CuCO₃-painted Containers and Root Pruning Affect Apple and Green Ash Root Growth and Cytokinin Levels

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Abstract. CuCO₃ at 100 g-liter⁻¹ in a paint carrier applied to interior container surfaces effectively prevented root deformation in container-grown *Malus domestica* Borkh. and *Fraxinus pennsylvanica* Marsh. seedlings. CuCO₃ treatments nearly doubled the number of white unsuberized root tips in both species. CuCO₃ treatment increased some measures of root and shoot growth before and after transplanting to larger untreated containers. Root pruning at transplanting tended to reduce root and shoot fresh and dry matter accumulation in *F. pennsylvanica* seedlings and shoot extension in *M. domestica* seedlings. In some cases, root pruning of *M. domestica* at transplanting from CuCO₃-treated containers increased root growth compared to unpruned CuCO₃-treated and untreated seedlings. Changes in growth induced by CuCO₃ and root pruning were not related to changes in *trans*-zeatin riboside-like activity in the xylem sap of apple.

Containerized production of landscape plants allows the planting season to be extended. *Fraxinus pennsylvanica* and *Malus* spp. and cultivars are common container-grown ornamental. *Malus domestica* cultivars are container-grown for use in home orchards and are used as rootstock for ornamental crabapple cultivars. However, due to the restricted root volume in containers, root system morphology is altered with kinked, circling, twisted, and matted roots forming at the medium-container wall interface. Following transplant, this root deformation has been reported to decrease vigor and mechanical stability and shorten the life span of trees (Nichols and Aim, 1983; Nussbaum, 1969; Preisig et al., 1979; Stone and Norberg, 1978).

Recommended corrective root pruning (Bush-Brown and Bush-Brown, 1980; Harris, 1983; Stone and Norberg, 1978) removes large portions of the root system, decreases shoot growth, reduces photosyn-

thesis and transpiration, and hastens senescence of leaves (Arnold, 1987; Arnold and Struve, 1989b, 1989c). CuCO₃ in a paint carrier applied to interior container walls effectively prevented root deformation in conifer (Burdett, 1978; Burdett and Martin, 1982; McDonald et al., 1984a, 1984b; Ruehle, 1985) and hardwood species, including green ash (Arnold and Struve, 1989a, 1989b; Struve et al., 1987, 1988), by inhibiting root elongation. This resulted in increased root system density in conifers (Burdett and Martin, 1982; McDonald et al., 1984a, 1984b; Ruehle, 1985) and increased the number of lateral roots formed on individual *F. pennsylvanica* roots (Arnold and Struve, 1989a). Plants grown in CuCO₃-treated containers exhibited increased growth while in treated containers and following transplanting compared to untreated controls (Arnold and Struve, 1989b; McDonald et al., 1984a). Root tips are a site of production of cytokinins that are translocated in the xylem and can influence shoot growth (Skene, 1975). The influence of CuCO₃-induced increases in root tip number on cytokinin level is unknown.

The objectives of this study were to determine if: 1) CuCO₃ treatments increased the number of white unsuberized root tips of apple and green ash on a whole-plant basis, 2) CuCO₃ and root pruning-induced growth differences during container production and following transplanting are related to changes in cytokinin levels in the xylem sap, 3) CuCO₃ is an effective agent for controlling root deformation in containerized apple trees.

On 2 May 1989, 60 one-year-old dormant bare-root seedlings of *M. domestica* (Lawyer's Nursery, Plains, Mont.) and 60 *F.*

pennsylvanica (State Nursery of Ohio, Zanesville, Ohio) were planted in Surface medium (AMCOR Applied Industrial Materials Corp., Deerfield, Ill.) in 3.5-liter plastic containers. Before planting, half of the containers used for each species had interior surfaces coated with 100 g CuCO₃/liter of flat white acrylic latex exterior house paint. Roots were pruned to a 20-cm length to fit in the containers. The 120 potted seedlings were arranged in a randomized complete-block design in a greenhouse. Weekly, 125 ppm N from 20N-8.7P-16.6K Peters water-soluble fertilizer and, monthly, trace elements (0.63 g STEM/liter of water, W.R. Grace, Fogelsville, Pa.) were applied. Seedlings were trained to a single stem and -staked. Temperatures were 24/18C day/night under natural photoperiods. At planting, 10 additional bare-root seedlings of each species were destructively harvested and xylary cytokinin was measured in *M. domestica*.

