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Research Note

Combined effect of curing followed by acetic acid vapour treatments improves postharvest control of *Penicillium digitatum* on mandarins

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ABSTRACT

In recent years several alternatives to the chemical control of postharvest decay have been examined but satisfactory levels of control, with a single system, have not been achieved yet. In the present study the results of an integrated postharvest approach are reported. Early and late harvested hybrid mandarin fruit "Fremont" and "Fairchild", inoculated with *Penicillium digitatum* (Pers.:Fr.) Saccardo, were cured at 36 °C for 36 h with 95% RH and then fumigated with 0, 5, 15, 25, 50, 75 and 100 μL/L of acetic acid (AAC) vapours for 15 min. Following the treatments, fruit was stored at 20 °C and 80% RH to simulate a marketing period, and after 2 weeks the decay incidence and the visual appearance were evaluated. Curing or fumigations performed alone reduced decay with respect to untreated fruit, but the best control was achieved with combined treatments. For early harvested fruit the lowest decay percentage was obtained by using 75 μL/L with 8.3% and 2.1% of rots for "Fremont" and "Fairchild", respectively, whereas for late harvested fruit the highest efficacy was observed using 50 μL/L (1.4% and 6.6%). Rind damage as pitting was observed only if fruit was treated with AAC alone at 100 μL/L.

1. Introduction

Postharvest chemicals such as imazalil or thiabendazole are currently used to control *Penicillium digitatum* (Pers.:Fr.) Saccardo in packing-houses but their continuous utilization, because of the restricted number of allowed fungicides, has led to the proliferation of resistant pathogen strains (Stange and Eckert, 1994). Furthermore the use of synthetic chemicals is becoming more difficult to justify, because of the increasing concerns for human health as well as environmental considerations (Schirra et al., 2000; Palou et al., 2008). New friendly technologies are therefore needed to reduce fungicide residues and the selection of resistant pathogen strains.

Many authors have reported on the effectiveness of curing in reducing the incidence of citrus green mould when performed at temperatures ranging from 30 to 36 °C for 48, 65 or 72 h (Ben-Yehoshua et al., 1987; Stange and Eckert, 1994; Plaza et al., 2003, 2004). However this method is not widely used in citrus packing-houses because of the long treatment durations and subsequent higher costs of heating large volumes of fruit, as compared to conventional treatments (Schirra et al., 2000). Nevertheless some authors report that the use of heat treatments should be reconsidered in order to reduce the use of chemicals and to apply safe practices against moulds and pests (Mulas and Schirra, 2007). The utilization of GRAS (Generally Recognized as Safe, according to US-Food and Drug Administration) compounds is also a justifiable alternative to replace chemicals since their use is allowed as food additives (European Union).

Several researchers have focused on the use of carbonic acid salts as alternatives to synthetic fungicides to control citrus green mould. The findings have shown lower efficacy in controlling decay caused by *Penicillium italicum* Wehmer compared to *P. digitatum*, and among *Citrus* species the worst results were obtained with mandarin fruit (Palou et al., 2001; Venditti et al., 2005). Acetic acid (AAC) is a valid candidate and its effectiveness in preventing postharvest fruit decay caused by *P. digitatum*, as well as many other decay-causing pathogens such as *Botrytis cinerea*, *Penicillium expansum*, *Monilinia fructicola* and *Rhizopus stolonifer* is well documented. Studies have been conducted on stonefruit (Sholberg and Gaunce, 1996), table grapes (Sholberg et al., 1996), citrus fruit such as oranges, lemons and grapefruit (Sholberg, 1998), apples (Sholberg et al., 2001), apricots and plums (Liu et al., 2002) and pears (Sholberg et al., 2004), but not yet on mandarins. In recent years several alternatives to the use of chemicals have also been exploited (Palou et al., 2008), but satisfactory levels of control with a single system, comparable to the effectiveness of synthetic fungicides, have not been achieved yet. A possible solution is an integrated postharvest approach, with curing at 36 °C for a shorter period (36 h) compared with that reported in previous papers, followed by AAC fumigation, in order to have a synergistic effect of the two treatments that enhance the efficacy of pathogen control.

