Altering Nutritional Factors After Harvest to Enhance Resistance to Postharvest Disease

William S. Conway

Research plant pathologist, USDA, ARS, Horticultural Crops Quality Laboratory, BARC-West, Beltsville, MD 20705.
Accepted for publication 15 March 1989.

Many postharvest storage disease problems arise as quiescent or latent infections that are held in check until the fruit matures. Others occur as a result of injuries during harvesting and handling operations and subsequent infection from fungal spores infesting the fruit surface or those encountered in the various types of dump tanks. These fungi, having bypassed the first layer of defense or the skin or peel of the fruit, may secrete enzymes that macerate or break down the cell walls of the organ, resulting in decayed or rotten fruit. If disease resistance in the host can be enhanced by strengthening or stabilizing the cell walls, making them more resistant to breakdown by macerating enzymes, losses due to decay may be reduced.

Divalent cations in general and calcium (Ca) in particular have been associated with physiological and pathological problems of stored fruit. There are a vast number of possible interactions that can affect Ca uptake and distribution, and we are unlikely to see the development of cultural practices in the near future that will completely eliminate Ca deficiency, without a direct application of Ca to the susceptible organ (1). Therefore, methods should be developed or improved for direct Ca treatments of fruit as has been done successfully for apple fruits (25). This paper discusses the factors involved in the uptake of calcium by postharvest treatments and the resulting effect this increased calcium has on fruit quality.

METHODS OF POSTHARVEST TREATMENT

Storage disorders resulting from low Ca in storage organs are thought to result from an inefficient distribution of Ca rather than poor Ca uptake, since leaves can have much more Ca than

storage organs from the same plant (2). Various methods of increasing the amount of Ca in these storage organs have been investigated. Increasing Ca content through fertilization and liming regimes would seem to be the obvious method of choice. However, as stated above, there are a vast number of possible interactions that can offset Ca uptake and further distribution, and fertilization and liming cannot guarantee increasing the Ca content by the required amount. It has, however, been shown to be effective in some cases (19). The direct application of Ca to the storage organ would appear to be the most effective method for increasing its Ca content. The Ca content of apples has been increased by foliar sprays, but not to the extent that results from postharvest dipping in $CaCl_2$ solutions (13,26). Dipping apples in solutions of $CaCl_2$ can increase the concentration of Ca in the fruit flesh (22). The addition of additives such as food thickeners to the dip solutions further increases Ca levels in apple fruit, probably by allowing the fruit to remain in contact with a film of Ca solution for a longer period of time (18). The use of temperature differentials, especially dipping warm apples into a cold solution of $CaCl_2$, also resulted in an additional uptake of the solution (17). Vacuum or pressure infiltration of $CaCl_2$ solutions proved superior to dipping for the control of bitter pit (caused by Ca deficiency) of apples in Australia and New Zealand (24). In studies comparing treatment of Delicious and Golden Delicious apples with solutions of $CaCl_2$ by dipping, vacuum infiltration, or pressure infiltration, the Ca concentration of fruit flesh was increased the most using pressure infiltration, followed by vacuum infiltration, with dipping being a distant third (6).

In another study comparing treatment of peaches with $CaCl_2$ solutions by preharvest spray application or postharvest pressure infiltration, there was a significant increase in Ca concentration of peach flesh after preharvest spray applications, but the increase due to postharvest pressure infiltration was twice that resulting from spraying (7). Vacuum infiltration of Ca solutions into potato tubers increased the Ca content in peel and medullar tissues significantly (19). Pressure infiltration of mangos with solutions of $CaCl_2$ failed to have any effect on the variables tested, but no determination of Ca concentration of flesh was made after treatment, so it was not known whether there was an increase in flesh Ca as a result of the treatment (28).

INFLUENCE OF FRUIT MATURITY ON CALCIUM UPTAKE

The level of control of both physiological and pathological disorders in stored fruits and vegetables by calcium is related to the amount of Ca taken up by the fruit. The potential amount of uptake, in turn, is influenced by the changes in the structure of the fruit as it matures during growth and development. Development of apple fruit was studied by several early workers (27,30,32). Cell division ceases within 4 wk of pollination, and all subsequent increase in fruit size is due to the enlargement of both cells and intercellular spaces. As the cells enlarge, they gradually separate as the middle lamella becomes gelatinous, and large, irregularly shaped intercellular spaces develop (31). Cell enlargement and increase in intercellular spaces continue throughout the life of the fruit on the tree (1).