On 15 Aug. 1989 (105 days from planting), 10 seedlings from treated and untreated containers of each species (40 plants) were harvested and cytokinin assayed in *M. domestica*. Amounts of xylem sap extracted from *F. pennsylvanica* were inadequate for cytokinin assay. All unharvested seedlings (80 plants) were transplanted to 6-liter untreated containers and placed in their previous positions in the greenhouse. At transplanting, half of the seedlings of each species and container treatment combination were not pruned according to recommended practices to control root deformation (Bush-Brown and Bush-Brown, 1980; Harris, 1983; Stone and Norberg, 1978). Four vertical cuts 2.5 cm deep, equally spaced, down the sides of the root ball were made and the matted roots in the lower 1.5 cm of the root ball were removed. The fresh weight (g) of removed roots was recorded. Root pruning half of the CuCO₃-treated seedlings was for comparison to like seedlings from untreated containers, not because CuCO₃-treated seedlings required corrective root pruning. On 2 Oct. 1989 (153 days after planting, 48 days after transplanting), all seedlings were harvested and cytokinin assayed in *M. domestica*.

Xylem sap was vacuum-extracted from *M. domestica* and assayed for cytokinin [pmol *trans*-zeatin riboside (*t*-ZR)-like activity per 0.1 ml xylem sap] as described by Belding and Young (1989) using an enzyme immunoassay kit (Phytodetek-*t*-ZR, Idetek, San Bruno, Calif.). The monoclonal antibodies used also react with zeatin riboside-5-monophosphate at 95.2% and zeatin at 47.3% of the reactivity of *t*-ZR (Werler, 1980), but very little with other derivatives. This analysis can be considered a very sensitive and specific bioassay for *t*-ZR-like binding activity, but not as a measure of actual endogenous *t*-ZR. Although *t*-ZR has been shown to be the primary cytokinin form in apple xylem (Jones, 1973), its occurrence relative to other forms was not measured in this study. Based on Pengelly's (1986) criteria, Young

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Table 1. Root growth of 1-year-old dormant bare-root *Malus domestica* and *Fraxinus pennsylvanica* seedlings while growing in CuCO₃-treated or untreated 3.5-liter containers (0 to 105 days from planting) and following transplanting to 6.0-liter containers (105 to 153 days from planting) with and without root pruning at transplanting.^{a,c}

Species	Days from planting	CuCO ₃ treated	Root pruned	Root fresh wt gain (g)		
				New root ^d	Inside original rootball	Total new and old roots
Apple	105	No	No	1.3 a	4.1 b	4.1 b
		Yes	No	1.6 a	6.3 a	6.3 a
	153	No	No	4.1 b	5.6 a	9.7 ab
		Yes	No	5.0 ab*	0.8 b	5.8 b
Green Ash	105	No	Yes	3.6 b	5.8 a	9.4 ab
		Yes	Yes	7.0 a*	6.9 a	13.9 a
		No	No	4.0 a	15.1 a	15.1 a
		Yes	No	5.5 a	15.2 a	15.2 a
	153	No	No	8.0 ab	41.1 ab	49.2 ab*
		Yes	No	13.5 a	51.3 a	64.8 a
		No	Yes	4.7 b	21.6 c	26.3 b*
		Yes	Yes	6.6 b	29.4 bc	35.5 b

^aValues are means of 10 observations.

^cMeans within a species and harvest date followed by the same letter are not significantly different at $P \leq 0.05$. Means within a species and harvest date followed by * are not significantly different at $P \leq 0.05$ but are at $P \leq 0.10$.

^dNew roots inside the original root ball at transplanting (day 105) and outside the original root ball at final harvest (day 153).

