Vitis 37 (1), 21–26 (1998)

The influence of silica fertilization on the resistance of grapevines to powdery mildew

by

R. Blaich and Hedwig Grundhöfer

Institut für Obst-, Gemüse- und Weinbau, Universität Hohenheim, Stuttgart-Hohenheim, Deutschland

Summary: Six different *Vitis* cultivars grown in recirculating nutrient solutions supplied with 0, 10 and 112 mg·l^{-1} SiO_2, respectively, were infected with spores of the powdery mildew fungus *Uncinula necator* and analyzed after 2, 4 and 6 weeks. Whereas the size of the silica endoskeletons induced by the powdery mildew was influenced by the SiO_2 concentration, the cultivar-specific resistance could not be increased. However, after 2 weeks all varieties showed a slightly but significantly enhanced resistance in the 112 mg·l^{-1} solution which disappeared during the following weeks. The results show silica to be essential for a normal powdery mildew resistance but make evident that the Oidium susceptibility of cultivars cannot be overcome by supplementary silica fertilization in the field, the Si contents of most soil solutions being far above the minimal requirements of the grapevine.

Key words: silica, fertilization, powdery mildew, resistance, variety.

Introduction

Although the induction of silica endoskeletons by the powdery mildew fungus *Uncinula necator* (Blaich and Wind 1989; Heintz and Blaich 1990) and observations in the field (Schaller *et al.* 1990; Bowen *et al.* 1992; Reynolds *et al.* 1996) indicated that silica might play a role in the oidium resistance of grapevines, fertilization with silica in the field or in hydroponic cultures with up to 50 mg·l^{-1} SiO_2 had no clear effects on natural infections (Lafos 1995). Therefore, we studied the effects of higher silica concentrations (up to 112 mg·l^{-1}) on the resistance and on the inducible endoskeletons of different *Vitis* cultivars after artificial infections with spores of *Uncinula necator*.

Material and methods

Grapevine cultivars with different degrees of powdery mildew resistance (Tab. 1) were grown on recirculating nutrient solution as previously described (Blaich and Grundhöfer 1997). A silica free solution was used as control. The effects of a 10 mg·l^{-1} solution were tested because this concentration is well below that of filtrates of aqueous soil suspensions prepared from different locations in the fields of our institute (around 60 mg·l^{-1} SiO_2) and also lower than the concentrations used by Lafos (1995) who found no clear effects of 25 and 50 mg·l^{-1} SiO_2 on spontaneously occurring powdery mildew infections in hydroponic cultures. Therefore, we tested a 112 mg·l^{-1} solution (near to saturation and more concentrated than aquous soil suspensions) to enhance possible effects.

Table 1

Grapevine cultivars used for the experiments

Cultivar	Parentage	Oidium resistance[1)]
Regent	interspecific	very high
Sirius	interspecific	high
Orion	interspecific	high
Staufer	interspecific	medium
Müller-Thurgau	*Vitis vinifera*	none

[1)] According to Eibach (1994)

Powdery mildew fungus (*Uncinula necator*) was collected in vineyards which had not been treated with fungicides. Spores were brushed from infected leaves onto leaves of plants grown in hydroponic culture. Inoculum density (10 - 100 spores per mm^2 and germination rate (30-50 %) as well as the proportions of infected leaf areas after 2, 4 and 6 weeks were estimated according to Stein *et al.* (1985).

Light microscopy: Suitable segments (about 25 mm^2) of grapevine leaves were kept in methanol at room temperature for 1-2 d until all chlorophyll had disappeared. The bleached leaves were heated in 10 % sulfuric acid for 10 min at 100 °C. After removing the acid by thorough washing with water the segments were dehydrated in an ethanol series (25, 50, 75, 100 %) and transferred into xylene via similar steps of an ethanol-xylene mixture. Leaf pieces were immersed for 20-30 min in a 0.1 % solution of methyl red (purified according to Blaich and Grundhöfer 1997) or cristal violet lactone or in xylene. After complete removal of the excess colour with xylene the specimens could be embedded in a suitable resin if necessary (method modified after Danayadan *et al.* 1983).