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The aims of the present work were to: (1) evaluate if this combined postharvest method can be effective in controlling green mould, particularly on mandarin fruit, and (2) investigate the influence of the harvesting period on the efficacy of the treatment.

2. Materials and methods

The experiment was carried out with hybrid mandarin fruit “Fairchild” (*Citrus reticulata* Blanco × Orlando tangelo) and “Fremont” (*Citrus reticulata* Blanco × Ponkan), handpicked from an experimental orchard, belonging to ISPA – CNR, located in southwest Sardinia, Italy (39°55′N), managed using standard horticultural practices. The mandarins were collected in two different periods, at the beginning of February as early harvested (EH) and at end of the same month as late harvested (LH). The fruit were delivered on the same day to the laboratory, dipped in a 0.2% sodium hypochlorite solution for 2 min, rinsed twice with distilled water and air-dried. After disinfection each fruit was wounded by injuring the flavedo and albedo at four equatorial points with a steel rod 3 mm wide and 3 mm deep and then held on the laboratory bench for 1 h to allow the wounds to dry.

The strain of *P. digitatum* was isolated from naturally decayed oranges and cultured on PDA (Fluka–Sigma–Aldrich Buchs - Schweiz) for 10 d at 23 °C. Spores were harvested from Petri dishes (9 cm diameter) and the conidial suspension passed through two layers of cheesecloth and then adjusted to a concentration of 1×10^4 conidia/mL with a haemocytometer. Inoculation was performed by injecting with a micropipette 20 μL of the suspension into each injury. After the inoculum had dried (60 min) the fruit were placed in plastic boxes with a wet filter paper on the bottom to ensure relative humidity close to saturation. Boxes were then covered with a plastic lid and held at 20 °C for 24 h (incubation period). Conidia concentration was established in order to attain between 80% and 90% decay in untreated fruit.

On the basis of preliminary experiments, curing was conducted by placing the boxes with the mandarins in a growth room 36 °C for 36 h with 95% RH and subsequently fruit were subjected to the AAC treatments for 15 min, at 0, 5, 15, 25, 50, 75 and 100 μL/L. Fumigations were performed by placing the mandarins in 20 L chambers equipped with a circulation fan (12 V–0.16 A). Each lid was provided with a rubber septum for AAC applications and sealed by an airtight siphon. Gradual and controlled evaporation of the AAC inside the chambers was obtained by heat delivered by a couple of electric resistances (10 Ω each) connected to a stabilized power supply. A voltage regulator (1–15 V of output) allowed control of the temperature of the resistances. The established amounts of glacial acetic acid (Carlo Erba, Milano, Italy) were injected with a microsyringe through the septum into heatproof glass vessels, placed on the electric resistances. Particular care was taken in setting the electric resistances voltage in order to retain the AAC temperature below the flash point. Once AAC was injected the circulation fans were turned on for 15 min. In addition to the combined curing and AAC fumigation sets, the fruit were divided into two groups of inoculated mandarins: the first was only fumigated with AAC vapours at 100 μL/L, while the second was the control. Each treatment was applied in three replicates of 21 mandarins each. The fruit were subsequently stored at 20 °C and 80% RH for 2 weeks, to simulate the marketing period. At the end of the experiment decay percentage was monitored as well as visual appearance for signs of phytotoxicity. Damage was rated on a 0–3 scale (0 no damage; 1 ≤25%; 2 between 25% and 50%; 3 >50% of fruit surface affected) (Lafuente et al., 1997).

Analysis of variance (ANOVA) was performed using the MSTAT-C software (Michigan State University, East Lansing, 1995), and when appropriate, mean separations was performed according to Duncan’s multiple range test at $P < 0.05$. Angular transformation of decay percentage values was performed prior to statistical analysis. The magnitude of the synergistic interaction between the two treatments was determined for the highest concentration of AAC (100 μL/L). The expected efficacy was calculated with the Abbott formula, as reported by Baider and Cohen (2003).

