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Effect of host and *Monilinia* spp. variables on the efficacy of radio frequency treatment on peaches

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ABSTRACT

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Currently, no chemical fungicides are allowed in the European Union to be applied to stone fruit after harvest. In previous work, radio frequency (RF) treatment for 4.5 min applied with fruit immersed in water at 40 °C was very promising for the control brown rot on peaches and nectarines. In the present study, the efficacy of this radio frequency treatment was studied employing different infection times, inoculum concentrations, fruit maturity levels and in naturally infected fruit. Generally, infection time and maturity level of fruit did not have a significant effect on the RF treatment efficacy and brown rot incidence was significantly reduced in fruit inoculated 0, 24 or 48 h before treatment and at all maturity levels evaluated in both peaches and nectarines. RF treatment significantly reduced brown rot incidence at all inoculum concentrations evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}). However, in peaches, the treatment efficacy was slightly less when the inoculum concentration was increased to 10^5 or 10^6 conidia mL^{-1}. In naturally infected fruit, brown rot incidence was significantly reduced from 92% among control fruit to less than 26% in peaches and complete brown rot control was achieved in nectarines. RF treatment did not have an effect on fruit firmness in the varieties tested, and even a delay of fruit softening was observed. Moreover, both external and internal fruit appearance was not affected by the treatment.

1. Introduction

Brown rot is the most important postharvest disease of stone fruit and is essentially caused by two species, *Monilinia laxa* (Aderh. et Rulh.) Honey and *Monilinia fructicola* (G. Wint.) Honey (De Cal et al., 2009). Stone fruit infection by *Monilinia* spp. can take place in the field during the growing season when conditions favor disease development. However, postharvest losses by brown rot that routinely occur during storage and transport (Hong et al., 1997) are typically more severe than preharvest losses, sometimes reaching high levels (59%) (Larena et al., 2005). Currently, no chemical fungicides are allowed in the European Union to be applied for postharvest treatment of stone fruit. In addition, public demands to reduce pesticide use and improve environmental and human health, as well as the development of resistance to widely-used synthetic fungicides by fungal strains, limits the preharvest application of chemical products in the field. These concerns, combined with a lack of effective postharvest treatments against *Monilinia* spp. have increased the need to develop new control methods.

Heat treatments have been widely studied for many years (Smith et al., 1964), however, heat treatments applied by immersion in hot water, vapor heat, hot air drying, curing or by hot water rinsing and brushing have been also investigated in recent years to control postharvest diseases in peaches (Casals et al., 2010b), oranges (Plaza et al., 2003), apples (Fallik et al., 1995) and lemons (Stange and Eckert, 1994). These conventional heat treatments are limited by the low thermal conductivity of fruit and thus necessitating prolonged heating in many cases. Casals et al. (2010b) reported a curing treatment at 50 °C for 2 h for controlling brown rot in peaches and nectarines. However, short treatment times are preferred from the viewpoint of commercial applications (Ikediala et al., 2002). Generally, hot water treatments are shorter where time exposures range between 20 s and 2.5 min and temperatures between 45 and 60 °C (Margosan et al., 1997; Karabulut et al., 2010), although their application may need to be combined with an alternative treatment to enhance effectiveness (Casals et al., 2010c; Sisquella et al., 2013b).

The need to achieve fast and effective thermal treatment has resulted in the increased use of radio frequency (RF) energy to heat foods. This electromagnetic energy directly interacts with the fruit interior to quickly raise the center temperature (Tang et al., 2000), because dielectric materials, such as most agricultural products, can

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store electric energy and convert electric energy into heat (Wang et al., 2001). The dielectric properties and specially the loss factor (ε''), influence both energy absorption and attenuation and describe the ability to dissipate energy in response to an applied electric field, which commonly results in heat generation (Ikediala et al., 2000). The amount of heat converted in the food is proportional to the value of the loss factor (Tang et al., 2000).

