1-1-2016

Effect of Rooting Media, Cultivars and Genotype on the Propagation of Blueberry

Tripti Saha

Effect of rooting media, cultivars and genotype on the propagation of blueberry

By

Tripti Saha

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Horticulture
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

August 2016

Effect of rooting media, cultivars and genotype on the propagation of blueberry

By

Tripti Saha

Approved:

Geoffrey C. Denny
(Major Professor)

Frank B. Matta
(Minor Professor)

Juan L. Silva
(Committee Member)

Hamidou F. Sakhanokho
(Committee Member)

Michael S. Cox
(Graduate Coordinator)

Jerry M. Phillips
(Department Head)

George Hopper
Dean
College of Agriculture and Life Sciences

Name: Tripti Saha

Date of Degree: August 12, 2016

Institution: Mississippi State University

Major Field: Horticulture

Major Professor: Geoffrey C. Denny

Title of Study: Effect of rooting media, cultivars and genotype on the propagation of blueberry

Pages in Study: 42

Candidate for Degree of Master of Science

The objectives of this research were to study: 1) the effects of five different propagation media containing composted materials on the rooting of semi hardwood blueberry cuttings and 2) the effects of four cultivars and one genotype on rooting responses of these cuttings. The media used were: peat moss and horticulture grade perlite as control, composted pine bark fines with only one-time application of ammonium nitrate, pine bark fines with composted cotton gin waste, hardwood with and composted chicken litter and pine bark with composted chicken litter. There were no significant differences among the media for rooting from semi hardwood cuttings. Cultivars or genotype, media, and the interaction of individual media and cultivar or genotype had effect on rooting responses. Climax and Tifblue showed higher number of rooting from cuttings compared to other lines. Climax had the highest number of callus and survival percentage among all.

DEDICATION

First and foremost, I would like to dedicate my work to my late respective parents, Mr. Nalini Ranjan Halder and Mrs. Pramila Halder for being my constant source of inspiration and motivation. I would also like to dedicate my work to my loving husband, Dr. Sukumar Saha, my two beautiful daughters, Sulagna Saha and Satabdi Saha and my son in law, Kamal Lamichhane for believing in me and encouraging me to pursue my graduate studies. I would also like to dedicate my work in memory of my late niece, Pradipta Chowdhury for her constant support, wisdom, and encouragement.

ACKNOWLEDGEMENTS

I would like to express my deepest and sincerest gratitude to my major advisor, Dr. Geoffery Denny for providing me guidance, support and help in my thesis. I would specially like to express my gratitude to Dr. Matta for providing funds, moral support, and helping me to regain confidence in myself throughout my research work. His insightful comments and constructive criticism has made it possible for me to reach this stage in my graduate and research career. I must express my heartfelt thanks and gratitude to Dr. Hamidou F. Sakhanokho, my graduate committee member. I will never forget that with great patience he helped a novice like me in my result analysis. I acknowledge the cheerful support and compassionate help of other graduate committee members, Dr. David H. Nagel and Dr. Juan L. Silva, I could never complete this research work without their help, mentorship and encouragement. Words are inadequate to express my thanks to Mrs. Susan S. Worthey for her endless support and help throughout the course of my graduate research. Thanks to all of my professors in the Department of Plant and Soil Sciences for providing me the academic knowledge and skills to further my career. Thanks to all my classmates for making my graduate program an unforgettable and enjoyable experience. Most importantly, I would like to acknowledge my family for their unconditional love, moral support, and belief in me throughout my graduate Master’s program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The commercial blueberry belongs to the genus *Vaccinium* under subgenus *Cyanococcus* of *Ericaceae* family which is composed of four different types. The lowbush blueberries (*V. angustifolium*), or wild blueberries, are dwarf, woody, deciduous shrubs present from New Hampshire up through Maine and into New Brunswick and Nova Scotia (Trehane, 2004). The taller shrubby northern highbush blueberry (*V. corymbosum*) distributed from North Carolina extending north into Canada and west up to Illinois, Indiana and Michigan (Trehane, 2004). Rabbiteye blueberries (*V. ashei*) are erect, spreading type of shrubs and better adapted in the south than northern highbush blueberry (Bowerman, 2012).

Vaccinium species are considered one of the important foods in the diet of North Americans because more species of blueberries are native to North America than any other continent of the world (The George Mateljan Foundation, 2016). Although lowbush blueberries are native to other parts of the world including Europe, the Mediterranean and Asia, *V. corymbosum*, highbush blueberries were originally found almost exclusively in North America (The George Mateljan Foundation, 2016).

The United States is the largest blueberry producer in the world since 1970. USA produced 214,000 tons of cultivated blueberries in 2011 which was more than double the production of Canada that ranked second in world blueberry production (USDA, 2012).

V. corymbosum, the highbush blueberry, is the origin of most commercially cultivated USA blueberry varieties. The economic value of blueberry reached $640.7 million, second to strawberries among berry crops in 2010 in the U.S. production (Geisler, 2012). Normally *V. ashei*, the rabbiteye blueberry, is the dominant type of blueberry grown in Mississippi. About 170 producers are involved in blueberry production in Mississippi. Mississippi ranked eighth among all U.S. states with an approximately 1900 acres of land in blueberry production and had an increase in products from 3,330 lbs/acre in 2012 to 3,840lbs/acre in 2014 (USDA, 2015).

Blueberry is considered as one of the highest sources of antioxidants in the U.S. diet among all fruits, vegetables, spices and seasonings. (The George Mateljan Foundation, 2016**)**. Recently the antioxidant properties have made blueberries very popular as an agent against cancer disease (North American Blueberry Council, NABC, 2000). Anthocyanin, which produce the blue color in blueberries, are water-soluble antioxidants which reduce heart disease and cancer in humans. Recently the George Mateljan Foundation (2016) provided a detailed report on the benefits of blueberry diet in human health. Blueberries are very high in fiber and low in saturated fat, cholesterol, sodium and very low in terms of their glycemic index with potential impact as diabetic diet. It is also popular as great low-calorie snack for diet conscious people who are trying to lose weight. Recently these high food values of blueberries caused an increase in popular demand for fresh blueberries availability throughout the year.

The *Vaccinium* species are normally heterozygous in their genetic makeup producing segregating plants with different phenotypes from parent when propagated by seeds. In addition, seedlings growing from blueberry seeds experience a juvenile stage without producing any flower in their life cycle. Therefore, blueberries are cultivated normally by vegetative propagation to maintain the genetic and phenotypic characteristics of the cultivar of interest and to achieve fruit bearing capacity quickly (Debnath, 2007). Sexual crossing of these species is normally used for seed propagation in breeding programs to produce hybrid plants by combining desired characteristics from different parents.

Blueberries are normally propagated from softwood or hardwood cuttings by selecting cuttings from healthy, disease-free mother plants in a suitable propagation medium. Stem cutting is the most commonly used method to propagate for blueberry plants. However, propagation of blueberry by stem cutting is difficult to root. The growth stages of stem cutting such as herbaceous, softwood, semi-hardwood, or hardwood of the stock plant are the most important factors influencing the rooting from stem cutting (Krewer and Cline, 2003).

Adventitious root formation from stem cutting is a post-embryonic organogenic developmental process in which roots are induced normally from determined or differentiated cells that have not been directed to form roots at positions where they form normally during developmental process (Diaz-Sala, 2014). Such de novo induction of roots in an ectopic position in stem cutting involve the induction of meristematic cells from adult somatic cells, usually induced by a stimulus such as wounding or hormone treatments, although it can also take place in some species in intact plants (Abarca and Diaz-Sala, 2009).

It is critical to provide growers an ideal rooting medium for cutting propagation that will not only be economical but also suitable and sustainable as a rooting medium for blueberry producers. Composted agricultural waste products such as cotton gin waste and poultry litter have been found to have merits for horticultural crops (Cole, 2003). The ideal pH level for blueberry propagation media is 4.2-5.5 (Krewer and Cline, 2003). Cotton gin waste has a higher pH than pine bark making it an ideal component for blueberry propagation media. Some media will need to have the pH adjusted to increase acidity with elemental sulfur or decrease acidity with dolomitic or calcitic lime.

Previous studies showed that adventitious root formation sometime is a rate limiting step in the clonal multiplication of some tree species because the efficiency of forming the roots declines rapidly with age (Diaz-Sala et al., 1996, Day et al., 2002, Abarca and Diaz-Sala, 2009). The bulk of evidences suggests rooting of some juvenile tissues in some trees is more dependent on forming root initials than on physical restriction in root emergence (Diaz-Sala, 2014).

Callus is normally a diverse population of cells with diverse regeneration capacity. Previous study documented that callus cells from adult tissues of woody species normally committed to form progenitor cells and exhibited lower proliferation rate and regeneration capacity than young juvenile tissues (Hackett et al., 1990). It has been shown also that in many tree species cambial cells or vascular parenchyma cells are the main progenitor cells to induce adventitious roots directly (Diaz-Sala, 2014).

