The role of hairs on the wettability of grapevine (*Vitis* spp.) leaves

by

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S u m m a r y : Hairs represent an important physico-chemical factor on abaxial leaf surfaces of grapevine and can influence the wettability of leaves. This was demonstrated by scanning electron micrographs and monitored with a new method using a torsion balance. Forces necessary to remove water droplets of distinct size from leaves were lower in case of pubescent (0,2-0,4 mN) compared to non-pubescent vine leaves (0,4-1,2 mN). Depending on the number of hairs and their surface structure the deposition and distribution of water droplets originating from dew and rain can be prevented due to weak adhesion. Thus, surface features enabling water droplets to pearl off may reduce the probability of infection by fungal spores.

K e y   w o r d s : downy mildew, low temperature scanning electron microscopy, Plasmopara viticola, pubescence, resistance, trichomes, Vitis, water adhesion.

Die Bedeutung von Haaren für die Benetzbarkeit von Rebblättern (*Vitis* spp.)

Z u s a m m e n f a s s u n g : Haare können als wichtiges Strukturmerkmal auf den Blattunterseiten von Reben die Benetzbarkeit beeinflussen, was anhand von Rasterelektronenmikroskop-Bildern aufgezeigt und mit Hilfe einer neuen Messmethode ermittelt werden konnte. Diejenigen Kräfte, die notwendig waren, Tropfen einer betimmten Größe von Blättern zu entfernen, waren geringer im Falle behaarter (0,2-0,4 mN) und größer im Falle unbehaarter Blätter (0,4-1,2 mN). Je nach Dichte und Oberflächenbeschaffenheit verhindern sie die Ablagerung und Verteilung von Wassertropfen aus Tau und Regen aufgrund der schwachen Adhäsion. Oberflächeneigenschaften, welche ein Wegrollen der Wassertropfen bewirken, können die Möglichkeit einer Infektion durch Pilzsporen reduzieren.

Introduction

Usually plant-environment interactions start at the outer surface of plants. Both, preformed and active defense mechanisms protect plants from biotic (*e.g.* pathogens) and abiotic (*e.g.* drought) stresses. Consequently, the physico-chemical properties of a plant surface greatly affect survival in a certain environment. Thus leaf surfaces of plants show considerable differences concerning water adhesion ranging from completely wettable to water repellent ("Lotus effect", Barthlott and Neinhuis 1997 a). Fundamental physico-chemical factors which govern the wettability of the leaf surface are the nature of the exposed chemical groups, the surface roughness and leaf orientation (Juniper and Jeffree 1982).

Usually wettability is expressed by the contact angles. The test consits of depositing a drop of water on a surface and determining the angle between the surface and the tangent to the drop at the solid-liquid-air meeting point. High hydrophobic surfaces show high contact angles and hydrophilic surfaces low contact angles. Roughness (*e.g.* wax or trichomes) increase the contact angle which varies mostly between 150 and 160 °C (Barthlott and Neinhuis 1997 b). Wettability can be measured only indirectly through phenomena which more or less clearly reflect the nature of molecular interactions. Furthermore droplet contact angle measurements have been shown to vary depending on various

factors, such as leaf turgor, osmotic changes within the leaf (Fogg 1944), temperature of the solid, liquid and the gas phase and drop size (Godd 1979; Ponter and Yekta-Ford 1985). Trichomes are obvious to certain leaf surfaces and there is an enormous variation in size, shape, type, frequency and distribution. Several authors have attempted to classify the type of plant trichomes (reviews: Hummel and Staesche 1962; Uphof 1962, and the literature therein; Johnson 1975).

Although the literature of plant ecology and evolution contains numerous references concerning the distribution and morphology of hairs or the possible role of hairs in the water balance, a relationship between hairs and plant defense has been poorly studied except for the roles of hairs in the interactions of the plant surface with insects (Juniper and Southwood 1986).

Only few reports about their role in plant-fungus interactions (especially for grapevine) exist. Nevertheless, leaf pubescence has been considered as a mechanism of plant defense to fungal pathogens (Steadman and Shaik 1988; Zailer *et al.*1990; Staudt and Kassemeyer 1995).

