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Rootstock susceptibility to *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp.

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Summary. Twenty healthy cuttings each were inoculated artificially with *Phaeomoniella chlamydospora, Phaeoacremonium inflatipes* and *Phaeoacremonium aleophilum*. After the formation of callus, they were planted in pots. Approximately one year after inoculation, disease occurrence was recorded as the length of brown vascular streaking from the base of plant toward the shoot tip. Discolored areas were cultured on potato dextrose agar amended with tetracycline hydrochloride (PDA-tet) and pathogens were re-isolated. Inoculation with *Pa. chlamydospora* showed that rootstocks 3309, 420A, 110R, 5C, Schwarzmann, St. George, and Salt Creek were least susceptible, while 99R, 39-16, Freedom, Riparia Gloire, 140Ru, 16-16 and 1103 were most susceptible. When inoculated with *Pm. inflatipes*, 16-16, 3309, AXR1, Salt Creek, 110R, 5C, Freedom and 140Ru were least susceptible, while 420A, St. George, 161-49, and Harmony were most susceptible. Inoculation with *Pm. aleophilum* showed that 1103, 420A, Harmony, and Salt Creek were least susceptible, while 110R, SO4, 39-16 and 161-49 were most susceptible. The susceptibility of the rootstocks and the occurrence of vine decline in the field in California did not appear to be well correlated because 3309, 101-14, 5C, and 110R were always most susceptible. But these rootstocks are the most widely planted, and hence the natural occurrence of the disease is probably skewed towards these rootstocks. However, since no resistance was detected in our study, even though there was a wide range of susceptibility, the degree of rootstock susceptibility may not be such an important factor in disease expression under natural conditions.

Key words: Petri disease, grapevine, rootstock.

Introduction

Esca (black measles) and Petri disesase are some of the most destructive diseases of grapevine. Esca is suspected of being caused by at least three and possibly more fungi in California: *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum, Pm. inflatipes* and *Fomitiporia punctata*, acting in combination or in succession. They attack the wood of the plant, which eventually dies. External symptoms can appear in severe or mild forms (Viala, 1926; Chiarappa, 1959; Dubos and Larignon, 1988, Gubler and Schnathorst, 1992, Sheck *et al.*, 1998). With mild esca, there is foliage alteration; infected grape clusters appear normal, but berries may not fill properly and generally do not reach maturity. The severe form of esca is also called apoplexy, characterized by the sudden wilting and death of bearing vines or cordons in midsummer.

Pa. chlamydospora and *Phaeoacremonium* spp. are also associated with a destructive disease of the woody tissue of young vines. This disease, known as Petri disease[(1)] (Crous *et al.*, 1996; Lari-

[(1)] At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that young grapevine decline, 'black goo', Petri vine decline will henceforth be called Petri disease.

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gnon and Dubos, 1997; Scheck *et al*., 1998; Mugnai *et al*., 1999; Crous and Gams, 2000) occurs worldwide. In California, *Pa. chlamydospora* and *Phaeoacremonium* spp. have been isolated from healthy vines and have been recovered from inoculated asymptomatic vines (Scheck *et al*., 1998). In Italy, *Phaeomoniella chlamydospora* has been isolated from healthy rooted cuttings (Bertelli *et al*., 1998). Petri disease has emerged as a significant problem in vineyard establishment. The disease affects grapevines during the first ten years after planting and is not specific to given scion/rootstock combinations. We suspect that these pathogens of young vines have existed for so many years, but that the planting and re-planting of large acreages due to Phylloxera infestations made the problem more acute and increased awarness of it.

Materials and methods

Rootstocks

Fifty cuttings each of Freedom, 99R, 39-16, R. Gloire, 140Ru, 16-16, 1103, Harmony, Dog Ridge, 44-53, SO4, 161-49, Salt Creek, St. George, Schwarzmann, 5C, 110R, 420A, 3309 and AXR were supplied by Sonoma Grapevine Nursery, CA, USA.

