Propagation

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Section Editor

Propagation of *Ilex vomitoria* 'Dare County' by Stem Cuttings

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Index Words: adventitious rooting, auxin, indole-3-butyric acid, native plants, yaupon holly.

Significance to Industry: Nontreated semi-hardwood stem cuttings of 'Dare County' yaupon holly will root at > 75%, whereas hardwood cuttings root poorly regardless of K-IBA treatment. Treatment of semi-hardwood cuttings with K-IBA will decrease percent rooting.

Nature of Work: *Ilex vomitoria* Sol. ex Ait. (Aquifoliaceae Bartl.) (yaupon, cassena, Christmas berry, or evergreen holly) is a dioecious, evergreen species native from southeast Virginia to central Florida and west to Oklahoma and Texas (2,11). Growth habit can vary from a small to large upright shrub or a small tree. New growth in the spring has a purple cast that is soon lost, becoming a lustrous, dark green.

Female plants of *I. vomitoria* are particularly attractive in mid-fall to spring as they often bear copious quantities of colorful fruit on 1-year-old wood. The fruit are globose drupes approximately 0.6 cm (0.25 in) in diameter with each fruit containing four pyrenes (nutlets) (11). Drupe color is normally red to scarlet, and there are selections with yellow or orange fruit (3).

The species is very adaptable as it will tolerate extremely dry to wet soils. It is also tolerant of salt spray and is frequently used in coastal landscapes. Owing to its adaptability to various site conditions and the large number of cultivars of various growth forms (3), *I. vomitoria* is a popular landscape plant in the southeast United States.

One outstanding cultivar of *I. vomitoria* is a female selection, 'Dare County' (syn. 'Virginia Dare'). 'Dare County' yaupon holly is a stiff and divergently large shrub or small tree. As a tree, it can reach heights > 7 m (23 ft) with a spectacular fall and winter fruit display of reportedly orange drupes that persist into spring. Despite having considerable landscape merit, little if any information has been published regarding propagation of this cultivar, other than it can be propagated by stem cuttings (7).

Cultivars of *I. vomitoria* are generally propagated vegetatively by stem cuttings, although as reported by Dirr and Heuser (4), rooting of cuttings can be "reasonably difficult." Also, the growth stages of stock plants that are most conducive to rooting appear to be semi-hardwood or hardwood (1,4) although 'Nana' yaupon holly can be propagated by softwood cuttings (5). Therefore, the following research was conducted to determine the influence of stock plant growth stage and auxin treatment on propagation of 'Dare County' yaupon holly by stem cuttings.

Two hundred terminal stem cuttings approximately 10 to 12 cm (3.9 to 4.7 in) in length were taken September 10 and December 17, 2009, and March 4, 2010, from a tree of 'Dare County' holly growing in a roadside planting of other *Ilex* L. (holly) sp. in Manteo (Roanoke Island), NC. The tree was multi-stemmed with an approximate height and width of 7.6 and 9.1 m (25 and 30 ft), respectively. Cuttings taken September 10, 2009, were semi-hardwood having stems that were tan to light gray in color. When pressure was applied to a cutting, the stem broke, sometimes with a snapping sound, but the pieces remained attached at the point where pressure was applied. Cuttings taken December 17, 2009 and March 4, 2010, were hardwood. Stems of the December 17 cuttings were similar in color and lignification to the semi-hardwood cuttings of September 10. The lower two-thirds of the stems of hardwood cuttings taken March 4, 2010, were light gray in color; the upper third reddish purple. When pressure was applied to the stem it broke with a snapping sound and the pieces held together at the point where pressure was applied.

The semi-hardwood cuttings and the December 17 hardwood cuttings consisted of the current season's growth, whereas the hardwood cuttings of March 4, 2010, consisted of growth which occurred the previous season in 2009. When cuttings of both stages were taken, prolific fruit development was observed on 1 year-old wood as flowering of *I. vomitoria* occurs in mid-April on this growth. However, the cuttings prepared for rooting did not include any of this older growth with fruit. Fruit color on September 10 was green. On December 17, fruit were yellow/gold in color, and on March 4, 2010, the fruit were orange. Following collection, cuttings of each growth stage were trimmed from the bases to lengths of 8 to 10 cm (3.1 to 3.9 in) and leaves removed from the lower 4 cm (1.6 in). Cuttings were then treated with solutions of the potassium (K) salt (K-salt) of indolebutyric acid (K-IBA) at 0, 2000, 4000, 6000, or 8000 $mg{\cdot}L^{-1}$ (ppm). Solutions were prepared by dissolving reagent grade K-IBA in distilled water.

