

# Effects of Calcium Salts on Growth, Polygalacturonase Activity, and Infection of Peach Fruit by *Monilinia fructicola*

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## ABSTRACT

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The effects of several calcium salts on growth, polygalacturonase (PG) activity, and infection of peach fruit by *Monilinia fructicola* were determined. All salts except calcium formate, calcium pantothenate, and dibasic calcium phosphate reduced growth of *M. fructicola* on amended potato-dextrose agar (PDA) after 7 days. Minimal growth occurred on PDA amended with calcium propionate. Calcium hydroxide, calcium oxide, calcium silicate, and calcium pyrophosphate reduced growth by approximately 65% compared with the control. Substances that were inhibitory on amended PDA were also inhibitory in potato-dextrose broth (PDB) but varied in effectiveness depending on whether PDB or PDA was used. The correlation between the amount of growth on PDA versus that in PDB was not significant. Fungal PG activity was inhibited by all salts used in this study except dibasic calcium phosphate and calcium tartrate. Greatest inhibition of PG was associated with calcium propionate followed by calcium sulfate, tribasic calcium phosphate, calcium gluconate, and calcium succinate. The activity of calcium salts was not affected by pH. PG activity was correlated with growth on PDA ( $r_s = 0.48$ ,  $P \leq 0.04$ ) but was not correlated with growth in PDB. When inoculum was sprayed on detached fruit, the incidence and severity of brown rot were least on fruit that had been dipped in solutions of calcium propionate or calcium silicate. When inoculum was applied as a localized drop to wounded fruit that had been dipped in a solution containing 1,200 mg of calcium per liter, brown rot severity was least for fruit treated with calcium oxide and calcium hydroxide. For nonwounded fruit and drop inoculations, calcium hydroxide was the most effective in reducing brown rot incidence, and all salts reduced rot severity similarly. There were significant correlations between growth in PDB and disease incidence and severity 4 days after inoculation at both calcium levels (600 and 1,000 mg per liter). Furthermore, disease incidence and severity at both levels of calcium were also correlated significantly with PG activity. Future investigations on brown rot and calcium salts should utilize PDB for in vitro investigations and spray mist inoculations for fruit studies.

Brown rot, caused by *Monilinia fructicola* (G. Wint.) Honey, is one of the most important diseases of stone fruit in the world's fruit-growing regions (5). Field losses of peaches, plums and prunes, sweet and tart cherries, and apricots can be extensive if conditions are favorable for disease development during the blossom period, after shuck fall, or during the preharvest and harvest periods (23). Disease control is achieved primarily through the use of fungicides to protect blossoms and ripening fruit, although orchard sanitation is an integral part of the disease-management program.

Postharvest losses of stone fruit caused by brown rot may be significant in some

years, and fungicide treatment of fruit after harvest is routine, especially if fruit is to be stored and/or shipped long distances. With the recent concern regarding pesticide residues on fruit (18), there is a need for alternative postharvest disease-management practices that will reduce risk to consumers. Increasing the calcium content of other fruits and vegetables with calcium salts has increased storage life, mainly as a result of the role of calcium in changing physiological and reducing pathological disorders (2,11). Most research on enhancing storage quality and reducing postharvest decay with calcium supplementation has been done with apples, even though peaches have a much shorter storage life (6-8,11). In this study, we investigated the effect of several calcium salts on in vitro growth and polygalacturonase (PG) activity of *M. fructicola* and infection and colonization of harvested peach fruit.

## MATERIALS AND METHODS

**Effect of various calcium salts on fungal growth.** Chemically pure calcium salts used in this study are shown in Table 1.

Salts were prepared in sterile, deionized, distilled water and were added to autoclaved, warm (45 to 55°C) 2% potato-dextrose agar (PDA) to provide a final concentration of 600 mg of calcium per liter, and the medium was poured into petri dishes 9 cm in diameter. The pH of the supplemented medium was determined in preparations that were not autoclaved. An agar disk 5 mm in diameter, taken from an active colony of *M. fructicola* originating from diseased nectarine at the University Experiment Farm, was placed in the center of each of four replicate dishes. Dishes were incubated in the dark at  $20 \pm 2^\circ\text{C}$ , and growth was assessed after 7 days by measuring two colony diameters at right angles to each other. The experiment was performed twice.

In an additional experiment, the fungus was grown in stationary flasks containing 2% potato-dextrose broth (PDB) amended with 600 mg of calcium per liter provided by the same salts (Table 1). Growth was assessed after 14 days by determining the dry weight of the mycelium. The experiment was performed twice with four replicate flasks per treatment. Data were analyzed with analysis of variance, and means were separated with the Waller-Duncan *k*-ratio *t* test (SAS Institute, Cary, NC).