Vegetative propagation using stem cutting without any hormone application will provide an efficient, cost-effective method for blueberry propagation. This will also help to maintain genetic purity, uniformity and gainful exploitation of appropriate pedigree of blueberry in commercial production. This research was targeted to develop a suitable method and media for blueberry propagation to meet the demand for high-quality planting materials in commercial production.

The specific objectives of this research were to:

1. Determine the effects of five different media including control on rooting, callus formation and survival of blueberry cuttings
2. Study the effects of four cultivars and one genotype on rooting, callus formation and survival of blueberry cuttings

CHAPTER II

LITERATURE REVIEW

Blueberry is under a large genus of *Vaccinium* and belongs to *Ericaceae* family. There are about 140 diverse species of *Vaccinium* in the Southeastern United States, which can be broadly grouped into six unique subgenera (Radford et al., 1968). *Oxycoccus*, one of the subgenera consist of cranberry species such as *V. macrocarpon* and *V. oxycoccus*. The creeping blueberry consist of *V. crassifolium* and *V. sempervirens* is under the subgenera of *Herpothamnus*. *V. erythrocarpum,* commonly known as southern mountain cranberry, belongs to the *Oxycoccoides* subgenus (Bowerman, 2012). *Batodendron* subgenus contains *V. arboreum* species, commonly known as the sparkleberry, only present in the Southeast USA. It is a perennial semi-evergreen species keeping its leaves through much or all of the winter and reaches tree size growing as tall as 10 meters (Radford et al., 1968). The *Polycodium* subgenus consists of multiple varieties of the species *V. stamineum,* or deerberry (Radford et al., 1968). The commercial blueberries can be divided into four broad groups. Lowbush blueberries (*V. angustifolium*), or wild blueberries, are woody, dwarf, deciduous shrubs, the northern highbush blueberry (*V. corymbosum*) is a tall shrub species, rabbiteye (*V. ashei*) plants are erect, spreading shrubs, the hybrids of two or sometimes three *Vaccinium* species is commonly known as Southern highbush blueberries (Bowerman, 2012). They are early ripening, similar to northern highbush, and have a low chilling hour requirement like rabbiteye. The southern highbush which has lower chilling hours allows producers to bring fruit to market faster than the rabbiteye types (Krewer and Walker, 2006).

Blueberry species in the section Cyanoccus including *V. angustifolium* (lowbush blueberry, 2n=4x=48) *V. corymbosum* (highbush blueberry, 2n=4x=48), *V. darrowii* (Darrow's evergreen blueberry 2n=2x=24, and *V virgatum* (syn. *V. ashei*) (rabbiteye blueberry, 2n=6x=72) are among the species that have been the most widely used in blueberry cultivar development. To a lesser extent, five additional section Cyanoccus species, *V. constableii* (Constable's blueberry, 2n=6x=72), *V. elliottii* (Steve Elliot's blueberry, 2n=2x=24), *V. myrtilloides* (Canada blueberry, 2n=2x=24), *V. pallidum* (hillside or dryland blueberry, 2n=4x=48 form), and *V. tenellum* (southern lowbush or small cluster blueberry, 2n=2x=24) have also contributed to the development of blueberry cultivars (Ballington, 2009; Draper, 1995). The contributions of *V. darrowii*, *V. elliottii* and *V. tenellum* were made possible by the production of higher ploidy clones of these diploid species through fertilization between unreduced gametes. The diploid tree-like blueberry *V. arboreum*, also called sparkleberry, in section Batodendron is native to the southern U.S, is very drought tolerant and, unlike many other blueberry species, grows well on soils with low organic matter and higher pH. It was recently hybridized with two diploid Cyanococcus species, *V. darrowii* and *V. fuscatum*, and the tetraploid species *V. corymbosum* (Chavez and Lyrene, 2010).

Blueberry breeders use interspecific hybridization in the development of both northern and southern types, blending species with different chilling requirements and cold tolerances. Extremely cold hardy cultivars have been produced by hybridization of highbush *V. corymbosum* (native to northern blueberry production areas of the U.S. such as Michigan and New Jersey) and lowbush *V. angustifolium* (native to Maine and eastern Canada) to develop “half-high” blueberries. Southern highbush cultivars with low chilling requirements have been developed through the introgression of the low-chilling southern diploid species *V. darrowii* into the *V. corymbosum* background to develop blueberry cultivars for the early fresh market.

At the global level, the production of blueberry has increased steadily from 50,000lbs in 1995 to 190,000 lb in 2010 (Villata, 2010). North America, the highest producer, supplies about 57% fresh market and processed 85% of blueberries. The southern states have the lowest amount of acreage in production, but this acreage is expanding rapidly due to high consumers' demands. Among the southern states, Georgia leads the region with production in 12,800 acres (USDA, 2015).

The most popular type grown in Mississippi is a rabbiteye for late market around the time of June . The southern highbush is also getting popular to give growers the advantage of earlier ripening fruit for early fresh market sales. Most of the blueberry production is south of U.S. highway I-20 and located in the southeast quarter of the Mississippi state (Stafne, 2012).

The increased demand has led blueberry producers to develop a method for quick propagation of elite blueberry lines. Most blueberry species are vegetatively propagated by means of hardwood, semi-hardwood and softwood cuttings, suckers and tissue culture (Krewer and Cline, 2003). Hardwood, softwood and semi-hardwood cuttings have been used with reasonable success in blueberry propagation with greater than 50% rooting efficiency (Mainland, 2006). A common method for propagation is blueberry stem cuttings. Mainland (1998) reported that hardwood, softwood and semi-hardwood cuttings can be used reasonably as successful method of propagation. He showed that hardwood and softwood cuttings propagated outside with intermittent mist provide an economical means of producing planting stock. Hardwood cuttings have rooted most successfully when put into the propagation bed in March in southeastern North Carolina, while softwood cuttings have rooted consistently when collected and stuck in early to mid August. According to this study hardwood cuttings of one highbush (HB), three southern highbush (SH) and two rabbiteye (RE) cultivars averaged 95% rooting in a medium of pine bark covered with a 1.0 cm layer of sawdust. However he observed that rooting exceeded 80% in all media combinations containing at least 50% bark or sawdust. It was also detected that both hardwood and softwood cuttings had the highest percentage rooting when taken from the terminal flush of shoot growth considering all cultivars together, however, SH rooted equally well when the cuttings had a combination of growth from the terminal and previous flush (Mainland, 1998). Removing the lower leaves of softwood cuttings before sticking into the sawdust medium increased rooting of HB and SH cultivars but reduced rooting of RE cultivars (Mainland, 1998).

Micropropagation or tissue culture is another method of propagating commercial blueberry plants, but it is seldom used due to the cost and the increase in genetic variance (Mainland, 1998; Miller et al., 2006). Micropropagating plants would incline to have a bushier growth habit which permits more flower buds per plant (Miller et al., 2006). This results in more fruit than those propagated by cuttings, with the average berry weight being about the same (El-Shiekh et al., 1996).

Krewer and Cline (2003) provided a detailed review on the proper methods of propagation by cutting in blueberry. They reported that contaminated rooting media (by

Cylindrocladium and other fungi) is the primary problems for propagation by cutting during the rooting. Proper sanitation in the cutting nursery is extremely important to avoid the contamination of cuttings and their survival. It is important to soak in previously used pots with 10% chlorine bleach solution (9 parts water to 1 part household bleach) to overcome the problems of contamination before planting the cuttings. It is very important to use fresh clean media and not use recycled media for rooting.

The root rot by *Phytophthora* is caused by swimming spores of the fungus on southern highbush blueberries , so it is good to avoid saturated conditions in the media during propagation. It is good to grow plants on gravel beds in the nursery to avoid *Phytophthora* contamination. In a propagation facility it is important to clean up all debris and spray the area with a 10% chlorine bleach solution before sticking cuttings in the second year (Krewer and Cline, 2003). It is recommended to test the water quality to make sure it is suitable for plant propagation. It is important to select cuttings only from the healthiest plants available and avoid selecting cuttings with leaf spots or diseases. It is recommended that the cuttings should be sprayed several times two weeks apart after sticking with a dilute foliar fungicide to avoid contamination from diseased leaves.

Cutting by softwood propagation requires a mist propagation system to keep a film of water on the leaves during the day and prevent cuttings from wilting. The mist is used in short intervals (5 to 10 seconds) every 2 to10 minutes, long enough to keep a thin film of water on the leaf surface and normally the system is turned off at night with proper care to avoid waterlogging in the rooting media (Krewer and Cline, 2003). However, the specific condition may vary according to the situation.

