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Effects of Auxin and Taxa on Rooting Performance of Vegetatively Propagated Wild Coffee (*Psychotria* sp.)

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Summary

Wild coffee (*Psychotria nervosa*), softleaf wild coffee (*Psychotria tenuifolia*), and Bahama wild coffee (*Psychotria ligustrifolia*) are evergreen shrubs with attractive foliage, fragrant white flowers, and colorful fruit. A cutting propagation study was conducted to evaluate the effects of auxin concentration on rooting of these three species, and a dwarf form, *P. nervosa* 'Little Psycho'. Percentage of rooting, root system quality, root length, and root number were determined for semi-hardwood cuttings treated with one of three indole-3-butyric acid (IBA) talc treatments: 0, 8000 or 16000 mg/kg (ppm) - placed under mist for 8 wk. Among all four taxa, at least one of these measures of rooting performance was improved with a talc formulation containing 8000 mg/kg IBA. The percentage of cuttings with roots was greatest for cuttings treated with auxin (88±4% and 88±2% for 8000 and 16000 mg/kg auxin, respectively) compared to cuttings not treated with auxin (control) (71±10%). Results of this study reveal that

all four *Psychotria* taxa evaluated can be easily rooted with or without auxin in a relatively short production cycle and show promise for widened commercial production and use in Florida. For improved root quality, application of auxin is recommended for more efficient liner propagation systems.

INTRODUCTION

Native plants have been historically overlooked in their value to urban landscapes and gardens. However, the appeal of using native plants in landscapes has increased (Kalaman et al., 2022) and homeowners are willing to pay more for a landscape that includes native plants (Gillis and Swim, 2020). The underutilization of native plants can be attributed to inefficient or unknown propagation systems, insufficient marketing and promotion, and limited availability in consumer markets (Wilson, 2020). Moreover, a majority of native species reported in natural areas are not available commercially (Tangren et al., 2022). Propagation knowledge is key to increasing the diversity within a native plant palette (Campbell-Martínez et al., 2022).

Psychotria, a member of the family Rubiaceae, is one of the largest genera of flowering plants with economic, medicinal, and ornamental importance. There are three *Psychotria* species native to Florida: wild coffee (*Psychotria nervosa)*, softleaf wild coffee (*Psychotria tenuifolia*), and Bahama wild coffee (*Psychotria ligustrifolia*) (Gann et al., 2022) with cold hardiness zones of 9a-11 (USDA, 2012) (Fig. 1A-D).

Characteristics common among these taxa include: leaves that are simple, oppositely arranged, with entire margins and prominent veins (**Fig. 1A-D**); white inflorescences that are cymose and fragrant (**Fig. 1E**); fruits occurring as drupes with a fleshy pericarp that turns red in the fall (**Fig. 1F**), each with 2-seeded, longitudinally ribbed seeds (**Fig. 1G**).

Native to Florida's peninsula, *P. nervosa* and *P. tenuifolia* occur in mesic and rockland hammocks. *P. nervosa* 'Little Psycho' is a dwarf cultivar first identified by Brightman Logan in a west-central Florida hammock and is now in commercial micropropagation (AgriStarts, 2022). *Psychotria ligustrifolia* is a rarer species native to mainland and Florida Keys Rockland hammocks. These *Psychotria* taxa can be readily distinguished from each other by their leaves and fruit size.

All four *Psychotria* taxa can be used interchangeably in the landscape under part shade to sun conditions, with minimal care once established. Yet, their availability is limited to a handful of native nurseries who typically propagate by seed when available. Even then, seed germination is inconsistent and erratic (S. Wilson, unpublished data). The overall goal of this study is to widen the year-round availability and use of *Psychotria* taxa in landscapes by developing practical methods for commercial cutting propagation. Specific objectives were to determine the effects of taxa and auxin concentration on optimal rooting responses of *Psychotria*.