To determine the effect of pH and the interaction of pH with calcium salts on the growth of *M. fructicola* in vitro, various calcium solutions (chloride, oxide, hydroxide, propionate, and silicate at 600 mg of calcium per liter) prepared with nanopure water were added to PDB, and the pH was adjusted to approximately 6, 7, 8, 9, or 10 by adding appropriate amounts of either 1 N HCl or NaOH. The control was PDB without calcium at each pH level. Inoculum consisted of one 3-mm-diameter plug per flask taken from a culture actively growing on PDA. Flasks were incubated at 22°C, and growth was determined 10 days after inoculation by filtering the medium through preweighed filters and then weighing the dried residual mycelium after 7 days at 40°C. The final pH of the culture filtrate was determined. The experiment was conducted three times with single replicates, data were analyzed with analysis of variance, and means were separated with the Waller-Duncan *k*-ratio *t* test.

**Effect of calcium salts on fungal PG activity.** Plugs (5 mm in diameter) of *M. fructicola*, grown on PDA, were transferred to stationary 250-ml flasks contain-

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ing 50 ml of 2% PDB that was either not amended or amended with one of 18 calcium salts to yield 600 mg of calcium per liter. The pH of the supplemented medium was determined before autoclaving and adjusted to 5.8 with either 0.1 N HCl or NaOH. Flasks were incubated for 10 days at  $20 \pm 2^\circ\text{C}$ , and then the filtrates were collected and analyzed immediately for PG activity (24). PG activity was tested on sodium polypectate at pH 4.5 in 0.1 M acetate buffer (24). Enzyme activity was determined spectrophotometrically at 510 nm and expressed as 100 times the increase in absorbance per hour per milliliter of broth per gram of dry weight of fungal mycelium. Each treatment had two replicates, and the experiment was conducted twice. Data were analyzed as described above. In addition, Spearman's rank correlation procedure was used to assess the relationship between PG activity and growth on PDA and PDB (SAS Institute, Cary, NC).

**Effect of calcium salts on infection of peaches.** *Spray inoculations of non-wounded fruit.* In August 1993, ripe peach fruit (cultivar Loring) from the University Experiment Farm were harvested and brought into the laboratory for dip treatment with several calcium salts (Table 2). The fruit had received no fungicide or insecticide sprays during the preharvest period. Of the 20 fruit, 10 were dipped for 5 min in calcium solutions prepared to yield either 600 or 1,000 mg of calcium per liter and 10 control fruit were dipped in distilled water for 5 min. After dipping, the

fruit were placed in paper trays within opaque plastic boxes and allowed to air dry. Fruit were misted with a suspension containing  $3 \times 10^6$  conidia of *M. fructicola* per milliliter determined with a hemacytometer. High humidity (>95% during the first 24 h of incubation) was maintained by the addition of wet paper towels to the boxes. Fruit were examined daily and rated for brown rot incidence and severity (percentage of fruit surface rotted). Average temperature during the incubation period was  $22 \pm 2^\circ\text{C}$ . The experiment was performed twice, data were subjected to analysis of variance, and means were separated with the Waller-Duncan *k*-ratio *t* test. Spearman's rank correlation procedure was used to examine the relationships between disease incidence and severity at 4 days after inoculation and mycelial growth on amended PDA and PDB and PG activity.

*Droplet inoculations of nonwounded and wounded fruit.* In July and August 1995, ripe peach fruit from cultivars Redhaven and Loring were harvested and dipped for 15 min in calcium solutions (hydroxide, oxide, propionate, and silicate) prepared at 1,200 mg of calcium per liter. Prior to dipping, each Redhaven fruit was wounded uniformly with a blunted nail that created a wound approximately 3 mm<sup>3</sup>. Fruit were inoculated approximately 1 h after being removed from the dip solutions by using a suspension containing  $1 \times 10^5$  conidia of inoculum per milliliter prepared as described above. Approximately 30  $\mu\text{l}$  of conidial suspension was placed in each

wound, and the fruit were incubated in the dark at  $25^\circ\text{C}$ . Lesion diameter (mean of two measurements at right angles to each other) was measured after 48 h. Controls included fruit that were wounded, dipped in water, and then inoculated and fruit that were wounded only (neither dipped nor inoculated). The experiment was conducted twice with three replicates of 10 fruit per treatment. Treatment of Loring fruit was similar to that of Redhaven, except that they were not wounded and fruit were inoculated with a 30- $\mu\text{l}$  drop of conidial suspension placed on the surface 5 mm below a mark made by an indelible pen. This experiment was conducted three times with three replicates of 10 fruit per treatment. In the first trial, inoculum was prepared in sterile distilled water and fruit were rated for infection and lesion diameter after 7 days. In the two other trials, inoculum was prepared in Miller's solution (15) and fruit disease incidence and severity were evaluated 4 days after inoculation. Miller's solution was used to improve the proportion of successful inoculations and thus reduce the amount of variability in incidence of infection among trials (19). Controls included fruit dipped in water and then inoculated with conidia and fruit dipped in water and then treated with 30  $\mu\text{l}$  of sterile distilled water. Mean fruit firmness (kg) and Brix (percentage of soluble solids) values were determined on a subsample of 10 fruit on each inoculation date with a firmness tester (Wagner Instruments, Greenwich, CT) and hand refractometer (Atago N1, Japan), respectively. The data were subjected to analysis of variance, and means were separated with the Waller-Duncan *k*-ratio *t* test.

