ETIOLOGY OF *BOTRYOSPHAERIA* STEM BLIGHT ON SOUTHERN HIGHBUSH BLUEBERRIES IN FLORIDA AND QUANTIFICATION OF STEM BLIGHT RESISTANCE IN BREEDING STOCK

By

AMANDA FAITH WATSON

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2008

To my family and friends for all their gifts of roots and wings

ACKNOWLEDGMENTS

I thank Jon Wright for his patience, love, kindness, humor, and strength throughout this process. I thank my parents for their guidance and support. I thank my sister for her humor and encouragement. I thank my major advisor Dr. Harmon and my committee members, for their instruction and patience. I thank Ms. Patricia Hill and Ms. Carrie Yankee for their willingness to help. I thank the Florida Blueberry Growers association for their funding and project support.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

ETIOLOGY OF *BOTRYOSPHAERIA* STEM BLIGHT ON SOUTHERN HIGHBUSH BLUEBERRIES IN FLORIDA AND QUANTIFICATION OF STEM BLIGHT RESISTANCE IN BREEDING STOCK

By

Amanda Faith Watson

December 2008

Chair: Philip Harmon
Major: Plant Pathology

The southern highbush blueberry (SHB) industry in Florida is an early-season high-dollar niche market increasing in acreage and market value. Stem blight caused by *Botryosphaeria dothidea* is a serious disease of SHB in Florida. In recent years, growers have reported increased economic losses due to stem blight and have reported differences in cultivar susceptibility.

In 2007, 360 samples of stems and crowns with stem blight symptoms were collected from SHB in Florida. *Botryosphaeria* spp. were isolated from 85% of samples collected. Phylogenic analysis of internal transcribed spacer region showed at least three spp. occur on SHB in Florida: *B. dothidea, B. rhodina,* and an unresolved clade consisting of *B. parva* and *B. ribis* species.

Environmental factors and genetic make-up were investigated as potential contributors to perceived differences in cultivar susceptibility. Progeny differed significantly by which parents were used to make the cross. Parents that produce stem blight resistant progeny were identified.

A technique was devised to screen for stem blight resistant progeny. There was no correlation between percent lesion length in either replicated 05 or 07 trials. Lack of repeatability was due in part to a limited number of replicates, and experimental modifications. *Botryosphaeria* was recovered from control plants, indicating plants were infected with the

fungus prior to inoculation. Disease-free material and more replicates need to be used for further experimentation.

CHAPTER 1
LITERATURE REVIEW

Breeding Southern Highbush Blueberries in Florida

The genus *Vaccinium* (Ericaceae) contains three major crops: blueberry, cranberry, and lingonberry. About 400 species are classified in the genus. Species are native to all continents except Australia and Antarctica. *Vaccinium* is divided into five groups: *Cyanococcus* (blueberry), *Myrtillus*, *Oxycoccus* (cranberry), *Vaccinium,* and *Vitis-idaea* (lingonberry). *Cyanococcus, Myrtillus,* and *Oxycoccus,* have a polyploidy series (2n=2x, 4x, 6x= 24, 48, 72) (60,111). Tretraploid highbush (*V. corymbosum*) and hexaploid rabbiteye (*V. ashei*) blueberries have been bred at the University of Florida. Breeding programs have not merged because the tetraploid x hexaploid crosses produce pentaploids, which have reduced male fertility and have dark fruit color (57).

Professor Ralph Sharp began the breeding program at the University of Florida (57). Superior northern highbush blueberry (NHB) cultivars from Michigan and New Jersey provided initial breeding stock. These cultivars were poorly adapted to Florida's subtropical climate; therefore, Florida native species were used to produce cultivars with better adaptation (57,58). Florida native species successfully incorporated into breeding stock have included diploid and tretraploid *V. corymbosum* spp. from north central Florida, *V. darrowi, V. elliottii,* and most recently *V. arboreum* (57). The complex crosses have produced varieties with low chill hour requirements that ripen a month ahead of the earliest rabbiteye blueberries (115).

Recurrent selection has been implemented to simultaneously change traits controlled by hundreds of genes (59). Recurrent selection is based on two principles. The first is heterozygous parents yield variable progeny. The second is that if progeny that are extreme in the expression

of certain characteristics are crossed; the second generation progeny will be variable, and some will be more extreme in the selected character than their parents (58).

The University of Florida breeding program has two main goals: selection of breeding stocks and cultivar selection. A total of 100 seedlings are grown from each individual cross for a total of 15,000 seedlings per generation. From the 15,000 seedlings, 200 are selected as parents for the next generation. The process is continued generation after generation.

Cultivar selection has four stages. Stage I consists of 15,000 seedlings planted in high density plots. After one year, stage I plants are rated for desirable fruit size, firmness, flavor, ripening time, and bush defects. The best 500 plants are selected, and evaluated in stage II. The rest of the plants are discarded. Stage II plants are rated for three years; the best 150-200 plants are numbered, and approximately 40 softwood cuttings are rooted from each plant. These best clones are planted in 15-plant plots using commercial spacing. The clones are rated over three years for survival, and bush and berry quality. The superior 12 to 15 stage III clones are vegetatively propagated and planted on multiple farms. The stage IV plants are evaluated for three to six years by the breeder and growers. On average, between one and two clones are selected for cultivar release each year (*Lyrene personal communication*).

Breeding for *Botryosphaeria* Resistance

Stem blight on SHB is caused by *Botryosphaeria dothidea*. Fungicide utilization for control of *Botryosphaeria* disease is inconsistent (11,15,24,47,50,83). Irrigation management and pruning practices have given little success combating the disease (71,73,81,87). Resistant cultivars produced though breeding efforts offers growers cost efficient control options with little or no added inputs.

Various levels of susceptibility to *Botryosphaeria* diseases have been noted in *Vaccinium* spp. dogwood, mango, and peach (25,32,36,75,86,90). Differences in cultivar susceptibility have

been attributed to cultivar genetics, plant stress, age of tissue used for inoculation, wound age, and inoculum virulence (30,32,75,86,113). Disease indexes, the use of fresh highly virulent isolates, and succulent stem tissue for inoculation have been reported to help standardize resistance screening methods (9,30,99).

Buckley (1990) concluded narrow since heritability was greater than broad since heritability for stem blight resistance (25). Both additive and non-additive genetic effects are involved in resistance which is conferred from the low bush blueberry (*V. angustifolium*) in populations from Michigan, New Jersey, and North Carolina (25). However, Gupton and Smith (1989) concluded there was a large nonadditive genetic variance, and SCA and GCA were equal, suggesting that only moderate progress could be made in stem blight resistance breeding (44).

Stem Blight of Blueberries

Blueberry stem blight is caused by *Botryosphaeria dothidea*. In the early stages of infection, leaves on affected branches appear yellow or reddish. Leaves turn brown and remain attached on stems girdled by *B. dothidea*. Pecan-brown discolored stem tissue typically occurs on one side of an affected branch. Discolored vascular tissue extends from a few inches to the length of the branch (116).

Botryosphaeria dothidea

Botryosphaeria dothidea is a filamentous ascostromatic ascomycete. Its pycnidial anamorph is *Fussicocum asculi* (3). The teleomorph is associated with stem blight; however it is infrequently encountered in nature. The anamorph frequently found on infected tissue is predominantly used for identification (97,105). *Botryosphaeria dothidea* can live as a saprophyte or endophyte, and is an opportunistic pathogen of wounded and stressed hosts (76,97,106) .

Colony characteristics are olive gray to violet black in color, with thick to wispy aerial mycelium that darkens with age. Margins are smooth, becoming crenulate with age. Conidia can be produced on media, and are similar to those produced in nature. Optimum temperature for growth is 25-28°C, and the growth range is 4 to 30°C (31,96).

Host Range

Botryosphaeria spp. have a broad host range, infecting many woody fruits, trees, and herbaceous plants (3). Twenty plant families, 34 genera, and 50 plant species are known to be susceptible to *B. dothidea*. Rosaceae, Juglandaceae, and Palmaceae are the most well known (100). *Botryosphaeria dothidea* also infects other economically important crops including apple, blueberries, eucalyptus, grapes, mangos, peach, and pistachios (22,69,80,90,98,110,116).

Disease Cycle

The disease cycle of *B. dothidea* is one of opportunism. Typically, *B. dothidea* persists as a soil saprophyte or as an endophyte (76,97,106). A latent infection period begins with host tissue colonization (117). For pistachios, latent infection periods are most frequent during the month with the most rainfall (76). Apple white rot infection occurs after petal fall and symptoms do not appear until 6-8 weeks before harvest (50)

Drought stress and wounding predispose a plant to infection by *Botryosphaeria* spp. (23,34,65,87). *Botryosphaeria dothidea* can enter host tissue through lenticels, stomata, or small openings in the bark (23,69,72,88,91,113). Resistance to *B. dothidea* is related to fungal development after infection rather than establishment (72). In apples and blueberries infection develops through open stomata or lenticels, the host cell layer beneath the epidermis undergoes cell division. The thickened periderm layer restricts *B. dothidea* to the outer portion of the lesion. After six weeks, small reddish brown lesions appear. The fungus does not move through the vascular tissue (23,72).

Invasion of wounded or succulent stems results in rapid breakdown of phloem and cortical tissues for almond, apple, blueberry, mango, melaleuca, and peach (17,23,43,72,90,91). After invasion, mycelium moves rapidly down the vascular tissue. Lateral movement occurs slowly through pits and intercellular spaces (23,43,72,91). Hyphae advance by colonizing all cell types, including callus parenchyma, cortical parenchyma, xylem ray parenchyma, trachieds, and vessels (17,91). Plant mortality results from partial or complete occlusions of the vascular tissue by tyloses and mycelium (23,72,90,91). Callus and lignified cells containing tannins do not restrict host colonization (17,91).

Partially submerged pycnidial stromata develop on stems colonized by *B. dothidea,* and are important sources of inocula for pistachios (69). Pycnidia mature after 12 days at temperatures ranging from 10-36°C for apple and pistachio (23, 69). Peak pycnidial production occurs at 30°C (23,69).

The epidemiology of *B. dothidea* has been researched for the following diseases: apple white rot, fungal gummosis of peach, as well as panicle and shoot blight of pistachio (8,17,19,23,31,61,69,70,73,76,81,88,113). Conidial production arises between 10-30°C four to six weeks after inoculation. Peak sporulation develops at 24°C (31,69). For apples, peaches, and pistachios, spore germination occurs four to six hours after inoculation at temperatures ranging from 25-35°C (23,70,113). In apple, conidial germ tubes consistently grow toward the wounded area of the stem suggesting a chemotatic response (23). Conidial germination declines with decreasing relative humidity for apple white rot infections. Germination is favored between 98-100% relative humidity. Less than 5% of conidia germinate at 95% relative humidity (105). Twelve hours of moisture is necessary for penetration of lenticels, stomata, fruit, and wounds of pistachios (69,73). Interrupted wetness periods of one hour or more irreversibly stop infection

for apple black rot and significantly reduce disease incidence for *B. obtusa* (8). For apple white rot infection air drying of twenty minutes significantly reduces conidial viability (105).

Inoculum can be found throughout a growing season for blueberry, peach, and pistachio (33,69,73,76,88,113). Pycnidia produced on pistachios during current or prior growing seasons provide inoculum for new infections throughout the year (69). Conidia have been detected from February to November in blueberries, peach, and pistachio orchards (33,69,113). The highest levels of conidial inoculum have been recorded between May to July for blueberry in North Carolina and from July to mid-August for peach in Georgia (33,88,113). Rainfall is required for spore dispersal. Light rain is more conducive for spore deposition and infection than heavy rain for dissemination in blueberry and peach orchards (33,113). The for pistachios number of continuous rainy days and increased summer temperatures are positively correlated with disease severity (73,76,88).

Plant Health and Disease Transmission

Plants are predisposed to disease when stressed. Drought limits photosynthetic production and the accumulation of carbohydrates aid the plant in disease defense (21,52,79). Pathogens responsible for stress-related diseases are usually facultative saprophytes, are latently present on host tissue, and attack when the host weakens (21,61,79). Susceptibility to *B. dothidea* increases as plant water potential (Ψ) decreases (34,61,87,95). Birch trees have a threshold between -12 to -13Mpa predisposing them to infection. Disease resistance to *B. dothidea* can be restored within 3-5 days after turgor pressure restoration. Susceptibility to disease is reversible between 14 to -18 MPa; Ψ greater than -18MPa birch trees are irreversibly predisposed to disease (34,95).

Protein synthesis, enzyme synthesis, and carbohydrate production decrease in drought-stressed plants (21). Hyphal growth inside healthy birch stems is irregular and contorted compared to large round hyphal growth inside stressed stems. Lytic activity on invading fungal

hyphae is suppressed in stressed plants (68). When aspens are drought stressed, catechol and salicin, compounds inhibitory to *Hypoxlon mammatum,* are suppressed (52). Stored carbohydrates are utilized due to limited photosynthetic production, and callus formation is limited during fungal invasion (79).

Higher plants cannot grow at Ψ below 0 Mpa, whereas many fungal pathogens can grow at Ψ below zero (1,21,40,46,65,78). Spore germination, germ tube elongation, and mycelia growth of *B. dothidea* increases from 0 to -2.0 MPa (65). Mycelia growth declines after -2.0MPa (40,65). Mycelial growth increases as water potentials decrease for other fungi including *Botrytis squamosa, Monilinai fructicola,* and *Macrophomina phaseolina* (1,46,78).

Management Options for Stem Blight

Cultural and management options for control of *Botryosphaeria* disease are similar in many cropping systems including apple, blueberries, grape, and peach. Fungicides have provided growers with short term crop protection and have limited disease incidence (11,45). Benomyl and strobilurin and DMI fungicides reduced external symptoms of *Botryosphaeria* blight; however, the infection was not prevented in apple,blueberry, cut flower, grape, and pistachio cropping systems (15, 24,29, 37,45,81). Root dip treatments for container-grown blueberry nursery plants limits *B. dothidea* development but does not provide long term control (29). Treatments of captan and difolatan improved peach tree fruit yield, and trunk diameter; however, infection was not prevented (11). An alternative control to traditional fungicides could be paclobutrazol (PBZ), a gibberellin inhibitor. PBZ reduced mycelial growth and spore germination for a broad range of woody pathogens including: *Armillaria gallica, Botryosphaeria dothidea,* and *Fusarium roseum* (47). PBZ enhanced tolerance to environmental stresses and has reduced foliar diseases including dollar spot (28, 47).

Fungicides have been more effective controls for *Botryosphaeria* fruit rots. Partial pressure infiltration of prochloraz and pyrimethanil controlled mango stem end rot (83). Fungicidal applications approximately 10wks after bud break have reduced fungal foliar and fruit diseases of cranberry (49). Late season applications of tebuconazole reduced latent apple white rot infections (50).

Fungicidal resistance has occurred in *Alternaria alternaria, Monilinia fruticola, Sclerotinia homeocarpa,* and *Venturia inaequalis* (17,41,102,121). The sensitivity of *B. dothidea* to tebuconazole and iprodione was evaluated (64,66). Resistant isolates were produced *in vitro* and retained high levels of virulence on pistachios. Tebuconazole retained efficacy while iprodione could not control mycelia growth of resistant isolates (64,66).

