Propagation of *Vaccinium arboreum* by Stem Cuttings for Use as a Rootstock for Commercial Blueberry Production

by

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Abstract

Commercial blueberries, including *V. corymbosum* and *V. ashei*, have very specific needs for optimum growth; hence, growing sites are limited. They require acidic soil (pH 4.0-5.5), good drainage, thorough aeration, and a constant moderate amount of moisture. To overcome these restrictions they could be grafted onto *V. arboreum*, a species adapted to less desirable growing conditions. Currently, *V. arboreum* plants are commercially propagated from seeds. Successful asexual propagation techniques will be necessary for rapid clonal propagation of selected cultivars of *V. arboreum*. The objective of this experiment was to identify an efficient way to propagate *V. arboreum* using stem cuttings. We found that IBA quick-dip concentration (0, 1000, 2500, 5000, or 7500 ppm IBA) did not influence rooting percentage of *V. arboreum*. The factors that influenced rooting the most were the source of the cutting and the cutting type (softwood, semi-hardwood, or hardwood). The greatest rooting success was observed with softwood cuttings; there was also success using semi-hardwood cuttings from plants that had been cut back in February 2010 and allowed to sprout new shoots. The results of this experiment can be used to determine the feasibility of using stem cuttings to commercially propagate *V. arboreum*.

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Table of Contents

List of Tables

List of Abbreviations

RTJ	Robert Trent Jones Golf Trail at Grand National
SCMS	Stone County, Mississippi
IBA	Indole-3-butyric acid
NAA	Alpha-naphthaleneacetic acid

Chapter I

Literature Review

Genus Vaccinium

The genus *Vaccinium* is both a large and complicated genus of the Ericaceae family. A large degree of variability can be seen throughout the genus, and some species have complicated, difficult-to-determine evolutionary histories. There are over 140 different species of *Vaccinium* in the Southeastern United States alone, and they can be divided into six unique subgenera (Radford et al., 1968). The first, *Oxycoccus*, is made up of cranberry species such as *V. macrocarpon* and *V. oxycoccus*. The *Herpothamnus* subgenus is comprised of creeping blueberries such as *V. crassifolium* and *V. sempervirens*. Commercial blueberries are located in the subgenus *Cyanococcus*. There are four main types of commercial blueberries. Lowbush blueberries (*V. angustifolium*), or wild blueberries, are dwarf, woody, deciduous shrubs found New Hampshire up through Maine and into New Brunswick and Nova Scotia (Trehane, 2004). The northern highbush blueberry (*V. corymbosum*) is a taller species of shrubby blueberry. They are typically found from North Carolina extending north into Canada and as far west as Illinois, Indiana and Michigan (Trehane, 2004). Rabbiteye blueberries (*V. ashei*) are erect, spreading shrubs and are more adept than northern highbush to growing conditions in the south. They require fewer chilling hours than northern highbush blueberries and are found in the southeastern United States from central Florida to eastern North Carolina and west to northern Arkansas and eastern Texas (Trehane, 2004). Southern highbush blueberry is a general term for hybrids of two, sometimes three *Vaccinium* species. They are early ripening, similar to northern highbush, and have a low chilling hour requirement like rabbiteye. In some hybrids, *V. darrowii* also provides heat and drought resistance (Trehane, 2004). There is only one species of the *Oxycoccoides* subgenus in the Southeast, which is *V. erythrocarpum*, or the Southern Mountain Cranberry. Also, there is only one species of the *Batodendron* subgenus in the Southeast, which is the sparkleberry, or *V. arboreum*. Finally, the *Polycodium* subgenus consists of multiple varieties of the species *V. stamineum,* or deerberry (Radford et al., 1968).

Vaccinium arboreum

Vaccinium arboreum has multiple common names including sparkleberry, farkleberry, tree-huckleberry and winter-huckleberry (Ballinger et al., 1982). It is the only species of *Vaccinium* that reaches tree size and can grow as tall as 10 meters (Radford et al., 1968). The breast-height trunk diameter can be as great as 35 cm (Lyrene, 1997). *Vaccinium arboreum* is a perennial semi-evergreen, and retains its leaves through much or all of the winter (Radford et al., 1968). The foliage is dark green, and in one western variety, *V. arboreum* var. *glaucescens*, glaucescent leaves are present. This variety, however, is not considered different enough from *V. arboreum* to be ruled a separate species (Ballinger et al., 1982). The root system is made up of coarse roots with a large taproot (Lyrene, 1997). It also has an erect, single trunk growth habit and can grow to resemble a small tree (Ballinger et al., 1982).

In addition to those mentioned previously, *V. arboreum* possesses several traits that would make it a desirable ornamental plant. The leaves are alternate and elliptic, turning a reddish-purple color in the fall (Radford et al., 1968). *Vaccinium arboreum* blooms in the late summer and has small, white flowers organized in an elongated raceme inflorescence (Brooks and Lyrene, 1998). *Vaccinium arboreum* is a very low-maintenance species due to its drought and soil pH range tolerance. Other attractive ornamental qualities include its exfoliating bark, similar to crapemyrtle (*Lagerstroemia indica* L.), and the fact that it is semi-evergreen. The fruit are small, black, shiny (Radford et al., 1968), very dry and leathery (Ballinger et al., 1982), and are often described as "gritty and inedible" (Brooks and Lyrene, 1998). The fruit, however, are edible and appreciated by some. Fruit are readily eaten by birds and would therefore be useful for attracting songbirds (Stockton, 1976).

Vaccinium arboreum is widespread throughout the Southeast, ranging from southern Virginia south to central Florida, and west to Texas, central Oklahoma and southeast Missouri (Brooks and Lyrene, 1998). It is capable of growing in coarse to medium textured soils with a soil pH tolerance of 4-7 (Radford et al., 1968). In addition, it is the only species of *Vaccinium* found in mafic or calcareous soils, and it is a shade-tolerant species (Brooks and Lyrene, 1998).

Vaccinium arboreum and commercial blueberry species such as *V. corymbosum* and *V. ashei* are in two different subgenera, and the relationship between them is therefore uncertain. If anthocyanins are of taxonomic importance, *V. corymbosum* and *V. ashei* are closely related to *V. arboreum* because the anthocyanins in the fruit are extremely similar. They both possess twelve anthocyanins within their fruit. This suggests that these commercial blueberry species are related to *V. arboreum* and would therefore have a greater chance of grafting success (Ballinger et al., 1982). Other factors that influence grafting success include the environmental conditions following grafting. The temperature can influence the rate of callus growth, with higher temperatures encouraging more rapid callus formation. This only occurs to a certain temperature, when any further increase in temperature may retard callus formation or cause cell death. The temperature tolerance depends on the species being grafted. The graft also must have high humidity for proper formation of callus. If cambium cells are exposed to drying air the cells will be killed and a graft union is less likely to form (Hartmann et al., 2002).

