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## Rooting Softwood Cuttings of *Acer griseum*: Promotion by Stockplant Etiolation, Inhibition by Catechol

Brian K. Maynard and Nina L. Bassuk

Department of Floriculture and Ornamental Horticulture, Cornell University, Ithaca, NY 14853

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Abstract. Effects of stockplant etiolation, stem banding, exogenous auxin, and catechol on the rooting response of softwood cuttings of paperbark maple (Acer griseum Pax.) were studied. Etiolated cuttings rooted better than light-grown cuttings, while stem banding did not affect rooting percentage (light-grown, 10%; light-grown + banding, 18%; etiolated, 41%; etiolated + banding, 37%). IBA did not promote, but catechol inhibited rooting (control, 31%; IBA, 37%; catechol, 17%; IBA + catechol, 21%). Root number was increased by IBA and unaffected by catechol. The distance from the cutting base to the first emerged root was measured as an indication of auxin toxicity. IBA interacted with etiolation and stem banding to increase this distance, which was greater in catechol-treated cuttings. Chemical names used: 1,2-benzenediol (catechol); 1H-indole-3-butanoic acid (IBA).

Paperbark maple is a desirable ornamental trees species that has presented a challenge to plant propagators for many years. Seed germination is normally poor (Fordham, 1969), grafting success is limited, and cuttings are generally considered difficult to root (Dirr and Heuser, 1987). However, several studies have reported some success with softwood cutting propagation of seedling material (Dirr and Heuser, 1987, p. 83). Of special interest is a study by Kling and Meyer (1983) in which cuttings from 2-year-old seedlings were soaked for 24 hr in 1H-indole-3 acetic acid (IAA) and the phenolic compound catechol. Up to 100% rooting was obtained after a 24-hr pre-sticking soak in a 2% aqueous ethanol (EtOH) solution of 1.1  $\times$  10<sup>-3</sup> M IAA plus 4.5  $\times$  10<sup>-3</sup> M catechol (193 and 495 mg·liter<sup>-1</sup>, respectively). Their study suggested that catechol might have acted as rooting cofactor which, in conjunction with exogenous IAA, promoted rooting of several difficult-to-root maple species.

Stockplant etiolation and stem banding with Velcro have been used to stimulate rooting of softwood cuttings from both young (1-year-old seedling) and mature (30-year-old specimen) stockplants of *Acer griseum* (Maynard and Bassuk, 1987). Though im-

provements in rooting response were demonstrated, the best rooting percentages observed were markedly lower than those of Kling and Meyer (34% vs. 100%).

The objective of the present study was to investigate a combination of the methods used in the studies described above, specifically, the influences of etiolation and exogenously applied auxin and catechol on adventitious root formation in cuttings. The study was designed as a  $2 \times 2 \times 2 \times 2$  factorial to test the effects of stockplant etiolation, Velcro-banding with IBA, and auxin or catechol soaks on the rooting of softwood basal stem cuttings of A. griseum. This study was undertaken to incorporate certain aspects of the work of Kling and Meyer (1983), not to replicate their study. Based on the assumption that IAA is more readily degraded than IBA in the course of a 24-hr soak (Dirr and Heuser, 1987, p. 37), we chose to substitute IBA for IAA.

Twenty 5-year-old field-grown seedling stockplants of *A. griseum* (Schichtel's Nursery, Orchard Park, N.Y.; provenance unknown) were potted into 2-liter plastic containers in a medium of 1 sandy loam soil: 1 sphagnum peatmoss: 1 perlite (by volume) and placed in cold storage at 5C for 8 weeks. On 1 Dec. 1987, all plants were placed in a greenhouse maintained at an average day/ night temperature of 2OC. Incandescent lamps (100 W), suspended ≈3 m above the stockplants and spaced 1 m apart, were used from 4:00 PM to 12:00 PM to extend the natural photoperiod to ≈16 hr (irradiance not deter-

mined). Stockplants were fertilized weekly with 200 mg 20N-10P-20K/liter. Ten of the stockplants were forced under black cloth (>98% shade) to produce typical etiolated growth (lack of color, lengthened internodes, succulence). Remaining stockplants were grown without shade. Two weeks later, shoots from one-half of the light-grown and etiolated plants were basally banded using 2.5-cm wide strips of Velcro coated with 0.8% IBA in talc, the standard procedure for stem banding developed by Maynard and Bassuk (1987). Etiolated plants were then acclimated to full sunlight over 4 days by first removing the cover on the north side, and then gradually rolling the cloth back each day until all 10 plants were in full sun. Basal shoot cuttings were taken 4 weeks later from positions throughout the stockplant crown and cut to a length of 7 to 10 cm with one pair of leaves. The terminal growth was removed. Banded shoots were cut immediately proximal to the Velcro bands and the bands removed. Four types of softwood cuttings were taken: 1) light-grown, nonbanded shoots (controls); 2) light-grown, banded shoots (banded); 3) etiolated shoots that had greened for 4 weeks (etiolated); and 4) etiolated and banded shoots, which yielded a cutting with an etiolated base (etiolated and banded).