Shaik (1985) and Steadman and Shaik (1988), for example, reported that a low-intensity development of rust pustules was correlated with bean leaf pubescence and that this correlation was not altered by rust races. The intensity of rust pustule development was lowest when inoculation was made on young, unexpanded leaves where hair density was highest.

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Fungal spores are deposited by air or in water drops. After spores have reached the leaf surface, this attachment and retention on the surface may be affected by the physical characteristics of the leaf. In general, most spores will neither germinate in the air nor develop under conditions of low relative humidity. The presence of liquid water on the leaf surface is the determining factor for the development of the majority of fungal pathogens in fungal diseases which usually require a period of leaf wetness to complete a cycle of infection (except for the powdery mildews). Slow desiccation leading to death is probably the fate of many spores. Some will only germinate in a pool of water and if leaves are dry at their surface, fungal infections are markedly reduced. In some *Vitis* species it appears that the degree to leaf wettability may be crucial in determining the susceptibility to downy mildew disease.

The present study was undertaken to evaluate the wettability of grapevine leaves as a function of the degree of hairness. For this purpose, 13 *Vitis* species and varieties with contrasting leaves were chosen and a new test which allows to determine the wettability was developed.

Material and Methods

During June and July 1998 the wettability or repellency of leaf surfaces was determined by measuring the adhesion of a water droplet at the surface. A sodium hydroxide/hydrogen peroxide-cleaned glass loop of 5 mm in diameter containing a water droplet of 15 µl was placed on the surface. The force necessary to remove the water droplet with the glass loop was measured with a torsion balance 0, 10, 30 s and 1, 5, 10, 30, 60 min after droplet placement. Apical leaves (5th to 7th insertion) of field-grown plants were monitored, except for *V. davidii* which was grown in the greenhouse. Each variety was measured 10 times at each point of time. Leaves of the following varieties and species were chosen: Kerner, Morio Muskat, Müller-Thurgau, Orion (Optima x Villard blanc), Phoenix (Bacchus x Villard blanc), Regent (Diana x Chambourcin), Riesling, *V. cinerea, V. davidii, V. doaniana, V. girdiana, V. labrusca, V. rotundifolia.*

Electron micrographs were performed using low temperature scanning electron microscopy. For this purpose water droplets were sprayed onto the leaf surface and immediately frozen above slushed liquid nitrogen on a copper block. Leaf pieces were transferred onto another precooled copper block (-180 °C), heated to -80 °C under vacuum to remove condensated water, cooled to -180 °C again, sputter-coated with gold under argon atmosphere and investigated with a Zeiss DSM 90 scanning electron microscope (Zeiss Oberkochen, Germany) operated at 15 kV.

In dew simulating experiments leaf pieces were fixed onto a cupper block and cooled to 4 °C, transferred into the gas atmosphere above hot water (50 °C, 30 s) and immediately frozen in liquid propane. After evaporation of the adhering propane under vacuum the leaf pieces were sputter-coated and investigated as described above.

To test the distribution of spores between water and an organic phase spores or spore suspensions were taken into a glass test tube, equivalent volums of water and pentane were added and the tube plugged with a silicone stopper. After vigorous vortexing for 10 s the two phases separated completely within 10 min.

