Vitis **28**, 153—160 (1989)

Bundesforschungsanstalt für Rebenzüchtung Geilweilerhof, Siebeldingen, BR Deutschland

Structural characters of epidermal cell walls and resistance to powdery mildew of different grapevine cultivars

by

CATHÉRINE HEINTZ and R. BLAICH

Strukturelle Eigenschaften von Epidermiszellwänden und Oidiumresistenz bei verschiedenen Rebsorten

Zusammenfassung: Untersuchungen an 12 unterschiedlich oidiumanfälligen Rebsorten ergaben bei jungen Blättern eine Korrelation zwischen Dicke von Cuticula plus Zellwand und Oidiumresistenz. Bei älteren Blättern, solchen von *in vitro*-Pflanzen und bei Beerenhäuten konnte keine Korrelation gefunden werden. Beschichten der Blattoberfläche mit einem Antitranspirant auf Polymetacrylat-Basis verhinderte in allen Fällen eine Sporulation, obwohl Infektionsstrukturen gebildet wurden.

Key words: oidium, resistance, variety of vine, leaf, berry, epidermis, cell wall, cuticle.

Introduction

In previous reports, it was shown that powdery mildew (*Uncinula necator, Oidium tuckeri*) may penetrate artificial membranes by mechanical force (HEINTZ 1986) and that a very important active defence mechanism of the epidermal cells of *Vitis* against *Oidium* is the formation of mechanical barriers in or near cell walls — e.g. papillae (HEINTZ and BLAICH 1989) and silica deposits (BLAICH and WIND 1989). Hence the thickness of the epidermal cell walls and cuticles might play a role as a passive character of resistance to powdery mildew in *Vitis* cultivars.

Materials and methods

Leaves and berries of 12 grapevine varieties (see Table 1) with different susceptibility to *Oidium* were taken from plants grown in the field, in the glasshouse or under *in vitro* conditions at different times during the growing season. Two types of leaves were used: 5th (young, just fully expanded) leaves and 8th (mature) leaves, counted from the top of the shoot.

As the thickness of leaf cell walls was less than 1 µm, measurements with the light microscope proved very difficult and required semithin sections of resin embedded leaves. Therefore, margins of broken leaf segments freeze dried at −60 °C were analyzed using a Zeiss DSM 950 stereoscan electron microscope (SEM). This method was more exact and far simpler than might be expected (for details of preparation see BLAICH *et ae.* 1984). Each value given in Tables 1 and 2 and Figs. 1 and 3 is based on 150 single measurements using 5 fragments of different leaves of one variety.

Culture of *Oidium* and *in vitro* grapevines as well as leaf disc tests for susceptibility to *Oidium* (indexed as intensity of sporulation) are described by STEIN *et al.* (1985).

Surface treatment with Protec (polymetacrylate normally used as an antitranspirant): leaf discs (mature and young leaves from field, *in vitro* and glasshouse plants, 10 discs/variant in triplicate) of all varieties were either dipped into the product or sprayed to obtain a thin but complete film, allowed to dry and then kept in petri dishes on damp filter paper. The surfaces were then inoculated with oidiospores as described previously (STEIN *et al.* 1985) and assessed after 12 d.

Results and discussion

Leaf cuticle: In the SEM it is rarely possible to differentiate between cuticle and cell wall (Fig. 2c), whereas in light microscopy the cuticle (about 0.4 µm) is at the limit of resolution. Therefore, the values in Tables 1 and 2 give the thickness of cuticle plus cell wall. In young leaves from field-grown plants a correlation between thickness of cell walls and susceptibility to *Oidium* could be demonstrated (Fig. 1). All *V. vinifera* cvs (susceptibility > 7) lie around 0.85 µm and all resistant varieties (< 2) above 1.1 µm. Mature field leaves, however, showed no significant correlation: They are generally more resistant in all varieties (see also DOSTER and SCHNATHORST 1985) and differences in thickness between susceptible and resistant varieties have diminished (Table 1). *In vitro* leaves uniformly have very thin walls (0.6 µm and less) and are much more sensitive to powdery mildew (see STEIN *et al.* 1985).

