Propagation

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Alleviating Seed Dormancy of Two Native Wildflowers: *Polygonella polygama* and *Polygonella robusta*

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**Index Words:** Germination, gibberellic acid, October flower, Sandhill wireweed

**Significance to Industry:** Native plants are widely recognized for their natural ability to adapt to tough conditions without substantial care once established. Native wildflowers, in particular, have an increasing role in ecological restoration, roadside beautification projects, and ornamental landscape use. The perennial nature, prolific white to pink flower spikes, and attractive foliage and form of *Polygonella polygama* (October flower) and *Polygonella robusta* (sandhill wireweed) suggest that these wildflowers could have significant ornamental and landscape potential, if an effective propagation method can be developed. To successfully germinate these seeds we must first find if they are dormant. The three types of dormancy tested for were physical (seed coat inhibits water entry), morphological (underdeveloped embryo), and physiological (physiological inhibiting mechanism of embryo that prevents radicle emergence) (1). Through controlled germination tests and seed sectioning, we determined that although seeds of both species did not possess physical nor morphological dormancy, physiological dormancy did exist. This was somewhat alleviated by application of Gibberellic acid, with the application of warm and cool stratification currently being studied.

**Nature of Work:** Native plant production faces many barriers including seed dormancy, collection, handling, and storage (1, 3). *Polygonella polygama* is a perennial plant occurring frequently throughout the sandhills, scrub and flatwoods of Florida. Its native distribution extends north to Virginia and south-west to Texas. A close relative, *P. robusta*, has a much narrower distribution, but produces showier flowers for longer periods of time. We believe that both species could have ornamental landscape potential and marketability, if feasible propagation and production practices can be developed.

Seeds deemed mature by brown coloration and ease of removal, were collected from natural populations of *P. polygama* (Wauchula, FL) and *P. robusta* (Hobe Sound, FL) on 15 Feb. 2008 and 10 April 2008, respectively. Seeds were cleaned and stored at room temperature prior to germination experiments. Pre-germination seed viability was examined on a subsample of seeds (four replications of 25 seeds) using 1.0% Triphenyltetrazolium chloride (TZ) for 48 h (4). Additional seeds (four replications of 25) were placed in petri-dishes and incubated in alternating light (12 hr photoperiod) or darkness at temperatures representing seasonal conditions throughout Florida: 22/11, 27/15, 29/19, and 33/24°C (72/52, 81/59, 84/66, and 93/75 °F). The experiment was carried out for 28 days, with data recorded weekly. A seed was considered germinated when radicle emergence was ≥ 2.0 mm (0.08 in).
To assess seed coat permeability, the increase in fresh mass was determined on four replications of 25 scarified or non-scarified seeds from both species. Mechanical scarification was achieved by nicking 1-2mm of the pedicel end of each seed. Increases in fresh mass were calculated over a 48 hr period using the formula $W_i = \frac{(W_i - W_n)}{W_n} \times 100$, where $W_i$ and $W_n$ are the masses of imbibed and non-imbibed tissues, respectively. To measure the potential for an underdeveloped embryo, the embryo:seed ratios were determined using light microscopy. Ten representative seeds of both species were sectioned (approximately 680nm) using a RMS microtome with a diamond knife and stained with 1.0% Toluidine Blue. The seed:embryo ratio was determined by measuring the distance between the distal and proximal length of the embryo and dividing this by the distance between the distal and proximal length of the seed. Inhibition of the embryo by physiological mechanisms was tested for using four replicates of 25 seed were subjected to five concentrations (0, 1, 10, 100, 1000 ppm) of Gibberellic acid (GA3) for 24 hr at 22/11°C (72/52 °F) with a 12-hr photoperiod. Germination was recorded once a week for four weeks.

Germination tests utilized a randomized complete block design. Treatments for initial germination were arranged in a 4 (temperature) × 2 (illumination) factorial. Germination data were transformed (arcsine of square root) prior to analysis and nontransformed data are presented. Data were analyzed using the GLM program (SAS 9.1,Cary, NC) with a significant $p$-value set to 0.05.

**Results and Discussion:** Although pre-germination viability was $77.4 \pm 6.3\%$ (mean ± SE) for *P. polygama* and $53.5 \pm 6.8\%$ for *P. robusta*, final germination did not exceed 24% (*P. polygama*) or 36% (*P. robusta*), regardless of temperature (Fig. 1). Analysis of variance detected a significant difference between temperature treatments for *P. polygama* ($p = 0.02$) in the light, though significant differences were not detected for *P. robusta* ($p = 0.09$) in the light. Both species had 0% germination in the dark treatment.

Initially fresh mass increased more rapidly in *Polygonella polygama* scarified seed than non-scarified. However, fresh mass increase was greater after 24 hr in the non-scarified seed. After 48 hr imbibition of *P. polygama* seed, fresh mass increased 74.2 ± 1.1% for non-scarified seed and 61.4 ± 3.8% for scarified seed. Though these final percentages are statistically different ($p = 0.04$), both treatments imbibed water regularly indicating no biological significance. *Polygonella robusta* also showed a marked fresh mass increase of 38.0 ± 3.6% for non-scarified seeds and 46.7 ± 4.7% for scarified seed after 48 hr, a difference not statistically significant ($p = 0.21$). Both species, regardless of treatment, imbibed water regularly (Fig. 2). In addition, neither macrosclerids nor osteosclerids were observed in seed coats of either species (Fig. 3), further indicating a lack of dormancy imposed by the seed coat. Light microscopy sectioning also revealed that both species had fully developed embryos. *P. polygama* had an embryo: seed ratio of 0.87:1.0 ± 0.04 and *P. robusta* had an even greater ratio of 0.95:1.0 ± 4.24.

Finally a common form of dormancy, physiological, was examined using GA3 (Fig. 4). Treatment of *P. polygama* seeds with 1000ppm GA3 increased germination to 34.7 ±
4.7% as compared to the other treatments ($p = 0.01$). *P. robusta* also responded with the highest germination of $58.3 \pm 7.8\%$ in the 1000ppm GA$_3$, though it was not significantly different from other concentrations ($p = 0.05$). Additional experiments have been implemented to determine the extent to which seeds require warm or cold stratification.