Table 2. Shoot growth of 1-year-old dormant bare-root *Malus domestica* and *Fraxinus pennsylvanica* seedlings while growing in CuCO₃-treated or untreated 3.5-liter containers (0 to 105 days from planting) and following transplanting to 6.0-liter containers (105 to 153 days from planting) with and without root pruning at transplanting.^{a,c}

Species	Days from planting	CuCO ₃ treated	Root pruned	New shoot		Total fresh wt gain (g)
				Length (cm)	Fresh wt gain (g)	
Apple	105	No	No	67 b	33.8 b	40.2 a*
		Yes	No	81 a	42.8 a	48.4 a*
	153	No	No	74 b	37.5 a	39.3 a
		Yes	No	88 a	39.9 a	40.3 a
Green Ash	105	No	Yes	73 b	36.3 a	37.1 a
		Yes	Yes	73 b	37.8 a	39.7 a
		No	No	30 a	33.4 a	50.7 a
		Yes	No	37 a	37.4 a	57.3 a
	153	No	No	34 a	41.7 ab*	45.1 ab
		Yes	No	38 a	44.8 a	54.0 a
		No	Yes	33 a	30.8 b*	27.2 b
		Yes	Yes	35 a	33.2 ab	39.7 ab

^aValues are means of 10 observations.

^cMeans within a species and harvest date followed by the same letter are not significantly different at $P \leq 0.05$. Means within a species and harvest date followed by * are not significantly different at $P \leq 0.05$ but are at $P \leq 0.10$.

(1989) found no interference with the immunoassay in apple xylem sap using the same extraction and assay procedures used in this study.

The following characteristics were measured on new tissues and those present at planting: fresh weight and dry weight of shoots, tap roots, and lateral roots, and shoot length. On seedlings harvested at planting and at transplanting to larger containers, the number of white root tips was counted in a randomly selected quarter (by volume) of the root ball. This number was multiplied by four to estimate the total number of new root tips. The statistical analysis for each species was run separately. For each species, a randomized complete-block design with five blocks containing two plant replications of each container treatment, root pruning, and harvest date combination (12 plants per block) was used. Statistical analysis used correla-

tion analysis and analysis of variance with paired t tests between treatments at each harvest date (SAS Institute, 1985). Fresh and dry weights were highly correlated (data not shown); thus, only fresh weights are presented.

Growing apple and green ash in CuCO₃-treated containers virtually eliminated circling, kinked, and matted roots at the medium-container wall interface, as previously reported for green ash (Arnold and Struve, 1989a, 1989b) and other hardwood (Struve et al., 1987, 1988) and coniferous species (Burdett, 1978; Burdett and Martin, 1982; McDonald et al., 1984a, 1984b, Ruehle, 1985).

Apple. Growing apple seedlings in CuCO₃-treated containers increased root growth (Table 1), primarily by increases in fresh weight of old (present at planting) roots. CuCO₃ treatments significantly ($P \leq 0.05$) increased

the number of new root tips present on apple seedlings at transplanting (day 105 from planting) compared to untreated plants, 1076 vs. 549. CuCO₃ treatments tended to increase root regeneration outside the original rootball following transplanting (Table 1, day 153). Root fresh weight gains within the original root ball were considerably less in unpruned seedlings transplanted from CuCO₃-treated containers than for those from untreated containers (Table 1, day 153). This difference was reflected in total root fresh weight increases (Table 1, day 153) as significantly less fresh weight accumulation in unpruned than in root-pruned CuCO₃-treated seedlings. While root pruning apple had little effect on root fresh weight gains of untreated seedlings, root pruning actually tended to stimulate root regeneration in seedlings grown in CuCO₃-treated containers (Table 1, day 153), particularly roots regenerated outside the original root ball.

Data collected at 105 days from planting (at transplanting) indicated that apple seedlings grown in CuCO₃-treated containers had significantly greater shoot length, fresh weight of new shoots, and total shoot fresh weight increases than seedlings grown in untreated containers (Table 2). After transplanting (Table 2, day 153), CuCO₃ treatments had no significant effects on increases in new shoot or combined new (since planting) and old (present at planting) shoot fresh weight gain. Root pruning had little effect on shoot fresh weight gain.

Levels of *t*-ZR in xylem sap were higher (35.9 p mol/0.1 ml) before budbreak (day 0, $P \leq 0.05$) than later in the growing season (13.9 p mol or less). Xylary cytokinin levels in apple have been reported to decrease shortly after budbreak occurs (Belding and Young, 1989; Young, 1989). No significant ($P \leq 0.05$) changes in response to CuCO₃ treatment or root pruning in *t*-ZR activity levels were found. No significant ($P \leq 0.05$) correlations between *t*-ZR levels and any root growth characteristic were found. Thus, increases in shoot growth in response to CuCO₃ and decreases in shoot growth in response to root pruning do not appear to be due to changes in xylary levels of *trans*-zeatin ribeside-like activity in apple.