Results

As a preliminary evaluation the wettability of *Vitis* leaves can easily be visualized by dipping the leaves in a 0.005 % aqueous solution of fluorescein. The staining solution remaining on the leaf surface can be detected by UV-light. The Morio Muskat leaf was extremely wettable by this solution whereas the dye adhered very poorly on the *V. labrusca* leaf. Only small areas and mainly the leaf veins were wettable (Fig. 1). More details of the water-surface relationship became visible using low temperature scanning electron microscopy (Fig. 2). The surface of the downy mildew susceptible variety Riesling was completely wettable. The droplets flattened and spread at the surface whereas at *V. doaniana* or *V. davidii* leaves the droplets remained spherical, and were unable to reach the leaf surface. Furthermore, the lipophilic hairs caused a separation and a deformation of the droplets (Fig. 2 e-g).
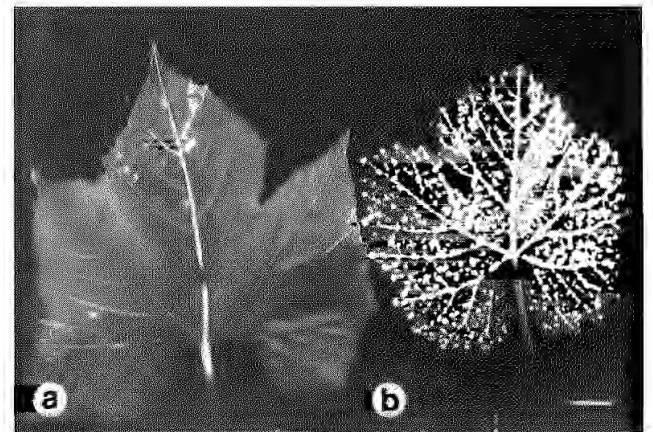

Fig. 1: Adhesion of fluorescein-dye indicating different wettability of hairy and non-hairy leaves; a) *Vitis labrusca*, b) *V. vinifera* cv. Morio Muskat; bar equals 2.5 cm.

Although leaves of the varieties Riesling and Siegerrebe also have hairs, they were completely wettable (Fig. 2 b) and unable to prevent water adhesion.

Fig. 3 summarizes the results of the dew experiments. At leaves of susceptible varieties condensed water droplets completely nestled against the hairs (Fig. 3 a) whereas the water droplets sticked very poorly to the hairs of wild species (Fig. 3 b). Hydrophobic spores were restricted to the surface and migrated passively by surface tension and gravitational droplets' forces to the water-leaf-interface (Fig. 3 c). After evaporation of the water spores adhered to the hairs which entrapped them (Fig. 3 d).