All the cuttings were wounded along one side (≈2 cm) by scraping to remove the bark, and the basal 1 cm of each cutting was soaked for 24 hr at room temperature in one of four solutions containing 2% ethanol: 2% ethanol only (control);  $4.9 \times 10^{-4}$  M IBA (100 mg·liter<sup>-1</sup>);  $9.1 \times 10^{-4}$  M catechol (100) mg·liter $^{-1}$ ); or 4.9  $\times$  10 $^{-4}$  M IBA and 9.1 × 10<sup>-4</sup> M catechol. Cuttings of each treatment were then distributed randomly into five groups of six cuttings each before insertion to a depth of ≈1 cm in a medium of 2 perlite : 1 sphagnum peatmoss (v/v) in a completely randomized design. Rooting took place under mist (6 sec every 4 min from 6:00 AM to 8:30 PM), with bottom heat (25C), and a 16-hr photoperiod (see above) provided by 100 W incandescent lamps suspended 1 m above the medium and spaced 1 m apart. Cuttings were evaluated for rooting after 38 days. Cuttings were considered rooted if they possessed one or more roots >1 mm long. Rooting percentage, the number of roots per rooted cutting, and the distance from the cutting base to the first emerged root were recorded. Data were analyzed using the PROC GLM option of SAS (SAS Institute, 1985); percentage data were transformed to arcsin square root before analysis (Snedecor and Cochran, 1980).

An analysis of variance (Table 1) shows main effects and interactions among treatments. All third- and fourth-order interactions were nonsignificant and were pooled in

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Table 1. Analysis of variance of a 2 × 2 × 2 × 2 factorial examining the effect of etiolation, stem banding, exogenous auxin, and catechol on the rooting of softwood cuttings of *Acer griseum*. Characteristics evaluated after 38 days included rooting percentage, number of roots per rooted cutting, and distance from cutting base to first emerged root. The analysis is based on a completely randomized design of five replicates of six cuttings per treatment. Rooting percentages were transformed to their arcsin<sup>1/2</sup> equivalents before analysis (Snedecor and Cochran, 1980).

Source State And Link	Partial sums of squares				
	df	Arcsin rooting percentage	Roots per rooted cutting	Distance to first emerged root	
Stockplant treatment	31319	5436.7***	762.2*	673.1**	
Etiolation (ET)	or (shorte1 7/2)	5125.5***	18.9	20.9	
Banding (BAND)	no dosf) Idaw	31.2	734.7**	487.8**	
ET × BAND	es. Ducculence	290.0	46.1	35.4	
Cutting soak	3	2023.7*	2082.0***	1884.0***	
IBA	1	184.7	1983.3***	2255.4***	
Catechol (CAT)	1	1847.6*	1.2	281.2*	
$IBA \times CAT$	1	2.2	44.5	138.8	
Interactions	9	1050.3	751.3	1512.3*	
$ET \times IBA$	ta odi otta	2.2	4.8	302.1*	
$ET \times CAT$	1	4.0	15.6	86.1	
$BAND \times IBA$	belautifd 1.478	105.9	584.0**	659.4**	
$BAND \times CAT$	laus Hut d ba	51.6	51.1	36.2	
Error mean square	63	188.5	67.2	68.4	

\*\*\*,\*\*,\*Significant at PR > F < 0.001, 0.01, or 0.05, respectively.

the residual error. The percentage of cuttings that formed roots was increased by stock-plant etiolation (14%, light-grown; 39%, etiolated) and decreased by a cutting presoak in catechol (34%, not soaked in catechol; 19%, soaked). Etiolated cuttings soaked in 2% EtOH (control) rooted the best (54%), while light-grown cuttings treated with catechol rooted poorly (4%). The IBA presoak and Velcro-banding treatments did not affect rooting percentage. No treatment interactions affecting rooting percentages were apparent.

In contrast to their effects on rooting percentage, etiolation and catechol had no effect on the number of roots produced per rooted cutting (Table 1). The IBA soak before sticking increased the mean number of roots produced to 9.1, while, in combination with Velcro-banding, the number of roots increased to 19.4. Velcro-banding alone increased the mean number of roots to only 3.1, while nontreated cuttings produced 2.7 roots.

In assessing rooting, we observed that roots frequently emerged above a rootless

Table 2. Effects of etiolation and banding stockplant treatments and an IBA cutting soak (4.9  $\times$  10<sup>-4</sup> M) on the distance (mm) from the cutting base to the first emerged adventitious root on softwood cuttings of *Acer griseum* at 38 days.

