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Stem Cutting Propagation and Micropropagation of Northern Bayberry

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Abstract. **Northern bayberry [*Morella* (formerly *Myrica*) *pensylvanica*] is an attractive, adaptable, semievergreen, northeastern North American native shrub that is sought for landscaping but difficult to propagate clonally. The impact of timing (June, July, or August) and concentration of indole-3-butyric acid [IBA (0, 2000, 4000 or 8000 ppm)] on propagation by stem cuttings was evaluated for genotypes of northern bayberry including the female cultivars Bobzam (Bobbee™) and UConn Compact and an unnamed male. Medium formulation and cytokinin type were evaluated for micropropagation of 'Bobzam' and 'UConn Compact'. Stem cuttings of 'Bobzam' and 'UConn Compact' rooted poorly (at ≤55% and ≤20%, respectively) at all timings and concentrations of IBA; however, rooting success of ≥85% was achieved for the unnamed male genotype when cuttings were taken in June. Micropropagation of 'Bobzam' was successful using Woody Plant medium with 4 mg·L^{-1} zeatin and explants taken from shoots that had expanded 12 to 18 cm on containerized stock plants. Initiated explants of 'Bobzam' required eight subcultures before they began to produce shoots consistently at a 2× multiplication rate and eventually reached a 3× multiplication rate. Micropropagation attempts using Murashige and Skoog medium, the cytokinins 6-benzylaminopurine, meta-topolin, and thidiazuron, or the cultivar 'UConn Compact' were unsuccessful. Microshoots of 'Bobzam' rooted at ≥80% either by in vitro prerooting or ex vitro rooting directly in trays. Rooted microcuttings easily acclimated to greenhouse conditions and grew rapidly when potted to 1.04-L containers and then into 5.68-L containers. The micropropagation protocol developed for 'Bobzam' can be used by propagators to expand production of this popular female cultivar.**

Northern bayberry [*Morella* (formerly *Myrica*) *pensylvanica*] is a semievergreen shrub native to North America, ranging from Ontario to Nova Scotia in Canada, south to North Carolina in the United States and west to Ohio (US Department of Agriculture, Natural Resources Conservation Service 2023). The plant has a dense, rounded, and suckering habit and reaches 1.5 to 3 m tall (Dirr 2009). Leaves are obovate to oblong, glossy, dark green, and fragrant when crushed. Northern bayberry can be found occupying diverse and frequently difficult habitats, to which few other species are adapted. These include sites with full sun and high wind exposure; wet, dry, and/or acidic soils; and coastal locations that receive sea salt spray (Hightshoe 1988). Northern bayberry has been found growing in road cuts, railroad banks, gravel pits, and disturbed sites stripped of topsoil. The plant's broad adaptability may be attributable to its ability to form root nodule associations with the nitrogen fixing bacteria, *Frankia* (Benson and Silvester 1993).

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Northern bayberry is dioecious, and plants produce small inconspicuous flowers in early spring (Dirr 2009). Female plants form attractive fruits that ripen in fall. Fruits are drupes, rounded in shape (3 to 5 mm diameter) and possess a wax coating that is white to pale gray in color and fragrant. Historically, the wax has been used to make candles and soap. To get good fruit set, 20% of bayberry plants in a landscape should be male. Fruits attract birds including chickadee, titmouse, pine siskin, and yellow-rumped warbler. If not taken by wildlife, then fruits will persist through winter.

Northern bayberry is a versatile and durable shrub that is sought by landscapers for use in areas as cold as USDA Hardiness Zone 4 (Dirr 2009). It is suitable for seaside gardens, roadside plantings, and other landscape sites where salt can be an issue. Plants will tolerate hot, sunny, infertile, and dry landscape locations, such as parking lot island plantings. Other landscape uses include mass plantings, screens, hedges (plants tolerate shearing and renewal pruning), naturalistic gardens, and as a neutral backdrop to set off more showy plants.

Many of the northern bayberry plants for sale are unsexed seedling-grown plants, but a small number of superior cultivars have been selected. The female cultivar Bobzam (Bobbee™) has a compact, dense form, and develops leaves that are larger than the species (Lake County Nursery 2023). The female cultivar Morton (Silver Sprite™) forms a dense, broad-oval mound, reaches 1.5 m tall, and produces gray-green leaves (Chicagoland Grows® 2023). Its male cultivar companion, which exhibits a similar form, is Morton Male (Male Silver Sprite™). These cultivars are not readily available in the US nursery trade because they are difficult to propagate by stem cuttings and micropropagation protocols have not been established. Recently, the female cultivar UConn Compact was selected for its compact habit. Although there has been grower interest in producing 'UConn Compact', the plant has not been licensed because it, like many genotypes, does not propagate with a high rate of success. There are few reports about clonal propagation of northern bayberry and no studies have been published in the scientific literature. Dirr and Heuser (2006) recommend taking softwood cuttings in mid-June, treating them with 5000 ppm indole-3-butyric acid (IBA) and using a quick draining medium with minimal mist. The objectives of this research were to evaluate the impact of timing and IBA concentration on propagation by stem cuttings, and medium formulation and cytokinin type on micropropagation, for genotypes of northern bayberry. This is the first report on the successful micropropagation of mature phase *Morella pensylvanica*. This reliable and efficient asexual propagation method will allow superior and desired female genotypes to be widely produced and available for landscape, wildlife, and conservation uses.

