Isopropyl Alcohol and Auxin Application Method Affect Phytotoxicity of Herbaceous Stem Cuttings©[1]

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ABSTRACT

In response to commercial propagators' inquiries regarding potential phytotoxicity of alcohol used in root-promoting solutions for cutting propagation, three experiments were conducted using stem cuttings of three herbaceous plant taxa. Solutions were prepared with three rates of isopropyl alcohol (0%, 25% or 50%) in combination with three rates of indole-3-butyric acid (IBA): 0, 1000, or 2000 ppm (Exp. 1); 0, 100, and 200 ppm (Exp. 2); or a mixture of IBA and 1-naphthalene acetic acid (NAA): 0+0, 500+250, or 1000+500 ppm IBA+NAA, respectively (Exp. 3) and applied to cuttings using the basal quick-dip method (Exps. 1 and 3) or total immersion method (Exp. 2). No stem or leaf burn occurred using the basal quick-dip method, whereas foliar and stem burn occurred on cuttings of *Pelargonium* ×*hortorum* 'Mary Helen' using the total immersion method with solutions containing alcohol (regardless of IBA rate). Results indicate that solutions containing up to 50% alcohol can be used safely when applied using either basal quick-dip or total immersion methods for stem cuttings of *Chrysanthemum* Mammoth™ and *Impatiens* 'Coral'.

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INTRODUCTION

Plant propagation by asexual methods (cuttings, grafting, layering, division, tissue culture, or other methods) is a fundamental activity in nursery plant production (Hartmann et al., 2011). Asexual propagation allows growers to produce new plants from production stock, maintain genetic characteristics of clonal plant selections, and meet consumer demand. The stem cutting method involves promoting initiation of adventitious roots on leafy (and sometimes leafless) stem pieces during the growing season (herbaceous, softwood, or semi-hardwood cuttings) or dormant season (hardwood cuttings) (Hartmann et al., 2011). Auxins are one of several naturally occurring phytohormones in plants and are involved with many plant responses, however, their most important role in plant propagation is to induce adventitious rooting in cuttings (Crawford, 2005). Commercial formulations of indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA), are used in nursery production to initiate rooting, increase rooting percentage, and increase quality and number of roots. These auxin-containing products (commonly referred to as "rooting hormones") are available in liquid, powder (talc), and water-soluble salt form (Blythe et al., 2007). The basal quick-dip method of auxin application is used most often due to its ease of application (Crawford, 2005). Immersion of whole cuttings has been reported to promote excellent rooting on herbaceous and other plant taxa when compared to powder formulations (Hartmann et al., 2011). Translocation of applied rooting hormones has been reported to occur acropetally in xylem with the transpiration stream, then laterally into surrounding tissues (Blythe et al., 2007). Isopropyl alcohol or ethyl alcohol can be used as solvents or carriers for IBA and/or NAA formulations to increase auxin intake. The acid form of auxin is relatively insoluble in water, but can be dissolved in a cosolvent, such as alcohol, before adding

water (Blythe et al., 2007). There have been anecdotal reports that use of alcohol can cause "stem burn" on cuttings; however, no formal research has been reported to adequately establish occurrence of tissue damage on stem cuttings with use of alcohol-based auxin solutions. The objective of this research is to assess potential phytotoxic effects of alcohol on stem cuttings from various plant taxa using methods of applications used in the nursery and floriculture industry. Presence and extent of tissue burn on cuttings of selected commonly grown, herbaceous crops treated with alcohol-based solutions were examined.

