Effect of IBA Treatments, Bottom Heat, Stock Plant Location, and Cutting Type on the Rooting of *Spigelia marilandica* Cuttings[1]

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Abstract

Spigelia marilandica (India pink, pinkroot), a herbaceous perennial native to the United States, is underused in the nursery trade, in part because it is difficult to propagate. We found from the first of two studies conducted during winter 2007 that stem-tip cuttings collected from greenhouse-grown stock plants submersed in 3000 or 6000 ppm (mg·liter^{-1}) potassium salt of indole-3-butyric acid (IBA) for one minute had greater percentage rooting (average 76.6) than cuttings not treated with IBA (46.9). Bottom heat [average 27.2C (81F)] compared to no bottom heat [23C (73F)] increased rooting percentage from 48 to 85%. The second winter 2007 study determined that rooting percentage according to method of application of 3000 ppm IBA was in the order: solution dip (60.1) > powder dip (49.8) ≥ solution submersion (46.6) ≥ control (42.7). During summer 2009, bottom heat and the same methods of application of 3000 ppm IBA described above were applied to terminal (stem tip) or subterminal cuttings collected from greenhouse- or field-grown stock plants. While IBA application method had no effect on rooting of cuttings from greenhouse-grown stock plants (average 54.1%), it affected cuttings from field-grown stock plants in the order: solution submersion (80.1%) > solution dip (54.4%) > powder dip = control (average 29.5%). Solution submersion cuttings from field-grown stock plants had much greater rooting (80.1%) than those from greenhouse-grown stock plants (47.2%). Control and powder dip cuttings from greenhouse-grown stock plants had greater rooting percentage than those from field-grown stock plants. Cutting type had no effect on rooting of field-grown cuttings (average 48.4%). Terminal cuttings from greenhouse-grown stock plants had greater rooting (73.3%) than subterminal cuttings (34.8%).

Index words: cuttings, India pink, indolebutyric acid, IBA, pinkroot, plant propagation, *Spigelia marilandica*.

Species used in this study: India pink [pink root (*Spigelia marilandica* L.)].

Chemicals used in this study: indole-3-butyric acid (IBA), Zero-Tol (hydrogen peroxide and peroxyacetic acid).

Significance to the Nursery Industry

Results of this study have shown that percentage rooting of *Spigelia marilandica* (India pink, pink root) stem tip cuttings was increased by treatment with 3000 ppm IBA and the use of bottom heat. For greenhouse-grown stock plants, percentage rooting of winter-collected stem tip cuttings was increased to 60.1% with solution dip treatment compared to 42.7% in control cuttings. When cuttings were collected from greenhouse-grown stock plants in the summer, IBA application method had no effect on rooting percentage, but average rooting was much higher (73.3%) from terminal cuttings than from subterminal cuttings (34.8%), or from winter-collected terminal cuttings (average 49.8%). While terminal or subterminal cuttings collected in the summer from field-grown stock plants had similar rooting percentage (average 48.4%), application of 3000 ppm IBA increased rooting percentage to 80.1% with solution submersion, 54.4% with solution dip, 36.7% with powder dip compared to the 22.2% rooting of non-treated cuttings.

Introduction

Spigelia marilandica L. (India pink, pinkroot), family Loganiaceae/Spigeliaceae, is a herbaceous perennial that is native to moist woodlands of the southeastern United States. It has a long flowering period extending from June to October in the mid-Atlantic region (1). Plants are 30–60 cm (12–24 in) tall with tubular flowers red on the outside and yellow on the inside which are displayed above medium green foliage. This species is a desirable ornamental plant for woodland gardens in cold hardiness zones 7 to 9, and partially sunny, moist borders in zones 5 and 6 (1). It is underused in the nursery trade due to difficulty in propagation (4).

