

ETIOLOGY AND MANAGEMENT OF RECENT OUTBREAKS
OF PEPPER ANTHRACNOSE IN FLORIDA

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2008

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To my wife, Cheryl, who has stood by me throughout this experience with patience, admiration, consideration, and love,

And to my children, daughter Jordan and sons Caleb and Canaan, who continue to provide for me the energy and entertainment needed to enjoy each day,

And to my mother, who unexpectedly and tragically passed on during the writing of this dissertation, but has always given me the inspiration and determination to make things happen, and who continues to live on and be with me in spirit.

ACKNOWLEDGMENTS

I would like to thank Dr. Ken Pernezny and members of my supervisory committee for their guidance and support. Also, I give special thanks to Dr. Paul Kuhn for all of his encouragement, wisdom, advice, and direction. Without his support for this project, it would not have been done.

I thank Dr. Charlie Mellinger of Glades Crop Care, Jupiter, Florida, for providing anthracnose-infected peppers from Hendry Co. Thanks also go to Dr. David Langston, University of Georgia, Tifton, for providing isolate GA01 and useful discussions; and to Dr. Robert McGovern, University of Florida, for providing isolates MG01-MG03, all used in this study. I also thank Midway Farms in St. Lucie Co., Florida, for allowing the sampling of pepper fields for anthracnose-infected pepper fruit; and to Leslie Fuquay, Syngenta Crop Protection (Greensboro, NC), for statistical analysis and recommendations.

I give special thanks to Dr. Sally Miller and Melanie Lewis-Ivey, of The Ohio State University, for hosting my visit to Wooster, Ohio and allowing me to use their laboratory. In addition to providing help and assistance, their work on pepper anthracnose provided the foundation for which my project was built. I am indebted to them for their graciousness and hospitality, and proud to have had the privilege to work in their laboratory.

Lastly, I thank Syngenta Crop Protection, for allowing me the opportunity and providing the resource to support this endeavor.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ETIOLOGY AND MANAGEMENT OF RECENT OUTBREAKS
OF PEPPER ANTHRACNOSE IN FLORIDA

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May 2008

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Major: Plant Pathology

In the last 4 to 6 years, anthracnose has become an increasingly serious disease on immature, green pepper fruit in Florida. This contrasts with earlier reports of anthracnose as strictly a ripe-rot disease of mature, colored pepper fruit. The species of *Colletotrichum* associated with anthracnose on both immature and ripe pepper in Florida were identified. Based on reactions with PCR-specific primers, 28 of 50 isolates associated with anthracnose lesions from Florida were identified as *C. acutatum*, including 22 of 22 recovered from immature, green fruit. Six of the *C. acutatum* isolates were associated with typical lesions on ripe, colored fruit, but only in fields where lesions on green fruit were also observed. In contrast, all 17 isolates identified by PCR as *C. gloeosporioides* were recovered from lesions found only on ripe, colored fruit from fields where no lesions on green fruit were initially observed. No isolates were identified as *C. capsici* or *C. coccodes*. Isolates of *C. gloeosporioides* grew up to twice as fast *in vitro* as isolates of *C. acutatum*, suggesting a way to tentatively differentiate pepper isolates without PCR testing. In addition, *C. gloeosporioides* produced conidia that were slightly larger than those produced by *C. acutatum*.

In field and laboratory pathogenicity tests, anthracnose isolate HB05 recovered from bell pepper was not pathogenic on tomato or strawberry when artificially inoculated on ripe and unripe fruit in the field. However, anthracnose lesions did form on detached, wounded fruit of all three crops in the laboratory. This result suggests that laboratory wound-inoculation studies might not be a reliable method to determine the natural host range of *Colletotrichum* spp. on various crops in the field.

Three fungicide field trials were conducted on pepper ('Revolution') artificially inoculated with an isolate of *C. acutatum* recovered from pepper (HB05) to evaluate azoxystrobin (Quadris 250SC), famoxadone plus cymoxanil (Tanos 50WG), copper hydroxide (Kocide 2000), mancozeb (Manzate 75WG), acibenzolar-S-methyl (Actigard 50WG), and fludioxanil plus cyprodinil (Switch 50WG) for control of pepper anthracnose. In one of the three trials, difenoconazole (Inspire 250EC) was included. All treatments provided significant control of anthracnose symptoms on fruit in comparison to the untreated control. Overall, azoxystrobin, fludioxanil plus cyprodinil, difenoconazole, and mancozeb provided the highest amount of uninfected, healthy fruit per plot, while famoxadone plus cymoxanil, copper hydroxide, and acibenzolar-S-methyl provided the least amount of healthy fruit per plot among all of the treatments. The name "early anthracnose" is proposed for the disease on immature, green fruit caused by *C. acutatum*.

CHAPTER 1 INTRODUCTION TO PEPPER ANTHRACNOSE

Peppers (*Capsicum* sp.) are herbaceous plants with fruit that are cultivated and consumed worldwide. The fruit are eaten as a fresh vegetable or dehydrated for use as one of the largest and most important spice commodities in the world. Pepper fruit or its extracts are widely used in foods, sauces, medicines, and cosmetics, and will continue to be an important vegetable and spice crop in many regions of the world.

Capsicum species originated in the tropical Americas and are believed to have been consumed by humans since about 7500 BC (MacNiesh, 1964). The plants are thought to be among the oldest cultivated crops in the Americas, with Native Americans reportedly growing and harvesting peppers between 5200 and 3400 BC (Heiser, 1976). Christopher Columbus is credited with bringing peppers to Europe on his return trip from the New World, after reportedly naming the fruit ‘red pepper’, due to its similarity in texture and taste to the unrelated black pepper, *Piper nigrum* (Bosland, 1996). *Capsicum* spread rather quickly throughout Europe and into Asia, becoming an increasingly important and prized spice for many civilizations. In the United States, pepper continues to be an important vegetable crop, not only for use as a spice but as an important component of the fresh vegetable market. During 2004, bell pepper was grown on over 23,000 hectares in the U.S. with a current market value of nearly \$600 million. Florida, which ranks 2rd to California among U.S. pepper-producing states, harvested over 7,400 hectares in 2004 with a current market value of over \$218 million (USDA annual agricultural statistics – www.usda.gov/nass).

The genus *Capsicum* belongs to the Solanaceae family, along with eggplant, petunia, potato, tobacco, and tomato, and currently consists of about 25 species. The most economically important domesticated species, *C. annum*, is the species to which most commercial cultivars

belong. The center of genetic diversity of this species is in Mexico, with a secondary center in Mesoamerica (Bosland, 1996). Four other domesticated species exist: *C. baccatum*, *C. pubescens*, domesticated in the Peruvian Andes, and *C. frutescens*, and *C. chinense*, domesticated in the Amazon Basin and Central America, respectively. *Colletotrichum frutescens* and *C. chinense*, are better known as tabasco and habanero pepper, respectively. The remaining wild species are rarely utilized by man, but are likely to contain a valuable reservoir of genes that could be used to produce plants with enhanced genetic traits such as increased yield or disease resistance.