**Literature Cited:**
Fig. 1. Final germination percent of *P. polygama* (top) and *P. robusta* (bottom) seeds incubated with light (12 hr photoperiod) at 22/11, 27/15, 29/19, and 33/24°C (72/52, 81/59, 84/66, and 93/75°F) for 28 days. Columns with the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan’s multiple range test. Error bars represent the standard error of the mean.
Fig. 2. Fresh mass increase as described by increase of fresh mass of *Polygonella polygama* (top) *Polygonella robusta* (bottom) seeds when scarified (solid circles) and non-scarified (open circles) at constant incubation of 27°C. Error bars represent standard error of the mean.
Fig. 3. Typical embryo to seed ratio magnified at 10x for *P. polygama* (A) and *P. robusta* (B). Fruit coat was magnified at 20x for *P. polygama* (C) and 40x for *P. robusta* (D). Seed coat was magnified at 20x for *P. polygama* (E) and *P. robusta* (F).
Fig. 4. Final germination percent of *P. polygama* (top) and *P. robusta* (bottom) seeds after soaking for 24 hours in varying concentrations of GA$_3$ and subsequent incubation at the constant temperature of 22/11$^\circ$C (72/52 $^\circ$F) for 28 days. Columns with the same letters are not significantly different at $\alpha = 0.05$ level according to Duncan’s multiple range test. Error bars represent standard error of the mean.
Influence of Nitrogen on Herbaceous Perennial Stock Plants: Quantity and Quality of Cuttings

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Index words: Propagation, nitrogen rate, *Gaura lindheimeri* 'Siskiyou Pink', *Dianthus* x 'Pixie Star' PPAF, *Perovskia atriplicifolia*, *Salvia* x *sylvestris* 'May Night'

Significance to Industry: Commercial propagators cite low numbers of viable cuttings per stock plant and/or cuttings that are slow to develop significant root mass for a number of perennial species. There is difficulty in achieving sufficient numbers of high-quality cuttings to meet demand for these popular perennial taxa: *Gaura lindheimeri* 'Siskiyou Pink', *Dianthus* x 'Pixie Star' PPAF, *Perovskia atriplicifolia*, and *Salvia* x *sylvestris* 'May Night'. In exploring stock plant management of herbaceous perennials, we quantified the effects of a range of nitrogen (N) rates as applied to stock plants on the number of cuttings (yield), rooting percentage, and subsequent root development of cuttings. We found cutting yield and rooting was maximized with the 100 and 150 mg·L⁻¹ N treatments. Little benefit was obtained from the higher rates, and the 50 mg·L⁻¹ N treatment produced the lowest number of potential cuttings across all taxa.

Nature of Work: Total nitrogen within plants and nitrate levels in cuttings influence carbohydrate allocation and formation of roots (1). Rowe and Blazich (5) found that increasing stock plant nitrogen levels influenced rooting percentage and adventitious root development of Loblolly pine. Druege et al. (2) established for chrysanthemum that increasing nitrogen encouraged adventitious root growth. In fact, manipulation of N-content of stock plants may be the only way to impact rooting of the cuttings using fertility; as adding fertilizer to the rooting substrate of cuttings of several perennial species did not influence rooting percentages (4).

Most work in the area of nutrient effects on herbaceous stock plant yield and rooting response has been done on important potted flowering crops such as chrysanthemum and geranium (2,3). Little research has been done on stock plant management of perennials. Our objective was to determine if manipulation of N rate would enhance the quantity of cuttings and quality of rooting for several perennials reputed to be difficult to propagate.

Stock plants. Plugs (72s) of *Gaura lindheimeri* 'Siskiyou Pink', *Dianthus* x 'Pixie Star' PPAF, *Perovskia atriplicifolia*, and *Salvia* x *sylvestris* 'May Night' were received May 22, 2008, irrigated with clear water for one week, then sorted for uniformity, and planted one per pot in quart pots (917 ml) and placed on the greenhouse bench. The planting substrate was peat and pine bark; the only nutrients added were 2.7 kg·m⁻³ dolomitic lime. Plants were arranged in a randomized complete block design, six replications per treatment per species.
Fertilizer treatments. Treatments began 20 days after planting and were comprised of five rates: 50, 100, 150, 200, and 250 mg·L⁻¹ N, in a custom complete fertilizer solution with 20P-200K-92Ca-40Mg-134S (all mg·L⁻¹ ) and micronutrients from Scotts S.T.E.M. (Soluble Trace Element Mix), added at a rate of 7 mg·L⁻¹. The 50 mg·L⁻¹ N treatment was developed using calcium nitrate, and all higher nitrogen rates were obtained via addition of the appropriate amount of ammonium nitrate to the 50 mg·L⁻¹ N rate. Volumetric substrate soil moisture measurements were taken daily (Delta HH2 Soil Moisture Meter). Plants were watered when volumetric substrate moisture fell below 25% for Dianthus and 30% for Gaura, Perovskia and Salvia. Substrate electrical conductivity (EC) was monitored weekly via direct-stick probe (Field Scout, Spectrum Technologies). Fertilizer solution was applied at each watering until substrate EC approximated the EC levels of the fertilizer solution at 70 DAT.

Propagation. At this point, the number of potential cuttings from each stock plant was determined. A cutting was comprised of at least two nodes, with the first node occurring below a recently matured leaf. The number of cuttings taken from each plant was based on the lowest number of available cuttings from a plant within a treatment group, and limited to a maximum of 10 cuttings. Cuttings were dipped in a solution of 1500 mg·L⁻¹ indole-3-butyric acid then stuck in 128-cell trays filled with a mixture of 70% of the previously-described peat/pine bark substrate and 30% perlite (v/v). Trays were placed under an intermittent mist system to allow rooting. Percent rooted cuttings were determined for Dianthus and Gaura cuttings 44 days after sticking (DAS) and 45 DAS for Perovskia and Salvia. Rooted cuttings were washed and total root volume and root surface area was determined with the WinRHIZO image analysis system. Data were analyzed by Analysis of Variance Procedure of SAS and subjected to regression analysis using SAS General Linear Models Procedure (version 9.2, SAS Institute, Cary, NC).

Results and Discussion

Dianthus. The 250 mg·L⁻¹ N treatment produced the greatest number of potential cuttings; though rooting percentage fell off drastically with the higher N rates (Table 1). Stock plants receiving the highest N treatment deteriorated rapidly after the cuttings were taken, indicating intolerance to long-term high soluble salts levels. The greatest root surface area occurred at 100 mg·L⁻¹ N though root volume was unaffected by rate.

Gaura. Nitrogen rate did not impact the number of potential cuttings (Table 2). Root surface area and volume was greatest with the 150 and 200 mg·L⁻¹ N treatment; rooting percentage was greatest with the 100 mg·L⁻¹ N treatment. However, at the higher rates (200 and 250 mg·L⁻¹ N), rooting percentages dropped off 20% and 25%, respectively, when compared to 100 mg·L⁻¹ N treatments.

Perovskia. Nitrogen rate significantly affected both number of potential cuttings and rooting performance (Table 3). The 100 mg·L⁻¹ N rate produced the greatest number of potential cuttings and also the greatest rooting percentage, though rooting percentage was 80% or better for all treatments. However, there was a strong linear decrease in root volume and surface area as the N rate increased from 50 mg·L⁻¹. Our
recommendation of 100mg·L⁻¹ N is based on the fact that the primary propagation issue for *Perovskia* (as noted by industry propagators) is achieving enough cuttings per plant.

