Foliar Auxin Application Improves Adventitious Rooting of Wall Germander Cuttings

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SUMMARY. Indole-3-butyric acid (IBA) is frequently used to promote adventitious root development in plant propagation from cuttings. We evaluated the effects of 0, 1000, or 3000 ppm IBA applied as a liquid foliar spray or talc-based basal dip on adventitious rooting of wall germander (*Teucrium chamaedrys*) cuttings. An initial experiment was conducted in Fall 2016, followed by a replication in Spring 2017. Two-dimensional root area, primary root count, root dry weight, and a rooting index (0 to 5 scale) were assessed as measures of root growth. By all metrics and for both experiments, cuttings benefited from IBA application and exhibited equal or greater root growth after the foliar spray treatment compared with the talc dip. In both experiments and for all metrics, the talc dip method achieved no greater root growth at 3000 ppm IBA than the foliar spray method at 1000 ppm IBA. These results suggest that for wall germander, IBA application by the foliar spray method is equal or superior to the talc dip method presently in widespread use in the horticulture industry.

Wall germander is a woody subshrub in the Lamiaceae family native to the Mediterranean Basin. It bears small, light pink flowers in early summer and is commonly grown as a low hedge or groundcover in mild climates (Armitage, 1997; Brickell and Cathey, 2004). Wall germander can be propagated from cuttings without adding auxin, but rooting is more rapid and uniform with the application of 1000–3000 ppm IBA (Dirr and Heuser, 2006).

Auxins are a class of plant growth regulators that affect many plant physiological processes, including rhizogenesis. The endogenous auxin indole-3-acetic acid plays the most noteworthy role, although there are several other endogenous auxins including IBA, and many synthetic analogs with auxinic effects (Taiz et al., 2015). For many years, talc- or water-diluted IBA formulations applied to the basal end of cuttings have been used in the nursery industry to induce rhizogenesis in cuttings for plant propagation (Hartmann et al., 2011).

The application of dilute liquid auxin directly to the foliage of cuttings could reduce production costs,

increase efficiency, and simplify compliance with workplace safety regulations (Blythe et al., 2004a). Workers exposed to even low concentrations of aerosolized talcum powder are legally required to wear personal protective equipment including a respirator, gloves, protective eyewear, long sleeves, and long pants (Brooker Chemical Corp., n.d.; OHP, Inc., 2010). The efficacy of foliar spray application of IBA to promote rhizogenesis has only been reported for a few species and the method has had mixed results (Blythe et al., 2002; Drahn, 2007). The method has been effective in the propagation of chrysanthemum (*Chrysanthemum pacificum*) and forsythia (*Forsythia ×intermedia* ‘Lynwood Gold’) cuttings but has been less successful with crape myrtle (*Lagerstroemia indica*), glossy abelia (*Abelia ×grandiflora*), and weeping fig (*Ficus benjamina*) cuttings (Blythe et al., 2003b, 2004a; Kroin, 1992). Commercial growers might consider foliar IBA rates and species efficacy proprietary information,

leading to anecdotal accounts that lack data. Furthermore, suppliers of unrooted cuttings might make recommendations for foliar auxin application without providing supporting data (Yoder Brothers, 2004). At present there is no detailed research on the effects of foliar auxin application in many plant species, including wall germander or any other species in the Lamiaceae. The objective of this study was to evaluate the foliar auxin spray method for rooting wall germander cuttings.

