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Effect of hot water treatments on eradication of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from dormant grapevine wood

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Summary. Hot water treatments were applied to eradicate *Phaeoacremonium inflatipes* and *Phaeomoniella chlamydospora* from dormant grapevine wood. A thirty-minute hot water treatment at 51°C did not eliminate these pathogens from dormant wood cuttings. Cuttings first inoculated with *Pa. chlamydospora* or *Pm. inflatipes* or both fungi, and then subjected to a hot water treatment were either incubated in crispers, or planted for six to eight weeks. Vascular discoloration was scored followed by isolation from the cuttings onto potato dextrose agar amended with 0.1 g l^{-1} tetracycline (PDA-tet). Isolations confirmed the presence of the pathogens in the inoculated, hot-water treated cuttings as well as in the inoculated, untreated control cuttings. This finding, along with earlier research on the direct effect of hot water on the mycelium of these species, leads to the conclusion that hot water treatments are ineffective in eliminating vine decline pathogens from dormant wood.

Key words: Petri disease, hot water, *Vitis vinifera*.

Introduction

Petri grapevine disease[(1)] caused by *Phaeomoniella chlamydospora* (Crous *et al*., 2000) (formerly *Phaeoacremonium chlamydosporum*), *Phaeoacremonium inflatipes,* and *Phaeoacremonium aleophilum* (Crous *et al*., 1996) has been documented in viticultural regions worldwide (Ferreira *et al*., 1994; Mugnai *et al*., 1999; Pascoe, 1999) and was first reported in as a significant disease of young vines in California in the 1990s (Scheck *et al*., 1998a). Since then, the disease has been reported in all major grape-growing regions of California. Grapevines infected with Petri disease show varying degrees of symptoms including stunted growth, shortened internodes, foliar chlorosis and necrosis, and vascular discoloration (Scheck *et al*., 1998b). Reports of this disease seem to coincide with the use of new rootstocks that are resistant to phylloxera (Scheck *et al*., 1998a), and economic losses due to replanting infected vineyards have raised alarm. The development of possible control strategies for eradication or management of this disease remains very important.

Phaeoacremonium spp. and *Phaeomoniella chlamydospora* are very complex fungi. Epidemiological studies have been difficult since they appear to be able to survive endophytically in symp-

[(1)] At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that the disease will henceforth be called Petri disease.

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tomless vines (Bertelli *et al*., 1998), epiphytically on grapevine tissue (Edwards *et al*., 2001; Gubler, unpublished), and as true vascular pathogens (Khan *et al*., 1999). It has also been reported that the spores of these fungi are aerially disseminated in California, (Eskalen *et al*., 2001) and that *Phaeoacremonium inflatipes* occurs in the soil (Rooney *et al*., 2001). Whiting *et al*. (2001) demonstrated that *Phaeoacremonium* spp. and *Pa. chlamydospora* were able to adapt to a wide range of water potentials, possibly as a survival strategy.

One possible means of control, the use of hot water treatments by nurseries to eliminate pests and pathogens from dormant grapevines, is widely used in many countries and is being increasingly used in California. Hot water treatments at 50°C for thirty minutes have been shown to be effective in eliminating or reducing pests such as nematodes (Lear *et al*., 1959), phylloxera (Messenger, 1948; Stonerod *et al*., 1996), and crown gall (Burr, 1989; Ophel *et al*., 1990) while not harming dormant buds. However the use of hot water to eliminate *Phaeoacremonium* spp. and *Pa. chlamydospora* pathogens from infected vines has shown varying results. Mycelial plugs of *Pm. inflatipes* and *Pa. chlamydospora* subjected to a thirty-minute hot water treatment at 51°C showed a slight reduction or no reduction in growth, suggesting that hot water treatments might not be successful in killing these pathogens (Whiting *et al*., 2001). The objective of this research was to determine if hot water treatments are effective in eliminating *Pa. chlamydospora* and *Pm. inflatipes* from dormant infected grapevines.