Radio frequency heating has been widely studied as a rapid pest control for nuts and dry products (Mitcham et al., 2004), cherries (Ikediala et al., 2002), oranges (Birla et al., 2005), apples (Wang et al., 2006), persimmons (Tiwari et al., 2008) and mangoes (Sosa-Morales et al., 2009). In contrast, little information is available about the possibility of applying RF heating to control postharvest diseases. Casals et al. (2010d) demonstrated the potential of the use of RF to control brown rot in peaches. In subsequent work, the improvement of this treatment by applying the RF with fruit immersed in water was investigated by Sisquella et al. (2013a) who reported that RF heating for 4.5 min in fruit immersed in water at 40 °C controlled *M. fructicola* in artificially inoculated peaches and nectarines. The response of a pathogen to heat can be influenced by several factors such as the moisture content of spores, age of the inoculum and inoculum concentration (Barkai-Golan and Phillips, 1991). Therefore, although these new conditions demonstrated high brown rot control, other factors require consideration before RF treatment can be implemented as a commercial treatment.

The aim of this study was to evaluate RF treatment with immersion of peach and nectarine fruit in water at 40 °C as a control for postharvest brown rot. We examined the effect of RF treatment on brown rot at different times after inoculation of the fruit, on fruit challenged with different inoculum concentrations, and with fruit at different maturity stages. The efficacy of the RF treatment also was tested with naturally infected fruit. In addition, the effect of RF treatment on fruit quality was evaluated in peaches and nectarines.

2. Materials and methods

2.1. Fruit

Experiments were conducted with 'Rome Star', 'Roig d'Albesa' and 'Placido' peaches (*Prunus persica* (L.) Batsch) and 'Fantasia', 'September Red' and 'PP-100' nectarines (*P. persica* (L.) Batsch var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control. Fruit free of visible wounds and rots and similar visual maturity were selected by hand immediately after harvest. Fruit not used at the time of harvest were stored at 0 °C until required for experimentation.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Centre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). The strain was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1%) at 4 °C in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA amended with acetone (J.T. Baker, 1%) and incubated in the dark at 25 °C for approximately two weeks. The isolate was inoculated onto peaches or nectarines by wounding the fruit (1 mm diameter and 2 mm depth) with a sterilized steel rod and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Fruit were then incubated at 25 °C and 85% RH in the dark for 5–7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test-tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to the desired concentration. Fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 μL of the desired conidial suspension.

2.4. Radio frequency heating system and suitable treatment conditions

A semi-industrial radio frequency equipment instrument (STALAM S.p.A, Nove, Vicenza, Italy) with 15 kW nominal maximum power and a frequency of 27.12 MHz was used to perform the experiments. The RF equipment is provided with two parallel electrodes of 150 cm × 100 cm. The electrode gap was adjustable over a range of 65–205 mm and the speed of the continuous conveyer belt ranged from 0.1 to 0.7 m min^{-1}.

Radio frequency conditions used in this study were those reported in a previous work (Sisquella et al., 2013a). The electrode voltage was set at 5800 V and the electrode gap was adjusted at 112 mm. Fruit at room temperature was introduced into a container (260 mm × 260 mm × 105 mm) with 2 L of water at 40 °C, so all fruit were completely submerged in the water. The containers were placed on the conveyor belt and RF treatment was applied for 4.5 min.

2.5. Effect of infection time on radio frequency efficacy

'Roig d'Albesa' peaches and 'PP-100' nectarines were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above and were maintained for 0, 24 or 48 h at 20 °C and 85% RH. After this time, fruit were immersed in water at 40 °C and then, RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each incubation time was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After treatment, fruit were incubated 5 days at 20 °C and 85% RH and then, the number of infected fruit was recorded.

2.6. Effect of inoculum concentration on radio frequency efficacy

'Roig d'Albesa' peaches and 'PP-100' nectarines were artificially inoculated with *M. fructicola* at 10^3, 10^4, 10^5 or 10^6 conidia mL^{-1} as previously described. Once wounds were dry, fruit were immersed in water at 40 °C and then, RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each inoculum concentration was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After treatment, fruit were incubated 5 days at 20 °C and 85% RH and then the number of infected fruit was recorded.

2.7. Effect of fruit maturity on radio frequency efficacy

In order to achieve different maturity levels, after harvest and before treatment, 'Roig d'Albesa' peaches and 'PP-100' nectarines were maintained for 24, 48 or 72 h at 20 °C and 85% RH. After this time, fruit were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above. Once wounds were dry, fruit were immersed in water at 40 °C and then RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each maturity level was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After