Materials and methods

Semi-hardwood terminal cuttings of wall germander were collected on 23 Sept. 2016 for the first experiment and 25 Mar. 2017 for the second experiment. Each cutting was ≈8 cm long, with two or three nodes. Exclusion criteria included the presence of flowers or flower buds, branches, disease symptoms, mechanical damage, or atypical growth. Cuttings were treated with 0, 1000, or 3000 ppm IBA, either as a foliar spray of water-diluted potassium-IBA salts (IBA Water Soluble Salts; Hortus USA Corp., New York, NY) until runoff or as a basal dip in talc-diluted IBA powder (Rhizopon; Phytotronics, Earth City, MO). Control treatments were deionized water for the foliar spray and 100% pure talc for the basal quick-dip. To mimic the proposed commercial application, foliar treatments were applied after cuttings were stuck, whereas talc treatments were applied before sticking. Cuttings were stuck in 2:1 sphagnum peat (Sunshine Select Canadian Sphagnum Peat Moss; Sun Gro Horticulture, Agawam, MA) and coarse perlite (NorCal, Richmond, CA) in square rose pots [2.25 inches top width, 3.25 inches depth, and 185 cm³ volume (SR225X; McConkey Co., Sumner, WA)]. Each pot was an experimental unit and received one cutting. The pots were placed in a greenhouse with daytime mist for

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
16.3871	inch ³	cm ³	0.0610
28,350	oz	mg	3.5274 × 10 ⁻⁵
1	ppm	µg·g ⁻¹	1
	°F	°C	(°C × 1.8) + 32

20 s every 3 min in a randomized complete block design. Mean atmospheric temperatures near the cuttings were $\approx 77 \pm 10$ °F and substrate temperatures were $\approx 76 \pm 10$ °F. In

Fall 2016, the first three blocks were harvested after 69 d on 1 Dec. 2016, whereas the remaining four blocks were harvested after 75 d on 7 Dec. 2016. In Spring 2017, two blocks were

harvested after 61 d on 25 May 2017, two blocks after 66 d on 30 May 2017, and three blocks after 68 d on 1 June 2017. The harvests were staggered when the initial blocks did not show sufficient root growth or when time constraints precluded a single harvest.

Roots were washed with a spray nozzle and then dried. A rooting index was assigned on a 0 to 5 scale: 0 = no roots, 1 = one or two roots, 2 = more than two roots but minimal rooting, 3 = moderate rooting but not sufficient for transplanting, 4 = sufficient roots for transplanting, and 5 = optimum roots for transplanting (Fig. 1). The roots were then excised from the cutting onto a transparent cellulose acetate sheet with care to avoid overlapping roots and scanned at 600 dpi against a white background (Perfection V19; Epson America, Long Beach, CA). Root tissue was oven dried at 72 °C for 48 h, then weighed to determine dry weight. Scans were analyzed with ImageJ (version 1.50a; National Institutes of Health, Bethesda, MD) to determine mean root two-dimensional area

Fig. 1. A 0 through 5 rooting index for wall germander cuttings (0 = no roots, 1 = one or two roots, 2 = more than two roots but minimal rooting, 3 = moderate rooting but not sufficient for transplanting, 4 = sufficient roots for transplanting, and 5 = optimum roots for transplanting).

Table 1. Analysis of variance table for rooting data from a repeated experiment with wall germander cuttings treated with 0, 1000, or 3000 ppm ($\mu\text{g}\cdot\text{g}^{-1}$) indole-3-butyric acid applied via a talc dip or a foliar spray ($n = 7$). The leftmost column contains the four root response metrics, each with the factors analyzed in the model: block, auxin rate, auxin application method, and the interaction between auxin rate and application method.

