

## Calcium in Potato Tuber Cell Walls in Relation to Tissue Maceration by *Erwinia carotovora* pv. *atroseptica*

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### ABSTRACT

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Increases in the concentration of calcium in nutrient solutions supplied to potato plants of the cultivars Superior and Russet Burbank resulted in tubers with increased concentrations of calcium. High-calcium tubers, when injected with either cell suspensions or pectolytic enzyme preparations from *Erwinia carotovora* pv. *atroseptica* and incubated under anaerobic conditions, were more resistant to tissue maceration than low-calcium tubers. Electrolyte leakage from sections of high-calcium tubers also was lower than that from sections of low-calcium tubers when immersed in pectolytic enzyme preparations. As the calcium content of cell walls in the medulla and peel of Superior tubers increased from 0.012 to 0.058% and from 0.073 to 0.445%, respectively, the amount of galacturonic acid recovered increased from 19.6 to 27.4% and from 13.9 to 21.3%,

respectively. Cell wall preparations from Russet Burbank tubers had consistently higher amounts of calcium than those from Superior tubers: 0.167–0.314% in the medulla and 0.120–0.610% in the peel. The amount of galacturonate recovered from these cell walls was similarly greater: 25.9–30.0% in the medulla and 20.9–24.2% in the peel. The percentage of wall protein did not vary, however. The reduction of *Erwinia* soft rot in high-calcium 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 calcium levels. Growth and spread of the pathogen through the tuber tissues are decreased as a result of this effect on tissue maceration.

Bacterial soft rot frequently causes severe losses of potatoes (*Solanum tuberosum* L.) during storage and transit in many sections of the United States as well as in other potato-growing regions of the world (25). A number of factors can influence the susceptibility of tubers to the major soft rot pathogen *Erwinia carotovora* under postharvest conditions (10,16,25). An important consideration is the mineral nutrition of the potato plant on which the tubers are produced. In particular, the severity of soft rot caused by *E. carotovora* pv. *atroseptica* (van Hall) Dye is inversely related to the concentration of tuber calcium (18–20). Calcium in potato tubers is mainly concentrated in the external suberized periderm (6) and the layers of cortical cells below the periderm (Fig. 1), with lowest concentrations in medullary tissue in the center of the tuber. In most plant tissues, calcium is generally concentrated in cell walls (11).

*E. carotovora* macerates tissue primarily by the action of the pectolytic enzymes polygalacturonase and pectic lyase on middle lamellae and cell walls (1). In addition, these enzymes induce electrolyte leakage and cell death, often before extensive tissue maceration has occurred (12,22,32). Maceration induced by bacteria in potato tubers can be mimicked by injection of sterile pectolytic enzyme preparations from cultures of *E. carotovora* pv. *carotovora* (16). The pectolytic enzymes and their substrates are greatly influenced by the concentration of calcium ions in vitro and in plant tissues (5,12,21,26,29,30).

Increased calcium deposition in infected plant tissues has been associated with a decrease in the activity of pectolytic enzymes and an increase in the resistance of pectic materials in cell walls to degradation (2–4,31,36). The objective of this research was to determine whether differences in the ability of *E. c.* pv. *atroseptica* and pectolytic enzyme preparations from this pathovar to macerate

tissues of tubers differing in calcium content were related to cell wall composition.

### MATERIALS AND METHODS

**Potato culture.** Plants of the potato cultivars Superior and Russet Burbank were grown in silica sand in 5-gal tin containers in a greenhouse. These were watered every fourth day with 3 L of a dilute solution of Miracid (Stern's Nurseries Inc., Geneva, NY) containing, per liter, 20 mg of nitrogen, 7 mg of phosphorus and potassium, 0.218 mg of iron, and 0.0335 mg of copper, manganese, and zinc. Magnesium chloride and sodium borate were added to produce concentrations of 37 mg/L of magnesium and 0.1 mg/L of boron. Calcium was supplied as calcium sulfate at concentrations of 2, 37, 100, 167, 300, and 500 mg/L. Each addition of nutrient solution provided sufficient liquid to flush the system and prevent salt accumulation. When symptoms of potassium and magnesium deficiency appeared after 2 mo, the concentrations of these two ions were increased to 21 and 55 mg/L with potassium citrate and magnesium sulfate, respectively. Fifteen plants of each cultivar were grown at each calcium concentration.

Tubers of the cultivar Superior were harvested after 118 days and those of Russet Burbank, after 140 days. From each cultivar, three groups of five tubers from each of the six calcium levels were immediately prepared for mineral analyses of peel and medullary tissues. The remaining tubers were stored at 4 C.