In Florida, peppers are an important commercial vegetable crop. They are grown throughout Florida, and are generally rotated with tomatoes or cucurbits. Like tomatoes, peppers are grown using the plastic mulch system with either drip or seep irrigation and in many cases are also staked and tied with string. Typically, pepper cultivation occurs twice a year in Florida, in both fall and spring seasons. Actual planting and harvesting dates depend on the specific location within Florida. The further south the location, the less likely for a frost to occur, and therefore the earlier they can be planted in the spring. Further north in the state, peppers are planted earlier in the fall and later in the spring. According to the USDA report '2002-2003 Acreage, Yield, Production and Value of Florida Vegetables (www.nass.usda.gov/fl/rtoc0v.htm), bell peppers accounted for 1,820 harvested hectares in the fall season and 5,170 hectares in the spring, with a total of 7,160 hectares harvested (out of 7,200 planted). The yields per hectare were 4,667 kg in the fall and 5,268 in the spring for an average of 4,968 kg per hectare. A total of 222 million kg were sold with a total annual value of nearly \$200 million. The 10-year statewide average yield for bell peppers as of 2003 is 5,400 kg per hectare (Maynard et al., 2003). This amount exceeds the reported value of cabbage, cucumbers, potatoes, snap beans, or

squash during the 2002-2003 vegetable season in Florida. Tomato was the only vegetable crop with a higher monetary value (\$546,699,000). This report does not include yields for jalapeno, habanero, or other specialty peppers, which are also grown in Florida, particularly in South Florida's Miami-Dade and Palm Beach Counties. Cultivars of these specialty peppers include: 'Aruba', 'Key West', and 'Key Largo' (Cubanelle-type peppers); and 'Habanero', 'Milta jalapeno', 'Xatapa jalapeno', 'Grande jalapeno', 'Hungariane', 'Hot Wax', 'Messilla', 'Long Thin Red Cayenne', and 'Large Red Thick Cayenne' as specialty hot peppers (Li et al., 2000). In recent years, bell peppers have not been grown on a significant scale in Miami-Dade Co.

As with most vegetable crops in Florida, peppers are susceptible to various diseases, pests, and disorders that can affect fruit quality and reduce yields. Some of the traditionally important diseases of pepper in Florida include bacterial spot, frog-eye leaf spot, Phytophthora blight, powdery mildew, and various viral diseases (Pernezny, et al., 2003). In some cases, cultivars of pepper have been developed with resistance to bacterial spot, Phytophthora blight, as well as some poty virus diseases (Bosland, 1996). Many of these diseases are controlled with various fungicides, such as maneb and copper for bacterial spot, mefenoxam, metalaxyl, and dimethomorph for Phytophthora blight, or azoxystrobin for powdery mildew (Maynard et al., 2003). All of these diseases can have severe consequences in terms of fruit quality and yield if not controlled by chemical or other means, such as crop rotation or soil fumigation.

Another increasingly important disease of pepper in Florida is pepper anthracnose, caused by *Colletotrichum* spp. (Roberts et al., 1998). This disease has been reported in Florida on various cultivars of *C. annuum* as well as an earlier report on *C. chinense*, or Jamaican Scotch bonnet pepper, in which incidence varied from 25 to 50% (McGovern and Polston, 1995). At least four different species of *Colletotrichum* have been reported in the U.S. to cause anthracnose

of pepper: *C. acutatum*, *C. capsici*, *C. coccodes*, and *C. gloeosporioides* (Hadden, 1989; Marvel et al., 2003; Roy et al., 1997). However, only three species have been reported to cause this disease in Florida, *C. capsici*, *C. coccodes*, and *C. gloeosporioides* (McGovern and Polston, 1995; Roberts et al., 1998). At the present time, *C. acutatum* has not been reported on pepper in Florida. Many of these species are likely pathogens or saprophytes of other important agricultural crops or weed species in Florida. For example, *C. gloeosporioides* has been reported to attack 470 host genera worldwide (Sutton, 1980).

The earliest report of anthracnose disease of pepper was by B. D. Halsted in 1891 (Halsted, 1891) where the causal agents were identified, perhaps mistakenly, as *Gloeosporium piperatum* and *Colletotrichum nigrum*. Since that time, much debate has revolved around the identification of species of *Colletotrichum* causing anthracnose on pepper and the potential host range of these species (Alexander and Pernezny, 2003; Hadden, 1989; Manandhar et al., 1995a; Roberts et al., 2001). Some of these species are likely to have wide host ranges among plants in the vicinity of pepper fields, and this information could be helpful in determining proper cultural techniques to reduce the incidence and severity of this disease in pepper.

Although anthracnose of pepper is becoming seemingly more prevalent and serious on pepper in the U.S., in parts of Asia it is considered the most important disease of pepper (Hong and Hwang, 1998; Jetiyanon et al., 2003; Manandhar et al., 1995b; Manandhar et al., 1995c; Qing et al., 2002). Therefore, much of the recent research regarding pepper anthracnose has been conducted in Korea, Thailand, and other Asian countries. In the U.S., reports of anthracnose have occurred in Louisiana (Hadden and Black, 1988), Mississippi (Roy et al., 1997); Virginia (Marvel et al., 2003); Georgia (David Langston, personal communication), Ohio (Lewis-Ivey et al., 2004), and Florida (McGovern and Polston, 1995, Roberts et al., 2001).

However, the disease is likely to occur in other southern states where warm temperatures and high humidity are prevalent, conditions that are reported to be conducive for disease development (Hong and Hwang, 1998; Kwon and Lee, 2002; Qing et al., 2002). Considering the disease is seed-borne (Grover and Bansal, 1970; Manandhar et al., 1995; Sangchote and Juangbhanich, 1984), over-winters in infected plant debris (Hadden, 1989; Kwon and Lee, 2002; Smith and Crossan, 1958), and the wide host-range of the fungal species involved (Bailey et al., 1992; Freeman et al., 2001; Hadden, 1989; Horowitz et al., 2002; Prusky and Plumbley, 1992), it seems likely that pepper anthracnose will only continue to increase in importance in the southeastern United States, including Florida.

In Asia, much effort has been conducted to evaluate whether ripe or unripe fruit are more likely to be the site of initial infection. In Florida, the disease has traditionally been associated with ripened fruit (Roberts et al., 2001, Ken Pernezny, personal communication), but in Georgia and Ohio, lesions on ripe and unripe (green) fruit have been reported (Lewis-Ivey et al., 2004; David Langston, personal communication). More recently, the disease on unripe fruit in Florida has been observed (Fig. 1-1). Interestingly, much work has been done that demonstrates ‘incompatible’ reactions of *C. gloeosporioides* infection on un-wounded ripened fruit, and ‘compatible’ reactions on un-wounded green or unripe fruit (Kim et al., 1999; Kim et al., 2001, Manandhar et al., 1995a; Manandhar et al., 1995b; Manandhar et al., 1995c; Oh et al., 1999a; Oh et al., 1999b). These compatible or incompatible interactions are based on the ability or inability of the fungus to cause disease when inoculated on the surface of either ripe or unripe fruit. Furthermore, inoculation with conidia after wounding the fruit does not differentiate infection between ripe or unripe fruit, as both stages of fruit are equally prone to infection using this method (Kim et al., 1999).

