Defining a Protocol for Vegetative Propagation of *Baptisia*, *Eupatorium* and *Thermopsis*[1]

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Abstract

We determined that highly successful vegetative propagation is possible for *Baptisia*, *Thermopsis* and *Eupatorium*. This is significant because vegetative propagation of these native genera has not been widely practiced in commercial ornamental production. Seed propagation has been utilized over vegetative propagation due to historically low rooting percentages and reduced crowning (bud formation at the root-shoot union) when vegetative propagation is employed. However, vegetative propagation can enable the production of ornamental nursery stock quicker and with greater uniformity in finishing time compared to seed propagation. Vegetative propagation can also facilitate the perpetuation of threatened or endangered species that have small or isolated populations (limited sexual reproduction) or can be used to build numbers of phenotypically superior individuals without wild digging. Four model species representing three native herbaceous genera that include multiple threatened or endangered species were used in the study. Four concentrations of K-IBA (potassium salt of Indole-3-butyric acid) were examined along with 28 and 56 days in a propagation environment to determine the best rooting percentage and root quality for each species. An economic analysis was conducted to test the efficacy of treatment with K-IBA versus double-sticking two untreated cuttings. In addition to developing a commercially viable propagation protocol for these three species, we also determined that treatment with K-IBA is not necessary for successful propagation of the majority of these species, but that more time in a commercial propagation environment leads to higher rooting percentages at a reduced propagation cost.

Index words: vegetative propagation, *Baptisia alba* (L.) Vent., *Baptisia australis* (L.) R. Br., *Eupatorium maculatum* L. var. 'Atropurpureum', *Thermopsis caroliniana* M.A. Curtis, threatened species, endangered species.

Significance to the Nursery Industry

Baptisia, *Eupatorium*, and *Thermopsis* spp. are genera that contain many species of popular native herbaceous perennial plants. Due to these genera's tolerance of minimally managed and non-irrigated landscapes, their popularity is increasing. However, many plants sold in the nursery trade historically have been wild-dug, principally because little published information is available regarding vegetative propagation within these genera. Due to increasing concerns over wild digging of plants, many growers attempt to utilize seed as a source for liner production, with minimal success. In production environments, seed germination can be extremely low and germination can be uneven; taking 6–12 months to attain liners. For that reason, this study aimed to increase the success of vegetative propagation within these genera while simultaneously shortening the propagation period from months to an industry standard 28 or 56 days. Additionally, these three genera contain several ecologically important species that are either federally endangered or threatened or on state 'special concern' listings, including the following species in the Southeastern United States: *Baptisia arachifera* Duncan [Fabaceae] (31), *B. megacarpa* Chapm. ex Torr. & A. Gray (6), *B. calycosa* Canby (14), *Thermopsis fraxinifolia* Nutt. ex M.A. Curtis [Fabaceae] (27), *Eupatorium frustratum* B.L. Rob. [Asteraceae] (14), and *E. villosum* Sw. (14). Utilizing related species from within these genera, we hope to define vegetative propagation protocols that can be applied in the future to related threatened and/or endangered species.

Introduction

Baptisia is typically a long-lived perennial genera that thrives in poor soils and in seasonally drought-prone environments. Several species of *Baptisia*, including *B. arachnifera*, *B. megacarpa*, and *B. australis* (L.) R. Br., are native to the Southeastern U.S. and the prairies of the Midwestern U.S. (6, 9). Depending on the species, *Baptisia* grows from USDA zones 3 to 9 (29) in a wide variety of soil moisture profiles and in soils with pH values of 6.5 and under. *Baptisia* prefer sun, but will tolerate some shade, especially in the Southern U.S. (9) and mature height varies from 0.6 to 1.2 m (2 to 4 ft). *Baptisia* blooms in a variety of colors, from white to yellow to violet. *Baptisia* provides nectar in the spring for bees (10), is a favorite food of several butterfly and moth species (26), and also fixes nitrogen in the soil (15). *B. tinctoria* (L.) R. Br. has been used as a substitute for indigo in dyeing fabric (4). *Baptisia* has also been used as a medicinal plant to reduce fevers and heal infections (18).