The cuttings were made after the first flush of growth occurred in May to June period. Usually soft wood cuttings root more easily than hardwood (Krewer and Cline, 2003). However, the wood of these cuttings was reasonably firm to prevent wilting. Bowerman (2012) reported that the source of the cutting and the cutting type (softwood, semi-hardwood, or hardwood) are important factors for rooting. The ideal cutting material should have flexible stems and terminal leaves half to full grown. Normally if the cuttings are selected from very young stems with the wood without large moisture reserves that would have risk of wilting. However, if the cuttings were made too late, the chance of good rooting would also be poor (Krewer and Cline, 2003).

Hardwood and softwood cuttings are the most economical and common methods used for blueberry propagation. The vegetative growth of blueberry occurs in flushes. The suitable stage is to have the stems flexible and terminal leaves ½ to full grown. If the cuttings are selected from very young stems, the wood will not have any moisture reserves and will wilt. The reverse occurs when the cuttings are made too late and the percentage of rooting is poor. The stem should be somewhat flexible and young enough to be pushed into the propagation media without breaking. Generally, the best cuttings are from the first flush of growth (Ingram et al., 2003). A second flush occurs in late July- early August and some growers take advantage of the late flush. Hardwood cuttings are made in the fall of the year (generally after the first frost) during January and February after sufficient chilling has occurred. The cuttings must be planted in appropriate time so that they can root before the return of cold weather.

Special precaution should be taken so that the cuttings do not wilt. This can be prevented by immediately placing them in a bucket of water or a wet burlap sack while taking the cuttings. Cuttings should be made from the upper part of the mother plant where the wood has a good diameter with sharp by-pass type clippers or florist scissors. The flower buds are removed and the very tip is discarded (Evans and Blazich, 1999). It is important that immediately after cutting to spread the cuttings under the mist system or cool them with ice water. If the cuttings must be transported, it is important to use an ice chest with slushy ice during transportation (Krewer and Cline, 2003). It is useful to wet the propagation media thoroughly before sticking the cuttings by placing the containers and media under the mist several days before cuttings will be stuck. It is appropriate to remove one-half the lower leaves from the cuttings, keeping 3 to 4 leaves to avoid excessive transpiration. It is not required for hormone treatment for most blueberry cultivars, but this may improve rooting and root development in certain cultivars. Placing media firmly around the base of the cuttings helps to avoid air pockets.

The cutting should start developing roots in about three weeks (Krewer and Cline, 2003). At this period the propagator must monitor watering most closely to maintain a well-drained rooting medium system. Most of the cuttings will initiate rooting by formation of a mass of callus tissue on the cut end. However, in some cases when the cuttings do not form callus, produce sparse, watery roots, and begin turning brown from the cut end upwards under over-watered saturated condition of the propagation media. This is the evidence of poor aeration in the propagation media and unsuitable for cuttings. Miller et al. (2006) reported that southern highbush (*V. corymbosum*) and rabbiteye (*V. ashei*) blueberries had the greatest rooting success using softwood cuttings, and northern highbush had a lower rooting percentage. 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 (Bowmen, 2012). Cuttings planted in the spring, are often ready for transplanting by August into grow-out beds or quart pots (if plants will be set in the field the next winter). Since this is a time of great heat, irrigation must be carefully monitored.

The effect of rooting media for blueberry propagation by cutting has been reviewed in detail in several previous studies (Worthey, 2015; Krewer and Cline, 2003). The rooting media must be porous to provide a well drained system. Composting helps in the conversion of raw organic waste materials into biologically stable, humic substances that make excellent soil amendments suitable for root growth (Thomasson and Willcutt, 1996). Mixtures containing 50% peat moss helps in extensive root formation, however media drainage must be carefully monitored to avoid contamination from diseases. Acidic rooting media is mostly suitable for cutting propagation. A survey of 18 producers in Georgia in 2005 regarding methods of blueberry propagation documented that ninety-eight percent of the respondents used pine bark as the media for propagation (Worthey, 2015). Mixtures of coarse sand, milled pine bark, and peat moss (1:1:1); peat and perlite (1:1); milled pine bark and perlite (1:1); milled pine bark, peat moss, and sand (8:1:1) and milled pine bark alone are known as suitable rooting media for propagation by cutting in blueberry.

The most common propagation media currently used in some southern states such as Georgia is milled, composted pine bark, since it is low cost and widely available. The components used in propagation media such as pine bark, the by-products of the forest industry. Other industries such as the fuel products and building industry have taken former waste products and found economical use as propagation media for cuttings. The manufacturing of Mississippi wood products generates several million tons of wood and bark waste every year (Borazjani, 2004), which could be useful source for propagation media of cuttings. Most of these products, being acidic and porous, will be economical and suitable with potential for well drained capacity as ideal for propagation media of cuttings (Cline and Mainland, 2008). The optimal peat content is 25-50% (¼ -1/2) of the propagation mix. If other components such as course sand, pine bark fines, or perlite are used as a 1:1:1 ratio, the composted materials could be substituted for the peat or pine bark in growing mixes. The components such as composted bio-solids, municipal solid wastes, and yard trimmings have been shown to improve vegetable, fruit, and field crop yields (Chen and McConnell, 2003). Such soil-less media will be ideal because of its consistency, excellent aeration, reproducible, and has low bulk density. Ingredients in a soilless media include peat moss, coir, rockwool, vermiculite, perlite, sand, shredded and milled bark, and some type of composted product will be suitable for cutting propagation .The lack of availability and the variability of components and quality are the draw backs of using compost as a component for germination or potting mixtures.

The modern technology provides an opportunity to produce a very uniform product of compost materials that will be sustainable as products in the propagation industry for regular use in horticultural crops (Jackson, 2005). Many facilities have the components set up in windrows with proper testing capacity to make sure the bacteria and moisture contents are optimal without quality break down of the organic constituents. Desirable physical characteristics include: free of disease pathogens, weeds, pests, nematodes; good water holding capacity; and drainage are maintained in such specially manufactured composted materials. Uniformity, excellent aeration, consistency and low bulk density are some of the benefits present in such soil less media compared to the composed media produced in firms (Jackson, 2005). Such propagation media should have the characteristics of more air space and better drainage capacity. Excess water in media often results in poor root initiation and development because of lack of oxygen near the root zones. This research to grow new plants from cutting would have several advantages to the blueberry growers because it will generate healthy plants that are genetically identical to the mother plants, thus overcome the difficulties of propagation by seeds, and the plants are expected to grow faster and flower sooner than a plant grown from seeds.

CHAPTER III

MATERIALS AND METHODS

Semi-hardwood cuttings of blueberry from Climax, Tifblue, MS794 and Desoto were used from plants free of disease in the spring of February 4, 2013, before any of the leaf buds open with just after a flush of current season growth. The cuttings were supplied from several sources including Southern Horticultural Laboratory of USDA/ARS, Poplarville, MS 39470, North Mississippi Research and Extension Center, Verona, MS 38879, and the Greenhouse of Plant and Soil Science Department, Mississippi State University, Mississippi State, MS 39762. A sharp knife was used to cut about one-year-old shoots with no side branches on them for cuttings. The wood of these cuttings was reasonably firm. These shoot cuttings, called whips, were used for rooting in a propagation media. The majority of the cuttings were used from terminal branches.

The branches were cut 30-90 centimeters long and subdivided into 12-13 cm sections (Figure 1). The flower buds were removed and the terminal tip was discarded. One-half the lower leaves from the cuttings were removed leaving 3 to 4 leaves to avoid excessive transpiration following the overall method of Krewer and Cline (2003). All of the fruit buds from the cuttings were removed leaving only the leaf buds pointing up. About half an inch of bark from the bottom of each section on two sides of the cutting was removed to help the cutting root more easily. The cuttings were placed vertically in each of the substrate in a standard propagation trays (Figure 1). The cuttings were inserted 7-10 cm deep into the propagation media. The media was pressed firmly around the base of the cutting and the cutting were placed in the propagation bed in a space of 5 x5cm (2 inches) apart. Appropriate spacing provided two benefits: aids in air flow to prevent disease and help in root retention quality during removal from propagation bed or pot of the cuttings (Harelson, 2009). After the cuttings were placed in media, mist was applied to keep them from drying. Special precaution was taken to avoid wilting of the cuttings. This was prevented by immediately placing the cutting in a bucket of water or a wet sack while planting the cuttings into the media. Each cutting was buried a little more than halfway keeping the trimmed side down, and press the rooting mixture around each cutting and watered the cuttings well right away at the time of planting. It is important to monitor watering very carefully when the cutting started initiating the root system. Maintaining a well-drained rooting medium system is very critical to prevent wilting and diseases (Krewer and Cline, 2003).

