IBA, Juvenility, and Position on Ortets Influence Propagation of Carolina Buckthorn from Softwood Cuttings[1]

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Abstract

Carolina buckthorn (*Rhamnus caroliniana* Walt.) has horticultural potential but is not often propagated or grown as a nursery crop. My objective was to determine how three concentrations of indolebutyric acid (IBA) affect foliar quality of cuttings, callus development, and rooting of terminal and subtending softwood cuttings from both juvenile and mature stock plants (ortets). Twelve cuttings were assigned to each of 12 factorial treatment combinations and held under intermittent mist for five weeks. Fewer than 10% of cuttings not treated with IBA rooted. Rooting ≥75% resulted when IBA at 3 or 8 g/kg (3000 or 8000 ppm) was applied unless the cuttings were from terminal positions on mature ortets. High quality and dry weight of leaves were associated with subtending cuttings that were juvenile and with terminal cuttings that were mature, but these foliar traits did not lead to particularly high rooting percentages. Juvenile cuttings with IBA at 8 g/kg (8000 ppm) formed the most primary roots and developed root systems with the greatest weight, and dry weight of roots on juvenile cuttings that were subtending (25.6 mg) was about four times that of juvenile, terminal cuttings (6.5 mg). The longest individual roots were on juvenile cuttings with IBA at 3 or 8 g/kg (3000 or 8000 ppm). Frequency of callus on the cut end of stems decreased as IBA concentration increased and was 94 and 75%, respectively, for cuttings from juvenile and mature ortets. Nearly all cuttings with IBA at 3 or 8 g/kg (3000 or 8000 ppm) developed callus on wounds that had been made along the sides of their stems. The frequency of side callus was greater for juvenile, subtending cuttings than for juvenile, terminal cuttings; the opposite was true for mature cuttings. These results confirm the sensitivity of stem cuttings of Carolina buckthorn to IBA. The data also demonstrate that use of subtending wood will improve rooting percentages of cuttings from mature ortets, and that use of subtending cuttings from juvenile plants and of IBA at 8 g/kg (8000 ppm) will increase the number and dry weight of roots.

Index words: IBA, juvenility, adventitious rooting, callus.

Significance to the Nursery Industry

Consumers need additional stress-resistant taxa of trees and shrubs that are ornamental during multiple seasons of the year. The value of species of *Rhamnus* L. (buckthorn), introduced to North America from other continents, is restricted by their potential to be invasive and by their roles in the life cycles of diseases and insects. Although rarely found in commerce, a species native on soils of limestone origin in North America, *Rhamnus caroliniana* (Carolina buckthorn, Indian cherry, or polecat-tree), forms small trees with attractive foliage and fruit. The species might merit increased production and use in the landscape if it can be propagated readily and confirmed to lack problems associated with other members of its genus. This study demonstrates to potential propagators of Carolina buckthorn that application of indolebutyric acid (IBA) to softwood cuttings, the developmental state of plants from which cuttings are taken (juvenile vs mature), and the position cuttings had been on the stock plants from which they were taken (ortets) influence the frequency and extent of rooting.

Introduction

Plants of Carolina buckthorn (*Rhamnus caroliniana* Walt.) form shrubs or small trees that may grow to about 11 m (35 ft) in height (6). The species is native to the southeastern and south-central United States, occurring as far north as extreme southern Ohio and central Missouri and south to central Florida and southern Texas. Throughout its range, plants are found both in low areas near water and on elevated ridges and glades (6, 7). This suggests the species may tolerate a wide range of soil moisture conditions, and its frequent occurrence on shallow soils over limestone (1, 4, 7) indicates tolerance of high soil pH. Relatively little attention has been given to the horticultural potential of Carolina buckthorn, and its production appears limited to a few specialty nurseries where plants are grown from seed (4). Its ornamental value may lead to a demand for Carolina buckthorn. Along their horizontally stratified branches are borne glossy, dark green leaves that become yellow, orange, and red in autumn (4). Equally impressive are the copious, three-seeded drupes that progress from green to light yellow to coral-pink and red before becoming blueberry-blue to black when ripe in late autumn (2, 4).

