Raising seed tuber calcium may impact its quality and performance

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Importance of Calcium in Plants

Calcium plays a critical role in plant growth, development and the maintenance/modulation of many cell functions (Poovaiah and Reddy, 1993). This becomes evident when considering the importance of calcium in membrane structure and function as well as the structure of the cell wall. Calcium in the extracellular solution is necessary for the maintenance of selective permeability, i.e. membrane integrity. Calcium also provides stable, but reversible, intermolecular linkages between pectic molecules, resulting in cell wall rigidity. The presence of extracellular calcium increases the bond between the cell wall and the outer face of the cell/plasma membrane (Palta, 1996). Furthermore, the role of calcium as a secondary messenger in plant response to many environmental and hormonal signals [touch, wind, gravity, light, cold, heat, auxin, gibberellic acid, abscisic acid, and fungal elicitors] has been documented (Poovaiah and Reddy, 1993), and the impact of these environmental (drought, cold, heat) and biotic stresses on plants can be mediated by changes in cytosolic calcium (Palta, 1996). There is some evidence that calcium application can mitigate heat (Tawfik et. al., 1996) and frost stress on potatoes (Vega et. al., 196).

Importance of Calcium to Potato

Calcium has also been linked to several key issues of quality in potatoes (*Solanum tuberosum* L.). It has been shown that storage rot, due to *Erwinia carotovora* pv. *atroseptica*, decreases as tissue calcium concentration increases (McGuire and Kelman, 1984, 1986). In addition, the incidence of internal disorders such as hollow heart, internal brown spot, and brown center decreased when the calcium concentrations in the tuber tissue were increased (Collier et. al., 1978 and Tzeng et. al., 1986). Thus, increasing tuber tissue calcium can significantly improve tuber quality and storability.

Storage organs, such as potato tubers, are naturally deficient in calcium. Since calcium moves with the water in the xylem, transpiration is the primary pathway for calcium transportation (Clarkson, 1984). Potato tubers, which are surrounded by moist soil, will have less transpiration as compared to above-ground parts of the plant. Consequently, low-transpiring organs, such as fruits and tubers, accumulate much less calcium per unit of fresh weight than leaves.

Potato tubers accumulate calcium directly from the surrounding soil via functional roots on the tuber and stolon (Kratzke and Palta, 1985). On a practical level, these results indicate that the placement of calcium is important for enhancing calcium uptake by the tuber (Kratzke and Palta, 1986). Maximum increases in tuber calcium occur when calcium is mixed into the hill where the tubers are developing (Simmons and Kelling, 1987 and Simmons et. al. 1988). It is, therefore, of critical importance to realize that if improvements in tuber quality are to be made by increasing tuber calcium, the supplemental calcium application must be made during the tuber production cycle. In our related studies

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we have shown that the tuber calcium level can be enhanced by the application of calcium during the bulking period.

Elevating the level of tuber tissue calcium during the seed production cycle may have an impact on the quality of the plant the seed piece produces. The objective of the studies reported here was to enhance seed tuber calcium during the seed tuber production cycle and study the impact of this calcium enhancement on performance the following year.

Experimental Methods

These studies were conducted over four seed production cycles and involved the following cultivars: Superior, Atlantic, Dark Red Norland, Snowden, and Russet Burbank. Calcium enhancement of seed tubers took place at a commercial seed production farm in northern Wisconsin, and the soil types were primarily silt loam (Superior, Atlantic, Dark Red Norland, Snowden) and Plainfield sand (Russet Burbank). The calcium content of these soils ranged from 1,060 lbs. Ca/acre to 2,680 lbs. Ca/acre (See Table 1). Nutrient applications were made three times during the tuber bulking period: hilling, hilling +3 weeks, and hilling + 6 weeks. The nutrients were dissolved in one gallon of irrigation water contained in a watering can and then hand applied to each 10 foot row. The total calcium delivered was 150 lbs. Ca/acre (50 lbs. Ca/application) from liquid calcium nitrate (9-0-0-11, Hydro Agri of North America) with nitrogen balanced across treatments using ammonium nitrate (34-0-0) delivered at the same application times (50 lbs. nitrogen/application). The total nitrogen for all plots was 225 lbs./acre, regardless of source.