Figure 1. Four taxa included in the study were: *Psychotria ligustrifolia* (A), *Psychotria nervosa* 'Little Psycho' (B), *Psychotria nervosa* (C), and *Psychotria tenuifolia* (D) displaying prominently veined, elliptical leaves. White, cymose, terminal inflorescences (E) are followed by ovoid drupes eventually turning red in color (F), and each containing 2 ribbed seed.

MATERIALS AND METHODS

Cuttings of all taxa were taken on the morning of 23 October 2020 from plantings located on the University of Florida Campus. A total of 54 semi-hardwood cuttings from each taxon were selected that had a terminal bud, a minimum of five nodes, was pest and disease-free, and lacked fruits. Cutting lengths were 8 to 9.5 cm for *P. ligustrifolia and P. tenuifolia,* 5 to 8.5 cm for *P. nervosa,* and 4.5 to 9.5 cm for *P. nervosa* 'Little Psycho'. The basal leaves were removed, and the 1.3 cm basal portion of each stem dipped in tap water prior to commercial talc rooting hormone containing either 0, 8000, or 16000 mg/kg (ppm) talc Indole-3-butyric acid (IBA; Hormex, Mainland, PA). For the control treatment (0 mg/kg IBA), cuttings were dipped into water only. After treatment application, cuttings were stuck into 6-cell trays (width 3.8 cm x length 3.8 cm x depth 5.8 cm) (T.O. Plastics, Clearwater, MN) filled with Metro-Mix 852 (6:3:1 bark:Canadian peat:perlite) (Sun Gro Horticulture, Agawam, MA). Each auxin treatment was applied to 6 cuttings replicated 3 times in a randomized complete block design. Overhead mist was provided every 5 min at 5 sec time intervals for 8 wks. The average, maximum, and minimum temperatures in the mist house were 25.1 ^{o}C, 36.4 ^{o} C, and 13.7 ^{o} C, respectively. Cuttings were checked weekly for root emergence, and foliage loss.

At the end of the experiment (after 8 weeks), rooting was evaluated by gently pulling cuttings out of individual cells to determine a root system quality value using a scale from 1 to 4 with 1 = alive cuttings with no roots; 2 = roots forming but do not hold medium; 3 = root ball partially holds plug medium; and 4 = fully formed root ball entirely holding the medium. Cuttings that did not survive the study were scored as a zero and removed from the means. Once a root quality value was determined, the substrate was gently removed from roots to measure root length (mean of the two longest roots) and record the number of roots (up to 25).

RESULTS

The data was analyzed in a two-step process, the first part was to test for interactions between taxa and auxin. Based on those results nonparametric tests were used to determine statistical differences in auxin, either across or for each taxon. This two-step method was used due to non-normality, right censoring, and boundary issues that made a traditional method inaccurate. In this analysis sub samples were assumed independent. Root system quality value, rooting percentage, root length, and root number data were analyzed using a linear mixed model with JMP v. 16 (SAS Institute Inc., Cary, NC).

The root system quality value reflects the combined effects of rooting percentage, root length and root number of all living cuttings. This overall assessment value (scale of 1 to 4) was influenced by auxin application and these effects differed among taxa. Root quality values for *P. ligustrifoli*a and *P. nervosa* (2.2 to 2.9 and 1.9 to 2.4, respectively) were similar among auxin treatments, whereas root quality values for *P. nervosa* ‘Little Psycho’ and *P. tenuifolia* were significantly improved with an auxin application (**Table 1**). As such, *P. nervosa* ‘Little Psycho’ had nearly twice the root quality score when treated with auxin compared to the control (1.6 vs 2.9 to 3.6; **Table 1**). *Psychotria tenuifolia* treated with 8000 mg/kg IBA also had higher root quality (3.6) than the control cuttings (2.7) and cuttings treated with 16000 mg/kg IBA had a similar root quality (3.1) compared to cuttings treated with 0 or 16000 mg/kg IBA (**Table 1**).