Source of variation	Fall 2016				Spring 2017			
	df	SS	MS	P	df	SS	MS	P
Two-dimensional root area (cm^2)								
Block	6	573.10	95.52	0.070	6	2,932.53	488.75	<0.001
Auxin rate	2	1,869.31	934.66	<0.001	2	968.59	484.30	<0.001
Application method	1	517.87	517.87	0.002	1	7.38	7.38	0.552
Auxin rate $\times$ application method	2	129.22	64.61	0.241	2	88.42	44.21	0.132
Error	30	1,300.31	43.34		30	611.32	20.38	
Primary roots (no.)								
Block	6	643.62	107.27	0.493	6	18,761.29	3,126.88	<0.001
Auxin rate	2	8,387.29	4,193.65	<0.001	2	27,564.14	13,782.10	<0.001
Application method	1	3,828.60	3,828.6	<0.001	1	1,000.60	1,000.60	0.112
Auxin rate $\times$ application method	2	808.62	404.31	0.044	2	5,090.05	2,545.03	0.004
Error	30	3,486.67	116.22		30	11,212.71	373.76	
Root dry wt (mg)								
Block	6	0.0015	0.0003	0.280	6	0.0129	0.0022	<0.001
Auxin rate	2	0.0063	0.0032	<0.001	2	0.0036	0.0018	<0.001
Application method	1	0.0013	0.0013	0.013	1	0.0000	0.0000	0.692
Auxin rate $\times$ application method	2	0.0004	0.0002	0.317	2	0.0004	0.0002	0.122
Error	30	0.0056	0.0002		30	0.0026	0.0000	
Rooting index (0 to 5 scale)								
Block	6	11.33	1.89	0.316	6	25.90	4.32	<0.001
Auxin rate	2	30.05	15.03	0.001	2	19.61	9.81	<0.001
Application method	1	9.52	9.52	0.018	1	0.38	0.38	0.440
Auxin rate $\times$ application method	2	4.62	2.31	0.237	2	1.33	0.67	0.355
Error	30	45.81	1.53		30	18.66	0.62	

SS = sum of squares; MS = mean squares.

(square centimeters) for each cutting. The contrast of the original images was first enhanced by 0.2%, then the images were converted to binary, with roots as black pixels and background as white pixels. Two-dimensional root area was defined as the total area of black pixels. The root scans were also used to assess primary root count, which was defined as the number of primary roots longer than 3 mm arising from the basal end of the cutting. This threshold was set because roots smaller than 3 mm were often difficult to distinguish from undifferentiated callus.

Data were assessed for normality via residual probability plots and analyzed with a three-factor analysis of variance followed by Tukey's honestly significant difference tests as applicable [$\alpha = 0.05$ (JMP version 12 Pro; SAS Institute, Cary, NC)].

Results

BLOCK EFFECTS. There were significant block effects for two-dimensional root area, primary root count, root dry weight, and rooting index in the Spring 2017 experiment. These effects may have been due to a moisture gradient resulting from air circulation fans blowing mist more heavily to one side of the bench than the other, from the staggered harvest dates, or from edge effects. However, because each block contained one of each treatment, the potential effects of these extraneous variables were evenly distributed among all the treatments. There were no secondary or tertiary interactions involving block effects.

TWO-DIMENSIONAL ROOT AREA. In the Fall 2016 experiment, auxin concentration ($P < 0.001$) and the auxin

application method ($P = 0.002$) had significant effects on two-dimensional root area (Table 1). Root area increased with increasing auxin concentration, and the foliar spray method led to larger root areas than the talc dip method at all concentrations (Fig. 2). In the Spring 2017 experiment, auxin concentration ($P < 0.001$), but not the application method, had a significant effect, with root area increasing as the auxin concentration increased from 0 to 1000 or 3000 ppm IBA. The largest root areas were achieved with a foliar spray of 1000 ppm IBA or a talc dip of 3000 ppm IBA (Table 2).

PRIMARY ROOT COUNT. In the Fall 2016 experiment, there was a significant interaction between auxin concentration ($P < 0.001$) and the auxin application method ($P < 0.001$) (Table 1). Root count increased with increasing auxin concentration, but the foliar spray at 1000 ppm led to root counts comparable with that of the talc dip at 3000 ppm (Fig. 3). The foliar spray method at 3000 ppm led to the highest root counts (Table 2). In Spring 2017, only auxin concentration had a significant effect ($P < 0.001$), with primary root count increasing as the auxin concentration increased from 0 to 1000 to 3000 ppm IBA (Fig. 4). The foliar spray method reached its maximum number of primary roots at 1000 ppm IBA, whereas the talc dip did so at 3000 ppm IBA (Table 2).

ROOT DRY WEIGHT. In the Fall 2016 experiment, auxin concentration ($P < 0.001$) and the application method ($P = 0.013$) had significant effects on root dry weight (Table 1). Increasing auxin concentration led to

Fig. 2. Two-dimensional root area data for wall germander cuttings receiving indole-3-butyric acid treatments of 0, 1000, or 3000 ppm applied as a foliar spray or talc dip. On each chart, treatments not sharing a letter in a year are significantly different according to a Tukey honestly significant difference test at $P < 0.05$ ($n = 7$); 1 ppm = 1 $\mu\text{g}\cdot\text{g}^{-1}$, 1 $\text{cm}^2 = 0.1550 \text{ inch}^2$.

