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Inducible silica incrusts in cell walls of *Vitis* leaves

by

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Induzierbare Silikatinkrustationen in Zellwänden von *Vitis*-Blättern

Zusammenfassung: In Wänden mehltaubefallener oder anderweitig mechanisch geschädigter Epidermiszellen von *Vitis*-Sorten wurden Silikatablagerungen gefunden — sowohl bei geschädigten wie bei benachbarten Zellen. Ganze Zellwände oder Teile davon waren derart verglast, daß nach Behandlung mit konz. H_2SO_4 + H_2O_2 bei 400 °C und Waschen mit konz. HCl reine, mechanisch widerstandsfähige Kieselskelette von Zellwänden zurückblieben. Sie bestanden aus Gruppen von 1—20 Zellen der oberen Epidermis mit anhängenden Teilen der entsprechenden Palisadenzellen beziehungsweise der unteren Epidermis (oft mit Stomazellen) und anhängendem Schwammparenchym. Auch sehr feine Strukturen der Zellwände (kleine Runzeln der oberen Epidermis, Abwehrpapillen, Reste von Oidiumhaustorien) waren verkieselt. Es scheint, daß pilzresistente Rebsorten größere Verkieselungen bilden als anfällige.

Key words: silicon, mineral, oidium, fungus, leaf, epidermis, parenchyma, cell wall, resistance.

Introduction

Relations between fungal resistance and silica content of Gramineae are well documented (LEUSCH and BUCHENAUER 1988). The association of silicate deposits with the penetration process of mildew was shown by KUNOH and ISHIZAKI (1976), ZEYEN *et al.* (1983), EBRAHIM-NESBAT *et al.* (1986), and CARVER *et al.* (1987).

Less information is available for other plant groups: Silicate fertilization reduced fungal diseases in *Cucumis sativus* (ADATIA and BESFORD 1986). Cytological observation of penetration sites of *Uromyces vignae* indicate a role of Si in the defense reaction of *Vigna* and *Phaseolus* (HEATH and STUMPF 1986). The methods applied in these studies gave, however, no indications on the amounts of silica involved.

In this paper evidence will be presented that defence reactions of *Vitis* involve the deposition of silica in high concentrations.

Material and Methods

Both mildew (*Uncinula necator*) infected and apparently healthy leaves from the *Vitis* cultivars Optima, Riesling (susceptible to powdery mildew), *V. riparia* × *V. rupestris*, Vidal, Orion, Phoenix, and Maréchal Foch (resistant) were prepared for examination in the scanning electron microscope (SEM) by one of the following methods:

(1) Freeze drying at −30 °C (this simple method led to shrinking of cells).
(2) Freeze drying at −60 °C after prefixation in 3 % glutaraldehyde to get high quality preparations.
(3) Heating to 600 °C on an aluminum stub (SEM object carrier) to ash the leaf while retaining the original position of the residues.

(4) Treatment with conc. H_2SO_4 and H_2O_2 at 400 °C, then repeated washing of the residues with water and conc. HCl.

Scanning electron microscopy (SEM) and energy disperse X-ray analysis (EDX) were carried out with a Zeiss DSM 950 microscope equipped with a Kevex 5100 X-ray energy spectrometer. The specimens analysed were sputtered with gold in an Edwards Sputter Coater or left untreated for EDX analysis.

Results and discussion

Studies of the penetration process of *Uncinula necator* (HEINTZ 1986 and 1988; HEINTZ and BLAICH in prep.) revealed an accumulation of fluorescing phenolic substances and an induction of peroxidase activity in the surrounding cell walls. According to interference contrast images of uncoloured 1 µm cuttings (increase of the optical density of fluorescing regions, Fig. 1) this lignification-like process seemed to be accompanied by the deposition of other substances in the cell walls which became less penetrable to organic solvents. EDX analysis was used in an attempt to identify accumulations of substances.

At lower magnifications all elements analysed were equally distributed in healthy leaves, but on infected leaves local accumulations of Si could be observed (Fig. 2 a, b).