**Table 1.** Colony diameter on potato-dextrose agar (PDA) after 7 days, mycelial dry weight in potato-dextrose broth (PDB) after 14 days, and polygalacturonase activity (relative activity [RA]/ml/mg of fungal dry weight) of *Monilinia fructicola* on media amended with various calcium salts (600 mg of calcium per liter)

| Treatment                   | pH   | Colony diameter on PDA (mm) | Dry weight in PDB (mg) | RA/ml/mg <sup>x</sup> |
|-----------------------------|------|-----------------------------|------------------------|-----------------------|
| Control                     | 4.3  | 70.0 b <sup>y,z</sup>       | 0.195 bc <sup>z</sup>  | 79.5 a                |
| Calcium acetate             | 5.8  | 45.7 I                      | 0.135 fgh              | 32.9 def              |
| Calcium chloride            | 4.4  | 56.5 g                      | 0.110 ijk              | 28.2 g                |
| Calcium formate             | 4.9  | 71.5 ab                     | 0.105 ijk              | 28.9 fg               |
| Calcium gluconate           | 4.8  | 62.5 de                     | 0.145 efg              | 21.6 h                |
| Calcium heptagluconate      | 4.8  | 63.5 d                      | 0.210 b                | 33.7 de               |
| Calcium hydroxide           | 11.1 | 21.2 k                      | 0.115 hij              | 30.8 efg              |
| Calcium lactate             | 4.8  | 61.5 e                      | 0.155 def              | 49.8 b                |
| Calcium nitrate             | 4.2  | 66.5 c                      | 0.125 ghi              | 44.5 c                |
| Calcium oxide               | 10.9 | 22.5 jk                     | 0.110 ijk              | 36.1 d                |
| Calcium pantothenate        | 5.5  | 69.8 b                      | 0.120 hi               | 30.1 efg              |
| Calcium phosphate, dibasic  | 5.0  | 72.2 a                      | 0.105 ijk              | 75.7 a                |
| Calcium phosphate, tribasic | 4.8  | 49.2 h                      | 0.245 a                | 19.0 h                |
| Calcium propionate          | 5.8  | 7.0 I                       | 0.090 klm              | 12.2 i                |
| Calcium pyrophosphate       | 4.4  | 23.2 j                      | 0.070 m                | 29.0 fg               |
| Calcium silicate            | 7.3  | 22.8 jk                     | 0.095 jkl              | 30.6 efg              |
| Calcium succinate           | 6.0  | 58.0 fg                     | 0.175 cd               | 22.6 h                |
| Calcium sulfate             | 4.2  | 58.5 f                      | 0.175 cd               | 18.5 h                |
| Calcium tartrate            | 4.3  | 58.8 f                      | 0.165 de               | 79.7 a                |

<sup>x</sup> Relative activity = thousandths of a second to reach 50% loss of viscosity of a 1% solution of substrate. Activity was tested on sodium polypectate at pH 5. Each value is the mean of four observations from the combined data of two experiments.

<sup>y</sup> Each value is the mean of eight observations from the combined data of four replicates from two experiments.

<sup>z</sup> Different letters in a column denote significant differences according to the Waller-Duncan *k*-ratio *t* test ( $P \leq 0.01$ ).

## RESULTS

**Effect of calcium salts on fungal growth.** All salts except calcium formate, calcium pantothenate, and dibasic calcium phosphate significantly reduced growth of *M. fructicola* on amended PDA after 7 days (Table 1). Calcium propionate was the most inhibitory, reducing growth on PDA by 90%. Calcium hydroxide, calcium oxide, calcium silicate, and calcium pyrophosphate reduced growth by approximately 65% of the control and were not significantly different from each other (Table 1). The least growth in liquid medium was with calcium pyrophosphate and calcium propionate, which reduced growth by 64 and 54%, respectively. In general, salts that were inhibitory in the amended agar medium were also inhibitory in liquid culture (Table 1), although several salts that were not inhibitory to growth on PDA were inhibitory in PDB and vice versa. Tribasic calcium phosphate stimulated growth of *M. fructicola* in liquid culture. The correlation between the amount of growth on PDA and that in PDB was not statistically significant.

