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The Influence of Ericoid Mycorrhizal Fungi on Rabbiteye Blueberry (Vaccinium Ashei)

Mark Linneaus McLaughlin

The influence of Ericoid Mycorrhizal fungi on Rabbiteye blueberry (*Vaccinium ashei*)

By

Mark Linneaus McLaughlin

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Mark Linneaus McLaughlin

Approved:

Frank B. Matta Professor of Plant and Soil Sciences (Major Professor)	David Nagel Extension Professor of Plant and Soil Sciences (Committee Member)
David M. Ingram Extension/Research Biochemistry, Molecular Biology, Entomology, and Plant Pathology (Committee Member)	Juan L. Silva Professor of Food Science, Nutrition and Health Promotion (Committee Member)
R. Crofton Sloan Assistant Research Professor of Plant and Soil Sciences (Committee Member)	J. Mike Phillips Professor and Department Head of Plant and Soil Sciences
William L. Kingery Professor of Plant and Soil Sciences (Graduate Coordinator)	George M. Hopper Dean College of Agriculture and Life Science

Name: Mark Linneaus McLaughlin

Date of Degree: December 15, 2012

Institution: Mississippi State University

Major Field: Horticulture

Major Professor: Frank B. Matta

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Candidate for Degree of Masters of Science

Ericoid Mycorrhizal fungi have been shown to form symbiotic associations with Ericaceous plants and increase nutrients uptake by the plant. The objective of this study was to assess the potential of two ericoid mycorrhizal isolates *Oidiodendron maius* and *Pezizella ericae* as potential sources of inoculant for rabbiteye blueberry (*Vaccinium ashei*) and to assess the variation in consistency and degree of ericoid mycorrhizal fungi colonization within rabbiteye blueberry (*Vaccinium ashei*). Field trials were conducted in 2012 in Verona, MS at the North Mississippi Research and Extension Center. In this study data was collected on fruit yield, leaf tissue nutrient concentration, leaf tissue nitrogen concentration, and percent colonization of root tissue. Ericoid mycorrhizal fungal isolates *Oidiodendron maius* and *Pezizella ericae* showed no influence on rabbiteye blueberry (*Vaccinium ashei*) during this study

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CHAPTER I

INTRODUCTION

Rabbiteye blueberry (*Vaccinium ashei*) production in Mississippi has steadily risen in acreage over the past six years. Production acreage has increased from 2000 acres in 2006 to 2700 acres in 2011 (USDA-NASS). Prior to 2006, national statistical data on blueberry production in the state of Mississippi was not available, but loss data is available. In 2010 Mississippi was ranked ninth out of thirteen states for total blueberry harvested acres, 2700 acres harvested, and tenth in total yield per acre (2960 pounds). Others states included in the national rankings are the following in their respective order 1) Michigan, 2) Georgia, 3) New Jersey, 4) Oregon, 5) North Carolina, 6) Washington, 7) California, 8) Florida, 10) New York, 11) Indiana, 12) Alabama, and 13) Arkansas (USDA-NASS, 2010).

Rabbiteye blueberries belong to the order of Ericales and are a member of the Ericaceae family within the genus Vaccinium and the section cyanococcus. Blueberry plants, like other ericales are acid loving, calcifuges that are naturally found on marginal land with low pH levels and high organic matter. While this may present some obstacles and disadvantages to some growers who wish to produce rabbiteye blueberries on typical agronomic soils, it is an adaptation that allows many of the native species of Vaccinium to exploit relatively hostile soil environments (nutrient poor, low pH).

Rabbiteye blueberries are a highly valued fruit crop produced in Mississippi and the Southern United States. Because of the blueberries high value the commercial blueberry industry has spurred research to increase production output while maintaining relatively minimal input cost. Factors such economic sustainability for the industry as well as environmental concerns has induced research into specific symbiotic relationships between specific fungal species and their plant host in the allocation of certain types for soil nutrients (Scagel, 2005).

