Reduced Severity of Erwinia Soft Rot in Potato Tubers with Increased Calcium Content

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ABSTRACT

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The severity of bacterial soft rot caused in potato tubers by *Erwinia carotovora* pv. *atroseptica*, was linearly and inversely related to the concentration of tuber calcium in laboratory and field experiments. Vacuum infiltration of $Ca(NO_3)_2$ solutions into potato tubers increased the calcium content of peel and medulla. When tubers inoculated with *E. carotovora* pv. *atroseptica* during infiltration were incubated in a mist chamber at 20 C for 60 hr, bacterial soft rot was significantly lower in calcium-infiltrated tubers than in untreated ones. Infiltration with solutions containing Mg^{++} and Sr^{++} also reduced the subsequent severity of tuber decay, but not as effectively as Ca^{++}; however, these divalent cations proved more effective than Na^{+} and K^{+}. A field trial was designed with different sources of nitrogen and calcium to establish a group of 18 subplots representing a range in concentrations of soil calcium. The tubers obtained from these plots in 1981 and 1982 had calcium contents ranging from 0.06 to 0.28% (dry weight basis) in the peel and from 0.01 to 0.06% (dry weight basis) in the medulla as determined by inductively coupled plasma - optical emission spectrometry. As the tuber calcium was increased, the percent surface area of the tubers decayed by *E. carotovora* pv. *atroseptica* under mist chamber conditions was reduced significantly each year. The results indicate that in certain soils with a low cation exchange capacity, tubers may be low in calcium and that increasing it may reduce the potential for bacterial soft rot in storage and transit.

Additional key words: blackleg, *Solanum tuberosum*.

Bacterial soft rots, caused by *Erwinia carotovora* pv. *atroseptica* (van Hall) Dye and *E. carotovora* pv. *carotovora* (Jones) Bergey et al are a major cause of potato tuber loss during storage and transit in many sections of the United States and in other regions of the world (32). A variety of factors influence tubers under postharvest conditions, resulting in changes that may affect their susceptibility to soft rot bacteria. Within tubers, these may include changes in water potential (19), in membrane permeability (35), and in intracellular concentrations of reducing sugars (29), polyphenol oxidase (21,37), and the phytoalexin rishitin (23). The concentration of oxygen within the tuber tissue is of major importance and can decrease rapidly if a film of water is maintained on the tuber surface for several hours (7). Under low oxygen conditions, the tuber is highly susceptible to attack by even small numbers of bacteria, whereas at atmospheric oxygen levels, large populations are required to initiate an infection (14). Pectic enzyme preparations of strains of *E. carotovora* similarly cause more extensive tissue degradation when the oxygen concentration is low than when it is at normal concentrations (24). The mineral nutrition of the potato plant also may influence soft rot development in tubers, but data on specific studies have not been presented.

A large number of calcium-related disorders of plants have been recognized, investigated, and reviewed in the literature (2,28,34). Most of the calcium in a potato plant is distributed into leaves and stems, frequently resulting in tubers that are low in calcium (11,15). Particularly in sandy soils with low cation exchange capacities, the calcium content of tubers may approach deficiency levels (*unpublished*).

Calcium is also unequally distributed in the tuber (6); the highest concentrations are in the cortex and periderm. The external, suberized periderm provides an initial line of defense against pathogen invasion and moderates the exchange of O_2, CO_2, and H_2O.

Increases in calcium deposition in cell walls of bean hypocotyls is correlated with increased resistance to the spread of *Rhizoctonia*

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solani (3,4). Calcium was considered to induce the increased resistance of the tissue to maceration by pectic enzymes of the fungus. Since Erwinia soft rots of Chinese cabbage and bean can be reduced by the calcium fertilization (31,33), the possible role of calcium nutrition of potato tubers in the development of bacterial soft rot warranted investigation.

The objectives of this research were to determine the concentration of calcium in potato tubers in relation to that of the entire plant, to determine whether this concentration of tuber calcium is related to the susceptibility of tubers to Erwinia soft rot, and to determine whether susceptibility to these bacteria could be reduced by increasing tuber calcium. Preliminary reports on this research have been published (25,27).

MATERIALS AND METHODS

Bacterial cultures. *E. carotovora* pv. *atroseptica* strain SR8 (Eca) was maintained as a stock suspension in distilled water at room temperature. Strain SR8, originally isolated from a potato tuber in Wisconsin, reacts positively with serogroup I antiserum of *E. carotovora* (13). When cultures were required, a portion of the stock suspension was pipetted into nutrient-dextrose broth containing (per liter): beef extract, 3 g; peptone, 5 g; and dextrose, 10 g. Shake cultures were grown at 20 C for 48 hr, then the cells were harvested by centrifugation, washed, and resuspended in sterile distilled water.

