

Possible Mechanisms by Which Postharvest Calcium Treatment Reduces Decay in Apples

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ABSTRACT

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Golden Delicious apples were pressure-infiltrated (68.95 kPa) with 0, 2, 4, 6, or 8% solutions of CaCl₂ and stored at 0 C. Another lot of fruits was stored in low (1%) oxygen storage (0 C). After 6 mo of storage, both lots were wound-inoculated with *Penicillium expansum* and incubated for 7 days at 20 C. The fruits treated with 4, 6, or 8% CaCl₂ and the fruits kept in low O₂ were equally firm. However, whereas the area of decay was reduced by only about 15% in fruits kept in low O₂, fruits infiltrated with 4, 6, or 8% CaCl₂ solutions had more than 50% less decay than nontreated fruits.

Fungal growth on media was not retarded when dehydrated PDA and PDB were amended with CaCl₂ to a concentration of calcium at least twice that encountered by the fungus in the fruit. There was no correlation between total phenolic compounds in the flesh and calcium concentration of the tissue. These results imply that the effect of calcium in reducing decay is indirect and may be due to the formation of cell wall components resistant to degradation by *P. expansum*.

Additional key words: *Malus domestica*, *Penicillium expansum*, phenolic compounds.

The majority of the efforts in the postharvest treatment of apples with calcium (Ca) salt solutions has been directed towards reducing losses caused by physiological disorders (2,3,9). More recent work (4,5,11), however, has shown that increased Ca content of fruit may also reduce fungus-induced decay. Apples with increased Ca content due to postharvest treatments were more resistant to decay by *Penicillium expansum* Link ex Thom. (4), but they were also firmer than low-Ca fruit (5), suggesting a possible negative relationship between decay and fruit firmness. This possible relationship was further indicated by the correlation between decay and water-soluble polyuronide content of fruit tissues (5).

Loss in firmness of apples can also be significantly reduced by controlled atmosphere storage at O₂ levels of 1.0–1.5% (1,15). This provides another method to influence firmness and independently test the effect of cell wall integrity and/or Ca on fungal decay.

Phenolic compounds have long been implicated as resistance factors in host-parasite interactions (10), but little is known of the effect that increased calcium content of apple tissue might have on phenolics or on any other postinfection inhibitor present in the fruit.

The objectives of these experiments were to determine: (i) whether maintenance of firmness is the mechanism by which Ca exerts its effect in decreasing fungal infection; (ii) whether Ca has a direct ionic effect that reduces fungal growth; and (iii) whether Ca has an effect on total fruit phenolics, which in turn could be resistance factors in host-parasite interactions.

MATERIALS AND METHODS

Cultivar Golden Delicious apples (*Malus domestica* Borkh.) were harvested from a commercial orchard in Pennsylvania. The apples were randomized and pressure-infiltrated (68.95 kPa) for 2 min with laboratory grade USP calcium chloride (CaCl₂; 76%) made up as 0, 2, 4, 6, or 8% solutions. Following treatment, the fruits were placed in storage (0 C). Another lot of apples from the

same orchard and harvested at the same time was placed in low O₂ (1%) storage, also at 0 C.

After 6 mo, both lots of fruits were removed from storage, wound-inoculated with *P. expansum*, and rated for decay development as previously described (5). Fifteen fruits were used for each treatment.

Ca content of the tissue of the apples pressure-infiltrated with the various concentrations of CaCl₂ was determined by mechanically removing the peel and outer flesh of the entire fruit to a depth of 2 mm. The next 3 mm of flesh was then removed, again using a mechanical peeler, and this layer was analyzed for Ca content, because it was to this depth that the apples were wounded for inoculation. The flesh from three apples made up one sample, and three samples from each treatment were analyzed. Following removal from the fruit, the flesh was immediately frozen in liquid nitrogen, freeze-dried, and ground. Tissue samples, 0.500 ± 0.005 g, were ashed at 500 C overnight and the residue dissolved in 5 ml 6 N HCl. The samples were then diluted and analyzed for Ca content with a Jarrell-Ash atomic absorption spectrophotometer. All Ca values were reported on a dry-weight basis.

Fruit firmness was measured on 15 fruits per treatment with a Magness-Taylor pressure tester by using an 11.1-mm-diameter tip that penetrated to a depth of 8.0 mm. Both lots of fruits were rated for firmness at the same time they were rated for disease severity.