Ericoid mycorrhizas are associations between ascomycetous (or rarely hyphomycetous) fungi and plant species belonging to the families Ericaceae, Epacridaceae, and Empetraceae (Smith and Read, 1997). Ericoid mycorrhiza is characterized by considerably uniform structure, similar to those in arbuscular mycorrhizas, but are usually more delicate (Peterson et al., 1980; Allaway and Ashford, 1996). The hyphae of ericoid mycorrhizal fungi penetrate a single layer of cortical cells of the roots and fill them with intercellular hyphal coils (Jansa and Vosatka, 2000). It is the hyphal coils that create the symbiotic interface between the fungi and its host plant. It is at this interface where the host plant receives mineral nutrients via fungal mycelium, while the heterotrophic fungus obtains carbon compounds from the host's photosynthesis (Azcon-Aguilar and Barea, 1997). Scagel (2005) was able to show that inoculations with EMFs in highbush blueberry (*Vaccinium corymbosum*) had positive effects on nutrient uptake and acquisitioning under controlled conditions. However, previous studies evolving inoculation of blueberries with EMFs are limited to highbush varieties and information on inoculation of southern grown species such as rabbiteye blueberries is relatively limited. A collective approach will be necessary in order to optimize a

rabbiteye blueberry inoculation program throughout the state of Mississippi and the Southern United States. A more thorough understanding of inoculation practices and which fungal species are appropriate to the soil environment of the Southern United States is needed.

The objectives of this study were to: 1) to examine if *Oidioidendron mauis* and *Pezizella ericae* can be utilized as potential sources of inoculate in rabbiteye blueberry (*Vaccinium ashei*). 2) Assess the variation, consistency, and degree of colonization of ericoid mycorrhizal fungi within rabbiteye blueberry (*Vaccinium ashei*).

CHAPTER II

LITERATURE REVIEW

Ericoid Mycorrhiza

A mycorrhiza is the symbiotic (generally mutualistic, but in some instances mildly pathogenic) association between the mycelium of a fungus and the roots of a vascular plant. Mycorrhizal associations occur within the rhizosphere, which is a narrow region of soil that surrounds the roots of plants and directly influences root secretion as well as associations with microorganisms. Mycorrhizas are considered crucial for the survival of plant species growing in nutrient-poor environments (Smith and Read, 1997). This is because ericoid mycorrhizal fungi possess the ability to mobilize nutrients from organic substances by secreting enzymes that break down simple and complex organic polymers (Read et al. 2004). However, mycorrhizal colonization varies widely within ecosystems, habits, species, and even individual plants (Allen 1991; Fitter and Merryweather 1992). Ericoid mycorrhizas (EMF) are symbioses between specialized soil fungus and the roots for many ericaceous plants, including highbush blueberries (*Vaccinium corymbosum*) (Sadowsky et al. 2012).

Ericoid mycorrhizas are characterized by the formation of intercellular hyphal coils in the epidermis of hair roots and hyphal extension of up to 1cm from the root surface (Read, 1984). Hair roots are finest absorptive roots of *Vaccinium* commonly exhibiting diameters less than 50 μm and are recognized as “hair roots” and not root

hairs. Ericoid mycorrhizal fungi possess the ability to mobilize nutrients from organic matter by releasing extracellular enzymes that break down simple and complex organic polymers making these nutrients available of uptake by their plant host(Read et al. 2004). Experiments have shown that the fungi forming ericoid mycorrhiza also have saprotrophic capabilities sufficient to enable competition with decomposers. Such experiments have uncovered the possibility of direct involvement of roots colonized by mycorrhizal fungi in the mobilization of N and P from the organic residues which are the major repository of both elements in most heathland ecosystems (Read, 1983; Read and Kerley, 1995). EMFs act as conduits (or passageways) for soil nutrients that would otherwise be less available to non-mycorrhizal plants. The first indication of this type of activity came from a study in which 15N labeled ammonium was fed to mycorrhizal and non-mycorrhizal plants of *Vaccinium* grown in sterile heathland soil (Stribley and Read, 1974). These experiments and observations lead to the understanding that despite having significantly greater yields and total nitrogen content, the mycorrhizal plants had lower 15N enrichment, demonstrating that dilution of labeled 15N by alternate N sources had occurred. In absence of nitrification, organic residues were the only realistic sources (Read, 1996).

