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**Research Note**

# The role of callose deposits during infection of two downy mildew-tolerant and two -susceptible *Vitis* cultivars

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Summary: Semithin sections of leaves from four cultivars were analysed for callose deposition after infection with *Plasmopara viticola*. Two of these cultivars are tolerant to this pathogen in the field (Orion, Phoenix), while the other two are susceptible (Kerner, Riesling).

Callose was not detectable during the first 5 days after experimental inoculation. During late stages of the infection cycle however, callose deposits could be found in tolerant as well as in susceptible grapes. During the fungal infection of tolerant plants hyphal development stops 3 to 4 days post infection indicating that mechanisms other than callose deposition are the main factors of tolerance.

Key words: Plasmopara viticola, downy mildew, callose, grapevine cvs Orion, Phoenix, Riesling, Kerner, disease tolerance.

**Introduction:** *Plasmopara viticola* is one of the major grapevine pathogens causing necrotic lesions of leaf tissues, flowers and berries. However, there are naturally tolerant *Vitis* species and newly-bred cultivars tolerant to the fungus. Several possible defense strategies of the plants are currently discussed. One of these involves a crucial role for the deposition of callose.

Callose is a plant glucan consisting of unbranched ß-1,3-D-glucose. This polymer constitutes cylinders surrounding the connective strands in sieve areas of the phloem (CURRIER 1957) and can be located in leaf and stem hairs of *Vitis*, in root hairs, epidermal cells as well as in parenchymatic tissues after parasitic attack (MANGIN 1892).

In many plant-fungus interactions deposition of callose after infection has been reported. Particularly during the defense reaction of tolerant hosts to fungal pathogens a crucial role has been attributed to the deposition of callose (AIST 1976). During interaction of *Plasmodiophora brassicae* with cabbage and *Bremia lactucae* with lettuce callose and cellulose are synthesized after induction by fungal penetration and layered onto the inside of the cell wall, outside of the cytoplasm (AIST 1976). Reduced or completely missing callose deposition is frequently observed in susceptible hosts. In potato cultivars susceptible to *Phytophthora infestans*, wall appositions are poorly developed, whereas fungal haustoria in tolerant hosts are entirely surrounded by callose (HOHL and STOSSEL 1976). In tomato enhanced callose deposition and stronger cell wall reinforcement follow infection of tolerant cultivars with *Cladosporium fulvum*, as compared to susceptible ones (LAZAROVITS and HIGGINS 1976 a, b).

Even after viral attack deposition of callose and cell wall thickening has been observed in and around viral lesions in bean (STOBBS and MANOCHA 1977) and tobacco (FAVALI *et al.* 1978). Early deposition of callose may therefore represent an important component of defense mechanisms against viruses prohibiting virus movement within the tissue.

As callose synthesis may be a generally important factor of plant defense, HEINTZ and BLAICH (1990) studied such deposits in grapevine following leaf infection with powdery mildew (*Uncinula necator*). Callose appositions were frequently observed in later stages of infection around the "neck" and sometimes also around the "body" of haustoria and localized to papillae.

Hence we investigated, whether callose plays a role also in the defense reactions of grapevine to downy mildew. Two *P. viticola*-tolerant and two -susceptible cultivars were studied.

**Materials and methods:** The 4th to 6th healthy looking leaves of the *Vitis vinifera* varieties Kerner, Riesling (both susceptible), as well as the fungus-tolerant varieties Orion (Optima x Seyve Villard 12-375) and Phoenix (Bacchus x Seyve Villard 12-375) were chosen from greenhouse-grown plants. The leaves were surface-sterilized with 1 % NaOCl and 0.05 % Tween 80 for 20 min, followed by three rinses with steril water. Subsequently they were held in petri dishes on steril water at 20-22 °C for several days.

Sporangia of *P. viticola* were collected during autumn 1995 and early summer 1996 from infected grapevine plants growing in the vineyards of the Institute for Grapevine Breeding Geilweilerhof. The material was dried under vacuum at room temperature, frozen and stored at -25°C. Clean sporangia of *P. viticola* were produced by allowing infection cycles to proceed after infection of axenic *in vitro* grapevine plants, cv. Vidal blanc.

Leaves were infected by application of 40 µl drops containing sporangia at a concentration of $5 \cdot 10^4 \cdot ml^{-1}$. After 1-7 d the leaves were fragmented into 1 $cm^2$ pieces, fixed in cold ethanol (94 %, -90 °C) and left at -25 °C until they had turned white.

