#### ORGANIC SOUTHERN HIGHBUSH BLUEBERRY PRODUCTION IN HIGH TUNNELS

by

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(Under the Direction of MARC VAN IERSEL)

#### ABSTRACT

Growers interested in producing early high quality yields of southern highbush blueberry (*Vaccinium corymbosum* L. x *darrowi*) in high tunnels face a lack of information regarding appropriate cultural methods. We sought to elucidate the optimal date to close the plastic covers over high tunnel blueberry plantings to hasten vegetative and reproductive growth of organic southern highbush blueberry. We also investigated inorganic nitrogen release from an organic fertilizer in a pine bark medium. High tunnels raised soil and daytime air temperatures during winter months, but the tunnels did not retain heat during night-time and did not provide freeze protection without the use of propane heaters. Initiation of flowering was advanced by 38 d in 'Emerald' and 39 d in 'Jewel' with the earliest tunnel closure date as compared to outdoor control plants averaged over the two year study. A late closure date (Jan 16), cultivars with short fruit development period (80-90) days, and careful tunnel management practices regarding freeze protection and pollination are recommended. Understanding nitrogen processes in pine barks can assist growers in nutrient management.

INDEX WORDS: blueberries, high tunnels, freeze protection, organic fertilizer, pine-bark beds, protected culture

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#### CHAPTER 1

#### INTRODUCTION AND LITERATURE REVIEW

High tunnels lend themselves nicely to organic horticultural practices and are quickly becoming indispensable components of many organic production systems (Lamont Jr, 2009). Success in out-of-season production of vegetables in high tunnels has spurred research into small fruit production in high tunnels (Demchak, 2009). Blueberries are an economically significant crop for Georgia. Production of out-of-season organic blueberries in high tunnels could be a financially lucrative endeavor for Georgia's growers. Overhead irrigation for freeze protection is typically used in South Georgia with early bloom southern highbush, but water resources are more limited in north Georgia. A key facet to determine in the development of best management practices for blueberries in high tunnels is to determine when to apply the plastic covers over the tunnels. This project sought to determine the optimal time to close high tunnels by experimenting with three different closure dates. This helped to determine what time is optimal for achieving the largest and highest quality crop. Since this was the first attempt at growing blueberries in high tunnels in Georgia, this work contributed to the development of best management practices for this type of production system. The plants were grown in a ground pine-bark medium. Little research has been conducted on the behavior of organic fertilizers in pine bark beds. Thus, as a second goal, this project examined nitrogen transformations occurring in the growth medium. These findings could assist growers to develop appropriate fertilization regimens for organic blueberry production.

This chapter provides an overview of what is currently known about 1) blueberry production 2) high tunnels 3) blueberry cultivation in high tunnels and 4) quantification of N mineralization in pine bark medium.

Chapter 2 describes the environmental conditions generated as result of the high tunnels and their effects on vegetative growth rates and flower initiation and development rates.

Chapter 3 describes the impacts of the high tunnels on yield, relative distribution of yield over time, and fruit quality characteristics such as fruit size, soluble solids, and anthocyanin content.

Chapter 4 describes the research conducted on N mineralization from an organic fertilizer in pine bark beds. Data from a field experiment using cups and resin exchange beads is discussed as well as an incubation experiment that was conducted in a controlled environment

Chapter 5 provides concluding remarks.

#### **Blueberry horticultural information**

Southern highbush blueberries (*Vaccinium corymbosum* L.) belong to the Ericaceae family, a large family of acid-soil-loving woody shrubs and trees. Southern highbush blueberries, are derived primarily from combinations of *Vaccinium corymbosum* L. (northern highbush), *Vaccinium astrale* Small and *Vaccinium darrowii* (Darrow's evergreen blueberry) (Brevis et al., 2008) . Georgia's blueberry plantings consist largely of rabbiteye blueberry (*Vaccinium ashei* Read). The Florida and Georgia southern highbush plants tend to have a lower chilling requirement than rabbiteye and require soils with a high level of organic matter to achieve satisfactory yields (Gough, 1994).

Demand for blueberries in the U.S and abroad has grown in recent years and has encouraged further plantings of this economically important horticultural crop. From 1996 to 2006, the area planted to blueberries in North America increased by 30%. Experts predict that the demand for blueberries will continue to grow and that the area planted to southern highbush berries will increase by 35% in the next ten years (Strik, 2006). The U.S currently leads worldwide production of blueberries with 55% of the world's production. Maine and Michigan produce the bulk of the berry crop, providing over 50% of US production. Forty percent is produced by New Jersey, Oregon, Georgia, and North Carolina. Smaller plantings in Indiana, New York, Alabama, Arkansas, and Florida contribute the remaining 10% (Fonsah et al, 2004).

Forcing blueberry into early production requires knowledge of its life cycle. This crop undergoes a period of dormancy in response to shortened photoperiods and low temperatures (Darnell, 1991). Prior to entering dormancy, buds are set which will provide the next year's flowering and vegetative growth. Chilling hours (temperatures below 7.7 °C) are required for normal development in the spring. Chilling requirements in southern highbush blueberry for flowering are documented and range from 200 to 800 hours (Gough et al., 1976; Spiers, 1976; Spiers and Draper, 1974). Once chilling requirements are met, the next requirement for growth and development is heat and is commonly calculated as growing degree days or heat units (Carlson and Hancock, 1991). By raising ambient air temperature, high tunnels rapidly accumulate growing degree days. The high day-time temperatures inside of high tunnels should encourage early rapid reproductive and vegetative development. Among the various environmental factors affecting blueberry growth and development, ambient air temperature is considered the most important High tunnels are known to increase air temperatures by 5-15 °C above ambient conditions (Kadir et al., 2006), which should advance growth and development of southern highbush blueberry crops during winter and early spring, when ambient temperatures may be suboptimal.

Research indicates that anthocyanin, a flavonoid molecule that is responsible for the blueberry's distinctive color, contributes significantly to blueberry's antioxidant properties (Beccaro et al., 2006; Moyer et al., 2002). Beccarro et al., 2006 reported a positive ( $r^2$ =0.88) correlation between total monomeric anthocyanin content and ferric-reducing antioxidant power (FRAP). Prior et al., (1998) noted a similar trend between anthocyanin content and oxygen radical absorbance capacity (ORAC).ORAC and FRAP are two commonly used indicators of the antioxidant strength of fruits and vegetables. They also reported that blueberries have high ORAC levels compared to many other fruits and vegetables. Considerable variation exists among blueberry species in antioxidant strength with the highest levels found in wild growing, lowbush plants (*Vaccinium angustifolium* Ait), bilberry (*V. myrtillus* L.), and rabbiteye (*V. ashei* Reade) (Prior et al., 1998). We selected anthocyanin content and soluble solids as important fruit quality characteristics and sought to discover what effect closure date of high tunnels has on their levels in southern highbush blueberries. Soluble solids indicate sugar levels which contribute to the sweet flavor of the blueberry.

#### Out of season fruit production

Driving the desire to produce out of season blueberries is the volatility of blueberry prices resulting from changes in supply. Berries that can reach the market one week to one month earlier than the bulk of the harvest will receive a premium price. As the harvest season progresses, prices of blueberries drop steadily (Williamson and Lyrene, 2004a). Since organic produce also receives a premium price, organic berries produced out of season will receive a very high price.

Positive findings have been made for high tunnel cultivation of small fruits such as strawberries, raspberries, and blackberries (Gaskell, 2004; Kadir et al., 2006; Kempler, 2002;

Xiao et al., 2001). California has seen a recent increase in acreage dedicated to high tunnel grown raspberries that reach the markets during periods of low production (Gaskell, 2004). Kadir et al. (2006) reported superior yields and quality of high tunnel grown strawberries as compared to field grown (Kadir et al., 2006). European raspberry growers also use high tunnels to produce high quality fruit for the fresh market (Gaskell, 2004).

#### High tunnel background

High tunnels differ from plastic greenhouses on several key points. First, high tunnels are typically covered with only a single layer of polyethylene rather than the two layers employed in most plastic-covered greenhouses. Secondly, they generally lack electricity so ventilation is achieved through the use of roll-up sidewalls, ventilation doors located at the ends of the tunnel, or passive roof ventilation. Although normally lacking a permanent heating system, portable heaters are often used to provide frost protection (Lamont et al, 2005). High tunnels are used widely throughout the Mediterranean, Japan, and Western Europe and are now gaining popularity in the U.S. (Lamont et al, 2005).

Research has documented many advantages of high tunnel cultivation. Early crop production (7 d to 21 d earlier than field), high yields per unit area (2 to 3 times higher than field) and production of cleaner and higher quality products are a few obvious advantages. (Lamont Jr, 2005). Less obvious are advantages such as reduced soil erosion, efficient water and fertilizer use, reduced weed pressure due to exclusion of weed seeds, and reduced pest pressure (Lamont Jr, 2005). High tunnels raise air (by 10 to 20°C (Kadir et al., 2006)) and soil temperatures (by 4 to 8°C (Reiss et al., 2004)) above ambient conditions. This warming of air and soil temperatures is what enables out-of-season production of many horticultural crops. Success with vegetables, such as tomato, melons, peppers, and eggplant, sparked interest in high tunnel production of small fruits (Waterer, 2003).

Blueberry (*Vaccinium* spp) is a high-value crop, particularly when grown organically and produced during periods of low production (Williamson and Lyrene, 2004a). Preliminary findings suggest that high tunnels could be used to promote early production of southern highbush blueberry (*Vaccinium corymbosum* L) (Bal, 1996; Baptista et al., 2006; Hicklenton et al., 2004; Ozeki and Tamada, 2006). However, many questions remain unanswered regarding optimal dates to close and open high tunnels, temperature management strategies, and region specific cultivar selection.

#### Blueberry production in high tunnels

Several proceedings papers document tentative positive findings regarding forcing of early blueberry flowering and fruiting in high tunnels. Ciordia et al. (2002) grew containerized blueberry plants inside of high tunnels in Northern Spain. They found that the southern highbush cultivar 'Bluetta' performed optimally in their climate. This cultivar fruited a full three weeks earlier than its outdoor counterparts and maintained a high level of fruit quality throughout the duration of the harvest.

