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Asexual Propagation of *Forestiera pubescens* (Nutt.) Using Stem Cuttings

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Abstract. **Desert olive (*Forestiera pubescens*), a large shrub or small tree native to the southwestern United States, exhibits ornamental value and has potential for broader application in managed landscapes. *F. pubescens* exhibits drought and flood tolerance making it well suited for use in urban settings and in green infrastructure, which experience variable, and often extreme, conditions. This taxon has demonstrated cold hardiness in US Department of Agriculture (USDA) zone 4b and may be useful as an option for the diversification of landscapes in the Upper Midwest. However, asexual propagation protocols for producing this taxon are nonexistent. If this species is to be adopted by the nursery trade and produced at a large scale, then propagation protocols need to be developed. The objective of this study was to investigate methods for asexual propagation of *F. pubescens* and explore the factors that influence rooting for different types of stem cuttings. We examined the rooting of *F. pubescens* when propagated using softwood, semihardwood, or dormant hardwood stem cuttings. Semihardwood cuttings were treated with varying concentrations of indole-3-butyric acid (IBA) or left nontreated to evaluate the effects of IBA on rooting. Rooting percentage, root length, and number of roots were evaluated. After 35 days under intermittent mist, 21%, 62%, 74%, and 87% rooting occurred with the nontreated controls [95% ethyl alcohol (ETOH)], 1000, 2000, and 3000 ppm IBA, respectively. Dormant hardwood cuttings were not a viable technique for propagating this taxon. Semihardwood cuttings placed in a 100% perlite substrate resulted in 97% rooting, whereas the use of a 50/50 peat/perlite substrate yielded 40% rooting. Results confirm that *F. pubescens* can be successfully propagated using stem cuttings. Cultivar specificity was determined to play a central role in the rooting response of this species. The application of IBA was not necessary for rooting to take place; however, the number of roots and root length were improved by treatment with IBA. Our findings support the use of semihardwood or softwood stem cuttings in 100% perlite substrate with an application of 3000 ppm IBA to achieve optimal rooting of *F. pubescens*.**

Underutilized nursery crops could serve as a means toward expanding species diversity within the green industry. *Forestiera pubescens* (Nutt.), more commonly known as desert olive, stretchberry, or New Mexico privet [Breen (n.d.); Jacobs 2009; Scianna and Hybner 2009], is a native shrub with a multitude of conservation applications and it may be used as an alternative landscape option that replaces invasive privets (*Ligustrum* L.). Formerly known as *Forestiera neomexicana* A. Gray (Nesom 2009), this taxon has been documented to have value in soil conservation applications including windbreaks, shelterbelts, and living snow fences (Scianna and Hybner 2009). *Forestiera pubescens* has demonstrated drought tolerance (Jacobs 2009) and flood tolerance (Tasker and Schulz 2017), making it applicable to landscapes in the Upper Midwest. *Forestier pubescens* grows naturally along stream courses, moist valleys, hillsides, and mesas at elevations of 3000 to 7000 ft (Jacobs 2009). Although considered drought tolerant, supplemental water and fertilizer improve performance in the landscape (Jacobs 2009). *Forestiera pubescens* can be grown in USDA zones 4 to 9 and reaches a mature height and spread of 0.9 to 3.0 m. [Breen (n.d.); Jacobs 2009]. Production of a beautiful yellow fall leaf color also adds to the horticultural merit of *F. pubescens*. There are no observed pest or disease problems reported with this taxon (Jacobs 2009).

The commercialization of emerging nursery crops and their introduction into cultivation requires an assessment of production limitations and solutions for growers. There is little known about the propagation of *F. pubescens*, and the only known publication on asexual propagation within this genus was determined by Geneve et al. (2019), who evaluated asexual propagation of swamp privet [*Forestiera acuminata* (Michx.) Poir.], a North American native plant, and close relative of *F. pubescens*, with nursery crop potential (Geneve et al. 2019). This work serves as a foundation for determining best practices with other species of *Forestiera*.

Although *F. pubescens* has largely been neglected in horticulture, the taxon was evaluated for landscape performance in Bridger, MT, USA (USDA zone 4) by Scianna and Hybner (2009). Seedlings of the cultivar Jemez were planted in a living snow fence and field windbreak in Bridger, MT. In this field trial, 'Jemez' plants were determined to perform well for landscape and conservation applications over a 33-year period, with no serious insect or disease problems (Scianna and Hybner 2009). The cultivar Jemez is the current recommended and preferred seed source for growing *F. pubescens* (Scianna and Hybner 2009) in Montana, especially for conservation and restoration purposes. Further, two specimens (accession 20050530) of this species [originally derived from the USDA-Agricultural Research Service (ARS) North Central Regional Plant Introduction Station (NCRPIS) via the NC7 trials] have been growing in the living collections of the Minnesota Landscape Arboretum (USDA zone 4b) for nearly 20 years; these plants demonstrate the suitability of the species to the climate and soil conditions of the Upper Midwest.

Due to its cold hardiness, adaptability, and resistance to abiotic and biotic stresses, *F. pubescens* merits horticultural attention and could support the development of resilient landscapes in the Upper Midwest. We hypothesized that it can be propagated by cuttings at different growth stages (semihardwood, dormant hardwood, softwood) using an exogenous application of the auxin-precursor (Strader et al. 2010, 2011), plant growth regulator IBA at standard levels typically used in the green industry for other staple nursery crop species (Table 1). The objective of this study was to develop an asexual propagation protocol for *F. pubescens* and explore the factors that optimize rooting for different types of stem cuttings.