Hartmann et al. (4) state that *S. marilandica* can be propagated by division or by stem cuttings under mist from greenhouse-grown stock plants. Seeds of this species have a low germination percentage and they are difficult to collect because the fruit have no distinct indicators of maturity and they explosively dehisce. Germination percentage decreases with seed storage, and so seeds should be sown as soon as they are mature (1). Seedlings also bloom only after their second season, further diminishing the attractiveness of this method to propagators who want to rapidly produce plants that are in flower (2). Additionally, plants may take six or more years before producing viable seed. Micropropagation (propagation by tissue culture) is expensive and requires that producers have the appropriate facilities including a suitable environment for microcutting survival. Propagation by stem tip cuttings is a widely practiced method of propagation that is relatively cheap, and requires minimal special equipment. Hence, *S. marilandica* is a good candidate for propagation by stem tip cuttings (3).

Exogenous indole-3-butyric acid (IBA) supplements the endogenous auxin, indole-3-acetic acid (IAA), to a concentration that ideally stimulates adventitious rooting of cuttings. Cullina (2) recommended taking 2-node stem tip cuttings from late spring growth of *S. marilandica* and applying

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2000 ppm liquid IBA to the cutting bases. Two-node stem tip cuttings treated with 2000–3000 ppm IBA were used by Bir and Barnes (1) with good rooting success. Foster and Kitto (3) found that using greenhouse-grown stock plants dramatically improved rooting in cuttings over those taken from outdoor stock plants. They suggested using bottom heat and using higher IBA concentrations than the 1000 ppm used in their research as possible treatments that should be investigated.

Application of IBA to cuttings can be achieved by several methods. The most common methods are as a talcum powder dip, a solution dip, or a solution submersion. Rooting percentage of *S. marilandica* is similar using either the potassium salt of IBA or IBA dissolved in alcohol (1). With ethanol-IBA solutions, the duration of dip or submersion treatments is critical so as to avoid alcohol damage with excessive exposure (4). We have found no reports on the most efficacious IBA application method for S. marilandica cuttings.

The objective of this study was to increase the percentage rooting of *S. marilandica* cuttings. The first two studies conducted during winter 2007 (December 2007–March 2008) examined the rooting responses of stem-tip cuttings from greenhouse-grown stock plants first to IBA concentration and bottom heat, and then to IBA application method. A third study conducted during summer 2009 (June–July) examined the rooting response of terminal (stem tip) cuttings or the woodier subterminal cuttings (proximal and adjacent to the stem tip cuttings) collected from greenhouse- or field-grown stock plants to method of IBA application.

Materials and Methods

Eighteen *S. marilandica* plants of unknown age and healthy in appearance, averaging 30 cm (12 in) in both height and diameter were transplanted during September 2007 from the University of Delaware Botanic Gardens into 7.5 liter pots (2 gal; one plant per pot) containing ProMix BX (Premier Horticulture, Rivière-du-Loup, Quebec, Canada) which then were top-dressed with 15 ml (1 tbsp)·pot^{-1} of slow-release fertilizer, Osmocote 14N-6.02P-11.62K (The Scotts Company, Marysville, OH). The plants were kept under mist for three days then moved to a greenhouse with a 16 h photoperiod provided by 1000W high pressure sodium lamps that supplemented solar radiation. Stems were headed back to 5 cm (2 in) above the medium surface to stimulate axillary growth. Pots were watered as needed and were solution-fertilized once a week with 200 mg·liter^{-1} (ppm) N from Peters Excel 21N-2.2P-16.6K (The Scotts Company).

Terminal (stem tip) cuttings with at least two nodes [about 7.5 cm (3 in) long] were collected from the stock plants. Flowers and the lowest pair of leaves were removed before surface sterilization by submersion in a Zero-Tol (H_2O_2 + peroxyacetic acid; BioSafe Systems, Hartford, CT) solution at the recommended 8 ml·liter^{-1} (1 fl oz·gal^{-1}) for 30 sec. Cuttings were allowed to dry without rinsing before application of IBA treatments.

The rooting medium, consisting of sphagnum peat:perlite (1:1 by vol) adjusted to pH 5.2 with 2 g·liter^{-1} (55 oz·yd^{-3}) pulverized dolomitic limestone, was placed in 809 inserts (each cell = 50 ml and 4 × 4 × 5 cm depth; 4.9 in^3 and 1.6 × 1.6 × 2 in depth). An 809 insert containing 1 cutting per cell (9 cuttings per insert) was an experimental unit. An 809 insert is a molded plastic unit, of which eight ('8') can be inserted into a standard flat, each insert having nine ('09') cells.

