Metalosate® Powered by Albion® Technology

Balchem® Plant Nutrition Research Paper

CALCIUM TRANSPORT AND ATPase ACTIVITY IN MICROSOMAL VESICLE FRACTION FROM 'MONTMORENCY' SOUR CHERRY FRUIT

J.L. Anderson and W.F. Campbell
Department of Plants, Soils and Biometeorology
Utah State University
Logan, Utah, 84322-4820
U.S.A.

Abstract

Calcium maintains plasma membrane integrity, osmoregulation and extends the post-harvest firmness of 'Montmorency' sour cherry, Prunus cerasus L. Experiments were conducted on plasma membrane vesicles from pre- and post-harvest Ca-treated sour cherry fruit to ascertain calcium transport and IC-stimulated ATPase activity. Cherry trees were sprayed with a solution of Calcium Metalosate(r) [an amino acid calcium chelate (AACa), Albion Laboratories, Inc., Clearfield, UT] and cherry clusters were dipped in AACa or calcium chloride (CaCl2). Samples from mechanically harvested trees and hand-harvested fruit from dip treatments were processed for membrane-enriched vesicle fractions. Membrane protein content was about 4-fold greater in Ca-treated fruit than in controls. In the absence of adenosine triphosphate (ATP), approximately 1 nmol of Ca2+ mg protein-1 was associated with the membrane vesicles after 15 mm and remained constant. Upon the addition of ATP and Mg2+, active Ca2+ uptake into the plasma membrane vesicles was observed. Addition of the Ca2+ ionophore A23187 caused an immediate release of accumulated free Ca2+. ATP-dependent Ca2+ was not inhibited by 10 (M oligomycin. ATPase activity and H+-pumping were both completely inhibited by orthovanadate, suggesting that the fractions were free from nonplasma membrane ATPases.

Key index words

Calcium, Fruit firmness, Sour cherry, Prunus cerasus, Plasma membrane.

1. Introduction

Fruit firmness is a major problem in marketing sour, Prunus cerasus L., and sweet, P. avium L., cherries. Softness in cherry fruit has been ascribed to over-maturity, excessive rainfall or irrigation immediately prior to harvest, excessive fruit set and damage that occurs during picking and handling (Poovaiah et al. 1988). Softening is also ascribed to solubilization of Ca2+ ions from the galacturonic acid cross-linkages, which are responsible for the middle lamella, the structure that binds cell walls (Hanson 1983).

Calcium is essential for the synthesis of enzymes and the functional macromolecular structure of cellular membranes, microtubules and microfilaments (Evans et al. 1991; Poovaiah 1985). The association of Ca^{2+} with cellular membranes, especially the linking of phospholipids with membrane proteins, is required to maintain membrane integrity and control membrane-associated functions (Hanson 1983, 1984). Calcium is also the major cation integral to the protein-pectin content of the middle lamella between plant cells and a "secondary messenger" involved in maintaining micromolar concentrations of cytoplasmic Ca2+ in plants as a response to environmental or hormonal signals (Briskin 1990; Briskin and Hanson 1992; Poovaiah and Reddy 1987).

One of the primary membranes in higher plant cells is the plasma membrane (PM). It is involved in many aspects of metabolism, physiology and regulation such as uptake of nutrients, intracellular pH and osmoregulation (Sze, 1985). However, the mechanism of solute uptake across the PM is difficult to study in intact organs, such as roots, leaves, cotyledons or fruits, because of the presence of different cell types, cell walls, and age gradients. This process is best studied in an in vitro system of PM vesicles isolated from specific plant cells. Moreover, ATP-dependent uptake of solutes by PM vesicles is most conveniently studied with sealed, inside-out vesicles since the active site of the PM ATPase is on the inner, cytoplasmic side of the PM (Sze 1985). Using this PM vesicle system, we sought to compare the effectiveness of foliar applications of AACa with calcium dips in maintaining firmness and quality of sour cherry fruit and the ion-stimulation of these processes on the membrane-bound ATPase activity in native vesicles isolated from control and Cab-treated sour cherries.