**Mineral analyses of plant material.** The mineral content of both peel and medullary tissue of tubers was determined by inductively coupled plasma-optical emission spectrometry by the Soil and Plant Analysis Laboratory of the University of Wisconsin-Madison. Five tubers were peeled with a standard vegetable peeler, producing a peel 2 mm thick containing the periderm and about 10 layers of cortical cells (Fig. 1); entire peels from five tubers were combined for each single peel sample. The five peeled tubers were then quartered lengthwise (from stem end to bud end), and one quarter from each was included in each medullary tissue sample. In this manner, three five-tuber samples of peel and medullary tissue

were prepared from each treatment. The tissues were completely dried at 65 C, then ground to pass a 40-mesh screen. Percentage of calcium content was determined on a dry weight (DW) basis.

**Isolation and characterization of potato tuber cell walls.** *Cell wall isolation.* Cell wall preparations were obtained from the peel and medullary tissues of tubers and analyzed to determine the content of galacturonate, calcium, and protein.

Two methods were used for isolating cell walls. The first, a modification of a procedure by Jarvis et al (13), was to peel the tubers and then freeze, at -20 C, about 150 g of medulla or 50 g of peel. The thawed tissue samples were then homogenized in 1% sodium deoxycholate in 5 mmol of sodium bisulfite (1 ml/g fresh weight) for 10 min in a Waring Blendor at high speed. *n*-Octanol (1 ml) was added to the homogenizing liquid to retard foaming. The pulp was poured into a 100-mesh sieve and rinsed with deionized water until the rinse water was clear, blended again for 5 min, then rewashed until no starch was detectable microscopically after the staining of a sample of the pulp with I<sub>2</sub>/KI. The second blending, or the use of dimethyl sulfoxide (27), was sufficient to remove detectable starch. The cell wall material was then freeze-dried.

The second method for cell wall isolation was designed to prevent loss of water-soluble, low molecular weight pectins (34). The separate tissue samples (100 g) were homogenized for 10 min in a Waring Blendor at high speed in 100 ml of 95% ethanol containing 0.2% ascorbic acid. The pulp was then boiled for 20 min in 95% ethanol and refiltered a number of times with alcohol to remove starch until the wash was clear. The material was blended again for 5 min, then resieved until no starch was detectable microscopically when a portion was stained with I<sub>2</sub>/KI. Finally, the tissues were air-dried in a Büchner funnel.

*Galacturonate content of cell walls.* The amount of galacturonate in cell walls was measured by the method described by Voragen et al (34). Dried peel or medullary cell wall material (0.25 g) was first moistened with 0.5 ml of 95% ethanol. Then, 200 ml of 50 mM sodium acetate buffer, pH 5.0, was added with 0.25 ml of polygalacturonase from *Aspergillus niger* (35 units; Sigma Chemical Co., P.O. Box 14508, St. Louis, MO) and 0.02 g of Macerace enzyme mixture from *Rhizopus* sp. (Calbiochem-Behring Corp., P.O. Box 12087, San Diego, CA). After the mixture was stirred for 24 hr at room temperature, the volume was increased with buffer to 250 ml, and a portion was filtered through Whatman No. 1 filter paper. The first few milliliters passing through were discarded, then 2 ml was diluted with distilled water to 10 ml; 2 ml of this portion was used for analysis of galacturonic

acid by the method of McCready and McComb (17). Concentrated H<sub>2</sub>SO<sub>4</sub> (12 ml) was placed into a large test tube and cooled below 5 C in an ice bath. The 2-ml sample was added, and the mixture was cooled, then reheated in boiling water for 10 min. After the mixture was cooled to room temperature, 1 ml of 1% carbazole in 95% ethanol was added and the preparation thoroughly mixed. After 30 min, the absorbance of the solution was measured at 520 nm on a Varian Techtron model 635 spectrophotometer. A standard curve for the range of 0-100 µg of anhydrous galacturonic acid was prepared for comparison.

*Protein content of cell walls.* Amino nitrogen determinations from cell wall material were made by the micro-Kjeldahl method for total nitrogen by the Soil and Plant Analysis Laboratory of the University of Wisconsin-Madison. Values were multiplied by 6.25 for conversion to percent crude protein.