IBA concentration and bottom heat (Winter 2007). Terminal cuttings were collected and prepared as described above in December 2007. Prepared cuttings were submerged for 1 minute in 0, 3000, or 6000 ppm IBA (respectively 0, 3.57, or 7.14 g·liter^{-1} K-IBA (0, 0.47 or 0.95 oz·gal^{-1}; Sigma, St. Louis, MO). Cuttings were immediately stuck in filled 809 inserts (1 cutting per cell) to a depth of 2.5 cm (1 in) so that at least one node was below the medium surface. One-half of the inserts was placed on an electrical resistance heat mat set to provide 27 ± 0.5C (81 ± 1F) at the cutting base [2.5 cm (1 in)]. Average temperature of cuttings bases without bottom heat was 21 ± 2C (70 ± 3.6F). The experiment was a three (IBA concentration) × two (bottom heat) factorial with four replications of nine cuttings per experimental combination arranged in split block design with bottom heat as main plots. All cuttings were watered before being placed under intermittent mist (10 minutes every 3 h from 0600 to 1800 daily) on December 13, 2007. Cuttings were harvested after 28 days. The cutting and medium from a cell was removed and gently swirled in a bucket of water to remove adhering growth medium. The percentage of rooted cuttings out of nine cuttings per 809 insert was calculated. The number of roots was counted and the average root length for each cutting was measured using a ruler. Percentage rooting data were transformed to arcsine$\sqrt{\%}$ to ensure normality, and IBA concentration was partitioned into trend components. All data were subjected to analysis of variance (anova).

IBA application method (Winter 2007). Terminal cuttings [144 cuttings: four IBA application methods × four replications × nine cuttings per replication (809 insert)] were collected as described above. IBA at 3000 ppm was applied to 36 cuttings by each of three application methods: cutting base dip in Hormodin 2, cutting base dip [basal 2.5 cm (1 in) of cutting for 1 minute] in K-IBA solution (solution dip), or solution submersion in K-IBA solution for 1 minute as described above (solution submersion). Thirty-six additional cuttings not treated with IBA served as controls. All inserts were placed on a heat mat as described above with treatments (809 inserts) arranged in randomized block design with four replications. Cuttings were subjected to the same conditions and harvesting techniques as described above. Transformed rooting percentage, and root number and length per cutting were subjected to anova.

Stock plant exposure, cutting type, and IBA application method (Summer 2009). Cuttings were collected from stock plants that had been in continuous active growth for 18 months in a greenhouse. The stock plants were headed back to 5 cm (2 in) two months before collection of cuttings in order to stimulate axillary growth. Field-grown stock plants were planted about 10 years ago at 30 cm (12 in) spacing in a wood-mulched Matapeake silt loam flower bed in part-sun. These plants received no maintenance other than removal of dead shoots in early spring. Cuttings from both greenhouse-grown and field-grown stock plants were collected on the same day (mid-June). Terminal cuttings contained at least two nodes [about 7.5 cm (3 in) long] and had their flowers removed. Subterminal cuttings (cuttings proximal and adjacent to the terminal cuttings), likewise, contained at least two nodes but were generally longer and more variable in length [7.5 to 20 cm (3 to 8 in) long] than the terminal cuttings owing to greater internode lengths. Cuttings were

Table 1. Effect of indolebutyric acid (IBA) concentration and bottom heat on rooting of *Spigelia marilandica* terminal stem cuttings (Winter 2007)[z].

Factor	Rooted cuttings [%, (deg.)][y]	Root number per cutting	Average root length per cutting (mm)
IBA (ppm)			
0	46.9 (43.2)b[x]	6.8b	16b
3000	78.1 (62.1)a	24.1a	25a
6000	75.0 (60.0)a	26.6a	23a
Bottom heat			
No	47.9 (43.8)b	11.1b	10b
Yes	85.4 (67.5)a	27.2a	32a
Significance[w]			
IBA ppm – linear	(*)	**	***
– quadratic	(**)	**	**
Bottom heat (BH)	(***)	***	*
IBA ppm × BH	(NS)	NS	NS

[z]Cuttings submersed in aqueous IBA solution for 1 minute. Bottom heat averaged 27.2C (81F) at cutting base compared to 23C (73F) without bottom heat.

