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Enhancing Tuber Calcium Concentration May Reduce Incidence of Blackspot Bruise Injury in Potatoes

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***Abstract*. Our previous research has provided evidence that in-season calcium applications can increase tuber calcium and improve tuber quality with reduced internal defects. To determine if increasing the tuber calcium concentration also mitigates tuber bruise incidence, five commercially relevant potato (*Solanum tuberosum* L.) cultivars ('Russet Burbank', 'Atlantic', 'Snowden', 'Superior', and 'Dark Red Norland') were grown during three seasons, 1999–2001. Three split applications of a calcium/nitrogen water soluble blend totaling 168 kg·ha^{-1}calcium were made starting at hilling. All plots, including controls, received an equal amount of total nitrogen in a season. Tubers were allowed to be bruised during normal machine harvest standard to commercial production in Wisconsin. Over 100 tubers from each replication (5–10 replications/treatment) were cut and examined for the incidences of bruise and internal brown spot. Paired samples of medullary tissue were taken for measuring calcium concentration. As expected, tuber tissue calcium concentration increased significantly, in all cultivars and in all years, with in-season calcium application. Bruise incidence varied among cultivars and seasons. Although tuber calcium concentration varied among seasons, 'Atlantic' and 'Snowden' consistently had the lowest calcium concentration, whereas 'Superior' and 'Dark Red Norland' consistently had the highest calcium concentration. Meta-analysis of pooled data for three years showed that blackspot bruise incidence was significantly reduced with calcium application in 'Atlantic', 'Burbank', and 'Snowden'. On the other hand, 'Dark Red Norland' and 'Superior' had low incidence of bruise and were unaffected by calcium applications. Regression analyses of pooled data from all cultivars for three years revealed a significant quadratic relationship between blackspot bruise and tuber tissue calcium as well as between blackspot bruise and internal brown spot. A linear to plateau plot of medullary calcium concentration versus blackspot bruise incidence revealed that bruise incidence is minimized between 200 and 250 μg/kg (dry wt)$^{-1}$ tuber calcium concentration. To our knowledge, ours is the first study providing evidence for reducing bruise by improving tuber calcium. Variations in the bruise incidences among cultivars generally followed tuber calcium concentration suggesting a genetic control. Given the role of calcium in improved membrane health and enhanced wall structure, and as a modulator of physiological responses, it is not surprising that internal brown spot and bruise incidences are reduced by in-season application to calcium-deficient cultivars.**

Machine harvest, handling, and storage of potato tubers can impart pressure and impact injury resulting in tuber blackspot bruise and reduced crop value. Tuber injury can promote premature aging (Workman and Holm, 1984) or elevated levels of respiration (Pisarczyk, 1982) leading to losses in processing quality, decreased seed piece performance, or susceptibility to soft rot (Bartz and Kelman, 1986). Factors that influence bruise incidence and severity include cold tuber temperatures and flaccidity during harvest or handling (Thornton and Timm, 1989) and harvesting practices such as use of padded rollers or optimizing soil conditions for minimal bruise (Brook, 1996). Tuber maturity at the time of harvest may also influence bruise rates with immature tubers showing less susceptibility (Pavek et al., 1985).

Mineral nutrition during the growing season may also influence tuber blackspot bruise susceptibility. Potassium deficiency was found to be associated with a higher blackspot bruise incidence and increased respiration levels in early studies (Mulder, 1949, 1955). The influence of potassium is not fully understood because Schenk (1981) tested several cultivars and concluded that higher potassium tended to reduce blackspot bruise but that it was not sufficient to explain incidence. Potassium deficiency has been associated with increased specific gravity, which in turn can render tubers more susceptible to blackspot bruising (Ophius et al., 1958). Nitrogen in excess, on the other hand, is associated with reduced dry matter and delayed maturity (Reeve et al., 1973), which renders tubers less susceptible to blackspot bruising. Early studies established that specific gravity and blackspot bruising are highly correlated (Massey et al., 1952). However, the role of specific gravity in bruise potential is thought to be limited in that it is probably more an indicator of tuber maturity than of blackspot bruise potential (Brook, 1996). Although some studies have implicated possible roles for minor nutrients such as copper (Kertesz, 1952) and zinc in bruise incidence (Mulder, 1956; Monday and Chaudra, 1981), these elements are not commercially recommended for reducing bruise incidence.

We have recently reported that by increasing tuber calcium, one can expect a reduction in the internal defects such as internal brown spot and hollow heart (Kleinhenz et al., 1999; Ozgen et al., 2006). During blackspot bruising, impact disrupts cells under the periderm allowing phenols to come in contact with polyphenoloxidase to ultimately produce the dark pigment melanin (Corsini et al., 1992). Because internal brown spot and bruise involve discoloration of tuber tissue, it follows that localized calcium deficiencies may also contribute to heightened bruise potential. In support of this, tyrosine concentration and phenolase (an enzyme whose activity is associated with bruise) activity were also found to be decreased by increases in tuber tissue calcium (Mapson et al., 1963). Localized deficiencies in calcium have also been associated with tissue necrosis such as brown center or internal brown spot (Bangerth, 1979; Collier et al., 1980; Levitt, 1942).

The objective of the present study was to investigate the potential for in-season supplemental calcium to reduce the incidence of blackspot bruise in field grown, machine-harvested potatoes. Tubers from five commercially significant cultivars that vary in bruise susceptibility were subjected to standard field-harvesting practices. By examining large numbers of tubers for blackspot bruising and internal defect and by obtaining paired samples for tissue calcium concentration analysis, we attempted to relate the incidence of blackspot bruise to tissue calcium concentration. Using a three-season study, we were able to assign the variability in blackspot bruise that can be explained by tuber calcium concentration in five cultivars. Data analyses also allowed us to predict the critical tuber calcium concentrations necessary to minimize blackspot bruising. Because both blackspot bruise and internal brown spot involve discoloration of the tuber tissue, we explored possible relationships

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between blackspot bruising and internal brown spot.

Materials and Methods

Plot establishment, nutrient sources, and application

Certified, A-grade (170–284 g) seed tubers (‘Atlantic’, ‘Superior’, ‘Snowden’, ‘Dark Red Norland’, and ‘Russet Burbank’) were cut into 56- to 84-g pieces, allowed to suberize for 3 d, and planted about 23 cm deep on a Plainfield loamy sand (sandy, mixed, mesic, Typic Udipsamment). At this location, soil pH was approximately six and cation exchange capacity was near 3 $cmol/kg^{-1}$ (Liegel et al., 1980). Experiments were conducted in 1999, 2000, and 2001 under linear irrigation at the University of Wisconsin Hancock Agriculture Research Station (Hancock, Wis.). Individual plots consisted of two 6.1-m experimental rows bordered by two 6.1-m guard rows. In-row seed tuber and between row spacing was 30 and 91 cm, respectively. Table 1 details pertinent seasonal, cultural, and environmental data.