Scanning electron microscopy: To isolate induced silica skeletons leaves were boiled in concentrated H_2SO_4 (around 330 °C) to which H_2O_2 was added dropwise and very cautiously. The residuals were washed repeatedly with water and concentrated HCl to remove the sulphates. After drying they were sputtered with gold in an Edwards Sputter Coater or left untreated for XRMA. SEM was carried out with a ZEISS DSM 950 microscope.

Correspondence to: Prof. Dr. R. Blaich, Institut für Obst, Gemüse und Weinbau, Universität Hohenheim, D-70599 Stuttgart-Hohenheim, Germany. Fax: +49-711-4593946. E-mail: rblaich@uni-hohenheim.de

Size classification of silica skeletons was carried out in a JIM system (PAMAS, Rutesheim, Germany) which allowed to determine the size of particles >2 µm by a laser device and sorting them into different size classes. The particles were prepared by acid digestion as described by BLAICH and WIND (1989).

Results

Infection experiments with *Uncinula necator* on grapevines grown in hydroponic cultures were repeated in 1990, 1991 and 1992 with identical results. Fig. 1 shows that 2, 4 and 6 weeks after infection all cultivars exhibited their characteristic varietal degree of resistance, Müller-Thurgau being the most susceptible, Regent the least susceptible. An influence of the SiO_2 content of the nutrient solution could be found only up to 2 weeks. Although the differences after two weeks were not very impressive, they were consistent for all cultivars in all three years: a silica supply of 112 mg·l^{-1} increased Oidium resistance in the first weeks slightly but significantly. However, this effect disappeared after 4 weeks and a concentration of 10 mg·l^{-1} SiO_2 in the nutrient solution was always sufficient to provide a degree of resistance which could not be further improved by 112 mg·l^{-1}.

Nevertheless, cytological investigations show that the size of induced silica skeletons is influenced by the silica content of the nutrient solution (Fig. 2). Whereas in leaves of plants grown on silica free solution no stainable areas were found, small incrustations showed up at 10 mg·l^{-1} SiO_2 (Fig. 2 b, e) and larger ones at 112 mg·l^{-1} (Fig. 2 a, d). Uninfected leaves showed no distinct stainable structures whereas leaves supplied with 112 mg·l^{-1} SiO_2 seemed to exhibit a stronger "background" stain, particularly in their teeth. The largest skeletons seemed to be produced by the cultivars Sirius and Regent (Fig. 2 c), whereas they appeared smaller in Müller-Thurgau, Orion and Staufer.

The isolation of skeletons from acid digests (Fig. 3) revealed that uninfected leaves or infected leaves from plants grown in SiO_2-free nutrient solution always seemed to dissolve completely in hot sulfuric acid whereas from plants grown on 10 and on 112 mg·l^{-1} SiO_2 particles and larger skeletons remained. In plants grown on the high concentration not only larger skeletons could be found (Fig. 3 a, b) but also more frequently palisade cells were silicified (Fig. 3 a, f) and often cell lumina were filled with granular, amorphous silica (Fig. 3 e). Very often silicified vascular elements were found (Fig. 3 c, d). Larger magnifications showed the average diameter of the grains composing these structures to be around 0.2 µm.

To support the impressions gained from the cytological investigations by a quantitative method we tried particle size analysis with the JIM system. This was carried out separately for leaves from the canes' top (1-10), center (11-20) and base (21 and more). The size distribution of insoluble silica structures from infected leaves of different varieties grown on two different SiO_2 concentrations is given in Fig. 4. Most skeletons are found in the size class 11.7-16.6 µm which