It is interesting to note that freshly harvested dry spores of *P. viticola* were completely hydrophobic. During germination they became hydrophilic and were completely excluded from the organic phase (Fig. 4).
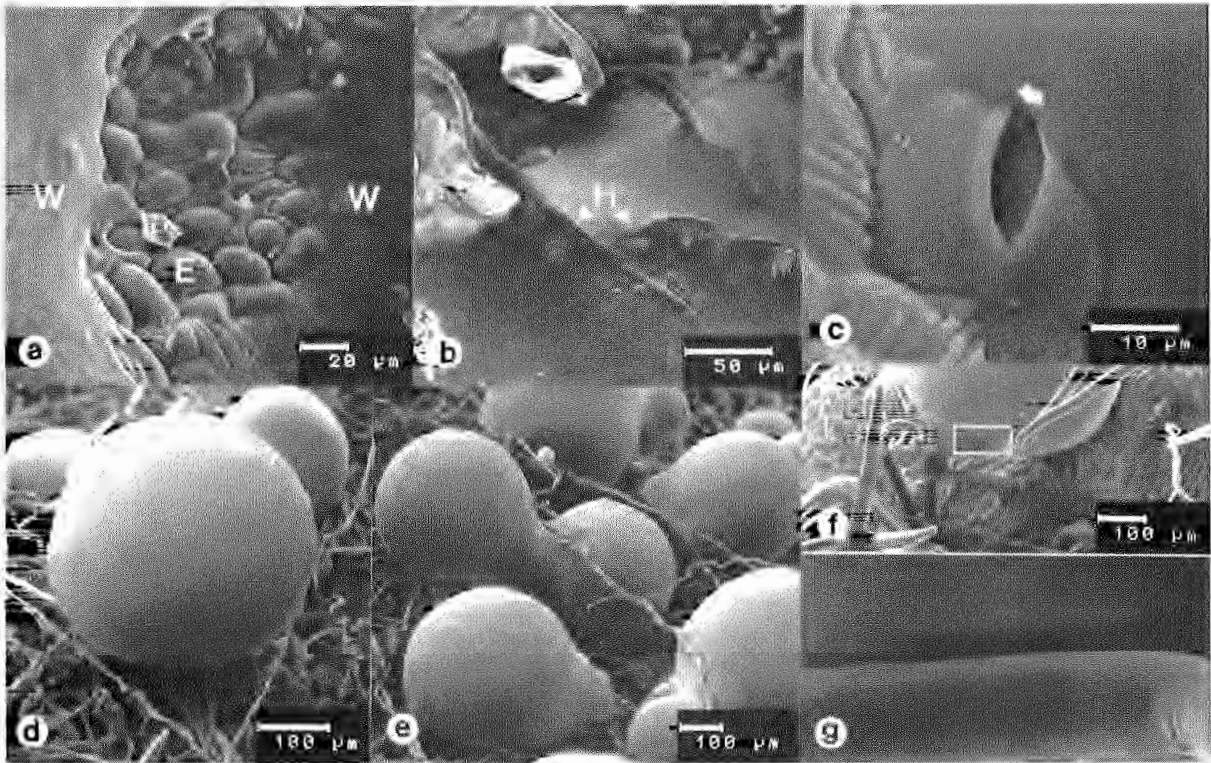
Fig. 2: Scanning electron micrographs showing water droplets on different *Vitis* leaves; **a**) RIESLING, showing a strongly wettable surface (E = epidermis, W = water). **b**) Riesling hairs (H) in a spread droplet of water indicating their wettability. **c**) The spread of a water droplet led to penetration of water into a stoma (arrow), which is also completely wettable. **d**) Hairy surface of a *V. doaniana* leaf. The sprayed droplet remained rounded and was held away from the surface. **e**) Leaf hairs of *V. doaniana* are completely hydrophobic leading to a separation of two water droplets which normally would merge. **f**) and **g**) *V. davidii*; both hydrophobic bristles and hairs are not wettable as depicted at higher (eightfold) magnification (g).
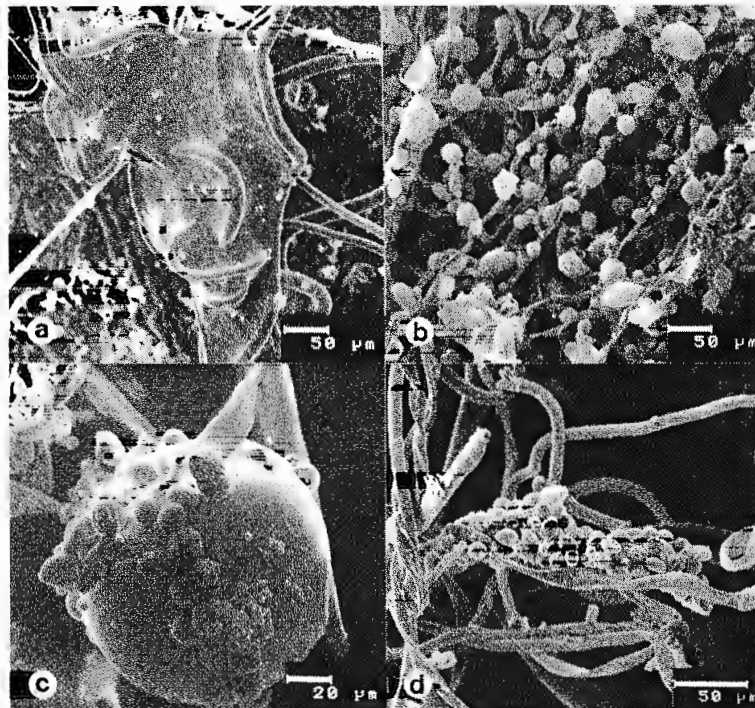

Fig. 3: **a**) and **b**) dew experiments; **a**) leaf hairs as well as the leaf surface of the variety Riesling are completely wettable; **b**) droplets on *V. labrusca* remained separated from the leaf surface. Globular structure of droplets indicate hydrophobicity of the hairs. **c**) Spores of *Plasmopara viticola* deposited on a "wet" *V. davidii* leaf. **d**) Leaf of *V. davidii*, spores left sticking to the hairs after evaporation of the water.