More recently, Kim et al., (2001) reported that a pepper esterase gene, designated *PepEST*, was highly expressed in ripe fruit, but not in unripe fruit. This gene has been cloned and demonstrated to prevent appressorium formation when the *PepEST* protein was amended with conidia of *C. gloeosporioides* and inoculated on compatible unripe fruit. Although the *PepEST* protein is reported to have no fungicidal activity, it inhibits appressorium formation in a dose-dependent manner (Kim et al., 2001). It has been proposed in this study that the recombinant *PepEST* protein affects one or more signal transduction pathways involved in appressorium formation, based on other reported experimental results with the rice blast fungus, *Magnaporthe grisea*. Another gene, designated *PepTLP* for ‘pepper thaumatin-like protein’, was isolated and found to be expressed in the ripe fruit but not unripe fruit upon fungal infection, leading to higher levels of *PepTLP* mRNA and *PepTLP* protein in the infected ripe fruit (Kim et al., 2002). The authors suggested that ripe pepper fruit are protected because of the presence of the *PepTLP* protein in the intercellular spaces of ripe fruit and the subsequent absence of fungal colonization. These studies add support to the fact that the fungus is more virulent on un-wounded unripe green fruit than on un-wounded ripe fruit. This concept is particularly interesting, due to the fact that in Florida most lesions are found on ripened fruit, which are thought to be more susceptible (Alexander and Pernezny, 2003; Roberts et al., 2001). It is increasingly clear from these reports that infections from *C. gloeosporioides* are likely occurring on the fruit prior to ripening, although visible lesions and evidence of disease may only occur after ripening.

Other work with *Colletotrichum* infection on other hosts, particularly on tropical fruits, has demonstrated the fungus can undergo a period of fairly long latency after initial infection. In these reports, the growth of the fungus is contained only within the epidermal layer until after fruit ripening, at which point the pathogen is then able to invade host cells and cause disease

(Bailey et al., 1992). *Colletotrichum gloeosporioides* on avocado fruit underwent appressorium formation and penetration into unripe avocado fruit, and the subsequent hypha appeared to rest beneath the cuticle until the fruit began to ripen (Prusky and Plumbley, 1992). At that point, the hyphae go on to invade the cell walls, causing cell death and forming the characteristic anthracnose lesions. In another study of *C. capsici* and *Glomerella cingulata* on pepper fruit, infection remained quiescent on immature fruits and only developed after the fruits became ripe (Adikaram et al., 1983), even though appressoria did germinate to form penetration pegs and penetrate the host surface within 65 hours after inoculation. In other studies, some of the appressoria of *C. musae* on banana adhered to the fruit surface, but remained quiescent and ungerminated, while other appressoria produced penetrating hyphae on the unripe fruits. The initially quiescent appressoria eventually did germinate, but resulted in lesions only after the fruit began to ripen (Muirhead and Deverall, 1981). Prusky and Plumbley (1992) provided many such examples of quiescence during *Colletotrichum* infection on various fruits such as avocado, banana, mango, and pepper. In this summary, the authors quote Verhoeff (1974) and Swinburne (1983) in defining a quiescent infection as ‘a quiescent or dormant parasitic relationship which, after a time, changes to an active one’. Some degree of quiescence or latency likely plays a role in *C. gloeosporioides* infection of pepper, and could be used to explain observations in Florida of lesion development only on ripened red pepper fruit by this particular species.

The ability of the pepper anthracnose fungus to infect unripe green pepper fruit and not ripened red fruit could provide valuable insight into strategies for management of this disease. In Florida, it is currently not understood at what stage of development the fungus invades the pepper fruit to create the lesions that form on ripened fruit. Based on the work by Kim et al., (2001) and others, it is apparent that the fungus is initiating infection on green fruit. This

knowledge, along with further research, could provide insights that could ultimately lead to major changes to our approach to management of this disease on pepper in Florida.

Another factor of potentially great importance for this disease in Florida is the source of inoculum. As previously mentioned, *C. gloeosporioides* has a very wide host range including weed species as well as many commercially cultivated crops in Florida, such as mango and strawberry. Anthracnose is currently considered to be the most important fungal disease of mango in Florida (Ken Pernezny, personal communication), and in some areas of south Florida, mango is grown in close proximity to pepper fields. Strawberry could also be a source or reservoir of inoculum. *Colletotrichum gloeosporioides* causes crown rot of strawberry and is believed to spread to commercial strawberry fields in Florida from local weed species (Legard, 2000). Certainly, in addition to agricultural crops, the possibility of alternate weed hosts can not be ruled out as a source of inoculum for peppers. The identification of such alternate hosts is complicated by the fact that the fungus may survive on weed or crop hosts without detectable disease symptoms. *Colletotrichum acutatum* recovered from strawberry was shown to survive on inoculated pepper, eggplant, and tomato as mycelia and appressoria that failed to germinate resulting in epiphytic growth without invasion of host cells (Horowitz et al., 2002). If penetration of the plant did occur, it was only after several days and was restricted to the intercellular areas of the first cell layer and did not necessarily cause any visible damage to the plant tissue. Freeman et al., (2001) recovered *C. acutatum* from healthy looking asymptomatic plants of the weed genera *Vicia* and *Conyza* and found they were highly pathogenic on strawberry. Both *C. acutatum* and *C. gloeosporioides* are important pathogens of pepper and strawberry. It is certainly quite possible that many potential ‘external’ sources of *C. gloeosporioides* inoculum occur that might initiate the disease in peppers.

In addition to the identity of an external inoculum source, such as weed species or other crops, little is known regarding ‘internal’ inoculum sources of the disease on pepper plants. Although it is already known the disease is seed-borne in pepper and can spread to healthy plants via infected crop debris, it is not currently well understood at what growth stage in peppers that *Colletotrichum* becomes established. In lychee (*Litchi chinensis*), a fruit tree crop grown in south Florida, anthracnose caused by *C. gloeosporioides* is considered the most important and destructive disease. It was recently shown by Davis (2003) that greater numbers of conidia were consistently detected on inflorescence tissues than on leaves. Later in the season, mature fruits that were picked and placed in a moist chamber developed lesions from which *C. gloeosporioides* was isolated. This confirms that the presence and accumulation of inoculum in the flowers likely contributes to the disease on fruit later in the season. This clearly could have implications on spray timing and other control measures. In peppers, it is not known whether inoculum in the flowers could contribute to disease development in the subsequent fruits, but such knowledge may be critical for implementation of successful and efficient control measures.

Lastly, knowledge of the specific species of *Colletotrichum* responsible for the disease is also of critical importance. Although *C. gloeosporioides* has been documented as a pathogen of pepper in Florida, *C. acutatum* has not been reported and may differ in host range, survival, and other epidemiological characteristics. In Ohio, *C. acutatum* is considered a more aggressive pathogen of pepper than other *Colletotrichum* species and is capable of causing yield losses up to 90% (Lewis-Ivey et al., 2004). In addition, it has been proposed that this species does not differentiate from ripe or unripe fruit and can cause lesions on both (Sally Miller, personal communication). If *C. actuatum* can be isolated from unripe pepper in Florida, this certainly would have significant implications on the understanding and management of this disease.

Therefore, determining the etiology of this disease is crucial to providing knowledge and implementing control measures for pepper anthracnose.

In this study, over fifty isolates of *Colletotrichum sp.* were recovered from infected fruit collected from various pepper-growing regions throughout southern Florida. In two of the fields that were sampled, anthracnose lesions were only observed on ripened fruit. In these locations, the peppers were not harvested and therefore allowed to ripen, and an abundance of lesions were detected on nearly every fruit throughout the entire field. In the remaining locations, lesions were found on green, unripe fruit from younger fields that were intended to be harvested. In these fields, anthracnose lesions were found only in specific areas, or loci, and were not predominant throughout the field. Using PCR and species-specific primers for both *C. gloeosporioides* and *C. acutatum*, the conserved ITS regions of DNA were identified in most isolates as either one or the other of these two species. The isolates recovered from the fields containing only ripened fruit were identified as *C. gloeosporioides*, while those recovered from green fruit were identified as *C. acutatum*. This was the first report of *C. acutatum* recovered from pepper in Florida, and this discovery highlights what could be a significant threat to the pepper industry in Florida and will most likely require enhanced management strategies that include the application of pesticides.