Integrated pest management (IPM) programs including orchard sanitation and irrigation management have effectively reduced disease incidence. Altering the trajectory angle of sprinklers from 23° to 12° and drip irrigation have reduced spore release, dispersal, and germination in pistachio orchards (70,71,73). Reducing irrigation time from 24 to 12 hours also reduced the incidence of panicle and shoot blight, and 24 hour irrigation periods are not recommended for apple due to increased disease incidence (70,81). Removal of blighted shoots from pistachio orchards removed sources of inoculum for current and prior seasons (45,73). Stems infected with *Botryosphaeria* are pruned out during peach dormancy and chipped to increase decomposition (11,22).

Taxonomy of *Botryosphaeria*

The genus *Botryosphaeria* was described by Cesati and De Notaris in 1863. *Botryosphaeria* originally included twelve species lacking a complete morphological description or a type species (39, 35, 96,101). Barr (1972) designated *B. dothidea* as the lectotype species for the genus because it was originally included in the initial description (96).

Botryosphaeria has high morphological plasticity and despite obvious similarities researchers described new species occurring on different hosts (82,96,97). Von Arx and Muller (1954) synonomized many *Botryosphaeria* species into either B. quercuum or B. dothidea species complexes. Differences in anamorph morphology prohibited researchers from accepting the synonymization of *B. ribis* with *B. dothidea* (48,89,90,91,101,120). Others accepted the grouping according to the International Rules of Nomenclature (116, 82).

Smith and Stanoz indicated paraphyly within the species complex *B. dothidea*; *B. ribis* was phylogenticially separated from *B. dothidea* (101). Cluster analysis and conidial morphology reinforced the separation of *B. ribis* from *B. dothidea*, and *B. parva* (48, 101). Based on multi-allelic data sets, Slippers (97) validated previous studies (48,101,120). *B. ribis* was no longer considered a synonym for *B. dothidea* (96). The study allowed for accurate identification of *Botryosphaeria* spp. associated diseases on commercial crops including grapes, mango, pome and stone fruits (97,98,109,110).

Botryosphaeria Anamorphs

Eighteen anamorph genera have been associated with *Botryosphaeria* including *Diplodia, Dothiorella, Fusicoccum, Lasiodiplodia, Phylosticta,* and *Sphaeropsis* (39,48,101). Denaman combined the anamorphs of *Botryosphaeria* into two main lineages: *Diplodia*, pigmented conidia, and *Fusicoccum* hyaline conidia (39). Zhou and Stanoz supported Denman's findings and proposed the conidial groups *Hyala* and *Brunnea* (120).

The conidial groupings of *Diplodia* and *Fusicoccum* were disputed. Zhou and Stanoz noted that *B. dothidea* and *B. corticis* were less closely related to other *Fusicoccum* spp compared with *Diplodia* taxa (119). Crous refuted the two anamorph lineages of *Botryosphaeria* and noted many intermediate conidial characters between *Diplodia* and *Fusicoccum* (35). Using single gene phylogeny, ten anamorph lineages were recognized within Botryosphaeriaceae

including an unresolved clade: *(Diplodia/Lasiodiplodia/Tiarosporella), Botryosphaeria (Fusicoccum anamorphs), Macrophomina, Neoscytalidium, Dothidotthia (Dothiorella anamorphs), Neofusicoccum* (*Botryosphaeria*-like teleomorphs, Dichomera-like synanamorphs), *Pseudofusicoccum* (*Fusicoccum* and *Diplodia*-like synanamorphs), *B. quercuum* (*Diplodia*-like anamorph), and *Guignardia* (*Phyllosticta* anamorphs) (35).

Higher Classification of *Botryosphaeria*

Luttrell granted formal taxonomic status to the subclass Locoascomycetes defined by a bitunicate ascus-wall and pseudothecia (3,54,55,94). All other filamentous ascomycetes were segregated to the Euascomycetes (13,56). Separation from the unitunitcate ascomycetes was widely accepted. The placement and number of orders within the groups was disputed.

Lutrell placed *Botryosphaeria* in the Pleosporales (56). vonArx and Müller did not support the placement of *Guignardia* and *Botrosphaeria,* two closely related genera into separate orders (Dothideales and Pleosporales). Instead one order, the Dothideales, was delimited containing two sub-orders and 24 families (3,35,39,51). *Botrosphaeria* remained in Botryosphaeriaceae and was relocated to the Dothideales (35,39). Barr agreeing with Luttrel, disagreed with the consolidation, created ten orders based on dicaryon and ascus type (10). By the end of the 1980s two systems of classification existed that of Barr & Luttrel, and vonArx & Müller.

Berbee and Spatafora rejected the monophyly of Loculoascomycota, and questioned class validity (13,55,103). Studies retained sister group status of the Dothideiales and Pleosporales, while the Chaetothyriales formed a sister group with Eurotiomycetes. The Loculoascomycetes were split into two classes, the Chaetothyriomycete (lichenized pyrenomycete) and the Dothideomycetes (51,54,55).

Disagreement concerning subdivision within the Dothideomycetes was unresolved. The weight of taxonomic characters was heavily disputed and included: centrum development, pseudothecia, and pseudoparaphyses characteristics (10,56,94). Two Dothideomycete lineages predominate: the pseudoparaphyste Pleosporomycetidae (Pleosorales) and aparaphysate Dothideomycetidae (Dothideales, Capnodiales, and Myriangiales) (94).

Botryosphaeriaceae did not group phylogentically within any of the previously described orders. Higher taxonomic classification has been enigmatic because of the intermediate morphology: pseudoparaphyses are present in immature and absent in mature fruiting bodies (3,10,51,54,94). A new order, Botryosphaeriales was created to accommodate phylogenic separation (94).

Table 1-1. Comparison of morphological characteristics of *B. dothidea, B. ribis,* and *B. parva* (96,82).

	B. dothidea	*B. ribis*	*B. parva*
Ascostroma			
Position	Erumpent through bark	Erumpent through bark	Erumpent through bark
Size	200-500 µm	100-400 µm	Unknown
Ascomata	Pseudothecia	Pseudothecia	Pseuodtheica
Color	Brown to black	Brown to black	Brown to black
Shape	Botryose aggregate of up to 100, sometimes solitary or globose	Botryose aggregate of 5-50, globose	Caespitose aggregate 5-50 (-100) per cluster
Size	n/a	175-250 µm	150-250 µm
Opening	Central ostiole, ¼ to ½ emergent	Central ostiole, papillate or not	Non-papillate or short conical papilla
Asci			
Description	8-spored, bitunicate, clavate	8-spored, bitunicate, clavate	8-spored, bitunicate
Shape	Filiform	Filiform	Ellipsoide to fusoid
Size	63-125 x 16-20 µm	80-120 x 17-20 µm	75-143 (-210) x 17-21 µm
Paraphyses	Peudoparaphyses 2-4 µm wide	Peudoparaphyses 2-4 µm wide	N/A
Ascospores			
Description	Unicellular, biseriate in ascus	Unicellular, biserate in ascus	Unicellular
Color	Hyaline, smooth with granular contents	Hyaline, smooth with granular contents	Hyaline, smooth
Shape	Fusoid to ovoid	Fusoid to ellipsoide	Broadly ellipsoide to fusoid
Size	(17-) 19-24 (-32) x (6-) 7-8 (-10) µm	(14-) 18-23 (-27) x 6-8 (-10) µm	(14-) 18-23 (-26) x (7-) 8-10 (-11) µm

Table 1-1 Continued.

	B. dothidea	*B. ribis*	*B. parva*
Anamorph			
General	Indistinguishable from pseudothecia	Indistinguishable from pseudothecia	Indistinguishable from pseudothecia
Pycnidia	N/A	Solitary or imbedded	Locule 100-150 μm
Condiogenous cells			
Color	Hyaline	Hyaline	Hyaline
Size	6-20 x 4-5 μm	6-22 x 2-5 μm	N/A
Shape	Holoblastic, subcylindrical	Holoblastic, subcylindrical	N/A
Conidia			
Color	Hyaline, smooth with granular contents, rarely becoming septate with age	Hyaline, smooth with granular contents, rarely becoming septate with age	Hyaline, becoming light brown and 1-2 septate with age
Size	(17-) 18-20 (-22) x 4-5 μm	(16-) 19-23 (-24) x 5-6 (-7) μm	(11-) 14-18 (-23) x 5-7 (-10) μm
Shape	Narrowly or irregularly fusiform	Fusiform	Ellipsoid

CHAPTER 2
QUANTIFICATION AND IDENTIFICATION OF *BOTRYOSPHAERIA* SPP. CAUSING STEM BLIGHT ON SOUTHERN HIGHBUSH BLUEBERRIES IN FLORIDA

Introduction

The Florida southern highbush blueberry (SHB) industry is an early-season high-dollar niche market increasing in acreage and market value (114). Commercial production has more than doubled since the early 1980s; currently Florida ranks 5th in the United States for commercial acreage (104,115). Fungal vascular diseases have become a growing problem for commercial blueberry growers. These pathogens will enter through flower buds, lenticels, stomata, and wounds and colonize the xylem and phloem (23,69,72,88,91,114). Infected bushes are then weakened and exhibit dieback on stems and branches. Severe infection results in bush mortality by partial or complete occlusion of vascular tissue in the crown. Symptoms include dead branches with attached leaves, and pecan brown discoloration extending the length of the affected branch (72,116). Vascular tissue is mottled in the crown of plants killed by dieback. Stem blight caused by *Botryosphaeria dothidea* (Moug.:Fr.) Ces & DeNot. is associated with these described symptoms (Fig 2-1) (116).

Botryosphaeria spp. have a wide host range and geographical distribution (100). These fungi are largely considered drought-stress opportunistic pathogens living as saprophytes or endophytes most of the time (39,73,76,97,106). Since the genus was founded in 1863 (Moug.:Fr.) Ces & DeNot., different *Botryosphaeria* species have been identified causing cankers and blights on woody hosts (18,27,43,45,53,82,90,100,109,116). Species identification has been difficult because multiple species have been found parasitizing the same host (17,19,20,110,109). Virulence of and symptoms caused by *Botryosphaeria* spp. have been reported to be different depending on cultivars and location (97, 109).

The teleomorph, *Botryosphaeria* is infrequently associated with disease symptoms (96,116). The anamorphs of *Botryosphaeria* occur frequently on infected tissue, and are primarily used for identification (48, 111,118). Anamorphic characteristics are continuous between species and have high phenotypic plasticity (48, 96). Often the connection between sexual and asexual states has not been made (118). Eighteen anamorph genera have been associated with *Botryosphaeria*. Currently, ten lineages are recognized within the *Botryosphaeriaceae* (35,96). Phylogenic studies using morphological characterization and genomic data have contributed to the clarification of *Botryosphaeria* taxonomy (35,48,96,101,120,121). Data have allowed for the positive and rapid identification of *Botryosphaeria* spp. parasitizing apples, grapes, mango, and pistachio (6,7,62,63,77,96,98,109,110).

Witcher and Clayton described *B. dothidea* as the causal agent of stem blight of blueberries (116). They noted the morphology strongly resembled *B. ribis,* which was annotated for *B. dothidea* under von Arx and Muller (116). Based on multi-gene phylogeny, *B. ribis* is considered a separate species from *B. dothidea* (96). To date, *B. dothidea* has been most commonly associated with stem blight and dieback infections; however, other fungi such as *Diplodia* spp., *Macrophoma* spp, and *Phomopsis* spp. have been found causing similar symptoms (2). The objective of the study was to determine the incidence of *B. dothidea* causing stem blight and dieback infections on SHB in Florida.

Materials and Methods

Plant Material Collection

Infected crowns and stems were sampled from two farms in Florida: one located in Alachua Co. and the second located 225.3km south in Polk Co. A farm-wide survey of disease was taken at each location at four-month intervals (Jan-Feb, Jun-Jul, and Oct-Nov.) in 2007;

thirty samples of each symptom were collected from both farms during a survey period. Other samples outside the survey included isolates from *Vaccinium ashei*, SHB samples from the Florida Extension Plant Disease Clinic, *Ilex* spp, and four isolates from a foliar ring spot symptom caused by *Botryosphaeria* on the SHB cultivars 'Millennia', and 'Star' (Table 3). Excess bark of blueberry samples was removed to expose discolored vascular tissue; margins were excised and cut into small pieces. Sample pieces were surface disinfected in 10% household bleach for one minute and washed with tap water. Blueberry samples were dried with a paper towel and plated on 85-mm petri dishes containing V8 agar (BD, Sparks MD) amended with 0.01mg of rifampicin (rif) and 0.25g of ampicillin sodium salt (amp). Cultures were incubated at 25°C for five days. *Botryosphaeria* isolates were obtained by transferring mycelia fragments from the margins of growing colonies.

Infected crowns and stems not plated were left in sample bags at room temperature for two weeks. After incubation all samples were checked for the presence of sporulating structures. The number of samples with sporulating structures per farm and sample period was counted. Sexual fruiting bodies were single-spored using serial dilutions plated onto potato dextrose agar (PDA) (BD, Sparks MD). Mycelia fragments from the margin of single colonies were excised. Isolates obtained are currently maintained in collection in the Department of Plant Pathology at the University of Florida.

DNA Extraction, Amplification and Phylogenic analysis

Genomic DNA from select colonies consistent with *Botryosphaeria* growth was extracted from pure cultures using Qiagen Dneasy Kit (Qiagen 69106 Gmbh, Germany). After extraction oligonucleotide primers ITS1 and ITS4 (Integrated DNA Technologies, Inc Coralville, IA) were used to amplify part of the internal transcribed spacer region including the 5.8S region of rDNA. Polymerase chain reaction (PCR) was completed by combining 10µL of REDExtract-N-Amp

PCRmix (Sigma, Saint Louis MI), 2µL of each primer, 2µL PCR grade water, and 4µL of purified fungal DNA. The reaction was carried out in a thermal cycler (Brinkman Instruments Inc., Westbury NY) as follows: denaturization 3 min at 94^{o}C followed by 35 cycles of denaturization at 94 ^{o}C for 60s, annealing at 55 ^{o}C for 60s, and extension at 72 ^{o}C for 2min. Five µL of each PCR product were separated by gel electrophoresis in 1.2% agarose gels (FisherScientific, Fair Lawn NJ) containing 1µL of ethidium bromide in a 1.0x tris-borate buffer (Sigma, St. Louis MI). Five µL of PCR products were placed on half of a 96 well PCR plate and were sent to University of Florida's Interdisciplinary Center for Biotechnology Research (ICBR) for bidirectional sequencing.

Sequences were edited using the software program Sequencher 4.6 (Gene Codes Corp. Ann Arbor MI), locally aligned using ClustalX 2.06-macosx and manually aligned using the computer software McClade 4.08 OSX. Phylogenetic analysis was completed using PAUP 4.0bl0 (PPC). Alignment gaps were treated as missing data. Representative isolates from the survey (Table 2-3) were compared using phylogenic analysis to related sequences published in GenBank (Table 2-4). The *Pestalotia* isolate from the *Ilex* spp. was used as the outgroup for phylogenic analysis. Maximum parsimony analysis was performed using the heuristic search option (TBR branch swapping). Bootstrap values were evaluated using 1,000 replicates and 100 random sequence additions, saving no more than 10 trees greater than 264 to test branch strength. Tree length, consistency index (CI), and retention index (RI) were recorded for all analyses.