Eupatorium species, including *E. hyssopfolium* L. and *E. coelestinum* L., are principally native to the Eastern U.S., although some species, *E. capillifolium* Lam. and *E. perfoliatum* L., are native as far west as Texas and North Dakota (9). *Eupatorium* species are endemic to USDA zones 3 to 9 (9, 29). *Eupatorium* prefers sunny locations, and although it will survive in dry soils, it will grow more vigorously in consistently moist soils. The tallest species, *E. fistulosom* Barratt (9), will grow up to 2.4 m (8 ft) tall but most Eupatorium species range from 0.3 to 1.2 m (1 to 4 ft) in height. Flower color is typically white, although *E. coelestinum* has blue flowers. *Eupatorium* provides nectar for many migratory butterflies (26) and several species have medicinal uses, especially for curing respiratory ailments and kidney stones (18).

Thermopsis is a wide-spread genera that contains species that are native to the Eastern U.S. (*T. fraxinifolia* Nutt ex M.A. Curtis and *T. caroliana* M.A. Curtis) as well as the Western U.S. (*T. montana* Nutt. and *T. californica* S. Watson) (3). Depending on the species, *Thermopsis* grows in USDA zones 3 through 9 (3, 29). It grows in sun or partial shade, in dry or moist soils, and is moderately heat tolerant. Species of *Thermopsis* grow up to 1.2 m (4 ft) tall, have yel-

[1]Received for publication March 21, 2013; in revised form April 16, 2013.

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low, pea-like flowers and are a good substitute for *Lupinus spp.* L. [Fabaceae] in hot climates, because the two share a similar plant architecture and yet *Thermopsis* performs well in hot, dry areas where *Lupinus spp.* grows poorly or does not survive (3).

Many nurseries propagate these genera from seed, albeit with variable germination timing and low germination rates. There are currently no formal published propagation protocols for vegetative propagation of these genera at commercially acceptable rates. Information on propagating these genera vegetatively from cuttings is anecdotal, from personal communications and general comments in non-scientific publication and books (9). Moreover, when propagation from cuttings is attempted, *Baptisia* species typically do not produce crowns (Fig. 1) and therefore will not emerge from dormancy the year following vegetative propagation. Production of these three genera from cuttings could reduce the time to market for liners compared to seed propagation and result in a more uniform (size) crop (9, 12, 13). This should result in the species being more attractive for commercial ornamental nurseries to produce, ensuring that the species would become more widely available to the public for landscape use and for restoration projects.

Many native species are currently propagated by ornamental nurseries from wild-collected seed. This practice reduces population numbers and can decrease genetic diversity of wild populations, particularly when poor seed collection methods are employed (8, 28). The lack of genetic variation required for sexual proliferation of a population would be exacerbated by removing phenotypically desirable plants from the wild population, potentially resulting in a furthering of inbreeding depression and the generational decline of localized populations (2). Vegetative propagation from stock plants at a nursery has the potential to eliminate this pressure on wild populations, yet care must be taken to increase genetic variability within a population during this process rather than decrease genetic variability (32). Additionally, for native plant populations too small to produce adequate amounts of viable seed, such as *B. arachnifera*, vegetative cuttings may be the only feasible propagation method (7). While vegetative propagation and re-introduction of genotypes into the wild can negatively impact the genetic diversity of a wild population, it has been successfully employed (19). Due to the lack of knowledge related to propagation protocols combined with the market potential of these 3 genera, we conducted trials to identify commercially viable vegetative propagation protocols. Our objective was to produce a protocol for each species that would allow commercial ornamental growers to realize at least a 65% success rate in vegetative propagation.

Fig. 1. ***Baptista australis*** **cuttings crowning (formation of bud at the root-shoot union) 56 days after cuttings were struck. Crowns forming above the uppermost roots are circled for emphasis.**