In addition, increased knowledge of the epidemiology of *C. acutatum* isolates recovered from pepper, such as their host range on other crops grown adjacent to peppers, would aid in the understanding and prevention of disease epidemics in pepper and other important crops in Florida, such as strawberry and tomato, both of which are host to more than one species of *Colletotrichum* (Freeman et al., 2001; Lewis-Ivey et al., 2004; Prusky and Plumbley, 1992). In this study, an isolate from pepper was used to inoculate field-grown strawberries, tomatoes and

peppers to determine if this species of *Colletotrichum* on pepper could spread to nearby strawberry or tomato fields and create further disease epidemics. In addition, since no data have been generated to evaluate certain fungicides for control of *C. acutatum* on pepper in Florida, various fungicides were evaluated in field trials inoculated with an isolate of *C. acutatum* recovered from pepper in Florida. Results show that the isolates of *C. acutatum* recovered from pepper are not pathogenic on either strawberry or tomato, and that several fungicides do provide good to outstanding control of this disease, even under heavy disease pressure. The results from this study are likely to aid in the understanding and management of pepper anthracnose in Florida and provide further insight into this potentially devastating disease. Hopefully, this discovery of *C. acutatum* as a pathogen of pepper in Florida will spark further research that will undoubtedly continue to enhance our understanding of this pathogen and provide management options needed to control this disease in the future.



Figure 1-1. Pepper anthracnose lesions on green, unripe bell pepper fruit recovered from Palm Beach Co., Florida in 2004. Traditionally, the disease was observed only on fully-sized ripened, colored fruit (usually red); however, the occurrence of anthracnose symptoms on developing green, unripe fruit was becoming increasingly common since the late 1990's in Florida pepper fields.

CHAPTER 2 ETIOLOGY OF RECENT OUTBREAKS OF PEPPER ANTHRACNOSE IN FLORIDA

Introduction

Florida is second only to California in production of peppers (*Capsicum annuum* L.) in the United States. Most acreage is planted to sweet bell pepper with the bulk of the production during the winter months (September to May) in the southeastern and southwestern areas of the state (Maynard et al., 2003). In the 2003 – 2004 season, 223,605, 454 kg of pepper were harvested from more than 6,880 hectares with a total annual value of \$175, 654, 000, second only to tomatoes in farm-gate value in the state.

Anthracnose has emerged as an increasingly significant disease of pepper in Florida in recent years (Roberts et al., 2001). It has been observed on both sweet bell peppers and specialty peppers, such as cubanelle, jalapeno, and scotch bonnet (*C. chinense*) (McGovern and Polston, 1995). The disease is characterized by sunken, necrotic lesions on the surface of pepper fruit and usually contain an abundance of tan or salmon-colored conidia. Traditionally, the disease in Florida has been primarily associated with ripened fruit that have already turned from green to the ripened color of the cultivar (usually red). Therefore, the causal agents were generally thought of as mostly “ripe-rot” pathogens (Alexander and Pernezny, 2003; Roberts et al., 2001). On immature, unripe green fruit, typical anthracnose symptoms were generally not observed, and therefore the disease was not considered a significant problem on bell peppers harvested as mature green fruit (by far the bulk of the harvested acreage in Florida). Within the last few years, however, the disease has been observed on immature green pepper fruit grown in Florida. A similar outbreak of anthracnose on immature, green fruit has occurred in Ohio (Lewis-Ivey et al., 2004). The causal agent of the Ohio epidemic was identified as *Colletotrichum acutatum* (Simmonds). Although at least four different species of *Colletotrichum* have been reported in

the U.S. to cause anthracnose of pepper, *C. gloeosporioides* (Penz.), *C. capsici*, *C. coccodes*, and *C. acutatum* (Alexander and Pernezny, 2003; Haden, 1989; Haden and Black, 1998; Lewis-Ivey et al., 2004; Marvel et al., 2003; Roy, 1996), *C. acutatum* has never been identified as a pepper pathogen in Florida (McGovern and Polston, 1995; Roberts et al., 2001).

In Ohio, *C. acutatum* was reported as more aggressive than other *Colletotrichum* species known to infect pepper, capable of causing losses in marketable yield of up to 100% (Lewis-Ivey et al., 2004). Probably of more significance, *C. acutatum* will attack both ripe, colored fruit and immature, green fruit, unlike other species that are strictly ripe-rot pathogens. Since the epidemics of anthracnose on unripe, immature green fruit in Florida have occurred at roughly the same time as those in Ohio, it is possible that *C. acutatum* is responsible for the disease in Florida as well.

Various methods previously have been described to determine the species of *Colletotrichum* causing pepper anthracnose, including both molecular (Kim et al., 2002; Lewis-Ivey et al., 2004) and morphological (Haden, 1989; Kim et al., 1999; Lewis-Ivey et al., 2004) techniques. Species-specific primers based on the rDNA internal transcribed spacer (ITS) regions of different species have been used to differentiate *C. gloeosporioides* and *C. acutatum* (Brown et al., 1996; Freeman et al., 2000; Lewis-Ivey et al., 2004; Mills et al., 1992; Sreenivasaprasad et al., 1996). Molecular methods generally have been preferred over morphological methods (Lewis-Ivey et al., 2004; Marvel et al., 2003), because the morphology between species often are quite similar and a certain degree of morphological variation is considered acceptable within a species of *Colletotrichum* (Sutton, 1992). Colony growth rate on artificial media in growth chambers has been used to differentiate between *C. acutatum* and *C. gloeosporioides* recovered from pepper (Haden, 1989). The other species reported to occur on

pepper, *C. coccodes* and *C. capsici*, are easily distinguished due to the production of abundant sclerotia in culture by *C. coccodes*, and by the distinct falcate or ‘curved’ shape of conidia produced by *C. capsici* (Bailey et al., 1992; Haden, 1989; Roy, 1996; Sutton, 1992).

The purpose of this study was (i) to isolate and identify the species of *Colletotrichum* causing pepper anthracnose in Florida from both ripened, colored fruit and immature, green fruit, and (ii) identify morphological characteristics useful for differentiation of isolates identified to species by PCR-DNA analysis.