When treating cuttings with K-IBA, the basal 2 cm (0.8 in) was dipped into the K-IBA solution for 2 sec followed by 20 min of air drying before inserting into the rooting medium. After auxin treatment, cuttings were inserted to a depth of 3 cm (1.2 in) in individual plastic Anderson bands (Anderson Tool & Die, Portland, OR) [6.0 x 6.0 x 12.7 cm (2.4 x 2.4 x 5.0 in)] held in deep propagation flats/trays [40.6 x 40.6 x 12.7 cm (16 x 16 x 5 in)] with 36 cells per flat (6 rows x 6 columns). The rooting medium was 1 peat : 1 perlite (by vol).

The trays were placed on a single raised bench under natural photoperiod and irradiance in a glass covered greenhouse on the campus of NC State University, Raleigh. Day/night temperatures were approximately 21.1 $\pm$ 2.8/18.3 $\pm$ 2.8C (70 $\pm$ 5/65 $\pm$ 5F). Intermittent mist operated 4 sec every 5 min from 7:00 am to 8:30 pm daily. The experimental design was a randomized complete block using 6 cuttings per treatment with six replications.

Fourteen weeks after the rooting studies were initiated, cuttings were harvested and data recorded. Data included the number and length of primary roots > 1 mm (0.04 in). Any cutting having one or more roots was classified as rooted. Data were subjected to analysis of variance procedures and regression analysis.

Results and Discussion: Nontreated semi-hardwood cuttings of ‘Dare County’ holly rooted at 78% (Table 1), whereas hardwood cuttings taken on two dates rooted in low percentages regardless of auxin treatment (data not presented). Combining all treatments, rooting of hardwood cuttings taken December 17, 2009 and March 4, 2010, was 11% and 15%, respectively, indicating such cuttings should not be used to propagate ‘Dare County’ yaupon holly. On the other hand, nontreated semi-hardwood cuttings appear to be an excellent means to propagate the cultivar. Treatment with K-IBA was either of no benefit or inhibited rooting. The only significant effect of auxin treatment was a linear decrease in percent rooting ($P \leq 0.05$) whereas root number and root length were unaffected (Table 1).

There are two principal auxins used, either alone or in combination, to stimulate adventitious rooting of stem cuttings, indolebutyric acid and naphthaleneacetic acid (NAA). Both compounds are available as free acids or K-salts, the former being soluble in an organic solvent such as ethyl, methyl, or isopropyl alcohol and the latter being water soluble. In this research, we used solutions of the K-salt of IBA for treating stem cuttings since there are reports alcohol may injure cuttings of *I. vomitoria* (4) and “NAA will burn the stem and cause defoliation (9).” Also, the range of K-IBA treatments selected to treat the cuttings was based on previous reports for the species (1, 4, 9).

The authors did not attempt to root softwood cuttings of ‘Dare County’ holly due to lack of sufficient cutting material. Also, most reports indicate *I. vomitoria* is best propagated by semi-hardwood or hardwood cuttings (1,4) although one report indicates ‘Nana’ yaupon holly is propagated commercially by softwood cuttings (5). It would be useful to investigate propagation of ‘Dare County’ holly by softwood cuttings because, if successful, this would provide another window of opportunity to root stem cuttings.

There appears to be considerable misunderstanding regarding the proper cultivar name of ‘Dare County’ holly and the color of mature fruit. The cultivar was discovered and selected by Bauers in 1978 from a population of six plants supposedly having orange colored fruit at maturity (7). The plant selected from the population reportedly had the most attractive fruit. The plants were growing at the Bodie Island National Seashore Park, Dare County, NC. When registered with The Holly Society of America in May 1985, the cultivar name was ‘Virginia Dare’ holly in honor of the first English child born 1587 in the Americas in what is presently Dare County, NC (7). Following registration with the Holly Society, it was determined the name ‘Virginia Dare’ had been used and published previously for a selection of *I. opaca* L. (American holly) (8). The name ‘Virginia Dare’ was not valid and Bauers chose the name ‘Dare County’ (6,7,8). Thus, ‘Virginia Dare’ is a synonym for ‘Dare County’, although many individuals still use the name ‘Virginia Dare’. There is also confusion regarding the color of mature fruit.