Materials and Methods

Stock plants. We obtained dormant stock plants of *B. alba*, *B. australis*, *E. maculatum*, and *T. caroliniana* as 7.8 liter (2 gal) containers from Goodness Grows Nursery in Lexington, GA, as mature, salable containers, in February 2011. Substrate for all containers consisted of 90% composted pine bark at a 0.8 cm (5/16 in) fine size and 10% washed builder's sand. Stock plants were placed in a glasshouse under 500W high pressure sodium (HPS) lights emitting 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (PPF) density until they broke dormancy in early to mid-March 2011 and were irrigated using Decagon NR5 node/Decagon 10-HS (Decagon Devices, Pullman, WA) soil moisture sensors to maintain a soil moisture between 35–40% volumetric water content ($m^3 \cdot m^{-3}$). Artificial lighting was timed to supplement natural light so that the plants received 12 hr of light. This mimicked the PPF density and day length observed in May, when plants naturally emerge from dormancy. Supplemental lighting continued until May 1. Night and day temperature was set to 18.3C (65F) and 23.9C (75F), respectively. Once the plants broke dormancy, they were fertilized with Harrell's 16-6-11, 4-month controlled release fertilizer (Harrell's Inc., Lakeland, FL) at a high bag rate to simulate commercial growing conditions and stimulate the growth necessary to provide cuttings.

Propagation. Spring trial. We took softwood cuttings of *Baptisia* and *Eupatorium* on March 6, 2011, and *Thermopsis* on April 2, 2011; 10 days after the initiation of new growth and after four nodes had appeared on a majority of vegetative shoots. *Baptisia* and *Eupatorium* cuttings were 7.6 to 10.2 cm (3 to 4 in) long. Cuttings of *Thermopsis* were 15.2 cm (6 in) long. We used four different concentrations of K-IBA (Hortus IBA water soluble salts, Phytotronics, Inc., Earth City, MO): 0, 500, 1000, and 1500 parts per million (ppm) for *Baptisia* and *Thermopsis.* Due to a limited number of *Eupatorium* propagules, we utilized two concentrations of K-IBA (0 and 1000 ppm) for this taxa. The cuttings were

dipped for 5 s in the K-IBA solution before being stuck into the rooting media. *Baptisia* and *Thermopsis* cuttings were stuck with two nodes under the media surface. We assumed that rooting would occur at the lowest node below the soil line with the crown forming at the upper node below the soil line, ensuring the plant would successfully crown and emerge from dormancy the following spring. We were not concerned with the number of nodes above or below the media surface when sticking *Eupatorium*, as crowning is not necessary to ensure this species breaks dormancy the proceeding spring. No bottom heat was applied, in accordance with typical commercial ornamental propagation infrastructure in the Southern U.S., where bottom heat is not employed.

The study included three replications of six vegetative cuttings at each K-IBA concentration for each species. All cuttings within a species were placed in a randomized complete block design. We used 38 round-cell trays to propagate *Baptisia* and *Eupatorium* and 3.8 liter (1 gal) pots for *Thermopsis* cuttings due to their excessive internode length (Griffin Greenhouse and Nursery Supply, Inc., Ballground, GA) filled with three parts Fafard Nursery Mix [Canadian Sphagnum Peat Moss (25%), processed pine bark, vermiculite, perlite, starter nutrients, wetting agent and Dolomitic limestone] to one part perlite. This mixture provided a well-drained rooting medium typical of media used in nursery propagation operations. Cuttings were placed on a mist bench under 6 s of mist every 10 min; an industry standard misting cycle in the Southeastern U.S.

Propagation. Summer trial. The objective of our second trial was to see if non-dormant, semi-hardwood cuttings collected in summer rooted better than those collected in spring. As in the first trial, we used a randomized complete block design with three repetitions of six cuttings for each concentration of K-IBA for each species, with all materials and methods identical to the spring trial. We took cuttings of all species on May 19, 2011. Unlike the spring trial, there were enough cuttings of *Eupatorium* to use all four concentrations of K-IBA.

Data measurement and analysis. We measured rooting percentage, length of longest root, number of root tips, and root volume 28 d and 56 d after cuttings were stuck. Root volume was measured by water displacement by removing cuttings from the medium, washing the roots, and placing the stem and roots that were below the soil line into a 150 ml beaker filled with water marked with 1 ml increments. We used this method because we wanted to be consistent in our non-destructive methodology (5, 17); as we anticipated conducting a simultaneous study of vegetative propagation for *Baptisia arachnifera* Duncan (data not shown) and did not wish to destroy the rooted cuttings of the endangered *B. arachnifera* in the data collection process. We analyzed the data in SAS (SAS Institute, Inc., Cary, NC) using Proc GLM with Tukey means separation to determine differences between each treatment and Proc Means to find the standard deviation for each treatment mean. Data for rooting percentages were transformed using arcsine transformation before analysis was run; results were back-transformed for reporting. Only significant interactions will be reported.