Carolina buckthorn can be propagated by germinating seeds (3, 4, 9), and there are reports of asexual propagation by inducing roots on stem cuttings. Dirr and Heuser (3) reported up to 100% rooting on stem cuttings of Carolina buckthorn depending on the concentration of IBA applied. A similar responsiveness of stem cuttings of the species to IBA was suggested by Nokes (9). She stated that IBA at 3 or 5 g/kg (3000 or 5000 ppm) will induce rooting of semi-hardwood cuttings but did not report data on the frequency of success. While promising, these reports lack statistical treatment of the results, and they do not describe the developmental state (juvenile vs mature) of the ortet(s), the type [terminal vs subtending (sub-terminal)] and length of stems, nor the environmental conditions under which cuttings rooted. Because

[1]Received for publication September 20, 2001; in revised form December 11, 2001. I gratefully acknowledge the donation of plant material by L. Anella and the technical assistance and data analysis provided by M.A. Kroggel and J.R. Stewart. Journal Paper No. J-19555 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 3603, and supported by Hatch Act and State of Iowa funds.

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of the apparent sensitivity of stem cuttings of Carolina buckthorn to IBA, and because both juvenility and how close the wood had been to a primary apex of the ortet influence the capacity to root cuttings of many woody species (5), my objective was to determine the effects of IBA, juvenility, and position of the cutting wood on the ortet on callus development and rooting of stem cuttings of Carolina buckthorn.

Materials and Methods

One three-year-old plant of Carolina buckthorn and 36 of its full siblings were used as ortets. I considered the older plant mature because it had flowered and developed the seeds from which I grew its siblings. The mature plant was grown from a seed collected in 1998 from an indigenous plant in Johnston Co., OK. The siblings were grown from seeds collected from the mature plant in 2000, cold-stratified for three months, and germinated in March, 2001. All plants were grown in a common medium in individual containers in a greenhouse in Ames, IA, without supplemental irradiance. All ortets had been fertilized once per week with N at 10.8 $mol \cdot m^{-3}$ (150 ppm) from Peters Excel All-Purpose 21N–$5P_2O_5$–$20K_2O$ and Peters Excel Cal-Mag Special 15N–$5P_2O_5$–$15K_2O$ (Scotts, Marietta, GA). About 63% of the N was from the Cal-Mag Special fertilizer.

Cuttings were taken on July 19, 2001. Thirty-six terminal, softwood cuttings, each with a 7-cm-long stem and an actively growing apex, were removed from the mature plant. Thirty-six subtending cuttings of the same length were taken from wood formed in 2001 that was basipetal to where the terminal cuttings had been taken. In addition, one terminal and one subtending cutting were removed from each of 36 juvenile siblings. Two wounds were made to opposing sides of the stem of all 144 cuttings by scraping off a 2-mm (0.08-in)-wide band of the epidermis on the basipetal 2 cm (0.8 in). Within each group (terminal/mature, subtending/mature, terminal/juvenile, subtending/juvenile) of 36 cuttings, 12 were assigned randomly to each of three concentrations [0, 3, and 8 g/kg (0, 3000, and 8000 ppm)] of applied IBA. Rhizopon AA #2 and #3 (Phytotronics, Earth City, MO) were applied to the basipetal 2 cm of each stem assigned to IBA at 3 (3000 ppm) and 8 g/kg (8000 ppm), respectively.

Each cutting was stuck singly into a plastic container that was filled with coarse perlite (Strong-Lite, Seneca, IL). The perlite had been flushed with tap water, and a dibble was used to create the holes into which stems were inserted. Each container had drainage holes, a top diameter of 6 cm (2.4 in), was 8 cm (3.1 in) deep, and had a volume of 160 cm^3 (9.8 in^3). The cut ends were 4 to 5 cm (1.6 to 2.0 in) below the surface of the perlite. In general, all leaves were retained on cuttings, but a basipetal leaf sometimes was removed if the blade could not be kept above the perlite after the stem was inserted. Cuttings in the 12 treatment combinations (a 2 × 2 × 3 factorial of juvenility, position of cutting on ortets, and IBA) were randomly assigned to containers arranged in a completely randomized design on a bench in a glass-glazed greenhouse. Tap-water mist from nozzles over the cuttings was delivered for 20 s every 15 min during the photoperiod during the first two weeks of treatment. Frequency of mist was reduced to every 20 min during the final three weeks of treatments (August 2 through August 23). A data logger equipped with thermocouples and a model LI190SB quantum sensor (LI-COR, Lincoln, NE) recorded air temperature and photosynthetically active radiation (*PAR*) every 15 min during treatments. Mean daily minimum/maximum air temperatures during the treatment period were 20/31C (68/88F). No supplemental irradiance was used, and mean *PAR* during the middle 5 hr of the photoperiod was 199 $mmol \cdot s^{-1} \cdot m^{-2}$.