MATERIALS AND METHODS

Plant material for cuttings of *Impatiens* L. (interspecific) 'Coral' and *Pelargonium* ×*hortorum* L.H. Bailey 'Mary Helen' were obtained from production plants at the South Mississippi Branch Experiment Station in Poplarville, Mississippi. Cuttings of *Chrysanthemum* L. Mammoth™ 'Yellow Quill' were obtained from Ball Horticultural Company (West Chicago, IL). All cuttings were freshly prepared to a uniform size appropriate for the taxon (**Table 1)** and the lowest basal leaves were removed from each cutting. All flowers and flower buds were removed from cuttings of *Impatiens* 'Coral'. All cuttings received a 1-sec basal dip to a uniform depth (Exps. 1 and 3) or a 5-sec total immersion (Exp. 2) in a solution at ambient temperature containing IBA (Hortus IBA Water Soluble Salts®; Phytotronics Inc., Earth City, MO) at 0, 1000, or 2000 ppm IBA (Exp. 1); 0, 100, or 200 ppm IBA (Exp. 2); 0 + 0, 500 + 250, and 1000 + 500 ppm IBA + NAA (as Dip 'N Grow) (Exp. 3) prepared with isopropyl alcohol to final rates of 0%, 25%, or 50% (by vol.), for a total of nine treatment combinations with 0% alcohol plus 0 ppm IBA or IBA + NAA as the control. Treated cuttings were inserted to a uniform depth into a peat moss and pine bark-based potting mix (Fafard 3B; Conrad Fafard, Agawam, MA) in individual cells of 50-cell propagation trays (PROP-50-RD; T.O. Plastics, Inc., Clearwater, MN) set in carrying trays (FG1020A; J&M Plastics Inc., Royse City, TX). Treated cuttings were assigned to cells using a completely randomized design with 33 cuttings

per treatment and placed under intermittent mist (10 seconds every 10 minutes during daylight hours) in a climate controlled greenhouse.

Maximum photosynthetically active radiation at the level of the cuttings was 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the winter and 522.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the summer. Daily minimum and maximum temperature range varied depending on time of year cuttings were taken (**Table 1**). Temperature and humidity were monitored with a HOBO Pro RH/Temp data logger (Onset Computer Corp., Bourne, MA) placed with the cuttings. Rooted cuttings of *Chrysanthemum* Mammoth™ 'Yellow Quill' were harvested 30 days after treatment (DAT) and cuttings of all other taxa were harvested 50 to 55 DAT (**Table 1**). After cuttings were harvested, rooting substrate was removed from roots with water and individual cuttings were visually assessed for stem and leaf burn [presence of tissue necrosis (yes/no) and extent (percentage of tissue affected)] and mortality. When limited mortality occurred within a treatment, only surviving cuttings were assessed for stem and leaf burn. Root systems were dried individually in a horizontal air flow oven (Model 1680; VWR International/Sheldon Manufacturing, Inc., Cornelius, OR) for a minimum of 48 hours at 50°C to constant weight and the root (or shoot) dry weight recorded. Data were analyzed using linear models (for continuous response data) and generalized linear models (binary response data) using the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC), with auxin rate and alcohol rate as qualitative treatment factors. If the interaction term was not significant ($p \geq 0.20$), the main effects were evaluated; otherwise, simple effects were evaluated. Comparisons of least squares means among three rates of alcohol and three rates of IBA (main effects) or comparisons among the three levels of one treatment factor at each level of the other treatment factor (simple effects) were made using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.10$). A significance level of 0.10 was selected to reduce the chance of a Type II error.

RESULTS AND DISCUSSION

Chrysanthemum Mammoth™ 'Yellow Quill': There was no stem burn or leaf burn observed on any of the chrysanthemum cuttings in all experiments, regardless of alcohol or IBA rate (**Table 2)**. Root dry weight (RDW) varied with alcohol and IBA concentrations in Exp. 1, with the one or two lowest IBA rates tending to produce the greatest RDW, particularly in solutions containing 0% or 25% alcohol, whereas neither treatment factor nor their interaction significantly affected RDW in Exps. 2 and 3. However, in Exp. 3, the greatest mean RDW was produced by cuttings treated with a solution containing no alcohol or IBA + NAA, but there was no consistent pattern (**Table 2**). The greatest mortality (~15%) occurred in Exp. 1 using 50% alcohol with 0 ppm IBA and 2000 ppm IBA compared to no mortality using 50% alcohol with 1000 ppm IBA (**Table 2**). Results indicate that solutions containing up to 50% alcohol can be used safely when applied using either basal quick-dip or total immersion methods for stem cuttings of *Chrysanthemum* Mammoth™.