Current blueberry propagation

Throughout the world, blueberry acreage is expanding rapidly. There has recently been an increase in demand for fresh blueberries available throughout the year (Isutsa et al., 1994). This increased demand has led to the need to quickly propagate elite blueberry cultivars. A common method for propagation is by using stem cuttings. Hardwood, softwood and semihardwood cuttings have all proven to be a reasonably successful method of propagation, with greater than 50% rooting (Mainland, 2006). Another method of propagation is by tissue culture, which can lead to as many as 95% of propagules rooting (Isutsa et al., 1994). Plants propagated using micropropagation tend to have a bushier growth habit, which allows for more flower buds per plant (Miller et al., 2006). This facilitates more fruit than those propagated by cuttings, with the average berry weight being about the same (El-Shiekh et al., 1996). Although this sounds ideal, there are drawbacks to using micropropagation. First, setting up and maintaining a micropropagation facility is extremely expensive and time-consuming. The increased rooting success may not outweigh the cost of setup. Also, in order to have success with micropropagation, the workers must be skilled in working in vitro (Miller et al., 2006).

Finally, even though micropropagation leads to a bushier plant that produces more fruit, a larger number of fruit would be lost if they are mechanically harvested. Mechanical harvesting involves a machine grasping the base of the plant and shaking to remove the berries, which are caught below the plant. A plant with a large number of low branches increases the chance of blueberries missing the collection platform and falling to the ground (Miller et al., 2006). Tissue culture is a successful way to propagate blueberries, but there are drawbacks to using this method.

An increase in demand for blueberries also increases the demand for suitable growing sites. Because of *V. corymbosum*'s very specific needs, there is a lack of space with the required qualities available for expansion (Ballington et al., 1990). Highbush blueberries require acidic soil, in the range of pH 4.0-5.5, with a high amount of organic matter, Fe, and N in NH_4+ form (Darnell and Hiss, 2006). They also need soil with good drainage, ample aeration, and a relatively consistent moderate amount of moisture (Ballinger et al, 1982). *V. corymbosum* has a fibrous, shallow root system that is sensitive to drought and wind damage (Lyrene, 1997). Since it is limited to such specific growing habitats, the increase in blueberry production is limited unless a way to overcome some of these factors is found.

Breeding with V. arboreum

There are many traits of *V. arboreum* that would be ideal to have in commercial blueberry species, and *V. arboreum* could be used as a gene source to incorporate these traits. *V. arboreum* flower late in the season, which would reduce the risk of crop loss due to spring frosts. Berries of *V. arboreum* ripen late in the growing season, from September to December. *V. arboreum* also grows in areas that are not desirable for commercial blueberries (Wenslaff and Lyrene, 2003). There are also traits of *V. arboreum* that would not be desirable in commercial blueberries. The berries are dark and shiny, and are barely palatable. They have a gritty texture due to large seeds and a low juice content. Also, the berry size is quite small, usually smaller than those of commercial blueberry species (Lyrene, 1997). Although it may be beneficial to have some of these traits in commercial blueberries, there are also drawbacks.

V. arboreum as a rootstock

One way to help increase the culture of *V. corymbosum* is to graft onto a plant more suited to less desirable growing conditions. A potential rootstock would be *V. arboreum*, which has the ability to grow in many areas that would be unsuitable for commercial blueberries. First, *V. arboreum* has a coarse root system with a long tap root (Lyrene, 1997). Because of this, it is a very drought-resistant species and is able to grow in areas dryer than *V. corymbosum* can tolerate (Ballinger et al., 1982). Next, *V. arboreum* is one of the few *Vaccinium* species that can grow in calcareous soils, meaning they can survive in conditions with a higher soil pH. *Vaccinium corymbosum* has a very limited soil pH range that it can tolerate, about 4.0-5.5 (Darnell and Hiss, 2006), but *V. arboreum* can tolerate soil pH from 4-7 (Radford et al., 1968). Also, *V. arboreum* has a greater capacity to survive in soils that have NO_3^- as the prominent form of nitrogen in the soil (Darnell and Hiss, 2006). It can also tolerate soils that have limited quantities of iron, a condition that is not ideal for *V. corymbosum. V. arboreum* is also more efficient in acquiring nitrate than *V. corymbosum* (Poonnachit and Darnell, 2004). Finally, *V. arboreum* has an erect growth habit consisting of a single trunk, which would minimize fruit loss when mechanical harvesting techniques are used (Ballington et al., 1990).

Overall, there are many advantages to using *V. arboreum* as a rootstock for *V. corymbosum* to aid in expanding the blueberry industry to meet higher demands.

V. corymbosum has been successfully grafted onto *V. arboreum*, which shows that there is a genetic affinity and that grafting is possible (Galletta and Fish, 1971). *V. ashei* has also been successfully grafted onto *V. arboreum* (Ballington et al., 1990). Several techniques have proven successful; these include early spring cleft, whip or side grafts, and late summer t-budding (Ballington et al., 1990). Although grafting *V. corymbosum* or *V. ashei* onto *V. arboreum* appears to be a great idea, there are drawbacks. First, it is costly and the inconsistency of results may make grafted blueberry plants economically unfeasible (Ballington et al., 1990). There is also a limited availability of suitable stock material, and propagating *V. arboreum* is a difficult task. In order to take advantage of the positive improvements that grafted blueberry plants would bring, a commercially acceptable way of propagating *V. arboreum* is needed.

Propagation

V. corymbosum and V. ashei

There are several successful methods of propagating *V. corymbosum* and *V. ashei* that could potentially be used to propagate *V. arboreum*. Cuttings- specifically hardwood and softwood- are the most widely used method of propagation in the commercial industry (Mainland, 1993; Miller et al., 2006). Miller et al. (2006) found that southern highbush (*V. corymbosum*) and rabbiteye (*V. ashei*) blueberries had the greatest rooting success when using softwood cuttings, and northern highbush had a lower rooting percentage but was still successful. Both softwood and hardwood cuttings taken from the terminal or middle position on the branch had higher rooting percentages than cuttings taken from the basal portion of the branch. While softwood cuttings usually have a higher rooting percentage, many propagators prefer to use hardwood cuttings because propagation can be done in the slower, dormant season and cuttings can be kept in a cooler until an appropriate time for sticking (Mainland, 1993). The traditional media used for cuttings is sphagnum peat, either alone or with varying proportions of sand or perlite (Pokorny and Austin, 1982; Shelton and Moore, 1981), but there are also alternatives to using peat that may save money. Pokorny and Austin (1982), for example, found that milled pine bark could be used as an alternative to sphagnum peat and actually increased the percentage of rooting and root quality in *V. ashei*.

Another method of increasing rooting success is to remove leaves from the bottom half of the cutting when working with softwood cuttings. This increased the percentage of cuttings that rooted in *V. corymbosum* (Mainland, 1993). Micropropagation is another method of propagating commercial blueberry plants, but it is seldom used due to the cost and the increase in genetic variance (Mainland, 1993; Miller et al., 2006).