When calcium salts were prepared at different levels of pH, analysis of variance showed that pH was not significant (data not shown). The fungus grew equally well across the pH range of 6 to 10. In this experiment, only calcium propionate reduced growth, causing a 50% reduction in mycelial dry weight (0.082 g) relative to the control (0.164 g;  $P \leq 0.05$ ).

**Effect of calcium salts on fungal PG activity.** PG activity was inhibited by all salts in this study, except dibasic calcium phosphate and calcium tartrate (Table 1). The greatest inhibition of PG was caused by calcium propionate (85%), followed by calcium sulfate, tribasic calcium phosphate, calcium gluconate, and calcium succinate. These four salts reduced PG activity by approximately 75%, which was significantly different from the reduction caused by calcium propionate (Table 1). Calcium chloride, calcium hydroxide, and calcium oxide reduced PG activity by 65, 61, and 55%, respectively. For the entire set of 18 salts, PG activity was correlated with growth on PDA ( $r_s = 0.48$ ,  $P \leq 0.04$ ) but was not correlated with growth in PDB. However, for the subset of calcium salts used in the spray inoculation experiments (described below), PG activity was correlated significantly with fungal growth in PDB ( $r_s = 0.80$ ,  $P \leq 0.02$ ).

**Effect of calcium salts on infection of peaches.** *Spray inoculations of non-wounded fruit.* When fruit were dipped in solutions containing 600 mg of calcium per liter, inoculated, and incubated for 4 days, the incidence and severity of brown rot were least on fruit treated with calcium propionate (Table 2), although these values were not significantly different from those of the calcium silicate treatment. After 8 days of incubation, fruit in these two treatments still exhibited the lowest incidence of brown rot; however, expansion of lesions that did develop (disease severity) was not

affected by calcium treatment at this time. After 12 days of incubation, only fruit treated with calcium propionate showed reduced incidence and severity of brown rot relative to the control ( $P \leq 0.05$ ) (Table 2).

When fruit were dipped in solutions containing 1,000 mg of calcium per liter, inoculated, and incubated for 4 days, the incidence of brown rot was least on fruit treated with calcium propionate (Table 2), although the results of this treatment were significantly different only from those of calcium pyrophosphate and the control. Disease severity after 4 days of incubation was reduced significantly by all treatments, except calcium pyrophosphate. At 8 days postinoculation, incidence of brown rot was least on fruit treated with calcium propionate and calcium silicate (Table 2). Disease severity was least on fruit treated with calcium propionate or calcium hydroxide. After 12 days, brown rot incidence was again least on fruit treated with calcium silicate and calcium propionate, and severity was least on fruit treated with calcium propionate and calcium silicate, although results of the latter treatment were not significantly different from those of the calcium hydroxide treatment (Table 2).

There were significant correlations between growth in PDB and disease incidence and severity after 4 days at both levels of calcium. For 600 mg of calcium per liter, the correlation coefficients were  $r_s = 0.92$  ( $P \leq 0.03$ ) and  $0.97$  ( $P \leq 0.005$ ) for incidence and severity, respectively. For 1,000 mg of calcium per liter, the correlation coefficients were  $r_s = 0.82$  ( $P \leq 0.09$ ) and  $0.97$  ( $P \leq 0.005$ ) for incidence and severity, respectively. Furthermore, disease incidence and severity after 4 days at both levels of calcium were also correlated significantly with PG activity. For the subset of six salts used in the fruit inoculations, growth in PDB was cor-

related significantly with PG activity ( $r_s = 1.0$ ). Disease incidence after 4 days on fruit treated with 1,000 mg of calcium per liter was correlated with growth on PDA ( $r_s = 0.90$ ,  $P \leq 0.04$ ).

*Droplet inoculations of nonwounded and wounded fruit.* Incidence of brown rot on wounded fruit (Redhaven) was 100% for all salts. In nonwounded fruit (Loring), mean disease incidence (for three trials) ranged from 86% in the water-dipped control to 29% for fruit dipped in calcium hydroxide. Calcium hydroxide reduced disease incidence compared with the control in trials 2 and 3 ( $P \leq 0.05$ ) but not in trial 1. Mean disease incidence with calcium silicate (45.6%) and calcium oxide (44.4%) was also significantly different from that of the control and was similar to that of calcium hydroxide. Disease incidence with calcium propionate (63.3%) was not significantly different from that of the control nor was it different from that of calcium silicate or calcium oxide. The salts performed consistently across all trials of the experiment (treatment  $\times$  trial interaction was not significant in the analysis of variance).