Materials and Methods

Expt. 1. Semihardwood cuttings (derived from partially mature woody tissue) were collected on 22 Jul 2022, from accession 20050530 at the University of Minnesota Landscape Arboretum (UMLA) in Chaska, MN (lat. 44.8°N, long. −93.6°W). Five-node terminal cuttings (~9 cm in length) were collected using a bypass pruner. Cuttings were immediately placed in a plastic bag with a moist paper towel to maintain hydration, transported to the University of Minnesota

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Table 1. Summary of six propagation experiments conducted to evaluate the effects of indole-3-butyric acid (IBA) concentration, substrate composition, cutting type, and basal heat on rooting of *Forestiera pubescens* and two cultivars (Silver Satin and Berry Girl). Experiments included semihardwood, dormant hardwood, and softwood cuttings. Treatments varied by IBA concentration, substrate type, and presence or absence of basal heat. Total number of cuttings per experiment (N) and per treatment (n) are shown. Accessioned plants were obtained by the University of Minnesota Landscape Arboretum (UMLA) or the US Department of Agriculture–Agricultural Resource Service Germplasm Resources Information Network.

Expt. no.	Cutting type	IBA treatments	Substrate(s)	Accession(s) / Cultivar(s)	Basal heat	Experimental units
1	Semihardwood	1000, 2000, 3000 ppm IBA[i]	100% perlite	UMLA 20050530	No	N = 336, n = 84
2	Dormant hardwood	1000, 3000, 8000 ppm IBA[i]	50/50 peat/perlite	Ames 27629, PI 303300	No	N = 100, n = 25
3	Dormant hardwood	3000 ppm IBA	Bark-based	Ames 27629, PI 303300	Yes[ii]	N = 128, n = 16
4	Semihardwood	3000 ppm IBA	50/50 peat/perlite, 100% peat, 100% perlite[iii]	UMLA 20050530	No	N = 160, n = 60 (100% perlite), n = 60 (50/50 peat/perlite), n = 40 (100% peat)
5	Softwood	1000, 3000, 5000 ppm IBA[i]	100% perlite	UMLA 20050530	No	N = 280, n = 70
6	Softwood	1000, 3000, 5000 ppm IBA[iv]	100% perlite	Silver Satin, Berry Girl[iv]	No	Silver Satin: N = 60, n = 15; 'Berry Girl': N = 60, n = 15

[i] Factor of evaluation: IBA treatments.
[ii] Factor of evaluation: Presence/absence of basal heat.
[iii] Factor of evaluation: Substrate.
[iv] Factors of evaluation: IBA treatments and cultivar.

Twin Cities St. Paul campus, treated, and inserted into substrate. Treatments included a nontreated control (95% ETOH), 1000 ppm IBA, 2000 ppm IBA, and 3000 ppm IBA (each dissolved in 95% ETOH solution) using a 3-second quick dip of the basal one-third of the cutting. The cuttings were then inserted into individual cells of 72-cell trays (T.O. Plastics, Otsego, MN, USA; 27.9 cm × 53.9 cm × 5.7 cm) filled with 100% perlite (Midwest Perlite, Appleton, WI, USA) and arranged using a completely randomized design with 14 experimental units [comprising six single cuttings each (subreplicates)] per treatment (N = 336, n = 84). Trays were placed in a mist bay greenhouse where intermittent mist sprayed for 8-second intervals, every 4.5 min, in Saint Paul, MN, USA, using a completely randomized design. Every 15 min, an Apogee® SQ-500 Full-Spectrum Quantum Sensor (Apogee Instruments®, Inc., Logan, UT, USA) recorded the average photosynthetically active radiation (PAR) of 128.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 889.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Greenhouse temperature and relative humidity were monitored by a HOBOconnect MX2302A data logger (Version: 1.6.1, Onset Computer Corporation, Bourne, MA, USA). The average temperature was 25.1 °C, with minimum and maximum values of 20.1 and 35.3 °C, respectively. Relative humidity averaged 72.3%, ranging from 30.7% to 97.6%. Rooting data were collected 35 d posttreatment with exogenous application of IBA. Individual cuttings were carefully removed from the cell, rinsed, and examined. The total number of roots as well as length of each root were recorded. A root was counted if it measured ≥0.25 cm in length.

Expt. 2. Dormant hardwood cuttings were collected on 23 Feb 2023 from the USDA-ARS NCRPIS in Ames, IA (lat. 42°N, long. −93.6°W). The cuttings were inserted into a 50% perlite (Midwest Perlite) and 50% peat (Berger® BP Series Professional Sphagnum Peat Moss; Saint-Modeste, Québec, Canada) mix on 2 Mar 2023. Cuttings were ∼23 cm in length and ∼3 mm in diameter. Fifty dormant hardwood cuttings were each collected from two accessions: Ames 27629 and PI 303300. Treatments included a nontreated control (95% ETOH), 1000 ppm IBA, 3000 ppm IBA, and 8000 ppm IBA (each dissolved in 95% ETOH) using a 3-second quick dip of the basal one-third of the cutting. Stem cuttings were placed into 50-cell count trays (T.O. Plastics; 27.9 cm × 53.9 cm × 6.0 cm) using a completely randomized design with five experimental units [comprising five single cuttings each (subreplicates)] per treatment (N = 100, n = 25). Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for 8-second intervals, every 4.5 min, in Saint Paul, MN, USA, using a completely randomized design. The average temperature was 22.8 °C, with minimum and maximum values of 10.3 and 31.7 °C, respectively. Relative humidity averaged 56.3%, ranging from 11% to 92.6%, as monitored by the HOBOconnect MX2302A (Version: 1.6.1, Onset Computer Corporation). Rooting data were collected at 50 d posttreatment with exogenous IBA; however, due to low rooting counts, data were collected again at 85 d posttreatment. After the initial assessment, cuttings were carefully re-inserted into the substrate to maintain rooting conditions. A root was counted if it measured ≥0.25 cm in length.

