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Response of Peach Seedlings to Calcium Concentration in Nutrient Solution[1]

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Abstract. **'Elberta' and 'Lovell' peach seedlings (*Prunus persica* (L.) Batsch) were grown in nutrient solutions for 36 days with 0.8, 2.5, 7.5, 22.5, 67.5, and 202.5 μM Ca. At 0.8 μM Ca root growth was reduced, roots became brown, appeared gelatinous, and decayed before any foliar Ca deficiency symptoms developed. The Ca concentration in the external solution increased from 0.8 μM to 4.5μM Ca due to exchange of previously adsorbed Ca and efflux of Ca from the roots. Leaves wilted severely within 24 hrs and plants began to defoliate 5 days after initiating the 0.8 μM Ca treatment. Calcium deficiency symptoms in 2.5 μM Ca developed in the leaves as marginal chlorosis followed by necrosis that spread to the leaf apexes. Shoot tips died on some peach seedlings. Roots in 2.5 μM Ca died back severely and little regrowth occurred. Elongation of main and secondary roots was restricted and roots that developed were swollen and stubby. Reduced root growth was the only indication of Ca deficiency in seedlings grown in 7.5 μM Ca. No Ca deficiency symptoms appeared on seedlings grown in Ca concentrations greater than 7.5 μM.**

Calcium deficiency in the shoots of annuals has been characterized by marginal chlorosis, curling, and necrosis of apical leaves and the collapse of petioles (5) generally in the young, meristematic area of plants (8). In some plants, the petioles of mature leaves collapse before leaves near the shoot apex show Ca deficiency.

Growth of primary and secondary roots and root hairs is reduced by Ca deficiency (6). Roots, grown 4 to 5 days without Ca, develop a swollen, stubby, and spatulate appearance. Calcium is essential for root growth (7) although the Ca concentration required for adequate growth is relatively low. A Ca concentration of 1 μM is needed for cell division and 10 μM for cell elongation in wheat roots (1).

Symptoms of Al toxicity have been reported for peach seedling roots (4). Since Al reduced the concentration of Mg, P, Zn, and Mn in peach tissue, the Al toxicity symptoms could have been attributed to a deficiency of one or more of these nutrients. However, based upon characterization of the symptoms that developed, tissue concentration of Ca, and Ca-uptake rates, these symptoms probably were caused by Ca deficiency. Thus our objectives in this study were to characterize the Ca deficiency symptoms in peach seedling roots and shoots and to determine the threshold Ca concentration below which Ca deficiency occurs in nutrient solution.

Materials and Methods

'Elberta' and 'Lovell' peach seeds were germinated and seedlings were grown for 21 days to a height of 12 to 16 cm in sand in the greenhouse during December and January. During the 21 days of growth, seedlings were fertilized once with Peters[4] 25-20-20 (25N-4.3P-8.3K) fertilizer. Seedlings were removed from the sand, roots were washed in distilled water for 2 hr, and then transplanted into 15-liter tanks. Each tank contained 2 seedlings of each cultivar. Nutrient concentrations were as follows: 0.25 mM KH_2PO_4, 0.5 mM KCl, 0.25 mM $MgSO_4$, 75 μM FeDTPA, 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, and 0.05 μM Mo.

When seedlings were transplanted, Ca concentration of 0.8, 2.5, 7.5, 22.5, 67.5, and 202.5 μM were initiated by adding $Ca(NO_3)_2$ to the nutrient solutions with N concentration adjusted with NH_4NO_3 to 0.5 mM total N. Temperature was maintained at 24 ± 5°C, and sunlight was supplemented with fluorescent light to produce a minimum of 4 klx at the canopy for a 16 hr day.

Solution pH was measured daily and maintained at 5.5 by adding HCl or NaOH. Nutrient concentration was monitored by removing 50 ml of solution from each tank and determining the concentration of the nutrient by standard methods every 2 days to prevent depletion of Ca and other nutrients. The volume of solutions in tanks were adjusted by adding nutrient solution to adjust the concentration to the original levels. The vigorously aerated nutrient solutions were changed every 7 days. During this period, the concentration of nutrients did not vary more than 5% from the original concentration.