V. arboreum

The current method for propagating *V. arboreum* is using seeds. They are more difficult to germinate than commercial blueberry species from section *Cyanococcus* (Lyrene and Brooks, 1995). When using methods similar to those used for germinating *Cyanococcus* species (placed uncovered in sphagnum peat in an unheated greenhouse and watered with intermittent mist for 3 hours a day for 2 months), sparkleberry seedlings grew poorly (Lyrene and Brooks, 1995). One method for increasing germination success was to soak the seeds overnight in a gibberellic acid solution (4g per liter of water) (Lyrene and Brooks, 1995). When propagating *V. arboreum* to use as a rootstock, seeds would not be ideal due to the genetic variance of each plant. An asexual propagation method would be needed to propagate specimens with ideal qualities to serve as a rootstock.

To date, there has been little success in propagating *V. arboreum*. There has also been very little research on the subject. Reese (1992) used semihardwood cuttings to try to propagate *V. arboreum*. Cuttings were taken from plants growing in their native habitat. The basal end of each cutting was cut at a slant to expose more cambium tissue. Several treatments were used including quick dips of various concentrations of IBA+NAA, a 24 hour soak in willow water, a 24 hour soak in water, willow water plus Hormodin III, water plus Hormodin III, only Hormodin III, a five-second quick dip in 95% ethanol, and a control (water). A 1:1 (volume) milled pine bark:perlite substrate was used in rhizotrons so that rooting could be observed. Cuttings were placed under intermittent mist for ten seconds every five minutes. After three months, data was taken on the rooting. The Hormodin III had the highest rooting percentage at 12.5% and the control (water) had the lowest rooting percentage with 0%. All of the treatments were statistically similar, and it was concluded that this would not be a commercially feasible way to propagate *V. arboreum* (Reese, 1992).

A second experiment was performed using hardwood cuttings. A similar set up was used, but the treatments were different. They included Hormodin III, mechanical wounding, and Hormodin III in addition to mechanical wounding, plus various combinations of IBA+NAA and wounding. When the cuttings were examined for rooting, only two in the entire experiment had rooted (Reese, 1992). This is also not a commercially feasible way to propagate *V. arboreum*.

Stockton (1976) tried to propagate *V. arboreum* using softwood stem cuttings. Four different concentrations of K-IBA dissolved in water were used (0, 10000, 15000, 20000) for a quick dip. A 2:2:1 (volume) peat:perlite:sand substrate was used. The cuttings were placed under intermittent mist for 8 seconds every 10 minutes during daylight hours. After 60 days, the cuttings were checked for rooting. Minimal to no success was seen in all of the treatments (Stockton, 1976), and therefore this is not an efficient way to root *V. arboreum*.

Stockton (1976) also tried to achieve rooting using rhizome cuttings. The rhizomes were taken later in the year, from July until November. The caliper of the rhizomes varied from 0.5 to 3 cm in diameter and 10-30 cm in length. The rhizomes were placed vertically with either the distal end up or the proximal end up and were placed at least 3 cm below the surface of the substrate. There was some success rooting, but this would not be a feasible way to propagate *V. arboreum* because the success rate is not high enough to make it worthwhile; furthermore, harvesting rhizomes results in the death of the stock plant (Stockton, 1976).

Deciduous azaleas and other hard to root species

Since propagating *V. arboreum* has been difficult, a review of rooting techniques for another hard to root species may be helpful to gain insight on how to root *V. arboreum*. One group of plants to consider would be deciduous azaleas, which are also in the Ericaceae family and are acid-loving plants.

The success rate of azalea propagation by stem cuttings depends on many factors such as the time of year, the specific cultivar, substrate, irrigation levels, bottom heat, and amount of light. Knuttel and Addison (1984) used ‘Royal Lodge’, ‘Visco Sepala’, ‘Sunset Boulevard’, ‘Satan’, ‘Crimson Tide’, and ‘Pink Jolly’ stock plants that were kept in a controlled temperature overwintering structure. This way, the temperature can be gradually raised to allow the plants to come out of dormancy early and cuttings, therefore, can be taken earlier in the year, around April-May (Knuttel and Addison, 1984). Young, herbaceous growth that is slightly firm was considered ideal cutting material (Knuttel and Addison, 1984). Cuttings should be slightly hairy and feel like they are about to snap when bent double (Nienhuys, 1980). Various concentrations of IBA and NAA, depending on what is recommended for the specific cultivar, can also be used to achieve a higher rooting percentage. Another way to aid in rooting is to provide bottom heat at 21.1-22.8°C to keep the medium warm (Knuttel, 1984; Mylin, 1982; Nienhuys, 1980). Light intensity is another factor that influences the rooting success of azalea cuttings (Read and Economou, 1983); an increase in rooting percentage was observed as light intensity decreased. Cuttings rooted with lower intensity light (10 $\mu Em^{-2}s^{-1}$) had a rooting percentage of 88.3%, compared to 65.8% when using high intensity light (75 $\mu Em^{-2}s^{-1}$) (Read and Economou, 1983). Using these techniques, alone or together, can result in higher rooting percentages for deciduous azalea cuttings.

Another hard-to-root, Ericaceous species is *Kalmia latifolia*. Williams and Bilderback (1980) used *K. latifolia* cuttings taken in September, October, and November. Hormone treatments included 0.1% fenoprop + talc, 1.0% K-IBA + Benomyle + talc, and a 0.5% K-IBA 10 s quick dip. Cuttings remained in a 1:1 peat:perlite substrate for 165 days. The month the cuttings were taken influenced the rooting percentage, with the cuttings in September having a significantly higher rooting percentage than those taken in October and November, which had statistically similar rooting percentages in all three treatments. The highest rooting percentage was observed in cuttings taken in September and treated with 0.1% fenoprop + talc with 55%. Rooting percentages observed overall ranged from 12-55% (Williams and Bilderback, 1980), which is not feasible for commercial production.

Plant Hormones and Plant Growth Regulators

Plant hormones and plant growth regulators (PGRs) are used in propagation of many species. A plant hormone is a naturally occurring chemical that is synthesized within the plant and is involved in the growth and development of that plant. The five major plant hormones are auxins, cytokinins, gibberellins, abscisic acid, and ethylene. In addition to naturally occurring hormones, other chemicals, both naturally occurring and synthetic, can induce a response in a plant. These chemicals are grouped together and are known as PGRs (Hartmann et. al., 2002).

Auxin is the most widely used plant hormone for the induction of adventitious roots in cuttings. It is naturally produced in leaf primordial, young leaves, and developing seeds in the form of the chemical indole-3-acetic acid (IAA). Auxin is important in the phenomenon of apical dominance by inhibiting lateral bud break. Another naturally occurring form of auxin is indole-3-butyric acid (IBA). There are also several synthetic forms of auxin, including indole-3-butyric acid-potassium salt (K-IBA). Usually, IBA is found in salt form, which is water-soluble; otherwise it is only soluble in alcohol, which could burn sensitive cuttings. Other synthetic forms of IBA include alpha-naphthaleneacetic acid (NAA), 2,4-dichloro-phenoxy-acetic acid (2,4D), and 2,4,5-tri-chloro-phenoxy-acetic acid (2,4,5T) (Hartmann et. al., 2002). By using an auxin application the rooting success may be increased.