Impatiens 'Coral': There was no stem burn or leaf burn present on any cuttings of *Impatiens* 'Coral' in any experiment, regardless of alcohol or IBA rate (**Table 1**). Shoot dry weight (SDW) was greatest in Exp. 1 using 0% alcohol with 2000 ppm IBA, but differences were not great enough to suggest that use of IBA has much, if any, impact on crop production. In Exp. 2, SDW was greatest on transplants grown from cuttings treated with solutions containing 50% alcohol compared to 0% and 25% alcohol, regardless of IBA rate. These results are consistent with Boyer et al. (2013) and Crawford (2005) who reported that alcohol allows for improved absorption of auxin, with increased rooting resulting in increased shoot growth. Root dry weight were greater on cuttings treated with the highest rate of IBA + NAA in Exp. 3, and also greater when treated with solutions containing 25% and 50% alcohol compared to solutions containing no alcohol (**Table 3**), results also consistent with Boyer et al. (2013) and Crawford (2005). Neither

treatment factor nor their interaction had any significant effect on mortality in any of the treatments. After 28 days, cuttings in Exp. 1 and Exp. 2 were transplanted to 10-cm square pots (SVT-450; T.O. Plastics, Clearwater, MN), placed into trays (450-S-15 PF; T.O. Plastics) to allow further shoot growth on the rooted cuttings [evaluated as shoot dry weight (SDW)]. Stem epinasty (an upward bending of stem at the base) was observed on the transplants in Exp. 2 that grew from cuttings treated with solutions containing 50% alcohol with 100 ppm and 200 ppm IBA (12.5% and 100% of plants, respectively) (**Table 3**). It has been reported epinasty can occur as a result of an increase in endogenous ethylene when exogenous auxin is applied (Taiz and Zeiger, 2010). These results were similar to those of Reid et al. (1981) with epinasty of poinsettia. Simple effects were assessed for stem epinasty due to a significant interaction between alcohol and IBA, with results suggesting that using 25% or 50% alcohol and 200 ppm IBA with the immersion method may increase ethylene production, causing an epinastic response. Also, general observation indicated reduced root development following transplanting on rooted cuttings that had been treated with solutions containing 25% and 50% alcohol (regardless of IBA rate). These responses may warrant additional research. Results indicate that solutions containing up to 50% alcohol can be used safely with either basal quick-dip or total immersion methods of application for stem cuttings of *Impatiens* 'Coral'. Treatment with IBA + NAA may also promote development of larger root systems compared with nontreated cuttings.

Pelargonium ×hortorum 'Mary Helen': No stem burn or leaf burn was observed on any of the cuttings in Exps. 1 and 3, regardless of alcohol or IBA rate. In Exp. 2, darkening of leaf and stem tissue occurred immediately following the total immersion in solutions containing 25% and 50% alcohol (regardless of IBA rate), indicating rapid damage of tissues by alcohol. There was stem burn and leaf burn present on cuttings in Exp. 2 using solutions containing 25% and 50% alcohol, with 100% stem burn occurring in the latter case, but no stem or leaf burn occurred using solutions with 0% alcohol. Although data analysis indicated a significance effect by IBA rate

and interactions between alcohol and IBA rate in causing stem and leaf burn, there was no consistent pattern; therefore, IBA rate likely had little or no effect on tissue burn (as was clearly the case with percentage of cuttings with leaf burn) (**Table 4**). In Exp. 1, there was no consistent pattern of RDW observed, suggesting that cuttings of *Pelargonium ×hortorum* 'Mary Helen' do not require treatment with an IBA solution to root successfully. In Exp. 2, RDW of surviving cuttings generally tended to be greater with increasing rate of IBA; whereas, in Exp. 3, the greatest RDW occurred with solutions containing 50% alcohol, regardless of IBA + NAA rate (**Table 4).** Mortality in Exp. 1 occurred with all treatments, being different among rates of IBA, but similar among rates of alcohol. Cuttings treated with 2000 ppm IBA had greater mortality compared to cuttings treated with 0 ppm IBA, but similar mortality to cuttings treated with 1000 ppm IBA. In Exp. 2, the greatest mortality occurred using solutions containing 25% or 50% alcohol (regardless of IBA rate). These results are consistent with Kroin (2011) who reported no tissue damage using foliar applied K-salt formulation of IBA - while addition of alcohol caused a decline in rooting percentage. In Exp. 3, cutting mortality was greatest when using 25% and 50% alcohol with 1000 ppm IBA + 500 ppm NAA indicating higher rates of auxin (and not alcohol) may affect cutting mortality. However, mortality was limited when using no alcohol with 1000 ppm IBA + 500 ppm NAA. When using 0% alcohol, mortality was greatest with 500 ppm IBA + 250 ppm NAA compared to 1000 ppm IBA + NAA and no IBA. Likewise, when using 50% alcohol no mortality occurred using 500 ppm IBA + 250 ppm NAA compared to 1000 ppm IBA + 500 ppm NAA and no IBA + NAA. These results indicate cutting mortality was affected by other factors, as noted by Hartmann et al. (2011).

