Postharvest Calcium Treatment of Apple Fruit to Provide Broad-Spectrum Protection Against Postharvest Pathogens

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ABSTRACT

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Apple fruit (*Malus domestica*) were pressure infiltrated with calcium chloride solutions at harvest during three separate growing seasons. In 1984, the calcium concentration of Golden Delicious and Delicious apples ranged from 170 to 1,600 and 200 to 2,000 μg/g, respectively. In 1988, the calcium concentration of Delicious fruit ranged from 200 to 1,050 μg/g; similarly, that of Golden Delicious apples was 193–1,046 μg/g in 1989. Calcium concentration was negatively correlated with decay caused by the three different fungal pathogens tested during these three years. However, calcium reduced decay to a greater extent (70%) in fruit inoculated with *Glomerella cingulata* than in fruit inoculated with *Penicillium expansum* (37%) or *Botrytis cinerea* (50%). Thus, calcium-induced resistance to postharvest fungal pathogens is broad in spectrum.

Increasing the calcium (Ca) content of apples (*Malus domestica* Borkh.) has been shown to alleviate many physiological storage problems (13) and reduce losses due to postharvest decay-causing organisms (15). Storage losses caused by *Gloeosporium* spp. in fruit that had been sprayed before harvest with Ca sprays were lower than in unsprayed controls (15). Infiltration of fruit with Ca solutions after harvest reduced blue mold rot caused by *Penicillium expansum* Link (6,9). These postharvest treatments increased both total and cell-wall-bound Ca (8).

Calcium-induced resistance to blue mold rot was due, at least in part, to a decrease in the maceration of cell walls by fungal polygalacturonase (PG) (7). The effect of Ca in fruit may also complement the efficacy of fungicide treatments. The addition of calcium chloride ($CaCl_2$) to benomyl enhanced the activity of the fungicide for decay control following postharvest treatment of apples (5).

The research presented herein was conducted to determine if postharvest Ca treatment reduces decay in apple fruit by postharvest pathogens other than *P. expansum*. Two fungi that produce pectolytic enzymes (similar to *P. expansum*), *Botrytis cinerea* Pers.:Fr. and *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, were used in these experiments. A secondary objective was to determine if Ca controlled the three pathogens differentially.

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MATERIALS AND METHODS

The experiment was conducted during three separate years. In each year, Delicious or Golden Delicious apples or fruit of both cultivars were harvested from commercial orchards and randomized independently. The fruit were then infiltrated under a pressure of 103 kPa for 2 min with 0, 2, 4, or 8% solutions (w/v) of $CaCl_2$ in distilled water ($CaCl_2 \cdot 2H_2O$) for Delicious and 0, 1, 2, or 4% $CaCl_2$ solutions for the Golden Delicious fruit. Following treatment, the fruit were placed on kraft paper and allowed to air-dry before storage at 0 C. After 6 mo (a typical storage period for these cultivars), the fruit were removed from 0 C and stored at 20 C overnight to warm to that temperature. All fruit were wound inoculated with the respective pathogens (10^5 spores per milliliter) as previously described (6).

Apples were rated for decay severity when lesion diameter of untreated fruit averaged approximately 32 mm, resulting in an area of decay of approximately 800 mm^2, calculated as previously described (6). During the 1984 study with *B. cinerea* (similar in virulence to *P. expansum*), the fruit were kept at 20 C for 7 days following inoculation before the area of decay was determined. However, in the 1989 study with *B. cinerea*, the area of decay was determined after 5 days. In the 1988 study with *G. cingulata* (a less aggressive pathogen than *P. expansum*), decay severity was evaluated after 16 days, whereas in the 1989 study, the area of decay was determined after 14 days. In 1989, the area of decay caused by *P. expansum* was determined after 7 days.

During each year, 40 fruit were used per treatment, and each treatment was replicated three times in a randomized incomplete block design. The three experiments were analyzed separately by year utilizing a general linear mixed models program (2). The best fitted quadratic regression was determined with the general linear models procedure of the Statistical Analysis System (14). Each experiment within each year was repeated once. Ca content from similarly treated but uninoculated fruit was determined as previously described (6). The flesh from four apples made up one sample, and five samples were analyzed from each treatment.