The effect of salt on disease severity was more variable than that on incidence. Infection and colonization of wounded fruit by *M. fructicola* progressed rapidly; thus, we chose to limit the time period to 2 days to accurately determine disease severity. For wounded fruit, all calcium salts reduced lesion diameter relative to the control; calcium oxide and hydroxide provided the most reduction in both trials of the experiment (Table 3). For nonwounded fruit, calcium hydroxide was the best treatment for reducing rot severity when conidia were suspended in water (trial 1); but when conidia were supplemented with nutrients to reduce variation in disease incidence (trials 2 and 3), all the salts inhibited rot development similarly (Table 3).

**Table 2.** Effects of various calcium salts (600 and 1,000 mg of calcium per liter) on incidence (%) and severity (%) of peach fruit infection by *Monilinia fructicola* 4, 8, and 12 days after inoculation with conidia by uniform mist application

| Treatment                     | 4 days postinoculation |                       | 8 days postinoculation |          | 12 days postinoculation |          |
|-------------------------------|------------------------|-----------------------|------------------------|----------|-------------------------|----------|
|                               | Incidence <sup>y</sup> | Severity <sup>y</sup> | Incidence              | Severity | Incidence               | Severity |
| 600 mg of calcium per liter   |                        |                       |                        |          |                         |          |
| Control                       | 65.0 a <sup>z</sup>    | 49.7 a                | 100.0 a                | 82.1 a   | 100.0 a                 | 100.0 a  |
| Calcium formate               | 50.0 ab                | 35.2 b                | 70.0 ab                | 76.2 a   | 100.0 a                 | 87.4 ab  |
| Calcium hydroxide             | 50.0 ab                | 30.6 b                | 90.0 ab                | 81.8 a   | 100.0 a                 | 100.0 a  |
| Calcium oxide                 | 65.0 a                 | 29.3 b                | 85.0 ab                | 82.3 a   | 100.0 a                 | 100.0 a  |
| Calcium propionate            | 10.0 c                 | 13.8 c                | 30.0 c                 | 45.7 a   | 85.0 b                  | 75.4 b   |
| Calcium pyrophosphate         | 65.0 a                 | 28.2 b                | 80.0 ab                | 72.9 a   | 100.0 a                 | 92.3 ab  |
| Calcium silicate              | 25.0 bc                | 21.6 bc               | 60.0 bc                | 64.7 a   | 100.0 a                 | 89.0 ab  |
| 1,000 mg of calcium per liter |                        |                       |                        |          |                         |          |
| Control                       | 65.0 a                 | 49.7 a                | 100.0 a                | 82.1 a   | 100.0 a                 | 100.0 a  |
| Calcium formate               | 30.0 abc               | 22.7 b                | 50.0 bc                | 66.1 ab  | 95.0 ab                 | 91.3 ab  |
| Calcium hydroxide             | 20.0 bc                | 24.0 b                | 50.0 bc                | 38.4 bc  | 100.0 a                 | 80.0 bc  |
| Calcium oxide                 | 40.0 abc               | 24.1 b                | 55.0 b                 | 56.3 ab  | 85.0 ab                 | 86.6 ab  |
| Calcium propionate            | 5.0 c                  | 13.2 b                | 25.0 d                 | 13.6 c   | 80.0 bc                 | 55.8 d   |
| Calcium pyrophosphate         | 50.0 ab                | 34.2 ab               | 70.0 b                 | 74.0 ab  | 100.0 a                 | 90.8 ab  |
| Calcium silicate              | 15.0 bc                | 20.4 b                | 30.0 cd                | 47.5 abc | 75.0 c                  | 67.2 cd  |

<sup>y</sup> Incidence = percentage of fruit exhibiting brown rot lesions; severity = percentage of fruit surface area with brown rot (infected fruit only). Each value is the mean of 20 observations from the combined data of two experiments. All inoculations were with  $3 \times 10^6$  conidia per milliliter.

<sup>z</sup> Different letters in a column denote significant differences according to the Waller-Duncan *k*-ratio *t* test ( $P \leq 0.05$ ).

## DISCUSSION

This study has demonstrated the general toxicity of calcium salts to growth of *M. fructicola* in vitro. Of the 18 salts tested on PDA, 15 were slightly to strongly inhibitory, reducing growth by 5 to 90%; in PDB, 14 salts were slightly to moderately inhibitory, reducing growth by 15 to 64%. Salts that were not inhibitory in the PDA assay caused inhibition in the PDB assay and vice versa. Sixteen of the 18 salts inhibited PG activity by 37 to 85%.