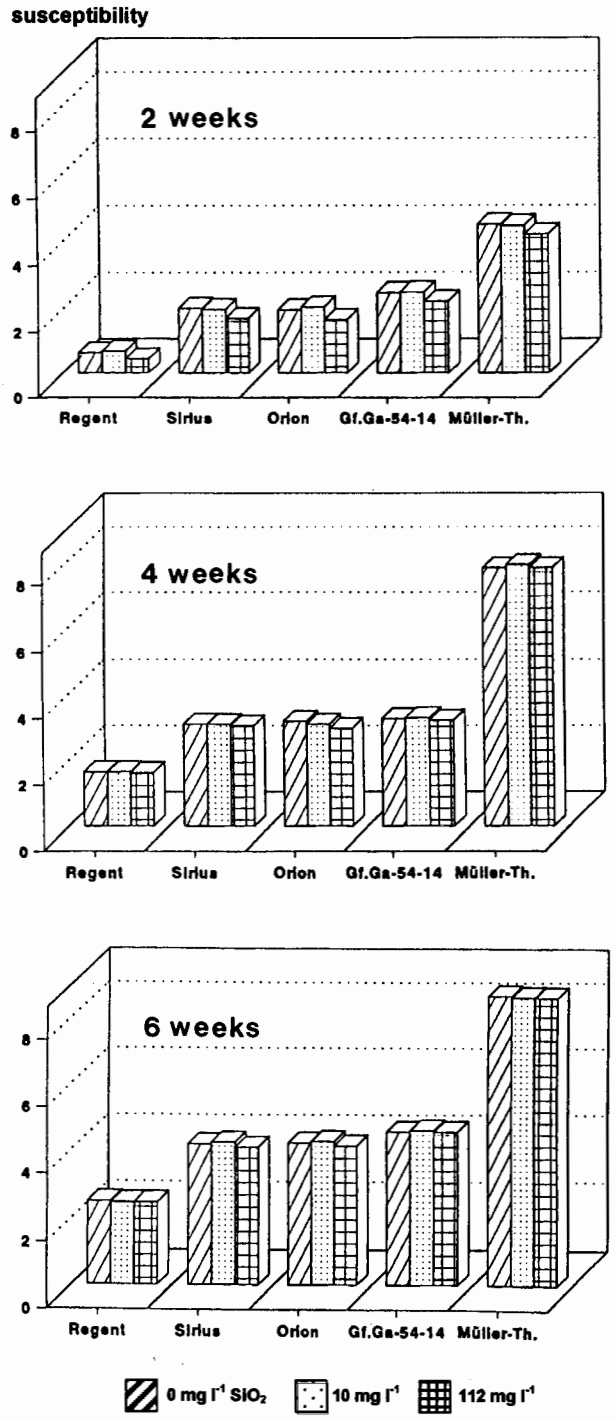

Fig. 1: Oidium susceptibility of several grapevine cultivars grown on recirculation nutrient solutions supplied with different amounts of silica (average of measurements of the years 1990 and 1991). Two weeks after inoculation all cultivars grown on 112 mg·l^{-1} silica are significantly ($p < 0.05$) less infected than plants from the two lower concentrations. Differences observed after 4 and 6 weeks were not significant. The well known differences between cultivars were not statistically analyzed. 0: not - 8: most susceptible.

means they consist of just one silicified epidermal cell. Evidently it is difficult to find varietal differences in these graphs and it is impossible to show correlations to the powdery mildew resistance of the cultivars, although Sirius (resistant) tended to produce higher proportions of large skeletons - in contrast to the susceptible Müller-Thurgau. A