Fungal strains

Four isolates each of *Pa. chlamydospora* (strain Pc1, Pc2, Pc36, Pc37), *Pm. inflatipes* (strain Pi36, Pi57, Pi85, Pi88), and *Pm. aleophilum* (strainPa9, Pa10, Pa11, Pa54) from the Davis Plant Pathology Department Collection, were collected from dried frozen culture on potato dextrose agar supplemented with 0.1 g l^{-1} tetracycline hydrochloride (PDA-tet). Two-week-old cultures were harvested in sterile distilled water. The suspension was passed through a double layer of cheesecloth. Final concentration of inoculum was adjusted to 10^7 conidia ml^{-1}.

Inoculations

Ten healthy cuttings of each rootstock were selected. The base of each cutting was pruned to produce a fresh wound. The cuttings were dipped into an inoculum suspension of *Pa. chlamydospora, Pm. inflatipes,* or *Pm. aleophilum* for 15 min. The cuttings were then placed upright in callusing media. After one month, the cuttings were removed from callusing medium and planted in 3.8-l pots in the greenhouse. Pots were arranged in a completely randomized design on 3 greenhouse benches. Approximately one year after inoculation, disease occurrence was recorded as shown by the length of brown vascular streaking from the base of the plant. The roots of each cutting were removed. The bark was peeled off and the length of the dark streak was measured. Discolored areas were cultured on PDA-tet medium and the pathogen reisolated.

Statistical analysis of the data was done using the Waller-Duncan test for means separation.

Results

Rootstocks were removed from the pots and bark was removed or cut longitudinally. Dark streaks in vascular tissue were examined on the peeled surface at the base of the plant and advancing upward (Fig. 1). In cross-section the streaks appeared as individual spots, sparsely distributed or grouped. Rootstocks susceptibility appeared to be correlated with the length of streaks in the wood.

Figures 2, 3 and 4 compare the rootstock response to *Pa. chlamydospora* and *Phaeoacremonium* spp.

When inoculated with *Pa. chlamydospora* (Fig. 2) rootstocks 3309, 420A, 110R, 5C, Schwarzmann, St. George, and Salt Creek were the least susceptible, while 99R 39-16, Freedom, R. Gloire, 140Ru, 16-16 and 1103 were the most susceptible. In response to inoculation with *Pm. inflatipes* 16-16, 3309, AXR1, Salt Creek, 110R, 5C, Freedom and 140Ru were the least susceptible, while 420A, St. George, 161-49, and Harmony were the most susceptible (Fig. 3). Inoculation with *Pm. aleophilum* showed that AXR, 1103, 420A, Harmony, and Salt Creek were the least susceptible, while 110R, SO4, 39-16 and 161-49 were the most susceptible (Fig. 4). Figures followed by the same letter are not statistically different (P=0.01*)* according to Duncan's multiple range test.

Discussion

The susceptibility of the rootstocks and the occurrence of Petri disease in California did not appear to be well correlated in this study, because 3309, 101-14, 5C, and 110R seem to show the disease most prevalently. However, these rootstocks

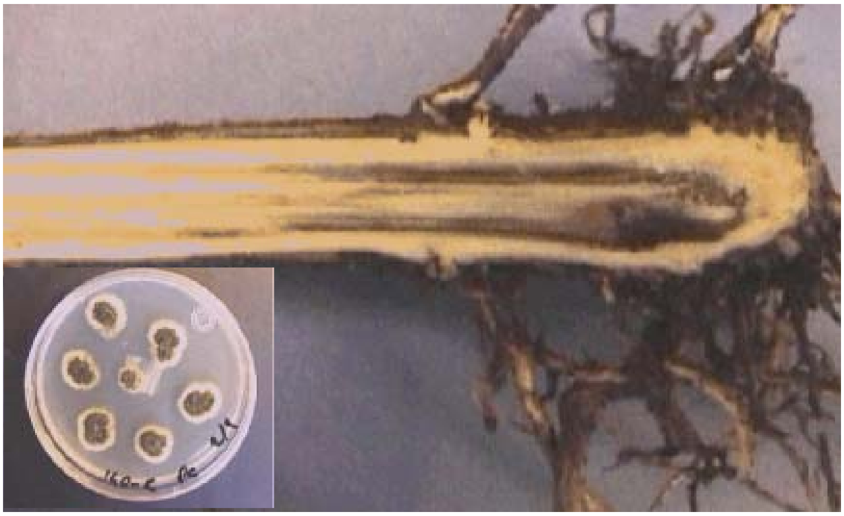

Fig. 1. Black streaking in vascular tissue of an inoculated rootstock, and re-isolation of *Phaeomoniella chlamydospora*.