Table 1

Thickness of epidermal cell walls including cuticle of different grapevine varieties · Young leaf: 5th, mature leaf: 8th, counted from the top of the shoot

Dicke der Epidermiszellwand einschließlich der Cuticula verschiedener Rebsorten · Junge Blätter in Position 5, ältere in Position 8, von der Triebspitze aus gezählt

Variety	Thickness of cell wall + cuticle (µm)			
	Field plants		*In vitro* plants	LSD (5 %)
	Young leaves	Mature leaves		
Riesling[1])	0.85	1.20	0.62	
Kerner[1])	0.85	0.90	0.52	
V 3125[1])	0.85	1.07	0.40	
Pollux	0.86	1.02	0.37	
Phoenix	1.11	1.18	0.62	
Gf. Ga-50-34	1.14	1.16	0.63	0.13
Sirius	1.05	1.12	0.52	
Gf. Ga-54-14	1.23	1.26	0.51	
Orion	1.03	1.06	0.40	
Gf. 67-198-3	1.10	1.06	0.55	
Maréchal Foch	1.16	1.24	0.44	
Vidal 256	1.46	1.47	0.44	
Mean	1.10	1.15	0.50	0.04

[1]) Varieties of *Vitis vinifera*.

C o a t i n g o f l e a v e s : The treatment of leaf surfaces with polymetacrylate (Protec) to produce an artificial thickening of the cuticle was successful for all varieties and all leaf types: The mean (all varieties) sporulation notes for untreated young leaves were 5.3 (for field plants) and 2.3 (for glasshouse plants), for mature leaves < 1. Protected leaves never showed sporulations. Cytological observations revealed that the fungus formed appressoria and that some penetration attempts were successful as indicated by the accumulation of callose around the penetration peg (fluorescence after staining with aniline blue; see HEINTZ and BLAICH 1989). This was, however, never sufficient to produce visible symptoms of sporulation on the leaf. SEM showed that the coat was very uneven and had thin areas and even holes (Fig. 2 e). The capability of the parasite to penetrate polymetacrylate membranes of < 1 µm is well known (HEINTZ 1986). These membranes were, however, formed using true solutions whereas the polymetacrylate layers on leaves were produced by drying an aqueous dispersion of small polymetacrylate beads which fuse due to capillary forces.

B e r r y c u t i c l e : Peripheral cell walls and cuticle of berries are much thicker than in leaves (for more details see CONSIDINE and KNOX 1979). The cuticle consists of 3 layers which may be differentiated under the fluorescence microscope (Fig. 2 f) and — in rare cases — by SEM (Fig. 2d). The changing thickness during maturation is demonstrated for a resistant and a susceptible variety (cv. Vidal 56 and cv. Kerner) in Fig. 3. It reached a maximum during the 2nd growth phase and decreased slightly until the end of maturation. The time when maximal thickness was reached differed between both varieties (Fig. 3); it seems also to depend on climatic conditions

Table 2

Thickness of the epidermal cell walls of berries of different grapevine varieties at one sample time (4th October 1984) · If possible, mildew infected berries were used for the analysis · These values are only intended to give hints for discussion; they are not suitable for further comparisions since varieties are different in maturity and wall thickness might have been measured before or after maximum (compare Fig. 3)

Dicke der Epidermiszellwand der Beeren verschiedener Rebsorten zu einem bestimmten Zeitpunkt (4. Oktober 1984) · Nach Möglichkeit wurden oidiumbefallene Beeren ausgewertet · Die Werte sollen lediglich als Diskussionsgrundlage dienen; für weitergehende Vergleiche sind sie nicht geeignet, da die einzelnen Sorten zu verschiedenen Zeiten reif werden und die Wanddicke deshalb vor oder nach dem Maximum gemessen worden sein könnte (vgl. Fig. 3)

Variety	Thickness of wall + cuticle (µm)	Sporulation intensity	Sugar content (g/l)
Riesling	5.0	2.3	57.7
Kerner	4.7	7.2	93.5
V 3125	4.3	3.8	40.0
Pollux	4.8	0.1	139.5
Phoenix	3.9	0.3	117.7
Gf. Ga-50-34	3.1	0	140.5
Sirius	4.6	2.1	101.2
Gf. Ga-54-14	4.7	0.2	84.5
Orion	4.4	2.7	101.2
Maréchal Foch	4.9	0	181.5
Vidal 256	6.6	0	93.5