Materials and methods

Dormant grapevine cuttings of three *Vitis vinifera* cultivars (Cabernet Sauvignon, Pinot noir and Thompson Seedless) were taken during the growing season before the experiments were conducted and kept dormant in cold storage until used. Grapevines were cut into small, one-to-two-bud pieces and surface sterilized in a 10% sodium hypochlorite solution for five minutes followed by two rinses in deionized water. Cuttings were recut to obtain freshly cut ends. Cuttings were then inoculated with either *Pa. chlamydospora*, *Pm. inflatipes* or both. Inocula of these isolates were previously shown to be effective pathogens. Inocula consisted of spore suspensions (10^6 spores ml^{-1}) containing a mixture of two California isolates from each or both fungal species (isolates pi67, pi71, pc48, pc91; University of California, Davis Plant Pathology collection). Cuttings were vacuum-inoculated using a lab bench vacuum apparatus (40 mm Hg). Rubber tubing was fitted around each cutting and connected to the vacuum apparatus. Ends of cuttings were immersed in spore suspensions for approximately 7–10 s. This allowed ample time for uniform inoculation throughout the cuttings' vascular system. Cuttings were also vacuum infiltrated with sterile water in the same manner to act as a control. Cuttings were then subjected to one of three treatments. Twenty cuttings were used for each treatment. Treatments included an inoculated, non-hot water treatment, an inoculated, immediate hot water treatment and an inoculated, delayed hot water treatment. Cuttings to be treated in hot water were wrapped together in cheesecloth and immersed in a 51°C hot water bath (Neslab GP-300 Constant Hot Water Bath, Union City, CA USA) for 30 min. Following the hot water treatment, cuttings were kept in a 23°C water bath for 30 min and then either placed directly in moist incubators or callused, planted in pots and grown for approximately 6–8 weeks before they were scored for discoloration and isolations were made. Cuttings were given a discoloration score from 0–5, 0 representing no discoloration and 5 severe discoloration. Isolations were taken from all cuttings and plated on PDA-tet to determine whether the fungi were still viable in the treated and untreated cuttings. A repetition for each experiment followed. Statistical analyses consisting of ANOVA and Duncan's Multiple Range Test ($P<0.001$) were performed on each data set.

Results

Moist chamber incubation: *Vitis vinifera* cv. Cabernet Sauvignon vines inoculated with *Pa. chlamydospora*

Results obtained from Cabernet Sauvignon cuttings inoculated with *Pa. chlamydospora* are shown in Fig. 1. *Pa. chlamydospora* was not recovered from any of the uninoculated control cuttings and little discoloration was seen. However, distinct vascular discoloration was seen in the

