Peracetic Acid and Chlorine Dioxide for Postharvest Control of *Monilinia laxa* in Stone Fruits

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ABSTRACT

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The effects of different concentrations of peracetic acid (PAA; 62.5, 125, 250, 500, and 1,000 µg/ml) and chlorine dioxide (ClO₂; 12.5, 25, 50, 100, and 200 µg/ml) on germination of *Monilinia laxa* conidia were tested. Conidia germination was related to the concentration of chemical product used, as well as duration of treatment. Complete inhibition of germination was observed with PAA at 500 µg/ml after 5 min of contact with conidia and with ClO₂ at 50 µg/ml after 1 min of contact with conidia. The results of in vitro tests were confirmed by inoculation of fruits with treated conidia. The PAA treatment also was effective 1 h after pathogen inoculation but only on plums, for which a 1,000 µg/ml treatment significantly reduced decay incidence by 50%. In a semi-commercial test, pathogen conidia dipped for 20 min in PAA at 250 µg/ml or ClO₂ at 10 µg/ml or for 5 min in PAA at 250 µg/ml were completely inhibited, and no brown rot was observed in inoculated wounded nectarines and plums.

Additional keywords: postharvest decay, sanitizing product

Brown rot caused by *Monilinia laxa* (Aderh. & Ruhl.) Honey is one of the most important diseases found on all commercially grown *Prunus* species in Italy (19). The pathogen develops on fruit in orchards, in packinghouses, during transit and marketing, and in the homes of consumers. Elsewhere, postharvest fungicide treatment of fruit is a routine practice, especially if the fruit is to be stored or shipped over long distances (5). In Italy, however, postharvest treatments are not authorized, hence brown rot control is mainly limited to preharvest measures that are unsatisfactory. Losses attributed to *M. laxa* are considerable, and under certain climatic conditions, brown rot results in substantial damage and market losses (14).

The demand for alternative postharvest disease management practices that could reduce consumer and environmental risks has increased in recent years because of the appearance of resistant strains (11,16) and increasing concerns about hygiene and public health. In the search for alternatives to chemical control, natural compounds with fungitoxic properties are of interest and could be useful for controlling postharvest fruit rots, including *Monilinia* spp. (8,13,22). Moreover, calcium hydroxide also is effective in reducing brown rot incidence, caused

by *M. fructicola*, on unwounded peach fruit (5).

The effectiveness of chlorine in decay prevention has been known for some time (2), although several limitations exist (i.e., the rapid drop in fungistatic activity of chlorine in the presence of organic substances that modify the pH of the solution). For this reason, chlorine dioxide (ClO₂) appears to be preferable, because it is more stable than chlorine and is not corrosive (16). ClO₂ has been used commercially for bacterial control of postharvest fungal decay (15) in pea corn (20), pear (18), and peach (17).

Limited data are available on the effects of peracetic acid (PAA) on postharvest fruit decay. PAA has been tested on tomatoes (10) and degreened oranges (6) with conflicting results.

Our study was conducted to evaluate the effects of several concentrations of ClO₂ and PAA on *M. laxa* conidia germination and control of fruit decay under laboratory and semicommercial conditions.

MATERIALS AND METHODS

Brown rot pathogen. *M. laxa* was isolated from decayed nectarines stored commercially. An isolate with high pathogenic activity on peaches, nectarines, and plums was grown as a monoconidial culture on V8 agar (V8A: 250 ml of V8 vegetable juice and 40 g of agar per 1,000 ml of distilled water). Petri dishes were incubated at 25°C with 12 h of dark/12 h of light cycles for 10 to 14 days. A conidial suspension was prepared by washing colonies grown on V8A in sterile water and Tween 80 (0.05%). Suspension concentrations were adjusted with a hemacytometer.

Fruits studied. Fruits used in this study included nectarines (cv. Stark Red Gold) and plums (cv. Black Ambert) obtained from a local packinghouse. Fruits were free of wounds and rot and were homogeneous in maturity and size. Fruits were stored at 1°C and inoculated within 5 days of harvesting.

