PATHOGENS ASSOCIATED WITH BLUEBERRY CUTTING FAILURE IN SOUTH GEORGIA NURSERIES AND THEIR CONTROL

by

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(Under the Direction of Phillip M. Brannen and Harald Scherm)

ABSTRACT

In 2007 and 2008, a survey of 18 blueberry propagators in south Georgia was conducted to determine the incidence of soilborne plant pathogens and the prevalence of propagation practices currently used. There was little consistency in propagation methods across nurseries, documenting a need for developing standardized best practices. Across 204 symptomatic blueberry cuttings sampled during four survey dates, 15.2, 4.9, 1.0, and 16.7% harbored species of *Cylindrocladium*, *Rhizoctonia*, Oomycetes, and *Fusarium*, respectively, although the pathogenicity of *Fusarium* remains to be confirmed. A link was established between propagation media reuse and presence or absence of *Cylindrocladium*. Fungicide efficacy trials were conducted on cuttings planted in propagation medium infested with *Cylindrocladium parasiticum* or *Rhizoctonia* sp. Fludioxonil proved to be the most effective against *Cylindrocladium*, whereas fludioxinil, flutolanil, and azoxystrobin were effective against *Rhizoctonia*. This survey provides the groundwork for future standardized recommendations for propagating diseases-free blueberry cuttings.

INDEX WORDS: *Cylindrocladium parasiticum*, *Rhizoctonia*, *Pythium*, *Phytophthora*, blueberry, *Vaccinium*, vegetative propagation.

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DEDICATION

This is dedicated to my mother and father who stood by me throughout my academic career and will finally be able to say their son has finished with school. At least for now…

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There are many people whom I would like to thank for their support during the last 3 years. First and foremost are my major professors Drs. Brannen and Scherm without whose patience and support my research would not have been possible. My other committee members Drs. NeSmith and Krewer have provided help and advice during my research. I would also like to thank Amy Savelle for her help with my lab work. And finally to all my friends in the department. You have made the last 3 years the best of my life. You all made the stress worth it.

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CHAPTER 1

INTRODUCTION

In the past 10 years, blueberries have become a major player in Georgia's fruit and nut crop industry. Harvested area has increased from a mere 1400 ha in 1996 to more than 4000 ha by 2006 (1,6). In 2004, blueberries eclipsed peaches in value and became the most valuable fruit commodity in the state (1). On a national scale, Georgia now ranks third behind New Jersey and Michigan in crop value and harvested acreage (13). As new acres have been planted, the demand for rooted cuttings has risen dramatically, prompting many growers to begin propagating cuttings for their own use and to sell. The methods being employed in propagating blueberries have remained largely unchanged for decades. The first published accounts of blueberry propagation were made in a USDA Bulletin by Dr. Frederick V. Coville in 1910 (5). In the interceding 98 years, very little has changed in blueberry propagation methods (4). Many recommendations have been made based on Coville's findings, but no preferred method has been established for either propagation or disease management. Growers have applied a wide range of methods which combine elements from many different sources resulting in wide variation in propagation methods.

In Georgia, softwood cuttings are the primary choice for propagation material. There are two opportunities for taking softwood blueberry cuttings: May to early June and late July to early August. Softwood cuttings are taken from apicial shoots from the current season's growth and should measure between 11.5 and 15 cm in length (4). Whereas most growers propagate using softwood cuttings, the methods of rooting can vary widely among operations (7). For example, propagation systems can be separated into two main types, open and closed; a closed system is

defined by an enclosed growing environment (such as a shade- or greenhouse), whereas an open system is exposed to the natural outdoor environment. Preliminary farm visits also indicated that a range of growing media, containers, and pest management practices are used in blueberry propagation in the state (P.M. Brannen, *personal communication*).

Propagation of blueberries in Georgia is still on the rise, and diseases affecting blueberry cuttings will continue to be a problem for a long time. Most growers are uncertain how to handle disease issues when they arise, and extension agents are hampered in their recommendations by the lack of chemical controls registered for use on blueberry cuttings. Four of the most commonly mentioned pathogens are species of *Pythium*, *Phytophthora*, *Cylindrocladium*, and *Rhizoctonia*, causing Pythium, Phytophthora, Cylindrocladium, and Rhizoctonia root rots, respectively (5,6,12).

The Oomycetes *Pythium* and *Phytophthora*, also known as water molds, are favored by high moisture levels and typically are associated with poorly drained soils or propagation media. The most likely Oomycetes to pose a threat to propagation systems are *Phytophthora cinnamomi*, which is a common pathogen of ericaceous plants, and *Pythium* spp., which until recently were not generally associated with root rot in blueberry (2). *Phytophthora cinnamomi* was first reported as a pathogen of blueberry in 1963 (11), but by 1967, 40% of the blueberry plantings surveyed in southeastern North Carolina were affected (8). The wide host range of *P. cinnamomi* and its broad distribution make it a serious threat to blueberry production nationwide. Symptoms manifest as brown to black lesions on the fibrous feeder roots. Later, wilting, reddening, and/or chlorosis of the leaves, tip dieback, and stunting of the plant can occur as the pathogen works its way to the larger roots and stem, which can eventually be girdled. Highbush (*Vaccinium*

corymbosum) and southern highbush (*V. corymbosum* interspecific hybrids) blueberry cultivars are the most susceptible to infection by this pathogen, whereas most rabbiteye (*V. virgatum*) cultivars are somewhat resistant (8). Symptoms of *Pythium*-related root rots are very similar but typically result in the loss of vigor as opposed to plant death. The life cycles of both pathogens are similar, as are the conditions favoring disease.

Rhizoctonia spp. are fairly common pathogens in propagation systems, especially in the ornamental industry. Whereas little has been formally written on *Rhizoctonia* as a pathogen of blueberry, several sources have reported *Rhizoctonia* spp. causing a disease on this crop. In an unpublished study by Cameron Whiting (*personal communication*) in 2005, six *Rhizoctonia* isolates were obtained from blueberry nurseries in south Georgia, three of which proved especially aggressive on blueberry cuttings. The University of Georgia Plant Diagnostic Clinic also has diagnosed *Rhizoctonia* as a disease-associated agent in 5 of 17 blueberry root rot samples submitted for diagnosis in 2006 (12). As such, *Rhizoctonia* must be considered a possible threat to blueberry propagation.

Cylindrocladium spp. are the imperfect form of Ascomycetes in the genus *Calonectria*. This pathogen was first reported as a disease-causing agent on North Carolina blueberries in 1973 (9). Attacking stems, leaves, and the developing roots of cuttings, it is one of the few pathogens that has been implicated in plant-to-plant spread of disease in blueberry cuttings (3). The first symptom of infection is the formation of a dark brown or black lesion on the stem base which will eventually girdle the cutting, resulting in wilting, leaf drop, and eventual mortality. Roots can also be attacked, resulting in darkened roots and root rot. Leaf infection results in brown leaf spots measuring 1 to 3 mm in diameter with red borders. Perithecia will occasionally

form on necrotic tissue; easily seen without the aid of magnification, these are bright orange and measure 260 to 400 μm in width (10). This disease spreads through rooting beds leaving circular areas of blighted cuttings measuring 0.3 to 1.2 m in diameter (3).

Currently, no fungicides are registered for the control of *Rhizoctonia* or *Cylindrocladium* on blueberry crops. Further complicating matters is the lack of consistency in propagation methods among growers. In order to understand the problem more fully, an investigation was made into the current state of Georgia's blueberry propagation industry to determine the most effective production methods for the region. The primary pathogens associated with cutting failure and their importance must be determined along with the chemical controls needed to manage them. Fungicides were tested for efficacy and will be submitted for registration so extension agents can make better recommendations to blueberry propagators.

Based on these needs of the blueberry propagation industry, the following objectives were pursued:

1. Document propagation methods used and problems occurring in blueberry nurseries in Georgia.
2. Identify pathogens associated with blueberry propagation in Georgia and determine their relative frequency and pathogenicity.
3. Evaluate fungicidal treatments for key pathogens associated with blueberry propagation in Georgia.
4. Develop an extension bulletin summarizing how to produce disease-free blueberry cuttings.

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CHAPTER 2

LITERATURE REVIEW

Blueberry propagation systems. There are two main types of propagation systems in use, the covered design and the open design. The open design is fairly simple; the cuttings are exposed directly to the environment, and rain is allowed to fall on the cuttings and supplemented with sprinkler systems. Windbreaks may be used to maintain the efficiency of mist systems under windy conditions, and tall stands of pine trees may provide protection from the afternoon sun. In a covered design, the cuttings are protected from rainfall and the sun by a propagation structure, which can range in complexity from simple shade cloth tents to complex greenhouses (7).

Propagators have traditionally used propagation beds for rooting cuttings. In North Carolina, most poor rooting results can be attributed to anoxic conditions in the soil due to media compaction and water-logging (6). Rooting beds must provide adequate drainage, and water levels must be maintained carefully to prevent anoxic conditions from developing, especially in media containing components with high water-holding capacity such as peat moss. This is typically achieved by constructing raised beds with raised edges between 15 to 20 cm high; wire screen is attached to the bottom to form a tray to hold the rooting medium. This can be placed on a bed of gravel, suspended above the ground on cement blocks, or raised on legs to chest level (11). In North Carolina, it is recommended that the beds be built atop a layer of coarse sand between 30.5 to 45.7 cm deep (14). The sand draws out excess moisture via capillary action. Whereas used commonly in Georgia, large cutting beds have been discouraged due to disease issues associated with the fact that at least part of the medium is typically reused when a new

batch of cuttings is started (14). Containers can also be used to grow cuttings, but the containers must be deep enough so the developing roots can avoid zones of saturation that form at the bottom. Typically, containers ~12 cm deep are sufficient. The most typical container utilized to grow cuttings is the black plastic "trade gallon" commonly used by ornamental plant producers. These containers are both inexpensive and reusable if sterilized with a 10% household bleach solution. Container-grown cuttings often fare better than those grown on beds since the growing medium is used only once, thus reducing the chance of pathogen infestation. Pathogen spread is largely limited within the container, as opposed to spreading freely throughout an entire bed. Containers with diseased cuttings can also be removed easily to eliminate the inoculum source.

Propagation media. Propagation media for blueberries must meet three requirements; they must be acidic, porous, and well drained. The medium must maintain a 100% humidity level around the base of the cutting, whereas allowing excess water to drain away. Until recently, the propagation medium of choice had been aged piles of sawdust from the lumber industry. As these piles have become rare over the years, other options have been explored (7). Coarse sand, milled pine bark, and peat moss mixed at a ratio of 1:1:1, a 1:1 ratio of perlite mixed with either peat moss or milled pine bark, or milled pine bark without any other components all can give good results (14). In Georgia the most commonly used medium for blueberry propagation is plain milled pine bark (14). With Georgia's large pine industry, this medium is readily available and relatively inexpensive, costing approximately $7 per cubic yard (10). Most growers obtain good results with this medium.

Propagation methods. Until the widespread use of misting systems, use of hardwood cuttings was preferred over other propagation methods. Mist systems allowed growers to

propagate with softwood stock without the fear of the cuttings drying out between watering. Another benefit of softwood cuttings is a shorter rooting period of 6 to 8 weeks as opposed to 6 months with traditional hardwood production. Use of softwood cuttings also usually results in better rooting percentages; 70 to 80% rooting can be expected with softwood cuttings, whereas only 60 to 70% of hardwood cuttings root (14). In addition, hardwood cuttings are more likely to develop stem blight (14), so softwood cuttings are now the dominant method of blueberry propagation in Georgia for the production of both southern highbush and rabbiteye blueberries (14). In south Georgia, softwood propagation can begin after the first flush of new growth between April and May, depending on the cultivar's chilling requirements. Most sources agree that blueberry cuttings taken during this period root more successfully than those taken later in the year (8,14). A second flush of growth occurs in late August or early September, giving Georgia growers a second chance for collecting propagation material (14). Even though rooting percentages are lower, many growers take advantage of this second window as they are occupied with the blueberry harvest during the spring flush. Softwood cuttings should be taken from the terminal new growth and be between 11.5 and 15 cm in length with leaves just reaching maturity (7). Terminal cuttings are preferred, but if propagation material is scarce, multiple cuttings can be made from the same shoot. Cuttings should be placed in a moist environment such as a bucket of water or wet burlap bag or other similar container as soon as they are cut to prevent wilting. Ideal cuttings should be rigid enough to withstand insertion into the growth medium without breaking while retaining some degree of flexibility. Before sticking, the cuttings should be stripped of all but the top three or four leaves to increase air flow (14), and cuttings should be

spaced no closer than 3.8 to 5 cm apart (7). After sticking, young cuttings require a thin film of water on their foliage to prevent desiccation, especially during high temperatures.

Many different methods of irrigation, from hand-watering to impact sprinklers, have been used in production of blueberry cuttings, but most growers use overhead misting systems to maintain the required humidity levels. The mist is usually applied for a 5 to 10-sec burst every 2 to 10 min (14). Mist settings will vary based on the propagation structures being used as well as ambient temperature and humidity. For example, closed structures require less frequent intervals whereas open systems require more, and high temperatures demand more frequent intervals as well. Once the cuttings have rooted, the frequency of irrigation can be reduced, and the importance of leaf wetness is superseded by the moisture level of the medium. The level of moisture in the medium can be tested by simply taking a handful of the medium and squeezing it as hard as possible. No more than two or three drops of water should be extracted from the medium, and additional moisture is a sign of overwatering (7). Using this technique, irrigation of the cuttings can be adjusted to proper levels.

Diseases in blueberry propagation and their control. Along with increased demand for cuttings has come increased awareness of diseases affecting propagation. The most common symptoms reported by growers are stunted root systems, root rots, failure to root, and cutting death. Whereas abiotic factors are known to contribute to these problems, such as overwatering and anoxic conditions in rooting media, diseases also play a part in propagation losses (14). Several soilborne pathogens are possible candidates for losses in propagation beds. Among these, species of *Cylindrocladium*, *Rhizoctonia*, and the Oomycetes *Pythium* and *Phytophthora* have been associated with poor rooting and/or death of blueberry cuttings. Propagation losses are

often poorly reported, but in certain instances they can exceed 50% or more depending on the disease and the growing conditions.

Cylindrocladium. *Cylindrocladium* spp. are the imperfect form of Ascomycetes in the genus *Calonectria*. The first report of this pathogen attacking blueberries came in 1973 in North Carolina (17). *Cylindrocladium* attacks stems, leaves, and roots of cuttings, and it is one of the few pathogens implicated in plant-to-plant spread of disease in blueberry cuttings (6). The first symptom of infection is the formation of a dark brown or black lesion on the stem at soil level. This lesion will eventually girdle the cutting, resulting in wilting, leaf drop, and eventual death. Roots can also be attacked, causing darkened roots and rot. Leaf infection results in brown leaf spots measuring 1 to 3 mm in diameter with red borders (17). Perithecia will occasionally form on necrotic tissue. The perithecia, easily seen without the aid of magnification, are bright orange and measure between 260 to 400 μm wide (17). This disease spreads through rooting beds leaving circular areas of blighted cuttings measuring 0.3 to 1.2 m in diameter (6). There are three infective propagules for this pathogen: conidia, ascospores, and microsclerotia. The conidia of *Cylindrocladium* associated with blueberry are cylindrical, hyaline, generally have three septations, and average 70.2 × 6.0 μm in size. Ascospores average 36.6 μm long by 6.0 μm in width, with one to three septations and constrictions at each septum (17). The primary survival structures for this fungus are microsclerotia. In most plant species, *Cylindrocladium* microsclerotia form primarily on the roots, but they have also been observed on leaves, stems, and flowers. Whereas no microsclerotia have been reported in blueberry tissue, long chains of enlarged hyphal cells, a precursor to microsclerotia formation, have been observed (16). *Cylindrocladium* is spread primarily through contaminated plant material or propagation

substrate. Reuse of propagation media has been implicated in the spread of *Cylindrocladium* in North Carolina rooting beds and is not recommended (6).