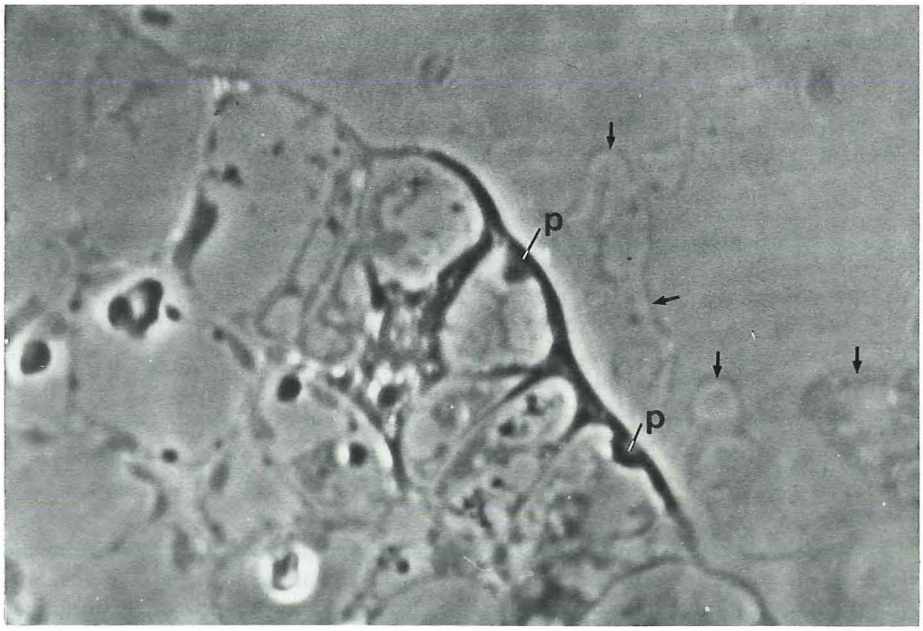

Fig. 1: Unstained interference contrast image of a transversal cutting (semithin 1 µm in acryl resin) through a region around a mildew-infected epidermal cell; without optical contrast no structures are visible. The arrows indicate fungal hyphae and infection structures. P = papilla.

Ungefärbtes Interferenzkontrastbild eines Semidünnschnittes (1 µm; Kunstharzeinbettung) durch eine Mehltauinfektion der Epidermis; ohne optischen Kontrast sind keine Strukturen sichtbar. Pfeile weisen auf Pilzhyphen und Infektionsstrukturen hin. P = Papille.

Higher magnifications revealed that they were always associated with such epidermal cells where mildew appressoria could be observed (Fig. 3 a—d). A simple freeze drying preparation allowing the collapse of cells indicated that such cells were less susceptible to shrinking (Fig. 3 a) — a fact indicating that silica incrusts might not be merely superficial but extend into deeper layers of the leaf. This could be corroborated by the examination of freeze fractures through infected regions which revealed that Si accumulations were present also in deeper cell walls of the epidermis and even in parts of the palisade cells (Fig. 3 c, d).

After acid treatment of infected leaves a white powder remained which was composed of nest-like cell skeletons typically composed of parts, or complete walls of lower or upper epidermis cells and frequently parts of the adjacent cells of the palisade or spongy parenchyma (Fig. 4a—e). Fig. 1 should help to understand the composition of these structures.

In preparations from mildew-infected leaves either traces of mildew penetration or penetration attempts could always be found near the center of these structures if their position on the SEM stub allowed the examination of their cuticular surface. In the