In a description of the cultivar published in 1985, mature fruit color was described as orange (7). However, a description of the selection included with the Holly Registration Certificate (Registration No. 6-85) submitted by Bauers and dated May 31, 1985, described the fruit as “translucent yellow/orange” (10). We have observed fruit color can vary yearly and the color of mature fruit is not always orange. As the fruit develop they are initially green and as they mature in fall, they develop a yellow/gold color which in late fall sometimes changes to orange. In other years, the fruit remain yellow/gold with only a trace of orange color. The variable color of the fruit from year to year is probably influenced by environmental/climatic factors. Regardless of the color of the fruit, the yearly fruit display is outstanding and extremely attractive.

Since June 2009 the authors have observed growth of the stock plant (tree) from which stem cuttings were taken in 2009 and 2010. To date no insect or disease problems have been observed with one exception. In December 2009 some leaf mining injury was observed and care taken not to remove cutting material having such injury. The injury was subsequently identified by the NC State Plant Disease & Insect Clinic as caused by *Phytomyza vomitoriae* Kulp [holly leafminer (Diptera: Agromyzidae)].

Adventitious roots of ‘Dare County’ holly, like other cultivars of *I. vomitoria* are fleshy, but fine textured and easily damaged even when rooted in a medium of 1 peat:1 perlite, as in this research. Since the roots are prone to injury, the authors strongly advise that direct rooting/sticking be used when propagating ‘Dare County’ yaupon holly by stem cuttings as this should reduce potential transplant shock.

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Table 1. Influence of K-IBA treatments on the rooting of semi-hardwood cuttings of 'Dare County' yaupon holly.

Treatment	Rooting (%)[z]	Mean Root Number [y]	Mean Root Length (mm)[y]
Nontreated	77.8	5.5	33.0
2000 ppm K-IBA	61.1	3.9	44.5
4000 ppm K-IBA	50.0	6.7	23.9
6000 ppm K-IBA	47.2	3.0	43.9
8000 ppm K-IBA	36.1	2.3	30.1
Linear	*	NS	NS
Quadratic	NS	NS	NS

[z] Each value is based on 36 cuttings.
[y] Each value is based on the number of cuttings which rooted for a particular treatment.
[NS, *]Nonsignificant or significant at $P \leq 0.05$, respectively.

Comparison of Two Water-soluble Forms of IBA for Rooting Cuttings

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Index Words: Auxin, Indole-3-butyric acid, K-IBA, Vegetative Propagation.

Significance to Industry: A question posed by commercial nursery growers as to whether rooting results obtained in cutting propagation using auxin solutions made with Hortus IBA Water Soluble Salts [an EPA-registered product which forms the potassium salt of indole-3-butyric acid (K-IBA) when dissolved in water] would be comparable to results obtaining using technical grade K-IBA (available for research use, but not EPA-registered for commercial use) prompted a study to examine this issue. Solutions were prepared using these two products at five rates of IBA: 500, 1000, 1500, 2000, and 3000 ppm. Subterminal (3-node) cuttings of *Ligustrum japonicum* 'Texanum' (Texas privet), single-node cuttings of Rosa 'Red Cascade' (rose), and subterminal (2-node) cuttings of *Trachelospermum jasminoides* (star jasmine) received a 1-sec basal quick-dip in one of the ten auxin solutions. Cuttings of all three crops showed no significant difference in rooting response between the two products. Results indicate that commercial propagators can switch from K-IBA to Hortus Water Soluble Salts for a basal quick-dip without an adjustment in IBA rate.

Nature of Work: In 1935, Thimann and Koepfli (4) reported the synthetic preparation of the auxin indole-3-acetic acid (IAA), a naturally occurring substance that had recently been found to have root-forming properties, and demonstrated its practical use in stimulating root formation on cuttings. Soon after, the synthetic auxins indole-3-butyric acid (IBA; now known to occur in plants) and 1-naphthaleneacetic acid (NAA) were shown to be more effective than IAA for rooting cuttings (5). IBA and NAA are currently the most widely used auxins for promoting root formation on stem cuttings (1). Auxin treatments are commonly used in commercial plant propagation to increase overall rooting percentages, hasten root initiation, increase the number and quality of roots, and encourage uniformity of rooting (2,3). Commercial root-promoting products (“rooting hormones”) are available in various formulations: liquid concentrates, water-soluble salts and tablets, gels, and powder (talc) (1).