There was a significant auxin effect for percent rooting (P<0.0001). However, rooting percentage did not statistically differ by taxa (P=0.0860) (rooting percentages were 87.0%, 87.0%, 85.2%, and 98.1% for *P. ligustrifolia*, *P. nervosa*, *P. nervosa* ‘Little Psycho’ and *P. tenuifolia*, respectively) nor was there a taxa × auxin interaction. The percentage of cuttings with roots was greatest for cuttings treated with auxin [88±4% and 88±2% for 8000 and 16000 mg/kg IBA, respectively] compared to cuttings not treated with auxin (control) (71±10%) (**Table 2**).

Table 1. Mean root system quality rating and root length ± standard error of four *Psychotria* taxa subjected to three auxin treatments [0, 8000, and 16000 mg/kg (ppm) indole-3-butyric acid (IBA)].

Taxa[z]	IBA conc.	Root system quality[y] (1 to 4 scale)	*n*[x]	Root length[w] (mm)	*n*[x]	Root no.[v]	*n*[x]
P. ligustrifolia							
	0 mg/kg	2.22 ± 0.24 a	18	23.21 ± 3.58 b	14	10.50 ± 2.16 b	14
	8000 mg/kg	2.83 ± 0.25 a	18	43.06 ± 4.83 a	16	19.81 ± 2.21 a	16
	16000 mg/kg	2.88 ± 0.18 a	18	33.82 ± 5.75 ab	17	18.82 ± 1.85 a	17
P. nervosa							
	0 mg/kg	1.89 ± 0.18 a	18	18.08 ± 4.28 a	13	7.31 ± 1.55 b	13
	8000 mg/kg	2.44 ± 0.23 a	18	27.72 ± 3.73 a	16	24.25 ± 0.49 a	16
	16000 mg/kg	2.44 ± 0.21 a	18	29.06 ± 3.52 a	17	17.00 ± 1.97 a	17
P. nervosa ‘Little Psycho’							
	0 mg/kg	1.61 ± 0.12 b	18	10.05 ± 1.36 c	11	7.73 ± 1.30 c	11
	8000 mg/kg	2.89 ± 0.18 a	18	32.06 ± 4.53 b	18	23.17 ± 0.91 b	18
	16000 mg/kg	3.59 ± 0.15 a	18	44.21 ± 2.03 a	17	25.00 ± 0.00 a	17
P. tenuifolia							
	0 mg/kg	2.67 ± 0.24 b	18	36.74 ± 4.95 b	17	14.76 ± 2.33 a	17
	8000 mg/kg	3.64 ± 0.18 a	14	55.25 ± 2.81 a	14	23.93 ± 0.94 a	14
	16000 mg/kg	3.06 ± 0.22 ab	18	43.78 ± 5.22 ab	18	22.00 ± 1.43 a	18

[z] For each taxa, means within a column followed by the same letter are not significantly different according to a Steel-Dwass test at P≥0.05.
[y] Cuttings were evaluated using a visual root system quality scale from 1 to 4 with 1 = alive cuttings with no roots; 2 = roots forming but do not hold medium; 3 = root ball partially holds plug medium; and 4 = fully formed root ball entirely holding the plug medium when removed from the tray.
[x] Total number (*n*) of cuttings included in analysis are designated in columns after each measured trait.
[w] Rooting lengths of the two longest roots were averaged.
[v] Root number was counting up to 25 roots.

Table 2. Mean percent rooting ± standard error based on the Agresti–Coull Percent estimates method of the combined four *Psychotria* taxa subjected to three auxin treatments [0, 8000, and 16000 mg/kg (ppm) indole-3-butyric acid (IBA)]. Means within a column followed by the same letter are not significantly different according to a Steel-Dwass test at P≥0.05. Total number (*n*) of cuttings included in analysis are designated in column after the rooting percent.

