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.

1 Dickinson, C.H., and Preece, T.F., Microbiology of aerial plant surfaces. Academic Press, London 1976.
2 Blakeman, J.P., Microbial ecology of the phylloplane. Academic Press, London 1981.
3 Andrews, G.L., and Sikorowski, P.P., J. Invertebr. Path. *22* (1973) 290.
4 Young, S.Y., Yearian, W.C., and Kim, K.S., J. Invertebr. Path. *29* (1977) 105.
5 Oertli, J.J., Harr, J., and Guggenheim, R., Z. PflKrankh. PflSchutz *84* (1977) 729.
6 Harr, J., Guggenheim, R., Boller, T., and Oertli, J.J., Cot. Fib. Trop. *35* (1980) 379.
7 Elleman, C.J., and Entwistle, P.F., Ann. appl. Biol. *100* (1982) 553.
8 Guggenheim, R., Boller, T., and Harr, J., in preparation.
9 Mooney, H.A., Gulmon, S.L., Ehleringer, J., and Rundel, P.W., Science *209* (1980) 693.

0014-4754/84/090935-03$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

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

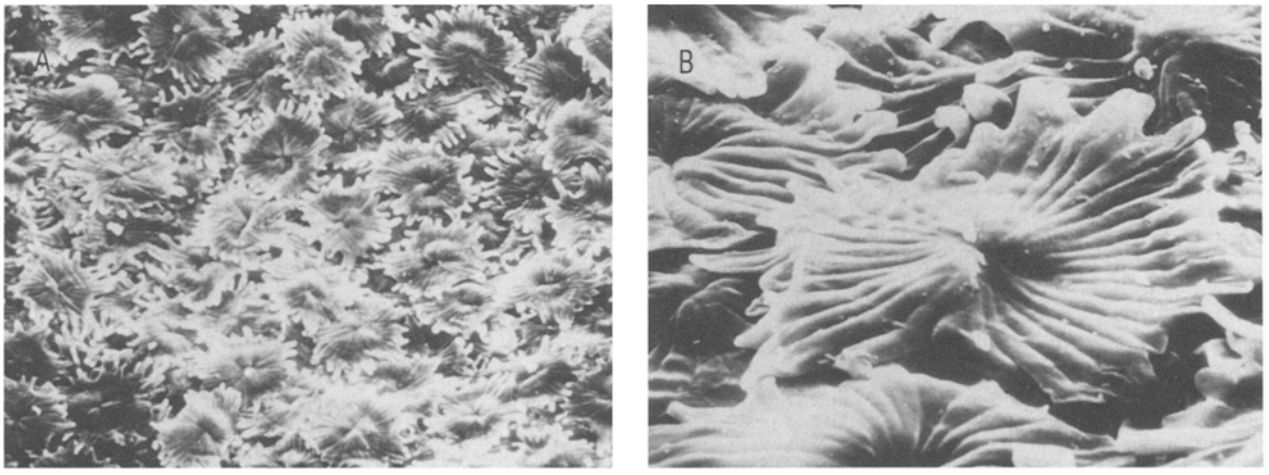
I. Kubo and A. Matsumoto[1]

Division of Entomology and Parasitology, College of Natural Resources, University of California, Berkeley (California 94720, USA), 7 November 1983

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.

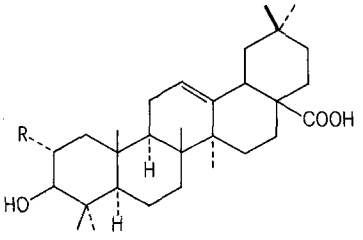
Scanning electron micrographs (fig.) show almost pure crystals of oleanolic acid on the leaf surface of *O. europaea*. Crystals (11 mg) were collected under magnification from the surface of one leaf of an average size (fresh weigth, 343 mg) with a spatula without damaging the cuticle, and were shown 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.

acid (**1**) from various spectral data (UV, IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR)[2,3]. Another triterpene present in very small amounts [$C_{30}H_{48}O_4$, m.p. 264–266 °C and $[\alpha]_D^{20}$+60.0° (c = 0.01, $CHCl_3$)] was another triterpene, maslinic acid (**2**), was also identified by spectral data (UV, IR, EI-MS, ^{1}H-NMR and ^{13}C-NM)[2,3]. The total yield of these triterpenes from the leaf surface is at least 3.2% of the total fresh leaf weight, and is comparable to the yield reported by Roncero and Janer[2] for the total leaf.