*Salvia*. Stock plants receiving 100 mg·L⁻¹ N and higher rates produced a significantly greater number of potential cuttings than the 50 mg·L⁻¹ N treatment (Table 4). Root surface area and volume was unaffected by rate. Percent rooting was variable, but did decrease with the higher N rates.

For the taxa studied, overall performance of stock plants and cuttings was generally maximized with the 100 and 150 mg·L⁻¹ N treatments. Little benefit was obtained from the higher rates, and the 50 mg·L⁻¹ N treatment produced the lowest number of potential cuttings across all taxa.

**Literature Cited**


Funding for this project was provided by the Fred C. Gloeckner Foundation, Inc. We also thank Yoder- Greenleaf and Fafard, Inc. for their support of Virginia Tech floriculture research.
Table 1. Effect of nitrogen rate on number of potential cuttings per plant (n=6), cutting root surface area, cutting root volume, and percent rooted (n=10) at 44 days after sticking (DAS) for *Dianthus x 'Pixie Star'*PPAF.

<table>
<thead>
<tr>
<th>mg·L⁻¹ N</th>
<th>Number of Cuttings @ 70DAT</th>
<th>Root Surface Area (cm²)</th>
<th>Root Volume (cm³)</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>16.2</td>
<td>3.02</td>
<td>0.11</td>
<td>30</td>
</tr>
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<td>100</td>
<td>25.5</td>
<td>3.17</td>
<td>0.12</td>
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</tr>
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<td>150</td>
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<td>2.25</td>
<td>0.08</td>
<td>45</td>
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<td>200</td>
<td>24.3</td>
<td>2.71</td>
<td>0.13</td>
<td>15</td>
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<td>250</td>
<td>31.2</td>
<td>1.34</td>
<td>0.05</td>
<td>25</td>
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Rate Effect

<table>
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<th>Effect</th>
<th>Number of Cuttings @ 70DAT</th>
<th>Root Surface Area (cm²)</th>
<th>Root Volume (cm³)</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>5.4</td>
<td>0.0011</td>
<td>0.0189</td>
<td>68</td>
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Significance denoted as: NS = Not Significant; *, **, *** = significant at the 0.05, 0.01, and 0.001 level, respectively.

Table 2. Effect of nitrogen rate on number of potential cuttings per plant (n=6), cutting root surface area, cutting root volume, and percent rooted (n=9) at 44 days after sticking (DAS) for *Gaura lindheimeri* 'Siskiyou Pink'.

<table>
<thead>
<tr>
<th>mg·L⁻¹ N</th>
<th>Number of Potential Cuttings @ 70DAT</th>
<th>Root Surface Area (cm²)</th>
<th>Root Volume (cm³)</th>
<th>Rooting (%)</th>
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<tr>
<td>50</td>
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<td>10.34</td>
<td>0.20</td>
<td>73</td>
</tr>
<tr>
<td>100</td>
<td>16.2</td>
<td>13.73</td>
<td>0.29</td>
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<td>150</td>
<td>13.7</td>
<td>16.33</td>
<td>0.32</td>
<td>74</td>
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<td>200</td>
<td>17.0</td>
<td>18.55</td>
<td>0.34</td>
<td>68</td>
</tr>
<tr>
<td>250</td>
<td>14.7</td>
<td>13.01</td>
<td>0.26</td>
<td>63</td>
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Significance denoted as: NS = Not Significant; *, **, *** = significant at the 0.05, 0.01, and 0.001 level, respectively.
Table 3. Effect of nitrogen rate on number of potential cuttings per plant (n=6), cutting root surface area, cutting root volume, and percent rooted (n=9) at 45 days after sticking (DAS) for *Perovskia atriplicifolia*.

<table>
<thead>
<tr>
<th>mg·L⁻¹ N</th>
<th>Number of Potential Cuttings @ 70DAT</th>
<th>Root Surface Area (cm²)</th>
<th>Root Volume (cm³)</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>10.0</td>
<td>10.99</td>
<td>0.28</td>
<td>80</td>
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<tr>
<td>100</td>
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<td>0.16</td>
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<td>7.60</td>
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<td>0.10</td>
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<td>250</td>
<td>16.2</td>
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Rate Effect
- **NS**

Linear LSD 5.8
- **NS**

Quadratic LSD 5.8
- **NS**

Significance denoted as: NS = Not Significant; *, **, *** = significant at the 0.05, 0.01, and 0.001 level, respectively.

Table 4. Effect of nitrogen rate on number of potential cuttings per plant (n=6), cutting root surface area, cutting root volume, and percent rooted (n=7) at 45 days after sticking (DAS) for *Salvia x sylvestris* 'May Night'.

<table>
<thead>
<tr>
<th>mg·L⁻¹ N</th>
<th>Number of Potential Cuttings @ 70DAT</th>
<th>Root Surface Area (cm²)</th>
<th>Root Volume (cm³)</th>
<th>Rooting (%)</th>
</tr>
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<tr>
<td>50</td>
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<td>4.41</td>
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<td>80</td>
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<td>100</td>
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<td>5.11</td>
<td>0.17</td>
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<td>150</td>
<td>21.3</td>
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<td>79</td>
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<td>29.2</td>
<td>7.12</td>
<td>0.18</td>
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<td>250</td>
<td>22.8</td>
<td>4.69</td>
<td>0.16</td>
<td>59</td>
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</table>

Rate Effect
- **NS**

Linear LSD 9.9
- **NS**

Quadratic LSD 9.9
- **NS**

Significance denoted as: NS = Not Significant; *, **, *** = significant at the 0.05, 0.01, and 0.001 level, respectively.
Propagation of Eastern Redbud (Cercis canadensis) by Stem Cuttings is Influenced by Clone and Date of Cutting Collection

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Index Words: Adventitious rooting, auxin, K-indolebutyric acid, Fabaceae

Significance to Industry: Although clones of eastern redbud [Cercis canadensis L. (Fabaceae Lindl.)] are typically propagated by budding or micropropagation, recent research suggests some clones can be propagated by stem cuttings. Results herein support this hypothesis as the clones ‘Appalachian Red’, ‘Ace of Hearts’, and to a lesser extent, ‘Hearts of Gold’ demonstrated good rooting potential. However, results indicate the optimum time for taking stem cuttings and optimum auxin treatment differ for each clone. Stem cuttings of ‘Appalachian Red’ rooted at 96% when taken in July, 15 weeks after budbreak (WAB), and treated with the potassium (K) salt (K-salt) of indolebutyric acid (K-IBA) at 5000 mg·L⁻¹ (ppm). Stem cuttings of ‘Ace of Hearts’ rooted at 75% when taken in May, 6 WAB, and treated with K-IBA at 5000 mg·L⁻¹, whereas stem cuttings of ‘Hearts of Gold’ rooted at 58% when taken in June, 8 WAB, and treated with K-IBA at 15,000 mg·L⁻¹. In contrast, stem cuttings of ‘Forest Pansy’ rooted poorly at all collection dates regardless of K-IBA treatment.