2. Materials and Methods

2.1. Plant Material

Three foliar sprays containing 4.7 L/ha of chelated AACa (5% Ca) were applied at 14 day intervals in June and July, 1994 to 'Montmorency' sour cherry trees at the Utah State University Farmington Field Station (FS USU) and at the Rowley orchard in Payson, Utah 2 to 6 weeks prior to harvest. Fruit from foliar-sprayed and unsprayed trees was mechanically harvested as per commercial industry practices and random samples placed in plastic bags. Fruit from comparable samples was harvested as above, machine-pitted, and graded by Federal inspectors for number of pits remaining and fruit character. Fruit clusters from untreated cherry trees were also dipped for 30 sec in 0.5% and 0.05% calcium as AACa or CaI_2 solutions, hand harvested 20 h later and placed in plastic bags. All cherry samples were transported to the laboratory on ice and processed for PM vesicles (Fig. 1).

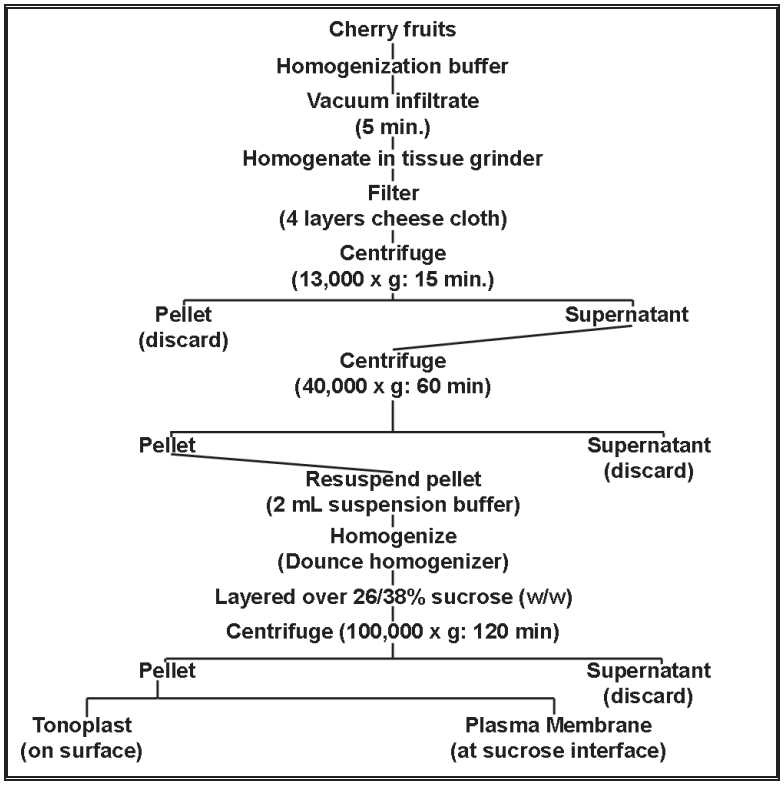

Figure 1. Flow Diagram for plasma membrane isolation from sour cherry fruit.

2.2. Isolation of Microsomal Membrane Fraction

After pitting, fruit pulp was placed in a buffer [250 mM sucrose, 250 mM potassium Iodide, 3 mM ethylenediaminetetra acetic acid (EDTA), 70 mM Tris-HCl (pH 8.0), 0.5% (w/v) polyvinylpyrrolidone (PVP_{40}), 2 mM Na_2ATP, 10% (v/v) glycerol, 0.5% (w/v) bovine serum albumin (BSA, fraction V powder), 2 mM phenylmethylsulfonyl fluoride (PMSF), 4 mM dithioerythreitol (DTE), and 15 β-mercaptoethanol], evacuated, and homogenized in an ice-cooled blender (Giannini et al. 1987). The PMSF, DTE, and β-mercaptoethanol were added fresh. The homogenate was strained through four layers of cheesecloth, and the effluent was centrifuged successively at 13,000 g for 15 mm (pellet discarded) and 40,000 g for 60 mm (supernatant aspirated). The membrane pellet was gently suspended in buffer [250 mM sucrose, 10% glycerol, 0.2% BSA, 1 mM DTE (added fresh), 1 mM PMSF (added fresh), and 2 mM bis-tris propane/4-morpholinoethanesulfonic acid (BTP/Mes), pH 7.0], layered onto the surface of a differential sucrose (26% and 38%) gradient and centrifuged at 100,000 g for 2 h. The high-density membrane pellet was resuspended in the same buffer and used immediately or frozen in liquid N2 and stored at -80° C.

2.3. Membrane Markers.

To Identify the PM and assess contamination by other membrane components during the membrane isolation procedure, K^+-stimulated ATPase and vanadate-sensitive ATPase were monitored (Briskin et al. 1987).