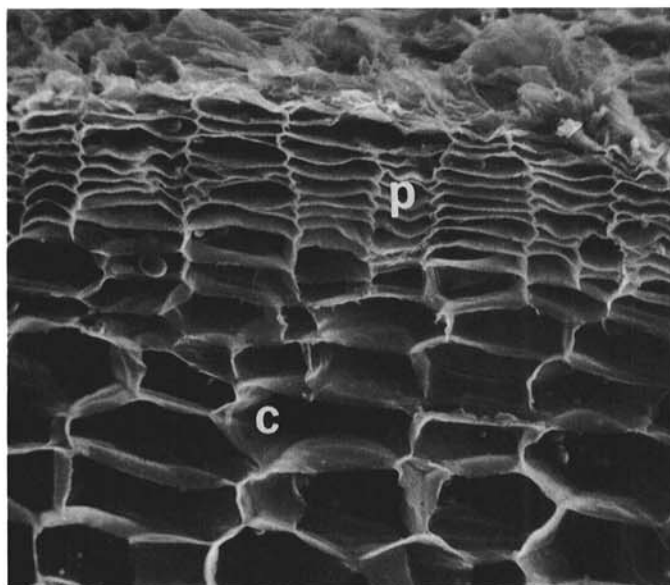
**Bacterial cultures.** *E. c. pv. atroseptica* strain SR8 was maintained as a stock suspension in distilled water at room temperature. Strain SR8 was originally isolated from a potato tuber in Wisconsin and reacts positively with serogroup I antiserum of *E. c. pv. atroseptica* (9). To prepare inoculum, 0.1 ml of the stock suspension was pipetted and spread on plates of nutrient dextrose agar containing, per liter, 3 g of beef extract, 10 g of peptone, 10 g of dextrose, and 15 g of agar. Transfers from these cultures were made to nutrient dextrose broth. Shake cultures were grown at 20 C for 48 hr. The cells were harvested by centrifugation, washed, and resuspended in sterile distilled water.

**Injection of bacterial cells and enzyme preparations.** Tubers of the cultivars Superior and Russet Burbank were evaluated 30 and 8 days after harvest, respectively, for soft rot susceptibility by a modification of the injection method of De Boer and Kelman (10). The tubers were washed, immersed in 0.05% sodium hypochlorite for 40 min, rinsed with deionized water, and allowed to air-dry. A suspension of *E. c. pv. atroseptica*, 10<sup>8</sup> cfu/ml, was serially diluted, and plastic pipet tips containing 0.1 ml of four bacterial dilutions plus a control of sterile water were injected at randomized sites on upper surfaces of 12 tubers of each cultivar. The tubers, containing one pipet tip from each dilution of the series, were then incubated at 20 C on racks in a 190-L drum tank from which the air was subsequently flushed and replaced with nitrogen. After 96 hr, the pipet tips were removed, the tubers were sectioned at the injection sites, and the diameters of the decayed areas were measured.

A sterile, partially purified culture filtrate of *E. c. pv. atroseptica* was also used for injections into tubers after a procedure similar to that described above and for studies of electrolyte loss by tuber sections. Cells were removed by centrifugation from a 5-day culture of *E. c. pv. atroseptica* grown in 500 ml of a pectate medium containing, per liter, 13.2 g of KH<sub>2</sub>PO<sub>4</sub>, 2.0 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0 g of yeast extract, and 8.0 g of sodium polypectate (NaPP; M. Burger Enterprises, 2225 Eton Ridge Road, Madison, WI) (12). Proteins from the supernatant were precipitated by adding ammonium sulfate to 95% saturation. The precipitate was collected by centrifugation, redissolved in distilled water, and dialyzed in distilled water for 12 hr with one change of distilled water. The portion of this partially purified pectic enzyme fraction that was not used immediately was stored at -20 C.

The activity of pectic lyase was measured by recording the increase in absorbance at 230 nm in a reaction mixture containing 1 ml of 0.2% NaPP in 0.2 M tris-HCl buffer (pH 8.0), 1 ml of distilled water, and 1 ml of culture filtrate preparation. The activity of polygalacturonase was determined by measuring the production of reducing sugars (compared with galacturonic acid standards) in a reaction mixture containing 1 ml of 0.2% NaPP in 0.2 M sodium acetate buffer (pH 4.5), 1 ml of distilled water, and 1 ml of culture filtrate preparation. The protein concentration of the preparation was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA). For studies on the effect of these pectolytic enzymes in tubers or on tuber slices, tests were completed with both the active preparation and a control inactivated by autoclaving at 121 C for 15 min and increased to its original volume with distilled water.

**Measurement of electrolyte loss from enzyme-treated tissues.**



**Fig. 1.** Transverse section through a potato tuber exposing the periderm (p) and cortex (c).  $\times 160$ . The 5-mm cube of tissue was dehydrated by freeze substitution, carbon-coated, and examined by scanning electron microscopy.

About 6 mo after harvest, rates of electrolyte loss from high- and low-calcium tuber tissues were determined after the immersion of tuber slices in the pectolytic enzyme preparation from *E. c. pv. atroseptica*. High-calcium Russet Burbank tubers used in these experiments contained 0.332% calcium in the peel and 0.115% calcium in the medulla; low-calcium tubers had values of 0.117 and 0.037%, respectively. The high-calcium Superior tubers had peel and medullary calcium concentrations of 0.283 and 0.062%, respectively, whereas low-calcium tubers contained 0.026 and 0.014%, respectively. Tuber sections, removed with a cork borer, were 5 mm in diameter and 2 mm thick. Peel and medulla sections were tested separately; medulla sections were removed from a depth of 2–6 mm. Twenty-five sections were immersed in 10 ml of active or autoclaved enzyme preparations with constant gentle stirring. The rate of electrolyte loss was measured with a conductivity bridge (model RC 16B2, Industrial Instruments, Inc., 89 Commerce Rd., Cedar Grove, NJ). Loss attributable to pectolytic activity was determined by subtracting readings of disks in the control series (autoclaved enzyme) from those in the active enzyme preparation.