Structure and development of ericoid mycorrhizal fungi (EMF) associations

Detailed investigations on the structure and molecular cytology of ericaceous infections are sparse at best. Most, if not all are from field collected material where neither the fungal partners nor the length of time since infection was known (Cairney and Ashford, 2002). Development of symbiosis is initiated when a fungal hypha contacts a compatible region of a hair root. The apical region of actively growing roots is usually

not infected. Hair roots of different order are reported to carry different infection levels and infection in those epacrids studied appears somewhat less than that reported in other Ericaceae. Extra-radical mycelium on the root surface is usually rather sparse in field-collected epacrids as in (*Calluna vulagris*) and (*Vaccinium myrtillus*) (Bonfante-Fasolo &Gianinazzi-Pearson, 1979; Bonfante-Fasolo et. al., 1981). Upon contact of the fungus and the hair root an appressorium-like structure is formed in some species. There is no information on what controls the formation of the appressorium-like structure as there is for some pathogens and Arbuscular mycorrhiza (AM) fungi, and as mention previously the appreossorium-like structure is not present or visible in all circumstances. Appressorium formation is followed by the development of a narrow penetration hypha. This usually grows through the outer tangential wall (or occasionally the outer part of the radial wall) and enters the periplasmic space or epidermal cell where it widens and forms a coil. Typically there is only a single penetration point. Halo formation around the penetrating hyphae is a sign that wall digestion has occurred (Cairney and Ashford, 2002). There is no information on the extent of control exerted by the root, as there is for AM fungi, where it is known that the penetration step and form that the intercellular hyphae take are under a complex genetic control by the plant (Harrison, 1999). Prior to penetration, a hypha encounters the surface mucilage and there are many images of hyphal profiles on the root surface completely enveloped in this mucilage (Allen et al., 1989; Steinke et al., 1996; Briggs and Ashford, 2001). It has been suggested that both the cell wall and the mucilage overlaying the hair roots are important in the reactions controlling the establishment of ericoid mycorrhizal associations (Bonfante-Fasolo, 1988). Once the hypha has penetrated the cell and is within the root, the fungal coil

begins to form inside the periplasmic space. It is, therefore, intercellular, but outside the epidermal cell plasma membrane and so, is considered an apoplasmic structure as far as the root is concerned. The epidermis is the only portion of the hair root which is colonized. The identity of the colonized surface cell layer as an epidermis is distinguishable if it is traced back to the root apex either in longitudinal section or whole section (Smith and Read, 1997). At this site (colonized surface epidermis cells) it is seen to have common origin with the very small root cap, both which arise from a layer of meristematic cells quite distinct from that giving rise to the cortex. The difficulty of identifying the mycorrhizal colonized layer as epidermis has arisen because the suberized cortex usually attaches poorly and the cells collapse so that it is not easily distinguishable from cell layers in whole roots or freehand cross sections. Once within the root cell the fungus remains in the first colonized cell and does not spread to adjacent cells (Cairney and Ashford, 2002). The production of fungal mucilage is suppressed once the hypha is in the root cell. The fungus does, however, continue to secrete wall material, as demonstrated by wheat germ agglutinin staining of N-acetyl-glucosamine residues of chitin (as well as other wall polysaccharides) in the region around the hyphal profiles of epidermal coils (Bonfante-Fasolo et al., 1987); Perotto et al., 1995). An electron-lucent gap containing dispersed material separates the fungal wall from the invaginated plant plasma membrane as in most electron microscopy images of ericoid mycorrhizas (Allen et al., 1989; Briggs and Ashford, 2001). The region has been termed an “interfacial matrix” (Smith and Read, 1997). The “interfacial matrix” is clearly the site across which nutrient exchange will occur and so it is important to know its structure and dimensions,

but it is not fully understood to what extent the gap is in artifact of specimen preparation (Cairney and Ashford, 2002).