A completely randomized design was used in all years with five replications per year for each cultivar and treatment, with the exception of 2000 in which all treatments were replicated 10 times for each cultivar. For the analysis of variance Tables (4–7), a widely accepted statistical method “meta-analysis” (Cochrane and Cox, 1957) was used that combined replications from identical experiments grown in the same field in different years; the total number of replications of the experiment was 20 replications per treatment per cultivar. Examples of the use of this type of analysis combining identical plant experiments from different years or laboratories is available in several references (Morgan et al., 2003; Shaw and Larson, 1999). Starter fertilizer (6N-24P-24K) pretreated with Admire was applied at a rate of 560 $kg·ha^{-1}$. At emergence, 392 $kg·ha^{-1}$ of ammonium sulfate (21N–0P–0K) was given. All additional nitrogen in the control plots was supplied as commercial-grade ammonium nitrate (34N–0P–0K). Total nitrogen was 255 $kg·ha^{-1}$ for all treatments. The balance of 138 $kg·ha^{-1}$ nitrogen was split equally into 3 applications starting at hilling using ammonium nitrate for control treatments and a combination of liquid calcium nitrate, solid calcium chloride, and urea designated “CUC” for the supplemental calcium treatment (Table 2). “CUC” combined treatment of calcium nitrate, calcium chloride, and urea with 50% calcium from $Ca(NO_3)_2$ and the other 50% calcium from $CaCl_2$ and N balanced with urea. Urea was obtained in solid form from Sigma (St. Louis, MO) and commercially available liquid calcium nitrate (9.0N-0P-0K-11.0Ca^{2+}; Hydro Agri of North America, Inc. Tampa, FL) from Hartung Bros., Arena, Wisconsin. For applications made at hilling, 3 weeks after hilling, and 6 weeks after hilling, half of the calcium was supplied as liquid calcium nitrate and half as laboratory-grade calcium chloride ($CaCl_2/2H_20$), from Sigma. For calcium, combinations of N and calcium, and control treatments, N alone were applied to two experimental rows within each experimental plot. Half the fertilizer was diluted and dispensed from a calibrated container containing 6 L solution in one direction uniformly to the top of the hill with minimal foliar contact. The second half of the fertilizer was applied in the other direction to equalize discrepancies as a result of watering. Irrigation was applied when needed to replace soil water lost resulting from evapotranspiration. Weed, disease, and insect pest pressures were maintained by using cultivation and agrichemicals common to commercial practices in the Central Sands region of Wisconsin.

Harvest, grading, tuber quality, and tuber calcium

To apply a bruising insult to a large number of tubers, the machine harvester served as the bulk sample bruising mechanism. A similar method has previously been used in which tubers were rotated in a drum for a preset time (Brook, 1996). Two to 3 weeks after chemical vine desiccation, tubers were removed from the two inner rows of each 4-row plot with a single-row Gallenburg harvester (Gallenburg Equipment Inc., Antigo, Wis.). It consisted of a Holland belted chain, weed chain for debris, and an adjustable star table to remove undersize tubers and dirt. The components were rubber-coated. During the harvest, tubers dropped 0.63 m into crates. At this time, tubers were rinsed free of soil and graded with an electronic grader within 1 d of harvest. Immediately after grading, 170- to 284-g tubers and tubers greater than 284 g were collected separately and transferred into a cold room maintained at 5 °C, 85% relative humidity in Madison for evaluation of tuber bruise, internal brown spot, and sampling for calcium concentration.

Table 1. Seasonal, cultural, and environmental information for experiments conducted at Hancock Agricultural Experiment Station (Waushara County, WI) from 1999 to 2001.

	Year			
Dates and parameters	1999	2000	2001-early[z]	2001–late[y]
Planting	26 Apr	2 May	7 May	7 May
Emergence (50%)	18 May	17 May	25 May	25 May
Hilling	4 Jun	9 Jun	12 Jun	12 Jun
Vine kill	25 Aug	3 Aug	13 Aug	17 Sept
Harvest	11 Sept	3 Sept	29 Aug	28 Sept
Soil calcium, mean (ug/g^{-1} dry weight)	380	285	400	400
Harvest temperatures	°C			
Air	18	18	21	6
Soil 7 cm	19	22	23	13
Soil 14 cm	20	26	23	13
Days of season	No. of days			
Length of season[x]	121	124	98	133
Maximum 4’ soil temp >32.2 °C	2	0	14	14
Maximum 4’ soil temp >26.7 °C	48	75	58	58
Accumulated heat units[w]	Heat units			
Planting	35	56	165	165
Emergence	182	217	324	324
Hilling	364	432	476	476
Final treatment[v] (H + 6)	1160	1120	1347	1347
Vine kill	1917	1606	1821	2324
Harvest	2199	1958	2102	2377
Total rainfall[u]	mm			
Planting	151	98	189	189
Harvest	713	606	563	680
Hilling	252	283	339	447
Final treatment (H + 6)	428	447	447	447
Vine kill	713	483	467	656
Between hilling and H + 6	176	164	108	108
Between planting and hilling	101	185	150	150
Between planting and H + 6	277	350	258	258
Between planting to vine kill	562	385	278	467
Between planting to harvest	563	508	374	374
Rainfall events >19 mm[t]	No. of events			
Planting to vine kill	8	10	4	6
Planting to hilling	1	4	2	2
Hilling to last treatment (H + 6)	2	4	2	2

[z]Harvested cultivars ‘Dark Red Norland’ and ‘Superior’ earlier in 2001.
[y]Harvested cultivars ‘Atlantic’, ‘Snowden’, and ‘Russet Burbank’ later in 2001.
[x]Growing season defined as planting to vine kill.
[w] Heat units base 10 °C. www.soils.wisc.edu/wimnet/awon/SelectDailyClim.html.
[v]H + 6 = 6 weeks after hilling and last split nutrient treatment and control.
[u]Independent of irrigation and cumulative from beginning of year.
[t]Number of days on which total precipitation exceeded 19 mm.

Table 2. Treatment source, timing, and rate of N and calcium applications.[z]

Treatment	Source	Nutrient: amount at each application ($kg \cdot ha^{-1}$) Nitrogen	Calcium	Timing[y]
Split ammon nitrate	NH_4NO_3	46	0	H,H + 3,H + 6
CUC 168 kg Ca^{2+} [x]	$Ca(NO_3)_2$	23	28	H,H + 3,H + 6
	H_2NCONH_2	23	0	
	$CaCl_2$	0	28	

[z]All plots received the same total amount of N (112 $kg \cdot ha^{-1}$ prehilling and 138 $kg \cdot ha^{-1}$ posthilling). Plots given the calcium treatment received 168 $kg \cdot ha^{-1}$ calcium. Beginning at hilling, nutrient applications were split into 3 equal applications. At each application for each plot, nutrients were dissolved into a total of 24 L of water and applied to the top of the hill with watering cans.

