Efficacy of Three Calcium Products for Control of Peach Brown Rot

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SUMMARY. The effectiveness of the calcium products Chelan, Power-Ca, and calcium chloride to reduce the development of *Monilinia laxa* on 'Andross' peach (*Prunus persica*) was investigated. The mycelium growth of *M. laxa* on potato dextrose agar modified with Chelan, Power-Ca, or calcium chloride compound at concentrations 1, 2, and 4 g·L⁻¹ was significantly reduced in comparison with the water. Chelan, Power-Ca, and calcium chloride applied as foliar sprays did not significantly affect the development of the pathogen on inoculated immature and mature peaches. However, dipping peaches in solutions containing one of the calcium products tested reduced significantly the percentage of *M. laxa* infection on inoculated fruit.

Fruit decay, incited by *Monilinia laxa*, is a major problem worldwide, limiting the shelf life of peaches for fresh consumption (Sharma and Kaul, 1989). It causes field losses of peaches, especially in locations where climate conditions are wet. The use of fungicides is the most common method of controlling this disease (Lalancette, 1997; Liguori and Bassi, 1998). However, recent concerns regarding pesticide residues on fruit means there is a need for alternative disease management practices that will reduce the risk to consumers (Tamm et al., 2004).

Previous work has shown that the application of calcium (Ca) such as calcium chloride enhances natural resistance of peach fruit to brown rot indirectly by enhancing host resistance (Biggs et al., 1997; Conway et al., 1987, 1994) and directly by inhibiting fungal growth, because Ca reduces polygalacturonase activity (Biggs et al., 1997). Calcium treatments could be an alternative method to

reduce losses of peaches from brown rot.

The primary goals of this study were to investigate the effect of three Ca products applied as sprays or postharvest dipping on the resistance of peaches to *M. laxa*. The products tested were Power-Ca [20% calcium oxide (CaO) + sugars + amino acids; Farma Chem SA, Sindos Thessaloniki, Greece], Chelan (15% CaO + amino acids; Nature A.E., Nea Efesos Pieria, Greece), and calcium chloride (36% Ca; Farma Chem SA). Power-Ca and Chelan are two novel products to agriculture. Calcium chloride was the standard and used as the control.

Materials and methods

Effect of calcium products on mycelium growth

Solutions of Ca products (Chelan, Power-Ca, and calcium chloride)

tested were prepared in sterile, deionized distilled water and were added to autoclaved, warm (45 to 55 °C) 2% w/w potato dextrose agar (PDA) adjusted with sodium hydroxide to pH 7.0 to provide concentrations of 1 (recommended by manufacturer), 2, and 4 g·L⁻¹. The medium was poured into 9-cm-diameter petri dishes. An agar disk, 6 mm in diameter, taken from an active colony of *M. laxa* (identification was based on cultural and morphologic characters) originating from diseased 'Andross' peach fruit, was placed in the center of each of five replicated dishes. Dishes were then incubated in a growth chamber at 23 °C for 7 d. The diameter of the resulting colony was recorded. Medium without any Ca product was used as the control.

Preharvest calcium treatments

Experiments were established in commercial orchards in Imathia province, Greece. Twelve-year-old 'Andross' peach trees, grafted on the peach rootstock 'GF677' [a hybrid of peach × almond (*Prunus amygdalus*)], were sprayed three times (30 May—15 d before pit hardening; 9 June—at pit hardening time; 24 June—15 d after pit hardening) with solutions of Chelan, Power-Ca, or calcium chloride at a concentration of 1 g·L⁻¹ (recommended by manufacturer). Applications were made during late evening [≈15 to 18 °C air temperatures and 40% to 50% relative humidity (RH)] by using a high-pressure sprayer (working at 30 bars) forwarding the solution at ≈5 m aboveground so that each tree (inner and outer fruit and foliage) received 3 L of solution. Unsprayed trees acted as the control. The experimental design was a randomized block design with five replicates, in

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.1	bar	MPa	10
29,574	fl oz	μL	3.3814 × 10 ⁻⁵
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.5400	inch(es)	cm	0.3937
25.4000	inch(es)	mm	0.0394
1.1209	lb/acre	kg·ha ⁻¹	0.8922
28.3495	oz	g	0.0353
0.001	ppm	g·L ⁻¹	1000
1	ppm	mg·L ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

which each replicate consisted of three trees.

