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Radio-calcium Uptake by 'Spartan' and 'Delicious' Apple as Influenced by Rootstock and BA + GA_3 to Activate Growth of Lateral Buds

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Abstract. **One-year old trees of 'Spartan' and 'Delicious' apple (*Malus domestica* Borkh.) on 4 clonal rootstocks were cut to 3 scion nodes and transferred to an aerated Long Ashton full nutrient solution containing ^{45}Ca. Treating the axillary buds with 6-benzylamino purine plus gibberellic acid (BA + GA_3) resulted in a small increase in number, fresh weight, and total length of new shoots. Total area of new leaves per tree was unaltered. The BA + GA_3 treatment significantly reduced the ^{45}Ca content of the "new shoots" fraction and the total amount of ^{45}Ca absorbed by the treated trees. 'Spartan' trees absorbed and transported more ^{45}Ca than did 'Delicious' and there was no significant interaction with growth regulators. Of the rootstocks, Malling Merton (MM) 111 was less efficient than Malling (M) 26, M 7, or MM 106 in transporting ^{45}Ca across the graft union to either scion cultivar. Growth-regulator-treated trees on M 26 roots absorbed significantly less ^{45}Ca than did comparably treated trees on MM 106 or MM 111 roots.**

The incidence of bitter pit and various internal breakdown disorders of apple fruits is inversely related to fruit Ca levels (3, 16). This observation has stimulated interest in identifying specific factors that influence fruit Ca levels and in devising horticultural practices to improve fruit Ca status.

The reason or reasons why low fruit Ca is such a common occurrence continues to elude researchers. It is now believed that the availability of Ca in the soil solution is seldom limiting (6, 13), and even when soil amendments do result in higher foliage Ca levels, they may not improve the Ca status of the fruit (7). The problem appears to be one of distribution.

There is evidence to suggest that Ca uptake and transport is in some way regulated by plant-growth substances (3). Auxin appears to promote Ca uptake and transport in herbaceous plants (5, 14). A similar relationship may exist in apple as evidenced by the effects of auxin transport inhibitors (12) and experiments involving induced parthenocarpy (2). Cytokinins also appear to promote Ca movement into treated tissues (11), whereas gibberellins may have the opposite effect (1, 14). Looney (10) observed that a mixture of BA + GA_{4+7} applied to fruits or fruits and foliage reduced fruit Ca levels slightly but consistently.

Since these same growth regulators can be used to promote branching of young trees (4, 17) and, formulated as Promalin, are being tested for this purpose across North America, the possible effects on Ca uptake and distribution should be understood.

Virus-free scions of 'Spartan' and 'Delicious' (Harrold Red strain) were bench-grafted onto virus-free M 26, M 7, MM 106, and MM 111 clonal rootstocks and subsequently grown in coarse silica sand in a glasshouse with supplemental light and heating. They were watered daily with a Long Ashton full nutrient solution (8) and by late March the single shoot on each tree was about 100 cm long. On April 26, May 3, May 12, May 15, May 21, and May 23, 32 trees (4 of each cultivar on each rootstock) were cut back to 3 basal leaves. Each tree was transferred carefully to a dark container containing 2.5 liters of continuously aerated Long Ashton full nutrient solution with either 23 or 46 μCi of ^{45}Ca (Fig. 1). In the experiments commencing May 3, May 15, and May 23, the 3 axillary buds were painted with a mixture of 2000 ppm BA, 5% dimethylsulfoxide (DMSO) and 1% Tween 20 (4). On each of 2 subsequent days, these same buds were treated with 500 ppm GA_3 plus 0.1% Tween 20.