Control of *Cylindrocladium* in propagation beds is difficult due to the buildup of microsclerotia in the medium. Formation of these survival structures makes sanitation a prime concern to propagators. Reuse of media is discouraged due to the possibility of heavy losses, and sticking cuttings into *Cylindrocladium*-infested propagation medium frequently resulted in 100% loss (6). Contamination of media by contact with native soil is another source of concern for the grower. *Cylindrocladium* is frequently associated with tulip poplar (*Liriodendron tulipifera*), sweet gum (*Liquidambar styraciflua*), and many other tree species native to Georgia (19). Media storage should be established away from trees, preferably on a cement slab to reduce the chance of contamination. If reusable containers are employed, they should be disinfested in a 10% household bleach solution before subsequent usage. Chemical control of *Cylindrocladium* in rhododendron, a related ericaceous plant species, is typically achieved by applications of thiophanate-methyl (Cleary's 3336, OHP 6672, and others), triflumizole (Terraguard), fludioxinil (Medallion), or pentachloronitrobenzene (Terraclor) (12,18) These fungicides are generally applied as a drench to control root and stem rots. In the past, this disease was controlled in azalea and rhododendron operations by fumigating the propagation beds with methyl bromide at a rate of 9.77 kg per 100 m^2 (18), and Cline (6) demonstrated that methyl bromide application works well in blueberry propagation beds in North Carolina. However, restrictions on usage make this option problematic, and its viability in the future is highly doubtful.

Rhizoctonia. *Rhizoctonia* spp. have a broad host range which includes the members of the blueberry genus *Vaccinium* (1) and several other ericaceous hosts, most notably the rhododendrons and azaleas. Whereas little has been written on *Rhizoctonia* as a pathogen of blueberry, several sources have mentioned *Rhizoctonia* spp. causing disease on this crop. In an unpublished study by Cameron Whiting (Valdosta State University) in 2005, six isolates of *Rhizoctonia* were obtained from blueberry nurseries in south Georgia, three of which proved especially aggressive on blueberry cuttings. The Plant Diagnostic Clinic at the University of Georgia has also diagnosed *Rhizoctonia* as a disease-associated agent in 5 of the 17 of blueberry root rot samples submitted for diagnosis in 2006 (23). As such, *Rhizoctonia* must be considered a possible threat to blueberry propagation. The symptoms of Rhizoctonia root rot are generally similar to those caused by *Cylindrocladium*. Infected plants develop reddish-brown lesions on the roots or stem just below the soil line, which, if given time, can girdle the stem and kill the plant. In rhododendron, *Rhizoctonia* can cause web blight in the canopy as well.

Rhizoctonia root rot is favored by high temperatures, high humidity, and high moisture content in the medium. These, unfortunately, are the conditions in which blueberries are propagated. *Rhizoctonia* primarily overwinters as sclerotia in the soil or on plant debris from previous crops. Sclerotia can survive in contaminated propagation media or recycled containers, so sanitation is a priority in the control of this disease. The sclerotia of *Rhizoctonia* are much larger than the microsclerotia of *Cylindrocladium*, and are highly variable in size, shape and color. Sclerotia form either from undifferentiated hyphae or, more frequently, small chains of thick-walled monilioid cells. These aggregates of barrel-shaped cells compact and divide to form a “loose type” sclerotium without the rind found in some of the other sclerotium-forming fungi.

Sclerotium formation is favored by high nutrient availability, high humidity, and good aeration. Light also seems to play a role in some isolates (21). Sclerotia can survive in the soil for years if conditions are favorable. When sclerotia germinate, hyphae grow toward young plants attracted by carbohydrates, phenols, organic acids, and other root exudates. Upon contact with the cutting/young plant, the fungus begins to colonize the plant surface, gaining a firm foothold before forming characteristic "T-shaped" branches whose swollen tips form infection pegs which penetrate the host tissue. In some cases, complex mats of hyphae form, resulting in an infection cushion which can produce multiple infection pegs. After penetration, the hyphae begin colonization of the epidermis and outer cortex layers, giving rise to stem or root lesions (13).

Control of *Rhizoctonia* is almost identical to that of *Cylindrocladium*, with sanitation being the most important component. Any cuttings showing symptoms or signs of infection should be culled. In addition, the cuttings surrounding the diseased area should be removed to prevent plant-to-plant spread of the pathogen. Fungicides for the control of this pathogen in other ericaceous crops include many of those recommended for *Cylindrocladium*. Applications of thiophanate-methyl, triflumizole, or pentachloronitrobenzene have been recommended in azalea and rhododendron propagation (12).

Oomycetes. Other candidates for disease-related propagation failures in Georgia are the Oomycetes. These members of the kingdom Stramenopila are favored by high moisture levels and typically are associated with poorly drained soils or media. The most likely Oomycetes to pose a threat to propagation are *Phytophthora cinnamomi*, which is a common pathogen of ericaceous plants, and *Pythium* spp., which until recently were not generally associated with root rot in blueberry. *Phytophthora cinnamomi* was first reported as a pathogen of blueberry in 1963,

but by 1967, 40% of the blueberry plantings surveyed in southeastern North Carolina were affected (15). The wide host range of *P. cinnamomi* and its broad distribution make it a serious threat to blueberry production nationwide. Symptoms manifest as brown to black lesions on the fibrous feeder roots. Later, wilting, reddening, and/or chlorosis of the leaves, tip dieback, and stunting of the plant can occur as the pathogen works its way to the larger roots and stem, which can eventually be girdled. Highbush and southern highbush blueberry cultivars are the most susceptible to infection, whereas most rabbiteye cultivars are more or less resistant to *Phytophthora* (20). Symptoms of *Pythium*-related root rots are very similar but typically result in a loss of vigor rather than plant death. The life cycles of both pathogens are similar, as are conditions favoring disease.

Phytophthora cinnamomi overwinters as chlamydospores in soil, media, and plant debris. These asexual spores can survive for up to 6 years until favorable conditions arise (9). Conducive conditions for disease development include temperatures of 20 to 32°C and an abundance of available moisture. Chlamydospores can either germinate directly or produce a sporangium. Sporangia of *P. cinnamomi* are slightly ovoid, nonpapillate, and highly variable in size. They can measure anywhere from 19 × 14 μm to 60 × 62 μm, but they average around 43 × 35 μm. Sporangia give rise to 15 to 20 biflagellate zoospores which are attracted to root exudates from the zone of elongation. Zoospores can remain motile in water for extended periods of time. Factors such as temperature, oxygen availability, or pH of the water all influence zoospore motility. In the case of *P. cinnamomi,* motility may last as long as 84 hours under optimum conditions (9). Eventually the zoospores lose their flagella and form cysts. The zoospores of *P. cinnamomi* can survive in pond water and be spread through irrigation systems (22). The surface

of encysted zoospores contains vesicles which produce sticky substances, allowing them to adhere to the roots of host plants. Within 30 min of encystment the zoospores germinate, forming a germ tube which invades the root and can spread through the vascular tissue. The internal mycelia produce more chlamydospores within the plant tissues. Because of its heterothallic nature, oospore formation is rare in *P. cinnamomi*. When compatible mating types are present, oospores measuring 31 to 50 μm in diameter are produced (9).

Pythium spp. survive in the soil as mycelium or as oospores, which either germinate directly forming cottony white mycelia or give rise to sporangia which in turn produce a saclike vesicle where 100 or more biflagellate zoospores are formed. Like the oospores, sporangia can also germinate directly. Zoospores undergo a short swarm phase, and they then round off and lose their flagella much the same as *Phytophthora*. The encysted spore produces a germ tube that can either penetrate directly the host's root tissue or form a vesicle and more zoospores. The size and shape of oospores and sporangia are highly variable among species and can be used for identification. Most *Pythium* species are primarily saprophytes and usually not pathogenic. However *Pythium* can be an opportunistic pathogen if presented with a stressed host plant. As with *P. cinnamomi*, the zoospores of *Pythium* can survive in pond water and be spread through irrigation systems (22).

Both cultural and chemical control methods must be employed to control *Pythium* and *Phytophthora*-incited diseases. Sanitation plays a large role in producing disease-free cuttings. In addition to protecting propagation media from potentially infested field soils, the cuttings should be taken from the upper portions of the plant. Shoots low to the ground are subject to rain splash and could become contaminated. Cuttings showing symptoms or signs of infection should be

discarded along with any surrounding plants to forestall an outbreak. This is difficult in a bed propagation situation, whereas container-grown cuttings facilitate the culling process. In a study of commercial blueberry plantings in Oregon, fields where these pathogens were detected were generally asymptomatic unless soil drainage was poor (5). This reinforces that propagation beds must be well drained, especially in open propagation systems where rainfall can not be controlled. Cuttings rooted in containers can also be subject to infection by pathogenic Oomycetes. If containers are placed on impermeable or poorly drained surfaces, standing pools of water allow zoospores to spread from container to container. To prevent this, containers should be placed either on raised benches or on beds of coarse gravel. Retention ponds can also be contaminated by runoff from infected fields or greenhouses, so deep wells are the best source of water for propagation. Water from retention ponds can be used if the water intake for the pump is positioned midway between the surface and the pond's bottom. Oomycete zoospores tend rise to the surface of ponds, whereas most other plant pathogens have spores that settle to the bottom. Water drawn from mid-water should remain relatively free of pathogens (22).

Options for chemical control of *Phytophthora* have been explored more thoroughly than for the other blueberry root rot pathogens. Fosetyl-Al (Aliette), potassium phosphite (ProPhyt, Fosphite), mono and di-potassium salts of phosphorous acid (K-phite, AgriFos**)** and mefenoxam (Ridomil Gold) are registered on blueberries and show good control of *Phytophthora* (2,4). Fortunately, these chemicals control *Pythium* as well (3). Forsetyl-Al and other phosphonate fungicides have similar modes of action; so mefenoxam is important in chemical rotation schedules.

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CHAPTER 3

SURVEY OF PRODUCTION PRACTICES USED IN BLUEBERRY NURSERIES IN GEORGIA

3.1 Introduction

With the rapid expansion of Georgia's blueberry industry in the past 10 years (1), the demand for rooted cuttings has increased exponentially. This has prompted many growers to begin propagating cuttings both for their own use and for sale to other growers. The first published accounts of blueberry propagation were made in a bulletin from the USDA Bureau of Plant Industry entitled "Experiments in Blueberry Culture" by Dr. Frederick V. Coville (4). When this work came to the attention of Elisabeth White, the daughter of a prominent cranberry producer in New Jersey, she contacted Coville concerning the possibility of commercial blueberry production in her area. The result was a long and successful partnership which gave birth to the modern commercial blueberry industry. In 1916, Coville published "Directions for Blueberry Culture, 1916," laying out the basic procedures for the propagation and production of blueberries as a horticultural crop (5). In the interceding 93 years, very little has changed in blueberry propagation methods (3). Many recommendations have been made based on Coville's work, but no preferred method has been established for either propagation per se or disease management during propagation. Thus, it is not surprising that growers and nursery operators in Georgia have applied a wide range of methods which combine elements from many different sources, resulting in wide variation in propagation methods (P.M. Brannen; *personal communication*).

Propagation of blueberries is achieved primarily by rooting either softwood or hardwood cuttings. For hardwood propagation, cuttings measuring 10 to 15 cm long with a diameter of 0.4 to 0.8 cm are taken from the previous season's growth in late January to early February (8). In contrast, there are two opportunities for taking softwood blueberry cuttings: May to early June and late July to early August. Softwood cuttings are taken from apicial shoots from the current season's growth and should measure between 11.5 and 15 cm in length (3). Another advantage of softwood is the speed at which rooting occurs; softwood cuttings can be rooted in a mere 6 to 8 weeks (8), compared with hardwood stock which can take as long as 6 months to produce a rooted cutting. Hardwood cuttings also generally have lower rooting percentages and are subject to stem blight, caused by *Botryosphaeria dothidea*, as compared with softwood cuttings.

In Georgia, softwood cuttings are the primary choice for propagation material, but many different propagation systems and methods are employed by growers (8). For example, propagation systems can be separated into two main types, open and closed; a closed system is defined by an enclosed growing environment, whereas an open system is exposed to the natural outdoor environment. Preliminary farm visits also indicated that a range of growing media, containers, and pest management practices are used in blueberry propagation in the state (P.M. Brannen, *personal communication*). In order to better understand the diverse methods of propagation found in Georgia and as a baseline for developing best management practices, a survey was conducted to document production and disease management methods currently in use.

3.2 Materials and Methods

Survey data was collected from 18 blueberry propagation nurseries located in Appling (*3*), Bacon (*5*), Brantley (*1*), Clinch (*5*), Pierce (*2*), and Ware (*2*) counties (key areas of blueberry production) in mid-June of 2007. During farm visits, producers were interviewed as to their current propagation methods in order to document the diversity in production methods, review current industry trends, and assess disease risk based on propagation practices. The survey consisted of the following questions:

1. How many cuttings do you take every year? How many are rabbiteye and how many are southern highbush? Are they hardwood or softwood?
2. What time of year do you take cuttings?
3. What percentage of your cuttings generally root?
4. Do you use rooting hormone?
5. Do you employ an open or a closed propagation system?
6. Do you use benches or grow at ground level?
7. Do you use containers? If so, what type?
8. Do you reuse containers? If so, do you sterilize the containers?
9. What kind of medium is being used for propagation?
10. Do you reuse media?
11. What is your source of irrigation water for your propagation system?
12. What type of irrigation system do you use? (mist heads, impact sprinklers, etc...)
13. What fungicides do you use and at what rates are they applied?
14. What is your spray schedule?

15. What disease(s) do you generally see in your propagation system?

The collected survey data was summarized (means for continuous variables, frequencies for categorical variables), and associations between selected practices (e.g., use of open or closed system vs. production in beds or containers) were analyzed using Chi-Square contingency tables.

3.3 Results and Discussion

As expected based on preliminary observations, this survey revealed a diversity of propagation methods. Growers in close proximity to each other tended to use similar methods, but they would still try to improve upon the ideas gleaned from neighboring nurseries. The resulting lack in uniformity makes identifying best practices leading to success more difficult. To illustrate the diversity present in the propagation systems surveyed, the production methods of four nurseries will be outlined for comparison.

Propagations system examples. A typical example of an open propagation system was located in Bacon County (Fig. 3.1). This nursery eliminated construction costs by growing propagated plants under a pine stand, which acted as a wind break and provided shade for the cuttings. Cuttings were produced in “trade gallon” (2.84 L) plastic containers on ground covered with a ground cloth barrier to suppress weed growth and help prevent ingress of pathogens from the soil into the pine bark rooting medium. Cuttings were taken in May to June. Impact sprinklers were used for irrigation, and the water source was a deep well. In 2006, this nursery produced 200,000 cuttings, two-thirds of which were rabbiteye and one-third southern highbush. The disease management program consisted of fungicide sprays every 7 to 10 days with a

rotation of trichloromethyl, pyraclostrobin, and thiophanate-methyl. The grower reported 90% rooting using this system, which concurred with observations at the site. However, poor site drainage in low areas tended to result in pooling water from the irrigation system, which led to lower rooting success in certain portions of the nursery. In years with heavy rain, this drainage issue could pose serious problems.

Another open growing system, located in Clinch County, used very different propagation methods (Fig. 3.2). As before, cuttings were propagated in "trade gallon" containers with milled pine bark as the medium, but the containers were grown in full sun on ground cloth under an overhead mist system connected to a deep-well water source. Cuttings were taken in September during the second flush of vegetative growth, and these were treated with trichloromethyl only when disease was observed. Both *Fusarium* and *Cylindrocladium* were recovered from this nursery (see Chapter 4). The grower reported producing 200,000 cuttings in 2006 with 80% rooting success. At this nursery, overcrowding was a major concern, as more than 20 cuttings were stuck in each container, and of these, approximately one-third had died. Overcrowding, along with sun exposure (overheating) on the sides of the containers, apparently was responsible for the failure of these cuttings. The estimated 80% rooting success seems overly optimistic considering issues observed.