Table 2. Adventitious rooting least square means data for wall germander cuttings receiving indole-3-butyric acid (IBA) treatments of 0, 1000, or 3000 ppm applied as a foliar spray or talc dip ($n = 7$).

IBA level (ppm) ^z	Application	Fall 2016				Spring 2017			
		Two-dimensional root area (cm²) ^z	Primary roots (no.)	Rooting index (0 to 5 scale) ^y	Root dry wt (mg) ^z	Two-dimensional root area (cm²)	Primary roots (no.)	Rooting index (0 to 5 scale)	Root dry wt (mg)
0	Talc dip	3.3 a ^x	4.7 a	2.3 a	9.4 a	11.7 ab	26.1 a	2.9 a	24.0 a
0	Foliar spray	7.4 a	11.6 a	2.7 ab	15.0 a	11.4 a	30.1 ab	3.1 a	27.3 ab
1,000	Talc dip	8.9 ab	13.4 a	3.0 abc	20.7 ab	18.9 bc	58.4 bc	4.0 ab	41.7 bc
1,000	Foliar spray	20.8 c	40.4 bc	4.6 bc	41.0 bc	23.7 c	97.6 d	4.6 b	49.0 c
3,000	Talc dip	19.1 bc	31.0 b	4.6 bc	38.0 bc	23.2 c	93.0 d	4.7 b	48.7 c
3,000	Foliar spray	24.1 c	54.4 c	4.9 c	45.6 c	21.2 c	79.1 cd	4.4 b	41.6 bc

^z 1 ppm = 1 $\mu\text{g}\cdot\text{g}^{-1}$; 1 $\text{cm}^2 = 0.1550 \text{ inch}^2$; 1 mg = 3.5274 $\times 10^{-5}$ oz.

^y 0 = no roots; 1 = one or two roots; 2 = more than two roots but minimal rooting; 3 = moderate rooting but not sufficient for transplanting; 4 = sufficient roots for transplanting; and 5 = optimum roots for transplanting.

^x Treatments not sharing any letters in a column are significantly different according to Tukey honestly significant difference tests at $P \leq 0.05$.

Fig. 3. Primary root count data for wall germander cuttings receiving indole-3-butyric acid (IBA) treatments of 0, 1000, or 3000 ppm applied as a foliar spray or talc dip (Spring 2017). Columns not sharing a letter are significantly different according to a Tukey honestly significant difference test at $P \leq 0.05$ ($n = 7$); 1 ppm = $1 \mu\text{g}\cdot\text{g}^{-1}$.

Fig. 4. Primary root count data for wall germander cuttings receiving indole-3-butyric acid (IBA) treatments of 0, 1000, or 3000 ppm (Fall 2016). Columns not sharing a letter are significantly different according to a Tukey honestly significant difference test at $P \leq 0.05$ ($n = 7$); 1 ppm = $1 \mu\text{g}\cdot\text{g}^{-1}$.

Fig. 5. Root dry weight data for wall germander cuttings receiving indole-3-butyric acid treatments of 0, 1000, or 3000 ppm applied as a foliar spray or talc dip. On each chart, treatments not sharing a letter in a year are significantly different according to a Tukey honestly significant difference test at $P \leq 0.05$ ($n = 7$); 1 ppm = $1 \mu\text{g}\cdot\text{g}^{-1}$, 1 mg = 3.5274×10^{-5} oz.

increasing root dry weight, and the foliar spray method led to larger root dry weight than the talc dip method (Fig. 4). However, at any given concentration, the advantage of the foliar method was not significant (Table 2). In the Spring 2017 experiment, only auxin concentration had a significant effect ($P = 0.004$), with root dry weight increasing as the auxin concentration increased from 0 to 1000 to 3000 ppm IBA (Fig. 5). However, for the foliar spray, the greatest root dry weights were achieved at 1000 ppm IBA, with weights declining at 3000 ppm IBA. Only the foliar spray at 1000 ppm IBA and the talc dip at 3000 ppm IBA yielded significantly greater root dry weights than the controls (Table 2).