## RESULTS

**Composition of potato tuber tissues and cell walls.** Sets of potatoes comprising a wide range of tuber calcium concentrations were obtained from the greenhouse study with different calcium fertilization regimes. Calcium ranged from 0.014 to 0.062% and from 0.031 to 0.115% (DW) in medullary tissue of Superior and Russet Burbank tubers, respectively. Calcium in the peel was also consistently lower in Superior tubers, 0.026–0.283%, than in Russet Burbank tubers, 0.047–0.332% (Tables 1 and 2).

The amounts of calcium, pectic materials, and protein recovered from the potato tuber samples differed for the two cell wall methods. With the use of sodium deoxycholate (13), almost 50% more wall calcium was recovered than with the ethanol procedure, but up to 12% of the galacturonates was lost. Extraction in 95% ethanol (34) recovered more water-soluble pectic materials than did the sodium deoxycholate method, but the ethanol precipitated cytoplasmic proteins (28); furthermore, this solvent is a weak acid and may have dissolved more wall calcium than the first method did. Accordingly, cell wall calcium and protein were measured in materials obtained by the first procedure and galacturonate concentrations were determined from cell walls prepared by the second method.

With increased calcium fertilization of Superior potato plants, the galacturonate component increased from 19.6 to 27.4% in the

medullary walls and from 13.9 to 21.3% in the peel (Table 1). Calcium in cell walls from medullary tissues also increased, from 0.012 to 0.058%, whereas that in the peel rose from 0.073 to 0.445%. The location of the calcium binding sites in these cell walls would require procedures such as radioautography or electron microprobe analyses, and these techniques have not been applied. However, assuming most of this calcium was bound to galacturonates (34), a maximum of 2.04% of this material in medullary walls and 20.16% in walls of peel tissues would be calcium digalacturonate.

One millimole of the calcium digalacturonate molecule would contain 40 mg of calcium bridging the 386.28 mg of galacturonate (193.14 mg × 2). The relationship of 40 mg:386.28 mg, therefore, establishes the level at which 100% of the galacturonates could be present as calcium salts. For example, when calcium and galacturonate components of the cell wall of peels of Superior tubers were 0.445 and 21.3%, respectively, this amount of calcium (4.45 mg/g) could maximally bind 42.97 mg of galacturonate. However, the total galacturonate concentration was 213.2 mg/g of cell wall, indicating that the calcium salt could be no more than 20.16% of the total.

Cell walls of Russet Burbank tubers contained greater percentages of galacturonates at low levels of calcium fertilization (Table 2) than did those of Superior tubers (Table 1); at the highest calcium level, however, galacturonate concentrations of both cultivars were similar. In cell walls of Russet Burbank tubers, the content of galacturonates ranged from 25.9 to 30.0% in the medulla and from 20.9 to 24.2% in the peel. Unlike that of the cultivar Superior, in which the galacturonate content of tubers continued to increase with an increase in the concentration of calcium in the nutrient solution supplied to the plants, the maximum content of this wall component in Russet Burbank tubers was reached in the middle ranges of the fertilization series. Wall calcium was also greater in Russet Burbank tubers: 0.167–0.314% in medullary tissue and 0.120–0.610% in the peel. The combination of increased galacturonates and calcium in these walls increased the possible maximum percentage of calcium digalacturonate from 6.23 to 10.89% in medullary tissues and from 5.54 to 25.17% in the peel, values consistently greater than those of the cultivar Superior.

By comparing the amount of calcium in the cell walls with that in the dried whole tuber, the percentage concentration of calcium within the walls could be estimated. For example, the cell walls of peels of Superior tubers from plants receiving 500 mg of Ca<sup>++</sup>/L in the nutrient solution accounted for 0.80 g of the 1.84 g of dried tuber peel, or 43.48% of the dry weight and 3.56 mg of calcium (0.80

TABLE 1. Calcium and pectic materials in cell walls of peel and medullary tissues from Superior potato tubers<sup>a</sup>