A good example of a closed propagation system was found in Pierce County. Cuttings were grown in a shade-house on benches under overhead mist (Fig 3.3). The grower used open propagation flats filled with finely milled pine bark as a propagation medium, and he reported production of 80,000 cuttings with equal proportions of highbush and rabbiteye and 90% rooting. Cuttings were taken during the first flush in late May to mid-June, and these were subjected to a

10-day spray schedule with rotations of trichloromethyl, mefenoxam, benzimidazole, and phosphonate-based fungicides. On-site observations confirmed high rooting percentages, and mostly asymptomatic cuttings were found at this nursery. Benches appeared to promote good air flow, and containers were well-drained while retaining enough moisture to prevent cuttings from drying out. Benches also made working with cuttings much easier, as no bending was required.

Another closed system in Bacon County used entirely different methods. The propagation house was constructed of fiberglass with canvas sides to allow cross-ventilation (Fig 3.4). Cuttings were propagated in raised bark bed troughs approximately 0.76 m high and 0.91 m wide which ran the length of the structure. The bottom of the bed was lined with drainage tile covered by a 30.5-cm sand layer. This was covered by a thick layer of finely milled pine bark which acted as the propagation medium. Cuttings were irrigated by a mist system controlled by a micro-leaf mist controller (Fig 3.5); this is a mechanical device that consists of a small section of light wire mesh at the end of a lever connected to a solenoid valve. As long as the mesh is wet, the solenoid remains in the off position, but as the mesh dries, the lever rises to activate the solenoid which turns the mist on. Cuttings were taken in June toward the end of the first vegetative growth flush, but sometimes a second crop of rooted cuttings was produced after the second flush in August to September. The fungicide program at this nursery consisted of weekly rotations of trichloromethyl and thiophanate-methyl. Both *Cylindrocladium* and *Rhizoctonia* were subsequently recovered from this nursery (see Chapter 4). The grower reported producing 350,000 cuttings with an 85% rooting percentage, which appeared overly optimistic in light of several issues observed. Among these, the propagation beds were severely overcrowded with little room for airflow among cuttings. The close proximity of cuttings also facilitated disease

spread through plant-to-plant contact and spread through the medium. The second factor that increased disease risk was the reuse of medium for a second crop of cuttings later in the year. Losses in these beds were severe, and diseased cuttings were prevalent, with ~30% of the propagation bed area showing diseased plants.

Prevalence of specific propagation methods. One factor complicating data analysis was the relatively small data base of nurseries available for survey (n = 18). Many blueberry growers simply purchase nursery stock rather than producing it themselves, especially in cases where the grower is establishing initial field production. Still, many observations made during the course of the survey and the raw data collected shed light on the current situation in south Georgia's blueberry nurseries (Table 3.1).

Types of cuttings produced. There are two major types of blueberries produced in south Georgia, rabbiteye (*Vaccinium virgatum*) and southern highbush (*V. corymbosum* interspecific hybrids) (11). Based on the results of this survey, nurseries are producing more rabbiteye cuttings than southern highbush, but the relative proportions currently may be shifting toward an increase in southern highbush. Of the 18 nurseries surveyed, 14 responded with the approximate number and type of blueberry grown. These 14 growers produced approximately 1.6 million cuttings in 2007, of which 75% were rabbiteye. Historically, approximately 90% of blueberry plantings in Georgia have been rabbiteye (9), but in recent years the popularity of southern highbush cultivars has increased due to their earlier harvest date and subsequent market advantage. This, in addition to the fact that southern highbush blueberries are often grown in high-density beds (thereby requiring a larger number of nursery plants for initial establishment),

may account for the lower proportion of rabbiteye cutting production compared with field estimates of relative rabbiteye acreage.

Regarding the timing of cutting collection, 12 growers took softwood cuttings during the first flush in May and June, whereas four utilized softwood cuttings from the second flush in August and September, and only two engaged in hardwood propagation (taking cuttings in February and March). The time the cuttings were taken was dependent on the schedule of the blueberry farm. Cuttings were usually taken immediately after harvest, without regard to the type of blueberry (i.e., rabbiteye or southern highbush). Most growers chose not to use rooting hormone as previous experience had shown very little benefit on their rooting percentages. This is consistent with observations from North Carolina (3).

Nursery size. For the purposes of this paper a small nursery was defined as one that propagated 70,000 or fewer cuttings per year whereas a nursery propagating more than 70,000 cuttings was defined as a large operation. A slight majority of nurseries (60.0%) surveyed were large operations (Table 3.1). This proportion will fluctuate slightly from year to year as some of the growers produce cuttings based on their own needs, hence demand could drop considerably after their new plantings have been established.

Open vs. closed systems. Open growing systems are exposed to the elements, although some employ man-made or natural wind breaks to prevent moisture loss. In closed structures, cuttings are grown in a greenhouse or shade-house where moisture can be controlled more easily. Based on the survey (Table 3.1), the two general production types were represented roughly equally, with closed systems being slightly more prevalent (52.7%). Recent summer droughts have allowed growers the luxury of almost total control of moisture levels, even in open

propagation systems. However, an open system may prove to be a liability in wet years, as was the case in Georgia when reports of reduced rooting percentages followed heavy rains in 2001 (8). Closed propagation systems are the best option for growers who plant rooted cuttings annually. Whereas the initial expense might be high, the reductions in risk due to better environmental control will likely pay off over time.

Chi-Square contingency table analysis revealed a significant association ($P = 0.003$) between nursery size and the use of open vs. closed propagation systems. Specifically, large-scale production (>70,000 cuttings per year) tended to be associated with the use of closed systems for propagation (Table 3.2). Indeed, of large-scale nurseries, 77.8% used closed systems, whereas none of the small-scale nurseries used closed systems. This relationship is not surprising, as most large-scale growers have more resources to invest in permanent or semi-permanent structures. Conversely, small-scale growers primarily grow cuttings on a temporary basis and have less capital to invest in infrastructure.

Use of benches. Slightly less than half (47.1%) of the surveyed nurseries produced the cuttings on benches rather than at ground-level (Table 3.1). Chi-square analysis showed a trend ($P = 0.09$) for an association between propagation system (open vs. closed) and the use of benches, whereby two-thirds of propagators who used closed systems also used benches (Table 3.2). Open systems, on the other hand, tended to favor growing the cuttings at ground level with only 25.0% utilizing benches. This trend is not surprising, as it is anticipated that growers who go to the expense of constructing a propagation structure will also be willing to devote resources to bench construction.

Container vs. bed production. The decision whether to grow cuttings in continuous propagation beds or in containers is a very important consideration. Survey results showed that the two types were represented roughly equally, with containerized production being slightly more prevalent (Table 3.1). Five of the eight nursery operators who utilized bed systems chose to build their beds on benches or at least raised off the ground on cinderblocks. Containers also were either placed on benches or left on the ground, but there was no statistical association between container use and bench use (Table 3.2). For ground-based containerized systems, some kind of barrier (usually ground cloth) was used in most cases to prevent direct contact of the containers with the soil. Of the containers chosen for propagation, the standard "trade gallon" was by far the most popular option, being used in six out of the nine container-using nurseries. These containers are inexpensive, reusable, and easily available; provide sufficient room for multiple cuttings per container; and are deep enough to prevent the formation of zones of saturation around the roots. The other containers in use were deep cell packs and large propagation flats, and these met requirements needed for good rooting as well. Of those nurseries using containers rather than beds, 77.8% reused their containers. Of concern was the fact that only 33.3% of these nurseries sterilize the containers before reuse, providing potential ingress points for pathogens to enter propagation systems.

Propagation media. Historically the medium of choice for propagating blueberries was aged sawdust from sawmills. Over the years, most sources of sawdust have disappeared, forcing propagators to choose substitutes. Raw pine bark is now the primary propagation medium used in blueberry nurseries in North Carolina (3). This survey shows raw pine bark was used in 89% of nurseries in Georgia; only two nurseries used other types of propagation media, with one

having access to aged sawdust whereas the other used a peat-bark-perlite mixture. The grower using the mixed medium used deep cell packs, which allowed him to use much less medium than those who used either trade gallon containers or bedding systems, while also making the best use of the more expensive medium.

The almost exclusive use of raw pine bark as a propagation material is one of the few consistent factors illuminated in this survey. However the quality of the bark varied greatly among operations. Composted pine bark is highly recommended as a propagation material, and it has been shown to suppress many pathogens, including species of *Phytophthora*, *Pythium*, and *Rhizoctonia* (6). Notwithstanding these benefits, composted bark is rarely used. Composted bark takes more time to produce, and it would be required in greater volume as compared with raw pine bark, rendering it more expensive. As the price of pine bark continues to rise, or if issues of quality become more pervasive, a shift may occur away from pine bark toward other propagation media.

The reuse of the propagation media is another issue encountered in this study, as almost one-third of nurseries reported reusing the medium (Table 3.1). As expected, growers who used containers were the least likely to reuse media, with only 11.1% (1 out of 9) of this subgroup doing so. Based on a Chi-Square analysis of the data, a trend ($P = 0.08$) toward the reuse of media by growers using bedding systems (as opposed to containerized production) was noted (Table 3.2). Bark decomposes and settles by the end of the growing season, and if the grower uses a ground-based, continuous propagating system, there is a great temptation to simply add fresh bark to the top of the bed without removing the bark from the previous year. As bark ages

and breaks down, drainage is compromised, and the disease-suppressive qualities of the bark are much reduced (7).

In an ideal propagation bed system, the bark must be removed and disposed of after each growing season. As this process would be time-consuming and expensive, the use of containers is advantageous. Growing in containers has additional benefits, besides simply allowing easy disposal of used media. Containers provide barriers against the spread of soilborne pathogens; in a continuous rooting bed, pathogens can spread rapidly throughout the bedding system through plant-to-plant contact (2), but when containers are used, the diseased plants can simply be removed with the container, rather than going through the arduous process of removing the infected cuttings from the bed and then digging out the infected bark in an attempt to prevent further spread.

Water sources and irrigation systems. Most nurseries (88.2%) used wells as a source of irrigation water, with the remainder using pond water (Table 3.1). As Oomycete diseases can be spread through contaminated pond water (10), the widespread use of wells is encouraging. One fact nursery operators must keep in mind is that most well water in the survey area has a high pH. As blueberry plants are acid-loving members of *Ericaceae*, the alkalinity of the water must be taken into consideration when irrigating this crop.

Nursery irrigation occurred primarily by mist systems, with 12 of the 18 propagators utilizing this system. The remaining propagators utilized large open systems using less expensive impact sprinklers, which appeared to work well, especially where adequate shade and protection from the wind were provided to help prevent moisture loss. As long as cuttings are provided with a constant film of water on their leaves for the first 3 weeks after collection and sticking, they

should root. However, nurseries using impact sprinklers are more vulnerable to desiccation since this system relies on fewer spray heads to cover a relatively large area when compared with an overhead mist system, and a single clogged sprinkler head can result in significant losses. Cuttings can suffer irreversible damage if left dry for as little as 30 min (3).

Cutting failure and fungicide use. Most nursery operators provided only a vague estimate of rooting success, often in the range of 80 to 90%. However, observations made during the course of the survey stood in contrast to the numbers estimated by the growers, with both under- and overestimation taking place. This data is not tracked closely by many growers, as would generally be the case in most ornamental nurseries.

A major reason for cutting failure was the tendency toward overcrowding cuttings in both container and bed propagation nurseries. The standard recommended spacing is between 3.8 and 5 cm to allow for adequate airflow and root growth (3). Many growers stuck cuttings with a spacing of 1.5 cm. In some extreme cases, growers placed as many as 25 cuttings in a single trade gallon container, which at most should hold 14. Overcrowding increases competition among cuttings for moisture and space for developing root systems. Indeed, many of the cuttings in overcrowded beds died, having never formed sufficient roots due to lack of water and space to grow. In several cases where samples were taken for the pathogen isolations (described in Chapter 4), cuttings were difficult to remove from beds or containers because roots had inter-grown with neighboring plants. In order to separate the cuttings, the young plants have to be separated by either cutting or tearing the root system. This leaves numerous potential entry points for disease on replanting. As described in Chapter 4, in situations where Cylindrocladium root rot caused severe losses, cuttings appeared to be planted too close.

Diseases can be an important cause of cutting failure, but when growers were asked what diseases they encountered in their nurseries, many reported having observed problems, but they could not pinpoint the causal agents responsible. This suggests a need to better educate blueberry propagators about the potential disease threats to their crop. Results of a pathogen survey in these nurseries, as well as associations between certain propagation methods and pathogen prevalence, are presented in Chapter 4.

Disease management in the surveyed propagation systems appeared to consist primarily of fungicide applications to control diseases that affect the upper portion of the cuttings, such as gray mold (*Botrytis cinerea*), Phomopsis twig blight (*Phomopsis* sp.), Septoria leaf spot (*Septoria albopunctata*), and anthracnose leaf spots (*Colletotrichum* spp. and *Gloeosporium minus*). The most commonly used fungicides were Captan/Captec products (containing trichloromethyl). Other active ingredients used by at least three of the 18 nurseries included mefenoxam, phosphonate products, pyraclostrobin, and thiophanate-methyl. Several additional compounds were used less commonly across nurseries (Fig. 3.6). Whereas many growers are using broad-spectrum fungicides that can control many fungal groups, including Oomycetes (phosphonates and mefenoxam) and *Rhizoctonia* spp. (azoxystrobin), it must be mentioned that none of these chemicals are registered for this usage pattern (i.e., for use in blueberry propagation). This is a major concern revealed by this survey. As very few chemicals are registered for use in blueberry propagation, much work needs to be done via education of the growers concerning potential threats to their crop and to get chemicals registered for legal use in this industry.

3.4 Conclusions

The foundation of any horticulture industry begins with healthy plants. A streamlined and standardized method of propagation is needed to maintain the health of the blueberry industry. In the future, as the expansion of new blueberry acreage subsides, propagation will still play an important role in the continued success of the industry. As better-yielding and disease-resistant cultivars are released and old plantings are replaced, the demand for high-quality cuttings will continue although not in such large quantities as is currently the case. The wide diversity in propagation systems paints a picture of a propagation industry not yet out of its infancy. Whereas much information is available on the production of blueberries, very little has been published concerning propagation and diseases associated with propagation systems. With the widespread use of fungicides not registered for use in blueberry propagation, much research is necessary to determine the key pathogens associated with cutting failure as well as optimal chemical control programs. Strong recommendations should be made against the reuse of propagation media as it has been associated with Cylindrocladium root rot (see Chapter 4). Recommendations supporting the use of containers over the traditional bedding system should be made as propagation beds encourage media reuse. Based on this survey, the groundwork for future standardized recommendations for the production of disease-free blueberry cuttings can be laid.

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Table 3.1. Prevalence of propagation practices in blueberry nurseries in south Georgia, based on a survey conducted in 2007.

Attribute or practice	Classes	*n*	Frequency (%)
Nursery size	Large (>70,000 cuttings per year)	9	60.0
	Small (≤70,000 cuttings per year)	6	40.0
System type	Open	8	47.1
	Closed	9	52.9
Bench use	+	8	47.1
	-	9	52.9
Cutting production	Container	9	52.9
	Bed	8	47.1
Reuse containers	+	7	77.8
	-	2	22.2
Sterilize containers	+	3	33.3
	-	6	66.7
Reuse medium	+	5	29.4
	-	12	70.6
Water source	Pond	2	11.8
	Well	15	88.2
Irrigation method	Sprinkler	6	33.3
	Mist	12	66.7

Table 3.2. Frequency (%)[a] of south Georgia blueberry nurseries (n = 18) with select propagation practices.

