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Short communication

Control of brown rot of stone fruits by brief heated water immersion treatments

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ABSTRACT

The effectiveness of brief (30 or 60 s) immersion in water at 24, 50, 55, 60, 65, or 70 °C was evaluated for the control of brown rot, caused by *Monilinia fructicola*, on California-grown peaches, nectarines, and plums. Inoculated fruits were treated and either stored at 20 °C for 5 days or at 0 °C and 95% RH for 30 days followed by 5 days at 20 °C to simulate commercial marketing conditions. Immersion in water at 55 °C for 60 s or at 60 °C for 30 or 60 s significantly reduced both decay incidence and severity among the remaining wounds that developed the disease. Water temperatures of 65 °C or higher were phytotoxic and caused moderate to severe surface injuries. Immersion in water at 60 °C for 60 s was effective for plums and it reduced the incidence of brown rot from more than 80% among control fruit to less than 2%. In nectarines, this treatment reduced decay incidence from 100 to less than 5% on fruit stored at 20 °C and from 73 to 28% on cold-stored fruit. Therefore, brief immersion in heated water can be an effective approach to manage postharvest brown rot of stone fruits, especially for the organic fruit industry.

1. Introduction

Brown rot caused by *Monilinia fructicola* (G. Wint.) Honey is the major postharvest disease of stone fruits in California and other producing areas worldwide (Ogawa and English, 1991). Fruit losses due to decay are estimated to be 5–10% when postharvest fungicides are used; without fungicide treatment, losses may reach 50% or more (Margosan et al., 1997).

Although fungicides such as fludioxonil and fenhexamid are registered in the United States for postharvest application to control decay of peaches and nectarines, there are important reasons to seek alternatives to chemical fungicides. Fungicide resistance has developed repeatedly in the past among *M. fructicola* populations (Ma et al., 2003). The presence of fungicide residues on the fruit can prevent their export to some foreign markets. In the European Union, Turkey, and other countries, postharvest application of synthetic fungicides to these fruit is prohibited (Karabulut and Baykal, 2004). There is an increasing demand for organically grown fruit and compliance with "organic" regulations entails restricted pre and postharvest fungicide use.

Several studies have shown that hot water treatments by themselves or in combination with other treatments can substantially control postharvest diseases of peaches and nectarines (Wells, 1971; Margosan et al., 1997). However, there are no prior reports employing hot water to control postharvest decay of plums. Treatments that have been evaluated were of relatively long duration (usually 1.5–3 min) at relatively low temperatures (45–52 °C). Treatments of long duration usually require very long tanks, and the costs and space they require are an issue that has hindered the commercial adoption of hot water treatments. While higher water temperatures could reduce duration of the treatment and facilitate the use of smaller tanks, the effectiveness of brief treatments for use on stone fruits has not been investigated thoroughly. Recently, 40 s dips in water heated to 60 °C were effective to control *Monilinia laxa* on artificially inoculated peaches and nectarines (Casals et al., in press). Furthermore, a 20 s duration treatment where peaches and nectarines passed through a drench of water heated to 55 or 60 °C over rotating brushes controlled brown rot effectively (Karabulut et al., 2002).

Our objective was to evaluate the efficacy of heated water treatments at relatively high temperatures (55–70 °C) for short durations (30–60 s) to control *M. fructicola* on artificially inoculated peaches, nectarines, and plums of commercial importance in California. The efficacy of the treatments and occurrence of visible injuries to the fruit were assessed after storage at 20 °C for 5 days or cold storage at 5 °C and 95% RH for 30 days.

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2. Materials and methods

Nectarines (*Prunus persica* (L.) Batsch. var. *nucipersica* (Suckow) Schenid) cvs. 'Summer Fire' and 'August Fire', peaches (*P. persica* (L.) Batsch.) cv. 'O'Henry', and plums (*Prunus salicina* Lindl.) cv. 'Casselman' were hand-harvested at commercial maturity in central California orchards and kept at 0 °C until use.