To determine whether a soluble inhibitor was present or was being maintained by increasing the concentration of Ca in the fruits, *P. expansum* was grown on juice extracted from the fruits treated with the various concentrations of CaCl₂. Juice was extracted from the fruits by first removing the peel and core and juicing the remaining tissue in a model 7001 Acme Challenger Juicerator (Acme Juicer Mfg. Co., Sierra Madre, CA). The extracted juice was clarified for 25 min at 10,000 g. The supernatant was then sterile-filtered in a 0.20-μm Nalgene sterilization filter unit (Nalge Co., Rochester, NY). Ten milliliters of the sterile juice was placed in sterile, 50-ml Erlenmeyer flasks and inoculated with 1 ml of a spore suspension of *P. expansum* (1 × 10⁶ spores per milliliter). Three apples per sample were juiced, and a total of four samples per treatment were used. The flasks were incubated for 3 days at 20 C. Mycelial biomass was determined by filtering the contents of each flask in a Buchner funnel through Whatman No. 1 filter paper (4.25-cm diameter) that previously had been dried for 4 hr at 85 C and weighed. The filter paper containing the mycelium was dried

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for 16 hr at 85 C and weighed.

The ionic effect of the calcium ion on fungal growth was determined in solid and liquid media. Dehydrated potato-dextrose agar (PDA), amended with CaCl₂ to produce 0, 300, 1,000, 3,000, or 10,000 ppm Ca, was used for the solid medium. The pH was adjusted to 6.0. Mycelial plugs 5 mm in diameter from 2-wk-old cultures of *P. expansum* were aseptically transferred to the center of petri plates containing the amended PDA. The fungi were then incubated for 4 days at 20 C. Fungal growth was determined by measuring the diameter of growth as the mean of its width and length. Dehydrated potato-dextrose broth (PDB), amended with CaCl₂ to produce 0, 300, 1,000, 2,000, 3,000, or 5,000 ppm Ca, was used for the liquid medium. The pH was adjusted to 6.0 in all treatments, and 10 ml of the Ca-amended PDB was placed in 50-ml sterile Erlenmeyer flasks and inoculated with a spore suspension of *P. expansum* (1 × 10⁶ spores per milliliter). Ten flasks at each CaCl₂ concentration were inoculated. The flasks were incubated for 2 days at 20 C. Mycelial biomass was determined by filtering and weighing as described previously.

The effect of Ca treatment on total phenolics in stored apples was also evaluated. Fruits that had been pressure-infiltrated with the various concentrations of CaCl₂ were removed from storage (0 C) after 6 mo. The peel and core of the apples were removed, the remaining apple tissue sliced, and 10 g immediately placed in 150 ml of boiling methanol for 18 min. The methanol extract was then decanted, and 150 ml of 50% methanol was added to the tissue and fragmented in a Waring blender for 2 min. This mixture was boiled for 20 min and combined with the initial methanol extract and clarified by centrifugation and 18 min at 10,000 g. The final volume of the extract was made up to 150 ml with methanol. Sections from three fruits were composited in each sample and four samples per treatment were analyzed. The quantitative estimation of total phenolics was made by the method of Swain and Hillis (12).

RESULTS

Fruit firmness after storage, whether fruits were treated with CaCl₂ or kept in low O₂, ranged between 60 and 85 N (N = Newton = 0.2248 lb) (Fig. 1). Firmness was increased by higher concentrations and by longer exposures to low O₂. At a similar firmness, however, area of decay was reduced only 15% by low O₂, but more than 50% with 4, 6, or 8% CaCl₂.

The relationship of area of decay to Ca content of the apple flesh is shown in Fig. 2. Ca concentration of fruit flesh ranged from about 100 ppm (μg Ca/g d.b.) in the nontreated fruits to more than 2,100 ppm in the fruits treated with 4, 6, or 8% CaCl₂, with a corresponding decrease in the area of decay of about 50%. These results indicate that the apples from any given lot of fruit can only take up a certain amount of CaCl₂ solution, and in this instance, increasing the concentration to much above 4% did not result in an appreciable increase in Ca concentration in fruit flesh or a more significant decrease in the area of decay. Fruits stored in low O₂ had essentially the same Ca concentration in the flesh as the apples treated with 0% CaCl₂.

When the fungus was grown on apple juice extracted from fruits treated with the various concentrations of CaCl₂, there was no significant correlation between mycelial biomass and percent CaCl₂ with which the fruits were treated. Approximately 25.3 mg of mycelium was produced regardless of the CaCl₂ content.

There was also no correlation between mycelial biomass and Ca concentration in Ca-amended PDA and PDB. Not only was fungal growth not inhibited by increased Ca content of the growth media, but concentrations of Ca up to 5,000 ppm in the media actually proved to be somewhat stimulatory to the growth of the fungus.

Total phenolic content ranged from 2.2 to 2.7 mg chlorogenic acid equivalents per gram of fresh tissue, but there was no correlation between total phenolic content of fruit and percent CaCl₂ with which the fruits were infiltrated.