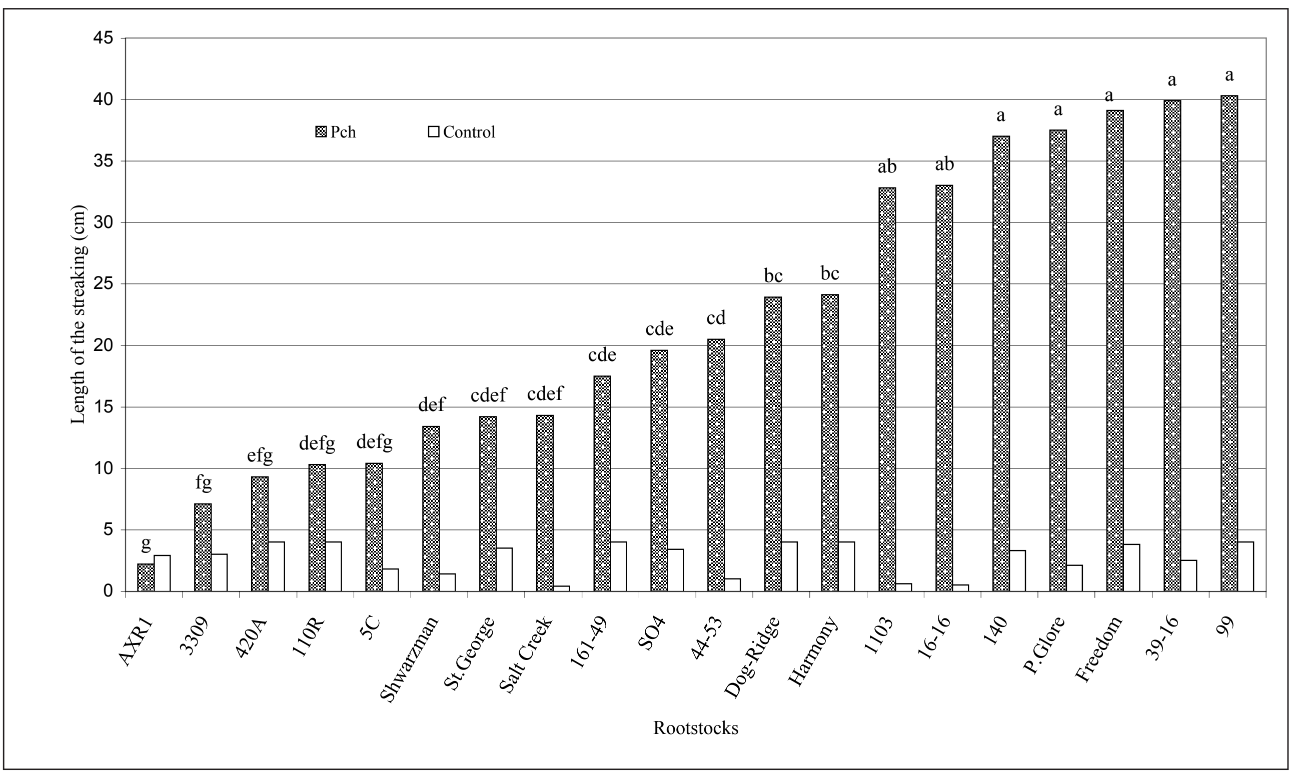

Fig. 2. Length of vascular streaking (cm) in *Phaeomoniella chlamydospora* (Pch) inoculated rootstock.