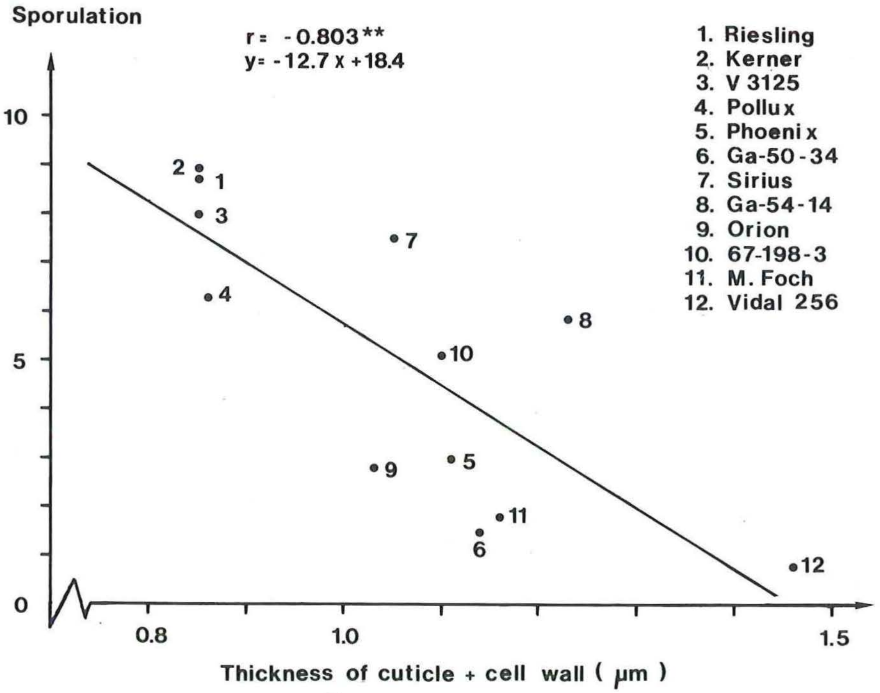
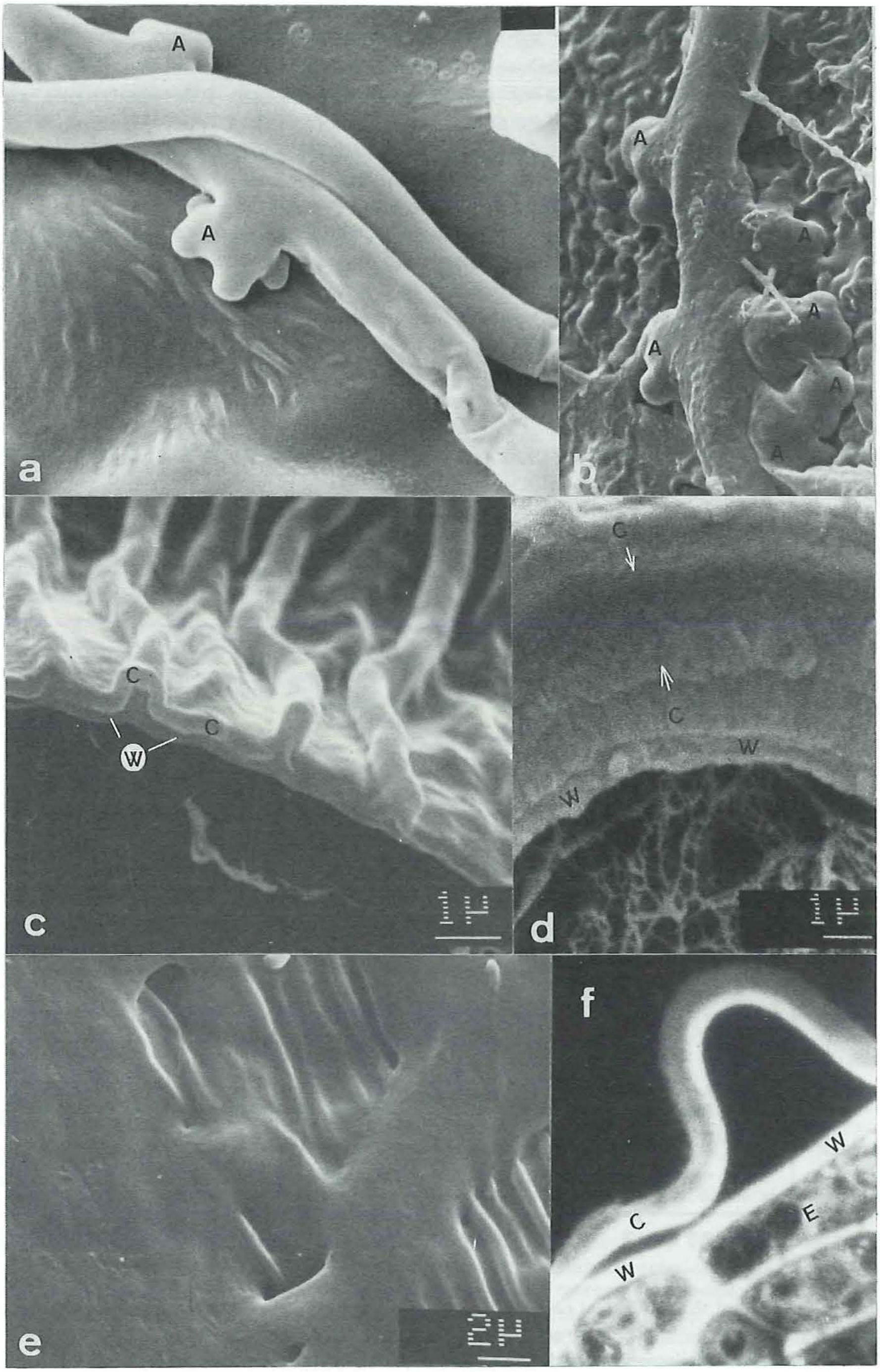