To gain a better understanding of the economics of hormone treatment to increase rooting percentages in *B. australis*, we investigated the labor expense when cuttings were 1) treated with K-IBA or 2) not treated with K-IBA but rather when two cuttings are stuck in the same container (double-stuck). Double-sticking cuttings is performed to compensate for lower rooting percentages observed when propagating without the use of rooting hormone(s).

Table 1. Rooting percentage of *Baptista, Eupatorium* and *Thermopsis* spp. in the spring trial, based on concentration of K-IBA. *Baptisia* and *Eupatorium* were propagated on March 6, 2011, and *Thermopsis* on April 2, 2011; 10 days after the initiation of new growth and after four nodes had appeared on a majority of vegetative shoots. Due to a low number of vegetative shoots, *Eupatorium* was only treated at 0 and 1000 ppm K-IBA.

	K-IBA (ppm)[z]			
Species	0	500	1000	1500
B. alba	79 ± 13a[y]	66 ± 27a	58 ± 20a	66 ± 27a
B. australis	87 ± 38a	96 ± 30a	77 ± 40a	77 ± 40a
E. maculatum	91 ± 36a	*	96 ± 30a	*
T. caroliniana	96 ± 30a	100 ± 19a	100 ± 0a	100 ± 0a

[z]Hormone concentration (K-IBA ppm) was analyzed separately within species using Tukey's LSD groupings at P = 0.05.

[y]Different letters represent statistically different means.

Results and Discussion

Data for the spring and summer trials was analyzed separately to attain means for rooting percentage by K-IBA treatment and days on the propagation bench. Measures of rooting quality (number of roots, root mass, and root length) were also analyzed separately for spring and summer trials. A summary of all results can be viewed in Tables 1 through 4.

Baptista. In the overall results for *B. alba*, there was no effect of K-IBA treatments on rooting percentage (P = 0.3489). However, there was an effect of the date of trial (P = 0.0013), and the number of days on the propagation bench (P = 0.0127), on rooting percentage. The highest rooting percentage was in the spring (Table 1) and after 56 days on the propagation bench (Table 2). There was also an interaction between date of trial and the number of days on the propagation bench (P = 0.0164) (Fig. 2a).

In the *B. alba* spring trial, there was no effect of hormone concentration (P = 0.3280) on percentage rooted. There was an effect (P = 0.0075) of days on the propagation bench on percentage rooted. The number of days on the propagation bench also affected rooting quality as measured by the number of roots (P = 0.01777) and root length (P = 0.0002). However, there was no effect of days on the propagation bench on mass of roots (P = 0.4959). In the *B. alba* summer trial, there was no effect of days on the propagation bench (P = 0.3910) or hormone concentration (P = 0.1571) on rooting percentage. There was also no effect of hormone concentration or days on bench on rooting quality measurements.

In the overall results for *B. australis*, there was an effect of days on the propagation bench (P < 0.0001), hormone concentration (P = 0.0012), and the interaction of date of trial and days on the propagation bench (P = 0.0103) (Fig. 2b) on rooting percentage. There was no effect of date of trial (P = 0.5491) on rooting percentage. There was an effect of days on the propagation bench on rooting quality as measured

Table 2. Measurement of rooting effectiveness of *Baptista*, *Eupatorium* and *Thermopsis* spp. in the spring trial at 28 and 56 days after propagules were struck. Means of rooting percentage, number of root tips, length of longest root and root mass were calculated at harvest and analyzed using Proc GLM.