2.4. Uptake of Calcium

Calcium uptake in the PM vesicle membranes was measured using radiolabeled $^{45}CaCl_2$. At timed intervals following the initiation of uptake, aliquots of the medium were removed and the PM vesicles collected by vacuum filtration onto membrane filters. Radioactivity associated with the filters was determined by liquid scintillation counting (Giannini et al. 1987).

2.5. Enzyme Assay

Total ATPase activity was determined in the presence of 3 mM ATP, 3 mM magnesium sulfate ($MgSO_4$), 35 mM Tris-Mes (pH 6.5), and 50 mM of the cationic salt. Cation stimulation was determined by subtracting the activity in the absence of monovalent cations from the activity in the presence of monovalent cations.

2.6. Protein Determination

The quantity of total protein was determined with bovine serum albumin (BSA) as a standard (Bradford, 1976).

3. Results and Discussion

Applications of pre-harvest foliar treatments of AACa or dips in AACa and $CaCl_2$ produced firmer sour cherry fruit and decreased susceptibility to impact damage. Low concentrations of Ca^{2+} salts, as pre-harvest sprays or post-harvest dips, have been shown to reduce bitter pit of apples (Mielke and Facteau 1988), cork spot of pear (Raese 1988; Raese and Drake 1992, 1993), splitting of sweet (Drake and Proebsting 1985; Callan 1986) and sour themes (Anderson et al. 1989; Anderson and Campbell 1991, 1992, 1995; Campbell and Anderson 1993; Campbell et al. 1992), and reduce winter freeze damage to pears (Raese and Drake 1992). Calcium, applied as 1-3% $CaCl_2$, also maintained cellular turgidity in processed sweet and sour themes (Drake and Proebsting 1985; Anderson et al. 1989; Anderson and Campbell 1992; Campbell et al. 1992).

Sealed PM vesicles were ascertained by electron microscopy, and the identity and purity of the membrane fraction was demonstrated by potassium nitrate (KNO_3) stimulation and sodium vanadate (Na_3VO_4) sensitivity (Fig. 2). Although PM vesicles took up calcium when incubated In the presence of guanosine triphosphate (GTP) and $MgSO_4$, both ATP substrate and $MgSO_4$ were required (Figs. 3 and 4). Application of 10 μL of the calcium ionophore after 20 min released the absorbed calcium. In addition, incubating the PM vesicles in erythrosin isothiocyanate (EITC) for 30 min at 25° C substantially decreased the amount of calcium uptake (Fig. 5).

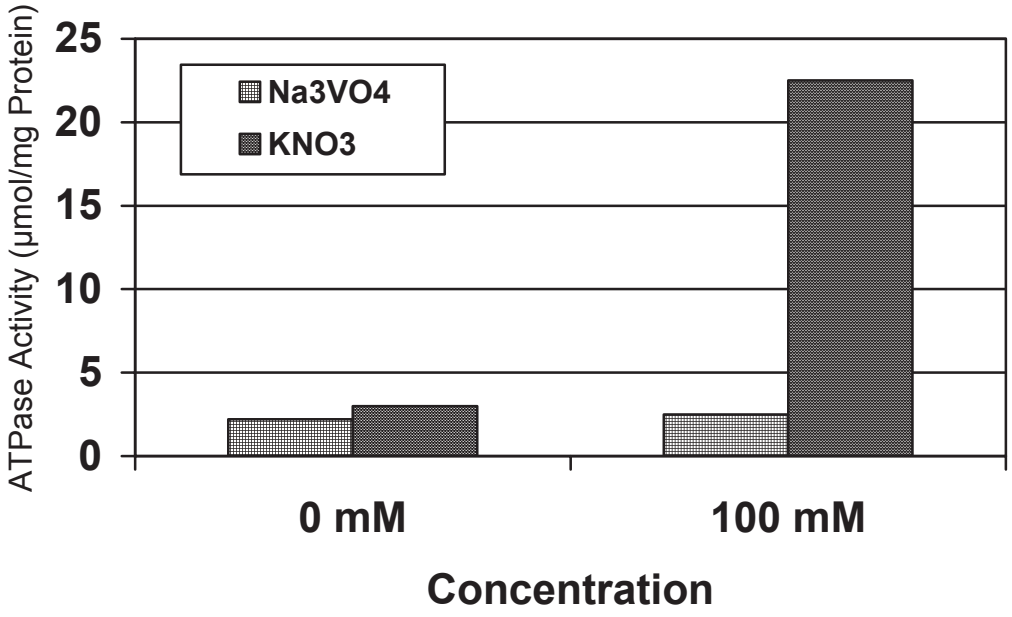

Figure 2. Effect of KNO_3VO_4 on sour cherry plasma membrane ATPase activity.

