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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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.

Exploring Methods to Enhance Rooting of *Vaccinium arboreum* Stem Cuttings

by

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Abstract

Sparkleberry (*Vaccinium arboreum* Marshall) has great potential in the fruit and ornamental industries, but is very difficult to vegetatively propagate. A series of rooting experiments were run using juvenile cuttings from mature plants. The first study was designed to test the effects of environment and substrate on softwood cuttings. The second study tested the effects of substrate and wounding on hardwood cuttings. These factors were compared in two environments. The two environments were a “mist tent” and a “sweat tent”. The second portion of this experiment used a completely randomized design to test the effects of wounding on softwood cuttings. The third study was designed to test the effects of ascorbic acid on softwood cuttings a 10 s quick dip or 2 hr soak in ascorbic acid with varying concentrations of IBA (0, 100, 1000, 2500, and 5000) on softwood cuttings. Rooting in all experiments ranged from 0 – 23%. There were no significant effects due to environment, substrate, wounding, ascorbic acid, IBA concentrations, or interactions between these factors. No factors were found to affect rooting or callus percentages. Thus far, auxin treatments, wounding, environment, and ascorbic acid applications have proven to be ineffective for enhancing adventitious root formation of *V. arboreum* cuttings. Further research is needed to identify beneficial treatments.

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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
K-IBA	Indole-3-butyric acid-potassium salt

Chapter I

Literature Review

Genus *Vaccinium*

Vaccinium is a large, complex genus of great economic and ecological importance to North America. As a member of the Ericaceae family, *Vaccinium* species prefer acidic soils. Plants in this genus have unique cultivation requirements that include a soil pH of 4.0 to 5.2 (Peterson, 1987), ample moisture (Spiers et al., 1985), and good drainage (Childers, 1983). Other members of the Ericaceae family include *Rhododendron* and *Kalmia*. Members of the genus *Vaccinium* generally have fibrous, shallow root systems. These characteristics make plants more susceptible to drought and wind damage (Lyrene, 1997). The genus *Vaccinium* has many sections, but *Cyanococcus* has the greatest economic importance. All commercial blueberries are located in this section.

There are four main types of commercial blueberries: lowbush, northern highbush, rabbiteye, and southern highbush. Lowbush blueberries (*V. angustifolium* Aiton), or wild blueberries, are dwarf, woody, deciduous shrubs found from New Hampshire through Maine, extending into New Brunswick and Nova Scotia (Trehane, 2004). The northern highbush blueberry (*V. corymbosum* L.) is a taller species of shrubby blueberry, that 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* Reade) are erect, spreading shrubs that are more adapted to southern growing conditions. 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 (*Vaccinium* sp) is a general term for hybrids of two to three *Vaccinium* species (Lyrene, 2004). Wild northern highbush plants were crossed with several low-chill *Vaccinium* species. Southern highbush ripen early and have a lower chilling hour requirement than rabbiteye.

Vaccinium arboreum

Vaccinium arboreum Marshall is a species native to the southeastern US. The species *arboreum* is widespread, ranging from Virginia to Florida, through the Midwest (Indiana, Illinois), as far west as Nebraska, and south to Texas (Duncan and Duncan, 1988). Historically *V. arboreum* hasn't been a cultivated species. *Vaccinium arboreum* has many common names that include sparkleberry, farkleberry, and tree huckleberry. *Vaccinium arboreum* is a shrub or small deciduous to semi-evergreen tree. It has an erect, single trunk growth habit and at maturity resembles a small tree (Ballinger et al., 1982). It is shade tolerant, and occasionally grows to 9 m; the trunk can reach 37.5 cm in diameter at 1 m, but more often forms a shrub 25 to 37.5 cm tall (Trehane, 2004). Sparkleberries are frequently found along fence rows, wooded edges, or in the understory of mature southeastern forests. *Vaccinium arboreum* tolerates a wider range of soil types and has a coarse root system compared to rabbiteye and highbush species. *Vaccinium arboreum* has a large taproot, which increases drought tolerance. *Vaccinium arboreum* often grows on soils with a pH of 6.0 to 6.5 with low organic matter, low iron availability, and nitrogen primarily in the NO_3 form (Lyrene, 1997). It was hypothesized (Poonnachit and Darnell, 2004) and then confirmed (Darnell and Hiss, 2006) that *V. arboreum* is able to assimilate iron and nitrate more efficiently than *V. corymbosum*, resulting in higher iron / nitrogen uptake.

Vaccinium arboreum has many traits that make it a desirable ornamental plant. Its leaves are alternate, elliptic and usually glossy, turning crimson to reddish purple in the fall, often staying on the tree through the winter (Radford et al., 1968). The leaves shed over a long period in the fall and produce a range of fall color at any given time (Trehane, 2004). Its leathery leaves are about 2.5 to 4 cm long, and 1.25 to 2 cm wide, shiny above and glaucous below. The bark on mature small trees can be an attractive gray, which exfoliates showing colorful patches of reddish brown, gray and orange (Dirr, 1983). The berries are 5 – 8 mm long and ripen in the fall. The berries are black and shiny, contrasting with the autumn foliage. They are mealy and insipid with several small hard seeds. The berries are of little appeal to humans, however, wildlife will readily consume the berries. The fruit often remain on the tree well into the winter (Duncan and Duncan, 1988).

Propagation of Cultivated Blueberry

Blueberries can be vegetatively propagated by a variety of methods such as softwood cuttings, hardwood cuttings, suckers, and tissue culture (Krewer and Cline, 2003). Successful vegetative propagation of cuttings is dependent on many factors such as rooting propensity of the species or cultivar, type of cutting (hardwood, softwood, or semi-hardwood), juvenility of the source plant, time of the year the cuttings are taken, vigor of the stock plant, position on the plant from which the cuttings were taken, and subsequent treatment of the cuttings (Dirr, 1986). These treatments would include type of substrate, amount of light, intermittent mist and other factors such as bottom heat, wounding, and application of different auxin formulations.

Both rabbiteye and southern highbush can be propagated from hardwood cuttings. Results are generally more erratic when using hardwood cuttings (Krewer and Cline, 2003).

Rooting percentages of hardwood cuttings can range from 0 to 100% when combined with wounding and auxin treatments (Kossuth et al., 1981). Hardwood cuttings are used due to the following reasons: 1) the propagation could be done during the dormant season, which is a less busy time of year in blueberry production; 2) ease of handling due to the decreased wilting problem that would normally require regular misting; and 3) an extended growth period, since the plants are rooted by early summer, unlike softwood cuttings that are just being stuck in early summer. This results in a plant more likely to survive its first winter, and a larger, more marketable 2-year old plant (Shelton and Moore, 1981). However, in many plant species, especially deciduous hardwoods, softwood cuttings are the only type that will root (Hartmann et al., 2002).

Softwood cutting propagation is the other common method of propagating rabbiteye and southern highbush blueberries. Rooting percentages can be as high as 80 - 100% (Abolins and Gurtaja, 2006). In North Carolina, the use of softwood cuttings has replaced much of the traditional hardwood propagation due to decreased rates of stem canker and quicker rooting times (6 – 8 weeks vs 6 months for hardwood) (Krewer and Cline, 2003). The genotype of a cutting can also make a difference in rooting percentages of softwood and hardwood cuttings. Kossuth et al. (1981) found rooting percentages of softwood cuttings varied between 19 – 100% between different highbush cultivars.