Figure 1 The overall propagation method used from semi-hardwood cutting of blueberry

A. The leaves were removed from the bottom part of the semi-hardwood cuttings B. The cuttings were planted within the propagation media in a tray C. At the end of the experiment the data was recorded for root responses after harvesting the cuttings from the propagation tray

Five different substrates (control, hardwood bark with composted chicken litter mixture, Pine bark and Nitrogen mixture, Pine bark and cotton gin waste mixture, Pine bark and chicken litter mixture were used in this experiment. All of the media components were received from Ms. Susan S Worthey of the North Mississippi Research and Extension Center, Verona, MS 38879. She received the media from several sources (Worthey, 2015). The substrate components for this study were obtained from various sources. Non-ammoniated pine bark fines were received from a King's Nursery, Pontotoc, MS. The ammoniated pine bark, composted hardwood- poultry litter media, and pine bark and composted cotton gin waste media were aquired from Penick Forest Products in Macon, Mississippi. The control media, consisting of of 50% peat and 50 % horticultural gradeperlite v/v, was obtained from BWI Companies Inc., Memphis, TN. This five treatments including control were replicated four times in a complete randomized block design. The experiment was conducted on site in a double layer polyethylene covered greenhouse structure at the North Mississippi Research and Extension Center, Verona, MS. The following specific treatments were used: Control: 50% peat moss, 50% horticulture grade perlite. 1:1 v/v 2. Loamus-composted pine bark fines with cotton gin waste3. Loamate- ammoniated composted pine bark fines (one time application of Ammonium nitrate), 4. Nutri-Mulch- composted hardwood bark sieved to ¼ screen to leave out large particles 5. Pine bark fines with composted chicken liter The substrates had the physical properties tested such as bulk density, pore space, air space, and water holding capacity at Environmental Labs, Starkville, MS or Mississippi State University Soil testing laboratory. The chemical properties of the media such as soil pH and electrical conductivity were measured using the North Carolina State University Pourthrough method (Whipker et al., 2001) and the physical and chemical properties data were provided by Ms. Susan S. Worthey (Worthey, 2015).

When the cuttings are grown in containers or trays for promoting root initiation, their roots are restricted to a small volume, consequently the demands made on the substrate for water, air, nutrients and support are much more intense than those made by field-grown plants which have an infinitely greater volume of soil in which to facilitate root growth (Bunt, 1988). Several factors of physical and chemical properties of the media would influence on the success of such propagation methods. A previous report outlined the best management practices for producing container grown plants (Yeager et al., 2007). This report suggested that desired physical properties for container substrates after irrigation and drainage (% volume basis): air space 10 to 30%, water holding capacity 45 to 65%, total porosity 50 to 80%, and bulk density 0.19 to 0.70 g/cm^3. This report suggested guide that substrates amended with controlled release fertilizers should have pH levels in the range of 5.0 to 6.0 (varies with species being grown) and have electrical conductivity (EC) levels between 0.2 and 0.5 mS/cm (mmhos/cm). Most of the physical and chemical properties used in the media of this research are within the suggested parameters of the best management practice guide for producing container grown plants (Yeager et al., 2007). Each propagation tray had 36 holes. Each block consisted of two propagation trays where the individual media was placed leaving a blank row between the treatments. Four cuttings were placed in each substrate. The cuttings were placed under intermittent mist system. The system would run for five hours daily from 9:00 am to 12:00 pm for a duration of 10 sec every ten minutes. The mist length was extended as the temperatures got warmer or cut off if the exterior temperatures were cool and cloudy. The semi hardwood cuttings were harvested after four months of planting. The data were recorded from individual cuttings as the dead versus alive number of cuttings, callus formation per cutting and root formation on individual cutting from individual medium (Figure 2).

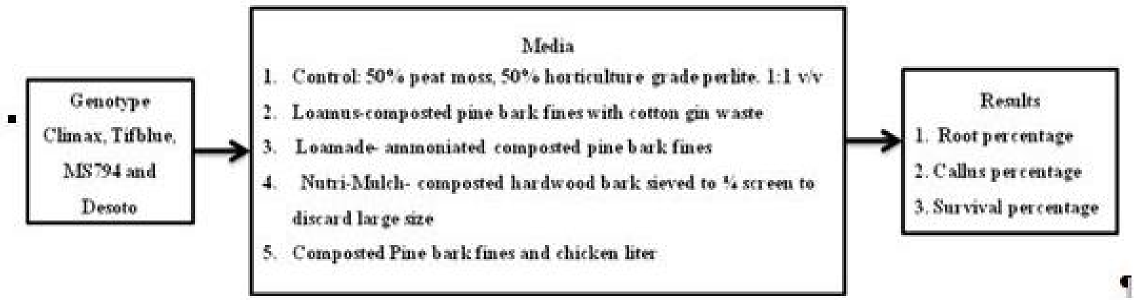

Figure 2 Schematic flow chart of protocols for Blueberry Propagation method used in the experiment

The data recorded during the experiments were used for average and the means were separated with Fisher's Protected LSD at the 0.05 significance level using SAS PROC MIXED (SAS Institute Inc., Cary, NC). An ANOVA analysis was used to study the genotype or cultivars, media and genotype or cultivar and media interaction effects respectively on root responses including rooting percentage, callus percentage and survival percentage.

The experiments were conducted three different times during the period of 2013 to 2016. In the first attempt of this experiment more than 50% cuttings were wilted to death because the automatic mist system at the greenhouse of Plant and Soil Science Department, Mississippi State University, was not maintained properly. However, precaution was taken to avoid this problem with the second batch of cutting sets at the greenhouse of Plant and Soil Science Department, Mississippi State University. It was observed that the cutting started to develop roots in about six to nine week period depending on the cultivar or genotype and media. The data were recorded in every week and final data record was collected for result analysis after harvesting. The third set of cuttings were planted at the North Mississippi Research and Extension Center, Verona, MS 38879. However, cuttings were only available from Climax and Tifblue among the four genotypes in the third set of experiment. Considering the complete data set from all of the cultivars and genotypes were available from all of the media only in the second set of experiment, the statistical analysis were conducted using only the data from the second set of observation.

CHAPTER IV

RESULTS AND DISCUSSION

Results were analyzed from the second of the three different experiments because the complete data set from all of the cultivars and genotypes were available from all of the media only in the second set of experiments as we already have mentioned in the previous material and method section. All of the cultivars and genotypes were able to develop root system and showed different root responses from semi-hardwood cuttings in all of the media.

Many of the cuttings initiated rooting by the formation of a mass of callus tissue on the cut end (Figure 3). After 6-8 weeks some of the cuttings formed callus, usually a mass of undifferentiated tissue which forms as part of a wound response (Figure 3). It was reported that adventitious root meristems (i.e., root primordia) were formed by lateral extension of callus tissues from cuttings of some forest tress (Cameron and Thomson, 1969), which ultimately initiated to form roots. Bowerman et al. (2013) observed callus percentages ranging from 30 to 85% with softwood cutting of blueberry when the experiment was intiated in May. However, Baker (2014) did not find any external factors to affect rooting or callus percentage. Results suggested that callus formation in some of these blueberry cuttings could be the potential source of the first stage of root primordia formation. It was observed that some of the cuttings did not form callus, but had sparse and watery roots. In addition, some of the cuttings began turning brown from the cut end upwards due to over-watered, saturated condition in some of the propagation media.

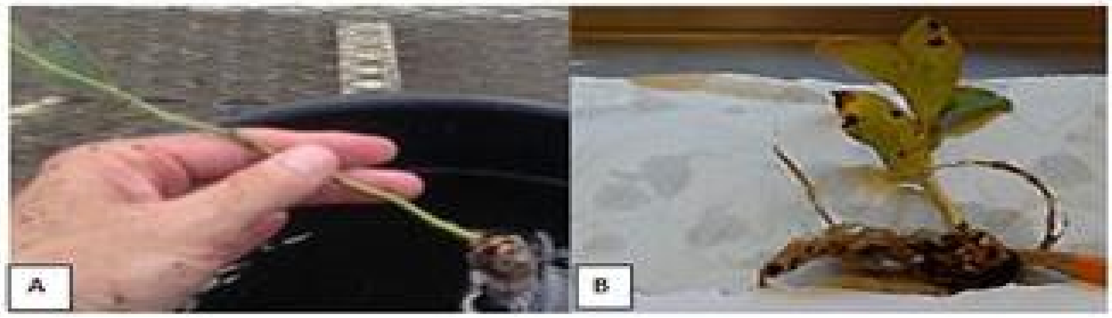

Figure 3 Some of the cuttings produced roots from friable callus.