Ca <sup>++</sup> in nutrient solution (mg/L)	Tuber calcium (% DW)	Cell wall GalU (%)	Cell wall calcium (%)	Ca(GalU)/GalU (%)	Cell wall calcium (% DW)/tuber calcium
Medullary tissue					
2	0.014 a <sup>z</sup>	19.6 a	0.012 a	0.59 a	8.1 d
37	0.019 b	20.5 ab	0.023 b	1.09 b	6.9 c
100	0.025 c	21.0 b	0.035 c	1.61 c	7.6 d
167	0.032 d	21.8 b	0.043 d	1.90 d	7.8 d
300	0.049 e	24.3 c	0.051 e	2.03 d	5.8 b
500	0.062 f	27.4 d	0.058 f	2.04 d	3.8 a
Peel tissue					
2	0.026 a <sup>z</sup>	13.9 a	0.073 a	5.07 a	31.5 a
37	0.114 b	14.3 b	0.095 b	6.44 b	36.0 a
100	0.142 c	15.4 c	0.136 c	8.52 c	51.9 b
167	0.175 d	16.7 d	0.196 d	11.37 d	53.2 b
300	0.242 e	17.8 e	0.296 e	16.06 e	60.8 c
500	0.283 f	21.3 f	0.445 f	20.16 f	68.4 d

<sup>a</sup>DW = dry weight, GalU = galacturonic acid, Ca(GalU) = calcium galacturonate.

<sup>z</sup>Within columns of one tissue, numbers followed by the same letter are not significantly different according to Duncan's multiple range test, *P* = 0.05.

TABLE 2. Calcium and pectic materials in cell walls of peel and medullary tissues from Russet Burbank potato tubers<sup>a</sup>

Ca <sup>++</sup> in nutrient solution (mg/L)	Tuber calcium (% DW)	Cell wall GalU (%)	Cell wall calcium (%)	Ca(GalU)/GalU (%)	Cell wall calcium (% DW)/tuber calcium	Cell wall crude protein (%)
Medullary tissue						
2	0.031 a <sup>z</sup>	25.9 a	0.167 a	6.23 a	59.0 c	ND
37	0.037 b	26.8 b	0.193 b	6.95 ab	67.2 d	5.9 a
100	0.042 c	29.5 d	0.196 b	6.42 a	58.6 c	5.8 a
167	0.065 d	30.0 d	0.255 c	8.21 b	44.2 b	4.9 ab
300	0.092 e	27.8 c	0.314 e	10.89 d	50.3 bc	5.3 a
500	0.115 f	27.4 bc	0.282 d	9.93 c	33.9 a	4.5 b
Peel tissue						
2	0.047 a <sup>z</sup>	20.9 a	0.120 a	5.54 a	64.8 a	ND
37	0.117 b	22.6 b	0.259 b	11.09 b	67.6 a	7.5 a
100	0.172 c	23.8 cd	0.324 c	13.13 c	65.2 a	8.1 a
167	0.252 d	21.6 b	0.418 d	18.68 d	75.4 b	8.5 a
300	0.295 e	24.2 d	0.551 e	21.96 e	75.0 b	8.3 a
500	0.322 f	23.4 c	0.610 f	25.17 f	71.8 b	8.0 a

<sup>a</sup>DW = dry weight, GalU = galacturonic acid, Ca(GalU) = calcium galacturonate, ND = not determined.

<sup>z</sup>Within columns of one tissue, numbers followed by the same letter are not significantly different according to Duncan's multiple range test, *P* = 0.05.

g × 0.445%). This 3.56 mg, compared with the 5.21 mg in the dried peel (1.84 g × 0.283%), was a recovery of 68.3% from cell wall material. When calcium concentrations in the nutrient solution were low, a greater relative percentage of calcium in the medullary tissues of both cultivars was deposited in cell walls. This ratio of cell wall Ca<sup>++</sup>/DW Ca<sup>++</sup> then declined with increasing availability of the nutrient. However, as the amount of calcium fertilization increased, an increased percentage of the total calcium was deposited in peel cell walls. When these ratios were averaged over the six levels of calcium fertilization, it became evident that about one and one-half times as much Ca<sup>++</sup> was deposited into peel walls and nearly four times as much into medullary walls of Russet Burbank tubers as was the case with Superior tubers.

The crude protein content of cell walls of Russet Burbank tubers was examined also (Table 2). The percentage of protein was greater in cell walls of the tuber peel than in those of the medulla. In both tissues, the percentage of crude protein in the cell wall remained constant as the calcium content increased.

**Injection of bacterial cells and enzyme preparations.** Tubers of Superior and Russet Burbank with high and low medullary concentrations of calcium were injected with serial dilutions of *E. c. pv. atroseptica*. After 96 hr in a nitrogen atmosphere, the diameter of decay at each injection site was measured (Table 3). Decay diameters were consistently larger in low-calcium tubers of both cultivars than in those with high levels of medullary calcium. In general, Superior tubers were more resistant to bacterial maceration than Russet Burbank tubers, although calcium levels were lower in the Superior than in the Russet Burbank tubers. This may be attributable to the difference in the dates of harvest and length of storage. The early cultivar, Superior, was stored in a cold room at 4 C for 22 days longer than Russet Burbank. In 1981, 1982, and 1983, when tubers of both cultivars were harvested from field plots and tested immediately, Superior tubers were consistently more susceptible to soft rot by *E. c. pv. atroseptica* than Russet Burbank tubers (20). A decrease in water potential during the

storage of the Superior tubers may have resulted in an increase in resistance to tissue maceration (14).

