Preharvest Calcium Applications Have Little Effect on Mineral Distribution in Ripe Strawberry Fruit

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Abstract. Supplemental Ca was supplied to 'Cardinal' and 'Fern' strawberry (*Fragaria* × *ananassa* Duch.) plants grown in an Enders clayey silt loam soil as a foliar spray of Ca glutarate, as soil incorporated gypsum, as fertigated calcium nitrate (CaNO_3), or as a combination of the above. Controls received no Ca. Individual fruits were partitioned into six parts: proximal, distal, inner and outer receptacle, and proximal and distal achenes. Mineral nutrient concentrations (dry mass basis) found in the inner and outer receptacle, and in achenes were, in descending order, K, P, Ca, Mg, Al, Na, Fe, Mn, Zn, B, and Cu; K, P, Ca, Mg, Na, Mn, Fe, Zn, Al, B, and Cu; and Ca, P, Mg, K, Na, Fe, Mn, Zn, Al, Cu, and B, respectively. Many nutrients, including Ca, tended to occur in greater concentrations in the proximal portion of the fruit than in the distal part. With the exception of Al, nutrient concentration gradients were lowest in the inner receptacle. Fruit Ca concentrations were highest in achenes and lowest in inner receptacle tissue. Differences among cultivars in Ca concentration were found in achenes but not in receptacle tissue. Calcium treatment had no effect on receptacle tissue Ca concentrations, regardless of cultivar, but CaNO_3 and combination treatments increased Ca concentrations in the achenes in the proximal half of 'Cardinal' fruit. Concentrations of all other nutrients except Mn were unaffected by supplemental Ca treatments.

Strawberry fruit are highly susceptible to bruising and postharvest decay. During fruit ripening, Ca participates in maintaining the integrity of the middle lamella (Clarkson and Hanson, 1980). However, cytoplasmic Ca also participates in a broad range of plant functions that could influence fruit decay, including regulation of calmodulin, Ca-dependent ATPases, protein kinases, and several glucanohydrolases (Gilroy, 1989; Poovaiah and Reddy, 1987). Activity of some of these enzymes, such as β 1,4-glucan synthase, can be increased 30-fold by increasing tissue Ca from 0 to 10^{-6} M, and have been implicated in host-plant disease resistance. Cell membrane leakage and tissue senescence are reduced or delayed by adequate or elevated tissue Ca levels, and Ca ions can inhibit the action of intra- and extracellular polygalacturonase. This latter attribute has been manipulated by postharvest physiologists to delay fruit softening and decay.

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In an attempt to increase strawberry fruit Ca concentrations, several preharvest treatments have been evaluated. Foliar application of CaNO_3 did not improve firmness in 'Sunrise' and 'Pocahontas' fruit, but did reduce the drip loss in thawed 'Pocahontas' processed fruit (Crivelli et al., 1973). Calcium glutarate sprays or soil applied gypsum, CaNO_3 , or a combination of soil and foliar Ca fertilizers reduced fruit decay, but did not firm fruit of 'Cardinal' and 'Fern' (Makus and Morris, 1989). Preharvest sprays of CaCl_2 have had variable effects on postharvest storage (Cheour et al., 1990; Eaves and Leefe, 1962), and strawberry fruit and plants can be deleteriously affected by CaCl_2 application (Cheour et al., 1990; Eaves and Leefe, 1962). In the latter studies, the role of Cl^- may have been overlooked, since Cl^- can suppress fungal colonization at concentrations even below 1% (Elmer, 1992).