CHAPTER III

MATERIALS & METHODS

Site Description

This study was conducted on a 134 m² field plot located on the North Mississippi Research and Extension Center in Verona, MS (34˚16' 31"N, 88˚72'42"). The site was selected because of the well-drained soil and accessibility to an irrigation system. Soil of the selected site is a Ora fine sandy loam (Fine-loamy, siliceous, semi active, thermic Typic Fragiudults).

Prior to planting, the field plot was sub-soiled using a Paratill subsoiler (Bingham Brothers, Lubbock, TX) and then a hipper roller was used for bed preparation in December of 2011. Rabbiteye blueberry cultivars 'Tifblue' and 'Climax' were planted into two rows, with each row consisting of a single cultivar, of 24 plants. Each row measured 120 ft. long with plants spaced 5 ft. apart within the row and spacing between rows was 12 ft. Rows were mulched after planting with shredded pine bark. Mulching each row was repeated in May after drip irrigation had been placed down each row. Blueberry plants were irrigated using 1/2" pressure compensating emitter tubing drip line irrigation (Rain Bird Corporation, Azusa, CA). Emitters in the drip line were spaced approximately 1 ft. apart and emitted 0.4 gallons per hour. Irrigation of the blueberry plants began in May of 2012 and was continued throughout the growing season and through September of 2012. Weekly irrigation was controlled by an automatic hose

faucet timer (Orbit Irrigation Products, Inc., North Salt Lake, UT). Plants were irrigated three times a week every other day for three hours. Each three hour irrigation session emitted 1.2 gallons of water per row foot.

Experimental treatments

Blueberry plants were obtained from Amber's Blueberry Farm in Waynesboro, Mississippi. Two cultivars were chosen with a total of 150 plants of each variety. The blueberry plants had been propagated from cuttings and replanted into one gallon black plastic pots containing a 1:1 ratio of pine bark to peat moss growing medium. The blueberry plants used in this study where approximately one year old at the time the plants were obtained. The blueberry plants remained in one gallon black plastic pots for approximately one month before being repotted into three gallon black plastic pots containing a growing medium with a 2:1:1 ratio of peat moss, perlite and sand. The two cultivars chosen to conduct the study were 'Tifblue' and 'Climax'. Both of which are rabbiteye blueberries (*Vaccinium ashei*) native to the southeast. Each one of these blueberry plants was then numbered and those numbers were then taken and drawn at random to be inoculated with one of four different inoculum solution/slurry treatments produced in this study. Blueberry plants used in this study were maintained outdoors at the Mississippi State University greenhouse, Mississippi State, MS from April of 2011 until planting December in of 2011.

Inoculum Production

Fungal isolates where acquired from American Type Culture Collection (ATCC, Manassas, VA). The isolates of ericoid mycorrhizal fungi chosen for the study were

Oidiodendron maius (ATCC 32425, isolated from soil in New York, USA), and *Pezizella ericae* Pearson et Read (ATCC 32985, isolated from ericaceous roots). The fungal isolate *Oidiodendron maius* was propagated in petri dishes containing Difco™ malt agar produced by Becton, Dickinson and Company (Sparks, MD). *Pezizella ericae* was also propagated in petri dishes, however, it was produced on Difco™ YM (yeast and mold) agar (Becton, Dickinson and Company). Agars for each isolate were chosen based on recommendations outlined in the product information sheet provided by American Type Culture Collection (ATCC). Isolates were plated on petri dishes containing their respective growth medium and then allowed to incubate at temperatures between 20-25˚C for approximately 25 days. After approximately 25 days, isolates were then transferred into a liquid culture medium to begin inoculum production. All transfers of isolates were performed aseptically and under a laminar flow hood to assure that the culture media would not be contaminated by outside organisms.