RESULTS

As the Ca content of the fruit increased, the severity of decay due to each of the pathogens decreased in both cultivars (Figs. 1–3). In 1984, the effect of cultivar on disease severity was significant, but the cultivar-by-Ca interaction was not significant. Therefore, the cultivars responded similarly with Ca treatment. In the 1989 experiment, the three pathogens produced lesions of different sizes, but each was affected similarly by the Ca treatment (Ca decreased the lesion size) and the pathogen-by-Ca interaction was not significant. Quadratic relationships were statistically significant between decay area and Ca content for all pathogens in all years tested. In 1984, the Ca content of the Golden Delicious fruit inoculated with *B. cinerea* ranged from 170 μg/g in the control to about 1,600 μg/g in the fruit treated with the 4% $CaCl_2$ solution. The area of decay was reduced from about 1,550 mm^2 in the control fruit to less than 550 mm^2 in the fruit treated with 4% $CaCl_2$ (65% reduction) (Fig. 1). Delicious apples inoculated with *B. cinerea* had a Ca con-

tent of about 200 μg/g in the control and over 2,000 μg/g in fruit treated with 8% $CaCl_2$. Decay was reduced from over 1,500 mm^2 in the control to about 400 mm^2 in the fruit treated with 8% $CaCl_2$ (74% reduction) (Fig. 1).

With the Delicious fruit in 1988, the Ca content of the untreated fruit was below 200 μg/g, whereas that in fruit treated with 8% $CaCl_2$ was over 1,000 μg/g (Fig. 2). This was significantly less Ca than that in the Delicious fruit infiltrated with similar $CaCl_2$ concentrations and inoculated with *B. cinerea* during 1984. Sixteen days elapsed following inoculation before the diameter of the lesions on the fruit inoculated with *G. cingulata* averaged approximately 30 mm. The area of decay was nearly 700 mm^2 in the control and only 250 mm^2 in the fruit treated with 8% $CaCl_2$ (Fig. 2), a reduction of approximately 65%.

The Ca content of the Golden Delicious apples treated with $CaCl_2$ solutions in 1989 (Fig. 3) was similar to that in Delicious apples treated in 1988. The Ca content averaged about 200 μg/g in the control and was increased to 1,046 μg/g in the fruit treated with 4% $CaCl_2$. The area of decay in the *P. expansum* control was over 900 mm^2, which was reduced to just under 600 mm^2 in the fruit treated with 4% $CaCl_2$, resulting in 37% less decay (Fig. 3). Similarly, the reduction in the area of decay in fruit inoculated with *B. cinerea* was about 50% (800 mm^2 reduced to about 400 mm^2). The area of decay of fruit inoculated with *G. cingulata* decreased from over 900 mm^2 to just over 250 mm^2, for a decay reduction of 70%. It took 7 days for the diameter of decay of the control fruit to average approximately 32 mm (the arbitrary evaluation point chosen) in the fruit inoculated with *P. expansum*, 5 days in the fruit inoculated with *B. cinerea*, and 14 days in the fruit inoculated with *G. cingulata*.

In 1984, there was some brown surface discoloration on the Golden Delicious fruit treated with 4% $CaCl_2$ and on the Delicious treated with 8% $CaCl_2$. In 1988, there was no surface injury on the Delicious fruit, but there was again some brown surface discoloration on the Golden Delicious fruit treated with 4% $CaCl_2$ in 1989. All injury was limited to the peel surface, and fruit not suitable for the fresh market would be acceptable for processing.

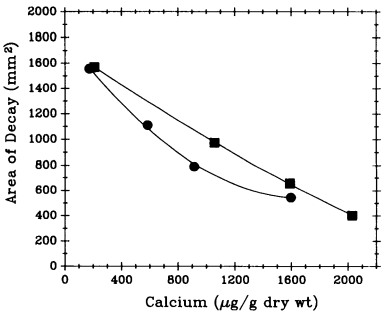

Fig. 1. Relation between area of decay and calcium concentration of apple tissue following pressure infiltration of fruit with calcium chloride solutions in 1984. Regression curves indicate Delicious (■) ($y = 1{,}805.29 - 1.49x + 0.0004x^2$, $R^2 = 0.99$) and Golden Delicious (●) ($y = 1{,}724.60 - 0.77x + 0.00006x^2$, $R^2 = 0.97$) inoculated with *Botrytis cinerea*. Data points indicate the concentrations of calcium chloride solutions (0, 2, 4, or 8% for Delicious, and 0, 1, 2, or 4% for Golden Delicious fruit from left to right, respectively).