	Nursery size		System type		Media reuse		Container use	
Attribute or practice	large	small	open	closed	+	-	+	-
Closed system	100	0.0						
Open system	25.0	75.0						
	(0.003)[b]							
Reuse growing medium	75.0	25.0	40.0	60.0				
Discard growing medium	54.5	45.5	50.0	50.0				
	(0.475)		(0.707)					
Propagate in containers	62.5	37.5	55.6	44.4	11.1	88.9		
Propagate in beds	57.1	42.9	37.5	65.5	50.0	50.0		
	(0.833)		(0.467)		(0.079)			
Grow on benches	71.4	28.6	25.0	75.0	12.5	87.5	37.5	62.5
Grow at ground level	50.0	50.0	66.7	33.3	44.4	55.6	66.7	33.3
	(0.398)		(0.086)		(0.149)		(0.229)	

[a] Frequencies in each row add up to 100%.
[a] Values in parentheses denote *P*-levels for associations at according to Chi-Square contingency table analysis.

Fig. 3.1. Open, containerized blueberry propagation system using impact sprinklers in Bacon County.

Fig. 3.2. Open, containerized blueberry propagation system in Clinch County (left). Note overcrowded containers (right).

Fig. 3.3. Closed, containerized blueberry propagation system (shade-house) in Pierce County, with cuttings produced in flats located on benches.

Fig. 3.4. Closed, bark bed-based blueberry propagation system in Bacon County.

Fig. 3.5. Micro-leaf mist controller in the blueberry propagation nursery shown in Fig. 3.4.

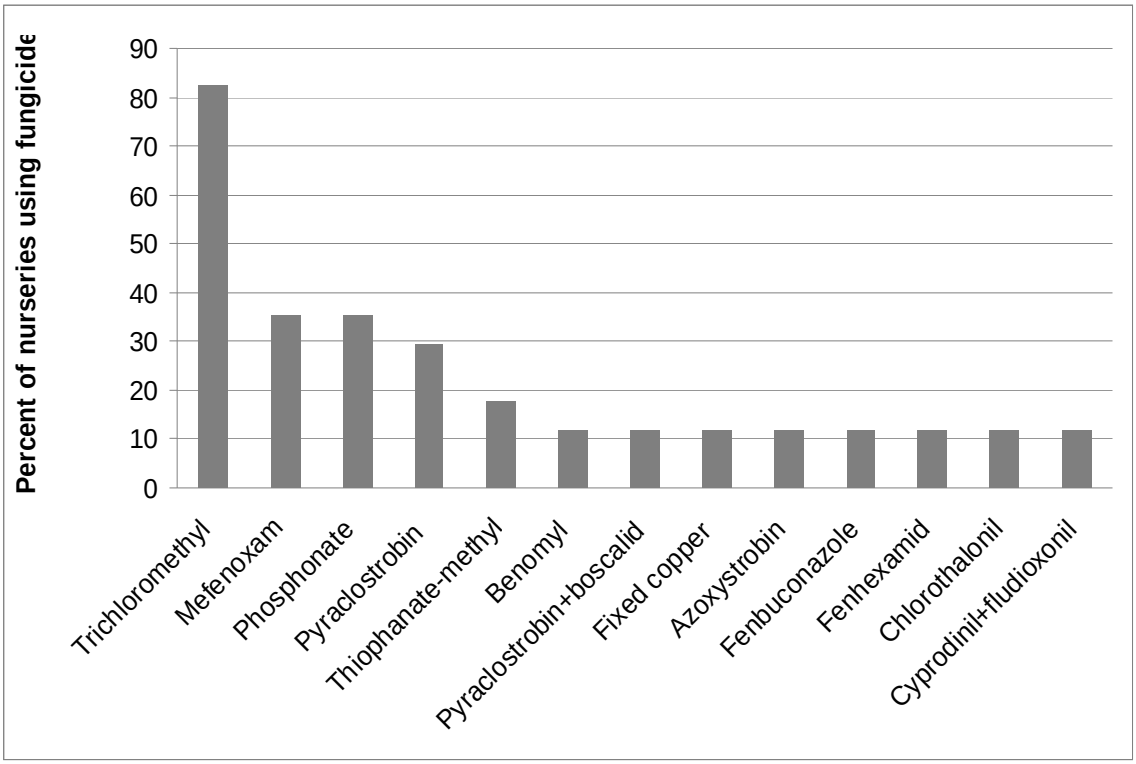

Fig. 3.6. Fungicide usage in surveyed blueberry propagation nurseries in south Georgia.

CHAPTER 4

SURVEY FOR THE PRESENCE OF POTENTIAL ROOT PATHOGENS IN BLUBERRY CUTTINGS COLLECTED FROM GEORGIA NURSERIES.

4.1 Introduction

A rapid increase in blueberry propagation in south Georgia has been accompanied by reports of increased disease in blueberry nurseries, but propagation diseases have not been addressed adequately by past research. Based on previous reports from other areas, the three main pathogenic fungi affecting blueberry cuttings are species of *Rhizoctonia* (Cameron Whiting, formerly at Valdosta State University, *personal communication*), *Cylindrocladium*, and the Oomycetes *Pythium* and *Phytophthora* (5).

Rhizoctonia has a broad host range which includes members of the blueberry genus, *Vaccinium*, and several other ericaceous hosts, most notably the rhododendrons and azaleas (1). Infected plants develop reddish-brown lesions on the roots or the stem just below the soil line which, if given time, can girdle the stem and kill the small plant. On rhododendron, *Rhizoctonia* can cause web blight in the canopy as well. Whereas little has been formally published on *Rhizoctonia* as a pathogen of blueberry, several sources have reported *Rhizoctonia* spp. causing a disease on this crop. In an unpublished study by Cameron Whiting (*personal communication*) in 2005, six isolates of *Rhizoctonia* were obtained from blueberry nurseries in south Georgia, three of which proved especially aggressive on blueberry cuttings. The University of Georgia Plant Disease Clinic has also diagnosed *Rhizoctonia* as a disease-causing agent in 5 of 17 blueberry root rot samples submitted for diagnosis in 2006 (17). As such, *Rhizoctonia* must be considered a

possible disease risk in blueberry propagation.

Cylindrocladium spp. are the imperfect form of Ascomycetes in the genus *Calonectria*. This fungus was first reported as a disease-causing agent on North Carolina blueberries in 1973 (13). Attacking stems, leaves, and the developing roots of cuttings, it is one of the few pathogens directly implicated in plant-to-plant spread of disease in blueberry cuttings (5). The first symptom of infection is the formation of a dark brown or black lesion on the stem base which will eventually girdle the cutting, resulting in wilting, leaf drop, and eventual mortality. Roots can also be attacked, resulting in darkened roots and root rot. Leaf infection results in brown leaf spots measuring 1 to 3 mm in diameter with red borders. Perithecia will occasionally form on necrotic tissue; easily seen without the aid of magnification, these are bright orange and measure 260 to 400 μm in width (14). This disease spreads through rooting beds leaving circular areas of blighted cuttings measuring 0.3 to 1.2 m in diameter (5).

Cylindrocladium produces three infective propagules: conidia, ascospores, and microsclerotia. In the field, blueberry-associated conidia of the species *C. parasiticum*, which has been previously reported to affect blueberry cuttings in North Carolina (13,14), are cylindrical, hyaline, generally have three septations, and average 70.2 × 6.0 μm in size. Ascospores average 36.6 μm long by 6.0 μm wide, with one to three septations and constrictions at each septum (14). The primary survival structures for this fungus are microsclerotia. In most plant species, *Cylindrocladium* microsclerotia form primarily on the root tissues, but they also have been observed on leaves, stems, and flowers. Whereas no microsclerotia have been reported in blueberry tissue, long chains of enlarged hyphal cells, a precursor to microsclerotia formation, have been observed (13). *Cylindrocladium* is spread primarily through contaminated plant

material or propagation substrate. Reuse of propagation media has been linked to the spread of *Cylindrocladium* in North Carolina rooting beds and is not recommended (5).

Other candidates for disease-related propagation failures in Georgia are the Oomycetes. These members of the kingdom Stramenopila are favored by high moisture levels and typically are associated with poorly drained soils or propagation media. The most likely Oomycetes to pose a threat to propagation are *Phytophthora cinnamomi*, which is a common pathogen of ericaceous plants, and *Pythium* spp., which until recently were not generally associated with root rot in blueberry (4). *Phytophthora cinnamomi* was first reported as a pathogen of blueberry in 1963 (16), but by 1967, 40% of the blueberry plantings surveyed in southeastern North Carolina were affected (12). The wide host range of *P. cinnamomi* and its broad distribution make it a serious threat to blueberry production nationwide. Symptoms manifest as brown to black lesions on the fibrous feeder roots. Later, wilting, reddening, and/or chlorosis of the leaves, tip dieback, and stunting of the plant can occur as the pathogen works its way to the larger roots and stem, which can eventually be girdled. Highbush and southern highbush blueberry cultivars are the most susceptible to infection by this pathogen, whereas most rabbiteye cultivars are somewhat resistant to *Phytophthora* (12). Symptoms of *Pythium*-related root rots are very similar but typically result in the loss of vigor rather than plant death. The life cycles of both pathogens are similar, as are the conditions favoring disease.

The purpose of this study was to identify the key pathogens associated with blueberry propagation in Georgia and to determine their relative frequency and importance to Georgia's blueberry propagation industry. To this end, three pathogen surveys were conducted in 18 blueberry nurseries in south Georgia during the 2007 growing season, followed by one early in

the 2008 growing season. Data on pathogen incidence at the nursery level were analyzed in relation to a survey of production practices used by these same nurseries

4.2 Materials and Methods

Sample collection. Samples were collected from 18 blueberry nurseries located in Appling, Bacon, Brantley, Clinch, Pierce, and Ware counties in four separate surveys during the 2007 and 2008 growing seasons. The first three surveys were conducted in 2007, and these were made on 12 to 14 June (corresponding to the first time period in which cuttings are normally taken), the second on 10 to 11 September, and the third on 25 to 26 October. A fourth survey to inspect and isolate from overwintered cuttings was made on 29 to 30 April 2008. Propagation sites, designated by number to maintain anonymity, were scouted visually for symptomatic cuttings. Cutting samples were selected based on symptom expression: defoliation, necrotic stem or root lesions, or stunting, regardless of blueberry variety or cultivar. However, the majority of collected cuttings were of the southern highbush blueberry type (*Vaccinium corymbosum* interspecific hybrids). At each site multiple samples were taken from at least four areas of apparent disease in each sampled bed. In the case of container-grown cuttings, samples were taken from at least four different containers at each site. If disease appeared in more than one propagation house/ bed or in sections with an obvious change in cultivar, separate samples were taken for up to two of these locations at each site. In the first survey, samples were taken from two separate locations at propagators 4, 6, 11, 12 and 17. In the second survey only grower 7 was sampled at two separate locations at the same site. Subsequent to the second survey, only one location per site was sampled. Cuttings for sampling were selected from the margins of areas of

symptomatic patches, placed in plastic bags, and stored in a cooler and cold room (5 to 6°C) until pathogen isolation. Preference was given to cuttings with root systems when available, as pathogen isolation was easier from rooted cuttings.

Not all growers had cuttings in production at the same time, so samples were not obtained from all propagators during each survey trip. In the first survey all of the cuttings collected had overwintered from the previous year. Fresh cuttings were in place at some locations but disease symptoms had not yet appeared on the new cuttings, hence these fresh cuttings were not sampled at this time. In the second survey samples were limited to the current season's cuttings which showed disease symptoms, whereby the age of the cuttings ranged from 1 to 3 months and most cuttings showed at least some evidence of rooting. The third survey included current season's cuttings which ranged from 2 to 4 months in age. By this date some of the cuttings had already been placed in grow-out beds or potted up, and fresh cuttings (not sampled at this time) had been put in place. The fourth survey consisted of overwintered cuttings from the previous growing season, similar to the first sample in 2007.

Pathogen isolation. From each of the four areas or containers sampled at each site and sampling date, one symptomatic cutting was selected and placed in a 400-mL beaker fitted with a wire screen over the mouth. Chlorinated tap water was allowed to gently run over the root systems for 5 min until particulate matter (mostly pine bark) adhering to the roots had been removed. Six root pieces (or stem sections if no roots were available) were removed from the margins of diseased tissues on each cutting, and each root piece was surface-disinfested by immersion in a 10% household bleach solution (0.5% NaOCl) for 30 sec, followed by a rinse in deionized water, and then followed by another surface-disinfestation in 70% EtOH for 15 sec.

The tissue pieces were then blotted dry on a sterile paper towel and plated on three media: PARP, a semi-selective medium for Oomycetes (8); Ko & Hora, a medium semi-selective for *Rhizoctonia* (9); and potato dextrose agar (PDA) amended with 0.20 g/L of streptomycin (SPDA), a general medium capable of supporting a wide variety of fungi. There were two tissue pieces from each cutting plated on duplicate Petri dishes for each of the three media. The dishes were incubated under diurnal light at room temperature (23 to 25^{o}C) and examined daily for fungal growth. When colonies were large enough to transfer, hyphal tips were plated on SPDA or corn meal agar (for Oomycetes). Isolates were identified to genus based on morphology. With the exception of Oomycetes, fungal isolates were stored on PDA slants; Oomycetes were transferred to V8 agar, and plugs of V8 agar were stored in sealed vials of sterile water.

Pathogen isolation frequencies were calculated at the cutting and nursery level at each sampling date. To determine isolation frequency at the cutting level, a cutting was considered positive for a given pathogen when that pathogen was detected on at least one of the six tissue pieces plated on any of the three media types on a particular survey date. Isolation frequency at the cutting-level was then expressed as a percentage of the total number of cuttings sampled from each survey. In order to calculate pathogen isolation frequency at the nursery level, a nursery was considered positive when there was at least one positive cutting (as described above) among the cuttings sampled from that nursery on a given date. Nursery-level isolation frequency was then expressed as a percentage of positive nurseries out of the total number of nurseries surveyed for each survey date.

Along with sampling, a survey was conducted among the nursery owners from which cuttings were collected regarding propagation practices, such as whether or not the propagation

system was open or closed, whether or not the grower reused propagation media, if containers were used or a bedding system was utilized, and if benches were used or if the cuttings were propagated at ground level (see Chapter 3). Results, in the form of binary values (e.g., presence or absence of a given pathogen species at the nursery level), were tabulated and subjected to Chi-Square contingency table analyses to determine trends and associations among propagation practices and pathogen presence (PROC FREQ in SAS v9; SAS Institute, Cary, NC).

4.3 Results

Many cuttings yielded saprophytic organisms, which were considered secondary for the purposes of this study, especially since they did not occur consistently with location or symptom. These included species of *Alternaria*, *Aspergillus*, *Colletotrichum*, *Paecilomyces*, *Penicillium*, *Pestalotia*, *Phlyctaena*, *Trichoderma*, and a few others which were not readily identified. Bacterial growth also was observed, especially on PARP medium.

In the first survey, which involved 10 nurseries, isolations were made from 55 cuttings that had overwintered from the previous year; 3.6% of cuttings yielded *Cylindrocladium*, 10.9% yielded *Rhizoctonia*, no Oomycetes were recovered, and *Trichoderma* was recovered from 23.6% of cuttings across the three media. Relatively high levels of *Fusarium* (29.1%) were also isolated in this survey, and they were recorded separately from the other fungi classified as saprophytes in this and all subsequent surveys since the pathogenic status of *Fusarium* on blueberry cuttings is unclear. Calculating the same data on the basis of the 10 nurseries included in the first survey period, *Cylindrocladium*, *Rhizoctonia*, Oomycetes, and *Fusarium* were present in 10.0, 20.0, 0, and 70.0% of nurseries surveyed, respectively (Tables 4.1 and 4.2).