Materials and Methods

Isolates

Fifty isolates were recovered from both ripe, colored and immature, green symptomatic pepper fruit from various commercial farms throughout Florida. An additional isolate from a diseased green bell pepper in 2005 from southern Georgia was provided by D. Langston (Table 2-1). Forty-seven of the Florida isolates were recovered from infected pepper fruit during the 2004 – 2005 vegetable season, and three isolates from pepper were originally recovered in the mid 1990’s by R. McGovern. Two isolates, Ca Mil-1 (*C. acutatum*) and GD (*C. gloeosporioides*) (Lewis-Ivey et al., 2004), were used as reference isolates in the PCR studies. The 50 isolates from Florida were collected from three different pepper-growing areas: Indian River and St. Lucie Co. in east-central Florida, Palm Beach Co. in southeast Florida, and Collier and Hendry Co. in southwest Florida (Table 2-1). Fungi were isolated by rinsing symptomatic fruit with de-ionized water and placing in a closed plastic container containing a moist paper towel (ca. 100% humidity) for 24 h. Conidia from lesions on the surface of the fruit were removed with a sterile loop, which was then streaked onto the surface of 10-cm-diameter Petri plates containing water agar (15 grams agar per 1000 mL distilled water), and allowed to grow for 12 to 18 h. Up to four germinating, single-spores per isolation were identified under a dissection microscope (40×),

removed with a sterile needle, and transferred to a Petri dish containing 25 mL of potato dextrose agar (PDA) and allowed to grow at 30°C for 7 d. One isolate per lesion was selected for storage and further study. In some cases, two isolates were obtained from different lesions on the same fruit. Isolates were transferred to PDA plates containing small (approximately 5-mm²) pre-cut sterilized pieces of filter paper (Whatman #4) placed directly on the surface, and incubated at 20°C for 14 d with continuous light. The individual pieces of colonized filter paper were then removed from the surface of the agar using sterile forceps, allowed to dry in empty Petri-dishes for 14 d, and placed in vials for long-term storage at -4°C. Isolates were recovered as needed by transferring filter paper units to PDA and incubating plates a minimum of 3 d at 20°C.

PCR Amplification

Polymerase chain reaction (PCR) amplification was used to putatively identify isolates as species of *C. acutatum* or *C. gloeosporioides* using species-specific primers as previously described (Lewis-Ivey et al., 2004; Mills et al., 1992; Sreenivasaprasad et al., 1996). The species-specific primers for *C. gloeosporioides* (CgInt; 5'-GGCCTCCCGCCTCCGGGCGG-3') (Mills et al., 1992) and for *C. acutatum* (CaInt2; 5'-GGGGAAGCCTCTCGCGG -3') (Sreenivasaprasad et al., 1996) from the ITS 1 region of the rDNA were used in combination with the conserved primer ITS 4. Before conducting PCR, the DNA of each isolate was extracted according to the protocol previously described (Lee and Taylor, 1990) and modified by Lewis-Ivey et al., (2004). Each 25 µL reaction mixture contained: 2.5 µL of extracted DNA (50 ng/µL), 0.125 of each 10 µM primer, 0.08 µl 10 mM dNTP, 0.5 µL Taq Polymerase (5 U/µL), 1.5 µL of 25 mM MgCl₂, 2.5 µL 10X polymerase buffer, and 16.9 µL sterile de-ionized water. The PCR was performed with a MJR PTC-100 thermocycler (MJ Research Inc., Waltham, MA) using the following temperature-cycle program: 5 min at 94°C, 30 cycles of 1.5 min at 94°C, 2 min at 55°C, and 3 min at 72°C, followed by a 10 min final extension at 72°C. The PCR

products (7 μ L) were mixed with 3 μ L of loading dye (5 mg bromphenol blue, 5 mL 5X TBE, 2g sucrose) and separated by horizontal gel electrophoresis in 1.5% agarose in 0.5X TBE buffer at 110 V for 150 min. Gels were then stained in dilute ethidium bromide (2 μ g/mL), visualized under UV light, and photographed using the Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 (Eastman Kodak Company, New Haven, CT). The PCR procedure was conducted three times for each isolate.

Growth Rate *in vitro*

Radial growth rate (mm) was determined for each isolate. Isolates were grown on PDA for 3 to 5 d and were transferred to each of three replicate PDA Petri-dishes using plugs made with a sterile #3 cork-borer. Plates were placed into a growth chamber (Enviro chamber, Detroit, MI) at 30°C in continuous darkness, and arranged within the growth chamber in a completely randomized design. At 5 d, the radius of each colony was measured and recorded. Mean growth rates were calculated for all isolates and were compared statistically using ANOVA ($P < 0.05$). The experiment was repeated once.

Conidial Measurements

Isolates VB07, VB09, MF05, MF08, MG01, HB01, HJ01, HC02, and GA01 were grown on PDA for 5 d under continuous fluorescent light at 25°C to promote sporulation. Conidia were suspended in sterile water using a sterile loop and mounted on a microscope slide. Length and width were measured for 25 conidia per isolate using an ocular scale at 700 \times magnification (10 \times ocular, 70 \times objective) using bright field microscopy (Leitz, Germany). The length and width were compared statistically using a one-sided t-test between the two species.

Results

PCR Amplification with Species-Specific Primers

Twenty-eight of the 50 pepper isolates from Florida were identified as *C. acutatum*, using the species-specific primer CaInt2 in conjunction with the ITS4 primer for *C. acutatum*. Seventeen isolates were identified as *C. gloeosporioides*, using the CgInt and ITS4 primers specific for *C. gloeosporioides*. The *C. gloeosporioides* isolates were recovered exclusively from ripe, colored pepper fruit in St. Lucie and Indian River Co. in 2004–2005, and in the mid 1990's in Collier Co. The *C. acutatum* isolates were recovered in 2004–2005 from anthracnose lesions on green, immature fruit, or on ripe, colored fruit in close proximity in the same fields. PCR reactions of several representative isolates are shown (Fig. 2-1) and compared with the previously published reference isolates, Ca Mil-1 (*C. acutatum*) and GD (*C. gloeosporioides*) (Lewis-Ivey et al., 2004). Five pepper isolates (VB01, VB03, VB04, VB05, and VB06) did not produce a PCR product with either primer mixture. The isolate from immature, green fruit in southern Georgia (GA01), also was identified as *C. acutatum* (Fig. 2-1).

Colony Growth Rate

In two separate tests, isolates identified by PCR as *C. gloeosporioides* grew significantly faster ($P < 0.0001$) than those identified as *C. acutatum* (Figs. 2-2 and 2-3). The 17 isolates of *C. gloeosporioides* grew an average of 5.91 mm/day in Test 1 and 5.93 mm/day in Test 2, while the 28 isolates of *C. acutatum* grew an average of 2.96 mm/day and 3.54 mm/day in Test 1 and Test 2, respectively. The five isolates that did not produce an identifiable PCR product (VB01, VB03, VB04, VB05, and VB06), grew at a mean rate of 5.85 mm/day (data not shown), consistent with that of the growth rate for isolates of *C. gloeosporioides*. The isolate identified by PCR as *C. acutatum* from green pepper fruit in Georgia (GA01) grew at a mean rate of 3.37 mm/day (data not shown), consistent with the known growth rate of isolates of *C. acutatum*.

Conidial Measurements

Conidial length and width were measured for five isolates previously identified by PCR as *C. gloeosporioides*, as well as four isolates of *C. acutatum*. The isolates designated as *C. gloeosporioides* had an average conidial size of $17.96 \times 6.37 \mu\text{m}$ (standard error = 0.146×0.041), whereas isolates designated as *C. acutatum* had an average conidial size of $16.79 \times 4.49 \mu\text{m}$ (standard error = 0.172×0.049). The length and width were statistically analyzed using a one-way ANOVA t-test, and both were significantly larger for *C. gloeosporioides* ($P < 0.0004$ and $P < 0.0001$, respectively).