Pathogenicity

Eight clones of the cultivar 'Misty' were arranged in a randomized complete block (RCB) design in greenhouse inoculation trials. Plants were pruned before inoculation. *Botryosphaeria* isolates were grown for three days on V8 agar amended with rif and amp. Four isolates were

chosen from each of the three clades from the ITS phylogeny: *B. dothidea,* possible *B. parva* or *B. ribis* species, and *B. rhodina*. MixFC-6 ITS sequence was similar to *B. rhodina* isolates in Genbank. Likewise 07-30 was similar to *B. dothidea*; while WsuF-29 and WWF-47 were similar to either *B. parva* or *B. ribis*. The positive control, isolate 04-40 was similar to either *B. parva* or *B. ribis.* A sterile agar plug of V8 amended with rif and amp was the negative control. Eight millimeter plugs were excised from the colony margin of each species, and placed on a pruned stem. Lesion lengths were measured in centimeters once every week for three weeks. Audpc values were calculated from lesions lengths. Data was analyzed in SAS (SAS Institute, Cary, N.C.) using a general linear model. Waller Duncan *k-ratio t*-test (k=100) was used to separate mean lesion length differences between isolates. Experiment was repeated thrice.

Results

Field Survey, Fungal Isolation, and Molecular Characterization

Colonies consistent with *Botryosphaeria* growth were isolated from, 99 out of 120 samples in the winter, 92 out of the 120 samples in the summer, and 115 out of 120 samples in the fall (Table 2-1). Incidence of *Botryosphaeria* spp. did not vary significantly between sample periods and locations. Overall, colonies consistent with *Botryosphaeria* growth were isolated from 85% of the 360 samples. Other fungal genera isolated from blueberry samples were *Alternaria* spp. *Pestalotia* spp., and *Phomopsis* spp.

Identification of isolates from colonies consistent with *Botryosphaeria* growth was based on ITS sequence data (Table 2-2). The total number of isolates sequenced from the winter, summer, and fall collection periods were 78, 63, and 78, respectively. Fungi isolated included: *B. parva* or *B. ribis*, *B. rhodina*, and other fungi such as *Alternaria* and *Phomopsis*. *Botryosphaeria* spp. were interspersed between crown and flag samples (Table 2-2, 2-3). However, *B. rhodina* was recovered at a larger percentage from crown samples. *B. dothidea* was

isolated twice from samples outside the survey area. Isolation frequency of *Botryosphaeria* spp. remained consistent and did not vary between sample periods.

One sample was found to have pycnidial fruiting bodies during the winter and one during the summer collection periods. The greatest numbers of fruiting structures were observed in the fall. Eleven samples had pycnidia, and two samples had perithecia on the farm in Alachua Co., Fl. Eighteen samples had pycnidia and two samples had perithecia on the farm in Polk Co, Fl. All samples found having perithecia, were either *B. parva* or *B. ribis* species (Fig 2-3). Winter and summer pycnidial fruiting bodies were *B. rhodina*. Pycnidial of *B. rhodina* were recovered from ten samples from Alachua Co., and from seventeen of the samples from Polk Co. Pycnidia of either *B. parva* or *B. ribis* were recovered once from each location in the fall.

Phylogenetic Characterization

ITS sequences of Floridian *Botryosphaeria* isolates were compared with homologous ITS sequences published in GenBank. Of the 533 nucleotides analyzed 101 characters were parsimony informative. Maximum parsimony analysis yielded one tree (length = 264, CI= .905 RI= .985). Botryosphaeria species having hyaline thin-walled conidia grouped within a clade; supported by a 94% bootstrap value (Fig 2-4). Species included *B. dothidea, B. corticis, B. parva,* and *B. ribis* and have *Fusicoccum* or *Fusicoccum*-like anamorphs. Intraspecific variation was present in the *B. dothedia* clade. *B. parva* and *B. ribis* isolates could not be resolved and grouped in a single clade with high intraspecific variation. *B. rhodina* isolates, *Diplodia* anamorphs, formed a sister clade to the *Fusiccocium* isolates, no intraspecific variation was present within the clade. The three clades were strongly supported with bootstrap values of 97, 100, and 100 percent.

Pathogenicity

Audpc values were significantly different ($p < 0.001$) between *Botryosphaeria* spp (Figure 2-5). *B. dothidea* AUDPC values were significantly lower than the other *Botryosphaeria* spp. The positive control, isolate 04-40, had a significantly higher AUDPC value than *B. parva* or *B. ribis* isolate WsuF-29, and *B. rhodina* isolate MixFC-6.

Discussion

This study constitutes the first attempt to assess the presence and diversity of fungal species causing stem blight and dieback infections in Florida. Based on partial sequence analysis of the ITS region, at least three *Botryosphaeria* species were isolated from crowns and branches of SHB from Alachua and Polk Co., Fl. *B. dothidea*, *B. parva,* and *B. ribis* were previously recognized as pathogens of SHB in Florida (2). The association of *B. rhodina* with SHB in Florida has not been reported.

Stem blight and dieback of Florida SHB has been attributed to *B. dothidea* and occasionally to other fungi such as *Diplodia* spp, *Macrophoma* spp, and *Phomopsis* spp (2). However, *B. parva, B. ribis*, and *B. rhodina* were recovered from stem blight and canker infections more often than *B. dothidea* or any other fungal genus (Table 2-1, 2-2), indicating the former species may be a more important cause of SHB mortality than previously recognized.

Difficulties distinguishing *Botryosphaeria* species are common because the group of fungal organisms has many taxonomic and nomenclatural ambiguities (39, 96). Teleomorphs of *Botryosphaeria* are infrequently encountered in nature, and are difficult to produce *in vitro* (8, 116). Species identification has been based on anamorph characteristics such as colony and conidial morphology (39,48,96,101,109). Differentiation based on conidial characteristics is difficult because characters vary with age and type of media (48) (Fig 2-2). *Botryosphaeria* spp. have overlapping host ranges, and consequently multiple species can parasitize the same host

(17,22,96,110,111). Results found herein support previous studies (39, 96,110,111), sexual states were infrequently recovered from sample material, and multiple *Botryosphaeria* species were found on SHB in Florida.

DNA sequence comparisons accurately identified *Botryosphaeria* spp. recovered from Florida SHB. Results of ITS phylogenetic analysis supports previous work classifying *Botryosphaeria* anamorphs into two groups: *Diplodia* and *Fusicoccum* (48, 39,109,120) (Fig 2-2). Current phylogenetic research supports multiple conidial lineages within *Botryosphaeriaceae* (35,98). However, species found on Florida SHB separated into two distinct groups. The phylogeny is not a complete sampling of the family; however, the differentiation between *Diplodia* and *Fusiccocium* conidia is important as a diagnostic tool allowing species differentiation.

No intraspecific variation was observed within the *B. rhodina* clade, indicating a uniform population, possibly due to limited sexual recombination. Intraspecific variation was present in the *B. dothidea* and the unresolved *B. parva/B. ribis* clades. The presence of isolates from different hosts and geographic locations could explain the variation. However, variation between Florida *B. parva/ B. ribis* isolates could either be due to sexual recombination, or that species could not be distinguished based solely on the ITS sequence data. Previous studies using single gene phylogenies, RFLP and RAPD makers have been unable to separate the species (6,7,98,101). The EF1-α region has been show to distinguish the two species (96,111). *B. parva* and *B. ribis* are difficult to differentiate molecularly and morphologically; pathogenicity is very similar (96,111). Currently, further molecular, morphological, and pathogenicity studies designed to help elucidate the *B. parva/B. ribis* clade are now underway.

Table 2-1. Incidence of colonies consistent with *Botryosphaeria* growth habit

Survey Period	No.	Percent
Winter		
Alachua Co.		
Flag	25	83%
Crown	27	90%
Subtotal	52	87%
Polk Co.		
Flag	18	60%
Crown	29	97%
Subtotal	47	78%
Winter Total	99	83%
Summer		
Alachua Co.		
Flag	24	80%
Crown	23	77%
Subtotal	47	78%
Polk Co		
Flag	24	80%
Crown	21	70%
Subtotal	45	75%
Summer Total	92	76%
Fall		
Alachua Co.		
Flag	30	100%
Crown	30	100%
Subtotal	60	100%
Polk Co.		
Flag	26	87%
Crown	29	99%
Subtotal	55	92%
Fall Total	115	96%

Table 2-2. Preliminary species identification of isolates consistent with *Botryosphaeria* growth habit; no samples outside the survey area were included. Preliminary identification was determined by comparing ITS region with isolates published in GenBank.

Survey	***B. parva-ribis***		***B. rhodina***		**Other**	
	No.	Percent	No.	Percent	No.	Percent
Winter						
Alachua, Co						
Flag	14	82%	3	18%	0	-
Crown	13	65%	7	35%	0	-
Subtotal	27	73%	10	27%	0	-
Polk Co						
Flag	17	85%	2	10%	1	5%
Crown	12	57%	9	43%	0	-
Subtotal	29	71%	11	27%	1	2%
Winter Total	56	72%	21	27%	1	1%
Summer						
Alachua, Co						
Flag	18	95%	1	5%	0	-
Crown	9	60%	4	27%	2	13%
Subtotal	27	79%	5	15%	2	6%
Polk Co						
Flag	11	69%	3	19%	2	12%
Crown	11	85%	2	25%	0	-
Subtotal	22	76%	5	17%	2	7%
Summer Total	49	78%	10	16%	4	6%
Fall						
Alachua, Co						
Flag	20	87%	3	13%	0	-
Crown	14	78%	4	22%	0	-
Subtotal	34	83%	7	27%	0	-
Polk Co						
Flag	14	70%	4	20%	2	10%
Crown	11	65%	6	35%	0	-
Subtotal	25	68%	10	27%	2	5%
Fall Total	59	76%	17	22%	2	2%

Table 2-3. Representative Isolates from sample collections used in phylogenic analysis

Species	Origin	Date Collected	Host	Abbreviation.
B. parva or B. *ribis*	Archer	Aug-04	SHB	A0440
B. rhodina	Apopka	May-05	*Illex cassine*	A05161
B. parva or B. *ribis*	Alachua	Jun-07	SHB	3010B
B. rhodina	Hawthorne	Jun-06	SHB	A0636
B. dothidea	Wildwood	Feb-07	SHB	A0730
B. dothidea	Archer	Aug-07	SHB	ArcherRingSpotM
B. parva or B. *ribis*	Archer	Aug-07	SHB	ArcherStarRingSpot
B. parva or B. *ribis*	Windsor	Aug-07	SHB	BBC2
B. rhodina	Floral City	May-07	SHB	FerrisFarm
B. parva or B. *ribis*	Waycross, GA	May-07	SHB	GAC1
B. parva or B. *ribis*	Waycross, GA	May-07	SHB	GAC3
Pestalotia	Gainesville	Jul-07	*Ilex* spp.	Holley1
B. parva or B. *ribis*	Hawthorne	May-07	*Vaccinium ashei*	rbe2
B. rhodina	Windsor	Dec-06	SHB	WDSP2
B. parva or B. *ribis*	Windsor	Aug-07	SHB	WindsorRingSpot-1
B. parva or B. *ribis*	Windsor	Aug-07	SHB	WindsorRingSpot-2
B. rhodina	Polk Co	Oct-07	SHB	MixFC151
B. parva or B. *ribis*	Polk Co	Oct-07	SHB	MixFC221
B. parva or B. *ribis*	Polk Co	Oct-07	SHB	MixFC42
B. parva or B. *ribis*	Polk Co	Oct-07	SHB	MixFC7
B. rhodina	Polk Co	Oct-07	SHB	MixFF1
B. parva or B. *ribis*	Polk Co	Oct-07	SHB	MixFF15
B. rhodina	Polk Co	Oct-07	SHB	MixFF19
B. parva or B. *ribis*	Polk Co	Oct-07	SHB	MixFF8
B. rhodina	Polk Co	Jul-07	SHB	MixSuC14
B. parva or B. *ribis*	Polk Co	Jul-07	SHB	MixSuC282
B. parva or B. *ribis*	Polk Co	Jul-07	SHB	MixSuC51
B. parva or B. *ribis*	Polk Co	Jul-07	SHB	MixSuF13

Table 2-3 Continued.

B. rhodina	Polk Co	Jul-07	SHB	MixSuF7
B. rhodina	Windsor	Nov-07	SHB	WFC21
B. parva or B. *ribis*	Windsor	Nov-07	SHB	WFC25
B. parva or B. *ribis*	Windsor	Nov-07	SHB	WFC6
B. parva or B. *ribis*	Windsor	Nov-07	SHB	WFF10
B. parva or B. *ribis*	Windsor	Nov-07	SHB	WFF29a3
B. parva or B. *ribis*	Windsor	Nov-07	SHB	WFF9
B. rhodina	Windsor	Nov-07	SHB	WFF92
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WsuC17
B. rhodina	Windsor	Jun-07	SHB	WsuC21
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WSuC5
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WsuC61
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WSuC9
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WSuF16
B. rhodina	Windsor	Jun-07	SHB	WsuF22
B. parva or B. *ribis*	Windsor	Jun-07	SHB	WSuF29
B. parva or B. *ribis*	Windsor	Jan-07	SHB	WWC38
B. rhodina	Windsor	Jan-07	SHB	WWC47
B. parva or B. *ribis*	Windsor	Jan-07	SHB	WWF37
B. rhodina	Windsor	Jan-07	SHB	WWF46
B. parva or B. *ribis*	Windsor	Jan-07	SHB	WWF47
B. rhodina	Windsor	Feb-07	SHB	WMixC35
B. parva or B. *ribis*	Polk Co	Feb-07	SHB	WmixC4
B. parva or B. *ribis*	Polk Co	Feb-07	SHB	WmixF13
B. parva or B. *ribis*	Polk Co	Feb-07	SHB	WmixF14
B. parva or B. *ribis*	Polk Co	Feb-07	SHB	WmixF15
B. rhodina	Polk Co	Feb-07	SHB	WmixF27