A. Some of the cuttings formed callus at the base. B. A cutting showed that root was developed from callus tissues.

Results from Analysis of variance (ANOVA) analysis suggested that there are several factors which influence the competence of a cutting to initiate roots and other parameters on root responses. Cultivar or genotype, treatment or propagation media and interaction of the genotype and propagation media had significant effect respectively on root responses including root percentage (Table 1), callus percentage (Table 2) and survival percentage (Table 3) suggesting cultivar or genotype, propagation media and interaction of the genotype and propagation media are important for root, callus and survival percentage respectively. Results also demonstrated that interaction of the genotype or cultivars with propagation media had significant differences on averages effects of root, callus and survival percentage respectively further confirming ANOVA results (Figure 4). The averages effects of root, callus and survival percentage respectively did not have any specific pattern according to genotype or cultivars suggesting interaction of the genotype or cultivars with propagation media played a role on root response parameters

Table 1 Analysis of variance of root percentage in cuttings of rabbiteye blueberries

Source	Degree of freedom	Sum of squares	Mean square	F value	Pr > F
Block	3	18.52	6.17	0.1	0.96
Cultivar*	3	2916.67	972.22	5.88	0.0001
Treatment*	4	1450.81	362.7	15.76	0.0002
Cultivar and Treatment Interaction*	12	4007.52	333.96	5.41	0.0001

* Significant from zero at ≤ 0.05 alpha level

Table 2 Analysis of variance of callus percentage in cuttings of rabbiteye blueberries

Source	Degree of freedom	Sum of squares	Mean square	F value	Pr > F
Block	3	12.73	4.24	1.35	0.2588
Cultivar*	3	52.08	17.36	5.53	0.0037
Treatment*	4	50.34	12.59	4.01	0.0011
Cultivar and Treatment Interaction*	12	118.63	9.89	3.15	0.0004

* Significant from zero at ≤ 0.05 alpha level

Table 3 Analysis of variance of survival percentage in cuttings of rabbiteye blueberries

Source	Degree of freedom	Sum of squares	Mean square	F value	Pr > F
Block	3	7.81	2.60	0.20	0.8938
Cultivar*	3	725.41	33.71	2.64	0.0351
Treatment*	4	134.84	241.80	18.90	0.0001
Cultivar and Treatment Interaction*	12	311.92	25.99	2.03	0.0228

* Significant from zero at ≤ 0.05 alpha level

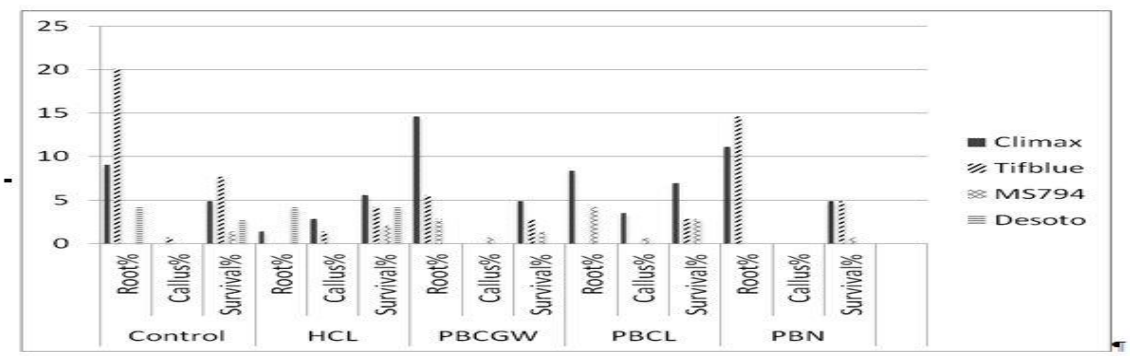

Figure 4 Average of interaction effects of the genotype\cultivars on propagation media on root responses

The results in graph showed that there is no specific pattern according to a specific media or genotype\cultivars on root responses including root percentage, callus percentage and survival percentage.respectively suggesting interaction effects of the genotype/cultivars with media.

Results on the effect of cultivar on callus production, survival rate, and rooting percentage based on LSD analysis showed that Climax and Tifblue were significantly higher in survival and rooting percentage from MS 794 and Desoto. Climax was also significantly different in Callus percentage from Tifblue, MS794 and Desoto (Table 4). However, the average of Climax was highest in all of the three parameters of root responses from cutting (Table 4). Desoto had the lowest average callus and survival percentage among all of the cultivars. The average rooting percentage from Tifblue cutting was the second highest among all of the cultivars (Table 4).

Table 4 Effect of cultivar on callus, survival rate, and rooting percentage in cuttings of rabbiteye blueberries

Cultivar	**Callus (%)**	**Survival (%)**	**Rooting (%)**
Climax	1.25 a*	5.42 a*	8.90 a*
Tifblue	0.42 b	4.44 a	8.10 a
MS 794	0.28 b	1.67 b	1.39 b
Desoto	0.00 b	1.40 b	1.68 b
LSD	0.64	1.29	2.83

*Means followed by different letters within the same column are not significantly different according to LSD test ($p \leq 0.05$).

It was observed that both Climax (Figure 5) and Tifblue (Figure 6) performed well compared to MS794 and Desoto on root, callus and survival percentage in almost all of the media. The pedigree history of the cultivars or genotypes used in this experiments showed that Climax and Tifblue had a common parent (GRIN, 2015). Climax (PI554700) was developed from a cross of Callway and Ethel, where as Tifblue (PI 554698) was developed from the cross of Ethel and Clara at Coastal Plant Experiment Station, Georgia State University, Georgia. Climax flower earlier than Tifblue, but Tifblue is very widely adapted covering over 50% of acreage in rabbiteye blueberries (GRIN, 2015). It could be possible that the common parent Ethel of Climax and Tifblue contributed to the genetic potential for higher root, callus and survival percentage of the cuttings compared to MS794 and Desoto who had a different pedigree history. Desoto (PI 641332) was developed from a cross of Woodard and Garden blue. It is a late low chilling hours rabbiteye blueberry cultivar. MS794 was developed from a cross of MS167 and MS536 and selection from crosses at Southern Horticultural Research Center, USDA/ARS, Poplarville, MS (personal communication Dr. Hamidou F. Sakhanokho, Southern Horticultural Research center, USDA/ARS).

It could be possible that some of the variation of root response results due to the differences in age or in physiological states of the initial cutting sources. Wagner et al. (1989) reported that from the same source of some trees the rooting potential in cuttings ranged from a low 10% to a maximum 87%. It has also been reported that time of collection of the cuttings in different seasons could also affect the rooting potential in clonal propagagtion (Bhella and Roberts, 1975). Climax had the highest average in rooting and survival percentage and did well compared to other genotype and cultivars in almost all of the media (Figure 5). Tifblue produced the second highest rooting percentage among the cultivars and genotypes (Figure 6). In a previous study Worthey (2015) used hardwood cutting of Climax and Tifblue in all of the same media except composted pine bark and chicken liter and observed that hardwood cuttings from Climax did not survive, but cuttings from Tifblue showed positive responses on rooting under the same condition in blueberry propagation. The differences on rooting responses of the cultivars in this experiment might be due to influence of some other external factors such as differences of physiological states or age or position of the parent material or different seasonal effects. Such external factors sometimes could confound the results on root responses such as rooting, survival and callus formation in blueberry propagation. For example, it has been suggested that it would be good to avoid shaded portions of the parent plant when taking cutting because the cutting will have lower levels of carbohydrates which might cause difficulties to root well (Whitcomb, 1978). It is important to ensure that healthy parent plant materials with vigorous growth should be used for cutting from proper place and appropriate time.

Figure 5 Climax stem cuttings showed response in rooting in different propagation media under the same condition

A. Climax cuttings from Control composted media containing (1:1 V/V) peat moss and horticulture grade perlite, B. Climax cuttings from Pine bark fines (75%) with cotton gin waste (25%) composted media, C. Climax cuttings from Pine bark fines with one time application of ammonium nitrate composted media, D. Climax cuttings from composted Pine bark fines and chicken liter.

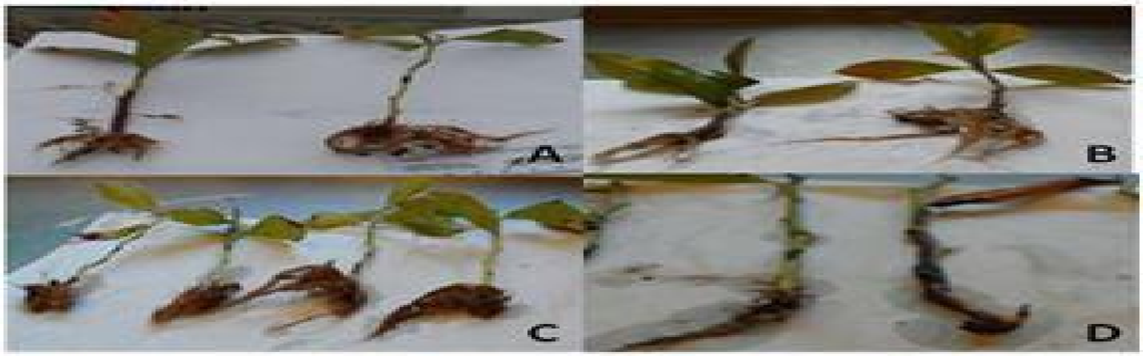

Figure 6 Tifblue stem cuttings showed response in rooting on different propagation media under the same condition

A. Tifblue cuttings in Control composted media containing (1:1 V/V) peat moss and horticulture grade perlite, B. Tifblue cuttings in Pine bark fines (75%) with cotton gin waste (25%) composted media, C. Tifblue cuttings in Pine bark fines with one time application of ammonium nitrate composted media, D. Tifblue cuttings in composted Hardwood bark fines and chicken liter

The results of rooting responses on callus percentage of control, PBN and PBCGW were significantly different from PBCL and HCL (Table 5). However, there were no differences among the media on survival and rooting percentage (Table 5). However, results based on average on rooting responses were numerically very different in all of the root response parameters of this experiment. The average of survival rate, and rooting percentage were highest in control compared to all other media. The average on rooting percentage on the propagation media of Pine bark fines with one time application of ammonium nitrate (PBN) was the second highest compared to three other media or treatments (Table 5).