Discussion

Colletotrichum acutatum has been identified as the causal agent of the recent epidemics of anthracnose on immature, green pepper fruit in Florida. This conclusion is based primarily on reaction of DNA from all isolates from lesions on immature, green fruit with PCR-specific primers for *C. acutatum*. This is the first report of an extensive collection of *Colletotrichum* isolates in the United States from immature pepper anthracnose lesions that definitively identify the pathogen as *C. acutatum*. All isolates identified by PCR as *C. gloeosporioides* were recovered from ripe, colored fruit, never from immature, green fruit. A few *C. acutatum* isolates were from mature, colored fruit, indicating that *C. acutatum* can attack pepper fruit during all stages of maturity. *Colletotrichum gloeosporioides*, on the other hand, seems to be strictly a ripe-rot pathogen on pepper. These results parallel those reported recently based on two isolates in Ohio (Lewis-Ivey et al., 2004). Pepper can now be added to the list of hosts for *C. acutatum* in Florida (Brown et al., 1996; Lahey et al., 2004; Legard, 2000; Peres et al., 2005; Timmer and Brown, 2000) and other locations (Adaskaveg and Förster, 2000; Bailey et al., 1992; Correll et al., 2000; Freeman, 2000; Freeman et al., 1998; Freeman et al., 2001; Peres et al., 2002). Although only one isolate from Georgia was included in our study, it seems likely that

anthracnose on immature, green peppers in Georgia is also caused by *C. acutatum*. More isolates from Georgia need to be recovered and identified to confirm this contention.

Growth rates in culture were clearly different for *C. acutatum* and *C. gloeosporioides*. Isolates of *C. gloeosporioides* grew 50 to 200 % faster than those of *C. acutatum*. These observations serve as the basis for a suggestion that colony growth rate under very specific conditions (30°C in complete darkness on PDA plates) can be used to tentatively separate these two species. Other researchers (Brown et al., 1996; Haden, 1989; Kim et al., 1986; Marvel et al., 2003; Sutton, 1992) have also suggested that colony growth rate of isolates can be of taxonomic significance. Tolerance to benomyl (Adaskaveg and Hartin, 1997; Bernstein et al., 1995; Freeman et al., 1998; Peres et al., 2004) also tends to vary between these two species. The difference in conidial size between the two species was less distinct than growth rate differences in our studies. However, conidia of *C. acutatum* were significantly smaller than conidia of *C. gloeosporioides* in both length and width. Differentiation between these two species on conidial size alone could prove difficult, due to size variation within an isolate and the similarity of spore shape between the two species. However, for laboratories without access to many modern molecular techniques, colony growth rates, conidial size, and other phenotypic characteristics may be very important for initial identification of fungal isolates.

On a particular host, *Colletotrichum* species may exist as a hemi-biotroph or necrotroph using the terminology of Bailey et al., (1992) and O'Connell et al., (2000), and more recently Dieguez-Uribeondo et al., (2005). When *C. gloeosporioides* attacks pepper fruit, its lifestyle is probably that of a hemi-biotroph. Most likely, it initially colonizes the space directly below the cuticle. Only as the fruit ripens, does it produce enzymes that kill tissue and allow for development of the typical sunken lesions characteristic of anthracnose. When immature, green

bell pepper fruit were inoculated with *C. gloeosporioides* in field plots, lesions did not form until 45 d later when the pepper fruit ripened and turned red (Harp, unpublished).

Colletotrichum acutatum, in contrast, seems to establish as a necrotroph soon after colonization of immature, green fruit, producing symptoms in 7 to 10 d, long before fruit turn color (Lewis-Ivey et al 2004). However, one cannot easily predict how a particular species will react. For example, *C. acutatum* acts as a hemi-biotroph, not a necrotroph, on apple, blueberry, and peach (Bernstein et al., 1995; Jones et al., 1996; Milholland, 1995; Peres et al., 2005; Zaitlin et al., 2000), and probably other crops (Prusky and Plumbley, 1992; Timmer et al., 1998).

Because most bell pepper in Florida is harvested at a mature green stage, until recently, anthracnose had been a problem only when crops were extended to the “colored fruit” stage. Indeed, in the past, some growers and extension personnel referred to anthracnose as “ripe-rot” to reflect its impact on ripe, colored fruit only. However, the recent emergence of *C. acutatum* as a pathogen of immature, green fruit raises the status of anthracnose to a potentially major disease problem throughout the industry. This likely means that anthracnose control measures must be initiated earlier and followed diligently throughout the crop cycle. This “new” anthracnose disease is sufficiently different from the traditional ripe-rot anthracnose to merit, in our opinion, a distinctive name. We propose the name “early anthracnose” for the disease of immature, green pepper fruit caused by *C. acutatum*.

The presence of “early anthracnose” on pepper in Florida could have dire consequences for pepper growers throughout the state. Florida’s humid and wet environment is most likely conducive to anthracnose diseases and could be cause for potentially dramatic yield losses. More research is needed on this pepper disease, such as pathogen host range and efficacy of fungicides.

Table 2-1. Isolates of *Colletotrichum* spp. recovered from pepper fields throughout Florida (sample no. 1 – 7) or Georgia (sample no. 8).

Sample no.	Isolate designation ^z	Location (Co.)	No. of Isolates	Host Sample Description	Species recovered
1	MF01-MF09	St. Lucie	9	Red bell ‘Olympus’	<i>C. gloeosporioides</i>
2	VB01-VB10	Indian River	10	Red jalapeno ‘Milta’	<i>C. gloeosporioides</i>
3	PB01-PB06	Palm Beach	6	Green bell ‘Brigadier’	<i>C. acutatum</i>
4	HB01-HB08	Hendry	8	Green/Red bell ‘Aristotle’	<i>C. acutatum</i>
5	HC01-HC09	Hendry	9	Green Cubanelle ‘Aruba’	<i>C. acutatum</i>
6	HJ01-HJ05	Hendry	5	Green/Red jalapeno ‘Tormenta’	<i>C. acutatum</i>
7	MG01-MG03	Collier	3	Red bell, Scotch bonnet, Thai	<i>C. gloeosporioides</i>
8	GA01	Tift (GA)	1	Green bell	<i>C. acutatum</i>

^zAll isolates were recovered in 2004 and 2005 except for those collected from Collier Co. (MG01 – MG03), collected in the mid 1990s by R. McGovern, University of Florida.