<u>Tuber calcium analysis:</u> The seed tubers were harvested at maturity and stored in cold conditions until planting the following spring. Prior to planting, the seed tubers were washed with deionized water to remove any remaining soil, and a longitudinal slice, approximately 0.25 inch, was taken from the middle of each tuber. Care was taken to ensure that the apical bud region and stolon attachment were removed as well as the skin and cortical tissue ring. Trimmed slices from each individual tuber were diced, dried in a forced air oven, ground to pass a 40 mesh screen, and ashed in a muffle furnace. Individual tuber samples were prepared for atomic absorption measurement by digesting the ash with 2N HCl and then brought to a standard volume with lanthanum chloride solution. The calcium content of each tuber sample was read using an atomic absorption flame spectrophotometer (Varian model Spectra AA20).

<u>Seed Piece Evaluation:</u> Seed pieces were evaluated at the Hancock Agriculture Experiment Station (Plainfield Sand, soil calcium ranging from 720-860 lbs./acre) for their performance. At planting each seed tuber to be evaluated was sliced with a potato chip slicer in the field and individual tubers were planted at two foot intervals to minimize intrarow competition between the plants. This also facilitated total tuber yield measurements from each individual seed piece. (Individual seed tubers were tracked at all times with a code number and tag, which was buried with each seed piece.) Tubers from each treatment (calcium nitrate or ammonium nitrate) during the seed production year were handled identically in the evaluation year and received exactly the same standard nutrient regime (i.e. no supplemental calcium was given during the evaluation year). Total yield evaluations were made on each plant by hand harvesting and weighing all of the tubers produced.

In 1998 our evaluation protocol was revised to include two samples of tuber calcium's effects on stem number and canopy size during the season. These two evaluations occurred at 31 and 70 days after planting. Data presented in this report includes data from the 1995, 1996, 1997 and 1998 growing seasons.

Results

We analyzed over 3,000 individual tubers during the past four seasons, and as a result of this analysis we conclude that:

It is possible to increase the calcium concentration of the seed tuber tissue, even in soils that test high in native calcium, with the supplemental application of calcium

Results of the seed tuber calcium concentration are given in Table 1. The mean tuber calcium varied from year to year for the same cultivar, indicating seasonal effects on the calcium accumulation in the tubers. In the three years of the study 11 out of the 12 trials showed a significant increase in tuber calcium concentration with the application of calcium nitrate (based on L.S.D. with α=0.05). In some cases this increase in calcium concentration was over 45% as compared to the ammonium nitrate treatment (Table 1, Russet Burbank). It is also important to note that this increase in calcium concentration was found even in soils that tested 1340 ppm of available calcium (Table 1, Superior 1995).

Supplemental calcium application results in a higher proportion of tubers with increased tissue calcium

In addition to the increase in the mean tuber calcium we found a dramatic shift in the proportion of tubers with a higher amount of calcium (Figure 1). For example, in the cultivar Atlantic, the percentage of tubers containing over 130 ppm calcium increased from 20% to 50% with supplemental calcium nitrate application (Figure 1). Similarly, in the cultivar Superior, the percentage of tubers containing over 190 ppm calcium increased from 40 to nearly 70%. Such results were also found for the Dark Red Norland, Snowden, and Russet Burbank cultivars.

Some cultivars consistently accumulate higher calcium than others

There are variations in the accumulation of calcium among cultivars and during different seasons. (Table 1). However similar differences are found between cultivars from season to season. For example, Superior and Snowden consistently had higher calcium than Atlantic and Dark Red Norland (Table 1). Thus, for different cultivars, there appears to be a different threshold of calcium uptake by the tubers. It is this level that may be critical for the improvement of tuber seed piece quality. These results also suggest that there is genetic variability for tuber calcium among various cultivars. Which means that there may be a possibility to improve tuber calcium and calcium related quality characteristics via a directed plant breeding program focused on maximizing tuber tissue calcium concentrations.

Cultivar response to soft rot

In 1995, 1996, and 1997 the incidence of decay due to soft rotting *Erwinia* bacteria was also examined under field conditions. It was observed that over the three years of study there was some consistency in the ranking of cultivars with regard to decay incidence (Figure 4). For example, Atlantic consistently showed the lowest incidence of decay under our experimental field conditions. Dark Red Norland responded with the highest incidence of decay consistently. This was true in all years where overall the disease pressure was low (for example 1995) and in years where the disease pressure was higher (1997) for our experimental protocol. These results suggests there may be a genetic component involved in this response and these genotypic differences could be exploited to improve cultivated potatoes.