DROPLET INOCULATIONS. In these experiments, immature fruit were harvested ≈ 125 d after full bloom (1 month after last application) from trees sprayed with one of the tested Ca products and from unsprayed trees and dipped in 10% domestic bleach (sodium hypochlorite 5.96%) solution for 10 min to be disinfected. After washing, scrubbing, and agitating in sterile, distilled water for 30 s, fruit were dried at room temperature (≈ 23 °C) and wounded uniformly (one wound per fruit) with a blunted nail that created a wound ≈ 3 mm in diameter. Fruit and solutions were at room temperature (≈ 23 °C). Fruit were inoculated at their base by using a suspension containing 5×10^5 conidia/mL. Approximately 40 μ L of conidial suspension was placed in each wound. Inoculated fruit were lightly enclosed in special plastic dishes (50 cm long, 29 cm wide, 10 cm high) and placed in a growth chamber at 24 to 26 °C with $\approx 90\%$ RH without light. Control fruit were inoculated with sterile, distilled water without conidia. Seven days later, lesion diameter (mean of two measurements at right angles to each other) was measured. There were three replicates of 20 fruit per treatment.

DIPPING INOCULATION. Mature fruit (20 from each tree) were collected 135 d after full bloom (≈ 1.5 month after last application) from trees sprayed with one of the tested Ca products and from unsprayed trees and disinfested as described previously. Fruit and solutions were at room temperature (≈ 23 °C). After drying at room temperature (≈ 23 °C), they were immersed for 1 min in a spore suspension of $\approx 3 \times 10^6$ conidia/mL *M. laxa* with 0.1% (v/v) Tween 20. Fruit immersed in water were used as the control. The inoculated fruit were left drying at room temperature before packing into cardboard boxes (different for each treatment) in one layer and stored at 2 to 4 °C with $\approx 90\%$ RH for 2 weeks. Rotting was subjectively determined using a scale of 0% to 100% with the extremes of the scale representing healthy fruit and fruit with obvious infection (average percentage of individual fruit covered with decay) over the entire fruit,

respectively. There were three replications of 20 fruit for each treatment.

The level of Ca in the leaves and the flesh of immature and mature fruit (five fruit from each treatment) was determined. Leaves and fruit samples were washed before ashing. Portions of ground fruit flesh (1.0 g) were reduced to ash for 5 h at 550 °C (peel retained). The cooled ash was dissolved in 5-mL aliquots of 2 N hydrochloric acid and brought up to 50 mL with distilled, deionized water. Solutions were filtered and analyzed for Ca by atomic absorption spectroscopy (Chapman and Pratt, 1961).

Postharvest calcium treatment

Untreated ‘Andross’ peaches without disease symptoms were collected at harvest (135 d after bloom) and disinfested as described previously (droplet inoculations). The fruit were then immersed for 30 min in solutions of Chelan, Power-Ca, or calcium chloride at concentrations of 2, 4, or 6 g·L⁻¹ formulation. Inoculations were made by using the same methodology described in the “dipping inoculation” experiment. Again, a scale of 0% to 100%, representing a range from healthy fruit to fruit with infection obvious over the entire fruit, was used to collect the results. Fruit and solutions were at room temperature (≈ 23 °C). There were five replications of 20 fruit for each treatment. Control fruit were dipped in sterile water. The level of Ca in the flesh of fruit (five fruit from each treatment) was determined as described previously.

Statistical analysis

All experiments were conducted for 2 consecutive years, 2004 to 2005. Analysis of variance was used to analyze data. Data expressed as percentages were analyzed after angular transformation to obtain normality. For analysis of data from combined experiments, Bartlett’s test (Bartlett, 1937) was used to demonstrate homogeneity of variances. Treatment means were separated by Duncan’s multiple range test ($P < 0.05$).