DISCUSSION

Because calcium ion in vitro did not reduce fungal growth, and because there was no growth reduction when *P. expansum* was

grown on juice extracted from calcium-treated fruit, it seems that the effect calcium has on reducing decay is indirect and that the intact fruit is necessary for this effect to be realized. Calcium did not influence total phenolic compounds in the cells, and it is unlikely that its effect is produced by influence on individual phenolic compounds.

A more plausible explanation of the role of calcium in reducing decay in stored fruit is that it may stabilize or strengthen cell walls of fruits, making them more resistant to decay by fungal enzymes. *P. expansum* produces polygalacturonases, which are involved in the rotting of apple fruits (3). The activity of hydrolases that degrade pectic acids is often reduced by calcium-induced changes in the substrate. Since calcium-treated apples remain firmer than low-Ca fruit (2), it appears that calcium may prevent the pectic enzymes from destroying pectin (6). It has been suggested (14) that calcium introduced into apples by postharvest treatment is incorporated into the cell wall in the same manner as native calcium, and it may stabilize the cell walls and maintain fruit firmness by resisting degradation by enzymes occurring naturally in the fruit. We assume that the Ca-induced changes in the cell wall also render the wall more resistant to enzymes produced by fungal pathogens, which in turn slows penetration of the fungus and decreases decay.

Although low-O₂ storage of apples maintains fruit firmness (1,7), the mechanism for maintaining this firmness is not known. Perhaps the native pectic enzymes of the fruits are not functional or their

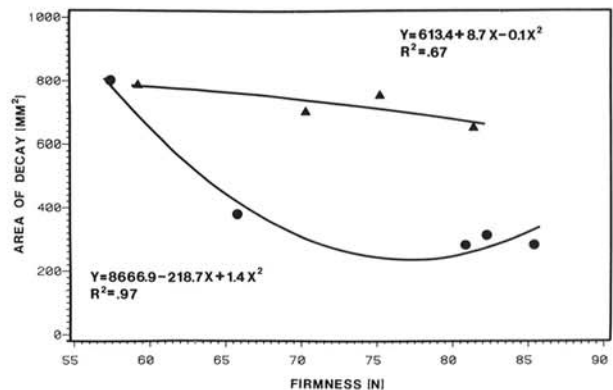


Fig. 1. Relationship between area of decay and fruit firmness when firmness was mediated by calcium chloride (CaCl₂) treatment (●) or low oxygen storage (▲). Data points for calcium treated fruit indicate the concentration of CaCl₂ solutions (0, 2, 4, 6, or 8%) from left to right, respectively. Data points for the low oxygen stored fruit indicate fruit stored in conventional cold storage for 6 mo, fruit stored in low oxygen storage for 2 mo and conventional cold storage for 4 mo, fruit stored in low oxygen storage for 4 mo and conventional cold storage for 2 mo, and fruit stored in low oxygen storage for 6 mo, from left to right, respectively.

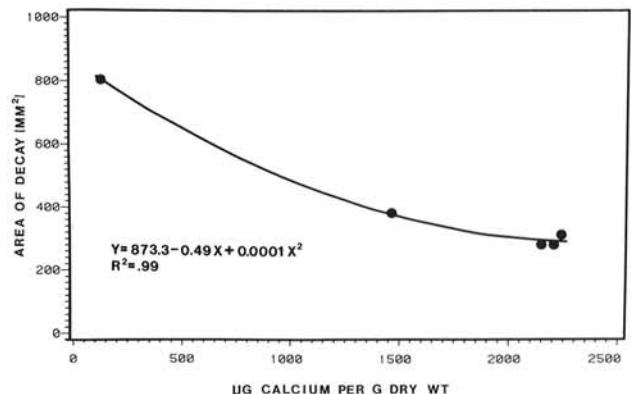


Fig. 2. Relationship between area of decay and calcium concentration of apple tissue when fruit were pressure infiltrated with calcium chloride (CaCl₂). Data points indicate the concentration of the CaCl₂ solutions (0, 2, 4, 6, or 8%) from left to right, respectively.

synthesis is slowed by low O₂. The effectiveness of the fungus in causing decay was decreased by over 15% over the firmness range from 60 to 85 N. This is the range in which firmer fruit is expected to have more hemicelluloses (13). Thus, it is possible that the decrease in fungal decay in low O₂ fruit is caused by structural changes in the cell wall. Although both systems tested maintain the same fruit firmness in storage at 0 C, Ca-mediated firmness resists decay better than O₂-mediated firmness. Although firmness, per se, may play a role in resistance to decay, it is probably the chemical composition of the cell wall by which firmness is mediated that confers greater resistance to decay by fungal enzymes.

Additional studies into the actual mechanism or mechanisms involved in Ca-mediated resistance to decay, then, must be undertaken.

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