Species	Measurement	Days on bench[z]	
		28	56
B. alba	Rooting percentage	42.00 ± 9.75b[y]	86.00 ± 5.09a
	Number of roots	2.75 ± 0.86b	9.87 ± 4.39a
	Root length (cm)	3.33 ± 0.06b	50.45 ± 4.39a
	H_2O displacement (g)	0.50 ± 0.11a	0.44 ± 0.19a
B. australis	Rooting percentage	54.00 ± 38.26b	100.00 ± 18.24a
	Number of roots	4.31 ± 1.61b	10.71 ± 3.36a
	Root length (cm)	18.92 ± 7.37b	90.47 ± 24.95a
	H_2O displacement (g)	0.69 ± 0.24a	0.87 ± 0.32a
E. maculatum	Rooting percentage	91.00 ± 36.20a	96.00 ± 30.12a
	Number of roots	9.23 ± 3.20b	19.47 ± 4.68a
	Root length (cm)	49.00 ± 21.27b	167.87 ± 18.20a
	H_2O displacement (g)	0.65 ± 0.21b	1.93 ± 0.42a
T. caroliniana	Rooting percentage	100.00 ± 18.24a	100.00 ± 18.24a
	Number of roots	17.24 ± 6.23b	33.15 ± 6.52a
	Root length (cm)	52.94 ± 17.88b	221.35 ± 32.52a
	H_2O displacement (g)	0.60 ± 0.25b	3.44 ± 1.62a

[z]Number of days from cutting initiation to harvest (28 d and 56 d) was analyzed separately within species using Tukey's LSD groupings at P = 0.05.
[y]Different letters represent statistically different means.