After 3 weeks, the trees were removed and the following tissues analyzed by scintillation counting: 1) all new shoot growth (new shoots); 2) the leaves present at the start of

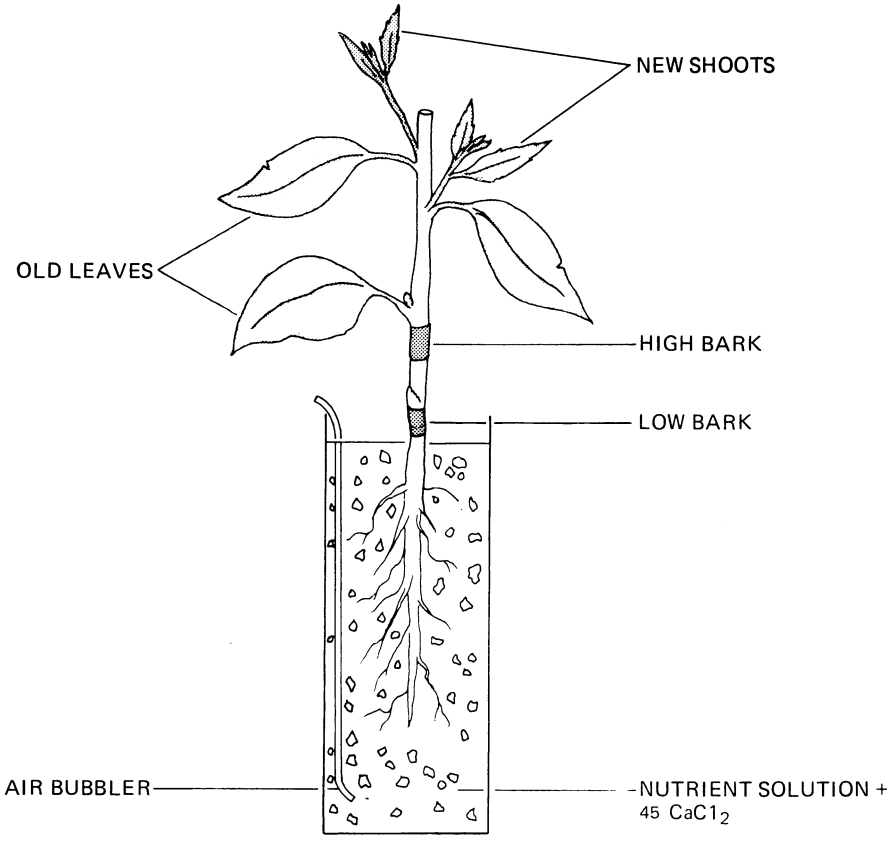

Fig. 1. A schematic representation of the treatment procedure and tree positions analyzed for ^{45}Ca after 20 or 21 days.

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Table 1. Growth and development of new shoots arising from axillary buds treated with BA + GA_3. Data for 2 cultivars and 4 rootstocks are pooled for each of the 6 experiments.

Experiment	Growth-regulator treatment	Date started	Experiment duration (days)	No. of new shoots per tree	Wt of new shoots (g/tree)	Length of new shoots		Leaf area	
						cm/shoot	cm/tree	cm^2/tree	cm^2/cm of shoot length
I	nil	Apr. 26	20	2.6	2.3	3.8	9.9	34.3	3.5
III	nil	May 12	21	2.8	2.1	4.0	10.9	38.7	3.5
V	nil	May 21	20	2.8	2.6	4.5	12.6	42.2	3.3
			Mean	2.7 B[z]	2.4 B	4.1 A	11.2 B	38.4 A	3.4 A
II	BA + GA_3	May 3	21	3.0	2.6	3.9	11.7	31.9	2.7
IV	BA + GA_3	May 15	21	3.0	2.6	4.1	12.2	37.8	3.1
VI	BA + GA_3	May 23	20	3.0	3.1	4.7	13.8	40.7	3.0
			Mean	3.0 A	2.8 A	4.2 A	12.5 A	36.8 A	2.9 B

[z]Mean separation by Duncan's multiple range test, 1% level.

the experiment (old leaves); 3) a ring of bark immediately above the graft union (high bark); and 4) a ring of bark just below the graft union (low bark) (Fig. 1). Total depletion of ^{45}Ca from the aerated solution was also determined.