Liquid medium culture

Each isolate was grown in sterile liquid culture of modified Melins-Norkrans agar medium (Molina and Palmer, 1982) produced by Bio-world (Dublin, OH). Liquid inoculum was produced using two, 2000 ml capacity erlenmeyer flasks. Each 2000 ml erlenmeyer flask was filled with 1000 ml of modified Melins-Norkrans agar medium, covered with aluminum foil and then autocalved for 15 minutes at 121˚C. After autoclaving liquid medium was allowed to cool to 25˚C under a laminar flow hood. Once cooled to proper temperature, liquid medium in each flask was then inoculated with either *Oidiodendron maius* or *Pezizella ericae.* In addition to, the two, 2000 ml erlenmeyer flasks filled with 1000 ml of modified Melins-Norkrans agar, two additional 1000 ml

erlenmeyer flasks filled with 500 ml of modified Melins-Norkrans agar medium were also made and inoculated in the fashion as previously mentioned. This was done to insure that there would be enough inoculum solution to treat every plant.

Inoculation of liquid medium was carried out aseptically and under a laminar flow hood. Transfer of isolates to liquid medium was done by using a #11 cork borer to cut two circular shaped agar plugs from the fungal isolates growing in petri dishes. Plugs (two for 1000 ml of liquid medium and one plug for 500 ml of medium) from each isolate were then transferred to liquid medium using a sterile syringe needle. Once inoculated, the flasks were then recovered with the aluminum foil that was used earlier in the autoclaving process. Liquid medium cultures of each isolate were allowed to then incubate for approximately 30 days at 20-25˚ C. During this 30 day incubation period cultures were checked daily for contamination from outside organisms. Liquid cultures were agitated by hand every 6 days; this was carried out by vigorously shaking the flasks back and forth on the laboratory counter top for approximately 60 seconds. Agitation or shaking of liquid cultures was done for two reasons: the first being that the fragmentation from agitating the liquid cultures helped fungal mycelium reproduction and secondly to help aerate the liquid culture medium.

Inoculum Preparation

Inoculum was prepared the day of application. Inoculum was prepared by separately taking each of the isolates grown in liquid culture medium and fragmenting it in a surface sterilized blender on the high setting for 60 seconds. After being fragmented in the blender, 14 ml of the liquid culture medium containing the fragmented mycelium was transferred via a large bore pipette into 36 ml of distilled and deionized water in a 50

ml screw cap vial. The blender was triple washed with soap and distilled water and then surface sterilized with a 1:1 solution of ethanol and distilled water between each use. This step was taking so that inoculum would not be mixed unintentionally (adapted from Scagel, 2005)

Inoculum Treatments

A total of four different inoculum treatments were used in this study. The blueberry plants in this study were treated with 50 ml solution/slurry of one of two EMF isolates, a mixture of both of the EMF isolates, or 50 ml of distilled and deionized water (noninoculated control).

Leaf Sampling

Leaf samples were collected once a month from April of 2012 until July of 2012. Leaf samples consisted of fifteen mature leaves taken at random from each treated plant. Leaf samples were taken from three randomly selected replications each month and each plant in the replication was sampled. Samples were placed into paper bags and labeled by replication and plant position within the replication from which the sample was taken. This was done so that later the leaf samples could be identified and matched with the inoculum that had been applied. Leaf samples were then sent to the Mississippi State University Soil Testing Laboratory for tissue analysis.