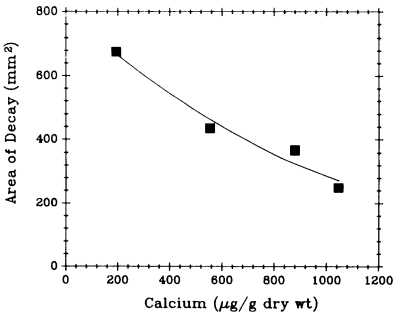

Fig. 2. Relation between area of decay and calcium concentration of Delicious apple tissue following pressure infiltration of fruit with calcium chloride solutions in 1988. Regression curve ($y = 802.03 - 0.73x + 0.0002x^2$, $R^2 = 0.97$) indicates fruit inoculated with *Glomerella cingulata*. Data points are the concentrations of calcium chloride solutions (0, 2, 4, or 8% from left to right).

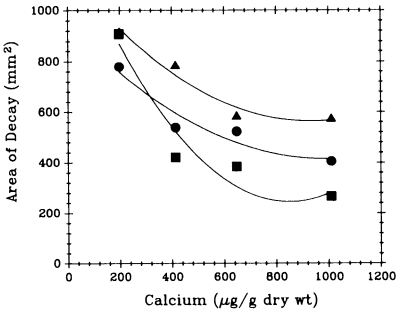

Fig. 3. Relation between area of decay and calcium concentration of Golden Delicious apple tissue following pressure infiltration of fruit with calcium chloride solutions in 1989. Regression curves indicate fruit inoculated with *Penicillium expansum* (▲) ($y = 1{,}103.98 - 1.04x + 0.00046x^2$, $R^2 = 0.99$), *Botrytis cinerea* (●) ($y = 979.82 - 1.06x + 0.00048x^2$, $R^2 = 0.97$), and *Glomerella cingulata* (■) ($y = 1{,}261.09 - 2.47x + 0.00151x^2$, $R^2 = 0.91$). Data points indicate the concentrations of calcium chloride solutions (0, 1, 2, or 4% from left to right).

DISCUSSION

The mechanism by which Ca reduces blue mold rot in apples caused by *P. expansum* is at least partially attributable to a decrease in maceration of cell walls by PG due to the improved structural integrity caused by an increase in Ca content (7). As the Ca content of the cell wall increased, enzyme activity, as measured by the release of uronic acid, decreased. This same mechanism, to a different extent, is postulated to be responsible for the reduction in gray mold rot in apples caused by *B. cinerea* and bitter rot caused by *G. cingulata*. However, Ca may also directly affect the activity of cell-wall-degrading enzymes. Millimolar concentrations of Ca inhibit PG activity in vitro, and it may be that these effects are similar in vivo (13).

The amount of Ca taken into the fruit from preharvest sprays (11) or postharvest treatments (10) can vary from year to year. These differences are affected by the growing conditions (11) as well as the maturity of the apple being treated and the cultivar (10).

In the tests reported here, the supplementation of the endogenous Ca in apples by postharvest infiltration of the fruit with $CaCl_2$ solutions reduced decay caused by *B. cinerea* and *G. cingulata* and, as reported previously (6,9,10), reduced decay caused by *P. expansum*. Since both *B. cinerea* and *G. cingulata* produce PG (12,17), Ca may inhibit the activity of PG produced by these fungi, either directly or by stabilizing the cell wall of the host and making it more resistant to breakdown. Ca, however, may differentially inhibit PG produced by various pathogens. Differential inhibition of pectolytic enzymes is well known among host proteinaceous inhibitors of fungal enzymes (1,3,4). PG produced by *P. expansum*, a highly aggressive pathogen of apple fruit, is the least affected by these inhibitors (1,3), whereas PG produced by *G. cingulata*, a relatively weakly aggressive pathogen, is most inhibited (3,4). The PG from *B. cinerea* was inhibited to a degree somewhere in between (1,4). This seems to be the order in which supplemental Ca inhibits the pathogenicity of these pathogens in apples and perhaps for the same reasons. Similar results have been found for pear fruit sprayed with $CaCl_2$ before harvest (16). The disease incidence caused by the highly aggressive *P. expansum* was not affected by these sprays, although that caused by the weaker pathogen *Phialophora malorum* was significantly reduced. PG from each of the apple pathogens in this study must be purified and the inhibitory effects of Ca tested against each individually to determine if the differential effect actually exists.

The effect of postharvest Ca treatment is broad in spectrum and the treatment may be useful against numerous pathogens. Whether the effect is on the activity of the PG produced by these fungi, as with *P. expansum* (7), and whether the effect on the PG produced by various pathogens is differential remains to be proven.

ACKNOWLEDGMENT

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