Vacuum infiltration. Vacuum infiltration of Eca in salt solutions involved suspending the bacteria at a concentration of 2×10^6 colony-forming units (cfu) per milliliter as determined by measuring absorbance at 600 nm with a Bausch & Lomb Spectronic 20 colorimeter. At 600 nm, an absorbance of 0.3 corresponds to 2×10^8 cfu of Eca per milliliter of water. Tubers of potato, *Solanum tuberosum* L. 'Superior,' produced under standard cultural practices in central Wisconsin, were first washed and surface sterilized by immersion in a solution of sodium hypochlorite (10% Clorox) for 40 min, rinsed with deionized water, and allowed to air dry. They were placed in anaerobe jars with lids modified for connection to a suction pump. The cell suspension of Eca was poured over the tubers, covering them completely but leaving a small airspace between the level of the liquid and the lid. With the lid in place, pressure in the jar was reduced to 100 mm of Hg. After 1 hr, normal atmospheric pressure was restored. A sample of the infiltrating solution was serially diluted; plated on crystal violet pectate agar selective for pectolytic, Gram-negative bacteria (12); and incubated at 20 C for 48 hr to determine the numbers of Eca that survived the infiltration procedure. The tubers, however, were kept submerged for an additional hour; then they were rinsed and one sample was placed in the mist chamber to determine tuber susceptibility to bacterial soft rot. A second sample was allowed to air dry and immediately was prepared for mineral analysis.

Mist chamber procedure. The mist chamber technique of Lund and Kelman (22) was used to determine tuber susceptibility to Erwinia soft rot. Previously inoculated tubers were placed in the chamber on shelves under which plastic sheets were attached to prevent excess water from dripping on the tubers below. A mist was maintained in the chamber to ensure a continuous water film on the tubers and tuber oxygen depletion. After 60 hr at 20 C, tubers were rated for the percent surface area decayed (%SAD) according to the visual acuity rating scale of Horsfall and Barratt (18). These numerical rating values were subjected to analysis of variance and then were converted to percentages. In addition, in some experiments, the tubers were weighed, the rotted tissue was removed by washing, and they were reweighed to determine the percent weight loss (%WL).

Plant mineral analyses. The mineral content of the various tissues of the potato plant was determined by inductively coupled plasma - optical emission spectrometry by the Wisconsin State Soil and Plant Analysis Laboratory. For determinations of calcium in leaf tissue, the top fully expanded leaf was collected from each of 10 plants at the flowering stage and these were bulked. For tuber analyses, 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; these were combined for each single-peel sample. The five peeled tubers were then quartered lengthwise (from stem to bud end), and one quarter from each was included in one medullar sample. In this manner, two samples were prepared from 10 tubers representing each treatment. The tissues were dried at 65 C then ground to pass a 40-mesh screen.

Field trials. Field experiments were designed to evaluate the uptake of calcium by tubers of cultivar Russet Burbank in a Plainfield sandy loam (Typic Udipsannent) adjusted to various concentrations of calcium. These tubers were then tested for susceptibility to bacterial soft rot. The calcium trials at Hancock, WI, in 1981 and 1982 involved a split-split plot design in randomized complete blocks with four replications. Two main plots were developed the first spring by pretillage broadcast applications of $CaSO_4$ at 11,200 kg/ha or $MgSO_4$ at 17,930 kg/ha, with a third main plot receiving no pretreatment. In the first split, one-third of each main plot received $CaSO_4$ at 210 kg/ha applied either in the row by the planter or as a sidedress at tuberization, or no application. In the second split, supplemental nitrogen was applied as $Ca(NO_3)_2$ at 220 kg N/ha (110 kg at emergence, 55 kg at tuberization, and 55 kg 1 wk after tuberization) or as $(NH_4)_2SO_4$ (110 kg at emergence, and 110 kg at tuberization).

The ratios of exchangeable cations in the soil were established in the main plots the first year (1981), and soil treatments at the same site in 1982 were designed to maintain these differences. The second year, all plots received K_2SO_4 broadcast at 500 kg/ha; the Ca main plot only was supplemented with $CaSO_4$ at 4,480 kg/ha. In the first and second splits, subplots received treatments identical to those applied the year before. Starter fertilizer (5-10-30, N-P-K) was applied at 655 kg/ha to all plots. Conventional herbicide, insecticide, and fungicide treatments were used throughout the experiments.

Analyses of exchangeable cations in the soil and of foliar levels of minerals were completed in midseason. Following the day of harvest, 40 tubers (10 tubers from each of four replications) from each of the 18 treatments were evaluated for soft rot susceptibility by immersion in an Eca suspension, 10^6 cfu/ml for 20 min. After 96 hr in the mist chamber, tubers were rated for %SAD according to the visual acuity rating scale of Horsfall and Barratt (18). These numerical rating values were subjected to an analysis of variance and then were converted to percentages. A second group of 40 tubers (10 from each treatment replicate) was analyzed for peel and medullar calcium by inductively coupled plasma-optical emission spectrometry.

