Calcium Infiltration of Golden Delicious Apples and Its Effect on Decay

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ABSTRACT

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Golden Delicious apples were treated with 0, 2, 4, 8, or 12% solutions of calcium chloride by dipping, vacuum infiltration (250 torr), or pressure infiltration (68.95 kPa). One lot of apples was inoculated with a conidial suspension of *Penicillium expansum* immediately after treatment, and a second lot was placed in storage for 5 mo at 0 C, removed, and inoculated in the same manner as the first lot. Inoculated apples were stored for 7 days at 20 C, then were rated for decay severity and analyzed for calcium content and water-soluble polyuronides. The smallest area of decay, the fewest water-soluble polyuronides, and the highest amount of calcium in tissues were found in the apples that were pressure infiltrated with 12% calcium chloride. However, apples stored for 5 mo after treatment by vacuum or pressure infiltration of 8 or 12% solutions of calcium chloride showed peel injury. The optimum treatment, which reduced the area of decay by 30% and resulted in no peel injury, was pressure infiltration of a 4% calcium chloride solution.

Additional key words: Malus domestica, Penicillium expansum.

The majority of efforts in the postharvest treatment of apples with low concentrations of calcium (Ca) salts has been aimed at reducing losses due to physiological disorders (1,12,14). Vacuum and pressure infiltration (15) of calcium salt solutions have been especially effective in getting Ca into the apple and reducing physiological problems.

The addition of $CaCl_2$ to benomyl increased the effectiveness of the fungicide in controlling decay following postharvest treatment (5). A more recent investigation (7) showed that increased Ca content of fruit by postharvest application may also reduce fruit decay. Delicious apples pressure infiltrated with an 8% $CaCl_2$ solution, stored for 3 mo, and inoculated with a spore suspension of *Penicillium expansum* Link ex Thom. had more than 40% less decay than nontreated fruits. No surface injury to the fruit was

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observed with the 8% $CaCl_2$ solution.

Ca also retards natural softening of apples in storage (12). This effect may be one of maintaining cell-wall integrity through the binding of carboxyl groups in the polygalacturonate polymers by calcium ions (13). The resulting cohesion is thought to retard the enzymic solubilization of polyuronides from the middle lamella (2). Because *P. expansum* produces enzymes that are involved in cellular degradation leading to typical decay symptoms (6), Ca may maintain cell-wall integrity in this case by retarding fungal enzyme activity.

The objectives of this study were to determine the effects of postharvest Ca treatment on the decay of Golden Delicious apples by *P. expansum*, to determine the effect of Ca treatment on water-soluble polyuronide content of the fruit, and to determine the optimum method of treatment and the optimum concentration of $CaCl_2$ solution that can be used without causing injury to the fruit.

MATERIALS AND METHODS

Golden Delicious apples (*Malus domestica* Borkh.) were harvested from a commercial orchard in Pennsylvania. The apples were randomized and treated with laboratory grade USP calcium chloride ($CaCl_2$, 76%), made up as 0, 2, 4, 8, or 12% solutions. Methods of treatment with each of the solutions included dipping, vacuum infiltration, and pressure infiltration. Dipping and pressure-infiltration (69.95 kPa) treatments involved placing the fruits in the respective solutions for 2 min. Vacuum infiltration was performed by placing the fruits in $CaCl_2$ solution for 2 min under 250 torr and then holding them in solution for 2 min after vacuum release. Following treatment, the fruits were placed on Kraft paper and allowed to drain dry for 1 hr. The fruits were then divided into two lots; one was inoculated 24 hr after the Ca treatment and the other was placed in storage (0 C). After 5 mo, the second lot was removed from storage, and the fruits were inoculated with *P. expansum* in the same manner as the first lot.

All inoculations were performed by first wounding the fruits on two sides to a depth of 2 mm by pressing them down on the head of a nail 2 mm in diameter. The fruits were then immersed for 15 sec in a conidial suspension (1×10^6 spores per milliliter) in nutrient broth containing 0.5% Tween-20. After additional holding at 20 C for 7 days, the apples were rated for severity of decay by measuring the diameter of the decayed area as the mean of its width and length and then computing total area of decay. Fifteen fruits were used for each treatment.

Ca content of the apple tissue was determined by removing the peel and outer flesh of the entire fruit to a depth of 2 mm with a mechanical peeler. The next 3 mm of flesh tissue was then removed, again using a mechanical peeler, and this layer was analyzed for Ca content since this was the depth to which the apples were wounded for inoculation. The flesh from three apples made up one sample, and three samples from each treatment were analyzed. After 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 of 6N HCl. The samples were then diluted and analyzed for Ca content with a Jarrell-Ash atomic absorption spectrophotometer. All Ca values are reported on a dry-weight basis.