Ca applied as postharvest treatments enters the fruit through epidermal openings, primarily lenticels (4). If the fruit is left on the tree past the prime harvesting date, Ca also enters through cracks in the epidermis (5). Growing conditions apparently are a primary factor in the development of cracks or other irregularities (14). Another study showed that cuticles isolated from fruit harvested in September of 1982 had far more cracks than did fruit harvested in September of 1981, and concluded that the development of cracks and other surface irregularities during the latter part of the growing season may combine to play a significant role in Ca penetration into the apple fruit (15). The development of cracks in late summer and early fall may contribute to late-season Ca sprays being more effective than early sprays. The varying amounts of cracking observed from year to year in the cuticle of the apple fruit may not only account for the varying effects of Ca sprays often observed between years, but also may implicate the relative importance of cracking as a pathway of Ca uptake (15). In a study to determine the influence of fruit maturity on Ca uptake in apples treated postharvest by pressure infiltration, fruit were harvested three times at 2-wk intervals. These harvests occurred 2 wk before the predicted prime harvest period, at the prime harvest period, and 2 wk later. Those fruit picked 2 wk after prime harvest and treated with an 8% solution of $CaCl_2$ had three times as much flesh Ca as fruit harvested 2 wk before prime harvest and similarly treated (9). This study also elucidated the major concerns of injury caused by the postharvest treatment of apples with solutions of $CaCl_2$. Little injury occurred on the fruit picked 2 wk before prime harvest. Surface injury occurred on fruit picked at prime harvest and treated with some of the higher concentrations of $CaCl_2$ solutions, but an examination of the cross section of the apple revealed that the injury was superficial and limited mainly to the peel, so the fruit would be quite suitable for processing. Fruit harvested and treated 2 wk after prime harvest took in excessive Ca from the 8% $CaCl_2$ solution. Injury, in the form of brown discoloration, extended 10 mm into the cortex, making fruit unsuitable for the fresh market and probably processing as well. Even dipping fruit in higher concentrations of $CaCl_2$ solutions may cause surface injury (22). Some of the injury may be due to poor drainage from the calyx and stem end cavity (23). By using an active infiltration procedure such as vacuum or pressure, the Ca can be forced into the fruit and that remaining on the surface can be rinsed off following treatment to reduce the likelihood of injury.

RELATIONSHIP OF CATIONS TO PLANT DISEASE RESISTANCE

The relationship between Ca ions and the cell wall has been shown to play a key role in disease resistance. Ca ions are bound to the pectins present in the cell wall (12). Few pectins, if any, are free of neutral sugars, notably rhamnose, and are composed of polygalacturonic acid residues in a chain with rhamnose insertions into the chain (21). The rhamnose insertion puts a marked kink in this chain. The resulting bunched configuration of the polygalacturonic chain allows spaces for the insertion of a series of cations, all of which may be filled because the binding of one ion causes chain alignment that facilitates the binding of the next (16). The formation of cation cross bridges between pectic acids or between pectic acids and other polysaccharides with acid groups may make the cell wall less accessible to enzymes produced by fungal pathogens that cause decay (29). There is a high affinity of the carboxylic groups for Ca ions, and the resulting effect on physiological or pathological processes is greater than for other cations routinely encountered in plant tissues. Alternately, the middle lamella exists as a gel and Ca is very efficient in promoting gelling in a pectic solution, while manganese (Mn) and magnesium (Mg) are practically without effect (29). When ion exchanges are performed in the cell walls, either between Ca and monovalent cations or between Ca and Mg, the walls have always exhibited a marked preference for Ca (11,13). Bateman (3) found that in bean tissue infected with *Rhizoctonia solani* Kühn, tissue maceration by polygalacturonase was greatly reduced in the presence of barium (Ba) and Ca; it was less inhibited by Mg, and was not significantly influenced by the monovalent cations potassium (K) or sodium (Na). When potato tubers were infiltrated with salt solutions of divalent cations and inoculated with *Erwinia carotovora* pv. *atroseptica*, bacterial soft rot was significantly lower in Ca-infiltrated tubers than in nontreated ones. Mg and strontium (Sr) also reduced the severity of tuber decay, but not as effectively as Ca, and all of the divalent cations proved more effective than Na or K (19). A continuation of the potato tuber-Ca study concluded that the reduction of Erwinia soft rot in high-Ca tubers can be attributed, in part, to the decrease in maceration by pectolytic enzymes that is related to the enhancement of structural integrity of cell walls and

membranes by increasing Ca levels (20).