Common rooting media for blueberries include combinations of peat, aged pine bark, sand, and perlite. Aged pine bark used alone has shown to be as effective as these various combinations and is less expensive (Pokorney and Austin, 1982). Abolins and Gurtaja (2006) found that a 2:1 peat/perlite substrate produced the best results with assorted highbush cultivars. It is important that the rooting media is porous and well drained. Rooting substrate should also be acidic (pH 4.0 – 5.5), especially if alkaline well water will be used (Krewer and Cline, 2003). Giroux et al. (1999) found that sub-irrigation with a low pH (4.5) water increased rooting in several Rhododendron species.

Tissue culture is a possible alternative to propagating hardwood and softwood cuttings. Success rate of tissue culture can be as high as 95% on lowbush blueberries (Nickerson, 1978). Despite the benefit of high rooting percentages, micropropagation facilities are expensive and require skilled operators. Aside from the high initial costs, one of the most commonly cited disadvantages of micropropagation is the production of genetic variants, or plants that look or behave differently than the parent. Historically, tissue culture resulted in more problems with genetic variance than conventional propagation (Cohen, 1980). Other research shows that plants derived from micropropagation have a bushier growth habit, and more flower buds per plant, often resulting in higher yields (El-Sheikh et al., 1996).

***Vaccinium arboreum* as a Rootstock**

The use of rootstocks to extend fruit culture in less than ideal soils is extremely common among the tree fruit crops such as pear, apple, peach, and plum (Hartmann et al., 2002). However, there has been little record of blueberry grafting, possibly due to the relatively short time the blueberry has been under commercial production. Furthermore, special problems can be encountered when grafting a multi-stemmed shrub. Previous work has shown that both highbush and rabbiteye cultivars can be grafted onto various *Vaccinium* species (Galletta and Fish, 1971). The work of Galleta and Fish (1971) suggests broad interspecific graft compatibilities within the *Vaccinium* genus. Selection of rootstocks for specific purposes appeared feasible in their studies. Several grafting methods have been successfully used on *V. arboreum,* including: whip, cleft, bench (whip method), patch, chip, and T-budding. A good union can be difficult to obtain because of the hardness of the wood and small stem diameter (Ballington et al., 1990). Stockton (1976) found that the time of year was instrumental to the success of the unions. Cleft and whip grafts were most successful early in the growing season. Plants budded during the middle of the growing season were unsuccessful. Galletta and Fish (1971) found that budding later in the growing season (late summer, fall) was more successful.

Vaccinium arboreum has many characteristics that would make it a great choice for a blueberry rootstock. *Vaccinium arboreum* is one of the few Ericaceous species that can tolerate calcareous soils. Calcareous soils have a high pH with increased levels of calcium and magnesium. Calcareous soils limit the availability of iron, and nitrogen is predominately available in the NO_3 form. *Vaccinium* species generally prefer the NH_4^+ form of nitrogen. *Vaccinium arboreum* also grows well on soils that are low in organic matter and have a pH as high as 6.2 (Brooks and Lyrene, 1998). *Vaccinium arboreum*'s wide range of suitable growing areas makes it versatile as a rootstock. Incorporation of wider soil tolerance could extend the range of blueberries as a cultivated crop (Brooks and Lyrene, 1998). Modern blueberry production in regions with higher pH levels is possible, but takes large amounts of soil amendments. Soil pH is lowered by incorporating sulfur into the soil, and the blueberries are planted into mounds of milled pine bark. The costly amendments greatly increase the initial cost and labor for new plantings. *Vaccinium arboreum* also has added drought resistance. The use of *V. arboreum* as a rootstock could solve many of the problems associated with expanding blueberry acreage in the southeastern U.S.

The root system of *V. arboreum* consists of coarse roots with a large taproot (Lyrene, 1997). Drought resistance could potentially expand blueberry acreage drastically. *Vaccinium* *arboreum*'s single trunk growth habit would give grafted plants an upright form, and a large trunk (up to 6 inches in diameter) up to the graft union. Above the graft union the scion reverts to its inherent multi-stemmed character (Reese, 1992). The end result would resemble that of an umbrella; this upright form would greatly facilitate several cultural practices like mowing, harvesting, and application of herbicides. Herbicides could be more easily applied to the soil beneath the plant canopy without damage. An offset mower could mow under the plant more easily. Mechanical harvesters could also be used with limited breakage and less waste associated with mechanical harvesting of multi-stemmed plants.

Mechanical harvest of blueberries continues to gain momentum in the industry, as the cost and availability of labor continues to be problematic for growers across the nation. Mechanical harvest using automotive harvesters has been shown to reduce labor from 520 to 10 h of labor per acre on highbush cultivars (Brown et al., 1983; Gough, 1994; Mainland, 1993). The benefits of mechanical harvest do not come without drawbacks. Machine harvested fruit typically have 22 to 30% less pre-storage firmness than hand-harvested fruit (Austin and Williamson, 1977). Fruit loss during harvest can also be as high as 30%, with significantly higher percentages of immature fruit picked as well. Cultivar selection has been shown to be very important to the quality of mechanically harvested fruit. Plant breeders are beginning to shift some focus to developing cultivars that are better suited for mechanical harvest (NeSmith et al., 2000).

Vaccinium arboreum has many traits that would be desirable to blueberry breeders. Soil suitability is probably the most limiting factor in the expansion of blueberry acreage. The broad soil adaptation of *V. arboreum* shows that it could be a good source of genetic material for incorporating into blueberry breeding programs (Lyrene, 1997; Darnell and Hiss, 2006).

Vaccinium arboreum has the ability to thrive on soils with less than ideal characteristics, as *V. arboreum* tolerates low organic matter, higher soil pH, high levels of N in the nitrate form, drought, and low iron. *Vaccinium arboreum*'s flowers have shorter corolla tubes and wider corolla openings than blueberry flowers. Flowers of some *V. arboreum* clones are more fragrant than flowers of rabbiteye and southern highbush. If *V. arboreum* flower characteristics were incorporated into blueberry cultivars, they might result in better insect pollination and improved fruit set. *Vaccinium arboreum*'s late flowering habit also makes it less subject to crop loss due to late freezes than cultivated blueberries (Lyrene, 1991).

Propagation of *Vaccinium arboreum*

The current method of propagation for *V. arboreum* is by seed. *Vaccinium arboreum* is more difficult to propagate by seed than most commercial blueberry species (Lyrene and Brooks, 1996). Stockton (1976) had a germination rate of 2%. Traditional blueberry seedling propagation involves placing seeds uncovered in sphagnum peat in an unheated greenhouse under mist. *Vaccinium arboreum* seedlings germinated poorly using this method (4-5%) (Lyrene and Brooks, 1998). Lyrene (1998) did find that cold stratification increased germination to 20-50%. Despite moderate success germinating *V. arboreum*, an asexual means of propagation is necessary to perpetuate desirable rootstock characteristics.

Despite *V. arboreum*'s advantageous qualities as a rootstock, it has proven difficult to propagate from stem cuttings. A variety of plant growth regulators have been used with little to no success. Stockton (1976) tested four levels of potassium salt of indole-3 butyric acid (K-IBA) on *V. arboreum* softwood cuttings with concentrations of 0, 10,000, 15,000, and 20,000 mg/L. Stockton tested the four concentrations on cuttings from four collection dates ranging from April

18 to May 15. The cuttings were taken from the same trees on four separate dates. The trees were located in three different counties in Texas. All cutting material was taken from water sprouts on one year old wood. Three leaves were left on each cutting and they were treated with a systemic fungicide. Cuttings were placed into a greenhouse with mist applied for 8 seconds every 10 minutes. Rooting percentages for all treatments were 0 to 12%. No difference in rooting percentage was observed using any of the treatments. Rhizome cuttings rooted with some success (69 %), but this is a questionable method for commercial propagation as it involves destructive harvest of the stock plant and is time consuming (Stockton, 1976).