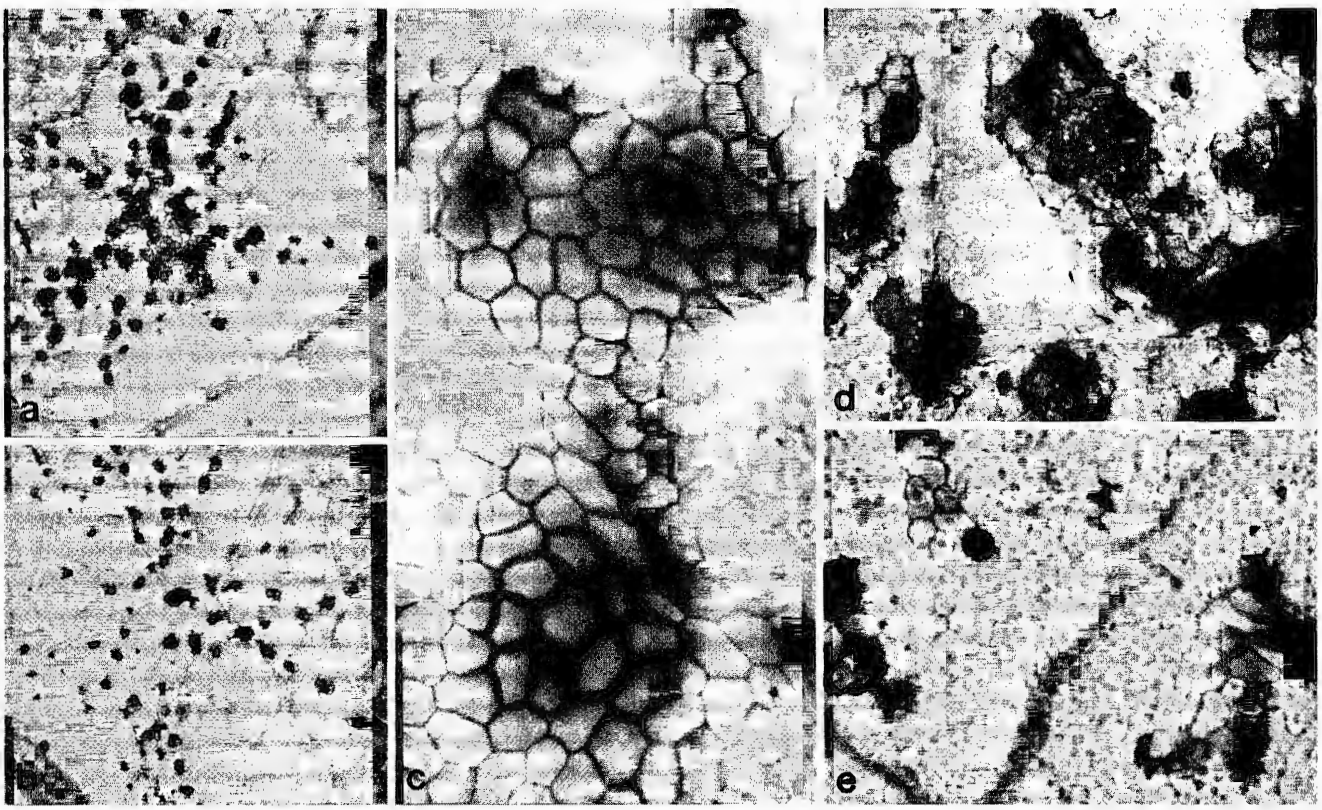

Fig. 2: Grapevine leaves infected with powdery mildew after bleaching and staining of silica; **a**, **b**: leaves of the resistant cultivar Staufer fertilized with 10 mg·l^{-1} and 112 mg·l^{-1} SiO_2, respectively (x 80); stained with methyl red; numerous stainable areas are arrayed along the paths of mildew hyphae removed by the staining procedure; **c**: very large structures in Regent fertilized with 112 mg·l^{-1} (x 600); anticlinal cell walls in the centre show appositions; **d**, **e**: leaves of the resistant cultivar Sirius grown on 10 and 112 mg·l^{-1} SiO_2, respectively; stained with cristal violet lactone (x 400).

statistical evaluation of the data from Fig. 4 is given in Tab. 2 which shows that particle size is influenced by the SiO_2 concentration in the nutrient solution, but that leaf age is more important.

Discussion

The problem of how many silica may be available to the plant in the "soil solution" has been discussed in detail by JONES and HANDRECK (1965). It may differ considerably but seems never to exceed 80 mg·l^{-1} (calculated as SiO_2) whereas pure monosilicic acid has a solubility of 150 mg·l^{-1}. MUNK (1981), citing earlier work, gives concentrations between 1 and 20 mg·l^{-1} but it is not always clear whether these values are related to Si or SiO_2. Actually LAFOS (1995) found 30 mg·l^{-1} Si in the soil solution (corresponding to around 60 mg·l^{-1} SiO_2) and used 25 and 50 mg·l^{-1} in his hydroponic cultures whereas in the soils of the Institut für Rebenzüchtung around 60 mg·l^{-1} were determined (BLAICH and GRUNDHÖFER 1997). To get a maximal effect on resistance we applied both, a higher and a lower concentration.