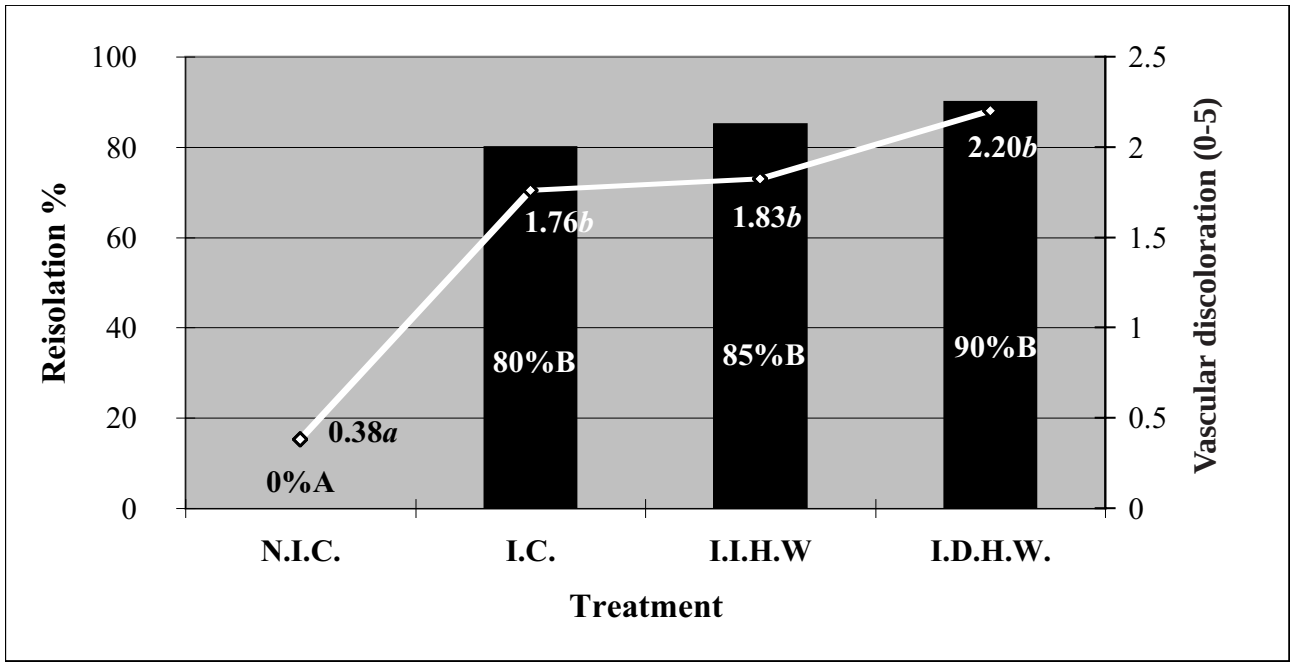

Fig. 1. Hot water treatment effect on colonization by *Phaemoniella chlamydospora* (histogram) and vascular discoloration (line) on artificially inoculated grapevines (*Vitis vinifera* cv. Cabernet Sauvignon). N.I.C., cuttings inoculated with sterile water only and not treated in hot water; I.C., cuttings inoculated with *Pa. chlamydospora* and not treated in hot water; I.I.H.W., cuttings inoculated with *Pa. chlamydospora* and immediately treated in hot water; I.D.H.W., cuttings inoculated with *Pa. chlamydospora* and treated in hot water 24–48 hours later. Means followed by the same letter are not significant by different according to Duncan's Multiple Range Test ($P<0.001$)

inoculated cuttings, both treated and untreated, which differed statistically from the control. No significant differences were seen in the length of the vascular discoloration or in reisolation of the pathogen between treated and untreated cuttings. These results indicate that the hot water treatments at 51°C for 30 min did not eliminate *Pa. chlamydospora* from dormant Cabernet Sauvignon cuttings.

Moist chamber incubation: *Vitis vinifera* cv. Pinot noir vines inoculated with *Pm. inflatipes*

Fig. 2 illustrates the results of Pinot Noir vines inoculated with *Pm. inflatipes*. Cuttings inoculated with *Pm. inflatipes* showed less discoloration than those inoculated with *Pa. chlamydospora*. Both uninoculated and inoculated cuttings had similar lengths of discoloration. Cuttings that received the delayed treatment had a significantly longer discoloration than uninoculated cuttings. Cuttings from the inoculated control group showed statistically similar lengths of discoloration to cuttings inoculated and immediately hot-water treated. Isolations from cuttings showed that none of the control cuttings were infected with *Pm. inflatipes*, and no statistical differences in reisolations existed between cuttings with hot-water treatments and those without. Although *Pm. inflatipes* was reisolated from fewer cuttings than *Pa. chlamydospora* in the previous experiment, hot water treatments still failed to in eliminate *Pm. inflatipes* from dormant Pinot noir cuttings.

Moist chamber incubation and growth in pots: *Vitis vinifera* cv. Thompson Seedless vines inoculated with *Pa. chlamydospora* and *Pm. inflatipes*

In addition to inoculating individual species of fungi this experiment involved inoculating mixtures of *Pa. chlamydospora* and *Pm. inflatipes* into cuttings. Hot water treatments were performed as