Pine straw with little or no nitrogen application was considered as an excellent mulch for blueberry establishment, since it provided good source of weed control (Krewer and Walker, 2006). Composts and poultry liter were used as fertilized sources in organic farming for blueberry production (Krewer and Walker, 2006). It should be noted that compost materials used in the media were secondary by products of some major Mississippi agricultural industries. Results from this experiment suggested that these media have potential to be equally effective like control media (Peat moss and horticultural grade perlite).

Table 5 Effect of propagation media on callus, survival rate, and rooting percentage in cuttings of rabbiteye blueberries

Treatments	**Callus (%)**	**Survival (%)**	**Rooting (%)**
Control*	0.17 b*	4.20 a*	8.33 a*
PBN**	0.00 b	2.60 bc	6..42 ab
PBCGW***	0.17 b	2.26 c	5.73 ab
PBCL****	1.04 a	3.13 abc	3.13 bc
HCL*****	1.04 a	3.99 ab	1.40 c
LSD	0.71	1.44	3.16

*Means followed by different letters within the same column are not significantly different according to LSD test ($p \leq 0.05$). *Control peat moss and horticulture grade perlite, **pine bark fines with only one-time application of ammonium nitrate, ***composted pine bark fines with cotton gin waste,
****hardwood bark and chicken manure.

Results on the chemical properties of the media showed that there are significant differences on the pH among the treatments (Table 6). For example, control had the lowest pH compared to all other treatments. There was significant difference in pH among the media. Hardwood bark with chicken manure had the highest pH among the media. It has been reported that acidic media is helpful for blueberry propagation (Cline and Mainland, 2008). It was reported that ideal pH for blueberry production is 4.2-5 (Krewer and NeSmith, 2006). All of the media used in this experiment had pH higher than 5. Holt et al. (1998) reported that azalea stem cuttings (Rhododendron spp.) had higher rooting percentages and produced larger root balls at pH 4.5 than at pH 7.5. Williams et al. (1985) observed that several Australian woody species produced roots in vitro in a medium with a pH of 4.0, but not when the medium pH was 5.5. It was reported that *V. arboreum* grows well on soils that are low in organic matter and have a pH as high as 6.5 (Lyrene, 1997). Previous study showed that highbush blueberries require acidic soil, in the range of pH 4.0-5.5, with a high amount of organic matter (Darnell and Hiss, 2006). However, all of the media used in this experiment had slightly higher pH than the suggested range. This could be due to the different proportion of byproduct composted materials used in this experiment. It will be interesting for future investigation to study rooting responses of Rabbiteye blueberries (*V. ashei*) cutting, the material used in this experiment, using media within the range of 4.2-5 pH. Our results showed that numerically control had the highest average in rooting percentage and hardwood bark with chicken manure had the lowest rooting percentage (Table 5). It is possible that higher acidic characteristic of the control media compared to others promoted survival and rooting percentage of blueberry cuttings in the experiment.

The electrical conductivity (EC) of composted pine bark fines with cotton gin waste (PBCGW) was the highest among the four treatments including control (Table 6). Major and minor nutrients, important for plant growth, dissolved in the soil water of propagation media take the form of either cations (positively charged ions) or anions (negatively charged ions) carrying electrical charge and thus determine the EC level of the propagation media. The soil water EC level is a good sign of the amount of nutrients available for the crops to absorb. Chen and McConnel (2003) reported that the EC rating in propagating most plants are in the range of 1-2.5 MS/cm. Normally a good soil EC level will be in the range of 200 µS/cm and 1200 µS/cm (1.2 MS/cm) (Agricultural Solutions LLC., 2016). Cation exchange capacity of substrates is also an indicator in all substrates to retain nutrients against leaching by irrigation water or rainfall (Jackson, 2005).

Table 6 The pH and electrical conductivity (EC) of the propagation media measured after the experiments

Treatments	**pH**	**EC (µS/cm)**
Control*	6.2d	244.75c
PBN**	6.8c	291.8b
PBCGW***	7.1b	338.94a
HCL****	7.7a	270.00bc

*Control peat moss and horticulture grade perlite, **pine bark fines with only one-time application of ammonium nitrate, ***composted pine bark fines with cotton gin waste, ****hardwood bark and chicken manure. Means followed by the same letters within a column are not different based on Fischer's protected LSD value at probability of 0.05 significance level. The chemical properties of PBCL was not recorded and discarded from the table.

Results of physical properties based on LSD analysis suggested that there were no significant differences in bulk density (g.cm^{-3}dry), pore space (%) and water holding capacity (%) among the four treatments except air space (%) (Table 7). It has been reported that a substrate with a bulk density ranging from 0.15 to 0.8 gcm–3 (dry weight), an air space of 10% to 20%, and water holding capacity between 20% and 60%, and total porosity of 50% to 75% by volume are considered appropriate for rooting or producing containerized plants (Bunt, 1988; Chen et al., 2003; De Boodt and Verdonck, 1972; Evans and Gachukia, 2007; Yu and Zinati, 2006,). Comparing the physical properties of the media (Tables 7) to the recommendations, all parameters were within the suggested ranges except air space (%). The air space percentage of the control media in the present study was significantly higher compared to all other media of the experiment (Table 7). The higher air space percentage in control media could be helpful to promote root growth as indicated by the highest average of survival and rooting percentage of blueberry cutting in control media.

Table 7 Physical properties of the propagation media prior to insert cuttings

Soil	Bulk Density ($g.cm^{-3}$ dry)	Pore Space (%)	Air Space (%)	Water Holding Capacity (%)
Control*	0.16c	0.66a	0.17a	0.49b
PBN**	0.39a	0.63ab	0.01b	0.61a
PBCGW***	0.23bc	0.59b	0.03b	0.59ab
HCL****	0.32ab	0.60b	0.01b	0.58a

**Pine bark fines with only one-time application of ammonium nitrate, ***composted pine bark fines with cotton gin waste, ****Pine Bark and Chicken litter, *****hardwood bark and chicken manure. The physical properties of PBCL was not recorded and discarded from the table.

Composting has now become one of the most popular methods to dispose the increasing amounts of agricultural wastes. This method provides a process for conversion of raw organic waste products into biologically stable, humic substances that provide an excellent alternative to soil amendments (Thomasson and Willcutt, 1996). Pine bark (PB) is considered a useful resource to make up 75 to 100% (by vol) of container substrates in the eastern U.S.for the nursery industry since the 1970s (Lu et al. 2006). Historically, the ideal propagation media for blueberry propagation was well composted saw dust, a cheap byproduct from the local sawmill or lumber yard. However, the acquisition of sawdust, peat moss, and the pine bark fines is more difficult now because of the competition with other industries. The byproducts of several local agro and poultry industries were used in the composted media of this study to test the feasibility of developing a cost-effective environmentally friendly propagation media for blueberry production. The shortage of pine bark for the horticulture industry has demanded to explore the feasibility as an alternative source of other materials such as hardwood bark (Boyer et al, 2008), cotton gin waste (Cole, 2003), and poultry litter (Tyler et al, 1993) for use in mulch, soil mixes, and soil amendment blends in blueberry propagation. The disposal of agricultural wastes remains a major concern for causing environmental pollution. The manufacturing of Mississippi wood products generates several millions tons of wood and bark waste every year (Borazjani et al, 2004), which could be useful source for propagation media of cuttings. Many of the manufacturing of wood industries in Mississippi produces several million tons of wood and bark waste that is dumped or burned (Borazjani et al., 2004). Cotton is grown as the single most important textile fiber crop in 90 countries including US which accounts for 21% of the total world fiber production (Lee et al, 2006)\. It is one of the major crops grown in Mississippi. Each year, 1.2 to 2.5 million metric tons of cotton gin by-products other than cottonseed are produced annually by U.S. cotton gins creating a significant disposal problem in the USA ginning industry (Buser, 2001, Fava 2004). The removal or disposal of cotton gin waste and cottonseed hulls is a serious problem for cotton gins and cotton oil meal processing plants throughout the U.S. (Cohen and Lansford, 1993). Cotton gin trash is rarely disposed in landfills because of the high cost involved in transportation and dumping fees for approved landfill sites (Cohen and Lansford, 1993). It was reported that 37% of surveyed gins disposed of cotton gin by-products either at a profit or no cost to the gins, whereas the other 63% of gins paid high cost for disposal (Kolarik et al., 1978 A, 1978 B). Cotton gin waste as compost material has also been shown to reduce dry matter weight by 50% and volume by 60%, making this material easier to handle and transport than the raw product (Mayfield 1991). Our results showed no significant differences among the media considering rooting and survival percentage suggesting the effect of pine bark fines with cotton gin waste, ammoniated composted pine bark fines, hardwood bark and pine bark fines with chicken litter would be equally effective in rooting responses like control containing peat moss with grade perlite.