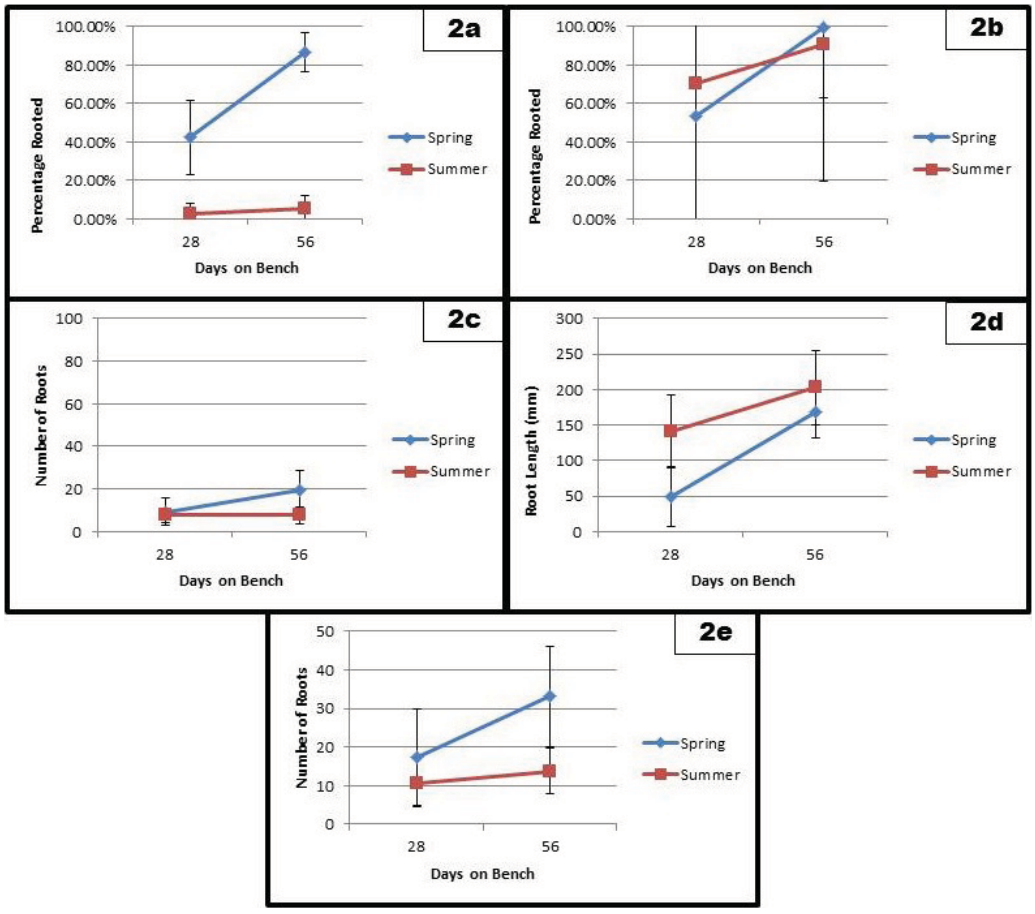

Fig. 2. (a) Interaction of date of trial and days on the propagation bench on rooting percentage for *B. alba*. Values are means with standard deviation as error bars; (b) Interaction of date of trial and days on the propagation bench on rooting percentage for *B. australis*. Values are means with standard deviation as error bars; (c) Interaction of date of trial and days on the propagation bench on number of roots for *E. maculatum*. Values are means with standard deviation as error bars; (d) Interaction of date of trial and days on the propagation bench on root length for *E. maculatum*. Values are means with standard deviation as error bars; (e) Interaction of date of trial and days on the propagation bench on number of roots for *T. caroliniana*. Values are means with standard deviation as error bars.

Table 3. Rooting percentage of *Baptista*, *Eupatorium* and *Thermopsis* in the summer trial, based on concentration of K-IBA. Vegetative propagules were harvested and treated on May 19, 2011.

Species	K-IBA (ppm)[z]			
	0	500	1000	1500
B. alba	6 ± 4a[y]	11 ± 0a	0 ± 0a	0 ± 0a
B. australis	91 ± 36ab	97 ± 30a	57 ± 39b	71 ± 40ab
E. maculatum	98 ± 25a	100 ± 19a	96 ± 30a	94 ± 34a
T. caroliniana	50 ± 38a	77 ± 40a	71 ± 40a	64 ± 40a

[z]Hormone concentration (K-IBA ppm) was analyzed separately within species using Tukey's LSD groupings at P = 0.05.

[y]Different letters represent statistically different means.

by root mass (P = 0.0191) and root length (P < 0.0001). Best rooting was in the spring trial at 56 days on the propagation bench (Table 2); second best rooting was in spring at 500 ppm K-IBA (Table 1). In the summer trial, rooting was best at 500 ppm K-IBA, but this was not better than the control treatment of no rooting hormone application (Table 3) or at 56 days on the propagation bench (Table 4).

In the *B.australis* spring trial, the only effect on rooting percentage was that of days on the propagation bench (P < 0.0001). Days on the propagation bench was also the only factor that influenced rooting quality as measured by number of roots (P = 0.0030); yet it had no significant effect on root mass (P = 0.3202). Days on the propagation bench also had an effect on root length (P < 0.0001), as did hormone concentration (P = 0.0262). In the *B.australis* summer trial, days on the propagation bench (P = 0.0463) and hormone concentration (P = 0.0219) had an effect on rooting percentage. There was also an effect of days on the propagation bench on rooting quality as measured by root mass (P = 0.0109) and root length (P = 0.0053).

Eupatorium. In the overall results for *Eupatorium*, the only factor that affected rooting percentage was days on the propagation bench (P = 0.0266). Date of trial (P < 0.0001), days on the propagation bench (P = 0.0001), and the interaction of date of trial and days on the propagation bench (P = 0.0002) (Fig. 2c) all had an effect on rooting quality as measured by the number of roots. That the interaction of date of trial and days on the propagation bench is significant is not surprising, since each factor in the interaction was also significant. Root length was also affected by date of trial (P < 0.0001), days on the propagation bench (P < 0.0001) and the interaction of date of trial and days on the propagation bench (P = 0.0141) (Fig. 2d). Days on the propagation bench also had an effect on root mass (P < 0.0001). Best rooting was at 56 days on the propagation bench in both spring and summer trials (Table 3 and 4).

In the spring trial for *Eupatorium*, rooting quality was affected by days on the propagation bench; including the number of roots (P = 0.0014), root mass (P < 0.0001), and root length (P < 0.0001). In the summer trial for *Eupatorium*, days on the propagation bench did affect rooting quality as measured by root mass (P < 0.0001) and root length (P < 0.0001); but not number of roots (P = 0.9121).

Thermopsis. In the overall results for *Thermopsis*, there was an effect of the date of trial on rooting percentage (P < 0.0001), but no effect from days on the propagation bench (P = 0.0542) or hormone concentration (P = 0.2077). Rooting quality as measured by number of roots was affected by date of trial (P < 0.0001), days on the propagation bench (P = 0.0002), and the interaction of date of trial and days on the propagation bench (P = 0.0140) (Fig. 2e). Days on the propagation bench was the only factor that affected root mass (P < 0.0001) and root length (P < 0.0001). Best rooting was in spring (Table 1 and 2).