3. Results

In early and late harvested fruit of both varieties subjected to curing, the levels of infected wounds ranged from 41.7% to 45.6%, with a substantial reduction in comparison to the control and with no significant differences between the two cultivars and the two harvesting periods (Table 1). The response of fruit fumigated only with AAC was different, according to the harvesting period. The incidence of decay in EH “Fremont” and “Fairchild” fruit decreased to 34.2% and 31.6%, respectively, but the same treatment carried out with LH mandarins produced worse results than curing performed alone, with decay of 57.3% and 49.2%.

The combined treatments in general significantly improved decay control on fruit, if compared with each of the two treatments performed alone. On LH mandarins the correlation between the increase in the GRAS compound concentration and the reduction of green mould, increased from the lowest treatment of AAC (5 μL/L) and achieved highest efficacy at 50 and 75 μL/L. The most effective control of decay was reached at 50 μL/L, with percentages of 1.4% and 6.6% for “Fremont” and “Fairchild”, respectively even if without significant differences between the two concentrations. Combining curing with AAC at 100 μL/L, resulted in poor pathogen control for both LH cultivars, with decay percentages increased to 25.0% and 30.0%. A similar pattern was observed on EH man-

Table 1

Percentages of wounds infected by *Penicillium digitatum*, after 14 d at 20 °C and 80% RH, in early and late harvested ‘Fremont’ and ‘Fairchild’ mandarins, treated with acetic acid (AAC) at 100 μL/L or cured at 36 °C for 36 h followed or not by a 15 min fumigation with different concentrations of AAC[a,b].

Treatments (μL/L)	Early harvested				Late harvested			
	Fremont		Fairchild		Fremont		Fairchild	
Control	89.6 a	A	88.5 a	A	92.7 a	A	91.7 a	A
AAC 100	34.2 c	BC	31.6 c	C	57.3 b	A	49.2 b	A
Curing + AAC 0	41.7 b	A	44.2 b	A	45.6 c	A	42.5 c	A
Curing + AAC 5	34.2 c	AB	31.6 c	B	37.1 d	A	37.5 d	A
Curing + AAC 15	26.7 d	A	20.1 d	A	15.7 f	A	23.3 f	A
Curing + AAC 25	22.5 d	A	14.6 e	A	11.7 f	A	20.0 f	A
Curing + AAC 50	12.5 e	A	4.7 f	BC	1.4 g	C	6.6 g	AB
Curing + AAC 75	8.3 e	A	2.1 g	A	2.5 g	A	9.2 g	A
Curing + AAC 100	13.3 e	AB	5.3 f	B	25.0 e	A	30.0 e	A

[a] Means reported in the table are the actual percentage values of decay.

[b] Different lower-case letters indicate, within the same column, means significantly different at $P < 0.05$ according to Ducan’s test; different capital letters, within the same row, indicate means significantly different at $P < 0.05$ according to Ducan’s test.

darins, in this case the best efficacy was achieved with 75 μL/L of AAC for the two varieties. On “Fremont” mandarins this treatment resulted in the lowest green mould incidence (8.3%), even if significant differences were not observed at concentrations of 50 or 100 μL/L. As for “Fairchild” mandarins, the lowest decay percentage was obtained at 75 μL/L of AAC, with a rot incidence of 2.1%. Lower but still good decay control was also obtained with 50 or 100 μL/L of AAC (4.7% and 5.3%, respectively) but with significant differences with respect to 75 μL/L. Regarding the visual assessment the mandarins of both varieties and harvesting periods, subjected to the combined treatments were rated “0” with no visible damage on the rind. Conversely, treatments performed with only AAC induced pitting on large areas of the surface, and subsequently fruit were rated “3”. The synergy factor (SF) calculated as the ratio between the observed and the expected efficacy was as a general rule greater than 1, indicating a synergistic interaction of the combined treatments.