Materials and Methods

Stem cutting propagation. Three genotypes, 'Bobzam', 'UConn Compact', and an unnamed male genotype were studied. Stem cuttings, 10 to 15 cm in length, were taken at the end of Jun, Jul, and Aug 2021 from established plantings at the University of Connecticut (UConn) Plant Science Research and Education Facility (Storrs, CT, USA). Cuttings were wounded on one side and dipped into 0, 2000, 4000, or 8000 ppm IBA in a 1:1 solution of ethanol and deionized water. Cuttings were stuck into 307.3-mL containers filled with a growing medium composed of one part sphagnum peatmoss (Fafard Inc., Agawam, MA, USA) one part horticultural-grade fine perlite (Whittemore Co., Lawrence, MA, USA) and one part horticultural-grade medium vermiculite (Whittemore Co.). Containers, each with one cutting, were set in flats, which held a total of 32 containers. An experimental unit consisted of a flat row with four containers. Experimental units were arranged in a randomized complete block design (RCBD) with five replications for each timing (June, July, and August) that cuttings were taken. Flats with cuttings were held in a greenhouse with set points of 21/17 °C day/night temperature threshold under intermittent mist that provided 10 s of mist every 6 min. Eight weeks after sticking, percent

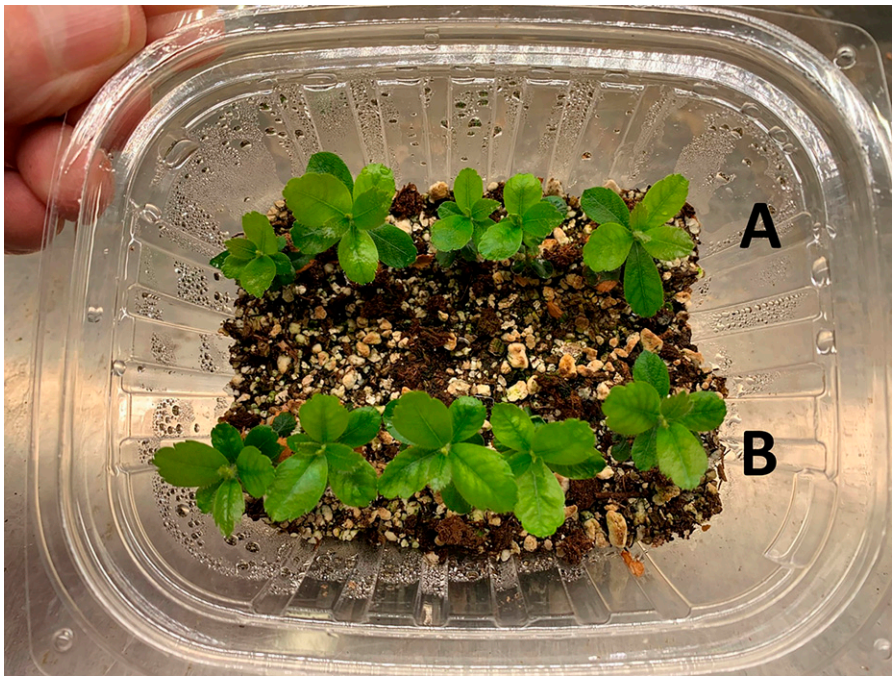

Fig. 1. A tray of rooted *Morella pensylvanica* 'Bobzam' Bobbee™ microcuttings, containing (**A**) a row of five prerooted microcuttings and (**B**) a row of five microcuttings directly stuck ex vitro.

rooting per experimental unit was recorded, and root number and root length per cutting were measured and averaged for the experimental unit. Roots had to be ≥2 mm to be counted, and root length was measured from the point of origin at the stem to the root tip, including all side branches. A subset of 30 rooted cuttings were potted into 1.04-L containers filled with growing medium composed of four parts pine bark (Fafard Inc.), two parts sphagnum peatmoss (Fafard Inc.), and one part sand. Containers were overwintered in a minimally heated hoophouse and the number of surviving plants was determined the following spring after grow out.

Micropropagation. Three studies were conducted. Study 1 included 'Bobzam' and 'UConn Compact' and studies 2 and 3 were conducted with only 'Bobzam'. In Feb 2022, dormant containerized stock plants were forced in a greenhouse with set points as described. When shoots had reached 4 to 6 cm long, tip cuttings, 2 to 3 cm long, were taken for initiating shoots in vitro for study 1. After leaves were removed, stems were disinfected by immersion in a solution of 0.54% (w/v) sodium hypochlorite for 15 min with intermittent agitation, followed by two rinses in sterile deionized water. Stem segments were then aseptically trimmed to remove damaged tissue and cultured in 25 mm × 150 mm glass test tubes containing 15 mL of medium. The treatment medium for study 1 was Woody Plant (WP) medium (Lloyd and McCown 1980) or Murashige and Skoog (MS) medium with vitamins (Murashige and Skoog 1962) supplemented with 4 mg·L^{-1} zeatin (ZT), 1 mg·L^{-1} 6-benzylaminopurine (BA), 5 mg·L^{-1} meta-topolin (MT), or 0.1 mg·L^{-1} thidiazuron (TDZ). All growth regulators were coautoclaved with the media. In addition, each treatment medium had 0.8% (w/v) agar (MilliporeSigma, St. Louis, MO, USA), 3% (w/v) sucrose, and pH 5.2 or 5.7. The experimental unit was a single test tube containing one shoot explant and tubes were arranged as a RCBD with 10 replications (blocks). Cultures were maintained at 25 °C (77 °F) with a 16-h photoperiod provided by cool-white, fluorescent lamps at an intensity of 40 μmol·m^{-1}·s^{-1}. Surviving explants were subcultured every 21 d. Explant survival was recorded after four subcultures. Experimental units containing a surviving explant were scored as 100% and units for which explants did not survive were scored as 0%.