Fig. 1: Correlation between the thickness of epidermal cell wall (including cuticle) of leaves of some grapevine cvs (see Table 1) and their susceptibility to powdery mildew.

Korrelation zwischen der Dicke der Zellwand (einschließlich Cuticula) der Blattepidermiszellen verschiedener Rebsorten (siehe Tabelle 1) und ihrer Oidiumanfälligkeit.

Fig. 2: a—e) SEM images, f) light microscopic image; A = appressorium, C = cuticle, W = periclinal wall of epidermal cell. — a) Two hyphae of *Oidium* on the leaf surface with typical single appressoria; note some wrinkles on the leaf surface. — b) Hyphae with a typical cluster of appressoria on berry surface. — c) Edge of a broken epidermal cell wall near a leaf vein where the epidermal surface forms numerous wrinkles; in this particular case, cell wall and cuticle can be differentiated, which is rare. — d) Edge of a broken epidermis cell of a berry (cv. Vidal 256) where cell wall and layers of cuticle can be differentiated. — e) Surface of a leaf coated with Protec; the polyacrylate layer is incomplete, leaving some holes and some areas where the space between wrinkles is not completely filled up. — f) UV microscopic image (405 nm) of 1 µm section of berry epidermis stained with thioflavin; the shrinkage of the underlying cells during preparation was stronger than that of the cuticle which (near an infection site) forms a ply separated from the cell wall; 2—3 layers can be differentiated.

a—e) REM-Bilder, f) Lichtmikroskopisches Bild; A = Appressorium, C = Cuticula, W = perikline Zellwand der Epidermiszelle. — a) Zwei *Oidium*-Hyphen auf einer Blattoberfläche mit typischen Einzelappressorien; man beachte einige Runzeln der Blattoberfläche. — b) Hyphe mit typischer Anhäufung von Appressorien auf Beerenhaut. — c) Kante an einer abgebrochenen Epidermiszellwand in der Nähe einer Blattader, wo die Epidermisoberfläche zahlreiche Leisten aufweist; ausnahmsweise lassen sich Zellwand und Cuticula differenzieren. — d) Bruchkante einer Beerenepidermiszelle (cv. Vidal 256); auch hier können Zellwand und geschichtete Cuticula ausnahmsweise differenziert werden. — e) Oberfläche eines Blattes, das mit Protec behandelt wurde; die Polyacrylschicht hat einige Löcher und dünne Stellen, an denen die Zwischenräume zwischen den Epidermisleisten nicht ganz ausgefüllt sind. — f) UV-Fluoreszenzbild (405 nm) eines kunstharzeingebetteten 1 µm dicken Schnittes einer Beerenepidermis; die Epidermiszellen sind während der Präparation stärker geschrumpft als die Cuticula, die sich (neben einer *Oidium*-Infektion) faltenartig von der peripheren Zellwand abgehoben hat: 2—3 Schichten können unterschieden werden.