RESULTS

Vacuum infiltration. Vacuum infiltration of calcium solutions into potato tubers increased the calcium content in peel and medullar tissues significantly (Table 1). The chloride, nitrate, sulfate, and gluconate salts of calcium were tested, but the low solubilities of $CaSO_4$ and $Ca(C_6H_{11}O_7)_2$ prevented the use of these two salts except at low concentrations. Prior to infiltration with solutions of $Ca(NO_3)_2$ and $CaSO_4$, the ratios of peel to medullar calcium in two different lots of cultivar Superior tubers were 4.7 and 6.6, respectively. Calcium infiltration preferentially enriched the outer cell layers of the tubers, increasing these ratios to 6.8 and 7.4, respectively.

Reduced air pressure (100 mm Hg) combined with increased $Ca(NO_3)_2$ salt concentrations at 0, 500, 3,000, 6,000, and 12,000 mg/L reduced the viability (cfu/ml) of cells of Eca from 5.1×10^6 to 3.0×10^6, 3.7×10^6, 2.0×10^6, and 10^4, respectively. These factors did not, however, significantly interfere with attempts to relate %SAD to tuber calcium. No significant difference in surface decay resulted when the bacteria were infiltrated into tubers at either 10^6 or 10^5 cfu/ml as suspensions in $Ca(NO_3)_2$ solutions (Table 2). This difference in concentration was approximately twice the decrease in numbers of cells following the infiltration procedure. Therefore, at 2×10^6 cfu of Eca per milliliter and with a Ca^{++} concentration at or below 6,000 mg/L, any reduction in %SAD was considered to be attributable to a host response associated with an increase in

calcium rather than to an adverse effect upon the bacterial inoculum.

Increasing the concentrations of calcium within the tuber peel and medulla by vacuum infiltration resulted in tubers that typically were less severely decayed after 60 hr in the mist chamber, although all had been uniformly inoculated with Eca (Table 3). Percent WL was reduced from 68.6 to 7.9% and %SAD from 93.3 to 15.0% over the range of calcium infiltrated, up to 6,000 mg/L. At the high calcium levels, these reduced levels of decay were not significantly different from those obtained with control tubers that were infiltrated only with water. At a Ca^{++} concentration of 12,000 mg/L, no decay was observed. At this high salt concentration the viability of bacterial cells in suspension was reduced (Table 3), and even the low background level of decay in this tuber sample was also eliminated. When the soft rot and tuber calcium level data were statistically analyzed, the correlation between the reduction in %SAD (transformed by arcsine of the square root) and the increase of tuber calcium resulting from vacuum infiltration of $Ca(NO_3)_2$ was evident (peel: $r = 0.981$; medulla: $r = 0.988$) (Fig. 1).

TABLE 1. Peel and medullar concentrations of calcium in cultivar Superior potato tubers following vacuum infiltration with calcium-containing solutions

	Calcium in tissue after infiltration[y,z] with:			
	$Ca(NO_3)_2$		$CaSO_4$	
Ca^{++} (mg/L)	Peel	Medulla	Peel	Medulla
0	0.102 a	0.022 a	0.132 a	0.020 a
250	0.165 b	0.034 b	0.173 b	0.028 b
500	0.164 b	0.036 b	0.201 c	0.031 bc
750	...	...	0.247 d	0.034 c
1,000	0.246 c	0.046 c	0.281 e	0.038 d
2,000	0.268 d	0.048 c	...	...
3,000	0.327 e	0.052 d	...	...
6,000	0.371 f	0.063 e	...	...
12,000	0.508 g	0.075 f	...	...

[y]Tubers immersed for 2 hr, the first hour of which was at 100 mm Hg air pressure; data are the means (percent, dry weight basis) for 10 tubers (... = not tested).

[z]Determined by inductively coupled plasma - optical emission spectrometry. Within columns, numbers followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 2. Percent surface area decayed (SAD) and weight loss (WL) of Superior tubers vacuum infiltrated with suspensions of *Erwinia carotovora* pv. *atroseptica* in a $Ca(NO_3)_2$ solution, 1,000 mg/L

	Bacterial population used to infiltrate[x]					
Decay severity	10^6	10^5	10^4	10^3	10^2	0
AD (avg. percentage)[y]	45 a	41 a	29 b	10 c	1 d	0 e
WL of decayed tissue[y]	43 a	41 a	21 b	10 c	0 d	0 d

[x]Colony-forming units per milliliter.