In strawberry plants, ^{45}Ca uptake occurred essentially through the root system (Norton and Wittwer, 1963). Factors such as increased (more negative) soil water potential, increased xylem water potential at night, nitrogen form (NO_3^- generally preferred to NH_4^+), or high soil K concentration reduced Ca uptake into strawberry plants (Bradfield and Guttridge, 1981; Chiu and Bould, 1976; Ganmore-Neumann and Kafkafi, 1985; Guttridge et al., 1981). Although NO_3^- increased root Ca as root-zone temperature increased from 10 to 32 °C, equimolar NO_3^- : NH_4^+ -N resulted in the best

plant growth (dry mass basis) and leaf area over a wide range of root-zone temperatures (Ganmore-Neumann and Kafkafi, 1983). Albregts and Howard (1978) reported that fruit Ca concentrations differed among cultivars and years and increased 50% to 100% over the January to April sampling period. Heavy soil texture and high fertilizer applications reduce Ca uptake (Bradfield and Guttridge, 1984). Penalosa et al. (1994) observed that when manure, with or without gypsum and dolomite, was added to a sandy soil where soil Ca and Mg were limiting, plant Ca in sap exudate and leaf dry matter was substantially increased.

Makus and Morris (1987, 1989) reported that preharvest supplementary Ca had no effect on fruit soluble solids, pH, titratable acidity, Color Difference Meter (CDM) color attributes, or shear, but consistently reduced fruit decay over multiple harvests, locations, and years. Leaf, but not whole fruit, Ca concentrations were significantly higher in plants given supplementary Ca fertilizers. We also have observed a modest reduction in fruit size and marginally improved yields from plants given supplementary Ca.

Calcium, applied to strawberry plants throughout the growing season as CaNO_3 or Ca-glutarate, or incorporated as gypsum into the soil, or the latter three combined, had little effect on strawberry whole fruit Ca concentrations, yet reduced fruit decay (Makus and Morris, 1987, 1989). Did supplemental Ca fertilizers alter fruit nutrient concentrations in a way which might affect fruit decay? The objective of this research was to determine the concentration and distribution pattern of Ca and other mineral elements within fruit harvested from two cultivars during the final year of this companion study.

Materials and Methods

A site near Booneville, Ark. (35°06' lat.), consisting of an Enders (clayey, mixed, thermic typic hapludults) soil of low fertility and having soil test values of 1400 kg-ha⁻¹ Ca was selected. 'Cardinal', a conic-shaped, firm-fruited, short-day cultivar, and 'Fern', a shorter, wedged-shaped, medium-firm, day-neutral cultivar, were fall-planted into a raised bed, double staggered row, hill system (71,750 plants/ha) in 1986. Treatments in 1988 consisted of 1) no supplemental Ca (control); 2) 21 foliar applications, incorporated into the fungicide cover sprays from 8 Apr. through 8 July, of Ca tri-hydroxy-glutarate ('Nutri-cal'; CSI Chemical Corp., Bondurant, Iowa) for a seasonal total of 30 kg-ha⁻¹ Ca; 3) pre-incorporated gypsum (renewed on 31 Mar. as a directed application over the bed) at a rate of 437 kg-ha⁻¹ Ca; 3) CaNO_3 (applied by trickle irrigation on 7 Apr., 13 May, 15 June, and 14 Sept.) for a total of 437 kg-ha⁻¹ Ca; and 5) a combination of the latter three treatments at 2.07 times the single treatment rate or 904 kg-ha⁻¹ Ca. Treatments not receiving CaNO_3 were adjusted to equivalent N rates by applying granular NH_4NO_3 on the same dates. Total

Table 1. Mineral distribution in fruit parts of 'Cardinal' and 'Fern' strawberries (dry mass basis) as affected by supplementary calcium treatments.

	K	P	Mg	Na	Al	Fe	Mn	Zn	Cu	B
	(μg·g ⁻¹)									
Main effects										
Cultivar (C)	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Treatment (T)	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
Fruit part										
Achene-proximal	1107 d ^c	3719 a	1587 ab	182 a	31 c	116 a	49 a	37 a	12.8 a	7.6 b
Achene-distal	1076 d	3764 a	1673 a	169 ab	34 c	114 a	55 a	38 a	11.6 a	6.9 b
Outer-proximal	23434 a	2166 b	1462 bc	152 bc	34 c	33 b	55 a	34 a	7.6 b	12.0 a
Outer-distal	20732 b	2109 b	1408 c	109 c	24 c	29 b	58 a	27 b	6.9 b	11.9 a
Inner-proximal	9994 c	935 c	781 d	149 bc	222 a	28 b	24 b	10 c	4.1 c	6.7 b
Inner-distal	9523 c	891 c	679 d	144 bc	203 b	24 b	26 b	9 c	3.0 c	6.6 b
Interaction										
C × T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