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

The method of extraction for water-soluble polyuronides was modified from Irwin (9). A 60-g sample of healthy cortical tissue from radial slices of fruit was blended for 30 sec in 120 ml of 0.2 M K_2CO_3 and then vacuum-filtered through 0.5-mm mesh nylon cloth. $MgCl_2$ solution (400 g/L) was added at the rate of 5 μl/ml of extract. Twenty-five milliliters of this solution was then centrifuged for 15 min at $10,000 \times g$. The pellet was suspended in 10 ml of absolute ethanol and centrifuged for 5 min at $10,000 \times g$. It was then resuspended in 25 ml of hot (100 C) water, cooled to 20 C in an ice bath, and clarified by centrifugation for 5 min at $10,000 \times g$. An aliquot of this solution was then assayed by the method of Blumenkrantz and Asboe-Hansen (3).

Both lots of fruit were also rated for calcium injury at the time of the disease-severity ratings. Injury rating was done by visually observing the fruit surface and assigning it a value between 1 (severe injury) and 5 (no injury). Ten fruits per treatment were examined, and the final rating was the mean of the ratings of three observers.

RESULTS

The effectiveness of treatment method and relation of flesh calcium concentration to decay were similar for both lots of apples, whether the apples were inoculated immediately after treatment or placed in storage for 5 mo and then inoculated. The results discussed relate mainly to data from the stored fruits because these findings are of greater interest.

The ability of $CaCl_2$ to reduce decay was dependent upon the method of treatment (Fig. 1). There was no decrease in decay severity in those apples dipped in the various $CaCl_2$ solutions. Vacuum infiltration was more successful, in that the area of decay was reduced more than 30% with the 12% $CaCl_2$ solution. Pressure infiltration proved the most effective way to reduce decay, as the 12% $CaCl_2$ solution reduced the area of decay by more than 50%, and even the 4% $CaCl_2$ solution decreased the decayed area by more than 30%.

The relationship of Ca content of the apple flesh to the area of decay is shown in Fig. 2. Concentration of Ca in apples dipped in $CaCl_2$ increased as the concentration of the solution increased (Fig. 2A), but not enough to reduce decay. Vacuum infiltration (Fig. 2B) of the 12% $CaCl_2$ solution increased Ca content to about 1,500 μg/g or about double that of the dip treatment in the same solution, resulting in a reduction of more than 30%. Pressure infiltration of the 12% $CaCl_2$ solution (Fig. 2C) increased Ca concentration of the flesh to 3,300 μg/g, more than twice that of the vacuum infiltration, and reduced the area of decay by more than 50%. A 4% $CaCl_2$ solution pressure-infiltrated into the fruits increased Ca content to about 1,500 μg/g, reduced the decayed area by more than 30%, and proved to be as effective as the 12% $CaCl_2$ solution vacuum-infiltrated into the fruit.

The relationship of fruit firmness to area of decay is given in Fig. 3. Firmness of fruit that was pressure-infiltrated with $CaCl_2$ solutions only are given, as the differences here are greatest. There was a direct relationship between area of decay and firmness, as there was between Ca content and area of decay, indicating that the effect of Ca in reducing decay may be related to an effect that Ca has on retarding fruit softening.

A direct relationship between area of decay and water-soluble polyuranide content of the fruit tissue, which also may be related to an effect that calcium has on maintaining fruit firmness, was

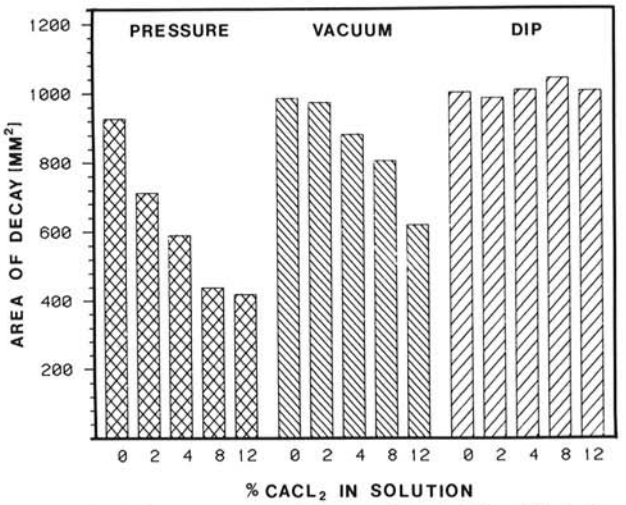

Fig. 1. Relationship between area of decay and method by which fruit were treated with calcium chloride ($CaCl_2$) solutions.