Nature of Work: Eastern redbud is a small flowering tree native to the eastern United States. Several unique clones exist and are commonly grown by the nursery industry (1). Typically, propagation is by budding or micropropagation, though propagation by stem cuttings would be more economical (7). However, propagation by stem cuttings traditionally has been considered unfeasible (3,7).

Previous research indicates some genotypes of redbud (Cercis L. sp.) can be propagated successfully by stem cuttings, but only if cuttings are taken during a short period after budbreak. Tipton (8) was able to propagate Mexican redbud [Cercis canadensis var. mexicana (Rose) M. Hopkins] by stem cuttings when cuttings were taken shortly after budbreak. Regression analysis predicted rooting of 88% for cuttings taken 4 WAB, while cuttings taken 8, 12, or 16 WAB did not root. Dillion and Klingaman (2) reported 94% rooting of stem cuttings of an unidentified clone of eastern redbud when cuttings were taken in May whereas cuttings taken in June rooted at 50%, and cuttings taken after June did not root.

Some clones, notably C. canadensis ‘Forest Pansy’, have been difficult to propagate by stem cuttings regardless of collection date. Murphy (6), working with hardwood cuttings, did not root a single cutting of ‘Forest Pansy’. Wooldridge et al. (unpublished data) were unable to root semi-hardwood cuttings of ‘Forest Pansy’. Because ‘Forest Pansy’ is a popular clone many nursery professionals have also attempted to propagate...
the cultivar by stem cuttings without success. The reputation of eastern redbud as
difficult to root likely resulted in part from work with ‘Forest Pansy’.

The aforementioned studies indicate stem cuttings taken soon after budbreak provide
the best chance for successful propagation. However, Wooldridge et al. (9) reported
semi-hardwood cuttings of ‘Flame’ eastern redbud taken 16 WAB rooted in higher
percentages (83%) than softwood cuttings (63%) taken 6 WAB. These findings suggest
that (A) cuttings should not always be taken soon after budbreak, and (B) for some
genotypes the window for successful rooting is greater than others. While semi-
hardwood stem cuttings of ‘Flame’ rooted more successfully, softwood cuttings also
rooted in relatively high percentages.

Previous research suggests the optimum time for collecting cuttings of eastern redbud
depends on the genotype. While reports of clonal effects on adventitious rooting are not
uncommon (4), reports of interactions between clone and optimum cutting collection
date are rare. Members of the genus Populas L. (poplars) appear to be influenced by
this interaction. Yu et al. (10) collected stem cuttings of hybrid aspen clones (Populas
tremula L. x P. tremuloides Michx.) in May and again in July. For most clones, cuttings
collected in May rooted better, but for a few clones, cuttings taken in July performed
better. Zalesny and Wiese (11) evaluated cuttings of several genomic groups of poplar
taken every 3 weeks from December to April. Collection date accounted for much of the
variation in root number and root dry weight. Kibbler et al. (5) conducted a study in
Australia on the effects of genotype on propagation of lemon myrtle (Backhousia
citriodora F. Muell) by stem cuttings. While most genotypes rooted in high percentages
from cuttings taken in spring, some genotypes rooted better when taken in autumn.

Eastern redbud may respond similarly as reported in the aforementioned studies and a
protocol for propagation of particular clones may differ slightly. Therefore, the following
research was conducted to determine the optimum growth stage to take stem cuttings
for propagation of four popular clones of eastern redbud. The research consisted of
taking stem cuttings of four popular clones of eastern redbud, ‘Ace of Hearts’,
‘Appalachian Red’, ‘Hearts of Gold’, and ‘Forest Pansy’, on seven dates following
budbreak during Spring and Summer 2007 and evaluating the clones for rooting
potential.

Results and Discussion: Rooting was affected by a clone and cutting date interaction,
indicating the optimum time to take cuttings was different for each clone. Cuttings of
‘Ace of Hearts’ taken 6 WAB rooted at 75% and 71% when treated with K-IBA at 5000
mg·L⁻¹ or 15,000 mg·L⁻¹, respectively. In contrast, cuttings of ‘Appalachian Red’ rooted at
96% and 93% when taken 15 WAB, the last date for that clone, and treated with K-
IBA at 5000 mg·L⁻¹ or 15,000 mg·L⁻¹, respectively. When taken 8 WAB and treated with
K-IBA at 5000 mg·L⁻¹ or 15,000 mg·L⁻¹, cuttings of Hearts of Gold rooted at 42% and
58%, respectively. Cuttings of ‘Forest Pansy’ rooted poorly regardless of collection date
or K-IBA treatment. Treatment of ‘Ace of Hearts’, ‘Appalachian Red’, and ‘Hearts of
Gold’, with higher K-IBA rates did not increase rooting percentages but often resulted in
more robust root systems. Propagation by stem cuttings may be feasible for some clones of eastern redbud, but separate protocols are necessary for each clone.

Literature Cited:
Cutting Propagation of Rose Using Basal and Foliar Applications of Wood's Rooting Compound

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Index Words: Adventitious rooting, auxin, root-promoting compounds, Rosa 'Red Cascade'

Significance to Industry: Foliar application of auxin is an acceptable alternative to a conventional basal quick-dip for rooting stem cuttings of some nursery crops. In two experiments, cuttings of Rosa 'Red Cascade' were treated with Wood's Rooting Compound (WRC) at selected rates of auxin using both methods of application. Cuttings receiving the control treatment of WRC at 1030 ppm IBA + 660 ppm NAA (a 10% dilution of the concentrated product) as a basal quick-dip produced the best overall rooting and initial shoot growth responses compared with cuttings receiving a basal dip with lower rates of auxin (IBA + NAA at 515 + 330 or 103 + 66 ppm), while use of a foliar spray application with these same three rates of auxin was detrimental. Results comparable to those of the control treatment were subsequently obtained using a foliar application with much lower rates of auxin (10.3 ppm IBA + 6.6 ppm NAA or less). Use of a foliar spray application of auxin with certain crops can lower production costs and reduce the number of employees who work with these chemicals.

Nature of Work: A variety of auxin formulations is available to the commercial propagator, including indole-3-butyric acid (IBA) as powders and water-soluble salts, as well as combinations of IBA and 1-naphthaleneacetic acid (NAA) as alcohol-based, liquid concentrates (6, 10). The basal quick-dip and powder application methods have historically been the most popular for treating stem cuttings with auxin (6, 10). In recent years, foliar application of auxin after sticking cuttings has been examined for propagation of a number of woody and herbaceous ornamental nursery crops (1, 2, 3, 5). The foliar spray application method has been reported to be useful in commercial practice (7, 8) as nursery professionals strive to reduce labor needs, production costs, and the number of employees that work with agricultural chemicals.

