High pH-values and secretion of ions on leaf surfaces: A characteristic of the phylloplane of Malvaceae

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Summary. All of 19 different species of Malvaceae have high pH-values, pH 9.5 or higher, on one or both surfaces of mature leaves. This is due to the secretion of magnesium and potassium carbonate and bicarbonate. The unusual pH and ionic composition may be important for parasites and pathogens living in the phylloplane of Malvaceae.
Key words. Ion secretion; leaf surface pH; Malvaceae; phylloplane.

The surface of plant leaves is an ecological niche, called the phylloplane, for microorganisms and small animals[1,2]. The phylloplane is usually characterized by a slightly acidic pH and a scarcity of inorganic ions[1,2]. Thus, dew collected from plant leaves is, in most cases, slightly acidic.

However, dew from cotton leaves, as an exception, is alkaline. Its high pH-value has been discovered and discussed primarily as a factor possibly contributing to the short persistence of virus-based insecticides on cotton[3,4]. Our continuous measurements of the leaf surface pH confirmed the exceptional behavior of cotton leaves[5]. On most plant species investigated, the pH-value of a freshly mounted water droplet fell immediately to about pH 5; on cotton leaves, in contrast, it rose to values of pH 10 or even higher. Later, we found the high surface pH of cotton leaves to be due to the excretion of substantial amounts of titratable base[6]. Leaf washings from a number of commercial cotton varieties were found to contain up to 3 mval titratable base per m^2 surface, with magnesium and potassium as principal balancing ions. The excretion of base and cations appeared to be connected to glandular trichomes occurring on the cotton leaf surface in variable numbers[7,8].

Here, we report that the phenomenon of a high pH-value on the leaf surface is widespread among different members of the Malvaceae. It is related to the secretion of the carbonates and bicarbonates of magnesium and, to a lesser extent, of potassium. This leads to an unusual ionic composition of the phylloplane of the Malvaceae, a factor that might be particularly important for the ecology of their parasites and pathogens.

Material and methods. Several plants of each species of the Malvaceae under study were grown from seed in a mixture of peat and sand (3:1, v/v) in a greenhouse at 20–30°C. Seeds were obtained from the seed collection of the Botanical Institute, University of Basel.

The surface pH of freshly detached leaves was recorded as previously described[5,6]; peak readings of pH were obtained within the first minutes of measurement and are presented in table 1. In order to obtain a detailed picture of the situation on the leaves of the test plants, pH measurements were taken on both leaf sides on leaves of 3 different ages, i.e. young (leaves not yet fully expanded or located at the apex), medium (leaves just fully expanded) and old (leaves fully expanded for several days or located at the stem base).

To obtain leaf exudates, a pair of freshly detached leaves was immersed in 150 ml of freshly demineralized water in a petri dish (15 cm diameter) and rinsed by gentle shaking for 1 min. Rinsing was continued in a 2nd portion of 150 ml of water for 10 min. The process was repeated with a total of 4 pairs of leaves, using the same rinsing solutions. The amount of base in the rinsing solutions was determined by titration with 0.01 N HCl, using methyl orange as end-point indicator, and the amount of cations was measured by atomic emission (Na and K) and by atomic absorption (Ca and Mg) spectroscopy. The data given represent the sum of the ions in the 2 rinsing solutions, expressed per leaf surface area as determined planimetrically. After the washing, the leaves were dried and ashed at 600°C overnight. The ash was dissolved in 150 ml 0.05 N HCl, and the cations were determined as in the exudates.