A second study conducted by Renquist (2005) in Douglas County, Oregon also examined the feasibility of growing highbush blueberries in high tunnels. In Oregon, the vast majority of the blueberry harvest occurs within a 45-day period between July 1 and August 15. The inundation of berries into the market causes the price to fall from \$2.42-\$2.64 in June to just \$1.54 per kilogram in July. The primary goal of this study was to compare different cultivars' responses to the high tunnel treatment. Across cultivars, harvest inside the tunnels occurred 1 to 3 weeks earlier than in the field. Two relevant conclusions were drawn from this study. First, each blueberry cultivar will respond slightly differently to being grown in tunnels. Second, the response of the crops to the tunnel treatments is affected greatly by variations in the yearly weather patterns (Renquist, 2005). These findings underscore the need for an enhanced information base regarding blueberry production in high tunnels.

A third study conducted by Bal (1996) in the Netherlands examined high tunnels for highbush blueberry production. The earliest berries were grown inside of heated greenhouses and fruited a full 2 months before field plants did. This indicates that flowering in highbush blueberry is primarily regulated by temperature and not by photoperiod. These researchers gained a 5-6 week advance in the ripening of fruits grown inside of unheated high tunnels. Inter-cultivar variation in earliness in responses to treatments was also observed (Bal, 1996). Many questions remain unanswered regarding optimal dates to close and open high tunnels, temperature management strategies, and region-specific cultivar selection.

#### Nitrogen processes in pine bark beds

Growing blueberries organically in pine bark beds inside of high tunnels represents a novel growing technique and more research is needed to develop accurate fertilizer recommendations. Organic fertilizers typically contain only small amounts of readily available nutrients for plant growth. Rather, mineral elements are often part of organic molecules that require microbial interactions to transform them into forms that plants can assimilate. The situation is further complicated by the high C:N ratio of pine bark media which can lead to microbial immobilization of soil nitrogen reserves (Havlin et al., 1999). Maintaining adequate nitrogen nutrition is critical to successful southern highbush blueberry production (Kozinski, 2006). Blueberry fertilization recommendations average 90 to120 kg/ha N per year (Kuepper

and Diver, 2004) but Williamson (2009) reported positive effects on yield and vegetative development with rates up to 365 kg/ha N per year for plants grown in pine bark beds.

Nitrogen cycling is complex and key processes, including mineralization, nitrification, immobilization, leaching, and de-nitrification, must be considered when developing a fertilizer regimen for organically grown blueberries in pine bark beds inside of high tunnels. One implication of high tunnel agriculture is the potential for a reduction in leaching and run-off of fertilizer due to better controlled watering. This could be advantageous to the grower in terms of reduced need for fertilizer application or detrimental due to build-up of salts if fertilizer application rates are not adjusted properly.

Mineralization is the conversion of nitrogen in organic molecules into ammonium ( $NH_4^+$ ) by soil microbes. Conversely, soil microbes may immobilize  $NH_4^+$  while breaking down organic residues with a high C:N ratio in a process called nitrogen immobilization. Nitrification is the conversion of  $NH_4^+$  into nitrate ( $NO_3^-$ ) by the soil bacteria *Nitrosomonas* and *Nitrobacter*. Nitrification is believed to be inhibited by low soil pH, particularly in clay soils containing aluminum and iron oxides (Lambers et al., 1998). To the contrary, Booth et al. (2005) reviewed over 100 studies reporting nitrification rates in a wide variety of soil types and found no correlation between pH and nitrification rates. In fact, soils that were high in organic matter (4-5%) and low in pH (<5) exhibited high rates of nitrification (Booth et al., 2005). Organic matter mitigates inhibition of nitrification to pH is likely to be different in soil and soil-less growing media. Thus, it appears that nitrification in acidic clay soils is inhibited by aluminum and iron oxides, rather than by the low pH itself (Haynes et al., 1986). Sources of nitrogen losses from soil include plant uptake, leaching, whereby the anion  $NO_3^-$  passes through the soil profile and

enters the subsoil where it may enter underground water supplies, and denitrification whereby soil nitrogen transforms into gaseous nitrogen forms (NO, N<sub>2</sub>O, N<sub>2</sub>) under hot, wet, anaerobic conditions (Havlin et al., 1999).

Aged ground pine bark provides an optimal growing environment for highbush blueberry for several reasons. First, blueberry thrives at a pH of 4.5 to 5.5 which is much lower than typical horticultural and agronomic crops. Aged pine bark usually has a pH between 4.5 to5. Second, blueberries require much greater levels of organic matter than most other crops and thrive with organic matter contents of 4 to 6% (Kuepper and Diver, 2004). Third, the porous nature of the bark enables it to drain well and provides a suitable environment for the fibrous blueberry roots. Since their roots lack root hairs, blueberry plants typically depend on ericoid mychorrhizal fungi to assist them in nutrient acquisition. Mychorrhizal growth is encouraged in growth media with high organic matter contents (Kuepper and Diver, 2004). Although pine bark maintains a relatively high cation exchange capacity (CEC, 10 to13 meq/100g), its ability to trap anions (anion exchange capacity) is poor (Krewer and Ruter, 2005). Thus, one would expect to find a greater amount of the anion NO<sub>3</sub><sup>-</sup> leaching from pine bark beds than the cation NH<sub>4</sub><sup>+</sup>. Little is known about the fate of organic fertilizers when added to aged pine bark beds.

The transformations occurring with soil nitrogen have implications for plant growth as well. Most plants uptake nitrogen as either  $NH_4^+$  or  $NO_3^-$ . Ericaceous plants like blueberry occur naturally on acidic soils in which  $NH_4^+$  is believed to be the predominant N form. This fact, combined with the low levels of nitrate reductase activity (an enzyme needed to reduce  $NO_3^-$  to  $NH_4^+$  within the plant) found in some Ericaceous plants, led researchers to conclude that Ericaceous plants preferentially uptake  $NH_4^+$  over  $NO_3^-$  (Kuepper and Diver, 2004; Lambers et al., 1998; Magalhäes and Huber, 1989). Early studies designed to test this hypothesis provided

evidence to support this hypothesis (Cain, 1952; Colgrove and Roberts, 1956; Spiers, 1978). Merhaut and Darnell (1996) reported no difference in vegetative growth in blueberry between plants fertilized with  $NH_4^+$  or  $NO_3^-$ , despite increase in effluent pH to 6 in the  $NO_3^-$  fertilized plants. Active nitrate uptake requires the simultaneous uptake of protons, causing an increase in soil pH that could be detrimental to blueberry root and shoot growth. Other studies have suggested that when pH of growing medium is tightly monitored, blueberry plants will uptake nitrogen and develop at similar rates regardless of the form ( $NO_3^-$  or  $NH_4^+$ ) applied (Ingestad, 1976; Peterson et al., 1988).

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#### CHAPTER 2

# HIGH TUNNEL MICROCLIMATE AFFECTS GROWTH AND DEVELOPMENT OF SOUTHERN HIGHBUSH BLUEBERRY

#### Abstract

Growers interested in producing early high quality southern highbush blueberries (Vaccinium corymbosum L.) in high tunnels face a lack of information regarding appropriate cultural methods. We sought to elucidate the optimal date to close the plastic covers over high tunnels to hasten vegetative and reproductive growth of organic southern highbush blueberry. December 15, Jan 2, and Jan. 16 were the three dates selected to close the high tunnels. High tunnels raised soil and daytime air temperatures during winter months, but the tunnels did not retain heat during night-time and did not provide freeze protection without the use of propane heaters. Initiation of flowering was advanced by 38 d in 'Emerald' and 39 d in 'Jewel' with the earliest tunnel closure date as compared to outdoor control plants averaged over the two year study. The two cultivars 'Emerald' and 'Jewel' differed in their date of first flower and in the days required from first flower to fruit ripening. Flowering of these cultivars was poorly synchronized in 2007 and much better in 2008. However, no fruit ripened in 2008 due to an unexpected freeze. Higher light interception by plants in high tunnels indicates the earlier vegetative growth compared to control plots. Cost-effective freeze protection methods and proper cultivar selection are critical to successful production of early southern highbush blueberries in high tunnels.

#### Introduction

In recent years many growers have sought methods to enhance out of season production of fruits and vegetables. Growers and researchers seek cultural methods which modify microclimatic conditions and enable season extension. High tunnels represent one such technology currently being explored for a wide variety of horticultural crops. A high tunnel is a passively ventilated plastic greenhouse covered with a single layer of polyethylene (Lamont Jr, 2009, Waterer, 2003).

Research has documented many advantages of high tunnel cultivation. Early crop production (7 d to 21 d earlier than field), high yields per unit area (2 to 3 times higher than field), and production of cleaner and higher quality products are a few obvious advantages. (Lamont , 2005). Less obvious are advantages such as reduced soil erosion, efficient water and fertilizer use, reduced weed pressure due to exclusion of weed seeds, and reduced pest pressure (Lamont, 2005). High tunnels raise air [by 10 to20°C (Kadir et al., 2006)] and soil temperatures [by 4 to8°C (Reiss et al., 2004)] above ambient conditions. This warming of air and soil is what enables out-of-season production of many horticultural crops. Success with vegetables, such as tomato, melons, peppers, and eggplant, sparked interest in high tunnel production of small fruits (Waterer, 2003).

Positive findings have been made for high tunnel cultivation of small fruits such as strawberries, raspberries, and blackberries (Demchak, 2009; Gaskell, 2004; Kadir et al., 2006; Kempler, 2002; Xiao et al., 2001). California has seen a recent increase in acreage dedicated to high tunnel grown raspberries that reach the markets during periods of low production (Gaskell, 2004). Kadir et al. (2006) reported superior yields and quality of high tunnel grown as compared to field grown strawberries (Kadir et al., 2006). European raspberry growers also use high tunnels to produce high quality fruit for the fresh market (Gaskell, 2004).