The number of new shoots developing per tree and their total fresh weight, length, and total leaf area were determined before this fraction was oven-dried (80°C for 12 hr), weighed again, and then ashed. All other tissues were simply weighed, dried, weighed again, and ashed. The ashed samples were transferred to glass scintillation vials with 2 ml of 0.5N HCl and made up to 15 ml with PCS solubilizer. Each sample was counted on two channels of a Beckman LS–100C soft-beta spectrophotometer. The quench-corrected results are expressed as disintegrations per minute (DPM) per unit of tissue dry weight.

The effects of growth-regulator treatment on shoot growth were significant but not dramatic (Table 1). There was a significant increase in the number of new shoots per tree and significant increases in total weight and length of new shoot growth. Since the total leaf area per tree was not significantly affected, the total leaf area per cm of shoot length was reduced significantly by the growth-regulator treatment.

Treatment with BA + GA_3 reduced ^{45}Ca in the new shoots and total ^{45}Ca uptake from the full nutrient solution (Table 2). With regard to the effect on new shoots, there was no significant interaction with cultivars or rootstocks. Regarding total ^{45}Ca uptake, a significant interaction with rootstocks was observed. Treated trees on M 26 roots absorbed relatively more ^{45}Ca than did comparably treated trees on the other 3 rootstocks. Interestingly, the average amount of nutrient solution used per tree was virtually identical for the treated and untreated trees (32.2 and 32.7 g per tree, respectively).

'Spartan' trees absorbed more ^{45}Ca from the nutrient solution than did 'Delicious' trees and significantly lower ^{45}Ca levels were detected in all 'Delicious' tissues sampled above the graft union (Table 2). In fact, these differences became steadily greater in more distal tissues.

The rootstocks also differed in the amount of ^{45}Ca absorbed and transported. The relative efficiency of growth-regulator-treated M 26 trees has already been mentioned. The MM 111 trees absorbed as much ^{45}Ca from the nutrient solution as did trees on other rootstocks, but the amount transported across the graft union was reduced greatly (Table 2).

The horticultural importance of these findings is difficult to assess. We believe that the growth-regulator results have a degree of practical relevance because BA + GA_3 did increase the number of lateral shoots, the commercial aim of such treatments. Our results suggest that these treatments may reduce the leaf area on induced shoots, reduce their Ca content (expressed on a tissue dry-weight basis), and perhaps even influence the absorption of Ca by the root system.

The low ^{45}Ca levels in the new shoots is probably related to their reduced leaf area. The average leaf area reduction, calculated per unit shoot length, was about 20%. The average reduction in ^{45}Ca was 26%.

The BA + GA_3 effect of reducing total ^{45}Ca uptake from the nutrient solution was substantial (about 33%), significant ($P = .01$), and very difficult to explain. There are very few reports relating any aspect of root activity to growth-regulator treatments applied to shoots and none concerned specifically with cytokinin–gibberellin mixtures. Thus, this result was totally unexpected.

A possible clue to the mechanism involved may be the observation that the M 26 trees, which showed little suppression of total ^{45}Ca uptake (Table 2), were the most responsive to the BA + GA_3 treatment with regard to new shoot growth: a 54% increase in fresh weight of new shoots compared to 8% to 14% for trees on the other 3 rootstocks. However, variability was such that these differences were not significant statistically.

The difference between 'Spartan' and 'Delicious' trees in the uptake and distribution of ^{45}Ca was not unexpected (J.L. Mason, unpublished). The fact that the 'Spartan' trees exhibited higher ^{45}Ca levels yet are more prone to Ca-related fruit disorders is consistent with earlier evidence suggesting that cultivars prone to such disorders are often characterized by having either an efficient mechanism for moving Ca to vegetative tissues or a high Ca requirement by these tissues (9, 15).

Rootstock effects on scion CA levels, while reported previously (9), are less predictable and few generalizations can be made. An interesting result of the present study was the

Table 2. Uptake and distribution of ^{45}Ca by young apple trees as influenced by growth regulators, cultivars, and rootstocks.