Table 2-4. *Botryosphaeria* sequences from Genbank used in phylogenic analysis

Isolate	Species	Host	Collector	Origin	Accession #
CBS119047	*B. corticis*	*V. corymbosum*	Oudemans PV	New Jersey	DQ299245
CAP234	*B. dothidea*	*Olea europaea*	Lazzizera C	Italy	EF638749
Bd.SC.PH-34.04	*B. dothidea*	*P. persica*	Schnabel G	South Carolina	DQ177876
CBS 116741	*B. dothidea*	*Populus nigra*	Phillips AJL	Portugal	AY640254
UCD1125NA	*B. parva*	*V. vinifera*	Urbez-Torres	California	DQ233612
CMW1130	*B. parva*	*Sequoia gigantean*	Swart S	South Africa	AY236945
CBS110301	*B. parva*	*V. vinifera*	Phillips AJL	Portugal	AY259098
EU249466*	*B. parva*	*E. lacrimans*	Dreaden TJ	Florida	EU249466
STE-U 4438	*B. parva*	*V. vinifera*	Hallen F	R.S.A	AY343467
CMW7799	*B. parva*	*Persica americana*	Pegg KG	Australia	AY615184
CM55	*B. rhodina*	*Theobroma cacao*	Rubini MR	Brazil	AY754002
WAC9853	*B. rhodina*	*V. vinifera*	Wood P	Australia	AY727849
UCD921SN	*B. rhodina*	*V. vinifera*	Urbez-Torres	Mexico	EU012370
CMW13496	*B. rhodina*	*Acacia mangium*	Mohali S	Venezuela	DQ103529
STE-U 4379	*B. ribis*	*P. cynaroides*	Saywood C	Zimbabwe	AF452525
CMW_14025	*B. ribis*	*Syzygium cordatum*	Pavlic D	South Africa	DQ316080
CMW7773	*B. ribis*	*Ribis sp.*	Slippers B	New York	AY236936
CMW7230*	*Botryosphaeria sp.*	*Eucalyptus*	Nakabonge G	Uganda	AY228098
CBS447.62	*L. pseudotheobromae*	*Citrus aurantium*	Smudlers C	Suriname	EF622081
CBS304.79	*L. pseudotheobromae*	*Rosa sp.*	Unknown	Netherlands	EF622079
CBS190.73	*L. theobromae*	*Persea Americana*	Bos WS	Tanzania	EF622068

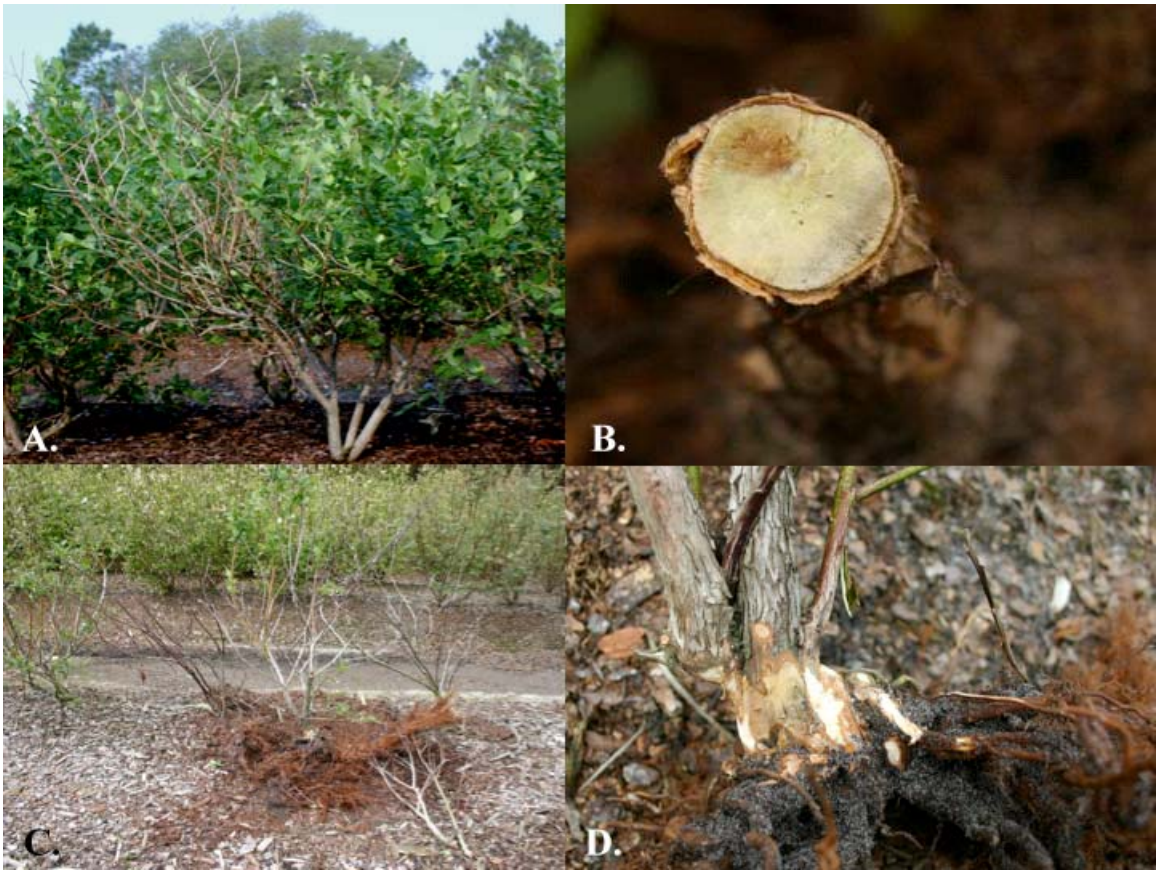

Figure 2-1. Symptoms of stem blight. A) Flagging symptom of stem blight. B) Pecan brown discoloration on one side of the vascular tissue associated with stem blight symptoms. C) Severe die-back infection. D) Discolored vascular tissue associated with stem blight infection of the crown.

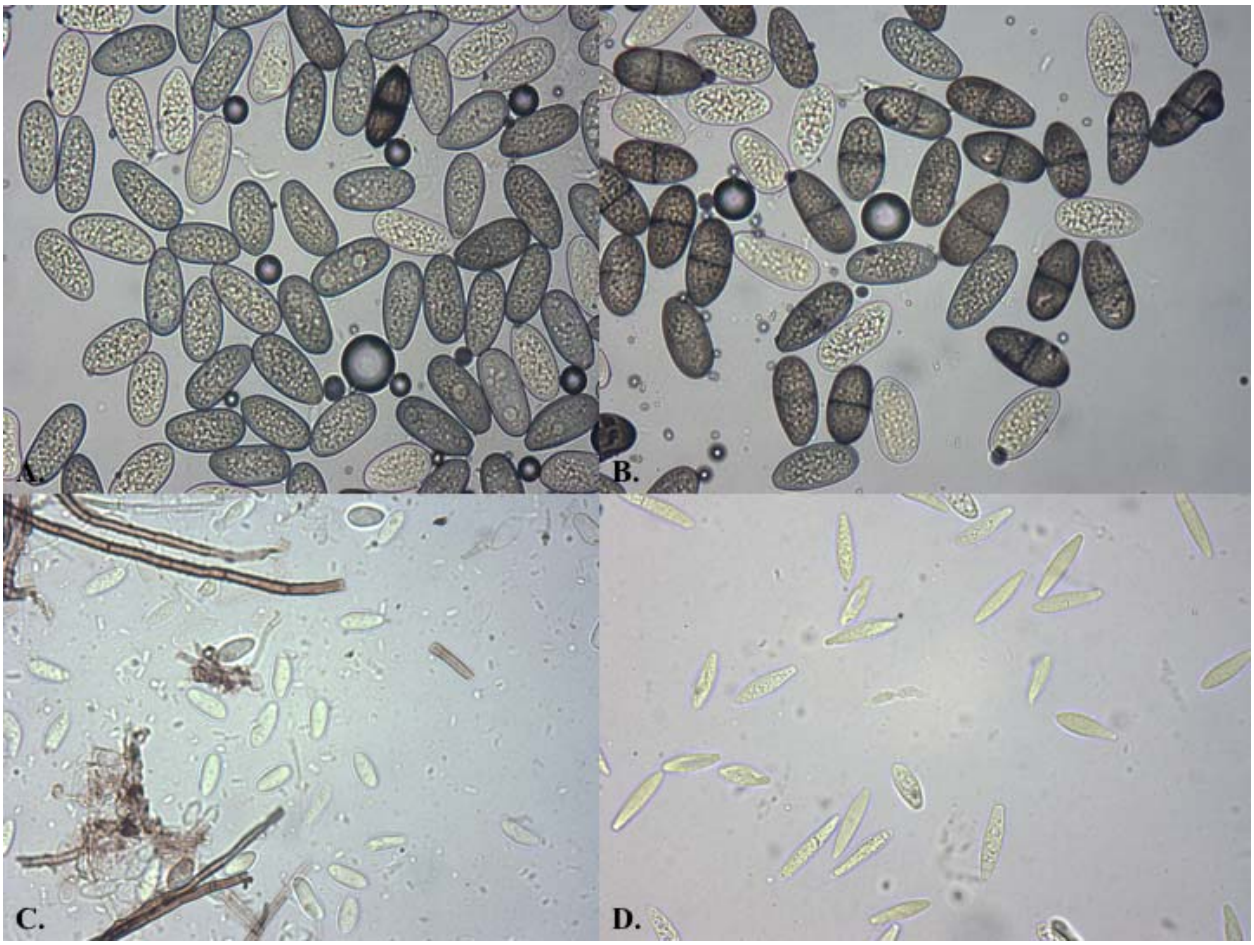

Figure 2-2. Conidial morphology of *Botryosphaeria* associated anamorphs., photographs were taken at 40x magnification. Conidia were produced directly on PDA. A) Immature *Lasiodiplodia theobromae* (teleomorph is *B. rhodina*) conidia (WmixC35). B) Mature *L. theobromae* (teleomorph is *B. rhodina*) conidia with dark brown longitudinal. striations (WmixC35) C) Conidia of a *Neofusicocum* anamorph of either *B. parva* or *B. ribis* (WWC38). D) Mature conidia of *Fusicoccum aesculi* (teleomorph is *B. dothidea* (ArcherRingSpotM).

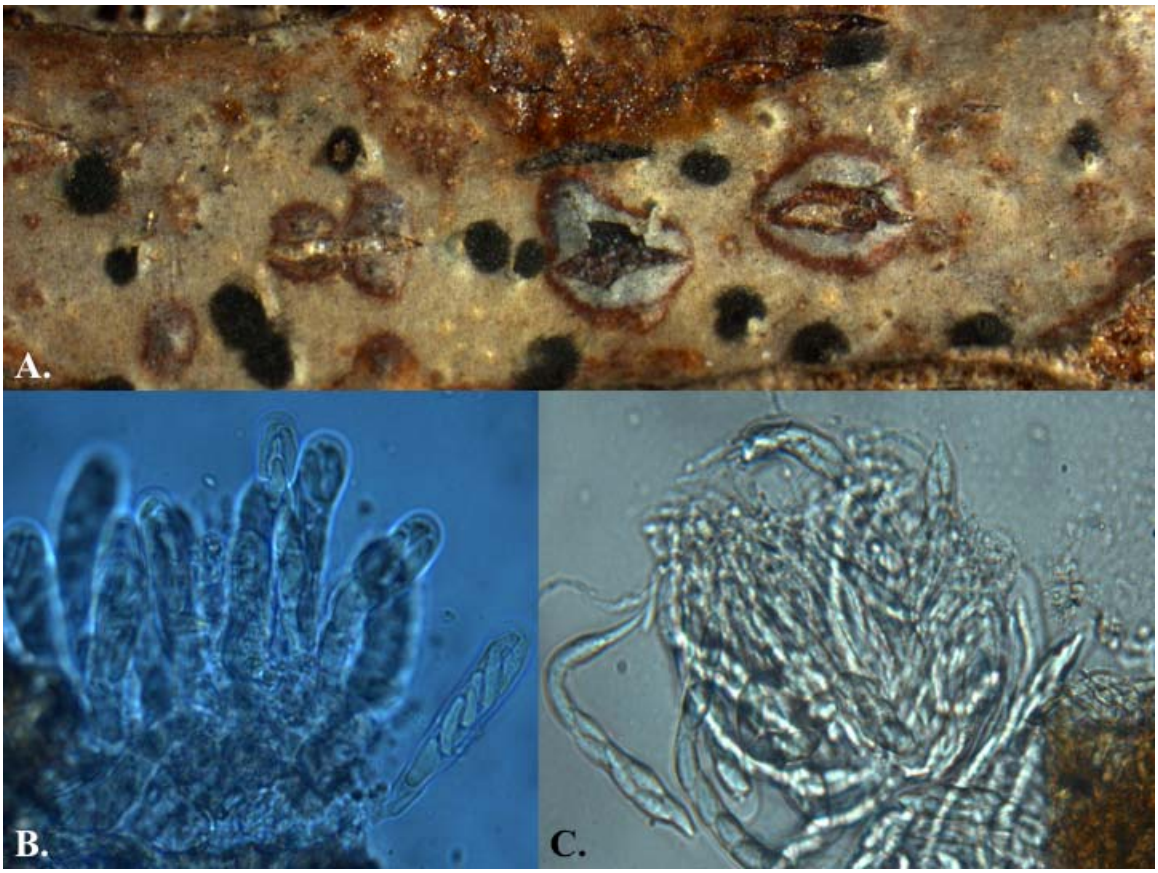

Figure 2-3. Light micrograph was taken with a dissecting microscope at 1.5x magnification. Micrographs of spores were taken with a compound microscope 40x magnification. A) Perithecia protruding from a plant stem. B) Immature acsi of *B. parva* or *B. ribis* from Polk county Florida (MixFC7). C) Asci of *B. parva* or *B. ribis* from Alachua county Florida (WFF29).

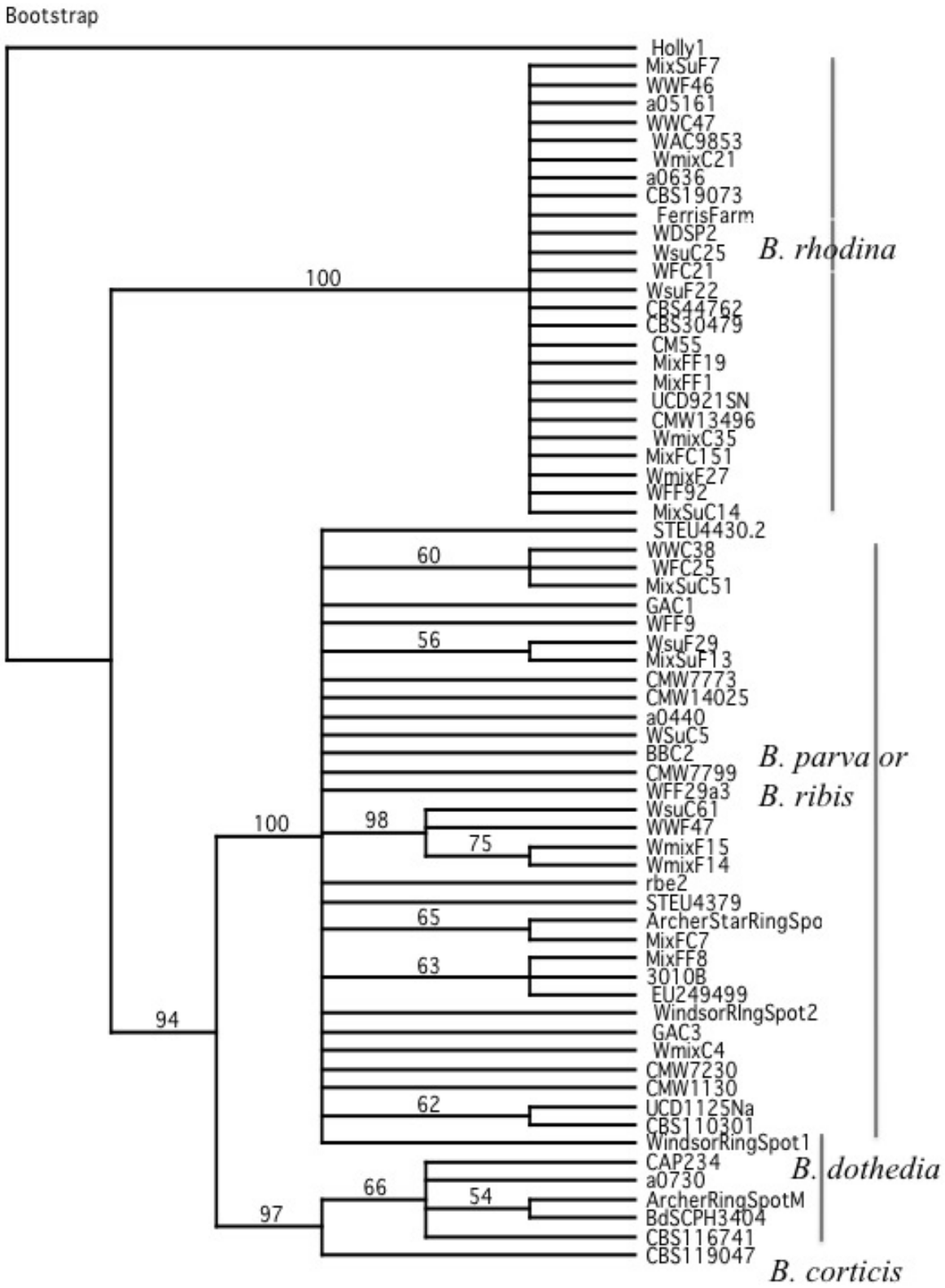

Figure 2-4. Single-gene ITS phylogeny using representative isolates from Alachua Co., GenBank, and Polk Co., FL.