Reese (1992) tested a variety of concentrations of IBA and NAA on semi-hardwood cuttings. Reese also tested willow water, Hormodin III, ethanol, and water. Cuttings from current season's growth were taken off several plants, and cuttings were placed under mist in a 1:1 pine bark/perlite media. Plants were given three months to root. Rooting ranged from 0 – 12.5%. No statistical differences on rooting percentages were found. Wounding was found to increase callus formation, however, it is unclear if callus formation is advantageous to rooting of *V. arboreum* cuttings.

Bowerman et al. (2013) tested softwood, hardwood and semi-hardwood cuttings with varying concentrations of IBA. Cuttings from two different locations were collected. Watersprouts from mature plants were collected from Robert Trent Jones Golf Trail at Grand National in Opelika (RTJ), Alabama, and Stone County Mississippi. Terminal and sub-terminal cuttings were tested. Concentrations ranging from 0 to 7500 mg/L IBA were applied. Cuttings were placed into a greenhouse with mist applied for 2 seconds every 10 minutes. IBA concentration did not have a significant effect on rooting percentages. The source and type of cutting did influence rooting percentage. Rooting for softwood experiments ranged from 26 –

43%. The rooting for semi-hardwood was 16 – 43%. Rooting for hardwood cuttings was 0 – 13%. The highest rooting percentage (43%) occurred with the use softwood terminal cuttings from RTJ Golf Course (Bowerman et al., 2013).

Deciduous Azalea and Other Difficult to Root Ericaceous Species

Vaccinium arboreum is one of many species in the Ericaceae family that is difficult to vegetatively propagate. Deciduous azalea (*Rhododendron sp*. L.) is another example of a species that is difficult to root. Deciduous azaleas are typically propagated by vegetative cuttings under mist. There are two problems with the propagation of deciduous azaleas by shoot cuttings: (1) rooting the cuttings, and (2) inducing new growth after rooting (Grzeskowiak and Grzeskowiak, 2003). Auxins have proven to positively affect rooting of deciduous azalea stem cuttings. Grzeskowiak and Grzeskowiak (2003) observed an increase in rooting percentages and root ball diameters among deciduous azaleas with the use of auxins (IBA); however optimal concentrations were variable. IBA was found to increase rooting percentages to 100%; it also enhanced root system quality, and shortened required rooting time (Dirr and Heuser, 1987). Dirr and Heuser (1987) suggested an auxin concentration of 4000 mg/L IBA, while Sommerville (1998) recommended a higher concentration of 8000 mg/L IBA. Knight et al. (2005) found that treating softwood cuttings with 10,000 mg/L K-IBA produced the highest number of rooted cuttings with the largest root systems. Auxins are one of many factors that can affect rooting percentages of deciduous azaleas. The time of year the cuttings are taken can be crucial to success. According to Grzeskowiak and Grzeskowiak (2003), cuttings taken after flowering have a higher degree of lignification. Anatomical examinations confirmed the negative relationship between the degree of lignification of the cuttings and their rooting ability. Optimum months for cutting propagation are from mid-May to mid-June (Knight et al., 2005).

Kalmia latifolia L. is another species in the Ericaceae family that is difficult to root. Traditional propagation is by seed. Seeds are generally sown in October or November. Seeds are typically cold stratified at 4.4 °C in sand for 3 months (Kujawski and Davis, 2001). Vegetative propagation can be done with softwood or hardwood cuttings. Bottom heat is common practice with *Kalmia* propagation. Cuttings are normally held at 22.8 to 23.9 °C. Softwood cuttings are normally taken as tip cuttings. Cuttings are traditionally taken in autumn, and treated with 5000 mg/L IBA and placed into a mist system. Hardwood cuttings are taken in January. *Kalmia* cuttings generally take 3 to 5 months to root. Certain cultivars of *Kalmia* can reach 100% rooting success (Radder, 1973).

Juvenility of stock plant material can be a crucial factor in propagation. Cuttings from juvenile plants are typically easier to root from stem cuttings than cuttings from older, mature plants in the adult growth phase (Preece, 2003). Maintaining juvenile stock plants for propagation provides a supply of cutting material, and can maximize rootability while maintaining healthy, uniform stock blocks (Hartmann et al., 2002; Howard, 1994). Several plant management tactics exist that increase rooting potential among difficult-to-root taxa, including severe winter pruning or hedging (Cameron et al., 2001; Howard, 1994). Hedging of young plants or serial hedging of older plants can maintain juvenile vegetative characteristics or reinvigorate stock. After hedging, adventitious bud break occurs at the base of plants, thereby producing an abundance of vigorously growing, non-flowering shoots, similarly to the juvenile growth phase (Macdonald, 1986). Therefore, hedging allows for preservation of the juvenile growth phase, which is correlated to an enhanced effect on rooting (Hartmann et al., 2002; Howard, 1994; Macdonald, 1986).

Other Factors Affecting Root Formation

Before the use of root-promoting growth regulators in rooting stem cuttings, many chemicals were used with limited success. The discovery that auxins stimulated the production of adventitious roots in cuttings was a milestone in propagation history (Hartman et al., 2002) Auxins are involved in a variety of activities in higher plants, such as the influence of stem growth, adventitious root formation, lateral bud inhibition, abscission of leaves and fruits, and activation of cambial cells. There are many types of auxin used for propagation today. Indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) are the most common types of auxin used. IBA and NAA can be very helpful in some difficult to root species.

Indole-3-acetic acid (IAA) is a naturally occurring auxin. IBA and NAA are synthetic forms of auxin. Both IBA and NAA are said to be more effective than IAA for rooting; however, some species do not respond well to either IBA or NAA. If a cutting does not respond to IBA then there are other options available. There is another form of auxin called indole-3-butyric acid-potassium salt (K-IBA). The potassium salt of IBA (K-IBA) is used on many difficult to root species such as *Rhododendron austrinum* Rehder and *Magnolia grandiflora* L. K-IBA can be dissolved in water without the use of alcohol or other organic solvents (Banko, 1983).

Wounding is another method to enhance rooting success in vegetative cuttings. Cuttings are often wounded when being prepared by the removal of leaves and branches. Additional wounding at the base of cuttings is common practice for genera like rhododendrons, junipers, and magnolias. Following wounding, callus production and root development are frequently greater along the margins of the wound. Wounded tissues stimulate cell division and production of root primordia. This is due to natural accumulation of auxins and carbohydrates in the wounded area and increased respiration rate due to the creation of a new "sink area" (Hartman et al., 2002). de Andrés et al. (2004) found wounding to greatly increase rooting of *Colutea istria* L. dormant hardwood cuttings. Wounding also increased rooting percentages exponentially in dormant hardwood cuttings of the Greek strawberry tree L. (*Arbutus andrachne*) (Al-Salem et al., 2001).