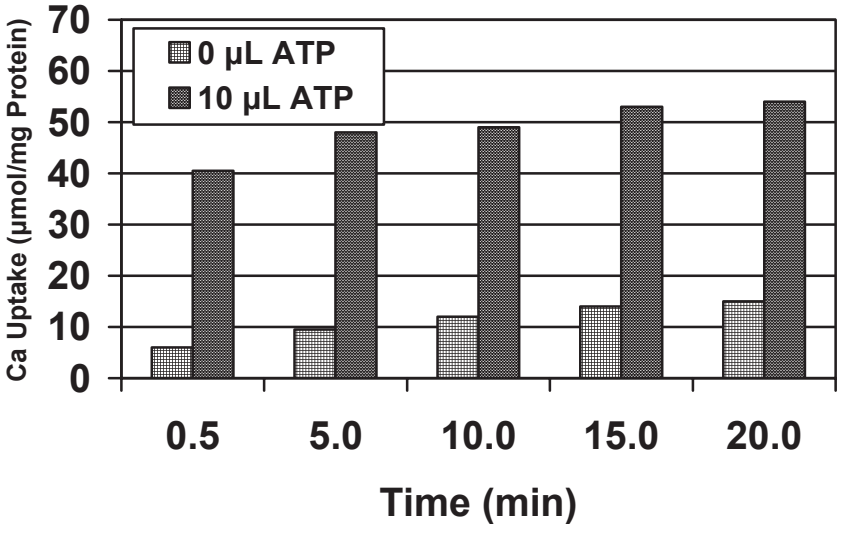

Figure 3. Effect of ATP on calcium uptake by plasma emebrane vesicles of sour cherry fruit.

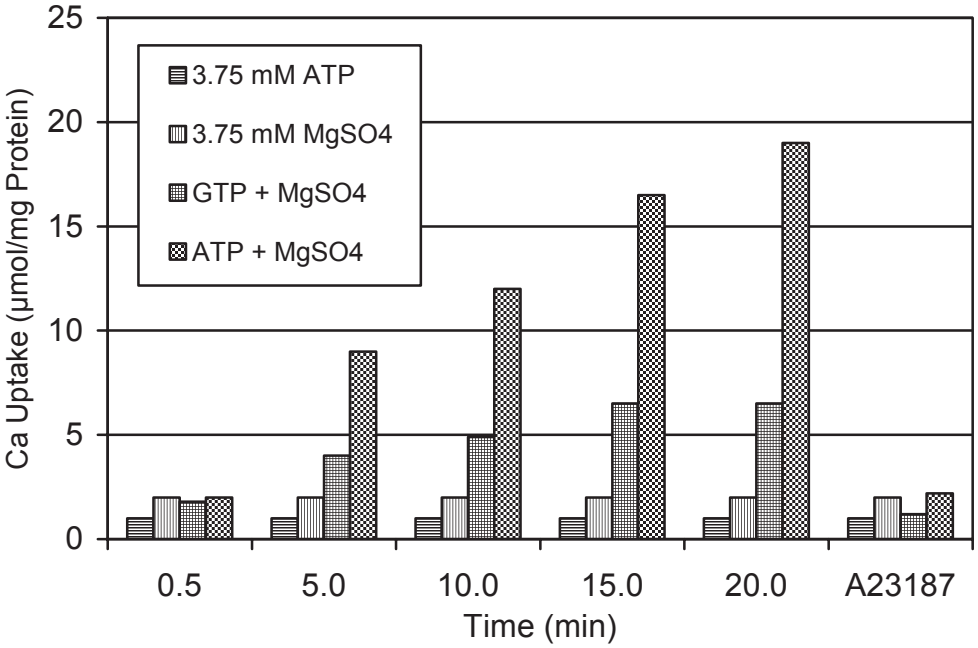

Figure 4. Effect of ATP, $MgSO_4$, GTP and A23187 on calcium uptake by plasma membrance vesicles of sour cherry fruit.