Conclusion

The allure of the positive changes that using *V. arboreum* as a rootstock could bring is the driving force behind further investigating methods of propagating *V. arboreum*. In addition, *V. arboreum* could also be used as an ornamental plant because of its attractive bark (similar to crapemyrtle), attractive fall foliage colors, semi-evergreen to evergreen habit, fruit desirable to birds, and tree-like growth habit. There are still a number of factors that could be explored, including semihardwood cuttings, rooting substrate, different hormone treatments or wounding, and other environmental factors such as the amount of light and moisture, that justify continuing forward with research on productive and commercially useful methods of propagating *V. arboreum*.

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Chapter II

Propagation of *Vaccinium arboreum* by Stem Cuttings for Use as a Rootstock for Commercial Blueberry Production

Introduction

In recent years, there has been an increase in consumer demand for fresh blueberries throughout the year, which also increases the demand for sites suitable for growing blueberries. Commercial blueberries, including highbush (*Vaccinium corymbosum* L.) and rabbiteye (*Vaccinium ashei* Reade), have specific requirements for optimal growth. As part of the Ericaceae family, commercial blueberries favor acidic soil, in the range of pH 4.0-5.5 (Trehane, 2004). Blueberries also need high amounts of organic matter within the soil, as well as iron and nitrogen in the NH_4+ form (Darnell and Hiss, 2006). Other soil characteristics include good drainage, aeration, and a relatively consistent moderate moisture content (Ballinger et al., 1982). In addition to having specific growing needs, commercial blueberries also have fibrous, shallow root systems that are sensitive to drought and wind damage (Lyrene, 1997). Because of all these limitations, suitable growing sites are in short supply unless the soil is adapted using costly amendments.

One way to overcome a poor growing environment is to use a rootstock that has the capability to grow where others cannot. A potential rootstock for commercial blueberries is the sparkleberry (*Vaccinium arboreum* Marsh), which has many desirable qualities that give it the ability to grow in many areas that would be unsuitable for commercial blueberries. *V. arboreum* is one of the few *Vaccinium* species that can tolerate calcareous soils, meaning they survive in conditions with higher soil pH levels, from pH 4 to pH 7 (Radford et al., 1968). *V. arboreum* is also able to grow in conditions where the prominent form of nitrogen in the soil is nitrate (NO_3^-) (Darnell and Hiss, 2006). It can tolerate soils with limited quantities of iron and is more efficient at acquiring nitrate than commercial blueberries (Poonnachit and Darnell, 2004). *V. arboreum* has a coarse root system with a long taproot (Lyrene, 1997), making it a very drought-resistant species and less likely to be uprooted due to wind (Ballinger et al., 1982). *V. arboreum* can grow well in soils with less than 2% organic matter (Lyrene, 1998). Finally, *V. arboreum* has an erect growth habit consisting of a single trunk that would minimize fruit loss when using mechanical harvesting techniques (Ballington et al., 1990). With the increased demand for blueberries as a healthy snack, *V. arboreum* could be used to increase blueberry production to meet the growing demands.

In the past, *V. corymbosum* has been successfully grafted onto *V. arboreum*, which shows a genetic affinity, therefore making grafting possible (Galletta and Fish, 1971). *V. ashei* has also been successfully grafted onto *V. arboreum* (Ballington et al., 1990), again showing a genetic affinity. Techniques that have proven successful include early spring cleft, whip or side grafts, and late summer t-budding (Ballington et al., 1990). One problem encountered was the production of suckers from the rootstock; these increased with the age of the plant (Eck, 1988).

The current method for propagating *V. arboreum* is using seeds. They are more difficult to germinate than commercial blueberry species from section *Cyanococcus* (Lyrene and Brooks, 1995). When using methods similar to those used for germinating

Cyanococcus species (placed uncovered in sphagnum peat in an unheated greenhouse and watered with intermittent mist for three hours a day for two months), sparkleberry seedlings grew poorly (Lyrene and Brooks, 1995). One method for increasing germination success was to soak the seeds overnight in a gibberellic acid solution (4g per liter of water) (Lyrene and Brooks, 1995). When propagating *V. arboreum* to use as a rootstock, seeds would not be ideal due to the genetic variance of each plant. An asexual propagation method would be needed to propagate specimens with ideal qualities to serve as a rootstock.

To use *V. arboreum* successfully as a rootstock, protocols for clonal propagation of the species in large quantities are needed. To date, there has been little research on the propagation of *V. arboreum*. Stockton (1976) tried to propagate *V. arboreum* using softwood stem cuttings and K-IBA quick-dips. Four different concentrations of K-IBA dissolved in water were used (0, 10000, 15000, and 20000 ppm). A 2:2:1 (volume) peat:perlite:sand substrate was used. Cuttings were placed under intermittent mist for 8 s every 10 m during daylight hours. After 60 days, cuttings were checked for rooting and minimal to no success was observed in all of the treatments (Stockton, 1976). Reese (1992) used semihardwood stem cuttings and different treatments to try to enhance rooting, including various levels of IBA+NAA, willow water, and Hormodin III. Cuttings were stuck in a 1:1 pine bark:peat substrate and placed in a rhizotron under intermittent mist. Similar to the previous study, little rooting was observed, with only a 0-12.5% rooting percentage recorded among the treatments. The control treatment had 0% rooting and all of the treatments were statistically similar, suggesting that none of the treatments influenced rooting success (Reese, 1992). A subsequent study using hardwood cuttings and different combinations of wounding and hormones resulted in only two rooted cuttings for the entire experiment. Hence, previous research suggests *V. arboreum* is a very hard-to-root species, with no indication of viable treatments to enhance rooting of stem cuttings.

In addition to stem cuttings, rhizome cuttings have been evaluated (Stockton, 1976). Rhizome sections were taken from July until November. The caliper of the rhizomes varied from 0.5-3 cm in diameter and 10-30 cm in length. Rhizomes were placed vertically with either the distal end up or the proximal end up and at least 3 cm below the surface of the substrate. Though percent rooting was not reported, some root formation did occur. Stockton (1976) concluded that this would not be an ideal way to propagate *V. arboreum* because of the low success rate and the likelihood of harvesting rhizomes resulting in the death of the stock plant.

Determining a viable way to propagate *V. arboreum* would benefit commercial blueberry production as a potential rootstock, as well as the selecting and marketing of *V. arboreum* as a landscape plant. Partly due to the difficulty of propagation, *V. arboreum* is seldom marketed as a landscape plant. However, *V. arboreum* can grow to be an aesthetically pleasing semi-evergreen small tree, with attractive fall color, exfoliating bark, and edible fruit. Since *V. arboreum* tolerates drought and a range of soil types, it is a good selection as an attractive woodland shrub/small tree for xeriscaping and native plant landscaping. A viable way to clonally propagate *V. arboreum* is necessary to allow for selection of plants with desirable ornamental and rootstock characteristics.