Chemical products. Pure PAA solution (Aldrich Chemical Company, Milwaukee, WI) and a chlorine-based product containing 2% ClO₂ (Acquabio-SL, Acquacolor, Bologna, Italy) were used in all trials. The exact concentration of available chlorine was measured spectrophotometrically during each treatment with a reagent kit (Spectroquant, Merck, Darmstadt, Germany).

Effects of PAA and ClO₂ on conidia germination. PAA and ClO₂ were added to *M. laxa* suspensions to obtain final pathogen concentrations of 6×10^5 conidia per ml. ClO₂ was added to obtain final concentrations of available chlorine of 12.5, 25, 50, 100, and 200 µg/ml, and PAA solution was added to obtain final concentrations of 62.5, 125, 250, 500, and 1,000 µg/ml. All mixtures were kept at $2 \pm 1^\circ\text{C}$. After 20 s and 1, 2, 5, and 10 min of conidia contact with sanitizing solution, 0.5 ml of suspension was taken from each concentrate and diluted 100-fold. Aliquots (0.1 ml) of suspensions were spread on petri dishes containing 25 ml of malt extract agar and incubated for 24 h at 20°C. A suspension of untreated conidia (6×10^3 conidia per ml) was used as the control. Ten duplicate samples from one dish were used per treatment. Conidia germination was determined by observing with a light microscope at least 300 conidia from both treatments and the control. Data were expressed as percent conidia germination inhibited compared with the control. The experiment was conducted twice.

Effects of PAA- and ClO₂-treated conidia inoculum on fruits. Nectarines and plums were wounded with a scalpel by removing $3 \times 3 \times 3$ -mm blocks of tissue. Wounds were made on the equator of each fruit: two wounds per nectarine and one wound per plum. Each wound was inoculated with 20 µl of conidia suspension (6×10^3 conidia per ml) that was treated with PAA and ClO₂ at the concentrations described above. Control fruits were inoculated with 20 µl of conidia suspension (6×10^3 conidia per ml) that was not treated with PAA or ClO₂. For nectarines, each test group consisted of 3 sets of 5 fruits each, for a total of 30 wounds. For plums, each test group consisted of 3 sets of 25 fruits each, for a total of 75 wounds. Fruits were

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placed on trays and wrapped in polyethylene bags. Lesion diameter was measured after 6 days at 20°C. The experiment was conducted twice.

Effects of PAA treatment on inoculated fruits. Nectarines and plums were wounded with a sterile needle (two wounds per fruit at the equator) and inoculated by dipping for 1 min in a *M. laxa* suspension of 8×10^3 conidia per ml. After inoculation, fruits were kept at 20°C for 1 h, dipped for 20 s or 1 min in PAA solution (500 or 1,000 µg/ml). Concentration used depended on test group. Control fruits were inoculated with *M. laxa* and, after inoculation, were dipped in water for 1 min under

Fig. 1. Inhibition of *Monilinia laxa* conidia germination when suspended in peracetic acid solution for varied exposure times and treatment concentrations. Aliquots (0.1 ml) of a 1:99 dilution of *M. laxa* suspension were spread on malt extract agar dishes and incubated for 24 h at 20°C. Ten samples from one dish were used per treatment. Conidia germination was determined by observing at least 300 conidia under a light microscope for both treatments and the control. Data were expressed as percent germination inhibition in treated conidia compared with the control.

Fig. 2. Inhibition of *Monilinia laxa* conidia germination when suspended in a chlorine dioxide solution at varied exposure times and treatment concentrations. Aliquots (0.1 ml) of a 1:99 dilution of *M. laxa* suspension were spread on malt extract agar dishes and incubated for 24 h at 20°C. Ten samples from one dish were used per treatment. Conidia germination was determined by observing at least 300 conidia under a light microscope for both treatments and the control. Data were expressed as percent germination inhibition of treated conidia compared with the control.

the same conditions used for treated fruits. Fruits were kept at 20°C for 6 days, and the percentage of infected wounds was determined. The single test group consisted of 3 sets of 25 fruits each, for a total of 150 wounds per group. The experiment was performed twice.