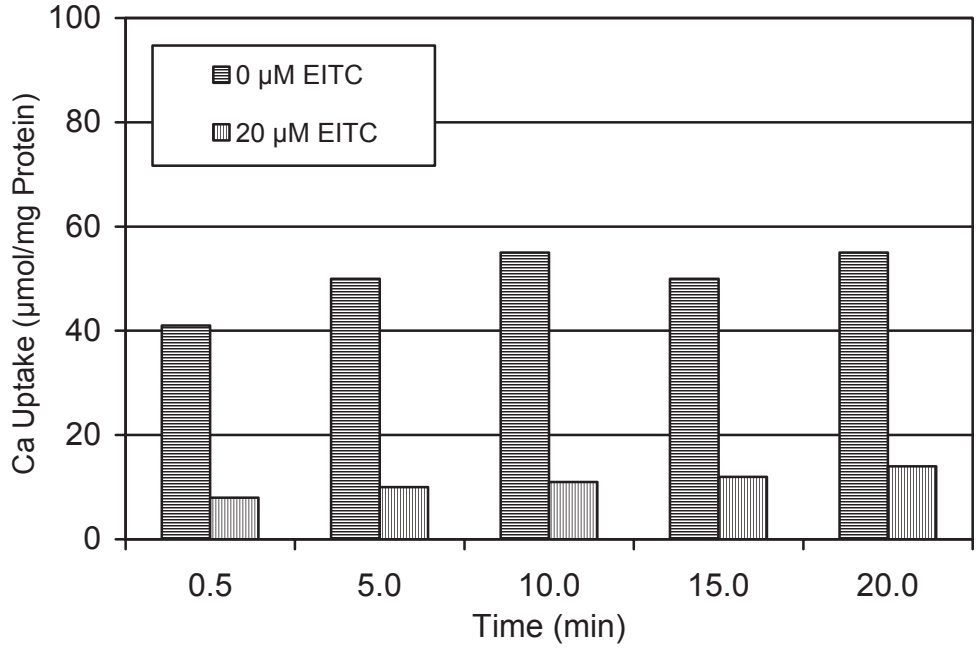

Figure 5. Effect of EITC on calcium uptake by plasma membrane vesicles of sour cherry fruit.

Pre-harvest foliar applications of AACa at two sites increased PM protein by 20% and 28% (Fig. 6). The amount of PM protein increased with the concentration of calcium (Fig. 7).

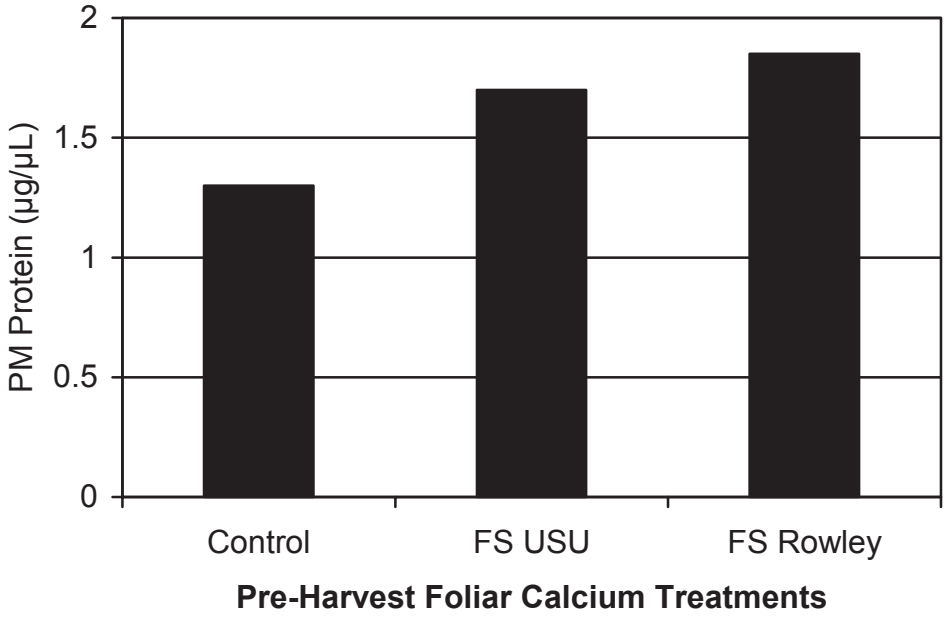

Figure 6. Foliar calcium treatments on plasma membrane protein in sour cherry fruit.