It must be kept in mind that the inoculum density we used to infect the plants with powdery mildew was far higher than would be expected in the field, a condition that might obscure minor differences of resistance. Nevertheless, the conclusion seems to be allowed that - while high silica concentrations may lead to a slight initial improvement of resistance - after some weeks even relative small amounts seem to be sufficient to fully establish the characteristic resistance potential of a grapevine cultivar (similar observations were made in cucumbers by ADATIA and BESFORD 1986). Evidently the capacity of the cell walls to integrate silica is limited. It seems, however, that the deposits found in cell lumina (Fig. 3 e) were not accumulated by the living cell. Since part of the powdery mildew resistance of the analyzed cultivars is due to hypersensitivity (HEINTZ and BLAICH 1990) the cell was probably already dead by this time - its lumen hence belonging to the apoplastic space. Anyhow, the control of powdery mildew in susceptible varieties by increasing the silica supply of normal soils seems not to be very promising as shown by LAFOS (1995) who found no effects of silica slag fertilization in vineyards and a doubtful effect of silica in hydroponic cultures. The observation that the influence of silica fertilization was restricted to the beginning of infections was made already by LEUSCH and BUCHENAUER (1988); in rye they found an initial growth inhibition of *Erysiphe graminis* supplied with Si fertilizer which disappeared after some weeks.

As already discussed in BLAICH and GRUNDHÖFER (1997), the bulk of silica in old leaves is insoluble in water. Optical microscopy of uninfected leaves or SEM of the residues of acid ashing showed no skeletons or particles, however the enhanced background stain of fertilized leaves seems to indicate its presence. ENGEL (1953) stated that most of the soluble silica in rye was only accessible after destroying the