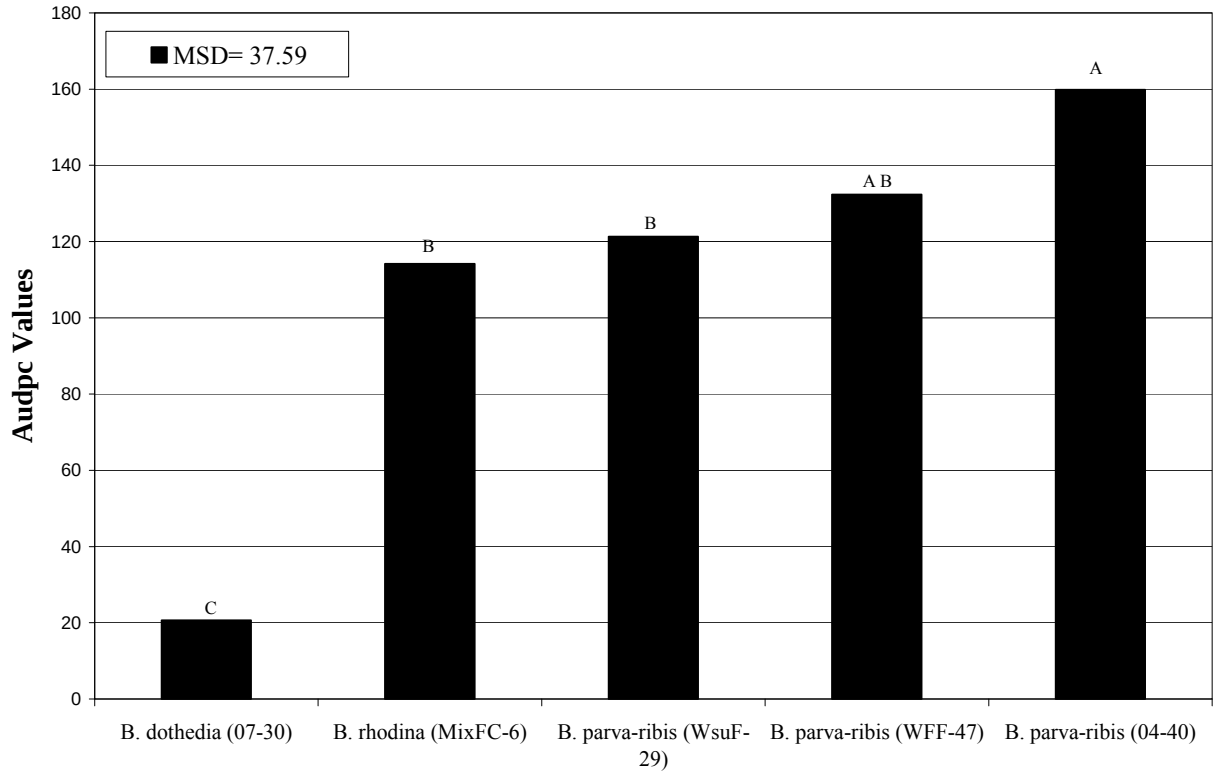

Figure 2-5. The audpc values for isolates used in pathogenicity study. Uninoculated control plants developed no symptoms and were not included. Columns topped with the same letter are not significantly different according to Waller Duncan *k-ratio t*-test (*k*=100) the minimum significant difference (MSD) = 37.59.

CHAPTER 3
SCREENING FOR AND QUANTIFICATION OF STEM BLIGHT RESISTANCE IN SOUTHERN HIGHBUSH BLUEBERRY BREEDING STOCK

Introduction

Stem blight on southern highbush blueberries (SHB) is caused by *Botryosphaeria* spp. in Florida. Stem blight costs growers time and money by causing mortality and by reducing yield. Growers have noticed that some cultivars have higher mortality rates than others suggesting a potential difference in cultivar susceptibility. Varying levels of susceptibility to *Botryosphaeria* pathogens have been noted in blueberry, dogwood, mango, and peach (25,32,36,75,86,90). Susceptibility has been attributed to cultivar, age of tissue used for inoculation, wound age, and inoculum virulence (30,32,86,114). The use of disease indexes, highly virulent isolates, and non-woody stem tissue have been reported to standardize resistance screening methods (9,30,100).

Buckley (1990) concluded that narrow sense heritability was greater than broad sense heritability for stem blight resistance (25,32). Both additive and non-additive genetic effects are involved in resistance which is derived from the low bush blueberry (*V. angustifolium*) in populations from Michigan, New Jersey and North Carolina. Buckley recommended that progeny could be screened for the identification of superior parents (25).

The UF breeding program uses recurrent selection which is based on two principles. The first is heterozygous parents yield variable progeny. The second is that progeny that are extreme in the expression of certain characteristics, and when crossed to produce a second generation progeny, will be variable, and some seedlings will be more extreme in character expression than their parents (58). Cultivar selection at UF has four stages. In stage I, 15,000 seedlings are planted in high density plots. After one year, stage I plants are rated for desirable bush defects, firmness, flavor, fruit size, and ripening time. The best 500 plants are selected and advanced to

stage II. The rest of the plants are discarded. Stage II plants are rated for three years; the best 150-200 plants are numbered and marked for asexual propagation, approximately 40 softwood cuttings are rooted from each plant. The best clones are planted in 15-plant plots using commercial spacing. The clones are rated over three years for survival, and for other bush and berry qualities. The superior 12 to 15 stage III plants are asexually propagated and planted on multiple farms. The stage IV plants are evaluated for three to six years by the breeder and growers. On average one or two plants are selected for cultivar release each year (*Lyrene personal communication*).

Progeny are diverse and have varying levels of stem blight mortality in stage III evaluation plots. Variation could be due to different levels of inoculum, variations in field conditions, or varying levels of resistance. If differences are due to resistance, then progeny with the same parents should be more similar in levels of resistance than progeny of different parents. If resistance has a strong genetic component, selection for resistance should be possible given an effective screening tool. Therefore, resistance to *Botryosphaeria* was quantified using blueberry clones being evaluated for cultivar potential, and a screening protocol was devised to select the most resistant seedlings.

Methods

Field Evaluation

Stage III 2003, 2004, and 2005 evaluation plots located in Windsor, FL were rated for disease. Each clone had 15 replicate plants per plot. The clones were rated on a 0 to 2 scale with healthy plants receiving a zero, symptomatic plants receiving a one, and dead plants receiving a two. The average disease score of each clone was determined by dividing plant ratings by fifteen. The disease score of each clone rated was assigned to both parents in the cross as a progeny disease score (PDS). PDS data for parents with three or less offspring replicates were

discarded. A general linear model in SAS (SAS Institute, Cary, N.C.) was used to analyze variance in data, and mean PDS of parents were separated by Waller-Duncan *k-ratio t*-test (*k*=100).

Clone Replicates and Inoculation

Genetically unique plants designated by pedigree number were clonally propagated by soft wood cuttings. Clones were separated by pedigree, randomized between and within pots by randomly selecting four unique clones which were planted per gallon pot in Canadian peat. Plants were cut with scissors below the top 3 or 4 leaves. Scissors were surface-sterilized with 95% ethanol between pots. Plant height was measured. Eight 3-day-old culture plates of *B. parva* or *B. ribis* isolate 04-40 were ground with 400ml of sterile water in a blender. The suspensions were sprayed onto sterile plates of media, and fungal growth was assessed. Plants were sprayed with one of the suspensions until runoff. Control plants were sprayed with a similar suspension of sterile media and water. A paper towel was moistened with sterile water and placed on top of the plants. Pots were bagged and placed in a 25°C incubator with 12h of light per day for two weeks.

Lesion lengths were measured in centimeters weekly for one month after the plants were removed from the incubator. Percent lesion length (PLL) was calculated by dividing lesion length by plant height. Average PLL was calculated for each clone evaluated. Variation in data was analyzed using a generalized linear model in SAS (SAS Institute, Cary, N.C.) with class variables of pot and clone, Waller-Duncan *k-ratio t*-test (*k* =100) assessed mean separations between clones. The experiment was repeated twice with unique clonal accessions from 2005 and 2007 selected by the breeder.

Results

Heritability Study

Progeny susceptibility assessed in 15-plant clonal field plots differed significantly depending on which parents were used to make the cross. *P-values* for 2005 and 2004 evaluation plots were < 0.05. In 2003, evaluation plots PDS were not significant ($p > 0.12$). Parents were ranked by PDS (Figs 3-1, 3-2, 3-3) from least to most susceptible.

Trials 1&2 (07 Clones)

Between-pot variation was significant ($p < 0.1$) in trials 1 and 2 (Fig 3-4 & 3-5). Clonal variation was not significant ($p > 0.1$). There was no correlation between average PLL and the clones used in trials one and two.

Trial 3&4 (05 Clones)

Average PLL was significant ($p < 0.05$) for trial 3 (Fig 3-6). For trial 4, pot and clonal variation was not significant ($p > 0.1$) at the time after plants were removed from the incubator (Fig 3-7). Clonal variation was significant ($p < 0.05$) one week after the end of the incubation period. Two weeks after the incubation period both pot and clone variables were significant ($p < 0.05$). Minimum significant difference (MSD) decreased when both variables became statistically significant. There was no correlation between average PLL and the clones replicated in both trials.

Discussion

Parents were identified from progeny lineages having varying degrees of resistance (Fig 3-1, and Fig 3-2). The 2003 plot was not significant because environmental factors were greater contributors to plant mortality in older plots (Fig 3-3). Resistance was a continuous gradient from the least to most susceptible parents. Results support Buckley's findings that pedigree will influence progeny stem blight resistance. Therefore, a reliable screening tool could be developed

to select progeny with superior stem blight resistance. Clonal susceptibility to stem blight was not replicated using either the ’05 or ’07 clones. Lack of repeatability was due in part to low numbers of clonal replicates used throughout the trials. A larger number of replicates per clone would help to reduce the standard errors used in comparing the clone means.

Differences between trials 1 & 2 contributed to variable results. Clones used in trial 1 were left in the incubator for three weeks instead of two unlike previous experiments. The three week incubation period left more time for infection many of the plants were dead by the end of the third week. Pots were not randomized in the incubator; some pots received less light because there were no lights on the bottom rack. Plants on the bottom rack were water soaked and greater disease incidence was observed. In trails three and four the incubator used had lights on the bottom shelf; all pots received equal amounts of light which helped to standardized the experiment.

Stem blight symptoms were evident on the control plants of trial four. No fungus grew on the petri plate sprayed with the control suspension. *Botryosphaeria* was re-isolated from the control plants having stem blight symptoms. These data suggest that cuttings used for trial 4 were already infected with *Botryosphaeria* before the trial began.

Clones did not display visible symptoms of stem blight prior to inoculation. This indicated a possible fungal latent infection period. Latent infection periods of *Botryosphaeria* have been reported for *Proteaceae* flowers, pistachio, and apple (39,50,73,76). Changes to propagation methods that could provide disease free material; and the addition of individual clonal replicates would help standardize the screening procedure.

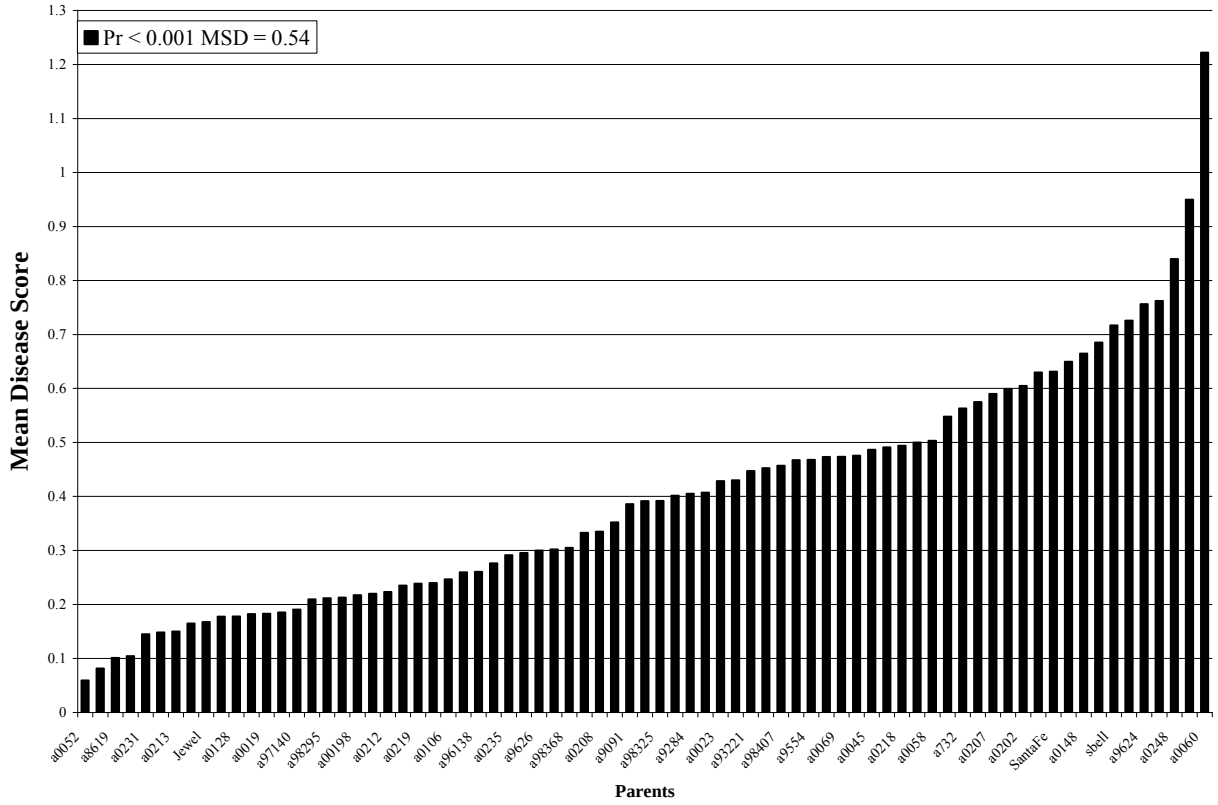

Figure 3-1. Mean progeny disease score of parents of the 2005 clone evaluation. Clones were dispersed randomly in the plot. The mean for each parent was based on 4 or more progeny clones. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k*-ratio *t*-test (*k*=100).