M. fructicola was isolated from an infected peach fruit and cultured on potato dextrose agar (Difco, Detroit, MI, USA). Spore suspensions were prepared by removing the spores from sporulating edges of 2–3 week old cultures with a bacteriological loop and suspending them in sterile distilled water. Spore concentrations were determined with a hemocytometer and adjusted to 10^5 spores per ml.

Fruit were surface disinfected with 100 μg/ml chlorine on rolling brushes, left to air dry at 20 °C, and inoculated by the method described by Palou et al. (2002). The shoulder of the disinfected fruit was wounded once with a probe tip (1 mm wide, 2 mm deep) and wound sites were inoculated with 10 μl of *M. fructicola* spore suspension (10^5 spores/ml). Inoculated fruits were kept at 20 °C for 12–16 h to achieve spore germination within the wound sites and simulate the infections occurring during harvest. After this period of time, fruits were dipped into water at 24 (control), 50, 55, 60, 65, or 70 °C for 30 or 60 s, depending on the experiment. The experiments were repeated twice. Treated fruits were air-dried at 20 °C, placed in fiberboard boxes, and stored at 20 °C and 90% RH for 5 days. Brown rot incidence (number of decayed wounds) and severity (lesion diameter) were determined after 3 and 5 days of incubation. After 5 days, heat injury was assessed for each treatment as visible pitting and/or brown discolored areas on the fruit surface. An injury index (0–3 scale) was established in which 0, 1, 2, and 3 corresponded to nonexistent, slight, moderate, and severe peel injury, respectively. Fruit into categories 2 and 3 were not marketable.

To evaluate the efficacy of hot water treatments on cold-stored fruits, an additional experiment using the same methodology was performed with 'August Fire' nectarines. Fruits were treated at 24 (control), 55, or 60 °C for 60 s and stored at 0 °C and 95% RH for 30 days followed by a shelf life period of 5 days at 20 °C and 90% RH. Decay incidence and severity and skin injury were determined at the end of the shelf life period.

In all experiments, each treatment was applied to 3 replicates of 20 fruit each. Analysis of variance (ANOVA) was applied to severity and arcsine-transformed incidence data and means were separated using Fisher's Protected LSD test ($P \leq 0.05$).

3. Results and discussion

Brief hot water treatments applied to fruit inoculated with *M. fructicola* and incubated at 20 °C significantly controlled brown rot (Figs. 1 and 2). Hot water treatment at 55 °C for 30 s reduced both the percentage of infected wounds (Fig. 1A) and the size of decay lesions (Fig. 2A) on 'Summer Fire' nectarines after 3 days of incubation at 20 °C. Nevertheless, after 5 days of incubation, this treatment and that of water at 50 °C for 30 s only caused a modest reduction in disease severity. On 'O'Henry' peaches treated with hot water at 55 and 60 °C for 30 s followed by incubation for 5 days, the percentages of infected wounds were 67 and 40%, respectively, while 90% of wounds among control fruit were infected (Fig. 1B). Disease severity on these fruit was also considerably reduced (Fig. 2B). In a second experiment with 'Summer Fire' nectarines, the longer treatment of 60 s at 60 °C almost controlled brown rot completely (Figs. 1C and 2C). In the experiments with 'Casselman' plums, hot water treatments at 55, 60, 65, and 70 °C for either 30 or 60 s significantly reduced disease incidence (Fig. 1D) and severity (Fig. 2D). However, treatments at 65 and 70 °C were unacceptable because they caused moderate to severe surface injuries among most of the fruit (injury categories 2 and 3). Among the non-injurious treatments, hot water applied at 60 °C for 60 s completely inhibited the disease and was significantly superior to other treatments.