Effects of PAA and ClO₂ treatments in a semicommercial test. Nectarines and plums were dipped in 50-L tanks containing *M. laxa* at a concentration of 2×10^3 conidia per ml. At the start of the test, control fruits were dipped for 1 min before adding sanitizing products to the tanks; ClO₂ or PAA was used at concentrations of 10 and 250 µg/ml respectively. Fruits were dipped for 1 min at 1, 4, 9, and 19 min after addition of chemicals. Fruits were incubated and examined as previously described. Each treatment consisted of 3 sets of 150 fruits each, for a total of 900 wounds. The experiment was performed twice.

Data analysis. A completely randomized design was used in each test. Statistical analysis was performed using the SYSTAT 5.2 software package (21). All data on the percentage of infected fruits were subjected to one-way analysis of variance. Before analysis of data, homogeneity of variance was tested by Bartlett's test. Mean separation was performed using the least significant difference test ($P = 0.05$).

RESULTS

Effects of PAA and ClO₂ on conidia germination. The effects of PAA and ClO₂ on *M. laxa* conidia germination were directly related to the concentration of chemical and duration of treatment. For PAA, the lowest concentration tested (62.5 µg/ml) produced only a small (22.7%) reduction in conidia germination, even after 10 min of exposure (Fig. 1). At 250 and 500 µg/ml, conidia growth was inhibited by 70.6% after 2 min and 59.3% after 1 min, respectively. The highest concentration of PAA (1,000 µg/ml) reduced conidia germination to 50.55% after only 20 s of treatment. To-

tal inhibition of conidia germination was observed after 5 and 10 min of exposure to PAA at 500 µg/ml.

For ClO₂, 100 µg/ml completely inhibited conidia germination, regardless of duration of dipping (Fig. 2). At 50 µg/ml, ClO₂ reduced the rate of germination by more than 50% after only 20 s of exposure. Lowering the concentration of ClO₂ to 25 µg/ml resulted in 56.9% inhibition of conidia germination after 1 min of exposure. The lowest concentration of ClO₂ (12.5 µg/ml) continued to effect germination after 5 min of treatment, resulting in 82.5% inhibition of germination.

Effects of PAA- and ClO₂-treated conidia inoculum on fruits. Fruits inoculated with treated conidia developed lesions with diameters varying according to the concentration of sanitizing chemical used in the treatment, duration of exposure to the treatment, and species of inoculated fruit. Fruits inoculated with conidia treated for 1 min with PAA at 1,000 µg/ml developed the smallest lesions (Fig. 3). In fact, under these treatment conditions there was almost total disease control in both nectarines (Fig. 3A) and plums (Fig. 3B). Nectarines inoculated with conidia treated for 20 s with ClO₂ at 100 µg/ml did not develop brown rot (Fig. 4A). Similar results were obtained for plums inoculated with conidia treated for 2 min with ClO₂ at 200 µg/ml (Fig. 4B).

Effects of PAA treatment on inoculated fruits. Treatment of previously inoculated nectarines with PAA did not result in any reduction in incidence of infected wounds, although there were some significant reductions in infected wounds in plums (Table 1). Dipping inoculated fruits for 20 s and 1 min in a PAA solution of 1,000 µg/ml significantly reduced the percentage of rot to 22.7 and 24%, respectively, whereas 50.7% of wounds in control fruits were infected. The disease incidence obtained after dipping inoculated fruit in PAA at 500 µg/ml was intermediate between disease incidence

Fig. 3. Effects of peracetic acid (PAA) treatment on conidia of *Monilinia laxa* inoculated into wounded nectarines (A) and plums (B). Wounds were inoculated with 20 µl of a conidial suspension (6×10^3 conidia per ml) previously treated with PAA at 0, 250, 500, and 1,000 µg/ml. Lesion diameters were recorded after 6 days at 20°C. Each point is the average of at least 30 wounds; vertical bars represent standard errors.

in the control and in the 1,000 µg/ml PAA treatment (Table 1).