IBA concentration [z]	Rooting percent	*n*
0 mg/kg	70.60 ± 9.53 b	72
8000 mg/kg	87.77 ± 4.38 a	72
16000 mg/kg	87.77 ± 2.29 a	72

[z] Rooting percentage did not differ by taxa (*P*=0.0861) and there was no taxa × auxin interaction (*P*=0.2849). Therefore, main treatment effects of auxin are reported for the combined taxa.

Root length and root number were influenced by auxin application and the effects of auxin differed among taxa (taxa × auxin interaction). Cuttings of *P. ligustrifolia* and *P. tenuifolia* treated with 8000 mg/kg IBA produced roots that were ~2.0 and 1.5 times longer (43 and 55 mm), respectively, than control cuttings (23 and 37 mm) while cuttings treated with 16000 mg/kg IBA had a similar root length (34 and 44 mm) compared to cuttings treated with 0 and 8000 mg/kg IBA (Table 1). For *P. nervosa*, auxin treatment did not improve root length regardless of auxin level (18 to 29 mm). However, for *P. nervosa* 'Little Psycho' auxin application did result in longer roots compared to the control and a higher auxin concentration further increased root length. *Psychotria nervosa* 'Little Psycho' cuttings treated with 16000 mg/kg IBA had the longest roots (44 mm), followed by 8000 mg/kg IBA (32 mm), while control cuttings had the shortest roots (10 mm) (Table 1). Root number was influenced by auxin application for three of the four taxa (Table 1). Auxin application increased root number compared to the non-treated control for *P. ligustrifolia*, *P. nervosa*, and *P. nervosa* 'Little Psycho' while root number for *P. tenuifolia* did not differ regardless of auxin concentration. For *P. ligustrifolia* and *P. nervosa* root number was similar among cuttings treated with 8000 or 16000 mg/kg IBA and these cuttings had more roots than control cuttings. *Psychotria nervosa* 'Little Psycho' had the greatest number of roots when treated with 16000 mg/kg followed by 8000 mg/kg, compared to the control.

DISCUSSION

Results herein show that all four *Psychotria* taxa can be propagated by stem cuttings within ranges considered acceptable for native nursery production (Cartabiano and Lubell, 2013; Green Isle Gardens Nursery, personal communication). While marginally acceptable rooting percentages (70%) were obtained without the application of auxin, rooting percentage was increased

~1.2 times and there was a positive effect on root quality, root number and root length for cuttings treated with 8000 mg/kg IBA with only minimal improvement with the use of 16000 mg/kg IBA. This study utilized fall cuttings and remarkably high IBA concentrations at 8000 or 16000 mg/kg, typically reserved for hard to root species (Davies et al., 2018). Spring and summer cuttings of a closely related Brazilian wild coffee (*P. nuda*) rooted to a comparable percentage but with a much lower IBA concentration (3000 mg/kg) (Nery et al., 2014). Likewise, our own subsequent anecdotal investigations revealed that *P. nervosa* can also be rooted in late spring using 3000 mg/kg talc IBA. Thus, it is probable that all four *Psychotria* taxa could have high rooting performance when treated with a similarly low IBA concentration and that propagation is not seasonally dependent.

Results also emphasize the value of assessing not only the rooting percentage of treated cuttings, but the overall root system performance, reflecting the combined effects of visual root quality (ability to hold medium), root length and root number. For example, *P. nervosa* 'Little Psycho' cuttings had 85.2% rooting, but very few cuttings held the medium in the absence of auxin. The application of auxin increased both root length and root number, resulting in higher quality root systems. Likewise, *P. tenuifolia* had 98.1% rooting but the addition of auxin improved visual quality (ability to hold medium) and length resulting in a finished liner sooner.

CONCLUSIONS

This serves as a first report of cutting propagation of four *Psychotria* taxa presented herein. Propagation results demonstrate these taxa are relatively easy to produce in a greenhouse under intermittent mist from fall stem cuttings leading to a finished liner within 8 weeks. Although clonal propagation of native plants such as *Psychotria* may not be ideal for restoration purposes, it serves as a reliable alternative to seed propagation for ornamental landscape use.

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