[y]H = hilling; H + 3, H + 6 = number of weeks after hilling.

[x]CUC = combined treatment of calcium nitrate, calcium chloride, and urea with 50% calcium from $Ca(N0_3)_2$ and the other 50% calcium from $CaCl_2$ and N balanced with urea.

All procedures described in this section were performed on individual tubers from each experimental row for a total of approximately 100 A grade tubers (170–284 g) and 10 to 30 large (greater than 284 g) tubers per replication per treatment. The total number of tubers rated from each treatment for each cultivar over 3 years was approximately 2000 A grade tubers and 500 large tubers. After a 2-month storage period, tubers were rated by halving lengthwise and the presence or absence of blackspot bruise and internal brown spot was noted. Blackspot bruise here is defined as a discolored (brown, grey, or black), hollow, or starch-filled anomaly generally external to the vascular ring, which was 3 mm or greater in diameter. A tuber was counted as bruised whether it had one or several bruises.

For calcium determinations, medullary tissue samples were taken and processed according to the procedure described by Kratzke and Palta (1986). Samples for calcium analysis were dried in an oven (49 °C) for 5 days, ground to pass a 40-mesh (0.635-mm) screen, weighed, ashed (450 °C, 8 hours), dissolved in 2 N HCl, and diluted with a lanthanum chloride ($LaCl_3/xH_2O$) solution and distilled–deionized water to obtain samples in 0.2 N HCl and in La at 2000 $\mu g/mL^{-1}$. Calcium concentration was determined by atomic absorption spectrophotometry (model SpectrAA-20; Varian Associates, Inc., Sunnyvale, Calif.). Tuber tissue calcium was measured by bulking 10 tuber samples, and this was repeated three times per replication. Sampling of three 10-tuber bulk samples from each experimental plot resulted in 60 bulked tuber calcium determinations or the sampling of 600 tubers for each treatment over 3 years.

Statistical analyses

Tuber calcium concentration and bruise incidence means for individual years, treatments, and cultivars were analyzed separately by model I. Treatment group mean values were compared with Fisher LSD (based on SAS GLM procedure, alpha = 0.05).

Model I: $y_{ij} = \mu + trt_i + e_{ij}$

The data were also combined for identical treatments over three seasons (1999, 2000, 2001) and analyzed for individual cultivars (model II) as well as for combined cultivars (model III) using the SAS MIXED procedure.

Model II: $y_{ijk} = \mu + trt_i + yr_j + trt{*}yrt_{ij} + e_{ijk}$

Model III: $y_{ijkl} = \mu + trt_i + yr_j + cult_k + yr{*}cult_{jk} + trt{*}cult_{ik} + trt{*}yr{*}cult_{ijk} + e_{ijkl}$

The last subscript in each model refers to the replication, otherwise

i = 1, 2 treatment, where
1 = ammonium nitrate,
2 = calcium/nitrogen blend CUC 150).

j = 1, 2, 3 year, where
1 = 1999, 2 = 2000, and 3 = 2001.

k = 1, 2, 3, 4, 5 cultivar, where
1 = Atlantic, 2 = Snowden,
3 = Dark Red Norland, 4 = Superior,
5 = Russet Burbank.

Treatment, year and cultivar were all modeled as fixed effects.

To ascertain the critical saturating calcium concentration at which bruise is minimized, data sets were also tested with a nonlinear method (model IV) using the SAS NLIN procedure. The point of convergence of the negative slope line with the plateau line is estimated to be the critical calcium concentration at which maximum reduction in bruising incidence is achieved, and above this level, additional calcium no longer significantly reduces blackspot bruising.

Model IV: Nonlinear segmented model, linear to plateau

for $x < x_0$ $y = a + bx$

for $x > x_0$ $y = c$

where: x_0 = point of convergence of 2 lines.

Table 3. Influence of calcium treatment on tuber tissue calcium concentration and on the incidences of bruise in 5 cultivars over 3 seasons.[z]

Cultivars	Tissue calcium $\mu g \cdot kg^{-1}$ dry weight for tuber grade 170–284 g		Incidence of bruise (%) Tuber grade: 170–284 g		Tuber grade: >284 g	
Year	Control	CUC[x]	Control	CUC	Control	CUC
Atlantic						
1999	119.2 a[y]	142.9 b	32.6 a	17.8 b	39.1 a	21.6 b
2000	131.6 a	160.5 b	45.2 a	34.8 a	65.1 a	48.1 b
2001	144.4 a	178.4 b	44.7 a	22.5 b	45.7 a	36.6 b
Russett Burbank						
1999	—	—	—	—	—	—
2000	173.8 a	223.6 b	30.3 a	21.7 a	55.2 a	40.4 a
2001	176.3 a	246.5 b	20.7 a	12.1 b	—	—
Dark Red Norland						
1999	215.3 a	288.7 b	3.4 a	4.2 a	13.6 a	4.8 a
2000	162.5 a	214.0 b	5.3 a	3.3 a	4.0 a	5.0 a
2001	260.9 a	320.8 b	3.7 a	3.1 a	15.0 a	11.1 a
Snowden						
1999	127.7 a	153.3 b	20.8 a	15.0 a	26.9 a	17.1 a
2000	127.7 a	162.1 b	27.7 a	25.5 a	42.1 a	35.2 a
2001	147.9 a	196.0 b	27.3 a	13.7 b	63.7 a[w]	37.4 a
Superior						
1999	212.0 a	263.3 b	9.1 a	5.8 a	17.3 a	9.9 a
2000	188.5 a	234.3 b	11.5 a	12.1 a	19.4 a	19.1 a
2001	295.1 a	338.4 b	6.1 a	4.8 a	24.1 a	17.7 a

[z]Blackspot bruise incidence is reported as the percent of approximately 100 tubers (170–284 g) evaluated for each of five replications in 1999 and 2000 and 10 replications in 2001. From each replication, three bulk samples of medullary tissue calcium from 10 tubers (170–284 g) were averaged for elemental calcium concentration. Mean separations for control and calcium treatment were made by LSD. Mean values having the same letter among the pair (control and CUC) are not statistically different based on the Fisher LSD in SAS General Linear Model procedure, SAS Institute, Inc., Cary, N.C. ($\alpha = 0.05$).

[y]Any two means within a row not followed by the same letter are significantly different at $P \leq 0.05$.