For study 2, tip cuttings 2 to 3 cm long were taken in Apr 2022 from the same stock plants of 'Bobzam' as for study 1, and at this time shoots were 12 to 18 cm long. Shoot tips were disinfected and stuck into test tubes as described for study 1. The treatment medium was WP with 0.8% (w/v) agar, 3% (w/v) sucrose, and pH 5.2 plus 2, 4, or 8 mg·L^{-1} ZT. The experimental unit was a test tube and tubes were arranged as a RCBD with 10 replications (blocks). Cultures were maintained as described for study 1 with subculturing done every 21 d for a total of 9 subcultures over 189 d. At each subculture time, microshoots were cut into 1 cm long explants to produce a combination of subapical nodal pieces with 4 to 6 nodes and apical tip pieces with 4 to 6 nodes. Explant survival was recorded at every subculture and cumulative number of shoots produced per starting explant was determined for the entire 189-d culture period. After cultures were 190 d old from initiation, they were grown in glass bottles (125 mL) with lids (B-Caps, Magenta, Lockport, IL, USA) containing 30 mL of WP medium supplemented with 0.8% (w/v) agar, 3% sucrose, 4 mg·L^{-1} ZT, and pH 5.2. Six explant pieces (a mix of nodal and tips) were stuck per culture jar at each subculture event.

For study 3, additional dormant plants of 'Bobzam' that were being held in a cooler at 38 °C were moved into the greenhouse for

Table 1. Rooting percent, root number and total root length for stem cuttings of *Morella pensylvanica* genotypes 'Bobzam' Bobbee™, male, and 'UConn Compact' taken at the end of Jun, Jul, and Aug 2021 and treated with 0, 2000, 4000, or 8000 ppm indole-3-butyric acid (IBA).

		June			July			August		
Genotype	IBA (ppm)	Rooting (%)	Root no.	Total root length (cm)	Rooting (%)	Root no.	Total root length (cm)	Rooting (%)	Root no.	Total root length (cm)
'Bobzam'	0	0 a[1]	—	—	55 a	3.8 a	32.4 a	5 a	0.7 a	11.7 a
	2000	15 a	0.82 a	7.1 a	35 ab	2.1 a	22.4 a	5 a	0.5 a	11.3 a
	4000	5 a	1.18 a	12.7 a	45 ab	4.3 a	40.0 a	5 a	0.7 a	1.6 a
	8000	0 a	—	—	20 b	4.0 a	22.9 a	0 a	—	—
Male	0	90 a	3.6 c	35.7 c	75 a	2.4 a	25.2 a	15 a	0.7 a	7.4 a
	2000	100 a	6.7 c	75.4 b	70 a	3.2 a	31.5 a	20 a	0.8 a	3.9 a
	4000	100 a	11.6 b	124.6 a	30 b	3.5 a	30.7 a	15 a	1.5 a	6.9 a
	8000	85 a	16.0 a	140.4 a	10 b	3.6 a	27.8 a	0 a	—	—
'UConn Compact'	0	0 a	—	—	15 a	1.2 a	11.8 a	0 a	—	—
	2000	10 a	2.0 a	4.8 a	20 a	1.0 a	5.7 a	0 a	—	—
	4000	0 a	—	—	10 a	0.6 a	3.0 a	0 a	—	—
	8000	0 a	—	—	0 a	—	—	0 a	—	—

[1] Mean separation within column within genotype, indicated by different letters, by Tukey's honestly significant difference test at $P \leq 0.05$.

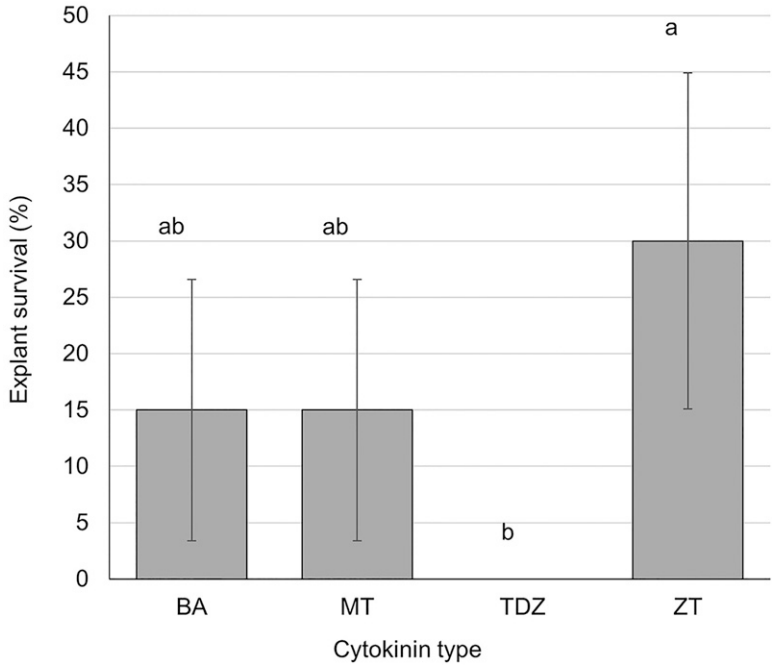

Fig. 2. Percent survival over four subcultures of explants prepared using shoots that were 4 to 6 cm long on stock plants of *Morella pensylvanica* 'Bobzam' Bobbee™ and initiated on Woody Plant (WP) medium supplemented with 4 mg·L^{-1} zeatin (ZT), 1 mg·L^{-1} 6-benzylaminopurine (BA), 5 mg·L^{-1} meta-topolin (MT), or 0.1 mg·L^{-1} thidiazuron (TDZ). Means (± *SE*) followed by different letters are statistically different based on Tukey's honestly significant difference test at $P \leq 0.05$.