The effect of calcium salts on fungal growth in PDB (but not on PDA) was correlated significantly with the incidence and severity of disease in the spray inoculation experiments (at both levels of calcium tested) but not in the droplet inoculation experiments. In addition, the effect of calcium salt on dry weight accumulation in the PDB tests was correlated with PG activity for the subset of salts used in the 1993 inoculation experiments. The only significant correlation with mycelial growth on PDA occurred in the 1993 experiment with disease incidence 4 days after inoculation at 1,000 mg of calcium per liter. On the basis of these results, it can be concluded that future tests should utilize growth in PDB, not on PDA, to determine preliminary toxicity levels. Calcium salts are more likely to remain in continuous contact with fungal mycelium in the PDB system, whereas in PDA, calcium could become depleted or rendered unavailable (i.e., converted to calcium oxalate) adjacent to mycelium.

The method used for fruit inoculations also influenced the results of our tests. When conidia were applied in an atomized suspension over the nonwounded fruit surface (1993 trials), results correlated with growth in PDB and PG activity. Conidia applied as droplets to wounded or intact fruit (1995 trials) provided different results regarding the relative efficacy ranking of the different salts, and there was no significant correlation to the in vitro test results. Perhaps the placement of discrete

droplets of inoculum at the calcium concentrations used overwhelmed inhibitory potential of the different salts. On the basis of these results, it was concluded that inoculum should be applied as a mist of conidia over the exposed fruit surface rather than as a discrete, localized droplet.

In previous studies with calcium salts, Conway et al. (7) found that peach fruit that had been sprayed with calcium chloride had 70% more calcium than untreated controls but did not resist decay, whereas fruit that were dipped and pressure infiltrated had two to four times more calcium and resisted infection but were physically injured by the pressure treatments (10). In our test, calcium chloride did not inhibit growth of *M. fructicola* on PDA as strongly as some other salts and because of this and problems reported by others (see below), was not selected for further tests on fruit. The effectiveness of calcium chloride against brown rot has been ascribed to the enhancement of host resistance by calcium (9). In our tests, calcium salts, including calcium chloride, reduced fungal growth and PG activity, suggesting the possibility that the observed effects of calcium in vivo may result partly from suppressed pathogen activity. Calcium chloride also inhibited colonization of excised peach twigs by *Leucostoma persoonii* (3). However, the association of calcium chloride with fruit injury (7) and foliar injury (22) may limit its use in commercial peach production.

Preharvest sprays of calcium nitrate have reduced decay during storage in peaches naturally infected with *Rhizopus stolonifer* (25). In our tests, calcium nitrate inhibited growth of *M. fructicola* only slightly on agar but moderately reduced dry weight and inhibited PG activity. Our results with calcium silicate and calcium formate were slightly different from those reported by Adaskaveg et al. (1), which showed no in vitro toxicity of calcium formate and calcium silicate to *M. fructicola*. On two of the three peach genotypes they

examined, calcium formate and calcium silicate controlled *M. fructicola* on fruit, and control of disease by calcium formate was equal to that of the fungicide iprodione. In the present study, we demonstrated in vitro toxicity of calcium silicate to *M. fructicola* in both agar and liquid culture and of calcium formate only in liquid culture. Both salts reduced PG activity with our *M. fructicola* isolate. However, for fruit treated with calcium formate, we were unable to reduce disease incidence relative to the control and reduced severity only after 4 days of incubation for both calcium concentrations.

The mode of action of the active calcium compounds in this system is unknown at present. It is possible that the Ca<sup>2+</sup> ion stimulates the synthesis of phytoalexins and/or phenols (14) or, alternatively, that Ca<sup>2+</sup> reduces the effectiveness of fungal PG enzymes by forming cation cross bridges between pectic acids in the plant cell walls, thus making the cell walls more resistant to digestion (9). However, the high concentrations of calcium that must be present in host cell walls in order to provide effective cross bridging in apple tissues (approximately 800 µg/g of dry weight) (4), although not measured, probably were not achieved in the present study.

Another hypothesis raised by our data concerns the effects of selected calcium compounds on PG activity, i.e., that Ca<sup>2+</sup> may act directly on the pathogen and cause reduced virulence or, in the extreme, fungistasis. Calcium propionate was the most effective treatment for reducing production of PG by *M. fructicola*; however, four other materials resulted in reduction of PG activity by approximately 75%. None of the calcium salts in the present study were fungicidal at the concentrations examined; however, the reductions in disease incidence obtained with calcium propionate in the mist inoculation experiments and with the other calcium salts in the droplet inoculation experiments were com-