In the *Thermopsis* spring trial, there was no effect on rooting percentage by days on the propagation bench (P

Table 4. Measurement of rooting effectiveness of *Baptista*, *Eupatorium* and *Thermopsis* spp. in the summer trial at 28 and 56 days after propagules were struck. Means of rooting percentage, number of root tips, length of longest root and root mass were calculated at harvest and analyzed using Proc GLM.

Species	Measurement	Days on bench[z]	
		28	56
B. alba	Rooting percentage	03.00 ± 2.78a[y]	06.00 ± 3.21a
	Number of roots	1.00[x]	6.00 ± 0.71[x]
	Root length (cm)	8.00[x]	25.50 ± 5.30[x]
	H_2O displacement (g)	0.00[x]	0.00 ± 0.00[x]
B. australis	Rooting percentage	71.00 ± 39.83b	91.00 ± 35.68a
	Number of roots	8.94 ± 4.04a	8.03 ± 3.11a
	Root length (cm)	40.17 ± 17.72b	98.15 ± 30.96a
	H_2O displacement (g)	0.33 ± 0.21b	1.14 ± 0.55a
E. maculatum	Rooting percentage	95.00 ± 31.52a	99.00 ± 22.01a
	Number of roots	7.62 ± 1.78a	7.73 ± 1.99a
	Root length (cm)	141.62 ± 25.61b	202.48 ± 26.26a
	H_2O displacement (g)	0.80 ± 0.22b	2.20 ± 0.61a
T. caroliniana	Rooting percentage	50.00 ± 37.55b	79.00 ± 39.27a
	Number of roots	10.58 ± 3.49a	13.76 ± 2.98a
	Root length (cm)	33.75 ± 11.46b	225.86 ± 39.67a
	H_2O displacement (g)	0.19 ± 0.16b	3.11 ± 1.22a

[z]Number of days from cutting initiation to harvest (28 d vs. 56 d) was analyzed separately within species using Tukey's LSD groupings at P = 0.05.

[y]Different letters represent statistically different means.

[x]Not enough degrees of freedom to execute Tukey test due to high mortality of cuttings. Standard deviation also not available for some results.

= 1.000) or hormone concentration (P = 0.1047). Days on the propagation bench did affect rooting percentage, as well as rooting quality as measured by the number of roots (P < 0.0001), root mass (P < 0.0001), and root length (P < 0.0001). In the *Thermopsis* summer trial, there was an effect on rooting percentage by days on the propagation bench (P = 0.0365), but not by hormone concentration (P = 0.5814). There was also an effect of days on the propagation bench on rooting quality as measured by root mass (P = 0.0023) and root length (P < 0.0001).

We definitively determined that vegetative propagation is possible at commercially acceptable levels for *Baptisia*, *Eupatorium*, and *Thermopsis*. Species in all 3 genera rooted at or above a threshold of 65% when propagated in spring as softwood cuttings, although *B. alba* needed 56 d on the bench to achieve that level (Table 2). Based on other studies, these rooting percentages are at or above commercially acceptable levels (16, 21, 22). Additionally, these rooting percentages (at or above 50%) will reliably result in at least one viable propagule per propagation container when cuttings are double-stuck.

K-IBA concentrations and time to rooting. With the exception of *B. australis*, there were no differences in rooting percentage or root quality among K-IBA concentrations between 0–1500 ppm. While surprising, this could lead to significant savings for growers that have been utilizing higher concentrations of rooting hormone with the expectation of improved rooting percentages.

Both species of *Baptisia* rooted best after 56 days under intermittent mist. In addition, *B. australis* cuttings had started crowning at 56 days (Fig. 1). The number of days in a propagation environment made no difference in rooting percentage for *Eupatorium* and *Thermopsis*. For all species, rooting quality as measured by length of longest root, root mass and number of root tips was best after 56 days on the bench. Although 56 days is a considerable period of time in a propagation environment, the investment in bench space could be well worth it in order to propagate a particularly desirable or endangered species and provide uniform timing in liner production compared to seed germination.