The *Cylindrocladium* species isolated during the survey was further identified to species based on morphology. Identification of this organism is based on the shape of the vesicle at the tip of the stipe, morphology and color of the perithecia (if present), and length and number of septations in the spores (6). For identification, cultures were grown on carnation leaf agar and incubated under a near-UV light source at 25°C. Microscopic measurements were taken of the diameter and shape of vesicles (n = 16) of conidiophores as well as the length and number of septations of conidia (n = 23). The isolate examined produced orange-colored perithecia prolifically in the surface of the medium. The vesicles were invariably sphaeropedunculate (light bulb-shaped). The average diameter of the measured vesicles was 8 µm with a standard deviation of 1.81 µm. Conidia were mostly 3-septate (70%) and measured 60 µm in length with a standard deviation of 1.73 µm. Using a key it was determined that the isolate was *C. parasiticum* (6). *Cylindrocladium parasiticum* produces abundant red to orange perithecia and its vesicles average between 7 to 10 µm in diameter. The conidia are straight and range from 45 to 90 µm in length, with an average around 62 µm (6).

In the second survey, 49 cuttings that had been stuck in the current season were collected from 11 nurseries. Here the results were much different: *Cylindrocladium* was recovered from 10.2% of samples, no *Rhizoctonia* was detected, and 4.1% of sampled cuttings yielded Oomycetes. *Fusarium* was isolated at a cutting-level incidence of 12.2%, whereas the incidence of *Trichoderma* was greatly reduced, appearing in only 4.1% of samples. On a per-nursery basis, pathogen prevalence was 18.2% for *Cylindrocladium*, 18.2% for Oomycetes, and 45.5% for *Fusarium* (Tables 4.1 and 4.2).

In the third survey, which again involved current-season cuttings, 44 cuttings from 11 nurseries were subjected to isolations, and the percentage of cuttings infected with *Cylindrocladium* increased to 36.4%, compared with 4.6% of cuttings infected with *Rhizoctonia*. No Oomycetes were isolated, and *Fusarium* was detected in 18.2% of cuttings. On a per-nursery basis, a relatively large percentage (54.6%) of propagators had *Cylindrocladium* present in their nursery. Only 9.1% had *Rhizoctonia*, whereas 63.6% of nurseries had *Fusarium*, which was the highest percentage of *Fusarium* recorded in this survey.

The fourth and final survey (n = 56 cuttings from 14 nurseries) was made in late April and, similar to survey 1, consisted of cuttings that had overwintered. This survey showed a reduction in the percentage of pathogen recovery for *Cylindrocladium* (14.3%), *Rhizoctonia* (3.6%), and *Fusarium* (7.1%). Again neither of the Oomycetes were recovered. When tabulating isolation data at the nursery level, isolation frequency was again reduced, but *Cylindrocladium* was still detected at 21.4% of the 14 sites surveyed. *Rhizoctonia* and *Fusarium* were detected in 7.1 and 14.3% of nurseries, respectively (Tables 4.1 and 4.2).

Across the 204 cuttings sampled during the four survey dates 15.2, 4.9, 1.0, and 16.7% harbored *Cylindrocladium*, *Rhizoctonia*, Oomycetes, and *Fusarium*, respectively. Across the 18 nurseries included in the survey 41.2, 17.6, 0.1, and 82.4% were infested with *Cylindrocladium*, *Rhizoctonia*, Oomycetes, and *Fusarium*, respectively, on at least one of the four survey dates.

A survey of propagation practices was conducted from the nurseries where the cutting samples were collected (see Chapter 3), and these practices were compared with nursery-level presence or absence of *Cylindrocladium* and *Rhizoctonia* using Chi-Square tests to determine whether any propagation practices were associated with disease (Table 4.3). Oomycetes were not

subjected to this analysis due their low incidence. The analysis revealed a statistically significant association between the reuse of propagation media and the presence of *Cylindrocladium* at the nursery level ($P = 0.04$). None of the other three evaluated practices (use of open production system, bed production, and propagation directly on the ground) were significantly associated with presence of *Cylindrocladium* or *Rhizoctonia*.

4.4 Discussion

At the beginning of this study, *Rhizoctonia* and the Oomycetes were anticipated to be the most common pathogens associated with blueberry cuttings in nurseries in Georgia. This was due in part to findings in an unpublished isolation survey conducted by Dr. Cameron Whiting (formerly at Valdosta State University). In her studies, *Pythium* spp. were isolated from 58 and 33% of root segments in March and May of 2004, respectively. In a third survey in July 2004, no *Pythium* was isolated; however, this was attributed to the use of stem segments instead of root segments for isolation. In these same surveys, *Rhizoctonia* was also detected in 14 (March), 35 (May), and 14% (July) of samples. *Cylindrocladium* was not isolated from either root or stem segments but was detected in mulch collected during the May survey. *Fusarium*, was isolated from 12% of root pieces in the March 2004 survey. In addition to Whiting's data, records from the UGA Plant Disease Clinic also support the idea of *Rhizoctonia* and *Pythium* being common pathogens of blueberry in Georgia. In 2006, 17 blueberry samples showing symptoms of root rot were submitted to the clinic, and five were diagnosed with *Rhizoctonia*, four with *Phytophthora*, and six with *Pythium*. Of the remaining samples, one was diagnosed with *Alternaria*, and in the other no cause of disease could be determined. Interestingly, *Cylindrocladium* was not found in

any of the submitted samples (17). In North Carolina, *Cylindrocladium* is considered the primary pathogen of blueberry propagation (5). Based on the current pathogen survey, it can now be reported that *Cylindrocladium* appears to be a primary pathogen in Georgia propagation systems as well.

While not observed in high incidence in the first survey, *Cylindrocladium* was more frequent in subsequent surveys, especially the third, where over half of the nurseries surveyed harbored this pathogen. This may be attributed to the buildup of sufficient disease severity for detection. The fourth survey showed a reduction in the percentage of pathogen recovery, and this might be explained by the fact that the cuttings were overwintered from the previous growing season, and most of the cuttings showing significant levels of disease had either died or been discarded. These same conclusions would apply to the first survey date in 2007.

The fact that *Cylindrocladium* could still be recovered from overwintered cuttings may be significant in the disease cycle of this pathogen. In azalea, *Cylindrocladium* enters nurseries primarily as leaf spots on cuttings from the field (11). Since little is known about the epidemiology of this pathogen in blueberry, more research is required to determine the initial source of inoculum, but the disease cycles on the two plant species may be very similar. Also, given the lack of fungicides registered for use on blueberry cuttings, this pathogen poses an important threat to the blueberry propagation industry. Fungicides that would suppress Cylindrocladium root rot are not registered for this use pattern on blueberry, so cultural management is currently the only option.

Rhizoctonia, although observed, was not found as often as expected in light of Whiting's unpublished study and Plant Disease Clinic records. Although *Rhizoctonia* was not often

recovered, a possible explanation for low numbers might exist, as *Trichoderma* spp. grew rapidly on all isolation media and quickly covered many of the dishes, obscuring any other fungi present. *Trichoderma* also has been used as a biocontrol agent against several pathogenic fungi, including *Rhizoctonia* (7). While not found in high incidence during this study, *Rhizoctonia* remains a threat to the industry and still warrants further study. Chemical controls are not readily available, also due to the lack of registered chemical compounds. Currently, Abound (22.9% azoxystrobin; Syngenta Crop Protection, Greensboro, NC), which is known to have activity against *Rhizoctonia*, is registered for use on blueberry, but it is not registered for this usage pattern during propagation. As with *Cylindrocladium*, this leaves producers with no legal recommendation for control other than sanitation and prevention.

Oomycetes, while found in very low numbers in this survey, should also not be dismissed as a threat. These pathogens may be present in much higher numbers than this study indicated. Several chemicals are registered for use against Oomycetes: Aliette WDG (80% fosetyl-Al; Bayer CropScience, Research Triangle Park, NC), ProPhyt (54.5 % potassium phosphate; Helena Chemical Co., Collierville, TN), Agri-Fos (45.8% mono- and di-potassium salts of phosphorous acid; Agrichem, Oak Brook, IL) and Ridomil Gold 4EC (49.0% mefenoxam; Syngenta Crop Protection) are all registered, and all have proven activity on Oomycetes (2, 3). These chemicals were in common use in the surveyed nurseries (see Chapter 3), which may have reduced Oomycete populations during the course of the survey.

Fusarium, while not confirmed as a pathogen of blueberry outside of a few disease reports (15), was found in sufficient numbers to arouse concern as to what role this organism might play in blueberry nurseries. In Argentina, *F. solani*, isolated from symptomatic plants, was

shown to cause lesions on the stem and roots of wounded 2-month-old plants inoculated with mycelial plugs (15). This raises the possibility that *Fusarium* may be a potential pathogen of rooted cuttings as well. Further research is needed to eliminate this pathogen as a potential threat.

A significant association between the reuse of propagation media and the presence of *Cylindrocladium* was established when pathogen presence-absence data at the nursery level was analyzed together with information from the production practices survey reported in Chapter 3. Based on what is known about Cylindrocladium root rot, an association with reused media is not surprising. Between bedding periods, *Cylindrocladium* can survive in the propagation medium as either mycelia or microsclerotia. This is especially important in continuous propagation beds, as many growers tend to add new pine bark to these beds seasonally rather than replace the medium completely. This can result in a perennial problem with this disease. Whereas survival of *Cylindrocladium* in peanut fields has been shown to be reduced in soils where temperatures remain at 4.4°C or lower for at least 4 weeks, the warm winters of south Georgia are likely conducive to survival of microsclerotia, especially in greenhouses where the temperature can remain warm even in the coolest of winters (10). The practice of adding new bark to the propagation beds in the spring also likely contributes to survival, as microsclerotia survive better when buried.

While the blueberry industry is small in comparison to many other crops, it contributes greatly to the economy of numerous small communities in the rural Southeast. As the traditional row crops of south Georgia have become less profitable, many farmers and investors have turned to blueberry production as an alternative. The foundation of any horticultural industry begins with healthy plants. This makes propagation one of the most important aspects that must be

addressed to ensure long-term success. One of the most important activities a grower can do is to keep detailed records of propagation operations. The location from where the cuttings were collected, along with the quantity, cultivar, and the date of collection should be recorded each growing season. Records should also include the date the cuttings were stuck, any disease that appears during propagation, along with the percentage of cuttings that root. This data can be very beneficial in determining the source of potential problems in the nursery, to include the optimal times for collecting cuttings, how to optimize the propagation system to reduce labor and costs associated with propagation, and for determining the location of diseased blocks of mother plants used for propagation.

Whereas blueberry research is increasing, very little is conducted on disease control in propagation settings. A streamlined propagation technique, with special attention to disease control, is of paramount importance to the continued growth of the industry. Based on this survey, the most important diseases can be targeted for future research, and the most efficient control methods can be determined.

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Table 4.1. Cutting-level isolation frequency (%)[a] of pathogens and saprophytes from blueberry cuttings, based on a pathogen survey conducted in blueberry nurseries in south Georgia in 2007 and 2008.

Organism	Survey 1 (*n* = 55) 12-14 June 2007	Survey 2 (*n* = 49) 10-11 Sept. 2007	Survey 3 (*n* = 44) 25-26 Oct. 2007	Survey 4 (*n* = 56) 29-30 April 2008
Cylindrocladium	3.6	10.2	36.4	14.3
Rhizoctonia	10.9	0	4.6	3.6
Oomycetes	0	4.1	0	0
Fusarium	29.1	12.2	18.2	7.1
Saprophytic fungi	87.3	89.8	88.6	98.2
Trichoderma	23.6	4.1	13.6	16.1
Bacteria	63.6	55.1	29.6	67.9

[a] Two tissue pieces from each cutting were placed on three different media (PARP, SPDA, Ko & Hora). If an organism was isolated from one the six tissue pieces, the cutting was considered positive.

Table 4.2. Nursery-level isolation frequency (%)[a] of pathogens and saprophytes from blueberry cuttings, based on a pathogen survey conducted in blueberry nurseries in south Georgia in 2007 and 2008.

Organism	Survey 1 (n = 10) 12-14 June 2007	Survey 2 (n = 11) 10-11 Sept. 2007	Survey 3 n = 11) 25-26 Oct. 2007	Survey 4 (n = 14) 29-30 April 2008
Cylindrocladium	10.0	18.2	54.6	21.4
Rhizoctonia	20.0	0	9.1	7.1
Oomycetes	0	18.2	0	0
Fusarium	70.0	45.5	63.6	14.3
Saprophytic fungi	90.0	100	100	100
Trichoderma	70.0	18.2	27.3	35.7
Bacteria	100	100	45.5	85.7

[a] Two tissue pieces from each cutting were placed on three different media (PARP, SPDA, Ko & Hora). If an organism was isolated from one of the six plated tissue pieces, the cutting was considered positive. A nursery was considered positive if at least one cutting sampled from that nursery was positive .

Table 4.3. Frequency (%)[a] of south Georgia blueberry nurseries (n = 18) with select propagation practices (see Chapter 3) in relation to presence or absence of two pathogens.

	Cylindrocladium		*Rhizoctonia*	
Attribute or practice	+	-	+	-
Open system (grown outdoors)	25.0	75.0	12.5	87.5
Closed system	55.6	44.4	22.2	77.8
	(0.201)[b]		(0.600)	
Reuse growing medium	80.0	20.0	60.0	40.0
Discard growing medium	25.0	75.0	8.3	91.7
	(0.036)		(0.119)	
Propagate in containers	33.3	66.7	11.1	88.9
Propagate in beds	50.0	50.0	25.0	75.0
	(0.486)		(0.453)	
Grow cuttings on benches	62.5	37.5	12.5	87.5
Grow cuttings at ground level	55.6	44.4	22.2	77.8
	(0.772)		(0.600)	

[a] Frequencies for each pathogen species in each row add up to 100%.
[b] Values in parentheses denote *P*-levels for associations according to Chi-Square contingency table analysis.

CHAPTER 5

CHEMICAL CONTROL OF CYLINDROCLADIUM AND RHIZOCTONIA ROOT ROTS IN BLUEBERRY PROPAGATION

5.1 Introduction

As the antioxidant-rich blueberry has found continued favor in the eyes of health-conscious consumers, the demand for this fruit has increased steadily. To meet this demand, growers have been quick to take advantage of this once largely ignored commodity. Nowhere is this more evident than in Georgia, where the harvested acreage has increased from a mere 1400 ha in 1996 to over 4000 ha by 2006 (3,4). In 2004, the blueberry quietly replaced the once vaunted peach as the most valuable fruit crop produced in the state (3). Nationally, Georgia is now the third-largest blueberry-producing state in terms of crop value and second-largest in harvested acreage (10). As more production area has been added, the demand for rooted cuttings has also risen dramatically, prompting many growers to begin propagating cuttings on a much larger scale, either for their own use or for sale.

Two of the most common soilborne pathogens of blueberry cuttings are species of *Cylindrocladium* and *Rhizoctonia* (see Chapter 4), causing Cylindrocladium and Rhizoctonia root rots, respectively. If allowed to go unchecked, these pathogens can result in considerable losses. *Rhizoctonia* is a fairly common pathogen in propagation systems, especially in the ornamental industry. Symptoms of infection by this pathogen include stem and root lesions, leaf spots, and defoliation. An aerial web blight of foliage can also occur under conditions of high

humidity. *Rhizoctonia* generally does not form spores in nature; instead it survives as mycelia on or in plant debris or in the soil as sclerotia.