**Table 3.** Brown rot incidence (%) and severity (lesion diameter [mm]) on peach cultivars Redhaven and Loring fruit<sup>w</sup> treated with various calcium salts and inoculated with conidia of *Monilinia fructicola* in droplets

| Treatment          | Wounded (Redhaven) <sup>x</sup> |         | Not wounded (Loring) <sup>x</sup> |          |           |          |           |          |
|--------------------|---------------------------------|---------|-----------------------------------|----------|-----------|----------|-----------|----------|
|                    | Severity                        |         | Trial 1                           |          | Trial 2   |          | Trial 3   |          |
|                    | Trial 1                         | Trial 2 | Incidence                         | Severity | Incidence | Severity | Incidence | Severity |
| Control (water)    | 14.5 a <sup>y,z</sup>           | 25.1 a  | 70.0 a                            | 75.2 a   | 93.3 a    | 30.4 a   | 93.9 a    | 73.6 a   |
| Calcium hydroxide  | 8.0 bc                          | 11.7 c  | 33.3 a                            | 22.3 d   | 13.3 b    | 2.8 b    | 40.0 b    | 5.7 b    |
| Calcium oxide      | 6.8 c                           | 12.5 c  | 56.7 a                            | 31.5 cd  | 20.0 b    | 1.7 b    | 56.7 ab   | 9.4 b    |
| Calcium propionate | 10.4 b                          | 16.6 b  | 56.7 a                            | 61.8 ab  | 53.3 ab   | 7.3 b    | 80.0 ab   | 18.3 b   |
| Calcium silicate   | 10.4 b                          | 17.9 b  | 60.0 a                            | 49.1 bc  | 26.7 b    | 5.8 b    | 50.0 b    | 17.1 b   |

<sup>w</sup> For wounded Redhaven fruit, Brix values were 10.1 and 10.0% and firmness 7.1 and 68 kg for trials 1 and 2, respectively; for nonwounded Loring peaches, Brix values were 11.2, 11.8, and 11.8% and firmness 6.7, 5.5, and 4.2 kg for trials 1, 2, and 3, respectively (values were determined from 10 fruits).

<sup>x</sup> Redhaven fruit were wounded prior to inoculation. Fruit were rated 2 days after inoculation. Loring fruit were not wounded and were rated 7 days (8/14) or 4 days (8/17, 8/24) after inoculation. Loring fruit in trial 1 were inoculated with conidia in distilled water, whereas those in trials 2 and 3 were inoculated with conidia in Miller's solution. All inoculations were with 1 × 10<sup>5</sup> conidia per milliliter.

<sup>y</sup> Each observation is the mean of 30 fruit.

<sup>z</sup> Letters denote significant differences according to the Waller-Duncan *k*-ratio *t* test (*P* < 0.05). Each value is the mean from 10 fruit sampled immediately after harvest.

parable or superior to those achieved with the fungicide iprodione in other tests (26).

Calcium propionate, the hemicalcium salt of propionic acid (a three-carbon organic acid), has been extensively used as a food additive and is well known as an inhibitor of certain molds and bacteria in stored grain (16,21,27), hay (13,17), and bread (4,12) and has the potential for widespread use in the preservation of other foods (4). Punja and Gaye (20) demonstrated the utility of calcium propionate dips in reducing black root rot, caused by *Chalara elegans*, on fresh market carrots. The  $pK_a$  of propionate is 4.87, and at a pH of 4.0, 88% of the compound is undissociated, while at a pH of 6.0, only 6.7% remains undissociated. The mechanism of action of calcium propionate is the result of fungistasis and is thought to be caused mostly by the molecule in its undissociated state (4). The lipophilic, undissociated molecule is readily soluble in cell membranes and may interfere with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system. In less acidic environments where a portion of the molecule is dissociated, the  $Ca^{2+}$  moiety may act to inhibit fungal growth and PG enzyme production in addition to possibly enhancing host resistance. Our results show that pH does not affect the activity of calcium propionate against *M. fructicola*.

More research is required to determine the best method to utilize some of the more effective calcium salts in this study. Short chain, organic acids could have a role as disease control agents for wound pathogens; however, individual compounds must be examined for activity against specific pathogens. If a compound is found to be fungistatic against an appropriate pathogen, this property could provide the time required for host defense mechanisms to provide more effective resistance. Alternatively, a substance such as calcium propionate that has no activity against yeasts could be used to supplement biological control by yeasts. Additional research should address optimal concentrations of calcium salts, the use of additives or synergists (i.e., organic acids), pH effects, and carriers that would maintain effective con-

centrations of materials for an effective time period.