Enhanced calcium may improve seed tuber performance

Tuber Yield: Seed tubers with enhanced calcium concentration tended to produce plants with higher tuber yield, although the results were not generally statistically significant (Table 2). In 7 of the 17 tests, we found yield increases greater than 5%, from tubers raised with supplemental calcium nitrate. Only in three cases was this yield increase statistically significant (based on the General Linear Models Procedure of LSMeans separation $p<0.20$). Interestingly tuber calcium and tuber yield was highest in 1996 for all the cultivars (Table 2). We are continuing this research to evaluate the potential of improved performance of seed tubers through an by increase in tissue calcium levels.

Stem Number: Total stems per plant was evaluated for each cultivar in 1996, 1997 and 1998 (not in 1995). We observed that in some cultivar/year combinations there was a decrease in stem number in seed pieces treated with calcium nitrate the previous season(Table 3). In Atlantic this trend was the same in both years, with 1997 showing a 6% decrease in stem number. Superior and Snowden also showed a decrease in one of the two years, which in the case of Snowden in 1996 was 10% fewer stems. In Dark Red Norland, the decrease in stem number was true for only one of the two years (13% reduction in 1996). In 1997 there was an increase in stems of ammonium nitrate treated seed tubers as compared to calcium nitrate treated seed tubers for Dark Red Norland. Additionally there was an increase in stems for Russet Burbank in 1997, the first year of study for the this cultivar.

These observations during 1996 and 1997 led us to further examine the relationship between calcium treatment during seed production and its effect on stem number the following season. In 1998 we made two observations of stem number at 31 and 70 days after planting. Total stems were counted at each of those evaluation times. Figure 2 shows the change in stem number that occurred from 31 to 70 days after planting. We observed a greater increase in stem number in seed tubers raised with ammonium nitrate as compared to seed raised with calcium nitrate. This was true for all cultivars except Burbank. The differences between the two treatments were much more pronounced for the Atlantic, Dark Red Norland and Snowden cultivars. The idea behind determining the change in stem number between 31 and 70 days after planting was to determine whether the plant is putting more biomass into the existing stems or into new stem production. These results indicate that less below ground branching occurred between the two evaluation times in seed pieces treated with calcium nitrate the previous season. These results also support the idea that perhaps calcium treated seed tubers were able to maintain a more vigorous mainstem with less below ground side branching.

In the 1998 season we also determined the increase in primary (mainstem) and secondary (other than mainstem) foliage growth between 31 and 70 days after planting (Figure 3). In general the secondary foliage growth was greater in seed pieces raised with ammonium nitrate as compared to calcium nitrate. These results also suggest that there is a trend towards decreasing stem numbers in seed tubers that were raised with calcium nitrate during the seed tuber production cycle.

It is not possible to precisely conclude the relationship between the seed tuber calcium level and the stem number because of the large variability for this parameter among different seed pieces within each treatment (i.e. ammonium nitrate as well as calcium nitrate). Clearly a number of factors contribute to the performance of tuber seed pieces. In addition to calcium level these factors include seed tuber size, age, number of buds per seed piece and storage conditions. In our studies storage conditions were uniform for tubers used, however there was some variation in seed piece size as well as in the number of buds per seed piece. Furthermore under field conditions variation in soil conditions (micro environment) around each seed piece can also create variability among seed pieces within a given treatment. An example of such variability is illustrated in Figure 5a,b. Each data point represents a different seed tuber. All the seed tubers from a given year were raised in the same field under the same cultural practices. Large variation in calcium level among seed tubers is expected. This is due to the fact that calcium is derived by the tuber from the surrounding soil (Kratzke and Palta, 1985 and 1986; Palta, 1996). Since the soil calcium varies from one location to the other in a given hill so to does the tuber calcium level. By previously tracking each seed tuber for calcium level and performance (e.g. tuber yield) we are able to generate unique data such as shown in Figure 5a. However these evaluations do not allow us to determine a threshold of seed tuber calcium required for optimum performance. For example, we found a trend for this relationship in 1996 (Figure 5a), but not in 1997 (Figure 5b). The large variability in tuber yield at any given seed tuber calcium level indicates other factors (seed size, number of buds per seed piece) need to be controlled in our evaluation. Furthermore soil conditions need to be uniform for such an evaluation.