Fig. 5 presents the results obtained by a torsion balance; they are in good agreement with the results of the simplified fluorescein test and the electron micrographs. It can be seen that there is a great difference between "hairy"
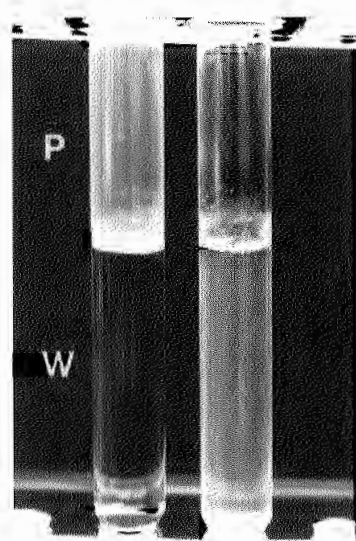
Fig. 4: Separation of *Plasmopara viticola* spores. Left side: Freshly harvested dry spores are all visible in the upper organic phase (P = pentane) indicating at first a hydrophobic surface . Right side: Soaked spores are all in the lower water phase (W) indicating changes of surface chemistry.

and "non-hairy" plants. The susceptible varieties Morio Muskat and Müller-Thurgau are extremely wettable and great forces were needed to remove the water droplets.

Some varieties showed a considerable similarity with regard to the adhesion of the water droplets; e.g. the values of Kerner (susceptible) and Phoenix and Regent (resistant) are nearly the same. Only Riesling differed from the other varieties shortly after wetting. Although Riesling leaves became wettable within rather a short time the adhesion of water droplets was lower than that of the resistant variety Regent. Interestingly, *V. rotundifolia* which is highly resistant to several fungi, especially the mildews, was more wettable than other varieties. However, all varieties showed similar values 1 h after droplet application.

In contrast to these cultivars the hairy species were clearly differentiated. The forces to remove the water droplets were at the same level but showed a greater difference 1 h after droplet application. It is interesting to note that all species investigated show field resistance against *Plasmopara viticola*.

Discussion

A sufficient attachment of fungal spores on the hydrophobic plant surface is necessary to initiate host colonization. Normally ungerminated fungal spores are hydrophobic, too, as is the case for *Plasmopara viticola* (Fig. 4). Although a hydrophobic surface is advantageous for the attachment of fungal spores the presence and uptake of water is essential for initiating the germination process and the release of zoospores. Spores may favour the uptake of water by changing their surface characteristics (Fig. 4). However a successful germination of *Plasmopara* spores depends on the deposition and evaporation of water from rain or dew. While spores are able to dry and soak several times still staying alive (KORTEKAMP *et al.* 1998), a spore's life at the exposed desiccating surface of a leaf may be very short.

A characteristic of hairy *Vitis* leaves is that the droplets remained spherical and that they had high contact angles with the leaf resulting in weak physical forces to remove them (schematic drawings in Fig. 6). High contact angles cause water droplets to "pearl off". This not only enhances the speed of drying of the surface, but there is also less surface area for fungal penetration and disease initiation. In the case of downy mildew a high contact angle will prevent spreading by swimming zoospores over a large area. Even dew may be sufficient. The importance of dew also lies in creating water pools in which many spores are able to germinate. Here again the detail of the leaf surface may be crucial. Dew droplets adhere very weakly to the hairs of very pubescent leaves, a dense pubescence (e.g. *V. labrusca*, Fig. 3) also leading to small contact areas. If the water droplets reach a critical size this may cause pearling off.

Furthermore, hairs and related structures act to prevent spores from reaching the leaf surface. In the simplest case this occurs by forming a screen on which spores are deposited. When spores entrapped on hairs germinate, the resulting germ tubes often get entangled, and as a result they do not reach the leaf surface and are unable to get an infection started (HEATH 1974; VON ALTEN 1983; KORTEKAMP and ZYPRIAN 1999).