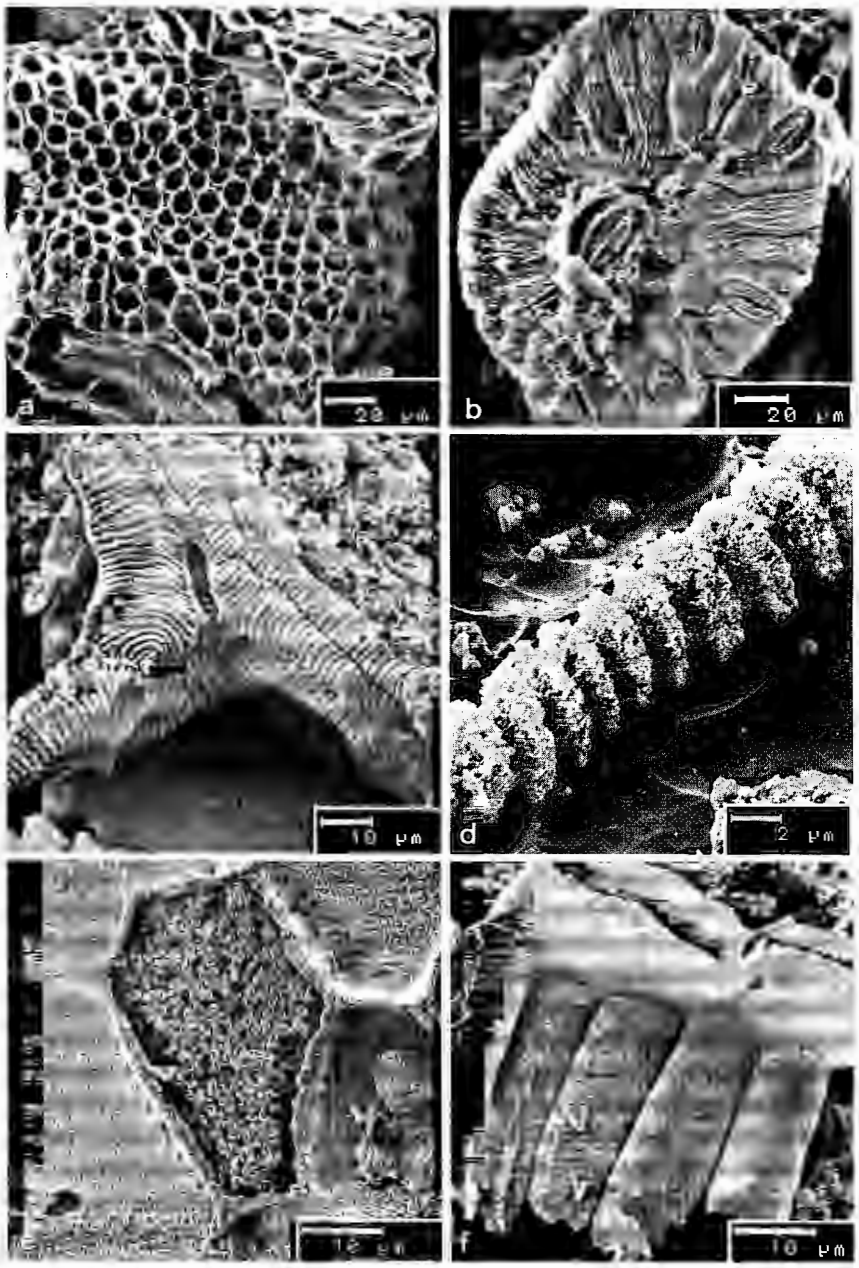

Fig. 3: SEM images of endoskeletons in grapevine leaves induced by powdery mildew and isolated by sulphuric acid ashing; grapevines grown on 112 $mg \cdot l^{-1}$ SiO_2; **a**: large skeleton from upper epidermis, palisade cells and vascular elements are visible (viewed from below, epidermal cells not visible); **b**: large skeleton from the lower epidermis comprising 5 stomata, at larger magnifications typical 0.5 µm penetration holes caused by oidium could be seen; **c** and **d**: silicified vascular elements; **e**: epidermis cell filled with granular silica; **f**: smaller element viewed from the side, two epidermal and 5 palisade cells are visible.

cellulose fibers. Either this is the case in the grapevine, too, or there exist very small particles (the structure in Fig. 3 e might be composed of such particles) which are washed away or dissolved during the acid ashing procedure. At higher magnifications the endoskeletons seem to be composed of grains whose size is well beyond visibility in the light microscope (Fig. 3 b). This technique therefore showed only larger structures with considerable size differences between the different levels of Si fertilization. It must be pointed out, however, that far more smaller particles are visible on scanning electron micrographs of acid digests of infected leaves - although while using this technique investigators tend to study and photograph preferentially larger structures. To get more objective data we used particle analysis to acid digests. Actually the proportion of larger skeletons increased with the amount of SiO_2 available in the leaf which in turn depended on both the leaf age and the silica content of the nutrient solution (BLAICH and GRUNDHÖFER 1997). According to our microscopic investigations the skeletons induced in resistant cultivars seemed to be larger than in susceptible ones but this could not be proved by particle analysis. However, this technique had been devised for other applications and it is not quite clear whether larger skeletons survive the procedures involved. Microscopical investigations of other defense reactions (autofluorescence, peroxidase and esterase activity, callose formation etc.) in grapevine leaves have shown too that induced areas are larger in resistant cultivars than in susceptible ones (HEINTZ