Percent colonization

Approximately 20 lateral hair root segments, 1-2 cm long, were selected from each blueberry plant, stained and mounted on microscope slides as follows: Roots and soil were collected at 0- to 15-cm depth below the canopy of the blueberry bush using a

soil sampling probe. Samples were taken during the months of June and July, 2012; three replications from both cultivars were sampled each time with every plant number in the replication being sampled. Soil cores containing root material were then placed in plastic zip lock bags, labeled, and placed in a cooler with ice and transported to the laboratory. Samples were then stored at 4˚C until washed, cleared and stained. Samples were washed individually in distilled water; root masses from each sample were excised and placed in histology capsules (Sakura Tissue-Tek 4090, Sakura Finetec, Torrance, CA, USA), cleared for 2 days in 10% KOH at room temperature, acidified in 2% HCl for 5 minutes, and stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol, and distilled water) (adapted from Kormanik et al., 1980; Grace and Stribley, 1991; Brundrett et al., 1996). Roots were mounted using PVLG mountant (Cunningham, 1972) on microscope slides and covered with cover slips. One slide was prepared for each planted sampled and was treated as a single unit and not as a subsample. Roots were aligned parallel to the long axis of the slides and observed at magnification x400(adapted from McGonigle et al., 1990).

Statistical Analysis

Statistical analysis was conducted using SAS 9.2 (SAS Institute, Cary, NC). The GLMMIX procedure in SAS 9.2 was utilized for analysis. Cultivar, EMF isolate treatment and EMF isolate by month interaction were tested for their effects at all sampling dates

CHAPTER IV

RESULTS AND DISCUSSION

Root Colonization

Colonization of roots by ericoid mycorrhizal fungi in rabbiteye blueberry cultivars 'Tifblue 'and 'Climax' was not visible in the root samples taken during the months of June and July in 2012 (figure 4.1). It was evident that no hyphal coils were shown to be present in the roots samples that were cleared, stained, and mounted on microscope slides for visual assessment. Furthermore, roots of noninoculated plants showed no visual differences compared to the inoculated plants. In a survey of commercial blueberry nursery plants, Scagel et al. (2005b) found that within a stock type and sampling date, the greatest differences in colonization were found between the northern highbush and rabbiteye cultivars. Low colonization during nursery production of blueberry may also be due to cultural conditions that inhibit colonization of fungi that are present whether plants had been inoculated or not (Haynes and Swift, 1985; Johansson, 2000). Others have found that ericoid mycorrhizal fungi colonization of blueberry varies significantly with cultivar, and the amount and type of soil organic matter present in the soil (Czesnik and Eynard, 1990; Eynard and Czesnik, 1989; Golldack et al. 2001; Powell, 1982).