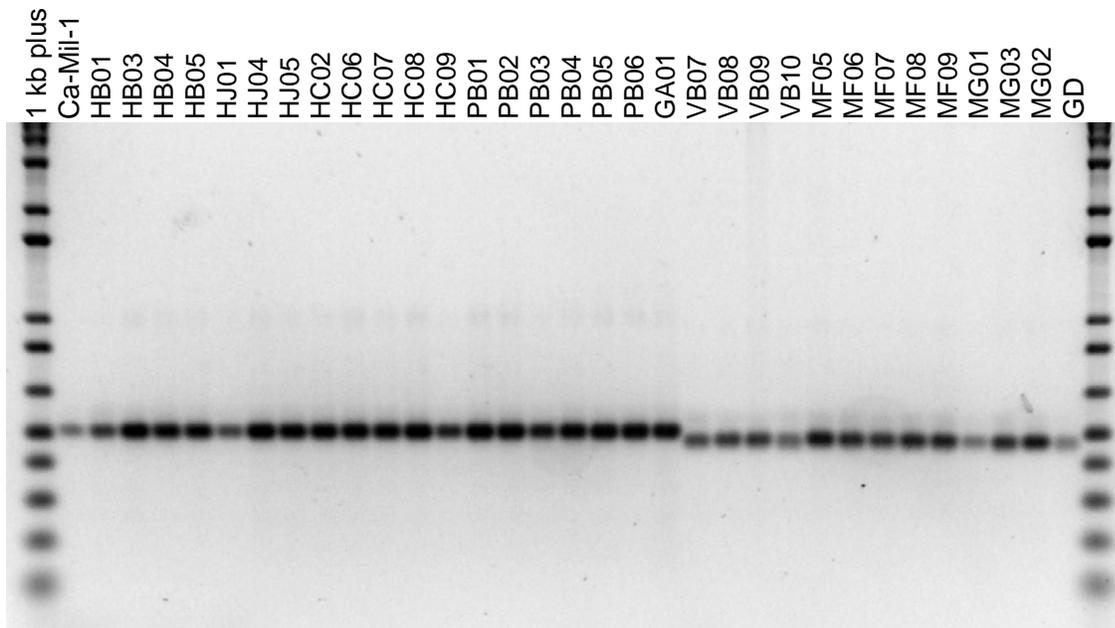


Figure 2-1. An agarose PCR gel of isolates of *Colletotrichum* spp. collected from Florida and Georgia that have produced amplified DNA fragments with either CaInt2 (20 isolates from left) or CgInt (13 isolates from right) species-specific primer. Ca Mil-1 and GD are reference isolates of *C. acutatum* and *C. gloeosporioides*, respectively.

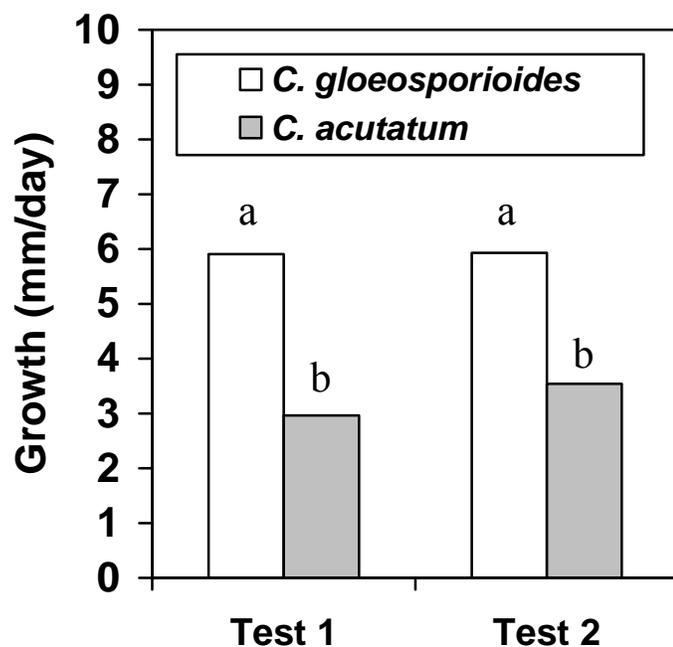


Figure 2-2. Average radial growth per day of 45 isolates representing the two species of pepper anthracnose isolates recovered from Florida as determined by PCR. Colony radius (mm) was measured for three colonies per isolate after mycelial plugs were allowed to grow on artificial media at 30°C for 5 days in two separate tests (Test 1 and 2). The species of each isolate was determined previously by PCR using species specific primers for *Colletotrichum gloeosporioides* (CgInt/ITS4) and *C. acutatum* (CaInt2/ITS4). Each of the 17 isolates designated as *C. gloeosporioides* grew at a significantly faster rate ($P < 0.0001$) in both tests than did the 28 isolates designated as *C. acutatum*.



Figure 2-3. Isolates of *Colletotrichum gloeosporioides* (top) and *C. acutatum* (bottom) recovered in 2004 from pepper fruit in Florida growing on potato dextrose agar in continuous darkness at 30°C for 5 days. Note that the isolates of *C. acutatum* grew slower than isolates of *C. gloeosporioides*.

CHAPTER 3
HOST RANGE OF PEPPER ANTHRACNOSE ISOLATES RECOVERED FROM PEPPER IN
FLORIDA

Introduction

Colletotrichum acutatum has been recently identified as an anthracnose pathogen of pepper in Florida (chapter 2). In that study, isolates of the anthracnose fungus were recovered from heavily infected peppers throughout Florida and identified as either *C. acutatum* or *C. gloeosporioides* using PCR amplification with ITS species-specific primers. Interestingly, the *C. gloeosporioides* isolates were recovered only from ripened, red fruit, whereas *C. acutatum* isolates were recovered primarily from unripe, green fruit. In the fields containing *C. gloeosporioides*, lesions were not observed until most or all of the fruit had ripened, after which an abundance of lesions appeared over a short period of time on nearly every fruit throughout the field (Fig. 3-1). However, in the fields where *C. acutatum* was recovered from green, unripe fruit (Fig. 3-2), anthracnose symptoms were found only within isolated loci. Heavy disease was observed on a few plants that were within close proximity to each other, but less disease was observed moving outward from that point. Within those loci, lesions were predominant on green fruit, ranging from newly-formed small fruit to fully-sized green fruit (Fig. 3-2). However, symptoms were also found on an occasional adjacent ripened, red fruit.

Colletotrichum gloeosporioides has a reportedly wide host range including many weed species (Bailey et al., 1992; O'Connell et al., 2000), and thus it is very likely that this species of *Colletotrichum* exists naturally in Florida, either symptomatically or asymptotically, on host plants that occur adjacent to pepper fields. This could help to explain the source and abundance of inoculum that must be necessary to cause the anthracnose symptoms on nearly every fruit that is commonly observed in fields of ripened pepper infected with *C. gloeosporioides* (Fig. 3-1). In contrast, *C. acutatum* has a much more limited host range (Bailey et al., 1992; Legard, 2000;

Peres et al., 2005), and is generally thought not to occur naturally on weed hosts to the same extent as *C. gloeosporioides* (Freeman et al., 1998; Freeman, 2000). As a result, widespread infection of *C. acutatum* in pepper fields was not observed (Bernstein et al., 1995; Harp et al., 2006; Lewis-Ivey et al., 2004; Marvel et al., 2003). Instead, symptoms appeared initially in localized foci, perhaps originating from a single to few infected plants or fruits.

On strawberry, where both *C. acutatum* and *C. gloeosporioides* cause anthracnose symptoms, *C. acutatum* has been isolated from lesions on nursery-grown transplants prior to being planted in the field (Legard, 2000; Peres et al., 2005). Most nursery-grown strawberry transplants that are planted in Florida are typically produced in other states (e.g. North Carolina or California), or in Canada. In these reports, the pathogen was apparently introduced on the transplants prior to arriving in Florida with the transplants serving as the primary inoculum in the field (Legard, 2000; Peres et al., 2005). In contrast, *C. gloeosporioides* has not been recovered from transplants and is believed to infect strawberry only after transplants are set in the field. The source of inoculum is probably weed hosts or infected debris (Freeman et al., 1998; Harp et al., 2003; Legard, 2000). In addition, research has been conducted in Florida that demonstrated the inability of *C. acutatum* isolates recovered from strawberry to survive in crop debris or soil for any extended period of time, such as during the summer months when strawberries are not grown (Legard, 2000). Therefore, it is unlikely in Florida that *C. acutatum* recovered from strawberry survived off-season in soil debris or a local alternative host (Bailey et al., 1992; Kwon and Lee et al., 2002; Legard, 2000; O'Connell et al., 2000). The same could be true with pepper, and this likely explains why the occurrence of *C. acutatum* on pepper is typically found in the field in randomly positioned loci, whereas the occurrence of *C. gloeosporioides* on pepper is found widespread throughout the field and detected at once on virtually every susceptible

(ripened) fruit within that field. Perhaps *C. acutatum* on pepper, as with strawberry, is seed-borne or somehow infects the nursery-grown transplants, whereas *C. gloeosporioides* on pepper originates from a local source, such as a weed host, and arrives on pepper after planting (Freeman et al., 1998; Freeman et al., 2001; Harp et al., 2003; Legard, 2000).