In a study to determine the effect of cations on the decay of stored apples caused by *Penicillium expansum* Link ex Thom. fruit were infiltrated with solutions of Ca, Mg, and strontium (Sr). All of the cations reduced decay, but Ca was significantly better than Mg or Sr, and Mg was not significantly different from Sr. Mg caused surface injury in the form of brown, circular, sunken lesions on the fruit surface that appeared to be similar to symptoms resembling bitter pit (10). A continuation of this study to determine the actual mechanism by which supplemental Ca reduced decay of stored apples was undertaken (8). Apples were pressure infiltrated at harvest with solutions of $CaCl_2$ and stored at 0 C. After 6 mo, fruit were removed from storage and cell walls were extracted and analyzed for Ca content. Polygalacturonase was purified from the decayed area of nontreated apples that had been inoculated with *P. expansum*. Cell wall Ca content was positively correlated with the percent Ca used in the infiltration solutions. Extracted walls with varying Ca content were then used as substrate for polygalacturonase of *P. expansum* to test the effect of wall Ca on in vitro enzyme activity. Significantly less product was formed when high-Ca cell walls were used as substrate compared to low-Ca cell walls. The results of this study support the conclusion that reduction in decay in apple caused by *P. expansum* is due, in part at least, to a decrease in maceration of cell walls by polygalacturonase as a result of improved structural integrity caused by an increase in Ca content. Thus, by increasing the amount of Ca in apple fruit, the level of defense in apple to enzymatic tissue maceration is increased and decay and resulting storage loss is reduced.

POSTHARVEST PATHOGENS AFFECTED BY SUPPLEMENTAL CALCIUM

As discussed previously, Ca seems to be more closely related to improved disease resistance than other cations associated with the cell wall. Various investigators have studied the effect of supplemental Ca on postharvest host-pathogen combinations and have used different means of increasing Ca in the hosts. In early work preharvest sprays of $Ca(NO_3)_2$ on apples reduced storage loss due to decay caused by *Gloeosporium perennans* Zeller et Childs (25). In a study on the effect of increased flesh Ca content of apples on storage decay caused by *P. expansum*, fruit were treated with solutions of $CaCl_2$ by dipping, vacuum, or pressure infiltration. The fruit did not take up enough Ca in the dipping treatment to affect severity of decay, but both vacuum and pressure infiltration increased Ca content of the fruit sufficiently to significantly reduce decay (6). Increasing the Ca content of potato tubers also reduced storage losses. Ca content was significantly increased by field fertilization or postharvest vacuum infiltration with $CaSO_4$ or $Ca(NO_3)$ and storage losses caused by *E. carotovora* were reduced significantly (19). In an attempt to reduce decay caused by *Colletotrichum gloeosporioides* Penz. (anthracnose), or *Diplodia natalensis* P. Evans and *Phomopsis citri* Fawc. (stem end rot), mangos were pressure infiltrated with solutions of $CaCl_2$. This treatment failed to control losses due to these pathogens. However, Ca content of mangos was not analyzed, and it is not known whether there was any actual increase of Ca content of the fruit (28). Peaches were sprayed preharvest or pressure infiltrated postharvest with solutions of $CaCl_2$ to increase Ca content. Both treatments increased Ca content of fruit flesh significantly, but only the pressure infiltration treatments increased Ca content sufficiently to reduce decay caused by *Monilinia fructicola* (Wint) Honey (7).

CONCLUSION

Methods that increase the Ca content of the host and reduce losses caused by postharvest pathogens are of unique importance because they are a means of increasing the effectiveness of a natural, internal resistance mechanism already present in the host. Rather than directly affecting the microorganism as is done with fungicides, addition of Ca stabilizes the cell wall and makes it more resistant to maceration by fungal pectolytic enzymes. Since Ca strengthens the host rather than kill the pathogen, there may be little selection pressure for the development of more aggressive strains of the pathogen. Also, Ca is available in the form of a common salt, which can be acquired as a food grade preparation. Increase of resistance of the host with Ca must be tested on a commercial basis to determine if it is as effective on a large scale as it has been demonstrated during experimentation. However, there are several potential difficulties with Ca that must be considered. Since Ca exists as a salt, the potential for surface injury of the treated commodity exists. Those working with apples have found some cultivars to be more susceptible to peel injury than others (6,9), and a peach cultivar was found to sustain surface injury from pressure infiltration of the distilled water control (7). Thus, the concentration of Ca that would afford significant protection against the pathogen but cause no superficial injury or poor taste must be determined for each commodity. Sanitation of solutions of Ca would have to be maintained to minimize the number of fungal spores entering the host during infiltration of a Ca solution.

Treatments of various storage organs with solutions of Ca salts has the potential to reduce storage losses due to pathological and physiological diseases and result in a high quality product for the consumer.