Cylindrocladium parasiticum causes symptoms very similar to those of *Rhizoctonia*, including stem and root lesions. *Cylindrocladium* forms both asexual conidia and sexual ascospores. In addition to these spore types, *Cylindrocladium* can also form microsclerotia which can survive in plant debris or in the soil. Whereas similar in structure and function to the sclerotia formed by *Rhizoctonia*, microsclerotia, as the name implies, are much smaller. *Cylindrocladium* has been implicated in plant-to-plant spread through direct contact, so avoiding overcrowding (correct spacing) is very important in managing this disease.

In this study we compared the efficacy of several fungicides against Cylindrocladium and Rhizoctonia root rots in a blueberry propagation setting. Our objectives were 1) to determine which fungicide classes provide the best efficacy for controlling these diseases, and 2) to establish whether any have adequate efficacy against both diseases simultaneously, allowing use in an integrated management scheme.

5.2 Materials and Methods

***Cylindroclium* isolation and pathogenicity test**. The *Cylindrocladium* isolate used in this study was obtained in the summer of 2006 from a propagation bed with a severe infestation (Bacon County, GA). Cuttings showing symptoms and signs of infection were placed in a 400-mL beaker fitted with a wire screen over the mouth. Chlorinated tap water was allowed to gently run over the root systems for 5 min until particulate matter (mostly pine bark) adhering to the roots had been removed. Several root segments showing evidence of infection were surface-

disinfested by immersion in a 10% household bleach (0.5% NaOCl) solution for 30 sec, and then rinsed in sterile deionized water. Cuttings were originally suspected of being infected with *Rhizoctonia*, so root pieces were plated on Ko & Hora medium which is semi-selective for *Rhizoctonia* (6). At the first signs of fungal growth, transfers were made to potato dextrose agar amended with .20 mg/L streptomycin (SPDA). The fungus was subsequently identified as *C. parasiticum* based on morphological characteristics on carnation leaf agar (see Chapter 4). In a preliminary test at the Athens Campus of the University of Georgia, this isolate proved to be especially virulent. After receiving sufficient chilling hours in the winter of 2007, several containerized southern highbush plants cv. 'Star' were placed in a greenhouse. After bud-break, the open flowers and flower buds were removed from the plants to encourage vegetative growth. Cuttings were taken from the plants as soon as the new shoots had reached about 10 cm in length. These cuttings were stuck into composted pine bark in twelve-celled flat liners and placed in a humidity chamber at 100% relative humidity in a greenhouse on 27 April 2007. Cuttings inoculated with the *C. parasiticum* isolate showed symptoms of stem lesions and defoliation within 4 days. At the end of 1 week all of the inoculated cuttings had died.

***Cylindrocladium* inoculum production (method 1)**. A V8-vermiculite growth medium (9) was utilized for inoculum production. The medium was produced by mixing V8 broth (163 mL V8, 652 mL deionized water, 1.63 g $CaCO_3$) with vermiculite (1.6 L). Ball regular-mouth half-pint (0.47-L) glass preserving jars were then filled with 100 mL of the mixture and sealed with a specially prepared lid containing a 1.5-cm hole fitted with a foam stopper. Jars were autoclaved for 30 min and then allowed to cool to room temperature. When cooled, each jar was

inoculated with three plugs of agar containing the pathogen. The inoculum was incubated for 2 weeks at room temperature.

***Cylindrocladium* inoculum production (method 2).** Mason jars filled with the V8-vermiculite medium tended to crack while being autoclaved, so an alternate method was devised using autoclavable plastic bags measuring 53.3 × 20.9 × 12 cm and fitted with a filter patch (spawn bags for mushrooms obtained from Myco Supply, Pittsburgh, PA). V8-vermiculite medium (1.6 L) was autoclaved separately in a metal vessel for 1 h, and then allowed to cool overnight. The medium was then packed into the spawn bags, autoclaved for an additional hour, and allowed to reach room temperature before being inoculated with approximately 48 plugs from a culture of the pathogen grown on SPDA. The bags were kept on a bench at room temperature for 2 weeks, being thoroughly mixed by shaking every 3 days.

Fungicide efficacy trials for *Cylindrocladium* management. The fungicides used in this study were selected based on their ability to control C. *parasiticum*. on ornamental crops, especially ericaceous plants such as azaleas and rhododendrons. For Cylindrocladium root rot management, the most promising chemistries include thiophanate-methyl (Cleary's 3336 WP), triflumizole (Terraguard SC), and fludioxinil (Medallion WSP) (5,8).

To determine the efficacy of these fungicides for control of Cylindrocladium root rot, four trials were conducted in a shade-house on the Griffin Campus of the University of Georgia (2007 and 2008). In 2007, cuttings were taken from mature, container-grown plants of southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrid) cv. 'Rebel'. This variety was chosen as its cuttings readily root, and southern highbush cultivars are generally more

susceptible to disease. The rooting medium chosen for this study was finely milled pine bark, which is the most commonly utilized rooting medium in the propagation systems of south Georgia (see Chapter 3). The pine bark medium was infested with *Cylindrocladium* to a level of ~300 colony-forming units (cfu) per mL by mixing the inoculum and bark in separate batches for each of the four replications. One replication required 6 L of bark/inoculum substrate. In order to achieve the desired concentration of *Cylindrocladium*, dilution-plating was conducted from the V8-vermiculite inoculum ahead of time. A half gram of inoculum was placed in a test tube containing 10 mL of sterile deionized water. The test tube was sealed and vortexed for 30 sec. After allowing the vermiculite to settle out of suspension (15 sec), the suspension was serially diluted by plating 0.1 mL of each dilution onto SPDA. Dishes were then incubated at room temperature for 24 h, after which fungal colonies were counted to determine inoculum potential. The amount of inoculum required to reach the desired inoculum density in the potting medium was then calculated based on this dilution plating data.

The bark and inoculum was mixed on-site by hand. For each replication in the first trial, 5.4 L of bark was mixed with 0.6 L of the inoculum to achieve a final inoculum density of 317 cfu/mL. For each replication in the second trial, 4.75 L of bark was mixed with 1.25 L of the inoculum to achieve a final inoculum density of 306 cfu/mL. The cuttings were stuck on 17 August and 2 October for trials 1 and 2, respectively; 12-compartment cell packs filled with the pine bark were utilized, and one cutting was stuck in each compartment. The experimental design was a split-plot with inoculation treatment as the main plot and fungicide treatment as the sub-plot. Main plots were: 1) *Cylindrocladium*-infested pine bark medium or 2) uninfested pine bark. Fungicide treatments (sub-plots) consisted of an untreated control (water drench), Cleary's

3336 WP (50% thiophanate-methyl; Cleary Chemical Corp., Dayton, NJ) at 60 g formulated product/ 100 L, Terraguard SC (42.14% triflumizole; Chemtura Corp., Middlebury, CT) at 62.5 mL formulated product/ 100 L, and Medallion 50 WSP (50% fludioxonil Syngenta Crop Protection, Greensboro, NC) at 15 g formulated product/ 100 L. Fungicides were applied the day after sticking (both trials) and again 2 weeks later (trial 2 only) by drenching 0.5 L of the fungicidal suspension to an experimental unit of 36 cuttings. Treatments were replicated four times in a randomized complete block.

Data was collected 29 and 28 days after planting for trials 1 and 2, respectively. Lesion incidence and lesion length were recorded for both trials; additionally, the incidence of stem girdling was recorded in trial 2. When girdling occurs, the likelihood of producing a healthy plant is greatly reduced. This measurement was not taken in trial 1 as all cuttings with lesions were girdled. As expected, non-inoculated cuttings did not develop disease symptoms in either trial; thus, only data from the inoculated main plots were subjected to analysis of variance and means separation by Fisher's Protected LSD test ($P = 0.05$).

In 2008, both trials (single fungicide application and two fungicide applications) were repeated with a few slight changes in methodology, the first being the use of spawn bags for inoculum production instead of Mason jars for the aforementioned reason. In 2008 the containerized ‘Rebel’ plants were not available, so ‘Rebel’ cuttings were obtained from a blueberry propagator in Manor, GA. The cuttings were taken in the early morning and stored in a cooler containing ice water until being stuck the following day. Since none of the uninoculated cuttings showed symptoms or signs of disease, fungicides were no longer applied to uninoculated cuttings. Uninoculated/ untreated cuttings were utilized as a negative control in these repeat

trials. Again, dilution-plating of the V8-vermiculite inoculum was used to determine inoculum density ahead of time. For each replication, 5.6 L of bark was mixed with 0.4 L of the inoculum to achieve a final inoculum density of 303 cfu/mL. Cuttings for the repeat of the one-application trial were stuck on 24 June and fungicides were applied immediately after planting. Plants were harvested destructively for assessment 27 days after planting, and the cuttings were stored overnight in a cold room for data collection the next day. The two-application trial was repeated on 19 May. Fungicides were applied immediately after planting and again 2 weeks later. Dilution-plating of the V8-vermiculite inoculum was used to determine inoculum density. For each replication, 5.6 L of bark was mixed with 0.4 L of the inoculum to achieve a final inoculum density of 288 cfu/mL. The experiment was terminated 28 days after planting and data was collected the following day.

***Rhizoctonia* pathogenicity test**. The *Rhizoctonia* isolate was provided by Dr. Cameron Whiting (formerly at Valdosta State University); in an unpublished experiment, it proved to be highly virulent on blueberries. This isolate was tested in a preliminary experiment in the same manner as the *Cylindrocladium* isolate using the V8-vermiculite medium. *Rhizoctonia* mycelium caused foliar web blight within 3 days, eventually resulting in almost complete defoliation of the cuttings and stem lesions covering most of the stem surface of cuttings. Within 7 days, all the cuttings inoculated with *Rhizoctonia* had died.

***Rhizoctonia* inoculum production.** For production of *Rhizoctonia* inoculum, V8-vermiculite medium (1.6 L) was autoclaved separately in a metal vessel for 1 h, as described previously, and then allowed to cool overnight. The medium was then packed into the filter-fitted autoclavable plastic bags (as described for *Cylindrocladium*), autoclaved for an additional hour,

and allowed to reach room temperature before being inoculated with approximately 48 plugs from a culture of the pathogen grown on SPDA. The bags were kept on a bench at room temperature for 2 weeks, being thoroughly mixed by shaking every 3 days.

Fungicide efficacy trials for *Rhizoctonia* management. In 2008, two fungicide efficacy trials were performed to evaluate control of Rhizoctonia root rot. Triflumizole (Terraguard SC) and fludioxinil (Medallion WSP), the two best-performing chemicals in the *Cylindrocladium* trials are labeled for use on *Rhizoctonia* in other crops, so they were chosen for continued testing; in addition, Prostar (70% flutolanil; Bayer CropScience, Research Triangle Park, NC) and Heritage (50% azoxystrobin; Syngenta Crop Protection) were applied at 45 g formulated product /100 L and 6.7 g formulated product /100 L final solution, respectively. The latter two fungicides are known to have activity against *Rhizoctonia* in azalea propagation and were selected for this reason (2,8). In addition to the fungicide treatments, untreated/ uninnoculated and untreated/ inoculated cuttings were used as control treatments in each replication. Again, 'Rebel' cuttings were obtained from a blueberry propagator in Manor, GA. The cuttings were taken in the early morning and stored in a cooler containing ice water until being stuck the following day. The pine bark medium was infested with *Rhizoctonia* by mixing the inoculum and bark in batches for each of the four replications. One replication required 7.5 L of bark /inoculum substrate. Dilution-plating on SPDA was conducted from the V8-vermiculite inoculum ahead of time, as described for *Cylindrocladium* above. The amount of inoculum required to reach the desired inoculum density was then calculated based on this dilution plating data.

The bark and inoculum was mixed on site by hand with each replication mixed separately. The experimental design for this experiment was the same as for the *Cylindrocladium*

experiments described previously. For each replication in trial 1, 7 L of bark was mixed with 0.5 L of the inoculum to achieve a final inoculum density of 138 cfu/mL. Cuttings for trial 1 were stuck on 24 June, and fungicides were applied immediately after planting and again 14 days after planting. For the second trial 7.2 L of bark was mixed with 0.3 L of the inoculum to achieve a final inoculum density of 351 cfu/mL. The experiment was terminated 27 days after planting, and the cuttings were stored overnight in a cold room. The next day, lesion incidence, lesion length, and percent girdling of the cuttings were recorded.

5. 3 Results and Discussion

***Cylindrocladium* control.** In the 2007 trials (Table 5.1), fludioxonil significantly reduced lesion incidence and lesion length. Neither triflumizole nor thiophanate-methyl reduced lesion incidence in trial 1 (although thiophanate-methyl did reduce lesion length), indicating that a single application of these materials was not sufficient for suppression of Cylindrocladium root rot. In trial 2, the additional fungicide application (2 weeks after the first application) clearly improved disease control for all fungicides. However, fludioxonil still performed statistically better than thiophanate-methyl and numerically better than triflumizole, as indicated by lesion incidence, lesion length, and girdling incidence. In the second set of trials conducted in 2008 (Table 5.2), fludioxonil again performed better than any other treatment. Triflumizole, however, did not perform as well as in the 2007 trials. There was no significant difference between the performance of thiophanate-methyl and triflumizole in either trial. In the two-application test, performance of thiophanate-methyl and triflumizole was not significantly different than the untreated control for either lesion incidence or girdling.

The differing results between the two tests may be due to the change in the source of cuttings. The cuttings used in the 2008 trials were taken from field-grown plants, as opposed to container-grown plants in 2007. The cuttings obtained from the field were more typical of what is used in the propagation industry. The cuttings were on the whole larger in caliper and were taken from softer wood. The containerized plants utilized in 2007 had fewer and shorter shoots from which cuttings could be taken, forcing the selection of cutting material slightly harder than normal. The harder cuttings may have been more resistant to infection than field-collected cuttings, thus allowing for the difference in lesion incidence.

Although fludioxonil is the more efficacious product, triflumizole may still be important for resistance management. Two-week rotations of fludioxonil and triflumizole should be evaluated as well as applications of both chemicals simultaneously. Whereas no phytotoxicity was observed during any of these experiments, detailed data should be collected regarding rooting percentage and application of fungicides. Additional research on all these products at higher rates is also warranted, especially with thiophanate-methyl which was used at the lower rate on the unrooted cuttings, in accordance to label recommendations. The labeled rate suggested for control of *Cylindrocladium* on rooted plants is much higher, and at a higher rate, it might provide protection equivalent to its counterparts in this fungicide efficacy test, provided that phytotoxicity is not a issue.

***Rhizoctonia* control.** The results of the *Rhizoctonia* trial showed azoxystrobin, flutolanil, and fludioxonil as the best choices for controlling this pathogen (Table 5.3). In all trials, azoxystrobin performed the best numerically. Azoxystrobin is a strobilurin fungicide, and resistance toward this family of materials is well documented in several fungal pathogens.

Whereas this material may provide control in the short term, other chemicals need to be assessed for use as rotation materials and possible replacements. Flutolanil and fludioxonil provided control statistically equivalent to that of azoxystrobin in all trials. All of these chemicals should be considered as part of a rotation for the control of Rhizoctonia root rot. The performance of fludioxonil also reinforces its potential importance to the blueberry industry, since fludioxonil may be used as a part of an integrated management system for the control of both Rhizoctonia and Cylindrocladium root rots. The poor performance of triflumizole in both *Rhizoctonia* trials is disappointing as it was the only other chemical to show promise for controlling *Cylindrocladium*.

One issue that needs to be addressed is the lack of uniformity between the two *Rhizoctonia* trials. Whereas the statistical rankings in the two trials were similar, control of the pathogen was more pronounced in the second trial. There could be several explanations for this. The environmental conditions between the two tests were slightly different, as the temperatures during the second trial were slightly cooler. The bark used in the trials was from a different source. The bark in trial 1 had been stored longer, and weed seeds were germinating throughout the experiment. Along with weed seeds, other organisms could have contaminated the medium. In trial 2, the medium came from a fresh load. The flats remained free of weeds, and the performance of all chemicals tested was improved over the previous trial. The results of the second trial showed almost complete disease control by all chemicals except triflumizole.