In tests with 'August Fire' nectarines, where treatments were followed by 30 days of storage at 0 °C plus 5 days at 20 °C to simulate marketing, immersion in water at 55 and 60 °C for 60 s significantly reduced both brown rot severity and incidence in comparison with control treatment at 24 °C (Table 1). Disease reductions were significantly better at 60 °C than at 55 °C. No visible surface injuries were associated with heat treatment on these fruit. These preliminary results with cold-stored nectarines should be confirmed in future research with other stone fruit species and cultivars. Although they were different nectarine cultivars, in this work decay control after treatment at 60 °C was lower on cold-stored fruit than on fruit stored at 20 °C. Presumably, this might be due to the low but unceasing metabolic activity, even at 0 °C, of both surviving fungal structures and fruit host during 30 days of storage plus 5 days of shelf life.

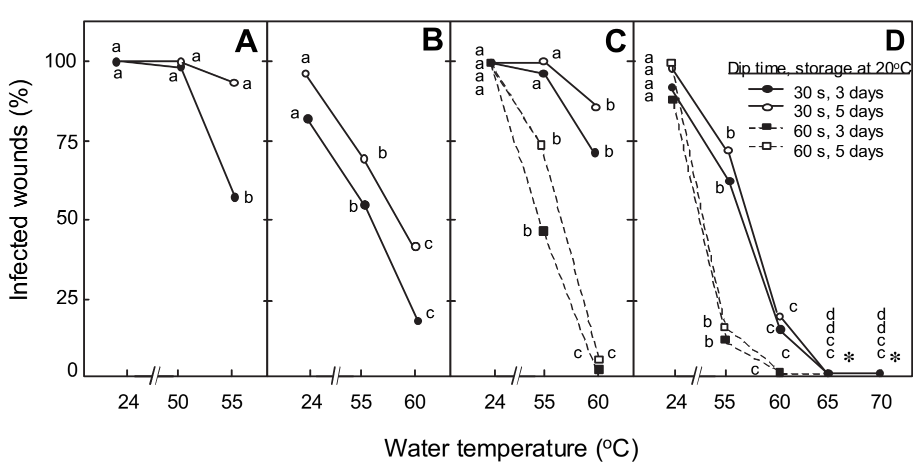

Fig. 1. The influence of hot water treatment for 30 or 60 s on the incidence of infected wounds on 'Summer Fire' nectarines (A and C), 'O'Henry' peaches (B), and 'Casselman' plums (D) inoculated before treatment with *Monilinia fructicola*. The fruit were stored at 20 °C and 90% RH after treatment and examined after 3 and 5 days. For each experiment, dip time and storage period, means with the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$) applied after an ANOVA to arcsine-transformed data. Actual values are shown. The * indicates moderate to severe skin pitting developed during storage.

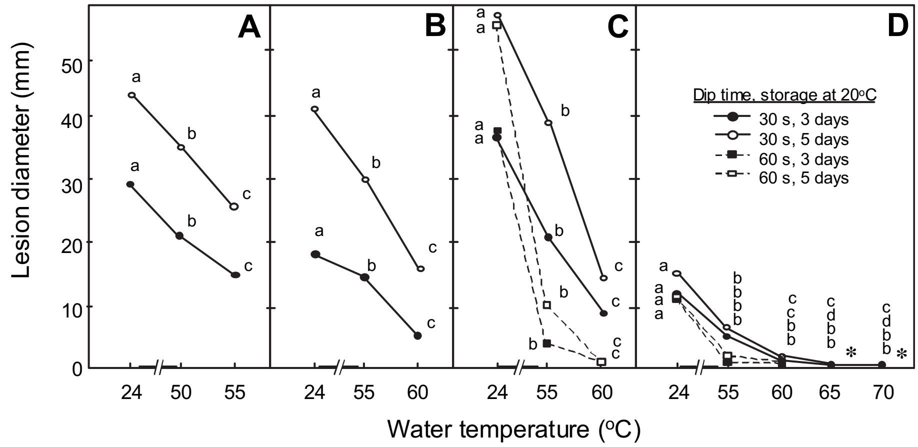

Fig. 2. The influence of hot water treatment for 30 or 60 s on the size of decay lesions on 'Summer Fire' nectarines (A and C), 'O'Henry' peaches (B), and 'Casselman' plums (D) inoculated before treatment with *Monilinia fructicola*. The fruit were stored at 20 °C and 90% RH after treatment and examined after 3 and 5 days. For each experiment, dip time and storage period, means with the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$). The * indicates moderate to severe skin pitting developed during storage.