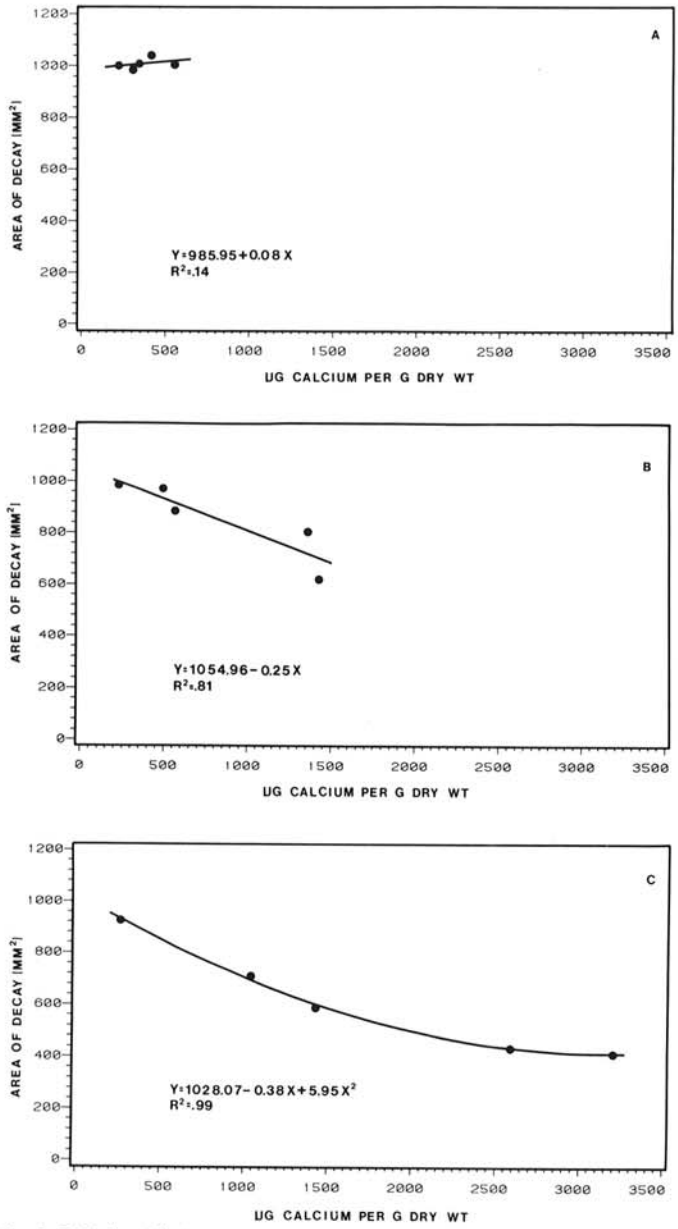

Fig. 2. Relationship between area of decay and calcium concentration of apple tissue when fruit were treated with calcium chloride ($CaCl_2$) solutions by **A)** dipping, **B)** vacuum infiltration, and **C)** pressure infiltration. Data points indicate the concentration of the $CaCl_2$ solutions (0, 2, 4, 8, or 12%) from left to right, respectively.

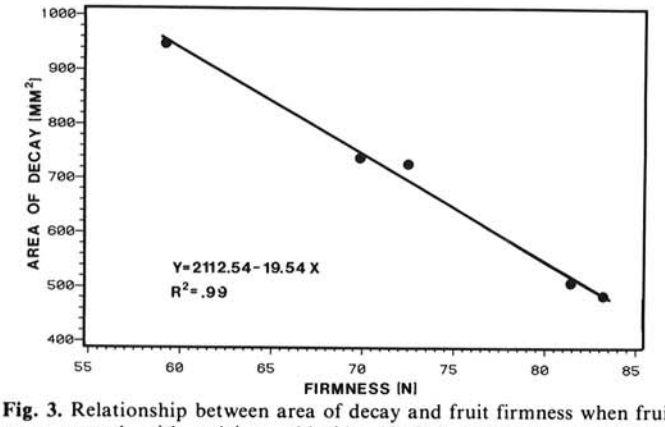

Fig. 3. Relationship between area of decay and fruit firmness when fruit were treated with calcium chloride ($CaCl_2$) solutions by pressure infiltration. Data points indicate the concentration of the $CaCl_2$ solution (0, 2, 4, 8, or 12%) from left to right, respectively.

determined (Fig. 4). Those fruits exhibiting a large area of decay also contained greater amounts of water-soluble polyuronides than fruits having less decay. Increasing calcium content of the fruit tissue, then, effectively retarded both decay and the solubilization of polyuronides.

The treatments causing substantial fruit injury were the 8 and 12% $CaCl_2$ solutions either vacuum- or pressure-infiltrated into the fruits (Table 1). Although cross-section examination of the fruits revealed that the injury was very superficial and limited mainly to the peel, the apples would not be suitable for market.