Treatment	Total depletion of ^{45}Ca from the nutrient solution (DPM × 10^6)		^{45}Ca in various tissues (DPM × 10^3/mg dry wt)			
			New shoots	Old leaves	Bark above graft union	Bark below graft union
Growth regulators						
Untreated	36.7 a[z]		38.7 a[z]	37.1 a	72.1 a	507.2 a
BA + GA_3	25.2 b		28.7 b	45.7 a	86.0 a	408.5 a
Cultivars						
Spartan	33.0 a[z]		44.1 a	51.7 a	94.7 a	499.0 a
Delicious	28.9 b		25.2 b	32.9 b	65.5 b	415.2 a
Rootstocks						
	Untreated	*BA + GA_3*				
M 26	40.0	33.1 a[z]	55.6 a	56.7 a	96.1 ab	418.3 a
M 7	32.2	24.2 b	39.5 a	46.3 a	80.8 b	447.9 a
MM 106	37.0	20.6 b	41.3 a	72.7 a	131.7 a	509.9 a
MM 111	40.7	22.9 b	13.6 b	15.1 b	37.6 c	390.7 a
Mean	36.7 A[y]	25.2 B				

[z]Mean separation within columns by Duncan's multiple range test, 5% level.
[y]Mean separation within one row by Duncan's multiple range test, 1% level.

relative inability of ^{45}Ca to move across the MM 111 graft union. Although a degree of stock/scion incompatibility must be suspected, there were no indications of poor growth by these scions and MM 111 is used widely and successfully as a rootstock for both cultivars. This observation remains unexplained.

Literature Cited

1. Bangerth, F. 1973. Investigations upon Ca related physiological disorders. Phytopathol. Z. 77:20–37.
2. Bangerth, F. 1976. A role for auxin and auxin transport inhibitors on the Ca content of artifically induced pathenocarpic fruits. Physiol. Plant. 37:191–194.
3. Bangerth, F. 1979. Calcium-related physiological disorders of plants. Annu. Rev. Phytopath. 17:97–122.
4. Broome, O.C. and R.H. Zimmerman. 1976. Breaking bud dormancy in tea crabapple [*Malus hypehensis* (Pamp.) Rehd.] with cytokinins. J. Amer. Soc. Hort. Sci. 101:28–30.
5. Chen, W.S. and S. Uemoto. 1977. Studies on calcium absorption in vegetable crops. II. Effect of NAA treatments on the mobility of calcium in the tomato plant. J. Japan. Soc. Hort. Sci. 45:362–368.
6. Fried, M. and R.E. Shapiro. 1961. Soil-plant relationships in ion uptake. Annu. Rev. Plant Physiol. 12:91–112.
7. Greene, G.M. and C.B. Smith. 1979. Effects of calcium and nitrogen sources on corking of apples. Comm. Soil Sci. & Plant Anal. 10:129–139.
8. Hewitt, E.G.J. 1966. Sand and water culture methods used in the study of plant nutrition. 2nd ed. Commonwealth Agr. Bureaux, Bucks., U.K.
9. Köksal, A.I. 1973. Wechselwirkungen zwischen Sorten, Unterlagen und Zwischenveredlungen beim Apfel. Gartenbauwissenschaft 38:287–310.
10. Looney, N.E. 1979. Some effects of gibberellins A_{4+7} plus benzyladenine on fruit weight, shape, quality, Ca content, and storage behavior of 'Spartan' apple. J. Amer. Soc. Hort. Sci. 104:389–391.
11. Shear, C.B. and M. Faust. 1970. Calcium transport in apple trees. Plant Physiol. 45:670–674.
12. Stahly, E.A. and N.R. Benson. 1976. Calcium levels of 'Golden Delicious' apples as influenced by calcium sprays, 2,3,5-triiodobenzoic acid, and other plant growth regulator sprays. J. Amer. Soc. Hort. Sci. 101:120–122.
13. Vang-Peterson, O. 1980. Calcium nutrition of apple trees: a review. Scientia Hort. 12:1–9.
14. Wieneke, J., O. Biddulph, and C.G. Woodbridge. 1971. Influence of growth regulating substances on absorption and translocation of calcium in pea and bean. J. Amer. Soc. Hort. Sci. 96:721–724.
15. Whitfield, A.B. 1964. The effects of stock and scion on the mineral composition of apple leaves. Rpt. E. Malling Res. Sta. for 1963. p. 107–109.
16. Wilkinson, B.G. and J.C. Fidler. 1973. Physiological disorders, p. 63–131. In: J.C. Fidler, B.G. Wilkinson, K.L. Edney, and R.O. Sharples (eds.). The biology of apple and pear storage. Commonwealth Agric. Bureaux, Bucks., U.K.
17. Williams, M.W. and H.D. Billingsley. 1970. Increasing the number and crotch angles of primary branches of apple trees with cytokinins and gibberellic acid. J. Amer. Soc. Hort. Sci. 95:649–651.