forcing in Aug 2022. When new shoots had reached 12 to 18 cm in length, shoot tips, 2 to 3 cm long, were taken for initiating shoots in vitro as described. The treatment medium was WP or Driver and Kuniyuki Walnut (DKW) medium (Driver and Kuniyuki 1984) supplemented with 0.8% (w/v) agar, 3% (w/v) sucrose, 4 mg·L^{-1} ZT, and pH 5.2. The experimental unit was a test tube and tubes were arranged as a RCBD with 10 replications (blocks). Shoots were subcultured every 21 d on nine occasions. Explant survival was recorded as described previously.

Microcutting rooting and acclimation. Rooting success of 'Bobzam' microcuttings was evaluated twice in time. Microcuttings (1.5 to 2 cm long) were taken from cultures growing in previously described 125-mL glass bottles with lids containing 30 mL of WP medium supplemented with 0.8% (w/v) agar, 3% (w/v) sucrose, 4 mg·L^{-1} ZT, and pH 5.2. For rooting, microcuttings were stuck directly ex vitro or prerooted in vitro before being transferred ex vitro. For prerooting, microcuttings were subcultured to glass bottles with lids containing 30 mL of WP medium with 0.8% (w/v) agar, 3% (w/v) sucrose, 1 mg·L^{-1} IBA, and pH 5.2 or 5.5. Seven microcuttings were stuck per culture bottle. After 14 d and when roots (≤2 mm long) were visible at the base of microcuttings, prerooted microshoots were transferred ex vitro to clear plastic trays with lids [473 mL (Dart Container Corp., Mason, MI, USA)]. Trays were filled one-third full of medium composed of four parts sphagnum peatmoss (Fafard Inc.), one part horticultural grade fine perlite (Whittemore Co.), and one part horticultural grade medium vermiculite (Whittemore Co.). Plastic trays had five drainage holes melted into the bottom of the tray. When prerooted microcuttings were transferred to salad trays, additional microcuttings that were not prerooted, were harvested, and bases were dipped in 1000 ppm IBA in talc (Hormodin #1; OHP, Mainland, PA, USA) and stuck directly ex vitro in the same trays. The experimental unit was a row of five microcuttings in a tray. A tray contained a total of 10 microcuttings, one row of five prerooted microcuttings and one row of five IBA treated unrooted microcuttings (Fig. 1). Each tray was a block and trays were arranged in a RCBD with 10 replications (blocks). Trays were maintained under 16-h photoperiod provided by white LED lamps at an intensity of 30 μmol·m^{-2}·s^{-1}.

After 28 d, rooted microcuttings were acclimated to greenhouse conditions by gradually decreasing the relative humidity around the microcuttings and gradually increasing the light levels. This was accomplished by melting five holes (diameter: 0.5 cm) in the tray lids. At this time cuttings were fertigated with a soluble fertilizer (Peters 20N–8.7P–16.6K; Scotts, Marysville, OH, USA) providing 100 ppm nitrogen (N). At 50 d after sticking, rooted microcuttings were removed from trays to determine rooting percentage, root number, and total root length. Roots had to be ≥2 mm to be counted and root length was measured from the point of origin at the stem to the root tip, including all side branches. Rooted microcuttings were then potted into 50-plug trays filled with medium composed of four parts pine bark (Fafard Inc.), one part sphagnum peatmoss (Fafard Inc.), and one part sand and covered with clear plastic propagation domes. After 7 d, 30 holes were melted into domes, plants were fertigated as described, and trays were transferred to the greenhouse and placed under 50% shade. After 10 d, 30 additional holes were melted into domes. After 10 more days, domes were removed, and after an additional 10 d, trays were exposed to full light greenhouse conditions. Plants were fertigated as described every 7 d. Plants in 50 plugs trays were grown in a greenhouse and were up potted into 1.04-L containers and then into 5.68-L containers as plant growth progressed using the same pine bark: sphagnum peatmoss: sand mix as described for 50 plug trays. Plants received 200 ppm nitrogen weekly once they were transitioned to 1.04- and 5.68-L containers.

Data analysis. Data were subjected to analysis of variance (PROC GLIMMIX) and mean separation with Tukey's honestly significant difference test ($P \leq 0.05$) using statistical software (SAS version 9.4; SAS Institute, Cary, NC, USA). For mean separation, percent rooting data from the stem cutting study was transformed using arcsine, and explant survival and number of shoots data from the micropropagation studies were log transformed.

Table 2. *Morella pensylvanica* 'Bobzam' Bobbee™ explant survival and cumulative number of shoots produced per explant over a 189-d period when initiated in vitro on Woody Plant (WP) medium with 2, 4, or 8 mg·L^{-1} zeatin (ZT) and on WP medium or Driver and Kuniyuki Walnut (DKW) medium with 4 mg·L^{-1} ZT.