We are hoping to pursue these studies in controlled conditions to: (a) precisely determine the relationship between seed tuber calcium and the seed piece performance; (b) determine a threshold calcium level for seed tuber to optimize its performance. Our results show this should be done for each cultivar since there are inherant variations in seed tuber calcium accumulation among different cultivars.

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Table 1. Mean calcium content of tuber medullary tissue and soil test calcium levels by cultivar and year. Due to experimental design and seasonal variability intercultivar and interyear comparisons are not valid.

Cultivar	Year	Mean Calcium Content[1] Ammonium Nitrate	Mean Calcium Content[1] Calcium Nitrate	LSD[2] (α=0.05)	Soil Test[3] (ppm Ca)
Superior	1995	168.5b	202.7a	13.8	1340
	1996	297.7b	309.4a	9.1	1070
	1997	172.9b	201.5a	21.4	970
Atlantic	1995	101.7b	122.3a	10.4	1070
	1996	220.5b	246.3a	8.3	820
	1997	110.4a	116.3a	11.8	910
Dark Red Norland	1995	128.2b	156.3a	16.6	770
	1996	187.3b	204.9a	6.7	870
	1997	98.2b	130.1a	15.2	530
Snowden	1996	229.8b	252.4a	7.2	820
	1997	177.5b	203.8a	19.5	580
Russet Burbank	1997	98.7b	142.8a	16.8	610

[1] ug Ca/g dry wt. (ppm) of medullary and pith tissue internal to cortical ring.
[2] Means within the same row, having the same letter are not significantly different.
[3] Ammoium acetate extractable soil Ca in parts per million (ppm).

Table 2. Total tuber yield by cultivar and year. Due to experimental design and seasonal variability intercultivar and interyear comparisons are not valid. LSMeans within the same row having the same letter are not significantly different (based on SAS General Linear Model procedure p<0.2000)

Cultivar	Year	Total Yield[1] Ammonium Nitrate Mean ±	SEM	Calcium Nitrate Mean ±	SEM
Superior	1995	1.50b	0.13	1.85a	0.11
	1996	2.37a	0.06	2.45a	0.06
	1997	1.34a	0.06	1.38a	0.06
	1998	2.13a	0.07	2.11a	0.07
Atlantic	1995	1.84a	0.13	1.77a	0.12
	1996	2.96b	0.09	3.15a	0.10
	1997	1.78a	0.07	1.80a	0.07
	1998	2.36	0.05	2.26a	0.05
Dark Red Norland	1995	1.37b	0.09	1.56a	0.09
	1996	2.65a	0.07	2.68a	0.08
	1997	1.36a	0.06	1.39a	0.06
	1998	2.15a	0.10	2.27a	0.10
Snowden	1996	2.89a	0.09	3.04a	0.09
	1997	1.47a	0.05	1.45a	0.05
	1998	2.00a	0.07	2.02a	0.07
Russet Burbank	1997	1.57a	0.06	1.66a	0.06
	1998	2.51a	0.12	2.63a	0.12

[1] kg/plant total tuber yield

Table 3. Stem number by cultivar and year. Due to experimental design and seasonal variability intercultivar and interyear comparisons are not valid. LSMeans within the same row having the same letter are not significantly different (based on SAS General Linear Model procedure p<0.2000)

Cultivar	Year	Stem Number[1] Ammonium Nitrate Mean ±	SEM	Calcium Nitrate Mean ±	SEM
Superior	1996	2.96a	0.10	2.95a	0.10
	1997	4.12a	0.17	3.99a	0.17
Atlantic	1996	2.61a	0.16	2.57a	0.16
	1997	3.39a	0.16	3.18a	0.16
Dark Red Norland	1996	3.15a	0.15	2.72b	0.15
	1997	3.45b	0.15	3.95a	0.15
Snowden	1996	3.56a	0.18	3.17b	0.18
	1997	4.24a	0.17	4.23a	0.17
Russet Burbank	1997	6.05b	0.28	6.60a	0.29