Blueberry (*Vaccinium* spp) is a high value crop, particularly when grown organically and produced during periods of low production (Williamson and Lyrene, 2004a). Previous findings suggest that high tunnels could be used to promote early production of southern highbush blueberry (*Vaccinium corymbosum* L.) (Bal, 1996; Baptista et al., 2006; Ciordia et al., 2002; Hicklenton et al., 2004; Ozeki and Tamada, 2006). They document early blueberry flowering and fruiting in high tunnels. Ciordia et al. (2002) reported a three week advancement in the ripening of containerized "Bluetta' plants grown in high tunnels. Baptista et al., 2006) reported a difference of 34 d in date of floral budbreak amongst 4 southern highbush cultivars grown inside of high tunnels. Renquist (2005) reported earliness of 1 to3 weeks inside of high tunnels, but that earliness ranged from seven to 21 days depending on cultivar.

Bal (1996) examined several different climate modifying technologies for highbush blueberry production in the Netherlands. Through careful cultivar selection and micro-climate enhancement he extended the blueberry season from 8 to 10 weeks to approximately 5 months. The earliest berries were grown inside of heated greenhouses and fruited a full 2 months before field plants. Ripening was advanced 5 to 6 weeks inside of unheated high tunnels. A delay in harvest date was achieved by spreading plastic rain-covers over the plants just before the berries start to change color. The rain cover slowed down the ripening process and fruits could be harvested two to three weeks later than field plants. Questions remain unanswered regarding optimal dates to close and open high tunnels, temperature management strategies, and regionspecific cultivar selection. These findings underpin the need for further knowledge regarding out of season blueberry production in high tunnels.

We explored the feasibility of production of southern highbush blueberry in high tunnels in the Southeastern United States. High tunnels will likely affect the growth and development of southern highbush blueberry and may advance the onset of flowering, speed floral and fruit development, and encourage early vegetative growth. The aim of the study was to determine the optimum tunnel closure date for generating large, early yields.

#### Materials and Methods

*Plant materials and site preparation.* A site was selected on the University of Georgia's certified organic farm located on the Horticulture farm in Watkinsville, GA. Eight plots  $(6 \text{ m} \times 12 \text{ m})$  spaced at intervals of 7 m were leveled. Clear plastic (5 mil) was then stretched over each plot for 15 d for solarization purposes to kill weeds and weed seeds. Six plots were selected to contain the high tunnels and two were designated as outdoor controls. The metal frames of the tunnels, 4.9 m x 11 m (Atlas Greenhouses, Alapaha, GA), were installed in the fall of 2006. Tunnels were designed with sidewalls that open to 1.5 m high to facilitate heat removal and ventilation.

The plots were then cultivated with a mechanical tiller to suppress weed growth. Two raised beds of ground, aged pine bark,  $(1.8 \text{ m} \times 9.5 \text{ m} \times 20 \text{ cm})$  were established within each plot. Planting occurred Nov. 1-7 2006. Each bed contained 20 plants (1 row of 10 'Emerald' and 1 row of 10 'Jewel' plants) for a total of 40 plants per tunnel. Plants were configured in a high density arrangement with 1 m between plants and 75 cm between staggered rows.

Fertilization was provided with a 5N-1.31P-3.32K organic granular fertilizer (5-3-4 McGeary Organics, Lancaster, PA) based on an application of 467 kg N/broadcast ha/year divided amongst five application dates. This high rate was used because of uncertainty regarding the release of mineral N from the organic fertilizer and the possibility of significant N

immobilization within the pine bark beds. Irrigation was provided with micro-jet sprinklers (54 L/hour) placed every 2 m in each bed. One hour of irrigation, three days per week, provided adequate moisture levels throughout fall, winter, and spring (76 L/m<sup>2</sup>/week). During summer months, irrigation was increased to 2 hours per week (152 L/m<sup>2</sup>/week). Frost protection on nights expected to be below 0°C was provided with portable propane heaters (Dayton 3VE42, 2344 W, Dayton, Detroit, MI) placed inside each tunnel. Pollination was facilitated by the introduction of colonies of bumblebees (Minipol, Class C hive, Koppert Biological Systems, Romulus, MI) into each tunnel upon the emergence of open blueberry flowers.

The four treatments consisted of three different times at which the tunnels were covered (Dec. 15, Jan. 2, and Jan. 16) with a single layer of 6 mil polyethylene greenhouse plastic (K50 clear, Klerks Hyplast Inc., Chester, SC) and a control which received no tunnel. Each treatment was replicated twice. Tunnel sidewalls were manually opened when outdoor temperatures exceeded approximately 16°C to facilitate heat removal. On May 15<sup>th</sup> of both years high tunnel sidewalls were opened and remained open for the duration of the growing season.

#### Data collection

*Environmental conditions.* Dataloggers (Hobo U-12, Onset Computer Corp., Bourne, MA) in radiation shields at 24 cm above the pine bark beds, recorded air temperature at the bottom of the canopy at hourly intervals in each plot. Attached soil temperature probes (TMC-1HD soil temperature sensor, Onset Computer Corp.) recorded pine bark bed temperature at 10 cm depth. Daily maximum and minimum values for each measurement were calculated.

*Flower developmental data.* In each plot, 5 plants of each cultivar were randomly selected. A branch section consisting of 3 inflorescences was then delimited. Inflorescences were observed once or twice per week and each flower was assigned a number based on its stage of

development. The numbering system for the flower and fruit development is based on the system of stage development identification developed by Michigan State University. Stage 1 occurs when individual flowers can be differentiated. Stage 5 occurs when the petals abscise from the ovary (normally 4 to7 days after anthesis (DAA) and stage 8 is a ripe fruit (Longstroth, 2002). Date of stage 1, date of stage 5, date of stage 8, and the number of days between these stages were determined.

*Vegetative growth.* Light interception was used as a measure of vegetative growth. Six random sites were chosen in each plot. Areas near end walls were excluded to avoid interference caused by the high tunnels structure. At solar noon on clear days (minimum photosynthetic photon flux (*PPF*) of 900  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>), a 1-m long line quantum sensor (LI-191, Li-Cor, Lincoln, Nebr.) was used to measure the *PPF* level above and below the plant canopy and the percent of light intercepted by canopy was calculated.

*Experimental design and statistical analysis.* The study employed a completely randomized design and a split-plot with four main treatments (tunnel closure date) and two replicates. The split in the design was the use of two cultivars within each tunnel. Treatment effects were analyzed using ANOVA (SAS 9.0, SAS Institute, Cary, NC). When ANOVA indicated significant effects (P < 0.05), means were separated using Duncan's multiple range test.

#### Results

*Environmental effects.* The high tunnels affected air and soil temperatures similarly in both years. Since temperatures in tunnels that were closed later quickly resembled those in earlier closed tunnels, only data from the 2006-2007 growing season and from the control and Dec. 15 treatments are shown. Maximum daily air temperature was consistently increased in all tunnels by 3 to15 °C, as compared to the outdoor control plot (Fig. 2.1). After opening tunnel sidewalls

May 15, air temperatures inside tunnels equalized with outdoor temperatures. Daily minimum air temperatures were not raised and temperatures inside tunnels actually dropped below ambient conditions on numerous nights (Fig. 2.1). Propane heaters successfully provided frost protection as they raised air temperatures within the tunnels 5 to 6 °C above ambient conditions. Daily minimum soil temperatures were increased by all tunnel treatments. Warming of the soil by 5 to 7 °C occurred within one to two weeks of tunnel closing. Tunnel soil temperatures remained 2 to 8 °C warmer than control plots throughout January, February, and March. In April, the control soil temperatures started to increase and equalized with tunnel soil temperatures by mid-May. During summer months (June-August), soil temperatures in control plots were 2-5 °C higher than in the tunnels (Fig. 2.2).

2007 was a difficult year for many fruit growers in the Southeastern US. A very warm March was followed by hard freezes on April 6 and 7 (-5°C) in an event known as the Easter freeze of 2007 (Warmund et al., 2008). These freeze events destroyed 100% of the developing fruit in the control plots, while the tunnel grown berries survived due to heating of the tunnels with propane heaters.

*Flower development*. There was an interactive effect of tunnel closure date and cultivar on the date of stage 1 in 2007 and 2008. In 2007 'Emerald' plants flowered earliest (Jan. 12) in the tunnels closed on Dec. 15 and Jan. 2 as compared to Jan 31 in outdoor control plots; an advancement of 19 days. Date of first 'Emerald' flower in the Jan. 16 closure treatment was not significantly earlier than in control plots. 'Jewel' flowered later than 'Emerald' in 2007 in all treatments by 30-50 d. Date of first 'Jewel' flower was advanced by 28 to 36 d in all tunnel treatments as compared to the control (Fig. 2.3).

In 2008, the Dec. 15 tunnel closure generated earlier 'Emerald' flowering than the control by 17 d, but stage 1 occurred later than in 2007 (Feb. 6 compared with Jan. 12). Date of first 'Jewel' flower was advanced by 20-24 d in all tunnel treatments as compared to the controls. The large difference in the date of stage 1 between the two cultivars in the first year (36 d) was reduced to 7 d in 2008 (Figure 2.3).

Treatment effects were observed in both years on the date of stage 5, just after petal drop and prior to fruit expansion. In 2007, 'Emerald' reached stage 5 in the Jan. 2 and Dec. 15 treatments 44 and 38 d earlier than the control, respectively. Although the date of first of flower for 'Emerald' was not earlier than the control in the Jan. 16 treatment, stage 5 was advanced by 30 d. Advancement of stage 5 was achieved in 'Jewel' with all tunnel treatments reaching stage 5 22-29 d earlier than control plots (Figure 2.3). In 2008 both cultivars also reached stage 5 earlier than their outdoor counterparts. In 'Emerald' date of stage 5 in the Dec 15 and Jan 2 treatments occurred at Julian day 57 and 60, respectively. This was significantly earlier than the Jan. 16 treatment (day 71) which also differed from the control (day 81) (Fig. 2.3).