[y]Tubers immersed for 2 hr, the first hour of which was at 100 mm Hg air pressure, followed by incubation in a constant mist chamber at 20 C for 60 hr. Within rows, numbers followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

Comparisons were made of the amount of soft rot resulting from vacuum infiltration of Eca suspended in solutions of other calcium salts. Treatment with each of the calcium compounds resulted in a reduction in soft rot, but the degree of reduction varied considerably (Fig. 2). Both $Ca(NO_3)_2$ and $CaCl_2$ reduce %SAD by approximately half compared with the control; however, $Ca(NO_3)_2$ was the most effective in reducing total weight loss. For this reason, it was selected for additional study. Calcium gluconate was least effective in reducing rot and also in penetrating the tuber during infiltration. The most obvious relationship was the reduction in %WL associated with an increase in medullar calcium. This increase in medullar calcium possibly reflected the solubility of the salt which facilitated the movement of calcium into the tuber and was dependent upon the anion.

It was necessary to determine whether this disease reduction was attributable to a specific action of calcium or whether it was merely a more general salt effect. Tubers of cultivar Superior were vacuum infiltrated with Eca suspended in solutions of sodium, potassium, strontium, magnesium, calcium, or aluminum nitrates. After 60 hr in the mist chamber at 20 C, the tubers were rated for %SAD (Fig. 3). The monovalent cations, Na^+ and K^+, were least effective in reducing decay when infiltrated with Eca into tubers, whereas the divalent cations, Sr^{++}, Mg^{++}, and Ca^{++}, produced a significantly greater response. Of these cations, Ca^{++} was the most effective. Although the greatest reduction in soft rot resulted from the treatment of tubers with $Al(NO_3)_3$, Al^{+++} ions at the concentration used are toxic to bacteria, and the host response may not have been as great as it appeared to be.

Relationship between tuber soft rot and calcium fertilization of potatoes in field experiments. The fertilizer treatments applied in the spring of 1981 were effective in producing subplots with a wide

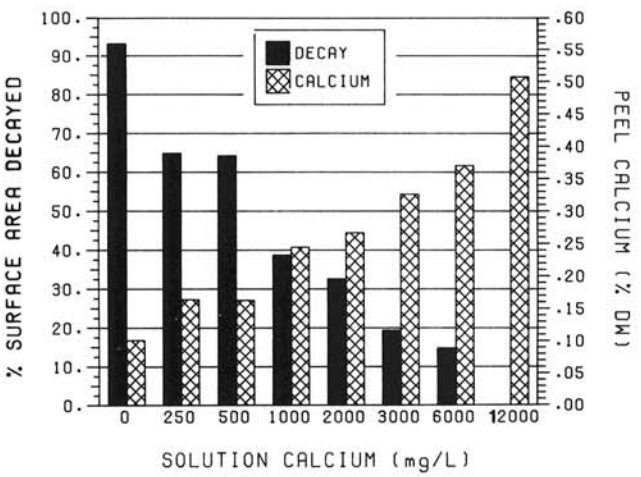

Fig. 1. Percent surface area decayed in relation to peel calcium content of Superior tubers vacuum infiltrated with *Erwinia carotovora* pv. *atroseptica* in $Ca(NO_3)_2$ solutions. Tubers immersed in a suspension containing 2×10^6 cfu/ml for 2 hr, the first hour of which was at 100 mm Hg air pressure, followed by incubation in a constant mist chamber at 20 C for 60 hr. Calcium determinations by inductively coupled plasma-optical emission spectrometry.

TABLE 3. Percent surface area decayed (SAD) and weight loss (WL) of potato cultivar Superior tubers vacuum infiltrated with suspensions of *Erwinia carotovora* pv. *atroseptica* (Eca) in solutions of $Ca(NO_3)_2$

	Calcium concentration (mg/L)								
Decay severity (%)	0[x] −Eca	0[y]	250	500	1,000	2,000	3,000	6,000	12,000
WL[z]	9.6 bc	68.6 a	59.0 a	53.6 a	21.4 b	23.5 b	11.4 bc	7.9 bc	0.0 c
SAD[z]	14.0 de	93.3 a	65.0 b	64.4 b	38.9 c	32.8 c	19.6 d	15.0 d	0.0 e

[x]Control = no Ca^{++} or inoculum added.

[y]Control = no Ca^{++} added.

[z]Tubers immersed for 2 hr in a suspension containing 2×10^6 cfu/ml for 2 hr, the first hour of which was at 100 mm Hg air pressure, followed by incubation in a constant mist chamber at 20 C for 60 hr; data are mean of 10 tubers. Within rows, numbers followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

range of soil calcium and magnesium (Table 4). Soil calcium in the upper 15 cm ranged from a low concentration, averaging 700 kg/ha in the $MgSO_4$-treated main plots that received nitrogen as $(NH_4)_2SO_4$, to an average of 3,420 kg/ha in the calcium main plots that received $Ca(NO_3)_2$. The ratio of soil Ca to Mg increased nearly eightfold across this range. These data, as well as others in this table, represent a mean of the three first-split $CaSO_4$ applications and do not show the complete range in values across all 18 treatments.