[y]Transformed (arcsine√%) rooting percentages used for statistical analysis.

[x]Means within a column and main effect followed by the same letter cannot be considered significantly different according to $LSD_{0.05}$.

[w]NS, *, **, *** Not significant or significant at $P \leq 0.05$, ≤ 0.01 or 0.001, respectively.

surface sterilized with Zero-Tol and were subjected to the same treatment methods for application of 3000 ppm IBA as described above (control, powder dip, solution dip or solution submersion). All cuttings were provided bottom heat. All other experimental details were identical to those described above. Transformed rooting percentage, and root number and length per cutting were subjected to anova.

Results and Discussion

IBA concentration and bottom heat (Winter 2007). Rooting percentage, root length, and root number were greater with 3000 or 6000 ppm IBA than with 0 ppm IBA, and with bottom heat than without bottom heat (Table 1). These results agree with the recommendations of Henny and Fooshee (5) to use bottom heat and Hormodin 2 (3000 ppm IBA) when rooting tip cuttings of *Aphelandra squarrosa*. The lack of a difference in rooting between 3000 and 6000 ppm IBA may be due to receptor saturation at the rooting site. *S. marilandica* tip cuttings may only need (or be able to respond to) 3000 ppm IBA during the rooting process, causing the additional IBA molecules at 6000 ppm to have no effect (7). Bottom heat is listed specifically as a treatment of stem cuttings of such herbaceous perennials as *Campanula persicifolia* (peach bells), *Fuchsia magellenica* hybrids, and *Lavandula* (lavender) species, but not for *S. marilandica* (4). Increased cellular activity at and near the cutting base with bottom heat leads to increased root initiation in certain species, as suggested by Wang (8). Higher temperatures at the rooting surface with bottom heat may increase the responsiveness of plant tissues to endogenous and exogenous auxins, thus magnifying their effects on cuttings by increasing receptor or transporter efficiency.

IBA application method (Winter 2007). Rooting percentage was affected in the order: solution dip > powder dip = solution submersion ≥ control dip (Table 2). Using either solution dip or solution submersion increased root number per cutting to an average of 16.6 from the average of 6.3 in powder dip or control cuttings. While solution dip resulted in the greatest average root length per cutting (23 mm; 0.9 in), solution submersion and powder dip resulted in an average 20 mm (0.8 in) root length compared to the 16 mm (0.6 in) average root length of control cuttings. The greater rooting percentage and average root length per cutting with solution dip than with powder dip may reflect improved transport of IBA into the cutting, because the chemical was already in solution as opposed to being present as a solid in powder dip. Also, a greater surface area of the cutting base exposed to IBA with solution dip than with powder dip may have permitted greater IBA uptake. Receptor saturation mentioned previously may also explain the differences observed between IBA solution dip and submersion. Both solution application methods may have saturated the rooting receptor sites with IBA so that any additional molecules transported to these sites from the distal portions of the cutting using the submersion method had no effect. Additionally, energy that would have contributed to rooting and root growth in the submersion method may have been used instead for active basipetal transport of IBA (7). Moore (6) found that different plant species responded differently to powder and liquid formulations of IBA, so our findings of greater rooting with solution submersion or dip for *S. marilandica* should not be taken as a recommendation to abandon powder dip use for other species.

Stock plant exposure, cutting type, and IBA application method (Summer 2009). The significant cutting location by IBA application method interaction revealed that for control cuttings (no IBA applied) and powder-dip cuttings, percentage rooting was greater when cuttings were collected from stock plants grown in the greenhouse than in the field (Table 3). Kitto and Foster (3), likewise, found that *S. marilandica* cuttings treated with powder dip (1000 ppm IBA) and col-

Table 2. Effect of application method of 3000 ppm indolebutyric acid (IBA) on rooting of *Spigelia marilandica* terminal stem cuttings (Winter 2007).