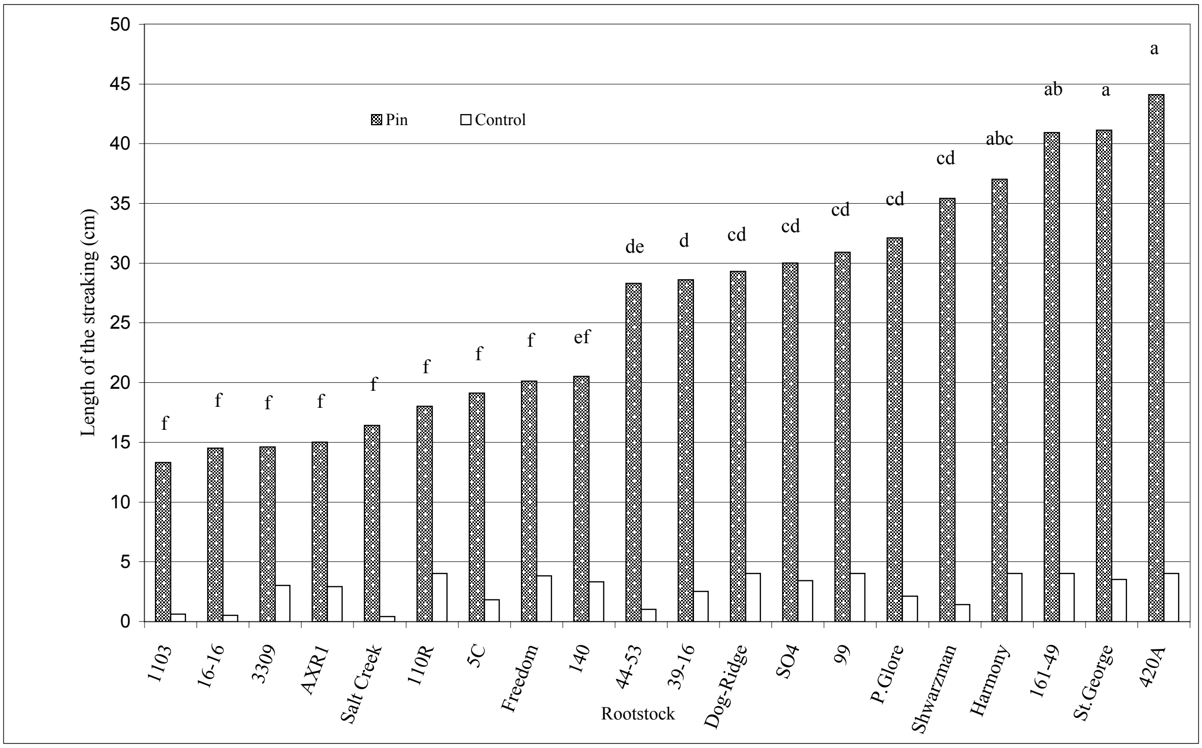

Fig. 3. Length of vascular streaking (cm) in *Phaeoacremonium inflatipes* (Pin) inoculated rootstock.