Results and discussion

Mycelium growth of *M. laxa* on PDA modified with Ca products was significantly reduced compared with the control, but not completely

inhibited even up to 4 g·L⁻¹ (Table 1). Of the three Ca products tested on PDA, 4 g·L⁻¹ Chelan resulted in the least mycelium growth, although the concentration of Ca in Chelan (15%) was the lowest compared with calcium chloride (36%) and Power-Ca (20%). It shows that something besides Ca in Chelan such as the amino acids contained in this product also has an effect, but no more information related to the composition of Chelan is given by the producer. Biggs et al. (1997) found that Ca salts significantly reduced the mycelium growth of *Monilinia fructicola* on modified PDA.

The incidence and severity of brown rot on immature and mature peaches sprayed with Chelan, Power-Ca, or calcium chloride and inoculated using two different methods was statistically similar to the untreated control (data not shown). Similarly, Conway et al. (1987) treated ‘Jerseyland’ peaches by 10 weekly preharvest calcium chloride sprays at rates of 30, 60, or 90 lb/acre and found no reduction of brown rot. In contrast, foliar applications with calcium chloride were effective in improving the resistant of apple (*Malus pumila*) to brown rot (Wojcik, 1999). Biggs et al. (1997) reported different effectiveness of Ca salts by field applications against *M. fructicola*.

Leaf and fruit flesh analysis showed no significant difference in Ca concentration between fruit sprayed with one of the Ca products tested and the untreated control (data not shown). In contrast, ‘Jerseyland’ peaches treated with a solution of calcium chloride by 10 weekly preharvest sprays at rates of 90 lb/acre had 70% more Ca in the flesh than the untreated control (Conway et al., 1987).

Postharvest dipping of mature peaches into Chelan, Power-Ca, or calcium chloride solutions for 30 min significantly reduced the infections from *M. laxa* (Table 2). The effectiveness of different Ca products used in this study was similar. These products reduced the percentage of fruit rots by more than half in comparison with the control (water only). Postharvest dipping of plums (*Prunus domestica*) in different Ca solutions has also been used by Vangdal and Brve (2002) to control brown rot. Conway et al. (1987) treated ‘Jerseyland’ peaches

Table 1. Mycelium growth of *Monilinia laxa* on potato dextrose agar (PDA) amended with commercial calcium formulations.

Treatment ^z	Rate of formulation (g·L ⁻¹) ^y	Diam of mycelium growth (mm) ^{y,x}
Control (PDA alone)	0	58.2 a ^w
Calcium chloride	1	52.0 b
Calcium chloride	2	50.4 b
Calcium chloride	4	46.7 bc
Chelan	1	47.6 bc
Chelan	2	40.2 d
Chelan	4	34.3 e
Power-Ca	1	52.0 b
Power-Ca	2	50.9 b
Power-Ca	4	42.7 cd

^zCalcium chloride (36% Ca; Farma Chem SA, Sindos Thessaloniki, Greece), Chelan [15% calcium oxide (CaO) + amino acids; Nature A.E., Nea Efesos Pieria, Greece], and Power-Ca (20% CaO + sugars + amino acids; Farma Chem SA).

^y1 g·L⁻¹ = 1000 ppm; 1 mm = 0.0394 inch.

^xValues are the means of two experiments; results were similar according to the Bartlett's test of homogeneity of variance, so data were combined.

^wValues in the same column followed by the same letters are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 2. Effect of postharvest dipping in calcium (Ca) solutions prepared from commercial formulations^z for 30 min on flesh Ca concentration and development of *Monilinia laxa* on inoculated mature 'Andross' peaches.

Treatment ^z	Rate of formulation (g·L ⁻¹) ^y	Ca (g·L ⁻¹)	Severity of fruit rots (%) ^x	Fruit Ca concn (mg·L ⁻¹) ^{y,x}
Control (water only)	0	0	36.36 a ^w	198 a ^w
Calcium chloride	2	0.56	15.28 b	273 b
Calcium chloride	4	1.12	13.25 b	296 cd
Calcium chloride	6	1.68	12.91 b	312 de
Chelan	2	0.30	16.00 b	277 b
Chelan	4	0.60	15.42 b	278 b
Chelan	6	0.90	14.22 b	326 e
Power-Ca	2	0.40	13.40 b	285 bc
Power-Ca	4	0.80	12.88 b	307 d
Power-Ca	6	1.20	12.08 b	318 e

^zCalcium chloride (36% Ca; Farma Chem SA, Sindos Thessaloniki, Greece), Chelan [15% calcium oxide (CaO) + amino acids; Nature A.E., Nea Efesos Pieria, Greece], and Power-Ca (20% CaO + sugars + amino acids; Farma Chem SA).