LITERATURE CITED

1. Bain, J. M., and Robertson, R. N. 1951. The physiology and growth in apple fruits. I. Cell size, cell number, and fruit development. Aust. J. Sci. Res. 4:79-91.
2. Bangerth, F. 1979. Calcium-related physiological disorders of plants. Annu. Rev. Phytopathol. 17:97-122.
3. Bateman, D. F. 1964. An induced mechanism of tissue resistance to polygalacturonase in *Rhizoctonia*-infected hypocotyls of bean. Phytopathology 54:438-445.
4. Betts, H. A., and Bramlage, W. J. 1977. Uptake of calcium by apples from postharvest dips in calcium chloride solutions. J. Am. Soc. Hortic. Sci. 102:785-788.
5. Clements, H. F. 1935. Morphology and physiology of the pome lenticels of *Pyrus malus*. Bot. Gaz. 97:101-117.
6. Conway, W. S. 1982. Effect of postharvest calcium treatment on decay of Delicious apples. Plant Dis. 66:402-403.
7. Conway, W. S., Greene, G. M., 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., Boyer, C. D., and Sams, C. E. 1988. Inhibition of *Penicillium expansum* polygalacturonase activity by increased apple cell wall calcium. Phytopathology 78:1052-1055.
9. Conway, W. S., and Sams, C. E. 1985. Influence of fruit maturity on the effect of postharvest calcium treatment of Golden Delicious apples. Plant Dis. 69:42-44.
10. Conway, W. S., and Sams, C. E. 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. J. Am. Soc. Hortic. Sci. 112:300-303.
11. Demarty, M., Morvan, C., and Thellier, M. 1978. Exchange properties of isolated cell walls of *Lemna minor* L. Plant Physiol. 62:477-481.
12. Demarty, M., Morvan, C., and Thellier, M. 1984. Calcium and the cell wall. Plant Cell Environ. 7:441-448.
13. Drake, M., Bramlage, W. J., and Baker, J. H. 1979. Effects of foliar calcium on McIntosh apple storage disorders. Commun. Soil Sci. Plant Anal. 10:303-309.
14. Faust, M., and Shear, C. B. 1972. Fine structure of the fruit surface of three apple cultivars. J. Am. Soc. Hortic. Sci. 110:166-171.
15. Glenn, G. M., Poovaiah, B. W., and Rasmussen, H. P. 1985. Pathways of calcium penetration through isolated cuticles of Golden Delicious apple fruit. J. Am. Soc. Hortic. Sci. 110:166-171.
16. Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., and Thom, D. 1973. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Lett. 32:195-198.
17. Lee, J. J. L., and Dewey, D. H. 1981. Infiltration of calcium solutions into Jonathan apples using temperature differentials and surfactants. J. Am. Hortic. Sci. 106:488-490.
18. Mason, J. L., Drought, B. G., and McDougald, J. M. 1974. Calcium concentration in apple fruit resulting from calcium chloride dips codified by surfactants and thickeners. HortScience 9:122-123.
19. McGuire, R. G., and Kelman, A. 1984. Reduced severity of Erwinia

soft rot in potato tubers with increased calcium content. Phytopathology 74:1250-1256.
20. McGuire, R. G., and Kelman, A. 1986. Calcium in potato tuber walls in relation to tissue maceration by *Erwinia carotovora* pv. *atroseptica*. Phytopathology 76:401-406.
21. Preston, R. D. 1979. Polysaccharide conformation and cell wall function. Annu. Rev. Plant Physiol. 30:55-78.
22. Reid, M. S., and Padfield, C. A. S. 1975. Control of bitter pit in apples with lecithin and calcium. N. Z. J. Exp. Agric. 7:379-381.
23. Scott, K. J., and Wills, R. B. H. 1977. Vacuum infiltration of calcium chloride: Method for reducing bitter pit and senescence of apples during storage at ambient temperatures. HortScience 12:71-72.
24. Scott, K. J., and Wills, R. B. H. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Aust. J. Agric. Res. 30:917-928.
25. Sharples, R. O. 1976. Post-harvest chemical treatments for the storage disorder of apples. Ann. Appl. Biol. 85:450-453.
26. Sharples, R. O., and Johnson, D. S. 1977. The influence of calcium on senescence changes in apples. Ann. Appl. Biol. 85:450-453.
27. Smith, W. H. 1950. Cell multiplication and cell enlargement in the development of the flesh of the apple fruit. Ann. Bot. N. S. 53:23-38.
28. Spalding, D. H. 1986. Evaluation of various treatments for control of postharvest decay of Florida mangos. Proc. Fla. State Hortic. Soc. 99:97-99.
29. Tepfer, M., and Taylor, I. E. P. 1981. The interaction of divalent cations with pectic substances and their influence on acid induced cell wall loosening. Can. J. Bot. 59:1522-1525.
30. Tetley, U. 1930. A study of the anatomical development of the apple and some observations on the "pectic constituents" of the cell walls. J. Pomol. 8:153-172.
31. Tetley, U. 1931. The morphology and cytology of the apple fruit, with special reference to the Bramley's Seedling variety. J. Pomol. 9:278-297.
32. Tukey, H. B., and Young, O. J. 1942. Gross morphology and histology of developing fruit of the apple. Bot. Gaz. 104:3-25.