IBA application method[z]	Rooted cuttings [%, (deg.)][y]	Root number per cutting	Average root length per cutting (mm)
Powder dip	49.8 (44.9)b[x]	7.3b	20b
Solution dip	60.1 (50.8)a	15.5a	23a
Solution submersion	46.6 (43.1)bc	17.7a	20b
Control	42.7 (40.8)c	5.2b	16c
Significance[w]	(**)	***	***

[z]IBA at 3000 ppm applied as powder (Hormodin 2) to the cutting base (powder dip), as K-IBA solution to the basal 2.5 cm (1 in) end of the cutting for 1 minute (solution dip), as K-IBA to the entire cutting for 1 minute by cutting submergence (solution submersion), or IBA not applied to the cutting (control).

[y]Transformed (arcsine√%) rooting percentages used for statistical analysis.

[x]Means within a column followed by the same letter cannot be considered significantly different according to $LSD_{0.05}$.

[w]**, *** Significant at $P \leq 0.01$ or 0.001, respectively.

Table 3. Effect of stock plant location (greenhouse vs field), cutting type (terminal or subterminal) within an axillary shoot, and application method of 3000 ppm indolebutyric acid (IBA) on the rooting of *Spigelia marilandica* cuttings (Summer 2009).

Factor	Rooted cuttings [% (deg.)][z]	
Stock plant location:	Greenhouse	Field
IBA application method[y]		
Powder dip	58.3 (50.6)b[x]	36.7 (36.7)cd
Solution dip	54.2 (47.8)b	54.4 (47.8)b
Solution submersion	47.2 (44.9)bc	80.1 (66.3)a
Control	56.6 (49.0)b	22.2 (27.6)d
Cutting type		
Terminal	73.3 (60.2)a	48.5 (44.1)b
Subterminal	34.8 (36.0)c	48.2 (45.1)b
Significance[w]		
Stock plant location (L)	(NS)	
Cutting type (C)	(***)	
L × C	(***)	
IBA application method (IBA)	(***)	
L × IBA	(***)	
C × IBA	(NS)	
C × L × IBA	(NS)	

[z]Transformed (arcsine√%) rooting percentages used for statistical analysis.

[y]IBA at 3000 ppm applied as powder (Hormodin 2) to the cutting base (powder dip), as K-IBA solution to the basal 2.5 cm (1 in) end of the cutting for 1 minute (solution dip), as K-IBA to the entire cutting for 1 minute by cutting submergence (solution submersion), or IBA not applied to the cutting (control)

[x]Means within an interaction followed by the same letter cannot be considered significantly different according to $LSD_{0.05}$.

[w]NS, *** Not significant or significant at $P \leq 0.001$, respectively.

lected in the Summer (June–August) had greater rooting percentage and root number and weight when stock plants were greenhouse-grown rather than field-grown. These authors suggested that the superior rooting of cuttings from greenhouse-grown stock plants than from field-grown stock plants, could be attributed to reduced physiological stress in the former. Although dipping the basal 2.5 cm (1 in) end of cuttings into IBA solution represented a much greater percentage of cutting length for terminal than for subterminal cuttings since subterminal cuttings were longer (greater intermodal lengths), this length difference between cutting type had no effect on rooting percentage of solution-dipped cuttings (Table 3). For solution-submersed cuttings, however, field-grown cuttings had a much greater percentage rooting (80.1) than greenhouse-grown cuttings (47.2). Without further experimentation, the reason(s) for this large differential in percentage rooting in response to the interaction of cutting location with solution-submersion treatment remain(s) unknown. While IBA application method had no effect on rooting percentage of cuttings from greenhouse-grown stock plants, rooting of cuttings from field-grown stock plants was in the order: solution submersion (80.1%) > solution dip (54.4%) > powder = control (average 29.5%). Thus, a strong interaction existed between stock plant location and IBA application method for summer-collected *S. marilandica* cuttings. Control cuttings from greenhouse-grown stock plants had less rooting when collected during the winter (42.7%, Table 2) than during the summer (56.6%, Table 3), with IBA treatments enhancing rooting in the former but not in the latter. Thus, an interaction also existed between the IBA effect on rooting and the season during which cuttings were collected.