In 2007, the two cultivars showed different fruit development periods (days stage 1-8). 'Emerald' required approximately 105 d to develop from a stage 1 flower to a ripe fruit (stage 8) while 'Jewel' required approximately 80 d (Figure 2.4). During 2008, fruit set was not achieved on tagged inflorescences (data not shown). In 2008, flower development never passed stage 5 within the tunnels. On March 25, the minimum temperatures dropped well below the predicted minimum (3 °C) and reached -5 °C inside of the high tunnels. Because the predicted minimum was 3 °C, the heaters were not turned on. Most flowers inside the tunnels were at stage 5 at that point, which is the most vulnerable stage for freeze damage, and fruit development within the

tunnels stopped at this point. Although bumblebee activity was very low in 2008, freeze damage appears the likely reason for a lack of fruit set.

*Vegetative development*. In 2007, there was no interactive effect of treatment and measurement date on light interception, so only main effects are shown (Fig. 2.5). All tunnel treatments had a higher average light interception (39%) as compared to the control (31%) (Fig. 2.5). As expected, light interception by the canopy increased throughout 2007 (Fig. 2.5). Light interception was low and stable in February and March, but increased to over 40% by early April, indicating the onset of rapid vegetative growth. In 2008 there was an interactive effect of treatment and measurement date on light interception. Light interception was similar in all treatments early in the growing season (Jan. 28 and March 1) and late in the season (June 25). Light interception was higher in all of the tunnel treatments compared to control on March 20 and May 1<sup>st</sup> (data not shown).

#### Discussion

The high tunnels affected microclimatic conditions in distinct, consistent ways that induced changes in the flowering and vegetative development of blueberry plants grown inside of them. The high soil and daytime air temperatures facilitated early floral and fruit development. An unforeseen result was the reduction in soil temperature in the tunnel treatments during the hot summer months when soil temperatures reached super-optimal levels (30-35°C) in the control plots. This reduction in soil temperature in the tunnels was likely due to increased shading of the pine bark beds by the larger plants inside of the tunnels. The reduction in soil temperature in tunnels may be beneficial since root and shoot growth of southern highbush blueberry is reduced at high substrate temperatures (Spiers, 1995). Spiers (1995) suggested that cultural practices that cool the soil during summer months may be beneficial for both southern highbush and rabbiteye

(*Vaccinium ashei*) blueberry growth. Once the tunnel walls were rolled up in late spring, air temperatures inside of tunnels did not differ from outdoor conditions. The light interception and visual observations revealed that the tunnel-grown plants were larger and bushier than the outdoor plants and the shade generated may have helped prevent against increased soil temperature during summer months.

The most striking of the environmental data was the lack of freeze protection generated by the tunnels. Anecdotal accounts suggested that the tunnels would retain heat at night but this was not the case. On numerous nights, minimum temperatures inside of high tunnels fell 1 to 5 °C below ambient. Albright (1989) modeled temperatures under row covers based on a variety of parameters, including long wave radiation transmittance properties of the cover, ventilation rates, and surface to volume ratio of the tunnel. He predicted that when long-wave transmittance was high, ventilation was low, and surface to volume ratios were high that temperatures under cover would drop below ambient due to radiative cooling and lack of convective air movement. The tunnels employed in this study meet the above criteria. This is consistent with our finding that high tunnels lose a greater amount of heat on clear nights than on cloudy nights, likely due to radiative heat loss (Ogden and van Iersel, 2008). Additionally, pine bark beds have been reported to cool air temperatures at plant level by as much as 2.9 °C (Lyrene and Williamson, 2004). The plastic cover employed in this study was not treated to block long wave radiation. Greenhouse plastics designed to block long wave radiation may be more effective at reducing radiative heat loss. However, we found that larger high tunnels with a longwave blocking plastic in South Georgia also did not provide freeze protection (Krewer et al, 2009)

The lack of freeze protection from the tunnels poses a significant problem for growers attempting early blueberry production at locations where freeze events during flower and fruit

development are common. Blueberry flowers become increasing susceptible to freeze damage as their development progresses. A stage 1 flower can withstand temperatures of -2.2 to -3.8 °C, while a stage 5 flower is susceptible to damage at -0.5 °C (Longstroth, 2002). Thus, provision of frost protection is critical to the successful production of early southern highbush blueberry. Numerous nights below freezing throughout the study forced the use of propane heaters to provide frost protection which added an additional expense. However, the option to heat is a clear advantage of high tunnels over outdoor production.

The tunnels effectively advanced the dates of first flower and petal drop of both cultivars as compared to their outdoor counterparts. There was much variation in flower development between the two growing seasons, likely due to weather differences. Previous researchers have reported similar variability (Hicklenton et al., 2004; Renquist, 2005). The number of days from flowering to ripening (80-100 d) was similar to those reported by Ciordia et al. (2006) for a variety of southern high blueberry cultivars grown in high tunnels. Edwards et al., (1970) reported reductions in fruit development rates during periods of low temperatures in rabbiteye blueberry (Edwards et al., 1970).

Poor synchronization of flower development between cultivars can be problematic in southern highbush blueberry production. Southern highbush blueberry cultivars possess varying degrees of self-incompatibility and all cultivars benefit from cross pollination. Benefits of cross pollination include rapid fruit development and enhanced seed production (Williamson and Lyrene, 2004b). Careful cultivar selection through further research should elucidate superior cultivars for advancement of flower initiation and fruit ripening. Interestingly, during the first year of the study, the cultivars were poorly synchronized in their flowering times. This likely resulted in little cross pollination between the two cultivars in 2007. Perhaps as a result, fruit set

was low in the Dec 15 closure (22%) while it increased to 68% in the Jan. 16 closure date (chapter 3). Interestingly, this large difference in time of flowering periods was not seen in 2008.

Vegetative growth indices are relevant for southern highbush blueberry for a variety of reasons. Most cultivars require greater a number of chilling hours for vegetative bud break than for reproductive bud-break (Gough, 1994). For this reason, floral bud-break normally precedes vegetative bud-break (Maust et al., 1999). If the reproductive chilling requirement is met, but the vegetative requirement is not, floral and fruit development may be negatively impacted due to a lack of leaves to provide needed sugars. Newly forming leaves initially act as a sink using up stored carbohydrate reserves, thus competing with fruits (Darnell, 1991). A delay in the onset of vegetative growth due to a lack of chilling would only exacerbate this problem. This situation may occur during mild winters in the Southeast US (Mainland, 1984; Williamson et al., 2002) and could be exacerbated by closing a high tunnel too early, potentially preventing the accumulation of chill hours. The situation is further complicated by the fact that some negation of chilling may occur if high temperatures occur during critical months (Lyrene and Williamson, 2004). Although the plants in the Dec. 15 and Jan. 2 closure dates flowered earlier than those in the Jan. 16 closure date, vegetative growth appears to have initiated at similar times in all tunnel treatments. This indicates that synchronization between vegetative and reproductive growth may be disrupted by using an early tunnel closure date.

#### Conclusion

High tunnels are promising for small fruit crops, including blueberry. High tunnels modified microclimatic conditions, which resulted in early flower development and advanced vegetative growth. Growth and development of southern highbush blueberry is closely tied to temperature, so microclimatic manipulation can be used to promote out-of –season production.
No benefit was gained by closing the tunnels on Dec. 15 as compared to Jan. 16. Higher costs of heating and possible interference with chilling are potential problems with early closure dates. Providing frost protection, ensuring pollination, and identifying appropriately synchronized flower initiation dates among cultivars remain challenges.

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## Figures and Tables



Figure 2.1. Effects of high tunnels on minimum and maximum air temperatures during the 2006-2007 growing season. Only two treatments are shown but similar trends were observed in all tunnels after closing them. Error bars indicate standard deviation. Note increase in maximum temperatures in tunnels compared to control but no increase in minimum temperatures inside of tunnels. Temperature differences ( $\Delta$ ) were calculated as daily maximum or minimum air temperature in tunnels minus maximum or minimum air temperature in control plots. Sidewalls of the tunnels were permanently opened on May 15.



Figure 2.2. Effects of high tunnels on minimum and maximum soil temperatures during the 2006-2007 growing season. Only two treatments are shown but similar trends were observed in all tunnels after closing them. Error bars indicate standard deviation. Note warming of soil temperatures above control plots during winter months and cooling of tunnel soil compared to

control during summer months. Temperature differences ( $\Delta$ ) were calculated as daily maximum or minimum soil temperature in the tunnels minus maximum or minimum soil temperature in control plots. Sidewalls of the tunnels were permanently opened on May 15.



Figure 2.3. The effect of tunnel closure dates on the flower development of 'Emerald' and 'Jewel' blueberries. Cultivar and tunnel closure date affected date of stage 1 and date of stage 5 during both years of study (P < 0.05). Means with the same letter are not different according to Duncan's MRT.



Figure 2.4. The effect of tunnel closure dates on time from floral initiation (stage 1) to ripe fruit (stage 8) of 'Emerald' and 'Jewel' blueberries. The two cultivars differed in the time required from floral initiation to fruit ripening (P < 0.05), but tunnel closure data had no effect. Fruit did not reach stage 8 in the control plots due to the 2007 Easter freeze. Means with the same letter are not different according to Duncan's MRT.



Figure 2.5. Average light interception during the growing season as affected by tunnel closure date (left) and changes in light interception during the growing season (right). Light interception was higher in all tunnel treatments as compared to the control (P < 0.05) and increased throughout the season (P < 0.05). Means with the same letter are not different according to Duncan's MRT.

### CHAPTER 3

## HIGH TUNNELS AFFECT YIELDS AND FRUIT QUALITY OF SOUTHERN HIGHBUSH BLUEBERRY

### Abstract

High tunnels have emerged as a major component of many small to medium size farms seeking season extension and out-of-season production of high value horticultural crops. Regionspecific research is needed to determine appropriate tunnel management practices and for production of large, early yields of high quality southern highbush blueberry. We sought to determine when to close single-bay high tunnels containing plantings of two low-chill requiring southern highbush cultivars ('Emerald' and 'Jewel') to achieve large, early yields. Three tunnel closure dates (Dec. 15, Jan. 2 and Jan. 16) and outdoor control plots were compared. In 2007, all tunnel treatments produced modest yields. Average total yield by treatment was Dec.15: 644 g/plant, Jan. 2: 1067 g/plant, and Jan. 15: 1593 g/plant, while fruit on control plants succumbed to freeze damage. A regression analysis ( $r^2=0.74$ , P=0.029) showed that 'Emerald' yield increased with later closure dates. Fruit weight was highest with Jan. 16 closure (1.26 g/berry) compared to Dec 15 (1.10g/berry) or Jan 2 closure (1.07 g/berry). Fruit set was low with the Dec. 15 closure (22%), which contributed to the low yields in this treatment, as fruit set and yield were positively correlated ( $r^2 = 0.89$ , P = 0.004). Tunnel closure date did not affect fruit quality, but 'Emerald' had higher levels of anthocyanins and soluble sugars than 'Jewel'. 2008 saw no fruit set in tunnel plots due to freeze damage and possibly poor pollination. A late closure date,

(Jan 15), cultivars with short fruit development period (80-90) days, and careful tunnel management practices regarding freeze protection and pollination are recommended.