A
A
a
A
A
A
A
A
A
b
C
C
W
1μ
c
C
C
W
W
1μ
d
2μ
e
f
W
E
C
W

since in Australia the maximum for *V. vinifera* cv. Muscat of Alexandria occurred during the 1st growth phase (CONSIDINE and KNOX 1979). CHRISTIN-BOURGUIGNON *et al.* (1986) found an analogous behaviour of the whole berry skin.

The other varieties were assessed only at one time. Reproducible inoculation of berries under laboratory conditions being extremely difficult, infected berries were collected in the field to obtain some additional hints on susceptibility. Because this, of course, was not possible for the highly resistant varieties and both *Oidium* susceptibility and wall thickness of berries are influenced by the state of maturity (Fig. 3), the data must be interpreted cautiously, although considerable varietal differences of the wall thickness of ripe berries were found (from 3.1 to 8.6 µm) which could be correlated (r = 0.63, significant at P = 5 %) to the wall thickness of mature field leaves.

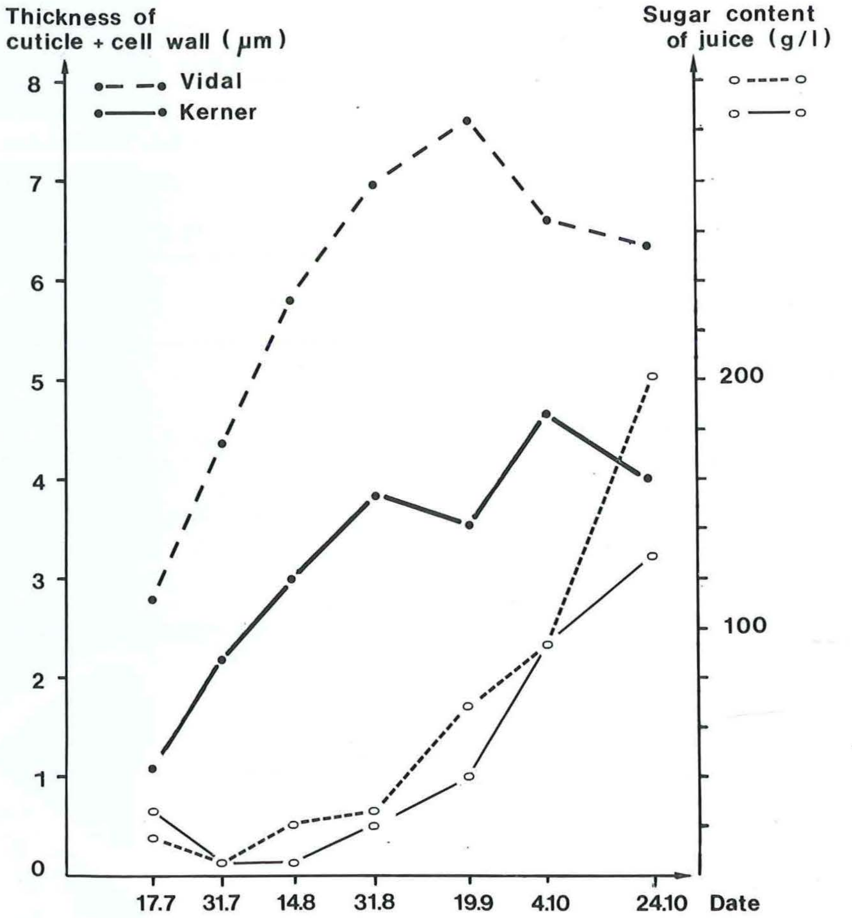


Fig. 3: Thickness of the epidermal cell wall (including cuticle) and sugar content of the berries of grapevine cvs Vidal 256 (resistant to mildew) and Kerner (susceptible) during maturation.
Dicke von Epidermiszellwand (einschließlich Cuticula) und Zuckergehalt von Beeren der Rebsorten Vidal 256 (oidiumresistent) und Kerner (anfällig) während der Reife.