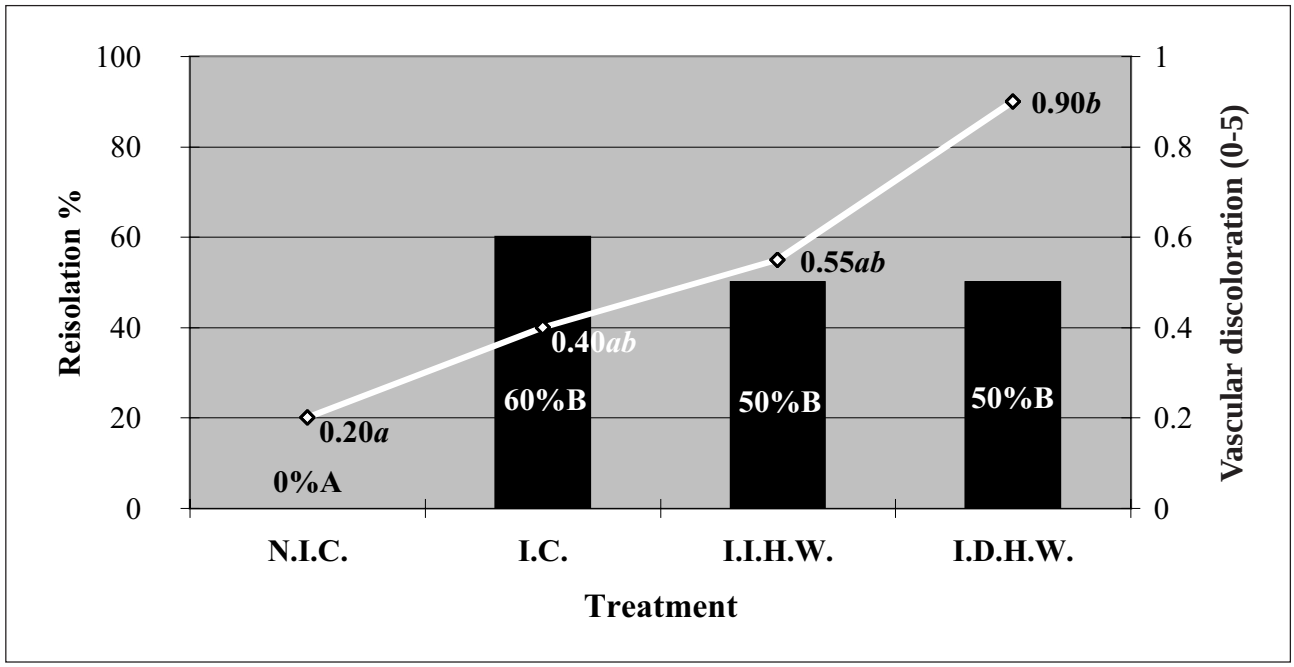

Fig. 2. Hot water treatment effect on colonization by *Phaeoacremonium inflatipes* (histogram) and vascular discoloration (line) on artificially inoculated grapevines (*Vitis vinifera* cv. Pinot noir). N.I.C., cuttings inoculated with sterile water only and not treated in hot water; I.C., cuttings inoculated with *Pm. inflatipes* and not treated in hot water; I.I.H.W., cuttings inoculated with *Pm. inflatipes* and immediately treated in hot water; I.D.H.W., cuttings inoculated with *Pm. inflatipes* and treated in hot water 24–48 hours later. Means followed by the same letter are not significantly by different according to Duncan's Multiple Range Test ($P<0.001$).

previously described. Vines were either incubated in moist chambers or planted in plastic pots. Results are shown in Fig. 3. No statistical differences existed between incubated and potted cuttings and they were therefore pooled for simplicity. Vascular discoloration was similar in all inoculated cuttings, but was significantly lower in uninoculated control cuttings. When isolations were done, none of the uninoculated control cuttings were infected with *Pm. inflatipes*, but a few (6%) were infected with *Pa. chlamydospora*. Infection may have occurred through contamination during inoculation or cuttings may have already been infected in the nursery. However, the reisolation percentages from the uninoculated control vines were significantly different from those of vines receiving all other treatments. In addition, no statistical differences in the reisolation of either fungus existed between inoculated cuttings with and without water treatments. The vascular discoloration means of these two groups of cuttings were also statistically similar. These results suggest that hot water treatments are ineffective in eliminating these fungi from dormant cuttings. Furthermore, symptom expression in the form of vascular discoloration appeared more severe when both fungi were present in the vines, though this cannot be determined for certain because different varieties of *Vitis vinifera* were used in the experiments.

Discussion

Contrary to results obtained by Crous *et al*. (2001), our results showed that hot water treatments of dormant cuttings at 51°C for thirty min did not eradicate or even reduce populations of *Pa. chlamydospora* and *Pm. inflatipes* in grapevines. This confirms Whiting *et al*. (2001) who found that hot water treatments were ineffective in reducing the mycelial growth of these fungi. *Pm. inflatipes*

