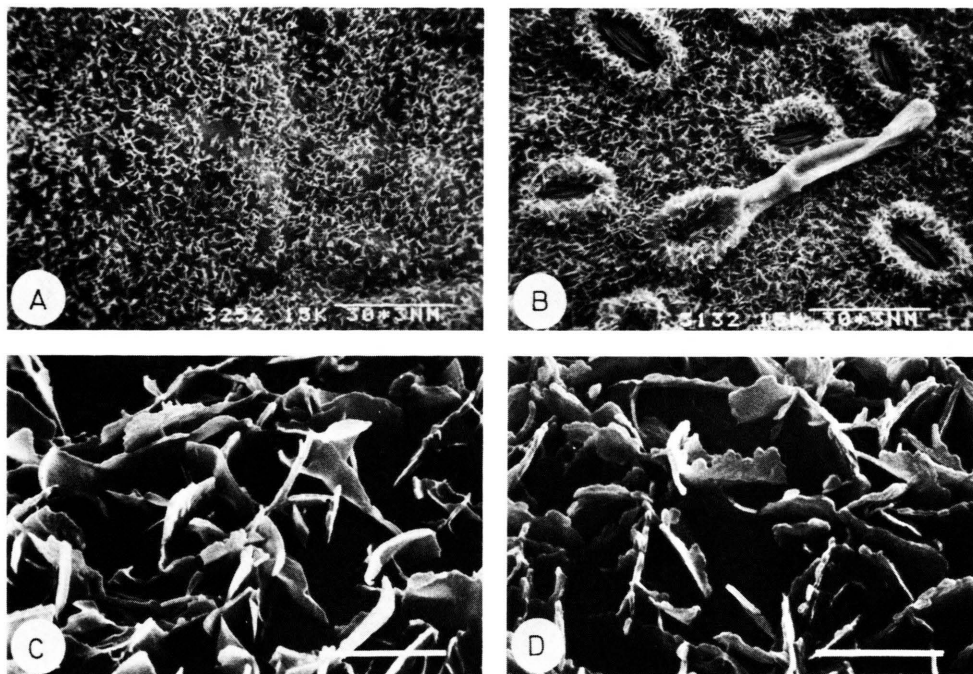


Fig. 5. *Q. robur* leaf surface structures of mature leaves in June 1990. A, adaxial epidermal cells with a dense arrangement of wax crystalloids. Bar = 30 μ m. B, abaxial epidermal cells with a dense arrangement of wax crystalloids showing condensation and orientation around the stomata. Bar = 30 μ m. C, wax crystalloids in the form of edged platelets on the adaxial leaf surface. Bar = 2.5 μ m. D, wax crystalloids in the form of edged platelets on the abaxial leaf surface. Bar = 2.5 μ m.

Discussion

Already the very young oak leaflets just emerging from buds contain a wax layer, but no wax sculptures or wax crystalloids are observed at that time. The epicuticular waxes of the initial stage were extracted and analyzed and showed a lipid composition which was quite different in yield and composition from those of mature leaves. In this wax no aldehydes were detected in the first preparation of 1990 (Fig. 7) [12].

After leaf unfolding a dynamic biosynthesis of wax lipids is started. Aldehydes, alcohols and fatty acids especially with chain lengths of C_{24} , C_{26} and C_{28} are synthesized. Tetracosanol becomes the main wax component, accounting for more than 40% of the wax (Fig. 7) [12].

This substance is therefore responsible for the development of wax crystalloids in the shape of platelets out of the continuous wax layer. These platelets are detected on both leaf surfaces for the first time 10 days after leaf unfolding (Fig. 2).

In May and June a dense and uniform arrangement of fringed, edged platelets develops from the wax layer on both sides of the leaf. In addition, an intensive condensation and orientation of platelets around the stomata is found on the abaxial leaf side (Fig. 5).

In 1991, leaf emergence is on 6th May, 12 days later than in 1990, due to a spell of cold weather in April. The temperature fell below 0 °C in the middle of April 1991. In that year dry weight of leaves was rather higher in the first preparations and wax from the sample of 6th May 1991 already contained traces of aldehydes. Increasing rates of aldehydes were found 10 days later. The activation of enzymes controlling the biosynthesis of aldehydes cannot therefore be retarded by the low temperatures experienced by the leaves emerging from their buds. Climatic factors can influence leaf development or the amounting and composition of wax produced in the initial stages. The development of wax crystalloids was again observed