The demand for potting media used in the southeastern United States horticultural industry has almost doubled in recent years, with pine bark and peat moss being the primary substrates used (Shumack et. al., 1991). The organic matter in the form of compost in the media will improve soil structure, reducing bulk density, and improving moisture percolation, thereby providing a more suitable root environment for plants growth (Raviv et al., 1998; Brady and Weil, 2000). For example, the fleshy cotton strip, also a component of cotton gin waste contain a significant amount of Nitrogen (N), Phosphorus (P), and Potassium (K), as well as numerous micronutrients (Hills et al., 1981). Cotton burrs have a carbon nitrogen (C:N) ratio of 22:1, eliminating the nitrogen tie-up that occurs when wood or wood based products are composted (Jackson, 2005). Thus C:N ratio allows cotton gin waste to be composted and then utilized as an amendment or growing media without causing severe nitrogen depletion to plants (Thomasson, 1990). The modern technology would provide an opportunity to use some of the secondary byproducts of the agro-industries to produce a very uniform product of compost materials that will be cost-effective and sustainable as products in the propagation industry providing positive responses on rooting (ability to provide adequate aeration, moisture retention, pH, and low electrical conductivity) without inhibition in root formation for regular use (Currey et al, 2013).

In conclusion, results showed no significant differences among the media considering rooting and survival percentage suggesting all of the media tested in this experiment would be equally effective in rooting responses like control containing peat moss with grade perlite which normally used in blueberry propagation media for cuttings. It is important to note that most of the products used in the media of this experiment are the byproducts of some major Mississippi industries such as poultry, forest and cotton industries. Climax and Tifblue performed well compared to MS794 and Desoto on rooting and survival percentage in all of the media. Climax showed highest in all of the three parameters of root responses from cutting. Sometimes influence of external factors such as differences of physiological states or age or position of the cutting materials from parents or different seasonal effects could confound the effect on root responses within the same source of cuttings in blueberry propagation. It was reported that rooting percentages in blueberry cuttings vary with the species and cultivar, time of the year, and age of the mother plant (Lyrene, 1980). The blueberry rooting percentage can be improved by treating cuttings with rooting hormones, such as indole butyric acid (Hartman and Kester, 1975). Future investigation such as cuttings from the same physiological states or different rooting substrate or hormone treatments, or use of other environmental factors such as light, moisture, temperature will help to overcome some of these challenges in developing a suitable commercially useful methods for blueberry propagation.

REFERENCES

Abarca, D., and Díaz-Sala, C. 2009. Reprogramming adult cells during organ regeneration in forest species. Plant Signal. Behav. 4: 1–3. doi: 10.4161/psb.4. 8.9238

Agricultural Solutions LLC. 2016. The why and how to testing the electrical conductivity of soils. https://www.agriculturesolutions.com/resources/92-the-why-and-how-to-testing-the-electrical-conductivity-of-soils [Accessed on January 30, 2016]

Baker, A.B. 2014. Exploring methods to enhance rooting of *Vaccinium* arboreum stem cuttings. M.S. thesis, Auburn University, Page 1-52.

Ballington, J.R. 2009. The role of interspecific hybridization in blueberry improvement. Acta Hort. 810:49–60.

Bhella, H.S. and. Roberts A.N. 1975. Seasonal changes in origin and rate of developmentof root initials in Douglas-fir stem cuttings. J. Amer. Soc. Hort. Sci.100:643-646.

Borazjani, H., Diehl, S.V., Stewart, H.S., and Brasher, K., 2004. Mississippi connection-Composting Research Targets Forest Products and Poultry Industry-University projects com furniture waste with litter, phenolic plywood, swine manure and mortalities. BioCycle- Journal of Composting and Recycling, 45(5):42-43.

Bowerman, J.R. 2012. Propagation of *Vaccinium arboreum* by stem cuttings for use as a rootstock for commercial blueberry production. M.S. thesis, Auburn University, Page 1-52.

Bowerman, J.R., Spiers J.D,, Coneva E., Tilt K.M., Blythe E.K., and Marshall D.A. 2013. Cutting type affects rooting percentage of vegetatively propagated sparkleberry (*Vaccinium arboreum*). J. Environ. Hort. 31:253–258.

Boyer, C., Rain G.B., Gilliam C.H., Gallagher T.V., Torbert, H.A., and Sibley J.L. 2008. Clean chip residual: A substrate component for growing annuals. HortTechnology 18:423-432.

Brady, N.C., R.R. Weil. 2000. Elements of the Nature and Properties of Soils. Prentice Hall. New Jersey.

Bunt, A.C. 1988. Media and Mixes for Container Grown Plants. Second Ed. Unwin Hyman, London.

Buser, M. 2001. Extruding cotton gin by-products to reduce chemical residues. J. Cotton Sci. 5:92–102.

Cameron, R. J., and Thomson G.V. 1969. The vegetative propagation of *Pinus radiata*: Root initiation in cuttings. Bot. Gaz. 130:242–51.

Chavez, D.J., Lyrene, P.M. (2009) Effects of self-pollination and cross-pollination of *Vaccinium darrow*ii (Ericaceae) and other low-chill blueberries. HortScience 44:1538–1541.

Chen, J.J and McConnell, D. 2003. Compost-formulated media for foliage plant production. University of Florida Extension bulletin ENH873.

Chen, J., McConnell D.B., Robinson C.A., Caldwell R.D., and Huang Y. 2003. Rooting foliage plant cuttings in compost formulated substrates. HortTechnology 13:110–114.

Cohen, T.M., and Lansford R.R.. 1993. Survey of cotton gin and oil seed trash disposal practices and preferences in the western U.S. Technical Report 12. Ag. Exp. Station. New Mexico St. Univ.

Cline, B. and Mainland, M., 2008. Blueberry propagation. Proceedings of the 42nd Annual Open House and Trade Show. North Carolina Blueberry Council, Clinton, NC. Pages 56-62.

Cole, D.M. 2003. Cotton gin compost as a horticultural substrate. M.S. Thesis, Auburn University, Auburn ,AL.

Currey, C.J., Gray,M., and Lopez,R., 2013. Parboiled rice hulls in propagation substrates. Greenhouse Grower, April. P. 32-34.

Darnell, R. L., and Hiss S. A.. 2006. Uptake and assimilation of nitrate and iron in two *Vaccinium* species as affected by external nitrate concentration. J. Amer. Soc. Hort. Sci. 131:5-10.

Day, M. E., Greenwood, M. S., and Díaz-Sala, C. 2002. Age-and size-related trends in woody plant shoot development: regulatory pathways. Tree Physiol. 22, 507–513. doi: 10.1093/treephys/22.8.507

Debnath, S.C. 2007. Influence of indole-3-butyric acid and propagation method on growth and development of in vitro- and ex vitro-derived lowbush blueberry plants. Plant Growth Regul. 51: 245-253.

De Boodt, M. and Verdonck O.. 1972. The physical properties of the substrates in horticulture. Acta Hort. 26:37–44.

Díaz-Sala, C., Hutchison, K. W., Golfbarb, B., and Greenwood, M. S. 1996. Maturation-related loss in rooting competence by loblolly pine stem cuttings: role of polar auxin transport and tissue sensitivity. Physiol. Plant. 97, 481–490. doi: 10.1111/j.1399-3054.1996.tb00507.x

Díaz-Sala, C. 2014. Direct reprogramming of adult somatic cells toward adventitious root formation in forest tree species: the effect of the juvenile–adult transition. Frontier in Plant Sci. 5:1-8.

El-shiekh, A.,Wildung, D.K., Luby, K.L., Sargent, K.L., and Read,J.P., 1996. Long-term effects of propagation by tissue culture or softwood single-node cuttings on growth habit, yield, and berry weight of 'Northblue' blueberry. Journal of American Society of Horticultural Science. 121:339-342.

Evans E. and Blazich F. 1999. Plant propagation by stem cuttings: instructions for the home gardener.. NC Cooperative Extension Resources. http://content.ces.ncsu.edu/plant-propagation-by-stem-cuttings-instructions-for-the-home-gardener. [Accessed on February 2, 2016]

Evans, M.R. and Gachukia M.M. 2007. Physical properties of sphagnum peat-based root substrates amended with perlite or parboiled fresh rice hulls. HortTechnology 17:312–315.

Fava, A. 2004. The waste with 2.5 million tons of potential. In Business 26:26–27.