Expt. 3. To test the effects of bottom heat, additional dormant hardwood cuttings grown at the Horticultural Research Center in Excelsior, MN, USA, were collected 23 Feb 2023 from the USDA-ARS-NCRPIS. The cuttings were ∼27.1 cm in length and 6.5 mm in diameter. There were 64 cuttings each from two accessions: Ames 27629 and PI 303300 (N = 128, n = 16). Cuttings were placed in 36-cell count trays (T.O. Plastics; 27.8 cm × 54.5 cm × 7.6 cm) with a bark-based potting substrate (Gertens® Mix; Gertens® Greenhouses & Garden Center, Inver Grove Heights, MN, USA) on 04 Mar 2023. For each treatment, a total of 16 cuttings were used per accession [four experimental units comprising four single cuttings each (subreplicates) with eight total unique treatment-accession combinations]. Each experimental unit was represented as a row of four cells in the tray, each cell stuck singly with a subreplicate cutting. Before sticking, cuttings were treated with or without exogenous IBA (3000 ppm IBA). Trays were assigned randomly to be provided with bottom heat that maintained a substrate temperature of ∼28 °C or without bottom heat. Propagules were rooted in a glass greenhouse in Excelsior, MN, USA, using a completely randomized design. Environmental sensing was conducted identically to Expt. 1. Average PAR measured 162.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.001 to a maximum of 1348.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 23.1 °C, with minimum and maximum values of 10.3 and 33.3 °C, respectively. Relative humidity averaged 56.1%, ranging from 11% to 92.6%. Rooting data were collected 90 d posttreatment with exogenous IBA. A root was counted if it measured ≥0.25 cm in length.

Expt. 4. The semihardwood cuttings from the first experiment underwent a dormancy period from 27 Oct 2022 to 2 Feb 2023. Once they produced semihardwood, two node (∼6 cm long and 3 mm in diameter) cuttings were taken to perform a second semihardwood experiment with exogenous

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IBA to evaluate the effects of substrate composition. The cuttings were ~6.2 cm in length and 3.1 mm in diameter. The cuttings were inserted in 50-cell count trays (T.O. Plastics; 27.9 cm × 53.9 cm × 6.0 cm) on 2 Jun 2023. Cuttings were treated with 3000 ppm IBA (dissolved in 95% ETOH) using a 3-second quick dip of the basal one-third of the cutting. The substrate treatments were 100% perlite (Midwest Perlite), 100% peat (Berger® BP Series Professional Sphagnum Peat Moss; Berger Peat Moss Ltd), and 50/50 peat to perlite (N = 160 cuttings: n = 60 cuttings in 100% perlite, n = 60 cuttings in 50/50 peat/perlite, n = 40 cuttings in 100% peat). Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for 8-second intervals, every 4.5 min, in Saint Paul, MN, USA, using a completely randomized design. Environmental sensing was conducted identically to Expt. 1. Average PAR measured 192.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.02 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.9 °C, with minimum and maximum values of 21.2 and 36.3 °C, respectively. Relative humidity averaged 55.2%, ranging from 20.8% to 90.4%. Rooting data were collected 40 d posttreatment with exogenous IBA. A root was counted if it measured ≥0.25 cm in length.

Expt. 5. Softwood cuttings were taken from the living collections (accession 20050530) at the UMLA in Chaska, MN, USA, on 14 Jun 2023. The propagules were three node cuttings (~11 cm long, 2 mm in diameter). The cuttings were inserted in 50-cell count trays (T.O. Plastics; 27.9 cm × 53.9 cm × 6.0 cm) on 14 Jun 2023, using a completely randomized design with 14 experimental units [comprising five single cuttings each (subreplicates)] per treatment (N = 280, n = 70). Cell trays were filled with 100% perlite (Midwest Perlite). Treatments included a nontreated control (95% ETOH), 1000 ppm IBA, 3000 ppm IBA, and 5000 ppm IBA (each dissolved in a 95% ETOH solution) using a 3-second quick dip of the basal one-third of the cutting. Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for 8-second intervals, every 4.5 min, in Saint Paul, MN, USA, using a completely randomized design. Environmental sensing was conducted identically to Expt. 1. PAR averaged 181.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.016 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.8 °C, with minimum and maximum values of 21.2 and 36.3 °C, respectively. Relative humidity averaged 57.8%, ranging from 27.3% to 90.4%. Rooting data were collected 35 d after treatment. A root was counted if it measured ≥0.25 cm in length.

Expt. 6. Container-grown plants of *F. pubescens* ('Berry Girl' and 'Silver Satin') were obtained from High Country Gardens (Hotchkiss, CO, USA). Softwood cuttings were collected from 'Berry Girl' and 'Silver Satin' and inserted into 50-cell count trays (T.O. Plastics; 27.9 cm × 53.9 cm × 6.0 cm) on 14 Jun 2023, using a completely randomized design. Cell trays were filled with 100% perlite (Midwest Perlite). Cuttings of 'Berry Girl' and 'Silver Satin' were two node cuttings, ~9 cm long and 1 mm in diameter. Treatments included a nontreated control (95% ETOH), 1000 ppm IBA, 3000 ppm IBA, and 5000 ppm IBA (each dissolved in a 95% ETOH solution) using a 3-second quick dip of the basal one-third of the cutting. There were three experimental units [comprising five single cuttings each (subreplicates) per treatment] for both 'Berry Girl' and 'Silver Satin' ('Berry Girl': N = 60, n = 15; 'Silver Satin': N = 60, n = 15). Propagules were placed in a mist bay greenhouse where intermittent mist sprayed for 8-second intervals, every 4.5 min, in Saint Paul, MN, USA, using a completely randomized design. Environmental sensing was conducted identically to Expt. 1. PAR measured 181.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, ranging from a minimum of 0.016 to a maximum of 1465.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average temperature was 25.8 °C, with minimum and maximum values of 21.2 and 36.3 °C, respectively. Relative humidity averaged 57.8%, ranging from 27.3% to 90.4%. Data were collected 35 d after treatment. A root was counted if it measured ≥0.25 cm in length.