Root formation in cuttings is affected by a variety of factors. Ascorbic acid is an extremely well known compound; however, the role of ascorbic acid in the plant's antioxidant system is still under investigation. Effects of antioxidants include deactivation of free radicals and other reactive oxygen species (ROS) formed in cell metabolism (Araujo et al., 2008; Arrigoni et al., 2002). Oxidation of the base of cuttings can affect the entire rhizogenic process. The use of antioxidant substances can help minimize the effects of oxidation (Wendling, 2002). Ascorbic acid has been found to reduce explant oxidation in tissue culture, and is involved during cell division and elongation of cells (Gonzales-Reyes et al., 1994; Kato and Esaka, 1999). While the use of ascorbic acid to enhance rooting has yielded mixed results, it has proven beneficial to the rooting of some species of plants. Siksnianas et al. (2006) increased rooting of gooseberries (*Ribes uva-crispa* L.) by incorporating ascorbic acid into IBA solutions. Struve and Lagrimini (1999) found a pre-treatment of ascorbic acid helped increase the propagation window of *Stewartia pseudocamellia* Maxim. semi-hardwood cuttings.

Vaccinium arboreum has many beneficial traits as a rootstock and landscape specimen. The potential for expansion of the blueberry industry is the driving force behind future research of *V. arboreum*. Successful vegetative propagation is essential to the development of *V. arboreum* cultivars with desired traits. Potential treatments that could enhance rooting of *V. arboreum* include determining favorable propagation environment factors such as substrate, air temperature, and substrate temperature. The adoption of successful strategies from previous research and other difficult to root species could potentially play a major role in successful propagation.

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Chapter II

Effects of Environment on the Propagation of *Vaccinium arboreum* Softwood Cuttings

Introduction

Vaccinium arboreum Marshall, or sparkleberry, is a species native to the southeastern US. The distribution of *V. arboreum* is widespread, ranging from Virginia to Florida, through the Midwest (Indiana, Illinois), as far west as Nebraska, and south to Texas (Duncan and Duncan, 1988). *Vaccinium arboreum* has many characteristics that would make it a great choice for a blueberry rootstock. Stockton (1976) found *V. arboreum* to be compatible with many rabbiteye cultivars. *Vaccinium arboreum* is one of the few Ericaceous species that can tolerate calcareous soils. Calcareous soils have a high pH with increased levels of calcium and magnesium. Calcareous soils limit the availability of iron, and nitrogen is predominately available in the NO_3 form. *Vaccinium* species generally prefer the NH_4^+ form of nitrogen. *Vaccinium arboreum* also grows well on soils that are low in organic matter and have a pH as high as 6.5 (Lyrene, 1997). *Vaccinium arboreum*'s wide range of suitable growing areas make it versatile as a rootstock. Incorporation of wider soil tolerance could extend the range of blueberries as a cultivated crop (Brooks and Lyrene, 1998). Modern blueberry production in regions with higher pH levels is possible, but requires large amounts of soil amendments. Soil pH is often lowered by incorporating sulfur and milled pine bark into the soil prior to planting. Costly amendments greatly increase the initial cost and labor for new plantings. *Vaccinium arboreum* also has added drought resistance. The use of *V. arboreum* as a rootstock could solve many of the problems associated with expanding blueberry acreage in the southeastern US.

Vaccinium arboreum has many traits that make it a desirable ornamental plant. Its leaves are alternate, elliptic and usually glossy, turning crimson to reddish purple in the fall, often staying on the tree through the winter (Radford et al., 1968). The leaves shed over a long period in the fall and produce a range of fall color at any given time (Trehane, 2004). The bark on mature individuals can be an attractive gray, which exfoliates showing colorful patches of reddish brown, gray and orange (Dirr, 1983). The berries are 5 – 8 mm long and ripen in the fall. The berries are black and shiny, contrasting with the autumn foliage. They are mealy and insipid with several small hard seeds. The berries are of little appeal to humans; however, wildlife will readily consume the berries. The fruit often remain on the tree well into the winter (Duncan and Duncan, 1988).

Despite *V. arboreum*'s advantageous qualities as a rootstock or landscape plant, it has proven difficult to propagate from stem cuttings. Stockton (1976) tested four levels of potassium salt of indole-3 butyric acid (K-IBA) on *V. arboreum* softwood cuttings with concentrations up to 20,000 mg/L. Rooting for all treatments was 0 to 12%. No difference in rooting percentage was observed using any of the concentrations of IBA. Reese (1992) tested a variety of auxin concentrations of IBA and NAA on semi-hardwood cuttings and found no statistical differences on rooting percentages. Cuttings from current season's growth were taken off several plants, and cuttings were placed under mist in a 1:1 pine bark/perlite media. Wounding was found to increase callus formation, however, it is unclear if callus formation is advantageous to rooting of *V. arboreum* cuttings. Bowerman et al. (2013) tested juvenile softwood, hardwood and semi-hardwood cuttings with varying concentrations of K-IBA. Concentrations ranging from 0 to 7500 mg/L K-IBA were applied. K-IBA concentration was not found to have a significant effect on rooting percentages. The source and type of cutting did influence rooting percentage. The highest rooting percentage (43%) occurred with the use softwood cuttings.

Both rabbiteye and southern highbush can be propagated from hardwood cuttings. Results are generally more erratic when using hardwood cuttings (Krewer and Cline, 2003). Rooting percentages of hardwood cuttings can range from 0 to 100% when combined with wounding and auxin treatments (Kossuth et al., 1981). Hardwood cuttings are used due to the following reasons: 1) the propagation could be done during the dormant season, which is a less busy time of year in blueberry production; 2) ease of handling due to the decreased wilting problem that would normally require regular misting; and 3) an extended growth period, since the plants are rooted by early summer, unlike softwood cuttings that are just being stuck in early summer. This results in a plant more likely to survive its first winter, and a larger, more marketable 2-year old plant (Shelton and Moore, 1981). However, in many plant species, especially deciduous hardwoods, softwood cuttings are the only type that will root (Hartmann et al., 2002).

Softwood cutting propagation is the other common method of propagating rabbiteye and southern highbush blueberries. Rooting percentages can be as high as 80 - 100% (Abolins and Gurtaja, 2006). In North Carolina, the use of softwood cuttings has replaced much of the traditional hardwood propagation due to decreased rates of stem canker and quicker rooting times (6 – 8 weeks vs 6 months for hardwood) (Krewer and Cline, 2003). The genotype of a cutting can also make a difference in rooting percentages of softwood and hardwood cuttings. Kossuth et al. (1981) found rooting percentages of softwood cuttings varied between 19 – 100% between different highbush cultivars.

Environment is particularly important for successful vegetative propagation. Humidity has proven to greatly impact rooting of vegetative cuttings in most species. Temperature also has many effects on vegetative cuttings. Bowerman et al. (2013) found the greatest success on *V. arboreum* softwood cuttings in high temperature environments. Common rooting substrate for blueberries include combinations of peat, aged pine bark, sand, and perlite. It is important that the rooting substrate is porous and well drained. Substrate pH should also be acidic, 4.0 – 5.5, especially if alkaline well water will be used (Krewer and Cline, 2003). The objective of this research was to determine the effects of two propagation environments on rooting success of juvenile *V. arboreum* stem cuttings placed in three different substrates.

Materials and Methods

The study was conducted at the Paterson Greenhouse Complex, Auburn University, Auburn, Alabama. Two environments were evaluated in this study: a "mist tent" and a "sweat tent". 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.

The two environments were created using 1.27 cm PVC frames covered with white polyethylene film. The "mist tents" sat on top of expanded metal frames that were left uncovered at the base for drainage, while the "sweat tents" were completely enclosed by white polyethylene film. Empty propagation flats were flipped upside down and placed under propagation flats with cuttings to raise the cuttings slightly off of the polyethylene film floor of the "sweat tent". The flats were raised to prevent water logging and "sweat tents" were periodically drained when necessary. Intermittent mist was applied in the "mist tent" for 4 s every 10 min from 6 am to 8 pm. The "sweat tent" was misted for 60 s at 8 am and again at 1 pm. The "sweat tent" created a high temperature and high humidity environment.