Results indicate that solutions containing alcohol can cause significant stem burn when applied to stem cuttings of *Pelargonium ×hortorum* 'Mary Helen' using the total immersion method; however, solutions containing up to 50% alcohol can be used safely when applied using the basal quick-dip method. Also, treatments of IBA + NAA appears to promote greater root development.

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Table 1. Taxa of herbaceous ornamental crops used to provide stem cuttings, with specifications on cutting preparation, propagation, and harvest.

Botanical Name	Cutting Type	Cutting Length (cm)	Cutting Source	Experiment Number	Propagation Date	Average Daily Min./Max. Greenhouse Temperatures (°F)	Depth of Insertion[z] (cm)	Harvest Date
Chrysanthemum Mammoth™ 'Yellow Quill'	Herbaceous, terminal	5	Purchased from supplier[y]	1	13 Jan. 2016	62±3 - 68±4	1	13 Feb. 2016
				2	13 Jan. 2016	62±3 - 68±4		13 Feb. 2016
				3	13 Jan. 2016	62±3 - 68±4		13 Feb. 2016
Impatiens 'Coral'	Herbaceous, terminal	5	Container-grown stock	1	8-Jul-15	72±4 - 88±5	1	4 Sept. 2015
				2	8-Jul-15	72±4 - 88±5		4 Sept. 2015
				3	11 Dec. 2015	62±3 - 68±4		28 Jan. 2016
Pelargonium ×*hortorum* 'Mary Helen'	Herbaceous, terminal	12.5	Field-grown stock	1	26 Oct. 2015	63±3 - 72±4	1	19 Dec. 2015
				2	26 Oct. 2015	63±3 - 72±4		19 Dec. 2015
				3	26 Oct. 2015	63±3 - 72±4		19 Dec. 2015

[z]Depth of insertion of the cutting into the rooting substrate.
[y]Ball Horticultural Company, West Chicago, IL.

Table 2. Stem and leaf burn (%), mortality (%), and root dry weight (g) of *Chrysanthemum* Mammoth™ 'Yellow Quill' observed using a basal quick-dip with selected rates of alcohol and IBA (Expt. 1) or IBA and NAA (Expt. 3) or using total immersion with selected rates of alcohol and IBA (Exp. 2).

Experiment 1		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		—	—	<.0001	<.0001
IBA		—	—	0.1859	<.0001
Alcohol*IBA		—	—	0.0119	<.0001
Alcohol (%)	IBA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0	0	0	0.0a	0.196a
0	1000	0	0	3.0a	0.182a
0	2000	0	0	0.0a	0.163b
25	0	0	0	0.0a	0.238a
25	1000	0	0	0.0a	0.154b
25	2000	0	0	0.0a	0.169b
50	0	0	0	15.2a	0.117a
50	1000	0	0	0.0b	0.135a
50	2000	0	0	15.2a	0.090b
		Means for Main Effects			
Alcohol (%)	IBA (ppm)				
0		0	0	0	0
25		0	0	0	0
50		0	0	0	0
	0	0	0	0	0
	1000	0	0	0	0
	2000	0	0	0	0

Experiment 2		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		—	—	0.2292	0.5097
IBA		—	—	0.8095	0.1468
Alcohol*IBA		—	—	0.079	0.2347
Alcohol (%)	IBA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0	0	0	0.0b	0.179
0	100	0	0	6.1a	0.172
0	200	0	0	0.0b	0.179
25	0	0	0	3.0ab	0.185
25	100	0	0	0.0b	0.184
25	200	0	0	6.1a	0.184
50	0	0	0	0.0a	0.168
50	100	0	0	0.0a	0.16
50	200	0	0	0.0a	0.199
		Means for Main Effects			
Alcohol (%)	IBA (ppm)				
0		0	0	0	0.177a
25		0	0	0	0.185a
50		0	0	0	0.176a
	0	0	0	0	0.177a
	100	0	0	0	0.172a
	200	0	0	0	0.188a