Statistical analysis. Analysis of variance (ANOVA) models were applied to each dataset to evaluate treatment effects on mean root length, using a one-way ANOVA or two-way ANOVA model dependent on the number of factors assessed. Where appropriate, data were transformed to meet model assumptions. Mean root length per stem cutting was calculated by dividing the total root length by the number of roots, with unrooted cuttings assigned a value of zero. To analyze the effect of treatment on the number of roots per stem cutting, negative binomial regression models were used due to overdispersion in the count data. Model fit for the negative binomial regressions was evaluated using the Pearson χ^2 dispersion ratio (χ^2/df) to assess overdispersion and McFadden's pseudo R^2 to gauge the proportion of variability explained by the model. For Expt. 4, a χ^2 test was used to assess whether the percent of rooted cuttings was different across treatments, and pairwise comparisons were performed using Fisher's exact tests with a Bonferroni correction for multiple comparisons. Mean separation among treatments was obtained by polynomial contrasts using Tukey's test and a 5% rejection level. For Expt. 6, to evaluate the significance of the main effects of treatment and cultivar on the number of roots, likelihood ratio tests (LRTs) were performed by comparing nested negative binomial regression models with and without the factor of interest. *P* values for these global effects were obtained using an ANOVA model with test = "LRT". Data were analyzed using R Statistical Software (Version 4.2.2; Vienna, Austria). The *ggpubr* package was used to generate figures for mean root length and root number, *emmeans* for group comparisons, *MASS* for fitting and estimating linear models, *multcompView* for generating compact letter displays, and *plotrix* for calculating standard errors.

Fig. 1. Typical semihardwood stem cuttings treated with (left to right): nontreated control [95% ethyl alcohol (ETOH)], 1000 ppm indole-3-butyric acid (IBA), 2000 ppm IBA, and 3000 ppm IBA (95% ETOH solution).

Results

Expt. 1. This study was implemented to determine if there was a difference in root length and number of roots produced using exogenous IBA in semihardwood stem cuttings. Semihardwood stem cuttings were rooted at 21%, 62%, 74%, and 87% for nontreated controls (95% ETOH), 1000 ppm, 2000 ppm, and 3000 ppm IBA, respectively (Fig. 1). Mean root length data met model assumptions with a significance at $P \leq 0.001$ (Table 1). All treatment groups had improved mean root length when compared with the nontreated control group (Table 2). The 3000-ppm IBA group had improved mean root length when compared with the 1000-ppm IBA group (Table 2). Compared with the nontreated controls, root length was improved by 342.6%, 485.2%, 641.9% for the 1000 ppm, 2000 ppm, and 3000 ppm IBA treatments, respectively.

For the number of roots per stem cutting, the exogenous IBA treatment groups were all different from the nontreated control, but not different from each other (Table 2). The estimated number of roots per stem cutting

Table 2. Expt. 1: Rooting responses (number of roots per stem cutting and root length) of semihardwood stem cuttings of *Forestiera pubescens* 35 d after treatment with a nontreated control [95% ethyl alcohol (ETOH)], 1000 ppm indole-3-butyric acid (IBA), 2000 ppm IBA, and 3000 ppm IBA (95% ETOH solution) via a 3-s quick dip. Roots were counted if they were ≥0.25 cm.

Treatment	Number of roots per stem	Mean root length (cm)
Control	0.7 ± 0.2 a[1]	0.2 ± 0.0 c
1000 ppm	5.4 ± 0.7 b	0.7 ± 0.1 b
2000 ppm	6.2 ± 0.7 b	0.9 ± 0.1 ab
3000 ppm	6.6 ± 0.5 b	1.2 ± 0.1 a

[1]Means ± standard error with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

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were 2.04, 2.17, and 2.24 for the 1000 ppm, 2000 ppm, and 3000 ppm IBA treatments, respectively, each significant at $P \leq 0.001$. The model showed good fit to the data (McFadden's pseudo $R^2 = 0.05$; $\chi^2/df = 1.05$), indicating no overdispersion. Compared with the nontreated controls, the number of roots per stem cutting was improved by 667.8%, 778.9%, 1237.6% for the 1000 ppm, 2000 ppm, and 3000 ppm IBA treatments, respectively.

Expt. 2. This study was implemented to determine if there was a difference in root length and number of roots produced using exogenous IBA in dormant hardwood stem cuttings. Results for mean root length were inconclusive from this study, as observed rooting was very low. Collected data did not fit model assumptions. At 50 d posttreatment, Ames 27629 rooted at 4%, 20%, 0%, and 24% for the nontreated control (95% ETOH), 1000 ppm, 3000 ppm, and 8000 ppm IBA, respectively. At 50 d, PI 303300 rooted at 0%, 0%, 4%, and 4% for control, 1000 ppm, 3000 ppm, and 8000 ppm IBA, respectively. The same propagules were again evaluated at 85 d posttreatment. At 85 d posttreatment, Ames 27629 rooted at 12%, 12%, 4%, and 36% for the nontreated control (95% ETOH), 1000 ppm, 3000 ppm, and 8000 ppm IBA, respectively. For Ames 27629, some previously rooted cuttings in the 1000-ppm IBA treatment group had deteriorated by 85 d, leading to an overall reduction in the recorded rooting percentage. At 85 d posttreatment, PI 303300 rooted at 8%, 16%, 24%, and 16% for the nontreated control, 1000 ppm, 3000 ppm, and 8000 ppm IBA, respectively. The 85-d posttreatment data were used for analysis, as this time point exhibited the highest rooting percentages across treatments compared with earlier time points.