## Introduction

Blueberry prices fluctuate throughout the growing season and growers receive premium prices for berries marketed during periods of low production. For example, Florida berries that reach the market earlier than the bulk of a given year's harvest receive higher prices (Williamson and Lyrene, 2004a). Freeze damage is a common problem with blueberry crops in the Southeastern United States. For instance a major spring freeze, known as the Easter freeze, destroyed much of the 2007 Southeast US small fruit crop (Warmund et al., 2008). Although high tunnels by themselves do not provide freeze protection (chapter 2), growing blueberries in high tunnels can provide security against spring freezes as tunnels can be closed and heated. Other high tunnel benefits include ease of picking during rainy weather, early yields, and reduced fungal pressure due to fruits and foliage staying dry during rain (Xiao et al., 2001).

High tunnels can be used to meet a variety of goals for the grower. We employed the tunnels to force early flowering and fruit development (chapter 2). To effectively promote early yields, the tunnels need to trap heat during the daytime to rapidly accumulate growing degree hours. Ozekei and Tamada (2006) reported 30 to 40 day advancement of blueberry flowering and fruiting inside of high tunnels as compared to outdoor grown plants. Other researchers have delayed harvest of southern highbush blueberry by using a rain cover (Bal, 1996) or row cover during fruit development. (Hicklenton et al., 2004).

Anthocyanin, a flavonoid molecule responsible for the blueberry's distinctive color, contributes significantly to blueberry's antioxidant properties (Beccaro et al., 2006; Moyer et al., 2002). Beccarro (2006) reported a positive correlation between total monomeric anthocyanin content and ferric reducing antioxidant power (FRAP), a commonly used measurement of antioxidant strength. Prior et al. (1998) noted a similar trend between anthocyanin content and oxygen radical absorbance capacity (ORAC). They also reported that blueberries have high ORAC levels compared to many other fruits and vegetables. Considerable variation exists among blueberry species in antioxidant strength with the highest levels found in wild growing, low bush plants (*Vaccinium angustifolium* Ait), bilberry (*V. myrtillus* L.), and rabbiteye (*V. ashei* Reade) (Prior et al., 1998). We selected anthocyanin content and soluble solids as important fruit quality characteristics and sought to discover what effect closure date of high tunnels has on their levels in southern highbush blueberries. Soluble solids indicate sugar levels which contribute to the sweet flavor of the blueberry.

We investigated the impacts of high tunnel closure date on yields, soluble solids content, anthocyanin content, and fruit size of southern highbush blueberry. Our objective was to determine the optimal closure date for the production of large, early yields and to quantify effects on fruit quality.

## Materials and Methods

*Plant material.* Six 53.5 m<sup>2</sup> high tunnels with manual side walls were constructed in 2006 on the USDA certified organic farm located on the Horticulture farm in Watkinsville, GA. Two raised beds of ground, aged pine bark, (1.8 m wide x 9.5 m long x 20 cm high) were established within each plot. Each bed contained 20 plants (1 row of 10 'Emerald' and 1 row of 10 'Jewel' plants) for a total of 40 plants per tunnel. Plants were configured in a high density arrangement

with 1 m between plants and 75 cm between staggered rows. Two-year old 'Emerald' and oneyear old 'Jewel' plants were planted in November 2006. Frost protection on nights predicted to be below 0°C was provided with portable propane heaters (Dayton 3VE42, 2344 W, Dayton, Detroit, MI) placed inside each tunnel. Pollination was facilitated by the introduction of bumblebees (Minipol, Class C hive, Koppert Biological Systems, Romulus, MI) into each tunnel upon the emergence of open blueberry flowers. A more detailed description of the plant material and production practices is given in Chapter 2.

*Yield data.* In 2007, all ripe fruits were harvested weekly for six weeks beginning April 23. Cultivars were harvested individually and total fresh weight was recorded. Fruit set was calculated as the number of flowers that produced a ripe fruit divided by the number of flowers on that inflorescence  $\times$  100%. Observed flowers and fruit for these measures were the same inflorescences used in the flower developmental data section of chapter 2.

*Fruit Size*. Approximately 150 g of fruit was weighed and photographed with a digital camera. The fruits were then frozen at -20°C for future quality analysis. Photographs were processed with image analysis software (Assess, APS, St. Paul, MN) to determine the number and diameter of the berries. About 100 berries were counted and measured per sub-sample. Average diameter and weight per berry were calculated.

*Soluble solids*. Ten g of frozen fruit were thawed, macerated and filtered through 2 layers of cheesecloth. Juice was then filtered through a Whatman number 1 filter paper under vacuum. The sample was analyzed for soluble solids with a digital refractometer (PR-32α, ATAGO USA, Bellevue, WA).

*Anthocyanin content*. Total monomeric anothocyanin content was determined using the pH differential method as described by Rodriguez-Saona and Wrolstad (2001) and Lee et al., (2005).

Absorbance at 520 nm was measured with a spectrophotometer (Spectronic Genesys 2, Thermo Fisher Sci., Waltham, MA). Since the anthocyanin composition of the fruit was unknown, the molecular weight of cyanidin-3-glucoside (449.2 g/mol) and a molar extinction coefficient of 26,900 were used in the calculations (Moyer et al., 2002). Anthocyanin concentrations are expressed as mg of cyanidin-3-glucoside equivalents per g fruit fresh weight.

*Experimental design and data analysis.* The study employed a completely randomized design with a split-plot (cultivar) with four main treatments, two replicates, and repeated measures. The four treatments consisted of three different dates (Dec. 15, Jan. 2, and Jan. 16) at which the tunnels were covered with a single layer of 6 mil polyethylene greenhouse plastic (K50 clear, Klerks Hyplast Inc., Chester, SC) and a control treatment which received no tunnel. The study was conducted in the 2006 – 2007 and 2007 - 2008 growing seasons. Treatment effects were analyzed using ANOVA and linear regression (SAS 9.0, SAS Institute, Cary, NC). When ANOVA indicated significant effects (P < 0.05), means were separated using Duncan's Multiple Range Test (MRT). In addition, the correlation between tunnel closure date on yield of both cultivars, fruit set and yield, and harvest data and fruit quality were tested using linear regression.

### **Results and Discussion**

*Yield.* Modest yields of 20.11 g/plant in Dec. 16 closure date, 38.22 g/plant in Jan. 2 closure date, and 61.55 g/plant in Jan. 16 closure date were achieved in 2007. Frost protection in the high tunnels was achieved with propane heaters during freeze events (Chapter 2), including the April 18, 2007 "Easter freeze" that destroyed much of the Southeastern US blueberry crop (Warmund et al., 2008). All developing fruits and flowers in control plots succumbed to freeze damage.

In 2007, early yields were achieved with the use of the high tunnels. Harvesting began on April 23 when an average of 40 g/plant was harvested in tunnels (Fig. 3.1). There was an interactive effect of tunnel closure date and harvest date on yield. Early yields (April 23 and 30, May 7) were similar in all tunnel treatments. Both the Jan. 2 and Jan. 16 closure dates resulted in higher yields on May 14 than the Dec. 15 closure date. On May 21, yield with the Jan. 16 closure date was higher than with the Dec. 15 closure date (Fig. 3.1).

There were no significant yield differences among tunnel treatments due to large variation between replicates, particularly with the Dec 15 closure date. One of the two tunnels of this treatment had very low fruit set and yield, likely due to low activity of the bumblebee colony in that tunnel. However, regression indicated that later tunnel closure resulted in higher yields for 'Emerald' ( $r^2$ =0.74, P=0.03), but not for 'Jewel' (Fig. 3.1).

Chapter 2 showed that, averaged over the two year study, flowering was advanced by 38 d in 'Emerald' and 39 d in 'Jewel' with the earliest tunnel closure date as compared to outdoor control plants. This suggests that if the fruits in the outdoor plots had not succumbed to freeze damage, they would have ripened 4 to 5 weeks later than in the high tunnels. The harvest time of these tunnel-grown berries in North Georgia corresponds to that of field-grown highbush blueberry production in South Georgia, which begins 3 weeks to one month earlier than in North Georgia (Ogden et al, unpublished data). Ciordia et al (2002) reported a three week harvest advancement by using unheated high tunnels over southern highbush blueberries in Spain. Bal (1996) reported similar advancement of ripening using high tunnels over southern highbush blueberry crops in the Netherlands.

Fruit set was highly correlated with yield (Fig. 3.2). This indicates that the poor fruit set seen in the Dec. 15 closure date contributed to the low yields in that treatment, whereas high fruit

set in the later closure dates resulted in better yields. Achieving high yields inside of high tunnels thus hinges on good fruit set.