Effects of PAA and ClO₂ treatment in a semicommercial test. On nectarines and plums, decay was significantly reduced when conidia remained in a PAA solution of 250 µg/ml for 2 min (Table 2). Exposure of conidia to ClO₂ at 10 µg/ml for 2 min reduced incidence of infected wounds by more than 55%. When exposure of conidia to chemicals was prolonged to 4 min, no decay developed on nectarines or plums dipped for 1 min in PAA at 250 µg/ml or for 19 min in ClO₂ at 10 µg/ml (Table 2).

DISCUSSION

Previously PAA has been used almost exclusively as a sanitizing agent in different sterilization processes, such as heat-sensitive equipment (3) and ultrafiltration membranes in water-treatment systems (1). The results of our in vitro trials confirmed considerable fungicidal activity against *M. laxa* by PAA. Germination of conidia was inhibited by PAA treatments; the degree of inhibition depended on PAA concentration and duration of exposure. The best results were observed with PAA at 1,000 µg/ml and an exposure time of at least 2 min.

When fruits were inoculated with *M. laxa* conidia exposed to PAA at 250 µg/ml for 5 min or 500 µg/ml for 2 min, no decay developed in either nectarines or plums. PAA at 50 µg/ml also had high fungicidal activity against *Aspergillus niger* and *Penicillium roqueforti* in tomatoes after 10 min of exposure (10) but not against blue mold, caused by *P. digitatum*, in oranges, even when PAA was used at 500 µg/ml (6).

Recently, chlorine dioxide has replaced chlorine in some sanitizing processes, because several disadvantages limit the use of chlorine, including its unpleasant odor. ClO₂ in in vitro trials was active against *M. laxa*. Exposure of conidia to ClO₂ at 50 µg/ml for 1 min completely prevented conidia germination, confirming the results obtained with other postharvest pathogens, such as *Botrytis cinerea*, *Mucor piriformis*, and *P. expansum* (18). However, when fruits were inoculated with treated conidia, treatment effects varied as a function of exposure time, concentration of ClO₂, and species tested.

Chlorine only kills by contact, not systemically, and is effective only on exposed fungal propagules, such as those suspended in water or on the surface of fruit. Chlorine does not kill pathogens under the fruit skin or after infection has occurred. Chlorine did not control decay caused by *M. piriformis* when used after inoculation (4). One hour after inoculation the fungicidal activity of PAA was significant on plums but not nectarines. The different fungicidal response on nectarines and plums may be explained by the decomposition rate of PAA, which depends on the nature and pH of the fruit surface (10).

Prolonged (20 min) exposure of *M. laxa* conidia to solutions of PAA (250 µg/ml) or

ClO₂ (10 µg/ml) caused devitalization of conidia, which was indicated by the absence of brown rot on nectarines and plums wounded and dipped for 1 min in sanitizing solution. The results obtained in the semicommercial tests appear to have practical applications. In the packinghouse, wash water used as the handling medium appears to be a source of inoculum for plums and nectarines fruits. Introducing a biologically active concentration of ClO₂ or PAA into the wash water could reduce the level of conidia with-

out the need for pesticides. Biocidal ClO₂ or PAA also could be successfully used during hydrocooling, because the technique involves leaving fruits for 30 to 45 min or more in chilled (4°C) water. This amount of time would allow both ClO₂ and PAA to act more effectively against *M. laxa*. Moreover, prerefrigeration of wash water, which limits weight loss and permits quick chilling of fruits, frequently can make fruits more susceptible to decay, especially if the fruit is not sold within a short time (19).