Fixed leaf fragments were embedded in methacrylat (Histocryl, The London Resin Co. Ltd., UK) mixed with isopropanol in different ratios: isopropanol:methacrylat 3:1, 1:1, 1:3 and methacrylat only. The embedded samples were hardened in an desiccator under high $CO_2$ atmosphere. A microtom (Microm, Heidelberg, Germany) was employed to obtain 1 µm slices. These sections were transferred into a water droplet on microscope slides, dried, smoothed at 80 °C and stained in two steps. The first step: toluidine blue (0.15 %, Merck, Darmstadt, Germany) in 5 % $NaHCO_3$, pH 9.0, to detect hyphae and haustoria. The second step: 1 min staining for callose with aniline blue (Merck; 0.15 % in 5 % $NaHCO_3$). The stained semithin sections were studied by epifluorescence microscopy. Toluidine blue- and aniline blue-stained sections exhibit a dark green colour under UV light (365 nm) while callose, when present, results in a light green fluorescence.

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**Results and Discussion:** Microscopic investigation of the thin sections showed, that *P. viticola* is able to infect all 4 cultivars studied. This was confirmed by scanning electron microscopy (data to be presented elsewhere). Infected leaves of all varieties - susceptible as well as tolerant ones - did not exhibit any fluorescence in the early stages of the compatible or incompatible interaction (the same holds for uninfected controls). Callose could not be detected, both hyphae and haustoria were free from and not surrounded by callose. Some dense material visible around haustorial necks does not contain callose (Figure).

This finding indicates that callose deposition does not occur early or in the middle (1 to 5 days post infection, dpi) of the interaction, neither in the tolerant cultivars Orion and Phoenix, nor in the susceptible Kerner and Riesling.

Stained callose, however, is visible rather late in the infection process (6th to 7th dpi) both in tolerant and susceptible plants. At this point, the asexual development of the fungus is almost completed, and sporangiophores start to grow out from the substomatal cavity under our experimental conditions. Propagation of *P. viticola in planta* is thus not inhibited by callose.

Both tolerant and susceptible grapevine cultivars are principally infected by *P. viticola*. In the tolerant varieties, progression of fungus growth stops at day 3-4 after infection. Infected areas turn necrotic and brownish, but hyphae between mesophyll cells are still clearly visible. This finding means that mechanisms different from callose deposition are involved in the defense of *Vitis* against *P. viticola*.

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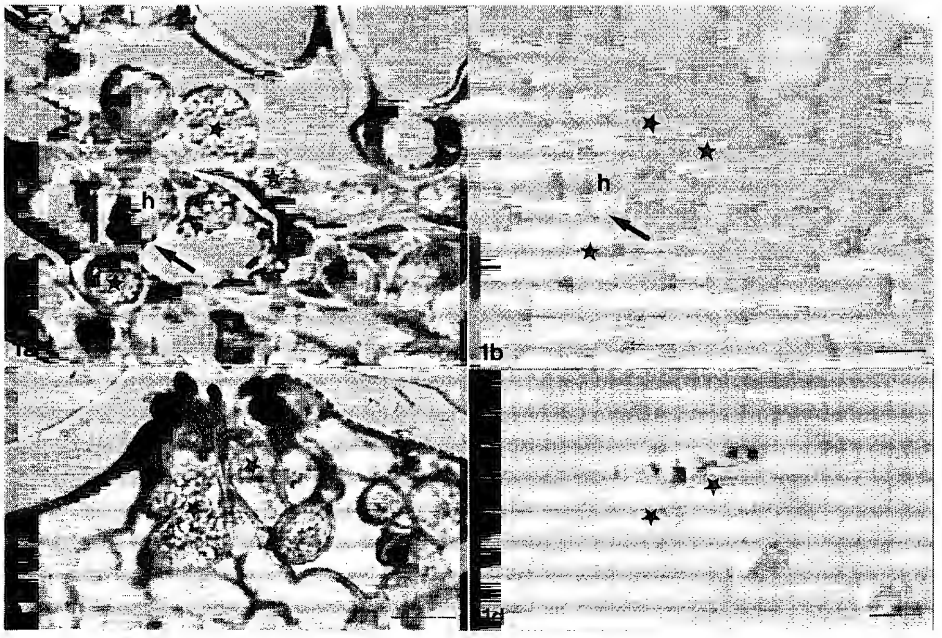


Figure: **a**. Semithin transverse section across a leaf of the cultivar Orion 4 dpi (days post infection) stained with toluidine blue. Hyphae (asterisks) in the intercellular space of mesophyll cells are clearly visible, in addition to a haustorium (h), although this cultivar shows field tolerance to the fungus. The haustorial "neck" is surrounded by some unidentified dense material (arrow). **b**. Same section stained with aniline blue for callose. No fluorescence is detectable around hyphae and haustorium. **c**. Semithin transverse section across a leaf of cultivar Kerner 7 dpi. Fungal hyphae (asterisks) in the substomatal cavity starting to form sporangiophores growing out from the stoma are noticeable. **d**. Same section stained for callose with aniline blue. Fluorescence typical for callose deposition is visible at the basis of sporangiophores. – Bars = 10 µm.

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