With the exception of *Eupatorium*, all species had superior rooting in spring rather than summer. This could be due to physiological factors, such as juvenility (11). Seasonality has proven to have a definite effect on vegetative propagation in many species. A study of vegetative propagation of *Leucaena* Benth. found that rooting percentages were higher in the summer when light intensity was higher and greenhouse temperatures were warmer; winter cuttings were also not as vigourous, even though they were taken at the same number of days after coppicing as summer cuttings (23). Cuttings of *Colutea istria* Mill. taken in winter rooted better than those taken in autumn, possibly due to the greater accumulation of carbohydrates in the winter cuttings (1). Physiological age of cuttings can also greatly influence the success of vegetative propagation. Juvenility was an important factor in the success of vegetative propagation of *Robinia pseudoacacia* L. and *Grewia optiva* J. R. Drumm. ex Burret, with highest rooting percentages coming from juvenile cuttings of the species (25).

Economics of double-sticking. We found that it took 2.9 labor hr to collect 1000 cuttings, 3.9 hr to prepare the cuttings, 0.8 hr to dip the cuttings in liquid K-IBA solution, and 2.5 hr to stick the cuttings; for a total of 10.1 labor hr. If the number of cuttings was doubled to accommodate a double sticking procedure and the step to dip them in K-IBA was eliminated, the process would take 18.6 hr. At a salary of $10.00/hr, it would cost $101.00 to propagate via cuttings treated with K-IBA and $186.00 to double-stick the cuttings and therefore eliminate the treatment with K-IBA. This does not take into account the cost of the K-IBA; a factor we omitted due to the variability in cost of commercially available rooting compounds. Since there is no clear statistical advantage to using K-IBA for any species and only a $0.008 USD per cutting increase in cost associated with double-sticking, the use of K-IBA for propagating these species is not necessary. There is also a cost associated with keeping cuttings on the bench. If a nursery does not have the ability or desire to keep cuttings on the bench for 56 days, double-sticking in order to increase the number of plants produced may be a good alternative for treatment with K-IBA for *B. alba* and *Thermopsis spp. Eupatorium* had a very high rooting percentage with all treatments, number of days on the bench, and at both times of the year, so both double-sticking and treatment with K-IBA are unnecessary for this species.

Genetic diversity. One potential means of increasing the heterogeneity of wild populations with small numbers of individuals is to infuse threatened populations with germplasm from disjunct (geographically) populations. With careful management, genetic diversity can be enhanced with the addition of outside germplasm. In the case of infusing heterogeneity via interplanting vegetatively produced plants, if the cuttings to be outplanted are from mother plants from a variety of sources and care is taken to match physiographic conditions of the source site and target site as closely as possible, genetic diversity and population health can be preserved. This technique has been used in clonal forestry (32). In this way, outplanting from plants produced from cuttings is no different than from plants produced by micropropagation, as has been done in some restoration projects (19). In fact, outplanting from cuttings of stock plants of various genetic lines can be used to enhance the genetic diversity of populations too small to maintain genetic diversity on their own. The minimum population size in sexually propagated species needed to avoid genetic drift has been estimated to be 500 plants (20). This population size far exceeds that of many individual populations of endangered native plants. It has been estimated that seed from at least 50 plants must be collected in order to ensure genetic diversity, but each species and population must be assessed to determine a safe amount of seed to be collected (24). Pressure to take whole plants or large numbers of seed, which would certainly be detrimental to genetic diversity, will be reduced by developing a protocol with a high rate of success for vegetative propagation as we have accomplished with this study.

Utilizing vegetative propagation, ornamental nurseries can produce these species from their own stock plants, reducing the pressure placed on wild populations associated with wild collecting of seed for propagation by ornamental nurseries. In addition, liners can be produced quicker and with a uniform finishing time utilizing cuttings rather than the increased variability in finishing time observed in seed propagated pants. Finally, a viable vegetative propagation protocol should allow for the selection and propagation of

wild-collected or nursery-selected genotypes or cultivars with commercially marketable ornamental traits without removing the genetic potential of that individual from a wild population of plants.

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