Sub-terminal softwood cuttings were collected from Stone County, Mississippi (Lat. 30° 80'N, Long. 89° 17' W, USDA hardiness zone 8b). Cuttings were harvested on Sept 8, 2012 and placed into a cooler in a mixture of ice and water. All cuttings were trimmed to 10 – 15 cm long and the basal portion of the cutting was cut to a 45° angle. The experiment was initiated on Sept. 9, 2012. Cuttings taken were juvenile cuttings arising from latent buds on mature plants that had been cut back to approximately 1 m in height in Feb 2012. This study was designed as a 3 x 2 complete factorial to test the effects of three substrates (100% perlite, 2:1 perlite/ peat, 1:1 perlite/ peat) in two different environments ("mist tent" and "sweat tent"). The experimental design was a split plot design with environment as a main plot factor and substrate as a sub-plot factor. There were four replications for each environment, and 8 replications for each substrate. Each substrate contained 2 sub-samples, with 6 cuttings per sub-sample. The mean day temperature in the "mist tent" was 18 °C (65 °F) ± 2.7 °C (5 °F). The mean night temperature was 16° C (62 °F) ± 2.7 °C (5 °F). The mean RH was 97%. The mean day temperature in the "sweat tent" was 22 °C (73 °F) ± 5 °C (9 °F). The mean night temperature was 18 °C (65 °F) ± 2.7 °C (5 °F). The mean RH was 99% during daylight hours. Maximum photosynthetically active radiation measured in the greenhouse on the cutting bench with a quantum meter (Model QMSS, Apogee Instruments, Inc., Logan, UT) was 750 µmol m^{-2} s^{-1}. The experiment was terminated on 20 December 2012. Air temperature and relative humidity were recorded every 60 min for the duration of the experiment using dataloggers (Watchdog A150 Temp/RH Logger; Spectrum Technologies, Inc., Aurora, IL). Additional data collected include number of cuttings that formed a callus, callus caliper (mm), number primary roots, and root length (cm).

Data was analyzed using generalized linear models with the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Rooting and callusing was analyzed using the binomial distribution and a logit link function, count data was analyzed using the negative binomial distribution and a log link function, and measurement data was analyzed using the normal distribution and the identity function.

Results and Discussion

Rooting percentages ranged from 2 – 8.3% (Table 2.1). Possibly due to the low number of cuttings that rooted, there were no significant effects of substrate, environment, or substrate × environment on rooting, number of roots, or total root length. Though callus percentage ranged from 29.1 – 54.1 among treatments, there were no effects of treatments on callus or callus caliper. Though Bowerman et al. (2013) observed the greatest rooting percentages with softwood cuttings (~40%), the cuttings were collected in May in that study while the cuttings for this experiment were collected in September; thus, air temperature and day length were much different than those experienced in this experiment. The time of year cuttings are collected, and/or the propagation environment of those cuttings may be important factors that contributed to the differences observed in rooting percentages in the present study compared to the study conducted on softwood cuttings by Bowerman et al. (2013).

Previous research has shown very little success with the vegetative propagation of *V. arboreum* cuttings to date. Most of the previous research conducted resulted in many treatments that do not affect rooting percentages. Reese (1992) found similar results with cuttings taken in August. Stockton (1976) also found similar results using softwood cuttings taken in April and May. The most significant finding reported by Bowerman et al. (2013) that softwood cuttings collected in May resulted in 43.3%. Genotype may be another possible factor effecting rooting.

Kossuth et al. (1981) found rooting percentages of softwood cuttings varied between 19 – 100% between different highbush cultivars.

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Table 2.1. Effect of environment and substrate on rooting of juvenile sparkleberry (*Vaccinium arboreum*) softwood cuttings.[z]

Substrate	Environment	Callus (%)	Callus caliper (mm)	Rooting (%)	Roots (no.)	Root length (cm)
1:1 Perlite/Peat	Mist	37.5	5.6	4.1	1.5	3.9
2:1 Perlite/Peat	Mist	33.3	4.3	2.0	1.0	3.5
100% Perlite	Mist	29.1	3.8	4.1	1.0	0.4
1:1 Perlite/Peat	Sweat	54.1	8.2	6.3	2.7	7.8
2:1 Perlite/Peat	Sweat	47.9	9.0	8.3	1.5	3.2
100% Perlite	Sweat	43.7	5.4	6.3	3.3	3.7
Significance[y]		NS	NS	NS	NS	NS

[z]Softwood cuttings taken 20 September 2012 from Stone County MS. Cuttings taken from water sprouts of mature plants. Experiment was terminated 20 December 2012.
[y]nonsignificant (NS)

Chapter III

Effects of Wounding on *Vaccinium arboreum* Hardwood and Softwood Cuttings

Introduction

Blueberries can be vegetatively propagated by a variety of methods such as softwood cuttings, hardwood cuttings, suckers, and tissue culture (Krewer and Cline, 2003). Successful vegetative propagation of cuttings is dependent on many factors such as rooting propensity of the species or cultivar, type of cutting (hardwood, softwood, or semi-hardwood), juvenility of the source plant, time of the year the cuttings are taken, vigor of the stock plant, position on the plant from which the cuttings were taken, and the handling and treatment of the cuttings (Dirr, 1986). Handling includes type of substrate, amount of light, intermittent mist and other treatments such as bottom heat, wounding, and application of different auxin formulations.

Juvenility of stock plant material can be a crucial factor in propagation. Cuttings from juvenile plants are typically easier to root from stem cuttings than cuttings from older, mature plants in most genera of plants (Preece, 2003). Maintaining juvenile stock plants for propagation provides a supply of cutting material, and can maximize rootability while maintaining healthy, uniform stock blocks (Hartmann et al., 2002; Howard, 1994). Several plant management tactics exist that increase rooting potential among difficult-to-root taxa, including severe winter pruning or hedging (Cameron et al., 2001; Howard, 1994). Hedging of young plants or serial hedging of older plants can maintain juvenile vegetative characteristics or reinvigorate stock. After hedging, adventitious bud break occurs at the base of plants, thereby producing an abundance of vigorously growing, nonflowering shoots, similar to the juvenile growth phase (Macdonald, 1986). Therefore, hedging allows for preservation of the juvenile growth phase, which is correlated to an enhanced effect on rooting (Hartmann et al., 2002; Howard, 1994; Macdonald, 1986).

Wounding is another method to enhance rooting success in vegetative cuttings. Cuttings are often wounded when being prepared by the removal of leaves and branches. Additional wounding at the base of cuttings is common practice for plants like junipers, magnolias, and some rhododendron species. Following wounding, callus and root development is frequently heavier along the margins of the wound. Wounded tissues stimulate cell division and production of root primordia. This is due to natural accumulation of auxins and carbohydrates in the wounded area and increases in respiration rates in the creation of a new "sink area" (Hartman et al., 2002). de Andrés et al. (2004) found wounding to greatly increase rooting of *Colutea istria* L. dormant hardwood cuttings. Wounding also increased rooting percentages exponentially in Greek strawberry tree (*Arbutus andrachne* L.*)* dormant hardwood cuttings (Al-Salem et al., 2001).