Experiment 3		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		—	—	0.3462	0.4367
IBA+NAA		—	—	0.0151	0.6767
Alcohol*IBA+NAA		—	—	0.3743	0.009
Alcohol (%)	IBA+NAA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0+0	0	0	6.1	0.179a
0	500+250	0	0	0.0	0.146b
0	1000+500	0	0	0.0	0.160ab
25	0+0	0	0	6.1	0.141a
25	500+250	0	0	0.0	0.155b
25	1000+500	0	0	0.0	0.170ab
50	0+0	0	0	0.0	0.147a
50	500+250	0	0	0.0	0.162a
50	1000+500	0	0	0.0	0.150a
		Means for Main Effects			
Alcohol (%)	IBA+NAA (ppm)				
0		0	0	2.0a	0
25		0	0	2.0a	0
50		0	0	0.0a	0
	0+0	0	0	4.0a	0
	500+250	0	0	0.0b	0
	1000+500	0	0	0.0b	0

When the interaction term in the model is significant ($p \leq 0.20$), simple effects means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.10$); otherwise, the treatment means are presented without letter groupings for informational purposes. When the interaction term in the model is not significant ($p > 0.20$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.10$).

Table 3. Stem and leaf burn (%), stem epinasty (%), mortality (%), and root or shoot dry weight (g) of *Impatiens* 'Coral' observed using a basal quick-dip with selected rates of alcohol and IBA (Exp. 1) or IBA and NAA (Exp. 3) or using total immersion with selected rates of alcohol and IBA (Exp. 2).

Experiment 1		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Shoot Dry Weight (g)	Stem Epinasty (%)
		Significance of Treatment Factors (P Values)				
Alcohol		—	—	0.3692	0.5939	0
IBA		—	—	0.3692	0.4632	0
Alcohol*IBA		—	—	0.4079	0.0344	0
		Treatment Means Grouped By Alcohol Rate				
Alcohol (%)	IBA (ppm)					
0	0	0	0	0.0	1.569a	0
0	1000	0	0	0.0	1.611a	0
0	2000	0	0	0.0	1.767a	0
25	0	0	0	0.0	1.726a	0
25	1000	0	0	3.0	1.639a	0
25	2000	0	0	0.0	1.843a	0
50	0	0	0	0.0	2.006a	0
50	1000	0	0	0.0	1.688b	0
50	2000	0	0	0.0	1.508b	0
		Means for Main Effects				
Alcohol (%)	IBA (ppm)					
0		0	0	0.0a	0	0
25		0	0	1.0a	0	0
50		0	0	0.0a	0	0
	0	0	0	0.0a	0	0
	1000	0	0	1.0a	0	0
	2000	0	0	0.0a	0	0

Experiment 2		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Shoot Dry Weight (g)	Stem Epinasty (%)
		Significance of Treatment Factors (P Values)				
Alcohol		—	—	0.2396	0.0351	<.0001
IBA		—	—	0.8147	0.9359	<.0001
Alcohol*IBA		—	—	0.0873	0.8013	<.0001
		Treatment Means Grouped By Alcohol Rate				
Alcohol (%)	IBA (ppm)					
0	0	0	0	0.0b	1.462	0.0a
0	100	0	0	0.0b	1.518	0.0a
0	200	0	0	6.1a	1.547	0.0a
25	0	0	0	0.0a	1.52	0.0a
25	100	0	0	0.0a	1.574	0.0a
25	200	0	0	0.0a	1.574	0.0a
50	0	0	0	6.1a	1.837	0.0c
50	100	0	0	3.0ab	1.653	12.5b
50	200	0	0	0.0b	1.721	100.0a
		Means for Main Effects				
Alcohol (%)	IBA (ppm)					
0		0	0	0	1.509b	0
25		0	0	0	1.556b	0
50		0	0	0	1.737a	0
	0	0	0	0	1.606a	0
	100	0	0	0	1.582a	0
	200	0	0	0	1.614a	0

Experiment 3		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors (P Values)			
Alcohol		—	—	0.9868	<.0001
IBA+NAA		—	—	0.839	0.0057
Alcohol*IBA+NAA		—	—	0.7823	0.2016
		Treatment Means Grouped By Alcohol Rate			
Alcohol (%)	IBA+NAA (ppm)				
0	0+0	0	0	0	0.191
0	500+250	0	0	9.1	0.224
0	1000+500	0	0	6.1	0.226
25	0+0	0	0	12.1	0.208
25	500+250	0	0	9.1	0.22
25	1000+500	0	0	15.2	0.22
50	0+0	0	0	18.2	0.244
50	500+250	0	0	6.1	0.272
50	1000+500	0	0	12.1	0.243
		Means for Main Effects			
Alcohol (%)	IBA+NAA (ppm)				
0		0	0	5.1a	0.214b
25		0	0	12.1a	0.216b
50		0	0	12.1a	0.253a
	0+0	0	0	10.1a	0.214b
	500+250	0	0	8.1a	0.239a
	1000+500	0	0	11.1a	0.230a

When the interaction term in the model is significant ($p \leq 0.20$), simple effects means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.10$); otherwise, the treatment means are presented without letter groupings for informational purposes.
When the interaction term in the model is not significant ($p > 0.20$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.10$).