[1] Total stems per plant

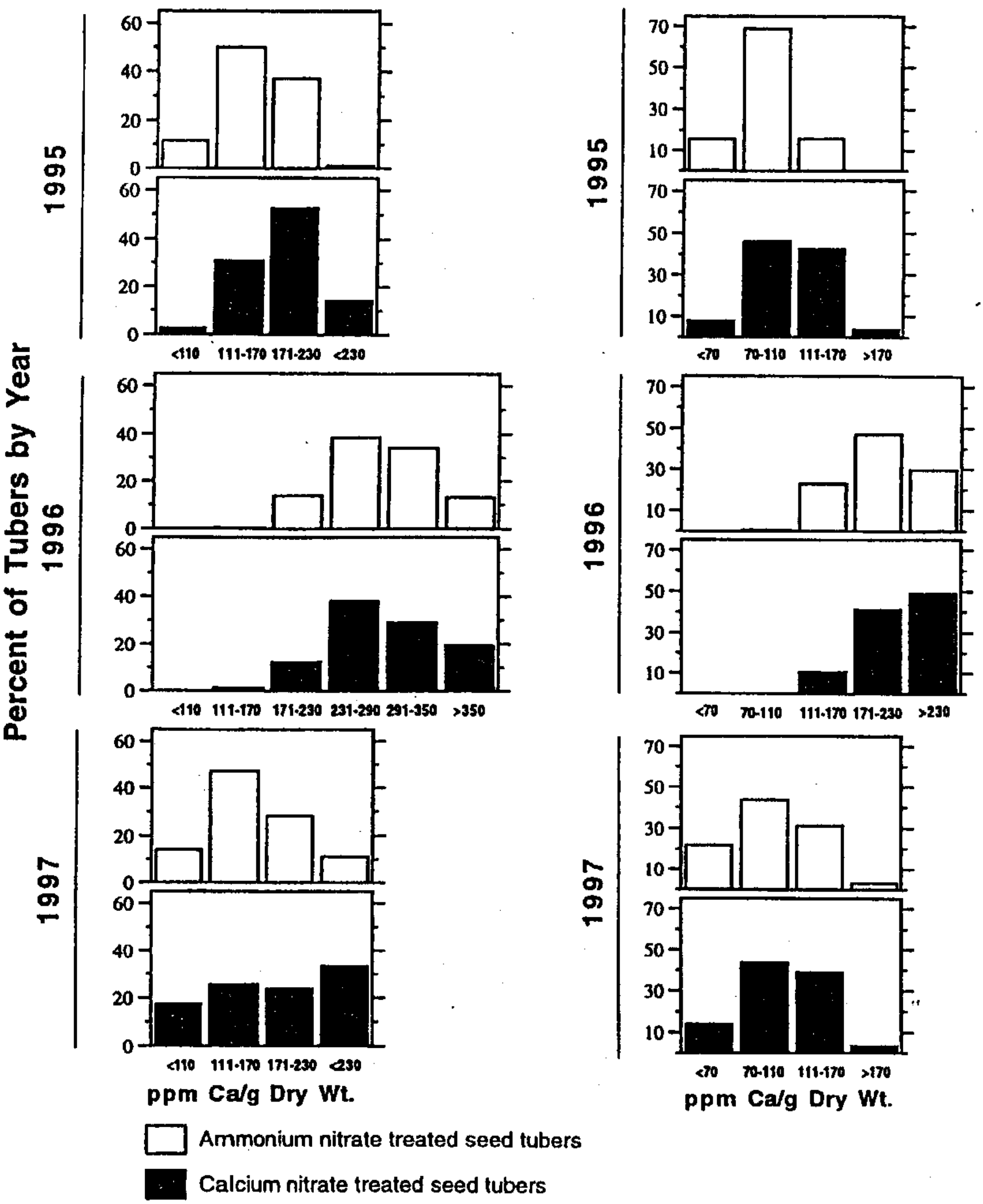

Figure 1a: Impact of supplemental calcium nitrate application on tuber calcium concentration of five potato cultivars (Superior, Atlantic, Dark Red Norland, Snowden, and Russet Burbank). Each tuber was sampled and analyzed for tuber calcium concentration individually (tissue internal to the cortical ring). Data are on the proportion (percentage) of tubers within various ranges of calcium concentration with ammonium nitrate and calcium nitrate applications. All values are expressed as ppm Ca/g dry weight.