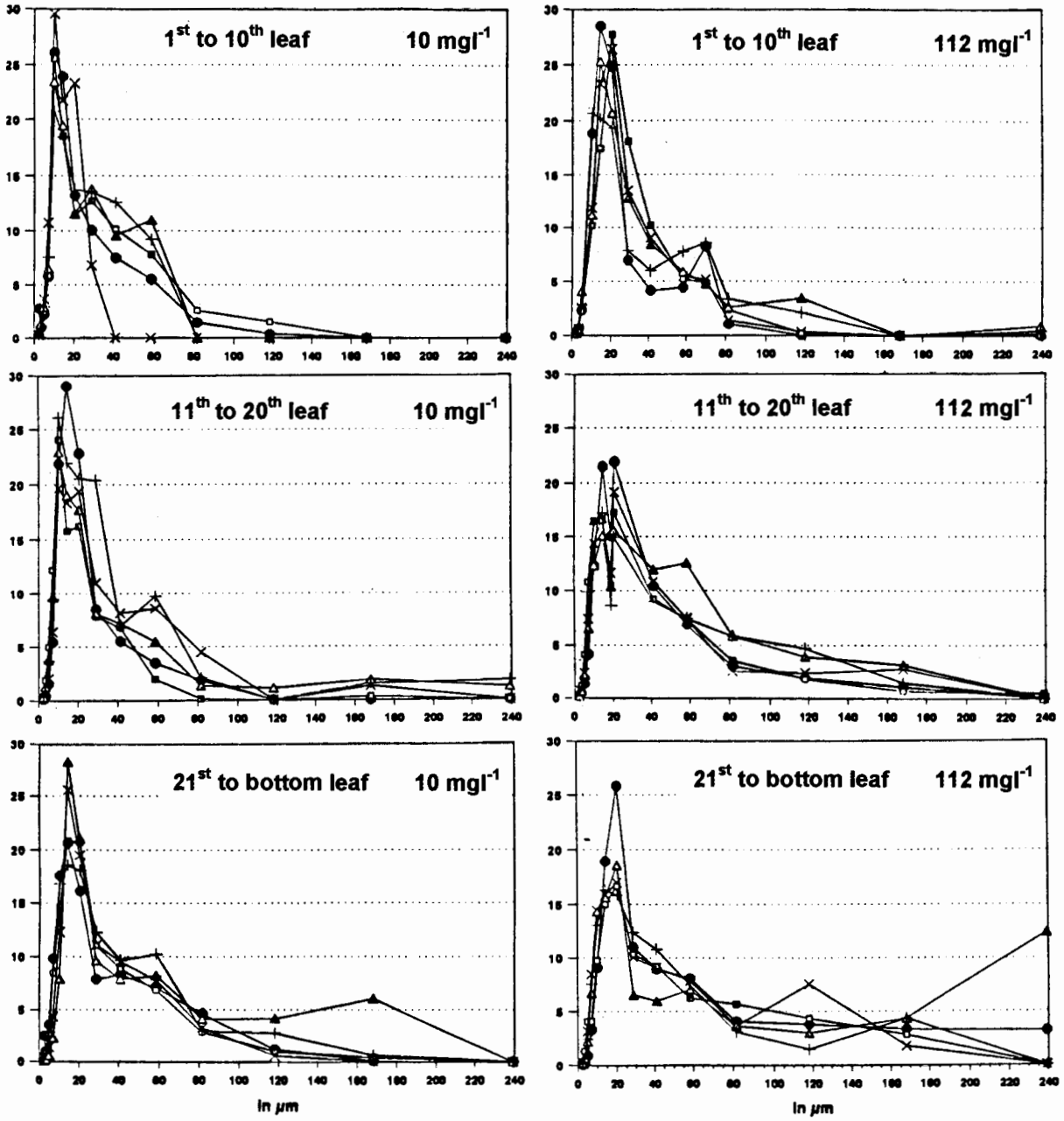

Fig. 4: Size classification of endoskeletons in grapevine leaves induced by powdery mildew infections and isolated by wet ashing. Grapevines were grown in the greenhouse on recirculation nutrient solutions containing 10 mg·l^{-1} SiO_2 (diagrams on the left) or 112 mg·l^{-1} SiO_2 (on the right). Leaves were harvested 6 weeks after infection and separated into 3 groups (1st to 10th leaf, 11th to 20th, and 21st to bottom leaf, counted from the top of the shoot). Abscissae: size classes; ordinates: relative amounts.

Table 2

Correlation of skeleton size with other parameters. Only particles >2 µm could be analyzed; significancies according to the Tukey-test (o: p> 0.05; +: p<0.05; ++: p<0.01; +++: p<0.001; df degrees of freedom)

Cause of variance	df	Size class between ... (µm)									
		2.0	4.0	5.7	8.2	11.7	16.6	23.7	68.4	138	197	232
Cultivar	4	o	+	+	o	o	+	o	o	o	o	
SiO_2 supply	2	+	o	+	+	o	+++	o	+	++	+	
Leaf age	2	+	o	o	o	+	o	o	+++	++	+++	

and BLAICH 1990; KORTEKAMP, in prep.) and it may well be that endoskeletons - probably being induced by the same elecitor mechanims - have a corresponding size.