Foliar applications of auxin for rooting cuttings of Rosa 'Red Cascade' have previously been evaluated using Dip 'N Grow (DNG) (Dip 'N Grow, Clackamas, OR) and the potassium salts of IBA (K-IBA) and NAA (K-NAA), with results showing similar or reduced rooting and initial shoot growth responses in comparison with a conventional basal quick-dip (5). The commercial product Wood's Rooting Compound (WRC) (Earth
Science Products, Wilsonville, OR) has not previously been evaluated for foliar auxin application. WRC contains IBA and NAA with isopropanol as a cosolvent (9) and has a higher NAA:IBA ratio (0.64:1) than DNG (0.5:1). The objectives of this study were to evaluate the response of cuttings of Rosa 'Red Cascade' to Wood's Rooting Compound using: 1) basal and foliar applications of auxin at rates typically used for a basal quick-dip and 2) foliar applications of auxin at levels that are lower than those normally used for a basal quick-dip.

Cutting material of Rosa 'Red Cascade' was collected from greenhouse-grown stock plants and prepared as 0.75-inch, single-node cuttings during spring of 2001. Cuttings were stuck into Fafard 3B (a blend of peat, perlite, vermiculite, and pine bark; Conrad Fafard, Inc., Agawam, MA) in cell trays. In Expt. 1, auxin solutions were applied to cuttings as either a 1-second basal dip (prior to sticking) or by spraying the cuttings to the drip point using a plastic hand-pump spray bottle (after sticking) using WRC diluted to IBA + NAA concentrations of 1030 + 660, 515 + 330, or 103 + 66 ppm, for a total of six treatments. In Expt. 2, cuttings in one treatment received a 1-second basal dip (prior to sticking) in WRC diluted to 1030 ppm IBA + 660 ppm NAA, while cuttings in the other five treatments were sprayed (as in Expt. 1) using WRC diluted to IBA + NAA concentrations of 41.2 + 26.4, 20.6 + 13.2, 10.3 + 6.6, 1.03 + 0.66, or 0.52 + 0.33 ppm. Deionized water was used for diluting the WRC. Cuttings receiving the basal dip in 1030 ppm IBA + 660 ppm NAA served as the control treatment in both experiments, representing a commonly used rate for commercial cutting propagation. Cuttings were stuck in the late afternoon, allowing sprayed cuttings to dry overnight. Cuttings were placed inside a high-humidity enclosure within a greenhouse for a rooting period of 23 days. There were 50 cuttings per treatment in each experiment. Rooting and initial shoot growth responses were compared among treatments using Fisher’s Exact Test (percentage rooted and percentage of rooted cuttings with shoots) and permutation tests (total root length) adjusted for multiple comparisons using the MULTTEST procedure of SAS (SAS version 9.1.3; SAS Institute, Inc., Cary, NC).

**Results and Discussion:** In Expt. 1, cuttings receiving a basal dip in 515 ppm IBA + 330 ppm NAA exhibited a lower total root length than cuttings receiving the control treatment of a basal dip in 1030 ppm IBA + 660 ppm NAA, while rooting percentages and percentages of rooted cuttings with shoots developing from the axillary buds were similar between the two treatments (Table 1). Cuttings receiving a basal dip using the lowest level of auxin (one-tenth the rate of the control treatment) showed reduced rooting, total root length, and shoot development in comparison to cuttings receiving the control treatment. These results indicate no advantage in reducing the auxin rate below the level of the control treatment when using a basal quick-dip for this cultivar.

Applying auxin to the cuttings as a foliar spray in Expt. 1 resulted in significantly fewer rooted cuttings and, on the cuttings that did root, reduced the size of the root systems and inhibited shoot development from the axillary buds (Table 1). A majority of the unrooted cuttings were dead (data not presented). Results demonstrate that levels of auxin that may be satisfactory for application to cuttings as a basal dip can result in phytotoxicity and inhibition of axillary shoot development when applied as a foliar spray.
Similar negative effects have been reported previously when auxin was applied to cuttings via the rooting substrate (4).

Evaluation of results from Expt. 1 led to selection of lower auxin rates for foliar spray treatments in Expt. 2, these selected rates being much lower than would typically be used for use with a basal quick-dip. In Expt. 2, all cuttings receiving the foliar spray treatments rooted, while all but one cutting rooted using the basal dip control treatment (Table 1). Compared with results using the control treatment, total root length and percentage of rooted cuttings with shoots were significantly less using foliar sprays with IBA + NAA at rates of 41.2 + 26.4 ppm and 20.6 + 13.2 ppm, but were similar using the three lowest rates of auxin (10.3 ppm IBA + 6.6 ppm NAA or less).

Results from this study indicate that foliar spray applications and basal quick-dips on cuttings of Rosa 'Red Cascade' using Wood's Rooting Compound provide comparable rooting and initial shoot growth responses provided that the foliar spray contains 1% or less of the auxin used in the 1030 IBA + 660 NAA ppm basal quick-dip. Spray application at rates normally used with a basal quick-dip can result in phytotoxicity or reduced root and shoot development.

**Literature Cited:**
Table 1. Root and shoot development responses of single-node, softwood cuttings of *Rosa* 'Red Cascade' treated with Wood's Rooting Compound [indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA)] as either a basal quick-dip or a foliar spray. Cuttings were rooted in Fafard 3B substrate\(^z\) in a warm, high-humidity rooting environment inside a greenhouse.