Geisler, M. 2012. Blueberries profile. Agricultural marketing resource center. University of Iowa. 2 October 2012. <http://www.agmrc.org/commodities__products/ fruits/blueberries-profile/[Accessed October 2, 2012].

GRIN. 2015. Germplasm Resource Informtion Network. http://www.ars-grin.gov/[Accessed March, 2016].

Hackett, W. P., Murray, J. R., Woo, H. H., Stapfer, R. E., and Geneve, R. 1990. Cellular, biochemical and molecular characteristics related to maturation and rejuvenation in woody species. *In Plant Aging: Basic and Applied Approaches,* eds R. Rodríguez, R. S. Tamés, and D. J. Durzan (New York: Plenum Publishing Corporation), Page 147–152.

Haralson, J.C.2009., Pathogens associated with blueberry cutting failure in south Georgia nurseries and their control. Master's thesis. Retrieved from Proquest Dissertations and Theses (Accession Order No. ATT 200905 MS)

Hartmann, H.T. and. Kester D.E. 1975. Techniques of propagation by cuttings, p. 271-313. In: Plant propagation: principles and practices. Prentice Hall, Englewood Cliffs, N.J.

Hills, D.J., Curley R.G., Knutson J.K., Seiber J.N., Winterlin W.L., Rauschkolb R.S., Pullman G.S., and Elmore C.L.. 1981. Composting treatment for cotton gin trash fines. Trans. Amer. Soc. Ag. Eng. 24:14-19.

Holt, T.A., Maynard B.K., and Johnson W.A.. 1998. Low pH enhances rooting of stem cuttings of Rhododendron in subirrigation. J. Environ. Hort. 16:4–7. http://www.smallfruits.org/Blueberries/production/03BlueberryPropagation Suggestions.pdf. page 1-14. [Accessed on 14 October 2012]

Ingram, D.L. Richard,W.H., and Thomas, H.Y., 2003. Growth media for container grown ornamental plants. Florida Cooperative Extension Service, Univ. of Florida, Bulletin 241.

Jackson, B.E. 2005. Cotton gin compost as an alternative substratefor horticultural crop production. M.S. Thesis. Auburn University, Auburn, AL

Kolarik,W.J., Smith M.L., 1978A. Economic Evaluation of South Plains (Texas) Ginning Waste as a Raw Material in the Production of Roofing Felt. Report prepared for Cotton Incorporated, Agreement No. 78-383.

Kolarik, W.J., Lalor W.F., Smith M.L., 1978B. Cotton Gin Waste in Texas. Cotton Gin and Oil Mill Press, pp. 14–16.

Krewer, G. K. and Cline, W.O, 2003., Blueberry propagation suggestions. http://www.smallfruits.org/Blueberries/production/03BlueberryPropagation Suggestions.pdf . [Accessed May 5,2013]

Krewer, G. and Walker R.. 2006. Suggestions for organic blueberry production in Georgia. http://www.smallfruits.org/blueberries/production/06organicblues.pdf [Accessed January 21, 2016]

Lee S., Kaittans C., Jansen R.K., Hostetler, Tallon L.J., Town C.D., and Daniell H. 2006. The complete chloroplast genome sequence of *Gossypium hirsutum*: organization and phylogenetic relationships to other angiosperms. **7**:61:2-12, doi:10.1186/1471-2164-7-61.

Lu, W., Sibley J.L., Gilliam C.H., Bannon J.S., and Zhang Y.. 2006. Estimation of U.S. bark generation and implications for horticultural industries. J. Environ. Hort. 24:29–34.

Lyrene, P.M. 1980. Micropropagation of rabbiteye blueberries. HortScience 15:80 81**.**

`North American Blueberry Council., 2000. P.O. Box 1036, Folsum, CA 95763. www.nabcblues.org/blueberry.com [Accessed June 3, 2104].

Lyrene, P.M. 1997. Value of various taxa in breeding tetraploid blueberries in Florida. Euphytica 94:15-22.

Mainland, C.M., 1998. Frederick Coville's pionering contributions to blueberry culture and breeding. In: Cline,W.O. and Ballington, J.R. (eds) Proceedings of the 8th North Carolina State University Research and Extension workers conference. North Carolina State University, Raleigh, North Carolina, pp.74-79.

Mainland, C. M. 2006. Propagation of Blueberries, p. 49-55. In: N. F. Childers and P. M. Lyrene (eds.). Blueberries: for growers, gardeners, promoters. E. O. Painter Printing Company, Inc., DeLeon Springs, Fla.

Mayfield, W. 1991. Gin trash utilization: what are the options? Proc. Beltwide Cotton Conf. 1:496-497.

Miller, S., Rawnsley E., George J, and Patel N. 2006. A comparison of blueberry propagation techniques used in New Zealand. Acta Hort. 715:397-401.

North American Blueberry Council., 2000. P.O. Box 1036, Folsum, CA 95763. www.nabcblues.org/blueberry.com [Accessed January 30, 2016].

Qiansheng Li, Chen J., Caldwell R.D., and Deng M. 2009. Cowpeat as a Substitute for Peat in Container Substrates for Foliage Plant Propagation. HortTechnology. 19:340-345.

Radford, A. E., Ahles H. E., and Bell C. R.. 1968. Manual of the vascular flora of the Carolinas. The University of North Carolina Press, Chapel Hill, NC.

Raviv, M., Medina S., Krasnovsky A., and Ziadna H.. 2002. Conserving nitrogen during composting. Biocycle. 43:48-51.

Shumack, R.L., Eakes D.J., Gilliam C.H., and Donald J.O. 1991. Using gin trash in composted soil ingredients. Proc. Beltwide Cotton Conf. 1:498-499.

Stafne, E.T. 2012. Blueberry production in Mississippi., 2012 North Mississippi Fruit Growers Association. Verona, MS.

Sugimoto, K., Jiao, Y., and Meyerowitz, E. M. 2010. *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway. *Dev. Cell* 18, 463–471. doi: 10.1016/j.devcel.2010.02.004

The George Mateljan Foundation. 2016. Blueberries. http://www.whfoods.com/genpage.php?tname=foodspice&dbid=8 [Accessed January 21, 2106].

Thomasson, J.A. 1990. A review of cotton gin trash disposal and utilization. Proc. Beltwide Cotton Prod. Res. Conf. 1:689-705.

Thomasson, J.A. and Willcutt M.H.. 1996. Wetting methods for initiating composting in cotton gin waste. Applied Eng. Ag. 12:417-425.

Trehane, J. 2004. Blueberries, cranberries and other Vacciniums. Timber press. Portland, Oregon and Cambridge, U.K.

Tyler H.H., Warren S.L., Bilderback T.E. and , Fonteno, W.C. 1993. Composted turkey litter: II. Effect on plant growth. J. Environmental Horticulture 11:137-141.

USDA NASS. 2012. Noncitrus Fruits and Nuts 2011 Summary. United States Department of Agriculture. National Agricultural Statistics Service. Cornell Univ. 31 October 2012. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1113> [Accessed January 21, 2106].

USDA NASS. 2015. Noncitrus Fruits and Nuts 2014 Summary. United States Department of Agriculture. National Agricultural Statistics Service. Cornell Univ. 31 October 2012. http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-07-17-2015.pdf. [Accessed January 21, 2106].

Villata, M., 2012. Trends in world blueberry production. http://www.growing produce.com/article.26272/2/trends-in-world-blueberry-production [accessed March 8, 2012].

Wagner, A.M., Fisher J.T. and Fancher G.A.. 1989. Vegetative propagation of 10-year-old blue spruce by stem cuttings. pp. 70- 75. In: Proceeding Intermountain Forest Nursery Assoc. Mtng., August 14-18, 1989, Bismarck, ND. USDA For. Ser. Gen. Tech. Rpt. RM-184.

Whipker, B.E., Cavins, T.J., and Fonteno, W.C. 2001. 1, 2, 3's of PourThru. Florex.005. page 1-4. North Carolina State University. http://www.ces.ncsu.edu/depts/hort/floriculture/Florex/PourThru%20Handout%20123s.pdf,[Accessed March 9th, 2016]

Whitcomb, C.E. 1978. Propagating woody plants from cuttings. Ag. Exp. Sta. Okla. State Univ. Bul. No. 733

Williams, R.R., Taji A.M., and Bolton J.A. 1985. Specificity and interaction among auxins, light, and pH in rooting of Australian woody species in vitro. HortScience 20:1052–1053.

Worthey,S.S. 2015. Propagation of blueberries in compost amended media. M.S. Thesis, Mississippi State University. Department of Plant and Soil Sciences. Mississippi State, MS.

Yeager, T. (editor). 2007. Best management practices: Guide for producing nursery crops. 2nd ed. The Southern Nursery Association, Atlanta, GA.

Yu, S. and Zinati G.M. 2006. Physical and chemical changes in container media in response to bark substitution for peat. Compost Sci. Util. 14:222-230.