^aMean separation within columns by Ryan–Einot–Gabriel–Welch Multiple F test, $P = 0.01$.

ns, **Nonsignificant and significant at $P < 0.01$, respectively.

yearly N rate was 176 kg·ha⁻¹ for all treatments. Superphosphate was applied 21 Mar. and 21 Apr. for a total of 60 kg·ha⁻¹ P. The 1987 fertilizer protocol was similar except that gypsum was applied preplant in Fall 1986. Fertilizer subplots were 3 m in length on 1.2-m row spacings.

In Spring 1988, ≈300 g of 'Cardinal' and 'Fern' fruit were sampled during their respective periods of peak fruiting. Mature fruit of 'Cardinal' had the following raw product attributes: CDM 'L', 'a', and 'b' values of 26.4, 35.1, and 14.7 (Std USDA #5 red plate), respectively; soluble solids concentration (SSC) of 7.2%; pulp pH of 3.51; titratable acidity (0.1 N NaOH) of 5.55 mL; and shear force pressure of 237 N (using a 150-g cell). Corresponding 'Fern' attributes were: 'L', 'a', and 'b' values of 23.9, 34.6, and 13.6, respectively; SSC 6.5%, pulp pH of 3.57, titratable acidity 4.41 mL, and shear force pressure 209 N. Average mass of 'Cardinal' and 'Fern' fruit used in the elemental analysis was 15.3 and 12.0 g, respectively.

Fruits were washed in tap water, immersed in 0.1 N HCl for 30 s, rinsed in tap water, then rinsed in distilled water three times. After calyx removal, fruit were kept frozen at -20° C. Fruit was partially thawed to aid in mechanical separation into proximal and distal halves, consisting of inner and outer receptacle tissue and achenes (six parts of the fruit). About 2 mm of the outer receptacle was removed to furnish the outer or dermal tissue sample. Fleishy portions were lyophilized; achenes were ground with mortar and pestle after drying at 70 °C. Mineral elements were analyzed by emission spectroscopy (Plank, 1992).

The strawberry is an aggregate fruit, and, in this study, the inner and outer, or interior and dermal, fleshy tissue or pulp, refer to the receptacle plus adjacent pith tissue in the case of inner pulp.

The experimental design was a randomized complete block split-plot, with nonrandom subsampling of fruit parts. The largest units were the blocks, in which the cultivars were planted in randomized plots. Random subplots within cultivar plots were treated with the various forms of Ca. Data from the four replications were analyzed by PROC GLM of SAS (version 6.12).

Table 2. Effect of supplemental calcium applications on 'Cardinal' and 'Fern' strawberry fruit calcium distribution (dry mass basis).

	Ca (μg·g ⁻¹)		
	Cardinal	Fern	$P > F$
Cultivar (C)	2226	2708	0.008
Treatment (T)			0.09
Control	2094 c ^z	2689 a	
Foliar	2108 bc	2646 a	
Gypsum	2211 a–c	2655 a	
CaNO ₃	2357 ab	2642 a	
Combination	2370 a	2895 a	
Fruit part			0.0001
Achene-proximal	5500 a	7200 a	
Achene-distal	3128 b	4516 b	
Outer-proximal	1790 c	1754 c	
Outer-distal	1309 d	1273 cd	
Inner-proximal	892 e	906 d	
Inner-distal	670 e	599 d	
Interaction			
C × T			0.76

^aMean separation within columns and sections (Treatment, Fruit part) by Ryan–Einot–Gabriel–Welch Multiple F test, $P = 0.01$. Cultivars analyzed separately.

Results and Discussion

The percentages by fresh mass of proximal-inner, distal-inner, proximal-outer, and distal-outer receptacle tissue and proximal- and distal-achenes for 'Cardinal' were 37.8%, 29.3%, 13.3%, 11.5%, 0.34%, and 0.46%, respectively (data not shown). Corresponding values for 'Fern' fruit were 41.8%, 32.0%, 11.6%, 10.6%, 0.32%, and 0.42%, respectively. Differences in mass between cultivar fruit parts were significantly higher only for proximal-outer receptacle tissue of 'Cardinal' ($P < 0.05$).