With this information, steps can be taken to register more fungicides for control of both *Cylindrocladium* and *Rhizoctonia* in blueberry propagation. While not considered a major crop, blueberries have a major impact on the economies of several Georgia counties, particularly in the southern part of the state. Fludioxinil provides at least one tool to control both threats to

propagation success; its ability to control both *Cylindrocladium* and *Rhizoctonia* makes it an excellent prospect for use in an integrated management system. Rotations or simultaneous applications of fludioxinil and triflumazole might provide some protection against fungicide resistance, as they both employ different modes of action. In the case of *Rhizoctonia*, azoxystrobin may be used as a rotation material if used sparingly, as it is subject to resistance development. More research needs to be conducted to discover other materials which can be used as rotation materials and as alternatives should the materials tested here prove not to be durable. One avenue to further this research is through the Interregional Research Minor Use Project 4 (IR-4), a research program which supports the research required by the Environmental Protection Agency (EPA) for obtaining proper labeling of a pesticide for use on minor crops (7). The status of blueberry as a specialty crop makes it a prime candidate for label expansions of triflumizole, fludioxonil, flutolanil, and azoxystrobin for use during propagation. Still, several issues must be addressed concerning the control of *Cylindrocladium* and *Rhizoctonia* in blueberry propagation, to include further chemical control trials, addressing the effectiveness of rotation schedules, and epidemiological studies to determine how this pathogen invades propagation systems.

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Table 5.1. Control of Cylindrocladium root rot on blueberry cuttings with fungicides, 2007.

	Lesion incidence (%)		Lesion length (cm)		Girdling incidence (%)
Fungicide and rate per 100 L[a]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 2
Untreated control	95.1 a[b]	92.4 a	3.8 a	3.4 a	88.2 a
Cleary's 3336 WP 60 g	96.5 a	60.4 ab	2.1 b	1.4 b	41.0 b
Terraguard SC 62.5 mL	93.0 a	42.6 bc	3.4 ab	0.6 bc	17.2 bc
Medallion 50WSP 15 g	44.0 b	8.4 c	1.0 c	0.1 c	2.8 c
LSD ($P = 0.05$)	14.2	39.0	0.7	1.2	27.9

[a] One and two drench applications were made in trials 1 and 2, respectively.
[b] Means within a column followed by the same letter are not significantly different according to a multiple t-test ($P = 0.05$).

Table 5.2. Control of Cylindrocladium root rot on blueberry cuttings with fungicides, 2008.

	Lesion incidence (%)		Lesion length (cm)		Girdling incidence (%)
Fungicide and rate per 100 L[a]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 2
Untreated control	97.0 a[b]	77.8 a	6.4 a	2.8 a	67.4 a
Cleary's 3336 WP 60 g	85.3 ab	67.4 a	3.4 b	1.8 b	54.2 a
Terraguard SC 62.5 mL	81.9 b	62.9 a	4.5 b	2.0 b	55.2 a
Medallion 50WSP 15 g	47.4 c	38.9 b	1.8 c	0.8 c	31.3 b
LSD ($P = 0.05$)	11.9	16.0	1.1	0.6	17.2

[a] One and two drench applications were made in trials 1 and 2, respectively.
[b] Means within a column followed by the same letter are not significantly different according to a multiple t-test ($P = 0.05$).

Table 5.3. Control of Rhizoctonia root rot on blueberry cuttings with fungicides, 2008.

	Lesion incidence (%)		Lesion length (cm)		Girdling incidence (%)	
Fungicide and rate per 100 L[a]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Untreated control	68.1 a[b]	74.3 a	2.7 a	1.5 a	57.6 a	29.9 a
Heritage 6.7 g	27.8 b	0.7 c	0.8 b	0.0 b	22.9 b	0.0 b
ProStar 45 g	36.1 b	2.1 c	1.4 ab	0.0 b	31.9 b	0.0 b
Terraguard SC 62.5 mL	59.7 a	25.7 b	2.1 ab	0.3 b	54.9 a	9.0 b
Medallion 50WSP 15 g	38.2 b	0.7 c	1.2 b	0.0 b	33.2 b	0.7 b
LSD ($P = 0.05$)	14.8	14.9	1.3	0.5	14.0	15.7

[a] Two drench applications were made at planting and 2 weeks later.
[b] Means within a column followed by the same letter are not significantly different according to a multiple t-test ($P = 0.05$).

CHAPTER 6

PROPAGATION OF DISEASE-FREE BLUEBERRY PLANTS FROM CUTTINGS

6.1 Introduction

Propagating disease-free blueberry plants is a major concern for the future of the blueberry industry in Georgia and the Southeast as a whole. Over the past decade, the Georgia blueberry industry has enjoyed a remarkable period of prosperity and has developed into a major source of income for many producers. To remain competitive, Georgia needs a constant supply of healthy plants for the replacement of older plantings and obsolete varieties as they are phased out in favor of newer, more desirable varieties.

Current blueberry propagation methods utilized in Georgia are highly variable, and there are few standardized practices that are used across the industry. Thus, both new and seasoned growers may find it difficult to determine the most suitable methods to adopt when deciding to manage their nursery. The following information presents a set of best practices for implementing a propagation system that will minimize cutting losses and improve the overall quality of plants produced.

6.2 Setting up a Propagation System

Several questions must be answered before propagation from cuttings can begin. How many plants need to be produced? Will cuttings be produced annually or only for a short time? Are the cuttings for sale or for use on-site? These factors will determine the ultimate selection of propagation methods.

System type. There are two basic system types which can be employed to produce rooted cuttings, open (outdoors) systems and closed (greenhouse or hoophouse) systems. In an open growing system, cuttings are exposed to the elements, and there are advantages and disadvantages in selecting such a system. Most importantly, from a cost management point of view, the open system reduces the initial investment required to begin production. The main disadvantage is a loss of the ability to control environmental conditions in the nursery; moisture levels are more difficult to control in open systems as plants are exposed to rain and ambient humidity. Excess rain can lead to the formation of zones of saturation around the root zone, and this should be taken into account when selecting containers and media (see below). Also, protection from wind is important, as drying of the leaves for periods as short as 30 min can permanently damage cuttings that have not yet rooted (4). An open system generally lends itself to growers who intend to grow cuttings in small numbers for use in their own operation as initial financial input is lower and the usable space for propagation can be adjusted at needed. However wind breaks, to prevent excessive drying, and shade will need to be provided; generally 40 to 63% shade should be sufficient for propagation (9).

Structures used for closed systems can vary greatly depending on the budget of the propagator. Both shade- and greenhouses can be used, allowing the grower to be in almost total control of the environment; moisture levels are controlled by irrigation, and shade can be controlled by the use of shade cloth or pigmented plastic greenhouse coverings. Again, similar to an open system, 40 to 63% shade should be provided. If long-term propagation is desired, a permanent structure provides the grower with a dedicated area that is much easier to control and where sanitation regimes can be implemented more easily.

Media. Blueberry is an ericaceous crop that requires an acidic substrate for proper growth. The medium must also allow for 100% relative humidity around the base of cuttings, while providing support against lodging (4).It must be porous enough to facilitate drainage, as anoxic conditions adversely affect rooting. In the past, growers have favored aged sawdust as a propagation material (4), and this medium was obtained free from the discard piles of sawmills. Over time, sawdust availability has dwindled, thus forcing growers to find other sources of propagation media. Currently, two main media types are recommended for blueberry propagation: artificial soilless mixes and pine bark.

In Georgia, the propagation medium of choice is pine bark. This medium is readily available and relatively inexpensive, although prices have been increasing recently. Several factors must be taken into account when using this medium. Bark should be finely milled to a uniform size. The pine bark should consist of particles (70 to 80%) that fall within arrange of 0.6 to 9.5 mm in diameter. The remaining particles should be no larger than 0.6 mm in diameter (10). The bark should be relatively free of wood chips. Bark is a hydrophobic substance that is relatively difficult to wet. Milling the pine bark into small, uniform pieces increases the water-holding capacity of the medium and facilitates easy entry of the cutting during sticking. Composted pine bark is preferred for this use, as the increased biological activity will act to suppress many soil-borne pathogens, including species of *Pythium*, *Phytophthora*, and *Rhizoctonia* (7). The naturally low pH of pine bark makes it very suitable for the acid-loving blueberry. However, contamination by foreign materials at sawmills can lead to problems. Lime ($CaCO_3$) is often spread over the ground at sawmills to prevent water-logging in bare areas with

high foot and vehicle traffic, and bark that is subsequently stored on this surface can be contaminated with lime which will raise the pH and result in poor rooting.

Another option for propagation is sterile soilless mix. While not commonly used for propagation in Georgia, there are several advantages to using a soilless mix, especially in closed systems. In a closed system, where moisture can be controlled, peat and perlite mixes perform well as a rooting medium. In open systems, peat-based media tend to become water-logged which leads to anoxic conditions in the medium and subsequent poor rooting (4). In many prepared mixes, lime is added to balance the acidity of the peat moss, so this must be kept in mind when selecting a propagation mix. There are several benefits to using a soilless mix, but the primary benefit is consistency. Bark is highly variable, especially when the source changes. If the grower mixes it themselves, a soilless mix should be consistent each and every year. Cuttings rooted in a peat-based medium will also produce more extensive root systems than those produced in a bark medium. Over the past few years the price of pine bark has increased steadily. If this trend continues, soilless mixes may become more common.

Container selection and bench use. Whereas many propagators in the past have used propagation beds, propagation in containers offers many important benefits. Containers must be at least 9 to 10 cm deep to insure that the cuttings do not come in contact with zones of saturation which can form at the bottom of shallow containers (9). Many types of containers have been used in the past for blueberry propagation; however, the most popular is the “trade gallon” black plastic container favored by the ornamental industry (Fig. 6.1). These containers are inexpensive, obtained readily, and reusable; they are also easy to handle when filled with medium, and they can support between 10 and 14 cuttings per container.

Another container which is highly recommended is the deep cell pack. These remove the guessing game from determining the correct spacing of cuttings and can reduce the amount of medium (in some cases by as much as half) needed to produce one cutting. A typical “trade gallon” container can support a maximum of 14 cuttings and has a volume of 2.8 L (0.2 L per cutting). In contrast, if a deep cell insert is used which can support 18 cuttings and has a volume of 2.32 L (0.13 L per cutting), the amount of medium used can be reduced by almost 50%. Whereas the inserts can not be reused, this fact can also work to the benefit of the grower; if the containers are discarded there is less likelihood of the spread of disease via contaminated containers. Depending on the needs of prospective customers, cuttings grown in deep inserts may be sold without being potted up, and the cost of the containers can be passed on to the buyer. One disadvantage to growing in flats is that they take up more space than potted cuttings. If space is limited, using pots may be the better option.

Another decision that must be made is whether or not to grow cuttings at ground level or on benches. Benches provide many benefits to the potential propagator. The most obvious benefit is the decreased likelihood of substrate saturation due to pooling water which can form on the ground. In cases of excess rain or accidental over-irrigation, zones of saturation in the medium will kill roots and promote disease (Fig. 6.2). Benches also make scouting for diseases much easier, as cuttings on benches are much closer to eye level, and disease problems are more readily observed early in their establishment. Also, it is easier to conduct work with cuttings on benches, as cuttings produced at ground level require more bending and lifting which is hard on the back. If cuttings will be produced annually, benches are highly recommended and well worth the expense. If cuttings are only grown on a temporary basis, they can be grown at ground level

if the proper precautions are made. Containers should be placed on a well-drained surface (preferably a layer of gravel) covered with landscape fabric. This will prevent pooling of water and act as a barrier to contamination from the native soil.

6.3 Propagation

Cutting selection and preparation. There are two opportunities to collect cuttings during the growing season. Late April to early May (first flush of vegetative growth) is the preferred timing for cutting collection in south Georgia; however, cuttings can also be taken early to mid-August after the second flush of growth has occurred in the early fall (9). Cuttings should be selected from designated mother blocks, and only vigorous plants with no disease (stem blight, leaf spots, viruses, bacterial leaf scorch, etc.) should be utilized. Records should be kept documenting the location and date where cuttings are taken, and bushes that appear off-type or stunted should be removed from the block and destroyed; if viral or bacterial symptoms are present, additional testing may be required to determine whether or not the mother block has been compromised.

Cuttings should be taken in the early morning while the plant stems are turgid and stored in containers (coolers or five-gallon buckets) filled with ice water until they can be stuck. Ice should not be allowed to make direct contact with the cuttings as it may cause damage to plant tissues (4,9). The cutting's stem must be stiff enough to withstand insertion in to the medium without breaking. Cuttings should be approximately 11.5 to 15 cm in length and should be selected from the terminal ends of new growth. If cutting material is in short supply, two or three cuttings can be made from the same shoot if it is long enough; however, these cuttings will

generally not root as well as terminal cuttings (4). Cuttings should be stripped of all but the top two or three leaves and be spaced 3.8 to 5 cm apart to provide adequate air flow as well as easy access to all parts of the plant and soil surface when applying fungicides or other products (Fig. 6.3) (4).

Irrigation. Irrigation is very important during the early stages of the rooting process. A constant film of water should remain on the leaves until roots are formed. Misting systems are highly recommended for this purpose (Fig. 6.4). Whereas it is possible to use other irrigation methods (impact sprinklers), mist heads provide the best coverage. Several different types of mist heads are on the market, but all generally fall into one of two categories: deflection nozzles and oil-burner heads. The deflection models are less likely to become clogged, but they tend to use more water (6). In either case, coverage must be uniform and consistent. If using mist in an open system, wind breaks should be employed as the fine spray from overhead mist is subject to the influences of wind (Fig. 6.5).

The simplest method for controlling irrigation is through use of a timer. Whereas conditions will vary between propagation systems, cuttings are usually grown under intermittent mists of 7 to 10 sec every 5 to 6 min. The timer on the irrigation system should be set to turn on 1 to 2 hours after sunrise and to turn off 1 to 2 hours before sunset. In general, night irrigation is not necessary, but if conditions are excessively hot, be sure to consider modifying the program. All propagation systems should be monitored frequently to prevent irrigation problems from developing. Mist heads are subject to clogging by algal or mineral deposits, and this could result in severe cutting losses due to desiccation. The growing medium should be monitored through the day for formation of saturated layers at the bottom of containers and for drying-out under

high temperatures. To conduct a simple test of misting frequency, squeeze a handfull of medium as hard as you can. If more than two or three drops of water is extracted, the medium is too wet (4). When the roots begin to form, the water requirement of the cuttings is much reduced and the frequency of watering can likewise be reduced.

Record-keeping. One of the most important activities a grower can do is to keep detailed records of propagation operations. The location from where the cuttings were collected, along with the quantity, variety, and the date of collection, should be recorded each growing season. Records should also include the date the cuttings were stuck, any disease that appears during propagation, and the percentage of cuttings that root. This data can be very beneficial in determining the source of potential problems in the nursery, including the optimal times for collecting cuttings, how to optimize the propagation system to reduce labor and costs associated with propagation, and for determining the location of diseased propagation (mother) blocks.

6.4 Diseases of Blueberry Cuttings and Their Management

Whereas problems caused by abiotic agents (irrigation issues, temperature, etc.) are responsible for poor rooting, several diseases can affect propagation success as well. The primary soilborne diseases of cuttings are caused by species of *Pythium* and *Phytopthora* (both of which belong to the fungus-like group of Oomycetes), as well as species of *Rhizoctonia* and *Cylindrocladium* (which are true fungi). In addition to root and stem rots, blueberry cuttings are also subject to various foliar diseases due to the high humidity environment required during propagation. Another issue which could have a significant negative impact on blueberry

propagation is the potential spread of viruses and other systemic pathogens through cuttings taken from infected plants.