Compared to previous reports (Wells, 1971; Margosan et al., 1997), the results of this work demonstrated that brief (30 or 60 s) immersion in heated (55 or 60 °C) water was at least as effective as immersion for 1.5–3 min at 45–50 °C to control brown rot on peaches and nectarines. Performance of these relatively brief heated water treatments was also very satisfactory on 'Casselman' plums. On plums, water temperatures of 65 °C or higher were phytotoxic and should be avoided. Although water at 60 °C for 60 s was the most effective application, immersion in either at 55 °C for 60 s, or 60 °C for 30 s, was nearly as effective and still could be of use. In most California packinghouses, a tank that accomplishes a 60 s immersion would be about 10 m in length, while a 30 s treatment would be accomplished in half this length that would make it more feasible. Another possible combination is 60 °C for 40 s (Casals et al., in press). Both our work and that by Casals et al. employed artificial inoculation and may underestimate the effectiveness of these treatments applied to naturally inoculated fruit. Smith (1962) reported that natural infections of *M. fructicola* or *Rhizopus stolonifer* on peaches were much better controlled by hot water treatment than those made artificially. The mode of action of hot water against *Monilinia* spp. is unclear, but it is accepted that it is not linked to a single event (Margosan et al., 1997). Presumably, both direct effects on the pathogen (cell damage) and indirect effects on the fruit host (induction of resistance mechanisms) are to variable degree responsible for the antifungal activity.

Hot water treatment is generally best used as a component of an integrated control system, where it is employed in combination with other treatments, such as conventional chemical fungicides (Wells, 1971), ethanol (Margosan et al., 1997), biological control (Karabulut et al., 2002; Karabulut and Baykal, 2004), or modified atmospheres (Karabulut and Baykal, 2004). In contrast, no benefits were reported by the addition to hot water of different soluble food additives or GRAS (generally regarded as safe) compounds to control brown rot (Palou et al., 2009). A feasible compound to add to hot water would be a well-regulated sanitizer such as chlorine. Phillips and Harris (1979) reported that brief treatment with cool chlorine alone often substantially reduced subsequent brown rot development on peaches.

Despite their good activity against *M. fructicola* and other stone fruit postharvest pathogens, shortcomings of hot water treatments applied alone are the lack of persistent antifungal activity, occasional mediocre efficacy, and the risk of injury to the fruit, especially in high-volume commercial applications where absolute control of treatment conditions is challenging. Our results indicate that short duration hot water treatments are a promising technological tool that improves their feasibility as part of integrated non-pesticidal alternative treatments for the control of brown rot of stone fruits. Furthermore, they could be an effective approach to manage postharvest decay for the organic fruit industry.

Table 1
Effect of a 60 s dip in hot water on the incidence and severity of brown rot on 'August Fire' nectarines inoculated with *Monilinia fructicola*. After treatment, the fruit were stored for 30 days at 0 °C and 95% RH, and then an additional 5 days at 20 °C and 90% RH.

Temperature (°C)	Lesion diameter (mm)	Infected wounds (%)[a]
24	22.1 ab[b]	73.3 a
55	13.2 b	58.7 b
60	6.4 c	28.0 c

[a] ANOVA was applied to arcsine-transformed data. Actual means are shown.
[b] Values in columns followed by the same letter are not significantly different according to Fisher's Protected LSD test ($P \leq 0.05$).

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