The objectives of this study were to determine whether cutting type (softwood, semihardwood, or hardwood), cutting position (terminal or subterminal), IBA concentration, or the interaction of these treatments influence rooting of *V. arboreum* stem cuttings. Previous experiments did not specify if the cuttings were taken from juvenile or mature wood. Only juvenile wood was used in this study, as juvenile wood is typically easier to root than mature wood for most species (Hartmann et al., 2002).

Materials and Methods

Cutting propagation material of *V. arboreum* was collected from two locations. Water sprouts from native, mature plants were collected from the Robert Trent Jones Golf Trail at Grand National (RTJ) in Opelika, Alabama (lat. 32°69'N, long. 85°44'W, USDA hardiness zone 8a). Juvenile cuttings arising from latent buds on mature plants that had been cut back to approximately 1 m in height in February 2010 were collected from Stone County, Mississippi (SCMS) (lat. 30°80'N, long. 89°17'W, USDA hardiness zone 8b). Softwood, semihardwood, and hardwood cuttings were collected, as well as subterminal and terminal cuttings. The softwood cuttings were lignified enough to stay upright when stems were stuck in substrate, but still flexible. Softwood cuttings from SCMS were taken the same day as semihardwood cuttings. The terminal cutting and that immediately below were used as softwood because they were still mostly flexible. The more lignified basal cuttings, at least 30 cm from the terminal end, were used for semihardwood cuttings.. Similar softwood and semihardwood cuttings were collected from RTJ, but the semihardwood cuttings were taken 47 days after softwood cuttings were collected. Hardwood cuttings were collected while the plants were dormant, before bud break.

Cuttings were trimmed to 10-12 cm long. Caliper of the softwood cuttings from RTJ and SCMS averaged 2.96 and 3.81 cm, respectively. Caliper of the semihardwood cuttings from RTJ and SCMS averaged 2.99 and 3.25 cm, respectively. Caliper of the hardwood cuttings from RTJ and SCMS averaged 2.82 and 3.07, respectively.

Auxin solutions were prepared using Hortus IBA Water Soluble Salts® (Hortus USA Corp.) and deionized water. The basal end of each cutting was cut at a 45° angle and received a 10-s basal quick-dip to a depth of 3 cm in either water (control) or a solution of 1000, 2500, 5000, or 7500 ppm IBA. Cuttings were then inserted to a depth of 3 cm into a cell in a 48-cell tray (Landmark Plastic Corporation, C-T1240, cell dimensions 5.7cm×3.7cm×6.6cm). A 1:1 peat:perlite substrate was used.

Hardwood cuttings from RTJ were taken and inserted on March 1, 2011. Hardwood cuttings from SCMS were taken on March 6, 2011 and inserted on March 8, 2011. Softwood cuttings from RTJ were collected and inserted on May 20, 2011. Softwood cuttings from SCMS were collected on June 19, 2011 and inserted on June 20, 2011. Semihardwood cuttings from RTJ were collected and inserted on July 6, 2011. Semihardwood cuttings from SCMS were collected on June 19, 2011 and inserted on June 21, 2011. After they were inserted, the cuttings were placed on a greenhouse bench in a 1.2 m wide by 2.4 m long by 0.9 m tall polyethylene covered enclosure at the Paterson Greenhouse Complex at Auburn University to ensure the relative humidity stayed at an appropriate level. Overhead mist was provided within the enclosure for 2 sec every 10 min.

Data was collected for RTJ and SCMS hardwood cuttings on August 19, 2011. Data was collected for RTJ softwood on September 15, 2011. Data was collected for SCMS softwood on October 14, 2011. Data for RTJ and SCMS semihardwood was collected on November 11, 2011. After collecting initial data, cuttings that had formed callus and not roots were re-inserted and checked again four weeks later for rooting. Only 3 cuttings out of all the cuttings with callus but no roots formed roots in that time period.

A completely randomized design was used with 30 cuttings (replications) per treatment. Rooting response (rooted or unrooted) was recorded for all cuttings, with a cutting considered rooted when any sign of adventitious roots were seen emerging from the stem. Additional data collected include number of primary roots emerging from the stem of each rooted cutting, total length of primary roots on each rooted cutting, number of rooted cuttings with new shoots, total shoot length on each rooted cutting, number of cuttings that formed callus, and callus caliper of cuttings with callus.

The treatment design was a 2 × 2 × 3 × 5 complete factorial design with four factors: 1) source (water sprouts from mature plants or sprouts arising from latent buds on cut back plants), 2) cutting position on the stock plant (terminal and subterminal), 3) cutting maturity (softwood, semihardwood and hardwood) and 4) IBA rate (0, 1000, 2500, 5000, and 7500 ppm), for a total of 60 treatment combinations. The experimental design was a completely randomized design. Data were analyzed using generalized linear models with the GLIMMIX procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). Rooting was analyzed using the binomial distribution and a logit link function, count data were analyzed using the Poisson distribution and a log link function, and measurement data were analyzed using the normal distribution and the identity function. Comparisons of least squares means were conducted using the Schaffer-Simulated adjustment for multiple comparisons. Correlations between callus and rooting were run using the Pearson Correlation test.

Results

In all the results, source refers to whether the cuttings came from water sprouts of mature, wild plants from RTJ, or from wild plants that had been cut back and allowed to sprout shoots from latent buds in SCMS. Type refers to whether the cutting is softwood, semihardwood, or hardwood. Position refers to whether the cutting is subterminal or terminal. IBA refers to the 10 s IBA quick-dip rate (ppm) to which the cutting was subjected.

In the original model, only softwood and hardwood cuttings were used. This was due to the fact that semihardwood, terminal cuttings were unavailable at one of the sources. Using only the softwood and hardwood cuttings allows for a 4-factor analysis. After sequentially removing nonsignificant four-way and three-way interactions, the only three-way interaction term that was significant was source×type×position; therefore, the way that source and type affect a cutting could vary depending on the position. Rooting percentages of subterminal cuttings were similar for softwood cuttings from both RTJ and SCMS (38.5% and 34.3%), but terminal softwood cuttings from SCMS had a lower rooting percentage (29.2%) than RTJ cuttings (43.3%) (Table 1). For hardwood cuttings, rooting percentages were statistically similar when using terminal cuttings, but when using subterminal cuttings RTJ cuttings had a higher rooting percentage than SCMS cuttings (Table 1). Because the effects of source and type could vary due to the position of the cutting, separate analyses were run for subterminal and terminal cuttings.

Subterminal cuttings

There were no significant effects of IBA rate on rooting percentage (Table 2) or any of the parameters (root number, root length, shoot number, shoot length, callus presence, and callus caliper) measured on subterminal cuttings (Tables 2-4). The source and type of cutting did influence the rooting percentage. The highest rooting percentage occurred when using softwood cuttings from RTJ with a rooting percentage of 38.6%. Similar rooting was observed in SCMS softwood and semihardwood with rooting percentages of 34.6% and 28.5% respectively (Table 5). None of the treatments significantly influenced the number of primary roots per rooted cutting (Table 5). Type of cutting influenced the length of primary roots. The longest roots were found on SCMS semihardwood cuttings with an average total root length of 23.3 cm (Table 5). SCMS softwood and hardwood and RTJ semihardwood cuttings had statistically similar root lengths, with average total root lengths of 18.3, 17.0, and 17.1 cm respectively (Table 5).