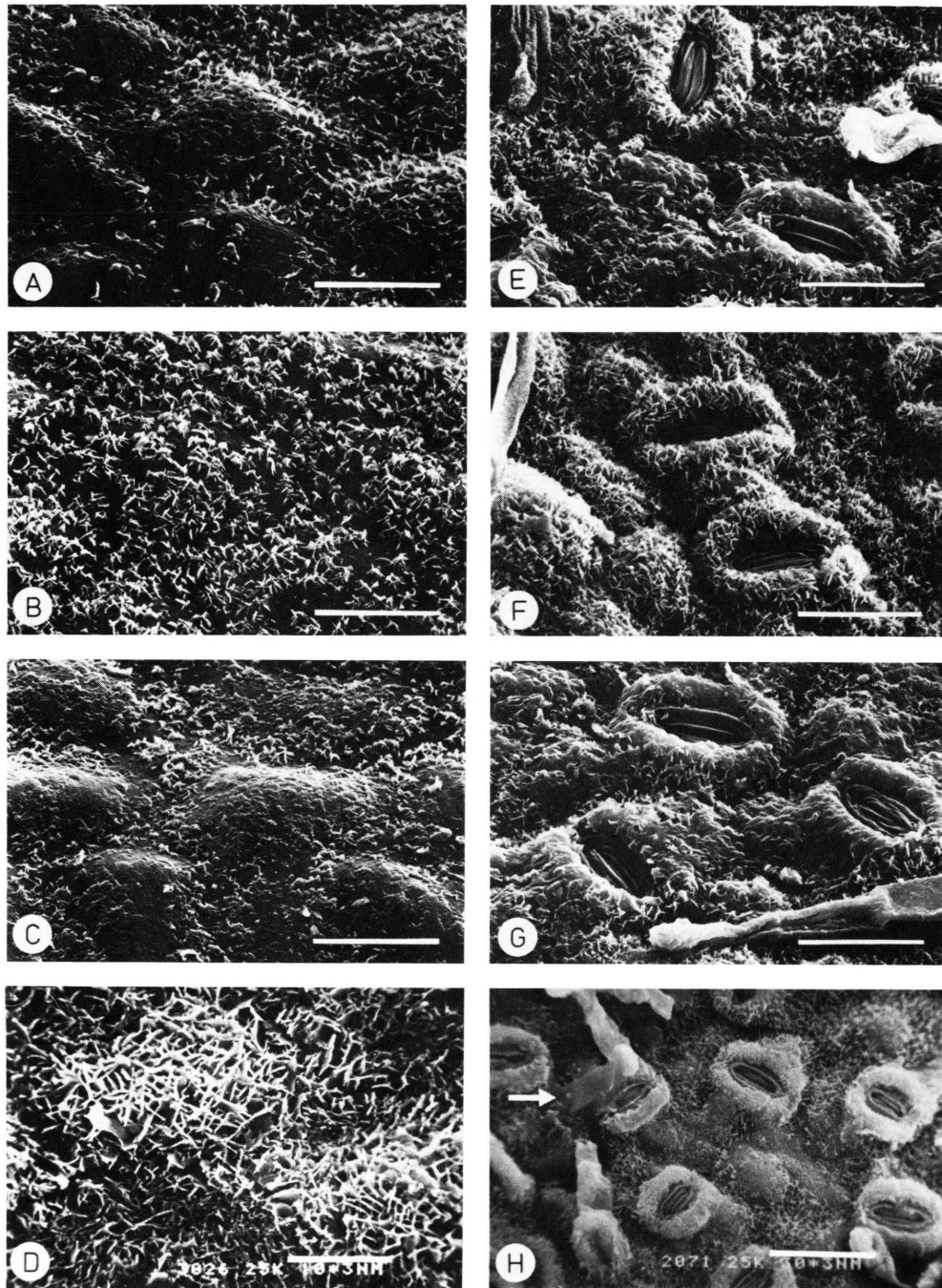


Fig. 6. *Q. robur* leaf surface structures of mature leaves. A, adaxial epidermal cells, with various surface structures close together. Bar = 20 μ m. B, adaxial epidermal cells, with parts of well-formed crystalloids (A). Bar = 20 μ m. C, adaxial epidermal cells with parts which have melted away (A). Bar = 20 μ m. D, adaxial epidermal cells in October after recrystallization. Bar = 10 μ m. E, abaxial epidermal cells, with different surface structures close together. Bar = 20 μ m. F, abaxial epidermal cells, with parts of well-formed crystalloids (E). Bar = 20 μ m. G, abaxial epidermal cells with parts which have melted away (E). Bar = 20 μ m. H, abaxial epidermal cells, wax crystalloids have been dissolved by essential oils from glands. Bar = 30 μ m.