^y1 g·L⁻¹ = 1000 ppm; 1 mg·L⁻¹ = 1 ppm.

^xValues are the means of two experiments. Data were analyzed by using analysis of variance after angular transformation (only percentage data) to obtain normality; results were similar according to the Bartlett's test of homogeneity of variance, so data were combined.

^wValues in the same column followed by the same letters are not significantly different according to Duncan's multiple range test ($P < 0.05$).

(pressure-infiltrated) after harvest with 2% or 4% solution of calcium chloride and found 40% and 60% less decay. The percentage of fruit rots was the same regardless of the rate of Ca applied. A hypothesis raised by data presented in Table 1 is that Ca²⁺ ions act directly on the pathogen and cause reduced virulence or, in the extreme, fungistasis. Another hypothesis raised by data presented in Table 2 is that Ca²⁺ ions activate the defense mechanism of peaches. This mode of action is not correlated with the rate of Ca applied, and more

investigation should be conducted on this area. According to Kohle et al. (1985), Ca²⁺ ions stimulate the synthesis of phytoalexins, whereas Conway and Sams (1984) reported that Ca²⁺ ions reduced the effectiveness of fungal polygalacturonase enzymes by forming cation cross bridges between pectin acids in the plant cell walls. Souza et al. (1999, 2001) reported that postharvest application of calcium chloride solution into wounds of 'Buiti' peaches promoted Ca incorporation into the fruit cell walls as well as the level

of enzymes (polyphenoloxidase); increased the levels of neutral sugars; reduced the degree of cell wall pectin esterification (but did not lead to increased lignin synthesis in cell walls); and reduced the infected area with brown rotting by 34% and the disease index of peaches by 29% as compared with water-treated fruit.

Flesh analysis showed that all Ca products increased fruit Ca content after postharvest dipping (Table 2). However, the different Ca products, with Ca concentrations ranging from 15% to 28%, resulted in fruit with similar Ca content. In addition, there is no correlation between the amount of Ca in the dip solution and final Ca concentration (calcium chloride, $r = 0.236$; Chelan, $r = 0.317$; Power-Ca, $r = 0.308$). A possible explanation is that peaches show different abilities to absorb Ca compounds from different Ca products. According to Conway et al. (1994), fruit that were dipped and pressure-infiltrated had two to four times more Ca and resisted infections from brown rot.

This study demonstrated the general toxicity of Chelan, Power-Ca, and calcium chloride to *M. laxa* in vitro. However, the mode of action of these Ca products is unknown at present, and more investigation in this area should be conducted.

All Ca products tested were ineffective in reducing the incidence of *M. laxa* on peaches when applied as foliar sprays (data not shown). This is probably related to the lack of Ca uptake into the tissue and indicated by the tissue analysis. It has been found that the effectiveness of Ca spray applications is strongly related to factors such as number and timing of applications, Ca concentration, form of Ca, and so on (Deyton et al., 2001; Raese and Drake, 2000; Wojcik, 1999, 2001). Additional research should address optimal concentrations of commercial Ca products, the use of additives or synergists, pH effects, and carriers that would maintain effective concentrations of materials for an effective period.

This study also demonstrated the possible use of the products Chelan, Power-Ca, and calcium chloride as an alternative to fungicidal control of *M. laxa*, which could be included in integrated fruit production (IFP) systems, reducing risk to consumers. Based on the results of this study,

for maximum effectiveness, applications should be made by dipping fruit into solutions, because spray applications were not effective. In seasons with low disease pressure, postharvest dipping of peaches in Ca solutions alone could be an appropriate measure for controlling *M. laxa*. In seasons with high disease pressure, many more applications of appropriate fungicides could be required. Application of postharvest dipping of peaches in Ca solution could be made in the packing house. According to the information given by producers, Chelan and Power-Ca are safer in terms of fruit injury and worker health (because they are natural products) than calcium chloride (an inorganic chemical) used as postharvest treatments and therefore more appropriate to be included in an IFP system.