<table>
<thead>
<tr>
<th>Application method; IBA + NAA (ppm)</th>
<th>Rooting (%)</th>
<th>Total root length (mm)</th>
<th>Rooted cuttings with shoots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1(^y):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal quick-dip; 1030 + 660 (control)</td>
<td>84</td>
<td>280</td>
<td>88</td>
</tr>
<tr>
<td>Basal quick-dip; 515 + 330</td>
<td>84(^{NSx})</td>
<td>190(^{**})</td>
<td>79(^{NS})</td>
</tr>
<tr>
<td>Basal quick-dip; 103 + 66</td>
<td>52(^{**})</td>
<td>84(^{***})</td>
<td>46(^{***})</td>
</tr>
<tr>
<td>Foliar spray; 1030 + 660</td>
<td>14(^{***})</td>
<td>131(^{**})</td>
<td>14(^{***})</td>
</tr>
<tr>
<td>Foliar spray; 515 + 330</td>
<td>8(^{**})</td>
<td>93(^{***})</td>
<td>0(^{***})</td>
</tr>
<tr>
<td>Foliar spray; 103 + 66</td>
<td>16(^{***})</td>
<td>76(^{***})</td>
<td>0(^{***})</td>
</tr>
<tr>
<td>Expt. 2(^y):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal quick-dip; 1030 + 660 (control)</td>
<td>100</td>
<td>255</td>
<td>96</td>
</tr>
<tr>
<td>Foliar spray; 41.2 + 26.4</td>
<td>98(^{NS})</td>
<td>105(^{***})</td>
<td>22(^{***})</td>
</tr>
<tr>
<td>Foliar spray; 20.6 + 13.2</td>
<td>100(^{NS})</td>
<td>178(^{***})</td>
<td>76(^{*})</td>
</tr>
<tr>
<td>Foliar spray; 10.3 + 6.6</td>
<td>100(^{NS})</td>
<td>204(^{NS})</td>
<td>96(^{NS})</td>
</tr>
<tr>
<td>Foliar spray; 1.03 + 0.66</td>
<td>100(^{NS})</td>
<td>222(^{NS})</td>
<td>100(^{NS})</td>
</tr>
<tr>
<td>Foliar spray; 0.52 + 0.33</td>
<td>100(^{NS})</td>
<td>233(^{NS})</td>
<td>100(^{NS})</td>
</tr>
</tbody>
</table>

\(^z\)A blend of peat, perlite, vermiculite, and pine bark.
\(^y\)Expt. 1 was initiated on April 17 and evaluated on May 10. Expt. 2 was initiated on May 14 and evaluated on June 6. There were 50 cuttings per treatment in each experiment.
\(^x\)Not significantly different (NS) or significantly different \([p \leq 0.05 (\text{*}), 0.01 (\text{**}), \text{or } 0.001 (\text{***})]\) from the control treatment. Significance was based on \(p\)-values obtained using Fisher's Exact Test (for percentage rooted and percentage of rooted cuttings with shoots) and permutation tests (for total root length) adjusted for multiple comparisons.
Effect of Sowing Depth and Soil Type on Germination and Initial Growth of Selected Wildflower Species

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Index Words: Coreopsis tinctoria, Gaillardia pulchella, Rudbeckia hirta, germination, native plants

Significance to Industry: Seeds of Coreopsis tinctoria Nutt., Gaillardia pulchella Foug., and Rudbeckia hirta L. were sown in three Alabama soil types at varying sowing depths. For R. hirta and C. tinctoria, germination and subsequent growth were highest when seeds were surface sown than when planted below the surface. For G. pulchella, germination and subsequent growth were highest when seeds were sown below the surface rather than above. G. pulchella had the most growth and highest germination rates of all species. Soil type, and in particular, soil physical properties, also influenced germination rates of all species.

Nature of Work: Wildflowers are an advantageous plant choice for many landscapes since they are low maintenance, are able to grow in a variety of climates and soils, and have low water input needs (3). Roadside plantings are becoming more common since they are not only aesthetically pleasing, but stabilize soils (4), and act as naturalizing agents (1). Wildflower ecotypes (from a particular region), especially, may be more competitive with weed species in local growing conditions (1), however, landscape establishment for many wildflowers has not been formally evaluated (5), especially in Alabama. Successfully establishing native wildflower species requires knowledge of planting requirements for good seed germination and subsequent growth (2). Therefore, the objective of this experiment was to compare germination responses of three native Alabama wildflower species sown at four depths in three Alabama soil types.

Seeds of Gaillardia pulchella, (Everwilde Farms, Bloomer, WI), Rudbeckia hirta, and Coreopsis tinctoria (Native American Seed Farm, Junction, Texas) were sown in three Alabama soil types at four depths. Trade gallon containers were filled with Maryvn loamy sand (fine-loamy, kaolinitic typic Kanhapludult), Tallassee loamy clay (fine-loamy, mixed, semiactive, thermic typic Hapludult), or Black Belt clay (very fine, smectitic, thermic oyaquic Hapludert). Twenty seeds of one species were sown in each container at one of four planting depths: 0 cm (surface), 0.3 cm (0.125 in.), 0.6 cm (0.25 in.), and 1.3 cm (0.50 in.) with three reps per depth (subsamples). Seeds of R. hirta and C. tinctoria were sown on 5 August 2008 and seeds of G. pulchella were sown on 12 August 2008. Treatments were in a 3 soil type x 4 sowing depth factorial. There were three single-container replications per treatment. The experiment was arranged in completely randomized design in greenhouses at Plant Science Research Center, Auburn University, Auburn, AL. The containers were misted one to two times a day as
needed. Germination counts were recorded every other day for two weeks and then weekly until experiment termination on 14 October 2008, 70 days after sowing (DAS) for *C. tinctoria* and *R. hirta*, and 63 DAS for *G. pulchella*. Roots and shoots were separated, and soil was rinsed from roots. Shoots and roots were dried at 66°C (150°F) for 48 hours, and total root (RDW) and shoot dry weight (SDW) per container were determined. Total leaf area (LA) per container was measured using a LI-3100C Area Meter (LI-COR, Lincoln, NE). Data were analyzed using generalized linear models procedures with means separation using LSD (*P*<0.05) (6).

**Results and Discussion:** Most germination in each species occurred within two weeks after sowing. However, the highest germination percentages within each species tended to be lower than expected (21-58%).

For *C. tinctoria* and *G. pulchella*, germination, LA, SDW, and RDW were highest when seeds were surface sown, with values generally decreasing with increasing sowing depth (Figs. 1,2,3,4). In most cases there was little to no germination or growth at the deepest sowing depth. This is likely due to small seed sizes, which would therefore be expected to germinate on the soil surface in natural habitats. For *G. pulchella*, germination, LA, SDW, and RDW were highest when sown below the surface. For all species, germination, LA, SDW, and RDW also tended to be highest in the Tallassee soil (Figs. 1,2,3,4). The pH of all the soils was similar so the difference is most likely due to the physical properties of the Tallassee soil, which had smaller particles and, therefore, greater seed-soil contact. Additionally, this soil drained slowly compared to other soils, thus facilitating germination of surface-sown seeds. In spite of this, germination and subsequent growth was observed in all soils, indicating that wildflowers can be established in various soil types throughout the state. However, care must be taken to ensure proper sowing depth for each species. Of all the species, *G. pulchella* had the most growth, suggesting it may be the most vigorous of those evaluated and would certainly make a good choice for roadside plantings. The somewhat low germination percentages would need to be taken into account when sowing; seeds are small and inexpensive, however, so compensation for low germination should not present a barrier to their use. All species of wildflowers evaluated in this work can be a valuable, low-maintenance components of a variety of landscapes including roadside, municipal, and residential. Development of local ecotypes and evaluation of performance in different soil types will contribute to their increased use and appreciation.