Uniform seedlings were selected for the experiment and representative samples harvested, weighed, and chemically analyzed to served as a comparison for treatment effects. Seedlings in 0.8 μM Ca were harvested 12 days after initiation of the experiment and those in the 2.5 μM Ca after 26 days. Seedlings from other Ca concentrations were harvested on day 36. Plants were separated into leaves, stems, and roots, and freeze dried. The freeze-dried samples were ground to pass a 40-mesh screen. The Ca concentration in the tissue was determined by atomic absorption spectrophotometry. Calcium uptake rates were calculated using the change in total elemental content and change in the fresh weight of seedling roots. The fresh weight of roots was used for this calculation rather than dry weight since the fresh weight of roots should better approximate the absorbing surface. For further detail of calculation methods see reference (3). The experimental design was 5 replicates of 6 Ca treatments in a randomized complete block. Data were subjected to an analysis of variance and there were no significant differences in main effects or interactions. The 0.8 μM and 2.5 μM Ca treatments were not included in the analysis of variance tables since they were harvested before the expt. was terminated.

Root volume was determined on peach seedlings using a water-displacement method. Relative growth rates (RGR) of seedlings were calculated according to the equation by Loneragen, Snowball, and Simmons (9):

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$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100$$

where W_1, and W_2 are the initial and final fresh weight of whole peach seedlings at time t_1 and t_2 in days.

Results and Discussion

The Ca deficiency symptoms of roots and shoots of 1-yr-old peaches were described previously (2). The symptoms described here relate only to our determination of the threshold Ca concentration in nutrient solution for Ca deficiency.

Root deficiency symptoms. The roots of both 'Elberta' and 'Lovell' seedlings grew poorly or very little from the time they were transplanted into 0.8 μM Ca until they were harvested. Within 2 days after initiation of the experiment, the solution Ca concentration had increased from 0.8 to 4.5 μM, from 2.5 to 6.3 μM, and from 7.5 to 12.8 μM. The efflux of Ca was observed for 3 additional days with no visible deficiency symptoms in the seedling shoots. The Ca treatments containing the 0.8 and 2.5 μM were changed on day 5 to adjust the Ca concentration to the original levels. After the nutrient solutions were changed, the roots in the 0.8 μM Ca became brown, flaccid, appeared gelatinous, and began to decay before any foliar Ca deficiency symptoms developed.

Seedling roots in 2.5 μM Ca began to show Ca-deficiency symptoms 19 days after the experiment was initiated. Root apexes appeared dead and new roots were initiated acropetally from the dead tips in healthy root tissue. These root tips appeared shorter and thicker than new root growth on seedlings not Ca deficient. Both 'Elberta' and 'Lovell' seedlings responded similarly to 2.5 μM Ca. Seedling roots grown in 7.5 μM Ca developed Ca deficiency symptoms 32 days after initiation of the experiment but root necrosis was mild compared to that in the lower Ca concentrations. These roots had fewer dead tips, dead sections were shorter and newly initiated tips were less stubby than those grown in 2.5 μM Ca.

One of the first symptoms of membrane damage in roots is the leaking of ions from the roots into the external solution (10). When we increased the Ca concentration in the nutrient solution to 22.5 μM, ion leakage was reduced and damaged membranes were apparently repaired. Stabilization of membrane structures in the plant root is one of the specific functions of Ca and other ions do not substitute completely for Ca (11).

The increase in Ca in the nutrient solution represented about 20% of the total Ca content of the peach seedlings. There are 2 possible reasons for the increase in Ca concentration. Before seedlings were subjected to Ca treatments we did not allow enough time for the Ca adsorbed on the roots to be removed. The second possibility is the efflux of previously absorbed Ca from the peach seedling roots. The increase in Ca in the nutrient solution was much too high to be accounted for entirely by efflux of Ca from peach roots. The increase in Ca was probably the sum of both efflux and exchange of previously adsorbed Ca.

Shoot-deficiency symptoms. One day after initiation of the experiment, shoots of seedlings grown in 0.8 μM had wilted leaves. These leaves began to abscise before they discolored or developed necrosis. Twelve days after the initiation of the experiment, leaves began to abscise and the seedlings were harvested.