Literature cited

- Bartlett, M.S. 1937. Properties of sufficiency and statistical tests. *Proc. Royal Stat. Soc. Ser. A* 160:268–282.
- Biggs, A.R., M.M. El-Kholi, S. El-Neshawy, and R. Nickerson. 1997. Effects of calcium salts on growth, polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola*. *Plant Dis.* 81:399–403.
- Chapman, H.D. and P.F. Pratt. 1961. *Methods of analysis for soils, plants and waters*. Univ. California Agr. Sci., Berkeley.
- Conway, W.S., G.M. Greene, and K.D. Hickey. 1987. Effects of preharvest and postharvest calcium treatments of peaches on decay caused by *Monilinia fructicola*. *Plant Dis.* 71:1084–1086.
- Conway, W.S. and C.E. Sams. 1984. Possible mechanisms by which postharvest calcium treatment reduces decay in apples. *Phytopathology* 74:208–210.
- Conway, W.S., C.E. Sams, and A. Kelman. 1994. Enhancing the natural resistance of plant tissues to postharvest diseases through calcium applications. *HortScience* 29:751–754.
- Deyton, D.E., C.E. Sams, and C.G. Milne. 2001. Influence of foliar and microjet application of Solubor and calcium on nutrient content of apple trees. *Acta Hort.* 594:569–573.
- Kohle, H., W. Jeblick, F. Poten, W. Blascheck, and H. Kauss. 1985. Chitosan-elicited cellulose synthesis in soybean cells as Ca²⁺-dependent process. *Plant Physiol.* 77:544–551.
- Lalancette, N. 1997. In search of the perfect brown rot fungicide. *Pennsylvania Fruit News* 77:62–66.
- Liguori, R. and R. Bassi. 1998. Cyprodinil (Chorus/registered): New fungicide for the defense of fruit trees from scab and *Monilia* spp. *Informatore Fitopatologico* 48:43–47.
- Raese, J.T. and S.R. Drake. 2000. Effect of calcium spray materials, rate, time of spray application, and rootstocks on fruit quality of ‘Red’ and ‘Golden Delicious’ apples. *J. Plant Nutr.* 23:1435–1447.
- Sharma, R.L. and I.L. Kaul. 1989. Host range of brown rot fungi (*Monilinia* spp.) in India. *Plant Dis. Res.* 4:177–178.
- Souza, A.B., M.F. de Chitarra, A.B. Chitarra, and J.C. Machado. 1999. Postharvest resistance of peach (*Prunus persica* cv. Biuti) to *Monilinia fructicola*: Induction of biochemical responses through the application of CaCl₂ at the site of injury. *Ciencia e Agrotecnologia* 23:865–875.
- Souza, A.B., M.F. de Chitarra, A.B. Chitarra, and J.C. Machado. 2001. Effects of calcium on wound healing and resistance of peach (*Prunus persica* (L.) Batsch cv. Biuti) to *Monilinia fructicola* (Wint.) honey. *Ciencia e Agrotecnologia* 25: 616–624.
- Tamm, L., A. Haseli, J.G. Fuchs, F.P. Weiber, and E. Wyss. 2004. Organic fruit production in humid climates of Europe: Bottlenecks and new approaches in disease and pest control. *Acta Hort.* 638:333–339.
- Vangdal, E. and J. Brve. 2002. Pre- and postharvesting Ca-treatment of plums (*Prunus domestica* L.). *Acta Hort.* 577:125–128.
- Wojcik, P. 1999. ‘Gloster’ apple yield and fruit quality as influenced by frequency of calcium chloride sprays. *J. Fruit Ornamental Plant Res.* 7:181–194.
- Wojcik, P. 2001. ‘Dabrowicka Prune’ fruit quality as influenced by calcium spraying. *J. Plant Nutr.* 24:1229–1241.