In 2008 there was no fruit set inside any of the high tunnels and a very small yield of late berries from the outdoor control plots. Lack of fruit set was attributed to freeze injury and low pollinator activity (chapter 2). In 2008, we saw little activity from bumblebee colonies in the high tunnels in Watkinsville, as well as in a larger high tunnel in South Georgia. Effective pollination by bumblebees is crucial for rapid fruit development, seed development, and large berry size (Sampson and Spiers, 2002). A freeze (-11 °C in unheated tunnels, -9 °C outdoors) on Jan 3 2008 was severe enough to damage swollen, undifferentiated 'Emerald' flowers. On March 25, temperatures dropped below predicted minimums and reached -5 °C inside of the high tunnels. The flowers were at a vulnerable development stage, just following petal drop and prior to fruit expansion, and no further fruit development was observed after this date. Thus freeze protection is a critical need for early blueberry production in high tunnels

Proper cultivar selection is also important for achieving high, early yields of southern highbush blueberries. The cultivars selected for present study had a very low chilling requirement (<300 hours) and normally would not produce fruit in North Georgia, because of early flowering and the likelihood of freeze damage. Use of regionally-adapted cultivars could prove beneficial. Although 'Emerald' flowered earlier than 'Jewel', its longer development time pushed ripening ahead to coincide roughly with that of 'Jewel'. Cultivars with a short fruit development period should enable early yields, while avoiding flowering during the coldest parts of winter. The two cultivars were selected for this study based on their good synchronization of flowering in South Georgia. Apparently, in 2007 the microclimatic conditions within the high

tunnels disrupted their normally well synchronized flowering times. This may have had a negative impact on pollination, fruit set, and yield.

*Fruit size*. The two cultivars were similar in size, averaging 1.14 g/berry with a diameter of 9.7 mm. Berries in the Jan 16 closure date were heavier (1.26 g/berry) than those in the Dec 15 (1.09 g/berry) and Jan 2 closure dates (1.07 g/berry). Blueberries averaged 1-2 gram/berry in previous studies (Hicklenton et al., 2004; Sampson and Spiers, 2002). The increase in fruit size with the Jan 16 closure date was possibly due to more effective cross pollination, due to better inter-cultivar synchronization of flowering. For instance, in 2007 the date of first flower occurred 30 and 39 d earlier in 'Emerald' than in 'Jewel' in the Dec. 15 and Jan 2 closure date, respectively, while this difference was reduced to 26 d in Jan 16 closure (chapter 2). Effective cross-pollination is associated with rapid fruit development and large berry size (Mainland, 1984; Sampson and Spiers, 2002). Fruit weight was low during the last two harvests (April 23and 28)), corroborating findings by Ciordia et al., 2002.

*Fruit quality.* Tunnel closure date had no discernible effect on soluble sugars. 'Emerald' had higher soluble sugars (12.81 °Brix) than 'Jewel' (11.5 °Brix). Soluble sugar content was consistent throughout the harvest period with a range of 11-13% (Table 3.1), values normal for southern highbush blueberries grown in high tunnels (Ozeki and Tamada, 2006).

Tunnel closure date also did not affect the concentration of anthocyanins in blueberry fruits. Higher anthocyanin concentrations were found in 'Emerald' (1.01 mg·g<sup>-1</sup>) than in 'Jewel' (0.83 mg·g<sup>-1</sup>) (Table 3.1). Previous researchers have reported a large variability in anthocyanin concentrations of southern highbush blueberry (0.25 - 4.95 mg·g<sup>-1</sup>) (Beccaro et al., 2006). Anthocyanin concentrations increased linearly throughout the harvest season ( $r^2 = 0.98$ , P < 0.001), from 0.66 mg·g<sup>-1</sup> on April 23 to 1.18 mg·g<sup>-1</sup> on May 28 (Fig. 3.3). These data suggest that

there may be some loss of quality in early ripened southern highbush blueberry, although other, non-quantified compounds such as phenolics, are also known to contribute blueberry antioxidant strength (Beccaro et al., 2006) and ORAC and FRAP were not calculated in our study.

## Conclusion

The latest closure date of Jan. 16 appears optimal for a variety of reasons. Highest yields of 'Emerald' were obtained in the Jan 16<sup>th</sup> closure date, In addition, fruit weight was highest in the last closure date. Plants inside of tunnels started to flower shortly after tunnel closure due to increased air and soil temperatures. An early closure (like Dec. 15) forces growers to provide frost protection during many nights, depending on the severity of the winter. Further cultivar trials should elucidate cultivars with well-synchronized flowering and short fruit development periods under high tunnel conditions. The high prices received for organic, out-of-season blueberries will undoubtedly stimulate further interest in this arena. Future research should focus on temperature management inside of the tunnels, freeze protection strategies, and cultivar selection.

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## **Figures and Tables**



Figure 3.1. The effect of high tunnel closure date on the temporal distribution (left, averaged over both cultivars) and total yield (right, averaged over tunnel closure and harvest dates) of two blueberry cultivars. There was an interactive effect of tunnel closure date and harvest date on yield/plant. Means within a harvest date with the same letter are not significantly different according to Duncan's MRT (P=0.05). Total yield of 'Emerald' increased with later tunnel closure dates, while 'Jewel' yield was not affected. Due to freeze damage, there was no yield in the outdoor control plots.



Figure 3.2. The effect of fruit set on yield of high tunnel grown blueberries. Percent fruit set correlated strongly with yields. Fruit set was low and yields were low with early tunnel closure dates while later closure dates generated higher percent fruit set and higher yields.



Figure 3.3. Fruit quality characteristics throughout the harvesting season. Harvest date affected soluble solids and anthocyanin concentration. Means with the same letter are not significantly different according to Duncan's MRT. Regression analysis showed that anthocyanin content increased linearly with harvest date.

Cultivar	Yield	Fruit set	Soluble solids	Anthocyanin concentration	Fruit size
	g/plant	%	°Brix	mg/g	g/berry
Emerald	45.87 a	0.226 b	12.8 a	1.01 a	1.14 a
Jewel	34.41 a	0.735 a	11.6 b	0.82 b	1.16 a

Table 3.1. A comparison of two blueberry cultivars with respect to fruit quality characteristics. Significant differences were detected with Duncan's multiple range test (P = 0.05) and are noted by different letters.

#### **CHAPTER 4**

# RELEASE OF INORGANIC NITROGEN FROM AN ORGANIC FERTILIZER IN THE FIELD AND UNDER CONTROLLED CONDITIONS

## Abstract

Aged pine bark is a popular medium for production of southern highbush blueberry (Vaccinium corymbosum L.). Cultural practices such as fertilization with organic fertilizers and the use of high tunnels are novel growing techniques aimed at producing high value small fruit crops during periods of low production. A better understanding of nitrogen cycling within this production system will assist in developing appropriate fertilization regiments for growers. The objective of this study was to quantify the release of inorganic nitrogen from an organic fertilizer in a pine bark medium. Buried cups with open tops and resin exchange beads at the bottom were employed in situ to determine the effects of the presence of a high tunnel (4.9 x 11m) on the release of inorganic nitrogen from an organic fertilizer in aged pine bark beds of southern highbush blueberry. Warm temperatures inside of high tunnels led to increased nitrogen availability early in growing season as compared to control. As expected, the pine bark retained nitrate poorly as indicated by high levels of leached nitrate found in resin beads. Organic fertilizer was also incubated in a growth chamber in either bark from existing beds or newly purchased aged pine bark. Bark age affected nitrification rates dramatically in controlled environment, as very little nitrate evolution occurred in newly purchased bark as compared to the field bark. Understanding nitrogen cycling within pine bark beds can assist blueberry growers to develop appropriate nutrient management strategies.

## Introduction

Growing blueberries organically in pine bark beds inside of high tunnels is a novel growing technique that needs research to develop accurate fertilizer recommendations. Organic fertilizers typically contain only small amounts of readily available nutrients for plant growth. Rather, mineral elements are often incorporated in organic molecules that require microbial interactions to transform them into forms that plants can assimilate. The situation is further complicated by the high C:N ratio of pine bark media which can lead to microbial immobilization of soil nitrogen reserves (Havlin et al., 1999). Maintaining adequate nitrogen nutrition is critical to successful southern highbush blueberry production (Kozinski, 2006). Blueberry fertilization recommendations average 90 to120 kg/ha N per year (Kuepper and Diver, 2004), but Williamson (2009) reported positive effects on yield and vegetative development with rates up to 365 kg/ha N per year for plants grown in pine bark beds. Nitrogen cycling is complex and key processes, including mineralization, nitrification, immobilization, leaching, and denitrification, must be considered when developing a fertilizer regimen for organically grown blueberries in pine bark beds inside of high tunnels. One implication of high tunnel agriculture is the potential for a reduction in leaching and runoff of fertilizer due to better controlled watering. This could be advantageous to the grower in terms of reduced need for fertilizer application or detrimental due to build-up of salts if fertilizer

application rates are not adjusted properly.

Mineralization is the conversion of nitrogen in organic compounds into ammonium ( $NH_4$ ) by soil microbes. Conversely, soil microbes may immobilize ammonium while breaking down organic residues with a high C: N ratio in a process called nitrogen immobilization. Nitrification is the conversion of ammonium into nitrate ( $NO_3^{-}$ ) by the soil bacteria *Nitrosomonas* and

*Nitrobacter*. Nitrification is inhibited by low soil pH, particularly in clay soils containing aluminum and iron oxides (Havlin et al., 1999; Lambers et al., 1998). To the contrary, Booth et al. (2005) reviewed over 100 studies reporting nitrification rates in a wide variety of soil types and found no correlation between pH and nitrification rates. In fact, soils that were high in organic matter (4-5%) and low in pH (<5) exhibited high rates of nitrification (Booth et al., 2005). Thus, it appears that nitrification in acidic clay soils is inhibited by aluminum and iron oxides, rather than by the low pH itself (Haynes et al., 1986). Processes that reduce the amount of plant-available N in soil include plant or microbial uptake, leaching, whereby the anion  $NO_3^-$  passes through the soil profile and enters the subsoil where it may enter underground water supplies, and denitrification whereby soil nitrogen transforms into gaseous nitrogen under hot, wet, anaerobic conditions (Havlin et al., 1999).

Aged ground pine bark provides an optimal growing medium for highbush blueberry for several reasons. First, blueberry thrives at pH values of 4.5-5.5, which are much lower than those required by typical horticultural and agronomic crops. Aged pine bark usually has a pH between 4.5 and 5. Second, blueberries require much greater levels of organic matter than most other crops and thrive with organic matter contents of 4 to 6% (Kuepper and Diver, 2004). Third, the porous nature of the bark enables it to drain well and provides a suitable environment for the fibrous blueberry roots. Since their roots lack root hairs, blueberry plants typically depend on ericoid mycorrhizal fungi to assist them in nutrient acquisition. Mycorrhizal growth is encouraged in growth media with high organic matter contents (Kuepper and Diver, 2004). Although pine bark has a relatively high cation exchange capacity (CEC, 10-13 meq/100g, its ability to trap anions (anion exchange capacity, AEC) is poor (Krewer, 2005). Thus, one would

expect to find a greater amount of the anion  $NO_3^-$  leaching from pine bark beds than the cation  $NH_4^+$ . Little is known about the fate of organic fertilizers when added to aged pine bark beds.