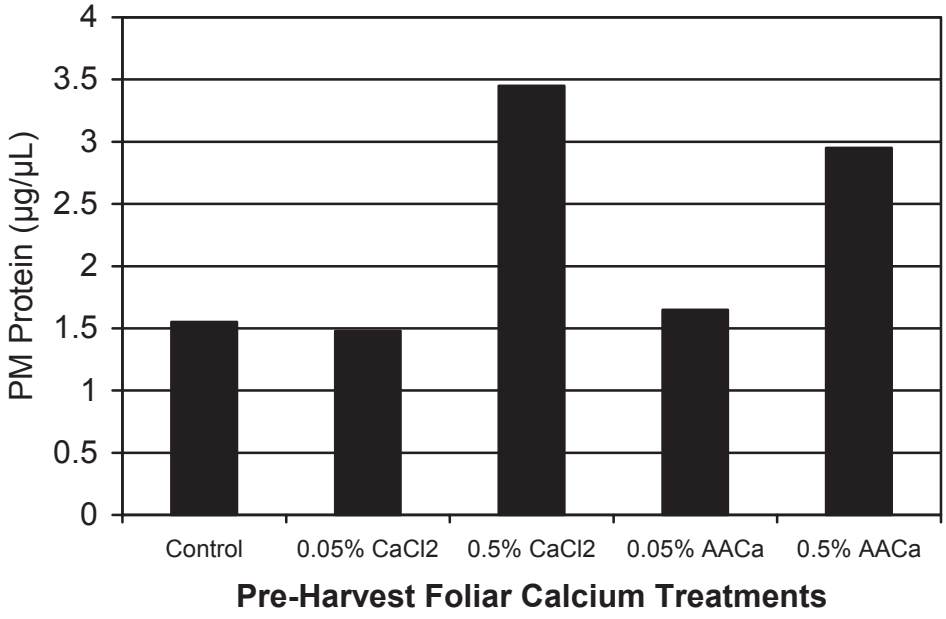

Figure 7. Pre-harvest calcium dip treatments on plasma membrane protein in sour cherry fruit.

Inhibitor-sensitive components of membrane-associated ATPase activity were calculated by subtracting the activity in the absence of monovalent cations from the activity in the presence of monovalent cations (Table 1). Activity appeared to be high when the monovalent cations were present.

Table 1
Effect of Monovalent Cations on Plasma Membrane ATPase Activity

Cation Added	Total Activity	Cation Stimulation	% of K Stimulation KCl Control	% Stimulation Total Activity
	Control (umol $mg.h^{-1}$)			
Tris	1.90			
Tris + KC1	2.20	0.30	100	16
Tris + RbC1	3.00	1.10	127	58
Tris + NaC1	1.80	0.10	33	-5
Tris + NH_4C1	2.00	0.10	33	5
Tris + CsC1	3.40	1.50	78	200
	AACa Dip			
Tris	2.60			
Tris + KC1	3.10	0.50	100	192
Tris + RbC1	2.50	0.10	20	-4
Tris + NaC1	3.20	0.60	117	23
Tris + NH_4Cl	3.80	1.20	46	42
Tris + CsC1	3.80	1.20	46	42

Senescence and moisture loss are crucial factors in post-harvest softening of sour cherry with subsequent loss of cellular turgidity and fruit quality. Calcium maintains osmoregulation and firmness, thereby extending the quality of sour cherry fruit (Anderson and Campbell 1991, 1992; Anderson et al. 1989). Ca^{2+} treated fruit was much firmer than untreated fruit and membrane protein content was greater in Ca^{2+}-treated fruit than in controls. $^{45}Ca^{2+}$ uptake by the plasma membrane vesicles was ATP dependent; the vesicles released the radiotracer when the calcium ionophore A23187 was added. ATPase activity was inhibited by orthovanadate, suggesting that the fractions were free from nonplasma membrane ATPases.

Isolating sealed PM vesicles allowed us to study the enzymes at sites of specific activity and indirectly indicated the factors that account for fruit turgidity and enhanced quality.

Fruit from cherry trees treated with foliar applications of AACa were superior to fruit from untreated trees as graded by Federal Inspectors. No pits were found in samples from treated trees. Results from field samples of the $CaCl_2$ dips have consistently produced firmer fruit for four years. Moreover, foliar application of AACa may offer a more acceptable way to apply Ca than soaking in the tanks of corrosive $CaC1_2$.