For the number of roots per stem cutting, treatment with 8000 ppm IBA improved the number of roots per stem cutting compared with the 3000 ppm IBA treatment group (Fig. 2). All other treatments were not different from each other. The estimated number of roots per cutting in the 8000 ppm IBA treatment group was 1.49 at a significance of $P = 0.04$. The model showed good fit to the data (McFadden's pseudo $R^2 = 0.03$; $\chi^2/df = 0.80$), indicating no overdispersion. Compared with the nontreated controls, the number of roots per stem cutting increased by 200% with treatment of 1000 ppm IBA, decreased by 42.9% with treatment of 3000 ppm IBA, and increased by 342.9% with treatment of 8000 ppm IBA.

Expt. 3. This study was implemented to analyze the effect of bottom heat on root length and number of roots in dormant hardwood stem cuttings. Dormant hardwood cuttings in both Ames 27629 and PI 303300 did not root successfully. Of the 128 cuttings between the two accessions, only five cuttings rooted. Four cuttings from Ames 27629 with IBA and bottom heat rooted, and one cutting from PI 303300 with IBA and bottom heat rooted.

Expt. 4. This study was implemented to analyze the effect of substrate on root length and number of roots in semihardwood stem cuttings. Semihardwood stem cuttings rooted at 97%, 40%, and 0% for substrate treatments of 100% perlite, 50/50 peat/perlite, and 100% peat, respectively. Mean root length data met model assumptions with a significance at $P \leq 0.001$ (Fig. 3). For mean root length, 100% perlite was different from all other treatment groups and had the most improvement for root length compared with the other treatment groups (Fig. 3). The 100% peat and 50/50 peat/perlite treatment groups were not different from each other (Fig. 3).

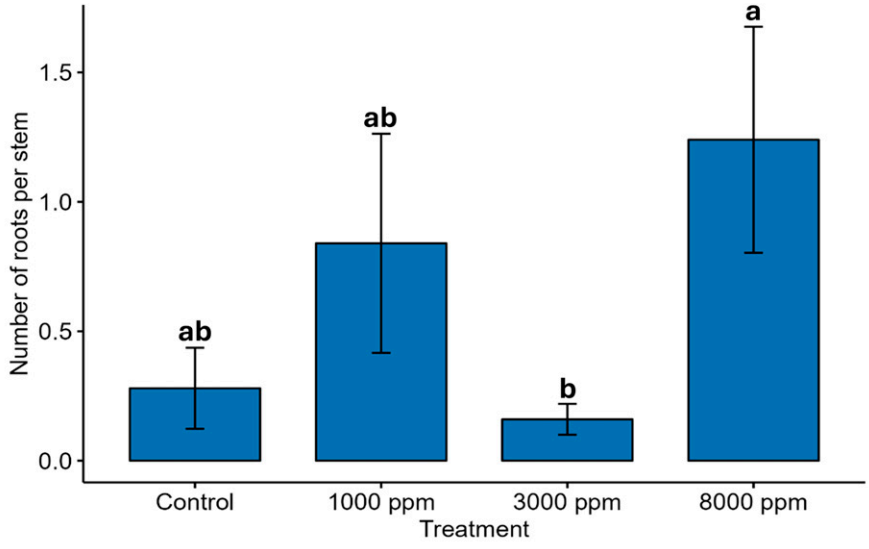

Fig. 2. Expt. 2: Mean number of roots (± standard error) of dormant hardwood stem cuttings of *Forestiera pubescens* 85 d after treatment with a nontreated control [95% ethyl alcohol (ETOH)], 1000 ppm indole-3-butyric acid (IBA), 3000 ppm IBA, and 8000 ppm IBA (95% ETOH solution) via a 3-s quick dip. Roots were counted if ≥0.25 cm in length. Means across species and treatments with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

Because of the complete absence of rooting in the 100% peat treatment group, root count data could not be reliably analyzed using a negative binomial model. Instead, rooting success (yes or no) was evaluated using a binomial approach. Each cutting was coded as rooted (1) or not rooted (0). The χ^2 test showed an association between substrate and rooting success ($P \leq 0.001$), indicating that the likelihood of rooting varied significantly across treatments. The pairwise Fisher's exact tests (with a Bonferroni correction) indicated that all treatments were significantly different from one another (adjusted $P \leq 0.001$) (Fig. 3). The model showed good fit to the data (McFadden's pseudo $R^2 = 0.14$; $\chi^2/df = 0.80$), indicating no overdispersion.

Expt. 5. This study was implemented to determine if there was a difference in root length and number of roots produced using exogenous IBA in softwood stem cuttings. Softwood stem cuttings rooted at 34%, 63%, 87%, and 81% for the nontreated control (95% ETOH), 1000 ppm, and 3000 ppm, and 5000 ppm IBA, respectively. Mean root length data were square root transformed to fit model assumptions with significance at $P \leq 0.001$ (Table 3). All treatment groups had improved mean root length when compared with the nontreated control group (Table 3). The 5000 ppm and 3000 ppm IBA treatment groups improved root length compared with the nontreated control and 1000 ppm IBA treatment group (Table 3). Compared with the nontreated controls, root length was improved by 83.8%, 236.9%, and 214.2% for the 1000 ppm, 3000 ppm, and 5000 ppm IBA treatments, respectively.

The number of roots per stem cutting was estimated at 1.05 for the 1000 ppm IBA treatment ($P \leq 0.001$), 1.88 for the 3000 ppm IBA treatment ($P \leq 0.001$), and 2.22 for the 5000 ppm IBA treatment ($P \leq 0.001$). All treatment groups had an increased number of roots per stem cutting when compared with the nontreated control group (Table 3). In addition, the 5000 ppm and 3000 ppm IBA treatment groups improved the number of roots per stem cutting compared with the 1000 ppm IBA treatment group (Table 3). The model showed good fit to the data (McFadden's pseudo $R^2 = 0.06$; $\chi^2/df = 0.95$), indicating no overdispersion. Compared with the nontreated controls, the number of roots per stem cutting was improved by 185.6%, 554.8%, and 824% for the 1000 ppm, 3000 ppm, and 5000 ppm IBA treatments, respectively.