This means that almost all oleanolic acid present is on the surface of the leaf. *O. europaea* may be protected against fungal attack at least to some extent by the simple mechanism of excreting these triterpenes onto the leaf surface. Although oleanolic acid itself does not exhibit antifungal activity, it acts as a barrier at the leaf surface, a site where the physiological environment is normally favorable for spore germination. The degree of surface wettability of the leaf is important, since spores cannot germinate and grow unless sufficient moisture is available. If a leaf is heavily coated with the non-water soluble oleanolic acid, moisture will not remain on the leaf surface. We consider this simple mechanism of excreting triterpenes onto the leaf surface to be part of a 'multichemical' defense against fungal attack[5].



1 R = H, oleanolic acid
2 R = OH, maslinic acid

1 We thank Mr K.A. Hoelmer for taking the electron micrographs and also Professor T. Kamikawa for NMR measurements.
2 Roncero, A.V., and Janer, M.L., Grasas y Aceites *20* (1969) 133.
3 Romualdo, C., Lorenzo, M., Pietro, M., and Lucio, P., Phytochemistry *13* (1974) 1551.
4 Kubo, I., Matsumoto, T., and Klocke, J.A., J. chem. Ecol. *10* (1984) 547.
5 Kubo, I., Matsumoto, A., and Takase, I., J. chem. Ecol. (1984) in press.

0014-4754/84/090937-02$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984

Age-dependent tolerance to *Baculovirus* in last larval instars of the codling moth, *Cydia pomonella* L., induced either for pupation or for diapause

F. Camponovo and G. Benz

Department of Entomology, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich (Switzerland), 22 August 1983.

Summary. LD_{50} values as well as time-dependent parameters of granulosis virus infections were determined at different times during the last larval instar (L_5) of the codling moth, *Cydia pomonella* L., induced either for pupation or for diapause. A significant increase of tolerance to virus was found in 48-h-old L_5 induced for pupation, and 24 h later in L_5 induced for diapause.
Key words. Baculovirus; granulosis virus; codling moth; tolerance, age-dependent; pupation; diapause; *Cydia pomponella.*

Although the larvae of Lepidoptera are usually very susceptible to infections with baculoviruses, several authors reported an increased tolerance to virus among different larval instars[1]. Multiplication of baculoviruses in pupae and adult insects is not common; only few cases have been reported[2]. In order to estimate the influence of the pupation on the susceptibility to *Baculovirus* infection, experiments were performed with L_5 of the codling moth induced for either uninterrupted development to pupation or for diapause as fully grown L_5[3]. The larvae were reared individually according to our standard method[4], either under continuous light in order to induce pupation or under a short day-regime with a light: dark cycle of 10:14 h, which induced diapause.

Experimental groups of 30–40 individuals were formed with synchronized L_5. Computing of age started as larvae moulted to the last instar. The age of larvae within a group varied by ± 2–4 h. Infections with subgroup B *Baculovirus*[5] (granulosis virus) were scheduled for 3-, 24-, 48- and 72-h-old L_5. Granulosis virus was purified by conventional methods[6]. A stock suspension containing 3×10^{11} granulosis capsules per ml was prepared with phosphate buffer ($^1/_{15}$ M; pH 7) and stored at 2 °C. Dilutions were made with destilled water. 1 µl virus suspension per larva was orally administered by means of microinjection[7]. Larvae not surviving the first 2 days after injection had probably been wounded when handled for infection and were discarded. The average time till death was recorded and the LD_{50} values were determined by probit analysis with a computer program[8].

LD_{50} values calculated from the dose-mortality responses for larvae infected at different times after the last larval molt are presented in table 1. The doses necessary to kill 50% of 3- and 24-h-old L_5 induced for pupation were similar. However, in the 48- and 72-h-old larvae about 2500 and 25000 times higher doses were necessary respectively. On the other hand, whereas

Table 1. LD_{50} values of the granulosis virus of *Cydia pomonella*. Effect of the age of last instar larvae (L_5) induced for either pupation (IP) or diapause (ID) on mortality and time parameters

Age of L_5 at infection		LD_{50}	95% confidence level Lower	Upper	Slope
IP	3 h	9.2×10^1	7.2×10^1	1.2×10^2	2.033
	24 h	7.6×10^1	4.1×10^1	1.1×10^2	1.332
	48 h	2.1×10^5	1.4×10^5	3.1×10^5	1.169
	72 h	2.1×10^6	1.1×10^6	3.6×10^6	1.380
ID	3 h	1.2×10^2	8.0×10^1	1.8×10^2	1.262
	24 h	1.1×10^2	5.7×10^1	1.9×10^2	1.238
	48 h	7.7×10^2	3.4×10^2	1.2×10^3	1.344
	72 h	2.0×10^5	8.8×10^4	7.3×10^5	0.758