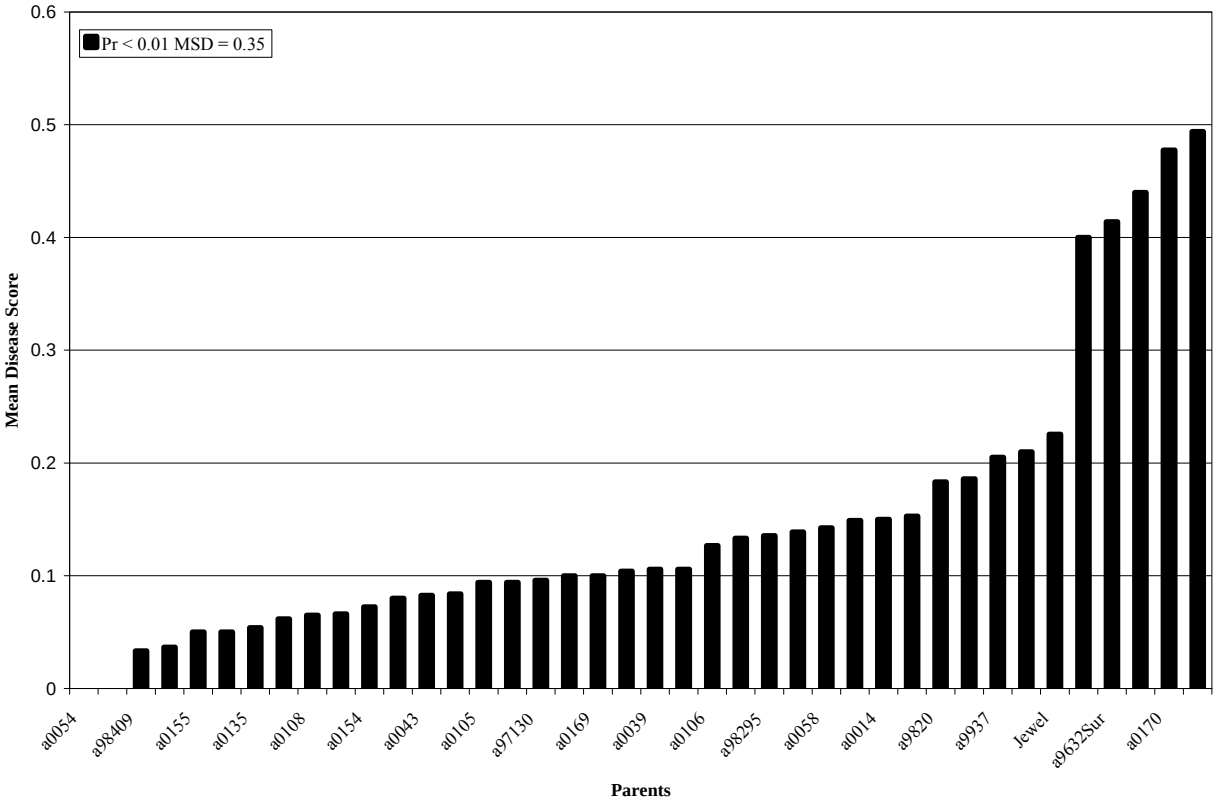

Figure 3-2. Mean progeny disease score of parents of the 2004 clone evaluation. Clones were dispersed randomly in the plot. The mean for each parent was based on 4 or more progeny clones. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k*-ratio *t*-test (*k*=100).

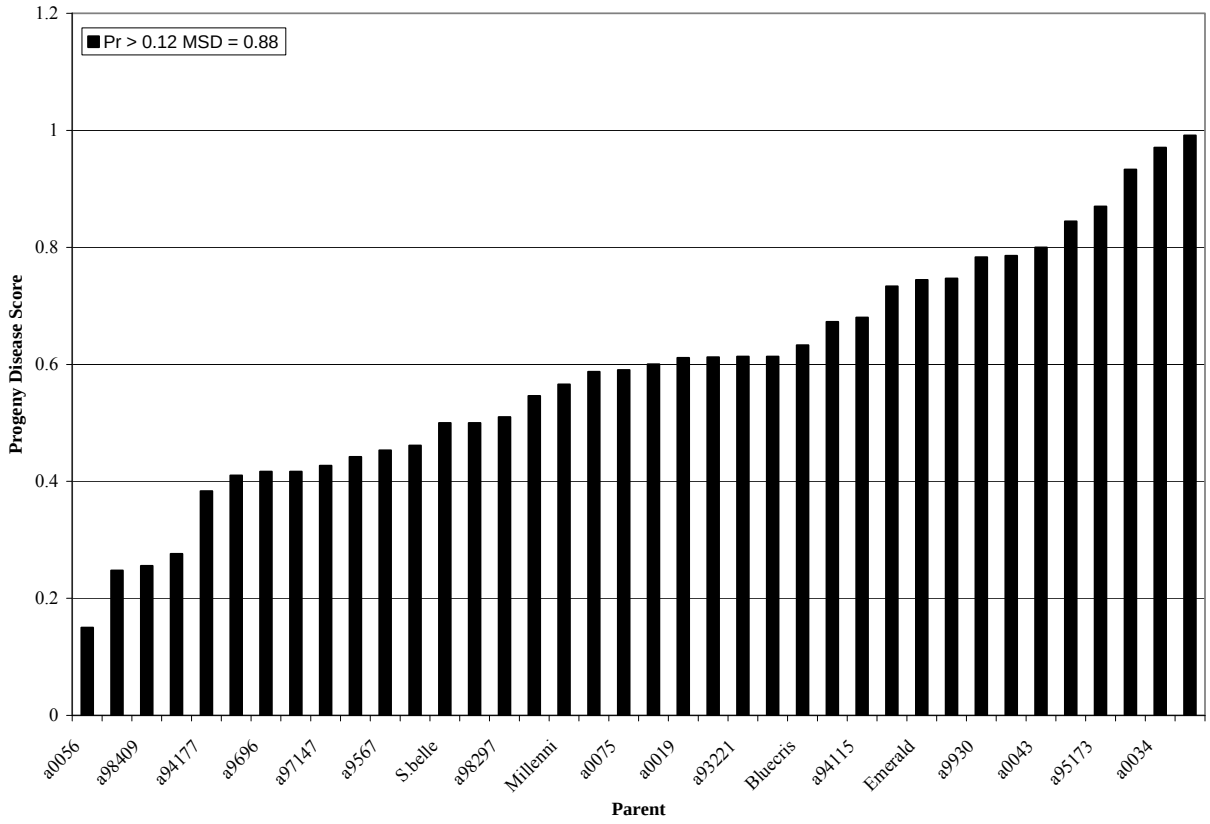

Figure 3-3. Mean progeny disease score of parents of the 2003 clone evaluation. Clones were dispersed randomly in the plot. The mean for each parent was based on 4 or more progeny clones. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k-ratio t*-test (*k*=100).

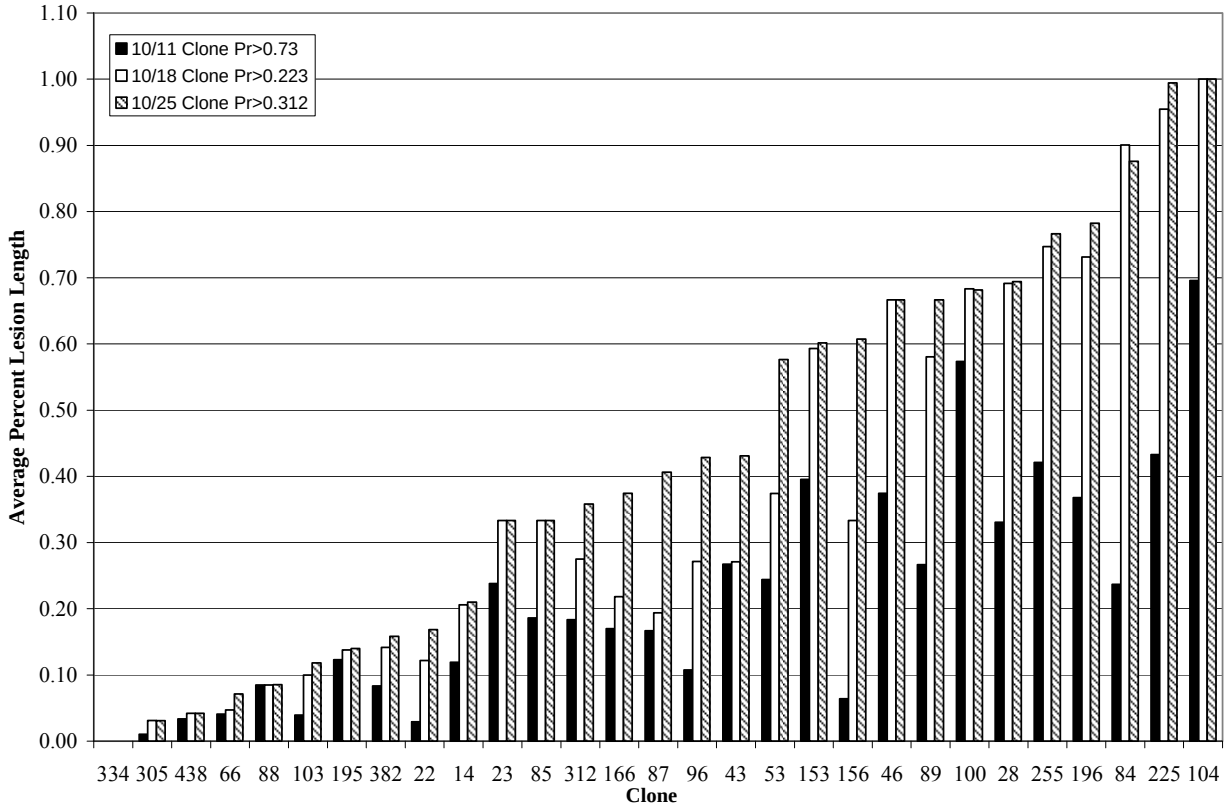

Figure 3-4. Trial 1 average percent lesion length of 07 clones inoculated with *Botryosphaeria* isolate 04-40. Lesion lengths were measured at three dates. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k-ratio t*-test (*k*=100).

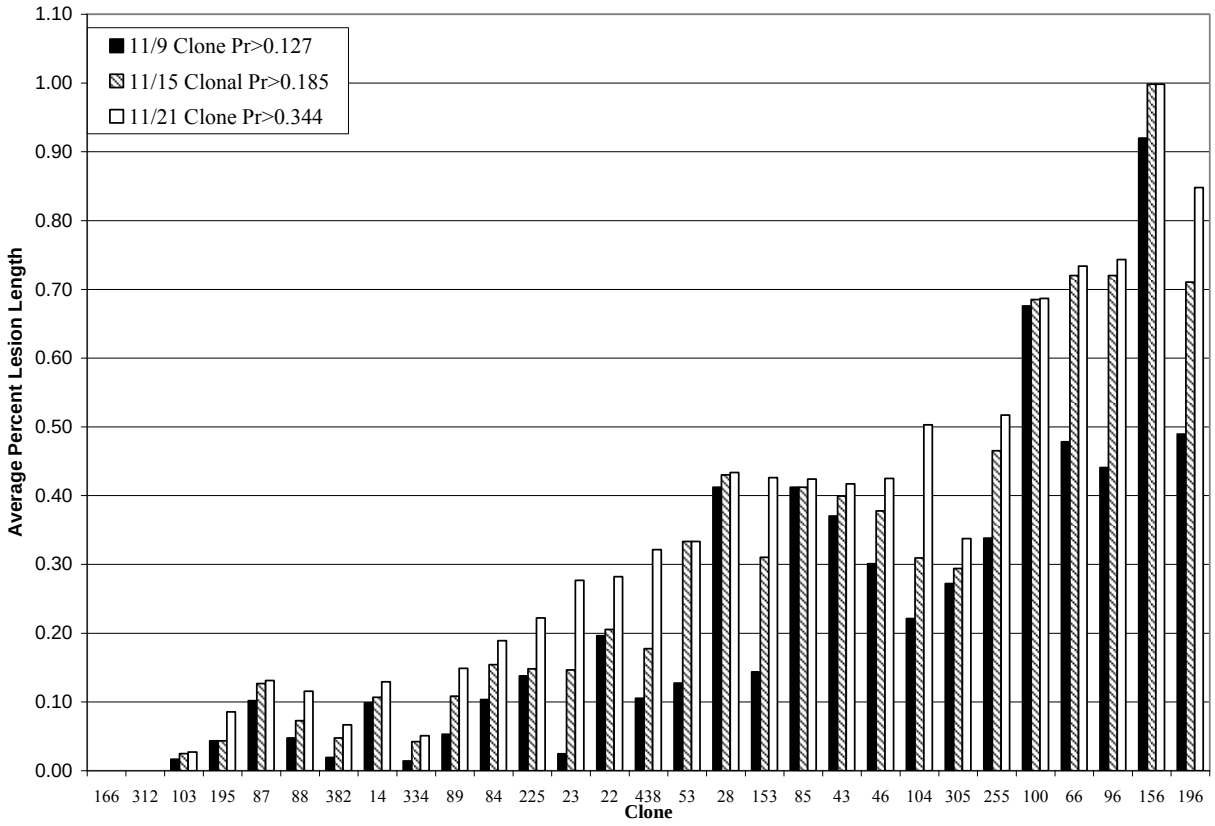

Figure 3-5. Trial 2 average percent lesion length of 07 clones inoculated with *Botryosphaeria* isolate 04-40. Lesion lengths were measured at three dates. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k-ratio t*-test (*k*=100).