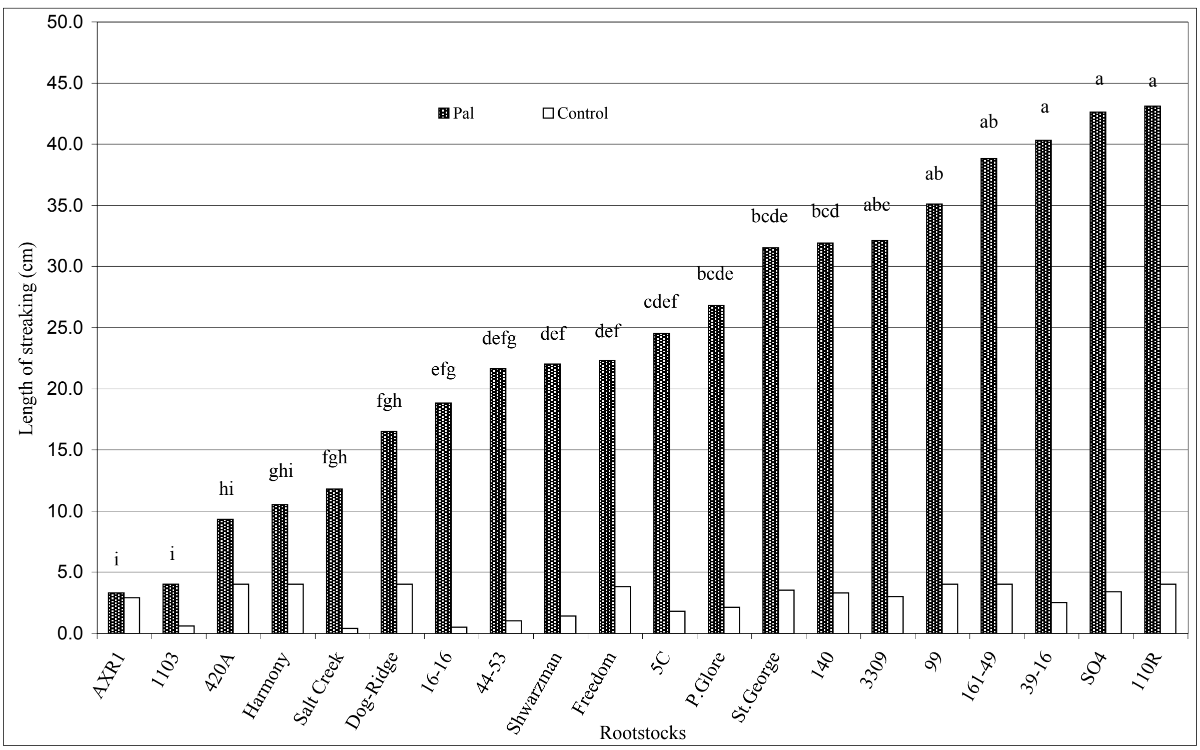

Fig. 4. Length of vascular streaking (cm) in *Phaeoacremonium aleophilum* (Pal) inoculated rootstock.

are the most widely planted in California and the natural occurrence may be skewed towards these rootstocks simply because of the great numbers planted. Anyway, since no resistance was detected in our study, even though there were wide variations in susceptibility, the degree of susceptibility may not be such an important factor in disease expression under natural conditions.

Pm. inflatipes and *Pm. aleophilum* produced a brown necrosis in sectors of the pith and vascular rootstock tissue. *Pa. chlamydospora* caused dark necrosis in young rootstocks similar to the discolored wood in standing vines as described by Larignon and Dubos (1997).

While our study showed that there were differences in susceptibility to *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., those differences may not be important since there was no true resistance to these pathogens. It may be that even a very slight susceptibility is already too much. In addition, we know that these fungi appear to be excellent endophytes and as such well adapted to colonization and growth in grapevine wood. They appear to be highly capable of infecting wood through various tissues, including the pith, vascular tissue, green cortical tissue, and berries (Gubler and Thind, unpublished). Prior to the early 1990s, most vineyards in California were planted on AXR1 or were own-rooted. The fact that AXR1 was always in the least susceptible group in this susceptibility study adds some basic understanding as to why there was no serious problem with Petri disease prior to planting on the new rootstock selection currently in use. With this there is good correlation, since prior to the major replanting in California vineyards to combat Phylloxera using rootstocks such 101-14, 5C, 3309 and 110R, there was no problem with decline of young vines. Though the fungi implicated in Petri disease have been documented in California and other vineyards worldwide for nearly a century, they caused no major problem until many of the rootstocks now used were introduced.

Research has shown that esca or black measles and Petri disease cause black or brown streaking in grapevine trunks (Chiarappa, 1959; Scheck *et al.*, 1998; Mugnai *et al.*, 1999; Chicau *et al.*, 2000; Crous and Gams, 2000; Khan *et al.*, 2000; Whiting *et al.*, 2000). Work in our lab has demonstrated that *Pm. aleophilum* and *Pm. infilatipes* when inoculated into rooted cuttings of cv. Thompson Seedless, Red Globe, Cabernet Sauvignon, Zinfandel and Chardonnay cause foliar symptoms identical to those observed naturally in vineyards in all California production areas. Recently fruit infection has also been found. While vines with Petri disease may recover and go on to produce a near-normal crop, the prevalence of infected vines may indeed result in an increase in esca symptoms in the future.

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