[x]CUC treatment consisted of calcium nitrate, calcium chloride and urea with a total season application of 168 kg/acre calcium.

[w]Analysis limited to four replications in this year with high variability.

Table 4. Analysis of variance for the effect of supplemental calcium treatment on incidence of tuber blackspot bruising for 5 commercial cultivars ('Atlantic', 'Russet Burbank', 'Dark Red Norland', 'Snowden', and 'Superior') following mechanical harvest in 1999, 2000, and 2001 seasons.[z]

		P value for bruise incidence	
Cultivar	Source	170–284 g	>284 g
'Atlantic'	Treatment	<0.0001[w]	<0.0001[w]
	Year	<0.0001[w]	<0.0001[w]
	Year × treatment	0.0443[y]	0.2117[NS]
'Russet Burbank'	Treatment	0.0045[x]	—
	Year	0.0014[y]	—
	Year × treatment	0.9857[NS]	—
'Dark Red Norland'	Treatment	0.3654[NS]	0.0697[NS]
	Year	0.8408[NS]	0.2569[NS]
	Year × treatment	0.2446[NS]	0.4700[NS]
'Snowden'	Treatment	0.0004[x]	0.1759[NS]
	Year	0.0042[x]	0.0130[y]
	Year × treatment	0.0284[y]	0.8095[NS]
'Superior'	Treatment	0.2081[NS]	0.5992[NS]
	Year	<0.0001[w]	0.0400[y]
	Year × treatment	0.3940[NS]	0.6564[NS]

[z]The data were analyzed using a generalized mixed model (MIXED) procedure of the SAS Statistical Software (SAS Institute, Inc., Cary, N.C.). Year was modeled as a random effect and treatments and cultivars as fixed effects. The effect of year in the analysis of variance is the value obtained using years as a fixed effect to see if years were significantly different in incidence of bruise. Blackspot bruise incidence is reported as the percent of approximately 100 tubers (170–284 g) evaluated for 20 replications (5 in 1999 and 2000 and 10 in 2001).

[NS,y,x,w]Nonsignificant or significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively.

Linear, quadratic, and third-order polynomial relationships were modeled for relationships between calcium concentration, bruise incidence, and internal brown spot using SAS REG procedure. In almost all cases, the best model was determined to be a quadratic fit. In Figure 1, quadratic, natural log, polynomial, and power relationships are used.

Results and Discussion

Response to in-season calcium application among 5 cultivars

All 5 cultivars ('Russet Burbank', 'Atlantic', 'Snowden', 'Superior', and 'Dark Red Norland') responded in all three seasons to the supplemental calcium treatment with significant increases in tuber calcium concentration (Table 3). Mean increase in tuber calcium concentration over 3 years was 21% for 'Atlantic' and 'Superior', 27% for 'Snowden', 30% for 'Dark Red Norland', and 34% for 'Russet Burbank' as compared with control. These results demonstrate that by supplemental calcium application we can dramatically improve the calcium level of the tubers after combined treatment with calcium nitrate, calcium chloride, and urea (CUC). This finding is consistent with previous studies which demonstrated that a modest application of soluble calcium during bulking is effective at increasing tuber calcium concentration in nonperiderm tissue (Kleinhenz et al., 1999; Ozgen et al., 2006).

Potato tubers are underground storage organs with low rates of transpiration. Because water and calcium move together, organs with low transpiration are susceptible to calcium deficiency (Palta, 1996). Our results are consistent with these previous studies and support the idea that tuber calcium concentration can be significantly enhanced by in-season application of water soluble calcium in the soil surrounding the tubers and that calcium must be placed near the tuber to enhance tuber uptake (Kleinhenz et al., 1999; Kratzke and Palta, 1985, 1986).

Seasonal variability in tuber calcium

Despite a consistent response to in-season applications, tuber calcium concentration varied among different seasons (Table 3). In general, tuber calcium concentration was highest in 2001 in all the cultivars (Table 3). Interestingly, variation in tuber calcium concentration among the different seasons was unrelated to soil calcium (Table 1). Overall, the lower tuber calcium concentration in the 1999 and 2000 seasons, across cultivars, could be the result of a higher incidence of leaching rain events in excess of field capacity (19 mm) in these seasons (Table 1). The central sands field used in our studies was typical of this area with low cation exchange and water-holding capacity. Furthermore, heat stress may have contributed to lower calcium uptake. The number of days in which soil temperatures exceeded 26.7 °C was greatest in the 2000 season (Table 1). Under heat stress, leaf transpiration increases further, thus reducing water transport to the tuber. Because calcium moves with water in the xylem, this may help explain lower tuber calcium values for the year 2000. In summary, tuber calcium variation among different seasons, irrespective of cultivar, can be perhaps best explained by variation in rainfall and temperature as opposed to soil calcium.

Tuber calcium variation among cultivars

Calcium concentrations varied among cultivars in all seasons (Table 3). Lowest overall calcium concentrations were noted for 'Atlantic' and 'Snowden' cultivars, intermediate in 'Russet Burbank', and highest in 'Dark Red Norland' and 'Superior'. These results suggest, on a cultivar level, a specific genetic control of calcium uptake and accumulation. Genetic variation among potato species have been reported (Bamberg et al., 1993).

Incidence of bruise among cultivars, tuber size, and treatments

Despite seasonal and cultivar differences in calcium accumulation, several cultivars responded in one or more years to supplemental calcium fertilization by reduced incidence of bruise sustained during mechanical harvest (Table 3). In general, the incidence of bruise was highest in the 2000 season and lower in 1999 and 2001 seasons. Bruise was generally higher in 2000 for 170- to 284-g tubers, but not for the larger ones for which the sample size was smaller (Table 3). Overall, the incidence of bruising was dependent on cultivar with 'Atlantic' having the highest incidence (Table 3). Cultivars ranked from lowest to highest incidence of bruise were 'Dark Red Norland', 'Superior', 'Snowden', and 'Russet Burbank' (generally equal) and 'Atlantic' (Table 3). The most dramatic reductions in bruise in 170- to 284-g tubers after supplemental calcium applications were in 1999 and 2001 for Atlantic with 45% and 50% reductions as compared with control, respectively (Table 3). Snowden also demonstrated large reduction (50%) in bruise in 2001 for 170- to 284-g tubers (Table 3). Means comparisons by LSD for 170- to 284-g tuber grade in individual years demonstrated significant decrease in bruise incidence in 2 of 3 for 'Atlantic', in 1 of 3 years for 'Snowden', and 1 of 2 years for 'Russet

Table 5. Analysis of variance for the effect of supplemental calcium treatment on incidence of tuber blackspot bruising for 5 commercial cultivars (Atlantic, Russet Burbank, Dark Red Norland, Snowden, and Superior) after mechanical harvest in 1999, 2000, and 2001 seasons.[z]

	P value for bruise incidence	
Source	170–284 g	>284 g
Treatment effects	<0.0001[w]	<0.0001[w]
Year × treatment	0.8691[NS]	0.0269[y]
Cultivar	<0.0001[w]	<0.0001[w]
Year × cultivar	0.0382[y]	<0.0001[w]
Treatment × cultivar	0.1760[NS]	<0.0001[w]
Year × treatment × cultivar	0.6337[NS]	0.1147[NS]

[z]Blackspot bruise incidence is reported as the percent of approximately 100 tubers (170–284 g) evaluated for 20 replications (5 in 1999 and 2000 and 10 in 2001). Data were analyzed (analysis of variance) using MIXED procedure (SAS Statistical Software, SAS Institute, Inc., Cary, N.C.).