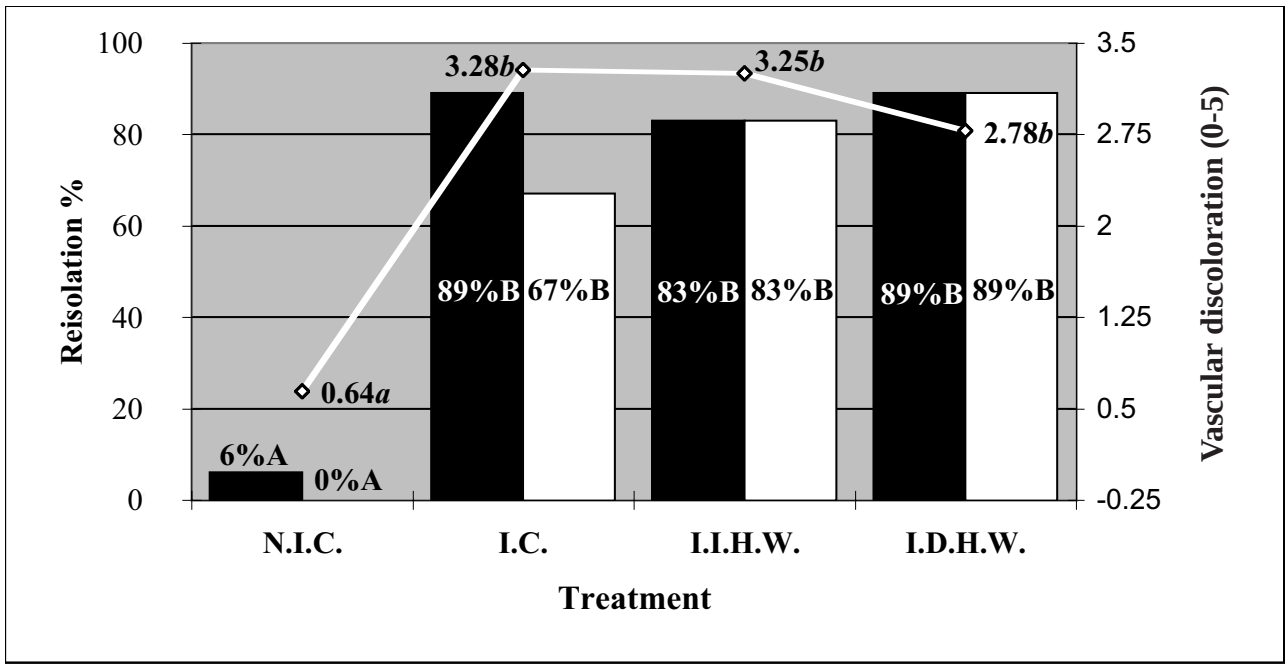

Fig. 3. Hot water treatment effect on colonization by both *Phaeoacremonium inflatipes* (white histogram) and *Phaemoniella chlamydospora* (black histogram) and vascular discoloration (line) on artificially inoculated grapevines (*Vitis vinifera* cv. Thompson Seedless). N.I.C., cuttings inoculated with sterile water only and not treated in hot water; I.C., cuttings inoculated with *Pa. chlamydospora* and *Pm. inflatipes* and not treated in hot water; I.I.H.W., cuttings inoculated with *Pa. chlamydospora* and *Pm. inflatipes* and immediately treated in hot water; I.D.H.W., cuttings inoculated with *Pa. chlamydospora* and *Pm. inflatipes* and treated in hot water 24–48 hours later. Means followed by the same letter are not significant different according to Duncan's Multiple Range Test ($P<0.001$).

mycelial plugs were able to survive for 120 min at 51°C and mycelial plugs of *Pa. chlamydospora* were able to survive for 60 min. Sporulation of both fungi was not affected by hot water (Whiting *et al.*, 2001). These results indicate that California isolates of *Pa. chlamydospora* and *Pm. inflatipes* are highly resistant. If mycelium of these fungi alone is unaffected by hot water treatment, it seems highly improbable that fungi protected in the vascular tissue of lignified grapevines are affected by such treatment. We believe it is possible that there is an initial shock to these fungi by the heat, which may explain why some researchers were unable to isolate the fungi immediately after treatment. However, the fungi clearly have the ability to overcome heat treatments and survive equally as well in hot water treated cuttings as in cuttings that were inoculated but not untreated. It is concluded that hot water treatments of grapevines infected with *Pa. chlamydospora* and *Pm. inflatipes* are not to be recommended as on effective control in California.

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