Data were collected on August 23. The quality of foliage on each cutting was rated as either 1, to represent dark green leaves that lacked any reduction in apparent health, or 0, which indicated some decline in apparent health, usually slight chlorosis or red, pink, or orange color on at least one leaf. Callus on the cut end of the stem and on the wounded areas along the sides of the stem was noted. I considered a cutting rooted if it had at least one root visible without magnification. The number of primary roots on each cutting was counted, and the length of the longest root was determined. All roots and leaves were removed from each cutting, dried at 67C (178F) for 2 days, and weighed. Data for all dependent variables were subjected to analysis of variance (ANOVA) for a completely randomized design, and least significant difference (LSD) values at the 5% level of probability were determined when appropriate. Individual cuttings were experimental units. The main effects of juvenility, position of cutting on ortets, and concentration of IBA applied, along with all potential interactions of these, were included as terms in the model. Data expressed as percentages were transformed to the arc sine of the square root before analysis. Non-transformed data in percentages and corresponding LSD values are shown. In all cases these were consistent with results of analyses of transformed values.

Results and Discussion

Among the 12 factorial combinations of treatments, rooting ranged from 0% (all cuttings on which no IBA was applied except cuttings that were mature and subtending) to 92% [juvenile, terminal cuttings treated with IBA at 3 g/kg (3000 ppm)]. Two interactions, IBA × juvenility and juvenility × position cuttings had been on ortets, were significant in the ANOVA for rooting percentage. Rooting was <10% with no IBA, and was >60% with IBA at 8 g/kg (8000 ppm), for cuttings from both juvenile and mature ortets (Fig. 1A). Rooting percentage of juvenile cuttings (88%) exceeded that of mature cuttings (17%) when cuttings were treated with IBA at 3 g/kg (3000 ppm) (Fig. 1A). Cuttings from subtending positions on the mature ortet rooted at a higher percentage than terminal cuttings from the mature ortet, but this was not true of juvenile cuttings (Fig. 2A).

All dependent variables that represented the size of root systems showed significant interactions of IBA and juvenility, and trends in these variables were similar. Root number (Fig. 1B) and dry weight (Fig. 1D) were highest for cuttings from juvenile ortets treated with IBA at 8 g/kg (8000 ppm), while IBA at 3 or 8 g/kg (3000 or 8000 ppm) on juvenile ortets maximized root length (Fig. 1C). Mature cuttings also were responsive to IBA, but only when 8 g/kg (8000 ppm) was used (Fig. 1). Another interaction, juvenility × position cuttings had been on ortets, existed for data on dry weight of roots. Mean dry weight of roots on terminal and subtending mature cuttings and of terminal, juvenile cuttings was similarly low, while subtending, juvenile cuttings developed root systems of higher weight (Fig. 2B). Data on rooting in Fig. 1 represent both rooted and unrooted cuttings, thus providing an inclusive representation of all experimental units. I also computed means and standard errors after unrooted cuttings were excluded from the data set to illustrate the number of