[NS,y,x,w]Nonsignificant or significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively.

Table 6. Quadratic relationship between incidence of blackspot bruise and tuber sample calcium concentration as well as with internal brown spot.[z]

	Quadratic relationship of bruise incidence with:			
	Internal brown spot		Tissue Ca^{2+}	
Sample	r^2	P > F	r^2	P > F
All years[y]	0.3572	<0.0001[t]	0.4972	<0.0001[t]
1999[x]	0.0827	0.0764[NS]	0.7456	<0.0001[t]
2000	0.2567	0.0003[t]	0.2145	0.0010[u]
2001	0.5720	<0.0001[t]	0.6936	<0.0001[t]
Cultivar[w]				
Atlantic	0.0357	0.1927[NS]	0.1210	0.0348[v]
Burbank	0.7099	<0.0001[t]	0.1342	0.0470[v]
Norland	–0.0288	0.6391[NS]	0.1202	0.0353[v]
Snowden	–0.0343	0.7052[NS]	0.1797	0.0097[u]
Superior	0.1836	0.0098[u]	0.2641	0.0018[u]

[z]Each data point consisted of a single replication analyzed for incidence of mechanical harvest blackspot bruising (100 tubers of 170–284 g size) and correlated with either tuber medullary tissue calcium concentration (average of three separate measurements of 10 bulked tubers) or with the incidence of internal brown spot in the same tubers. Relationships were analyzed using SAS REG procedure (SAS Statistical Software, SAS Institute, Inc., Cary, N.C.).

[y]Relationship analyzed for all samples from all cultivars, years, and treatment.

[x]Relationship analyzed for all samples from all cultivars and treatments from each year.

[w]Relationship analyzed for all treatments and all years for each cultivar.

[NS,v,u,t]Nonsignificant or significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively.

Burbank' (Table 3). Interestingly, 'Dark Red Norland' and 'Superior', which had higher calcium concentrations compared with the remaining cultivars, demonstrated the lowest incidences of bruising, and these cultivars did not respond to supplemental calcium for 170- to 284-g tubers in terms of reduced blackspot bruising. Data analyses showed a significant effect of year for each cultivar except 'Dark Red Norland' with significant interaction of year and treatment in 'Atlantic' and 'Snowden' (Table 4).

Overall blackspot bruising was substantially higher in the larger size grade of tubers as compared with 170- to 284-g size grade. Larger tubers, greater than 284 g, responded to the calcium treatments but results were only statistically significant in 'Atlantic' (Table 3). In general, Table 3 also shows that the incidence of blackspot bruising was higher in larger grade tubers in all years except 2000 for 'Dark Red Norland'. Larger tubers generally have higher rates of bruising as a result of greater weight because more energy is absorbed at the point of impact as compared to smaller tubers (Brook, 1996). Interestingly, only the larger grade tubers of 'Atlantic' responded to supplemental calcium with a reduction in bruise Tables (3 and 4).

Large variation in the bruise incidence among cultivars was expected given the report by Brook (1996). Historically, 'Russet Burbank' has been reported to be susceptible to bruise, whereas 'Atlantic' to be having moderate resistance (Brook, 1996). We found 'Russet Burbank' was moderately susceptible, but 'Atlantic' was highly susceptible. This difference from earlier reported results could be the result of differences in bruise testing method (impact, abrasive peel, and homogenation) (Dean et al., 1993), soil type, and soil water-holding capacity or differences in handling equipment during harvest. In agreement with previous reports (Brook, 1996), we found that 'Superior' and 'Dark Red Norland' are relatively resistant to blackspot bruise (Table 3). Our comparison of tuber bruise susceptibility based on size (Table 3) also confirmed previous reports that larger tubers had higher rates of blackspot bruising (Hughes, 1980). This was especially true for 'Atlantic'. The lack of treatment effect in larger grade tubers in cultivars other than 'Atlantic' could be, in part, the result of smaller number of tubers (approximately 30 per replication) analyzed for the larger grade and relatively lower incidence of bruising. 'Atlantic' may also have had a significant treatment effect for larger tubers because the level of blackspot bruising was generally high in this cultivar for both grades.

Generally, higher rates of tuber bruising observed in our studies, as compared with previous reports (Brook, 1996), can be attributed to the fact that tubers from sandy soil can be more susceptible because of higher dry matter and lower potassium (Ophius et al., 1958). We found 'Dark Red Norland' and 'Superior' to consistently have very low incidences of bruising. A possible explanation may be that these earlier maturing cultivars have lower specific gravity than later maturing cultivars such as 'Atlantic' and 'Russet Burbank', and hence lower bruise susceptibility (Brook, 1996). In our harvester, rows were individually harvested resulting in more contact of tubers with equipment and also tubers were dropped approximately 0.6 m into the crates from the rollers, which could have created a higher than usual bruise rate than experienced in a commercial harvest. Furthermore, the sandy soil in our location separated easily from the tubers leaving them in direct contact with the rollers and harvester mechanism (Brook, 1996). Variation among seasons can be partially explained by soil conditions preceding harvest. A lower bruise incidence in 1999 may in part be because the harvest was preceded by an extensive dry period without rain or supplemental irrigation, which may have led to slightly dehydrated tubers, which are known to be less susceptible to bruising (Brook, 1996). The other years such as 2000 had more wet soil conditions at harvest, which may have led to higher hydration status of tubers and hence a higher propensity for bruising (Brook, 1996).