All data presented are based on at least 3 replicates. Reproduc-

Table 1. Surface pH-values on leaves of different Malvaceae

Species	Variety	Upper leaf surface			Lower leaf surface		
		Young	Medium	Old	Young	Medium	Old
Abelmoschus esculentus		7.0	7.0	7.0	10.4	10.5	10.7
Anoda cristata		5.7	6.3	6.8	7.3	9.9	9.5
Gossypium arboreum	Karunganni	6.8	9.9	9.6	6.3	9.4	10.5
	Mollisoni	5.8	9.8	11.0	7.1	10.6	11.2
	Soudanense	5.9	7.3	10.7	5.9	10.4	11.4
Gossypium herbaceum	Kumpta	8.8	9.8	10.3	10.0	10.7	10.7
	Wagad	6.3	8.0	8.9	7.3	10.7	10.8
	Wightianum	6.6	8.2	9.8	6.8	10.7	10.8
Gossypium hirsutum	C 2	10.6	10.5	10.5	10.3	10.5	10.8
	C 8	10.5	10.5	10.5	10.4	10.6	10.9
Hibiscus manihot	manihot	6.0	7.4	8.6	5.4	9.0	10.1
Hibiscus moscheutos		6.8	8.5	9.8	7.9	9.4	10.0
Hibiscus rostellatus		5.5	7.0	9.5	5.3	6.3	6.8
Hibiscus trionum		9.0	9.5	9.5	7.0	8.8	10.1
Kitaibelia vitiifolia		10.8	11.0	11.0	10.8	11.0	10.9
Malva silvestris		6.7	8.5	10.5	7.8	9.6	10.8
Malva verticillata	typica	9.5	9.7	9.9	10.4	10.5	10.5
	crispa	7.5	8.6	10.3	7.6	10.5	10.8
	neuroloma	7.0	7.7	9.9	9.3	9.9	10.5
	sinensis	7.8	9.3	10.4	7.9	10.3	10.8

Table 2. Ionic composition of leaf washings and of leaf ash from different Malvaceae

Species, variety		Leaves washed[a]	Ionic composition of leaf washings: Titratable base mval m^{-2}	Total cations mval m^{-2}	% of total cations: Mg	K	Ca	Na	Ionic composition of leaf ash: Total cations mval m^{-2}	% of total cations: Mg	K	Ca	Na
Abelmoschus esculentus		2–3	1.03	1.54	39	30	25	6	26.4	24	23	52	1
Abutilon avicenae		3–5	0.60	1.40	12	43	33	12	30.1	16	16	67	1
Abutilon theophrasti		3–5	0.40	1.22	22	42	31	5	36.7	15	17	67	1
Anoda cristata		4–5	0.36	0.69	18	24	46	12	23.7	21	27	51	1
Gossypium arboreum	Karunganni	3–5	0.52	0.63	59	16	18	7	34.9	17	25	56	2
	Mollisoni	3–5	2.88	2.72	61	21	12	6	34.2	11	26	61	2
	Soudanense	3–5	0.85	0.69	54	22	13	11	40.5	14	21	64	1
Gossypium herbaceum	Wagad	5–6	1.09	1.19	35	42	13	10	33.2	14	23	61	2
	Wightianum	3–5	0.81	0.69	52	23	14	11	38.0	18	26	55	1
Gossypium hirsutum	C 2	4–6	2.56	1.91	38	42	15	5	34.6	15	23	61	1
	C 8	2–4	1.42	1.16	39	41	15	5	33.8	17	23	59	1
Hibiscus rostellatus		3–4	0.18	0.60	5	14	76	5	23.3	15	30	54	1
Kitaibelia vitiifolia		3–4	3.89	3.31	56	22	21	1	32.1	13	21	65	1
Malva neglecta		4–5	1.89	1.97	58	15	26	1	32.8	14	29	55	2
Malva nicaensis		4–5	1.65	1.90	50	12	35	3	35.5	12	28	59	1
Malva parviflora		4–5	0.98	0.95	41	9	47	3	31.3	13	21	65	1
Malva pusilla		4–5	1.04	0.93	48	6	45	1	29.6	13	23	63	1
Malva silvestris		8–10	0.65	0.57	47	11	36	6	16.5	22	18	55	5
Malva verticillata crispa		4–5	1.05	1.41	50	9	38	3	30.1	12	27	59	2
Malva verticillata sinensis		2–3	0.13	0.46	23	19	54	4	26.0	15	33	51	1
		4–5	0.31	0.49	19	14	64	3	25.7	12	32	54	2
		6–7	0.58	0.83	31	10	56	3	27.2	10	24	63	3
Modiola caroliniana		5–7	0.14	0.92	9	16	63	12	36.6	7	12	79	2

[a] Leaves numbered from the apex (top leaf = 1). Leaf age is 'medium' with the exception of *Malva verticillata* sinensis, where the 3 stages correspond with 'young', 'medium' and 'old', respectively.

ibility generally was excellent; however, no statistical test was applied to the data gathered.