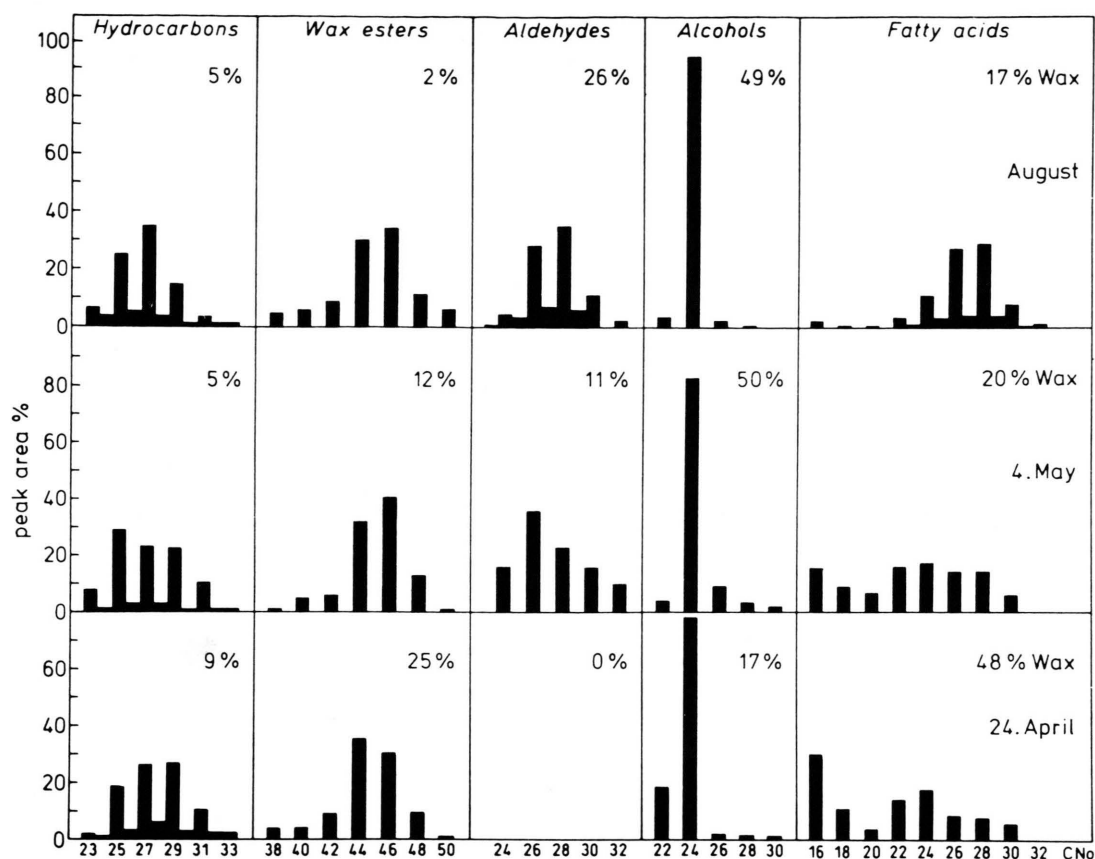


Fig. 7. Composition and distribution patterns of epicuticular waxes of *Q. robur* leaves on 24th April, 4th May and in August 1990.

10 days after leaf unfolding on 16th May 1991. Wax amount and composition reach the same mean values and standard deviations for July to November in both years [12]. Only values for leaf size were higher in 1991 ($\bar{X}_{16} = 47.5 \pm 5.6 \text{ cm}^2$) than in 1990 ($\bar{X}_{24} = 30.9 \pm 3.1 \text{ cm}^2$).

In both July 1991 and 1990 very hot days, with temperature in excess of 30°C occurred [14]. At that time we observed for the first time that a melting away of parts of the wax crystalloids occurred. First a rounding of the edges of the platelets was seen, followed by melting of the whole platelets and finally lumps and seals of wax were observed. Wax crystalloids and melted waxes are always seen on the same leaf on both surfaces. The transitions between both wax shapes are gliding without sharp limits. In a small area both wax shapes are found close together (Fig. 6). A melting away of the wax crystalloids is never observed for the

whole leaf, only distinct parts of the leaf are affected. These leaf parts may be those which are directly exposed to the sun while others are in the shade. These wax variations may be caused by external climatic factors such as increases in temperature to more than 30°C . In October and November when temperatures decrease, a recrystallization of wax platelets is observed.

On the other hand internal factors may cause the platelets to disappear. On the abaxial leaf surface glandular trichomes are found. These glands contain essential oils, which can be isolated by steam distillation in a concentration of about 0.025% of fresh weight from *Q. robur* leaves [Gülz, unpublished]. Essential oils from leaves of *Q. agrifolia* have been described recently [17].