The source and type of cutting both influenced the number of new shoots on rooted cuttings (Table 6). Hardwood cuttings from both SCMS and RTJ had more new shoots, 1.5 and 2.4 respectively, than softwood and semihardwood cuttings from either source (Table 6). The type of cutting and the source×type interaction both significantly influenced the length of new shoots. The longest shoots were observed on the RTJ hardwood cuttings with an average total combined shoot length of 5.3 cm (Table 6).

Source, type, and source×type interaction all significantly influenced the percent of cuttings that formed callus. The RTJ softwood cuttings had the highest percent of cuttings with callus formation with 82.4% (Table 6). Cutting source and type also influenced the callus caliper. RTJ hardwood cuttings had the greatest callus caliper with an average diameter of 7.1 mm. Statistically similar calipers were observed in RTJ softwood and SCMS hardwood cuttings with average diameters of 6.5 and 6.8 mm, respectively (Table 6).

Terminal cuttings

There were no significant effects of IBA rate on rooting percentage (Table 7) or any of the parameters measured on terminal cuttings (Table 7-9). The source and type of cutting both significantly influenced rooting percentage. The highest rooting percentage was observed on the RTJ softwood cuttings with 43.3% rooting. Softwood cuttings from SCMS had 29.2% rooting, which was greater than hardwood cuttings from both sources (2.0%) (Table 10). None of the treatments influenced the number of primary roots on rooted cuttings or the total combined length of primary roots (Table 10).

Shoot number was significantly influenced by the source and type of cutting. Softwood and hardwood cuttings from both RTJ and SCMS all had less than one shoot per cutting (Table 11). None of the factors significantly influenced shoot length (Table 11).

The source and type of cutting significantly influenced the percent of cuttings that formed callus. The highest percentage of cuttings with callus was observed in the softwood cuttings from both RTJ and SCMS with 73.4% and 33.9% of cuttings forming callus, respectively (Table 11). The callus caliper was not significantly influenced by any of the treatments (Table 11).

Correlations

There were no strong correlations between callus and rooting when the data were sorted by IBA treatment rate alone (Table 12). The data were also sorted by IBA rate and position, IBA rate and source, and IBA rate and cutting type. The strongest correlation between rooting and callus was seen in the cuttings from SCMS treated with 7500ppm

IBA. The correlation coefficient was 0.88. Overall the correlation coefficients ranged from 0.24 to 0.88, with most between 0.40 and 0.69 (Table 12).

Discussion

The IBA rate did not have a significant influence on the rooting percentage of any of the cuttings. This conclusion was also observed in previous research by Stockton (1976) with only IBA quick-dips. Reese (1992) found that combinations of IBA+NAA quick-dips also did not influence the rooting percentage, nor did treatments with Hormodin III. Some other Ericaceous plants, such as *Leucothoe racemeosa* 'Rainbow', are also are not affected by IBA rate (Scagel, 2005). No difference in rooting percentage was observed when treating cuttings to a 5 s quick dip of control water versus a 5 s quick dip in a 1.03% IBA, 0.66% NAA solution (Scagel, 2005). The fact that IBA rate did not affect rooting percentage of *V. arboreum* may be a characteristic of Ericaceous species.

Based on the statistical analysis, position (subterminal or terminal) alone did not influence rooting percentage. However, the source×type×position interaction term was significant, meaning that the effects of source and type on the rooting of a cutting could change based on the position of that cutting. For example, subterminal softwood cuttings from RTJ and SCMS had statistically similar rooting percentages, but terminal softwood cuttings from SCMS did not root as well as those from RTJ. Similarly, terminal hardwood cuttings from RTJ and SCMS had statistically similar rooting percentages, but subterminal hardwood cuttings from RTJ had a higher rooting percentage than those from SCMS (Table 1). As previously mentioned, this was also the basis for running separate analyses on subterminal and terminal cuttings.

Type of cutting greatly affected the rooting success of *V. arboreum*, with softwood cuttings rooting more readily than hardwood cuttings. In previous research there was virtually no success rooting *V. arboreum* using softwood, semihardwood or hardwood cuttings (Reese, 1992; Stockton, 1976). For this experiment, the greatest rooting percentage occurred using softwood cuttings. The percent rooting of softwood cuttings ranged from 29.2-43.2%, which is a great improvement compared to any previous research.

The source (RTJ or SCMS) of the cutting influenced the rooting percentage of semihardwood cuttings. Cuttings from SCMS had a similar rooting percentage to softwood cuttings, and the cuttings from RTJ had a low rooting percentage similar to hardwood cuttings (Table 5). This may be due to the quality of the cuttings obtained. The greater number of sprouts from the plants that had been cut back at the SCMS location allowed us to be more selective, and the cuttings may have been closer to softwood cuttings than the cuttings from RTJ. The cutting material from RTJ was limited, forcing the use of some less than ideal cuttings, and the cuttings had a wider range of lignification. As in previous studies, the hardwood cuttings had very little success. Hardwood cuttings for many species are generally less successful than softwood and semihardwood cuttings (Hartmann et al., 2002).

Other factors important in a rooted cutting include the number or primary roots. In this experiment there were no factors that significantly influenced the number of primary roots that a rooted cutting produced.

In nearly all of the combinations looked at, there were no strong correlations between the formation of callus and the formation of roots. The highest correlation coefficient was observed in SCMS cuttings treated with 7500 ppm IBA with a coefficient of 0.88 (Table 12). This could suggest that this treatment combination produces the most cuttings with callus and roots. Even though the correlation is strong, however, it does not confirm that the increase in callus directly caused the increase in rooting observed. There may be other factors that influence this relationship and played a part in the increased callus and rooting. Overall, most of the correlation coefficients were between 0.40 and 0.69 (Table 12). All of the coefficients were positive, meaning that callus and rooting increase as the other increases. Again, this does not mean that one necessarily directly influenced the other.

Since virtually no rooting success of *V. arboreum* was observed in previous research (Reese, 1992; Stockton, 1976), the fact that we observed 29.2-43.2% rooting in softwood cuttings was encouraging. Although this may not be a commercially feasible way to propagate *V. arboreum*, it demonstrates that rooting is possible and that the methods could potentially be improved. Previous research did not mention whether juvenile or mature cuttings were used in the experiments (Reese, 1992; Stockton, 1976). Only juvenile wood was used in this study, which may explain the greater rooting success. The next steps would be to discover ways to increase rooting to a commercially acceptable success rate. Some ideas that could be explored include the use of bottom heat, which is helpful when rooting similar hard-to-root species like deciduous azaleas. Bottom heat of 21.1-22.8°C has been used to successfully improve rooting percentage of deciduous azaleas (Knuttel, 1984; Mylin, 1982; Nienhuys, 1980). Also, a substrate other than peat:perlite may prove to be beneficial. One of the most difficult parts of this experiment was keeping the cuttings from drying out without over saturating the substrate. The use of pine bark or sand, or a combination of many components, could help with drainage and could decrease water logging. Light intensity is another factor that influences the rooting success of azalea cuttings (Read and Economou, 1983). An increase in rooting percentage was observed as light intensity decreased. Cuttings rooted with lower intensity light (10 $\mu Em^{-2}s^{-1}$) had a rooting percentage of 88.3%, compared to 65.8% when using high intensity light (75 $\mu Em^{-2}s^{-1}$) (Read and Economou, 1983). Another technique to increase rooting success may be to use a wounding technique that would expose more cambial tissue and encourage rooting. In addition to cuttings, other propagation methods could be explored such as mound layering. Overall, the rooting success observed in this experiment was encouraging and demonstrates the need for the further research of *V. arboreum* asexual propagation.