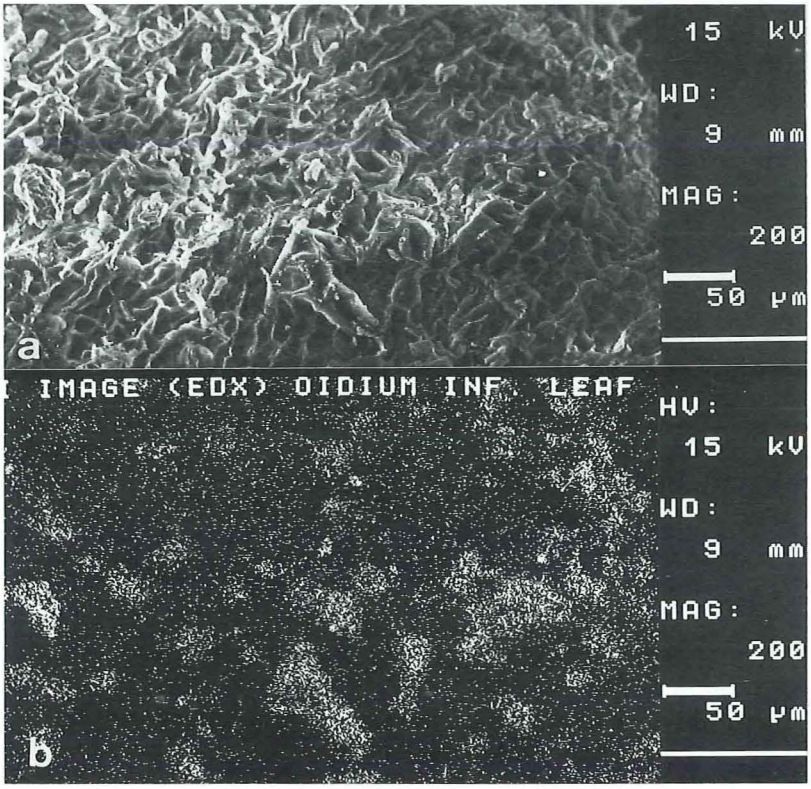

Fig. 2: a) Scanning electron microscope (SEM) image of the residues (ash) of an infected *Vitis* leaf after heating (on the REM carrier stub) to 600 °C. — b) Si-EDX image corresponding to (a).

a) Rastermikroskop- (REM-)Bild von Aschenresten eines infizierten Rebblattes nach Erhitzung auf 600 °C auf einem Aluminiumobjektträger. — b) Röntgenanalysebild (EDX) für Silizium von (a).

first case (Fig. 4 d) the diameter of the perforations was around 0.3 µm (which corresponds to the penetration pegs of *Uncinula necator*). Within the cell the holes seemed to continue into structures of different forms; sometimes even complete haustoria seemed to be silicified (Fig. 4 g). Unsuccessful penetration attempts showed up as shallow prints on the cuticle (Fig. 4 a, b) and as typical papillae on the inner side of the peripheral cell wall (Fig. 4 f). Sometimes perforations or prints and papillae could be seen to correspond.

Although quantitative analyses of the total silica were not made, it was evident that the total amount of unsoluble silica obtained after ashing depended on the age of the leaf. Silica structures remained after ashing of healthy leaves (without evident signs of fungal infections) of every age but those from young leaves (3rd to 4th well-developed leaves counted from the top of the cane) showed only amorphous structures. Silica from older, uninfected leaves exhibited the typical form of the cell wall skeletons obtained from mildew infected leaves. In these cases, however, the structures seemed