Terminal cuttings resulted in a greater rooting percentage (73.3) than subterminal cuttings (34.8) when they were collected from greenhouse-grown stock plants (Table 3). Bir and Barnes (1) likewise found that terminal *S. marilandica* cuttings from greenhouse-grown stock plants rooted at a higher percentage than woodier subterminal cuttings. By not harvesting subterminal cuttings, more nodes would remain on the stock plants to produce more axillary shoots from which terminal cuttings could be collected which had more than twice the rooting percentage as subterminal cuttings. For cuttings collected from field-grown stock plants, cutting type had no effect on rooting percentage (average 48.4). Thus, collecting both terminal and subterminal cuttings during the summer from field-grown stock plants would be justified. It should be mentioned that Bir and Barnes (1) advised that collecting *S. marilandica* cuttings from field-grown stock plants before late spring, rather than in the summer, resulted in the most vigorous liners the following spring. This observation reflected the longer period for growth rather than an effect of photoperiod on cutting response, since Bir and Barnes (1) noted that long or short photoperiods had no effect on the rooting response of cuttings directly or on rooting response

Table 4. Effect of stock plant location (greenhouse vs field), cutting type (terminal or subterminal) within an axillary shoot, and application method of 3000 ppm indolebutyric acid (IBA) on the number and length of roots on *Spigelia marilandica* cuttings (Summer, 2009).

Treatment	Number of roots per cutting	Average root length per cutting (mm)
Stock plant location		
Greenhouse	18.1a[z]	32a
Field	13.0b	25b
Cutting type		
Terminal	16.8a	30a
Subterminal	14.2b	28b
IBA treatment[y]		
Powder dip	11.8c	24b
Solution dip	23.1a	36a
Solution submersion	19.5b	35a
Control	7.7d	20c
Significance[x]		
Location (L)	***	***
Cutting type (C)	*	*
L × C	NS	NS
IBA treatment (I)	***	***
L × I	NS	NS
C × I	NS	NS
C × L × I	NS	NS

[z]Means followed by the same letters within a main effect in a column cannot be considered different according to $LSD_{0.05}$.

[y]IBA at 3000 ppm applied as powder (Hormodin 2) to the cutting base (powder dip), as K-IBA solution to the basal 2.5 cm (1 in) end of the cutting for 1 minute (solution dip), as K-IBA to the entire cutting for 1 minute by cutting submergence (solution submersion), or IBA not applied to the cutting (control).

[x]NS, *, *** Not significant or significant at $P \leq 0.05$ or 0.001, respectively.

of cuttings collected from stock plants subjected to these regimes. Given that *S. marilandica* flowers from June to October in the mid-Atlantic region (1), this species appears to be day neutral with respect to both flowering and the rooting of cuttings.

The number and length of roots per cutting were greater in cuttings from greenhouse-grown stock plants than field-grown stock plants, and in terminal cuttings than in subterminal cuttings (Table 4). Kitto and Foster (3) also noted that cuttings from greenhouse-grown stock plants had more roots and greater root mass than those from field-grown stock plants. All IBA treatments resulted in more and longer roots than occurred in control cuttings. Solution dip or submersion resulted in more and longer roots per cutting than powder dip treatment. Summer cuttings, as opposed to mid-spring cuttings, should be over-wintered in minimally heated greenhouse to survive in reasonable percentages (1).

Results of this study show that percentage rooting of *S. marilandica* cuttings is affected by IBA concentration, bottom heat, location of stock plants (field or greenhouse), the interaction of location of stock plants with method of IBA application, and the type of cutting (terminal or subterminal). The greatest rooting percentage for winter cuttings from greenhouse-grown stock plants occurred with bottom heat and 3000 or 6000 ppm IBA applied as a solution dip to the bottom 2.5 cm (1 in) of the cutting. Using 6000 ppm IBA would result in unnecessary cost. For summer collected cuttings, application of 3000 ppm IBA failed to increase rooting percentage of cuttings from greenhouse-grown stock plants, but increased rooting percentage of cuttings from field-grown stock plants when applied as a solution dip or solution submersion. Terminal cuttings had more than double the rooting percentage of subterminal cuttings from greenhouse-grown stock plants, but cutting type had no effect on rooting percentage of cuttings from field-grown stock plants.

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