Table 7. Critical saturating calcium concentration at which bruise reduction no longer responds to supplementary calcium.[z]

Sample	Point of convergence ($\mu g/g^{-1}$ dry weight)	P > F
All years[y] (all cultivars)	251.9	<0.0001[s]
1999[x] (all cultivars)	195.1	<0.0001[s]
2000 (all cultivars)	—[w]	—
2001 (all cultivars)	242.5	<0.0001[s]
Cultivar[v]		
Atlantic (all years)	197.3	0.0710[NS]
Burbank (all years)	—	—
Norland (all years)	136.2	0.6588[NS]
Snowden (all years)	147.2	0.0775[NS]
Superior (all years)	—	—

[z]The point of convergence of the negative slope line with the plateau line is estimated to be the critical calcium concentration above which additional calcium no longer significantly reduces blackspot bruising. Convergence of linear to plateau relationships between tissue concentration of calcium and the incidence of blackspot bruise was determined using nonlinear method SAS NLIN procedure, SAS Statistical Software (SAS Institute, Inc., Cary, N.C.). For each replication, approximately 100 tubers (170–284 g) were analyzed for incidence of mechanical harvest blackspot bruising and from the same tubers medullary tissue calcium concentration was determined from the average of three separate measurements (10 bulked tubers).

[y]Relationship analyzed for all samples from all cultivars, years, and treatment.

[x]Relationship analyzed for all samples from all cultivars and treatments from each year.

[w]Convergence of linear relationships failed to converge.

[v]Relationship analyzed for all treatments and all years for each cultivar.

[NS,v,t,s]Nonsignificant or significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively.

Response of cultivars to calcium for bruise incidence

Data were combined for identical treatments over three seasons in Tables 4 and 5. Combined-year data analyses demonstrated a significant effect of calcium treatment on reduction of blackspot bruise incidence for cultivars 'Atlantic', 'Russet Burbank', and 'Snowden' Tables (4 and 5). These results across multiple seasons parallel the previously presented results in the LSD (Table 3). Table 4 shows that in analysis of individual cultivars, the treatment effect was significant only for the following cultivars with lower calcium levels in tuber tissue: 'Atlantic',

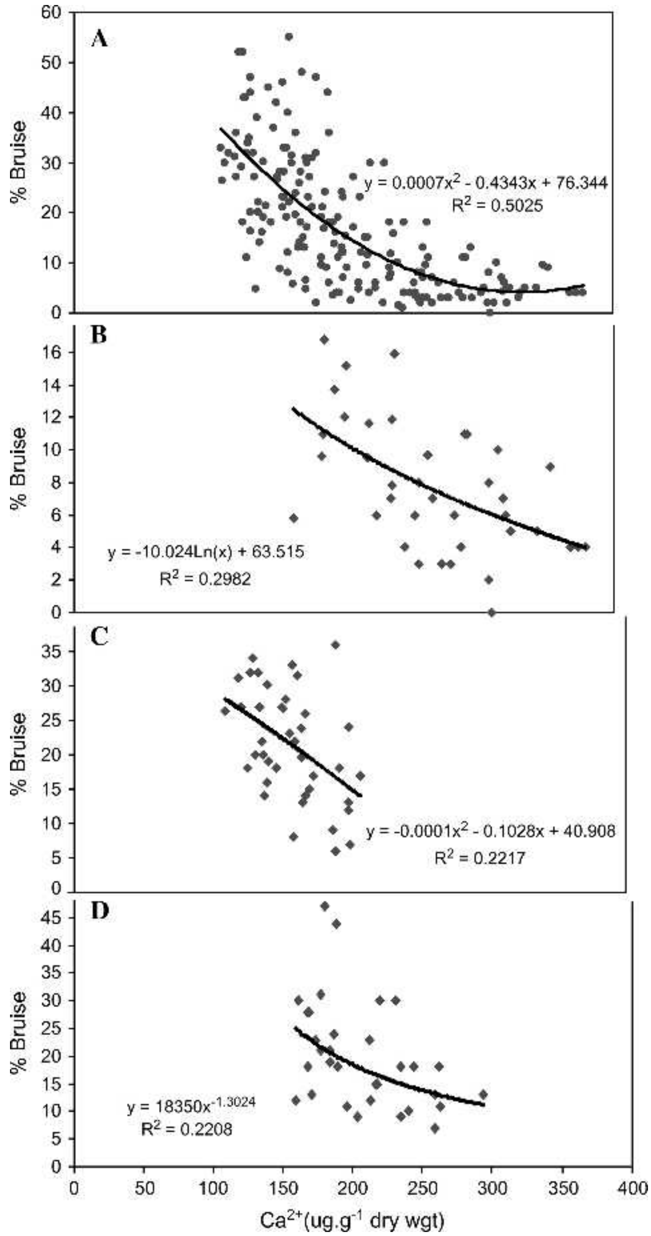

Fig. 1. Relationships between incidence of bruise during mechanical field harvest and calcium concentration of tuber medullary tissue over three seasons for all cultivars combined (**A**) and individual cultivars over 3 years for 'Superior' (**B**), 'Snowden' (**C**), and 'Russet Burbank' (**D**). Figures 1A–D are quadratic, natural log, polynomial, and power relationships, respectively. Each point represents a specific replication (plot) from which 100 tubers (170–284 g) were analyzed for incidence of mechanical harvest blackspot bruising and the average of three separate measurements of tuber medullary tissue calcium concentration for elemental calcium (10 tuber bulked for each sample). The data were analyzed across three seasons 1999, 2000, and 2001 with the SAS REG procedure (SAS Statistical Software, SAS Institute, Inc., Cary, N.C.).

'Russet Burbank', and 'Snowden'. Conversely, there was no significant effect of treatment for the highest calcium level cultivars, 'Superior' and 'Dark Red Norland' (Tables 3 and 4). There were significant treatment effects for both tuber sizes across all cultivars and years (Table 5). For the larger tuber grade, significant treatment effect was seen only for 'Atlantic' (Table 4).

Year*treatment interaction across all 5 cultivars was significant only in >284 g grade (Table 5). There was a significant effect of year in all cultivars except 'Dark Red Norland' (Table 4), the cultivar with highest calcium levels (Table 3). However, the year*treatment interaction was significant only for 'Snowden' and 'Atlantic' (Table 4), the lowest calcium accumulating of the cultivars (Table 3). For larger tuber size, year* treatment interaction was not significant when analyzed individually (Table 4); however, this interaction was significant when analyzed across all cultivars (Table 5). Cultivar and year*cultivar interaction was significant over years for both grades (Table 5). However, the treatment*cultivar effect was significant only in 170 to 284 g grade (Table 5). The year*treatment*cultivar interaction was not significant for either tuber grade (Table 5).

Our results show that the effectiveness of calcium for mitigation of blackspot bruise is tuber calcium concentration-dependent. We found a significant quadratic relationship between tuber calcium concentration and the incidence of blackspot bruise (Table 6). Analyzed for all cultivars and overall 3 years, the quadratic relationship between tuber tissue calcium concentration and blackspot bruise was found to be significant (Table 6 and Fig. 1). Table 6 shows that the relationship between blackspot bruise and tuber tissue calcium concentration, analyzed also for individual years across all cultivars, was found to be significant in all three seasons (Fig. 1A). Additional significant (nonquadratic) relationships between tuber calcium level and the incidence of bruise for 3 individual cultivars (Fig. 1B–D) also add support that raising tuber calcium may lead to a reduction in the incidence of black spot bruising.