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Table 1. Comparison of softwood and hardwood, subterminal and terminal rooting percentages of *V. arboreum* stem cuttings from two locations.

		Rooting (%)	
Source	Type	Subterminal	Terminal
RTJ[z]	Softwood	38.5 a[x]	43.3 a
SCMS[y]	Softwood	34.3 a	29.2 b
RTJ	Hardwood	10.3 b	1.9 c
SCMS	Hardwood	0.6 c	1.8 c

[z]Robert Trent Jones Golf Trail, Opelika, AL, cuttings from watersprouts.

[y]Stone County, MS, cuttings from plants that had been cut back Feb, 2010 and resprouted.

[x]Shaffer-Simulated grouping for source*type least squares mean (Alpha=0.05).

Table 2. Influence of IBA rate on rooting percentage, number of roots, and root length of subterminal *V. arboreum* stem cuttings.

	SCMS[z]			RTJ[y]		
IBA Rate (ppm)	Softwood	Semihard	Hardwood	Softwood	Semihard	Hardwood
			Rooting (%)			
0	26.6[x]	36.6	0.0	33.0	3.3	11.6
1000	30.0	16.6	0.0	36.6	6.6	8.3
2500	36.6	43.3	3.3	35.0	10.0	11.6
5000	36.6	23.3	0.0	36.6	16.6	13.3
7500	43.3	23.3	0.0	51.6	10.0	8.3
Significance[w]	NS	NS	NS	NS	NS	NS
			Roots (no.)			
0	2.0	2.4	0.0	2.3	1.0	2.0
1000	1.9	3.4	0.0	2.9	1.0	1.8
2500	1.8	1.6	1.0	2.5	3.0	1.7
5000	2.3	2.7	0.0	1.6	1.6	2.0
7500	1.8	3.1	0.0	2.7	1.0	2.0
Significance	NS	NS	NS	NS	NS	NS
			Root Length (cm)			
0	18.4	22.9	0.0	16.8	11.6	14.5
1000	19.0	22.5	0.0	15.3	12.7	8.5
2500	17.7	18.0	16.5	16.7	29.7	9.7
5000	22.2	27.7	0.0	12.1	16.6	11.3
7500	15.2	29.7	0.0	18.4	9.8	7.9
Significance	NS	NS	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 3. Influence of IBA rate on shoot number and total shoot length of subterminal *V. arboreum* stem cuttings.

	SCMS[z]			*RTJ*[y]		
IBA Rate (PPM)	Softwood	Semihard	Hardwood	Softwood	Semihard	Hardwood
			Shoots (no.)			
0	<1.0[x]	1.4	0.0	<1.0	2.0	2.6
1000	<1.0	1.0	0.0	<1.0	<1.0	2.0
2500	<1.0	1.2	1.5	<1.0	<1.0	2.7
5000	<1.0	1.3	0.0	<1.0	<1.0	2.2
7500	<1.0	2.1	0.0	<1.0	0.0	2.8
Significance[w]	NS	NS	NS	NS	NS	NS
			Length (cm)			
0	0.4	3.5	0.0	0.7	2.3	6.0
1000	0.6	1.5	0.0	0.7	2.0	7.8
2500	0.7	2.1	2.2	0.5	0.7	4.8
5000	1.4	3.7	0.0	0.3	1.0	3.3
7500	1.1	4.6	0.0	0.1	0.0	5.9
Significance	NS	NS	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 4. Influence of IBA rate on percent of cuttings with callus and callus caliper of subterminal *V. arboreum* stem cuttings.

	SCMS[z]			RTJ[y]		
IBA Rate (ppm)	Softwood	Semihard	Hardwood	Softwood	Semihard	Hardwood
			Callus (%)			
0	30.0[x]	46.7	8.3	71.7	23.3	50.0
1000	66.7	23.3	0.0	85.0	33.3	41.7
2500	36.7	53.3	5.0	83.3	33.3	48.3
5000	56.7	33.3	3.3	85.0	40.0	46.7
7500	53.3	26.7	1.7	83.3	20.0	33.3
Significance[w]	NS	NS	NS	NS	NS	NS
			Callus Caliper (mm)			
0	4.7	2.9	5.1	5.7	4.3	6.5
1000	3.7	3.7	0.0	6.3	5.4	6.9
2500	6.0	4.5	6.7	6.7	6.6	6.1
5000	4.9	4.7	11.0	6.5	4.7	7.0
7500	5.7	6.7	3.1	7.3	5.8	9.1
Significance	NS	NS	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 5. Effect of cutting source and cutting type on percent rooting, number of roots and root length of subterminal *V. arboreum* stem cuttings.

Source	Type	Rooting (%)	Roots (no.)	Root Length (cm)
RTJ[z]	Softwood	38.6 a[x]	2.2 a	16.0 bc
SCMS[y]	Softwood	34.6 a	1.9 a	18.3 ab
RTJ	Semihardwood	9.2 b	1.6 a	17.1 abc
SCMS	Semihardwood	28.5 a	2.4 a	23.3 a
RTJ	Hardwood	10.6 b	1.9 a	10.7 c
SCMS	Hardwood	0.7 c	1.0 a	17.0 abc

[z]Robert Trent Jones Golf Trail, Opelika, AL, cuttings from watersprouts.

[y]Stone County, MS, cuttings from plants that had been cut back Feb, 2010 and resprouted.

[x]Shaffer-Simulated grouping for source*type least squares mean (Alpha=0.05).

Table 6. Effect of cutting source and cutting type on shoot number, shoot length, percent with callus and callus caliper of subterminal *V. arboreum* stem cuttings

Source	Type	Shoots (no.)	Shoot Length (cm)	Callus (%)	Callus Caliper (mm)
RTJ[z]	Softwood	0.2 c[x]	0.4 c	82.4 a	6.5 ab
SCMS[y]	Softwood	0.4 bc	0.8 c	48.5 b	4.9 c
RTJ	Semi-Hardwood	0.5 bc	0.9 c	29.6 c	5.4 bc
SCMS	Semi-Hardwood	1.3 b	3.1 b	36.3 bc	4.4 c
RTJ	Hardwood	2.4 a	5.3 a	43.8 b	7.1 a
SCMS	Hardwood	1.5 ab	2.5 bc	3.5 d	6.8 ab

[z]Robert Trent Jones Golf Trail, Opelika, AL, cuttings from watersprouts.