Is is noteworthy that a dense covering often develops on young leaves. During leaf growth, the distance between
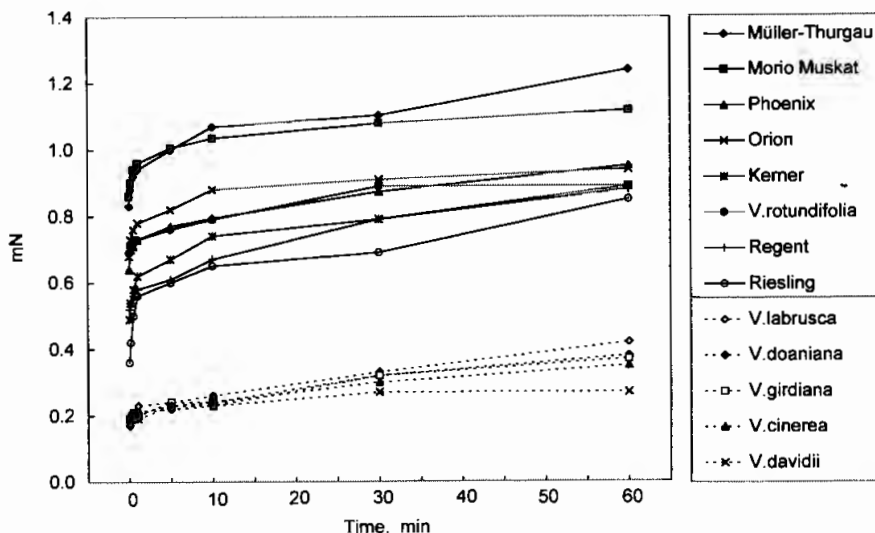

Fig. 5: Forces necessary to remove the water droplets as a function of time. Results obtained by weighing with a torsion balance.
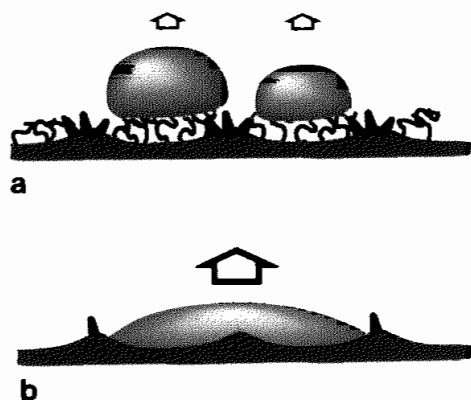
Fig. 6: Schematic drawings of contact behaviour and distribution of water droplets on a hairy leaf (a) and a non-hairy leaf (b). The forces necessary to remove the water droplets are symbolized by arrows.

trichomes is expanding, and unless no new hairs are formed a sparse pubescence will result in fully expanded leaves. On the other hand, in case trichome formation keeps pace with the expanding surface a dense pubescense is always present. Although trichomes are present during the whole life span of leaves the fact that the number of hairs per unit area decreases with senescence may be one reason for the enhanced susceptibility at the end of summer.

Leaves of some susceptible varieties, *e.g.* Riesling, also have hairs but the leaf surface is incompletely covered allowing water droplets to get into contact with the cuticle. Furthermore, the hairs do not seem to be water repellent, since they became strongly wettable within a few seconds. In general, it seems that the purely mechanical effects of the pubescence depend on some major characteristics, such as density, length and exposed chemical groups. Thus, the effect of hairs on disease resistance has to be viewed from two different perspectives: the nature of the individual hairs and the nature of the pubescence as a whole unit. A dense layer of hydrophobic hairs, as can be seen in Fig. 5, means that during intense rainfall these leaves remain water repellent whereas varieties with a poor pubescence, such as Riesling, become soon wet.

Interestingly, *V. rotundifolia* does not show water repellency but is highly resistant against downy mildew and other fungal diseases, indicating that this resistance is caused by a cellular reaction. In addition to cell reactions or exposed hairs, wax or compounds associated with wax may also play a role in disease resistance. The presence of duvatrienediols on tobacco leaf surfaces was associated with induced as well as preformed resistance against *Peronospora tabacina* (TUZUN *et al.* 1989).