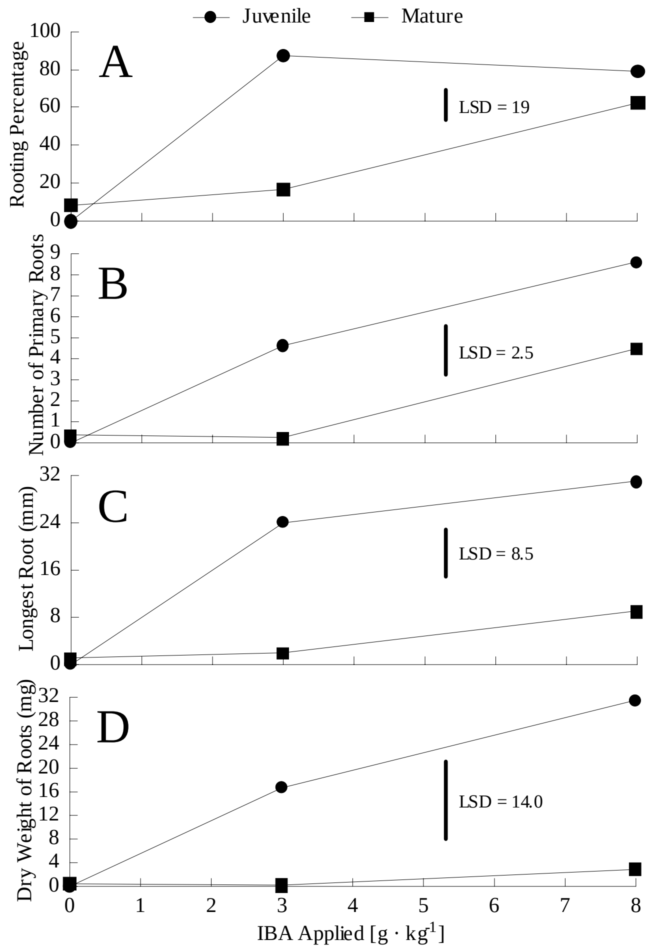

Fig. 1. Influence of indolebutyric acid (IBA) on (A) rooting percentage, (B) number of primary roots, (C) the longest individual root, and (D) the dry weight of roots formed on stem cuttings of *Rhamnus caroliniana* after five weeks under intermittent mist. Each symbol represents a mean of 24 experimental units (individual cuttings) that were collected from either multiple juvenile ortets or their parent, one mature ortet. Analysis of variance of each of these variables showed an interaction between the main effects of IBA and developmental state. Means are averaged over values from cuttings that were at both terminal and subtending positions on ortets. The vertical line for each dependent variable represents Fisher's least significant difference (LSD) at the 5% confidence level.