Expt. 6. This study was implemented to determine if there was a difference between cultivars for root length and the number of roots produced using exogenous IBA in softwood stem cuttings. Softwood stem cuttings rooted at 33%, 53%, 83%, and 60% for the nontreated control (95% ETOH), 1000 ppm, 3000 ppm, and 5000 ppm IBA, respectively, when pooled across cultivar. The cultivar Berry Girl rooted at 61.7% whereas the cultivar Silver Satin rooted at 53.3%. A two-way ANOVA analysis (treatment × cultivar) was used to look at cultivar differences as well as

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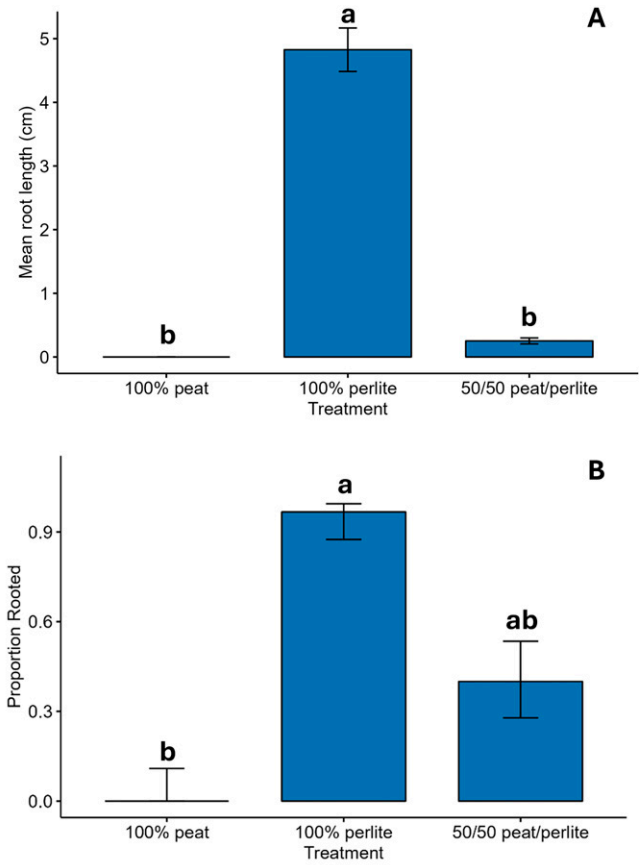

Fig. 3. Expt. 4: Rooting responses (mean root length and proportion rooted) of semihardwood stem cuttings of *Forestiera pubescens* 40 d after treatment with different substrate compositions: 100% peat, 100% perlite, and 50/50 peat/perlite. Roots were counted if ≥0.25 cm in length. Means (**A**) mean root length (cm) ± standard error. Different letters indicate differences among treatments based on Tukey's honestly significant difference test ($P \leq 0.05$). (**B**) Proportion rooted. Error bars represent 95% confidence intervals based on binomial proportion estimates. Different letters indicate differences among treatments based on pairwise Fisher's exact tests with a Bonferroni correction ($P \leq 0.05$).

the differences in treatment. The ANOVA results detected an interaction indicating that the effect of IBA treatment on root length differed between cultivars, $P = 0.0454$ (Fig. 4). The 1000 ppm and 3000 ppm IBA treatment groups of the cultivar Berry Girl improved mean root length compared with the nontreated control treatment group of the cultivar Silver Satin (Fig. 4). All other treatments were not different from each other (Fig. 4). For 'Berry Girl', compared with the nontreated controls, mean root length increased by 42.6% at 1000 ppm IBA, and decreased by 4.1% and 30.8% at 3000 ppm and 5000 ppm IBA, respectively. For the cultivar Silver Satin, compared with the nontreated controls, mean root length was improved by 98.7%, 360.9%, and 312.9% for the 1000 ppm, 3000 ppm, and 5000 ppm IBA treatments, respectively.

A two-way negative binomial analysis (treatment × cultivar) was initially conducted to examine cultivar differences as well as the differences in treatment; however, because the interaction term was not significant, it was removed from the model. The main effect of treatment ($P \leq 0.001$) estimated the number of roots per stem cutting at 1.08 for cuttings treated with 1000 ppm IBA ($P = 0.009$), 2.03 for cuttings treated with 3000 ppm IBA ($P \leq 0.001$), and 1.98 for cuttings treated with 5000 ppm IBA ($P \leq 0.001$). All exogenous IBA treatments in this experiment improved the number of roots per stem cutting when compared with the nontreated control group (Fig. 5). The 5000 ppm and 3000 ppm IBA treatment groups improved the number of roots per stem cutting compared with the 1000 ppm IBA treatment group (Fig. 5). The main effect of cultivar ($P = 0.001$) indicated that 'Silver Satin' associated with fewer roots per cutting compared with 'Berry Girl' ($P \leq 0.001$) (Fig. 5). The model showed good fit to the data (McFadden's pseudo $R^2 = 0.05$; $\chi^2/df = 0.66$), indicating no underdispersion. Compared with the nontreated controls, the number of roots per stem cutting was improved by 177.5%, 589.2%, and 458.3% for the 1000 ppm, 3000 ppm, and 5000 ppm IBA treatments, respectively, when pooled across the cultivars Berry Girl and Silver Satin.