IBA soak*	Distance (mm) base to first root				
	Etiolationz		Stem banding <sup>y</sup>		
	Without	With	Without	With	
Without	4.4	3.1	3.0	3.8	
With	9.9	18.4	22.0	10.4	

<sup>2</sup>Etiolation achieved by forcing shoots in darkness for 2 weeks, followed by gradual acclimation to full sunlight.

yStem banding applied using Velcro strips 2.5 cm wide coated with IBA (0.8% in talc) placed on the stem at the site of the cutting base for 1 month. \*Cuttings bases soaked to a depth of 1 cm for 24 hr at room temperature.

zone of either living or necrotic tissues. This phenomenon suggested a toxicity syndrome that has been reported by researchers using high levels of auxin in the propagation of cuttings (Chong, 1981; Dirr and Heuser, 1987). Therefore, the distance from the cutting base to the first emerged root was measured as an indicator of the degree of rooting inhibition caused by treatments to the cutting base. The distance from the cutting base to the first emerged root was generally greater in IBA-treated cuttings (Table 2). This distance was less in IBA-treated cuttings prepared from banded shoots, while stockplant etiolation interacted with the IBA soak to increase the distance from the cutting base to the first emerged root. Soaking the cuttings in catechol, independent of other treatments, increased this distance (8.7 mm without catechol; 13 mm with).

Rooting percentages observed in this study were much lower than those of Kling and Meyer (1983). Considering equivalent treatments, light-grown, nonbanded shoots soaked in IBA plus catechol, in this work, yielded 11% rooting, as opposed to 60% rooting in the previous study. Reasons for this difference might include the stockplant age (5-vs. 2-year-old seedlings), the type of auxin used (IBA vs. IAA), or differences in cultural conditions. We used the intermediate level of the auxin/catechol factorial examined in the study by Kling and Meyer (1983) in anticipation of observing an etiolation/banding response. Indeed, a significant improvement in rooting was observed when shoots were grown initially in darkness. However, in contrast to previous etiolation work with A. griseum (Maynard and Bassuk, 1987), etiolation and banding did not interact to increase rooting percentage, perhaps because of the generally improved rooting of etiolated cuttings in the present study. Comparing equivalent treatments, the highest rooting response achieved in the present study was 50% (etiolated or etiolated and banded cuttings soaked in IBA before sticking), as opposed to 34% in our previous report for cuttings that had been etiolated and Velcro-banded with IBA on the band. In the present study, catechol reduced rooting, in contrast to the finding of Kling and Meyer (1983). However, lack of an auxin effect on rooting in our study was consistent with the observations of Kling and Meyer (1983), who observed nonsignificant increases of 0% to 20% with IAA treatment.

The number of roots produced per rooted cutting did not appear to respond catechol or IAA in the study by Kling and Meyer (1983), but we found an IBA response that yielded, on average, between 9.1 (without banding) and 19.4 (with banding) roots per cutting. The interaction of IBA and banding might represent a response to the total amount of IBA applied to the cutting base, as the combination of Velcro-banding with IBA and an IBA soak corresponds to two exposures of the cutting base to auxin before sticking. A significant response of root number to Velcro-banding was seen only in IBA-treated cuttings.

Though IBA generally increased the distance from the cutting base to the first emerged root, the IBA response was complicated by interactions occurring between IBA and both etiolation and banding. Banded cuttings soaked in IBA produced roots nearer the cutting base than did nonbanded cuttings. This result is in contrast to what might be anticipated regarding the effect of auxin concentration on rooting, as there was apparently no effect of exposing the cutting to IBA twice (i.e., IBA on a band and an IBA soak) on the distance of rooting from the base. Perhaps some other aspect of the banding treatment, such as wounding or the more complete exclusion of light, promoted rooting closer to the cutting base, despite the presence of a higher auxin level. Etiolated cuttings soaked in IBA rooted farther from the cutting base, suggesting that, initally, etiolated stem tissues might have been more sensitive to IBA, e.g., exogenous IBA might supplement an already greater endogenous titer of IAA in etiolated stem tissues. Catechol also increased the distance from the cutting base at which rooting occurred, independent of other treatments, suggesting an inhibition of rooting unrelated to exogenous auxin application.

In conclusion, stockplant etiolation improved the rooting percentage of softwood basal stem cuttings from 5-year-old plants of Acer griseum. Stem banding alone had little effect on rooting percentage or the number of roots formed, while stem banding interacted with the application of hormone at sticking to increase the number of roots produced per cutting, suggesting an effect of the number of hormone applications on root initiation. IBA applied as a pre-cutting soak had no effect on rooting percentage, but increased the number of roots formed per cutting and the distance from the cutting base at which rooting occurred. Catechol acted to reduce rooting potential by reducing the percentage of cuttings that rooted and by inhibiting the development of roots near the cutting base. No interactions were observed between stockplant etiolation or banding of stems and exogenously applied catechol.

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