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

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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 submerged 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 27.2°C (81°F)) compared to no bottom heat [23°C (73°F)] 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
were top-dressed with 15 ml (1 tbsp)·pot⁻¹ of slow-release fertilizer (2 gal; one plant per pot) containing ProMix BX (Premier Horticulture, Rivière-du-Loup, Quebec, Canada) which then was planted into 7.5 liter pots and diameter were transplanted during September 2007 from healthy in appearance, averaging 30 cm (12 in) in both height and diameter. These plants received no maintenance other than removal of dead shoots in early spring. Cuttings from both greenhouse- or field-grown stock plants were collected and prepared as described above. The plants that had been in continuous active growth for 18 months in a greenhouse. The stems 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 selected 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 analysis of variance (anova).
Table 1. Effect of indolebutyric acid (IBA) concentration and bottom heat on rooting of Spigelia marilandica terminal stem cuttings (Winter 2007).^1

| Factor                  | Rooted cuttings [% (deg.)]^1 | Root number per cutting | Average root length per cutting (mm) |
|-------------------------|-----------------------------|-------------------------|-------------------------------------|
| IBA (ppm)               |                             |                         |                                     |
| 0                       | 46.9 (43.2)b                | 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^2

- IBA ppm – linear (**) ***
- IBA ppm – quadratic (***) *** *
- Bottom heat (BH) (***) *** *
- IBA ppm × BH (NS) NS NS

^1Cuttings submerged in aqueous IBA solution for 1 minute. Bottom heat averaged 27.2°C (81°F) at cutting base compared to 23°C (73°F) without bottom heat.
^2Transformed (arc sine%) rooting percentages used for statistical analysis.
^3Means within a column and main effect followed by the same letter cannot be considered significantly different according to LSD0.05.

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.

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). Kito and Foster (3), likewise, found that S. marilandica cuttings treated with powder (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 | Rooted cuttings [% (deg.)]^1 | Root number per cutting | Average root length per cutting (mm) |
|------------------------|-------------------------------|-------------------------|-------------------------------------|
| Powder dip             | 49.8 (44.9)b                 | 7.3b                    | 20b                                 |
| Solution dip           | 60.1 (50.3)a                 | 15.5a                   | 23a                                 |
| Solution submersion    | 46.6 (43.1)bc                | 17.7a                   | 20b                                 |
| Control                | 42.7 (40.8)c                 | 5.2b                    | 16c                                 |

Significance^3

- Powder dip (**) ***
- Solution dip (**) ***
- Solution submersion (***) ***
- Control (NS) NS

^1IBA 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 submersion (solution submersion), or IBA not applied to the cutting (control).
^2Transformed (arc sine%) rooting percentages used for statistical analysis.
^3Means within a column followed by the same letter cannot be considered significantly different according to LSD0.05.

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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, 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 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 | Average root length |
|-----------|-----------------|---------------------|
| Stock plant location | Field | Greenhouse |
| Powder dip | 11.8c | 13.0b |
| Solution dip | 23.1a | 19.5b |
| Solution submersion | 19.5b | 17.5a |
| Control | 7.7d | 16.8a |

**Significance**

- Location (L): ***
- Cutting type (C): *
- L × I: NS
- IBA treatment (I): NS

*Means followed by the same letters within a main effect in a column cannot be considered different according to LSD 0.05.

1IBA 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).
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 sub-terminal 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 sub-terminal). 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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