This increased availability of soil calcium resulted in an increase in uptake of the cation by the Russet Burbank potato plants. By far, the greatest amount of calcium was deposited in the foliage (0.190 to 1.188%, dry weight basis [DW]), and in no treatments were the leaves deficient in this mineral. Tuber calcium and also that in both the peel and in the medullar tissues increased as soil calcium was increased. Peel calcium ranged from 0.057 to 0.277% (DW) and medullar calcium from 0.011 to 0.062% (DW) across all 18 treatments.

Accompanying the increase of tuber calcium was a corresponding decrease in the %SAD measured 96 hr after tubers inoculated with Eca were placed in a mist chamber for optimal disease development (Table 5). Again, the values in this table represent only the means of first-split $CaSO_4$ treatments. Among all eighteen treatments, %SAD was reduced more than 50%, from 43.5% in tubers from the Mg subplot that received no supplemental calcium in either the first or second splits, to 19.4% in tubers of the Ca subplot that received $CaSO_4$ in the first split and $Ca(NO_3)_2$ in the second. The correlations between %SAD (transformed by arcsine of the square root) and peel and medullar calcium were $r = -0.933$ and $r = -0.948$, respectively.

Results in 1982 were similar to those obtained in 1981 for both tuber calcium and tuber susceptibility to bacterial soft rot. The range in concentrations of tuber peel calcium was less than that obtained the year before, from 0.080 to 0.148% (DW), as was that of medullar calcium, which ranged from 0.020 to 0.046% (DW). Percent SAD was again reduced the second year from 82.5% in the most susceptible tubers from the Mg subplot lacking any calcium fertilization, to 48.5% in tubers from the subplot that received the highest amounts of calcium (Table 5). Surface decay was inversely correlated with both tuber peel and medullar calcium (Fig. 4). In 1982, the correlation between %SAD and peel calcium was $r = -0.917$, whereas that with medullar calcium was $r = -0.880$.

DISCUSSION

Based upon analyses of large numbers of plants of many species, a calcium content from 0.2 to 4.0% (DW) has become accepted as the typical range for this macronutrient in leaves and stems of

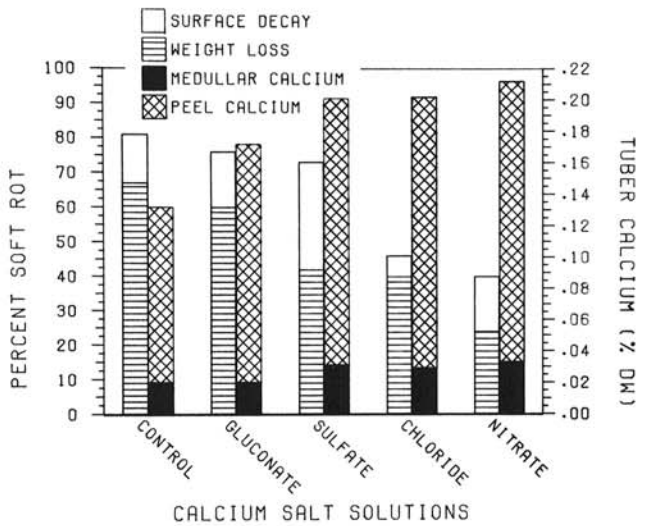

Fig. 2. Percent surface area decayed and weight loss in relation to peel and medullar calcium content of cultivar Superior potato tubers vacuum infiltrated with *Erwinia carotovora* pv. *atroseptica* in solutions of calcium salts. Tubers immersed in a suspension containing 2×10^6 cfu/ml for 2 hr, the first hour of which was at 100 mm Hg air pressure, followed by incubation in a constant mist chamber at 20 C for 60 hr. Calcium determinations by inductively coupled plasma-optical emission spectrometry.