The transformations occurring with soil nitrogen have implications for plant growth as well. Most plants uptake nitrogen as either  $NH_4^+$  or  $NO_3^-$  Ericaceous plants like blueberry occur naturally on acidic soils in which  $NH_4^+$  is believed to be the predominant N form. This fact, combined with the low levels of nitrate reductase activity found in some Ericaceous plants (an enzyme needed to reduce  $NO_3^-$  to  $NH_4^+$  within the plant), led researchers to conclude that Ericaceous plants preferentially uptake  $NH_4^+$  over  $NO_3^-$  (Kuepper and Diver, 2004; Lambers et al., 1998; Magalhäes and Huber, 1989). Early studies provided evidence to support this hypothesis (Cain, 1952; Colgrove and Roberts, 1956; Spiers, 1978). However, Merhaut and Darnell (1996) reported no difference in vegetative growth between blueberry plants fertilized with  $NH_4^+$  or  $NO_3^-$ , despite increase in effluent pH to 6 in the  $NO_3^-$  fertilized plants. Active nitrate uptake requires the simultaneous uptake of protons, which causes an increase in soil pH and could be detrimental to blueberry root and shoot growth. Other studies have suggested that when pH of growing medium is tightly monitored, blueberry plants will uptake nitrogen and develop at similar rates regardless of the form applied (NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>) (Ingestad, 1976; Peterson et al., 1988). In this study, we aimed to quantify the release of inorganic nitrogen from an organic fertilizer in pine bark beds, both in the field and under controlled conditions inside of a growth chamber.

### Materials and Methods

*Experiment 1: In situ N transformations.* A site was selected on the USDA-certified organic farm located on the horticulture farm in Watkinsville, GA. High tunnels with manual side walls, each 53.5 m<sup>2</sup>, were constructed in 2006. Two raised beds of ground, aged pine bark (pine bark soil

conditioner, Sun Gro Horticulture, McCormick, SC) (1.8 m wide x 9.5 m long x 20 cm high) were established within each plot. Each bed was planted with two-year old 'Emerald' plants and one-year old 'Jewel' in October 2006 for a total of 40 blueberry plants per tunnel. Plants were configured in a high density arrangement with 1 m between plants and 75 cm between staggered rows. Identical control plots without tunnels were also established. Fertilization was provided with a 5N-1.31P-3.32K organic granular fertilizer (5-3-4 McGeary Organics, Lancaster, PA) at a rate of at 467 kg N/ha. Fertilizer analysis revealed the fertilizer contained 43 mg NO<sub>3</sub><sup>-</sup>-N/kg and 122 mg NH<sub>4</sub><sup>+</sup>-N/kg. Fertilizer was applied throughout year at a rate of 2 kg per bed (48.47 kg N/ha) on Oct. 15 2006 and Jan. 30 2007, 3 kg per bed (87.5 kg N/ha) on April 25, 4 kg per bed (117 kg N/ha) on June 29, and 4 kg per bed on August 28. Irrigation was provided with micro-jet sprinklers (54 L/hour) placed every 2 m in each bed. One hour of irrigation, three days per week, provided adequate moisture levels throughout fall, winter, and spring (76 L/m<sup>2</sup> per week).

To evaluate mineralization and nitrification of fertilizer-derived N, 32 1.3 L plastic cups were prepared by puncturing 4 drainage holes in each cup and placing a pantyhose bag, containing 15 g of cation and anion resin exchange beads, at the bottom of each cup (Rexyn 300 H-OH Analytical grade, Fisher Scientific, Pittsburgh, PA). The resin beads had a total exchange capacity of 2.78 meq/g. The study employed two treatments; cups installed in high tunnels or in outdoor control plots with four replicates of each treatment. On Jan 29 2007, all cups were installed into the pine bark beds by digging out a small hole in the pine bark and filling the cups with it, and placing the cups on the surface of the soil underneath the beds so that the top of each cup was even with the surface of the bed. Cups did not have lids on them. Substrate temperature at 10 cm depth was monitored in all the beds with dataloggers (Hobo U-12, Onset Computer

Corp., Bourne, MA) with external soil temperature probes (TMC-1HD, Onset Computer Corp.) during the duration of the study. Cups were removed in sets of 8 (4 replicates of the two treatments) on April 9, June 5, August 21, and October 25. Upon removal, cups and contents were placed inside of sealed plastic bags and stored at 20°C until extracted (1-3 d later).

Bark and resin beads from the cups were extracted separately. Inorganic nitrogen in the bark represented plant-available nitrogen, while that contained in the resin represented the inorganic nitrogen leached from the beds. The bark was removed from each cup and placed into 4-L containers with 2.5 L of 1M KCl. Containers were then shaken for 30 minutes on an oscillating shaker (Eberbach, Ann Arbor, MI). 15 mL of extract was filtered through a Whatman number 1 filter paper and frozen at -20°C. To extract the resin beads, 2 M KCl was used due to the high exchange capacity of the resin beds. Pantyhose bags were opened and beads were placed in 1-L containers with 800 mL of 2M KCl. Containers were then shaken for 30 minutes and 15 mL of extract was filtered through a Whatman number 1 filter paper and frozen at -20°C. Upon completion of all extractions, samples were analyzed in Ecology Soil Science Laboratory at the University of Georgia. Values from the laboratory were reported in mg/L solvent and were converted to mg N L/bark by multiplying by the amount of solvent (2.5 L for bark, 0.8 L for resin) and dividing by the volume of the cup (1.3L).

The study employed a completely randomized design with two main treatments of outdoor plots versus high tunnels, four replicates, and repeated measures. Treatment effects were analyzed using ANOVA (SAS 9.0, SAS Institute, Cary, NC). Due to unequal variance found in the data, a logarithmic transformation of the data was performed. When ANOVA indicated significant effects (P < 0.05), means were separated using Duncan's mean separation test.

*Experiment 2: Incubation study.* Newly purchased aged pine bark (pine bark soil conditioner, Sun Gro Horticulture, McCormick, SC) and pine bark from the raised beds of an existing planting of southern highbush blueberry (field bark) were used. These were the same beds as in expt. 1. The water holding capacity (WHC) of both pine bark substrates was determined. Ten 15 cm plastic pots (1250 mL) were filled with either the newly purchased pine bark or the pine bark from the existing beds, saturated with water, and then allowed to drain for 3 h. After 3 h, samples were weighed and placed in forced-air drying oven at 60°C for 3 d. Samples were then weighed to determine the water holding capacity (WHC) by dividing the volume of water by the volume of the sample. The two barks differed in their ability to hold water as the aged bark had a WHC of  $0.30 \text{ L'L}^{-1}$ , while the field bark had a WHC of  $0.41 \text{ L'L}^{-1}$ . The dry bulk density of both substrates was 167 gL<sup>-1</sup>for the field bark and 185 gL<sup>-1</sup> for the aged bark.

Samples of 132 g aged bark at WHC and 174 g of field bark at WHC we were placed into 0.95 L mason jars. Given the different WHC of the two media, these values corresponded to a sample size of approximately 50 g of dry bark. Jars were weighed weekly to determine the water content of the medium. When the water content dropped below 60% of WHC (approximately every 14 d), water was added to bring the water content back to 70% of WHC. Seventy percent of WHC corresponded to a volumetric water content (VWC) of 0.21 L<sup>-1</sup> for the aged bark and 0.29 L<sup>-1</sup> for the field bark.

The study employed four treatments consisting of freshly purchased bark without fertilizer, freshly purchased bark with fertilizer, field bark without fertilizer, and field bark with fertilizer and each treatment was replicated three times. The organic fertilizer (5N-1.31P-3.32K organic granular fertilizer, 5-3-4 McGeary Organics, Lancaster, PA) was applied at a rate of 1 g/jar. Pine bark bed volume in the field study was 3420 L and we applied about 4 kg of fertilizer

per bed per application. That corresponds to 1.16 g/L, so we applied 1 g of fertilizer to the jars to mimic field applications. Once the VWC of all jars dropped below 70% of WHC, fertilizer was applied and water was added to return the VWC to 70% of WHC. The jars were sealed with lids with small pierced holes to facilitate gas exchange. Fertilizer was mixed in thoroughly with the pine bark at application. Jars were incubated inside of a growth chamber in darkness, high humidity (80% RH) and 25 °C. Jars were removed from the growth chamber and extracted after 0, 3, 7, 14, 21, 35, 59, 73, 87, and 101 d.

Extraction occurred by adding 600 mL of 2 M KCl to each jar and then shaking for 30 minutes. 15 mL samples of the extract were removed, filtered under vacuum, with Whatman number 42 filter paper and frozen at -20 °C. Samples were then analyzed by colorimetric assay for  $NO_3^-$  and  $NH_4^+$ . Values from the laboratory were reported in mg/L solvent and were converted to mg N per L bark by multiplying by the amount of solvent (600 mL) and dividing by the volume of bark (0.189 L).

The study employed a completely randomized design and a split-plot with two main treatments of bark type: field bark or recently purchased aged pine bark. The split in the design was whether or not the jars received fertilizer. Jars that did not receive fertilizer were considered controls and the amounts of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> found in these control jars were subtracted from the amount of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in the fertilized jars. This was done to account for the presence of fertilizer in the field bark applied prior to sampling bark for this experiment and thus to estimate the amount of mineralized N from the organic fertilizer. Treatment effects were analyzed using ANOVA (SAS 9.0, SAS Institute, Cary, NC). Due to unequal variance, a logarithmic transformation of the data was performed. When ANOVA indicated significant effects (P < 0.05), means were separated using Duncan's Multiple Range Test (MRT).