Vaccinium arboreum Marshall is a difficult species to propagate from stem cuttings. A variety of plant growth regulators have been used with little to no success. Stockton (1976) tested four levels of potassium salt of indole-3 butyric acid (K-IBA) on *V. arboreum* softwood cuttings with concentrations of 0, 10,000, 15,000, and 20,000 mg/L. Stockton tested the four concentrations on cuttings from four collection dates ranging from April 18 to May 15. All cutting material was taken from water sprouts on one year old wood. Rooting percentages for all treatments were 0 to 12% and there were no differences between treatments. Reese (1992) tested a variety of auxin concentrations of IBA and NAA on semi-hardwood cuttings. Reese also tested wounding, willow water, Hormodin III, ethanol, and water. Little rooting was observed in all treatments. Rooting ranged from 0 – 12.5%. No statistical differences on rooting percentages were found. Wounding was found to increase callus formation; however, it is unclear if callus formation is advantageous to rooting of *V. arboreum* cuttings.

Bowerman et al. (2013) tested softwood, hardwood and semi-hardwood cuttings with varying concentrations of K-IBA. Concentrations ranging from 1000 to 7500 mg/L K-IBA were applied. K-IBA concentration was not found to have a significant effect on rooting percentages. The source and type of cutting did influence rooting percentage. Rooting for softwood cuttings ranged from 26 – 43%. The rooting for semi-hardwood cuttings was 16 – 43%. Rooting for hardwood cuttings was 0 – 13%. The highest rooting percentage (43%) occurred with the use softwood terminal cuttings (Bowerman et al., 2013).

The objective of this research is to examine the effects of wounding on rooting of *V. arboreum* stem cuttings. Cutting juvenility is an important factor for successful propagation of difficult to root species. Bowerman et al. (2013) found the greatest success with juvenile softwood cuttings (43%), however, both softwood and hardwood cuttings are used in traditional blueberry propagation.

Materials and Methods

Two studies were conducted at the Paterson Greenhouse Complex, Auburn University, Auburn, Alabama. Rooting response (rooted or unrooted) was recorded for all cuttings, with a cutting considered rooted when any sign of adventitious roots was seen emerging from the stem. All cuttings were trimmed to 10 – 15 cm long. The basal portion of all cuttings was cut at a 45° angle. Air temperature and relative humidity were recorded every 60 min for the duration of the experiment using data loggers (Watchdog A150 Temp/RH Logger; Spectrum Technologies, Inc., Aurora, IL).

Two environments were created using 1.27 cm PVC frames covered with white polyethylene film. The "mist tents" sat on top of expanded metal frames that were left uncovered at the base for drainage. "Sweat tents" were built on the same frame except for the bench was covered with polyethylene film as well. This created a sealed environment with higher humidity and temperatures. Cuttings were wounded by scraping the 2 opposing sides of the base of each cutting with a knife to a length of approximately 3 cm. Additional data collected include stem caliper, number of cuttings that formed a callus, callus caliper (mm), number primary roots, and root length (cm).

Data was analyzed using generalized linear models with the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Rooting and callusing was analyzed using the binomial distribution and a logit link function, count data was analyzed using the negative binomial distribution and a log link function, and measurement data was analyzed using the normal distribution and the identity function.

Experiment 1

The first experiment was initiated on 28 February 2013 using hardwood cuttings arranged in a completely randomized design. Sub-terminal cuttings were collected from RTJ Golf Course in Opelika, Alabama (lat. 32° 69' N, long. 85° 44' W, USDA hardiness zone 8a). Cuttings were taken from water sprouts on mature plants. The experiment was designed in a 2 x 2 factorial to test substrate (Fafard® 3B mix [Sun Gro Horticulture Ltd., Agawam, MA] and a 2:1 perlite/peat mix) and ± wounding in two separate environments ("mist tent" and "sweat tent"). Intermittent mist was applied to the "mist tent" for 8 s every 20 min from 6 am to 8 pm. "Sweat tents" were misted for 60 s at 8am and 1pm. There were 20 replications per treatment, with each cutting considered a replication. Cuttings were inserted to a depth of 3 cm into 48 cell trays. The mean day temperature in the "mist tent" was 24 °C (76 °F) ± 5 °C (9 °F). The mean night temperature was 22 °C (72 °F) ± 2.7 °C (5 °F). The mean RH was 92%. The mean day temperature in the "sweat tent" was 28 °C (83 °F) ± 8.3 °C (15 °F). The mean night temperature was 23 °C (74 °F) ± 4.4 °C (8 °F). The mean RH was 99%. Study 1 was terminated on May 31, 2013.

Experiment 2

Softwood cuttings were collected from plants in Stone County, Mississippi (Lat. 30° 80'N, Long. 89° 17' W, USDA hardiness zone 8b). Cutting material was taken on 27 May 2013 and the experiment was initiated the following day. Experiment 2 was arranged in a completely randomized design to test the effects of wounding vs. non-wounding on softwood cuttings. There were 2 treatments with 48 cuttings per treatment. Each cutting was considered a replication. Both trays were placed into a "mist tent". "Mist tents" were misted for 4 s every 10 min. Mist was applied during daylight hours. Cuttings were inserted to a depth of 3 cm into 48-cell trays (Landmark Plastic Corporation, Akron, OH). A Fafard 3B mix substrate was used (Sun Gro Horticulture Ltd., Agawam, MA). Experiment 2 was harvested on September 24, 2013.

Results and Discussion

Experiment 1 was designed to test wounding and substrate on rooting percentages of juvenile hardwood *V. arboreum* cuttings in two different propagation environments ("mist tent" and "sweat tent"). Very few cuttings rooted and there were no effects of substrate, wounding, or substrate × wounding on rooting characteristics in either propagation environment (Tables 3.1

and 3.2). Rooting percentages ranged from 0 – 5% in the "mist tent" environment (Table 3.1) and from 0 -10% in the "sweat tent" environment (Table 3.2). Though not significant due to the very low number of cuttings that rooted, rooting percentage tended to be slightly higher in the 2:1 perlite/peat mix in both environments, perhaps due to increased water drainage. Previous research has demonstrated that hardwood cuttings of *V. arboreum* are very difficult to root. Bowerman et al. (2013) observed the lowest rooting percentages when using hardwood cuttings compared to softwood and semi-hardwood. Cuttings were not wounded in that study, and rooting percentages ranged from 0.7 – 10.6% for sub-terminal hardwood cuttings compared to 34.6 – 38.6% for softwood cuttings.

Substrates were included in the first experiment to allow for differences in water-holding capacities in case water availability was an issue for root formation. However, substrate or substrate x wounding did not affect rooting percentages, as very few cuttings rooted in any of the experiments. The percentage of cuttings with callus formation ranged from 0 – 19% (Tables 3.1-3.3) in both experiments.

Rooting percentages in the second experiment were 2% for both treatments (Table 3.3). Wounding had no significant effects on rooting percentages or number of roots. Callus percentage was between 8 – 19 %. Callus percentage, callus caliper, and length of roots tended to be numerically greater for the wounded cuttings, though results were not significant because of variability. Reese (1992) had callus percentages from 0 – 90% on wounded semi-hardwood cuttings taken in August. Reese also found that wounding did not increase rooting percentages.