Some growers have used the potassium (K) salt of IBA (K-IBA) over the years for preparation of water-based IBA solutions. Technical grade K-IBA is commonly used in plant propagation research; however, this product, previously purchased by growers from scientific supply companies, is no longer available for sale for use as a root-promoting compound due to lack of Environmental Protection Agency (EPA) registration. One EPA-registered product that can be used as an alternative to K-IBA is Hortus IBA Water Soluble Salts (containing 20% IBA, a pH buffer, and proprietary ingredients; Phytotronics Inc., Earth City, MO), which produces K-IBA when the product is dissolved in water.

Commercial nursery growers have questioned whether rooting results obtained using auxin solutions made with Hortus IBA Water Soluble Salts are comparable to results obtained using technical K-IBA. The current study was conducted to examine this issue using cuttings of three commonly grown nursery crops.

Solutions of K-IBA (Sigma, St. Louis, MO) and Hortus IBA Water Soluble Salts were prepared at five rates of IBA: 500, 1000, 1500, 2000, and 3000 ppm (total of ten treatments). Subterminal (3-node, 3.5-inch) cuttings of *Ligustrum japonicum* 'Texanum' (Texas privet), single-node (1-inch) cuttings of Rosa 'Red Cascade' (rose), and subterminal (2-node, 2.75-inch) cuttings of *Trachelospermum jasminoides* (star jasmine) were prepared on July 8, 2012, received a 1-sec basal quick-dip in one of the ten auxin solutions (30 cuttings per treatment), stuck in Sunshine Redi-earth Professional Growing Mix in 50-cell plug trays, and rooted in a greenhouse under intermittent mist for 6 to 7 weeks.

Upon harvest, root systems were washed to remove rooting substrate, then scanned and analyzed with WinRHIZO software (Regent Instruments Inc., Quebec, Canada) to determine total root length. Roots emerging from rooted cuttings were counted. Linear models were used to analyze total root length data with the GLIMMIX procedure of SAS. Generalized linear mixed models were used to analyze root count data with the Poisson distribution (rose) or negative binomial distribution (Texas privet and star jasmine) with the GLIMMIX procedure of SAS. Models included auxin rate (quantitative) and IBA source (qualitative). There were no significant interactions between auxin rate and IBA source.

Results and Discussion: Upon harvest, cuttings of Texas privet exhibited no significant difference in number of roots or total root length between the two products or among the different rates of IBA (Table 1). Cuttings of rose exhibited no significant difference in number of roots and a marginally significant increase in total root length using the Hortus product compared with technical K-IBA; number of roots and total root length showed highly significant and marginal increases, respectively, with increasing rate of IBA with both products. Cuttings of star jasmine exhibited no significant differences in number of roots or total root length between the two products, but significant increases with increasing rate of IBA with both products. Results indicate that commercial propagators can switch from K-IBA to Hortus Water Soluble Salts for a basal quick-dip without an adjustment in IBA rate.

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Table 1. Rooting response of cuttings of *Ligustrum japonicum* 'Texanum' (Texas privet), *Rosa* 'Red Cascade' (rose), and *Trachelospermum jasminoides* (star jasmine) obtained using a basal quick-dip in solutions of the potassium salt of IBA (K-IBA) or Hortus IBA Water Soluble Salts (WSS) (each prepared at 500, 1000, 1500, 2000, and 3000 ppm IBA) and rooted under intermittent mist in a greenhouse (n=30).

		Roots (no.)		Total root length (cm)	
	IBA rate (ppm)	K-IBA	WSS	K-IBA	WSS
Ligustrum japonicum 'Texanum'	500	8.1	7.9	80	93
	1000	8.3	7.7	88	81
	1500	8.3	8.2	91	88
	2000	7.7	8.3	85	86
	3000	8.3	7.6	91	92
Significance[z]:					
Auxin type		NS		NS	
Auxin rate		NS		NS	
Rosa 'Red Cascade'	500	3.1	3.4	51	57
	1000	3.6	3.2	52	61
	1500	3.6	3.8	56	63
	2000	4.1	4.2	60	60
	3000	4.7	5.6	59	65
Significance:					
Auxin type		NS		*	
Auxin rate		***		*	
Trachelospermum jasminoides	500	3.3	4.1	87	95
	1000	4.5	4.4	112	108
	1500	5.5	5.1	115	101
	2000	5.5	4.8	130	127
	3000	6.0	5.7	169	151
Significance:					
Auxin type		NS		NS	
Auxin rate		***		***	

[z]Not significant or significant at $\alpha = 0.05$ (*), 0.01 (**), or 0.001 (***).