Discussion

Propagation success of *F. pubescens* stem cuttings varied notably by cutting type, substrate, and cultivar, with exogenous IBA playing a critical role in enhancing rooting responses. Although many factors affect propagation success, the goal of achieving 80% rooting serves as a useful benchmark for a plant to be considered by some producers as commercially viable in nursery production (Griffith Gardner et al. 2019). This benchmark was met for semihardwood cuttings in 100% perlite substrate with application of 3000 ppm IBA as well as for softwood cuttings, including the cultivars of Berry Girl and Silver Satin under the same conditions. In line with *F. pubescens*, collection of stem cuttings of box huckleberry [*Gaylussacia brachycera* (Michx.) Torr. & A.Gray] collected in late spring to early summer will produce the highest rooting percentage (Kidwell-Slak et al. 2014). This timing of stem cutting collection corresponds with softwood and/or semihardwood production. However, this goal was not met in either experiment involving dormant hardwood cuttings. Bottom heat treatments also failed to improve rooting in dormant hardwood cuttings, reinforcing the conclusion that dormant hardwood cuttings are less conducive for vegetative propagation of *F. pubescens*.

Some members of the Oleaceae family have reportedly responded well to bottom heat application (Dirr and Heuser 2006). For example, early December cuttings of *Osmanthus americanus* (L.) A.Gray taken in Georgia and treated with 8000 ppm IBA-talc rooted at 75% after 8 weeks when placed in perlite under mist and bottom heat (Dirr and Heuser 2006). Likewise, semihardwood cuttings of *Syringa ×swegiflexa* J.S.Pringle taken in June in Illinois rooted successfully in a 1:1 (vol/vol) peat:sand substrate with application of 4000 ppm IBA-talc, bottom heat (26.7 °C), and mist (Hume and Owens 1970). Future research could explore whether adjusting bottom heat temperature improves rooting outcomes for dormant hardwood cuttings of *F. pubescens*, which underperformed under the conditions tested in this study.

Woody plants show considerable variation in their responses to asexual propagation techniques (Stokes et al. 2023). *Shepherdia canadensis* (L.) Nutt., an extremely hardy

Table 3. Expt. 5: Rooting responses (number of roots per stem cutting and root length) of softwood stem cuttings of *Forestiera pubescens* 35 d after treatment with a nontreated control [95% ethyl alcohol (ETOH)], 1000 ppm indole-3-butyric acid (IBA), 3000 ppm IBA, and 5000 ppm IBA (95% ETOH solution) via a 3-s quick dip. Roots were counted if there were ≥0.25 cm. To fit model assumptions for mean root length (cm), data were square root transformed; nontransformed data are presented.

Treatment	Number of roots per stem	Mean root length (cm)
Control	1.0 ± 0.2 a[1]	0.8 ± 0.2 c
1000 ppm	3.0 ± 0.5 b	1.4 ± 0.2 b
3000 ppm	6.8 ± 0.9 c	2.5 ± 0.2 a
5000 ppm	9.6 ± 0.8 c	2.4 ± 0.2 a

[1]Means ± standard error with the same letter (within a column) are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

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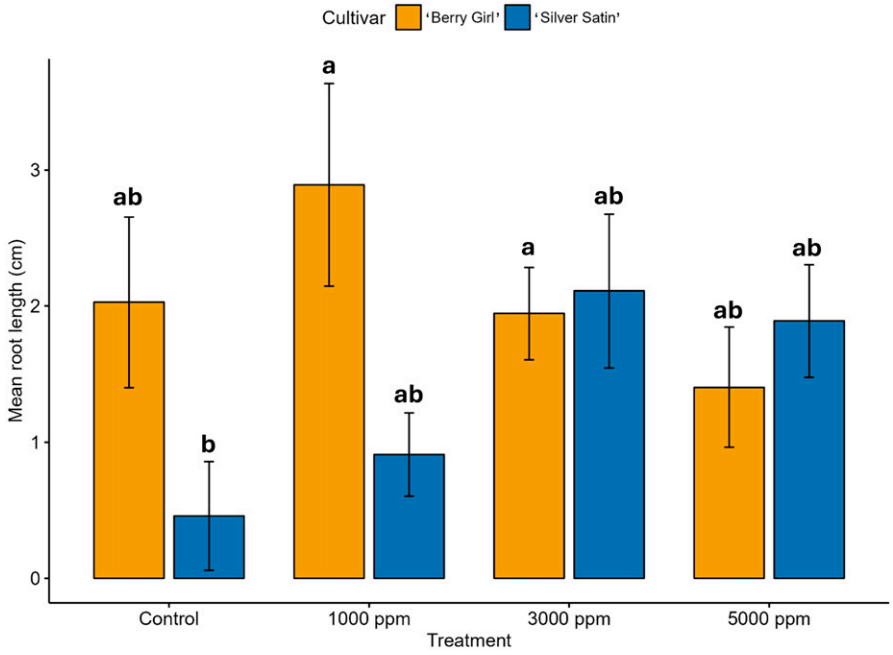

Fig. 4. Expt. 6: Mean root length (± standard error) of softwood stem cuttings of *Forestiera pubescens* 35 d after treatment with a nontreated control (95% ETOH), 1000 ppm indole-3-butyric acid (IBA), 3000 ppm IBA, and 5000 ppm IBA (95% ETOH solution) via a 3-s quick dip. Roots were counted if ≥0.25 cm in length. Means across treatments and cultivars with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

shrub species, was successfully propagated asexually using stem cuttings. Hardwood cuttings of *S. canadensis* rooted best with the application of 3000 ppm IBA, and rooting was inhibited by the application of 8000 ppm IBA (Krishnan and Hughes 1991). Similar to our findings, asexual propagation of *Comptonia peregrina* (L.) Coult. with the application of 3000 ppm and 8000 ppm IBA improved rooting percentage compared with nontreated stem cuttings (Griffith Gardner et al. 2019). For *F. pubescens*, 3000 ppm IBA was also the optimal level of exogenous IBA out of the range of treatments used in semihardwood and softwood cuttings. When looking at using higher levels of exogenous IBA, 8000 ppm IBA was used for dormant hardwood cuttings, which did not evoke any significant rooting across treatments. Further studies are necessary to determine a maximum threshold of exogenous IBA used on semihardwood and softwood cuttings of *F. pubescens* that inhibits root growth or reduces root length or number of roots per stem cutting.