Medium	ZT (mg·L^{-1})	Explant survival (%)	Cumulative no. shoots
ZT study (189-d duration)			
WP	2	80 a[i]	7.2 b
WP	4	60 a	23.3 a
WP	8	50 a	7.3 b
Medium study (126-d duration)			
WP	4	90 a	4.4 a
DKW	4	20 b	1.0 a

[1] Mean separation within column within study, indicated by different letters, by Tukey's honestly significant difference test at $P \leq 0.05$.

Results and Discussion

Growers report stem cutting propagation of bayberry to be challenging because rooting success varies dramatically among genotypes and is inconsistent between years. Our findings for stem cutting propagation of the female cultivars Bobzam and UConn Compact and a male genotype corroborate these reports. Although the male genotype rooted well, at ≥85% when cuttings were taken at the end of June, the two female cultivars rooted poorly

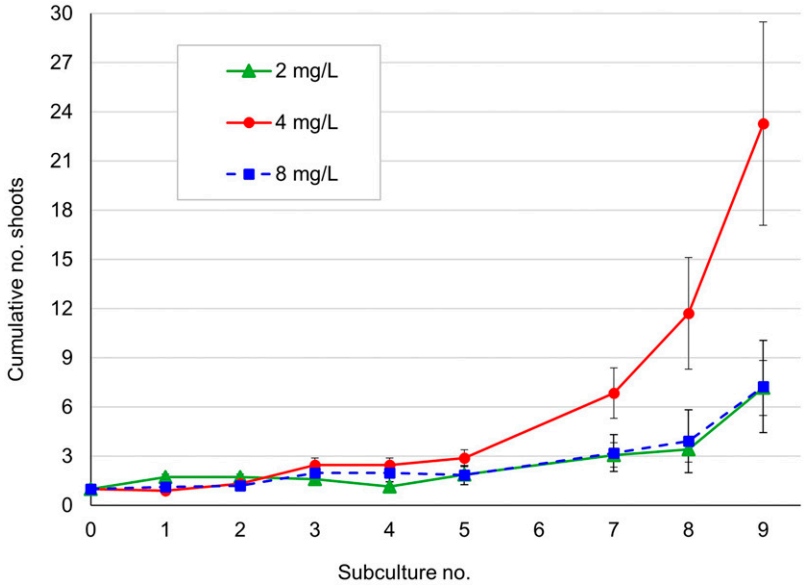

Fig. 3. Cumulative number of shoots that had been produced by each subculture time from *Morella pensylvanica* 'Bobzam' Bobbee™ cultured on Woody Plant (WP) medium supplemented with 2, 4, or 8 mg·L^{-1} zeatin (ZT). Vertical bars indicate ± *SE*.

overall (Table 1). Only one male genotype was included in this study, but it would be interesting to investigate whether males as a group hold greater rooting potential than females. Unfortunately, female plants are those in the greatest demand and for which easy rooting is most desired. 'Bobzam' cuttings taken in July and treated with IBA rooted at 35% to 55%, which is insufficient for large scale commercial nursery production (Cartabiano and Lubell 2013; Cumming 1976). Only a small number of 'Bobzam' cuttings taken in June and August and 'UConn Compact' cuttings taken at all three timings rooted. Zou et al. (2022) found that *Myrica rubra* stem cuttings could be rooted in May, June, and July, but rooting success was only 25% to 30%. It is interesting that southern wax myrtle [*Myrica* (*Morella*) *cerifera*], which is closely related to *M. pensylvanica*, could be easily rooted at 85% or higher using cuttings collected in early August (Blazich and Bonaminio 1984), although the sex of the cutting source was not provided. As IBA concentration increased, so did the number of roots from June cuttings of the male genotype. Similarly, more roots were produced for stem cuttings of southern waxmyrtle when the IBA concentration was increased from 0 to 4000 ppm (Blazich and Bonaminio 1984). There were no rooting trends for timing and/or IBA concentration across bayberry genotypes. Rooted bayberry cuttings that were overwintered in an unheated pit greenhouse survived and grew well the following spring with a 100% success rate. The stem cutting methods evaluated would not translate to a usable propagation protocol that