When sterile, partially purified culture filtrates of *E. c. pv. atroseptica* containing pectate lyase and polygalacturonase activities were injected into surface-disinfested whole Russet Burbank tubers and the tubers were then held in anaerobic conditions, maceration was more extensive in low-calcium tissue than in high-calcium tissue (Table 4). The activity of pectic lyase in this preparation was 3,100 μmol of product formed per minute per milligram of protein and that of polygalacturonase was 3.9 μmol of product formed per hour per milligram of protein. Control preparations in which the enzymes were inactivated by autoclaving did not macerate tuber tissue.

**Electrolyte loss from tuber slices in pectolytic enzyme preparations.** About 6 mo after the tubers were harvested, tissue disks from Superior and Russet Burbank tubers were immersed in the culture filtrate of *E. c. pv. atroseptica* containing partially purified pectolytic enzymes. Rate of electrolyte loss was higher from peel and medullary tissues of the low-calcium tubers than from those of the high-calcium tubers (Figs. 2 and 3). Leakage also occurred over time from tissue sections held in the autoclaved inactive enzyme preparation, but at a rate approximately one-half that from tissue treated with pectolytic enzymes.

In these experiments, medullary tissue of Superior tubers was more affected by the action of the enzymes than was that of Russet Burbank tubers. This result was similar to those of inoculations of tubers harvested from field plots.

## DISCUSSION

Potato tubers harvested from sandy soils with low cation-exchange capacities frequently are low in calcium and more susceptible to *Erwinia* soft rot than those high in calcium (19,20). Furthermore, low-calcium tubers of any one cultivar are also more susceptible to maceration by pectolytic enzyme preparations from *E. c. pv. atroseptica* than are high-calcium tubers of the same cultivar. Differences among cultivars in resistance to bacterial soft rot depend on a complex of factors, however, and not exclusively on calcium status.

Previous studies have indicated that increased calcium levels in plant tissues result in increased resistance to tissue maceration. Localized viral infections are characterized by elevated calcium concentrations in the tissue around the lesions, and these high-calcium tissues are more resistant to maceration by pectic enzymes than healthy tissue (36). Similarly, calcium deposition in bean hypocotyls increases in zones surrounding tissue infected by *Rhizoctonia solani* and as tissues age (2,4). Formation of ionic bonds and cross-linkage of the galacturonate units of pectates by calcium was suggested as the basis for increased resistance of the substrate to hydrolysis by polygalacturonase, both in vitro and in vivo (4,5). In addition, the increased supply of cations, resulting from increased transpiration at the infection site, may displace pectinmethylesterase from the cell wall. Calcium activates the esterase, which is then involved in the shift of pectins to pectate salts that are more resistant to hydrolysis (2,5).

The precise location of the calcium that accumulates around lesions and presumably retards the spread of *Rhizoctonia* infections in bean leaves was not determined, however (31). Such accumulations could have been cytoplasmic and thus unrelated to the inability of fungal enzymes to macerate cell walls, as was originally suggested. Our data, however, support the concept that calcium localized in cell walls may indeed inhibit tissue maceration.

Infection of bean by *Sclerotium rolfsii* progresses in a different manner (3). This pathogen produces oxalic acid that precipitates calcium in the cell walls; cross-linkages of calcium with galacturonate are reduced and activity of polygalacturonase is enhanced. Oxalic acid also lowers the pH of the tissue to a level more favorable for hydrolase activity.

Changes in calcium levels can also affect cell wall composition. Thus, calcium starvation in cucumber roots leads to a decrease in the total amount of pectic polymers in cell walls (15). The percentage of acidic polymers (such as those composed of pectic

TABLE 3. Tissue maceration resulting from injection of cell suspensions of *Erwinia carotovora* pv. *atroseptica* into high- and low-calcium Superior and Russet Burbank potato tubers

Cultivar	Medullary tuber calcium (% dry weight)	Maceration diameters (mm) <sup>y</sup> after injection with bacteria at (cfu/ml):				
		0	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>6</sup>	10 <sup>8</sup>
Superior	0.014	0.00 a <sup>z</sup>	1.71 b	4.57 c	6.29 c	ND
	0.062	0.00 a	0.50 a	1.67 b	5.50 c	ND
Russet Burbank	0.037	0.00 a	2.16 b	6.67 d	8.83 e	11.67 f
	0.115	0.00 a	1.08 ab	5.08 c	6.67 d	7.58 de

<sup>y</sup> Mean of 12 tubers. Superior and Russet Burbank tubers were injected 30 and 8 days after harvest, respectively, and held 96 hr in a nitrogen atmosphere at 20 C. ND = not determined.