4. Discussion

The increasing demand of products with low levels of chemicals has multiplied studies on the use of postharvest alternative methods for decay control, such as curing or GRAS compounds. In our experiment the duration of the heat treatment was reduced in comparison with previous studies (Ben-Yehoshua et al., 1987; Stange and Eckert, 1994; Plaza et al., 2004) and as a result, when fruit were only cured, we did not gain such an effective control of the pathogen. It is likely that the reduced decay control could be ascribed to the shorter curing period (Stange and Eckert, 1994). Furthermore it is important to underline that curing performed alone decreased the incidence of infection to similar percentages with no significant differences between the two varieties and harvesting periods.

With regard to utilization of GRAS compounds on citrus fruit, in recent years many authors have focused their studies particularly on the use of sodium carbonate and bicarbonate, finding a lower efficacy in controlling penicillium moulds on mandarin as compared to other *Citrus* species (Palou et al., 2001; Venditti et al., 2005). The different behaviour between mandarin fruit and other *Citrus* sp., may be overcome with the use of AAC vapours. The effectiveness of the treatments with this GRAS compound has been reported by many authors (Sholberg and Gaunce, 1995, 1996; Sholberg et al., 1996, 2004; Sholberg, 1998; Liu et al., 2002). In this trial, a different technique of AAC application was tested, with shorter time of fumigation and higher concentrations. When mandarins were treated with AAC at a concentration of 100 μL/L without curing, different efficacy was found according to the harvesting period: better control of the pathogen, with respect to the cured fruit, was obtained for both EH mandarins, while the LH fruit decayed more. These results seem to be consistent with those achieved by Sholberg (1998) where stonefruit treated with AAC, decay control appeared to be associated with fruit maturity. It is likely that the different behaviour, according to the harvesting periods could be explained by treatment damage on the peel. Phytotoxicity symptoms, caused by the treatment with AAC were also observed on different fruit, such as pitting on cherries, streaking and browning on stonefruit (Sholberg, 1998) and pears (Sholberg et al., 2004). The results obtained by combining the curing with AAC showed in general enhanced control of the pathogen, compared with each of the two treatments performed alone. Particularly for treatments with AAC at 100 μL/L the SF was calculated and as a general rule its value was greater than 1, showing the synergistic effect of the two treatments.

The interactions between heat treatment and decay-causing agents are well documented (Schirra et al., 2000). Curing can control postharvest diseases by a direct effect on the pathogen and by modulating fruit responses enhancing host resistance. In our experiment in addition to the reported effects, it is possible to assume that curing has also a role in preventing the development of injury on the fruit surface caused by the AAC treatment. This protective effect is particularly evident on LH mandarins, that were more prone to damage that was almost identical to symptoms caused by chilling injury. On “Fortune” mandarins it was pointed out that the surface of fruit affected by severe symptoms of pitting, consisted of depressed zones and the epicuticular wax morphology showed few wax platelets and was devoid of crystalline structures (Vercher et al., 1994). Curing or heat treatments can also reduce sensitivity of fruit to chilling injury such as in grapefruit (Sapitnitskaya et al., 2006). Many authors report that heat treatments produce important structural changes of epicuticular waxes. Scanning electron microscopy of cured cactus pear clearly showed that the skin surface appeared relatively homogeneous with gaps, micro-wounds and stomata partially or completely filled with melted wax (Schirra et al., 1999). It is possible to assume that the role of curing in enhancing the efficacy could be ascribed not only to a direct or indirect impact, but also to a protective effect on the fruit surface against physiological disorders caused by the AAC. According to Mulas and Schirra (2007) “it is unlikely that heat treatments can exert extensive and fully effective mould control. Heat therapy is to be seen more as a synergic practice, with respect to the use of traditional or new fungicides, as well as a way to enhance the effect of alternative products generally recognized as safe (GRAS)”. In our experiment the epicuticular wax melting and remodelling on the surface of the mandarin fruit played a key role, allowing AAC to exert its efficacy against the pathogen, avoiding treatment damage on the rind.

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