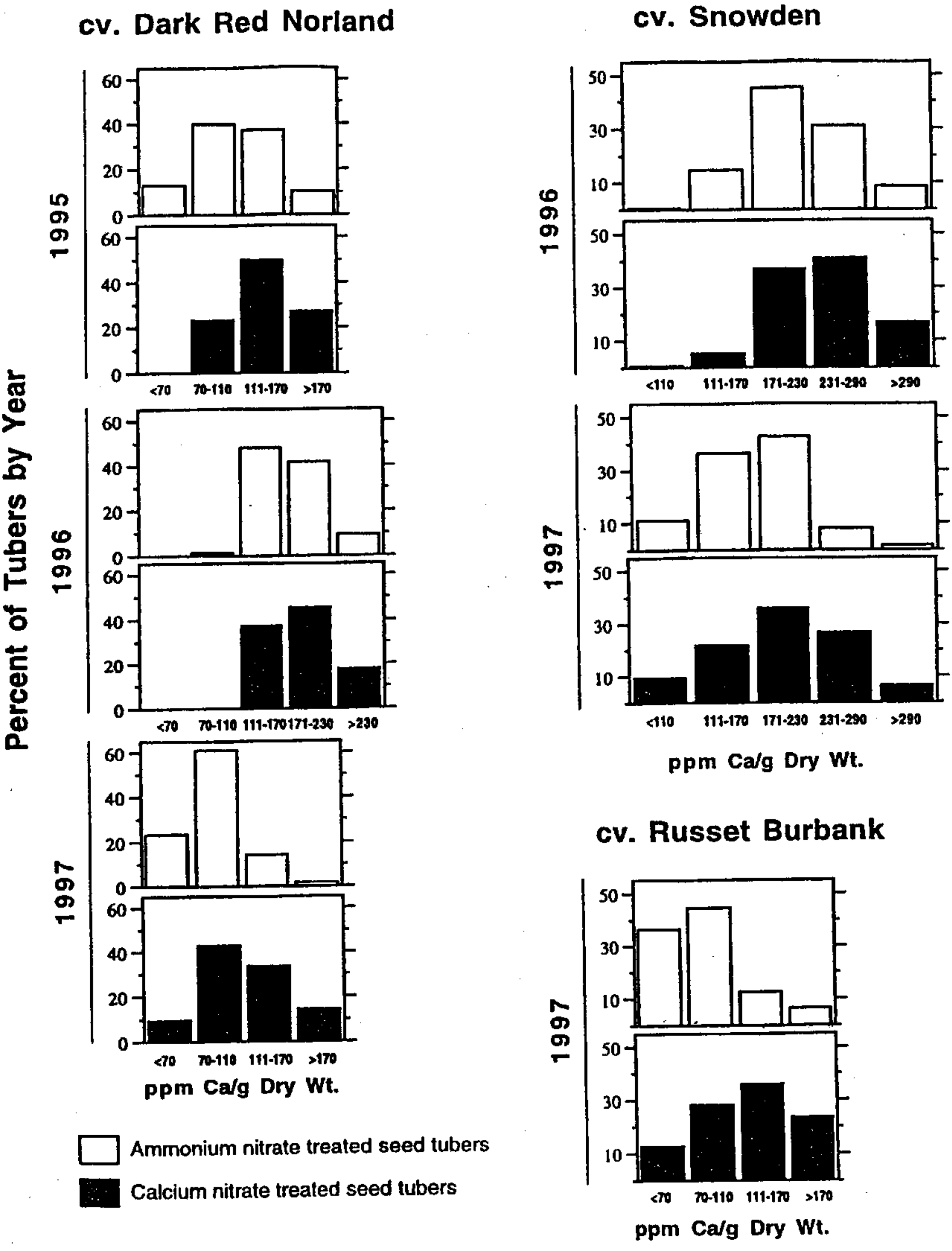

Figure 1b: Impact of supplemental calcium nitrate application on tuber calcium concentration of five potato cultivars (Superior, Atlantic, Dark Red Norland, Snowden, and Russet Burbank). Each tuber was sampled and analyzed for tuber calcium concentration individually (tissue internal to the cortical ring). Data are on the proportion (percentage) of tubers within various ranges of calcium concentration with ammonium nitrate and calcium nitrate applications. All values are expressed as ppm Ca/g dry weight.

Figure 2: Increase in stem number from 31 to 70 days after planting. Tubers were grown under standard cultural practices and were evaluated by hand. Stem numbers were counted as individual stems that emerge from the hill from a given seed piece. All values are expressed as stem number per plant.