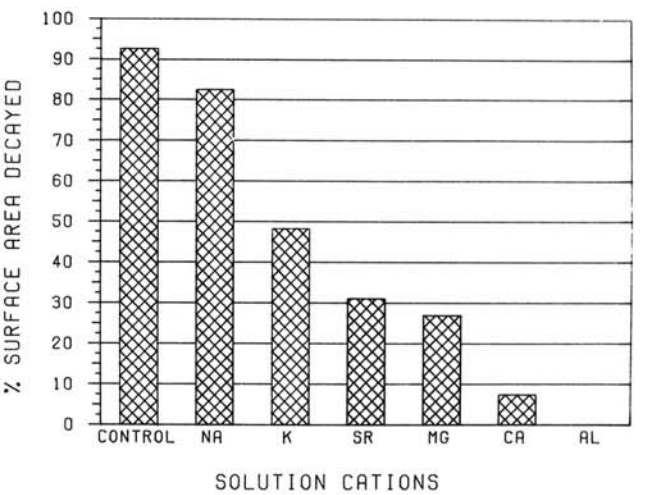

Fig. 3. Percent surface area decayed of cultivar Superior potato tubers vacuum infiltrated with suspensions of *Erwinia carotovora* pv. *atroseptica* in nitrate solutions of various cations, all at 0.08 M. Tubers immersed in a suspension containing 2×10^6 cfu/ml for 2 hr, the first hour of which was at 100 mm Hg air pressure, followed by incubation in a mist chamber at 20 C for 60 hr.

TABLE 4. Soft rot susceptibility of potato cultivar Russet Burbank tubers from field plots with various fertilizer amendments in relation to the calcium content in potato tissues and in soil (1981 trial)

	Plot treatments					
	Mg[u]		None		Ca	
	AS[v]	CN	AS	CN	AS	CN
Soil Ca[w,z]	700 a	1,460 c	1,010 b	1,400 c	3,360 d	3,420 d
Soil Ca/Mg ratio	0.9 a	1.5 b	2.7 c	3.4 d	7.7 e	7.9 e
Foliar Ca[x] (%)	0.258 a	0.549 b	0.549 b	0.944 d	0.618 c	1.061 e
Peel Ca[x] (%)	0.092 a	0.109 a	0.104 a	0.160 b	0.176 b	0.246 c
Medullar Ca[x] (%)	0.018 a	0.020 a	0.025 ab	0.029 b	0.044 c	0.050 d
Surface area decayed[y] (%)	35.8 a	30.8 b	31.7 ab	28.3 b	25.6 c	21.7 d

[u] Main plots receiving Mg (as $MgSO_4$) at 17,930 kg/ha, Ca (as $CaSO_4$) at 11,290 kg/ha, or none = no pretreatment.
[v] Nitrogen source, plots receiving 220 kg N per hectare as AS = $(NH_4)SO_4$ or CN = $Ca(NO_3)_2$.
[w] Soil calcium (upper 15 cm) kg/ha.
[x] By inductively coupled plasma-optical emission spectrometry.
[y] Percent surface area decayed after immersion for 20 min in a suspension of *Erwinia carotovora* pv. *atroseptica* (10^6 cfu/ml) followed by incubation in a constant mist chamber at 20 C for 96 hr; mean of 120 tubers.
[z] Within rows, numbers followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

plants (5). Our data indicate that the calcium content of the potato tuber peel is often half of this minimum value of 0.2%, whereas medullar calcium is approximately one fifth the concentration of that in the peel. Although the calcium concentration in potato tubers generally is lower than in leaves and stems, the concentration at which they may be considered to be deficient has not been clearly defined. There is evidence, however, that as the content of calcium declines in tuber medullar tissues from 0.018 to 0.014% (DW), the incidence of physiological necrosis, termed internal rust spot, internal brown spot can double (11).

Increasing the concentration of calcium within the potato tuber, either artificially by vacuum infiltration or by increased calcium fertilization of the growing plant, resulted in a significant increase in tuber resistance to Erwinia soft rot. Vacuum infiltration proved to be a very effective method of increasing tuber calcium concentrations. Uninoculated tubers subjected to this treatment did not appear to be damaged and, when surface-sterilized prior to treatment, remained free of decay for up to 1 wk under mist chamber conditions. Although reduced air pressure and increased salt concentration combined to reduce the viability of cells of Eca, such population reductions in the low calcium solutions were too small to influence tuber susceptibility significantly. The soft rot reduction associated with vacuum infiltration into potato tubers of Eca in calcium solutions, therefore, was considered to be attributable primarily to calcium effects on tuber tissue and not to an adverse effect upon the viability of the bacterial pathogen.

Results of field studies in each of 2 yr confirmed the initial observations of an inverse correlation between the content of calcium and the severity of bacterial soft rot in tubers of cultivar Russet Burbank. Data from field trials in 1983 involving different sources and concentrations of calcium have provided additional evidence that severity of bacterial soft rot is correlated inversely with calcium content of Russet Burbank tubers (*unpublished*). Increased calcium fertilization effectively raised tuber (and foliar) calcium levels; however, the ratio between concentrations of calcium and magnesium in the soil appeared to be a better basis for predicting tuber calcium than concentration of soil calcium alone.