Previous research has shown very little success with the vegetative propagation of *V. arboreum* cuttings to date. Most of the previous research conducted resulted in many treatments that do not affect rooting percentages, with the most significant finding reported by Bowerman et al. (2013) that softwood cuttings collected in May resulted in 43.3%

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Table 3.1. Effect of wounding and substrate type on rooting, callus formation, and new growth of sparkleberry (*Vaccinium arboreum*) hardwood cuttings in "mist tent"[z] environment.[y]

Substrate	Wound	Callus (%)	Rooting (%)	Roots (no.)	Root length (cm)
Fafard® 3B mix	N	0	0	*	*
Fafard® 3B mix	Y	0	5	3	2.0
2:1 Perlite / Peat	N	15	0	*	*
2:1 Perlite / Peat	Y	5	5	2	2.5
Significance[x]	NS	NS	NS	NS	NS

[z]"Mist tents" were covered with white polyethylene plastic and placed into intermittent mist 4 s every 20 min.

[y]Hardwood cuttings were taken 28 February 2013 from Robert Trent Jones Golf Course in Opelika, AL, cuttings taken from water sprouts of mature plants. Cuttings were harvested on 31 May 2013.

[x]nonsignificance (NS)

Table 3.2. Effect of wounding and substrate type on rooting, callus formation, and new growth of sparkleberry (*Vaccinium arboreum*) hardwood cuttings in "sweat tent"[z] environment.[y]

Substrate	Wound	Callus (%)	Rooting (%)	Roots (no.)	Root length (cm)
Fafard® 3B mix	N	0	0	*	*
Fafard® 3B mix	Y	0	0	*	*
2:1 Perlite / Peat	N	5	10	4.5	4.2
2:1 Perlite / Peat	Y	10	5	4.0	5.0
Significance[x]	NS	NS	NS	NS	NS

[z] "Sweat tents" were constructed using white polyethylene plastic on all sides with mist provided for 60 s at 8am and 1 pm.

[y]Hardwood cuttings were taken on 28 February 2013 from Robert Trent Jones Golf Course in Opelika, AL. Cuttings were taken from water sprouts of mature plants. Cuttings were harvested 31 May 2013

[x] nonsignificant (NS)

Table 3.3. Effect of wounding[z] on juvenile sparkleberry (*Vaccinium arboreum)* softwood cuttings[y].

Treatment	Callus %	Callus caliper (mm)	Rooting (%)	Root (no.)	Root length (cm)
Non-wounded	8	1.8	2	1.0	0.2
Wounded	19	3.0	2	1.0	3.0
Significance[x]	NS	NS	NS	NS	NS

[z]Cuttings were wounded by scraping the 2 opposing sides of the base of each cutting with a knife to a length of approximately 3 cm.
[y]Softwood cuttings taken from Stone County MS. Experiment was initiated on 28 May 2013. Mature plants were cut back to a height of 1 m. Juvenile shoots from latent buds were used for propagation material. Experiment was terminated on 24 September 2013.
[x]Nonsignificant (NS)

Chapter IV

Effects of Ascorbic Acid on *Vaccinium arboreum* Softwood Cuttings

Vaccinium arboreum Marshall has many benefits as a potential rootstock. *Vaccinium arboreum* can tolerate a wide range of soil conditions including drought, high pH, low organic matter, NO_3 as the predominant N source, and low amounts of iron (Darnell and Hiss, 2006). *Vaccinium arboreum* also has many desirable ornamental qualities including exfoliating bark, shade tolerance, fall color, and berries for attracting wildlife. Despite *V. arboreum's* advantageous qualities as a rootstock and ornamental, it has proven difficult to propagate from stem cuttings.

Rooting hormones have been ineffective in stimulating adventitious root formation in previous research on *V. arboreum*. Stockton (1976), Reese (1992), and Bowerman (2013) found rooting hormones to have no effect on root or callus formation. Antioxidants are frequently used in tissue culture to prevent the oxidation of metals in the medium (Taiz and Zeiger, 2004). Effects of antioxidants include deactivation of free radicals and other reactive oxygen species (ROS) formed in cell metabolism (Araujo et al., 2008; Arrigoni et al., 2001). Oxidation of the base of cuttings can affect the entire rhizogenic process. The use of antioxidant substances can help minimize the effects of oxidation (Wendling, 2012). Ascorbic acid has been found to reduce explant oxidation in tissue culture, and is involved during cell division and elongation of cells (Gonzales-Reyes et al., 1994; Kato and Esaka, 1999). While the use of ascorbic acid to enhance rooting has yielded mixed results, it has proven beneficial to the rooting of some species of plants. Siksnianas et al. (2006) increased rooting of gooseberries (*Ribes uva-crispa L.)* by incorporating ascorbic acid into IBA solutions. Struve and Lagrimini (1999) found a pre-treatment of ascorbic acid to help increase the propagation window of *Stewartia pseudocamellia* Maxim. semi-hardwood cuttings.

Auxins are involved in a variety of activities in higher plants, such as the influence of stem growth, adventitious root formation, lateral bud inhibition, abscission of leaves and fruits, and activation of cambial cells (Hartmann et al., 2002). There are many types of auxin used for propagation today. Indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) are the most common types of auxin used. Indole-3-acetic acid (IAA) is a naturally occurring auxin. IBA and NAA are synthetic forms of auxin. Both IBA and NAA are said to be more effective than IAA for rooting; however, some species do not respond well to either IBA or NAA. If a cutting does not respond to IBA then there are other options available. The potassium salt of IBA (K-IBA) is used on many difficult to root species such as *Rhododendron austrinum* Rehder and *Magnolia grandiflora* L. K-IBA can be dissolved in water without the use of alcohol or other organic solvents (Banko, 1983).

Bowerman (2013) tested softwood, hardwood and semi-hardwood cuttings with varying concentrations of K-IBA. Juvenile terminal and sub-terminal cuttings were collected from two different locations and subjected to K-IBA concentrations ranging from 0 to 7500 mg/L. Cuttings were placed into a greenhouse with mist applied for 2 seconds every 10 minutes. K-IBA concentration was not found to have a significant effect on rooting percentages. The source and type of cutting did influence rooting percentage. The highest rooting percentage (43%) occurred with the use softwood terminal cuttings (Bowerman, 2013).

The objectives of this research are to determine if ascorbic acid treatments can enhance root formation in *V. arboreum* stem cuttings in combination with K-IBA concentrations. Auxin applications thus far have been ineffective; however, ascorbic acid has been found to prevent the oxidation of root promoting hormones such as auxin (Araujo et al., 2008; Arrigoni et al., 2001).

Materials and Methods

Softwood cuttings were taken on 27 May 2013 from Stone County, Mississippi, US hardiness zone 8b. Plant material was placed in a cooler and kept moist with ice and water overnight. The experiments were initiated the following day. Cuttings taken were 10 to 15 cm long. The cuttings were cut at a 45° angle at the basal end. Auxin solutions were prepared using Hortus IBA Water Soluble Salts® (Hortus USA Corp, Earth City, MO) and deionized water. Data recorded for all three studies included rooting response (rooted or unrooted), number of primary roots emerging from the stem, total length of primary roots, number of cuttings that formed callus, and callus caliper. Cuttings were placed onto a greenhouse bench at the Paterson Greenhouse Complex at Auburn University. Cuttings were inserted to a depth of 3 cm into 48-cell trays (Landmark Plastic Corporation, Akron, OH). The substrate used was a Fafard 3B mix (Sun Gro Horticulture, Seba Beach, AB, Canada). "Mist tents" were created utilizing 1.27 cm PVC frames that were covered with white polyethylene plastic. These frames were placed onto expanded metal frames. Intermittent mist was applied to cuttings for 8 s every 20 min from 6 am to 8 pm. Air temperature and relative humidity were recorded every 60 min for the duration of the experiment using dataloggers (Watchdog A150 Temp/RH Logger; Spectrum Technologies, Inc., Aurora, IL). Experiments were harvested and final data was collected on September 24, 2013.