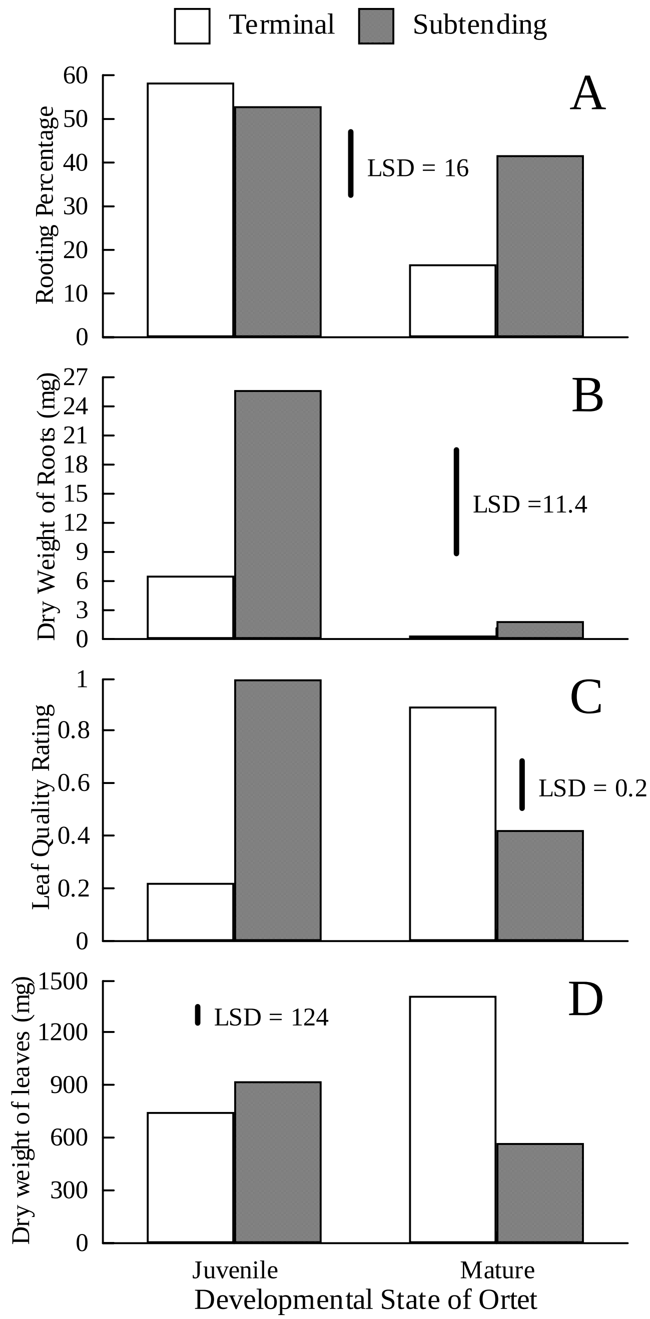

Fig. 2. (A) Rooting percentage, (B) dry weight of roots, (C) leaf quality rating, and (D) dry weight of leaves of terminal (unshaded bars) and subtending (shaded bars) stem cuttings from both juvenile and mature *Rhamnus caroliniana* after five weeks under intermittent mist. Leaves that were dark green and appeared to have retained their health during treatment were ascribed a quality rating of 1, whereas any change in color or other indication of senescence led to a quality rating of 0. Analysis of variance of each of these variables showed an interaction between the main effects of developmental state and position the cutting had been on the ortets. Cuttings were treated with indolebutyric acid (IBA) at 0, 3, and 8 g/kg (0, 3000, and 8000 ppm). Each value is a mean of 36 experimental units (individual cuttings), 12 from each IBA treatment. The vertical line for each dependent variable represents Fisher's least significant difference (LSD) at the 5% confidence level.

roots, the length of the longest root, and the dry weight of all roots propagators can expect to achieve among their rooted cuttings only. While only nine of the 12 juvenile, subtending cuttings treated with IBA at 8 g/kg (8000 ppm) rooted, the mean dry weight of roots from those nine cuttings was nearly twice the root dry weight associated with the rooted cuttings from any other treatment combination (Table 1).

Independent variables that influenced frequency of callus formation differed depending on the location of the callus. Frequency of callus on the cut end of stems was higher for cuttings from juvenile (94%) than from mature (75%) ortets ($P > F = 0.0009$). IBA decreased the frequency of terminal callus; 92, 88, and 75% of cuttings treated with IBA at 0, 3, and 8 g/kg (0, 3000, and 8000 ppm), respectively, had terminal callus (LSD = 14%). A three-way interaction of IBA, juvenility, and position cuttings had been on ortets existed

Table 1. Number of roots, length of the longest single root, and dry weight of all roots formed on softwood cuttings of *Rhamnus caroliniana* in each of the 12 factorial treatment combinations. Values for each dependent variable are means and standard errors (SE) representing only the cuttings that formed at least one root. These data complement those in Fig. 1 and Fig. 2, which show means representing all 144 cuttings in the experiment regardless of whether they rooted.

Developmental state of cuttings	Position of cutting on ortet	IBA applied [g/kg (ppm)]	Rooted cuttings (n/12)	Mean and (SE) for rooted cuttings only: Root no.	Longest root (mm)	Dry weight of all roots (mg)
Juvenile	Terminal	0 (0)	0	0	0	0
Juvenile	Terminal	3 (3000)	11	5 (1)	23 (4)	6 (2)
Juvenile	Terminal	8 (8000)	10	11 (2)	32 (5)	17 (4)
Juvenile	Subtending	0 (0)	0	0	0	0
Juvenile	Subtending	3 (3000)	10	6 (2)	33 (8)	34 (16)
Juvenile	Subtending	8 (8000)	9	11 (3)	47 (10)	65 (24)
Mature	Terminal	0 (0)	0	0	0	0
Mature	Terminal	3 (3000)	1	1	16	1
Mature	Terminal	8 (8000)	5	3 (1)	8 (4)	3 (1)
Mature	Subtending	0 (0)	1	6	24	9
Mature	Subtending	3 (3000)	3	1 (0)	9 (4)	1 (1)
Mature	Subtending	8 (8000)	10	9 (2)	18 (4)	6 (2)

for data on the frequency of callus along the wounded sides of stems. Nearly all cuttings treated with IBA at 3 or 8 g/kg (3000 or 8000 ppm) developed lateral callus (Table 2). In contrast to the results for terminal callus, lateral callus developed at lower frequencies among cuttings not treated with IBA, and the effect of the position cuttings had been on ortets on side callus differed depending on juvenility. Frequency of lateral callus was greater for subtending cuttings than for terminal cuttings from juvenile ortets, but the opposite was true for mature cuttings (Table 2).