From a practical point of view the final answer to these questions is not very important. Our results indicate that, although a basic amount of silica is essential for a normal powdery mildew resistance, this cannot be further increased by SiO_2 fertilization in the field since the Si contents of most soils are far above the minimal requirements of the grapevine. The efficiency of silica sprays against fungal infections, if any, (REYNOLDS *et al.* 1996) ought thus to be due to other mechanisms.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft. The experiments were carried out at the Institute for Grapevine Breeding of the Federal Centre for Breeding Research on Cultivated Plants. We are very much indebted for advices and technical assistance, particularly to CLAUDIA WELSCH and R. WIND and we owe special thanks to M. KLOTZ and K. HABEN (Pamas, Rutesheim) for allowing us to use the JIM system and for their kind support.

References

ADATIA, M. H.; BESFORD, R. T.; 1986: The effects of silicon on cucumber plants grown in recirculating nutrient solution. Ann. Bot. **58**, 343-351.

BLAICH, R.; GRUNDHÖFER, H.; 1997: Uptake of silica from soil and recirculating nutrient solutions by grapevines. Vitis **36**, 161-166.

- -; WIND, R.; 1989: Inducible silica incrusts in cell wall of *Vitis* leaves. Vitis **28**, 73-80.

BOWEN, P.; MENZIES, J.; EHRET, D.; 1992: Soluble silicon sprays inibit powdery mildew development on grape leaves. J. Amer. Soc. Hort. Sci. **117**, 906-912.

DANAYADAN, P.; KAUFMANN, P. B.; FRANKLIN, C.J .; 1983: Detection of silica in plants. Amer. J. Bot. **70**, 1079-1084.

EIBACH, R.; 1994: Investigations about the genetic resources of grapes with regard to resistance characteristics to powdery mildew (*Oidium tuckeri*). Vitis **33**, 143-150.

ENGEL, W.; 1953: Untersuchungen über die Kieselsäureverbindungen im Roggenhalm. Planta **41**, 5385-5390.

HEINTZ, C.; BLAICH, R.; 1990: Ultrastructural and histochemical studies on interactions between *Vitis vinifera* L. and *Uncinula necator* (SCHW.) BURR. New Phytol. **115**, 107-117.

JONES, L. H. P.; HANDRECK, K. A.; 1965: Studies of silica in the oat plant III. Uptake of silica from soils by the plant. Plant and Soil **23**, 79-96.

LAFOS, K.; 1995: Die Aufnahme und Verteilung von Silicium in Reben (*Vitis* spp.). Diss. Geisenheimer Berichte No. 22. Veröff. Forschungsanstalt Geisenheim.

LEUSCH, H. J.; BUCHENAUER, H.; 1988: Si-Gehalte und Si-Lokalisation im Weizenblatt und deren Bedeutung für die Abwehr einer Mehltauinfektion. Kali-Briefe (Büntehof) **19**, 13-24.

MUNK, H.; 1981: Zur Bedeutung silikatischer Stoffe bei der Düngung landwirtschaftlicher Kulturpflanzen. Landwirtsch. Forsch. **35**, 264-277.

REYNOLDS, A. G.; VETO, L. J.; SHOLBERG, P. L., WARDLE, D. A.; HAAG, P.; 1996: Use of potassium silicate for the control of powdery mildew (*Uncinula necator* [SCHWEIN] BURRILL) in *Vitis vinifera* L. cultivar Bacchus. Amer. J. Enol. Viticult. **47**, 421-428.

SCHALLER, K.; LÖHNERTZ, O.; MUSKAT, S.; 1990: Anreicherung von Silizium in den Blättern von Müller-Thurgau und Silvaner im Verlaufe zweier Vegetationsperioden in Abhängigkeit von der Silikatdüngung. Wein-Wiss. **45**, 128-134.

STEIN, U.; HEINTZ, C.; BLAICH, R.; 1985: Die *in vitro*-Prüfung von Rebsorten auf Oidium- und Plasmopara-Resistenz. Z. Pflanzenkrankh. Pflanzensch. **94**, 355-369.

Received October 17, 1997