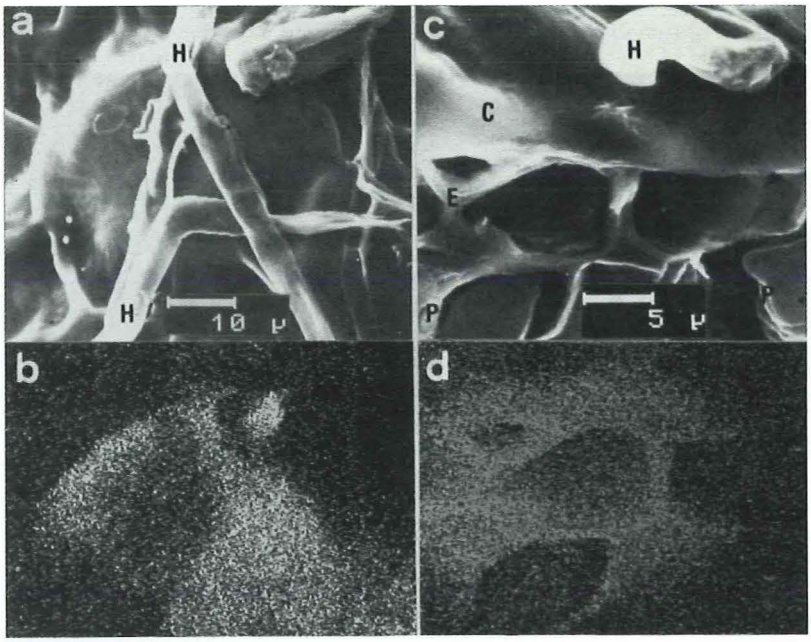

Fig. 3: SEM and corresponding Si-EDX images of *Vitis* leaves infected with *Uncinula necator*; the SEM images are poor because (for the sake of EDX analysis) the specimens could not be gold sputtered. C = epidermis with cuticle, E = epidermal cell, H = hypha, P = palisade cell. — a) Surface of freeze dried leaf; the central region seems to be less shrunk. — b) EDX image of Si corresponding to (a). — c) Freeze fracture through an infected region similar to that shown in (a); upper half: surface of epidermis, lower half: open epidermal cells and part of palisade parenchyma viewed from the side. — d) EDX image for Si corresponding to (c).

REM- und zugehörige Si-EDX-Bilder von *Uncinula-necator*-infizierten *Vitis*-Blättern; die REM-Bilder sind schlecht, da wegen der EDX-Analyse keine Goldbedampfung vorgenommen werden konnte. C = Epidermis mit Cuticula, E = Epidermiszelle, H = Hyphe, P = Palisadenzelle. — a) Oberfläche eines gefriergetrockneten Blattes; die Zone in der Mitte erscheint weniger eingefallen. — b) Si-EDX-Bild zu (a). — c) Gefrierbruch durch eine infizierte Stelle ähnlich (a); obere Hälfte: Epidermisoberfläche, untere Hälfte: aufgebrochene Epidermiszellen und Teile des Palisadenparenchyms von der Seite gesehen. — d) Si-EDX-Bild von (c).

to witness a general 'defense history' of the leaf because, although it was rarely possible to detect typical traces of *Uncinula* (perforations, papillae, haustoria), sometimes larger lesions were observed which we tentatively interpreted as penetration attempts by other fungi or stabs by insects or mites. In some cases one or more cells in the center were destroyed (Fig. 4 i). On the other hand the cuticular surface of some cell skeletons showed no signs of damage, so it might be supposed that in old leaves spontaneous incrustations occur.

Comparison of mildew-infected leaves of cultivars with strongly different susceptibility showed that resistant leaves formed larger and thicker cell nests. Vitrified papillae or haustoria were observed most frequently in susceptible cultivars where often only the peripheral parts of the epidermal cells were involved so that it was possible to look into the cells through their non-vitrified lower side (Fig. 4 f). In resistant cultivars the view into epidermis cells was mostly blocked by caps of palisade cells; silicified infection structures could then be seen only if the structures were accidently broken (Fig. 4 g). Methods for a classification of the silica structures by image analysis are being developed but at the moment we are still not able to support our observations by quantitative measurements.

Although nothing is known on the metabolism of silica in *Vitis*, we suppose that its transport into the leaves should be a passive process leading to accumulations in older leaves. It still remains unclear how the plant cells manage to keep it in solution. ENGEL (1953) reported that in rye 60 % of the total amount of silica is kept soluble as a galactose complex. Although freeze-dried preparations of *Vitis* showed that the vacuoles of epidermal cells contain high concentrations of soluble substances (normally removed by histological standard procedures), EDX analysis of such cells did not indicate Si accumulations. It must be kept in mind, however, that EDX images are obtained only from relatively high concentrations of elements at the surface of the specimen. If one assumes that the vacuoles of epidermal cells in old leaves contain an oversaturated silica solution this would explain that slight irritations which would not necessarily leave visible traces could be sufficient to start a general precipitation-like silica incrustation which seems to be completed within a day.