Results. Table 1 presents the leaf surface pH of 20 species and varieties of Malvaceae. All the species investigated had a high leaf surface pH, at least on one surface of mature leaves.

With the exception of *Hibiscus rostellatus* and *Hibiscus trionum,* the lower leaf surface had a higher pH than the upper one. An extreme example for this is *Abelmoschus esculentus,* which, on the lower leaf surfaces, had pH values 3.5 units higher than on the upper ones on young as well as on older leaves.

The 2 varieties of the amphidiploid cotton species *Gossypium hirsutum* tested, as well as *Kitaibelia vitiifolia* and *Abelmoschus esculentus,* had constant pH-values, independent of leaf age. In most cases, however, leaf surface pH-values increased with leaf age. Of all Malvaceae investigated, the pH-values found on leaves of *Kitaibelia vitiifolia* were highest, rarely being below pH 11 even on young leaves.

Measurements of the ionic compositions of leaf washings and of the whole tissue are given in table 2. The amount of titratable base per m^2 of leaf surface, mostly in the form of bicarbonate and carbonate as shown in titration experiments, differed widely among the species and varieties investigated. Typically, those with a relatively low surface pH-value had relatively low amounts of base, and *Kitaibelia vitiifolia* – with the highest pH-value – had also the highest amount of titratable base. For *Malva verticillata,* the washings from leaves of different ages were compared. The increase of titratable base observed paralleled the increase in surface pH. From *Abelmoschus esculentus,* the upper and lower surfaces were washed separately for 1 min each. The upper surface, with a low pH-value, yielded 0.02 mval base per m^2, and the lower surface, with a high pH-value, yielded 0.70 mval base per m^2.

In general, the amount of excreted cations matched the amount of excreted base fairly well. However, in some cases *(Anoda cristata, Abutilon avicenae, Hibiscus rostellatus, Modiola caroliniana),* there were considerably more cations in the leaf washings, with Ca usually predominating. We did not try to determine the anion associated with this surplus of cations.

Among the different cations, Mg was usually the most abundant, particularly in the washings with large amounts of base. There, Mg often amounted to more than 50% of the total cations. Thus, the cation composition of the excretion was very different from that of the whole leaf tissue, where Mg accounted for only about 15% of the total cations (table 2). A considerable amount of the foliar Mg may be excreted in such cases: in *Kitaibelia vitiifolia,* for example, about one third of the foliar Mg was present in the leaf washings.

Discussion. Elevated surface pH-values seem to be a general characteristic of the leaves of Malvaceae: All members of this family investigated here, representing 9 genera from 3 subfamilies, had pH values above 9.5 on at least one of the surfaces of mature leaves. The different species differed with regard to the temporal and spatial expression of high pH-values on their leaves. Cultivars of *Gossypium hirsutum* were found to have very high pH-values (pH 10.5) on both surfaces of all leaves, in accordance with previous work[6,7]. However, this was rather an exception. Only *Kitaibelia vitiifolia* behaved similarly. In all other species investigated, leaf surface pH rose with the age of the leaf and, in most cases, it was higher on the lower than on the upper leaf surfaces.

The high pH values correlated well with the high amounts of titratable base (primarily in the form of carbonate and bicarbonate) and of cations found in the leaf washings. In most cases, magnesium was the predominant cation in the exudate.

This is surprising in view of the much lower proportion of magnesium in leaf tissue; it indicates a special secretion mechanism for magnesium and, by analogy, of carbonate and bicarbonate in the Malvaceae. On the structural level, the high surface pH-values and the excretion of basic salts is correlated with the presence of glandular hairs[8]. Such glands have been found to be responsible for the high pH on the surface of *Gossypium hirsutum* leaves[6,7].

It is tempting to speculate about the function of the alkaline excretion on leaf surfaces of Malvaceae. It has been suggested[7] that, in cotton, under the semi-arid conditions of its natural habitat, the excretion might function like that of salt glands, where ions are pumped out in order to maintain the internal osmotic pressure, or that the excretion might be responsible for water uptake from the atmosphere, as has been suggested for the excretion product of a desert shrub, *Nolana mollis*[9]. However, our results show that the characteristic of alkaline secretion is not restricted to Malvaceae of semi-arid or arid habitats.