It can be concluded that a dense pubescence on the abaxial side of the leaves enhances the resistance features of *Vitis* against downy mildew, whereas the lack of leaf hairs does not mean susceptibility. It seems that hairs serve as an outer line of defense by presenting a physical barrier. Within the leaf, although physically safer on the microbe's perspective, the growing hyphal tip may be impeded by the induction of physical or chemical barriers.

References

BARTHLOTT, W.; NEINHUIS, C.; 1997 a: Purity of the sacred lotus or escape from contamination in biological surfaces. Planta **202**, 1-7.
- -; - -; 1997 b: Characterisation and distribution of water-repellent, self-cleaning plant surfaces. Ann. Bot. **79**, 667-677.
FOGG, G. E.; 1944: Diurnal fluctuation in a physical property of leaf cuticle. Nature **3912**, 515.
GOOD, R. J.; 1979: Contact angles and the surface free energy of solids. Surface Colloid Sci. **11**, 1-29.
HEATH, M. C.; 1974: Light and electronmicroscope studies of the interactions of host and non-host plants with cowpea rust *Uromyces phaseoli*. Physiol. Plant Pathol. **4**, 403-414.
HUMMEL, K.; STAESCHE, K.; 1962: Die Verbreitung der Haartypen in den natürlichen Verwandtschaftsgruppen. In: W. ZIMMERMANN und P. G. OZENDA (Eds.): Handbuch der Pflanzenanatomie, Bd. 4, Teil 5. Histologie, 207-250. Gebrüder Borntraeger, Berlin.
JOHNSON, H .B.; 1975: Plant pubescence: An ecological perspective. Bot. Rev. **41**, 233-258.
JUNIPER, B. E.; JEFFREE, C. E.; 1982: Plant Surfaces. Edward Arnold, London.
- -; SOUTHWOOD, T. R. E. (Eds.); 1986: Insects and the Plant Surface. Edward Arnold, London.
KORTEKAMP, A.; WIND, R.; ZYPRIAN, E.; 1998: Investigation of the interaction of *Plasmopara viticola* with susceptible and resistant grapevine cultivars. J. Plant Dis. Prot. **105**, 475-488.
- -; ZYPRIAN, E.; 1999: Leaf hairs as a basic protective barrier against downy mildew of grape. J. Phytopathol. **147**, 453-459.
PONTER, A. B.; YEKTA-FORD, M.; 1985: The influence of environment on the drop size-contact angle relationship. Colloid Polym. Sci. **263**, 673-681.
SHAIK, M.; 1985: Race-nonspecific resistance in bean cultivars to races of *Uromyces appendiculatus var. appendiculatus* and ist correlation with leaf epidermal characteristics. Phytopathology **75**, 478-481.
STAEDMAN, J. R.; SHAIK, M.; 1988: Leaf pubescence confers apparent race-nonspecific rust resistance in bean *(Phaseolus vulgaris)*. Phytopathology **78**, 1566.
STAUDT, G.; KASSEMEYER, H. H.; 1995: Evaluation of downy mildew resistance in various accessions of wild *Vitis* species. Vitis **34**, 225-228.
TUZUN, S.; REUVENI, M.; SIEGEL, M. R.; KUC, J.; 1989; The effect of removing leaf surface components with acetone from immunized and nonimmunized resistant tobacco plants on their susceptibility to blue mold. Phytopathology **79**, 1024-1027.
UPHOF, J. C. T.; 1962: Plant hairs. In: W. ZIMMERMANN und P. G. OZENDA (Eds.): Handbuch der Pflanzenanatomie. Bd. 4, Teil 5, Histologie. Gebrüder Borntraeger, Berlin.
VON ALTEN, H.; 1983: The effect of temperature, light and leaf age on the frequency of appressoria formation and infection with *Uromyces phaseoli*. Phytopathol. Z. **107**, 327-335.
ZAITER, H. Z.; COYNE, D. P.; STAEDMAN, J. R.; BEAVER, J. S.; 1990: Inheritance of abaxial leaf pubescence in beans. J. Amer. Soc. Horticult. Sci. **115**, 1158-1160.