**Literature Cited:**


Figure 1. Germination percentage of (A) Coreopsis tinctoria, (B) Rudbeckia hirta, and (C) Gaillardia pulchella 14 days after sowing.
Figure 2. The effect of sowing depth and soil type on leaf area (LA) of (A) *Coreopsis tinctoria* and (B) *Rudbeckia hirta*, 70 days after sowing, and (C) *Gaillardia pulchella*, 63 days after sowing.
Figure 3. The effect of sowing depth and soil type on shoot dry weight (SDW) of *Coreopsis tinctoria* and (B) *Rudbeckia hirta*, 70 days after sowing, and (C) *Gaillardia pulchella*, 63 days after sowing.
Figure 4. The effect of sowing depth and soil type on root dry weight (RDW) of (A) *Coreopsis tinctoria* and (B) *Rudbeckia hirta*, 70 days after sowing, and (C) *Gaillardia pulchella*, 63 days after sowing.
**In Vitro Regeneration of *Rhododendron* 'Fragrantissimum Improved'**

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**Index Words:** Micropropagation, thidiazuron, α-naphthaleneacetic acid, indole-3-acetic acid

**Significance to Industry:** *Rhododendron* 'Fragrantissimum Improved' is a wide hybrid that exhibits attractive exfoliating bark, lush foliage, and clusters of large, white and pink, pleasantly fragrant flowers. This combination of traits makes 'Fragrantissimum Improved' appealing to breeders for the development of novel cultivars for use in the landscape. Like many wide hybrids, however, 'Fragrantissimum Improved' is sterile. *In vitro* chromosome doubling can be used to develop new allopolyploids with restored fertility, providing new opportunities to use these plants in breeding programs (7, 9). This approach first requires the development of an *in vitro* regeneration system. In this study we developed an effective *in vitro* regeneration protocol from leaves of *R.* 'Fragrantissimum Improved' that was optimized using 8.8 µM thidiazuron (TDZ) and 10 µM α-naphthaleneacetic acid (NAA).

**Nature of Work:** The development of *in vitro* regeneration protocols requires the optimization of plant growth regulators (PGR). For *Rhododendron* species, thidiazuron has been effective in stimulating shoot regeneration and several studies have successfully utilized it for *in vitro* production of shoots of difficult to propagate species (1, 4, 5, 6). For example, shoot regeneration of *R.* 'P.J.M.' was 250 times higher on media containing TDZ and IBA (indolebutyric acid) than on media containing 2iP (2-isopentyl adenine) and IBA (6). In contrast, shoot production of *R.* catawbiense 'English Roseum' was optimized using 4 µM IAA (3-acetic acid) combined with 15 µM 2iP (8). Therefore, the objective of this study was to develop an *in vitro* regeneration system by determining the optimal PGR concentrations for *in vitro* callus formation and shoot regeneration from leaves of *R.* 'Fragrantissimum Improved'.

Recently expanded leaves of *R.* 'Fragrantissimum Improved' were collected from glasshouse-grown stock plants maintained at the Mountain Horticultural Crops Research Station in Mills River, NC. The leaves were rinsed under tap water for 4.5 hrs, then washed in 20% commercial bleach (5.25% NaClO) containing a few drops of Tween 20 for 25 minutes. Before being cultured on regeneration media, plants were rinsed three times in sterile, distilled water for 5 minutes per rinse.

The effect of hormone concentration on callus production and shoot regeneration was tested using TDZ (0, 5, 10, 15 and 20 µM) in combination with either NAA or IAA (0, 2.5, 5 and 10 µM). The basal medium consisted of MS salts and vitamins supplemented with 3% sucrose and 0.8% agar (pH 5.75-5.80 prior to autoclaving). Cultures were
maintained in the dark at 23°C (73.4°F). Experiments were conducted as two separate completely randomized factorial designs, using either IAA or NAA as the auxin source. The 20 possible PGR treatment combinations between each auxin and cytokinin were replicated with 8 petri dishes, each containing 5 leaf segments (subsamples). After 8 weeks, data were collected for the number of leaf segments producing callus tissue and shoots. Responses to PGRs were analyzed using multiple regression analyses (SAS version 9.1; SAS Institute, Cary, NC).

**Results and Discussion:** In *vitro* callus formation and shoot regeneration protocols were successfully developed from leaf segments of *R. 'Fragrantissimum Improved'* (Table 1). Leaf segments formed callus on media containing TDZ in combination with either NAA or IAA (Table 1; IAA data not shown). Shoot formation readily occurred on leaf segments exposed to NAA, but shoot production was limited on media containing IAA. Of the IAA treatments, only the combinations 10 μM TDZ and 2.5 μM IAA (0.08% segments forming shoots), 5 μM TDZ and 2.5 μM IAA (0.03% segments forming shoots), and 5 μM TDZ and 10 μM IAA (0.06% segments forming shoots) produced shoots (data not shown). This is consistent with previous observations that IAA did not promote organogenesis in *R. ‘Little John’* (Dr. Darren Touchell, personal observations). Therefore, our analysis focused on the effect of NAA and TDZ on callus formation and shoot regeneration.

Regression analysis indicated that the interaction between the concentrations of TDZ and NAA significantly affected shoot formation (*P*<0.01). From the regression model, a surface response was generated to highlight the interaction between TDZ and NAA and its affect on shoot regeneration (Figure 1). The optimal range of PGR concentrations to maximize shoot production in *R. 'Fragrantissimum Improved' was 8.8 μM TDZ in combination with 10 μM NAA (Figure 1). This is similar to previous research, which found concentrations of 0.1-10 μM TDZ was optimal for shoot regeneration of *R. ‘P.J.M.’* (6). Following the experiment, shoots were transferred to Anderson’s media supplemented with 10 μM 2iP and 4 μM IBA for elongation. The *in vitro* regeneration protocol for *R. 'Fragrantissimum Improved' developed in this study will be used in future experiments in attempts to develop new allopolyploids with restored fertility.

**Literature Cited:**


Table 1. Percentage of leaf segments (out of five subsamples) of *R. 'Fragrantissimum Improved'* producing callus or shoots when cultured on media with different concentrations of TDZ and NAA.

<table>
<thead>
<tr>
<th>TDZ (μM)</th>
<th>NAA (μM)</th>
<th>Segments with callus (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Segments with shoots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>0</td>
<td>2.5</td>
<td>20 ± 12</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>70 ± 30</td>
<td>2 ± 8</td>
</tr>
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<td>10</td>
<td>2.5</td>
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<td>15</td>
<td>2.5</td>
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<td>72 ± 10</td>
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<td>46 ± 32</td>
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<td>48 ± 30</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>66 ± 36</td>
<td>18 ± 16</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means (n=8) ± SEM.
Figure 1. Percent of leaf segments per dish (out of 5) producing shoots in response to α-naphthaleneacetic acid (NAA) and thidiazuron (TDZ). Percent of leaf segments producing shoots = -1.34 + (1.708*TDZ) - (0.107*TDZ^2) - (1.988*NAA) + (0.47*NAA^2) + (0.76*TDZ*NAA) - (0.0039*TDZ^2*NAA^2); P<0.0001; r^2 = 0.62.
In Vitro Shoot Regeneration from Leaves of Hypericum sp.