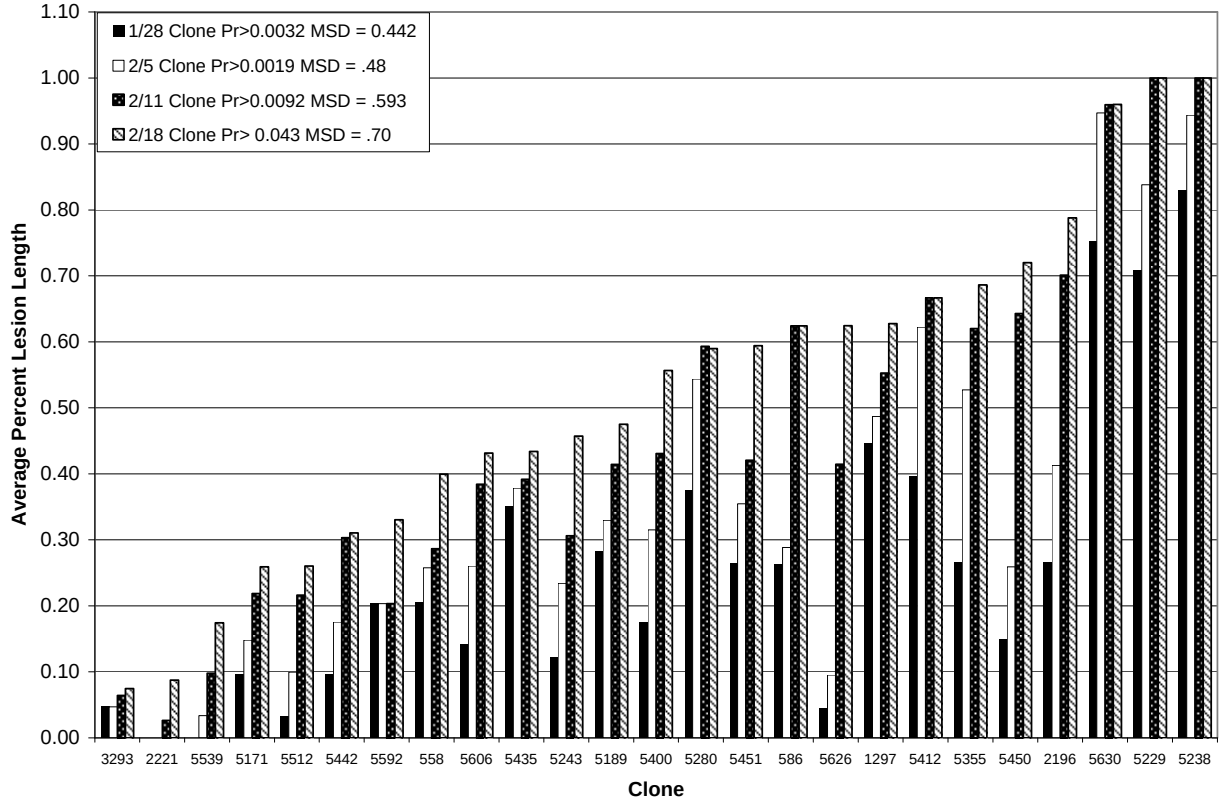

Figure 3-6. Trial 3 average percent lesion lengths of 05 clones inoculated with *Botryosphaeria* isolate 04-40. Lesion lengths were measured at four dates. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k-ratio t*-test (*k*=100).

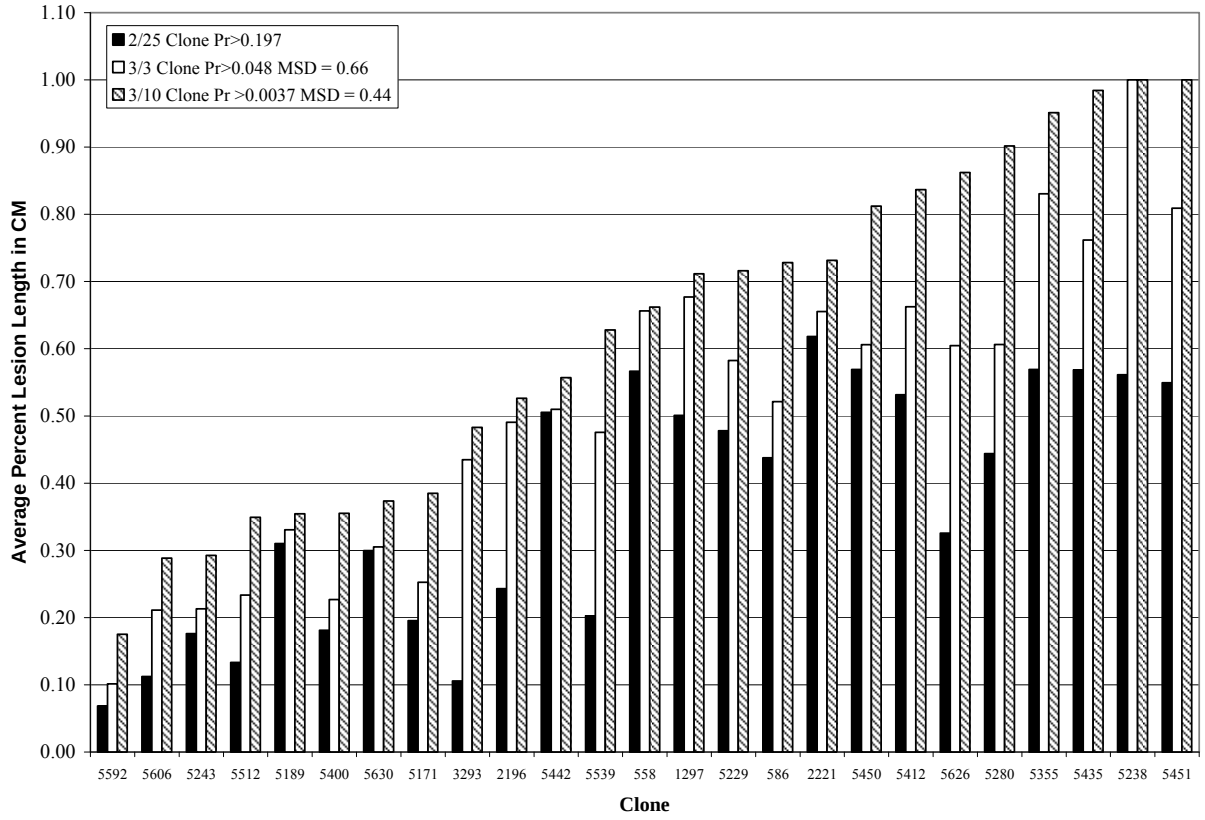

Figure 3-7. Trial 4 average percent lesion lengths of 05 clones inoculated with *Botryosphaeria* isolate 04-40. Lesion lengths were measured at three dates. Pr is the ANOVA *p-value* and MSD is the minimum significant difference according to the Waller Duncan *k-ratio t*-test (*k*=100).

LIST OF REFERENCES

1. Alderman, S.C. and Lacy, M.L. 1984. Influence of temperature and moisture on growth and sporulation of *Botrytis squamosa*. Can. J. Bot. 62: 2793-2797.

2. Alfieri Jr., S.A., Landon, K.R., Kimbrough, J.W., El-Gholl, N.E., and Wehlburg, C. 1994. Bulletin No. 14 Diseases and Disorder of Plants in Florida. DPI, Gainesville, Fl. 1-1115.

3. Alexopolis, C.J., Mim, C.W., and Blackwell, M. 1996. Introductory Mycology. John Wiley and Sons Inc. New York.

4. Alves, A., Correia, A., Luque, J., and Phillips, A.J.L. 2004. *Botryosphaeria corticola* sp. nov. on Quercus species, with notes and description of *Botryosphaeria stevensii* and its anamorph *Diplodia mutila*. Mycologia 96: 598-613.

5. Alves, A., Crous, P.W., Correia, A., and Phillips, A.J.L. 2008. Morphological and molecular data reveal cryptic species in *Lasiodiplodia theobromae*. Fungal Div. 28: 1-13.

6. Alves, A., Phillips, A., Henriques, I., and Correia, A. Evaluation of amplified ribosomal DNA restriction analysis as a method for the identification of *Botryosphaeria* species. FEMS Microbiol. Letters 245: 221-229.

7. Alves, A., Phillips, A.J.L., Henriques, I., Correia, A. 2007. Rapid differentiation of species of *Botryosphaeraiaceae* by PCR fingerprinting. Res. in Microbiol. 158: 112-121.

8. Arauz, L.F. and Sutton, T.B. 1990. Effect of interrupted wetness period on spore germination and ale infection by *Botryosphaeria obtusa*. Phytopathology 80: 1218-1220.

9. Ballington, J.R., Rooks, S.D., Milholland, R.D., Cline, W.O., and Meyers, J.R. 1993. Breeding blueberries for pest resistance in North Carolina. Acta Hort. 346: 87-94.

10. Barr, M.E. 1987. Prodromus to class Loculoascomycetes. Hamilton I. Newell, Inc., Amherst Mass.

11. Beckman, T.C., Pusey, P.L., and Bertrand, P.F. 2003. Impact of fungal gummosis on peach trees. HortScience 38(6): 1151-1153.

12. Beckman, T.G. and Reilly, C.C. 2006. Relative susceptibility of ornamental peach cultivar to fungal gummosis (*Botryosphaeria dothidea*). American Pomological Society 60(3): 149-154.

13. Berbee, M.L. 1996. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol. Biol. Evol. 13(3): 462-470.

14. Berbee, M.L. and Taylor, J.W. 1995. From 18S ribosomal sequence data to evolution of morphology among the fungi. Can. J. Bot. 73(Suppl. 1): S677-S683.

15. Bester, W., Crous, P.W., and Fourie, P.H. 2007. Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* species. Austral. Plant Pathol. 36: 73-77.

16. Biggs, A.R. 1994. Mycelial growth, sporulation, and virulence to apple fruit of *Alternaria alternara* isolates resistance to iprodione. Plant Dis. 78: 732-735.

17. Biggs, A.R., and Britton, K.O. 1988. Presymptom histopathology of peach trees inoculated with *Botryosphaeria obtuse* and *B. dothidea*. Phyophathology 78: 1109-1118.

18. Biggs, A.R. and Miller, S.S. 2004. Relative susceptibility of selected apple cultivars to fruit rot caused by *Botryosphaeria obtusa.* HortScience 39(2): 303-306.

19. Britton, K.O. and Hendrix, F.F. 1986. Population dynamics of *Botyrosphaeria* spp. in peach gummosis cankers. Plant Dis. 70: 134-136.

20. Bitton, K.O., Hendrix, F.F., Pusey, P.L., Okie, W.R., Reilly, C.C., and J.W. Daniell. 1990. Evaluating the reaction of peach cultivar to infection by three *Botryosphaeria* species. HortScience 25(4): 467-470.

21. Boyer, J.S. 1995. Biochemical and biophysical aspects of water deficits and the predisposition to disease. Annu. Rev. Phytopathol. 33: 251-274.

22. Brown, E.A., II and Britton, K.O. 1986. *Botryosphaeria* diseases of apple and peach in the Southeastern United States. Plant Dis. 70: 480-484.

23. Brown, E.A and Hendrix, F.F. 1981. Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. Phytopathology 71: 375-378.

24. Brown-Rytlewski, D.E. and McManus, P.S. 2000. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on Apple and Management of Stem Cankers with Fungicides. Plant Dis. 84: 1031-1037.

25. Buckley, Blair III. 1990. Occurrence of Stem Blight Resistance in Blueberry. Department of Horticultural Science NCSU. LD3921 Hort. B88.

26. Burgess, T.I., Barber, P.A., Mohali, S., Pegg, G., de Beer,W., and Wingfield, M.J. 2006. Three new *Lasiodiplodia* spp. from the tropics recognized based on DNA sequence comparisons and morphology. Mycologia 98: 423-435.

27. Burgess, T.I., Taylor, A., Hardy, G., and Wood, P. 2005. Identification and pathogenicity of *Botryosphaeria* species associated with grapevine decline in Western Australia. Australas. Plant Pathol. 34: 187-195.

28. Burpee, L.L., Green, D.E., and Stephens, S.L. 1996. Interactive effects of plant growth regulators and fungicides on epidemics of dollar spot in creeping bentgrass. Plant Dis. 80: 1245-1250.

29. Cline, W.O. and Milholland, R.D. 1992. Root dip treatments for controlling blueberry stem blight caused by *Botryosphaeria dothidea* in container-grown nursery plants. Plant Dis. 76: 136-138.

30. Cline, W.O., Milholland, R.D., Rooks, S.D., and Ballington, J.R. 1993. Techniques for breeding for resistance to blueberry stem blight caused by *Botryosphaeria dothidea*. Acta Hort. 346: 107-109.

31. Copes, W.E., and Hendrix, E.F. 2004. Effect of temperature on sporulation of *Botryosphaeria dothidea, B. obtusa*, and *B. rhodina*. Plant Dis. 88: 292-296.

32. Creswell, T.C. and Milholland, R.D. 1987. Responses of blueberry genotypes to infection by *Botryosphaeria dothidea.* Plant Dis. 71: 710-713.

33. Creswell, T.C. and Milholland, R.D. 1988. Spore release and infection periods of *Botryosphaeria dothidea* on blueberry in North Carolina. Plant Dis. 72: 342-346.

34. Crist, C.R. and Schoeneweiss, D.F. 1974. The influence of controlled stresses on susceptibility of European white birch stems to attack by *Botryosphaeria dothidea*. Phytopathology 65: 369-373.

35. Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O.., Philps, A.J.L., Alves, A., Burgess, T., Barber, P., and Groenwald, J.Z. 2006. Phylogenetic lineages in the *Botryosphaeriaceae*. Studies in Mycol. 55: 235-253.

36. Daniel, J.W. and Chandler, W.A. 1982. Field resistance of peach cultivar to gummosis disease. HortScience 17(3): 375-376.

37. Denman, S., Crous, P.W., Groenewald, J.Z., Slippers, B., Wingfield, B.D., and Wingfield, M.J. 2003. Circumscription of *Botryosphaeria* species associated with *Proteaceae* based on morphology and DNA sequence data. Mycologia 95: 294-307.

38. Denman, S., Crous, P.W., Sadie, A., and Wingfield, M.J. 2004. Evaluation of fungicides for the control of *Botryosphaeria protearum* on *Protea magnifica* in the western cape province of South Africa. Austral. Plant Pathol. 33: 97-102.

39. Denman, S., Crous, P.W., Taylor, J., Kang, J., Pasco, I., and Wingfield, M. 2000. An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. Studies in Mycol., No.45: 129-140.

40. Desprez-Loustau, M.L., Marcais, B., Nageleisen, L.M., Piou, D., and Vannini, A. 2006. Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. 63: 597-612.

41. Detweiler, A.R., Vargas, J.M., and Danneberger, T.K. 1983. Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. Plant Dis. 67: 627-630.

42. Ehlenfeldt, M.K., and Stretch, A.W. 2001. Resistance to blight by *Monolinia vaccinii-corymbosi* in diploid and polyploidy *Vaccinium* species. HortScience 36(5): 955-957.

43. English, H., Davis, J.R., and DeVay, J.E. 1974. Relationship of *Botryosphearia dothidea* and *Hendersonula toruloides* to a canker disease of almond. Phytopathology 65: 115-122.

44. Gupton, C.L. and Smith, B.J. 1989. Inheritance of tolerance to stem blight in *Vaccinium* species. HortScience 24: 748 (abstr).

45. Holtz, B.A. 2002. Plant protection for pistachio. HortTechnology 12(4): 626-632.

46. Hong, C. and Michailides, T.J. 1999. Mycelial growth, sporulation, and survival of *Monilinia fructicola* in relation to osmotic potential and temperature. Mycologia 95: 871-876.

47. Jacobs, K.A and Berg, L.C. 2000. Inhibition of fungal pathogens of woody plants by the plant growth regulator paclobutrazol. Pest Mang. Sci. 56: 407-412.

48. Jacobs, K.A and Rehner, S.A. 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. Mycologia 90(4): 601-610.

49. Jeffers, S.N. 1991. Seasonal incidence of fungi in symptomless cranberry leaves and fruit treated with fungicides during bloom. Phytopathology 81: 636-644.