The quality ratings and dry weights of leaves on terminal and subtending cuttings differed, and the pattern of difference depended on whether the cuttings were juvenile or mature. Leaves on subtending cuttings from juvenile ortets and on terminal, mature cuttings had the highest mean quality ratings, while the quality of leaves on terminal cuttings from juvenile ortets was low (Fig. 2C). Leaves of all cuttings were dark green and undamaged when treatments began. Cuttings that showed slight chlorosis or other changes in color developed those symptoms during the latter half of the treatment period. Leaves abscised from only two cuttings during treatments, and each of those cuttings lost only one leaf. Terminal and subtending mature cuttings had the highest and lowest mean leaf dry weight, respectively (Fig. 2D).

Table 2. Percentage of stem cuttings of *Rhamnus caroliniana* that developed callus along the sides of the stems, which were wounded before application of indolebutyric acid (IBA) at three concentrations. Individual values represent 12 cuttings in each of the 12 factorial combinations of three treatment main effects: IBA, juvenility, and position cuttings had been on ortets. Analysis of variance showed a three-way interaction of these main effects ($P > F = 0.0013$). The least significant difference between any two values is 22, and values differ significantly when followed by different letters.

IBA applied [g/kg (ppm)]	Cuttings with callus along wounded sides of the stem (%): Juvenile, Terminal	Juvenile, Subtending	Mature, Terminal	Mature, Subtending
0 (0)	33c	67b	58b	8d
3 (3000)	92a	100a	100a	92a
8 (8000)	100a	100a	100a	100a

These results substantiate earlier reports of rooting stem cuttings of Carolina buckthorn and provide new information on factors that influence rooting success. My finding that rooting can be accomplished with stem cuttings of this species is consistent with earlier reports (3, 9). Dirr and Heuser (3) achieved up to 100% rooting of an unreported number of cuttings from unspecified positions on ortet(s) of unreported maturation state. Nokes (9) stated that semi-hardwood cuttings collected late in the growing season could be rooted, but she did not report the frequency of success. Both previous reports are consistent with my findings concerning the importance of IBA to rooting success. Nokes (9) recommended IBA at 3 to 5 g/kg (3000 to 5000 ppm), while Dirr and Heuser (3) reported 27, 97, and 100% rooting eight weeks after treatment with IBA in solution at 0, 1, and 5 g/kg (0, 1000 and 5000 ppm), respectively.

This study demonstrates ways to increase the frequency and extent of rooting of Carolina buckthorn beyond the previously reported effectiveness of IBA applications (3, 9). The developmental state of the ortet (juvenile vs mature) and the position of cuttings on the ortet affected the frequency of rooting and the size of root systems. Ease of rooting commonly is associated with juvenility (3, 5), and success with rooting stem cuttings from different positions on ortets has been observed previously. For example, terminal softwood cuttings of 14-year-old trees of *Olea europaea* L. (olive) rooted at about half the frequency of cuttings from positions basipetal to the terminal wood (8), and rooting percentage of stem cuttings of *Vaccinium corymbosum* L. (highbush blueberry) increased as the origin of the cutting on ortets became more basipetal (10). The differences in rooting of terminal and subtending cuttings of Carolina buckthorn, and the dependence of the pattern of this difference on juvenility, might be due to differences in carbohydrate status and/or chemicals that inhibit rooting or act as rooting co-factors in tissues along the axis from the apex to the base of a shoot (3, 5).

Propagators seeking to achieve the highest rooting percentages with softwood cuttings of Carolina buckthorn should collect cuttings from juvenile plants and treat them with IBA in talc at 3 or 8 g/kg (3000 or 8000 ppm); averaged over terminal and subtending cuttings, this resulted in 79 to 88% rooting (Fig. 1A). IBA at 8 g/kg (8000 ppm) on juvenile cuttings will increase the number (Fig. 1B) and weight (Fig. 1D) of roots. The high leaf quality retained by subtending cuttings from juvenile ortets (Fig. 2C) did not evoke a rooting percentage higher than that of terminal, juvenile cuttings (Fig. 2A), but the dry weight of roots on subtending cuttings (25.6 mg) was about four times that of terminal cuttings (6.5 mg) (Fig. 2B). Thus, selection of only subtending cuttings from juvenile plants, along with use of IBA at 8 g/kg (8000 ppm), should maximize the extent of rooting. Use of only subtending cuttings is particularly important when ortets are mature because of the low rooting percentage of mature, terminal cuttings (Fig. 2A). IBA at no less than 8 g/kg (8000 ppm) should be used on mature cuttings (Fig. 1). Subsequent research could address whether higher concentrations of IBA, use of different auxins, or alternative methods of application would be advisable.

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