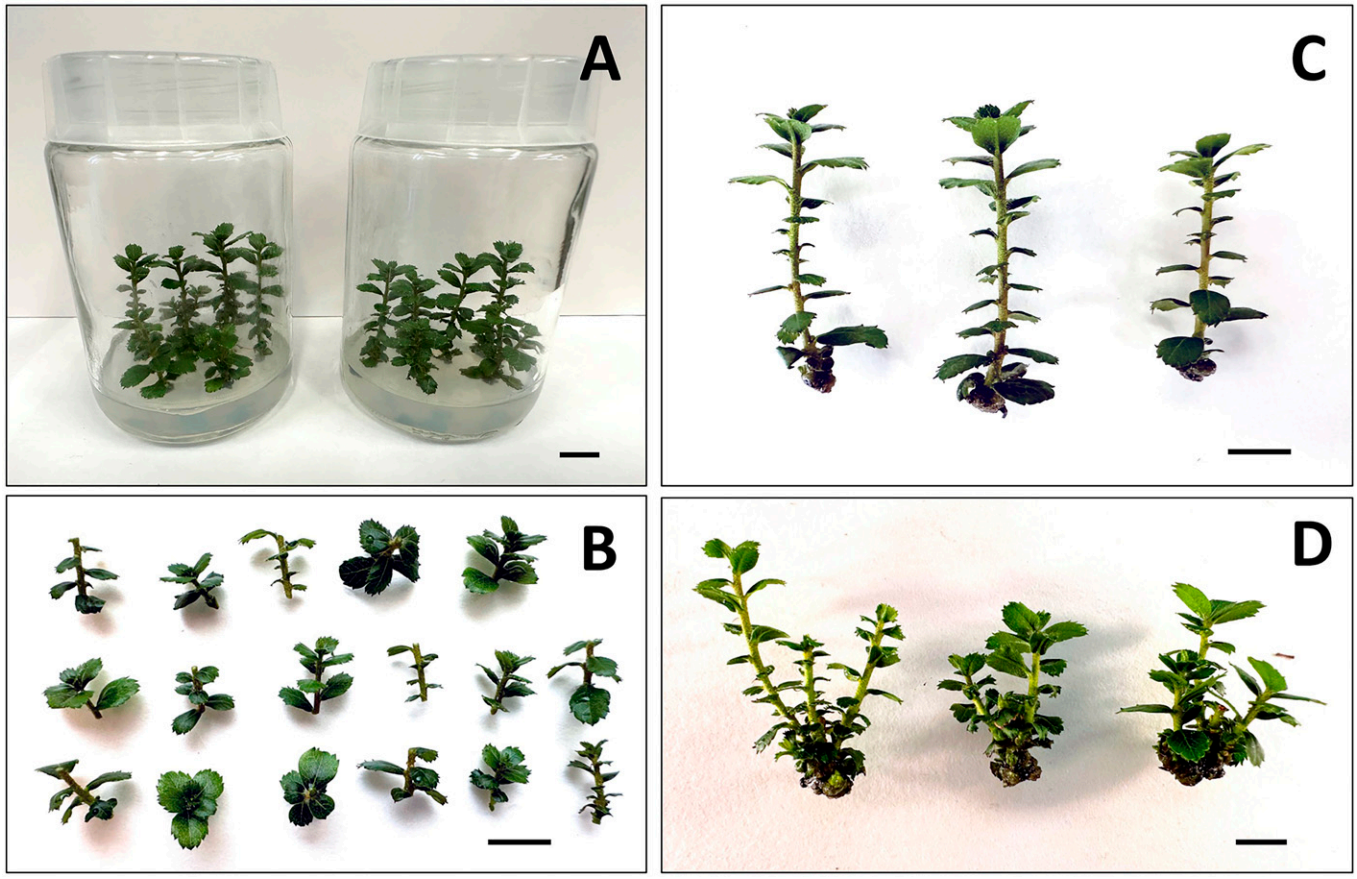

Fig. 4. Established shoot cultures of *Morella pensylvanica* 'Bobzam' Bobbee™ on Woody Plant (WP) medium supplemented with 4 mg·L^{-1} zeatin (ZT). (**A**) Bottles of shoots produced by the end of a 3-week subculture cycle from tip and nodal explants. (**B**) Shoot contents of a single bottle cut into appropriate tip and nodal explants ready to be placed on fresh culture media. (**C**) Resulting shoot extension of tip explants following 3 weeks of growth. (**D**) Resulting multiple shoot production of nodal explants following 3 weeks of growth. Horizontal black lines in all photos are 1 cm long.

Table 3. Ex vitro rooting percent, root number, and total root length of microcuttings of *Morella pensylvanica* 'Bobzam' Bobbee™ prerooted in vitro on medium with 1 mg·L^{-1} indole-3-butyric acid (IBA) or dipped in 1000 ppm IBA in talc (Hormodin #1; OHP, Mainland, PA, USA) and stuck directly ex vitro.

Time replication	Rooting treatment	Rooting (%)	Root no.	Total root length (cm)
1	Prerooted in vitro	98 a[1]	4.4 a	6.9 a
	Stuck directly ex vitro	100 a	4.3 a	5.5 a
2	Prerooted in vitro	70 a	2.4 a	2.4 a
	Stuck directly ex vitro	60 a	2.2 a	2.2 a

[1] Mean separation within column within time replication, indicated by different letters, by Tukey's honestly significant difference test at $P \leq 0.05$.

growers could apply to a range of bayberry cultivars.

As an alternative to stem cutting propagation, we were successful in establishing a micropropagation protocol for 'Bobzam', which propagators can use to expand production of this popular cultivar. This is the first report on the successful micropropagation of *Morella pensylvanica*. In the first micropropagation study, 'Bobzam' and 'UConn Compact' were initiated in vitro, but 'Bobzam' shoots on MS medium and 'UConn Compact' shoots on MS and WP media did not survive. 'Bobzam' shoots on WP medium survived for four subcultures, except for those on medium with TDZ, and began the miniaturization and acclimation process toward steady in vitro shoot multiplication (Fig. 2). WP media was also found to be a suitable medium for box myrtle [*Myrica esculenta* (Bhatt and Dhar 2004)] and red bayberry [*Myrica rubra* (Asghari et al. 2013)]. Shoots of 'Bobzam' on WP medium supplemented with ZT had the best shoot extension, leaf expansion, and color based on visual observation of the cultures; however, there were no statistical differences in explant survival over four subcultures among medium with ZT, BA, or MT.