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Growth Inhibition from Guava Root Exudates

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Abstract. **Root exudates of guava (*Psidium Guajava* L. cv. Beaumont) grown in sand culture were collected on columns of XAD-4 resin attached to the nutrient solution circulation system of sand-cultured plants. The compounds were eluted from the resin columns with methanol and the eluates were concentrated. The root exudates were inhibitory to the radicle growth of both lettuce (*Lactuca sativa* L. cv. Anuenue) and bristly foxtail (*Setaria verticillata* L. Beauvois) and lettuce seed germination was inhibited. Fractionating the root exudates resulted in the neutral and acidic fractions being inhibitory, the basic fraction having no effect. Methanolic extracts of oven-dried guava roots were also inhibitory.**

Guava is a crop of emerging importance in Hawaii and other tropical areas; its fruit is used in Hawaii primarily for puree. In one experimental field recently treated with glyphosate N-(phosphonomethyl) glycine to control existing vegetation, rings devoid of weeds were observed around each tree (Fig. 1) several weeks after glyphosate application. The primary weed species present was garden spurge (*Euphorbia hirta* L.). It appeared that substances produced by the guava root system or leachates washed from leaves by rain might be inhibitory to the reestablishment of weeds.

This study was conducted to determine if guava roots produce substances inhibitory to plant growth. The most common method of testing for allelochemicals has been through aqueous, alcoholic, or ether extractions of various plant parts. However, solvent extraction of plant tissue may be meaningless since many phototoxic substances are normally compartmentalized in vacuoles or organelles and may not be released into the environment naturally. A technique recently developed by Tang and Young (7) allows for the continuous collection of organic substances from undisturbed living roots growing in a sand culture. We studied the inhibitory potential of exudates from living roots as well as extracts of ground samples of dried roots.

Three 3-to-4-year-old rooted guavas (about 1 m tall) were depotted, desoiled, washed, and replanted in inverted, bottomless, one-gallon, brown-glass solvent bottles. The potting technique was similar to that used by Tang and Young (7) except the medium was crushed basaltic sand (mansand). A 4-cm layer of washed, 2-cm-size, crushed basaltic rock was placed in the bottom of each bottle. Mansand, which contained 90% of particles between 0.25 mm and 2 mm, was washed with running water for about 45 min. until the effluent was clear and was then placed around the roots of each guava tree. The containers were wrapped in aluminum foil to prevent photoconversions and irrigated with 1/20-strength Hoagland's solution (2) at a rate of 100 ml/pot/day. The plants were grown for 2 months before collection proceeded. Additional water was supplied as needed. Two pot controls (minus guava trees) were treated identically.

Guava root exudates were collected from the nutrient circulation system on columns of XAD-4 resin. After 3 days, resin columns from the guava plants and the pot controls were washed with distilled water, eluted with glass-distilled methanol, concentrated, and bioassayed as described by Tang and Young (7). This technique allows for the continuous collection of exudates from an intact, undisturbed living system. Initially, exudate was collected from 10- and 15-ml resin columns and assayed directly. After inhibitory activity was observed, the exudate was fractionated into neutral, acid, and basic fractions as described by Tang and Young (7). All eluates were concentrated to 0.33 ml/resin column. By determining the collection period and final volume of the extracted exudate, it was calculated that 5 ml of eluate was produced by one guava plant/hr.

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