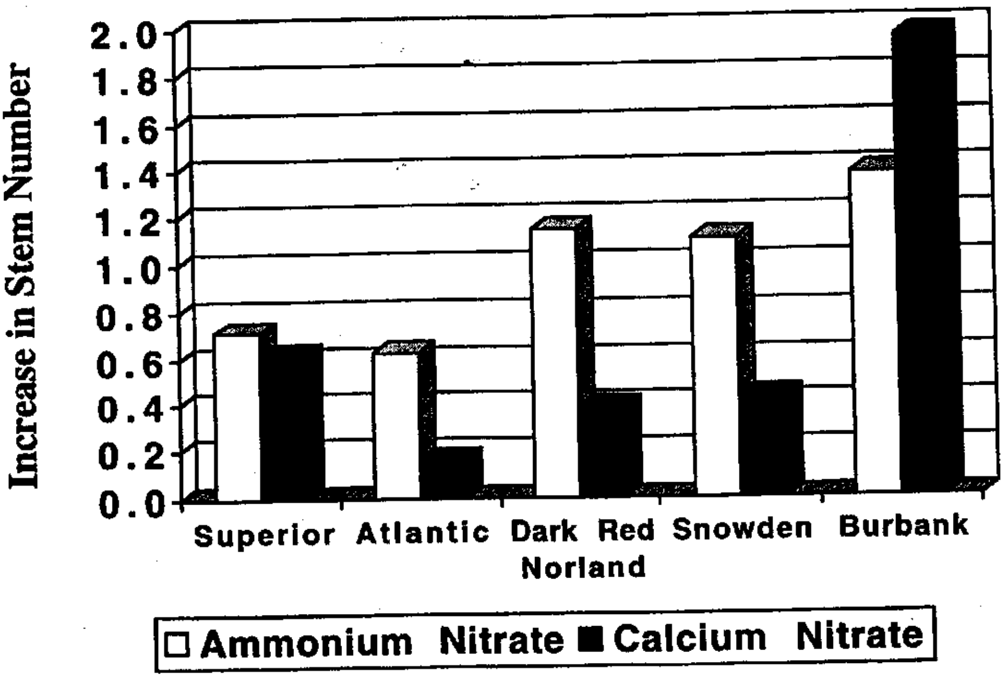

Figure 3: Increase in secondary foliage from 31 to 70 days after planting. Tubers were grown under standard cultural practices and were evaluated by hand. secondary foliage refers to any canopy weight (stems and leaves) which was not part of the most vigorous main stem. All values are expressed as grams per plant.