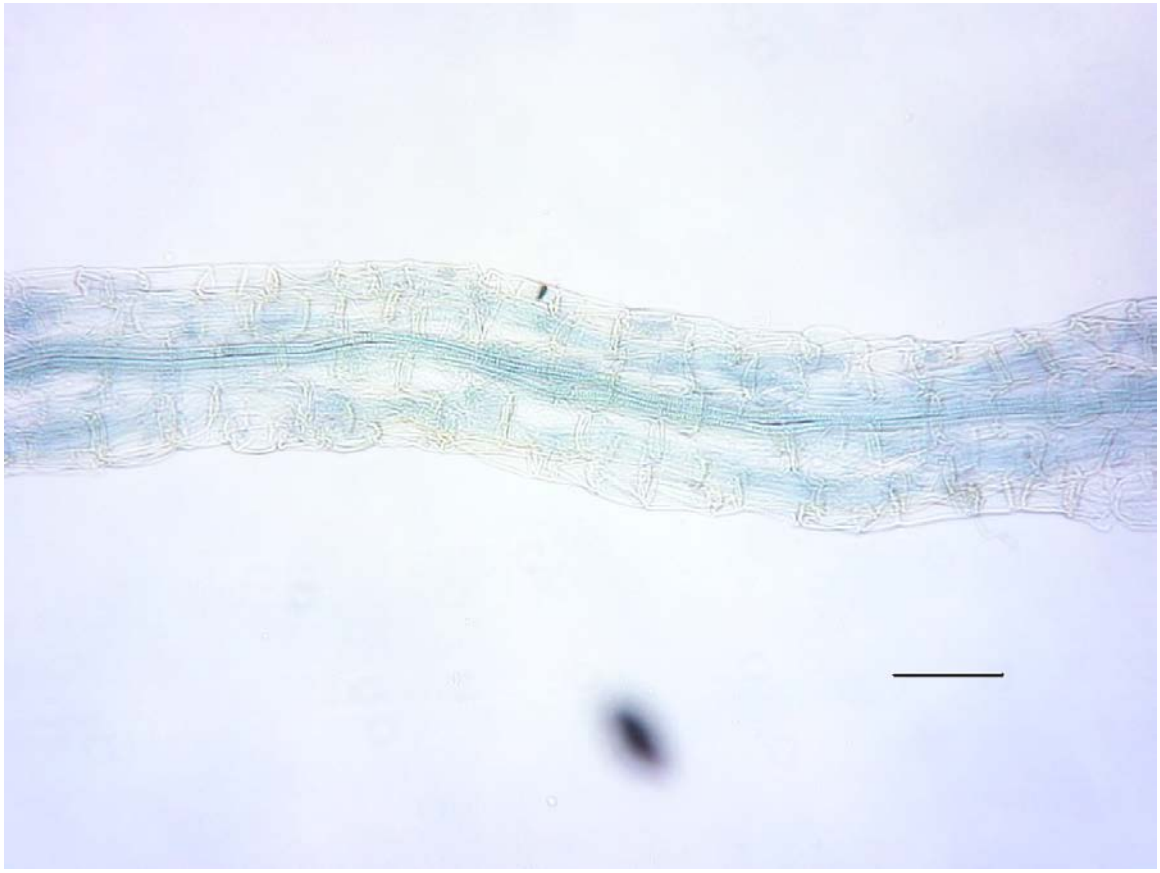

Figure 4.1 Root of rabbiteye blueberry (*Vaccinium ashei* 'Climax') stained with trypan blue. Scale bar = 35 μm.

Cultivar and Treatment Variationin Nutrient Concentration

Blueberry Fruit Yield by Cultivar and Treatment

The influence of EMF and cultivar on blueberry yield is shown in Table 4.1. A significant difference was found between cultivars. 'Tifblue' had a significantly greater yield compared to 'Climax'. However, treatments did not influence fruit yield. Higher blueberry fruit yield in 'Tifblue' is believed to be due to environmental factors and physiological traits or to genetic yield potential.

Nutrient Concentration in Leaf Tissue

The effect of cultivar on nutrient concentrations in leaf tissue is shown in Table 4.2. No significant differences were found between cultivars for nutrient concentration.

The effect of treatment on nutrient concentrations in leaf tissue is shown in Table 4.3. No significant differences were found between treatments for nutrient concentration.

Nitrogen Concentration in Leaf Tissue

Summary statistics for nitrogen concentration in leaf tissue of rabbiteye blueberry (*Vaccinium ashei* R.) are reported in Table 4.4. When analyzed as cultivar by month sampled, no significant difference in nitrogen concentration was found between the cultivar and the month in which the sample was taken. However, when treatments by month were analyzed there was a significant difference in nitrogen concentrations of leaf tissue between treatments and the month in which the sample was taken. Treatments were found to be significant during the months of April and June of 2012. However, this cannot be confirmed to be a direct interaction between the plant and the treatment because no percent of colonization could be visibly confirmed. Possible explanations suggest that the interactions taking place during the months of April and June are due to nitrogen mobilization. Nitrogen is a very mobile element within the plant. As older leaves yellow and die, the N is exported to younger developing leaves and shoots (Hopkins, 1995). It is, therefore, not surprising that N concentration with the leave tissue sampled fluctuated between the sampled months.

Table 4.1 Influence of ericoid mycorrhizal fungi (EMF) and cultivar on blueberry yield.