We have found large variations for bruising incidence among cultivars. Cultivar 'Atlantic' had the highest incidence followed by 'Russet Burbank' and 'Snowden' (Table 3). 'Superior' and 'Dark Red Norland' consistently had the lowest bruise incidence irrespective of season (Table 3). These variations in the incidences among cultivars generally followed tuber calcium concentration, suggesting a genetic basis for a relationship between tuber calcium and bruise incidence (Fig. 1A). Interpretation of analysis of variance results suggest that treatment effect was most significant in lowering bruise incidence for the lower calcium accumulating cultivars such as 'Atlantic' (Table 4). Furthermore, cultivars 'Dark Red Norland' and 'Superior' that accumulated adequate or approaching adequate tuber tissue calcium concentration were less responsive to supplemental calcium treatment (Table 4). The significant year effect suggests that environmental factors such as soil and air temperatures, irrigation, leaching, and other soil conditions may play significant roles in the uptake levels of calcium into tubers during bulking as well as a role in bruising incidence.

Threshold tuber calcium and incidence of bruise

By statistically measuring the convergence of a linear to plateau relationship, we have determined that across cultivars the concentration at which tubers substantially reduce response to calcium is approximately 250 ppm. Theoretically, the intersection of two linear relationships occurs at the saturating threshold calcium concentration where the calcium concentration plateaus (Fig. 2). These data are not intended to show a cause-and-effect relationship, but instead to demonstrate that variation among five cultivars and three seasons can be explained, in part, by tuber calcium. Analysis of all cultivars overall years yielded a significant convergence (Fig. 2, Table 7) at 252 ppm calcium concentration. Again, this does not mean that when tuber calcium concentration is reached close to 250 ppm, the bruise will be minimal in any cultivar. This analysis suggests that seasons or the cultivars that allow tubers to accumulate calcium close to 250 ppm will tend to have a lower incidence of blackspot bruising. In Table 7, analysis for point of convergence of all cultivars in individual years demonstrated saturation of effect at 195 ppm calcium in 1999 and 242 ppm in 2001. Only 2001 demonstrated a significant quadratic relationship between tuber calcium and incidence of blackspot bruise. Points of convergence for 'Dark Red Norland', 'Snowden', and 'Atlantic' were 136, 147, 197 ppm, respectively, although these results were not statistically significant.

Analysis for point of convergence by combining data from all cultivars in an individual year demonstrated that seasonal variation may affect optimal tuber calcium concentrations resulting in minimized blackspot bruising. It is important to note that no significant point of convergence was generated in the analysis of any individual cultivar. 'Russet Burbank' data were limited to 2 years and may have contributed to the failure of convergence, whereas 'Dark Red Norland' and 'Superior' were less responsive to calcium presumably as a result of their inherent high medullary tissue calcium concentrations.

Possible mechanism by which calcium may reduce blackspot bruise incidence

We do not know the exact mechanism by which tuber tissue calcium may be related to bruise incidence. In addition to strengthening the cell wall and membrane structure (Palta, 1996) tuber tissue calcium may influence the biochemical aspects of the tissue (Mapson et al., 1963). Biochemically, blackspot bruise is the accumulation of melanin following injury of cells resulting in black, brown, or grey pigmentation (vanMiddelem et al., 1953). Calcium may affect the level of tyrosine or phenolase present in the tuber tissue (Mapson et al., 1963). These authors found a decrease in the tyrosine and phenolase in tuber tissue of those given calcium fertilization during the season (Mapson et al., 1963). Tyrosine found free in the cell is correlated to bruise formation (Sabba and Dean, 1994), and the bruise incidence reaches a level of saturation when free tyrosine concentration in fresh tubers reaches 175 to 200 μg/g (Corsini et al., 1992). Another potential component for regulation by calcium is polyphenols, which lead biochemically to melanin formation (Stark et al., 1985). Clark et al. (1957) reported a bruise-susceptible cultivar to have more polyphenols than resistant cultivar, and Stark et al. (1985) reported positive correlation between blackspot bruise susceptibility and total phenolic content. Calcium's role as a secondary messenger controlling the functions of the enzymes and cellular metabolism is well documented (Palta, 1996). Further studies may help shed light on such a possible role of tuber tissue calcium in bruise incidence.

Bruises are usually found either in the perimedullary tissue just beneath the vascular ring or in the cortical area (Brook, 1996). In our study, most blackspot bruising occurred in the cortical area, whereas deeper pressure blackspot bruising would more typically occur in the medullary tissue as a result of pressure exerted in potato storage piles. A possible mechanism of calcium may be to interact with pectate molecules to strengthen the cell wall, but it appears that cell walls need not be ruptured for tissue to blacken. Reeve (1968) found blackened tissue associated with a bruise but no evidence of structural damage to the tissue, and concluded that instead lipoidal membranes were disturbed. In addition to maintaining membrane integrity, a strong relationship between early- versus late-maturing cultivars and blackspot bruise susceptibility suggests a role for cell wall strength or thickness in mitigating bruise susceptibility (Bangerth, 1979).

Supplemental calcium nutrition could also facilitate nitrogen use or potassium uptake, both of which are positively correlated with reduced blackspot susceptibility. Calcium may also affect lenticel and skin structure, which are also important determinants that lead to variation in blackspot bruise susceptibility (Brook, 1996). Tensile properties are found to vary in the skin–cortex combination as well as the in pith and medullary areas (Huff, 1971).

Relationship between incidence of bruise and internal brown spot

To investigate a possible relationship between internal defects involving discoloration of the internal tuber tissue, a regression was run across all years and cultivars between blackspot bruise and internal brown spot defect. A significant quadratic relationship was found between these two measured defects (Fig. 2). Furthermore, an analysis of data demonstrated that quadratic relationships between blackspot bruise incidence and internal brown spot were significant when analyzed across all cultivars and three seasons (Table 6 and Fig. 3). These relationships were also significant across all cultivars

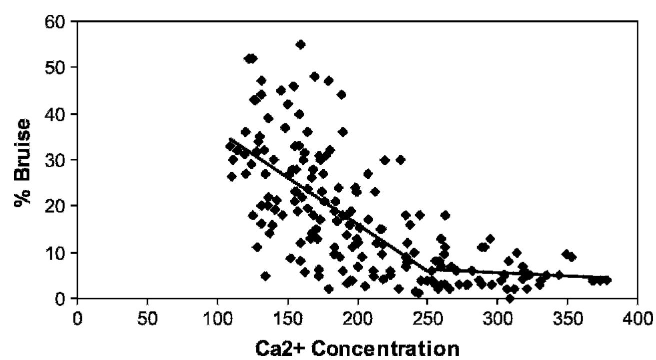

Fig. 2. Linear to plateau analysis of relationship between incidence of blackspot bruise (%) during mechanical field harvest and tuber calcium concentration (Ca2+) for five cultivars and three seasons. Each point represents a specific replication (plot) from which 100 tubers were analyzed for blackspot bruising incidence and the average of three samples for medullary tissue calcium concentration (10 tubers bulked for each sample). The data were analyzed across three seasons 1999, 2000, and 2001 with the SAS REG procedure (SAS Statistical Software, SAS Institute, Inc., Cary, N.C.).