ROOTING INDEX. In the Fall 2016 experiment, auxin concentration ($P = 0.001$) and the auxin application method ($P = 0.018$) had significant effects on rooting index (Table 1). The rooting index increased with increasing auxin concentration, and the foliar spray method yielded larger rooting indices than the talc dip method on average (Fig. 6). However, the differences due to the application method were not significant when comparing cuttings at the same concentration (Table 2). In the Spring 2017 experiment, only auxin concentration had a significant effect ($P < 0.001$), with higher concentrations yielding higher rooting indices. The foliar spray method reached a maximum rooting index at 1000 ppm, whereas the talc method reached its maximum at 3000 ppm. However, there were no significant

differences in rooting index among the auxin-treated plants, regardless of method of application or concentration (Table 2).

Discussion

Previous studies of foliar auxin application with other species have shown inferior or equal root response compared with the basal quick-dip method (Blythe et al., 2003a, 2003b, 2004a, 2004b). In our study, wall germander cuttings exhibited equal or superior response to the foliar spray method for all metrics and at both 1000 and 3000 ppm IBA. Consonant with Dirr and Heuser (2006), this study demonstrated improved rooting of wall germander cuttings in response to 1000 or 3000 ppm IBA application, with lesser rooting at 0 ppm IBA.

There were several differences in the rooting response between the Fall 2016 and Spring 2017 experiments. First, the distinct advantage of the foliar spray seen in all four root-response metrics in Fall 2016 was present only in the primary root count in Spring 2017. For the other three metrics, the foliar spray and talc dip methods were comparable. Second, in Fall 2016, there was a consistent increase in root growth with increasing auxin concentration for both the foliar spray and talc dip methods, although the increase in root growth from 0 to 1000 ppm IBA was always greater than that from 1000 to 3000 ppm IBA. By contrast, in Spring 2017, the foliar spray reached its maximum efficacy at 1000 ppm IBA in each of the four metrics, declining slightly at 3000 ppm IBA, whereas the talc dip root response increased with increasing auxin concentration, reaching similar maximum efficacy at 3000 ppm IBA.

These differences may arise from several sources. Plant material was at a different phenological stage in the fall vs. the spring. The soil moisture around the parent plants was much lower in September than in March. The daylength and sunlight intensity were greater and increasing in the spring, whereas they were lower and decreasing in the fall. Outdoor temperatures followed inverse patterns over the two experiments' growth periods.

Although the differences between the spring and fall experiments might prompt questions about seasonal changes in foliar auxin

Fig. 6. Root index data (0 = no roots, 1 = one or two roots, 2 = more than two roots but minimal rooting, 3 = moderate rooting but not sufficient for transplanting, 4 = sufficient roots for transplanting, 5 = optimum roots for transplanting) for wall germander cuttings receiving indole-3-butyric acid treatments of 0, 1000, or 3000 ppm applied as a foliar spray or talc dip. On each chart, treatments not sharing a letter in a year are significantly different according to a Tukey honestly significant difference test at $P \leq 0.05$ ($n = 7$); 1 ppm = $1 \mu\text{g}\cdot\text{g}^{-1}$.

uptake, transport, and metabolism, they do not negate the potential advantages of using the foliar spray method. Regardless of season, our results suggest that a foliar spray treatment is at least as effective as a talc dip treatment for propagating wall germander cuttings. Because the foliar spray method offers other advantages of improved efficiency, employee well-being, and cost, it need only yield comparable root growth to be beneficial to growers. Based on these results, we recommend that growers adopt the foliar spray method for wall germander and increase trialing of the method with other species. Because of the similarity in root response between wall germander cuttings treated with 1000 and 3000 ppm IBA, we recommend testing of lower auxin

concentrations to determine the lowest effective rate.

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