Rhizoctonia root rot. *Rhizoctonia* is a fairly common soilborne pathogen in propagation systems for many different crops, especially in the ornamental industry. The typical symptoms caused by this pathogen are stem and root lesions as well as defoliation. In cases where humidity is very high, the pathogen can cause an aerial blight of foliage (Fig. 6.7). This condition is typified by web-like strands of mycelium forming between leaves and stems. Conditions that favor *Rhizoctonia* are high humidity, excessive soil moisture, and overcrowding (8). Unlike most other fungi, *Rhizoctonia* does not generally form spores in nature; instead, this pathogen survives either as mycelia on plant debris or in the soil as sclerotia (a survival structure formed from compacted mycelia). This pathogen can cause serious damage in propagation systems where conditions are ideal for disease development.

Cylindrocladium root rot. *Cylindrocladium parasiticum*, one of the most frequent pathogens in blueberry propagation (5), causes symptoms similar to those of *Rhizoctonia*. This pathogen also causes stem lesions at the crown of the plant which can girdle and kill cuttings and prevent rooting from occurring. If roots have already formed, the pathogen will attack the roots, causing lesions and eventual rotting of the root system which can also result in the death of the cutting. This pathogen will cause defoliation; however, unlike *Rhizoctonia*, it will not generally cause web-blight within the canopy. What sets this pathogen apart from *Rhizoctonia* is its ability to reproduce using spores. *Cylindrocladium* forms two kinds of spores: conidia (asexual spores) and ascospores (sexual spores). Under a microscope the conidia form on structures called conidiophores (Fig. 6.8), which together look like small cylindrical bundles of rods surrounding

a central stipe tipped with a bulbous vesicle. The sexual ascospores are formed in specialized fruiting bodies called perithecia which are bright orange and - although small (pinhead sized) - are visible to the naked eye (Figs. 6.9). In addition to spores, *Cylindrocladium* can also form small survival structures called microsclerotia which can survive in plant debris or in the soil. Whereas similar in structure and function to the sclerotia formed by *Rhizoctonia*, microsclerotia, as the name implies, are much smaller and can only be seen with magnification. These structures can survive in the propagation medium between crops, making the reuse of medium highly risky. In nurseries where medium has been reused, losses up to 100% have been reported (5). *Cylindrocladium* has been implicated in plant-to-plant spread through direct contact, so avoiding overcrowding (correct spacing) is very important in controlling this disease (5).

Oomycetes. Another common group of pathogens in propagation systems are the Oomycetes, particularly *Pythium* spp. and *Phytophthora cinnamomi.* These organisms are favored by excessively wet media and high humidity and are also commonly known as water molds. They cause stunting, poor root growth, defoliation, and root and crown rots on young plants. Under a microscope, the mycelia of these pathogens are colorless and lack crosswalls. Oomycetes have a relatively complex life cycle which encompasses both sexual and asexual components. Sexual reproduction results in the formation of a structure called an oospore. Oospores are thick-walled and act as a survival structure for the organism. A functionally similar asexual survival structure called a chlamydospore is formed by some species of oomycetes (especially *P. cinnamomi*) within the roots of infected plants (3). Sporangia are asexual spore-containing structures that release zoospores, which can swim actively in a film of water using whip-like flagella. Zoospores have the ability to detect the exudates secreted by plant roots and

move toward a potential host based on the concentration gradient formed by root exudates. Oomycetes can be spread by splashing of spores, transfer of contaminated plant material or propagation medium, and also by irrigation from a water source contaminated by zoospores.

Managing soilborne diseases. The wet, humid conditions used in the production of cuttings are ideal for soilborne pathogens to spread and survive. There are two basic options for disease control in propagation systems: chemical application and implementation of good sanitation practices. Currently there are no chemicals registered specifically for the control of soilborne pathogens on blueberry cuttings, which leaves sanitation as the primary and only legal means of disease control. However, research is being conducted that will eventually result in the registration of fungicides that can be used to supplement good sanitation programs.

During propagation, there are three areas where pathogens can enter the nursery. The first is through contamination from the physical propagation tools and facilities. All tools used in collecting cuttings should be cleaned before, after and (if possible) periodically during use. A simple 10% household bleach solution (1 part bleach to 9 parts water) should be sufficient to kill potential pathogens before they come in contact with collected cuttings. The area where the cuttings are prepared for sticking should be made of a material that is cleaned easily and should be wiped down before and after each propagation session. Preferably, this staging area will be located away from high-traffic areas and on either concrete slab or bed of gravel or other well-drained material rather than soil which could carry potential pathogens. All plant material remaining after propagation has been completed should be removed and disposed of in an area far removed from the propagation area. If containers are to be reused, they should be soaked in a 10% bleach solution for 30 min before being reused. This is especially important if the nursery

has had a history of soilborne diseases. All of the pathogens mentioned here can be spread by contaminated soil or propagation medium (6). In the case of Oomycetes, contaminated irrigation water must also be considered. If pond water is used, care must be taken in the placement of the intake valve from the pond. Most fungal spores sink to the bottom of collection ponds, whereas zoospores of Oomycetes tend to rise to the pond surface. If the intake valve for the pond is located mid-water, the likelihood of contamination is greatly reduced (14).

The next possible pathogen entry point is by use of contaminated propagation medium (6). Media should never be reused for propagation as inoculum can build up to lethal levels in a relatively short period of time. This is especially true in the case of *Cylindrocladium* (5).

The final entry point of pathogens is through infected plant material (6). All mother plants should be checked for the presence of pathogens prior to cutting and sticking, but any cutting showing symptoms or signs of infection should be discarded and be removed from the propagation area as soon as possible.

Leaf spots and blights. In addition to root and stem rots, blueberry cuttings are also subject to various foliar diseases due to the high humidity environment required during propagation. Several fungal pathogens have been shown to cause leaf spots including *Alternaria tenuissima, Gloeosporium minus,* and *Phyllosticta* spp.; however, based on communications with producers and extension agents, two of the most frequently reported leaf diseases in south Georgia's blueberry nurseries are Septoria leaf spot and Botrytis blight. Septoria leaf spot is caused by *Septoria albopunctata* and produces leaf spots that are circular and range in color from white to tan with a red or purplish border. Severe infections can lead to poor growth, defoliation, and eventual death of cuttings. On the upper surface of the leaf, centered in each spot, one or

more pycnidia (asexual fruiting bodies) are usually present and visible with a good hand lens. Conidia of this pathogen are hyaline, filiform (very long and slender), and composed of 5 to 11 cells (11). In the field, Septoria leaf spot is usually most prevalent and severe during late summer and fall (12), but some spots begin to appear in early May prior to the onset of the propagation season (11), when they are difficult to see or still in the latent phase. In production fields, this pathogen is controlled by fungicide sprays during the summer and fall, but currently no chemicals are registered for use on blueberry cuttings. All cuttings taken for propagation should be inspected for leaf spots, which may be very faint at the time spring cuttings are taken, before sticking, and any cutting showing symptoms or signs of infection should be discarded.

Botrytis blight (caused by the fungus *Botrytis cinerea*) is a common disease in many crops and has a wide host range (8). This pathogen causes a blight of the flowers, twigs, and young succulent tissues of blueberries (2). *Botrytis,* while generally a weak pathogen under normal conditions, can result in severe losses during propagation due to the high humidity and tenderness of the tissue of cuttings (8). Commonly referred to as gray mold, *Botrytis* takes this common name from the appearance of its conidia, which en mass appear as grayish brown tufts on the surface of infected tissue. Under the microscope, the conidia are single-celled, ovate, and borne in clusters at the tip of highly branched, darkly pigmented conidiophores. In the field, this pathogen is controlled by fungicidal sprays, But no chemicals are currently registered for use on blueberry cuttings. As excessive humidity plays a key role in infection by and dissemination of this pathogen, irrigation should be reduced as soon as possible (when sufficient roots have formed). Sanitation of the propagation area is also of the utmost performance. All plant debris

should be removed, and work surfaces should be sterilized using a 10% household bleach solution before each propagation session.

Viruses. Another issue which could have a significant negative impact on blueberry propagation is the potential spread of viruses through cuttings taken from infected plants. Viruses are generally systemic, and therefore most if not all cutting taken from an infected mother plant will also carry the virus, thereby establishing the disease in new plantings. Currently there are nine viruses which have been confirmed to infect blueberries in North America (3). While not all of these cause severe disease, some can have serious effects on the crop, including poor growth, yield reductions, slow decline, and premature plant death. Typical leaf symptoms of viral infection include mosaic, mottling, ring spots, leaf-rolling, and elongated strap-like leaves (shoestringing). Any plants showing such symptoms of viral infection should be avoided when making cuttings, but it must be kept in mind that infected mother plants may not always show symptoms due to a latent period between infection and symptom development or because leaf symptoms do not manifest themselves until later in the season, after cuttings have been taken.

In the last few years, *Blueberry red ringspot virus* (BRRSV) has come to the attention of both growers and researchers alike when a number of highbush or southern highbush blueberry plantings in Georgia and North Carolina began showing symptoms and tested positive for presence of the virus. According to the literature, this virus is strictly transmitted via propagation, and no insect vector appears to be involved. As such, BRRSV is an excellent indicator that propagation practices need to be improved with respect to the production of disease-free propagation material. As the name implies, BRRSV causes red ringspots that are 3 to 6 mm in diameter on the leaves and stems; and in some cultivars the fruit may show symptoms

as well. Ringspots are most evident on the upper surface of the leaves (13), but they are not visible on the new growth at the time when spring cuttings are taken. At that time, dark red to purple or tan ringspots on stems produced during the previous year are the best indication that mother plants are infected. Overall, the yield losses associated with BRRSV appear to be limited, but as stated above, presence of the disease is a good indicator that disease spread throiugh cuttings has occurred and that propagation practices need to be improved.

Another disease or disorder that is likely to be systemic and hence transmitted via propagation is blueberry necrotic rig blotch disorder, a new, emerging problem in south Georgia and North Carolina. First noticed in 2006, this disorder gradual became more apparent over the following 2 years. Indeed, in a 2008 survey of Georgia counties, more than half of the 45 farms surveyed had symptomatic plants, sometimes at a very high incidence (1). Symptoms of this disorder are similar to those of BRRSV, except that the ringspots are larger, darker, more irregular and blotchy in appearance, and much thicker than those caused BRRSV. Additionally the ring blotches are apparent on both sides of the leaves unlike the ringspots of BRRSV (1). Anecdotal evidence indicates that yield losses associated with this disorder can be substantial, presumably due to premature defoliation on severely affected bushes. Although at this time it is not yet proven that necrotic ring blotch is caused by a virus (hence the designation as a disorder rather than a disease) or that it is truly systemic, plants showing symptoms should not be used for propagation to prevent the potential further spread of this problem.

6.5 Conclusions

The great amount of variation in the methods of blueberry propagation in south Georgia makes determining which methods work best very difficult. With the practices laid out in this guide, a new propagator will have the best chance of successfully producing cuttings that are of high quality and free of disease. Growers should avoid the reuse of media, and propagation containers should be sterilized before being reused. If possible, sticking of cuttings should be done on benches above ground level to prevent contamination by soil, and the work surface should be cleaned and all plant debris removed before and after each propagation session. After sticking, cuttings should be scouted frequently for disease. All cuttings showing any signs of disease should be culled immediately and disposed of well away from all propagation operations. These preventative steps are usually less expensive and more effective than addressing a problem after it reaches critical levels. Generally, by the time disease is visible in a nursery, it is too late to avoid serious losses. In the future, chemical controls will likely play a larger role in the control of soilborne and foliar diseases of blueberry cuttings. However, as no fungicides are currently registered in blueberry propagation, good sanitation practices are the best line of defense against disease. As research progresses and fungicides are registered, an integrated disease management system should be implemented to ensure a constant supply of high-quality, disease-free blueberry plants.

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Fig. 6.1. Blueberry cuttings in "trade gallon" plastic container.

Fig. 6.2. Water pooling below benches after over-irrigation (**a**) and on ground after rain (**b**).

Fig. 6.3. Blueberry cuttings properly stripped and spaced.

Fig. 6.4. Mist irrigation system.

Fig. 6.5. Open blueberry propagation system with a wind break.

Fig. 6.6. Mycelium of *Rhizoctonia*. Note right-angle branching.

Fig. 6.7. Web blight on blueberry cutting stem and leaves caused by *Rhizoctonia*

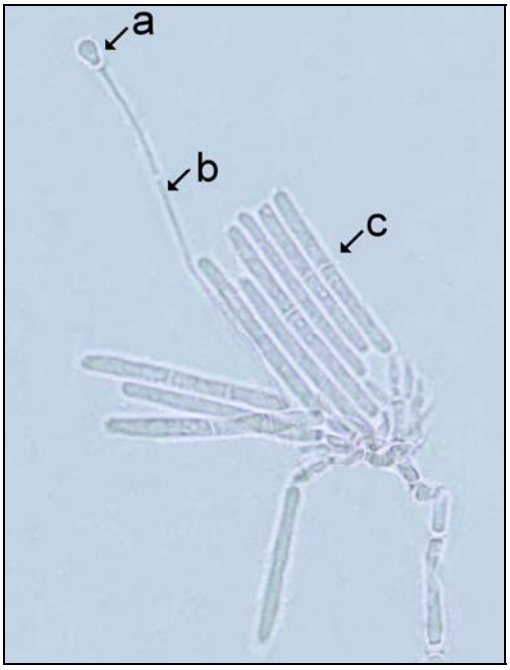

Fig 6.8. *Cylindrocladium* conidiophore with Vesicle (**a**), stipe (**b**), and conidia (asexual spores) (**c**).

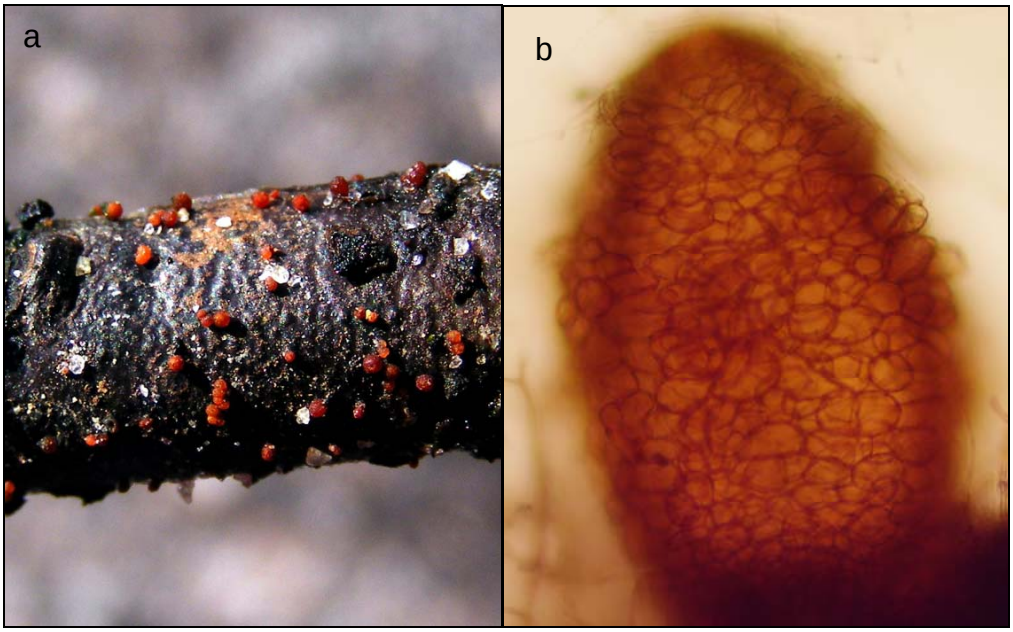

Fig 6.9. Perithecia (sexual fruiting bodies) of *Cylindrocladium parasiticum* on blueberry stem (**a**) and perithecium magnified (**b**).