Table 4. Stem and leaf burn (%), mortality (%), and root dry weight (g) of *Pelargonium ×hortorum 'Mary Helen'* observed using a basal quick-dip with selected rates of alcohol and IBA (Exp. 1) or IBA and NAA (Exp. 3) or using total immersion with selected rates of alcohol and IBA (Exp. 2).

Experiment 1		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		—	—	0.8837	0.9573
IBA		—	—	0.0087	0.0002
Alcohol*IBA		—	—	0.606	0.0003
Alcohol (%)	IBA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0	0	0	9.1	0.171a
0	1000	0	0	0	0.143b
0	2000	0	0	21.2	0.186a
25	0	0	0	6.1	0.177a
25	1000	0	0	6.1	0.130b
25	2000	0	0	15.2	0.185a
50	0	0	0	6.1	0.126b
50	1000	0	0	6.1	0.177a
50	2000	0	0	12.1	0.192a
		Means for Main Effects			
Alcohol (%)	IBA (ppm)				
0		0	0	10.1a	0
25		0	0	9.1a	0
50		0	0	8.1a	0
	0	0	0	7.1ab	0
	1000	0	0	4.0b	0
	2000	0	0	16.2a	0

Experiment 2		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		<.0001	<.0001	<.0001	<.0001
IBA		0.0091	0.623	1	0.0001
Alcohol*IBA		0.0008	0.7503	0.1666	0.004
Alcohol (%)	IBA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0	0.0a	0.0	6.1a	0.162b
0	100	0.0a	0.0	6.1a	0.166b
0	200	0.0a	0.0	12.1a	0.192a
25	0	87.9a	100.0	87.9a	0.038b
25	100	90.9a	97.0	90.9a	0.170a
25	200	63.6b	97.0	63.6b	0.132a
50	0	100.0a	100.0	100.0a	—
50	100	100.0a	100.0	100.0a	—
50	200	100.0a	100.0	100.0a	—
		Means for Main Effects			
Alcohol (%)	IBA (ppm)				
0		0	0.0b	0	0
25		0	98.0a	0	0
50		0	100.0a	0	0
	0	0	66.7a	0	0
	100	0	65.7a	0	0
	200	0	65.6a	0	0

Experiment 3		Cuttings with Stem Burn (%)	Cuttings with Leaf Burn (%)	Mortality (%)	Root Dry Weight (g)
		Significance of Treatment Factors		*(P Values)*	
Alcohol		—	—	0.9996	<.0001
IBA+NAA		—	—	0.9994	0.0064
Alcohol*IBA+NAA		—	—	0.0112	0.0019
Alcohol (%)	IBA+NAA (ppm)	*Treatment Means Grouped By*		*Alcohol Rate*	
0	0+0	0	0	0.0b	0.150a
0	500+250	0	0	21.2a	0.203b
0	1000+500	0	0	6.1b	0.177b
25	0+0	0	0	3.0b	0.202a
25	500+250	0	0	6.1b	0.174b
25	1000+500	0	0	42.4a	0.212a
50	0+0	0	0	9.1ab	0.205b
50	500+250	0	0	0.0b	0.265a
50	1000+500	0	0	18.2a	0.247a
		Means for Main Effects			
Alcohol (%)	IBA+NAA (ppm)				
0		0	0	0	0
25		0	0	0	0
50		0	0	0	0
	0+0	0	0	0	0
	500+250	0	0	0	0
	1000+500	0	0	0	0

When the interaction term in the model is significant ($p \leq 0.20$), simple effects means followed by the same letter are not significantly different using the Shaffer-Simulated adjustment for multiple comparisons ($\alpha = 0.10$); otherwise, the treatment means are presented without letter groupings for informational purposes. When the interaction term in the model is not significant ($p > 0.20$), main effects means for rates within each treatment factor followed by the same letter are not significantly different using the Shaffer-Simulated method for multiple comparisons ($\alpha = 0.10$).