Fig. 4. Effects of chlorine dioxide (ClO₂) treatment on conidia of *Monilia laxa* inoculated into wounded nectarines (A) and plums (B). Wounds were inoculated with 20 µl of a conidial suspension (6×10^3 conidia per ml) previously treated with ClO₂ at 0, 50, 100, and 200 µg/ml. Lesion diameters were recorded after 6 days at 20°C. Each point is the average of at least 30 wounds; vertical bars represent standard errors.

Table 1. Effect of peracetic acid (PAA) treatment on stone fruits^y inoculated with *Monilia laxa*

PAA concentration (µg/ml)	Exposure time (s)	Infected wounds (%) ^z	
		Nectarine	Plum
0	...	23.8 a	50.7 a
500	20	23.1 a	35.3 b
500	60	18.8 a	39.3 b
1,000	20	16.3 a	22.7 c
1,000	60	21.3 a	24 c

^y Wounded fruits were inoculated by dipping for 1 min in a *M. laxa* suspension (8×10^3 conidia per ml). After inoculation (1 h), fruits were dipped for 20 s or 1 min in PAA solution at 500 or 1,000 µg/ml. Fruits inoculated with the pathogen and dipped in water served as controls. All fruits were stored at 20°C for 6 days. A single test group consisted of 3 sets of 25 fruits each, for a total of 150 wounds per group. Data from two experiments were pooled.

^z Values in each column that are not significantly different based on the LSD test ($P = 0.05$) are followed by the same letter.

Table 2. Effect of peracetic acid (PAA) and chlorine dioxide (ClO₂) treatments on stone fruits inoculated with *Monilia laxa* under semicommercial conditions

Time exposed to conidia (min)	Infected wounds ^{y,z} (%)			
	Nectarine		Plum	
	PAA	ClO ₂	PAA	ClO ₂
0	35.6 a	35.6 a	65.6 a	65.6 a
2	6.6 b	15.6 b	10 b	26.7 b
5	0 c	6.7 bc	0 b	6.8 bc
10	0 c	5.7 bc	0 b	7.8 bc
20	0 c	0 c	0 b	0 c

^y Control fruits were dipped for 1 min in a *M. laxa* suspension (2×10^3 conidia per ml), to which ClO₂ (10 µg/ml) or PAA (250 µg/ml) was added. Treated fruits were dipped for 1 min at 1, 4, 9, and 19 min after chemical product was added. All fruits were stored for 6 days at 20°C. A single test group consisted of 3 sets of 150 fruits each, for a total of 900 wounds per group. Data from two experiments were pooled.

^z Values in each column that are not significantly different based on the LSD test ($P = 0.05$) are followed by the same letter.

Chlorine dioxide is inactivated by organic matter (9), although to a lesser degree than other chlorine compounds, and progressively deteriorates, with a consequent drop in antimicrobial activity, making frequent monitoring of pH and chlorine concentration necessary. ClO_2 is not corrosive on packing-house equipment, and it is effective over a wide pH range. However, the 10 $\mu\text{g}/\text{ml}$ concentration necessary to obtain significant fungicidal activity would be too expensive, and therefore, effective lower concentrations are desirable (18).

PAA has greater stability, faster biocidal properties, is not pH dependent (7), and has lower toxicity than ClO_2 . For example, the oral 50% lethal dose in rats is 1,540 mg/kg (12). Problems associated with corrosiveness could be reduced by use of commercial formulations containing a lower concentration of PAA and no sulfuric acid, making it suitable for use with stainless-steel equipment (3).

Sanitizing solutions can be integrated easily with hydrocooling systems to reduce post-harvest fruit decay. However, the low temperature used in hydrocooling reduces the efficacy of both ClO_2 and PAA. As with other chemical processes, sanitation takes place at a slower rate as temperatures fall, although it is not completely inhibited. The current study provides useful information for determining the optimal conditions for control of brown rot on stone fruits by biocidal PAA and ClO_2 products. However, further research is required to better define effective procedures.

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