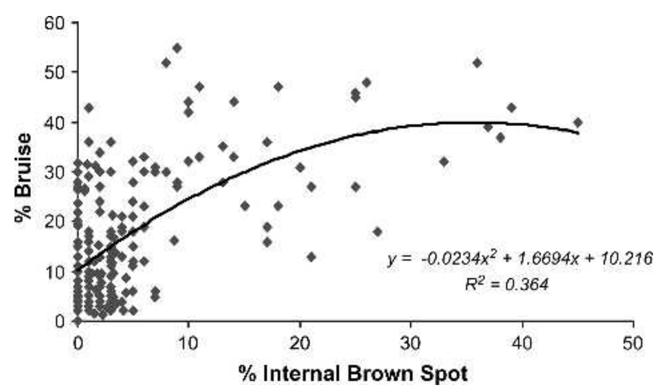

Fig. 3. Quadratic relationship between incidence of bruise during mechanical field harvest and incidence of internal brown spot. Each point represents a specific replication (plot) from which 100 tubers (170–284 g) were analyzed for incidence of mechanical harvest blackspot bruising and the average of three separate measurements of tuber medullary tissue calcium concentration for elemental calcium (10 tuber bulked for each sample). The data were analyzed across three seasons 1999, 2000, and 2001 with the SAS REG procedure (SAS Statistical Software, SAS Institute, Inc., Cary, N.C.).

for the 2000 and 2001 seasons. A quadratic relationship was also found to be significant for 'Russet Burbank' and 'Superior' across all years (Table 6). These are the cultivars for which tuber calcium concentrations were intermediate to high (Table 3).

Localized deficiencies in calcium have been associated with medullary and cortical cell death, or tissue necrosis as sometimes called brown center or internal brown spot (Bangerth, 1979; Collier et al., 1980; Levitt, 1942). Because both the internal brown spot and bruise involve similar discoloration of tuber tissue, we suggest that localized calcium deficiencies may also contribute to heightened bruise potential. In support of this, tyrosine and phenolase (an enzyme whose activity is associated with bruise) were also found to be decreased by calcium fertilization (Mapson et al., 1963). In these experiments by Mapson et al. (1963), potatoes were grown in vermiculite in boxes in a greenhouse and the calcium treatment received extra calcium nitrate to increase the nitrogen to three times that of control. Although this experiment suggests an effect of calcium on tyrosine, it is difficult to conclude the effect of calcium without control and treatment having equal nitrogen.

We have recently reported mineral nutrition studies suggesting that by increasing tuber calcium concentration, one can expect a reduction of internal defects such as internal brown spot and hollow heart with most dramatic reduction in internal defects in 'Atlantic', 'Russet Burbank' (Kleinhenz et al., 1999). The cultivar 'Atlantic' has been found to be susceptible to internal defects including necrotic lesions (such as internal heat necrosis) and internal brown spot (Ehlenfeldt, 1992; Rex and Mazza, 1989; Sterrett et al., 1991; Wannamaker and Collins, 1992). Consistent with our previous studies (Kleinhenz et al., 1999) and our present study (Table 3) with 'Atlantic', Sterrett and Henninger (1991) found significant reduction in internal heat necrosis by calcium application in this cultivar. However, the quadratic relationship between internal brown spot and incidence of bruise was not statistically significant in our study for this cultivar (Table 6). This may, in part, be the result of a very narrow range of tuber calcium concentration in this cultivar. Furthermore, these results may also suggest that factors that lead to enhanced incidences in internal brown spot may not be the same as those that lead to enhanced bruise incidence in this cultivar.

Conclusion

To our knowledge, this study is the first to suggest that calcium applications can mitigate blackspot bruising in commercially significant potato cultivars. This finding was demonstrated not only in individual cultivars in single years, but also across multiple years and across multiple cultivars. Another important finding of our study is that cultivars vary significantly in response to bruise mitigation by calcium. Cultivars with the lowest inherent calcium tuber tissue calcium concentrations responded strongest to supplemental in-season calcium applications. Specifically, cultivars 'Dark Red Norland' and 'Superior' demonstrated the lowest propensity for bruise, 'Atlantic' had large bruise potential, and 'Snowden' and 'Russet Burbank' intermediate in response. Conversely, the lowest overall calcium concentrations were noted for 'Atlantic' and 'Snowden' cultivars, intermediate in 'Russet Burbank', and highest in 'Dark Red Norland' and 'Superior'. These variations in the incidences among cultivars generally followed tuber calcium concentration, suggesting a genetic basis for a relationship between tuber calcium and bruise incidence (Fig. 1A). Furthermore, the cultivars ranked similarly for tuber calcium concentration in all years. These results suggest, on a cultivar level, a specific genetic control of calcium uptake and accumulation. Seasonal variations in tuber calcium concentration suggest environmental control for tuber calcium uptake and accumulation.

The threshold calcium concentration above which tuber blackspot bruising response abates was analyzed by a linear to plateau relationship. Theoretically, by statistically measuring the convergence of linear to plateau relationship, we have determined that across cultivars, for all seasons, the bruise incidence is dramatically reduced when the tuber calcium concentration approaches approximately 250 ppm; however, conclusive points of convergence for individual cultivars were not attainable as a result of data variability.

The results of our study suggest that in-season application of water-soluble calcium during the bulking period significantly raises tuber tissue calcium concentrations and may lead to a concomitant reduction in tuber blackspot bruising. We do not know the exact mechanism by which calcium may reduce the incidence of tuber bruising. The role of calcium in improved cell membrane health and wall structure of the potato tuber has been suggested (Palta, 1996). Tuber tissue being a low transpiring organ is generally deficient in calcium (Bangerth, 1979; Kratzke and Palta, 1985). Thus, increasing tuber tissue calcium concentration may improve tissue health that in turn may help mitigate bruise injury or enhance the ability of the tuber tissue to recover from this injury. Further research may help elucidate the mechanisms by which calcium may play a role in tuber tissue health.

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