Concluding remarks

In *Vitis*, penetration by *Uncinula necator* induces the formation of papillae and fluorescing halos indicating the deposit of phenolics associated with peroxidase activity in the surrounding cell walls, callose deposits, and hypersensitivity reactions (HEINTZ 1988; BLAICH and HEINTZ in prep.). Unsuccessful attacks are sufficient to induce these reactions. We feel that they play an important role within an unspecific defense mechanism which is more active in mildew resistant *Vitis* cultivars and less active in susceptible ones. The presence of such rapid defense mechanisms is now generally accepted for many higher plants (references in JAHNEN and HAHLBROCK 1988). Although it has been shown several times that silica may also be involved in resistance, our results for *Vitis* seem to be the first to demonstrate that the amounts of it may be large enough to form mechanical barriers strong enough to protect a number of cells against further attacks by downy mildew.

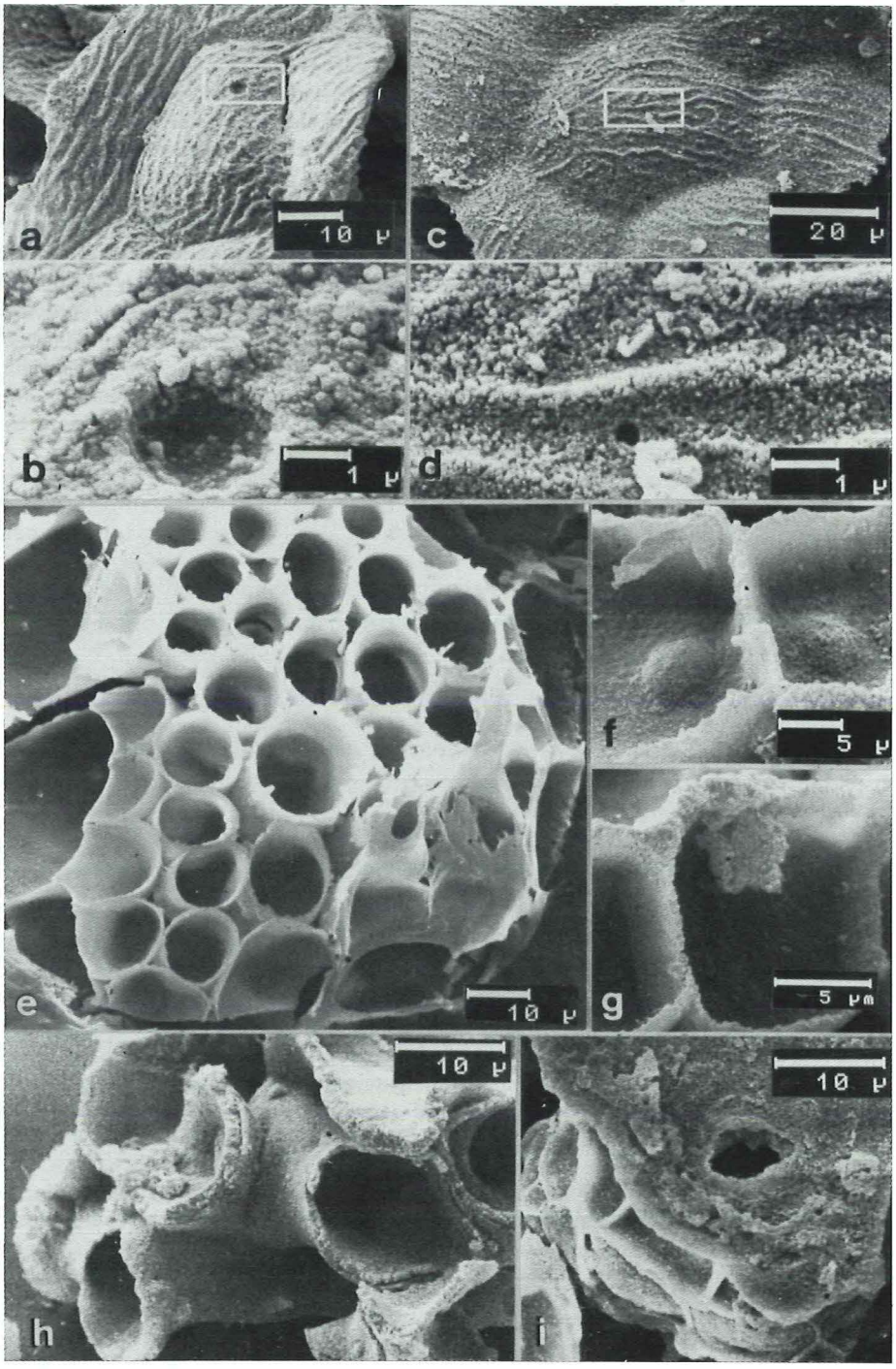
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Fig. 4: Silica structures remaining after hot, oxidative acid treatment of *Vitis* leaves, a-h) from mildew infected leaves, i) from healthy leaf. By EDX analysis only Si accumulations could be found (elements below Mg e.g. O, C, or N could not be analyzed). — a) Part of upper epidermis composed of (parts of) 7 cells with wrinkles and trace of penetration attempt (in the white frame). — b) Magnification of white frame in (a). — c) Structure similar to (a) with complete penetration. — d) Magnification of white frame in (c). — e) Structures according to (a) and (c) viewed from the opposite side (from below); silicified caps of approximately 30 palisade cells cover around 12 epidermal cells which are only visible at the margin. — f) Outer cell wall of two adjacent epidermal cells with two papillae viewed from below (inside, the bottom of the cells is not silicified). — g) Completely silicified epidermal cell with haustorium (?), side view; the cell is broken open during preparation. — h) Silicified cell group from the spongy parenchyma. — i) Silification of upper epidermis around a hole (caused by insect?).