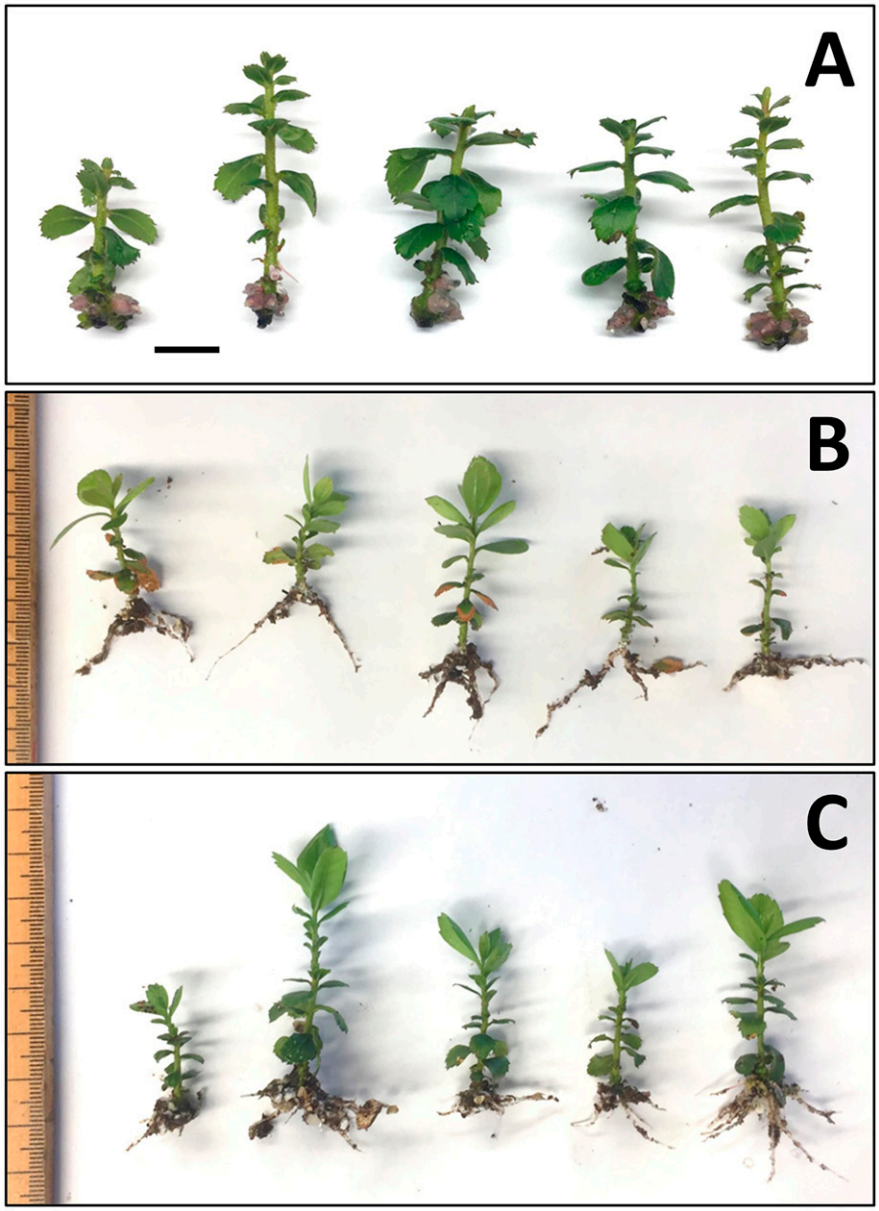

Fig. 5. Microcuttings of *Morella pensylvanica* 'Bobzam' Bobbee™. (**A**) Prerooted in vitro on Woody Plant (WP) medium with 1 mg·L^{-1} indole-3-butyric acid (IBA) showing root primordia (black horizontal line is 1 cm). (**B**) Prerooted in vitro, then transferred ex vitro to trays and excavated to show developed roots. (**C**) Dipped in 1000 ppm IBA in talc and directly stuck ex vitro and excavated to show developed roots.

In micropropagation study 2, WP medium supplemented with ZT at 4 mg·L^{-1} was optimal because this rate resulted in more cumulative shoots than rates of 2 or 8 mg·L^{-1} ZT (Table 2; Fig. 3). Our observations of cultures from studies 1 and 2 indicate that the time at which explants are collected impacts success of shoots in vitro. Explants collected from young, recently expanded shoots from stock plants, as in study 1, did not perform as well as shoots that had 28 to 35 additional days to grow out before explants were collected, as in study 2. Explant survival rates as high as 90% (WP medium with 4 mg·L^{-1} ZT) could be achieved for *M. pensylvanica* 'Bobzam' (study 3), which are comparable to rates of establishment reported for *M. rubra* (Asghari et al. 2013) and higher than rates reported for *M. esculenta* (Bhatt and Dhar 2004). Bhatt and Dhar (2004) found that phenolic compounds from explants inhibited culture establishment and were only partially controlled by protectants. With *M. pensylvanica* 'Bobzam' we did observe a modest amount of purple-black staining of media immediately next to explants due to phenolic exudates but it did not seem to negatively affect the plant tissue. Explants of *M. pensylvanica* 'Bobzam', which died following initiation typically first turned black on the above-media portions of the explant, while stem portions in contact with the media phenolics initially remained green.

Initiated explants in study 2 required seven subculture periods (a minimum of 147 d) before they exhibited a steady state of shoot proliferation (Figs. 3 and 4). Explants were producing shoots at a 2× multiplication rate at subculture periods 8 and 9. Shoot explants grown in bottles beyond 190 d from initiation reached full miniaturization and a steady state of 3× multiplication rate every 3 weeks. In comparison, *M. rubra* produced five shoots per explant over 9 weeks (Asghari et al. 2013), while *M. pensylvanica* 'Bobzam' produced approximately nine shoots per explant over a 9-week time period.