Cultivars. Mean Ca and B fruit nutrient concentrations were 18% and 15% higher in 'Fern' than in 'Cardinal', respectively. Other fruit nutrient concentrations were not significantly effected by cultivar (Tables 1 and 2).

Treatments. The CaNO₃ and combination treatments significantly improved fruit Ca concentrations in 'Cardinal' but not 'Fern' fruit (Table 2). Manganese concentrations were higher in 'Cardinal' fruit from plants receiving gypsum compared with plants that received CaNO₃ or the combination treatment (Table 1). The significance of this observation is arguable, since the paired Ca and sulfate ions from gypsum have no effect on soil pH in most soils, and should not influence Mn availability. The combination treatment had similar

gypsum application rates but the fruit Mn concentrations were lower in these fruit than in those from plots treated with gypsum only (data not shown).

Fruit parts. The outer receptacle tissue contained higher concentrations of K, P, Ca, Mg, Mn, Zn, Cu, and B than did the inner receptacle tissue (Tables 1 and 2); Al concentrations were 7-fold higher in the inner receptacle tissue. In achenes, Ca was found in highest concentrations, followed by P, Mg, K, Na, Fe, Mn, Zn, Al, Cu, and B. Fruit Ca gradients existed in two directions, being higher in proximal and outer tissues (Table 2). When the statistical model included only one type of fruit part (i.e., achene, inner, or outer receptacle), proximal/distal gradients were found in achenes for Ca, Mg, Mn, Cu, and B; inner receptacle tissue for P, Al, Zn, Cu and K, B, and Mn ('Fern', only); and outer receptacle tissue for K, Ca, Na, Zn, and Al (data not shown). Although these results are not totally unexpected, we believe this is the first report to document mineral nutrient gradients in strawberry fruit.

Davies and Dennis (1983) reported that 'bound' Ca accounted for 18 to 49% of total fruit Ca concentrations in 8 × 20-mm core sections taken at right angle to the axis of 'Cambridge Favourite' fruit. The bound fraction was correlated with fruit polygalacturonase activity ($r = -0.84$, $P < 0.01$).

There were no interactions between cultivars and calcium fertilizers. When individual fruit parts were analyzed across treatments by comparing control vs. all calcium treatments, only 'Cardinal' achenes in the proximal half of the fruit were higher in Ca compared to the distal half due to the application of calcium fertilizers. Other treatment contrasts of fruit parts in either cultivar were not significant for fruit Ca (data not shown).

El Ghauth et al. (1991) reported that strawberry achenes, but not receptacles, were a source of antifungal glucanohydrolases. The level of these enzymes may be modulated by cytosolic Ca (Gilroy, 1989). Hortynski et al. (1991) found that achene number in strawberry fruit was correlated ($r = 0.73$, $P = 0.01$) with fruit firmness. In our present study, fruit from 'Cardinal' had 38% greater achene mass per fruit than did fruit of 'Fern'. Supplemental Ca treatments had no effect on achene mass (data not shown). In a companion study using the same treatments, plots and multiple harvests, 'Cardinal' fruit were firmer, and had less decay upon holding at 21 °C for 3 d than did 'Fern' fruit (Makus and Morris, 1987). Supplemental Ca fertilizer treatments increased Ca concentrations in leaf blades but did not appear to affect those in whole fruit. The 'Cardinal' cultivar accumulated more Ca in the leaf blades than did 'Fern'.

In this study, we observed that downward and outward gradients exist along the strawberry fruit axis for most mineral nutrients. With the exception of Mn and Ca, supplemental Ca fertilizers did not affect concentration or distribution of these nutrients in the fruit. Only 'Cardinal' responded to soil applied Ca, and the increase in fruit Ca measured was primarily in the achenes of the proximal half of the fruit. Foliar applied Ca did not affect fruit Ca concentrations.

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