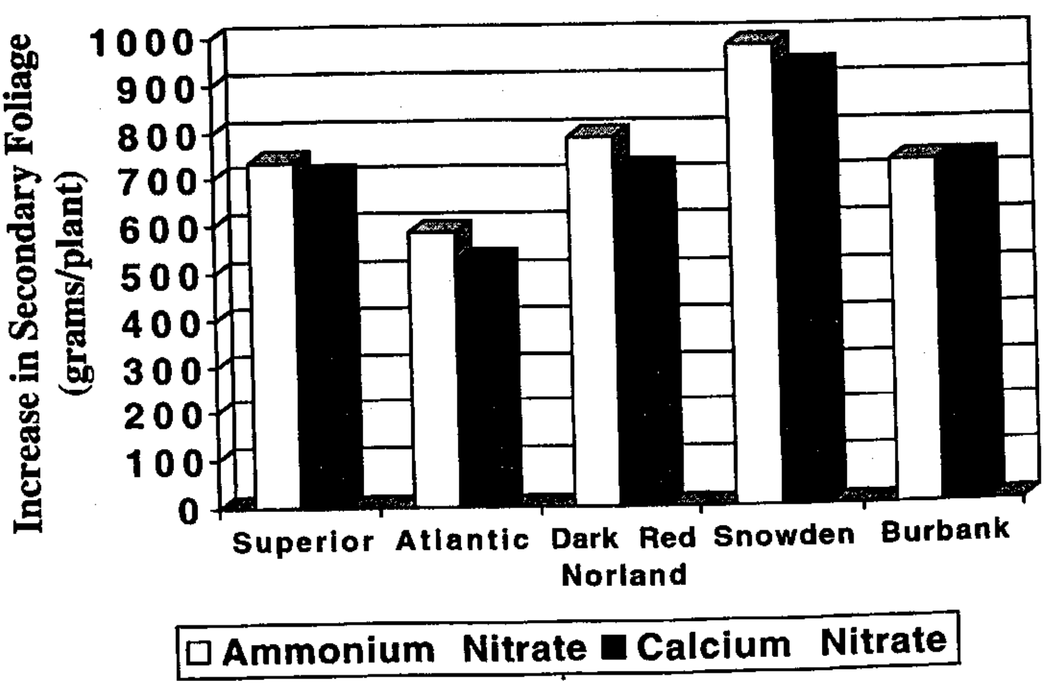

Figure 4: Incidence of decay for all cultivars in 1995, 1996 and 1997. Individual seed pieces were visually rated on a 5 point scale (1 0-10% decay, to 5 90-100% decay). Incidence refers to any seed piece having greater than 10% decay.

Incidence of Decay

Year	Rank								
1995	Atlantic (13%)	<	Superior (26%)	<	Dark Red Norland (50%)				
1996	Atlantic (19%)	<	Snowden (39%)	<	Superior (60%)	<	Dark Red Norland (94%)		
1997	Atlantic (37%)	<	Superior (69%)	<	Russet Burbank (84%)	<	Snowden (95%)	<	Dark Red Norland (99%)

Figure 5a: Superior, 1996, calcium nitrate treated. Performance plot of total tuber yield (Kg tubers/plant) per plant versus tuber calcium conten (ppm Ca/g dry weight). Individual seed pieces were planted on two foot centers to minimize interrow competition. All plants were hand harvested for total yield. Calcium analysis was performed as previously described.

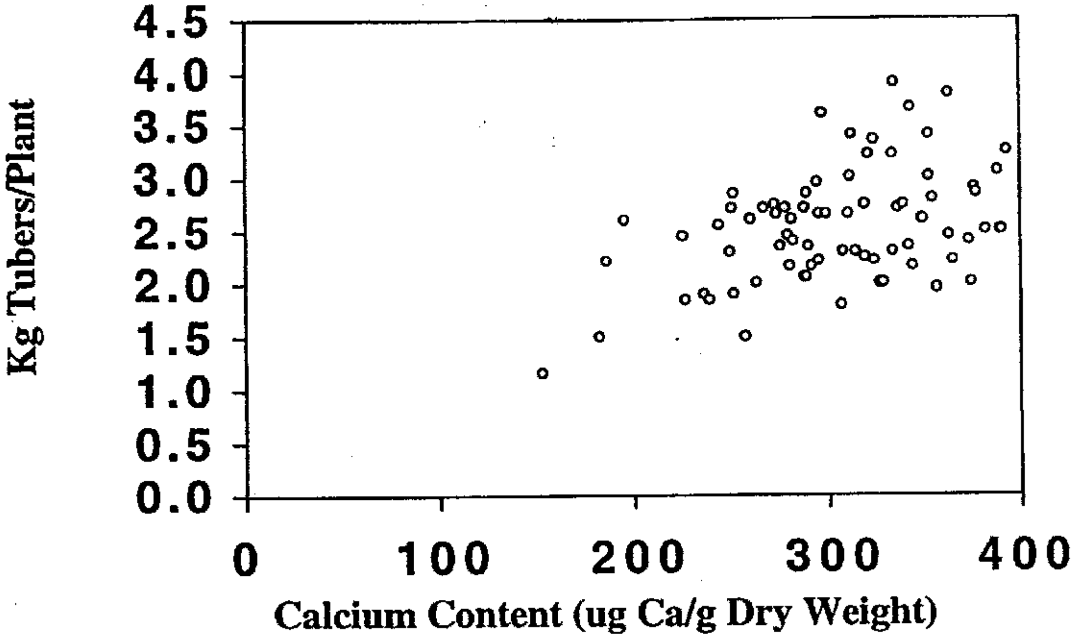

Figure 5a: Superior, 1996, calcium nitrate treated. Performance plot of total tuber yield (Kg tubers/plant) per plant versus tuber calcium conten (ppm Ca/g dry weight). Individual seed pieces were planted on two foot centers to minimize interrow competition. All plants were hand harvested for total yield. Calcium analysis was performed as previously described.