<sup>z</sup> Within cultivars, numbers followed by the same letter are not significantly different according to Duncan's multiple range test, *P* = 0.05.

TABLE 4. Tissue maceration resulting from injection of sterile pectolytic enzyme preparation of *Erwinia carotovora* pv. *atroseptica* into low- and high-calcium Russet Burbank potato tubers

Medullary tuber calcium (% dry weight)	Maceration diameters (mm) <sup>x</sup>	
	Active culture filtrate <sup>y</sup>	Autoclaved culture filtrate
0.037	8.9 a <sup>z</sup>	0.0
0.115	3.8 b	0.0

<sup>x</sup> Mean of 12 tubers. Tubers were injected with 0.1 ml of filtrate, then held 96 hr in a nitrogen atmosphere at 20 C.

<sup>y</sup> Pectic lyase activity, measured at pH 8.0 by the increase in absorbance at 230 nm, was 3,100 μmol of product formed per minute per milligram of protein. Polygalacturonase activity, measured by the increase in reducing sugars at pH 4.5, was 3.9 μmol of product formed per hour per milligram of protein.

<sup>z</sup> Numbers followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

acid as opposed to those made of neutral pectin or pectate) is greater with less calcium available, but side chains of arabinogalactan and rhamnogalacturon are fewer and shorter. In addition to those structural changes, the activity of endogenous polygalacturonase is increased substantially by calcium starvation. This increased enzyme activity is one possible cause of the observed changes in the cell wall.

In both Russet Burbank and Superior cultivars, the amount of uronic acid recovered from cell walls of medullary and peel tissues increased as the calcium content of those walls increased. Within medullary tissues of potato tubers, this would be mainly galacturonate (27). The amount of this galacturonate recovered from medullary cell walls (19.6–30.0%) was similar to concentrations reported by other investigators (13,27,34). The percentage of galacturonate in walls of tuber peels had not been reported previously and was consistently lower than that in medullary walls. This may be attributable to higher concentrations of suberin, lignin, and protein in the peel than in medullary tissue.

Galacturonic acid precipitates calcium to form calcium pectate, a major component of middle lamellae. Although calcium pectate cannot be measured directly, the quantity of this material in cell walls and middle lamellae can be estimated indirectly after the elimination of other components that bind calcium. Crystals containing inorganic calcium likely would not remain after the extensive extraction procedures that were used, and no crystals were observed microscopically. Organic acids such as citric or tartaric acid also were absent from such preparations. If calcium binds to any of the neutral sugars that comprise 69% of the medullary wall materials (28), such attachments must be few in number.

If the calcium that increased in the cell wall as calcium fertilization of tubers was increased was bound predominantly to pectic acids, the amount of calcium pectate should increase significantly in both medullary and peel tissues of high-calcium tubers. More galacturonate was recovered from high-calcium walls, but whether this apparent increase was due to increased synthesis of pectic material or to a conversion from soluble pectins that would otherwise be lost in preparation of the wall material has not been determined. In either case, the structural integrity of potato tuber tissue would presumably be enhanced by increased calcium fertilization in both cultivars tested (11).

Therefore, in addition to galacturonate (at approximately 27%), calcium could be bound only to cell wall proteins and lignin (at medullary concentrations of 2–4% and 1%, respectively) or possibly contaminating membrane fragments. The phosphorus content of wall preparations was quite low, indicating only a minor contamination, if any, by phospholipids. In medullary tissue, lignification of walls was very slight and lignin could not be involved appreciably in binding of calcium. Cell walls extracted in ethanol contained nearly twice as much protein (mostly cytoplasmic contaminants) as those extracted in sodium deoxycholate but less than half the calcium. This indicated that these proteins did not adsorb free calcium during wall extraction. Protein-binding of calcium may play some role, however. This may have been greater in the peel, where, with a hundredfold increase in lignin content,  $Ca^{++}$ -protein-lignin-polysaccharide complexes may have important structural significance (24).

Evidence for degradation of potato cell walls by the action of a protease from a strain of *Pseudomonas fluorescens* has been presented by Wang and Kelman (35). A proteolytic enzyme of *E. carotovora* may be involved in maceration of potato tissues, but evidence is lacking that it is a principal agent of attack (33).

When the concentration of calcium in the entire cell wall structure of a tuber was calculated and compared with that determined by dry weight analysis of the whole tuber, the ratio declined in the medulla but increased in the peel as more calcium was applied to the plant. These trends were similar for both Superior and Russet Burbank tubers (Tables 1 and 2). The increased ratio in the peel would be expected for an ion that has an abundance of binding sites in the wall but is actively excluded from the cytoplasm of a cell when above certain concentrations.