Contrary to the results of this study, semihardwood cuttings of swamp privet (*F. acuminata*) rooted above the commercial nursery crop rooting goal with 90% rooting for both nontreated cuttings and cuttings treated with potassium salt of indole butyric acid (K-IBA) (Geneve et al. 2019). Peterson et al. (2020) also found that application of K-IBA is not always necessary for adventitious rooting, with sweetgale (*Myrica gale* L.) root rating, root dry weight, and root length all reduced by the application of K-IBA (Peterson et al. 2020). Within the Oleaceae family, *Jasminum* spp. also rooted readily from softwood cuttings, with *Jasminum nudiflorum* Lindl. softwood cuttings reported as rooting 90% to 100% without treatment; however, application of auxin-class plant growth regulators was reported to generally improve the rooting of this species (Dirr and Heuser 2006). In contrast, *Fraxinus* spp., another member of the Oleaceae family, has been reported to be "virtually impossible" to root via stem cuttings even from juvenile trees (Dirr and Heuser 2006). These contrasting rooting responses among woody taxa underscores the complexity of adventitious rooting and the necessity of developing tailored propagation strategies for species such as *F. pubescens*.

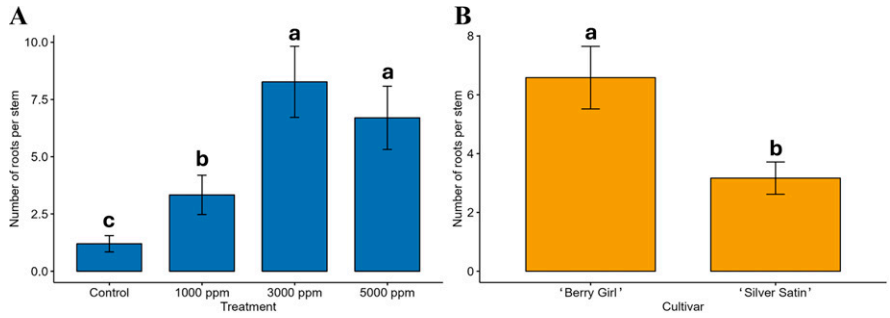

Fig. 5. Expt. 6: Mean number of roots (± standard error) on softwood stem cuttings of *Forestiera pubescens* 35 d after treatment. (**A**) Effect of rooting hormone concentration: cuttings were treated with a nontreated control [95% ethyl alcohol (ETOH)], 1000 ppm indole-3-butyric acid (IBA), 3000 ppm IBA, or 5000 ppm IBA (95% ETOH solution) via a 3-s quick dip. (**B**) Effect of cultivar: Berry Girl and Silver Satin. Roots were counted if ≥0.25 cm in length. Means across treatments and cultivars with the same letter are not different according to Tukey's honestly significant difference test ($P \leq 0.05$).

With *F. pubescens*, substrate influenced rooting. This phenomenon is observed across woody-plant taxa. For example, asexual propagation of half-highbush blueberries (*Vaccinium corymbosum* L. ×*angustifolium* Aiton) and lowbush blueberries (*Vaccinium angustifolium* Aiton) by stem cuttings showed that substrate can have a significant effect on rooting (Schwab et al. 2021). Stem cuttings of half-highbush blueberries rooted most successfully in a traditional soilless substrate of 1:3 (vol/vol) peat to perlite, outperforming the less conventional substrates tested. For lowbush blueberries, the same 1:3 (vol/vol) peat:perlite mix was the second most effective rooting medium. For mountain fly honeysuckle [*Lonicera villosa* (Michx.) Shult.], an underutilized plant with horticultural potential, peat was deemed not viable as a substate for vegetative propagation (Hayes and Peterson 2019). Mountain fly honeysuckle produced the best rooting response in 100% perlite compared with a mix with the addition of peat in the substrate (Hayes and Peterson 2019). The root size was diminished as the concentration of peat increased in an overhead mist irrigation system (Hayes and Peterson 2019). In the Oleaceae family, *Osmanthus heterophyllus* (G.Don) P.S.Green rooted at 75% when late summer stem cuttings (semihardwood) were treated with a 3000 ppm K-IBA for 20 s, placed in a 1:1 (vol/vol) sphagnum peatmoss:perlite substrate under mist, and maintained under a 16-h photoperiod (Dirr and Heuser 2006). In our study with *F. pubescens*, even with the utilization of traditional soilless substrates, peat was seen to not be a viable option for rooting of stem cuttings and even in combination with perlite, rooting results were not optimal. The superior performance of 100% perlite substrate indicates that well-drained, aerated substrates are critical for rooting semihardwood cuttings of *F. pubescens*.

The results of both semihardwood experiments demonstrated that semihardwood cuttings treated with 3000 ppm IBA and grown in 100% perlite growing medium maximizes overall rooting percentage, root length, and the number of roots. It is not recommended that growers attempt to root dormant stem cuttings of *F. pubescens*. The softwood IBA experiment shows that softwood cuttings root best with either 3000 ppm or 5000 ppm IBA for the maximization of root length and the number of roots per stem cutting. The cultivar study indicates that the effect of IBA on rooting varies between cultivars when using

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softwood cuttings, highlighting the importance of customizing propagation protocols for each genotype to improve efficiency. Optimization of a rooting protocol for *F. pubescens* can better facilitate the introduction of this adaptable species into the nursery industry to replace invasive privets in the *Ligustrum* genus and expand biodiversity of managed landscapes, potentially increasing system resiliency in response to more frequent and severe weather events (Pooler et al. 2024).

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