Essential oils dissolve waxes and their crystalloids when they are pressed out of the glands. A solution or melting away of platelets is not only

observed on the abaxial leaf side where glands are found, but also on the adaxial surface, where no trichomes are present. On this adaxial leaf side essential oils cannot cause the disappearance of wax platelets. Various factors may be responsible for the solution or the melting away of platelets on oak trees. Besides an increasing of temperature, other environmental factors or air pollution with organic solvents can be discussed for these observations, but we have found no concrete evidence in support of these factors.

Throughout the vegetation period, the chemical composition and ultrastructure of leaf surface waxes is correlated with leaf development for three deciduous broadleaf trees *T. tomentosa* [7, 11], *F. sylvatica* [8, 9] and *Q. robur* [12] in the last three years. These studies revealed that many factors such as the moment of leaf emergence, duration of leaf development, wax composition, surface wax structures, wax crystalloids and leaf morphology may vary for different plants.

Several general observations were also made: Folded leaves in buds already contain a wax layer without any wax sculptures or crystalloids. Waxes of this initial developmental stage are quite different in yield, composition and surface structure to those of mature leaves.

After unfolding of the leaf, a dynamic biosynthesis of wax lipids begins. Wax lipids with very long chain lengths in particular are synthesized. The synthesis of wax esters however is inhibited.

About 10 to 15 days after leaf unfolding the

de novo biosynthesis of distinct lipids is started. At this time aldehydes are found for the first time in waxes of *F. sylvatica*, *Q. robur* and *T. tomentosa* and additionally β -amyrenyl acetate in *T. tomentosa*.

10 to 15 days after leaf unfolding wax sculptures (*F. sylvatica*) or wax crystalloids (rodlets for *T. tomentosa* and platelets for *Q. robur*) developed out of the continuous wax layers.

After 5 to 12 weeks wax biosynthesis is completed. From July to November wax amount, composition and surface structures remained nearly constant.

Climatic factors can influence leaf development in the initial stages, but do not affect the final values.

The presence of one particular wax lipid in a relatively high concentration within the mixture of leaf waxes, leads to the formation of wax crystalloids with a distinct, characteristic shape. There is a close correlation between particular compounds and the specific micromorphology of the wax crystalloids they form.

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- [1] P. E. Kolattukudy, *Chemistry and Biochemistry of Natural Waxes*, Elsevier, Amsterdam 1976.
- [2] D. F. Cuttler, K. L. Alvin, and C. E. Price, *The Plant Cuticle*, Academic Press, New York 1982.
- [3] M. Riederer, *Naturwissenschaften* **78**, 201–208 (1991).
- [4] C. F. Jeffree, E. A. Baker, and P. J. Holloway, in: *Microbiology of Aerial Plant Surfaces* (C. A. Dickinson and T. F. Preece, eds.), pp. 119–159, Academic Press, London 1976.
- [5] W. Barthlott, in: *Scanning Electron Microscopy in Taxonomy and Functional Morphology* (D. Claugher, ed.), pp. 69–83, Clarendon Press, Oxford 1990.
- [6] R. B. N. Prasad and P.-G. Gülz, *Z. Naturforsch.* **45c**, 813–817 (1990).
- [7] P.-G. Gülz, R. B. N. Prasad, and E. Müller, *Z. Naturforsch.* **46c**, 743–749 (1991).
- [8] P.-G. Gülz, R. B. N. Prasad, and E. Müller, *Z. Naturforsch.* **47c**, 190–196 (1992).
- [9] R. B. N. Prasad and P.-G. Gülz, *Z. Naturforsch.* **45c**, 805–812 (1990).
- [10] G. Schneider, *Doctoral Thesis*, München 1990.
- [11] R. B. N. Prasad, E. Müller, and P.-G. Gülz, *Phytochemistry* **29**, 2101–2103 (1990).
- [12] P.-G. Gülz and E. Müller, *Z. Naturforsch.* **47c**, 800–806 (1992).
- [13] M. Riederer and G. Schneider, *Planta* **180**, 154–165 (1990).
- [14] Deutscher Wetterdienst, Wetteramt Essen, Klimadaten 1991, März bis November.
- [15] J. W. Hardin, *Bull. Torrey Bot. Club.* **106**, 313–325 (1979).
- [16] S. Hennig, *Doctoral Thesis*, Bonn 1989.
- [17] M. A. Palma-Fleming and R. E. Kepner, *Phytochemistry* **22**, 1503–1505 (1983).