Green ash. While root fresh weights of green ash seedlings grown in CuCO₃-treated containers were not affected (Table 1, day 105), the fibrosity of the root system was drastically increased. Seedlings grown in CuCO₃-treated containers had more ($P \leq 0.05$) (2046 vs. 1379) new root tips present at transplanting (day 105 from planting) than untreated plants. CuCO₃ treatment tended to increase root regeneration outside the original rootball following transplanting (Table 1, day 153). Root fresh weight gains inside the root ball and in total root growth followed a similar pattern as new root regeneration outside the original root ball (Table 1). Root pruning green ash tended to reduce root regeneration both inside and outside the original root ball (Table 1, day 153).

Green ash seedlings tended to have similar shoot growth responses as apple seedlings

grown in CuCO_3 -treated containers (Table 2, day 105) relative to untreated seedlings, although differences for ash were not significant. Seedlings transplanted from CuCO_3 -treated containers tended to have greater shoot elongation than those transplanted from untreated containers (Table 2, day 153), but root pruning negated the increased response of CuCO_3 -treated seedlings. Root pruning tended to reduce both new and old shoot fresh weight gain of green ash seedlings (Table 2, day 153). Changes in old shoot fresh weight gain would likely indicate changes in cambial growth among treatments.

General. Growing seedlings in CuCO_3 -treated containers increases root system fibrosity in conifers (Burdett and Martin, 1982; McDonald et al., 1984a, 1984b) and increases the number of lateral roots formed on individual green ash roots (Arnold and Struve, 1989a). This study shows quantitatively that, on a whole-plant basis, CuCO_3 treatments induce sizeable increases in the number of new roots regenerated in two deciduous hardwoods. CuCO_3 treatments have been shown to increase root penetration outside the original root ball in conifers (McDonald et al., 1984a) and in hardwoods (Arnold and Struve, 1989b). The present study confirmed these results for green ash and extended them to apple.

In general, root pruning had fewer adverse effects on vegetative growth of apple than on green ash seedlings following transplanting (Tables 1 and 2, day 153). In some instances, root pruning actually stimulated root growth of CuCO_3 -treated apple seedlings (Table 1, day 153). These differences in response to root pruning may be due in part to differences in the amount of the root system removed by recommended practices for correcting root system deformation. In apple, on a fresh weight basis, root pruning removed 27% (3.53 g) of the root system of untreated seedlings compared to 19% (2.86 g) in CuCO_3 -treated seedlings, whereas in green ash, root pruning removed a much larger percentage (50%; 9.06 g) of the untreated seedlings' root system, than for the CuCO_3 -treated system (20%; 3.70 g). The fresh weight of removed roots of root-pruned green ash seedlings transplanted from untreated containers was negatively correlated with new shoot length ($R = -0.63$, $P \leq 0.05$), but positively correlated with new shoot fresh weight ($R^2 = 0.68$, $P \leq 0.05$) in those transplanted from CuCO_3 -treated containers. Had root pruning occurred later in the season, when more roots were deflected along the medium-container wall interface in un-

treated apple seedlings, they might have responded to root pruning similarly to green ash. Regardless, root pruning of CuCO_3 -treated plants was not necessary to correct root deformation, while it was necessary in plants grown in untreated containers. Avoiding root deformation by the use of CuCO_3 -treated containers results in equal or greater root regeneration and shoot growth (Tables 1 and 2), both before and after transplant, than does growing seedlings in untreated containers and root pruning to correct root deformation at transplanting.

New information provided by this study shows that CuCO_3 -treated containers can effectively control root deformation in young apple seedlings and result in a quantitative increase in the number of new roots regenerated on a whole-plant basis in green ash and apple seedlings. Even though the number of new root tips was nearly doubled in both species by CuCO_3 treatments, there were no significant correlations between the number of new root tips and xylary cytokinin levels. Also, xylary cytokinin levels were not significantly altered by root pruning or CuCO_3 treatment. While root pruning generally had adverse effects on vegetative growth of both species, the need for this treatment can be avoided by growing seedlings in CuCO_3 -treated containers. CuCO_3 treatments increased some vegetative growth characteristics of apple and green ash seedlings while they were in CuCO_3 -treated containers and following transplanting.

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