Another possibility is a function in the protection from parasites and pathogens, as we have suggested for cotton[6]. The high pH of the leaf surface might render the leaves unsuitable for growth of non-specialized pathogens and parasites. In particular, this might hold for fungi since many fungal spores are known to be unable to germinate in alkaline conditions[6]. At any rate, the high pH and the unusual ionic composition found on the leaf surface of many Malvaceae are important ecological factors which are likely to render the phylloplane very different from that of most other plant families.

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Secreted oleanolic acid on the cuticle *Olea europaea* (Oleaceae); a chemical barrier to fungal attack

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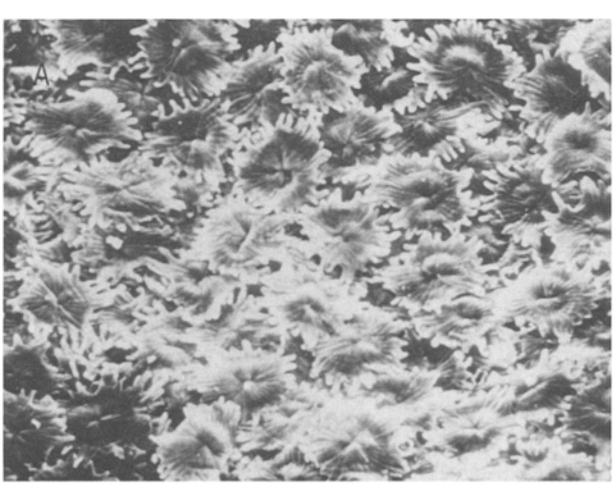
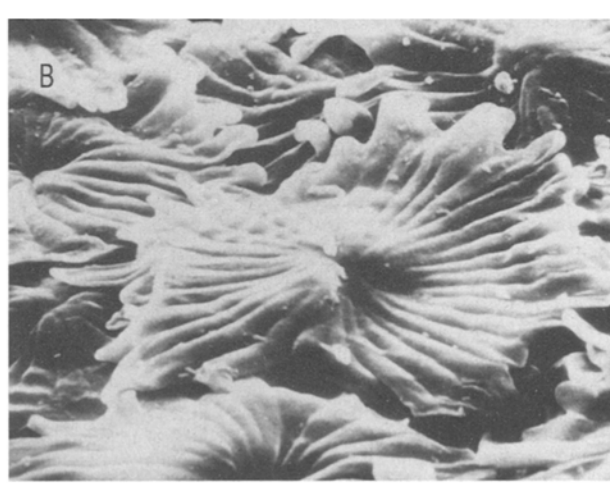
Summary. The leaf of the bitter olive *Olea europaea* (Oleaceae) is heavily coated by almost pure oleanolic acid, which forms part of a 'multichemical' defense against fungal attack.

Key words. Olea europaea; cuticle; oleanolic acid; fungal attack.

The presence of a large amount of oleanolic acid (**1**) in the foliage of bitter olive *Olea europaea* (Oleaceae) has long been known[2,3]. During our study of defense mechanisms[4] of this plant[5], which is known to be rarely attacked by fungi and insects, we found that oleanolic acid might play an important role in the defense against fungal attack.

Scanning electron micrographs (fig.) show almost pure crystals of oleanolic acid on the leaf surface of *O. europaea*. Crystals (11 mg) were collected under magnification from the surface of one leaf of an average size (fresh weigth, 343 mg) with a spatula without damaging the cuticle, and were shown to have only one major component when investigated using TLC ($CHCl_3$-MeOH, 20:1, v/v). The crystals were purified by silica gel column chromatography ($CHCl_3$-MeOH) to give 2 triterpenes. The major compound [$C_{30}H_{48}O_3$, m.p. 300–302°C and $[\alpha]_D^{20}$+86.3° (c = 0.13, $CHCl_3$)], was identified with oleanolic





The electron micrographs of the abaxial surface of the leaf of *O. europaea*. A, × 31; B, × 429.