## Results

*Experiment 1: Field Study.* Higher amounts of NO<sub>3</sub><sup>-</sup> and total N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) were found in the bark of the tunnel treatment as compared to the control, irrespective of sampling date, while  $NH_4^+$  concentrations in the bark did not differ between the treatments. There was a two-way interaction between treatment and sample date that affected leached NO<sub>3</sub><sup>-</sup>-N, leached NH<sub>4</sub><sup>+</sup>-N, total N leached, and total inorganic NO<sub>3</sub><sup>-</sup>-N (leached + bark). Higher amounts of total leached N were found in the resin of the tunnel treatment (Fig. 4.1) than in the outdoor control on April 9, which was largely caused by more leached NO<sub>3</sub><sup>-</sup>-N in the high tunnels. After April 9, said parameters were similar in the two treatments for remainder of study. At all dates, the leached N contained more NO<sub>3</sub><sup>-</sup>-N than NH<sub>4</sub><sup>+</sup>-N. The amount of leached NH<sub>4</sub><sup>+</sup>-N decreased as the growing season progressed in both treatments.

The higher amount of  $NO_3$ <sup>-</sup>-N in the bark of the tunnel treatment resulted in a higher ratio of  $NO_3$ <sup>-</sup>-N to  $NH_4^+$ -N (Figure 4.1). This ratio was higher in the tunnel treatment than in the control in the bark, leached, and total (bark +leached) N. Despite the presence of  $NO_3^-$ -N, soil pH was not increased as all bark samples maintained pH values between 4.8 and 5.1 throughout the study (data not shown).

Substrate temperatures in the high tunnel were higher than in the control from January to May and then dropped below those in the control plots during summer months, likely due to a reduction in solar radiation reaching the soil because of the plastic covers and the larger plant canopies in the tunnels (Figure 4.2).

*Incubation study.* There were two-way interactions between bark type and sampling date for the four parameters tested ( $NO_3^-$ ,  $NH_4^+$ , total N and  $NO_3^-$ :  $NH_4^+$ ). Fertilizer mineralization in pine bark medium under controlled environmental conditions differed in the two types of bark

employed in the study. In the aged bark, mineralized N consisted primarily of NH4<sup>+</sup> throughout the 101 d of the study, with very little nitrate appearing. Unlike the aged bark, , inorganic N release in the field bark consisted of both  $NO_3^-$  and  $NH_4^+$ , with the highest ammonium levels occurring early and the highest nitrate levels at 20 d and later (Fig. 4.3). An initial peak of ammonium occurred in the field bark at day 7 and then the  $NH_4^+$ -concentration dropped below that in the aged bark on day 35 and remained similar for the remainder of the study. The strong initial peak seen in ammonium concentration at day 7 was followed by a peak in nitrate concentration at day 21. Nitrate concentration remained higher in the field bark as compared to the aged bark for the remainder of the study and this difference was significant on 6 of the 10 sampling dates. This difference between inorganic N release and subsequent nitrification in the two types of bark is clearly illustrated by the  $NO_3$ :  $NH_4^+$  ratio which was higher in the field bark than in the aged bark on 8 of the 10 sampling dates (Figure 4.3). Approximately 50 mg of N were added to the fertilized jars at the onset of the experiment and there was approximately 16.4 mg N in jars present at end of study which indicates that 33% of the N added was mineralized during the 103 days of the study (data not shown).

## Discussion

Previous research has noted that the pine bark beds leach a larger amount of NO<sub>3</sub><sup>-</sup>-N than NH<sub>4</sub><sup>+</sup> (Krewer, 2005). This was likely due to pine bark's relatively low AEC and relatively high CEC (Krewer, 2005). The higher concentration of inorganic nitrogen extracted from the bark and resin beads on the first sampling date in the tunnel treatment as compared to the control treatment was likely due to increased substrate temperatures generated by the tunnel enclosures. Shaw and Harte (2001) reported a 60% increase in N mineralization rates inside of heated chambers, which increased soil temperatures by 1.2 °C, placed over in-situ sub alpine vegetation.

Although Shaw and Harte (2001) did not find a correlation between temperature and nitrification, an increase in mineralization rates early in the growing season in the present study would have provided  $NH_4^+$ , which is a prerequisite for nitrification (Havlin et al., 1999; Shaw and Harte, 2001). The apparent occurrence of nitrification in the pine bark beds, with a pH of 4.8, supports Booth's (2005) assertion that high rates of nitrification may occur at low pH in soils high in organic matter.

In the incubation study, the most striking difference in the release of inorganic nitrogen between the two types of bark was the lack of nitrate in the newly purchased bark. Similarly, nitrate concentrations were higher in aged pine bark as compared to fresh bark after applying fertilizer N at different concentrations (Altland and Buamscha, 2008). Both barks, however, in the present study would be considered aged bark. Nitrifying bacteria had apparently colonized the pine bark from the existing pine bark beds sufficiently to nitrify much of the ammonium. Based on the fertilizer analysis, only 0.043 mg NO<sub>3</sub><sup>-</sup>N was added to the fertilized treatments while a total of 18.77 mg NO<sub>3</sub> -N/L appeared in the field bark and 0.964 mgNO<sub>3</sub> -N/L in the aged bark. Thus, the  $NO_3$ -N found in the field bark in the present study suggests that nitrification was occurring in the pine bark beds. The aged bark may not have been exposed to such microorganisms prior to this study, resulting in very low  $NO_3^-$  concentrations. Blueberry apparently grows well with either form of N (Cain, 1952; Merhaut and Darnell, 1995; Spiers, 1978), but this lack of nitrification in the aged bark could be an issue with other crops that are susceptible to ammonium toxicity, like tomato (Magalhäes and Huber, 1989). The finding that only 33% of the N contained in the fertilizer was mineralized during the 103 d of the study suggests that further research is needed to determine mineralization rates, N availability, and optimal fertilizer rates for organically blueberries grown in pine bark beds.

## Conclusion

Examining inorganic nitrogen release from an organic fertilizer in aged pine bark medium in situ and in a controlled revealed several interesting trends. First, nitrification occurred readily in the field experiment and in the field bark tested in a controlled environment, but not in aged bark that had not yet been exposed to field conditions. Analysis of leached N revealed higher amounts of leached NO<sub>3</sub><sup>-</sup> than NH<sub>4</sub><sup>+</sup>. Multiple applications of fertilizer during periods of maximum plant uptake should minimize NO<sub>3</sub><sup>-</sup> losses. Presence of high tunnel closures promoted warm soil temperatures early in the growing season which resulted in increased mineralization and nitrification. Nitrogen release was found to be affected by bark type, as very little nitrification occurred in aged pine bark that had not been exposed to field conditions. Inoculation with field soil may be desirable for growing in pine bark media that are not in contact with soil if the crop requires NO<sub>3</sub><sup>-</sup>. Understanding nitrogen cycling can assist growers in nutrient management. Further research should elucidate optimal timing and quantification of organic fertilizer application to pine bark beds of highbush blueberry.

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Figure 4.1. Nitrogen release from organic fertilizer in pine bark beds in situ. A \* indicates a one way treatment effect (P<0.05) with significantly higher values in the tunnel treatment compared to the control for the given parameter. Total inorganic N (graphs on right) represents the bark N + leached N. Total N represents  $NO_3^- + NH_4^+$  extracted. Where ANOVA indicated a significant two way interaction between treatment and extraction date (*P* < 0.05), letters indicating significance were assigned according to Duncan's mean separation test.



Figure 4.2. Soil temperatures as affected by high tunnels. Tunnel closure trapped heat during the daytime and warmed soil temperatures above control treatment during winter months. During summer months soil temperature in tunnels was cooler than in control likely due to reduced solar radiation by shading caused by larger plant canopies in tunnels and plastic covering of tunnels.



Figure 4.3. Nitrogen release from organic fertilizer in two types of pine bark media in growth chamber. ANOVA indicated significant two way interaction between bark type and extraction date (P < 0.05) and letters indicate significance according to Duncan's mean separation test. Graph of ratios indicates that very little nitrate evolution occurred in the aged bark that had not yet been exposed to field conditions.

## **CHAPTER 5**

## CONCLUSION

High tunnels are promising for production small fruit crops, including blueberry. High tunnels modified microclimatic conditions, which resulted in early flower development and advanced vegetative growth. Growth and development of southern highbush blueberry is closely tied to temperature, so microclimatic manipulation can be used to promote out-of-season production. No benefit was gained by closing the tunnels on Dec. 15 as compared to Jan. 15. Higher costs of heating and possible interference with chilling are potential problems with early closure dates. The latest closure date of Jan. 15 appears optimal for a variety of reasons. Highest yields of 'Emerald' were obtained in the Jan 15 closure date. Secondly, weight per fruit was highest in the last closure date. Plants inside of tunnels started to flower shortly after tunnel closure due to increased air and soil temperatures. An early closure (like Dec. 15) forces growers to provide frost protection during many nights, depending on the severity of the winter. Further cultivar trials should elucidate cultivars with well-synchronized flowering and short fruit development periods.

Examining inorganic nitrogen release from an organic fertilizer in aged pine bark medium in situ and in a controlled revealed several interesting trends. First, nitrification occurred readily in the field experiment and in the field bark tested in controlled environment. Analysis of leached N revealed higher amounts of leached NO<sub>3</sub><sup>-</sup> than NH<sub>4</sub><sup>+</sup>. Multiple applications of fertilizer during periods of maximum plant uptake should minimize NO<sub>3</sub><sup>-</sup> losses. Presence of high tunnel closures promoted warm soil temperatures early in the growing season which resulted in increased mineralization and nitrification.

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Nitrogen release was found to be affected by bark type as very ilttle nitrification occurred in aged pine bark that had not been exposed to field conditions. Inoculation with field soil may be desirable for growing in pine bark media that is not in contact with soil if the crop requires NO<sub>3</sub><sup>-</sup>. Understanding nitrogen processes can assist growers in nutrient management. Further research should elucidate optimal timing and quantification of organic fertilizer application to pine bark beds of highbush blueberry.

The high prices received for organic, out-of-season blueberries will undoubtedly stimulate further interest in this arena. Future research should focus on temperature management inside of the tunnels, freeze protection strategies, and cultivar selection.

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