The optimum treatment in these tests, then, was a 4% $CaCl_2$ solution pressure-infiltrated into the apples, resulting in a 30% reduction in decay. Although the vacuum infiltration of a 12% $CaCl_2$ solution was equally effective, and pressure infiltration of an 8 or 12% $CaCl_2$ solution was even more effective in reducing decay, the surface injury caused by these treatments made them unsuitable for treating Golden Delicious apples for market.

DISCUSSION

The mechanism by which calcium retards fungal decay may be similar to the effect that calcium has on the mechanism that delays ripening or senescence and the resulting softening of the fruit. The breakdown of pectic substances in the middle lamellae and cell walls, and the resultant loss of cell-wall integrity, may be a key step in the initiation of many of the changes that occur during the fruit-ripening process (17,18). Pectinesterase (PE) and polygalacturonase (PG) apparently are the major enzymes that cause the breakdown of pectic substances in fruits. The work of Jansen and MacDonnell (11) suggests that, in vivo, PE first hydrolyzes methyl groups from the pectin chain, allowing PG to cleave the glycosidic linkage between de-esterified galacturonic acid units.

Free carboxyl groups on the polygalacturonate polymers are important in maintaining cell-wall integrity, probably through the cooperative binding of calcium ions (13). Polyuronides, solubilized during the ripening of apple fruit, are thought to be derived from the middle lamella of the cell wall (10). These molecules may arise from a loosening of the polyuronide network during enzymic hydrolysis (2).

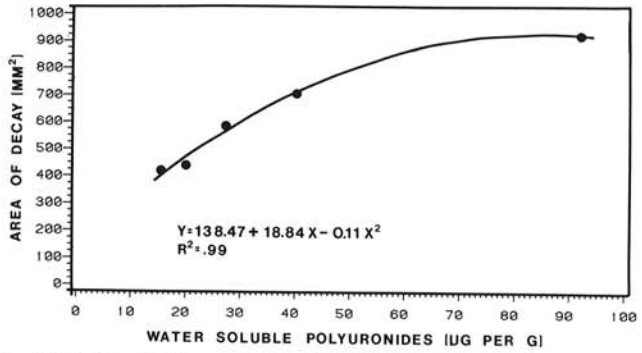

Fig. 4. Relationship between area of decay and water-soluble polyuronide content of apple tissue when fruit were treated with calcium chloride ($CaCl_2$) solutions by pressure infiltration. Data points indicate the concentration of the $CaCl_2$ solution (12, 8, 4, 2, or 0%) from left to right, respectively.

TABLE 1. Effect of treatment method and $CaCl_2$ solution concentration on Ca injury to apple fruit

Treatment method	Calcium concentration (%)				
	0	2	4	8	12
Pressure	5.0[a]	5.0	5.0	3.2	1.2
Vacuum	5.0	5.0	5.0	3.8	2.5
Dip	5.0	5.0	5.0	5.0	5.0

[a] Rating: 1 = severe injury, 5 = no injury.

Wills et al (22) found that ripening of tomatoes could be delayed substantially by vacuum infiltration of $CaCl_2$ solutions. They concluded that calcium inhibits ripening by affecting key reaction systems that control the onset and maintenance of ripening. Wills and Rigney (21) explained this function of calcium in delaying ripening when they found that a large increase in calcium in tomato fruit inhibited the activity of PE and PG and thereby reduced the rate of cell-wall breakdown.

Earlier, Weintraub and Ragetli (19), in working with tobacco leaves, found that cell walls containing primarily calcium pectate resisted degradation by pectic enzymes more readily than did tissues that contained primarily pectic acid interspersed with calcium pectate. Buescher and Hobson (4) reported that resistance to PG declines during ripening in tomatoes, but that Ca inhibits this degradation by PG of the cell wall and middle lamellae. Because apples treated with calcium remained firmer than low-Ca fruit (1), Faust (8) concluded that the calcium in calcium-treated apples may prevent the pectic enzymes from destroying pectin. Wienke (20) suggested that most calcium applied to the surface of apples penetrates through the lenticels and is localized in the cell wall, possibly bridging with the plasmalemma, and that the localization of native calcium and calcium originating from postharvest treatments is the same. Sharples (16) stated that the effects of calcium on cell-wall metabolism and on the structure of the apple not only confer greater resistance to changes that precede softening, fungal invasion, and the development of disorders, but also may delay the general rate of senescence of the tissue.

Enzymatic activity is also involved in the rotting of fruits by fungal organisms. *P. expansum* produces polygalacturonases that are involved in cell-wall degradation leading to the typical rotting symptoms (5). So, in much the same manner that calcium causes a resistance to the changes brought about by those enzymes that occur naturally in the fruit, it may also increase resistance to decay due to the enzymes produced by fungal pathogens. The proper selection of treatment method and calcium chloride concentration is important to ensure maximum decay reduction and minimum fruit injury.

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