A third micropropagation study comparing WP and DKW media found that 'Bobzam' survived better (90% vs. 20%) on WP medium than on DKW medium (Table 2). DKW medium, like MS medium, is not an appropriate medium for use with *M. pensylvanica*.

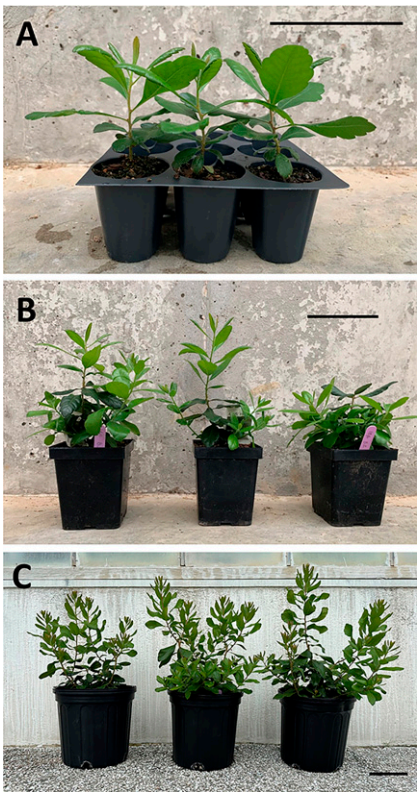

Fig. 6. Plants of *Morella pensylvanica* 'Bobzam' Bobbee™. (**A**) Rooted microcuttings in 50-plug trays. (**B**) Small plants in 1.04-L containers each potted from a single plug. (**C**) Finished plants in 5.68-L containers each potted from a single 1.04-L container small plant. Horizontal black lines are 10 cm.

Rooting success was similar for microcuttings prerooted in vitro and those stuck directly ex vitro (Table 3; Fig. 5). Rooting success was ≥98% for time replication 1. In time replication 2 microcuttings contracted a fungal pathogen but still rooted at 60% to 70%. Rooted microcuttings in 50-plug trays were easy to acclimate to greenhouse conditions (Fig. 6A). Plugs potted in 1.04-L containers grew rapidly and in 70 d had filled the containers (Fig. 6B). Liners from 1.04-L containers that were potted into 5.68-L nursery pots grew into salable landscape plants in ∼60 d (Fig. 6C).

In conclusion, genotype impacts the success of bayberry propagation by stem cuttings and micropropagation. Micropropagation of 'Bobzam' may be accomplished using explants taken from shoots that have expanded 12 to 18 cm in length and WP medium supplemented with Z at 4 $mg \cdot L^{-1}$. Initiated explants will require a minimum of eight subcultures before they begin to consistently multiply shoots. After the eighth subculture, propagators can expect a 2× to 3× shoot multiplication rate at every 3-week subculture. Microshoots can be expected to root at >80% by either in vitro prerooting or ex vitro rooting directly in trays. Micropropagation is a method that growers may use to successfully clone some desirable female genotypes of bayberry.

References Cited

Asghari S, Abbas SJ, Chen L, He X, Qin Y. 2013. Micropropagation of *Myrica rubra* Sieb. and Zucc. using shoot tips and nodal explant. Afr J Agric Res. 8:1731–1737. https://doi.org/10.5897/AJAR10.1160.

Benson DR, Silvester WB. 1993. Biology of Frankia strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev. 57:293–319. https://doi.org/10.1128/mr.57.2.293-319.1993.

Bhatt ID, Dhar U. 2004. Factors controlling micropropagation of *Myrica exculenta* buch. – Ham. Ex D. Don: A high value wild edible of Kumaun Himalaya. Afr J Biotechnol. 3:534–540. https://doi.org/10.5897/AJB2004.000-2097.

Blazich FA, Bonaminio VP. 1984. Propagation of southern waxmyrtle by stem cuttings. J Environ Hortic. 2:45–48.

Cartabiano JA, Lubell JD. 2013. Propagation of four underused native species from softwood cuttings. HortScience. 48:1018–1020. https://doi.org/10.21273/HORTSCI.48.8.1018.

Chicagoland Grows®. 2023. Chicagoland Grows® plant introduction program. http://www.chicagolandgrows.org/about.php. [accessed Jul 2023].

Cumming WA. 1976. Evaluation and propagation of saskatoons. Proc West Can Soc Hort. 25:76–80.

Dirr MA. 2009. Manual of woody landscape plants (6th ed). Stipes Publishing, Champaign, IL, USA.

Dirr MA, Heuser CW. 2006. The reference manual of woody plant propagation; from seed to tissue culture (2nd ed). Varsity Press, Athens, GA, USA.

Driver JA, Kuniyuki AH. 1984. In vitro propagation of Paradox walnut rootstock. HortScience. 19:507–509. https://doi.org/10.21273/HORTSCI.19.4.507.

Hightshoe GL. 1988. Native trees, shrubs and vines for urban and rural America. Wiley, New York, NY, USA.

Lake County Nursery. 2023. https://www.lakecountynursery.com/our-plants/lcn-selections/. [accessed Jul 2023].

Lloyd G, McCown BH. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb Proc Int Plant Prop Soc. 30:421–427.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 15:473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

US Department of Agriculture, Natural Resources Conservation Service. 2023. The PLANTS database. National Plant Data Team, Greensboro, NC, USA. http://plants.usda.gov. [accessed Jul 2023].

Zou Y, Zhang D, Hutzell Z. 2022. Windows of opportunity for rooting woody stem cuttings. Comb Proc Int Plant Prop Soc. 72:207–214.