Conclusions

Although the existing correlation between cuticular thickness and resistance to powdery mildew of young grapevine leaves does not prove the role of thickness as a preformed resistance character, this fact corroborated earlier assumptions from experiments with artificial membranes (HEINTZ 1986) which became unpenetrable at a thickness corresponding to the dimension of the cuticle of resistant varieties. Of course, we are aware that the mechanical properties of polyacrylate membranes and cutin structures may be quite different and even between the cuticle of the leaf and that of the berry there must be considerable differences because berries with cuticles of serval μm thickness may be susceptible. Such differences are recognized by *Oidium* because the density of appressoria is much higher on berries (Fig. 2 b) than on leaves (Fig. 2 a) — a phenomenon that has been already described for appressoria on leaves of resistant cultivars by DOSTER and SCHNATHORST (1985). Thus it is certainly not mere thickness but also other properties which determine resistance to mildew. It has been shown by BLAICH *et al.* (1984) that in the surface of berries natural perforations are formed during maturation which start from preformed weak zones. The high density of appressoria might simply reflect numerous penetration attempts to find such weak zones. On the other hand, it is well known that resistance is determined by a number of overlapping characters which may replace each other in different cultivars (HEINTZ and BLAICH 1989 and in preparation). As AUST and HOYNINGEN-HUENE (1986) point out, every barrier which slows down the penetration process may provide the time necessary for other mechanisms (formation of papillae, deposition of cell wall incrusts).

Summary

The analysis of 12 grapevine cultivars differing in susceptibility to powdery mildew revealed a correlation between the thickness of cuticle plus cell wall of young leaves and resistance to mildew. Neither in mature leaves or in leaves of *in vitro* plants or in berries such correlations could be established. Coating of the leaf surface with a polymetacrylate antitranspirant prevented sporulation in all cases, although infection structures were formed.

This work was supported by the Deutsche Forschungsgemeinschaft

References

AUST, H. J.; HOYNINGEN-HUENE, J.; 1986: Microclimate in relation to epidemics of powdery mildew. Ann. Rev. Phytopathol. **24**, 491—510.

BLAICH, R.; STEIN, U.; WIND, R.; 1984: Perforationen in der Cuticula von Weinbeeren als morphologischer Faktor der Botrytisresistenz. Vitis **23**, 242—256.

BLAICH, R.; WIND, R.; 1989: Inducible silica incrusts in cell walls of *Vitis* leaves. Vitis **28**, 73—80.

CHRISTIN-BOURGUIGNON, F.; MAUJEAN, A.; AUDRAN, J. C.; 1986: Pellicule et résistance de la baie de raisin à la pourriture grise chez deux cépages champenois. Vigneron Champenois **3**, 143—148.

CONSIDINE, J. A.; KNOX, R. B.; 1979: Development and histochemistry of the cells, cell walls, and cuticle of the dermal system of fruit of the grape, *Vitis vinifera* L. Protoplasma **99**, 347—365.

DOSTER, M. A.; SCHNATHORST, W. C.; 1985: Effects of leaf maturity and cultivar resistance on development of the powdery mildew fungus on grapevines. Phytopathology **75**, 318—321.

HEINTZ, CATHÉRINE; 1986: Infection mechanisms of grapevine powdery mildew (*Oidium tuckeri*): Comparative studies on the penetration process on artificial membranes and leaf epidermis. Vitis **25**, 215—225.

HEINTZ, CATHÉRINE; BLAICH, R.; 1989: Ultrastructural and histochemical studies on host-parasite relations between *Vitis* spp. and *Uncinula necator*. (Submitted).

STEIN, U.; HEINTZ, CATHÉRINE; BLAICH, R.; 1985: Die *in-vitro*-Prüfung von Rebsorten auf Oidium- und Plasmopararesistenz. Z. Pflanzenkrankh. Pflanzensch. **4**, 355—369.

Eingegangen am 20. 6. 1989

Prof. Dr. R. BLAICH
BFA für Rebenzüchtung
Geilweilerhof
D 6741 Siebeldingen