The concentrations of tuber calcium varied considerably over the 2 yr of the field study, with higher tuber calcium levels being obtained the first year. During the filling stage, tubers receive the greatest amount of their nutrients through the phloem. Because calcium moves throughout the plant almost exclusively in the xylem and preferentially to transpiring tissues, environmental factors such as high air temperatures and low humidity, which increase transpiration, will result in movement of greater amounts of calcium into the foliage than will less extreme conditions. Such factors can also result in movement of water out of the tuber. With a return to cool night temperatures, high humidity, and ample soil moisture, the water deficit in the tuber can be restored with the solution comparatively high in calcium being carried directly from the roots through the xylem (16). Cycles of such extreme conditions, which are more common in western states than in Wisconsin, may cause the tuber alternately to expand and contract, resulting in an increase in calcium during each cycle. In 1981, the irrigated plots at Hancock were exposed to an extended period of hot, dry weather; this may in part account for the increased concentrations of calcium in the tubers of the high calcium plots that year. In addition, a greater amount of magnesium was present in 1981 than in 1982 in plots that received heavy applications of $MgSO_4$. By the second year, this had partially leached out of the root zone, and additional applications were not made. Greater magnesium antagonism in 1981 may have reduced calcium uptake by roots and its transport to tubers in those plots relative to the same plots in 1982.

In both years of the field study, bacterial soft rot in tubers of cultivar Russet Burbank, as measured by %SAD, was reduced approximately half at the highest level of calcium fertilization. Disease severity was lower the first year when the tubers generally had a greater calcium content. It should be emphasized that the conditions for soft rot development under which these evaluations were performed were much more extreme than those encountered during commercial storage or transit. A constant mist produces a continuous film of water over the tuber surface; under such conditions, the tubers become anaerobic within 2.5 hr at 21 C (7) and disease severity is greatly enhanced. The decrease in bacterial soft rot related to increases in concentrations of tuber calcium should be much more pronounced under environmental conditions that are not as favorable to the pathogen.

The reduction in bacterial soft rot was highly correlated with the calcium increase in both peel and medullar tissues of the tuber. An increase in one tissue was related to a corresponding rise in the other. Direct movement of calcium into the tuber from the soil, however, may have helped enrich the peel and accounted for the reduced gradient toward the center (20). The periderm provides the first barrier against infection, and high concentrations of calcium may make this tissue more resistant to penetration. For decay to develop, however, the cell layers immediately below the periderm also must be invaded.

A considerable part of the calcium in a plant is localized in cell walls; its concentration in this region is approximately 10^{-3}M (10). Calcium, being a divalent cation, has the ability to bridge two galacturonates via their carboxylate groups, and calcium pectate is a principle component of the middle lamellae (9). In addition, binding of proteins to polysaccharides through phenolic acid and calcium bridges has been shown to have a strengthening influence on cell walls (30). Calcium also binds anionic groups of the

TABLE 5. Soft rot susceptibility of potato cultivar Russet Burbank tubers from field plots with various fertilizer amendments (1982 trial)

	Percent surface area decayed on tubers from plots treated with:						
	Mg[x]		None		Ca		First-split means
	AS[z]	CN	AS	CN	AS	CN	
0[y]	82.5 a	77.9 abc	83.3 ab	66.3 def	62.4 ef	59.3 fg	72.0 a
S	71.4 cde	72.1 cde	79.2 abc	70.5 cde	59.3 fg	58.7 fg	68.5 b
R	66.4 def	75.1 cd	74.4 bcd	67.0 def	50.9 g	48.5 g	63.7 c
Column means	73.4 b	75.0 ab	79.0 a	67.9 c	57.5 d	55.5 d	
Main plot means	Mg 74.2 a		none 73.4 a		Ca 56.5 b		
N source means	AS 70.0 a		CN 66.2 b				

[w] Percent surface area decayed after immersion for 20 min in a suspension of *Erwinia carotovora* pv. *atrosentica* (10^6 cfu/ml) followed by incubation in a constant mist chamber at 20 C for 96 hr; mean of 40 tubers. Numbers followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

[x] Main plots receiving Mg: $MgSO_4$ at 17,930 kg/ha; Ca: $CaSO_4$ at 11,200 kg/ha; or 0 = no pretreatment.

[y] First split receiving 210 kg/ha $CaSO_4$. R = in the row, S = as a sidedress, or 0 = no application.

[z] Nitrogen source plots receiving 220 kg/ha N as AS = $(NH_4)_2SO_4$; or CN = $Ca(NO_3)_2$.