Although increasing calcium fertilization resulted in increased deposits of calcium in medullary cell walls, a greater percentage was located elsewhere and subsequently lost in preparation of these walls (23,31). In addition to crystals, other possible loci for calcium include membranes of mitochondria (37), amyloplasts (7), and idioblasts (8), as well as plasma membranes. Calcium bound to these membranes is difficult to measure because the ion is extensively leached from such structures in all histological procedures (23). However, the reduction of electrolyte leakage from high- vs. low-calcium tissues following treatment with culture filtrates of *E. c. pv. atroseptica* does indicate improved stability of

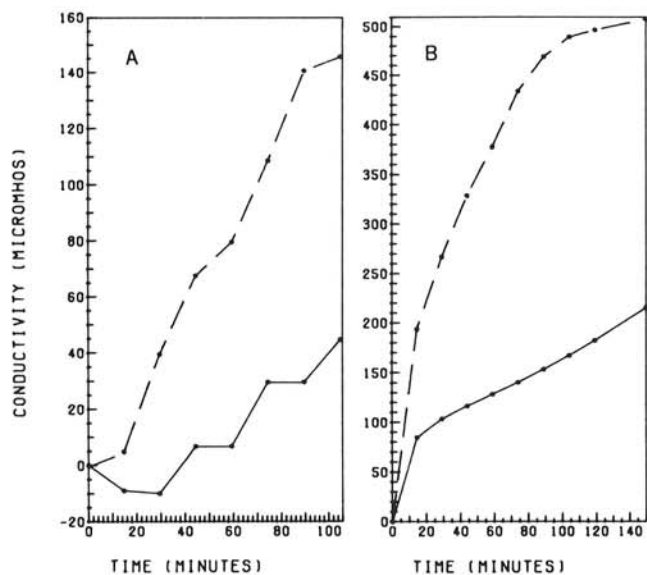


Fig. 2. Electrolyte leakage from slices of low-calcium (---) and high-calcium (—) A, peel and B, medullary tissues of Superior potato tubers immersed in a pectolytic enzyme preparation from *Erwinia carotovora* pv. *atroseptica*. Low-calcium tubers contained 0.026 and 0.014% calcium in the peel and medulla, respectively; high-calcium tubers contained 0.283 and 0.062% calcium in the peel and medulla, respectively. Pectic lyase and polygalacturonase activities were 3,100 and 3.9  $\mu$ mol of product formed per minute and per hour, respectively, per milligram of protein.

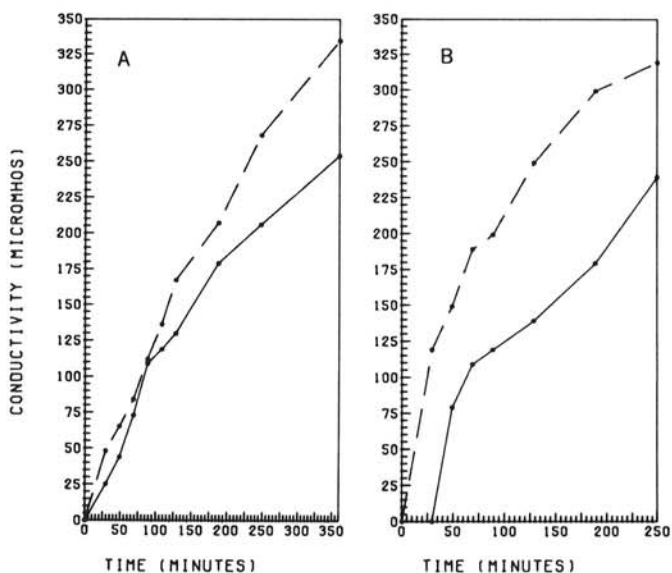


Fig. 3. Electrolyte leakage from slices of low-calcium (---) and high-calcium (—) A, peel and B, medullary tissues of Russet Burbank potato tubers immersed in a pectolytic enzyme preparation from *Erwinia carotovora* pv. *atroseptica*. Low-calcium tubers contained 0.117 and 0.037% calcium in the peel and medulla, respectively; high-calcium tubers contained 0.322 and 0.115% calcium in the peel and medulla, respectively. Pectic lyase and polygalacturonase activities were 3,100 and 3.9  $\mu$ mol of product formed per minute and per hour, respectively, per milligram of protein.

plasma membranes, possibly resulting from increased calcium bridging of membrane components.

Since calcium improves the structural integrity of both the plasmalemma and cell wall materials (11), soft rot by *E. c. pv. atroseptica* may be reduced in high-calcium tubers because maceration by pectolytic enzymes is reduced and the multiplication and intercellular spread of the pathogen through the tissues is impeded. Soft rot *Erwinia* macerate tissue primarily by the action of pectolytic enzymes. Thus, any level of defense a potato tuber may possess against enzymatic tissue maceration may determine in part its degree of resistance to the pathogen.

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