Nineteen days after the experiment was initiated, we observed marginal chlorosis on seedling leaves from the 2.5 μM Ca treatment. The affected leaf margins were rolled downward. Newly-developed leaves tended to be smaller and marginal edges were rolled downward. These symptoms were previously reported (4) as symptoms of Al toxicity in peach seedlings. Chlorosis always preceeded necrosis of the leaf margin in Ca deficient leaves. No severe wilting was observed on seedlings grown at Ca concentrations greater than 0.8 μM. There were no differences between seedlings of each cultivar in the development of foliar symptoms among the same Ca treatments. Seedlings treated with 2.5 μM Ca were harvested 26 days after initiation of the experiment. We observed no foliar Ca deficiency symptoms at Ca concentrations greater than 2.5 μM during the experimental period.

Growth. The growth of 'Lovell' and 'Elberta' seedlings was greatly restricted at 0.8 μM Ca (Table 1). Increasing the Ca concentration to 2.5 μM increased the terminal length, number of laterals, trunk cross-sectional area, and root volume as compared with the lower Ca concentrations. However, when the CA concentration in the nutrient solution exceeded 7.5 μM, we found no further significant increase in any of the growth parameters for either cultivar.

The increase in dry weight of roots was small with 'Lovell' and 'Elberta' seedlings at the 2 lowest Ca treatments (Table 1). The increase in dry weight of roots at and above 7.5 μM Ca concentration was much less for 'Elberta' than for 'Lovell' seedlings although no foliar Ca deficiency was evident. Apparently, the 'Lovell' seedlings are more efficient than 'Elberta' seedlings in the production of root tissue at the lower CA concentration.

Tissue Ca concentration. Before starting the experiment, the concentration of Ca in 'Lovell' seedling leaves plus stems averaged 5,750 μg/g (dry weight) and the roots averaged 1,377 μg/g (dry weight), whereas for 'Elberta' seedlings the compar-

Table 1. The influence of calcium on growth of 'Lovell' and 'Elberta' seedlings.

Ca concn (μM)	Seedlings	Increase terminal length (cm)	Increase in laterals (no.)	Increase trunk area (mm^2)	Increase in root vol (cc)	Increase in dry wt (g/2 plants) Leaves	Stems	Roots
0.8^z	'Lovell'	0	0	0	0	0.69^y	–	0.15
	'Elberta'	0	0	0	0	0.70^y	–	0.14
2.5^x	'Lovell'	51	4.2	6.45	3.4	2.64	1.08	0.86
	'Elberta'	40	4.3	6.32	4.7	2.12	0.74	0.78
7.5	'Lovell'	108	7.5	8.32	13.5	4.45	1.61	1.52
	'Elberta'	95	9.3	8.36	11.2	4.23	1.36	1.07
22.5	'Lovell'	119	7.0	8.70	14.8	4.19	1.58	1.72
	'Elberta'	125	7.9	8.26	13.9	5.05	1.79	1.57
67.5	'Lovell'	130	7.1	9.62	14.4	4.76	1.89	1.70
	'Elberta'	111	7.7	8.54	14.6	3.83	1.44	1.34
202.5	'Lovell'	149	7.0	9.91	15.8	4.92	2.15	1.63
	'Elberta'	123	6.4	10.23	15.8	4.29	1.81	1.47

^{z}Seedlings harvested 12 days after transplanting.
^{y}Stems combined with leaves.
^{x}Seedlings harvested 26 days after transplanting.

Table 2. Influence of calcium concentration in nutrient solutions on calcium concentration, relative growth rates, and calcium uptake rates of peach seedlings.