4. Acknowledgement

This research was supported in part by the Cherry Marketing Institute, Albion Laboratories, Inc., and the Utah Agricultural Experiment Station, Utah State University, Logan, Utah 84322-4810. Approved as journal paper no. 4676.

References

Anderson, J.L, and Campbell, W.F. 1991. Calcium chloride - What can it do for the cherry grower? Proc. Utah State Hort. Assn. 19-22.

Anderson, J.L, and Campbell, W.F. 1992. Effect of calcium treatments on sour cherry fruit quality. Albion International Nutritional Seminar. Feb. 22, 1992. Salt Lake City, UT.

Anderson, J.L, and Campbell, W.F. 1995. Effect of calcium treatments on 'Montmorency' sour cherry fruit quality. ISHS Intl. Cherry Symposium, Budapest, Hungary. Acta Hort. (in press)

Anderson, J.L, Seeley, E.J., and Olsen, R.L. 1989. Effects of calcium chloride on 'Montmorency' sour cherry quality. Abstr. Ann. Mtg. Amer. Soc. Hort. Sci. p. 58.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Briskin, D.P. 1990. Ca^{2+}-translocating ATPase of the plant plasma membrane. P1. Physiol. 94:397-400.

Briskin, D.P., and Hanson, J.B. 1992. How does the plant plasma membrane H^{+}-ATPase pump protons? J. Expt. Bot. 43:269-289.

Briskin, D.P., Leonard, R.T., and Hodges, T.K 1987. Isolation of the plasma membrane: Membrane markers and general principles. Methods Enzymol. 148:542-558.

Callan, N.W. 1986. Calcium hydroxide reduces splitting of 'Lambert' sweet cherry. J. Amer. Soc. Hort. Sd. 111:173-175.

Campbell, W.F., Anderson, J.L, and Walker, D.R. 1992. Calcium uptake in sealed plasmalemma vesicles isolated from post-harvest $CaCl_2$-treated sour cherries. HortScience 27:677.

Campbell, W.F. and Anderson, J.L. 1993. Characterization of $Ca2^{2+}$- translocating ATPase from plasma membranes of sour cherry. HortScience 28:228.

Drake, S.R., and Proebsting, E.L, Jr. 1985. J. Amer. Soc. Hort. Sci. 110:162-165.

Evans, D.E., Briars, S.A., and Williams, LE. 1991. Active calcium transport by plant cell membranes. J. Exptl. Bot. 42:285-303.

Giannini, J.L., Gildensoph, L.H., and Briskin, D.P. 1987. Selective production of sealed plasma membrane vesicles from red beet (Beta *vulgaris* L.) storage tissue. Arch. Biochem. Biophys. 254:621-630.

Hanson, J.B. 1983. The roles of calcium in plant growth. pp. 1-24. In: Randall, D.D, Blevins, D.G., and Larson, R. (ads.). Current Topics in Plant Biochemistry and Physiology. Vol. I. Univ. of Missouri, Columbia.

Hanson, J.B. 1984. The functions of calcium in plant nutrition. In: Advances in Plant Nutrition, Tinker, P.B. and Lauchli, A., (eds.), 1:149-208. Praeger Scientific, New York.

Mielke, E.A., and Facteau, T.J. 1988. An overview of calcium and its interactions in fruit trees. Proc. Wash. State Hort. Assn. 84:236-246.

Poovaiah, B.W. 1985. Role of calcium and calmodulin in plant growth and development. HortScience 20:347-351.

Poovaiah, B.W., and Reddy, A.S.N. 1987. Calcium messenger system in plants. CRC Crit. Rev. P1. Sci. 6:47-1 03.

Poovaiah, B.W., Glenn, G.M., and Reddy, A.S.N. 1988. Calcium and fruit softening: Physiology and biochemistry. Hort. Rev. 10:107-152.

Raese, J.T. 1988. Calcium: Effects on apple and pear disorders and fruit quality. Proc. Wash. State Hort. Assn. 84:247-257.

Raese, J.T., and Drake, S.R. 1992. Calcium sprays important in winter freeze damaged pears. Good Fruit Grower 43:11-12.

Raese, J.T., and Drake, S.R. 1993. Maintenance of calcium levels a must for pome fruit quality. Good Fruit Grower 44:31-33.

Sze, H. 1985. H'-translocating ATPases: advances using membrane vesicles. Ann. Rev. P1. Physiol. 36:175-208.