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Index Words:  Hypericum frondosum, Hypericum galioides, Hypericum kalmianum, micropropagation, benzylamino purine, indoleacetic acid, meta-topolin

Significance to Industry:  Species in the genus Hypericum L. have valuable ornamental merit and considerable potential for breeding and improvement. Breeding efforts at the N.C. State Mountain Horticultural Crops Research and Extension Center have produced the hybrid Hypericum H2003-004-016 from crosses including H. frondosum ‘Sunburst’, H. galioides ‘Brodie’, and H. kalmianum. To further enhance the ornamental qualities of this hybrid, an in vitro shoot regeneration protocol was developed as a foundation for future ploidy manipulations and mutation treatments. The greatest regeneration of shoots per leaf segment was achieved at concentrations of 5 μM benzylamino purine (BA) and 2.5 μM indoleacetic acid (IAA).

Nature of Work:  The genus Hypericum L. contains approximately 370 species worldwide. H. frondosum, H. galioides, and H. kalmianum have desirable ornamental characteristics and environmental tolerances that make them promising species for breeding and improvement. All three species demonstrate broad adaptability and have showy, golden flowers. In addition to attractive flowers, H. frondosum also has bluish-green foliage and a compact growth form. Although generally more open in habit than the other two species, H. kalmianum has the desirable characteristic of being cold-hardy to USDA zone 4. Hypericum galioides is particularly tolerant to the hot, humid conditions in the southeast United States. At the N.C. State Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, NC, these three species have been crossed, through multiple generations, to develop hybrid H2003-004-016. The hybrid exhibits a dense, compact growth form, narrow, bluish foliage, and an abundance of showy, golden flowers.

Tissue culture techniques can be a useful tool in furthering the improvement of ornamental features. The development of in vitro regeneration systems provides an ideal platform for further improvements through ploidy manipulations, mutation treatments, and transgenic applications. Previous in vitro regeneration studies of Hypericum species have included H. perforatum (3,4,5,6), H. heterophyllum (1), and H. frondosum (8). Benzylamino purine (BA) is a commonly used cytokinin for the in vitro regeneration of Hypericum. However, a newer cytokinin, meta-topolin (mT), has shown promise in the regeneration of Spathiphyllum floribundum (9), Musa AAB (7), Aloe polyphylla (2), and Pelargonium × hederaefolium ‘Bonete’ (10). In this study, mT and BA were investigated in order to develop a reliable and efficient shoot regeneration protocol from leaves of Hypericum H2003-004-016.
Young leaves of *Hypericum* H2003-004-016 were collected from glass house grown plants at the MHCREC and surface sterilized under a laminar flow hood for 17 min. in 20% commercial bleach, followed by three rinses of 5 min. each in sterile deionized water. The leaves were then sectioned with a scalpel into 5 mm (0.2 in.) long pieces and placed abaxial side down on petri dishes containing medium composed of Murashige and Skoog basal salts and vitamins, 3% sucrose, and solidified with 0.8% agar. Media was supplemented with either BA or mT at concentrations of 5, 10, and 15 μM in combination with indoleacetic acid (IAA) at concentrations of 0, 1.25, 2.5 or 5 μM. Plates were incubated in the dark at 23°C (73.4°F).

Each cytokinin (BA or mT) was treated as a separate experiment with a completely randomized factorial design. There were at least seven replicates (plates) per treatment and five subsamples (leaf segments) per replicate. After approximately five weeks, data were collected on the percentage of regenerative callus and number of shoots produced per callus by each treatment. Data were subjected to multiple regression analyses (Proc GLM, SAS version 9.1; SAS Institute, Cary, NC).

**Results and Discussion:** Production of regenerative callus and shoots was successfully achieved *in vitro* for *Hypericum* H2003-004-016. Shoots were induced in all treatments combining BA with IAA. In the mT treatments, all treatments produced shoots except for 5 μM mT + 0 μM IAA, 10 μM mT + 0 μM IAA, 15 μM mT + 0 μM IAA, and 15 μM mT + 1.25 μM IAA.

Regression analysis of treatments utilizing BA in combination with IAA demonstrated that BA concentration, IAA concentration, and the interaction between BA and IAA had a significant effect on the production of regenerative callus and number of shoots \( (P<0.05) \). From the regression models, a surface response plot was generated to show the response and interaction between PGRs and their affect on regenerative callus production and number of shoots (Figures 1 and 2). The optimal treatment in the BA experiment was 5 μM BA + 2.5 μM IAA and produced approximately 18 shoots per callus.

Regression analysis of treatments utilizing mT in combination with IAA indicated that mT and IAA concentrations had a significant effect on the production of regenerative callus \( (P<0.05) \) and number of shoots per callus \( (P<0.10) \). From the regression models, a surface response plot was generated to show the response and interaction between PGRs and their affect on production of regenerative callus and number of shoots (Figures 3 and 4). The optimal treatment in the mT experiment was 5 μM mT + 5 μM IAA and produced approximately 10 shoots per callus.

Protocols developed by this study will be used in the future experiments focused on the development of allopolyploids and induced mutants with more diverse and improved ornamental characteristics.
Literature Cited:
Figure 1. Effect of benzylamino purine (BA) and indoleacetic acid (IAA) on percentage of leaf segments producing regenerative callus.
\[ y = 0.082 + 0.037 \times BA + 0.398 \times IAA - 0.0415 \times IAA^2 - 0.0123 \times BA \times IAA, \quad P < 0.001, \quad r^2 = 0.83 \]

Figure 2. Effect of benzylamino purine (BA) and indoleacetic acid (IAA) on number of shoots per leaf segment.
\[ y = 6.25 - 0.0014 \times BA + 8.58 \times IAA - 1.14 \times IAA^2 - 0.24 \times BA \times IAA, \quad P < 0.05, \quad r^2 = 0.61 \]
Figure 3. Effect of meta-topolin (mT) and indoleacetic acid (IAA) on percentage of leaf segments producing regenerative callus.
(y=0.38 – 0.037*mT + 0.34*IAA – 0.041*IAA², P<0.05, r²=0.81)

Figure 4. Effect of meta-topolin (mT) and indoleacetic acid (IAA) on number of shoots per leaf segment.
(y=3.49 – 0.21*mT + 1.49*IAA, P<0.05, r²=0.49)