Silikatstrukturen, die nach heißer, oxidativer Säureveraschung von Rebblättern zurückbleiben, a-h) von oidiuminfizierten Blättern, i) von einem gesunden Blatt. EDX-Analyse zeigte nur Anhäufung von Si (leichtere Elemente als Mg, z.B. O, C oder N, konnten allerdings unter unseren Bedingungen nicht nachgewiesen werden). — a) Teil der oberen Epidermis bestehend aus (Teilen von) 7 Zellen mit Runzeln und Spuren eines Eindringversuches (im weißen Rahmen). — b) Ausschnittvergrößerung des weißen Rahmens. — c) Struktur ähnlich (a), mit vollständiger Penetration. — d) Ausschnittvergößerung von (c). — e) Strukturen ähnlich (a) oder (c) von der Unterseite aus betrachtet; verkieselte Kappen von etwa 30 Palisadenzellen verdecken etwa 12 Epidermiszellen, die nur am Rand sichtbar sind. — f) Periphere Zellwand zweier benachbarter Epidermiszellen, jede mit Papille (von unten/innen betrachtet, Zellboden hier nicht verkieselt). — g) Vollständig verkieselte Epidermiszelle mit Haustorium (?), Seitenansicht; die Zelle ist während der Präparation aufgebrochen. — h) Verkieselte Zellgruppe aus dem Schwammparenchym. — i) Verkieselung der oberen Epidermis um ein Loch (durch Insekt verursacht?).

Summary

Epidermal cells of *Vitis* cvs attacked by powdery mildew or damaged mechanically exhibited silica deposits in the walls of the attacked and surrounding cells. Entire or parts of cell walls were vitrified to such an extent that pure, mechanically resistant silica skeletons remained after a treatment with conc. H_2SO_4 + H_2O_2 at 400 °C and washing with conc. HCl. They consisted of groups of 1—20 cells of the upper epidermis with adhering parts of the corresponding palisade cells or of the lower epidermis (including stomatal cells) with adhering spongy parenchyma. Not only cell walls but also wrinkles of the upper epidermis, defense papillae and fungal haustoria were silicified. Silica accumulations were greater in resistant than in susceptible cultivars.

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