Data was analyzed using generalized linear models with the GLIMMIX procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Rooting and callus formation was analyzed using the binomial distribution and a logit link function, count data was analyzed using the negative binomial distribution and a log link function, and measurement data was analyzed using the normal distribution and the identity function.

Experiment 1 was a 2 x 5 factorial with 30 replications, with each cutting as a replication, arranged in a completely randomized block design. Cuttings received a 10 s basal quick-dip to a depth of 3 cm in either water (control) or a solution of ascorbic acid (NOW Foods, Vitamin C Crystals, Bloomingdale, IL) dissolved in water to a concentration of 2%. The cuttings were allowed to dry for 5 min, followed by a 10 s basal quick dip in 0 (water), 100, 1000, 2500, or 5000 mg/L IBA. The mean day temperature for the propagation environment was 27 °C (81 °F) ± 2.8 °C (5 °F). The mean night temperature was 24 °C (75 °F) ± 2.5 °C (4 °F). The mean day RH was 91% ± 4.5%. The mean night RH was 99%.

Experiment 2 was a 2 × 5 factorial with 30 replications, with each cutting as a replication, arranged in a completely randomized block design. The basal end of the cuttings were placed in either water (control) or a solution of 2% ascorbic acid (NOW Foods, Vitamin C Crystals, Bloomingdale, IL) to a depth of 3 cm for 2 h. The cuttings were allowed to dry for 5 min, followed by a 10 s basal quick dip in 0 (water), 100, 1000, 2500, or 5000 mg/L IBA. The mean day temperature in the propagation environment was 25 °C (78 °F) ± 1.7 °C (3 °F). The mean night temperature was 22 °C (72 °F) ± 1.6 °C (3 °F). The mean day RH was 91% ± 6.8%. The mean night RH was 99%.

Results and Discussion

In experiment 1, rooting percentages ranged from 3 – 23% (Table 4.1). There were however, no significant effects of ascorbic acid or IBA concentration. Callus percentage ranged from 3 – 20% among treatments. Ascorbic acid and IBA had no effects on callus percentage or callus caliper. Ascorbic acid has been shown to be beneficial to rooting of some species. Siksnianas et al. (2006) increased rooting of gooseberries (*Ribes uva-crispa L.)* by incorporating ascorbic acid into IBA solutions. Ascorbic acid is also beneficial for reducing explant oxidation in tissue culture (Gonzales-Reyes et al., 1994; Kato and Esaka, 1999). However, the effects of ascorbic acid on vegetative propagation are widely unknown.

Reese (1992) had rooting percentages from 0 – 12% and callus percentages from 5 – 55% on softwood cuttings taken in May. Bowerman et al. (2013) observed the greatest rooting percentages using softwood cuttings (~40%). Bowerman et al. (2013) had callus percentages ranging from 30 to 85%. The experiment was initiated in May. The environmental conditions were slightly different from the current experiment. Bowerman et al. (2013) had a mean day temperature of 34 °C (93 °F) ± 6 °C (10 °F). The mean day temperature in both experiments was 27 °C (81 °F) ± 2.8 °C (5 °F). The mean day temperature was 7 °C (12 °F) cooler in the present studies. The lower day temperatures could potentially increase substrate moisture levels. Excess moisture could have been responsible for the lower rooting percentages found in both experiments.

Experiment 2 had rooting percentages ranging from 3 – 10 % (Table 4.2). There were no significant effects of ascorbic acid or IBA on rooting percentages. Callus percentage ranged from 3 – 20% among treatments. There were no significant effects on callus percent or callus caliper. IBA concentration had no significant effect on number of roots, new leaves, new shoots, or shoot length. The method of application for ascorbic acid had no significant effects on rooting; there were also no significant effects of the interaction between the abscorbic acid and the concentration of IBA.

All previous research to date has yielded poor results. Bowerman et al. (2013) reported the highest yields (43%) using softwood cuttings. Softwood cuttings have yielded the highest rooting thus far. All plant growth regulator treatments have proven to be ineffective. These results are consistent with the work of Stockton (1976), Reese (1992), and Bowerman et al. (2013). Environment, wounding, and ascorbic acid applications have not shown to enhance rooting in softwood or hardwood cuttings. Bottom heat is one promising area for *V. arboreum* propagation. Bottom heat has been beneficial for rooting some members of the Ericaceae family. Another possible alternative to stem cutting propagation would be tissue culture. These possibilities of enhancing rooting of *V. arboreum* may warrant further research.

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Table 4.1. Effect of ascorbic quick dip and IBA applications on juvenile sparkleberry (V*accinium arboreum)* softwood cuttings[z].

Quick dip[y]	IBA[x] (mg L^{-1})	Callus (%)	Callus caliper (mm)	Rooting (%)	Root (no.)	Root length (cm)
Water	0	20	1.4	7	1.5	0.4
Water	100	10	4.0	10	3.0	5.0
Water	1000	3	0.0	7	1.0	2.9
Water	2500	13	1.7	17	4.2	3.2
Water	5000	17	1.5	23	3.0	2.3
Ascorbic acid	0	3	2.5	7	3.5	2.5
Ascorbic acid	100	20	4.3	13	1.3	3.3
Ascorbic acid	1000	13	2.5	6	2.0	2.4
Ascorbic acid	2500	13	2.6	3	6.0	6.5
Ascorbic acid	5000	17	3.1	10	3.7	3.0
Significance[w]		NS	NS	NS	NS	NS

[z]Softwood cuttings taken from Stone County MS. Experiment was initiated on 28 May 2013. Mature plants were cut back to a height of 1 m. Juvenile shoots from latent buds were used for propagation material. Experiment was terminated on 24 September 2013.
[y]Basal portion of cuttings were submerged for 2 hr, then allowed to dry for 5 min.
[x]Cuttings were dipped into IBA solutions for 10 s. IBA solutions were prepared using Hortus IBA Water Soluble Salts® (Hortus USA Corp, Earth City, Missouri) and deionized water.
[w]nonsignificant (NS)

Table 4.2. Effect of ascorbic acid soak and IBA applications on juvenile sparkleberry (V*accinium arboreum)* softwood cuttings[z].

Soak[y]	IBA[x] (mg L^{-1})	Callus (%)	Callus caliper (mm)	Rooting (%)	Root (no.)	Root length (cm)
Water	0	20	3.0	10	2.3	5.1
Water	100	10	2.7	10	2.7	3.1
Water	1000	7	2.5	10	2.3	0.9
Water	2500	10	2.2	7	2.0	5.1
Water	5000	13	2.9	3	1.0	3.0
Ascorbic acid	0	13	1.7	3	2.0	8.5
Ascorbic acid	100	7	1.9	7	2.5	6.4
Ascorbic acid	1000	20	2.0	7	1.0	2.8
Ascorbic acid	2500	13	2.6	3	2.0	4.0
Ascorbic acid	5000	3	1.6	3	1.0	2.5
Significance[w]		NS	NS	NS	NS	NS

[z]Softwood cuttings taken from Stone County MS. Experiment was initiated on 28 May 2013. Cuttings taken from water sprouts of mature plants. Experiment was terminated on 24 September 2013.
[y]Cuttings were submerged for 10 s, then allowed to dry for 5 min.
[x]Cuttings were dipped into IBA solutions for 10 s. IBA solutions were prepared using Hortus IBA Water Soluble Salts® (Hortus USA Corp) and deionized water.
[w]nonsignificant (NS)