50. Kim, D.H. Uhm, J.Y. 2002. Effect of application timing of ergosterol biosynthesis-inhibiting fungicides on the suppression of disease and latent infection of apple white rot cause by *Botrosphaeria dothidea*. J. Gen. Plant Pathol. 68: 237-245.

51. Kirk, P.M., Cannon, P.F., David, J.C., and Staplpers, J.A. 2001. Ainsworth and Bisby's Dictionary of the Fungi. Biddles Ltd. Wallingford, U.K.

52. Kruger, B.M. and Manion, P.D. 1994. Antifungal compounds in aspen: effect of water stress. Can. J. Bot. 72: 454-460.

53. Lazzizera C., Frisuloo, S., Alves, A., and Phillips, A.J.L. 2008 Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in Southern Italy. Plant. Pathol. ***in press.***

54. Lumbsch, H.T. and Huhndorf, S.M. 2007. Whatever happened to the pyrenomycetes and loculoascomycetes? Mycol. Res. 111: 1064-1074.

55. Lumbsch, H.T. and Lindemuth, R. 2001. Major lineages of *Dothideomycetes* (*Ascomycota*) inferred from SSU and LSU rDNA sequences. Mycol. Res. 105(8): 901-908.

56. Luttrell, E.S. 1951. Taxonomy of the Pyrenomycetes. Univ. Missouri Studies, Sci. Ser. 24: 1-120.

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

58. Lyrene, P.M. 2002. Breeding Southern highbush blueberries in Florida. Acta Hort. 574: 149-152.

59. Lyrene, P.M. 2005. Breeding low-chill blueberries and peaches for subtropical areas. HortScience 40: 1947-1949.

60. Lyrene, P.M., Vorsa, N., and Ballington, J.R. 2003. Polyploidy and sexual polyploidization in the genus *Vaccinium*. Euphytica 133: 27-36.

61. Ma, Z., Boehm, E.W.A., Luo, Y., and Michailides, T.J. 2001. Population structure of *Botryosphaeria dothidea* from pistachio and other hosts in California. Phytopathology 91: 665-672.

62. Ma, Z. and Michailides, T.J. 2002. A PCR-based technique for identification of *Fusicoccum* sp. from pistachio and various other hosts in California. Plant Dis. 86: 515-520.

63. Ma, Z. and Michailides, T.J. 2002. Characterization of *Botryosphaeria dothidea* isolates collected from pistachio and other plant hosts in California. Phytopathology 92: 519-526.

64. Ma. Z., Morgan, D.P., Felts, D., Michailides, T.J. 2002. Sensitivity of *Botryosphaeira dothidea* from California pistachio to tebuconazole. Crop Prot. 21: 829-835.

65. Ma, Z., Morgan, D., and Michailides, T. 2001. Effect of water stress on Botryosphaeria blight of pistachio caused by *Botryosphaeria dothidea*. Plant Dis. 85: 745-749.

66. Ma. Z., Young, L., and Michailides, T.J. 2001. Resistance of *Botryosphaeria dothidea* from pistachio to iprodione. Plant Dis. 85: 183-188.

67. Maddison D.R. and Maddison W.R. 2000. McClade 4: Analysis of phylogeny and character evolution. Sunderland, Massachusetts: Sinauer Associates.

68. McPartland, J.M. and Schoeneweiss, D.F. 1984. Hyphal morphology of *Botryosphaeria dothedia* in vessels of unstressed and drought stressed stems of *Betula alba*. Phytopathology 74: 358-362.

69. Michailides, T. 1991. Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on pistachio. Phytopathology 81: 566-573.

70. Michailides, T.J. and Morgan, D.P. 1992. Effects of temperature and wetness duration on infection of pistachio by *Botryosphaeria dothidea* and management of disease by reducing duration of irrigation. Phytopathology 82: 1399-1406.

71. Michailides, T.J. and Morgan, D.P. 1993. Spore release by *Botrosphaeria dothidea* in pistachio orchard and disease control by altering the trajectory angle of sprinkler. Phytopathology 83: 145-152.

72. Milholland R.D. 1972. Histopathology and pathogenicity of *Botryosphaeria dothidea* on blueberry stems. Phytopathology 62: 654-660.

73. Mila, A.L., Driever, G.F., Morgan, D.P., and Michailides, T.J. 2005. Effects of latent infection, temperature, precipitation, and irrigation on panicle and shoot blight of pistachio in California. Phytopathology 95: 929-932.

74. Morgan-Jones, G. and White, J.F., Jr. 1987. Notes on Coelomycete. II. concerning the *Fusicoccum* anamorph of *Botryosphearia ribis*. Mycotaxon 30: 117-125.

75. Mullen, J.M., Gilliam, G.H., Hagan, A.K., and Morgan-Jones, G. 1991. Canker of dogwood caused by *Lasiodiplodia theobromae*, a disease influenced by drought stress or cultivar selection. Plant Dis. 75: 886-889.

76. Ntahimpera, N., Driever, G.F., Felts, D., Morgan, D.P., and Michailides, T.J. 2002. Dynamics and pattern of latent infection caused by *Botryosphaeria dothidea* on pistachio bunds. Plant Dis. 86: 282-287.

77. Ogata, T.., San, T., and Harada, Y. 2000. *Botryosphaeria* spp. isolated from apple and several deciduous fruit trees are divided into three groups based on the production of warts on twigs, size of conidia, and nucleotide sequences of nuclear ribosomal DNA ITS regions. Mycoscience 41: 331-337.

78. Olaya, G. and Abawi, G.S. 1996. Effect of water potential on mycelial growth and on production and germination of sclerotia of *Macrophomina phaseolina*. Plant Dis. 80: 1347-1350.

79. Old, K.M., Gibbs, R, Craig, I., Myers, B.J., and Yuan, Z.Q. 1990. Effect of drought and defoliation on the susceptibility of eucalypts to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. Aust. J. Bot. 38: 571-81.

80. Pavlic, D., Slippers, B., Coutinho, T.A., and Wingfield, M.J. 2007. *Botrosphaeria* spp. form native *Syzygium cordatum* and introduced Eucalyptus tress in South Africa. South African J. of Sci. 103: VI-VII (abst)

81. Parker, K.C. and Sutton, T.B. 1993. Effect of temperature and wetness duration on apple fruit infection and eradicant activity of fungicides against *Botryosphaeria dothidea*. Plant Dis. 77: 181-185.

82. Pennycook, S.R. and Samuels, G.J. 1985. *Botryosphaeria* and *Fusicoccum* speciea associated with ripe fruit rot of *Actinida deliciosa* (Kiwifruit) in New Zealand. Mycotaxon 24: 445-458.

83. Plant. M.R.R., Joyce, D.C., Ogle, H.J., and Johnson, G.I. 2002. Mango stem-end rot (*Botryosphaeria dothidea*) disease control by partial-pressure infiltration of fungicides. Austral. J. Exp. Agric. 42: 625-629.

84. Phillips, A.J.L., Alves, A., Correia, A., and Luque, J. 2006. Two new species of *Botryosphaeria* with brown, 1-septate ascospores and *Dothiorella* anamorphs. Mycologia 97: 513-519.

85. Phillips, A.J.L., Oudemans, P.V., Correia, A., and Alves, A. 2006. Characterization and epitypification of *Botryosphaeria corticis* the cause of blueberry cane canker. Fungal Div. 21: 141-155.

86. Polashock, J.J. and Kramer, M. 2006. Resistance of blueberry cultivars to Botryosphaeria stem blight and Phomopsis twig blight. HortScience 41(6): 1457-1461.

87. Pusey, P.L. 1989. Influence of water stress on susceptibility of nonwounded peach bark to *Botryoshaeria dothidea*. Plant Dis. 73: 1000-1003.

88. Pusey, P.L. and Bertrand, P.F. 1993. Seasonal infection of nonwounded peach bark by *Botryosphearia dothidea*. Phytopatholoogy 83: 825-829.

89. Ramos L.J., Davenport T.L., McMillan R.T., Jr., and Lara, S.P. 1997. The resistance of mango (*Mangifera indica*) cultivar to tip dieback disease in Florida. Plant Dis. 81: 509-514.

90. Ramos, L.J., Lara, S.P., McMillan, R.T., Jr., and Narayana, K.R. 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. Plant Dis. 75: 325-318.

91. Rayachhetry, M.B., Blakeslee, G.M., and Miller, T. 1996. Histopathology of *Botryosphaeria ribis* in *Melealeuca quinquenervia*: pathogen invasion and host response. Int. J. Plant Sci. 157(2): 219-227.

92. Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W.V., Maki, C.S., Araugo, W.L., dos Santos, D.R., and Azevedo, J.L. 2005. Diversity of endophytic fungal community of cacao (*Theobroma cacao*) and biological control of *Crimipellis perniciosa*, causal agent of witches' broom disease. Int. J. Biol. Sci. 1: 24-33

93. Schabel, G., Chai, W., and Cox, K.R. 2006. Identifying and characterizing summer disease on 'Babygold' peach. Plant Health Prog. Doi:10.1094

94. Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Spatafora, J.W., and Crous, P.W. 2006. A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. Mycologia 98(6): 1041-1052.

95. Schoeneweiss, D.F. 1981. The role of environmental stress in diseases of woody plants. Plant Dis. 65: 308-314.

96. Slippers, B., Crous, P.W., Denman, S., Countinho, T.A., Wingfield, B.D., and Wingfield, M.J. 2004. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96: 83-101.

97. Slippers, B., Johnson, G.I., and Cous, P.W. 2005. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. Mycologia 97: 99-110.

98. Slippers, B., Smit, W.A., Crous, P.W., Countinho, T.A., Wingfield, B.D., and Wingfield, M.J. 2007. Taxonomy, phylogeny and identification of *Botryosphaeriaceae* associated with pome and stone fruit trees in South Africa and other regions of the world. Plant Pathol. 56: 128-139.

99. Smith, B. 2004. Susceptibility of southern highbush blueberry cultivar to *Botryosphaeria* Stem Blight. Small Fruits Rev. 3 No. 1/2: 193-201.

100. Smith, C.O. 1934. Inoculations showing the wide host range of *Botryosphaeria ribis*. J. Agric. Res. 49: 467-476.

101. Smith, D.R. and Stanosz, G.R. 2000. Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. Mycologia 93: 505-515.

102. Smith, V.F., Parker, D.M., Köller, W. 1991. Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole, Baseline sensitivity and implication for resistance monitoring. Phytopathology 81: 392-396.

103. Spatafora, J.W. 1995. Ascomal evolution of filamentous ascomycetes: evidence from molecular data. Can. J. Bot. 73(Suppl. 1): S811-S815.

104. Strik, B.C. and Yarborough, D. 2005. Blueberry production trends in North America, 1992 to 2003, and predictions for growth. HortTechnology 15: 391-398.

105. Sutton, T.B. and Arauz, L.F. 1991. Influence of temperature and moisture on germination of ascospore and conidia of *Botryosphaeria dothidea*. Plant Dis. 75: 1156-1159.

106. Swart, L., Crous, P.W., Petrini, O. and Taylor, J.E. 2000. Fungal endophytes of *Proteaceae*, with particular emphasis on *Botryosphaeria proteae*. Mycoscience 41: 123-127.

107. Swaford D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). 4.0 Beta. Sunderland, Massachusetts: Sinauer Associates.

108. Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., and Higgins, D. The Clustal_X Windown Interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876-4882.

109. Úrbez-Torres, J.R., Leavitt, G.., Guerrero, J.C., Guevara, J., and Gubler, W.D. 2008. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agent of Bot canker disease of grapevines in Mexico. Plant Dis. 92: 519-529.

110. Úrbez-Toorres, J.R., Leavitt, G.M., Voegel, T.M., and Gubler, W.D. 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. Plant Dis. 90: 1490-1503.

111. Vander Kloet, S.P. and Lyrene, P.M. 1987. Self-incompatibility in diploid, tetraploid, and hexaploid *Vaccinium corymbosum.* Can. J. Bot. 65: 660-665.

112. van Niekerk JM, Crous PW, Groenewald JZ, Fourie PH, Halleen F. 2004. DNA phylogeny, morphology, and pathogenicity of *Botryosphaeria* species on grapevines. Mycologia 96: 781-798.

113. Weaver, D.J. 1978. Role of conidia of *Botryosphaeria dothidea* in the natural spread of peach tree gummosis. Phytopathology 69: 330-334.

114. Williamson, J.G. and Lyrene, P.M. 2004. The Florida blueberry industry: a decade of growth. Proceedings Florida State Hort. Soc. 117: 234-235.

115. Willamson, J.G., Lyrene, P.M., Weitt, T.D., and Ruppert, K.C. 2004. Alternative opportunities for small farms: blueberry production review. RF-AC008. UF/IFAS Extension EDIS.PUB.

116. Witcher, W. and Clayton, C.N. 1962. Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). Phytopathology 53: 705-712.

117. Wiehe, P.O. 1952. Life Cycle of *Botryosphaeria ribis* on *Aleurites Montana*. Phyopathology 42: 521-525.

118. Worral, J.J., Correll, J.C., and McCain, A.H. 1986. Pathogenicity and teleomorph-anamorph connection of *Botryosphaeria dothidea* on *Sequoiadendron giganteum* and *Sequoia sempervirens*. Plant Dis. 70: 757-759.

119. Zhou, S. and Stanosz, G.R. 2001. Primers for amplication of mt SSU rRNA, and a phylogentic study of *Botryosphaeria* and associated anamorphic fungi. Mycol. Res. 105(9): 1033-1044.

120. Zhou, S. and Stanosz, G.R. 2001. Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analysis of ITS and 5.8S rDNA sequences. Mycologia 93: 515-526.

121. Zehr, E.I., Luszcz, L.A., Olien, W.C., Newall, W.C., and Toler, J.E. 1999. Reduced sensitivity in *Monolinia fructicola* to propiconazole following prolonged exposure in each orchards. Plant Dis. 83: 913-916.

BIOGRAPHICAL SKETCH

Amanda Faith Watson received a Bachelor of Science in biological science from Clemson University, May 2006. While attending Clemson, Amanda participated in organizations such as the Clemson Wesley Foundation, Sigma Alpha, and Tiger Band. She worked for two summers at the Outdoor Lab in Clemson. Her undergraduate research project was under Dr. Steven Jeffers. There she fulfilled Koch's postulates on foliage blight of hostas caused by *Phytophthora nicotianae*. While working in lab, she also helped with *Phytophthora ramorum* screening, and the maintenance of Clemson's *Phytophthora* collection. Upon graduation Amanda went to the Plant Pathology Department at the University of Florida to complete her Master of Science degree. There she worked on the etiology of stem blight of southern highbush blueberries (SHB) caused by *Botryosphaeria,* and the quantification of resistance in SHB breeding stock under Dr. Phil Harmon. While completing her masters Amanda presented her work at the Florida Phytopathological Society meeting, and at the Florida Blueberry Growers Association annual meetings Fall 2007 and Spring 2008. She was also an editor of the Plant Pathology news letter, and vice president of the Plant Pathology graduate student association. Currently, Amanda plans to continue working on stem blight of blueberries under Dr. Phil Harmon.