Ca concn (µM)	Seedlings	Ca (µg/g) Leaves	Stems	Roots	Relative growth rates (g/100g fr wt/day)	Calcium uptake rates (µMoles/g fr root per day)
0.8[z]	'Lovell'	4812[y]	–	536	0	–
	'Elberta'	4500[y]	–	586	0	–
2.5[x]	'Lovell'	1537	681	410	5	0.58
	'Elberta'	1424	851	494	7	0.53
7.5	'Lovell'	1113	680	463	10	1.06
	'Elberta'	813	750	510	9	0.83
22.5	'Lovell'	1313	1187	587	10	1.21
	'Elberta'	1275	907	600	10	1.30
67.5	'Lovell'	2213	1825	1238	10	2.24
	'Elberta'	2288	1445	1303	10	1.85
202.5	'Lovell'	3175	3400	1438	10	3.36
	'Elberta'	4125	3250	1575	11	3.30

[z]Seedlings harvested 12 days after transplanting.
[y]Stems combined with leaves.
[x]Seedlings harvested 26 days after transplanting.

able values were 7,562 and 2,083 µg/g (dry weight). Leaf Ca concentration was the lowest for the 2.5 and 7.5 µM Ca treatment (Table 2). The root and stem Ca concentration was increased most noticeably when the Ca concentration in the nutrient solution was 67.5 µM and higher, which indicates that roots only accumulate Ca when the Ca concentration in the nutrient solution exceeds a given Ca concentration.

The Ca concentration in the leaves plus stems was highest at 0.8 µM Ca and decreased as Ca concentration was increased to 2.5, 7.5 and 22.5µM (Table 2). The leaf Ca concentration increased at 67.5 and 202.5 µM Ca, but it did not exceed the Ca concentration at 0.8 µM. Because the growth of seedling in 0.8 µM Ca was very poor, the leaf Ca reflected the Ca concentration of the seedlings as they had been grown with Peters[4] fertilizer before the experiment was initiated.

Relative growth rates. Seedlings did not grow in 0.8 µM Ca (Table 1), therefore the RGR (Table 2) was zero. The RGR of both cultivars remained constant at Ca concentratons greater than 2.5 µM. Our data suggest that peach seedlings can tolerate a wide range of Ca concentration in the nutrient solution with little effect on RGR or the tissue Ca concentration.

Ca uptake rates. The increase in Ca uptake rates was similar for 'Lovell' and 'Elberta' seedlings. The Ca uptake rates increased most dramatically between the 2.5 and 7.5 µM Ca treatments (Table 2). At 7.5 µM Ca, the constant increase in growth (Table 1), and the constant RGR and tissue Ca concentration (Table 2) indicate that 7.5 µM Ca is adequate to produce maximum growth of peach seedlings under greenhouse conditions.

We observed 2 responses with 'Lovell' and 'Elberta' peach seedlings. The first was at low Ca concentrations (0.8 to 7.5 µM) and resulted in an increase in the RGR with a decrease in the tissue Ca concentration (Table 1). When the nutrient solution Ca concentration was increased above 7.5 µM (7.5 to 202.5 µM), tissue Ca concentration was markedly increased without affecting the RGR of either cultivar (Table 2). The Ca concentration that resulted in an uptake rate of 1.0 µM/g fresh weight root/day is sufficient to produce maximum growth of both cultivar seedlings under greenhouse conditions.

In a previous report (4), the symptoms induced by high Al concentrations were called Ca-deficiency in peach seedlings. This conclusion was based on symptoms developed in the roots and leaves. In a later report (3) we concluded from tissue analysis and uptake rates that the symptoms resulted from an Al-induced Ca-deficiency because Al inhibited Ca-uptake but did not influence Ca-translocation. The concentration of several nutrients was reduced in the leaves, stems, and roots by high Al concentration in the nutrient solution, and it was not possible to attribute the Al toxicity entirely to Ca-deficiency in these studies.

From the Ca-deficiency symptoms developed on roots and shoots of 'Lovell' and 'Elberta' seedlings, it appears that Al-toxicity symptoms reported in the previous study with 'Elberta' seedlings may have instead been Ca-deficiency. The root growth at 7.5 µM Ca resembled that of Fig. 3 in reference (4). The only difference between Al-toxicity symptoms and Ca-deficiency in the leaves was that we found no collapse of the midrib in these Ca treatments. These data with peach seedlings support other evidence with annuals (8) that a relatively low Ca concentration is needed at the root surface for maximum growth and it appears that 7.5 µM Ca is adequate for peach seedlings grown under greenhouse conditions.

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