[y]Stone County, MS, cuttings from plants that had been cut back Feb, 2010 and resprouted.

[x]Shaffer-Simulated grouping for source*type least squares mean (Alpha=0.05).

Table 7. Influence of IBA rate on rooting percentage, number of roots, and total root length of terminal *V. arboreum* stem cuttings.

	SCMS[z]		*RTJ*[y]	
IBA Rate (ppm)	Softwood	Hardwood	Softwood	Hardwood
		Rooting (%)		
0	36.7[x]	0.0	36.7	3.3
1000	20.0	3.3	60.0	0.0
2500	26.7	6.6	36.7	3.3
5000	23.3	0.0	40.0	3.3
7500	40.0	0.0	43.3	0.0
Significance[w]	NS	NS	NS	NS
		Roots (no.)		
0	1.8	0.0	2.3	1.0
1000	2.3	1.0	2.9	0.0
2500	1.8	3.0	2.5	2.0
5000	2.3	0.0	1.6	3.0
7500	2.4	0.0	2.7	0.0
Significance	NS	NS	NS	NS
		Root length (cm)		
0	20.7	0.0	13.2	10.8
1000	20.8	1.7	16.1	0.0
2500	14.7	5.7	15.6	15.9
5000	22.1	0.0	19.4	29.4
7500	21.7	0.0	20.1	0.0
Significance	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 8. Influence of IBA rate on number of shoots and shoot length of terminal *V. arboreum* stem cuttings.

	SCMS[z]		*RTJ*[y]	
IBA Rate (ppm)	Softwood	Hardwood	Softwood	Hardwood
		Shoots (no.)		
0	<1.0[x]	0.0	0.0	2.0
1000	<1.0	<1.0	0.0	0.0
2500	<1.0	1.5	0.0	1.0
5000	<1.0	0.0	0.0	0.0
7500	<1.0	0.0	0.0	0.0
Significance[w]	NS	NS	NS	NS
		Shoot length (cm)		
0	1.2	0.0	0.0	1.0
1000	0.2	1.0	0.0	0.0
2500	0.0	3.0	0.0	1.1
5000	0.0	0.0	0.0	0.0
7500	0.0	0.0	0.0	0.0
Significance	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 9. Influence of IBA rate on percent with callus and callus caliper of terminal *V. arboreum* stem cuttings.

	SCMS[z]		*RTJ*[y]	
IBA Rate (ppm)	Softwood	Hardwood	Softwood	Hardwood
		Callus (%)		
0	33.3[x]	0.0	60.0	16.6
1000	40.0	0.0	73.3	20.0
2500	30.0	0.0	76.7	10.0
5000	26.7	0.0	76.7	26.7
7500	40.0	0.0	80.0	3.3
Significance[w]	NS	NS	NS	NS
		Callus Caliper (mm)		
0	4.0	0.0	0.0	4.1
1000	5.5	0.0	0.0	8.4
2500	5.2	0.0	0.0	7.4
5000	5.2	0.0	0.0	6.4
7500	6.7	0.0	0.0	2.2
Significance	NS	NS	NS	NS

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]n=30.

[w]nonsignificant (NS).

Table 10. Effect of cutting source and cutting type on percent rooting, number of roots and root length of terminal *V. arboreum* stem cuttings.

Source	Type	Rooting (%)	Roots (no.)	Root Length (cm)
RTJ[z]	Softwood	43.3 a[x]	2.2 a	16.9 a
SCMS[y]	Softwood	29.2 b	2.0 a	20.1 a
RTJ	Hardwood	2.0 c	2.0 a	19.3 a
SCMS	Hardwood	2.0 c	2.7 a	6.4 a

[z]Robert Trent Jones Golf Trail, Opelika, AL, cuttings from watersprouts.

[y]Stone County, MS, cuttings from plants that had been cut back Feb, 2010 and resprouted.

[x]Shaffer-Simulated grouping for source*type least squares mean (Alpha=0.05).

Table 11. Effect of cutting source and cutting type on shoot number, shoot length, percent with callus and callus caliper of terminal *V. arboreum* stem cuttings.

Source	Type	Shoots (no.)	Shoot Length (cm)	Callus (%)	Callus Caliper (mm)
RTJ[z]	Softwood	0.0 a[x]	0.0 a	73.4 a	5.8 a
SCMS[y]	Softwood	<1.0 a	0.3 a	33.9 a	5.4 a
RTJ	Hardwood	<1.0 a	0.6 a	15.2 b	6.4 a
SCMS	Hardwood	<1.0 a	0.7 a	0.0 b	*[w]

[z]Robert Trent Jones Golf Trail, Opelika, AL, cuttings from watersprouts.

[y]Stone County, MS, cuttings from plants that had been cut back Feb, 2010 and resprouted.

[x]Shaffer-Simulated grouping for source*type least squares mean (Alpha=0.05).

[w]There were no cuttings with callus in SCMS Hardwood, therefore no caliper data.

Table 12. Correlation between rooting and callus presence on *V. arboreum* stem cuttings.

Sorted by First Factor	Second Factor	Correlation Coefficient[x]
IBA 0 ppm	none	0.57
IBA 1000 ppm	none	0.50
IBA 2500 ppm	none	0.51
IBA 5000 ppm	none	0.55
IBA 7500 ppm	none	0.68
IBA 0 ppm	subterminal	0.52
IBA 0 ppm	terminal	0.70
IBA 1000 ppm	subterminal	0.47
IBA 1000 ppm	terminal	0.59
IBA 2500 ppm	subterminal	0.49
IBA 2500 ppm	terminal	0.55
IBA 5000 ppm	subterminal	0.53
IBA 5000 ppm	terminal	0.60
IBA 7500 ppm	subterminal	0.67
IBA 7500 ppm	terminal	0.72
IBA 0 ppm	SCMS[z]	0.75
IBA 0 ppm	RTJ[y]	0.47
IBA 1000 ppm	SCMS	0.61
IBA 1000 ppm	RTJ	0.42
IBA 2500 ppm	SCMS	0.68
IBA 2500 ppm	RTJ	0.43
IBA 5000 ppm	SCMS	0.71
IBA 5000 ppm	RTJ	0.46
IBA 7500 ppm	SCMS	0.88
IBA 7500 ppm	RTJ	0.57
IBA 0 ppm	Hardwood	0.40
IBA 0 ppm	Softwood	0.58
IBA 1000 ppm	Hardwood	0.24
IBA 1000 ppm	Softwood	0.40
IBA 2500 ppm	Hardwood	0.38
IBA 2500 ppm	Softwood	0.44
IBA 5000 ppm	Hardwood	0.44
IBA 5000 ppm	Softwood	0.49
IBA 7500 ppm	Hardwood	0.35
IBA 7500 ppm	Softwood	0.60

[z]Stone County, MS, cuttings taken from plants cut back in Feb, 2010.

[y]Robert Trent Jones Golf Course in Opelika, Al, cuttings taken from water sprouts of mature plants.

[x]Pearson Correlation Coefficients.