TREATMENT	YIELD (GRAMS)
Control	90.67a
PE & OM	65.78a
Oidiodendron maius (OM)	68.21a
Pezizella ericae (PE)	53.37a
CULTIVAR	
Tifblue	128.57a
Climax	10.45b

Means followed by the same letter are not significantly different, according to LSD at α =0.05.

Table 4.2 The effect of cultivar on nutrient concentration in leaf tissue of rabbiteye blueberry (*Vaccinium ashei)*

Cultivars	N	P	K	CA	MG	SU	MN	ZN
	---------- Percent ----------						------ PPM ------	
Climax	1.85a	.09a	.44a	.54a	.19a	.09a	208.5a	23.1a
Tifblue	1.72a	.09a	.44a	.46a	.17a	.08a	164.6a	21.3a

Means followed by the same letter in cultivar are not significantly different, according to LSD at α=0.05.

Table 4.3 The effect of treatment on nutrient concentration in leaf tissue of rabbiteye blueberry (*Vaccinium ashei)*

Treatment	N	P	K	CA	MG	SU	MN	ZN
	----------Percent----------						--------PPM-------	
Control	1.74a	.09a	.44a	.52a	.18a	.09a	202.5a	22.5a
Oidiodendron maius & Pezizella ericae	1.87a	.1a	.42a	.52a	.19a	.09a	189.0a	23.1a
Oidiodendron Maius	1.82a	.09a	.46a	.45a	.16a	.09a	174.8a	21.9a
Pezizella ericae	1.72a	.09a	.45a	.51a	.18a	.08a	177.7a	21.3a

Means followed by the same letter in treatment are not significantly different according to LSD at α=0.05.

Table 4.4 Nitrogen concentration of leaf tissue of rabbiteye blueberry (*Vaccinium ashei*) as influenced by month, cultivar, and treatment

Cultivar	April	May	June	July
Tifblue	1.64ns	1.74ns	1.73ns	1.79ns
Climax	1.68ns	1.95ns	1.92ns	1.87ns
Treatment				
Control	1.45c	1.75ns	1.96a	1.82ns
Oidiodendron maius & Pezizella ericae	1.88a	2.03ns	1.73b	1.84ns
Oidiodendron maius	1.73b	1.83ns	1.91b	1.81ns
Pezizella ericae	1.57b	1.77ns	1.69c	1.86ns

Nonsignificant (ns) or significant at α=0.05 as indicated by small letter

CHAPTER V

SUMMARY AND CONCLUSIONS

Inoculation of rabbiteye blueberry (*Vaccinium ashei*) cultivars 'Tifblue' and 'Climax' with *Oidiodendron maius* and *Pezizella ericae* showed no differences in fruit yield, leaf tissue nutrient concentration, leaf tissue nitrogen concentration, or percent root colonization throughout the study. Although differences in blueberry yield between the two cultivars is apparent, this is most likely due to the differences between the two cultivars physiology and genetic potential. No direct correlation is apparently definitive to suggest that the inoculum treatments had any measureable effect on fruit yield or nutrient concentration within leaf tissue.

The month to treatment interaction that was found can most likely be attributed to the mobilization of nitrogen within the plant and not a treatment effect, since nitrogen is a very mobile element within the plant. As older leaves yellow and die, the N is exported to younger developing leaves and shoots (Hopkins, 1995). Furthermore, it is possible that the absence of EMF measured in this study was due to cultural conditions and practices that inhibit colonization. Absences of colonization may also be related to seasonal changes in plant development and plant age (Scagel, 2005). Absence of EMF colonization could also be the result of seasonal variation in root growth of the different cultivars. These results suggest that the two EMF isolates used in this study *Oidiodendron maius* and *Pezizella ericae* do not form symbiotic relationships with

rabbiteye blueberry (*Vaccinium ashei* R.) cultivars ‘Tifblue’ and ‘Climax’ when inoculated with inoculum slurry in a nursery production system and transplanted to the field. Furthermore, specific plant age and fungal isolate specificity may be factors which prevent fungal plant interaction to take place.

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