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#### LITERATURE CITED

1. Adaskaveg, J. E., Ogawa, J. M., Feliciano, A. J. 1992. Comparisons of calcium-based and film-forming materials for control of brown rot of peach caused by *Monilinia fructicola*. (Abstr.) Phytopathology 82:1158.
2. Bateman, D. F., and Lumsden, R. D. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility of an isolate of *Rhizoctonia solani*. Phytopathology 55:734-738.
3. Biggs, A. R., and Peterson, C. A. 1990. Effect of chemical application to peach bark wounds on accumulation of lignin and suberin and susceptibility to *Leucostoma persoonii*. Phytopathology 80:861-865.
4. Byrde, R. J. W. 1969. Nonaromatic organics. Pages 531-578 in: Fungicides, An Advanced Treatise. Vol. 2. D. C. Torgeson, ed. Academic Press, New York.
5. Byrde, R. J. W., and Willets, H. J. 1977. The Brown Rot Fungi of Fruit. Pergamon Press, New York.
6. Conway, W. S. 1982. Effect of postharvest calcium content on decay of Delicious apples. Plant Dis. 66:402-403.
7. Conway, W. S., Greene, G. M., II, and Hickey, K. D. 1987. Effects of preharvest and postharvest calcium treatments of peaches on decay caused by *Monilinia fructicola*. Plant Dis. 71:1084-1086.
8. Conway, W. S., Gross, K. C., and Sams, C. E. 1987. Relationship of bound calcium and inoculum concentration to the effect of postharvest calcium treatment on decay of apples caused by *Penicillium expansum*. Plant Dis. 71:78-80.
9. Conway, W. S., and Sams, C. E. 1984. Possible mechanisms by which postharvest calcium treatment reduces decay in apples. Phytopathology 74:208-210.
10. Conway, W. S., Sams, C. E., Brown, G. A., Beavers, W. B., Tobias, R. B., and Kennedy, L. S. 1994. Pilot test for the commercial use of postharvest pressure infiltration of calcium into apples to maintain fruit quality in storage. HortTechnology 4:239-243.
11. Conway, W. S., Sams, C. E., McGuire, R. G., and Kelman, A. 1992. Calcium treatment of apples and potatoes to reduce postharvest decay. Plant Dis. 76:329-334.
12. Doores, S. 1983. Organic acids. Pages 75-107 in: Antimicrobials in Food. A. L. Branen and P. M. Davidson, eds. Marcel Dekker, New York.
13. Draughon, F. A., Mobley, D. C., Safley, L. M., Jr., and Backus, W. P. 1982. Effect of calcium propionate and sodium diacetate on fungi in stillage. J. Food Sci. 47:1018-1019.
14. Kohle, H., Jeblick, W., Poten, F., Blaschek, W., and Kauss, H. 1985. Chitosan-elicited callose synthesis in soy bean cells as a  $Ca^{2+}$ -dependent process. Plant Physiol. 77:544-551.
15. Miller, H. J. 1944. The use of *Venturia inaequalis* and *Sclerotinia fructicola* with pure chemical stimulants in slide-germination tests of fungicides. (Abstr.) Phytopathology 34:1009.
16. Milward, Z. 1976. Further experiments to determine the toxicity of propionic acid to fungi infesting stored grain. Trans. Br. Mycol. Soc. 66:319-324.
17. Nash, M. J., and Easson, D. L. 1977. Preservation of moist hay with propionic acid. J. Stored Prod. Res. 13:65-75.
18. National Research Council, Board on Agriculture, Committee on Scientific and Regulatory Issues Underlying Pesticide Use Patterns and Agricultural Innovation. 1987. Regulating Pesticides in Food, The Delaney Paradox. National Academy Press, Washington, DC.
19. Northover, J., and Biggs, A. R. 1995. Effect of conidial concentration of *Monilinia fructicola* on brown rot development in detached cherries. Can. J. Plant Pathol. 17:205-214.
20. Punja, Z. K., and Gaye, M.-M. 1993. Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis. 77:989-995.
21. Raeker, M. O., Bern, C. J., Johnson, L. A., and Glatz, B. A. 1992. Preservation of high-moisture maize by various propionate treatments. Cereal Chem. 69:66-69.
22. Roberts, J. W., and Dunegan, J. C. 1932. Peach brown rot. USDA Tech. Bull. 328.
23. Robson, M. G. 1988. Some aspects of calcium nutrition on peach growth and postharvest fruit quality. Ph.D. thesis. Rutgers University, New Brunswick, NJ.
24. Sherwood, R. T. 1966. Pectin lyase and polygalacturonase production by *Rhizoctonia solani* and other fungi. Phytopathology 56:279-286.
25. Singh, B. P., Gupta, O. P., and Chauhan, K. S. 1982. Effect of preharvest calcium nitrate spray on peach on the storage life of fruits. Indian J. Agric. Sci. 52:235-239.
26. Smilanick, J. L., Hoskinson, S., and Michailides, T. J. 1996. Evaluation of postharvest fungicides for control of brown and Rhizopus rots, 1995. Fungic. Nematicide Tests 51:50.
27. Tsai, W.-Y., Shao, K.-P. P., and Bullerman, L. B. 1984. Effects of sorbate and propionate on growth and aflatoxin production of sublethally injured *Aspergillus parasiticus*. J. Food Sci. 47:86-90.