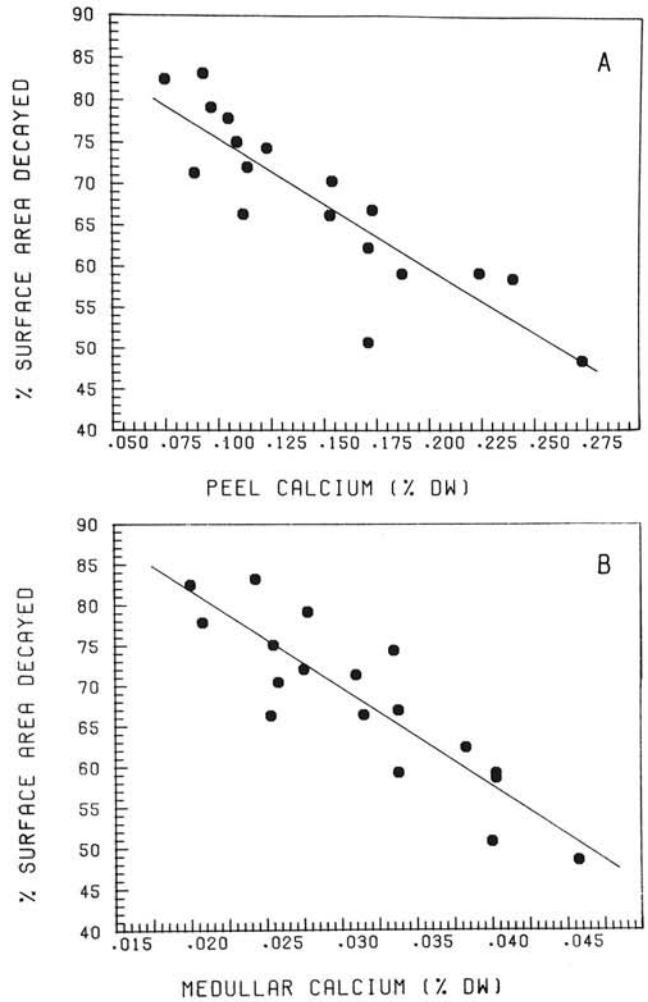

Fig. 4. Percent of potato tuber surface area decayed in relation to the percentage of **A,** tuber peel calcium and **B,** tuber medullar calcium of Russet Burbank tubers from the 1982 calcium trial inoculated with *Erwinia carotovora* pv. *atroseptica*. Tubers were immersed for 20 min in an inoculum suspension containing 10^6 cfu/ml, followed by incubation in a mist chamber at 20 C for 96 hr. Calcium was measured by inductively coupled plasma-optical emission spectrometry.

membrane to form bridges between structural components, thereby maintaining selective permeability, structural integrity, and cellular compartmentalization (9). Stress-induced leakiness of cells, caused by low temperatures or oxygen deficiency, can also be prevented or reversed by calcium treatments (8). Perhaps equally important, calcium appears to bind constituents of the plasmalemma loosely to the cell wall (17).

Since calcium improves the structural integrity of both cell wall components and cell membranes, the reduction in the severity of Erwinia soft rot in high-calcium potato tubers may be explained on the basis of the delay in the rate of maceration resulting from this structural enhancement. Soft rot *Erwinia* macerate host tissues primarily by the action of pectolytic enzymes that can be greatly influenced by calcium concentrations in vitro (36). When partially purified sterile preparations of pectic enzymes from Eca were injected into whole tubers and these were placed under anaerobic conditions, high calcium tubers showed less maceration than those with low levels of this element (26). In this study, a high inverse correlation between bacterial soft rot susceptibility and calcium content of medulla tissue of potato cultivars was demonstrated. It is recognized, however, that other physical and physiological relationships exist that may prove to be as important as the effect of calcium on pectolytic enzyme activity. Reduced respiration of tubers infiltrated with calcium has been noted (1).

Although more extensive survey data are needed, Erwinia soft rot appears to be a more severe postharvest problem in tubers produced in sandy soils than in those produced in loam soils. Tubers grown in soils with low cation exchange capacities are frequently low in calcium, although the soil apparently contains an ample supply for normal plant growth, and leaves are not deficient.

Increases in soil calcium resulted in potato tubers that had higher calcium content and were more resistant to bacterial soft rot. It is possible, however, that increases in soil calcium may enhance development of potato scab (caused by *Streptomyces scabies*). Although researchers disagree as to the specific role of nutritional factors on this disease, most studies have related increases in peel calcium with increased disease. The specific effects of various soil treatments upon the soil microflora are not known. No obvious increases in potato scab were observed on high-calcium tubers relative to low-calcium tubers in any of these field trials. If soils became more extensively modified through calcium fertilization, however, changes could occur that might result in an increase of other soilborne pathogens.

Calcium fertilization does not provide a means for complete control of Erwinia soft rot, but simply lowers the severity of the disease. However, the potential exists for calcium to reduce not only this disease, but others as well, in particular those diseases caused by pathogens that macerate tissues primarily with pectolytic enzymes. Recognition of the role of calcium in reducing bacterial decay of other fleshy vegetable and fruit crops warrants additional study.

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