Since *C. acutatum* infects immature, unripe green fruit, we are calling this disease ‘early anthracnose’. The implications for pepper growers are more significant than for ripe-rot anthracnose, caused by *C. gloeosporioides*, which only appears to cause symptoms on ripened, red fruit. Most pepper fruit harvested in Florida are harvested as fully-sized green fruit, and therefore anthracnose caused by *C. gloeosporioides* poses little or no threat under these circumstances. *Colletotrichum gloeosporioides* infection of pepper has been previously described in Florida (McGovern and Polston, 1995; Roberts et al., 2001) and overall accepted as a ‘ripe rot’ disease on pepper and other crops (Alexander and Pernezny, 2003; Maynard et al., 2003; Milholland, 1995). The possibility of *C. gloeosporioides* cross-infection from pepper to other crops has not been investigated, and likely would be of little consequence considering the wide host range and abundant inoculum source of this species already present in nature. However, with the recent introduction of *C. acutatum* on pepper, it is possible that early anthracnose of pepper could have disease management implications for other crops grown in Florida, especially those that grow in proximity to pepper and are known hosts of *C. acutatum*, such as strawberry (Legard, 2000) and tomato (Correll et al., 2000; Guerber et al., 2003).

In this study, an isolate of *C. acutatum* (HB05) was recovered from green bell pepper grown in Hendry, Co., Florida and used to artificially inoculate field-grown strawberries and tomatoes during the spring of 2006 and 2007. In addition, this isolate was tested for the ability to cause lesions on detached strawberry and tomato fruit harvested from the same fields used in

2007 by wound-inoculation in the laboratory. If anthracnose symptoms were to be observed on inoculated strawberries or tomatoes, this could have significant implications for disease management on tomatoes, strawberries, or other crops susceptible to *C. acutatum* that grow in Florida adjacent to pepper fields. Additionally, it could lead to insight regarding the source of inoculum on peppers, and also provide cultural recommendations for managing this disease on all such crops. The purpose of this study was to evaluate if *C. acutatum* recovered from pepper is pathogenic to two important crops grown in Florida, tomato and strawberry, under both field and laboratory conditions.

Materials and Methods

Host Range Field Trials

Two host-range field trials were conducted in field plots located at the Syngenta Vero Beach Research Center, Indian River Co., Florida, during consecutive growing seasons for strawberry, tomato, and pepper. The first field trial was initiated in the fall of 2006 (Field Trial 1), and the second trial in the fall of 2007 (Field Trial 2). In addition, fruits were harvested from the same fields used in Field Trial 2 (outside of the testing area) to conduct a wound-inoculation study in the laboratory (Laboratory Trial 1).

Plants

In Field Trial 1, strawberry plants ('Camerosa' and 'Chandler') were obtained as bare-root transplants produced in Canada and provided courtesy of Carl Grooms, Inc., Plant City, FL, and hand-transplanted on 29 October, 2006. The transplants were planted in double rows on raised beds under plastic mulch and single center drip-tube irrigation. The cultivars, 'Camerosa' and 'Chandler', were planted together in the same plot (six plants each per plot with 12 plants total per plot), and the trial consisted of three replications. Tomatoes ('FL 47') and peppers ('Revolution') were planted on 01 March, 2007, on raised beds with plastic mulch and drip

irrigation. Pre-plant fungicides, mefenoxam (Ridomil Gold SL, 1.2 L / Ha) and PCNB (Terraclor Super X, 7.1 L / Ha), were broadcast-incorporated into the soil along with diazinon (Diazinon AG500, 2.4 L / Ha) for soil fungi and insect control, respectively. A rotational spray program of spinosad (Spintor, 0.44 L / Ha), emamectin benzoate (Proclaim, 0.29 L / Ha), and lambda-cyhalothrin (Warrior, 0.29 L / Ha) were applied on all crops on a 7 to 14-day interval for insect control. In Field Trial 2, strawberry plants were obtained as plugs from Norton Creek Farms (Fischer, NC), and hand-planted on 22 October, 2007. Peppers ('Revolution') and Tomatoes ('FL 47') were planted on 10 October, 2007, and all crops in Field Trial 2 were planted and maintained under the same cultural conditions as in Field Trial 1.

Inoculum for Field and Laboratory Evaluations

Isolate HB05 was grown on PDA at 20°C under continuous lighting for 7 days as previously described (Chapter 2; Harp et al., 2006). Conidia were harvested by flooding cultures with de-ionized water and using an 'L-shaped' glass rod to remove conidia into solution. The conidial suspension was adjusted to a concentration of 2.5×10^4 conidia per mL using a hemacytometer. For the field inoculations, the conidial concentration was prepared in 5 L of de-ionized water, whereas for the laboratory inoculations, approximately 50 mL of inoculum was prepared.

Field Treatment Plots

In each crop, three treatment plots were arranged in a randomized, complete-block design and three replications. The treatments consisted of un-inoculated, inoculated, and water-sprayed control plots using the same de-ionized water used in the inoculated plots except without conidia. In Field Trial 1, the row spacing for each crop was 1.5 m, and the plant spacing was 45.7cm for tomatoes and peppers (8 plants per plot), and 30.5 cm for strawberries (12 plants per plot). The

plot sizes were 3 x 1.5 m for tomatoes and peppers, and 3.6 x 1.5 m for strawberries. In Field Trial 2, the row spacing for each crop was 1.5 m, and the plant spacing was 60.9 cm for tomatoes and peppers (15 and 8 plants per plot, respectively), and 45.7 cm for strawberries (15 plants per plot). The plot sizes were 6 x 1.5 m for tomatoes, 4.6 x 1.5 m for peppers, and 9.1 x 1.5 m for strawberries. The plot sizes and relevant planting details for each crop in Field Trial 1 and Field Trial 2 are summarized in Table 3-1.

Laboratory Detached Fruit

Tomato, strawberry, and pepper fruit were obtained from the border rows of the same fields used in Field Trial 2. These rows were sprayed with the same insecticide maintenance treatments as the plots within Field Trial 1 and Field Trial 2 but did not receive any fungicide treatments or inoculations. The fruit were collected on 19 January 2008, and washed with de-ionized water in the laboratory. A total of 40 strawberry fruit and 20 tomato fruit were collected that represented all stages of fruit development from small, unripe green fruit to fully-sized, ripened red fruit. Out of the 40 strawberry fruit, 20 were injected with the conidial suspension (see ‘Laboratory inoculations’) and 20 injected with water. Out of 20 tomato fruit, 10 were injected with the conidial suspension and 10 were injected with water. Eighteen pepper fruit were also collected that ranged from medium-sized, unripe green fruit to fully-sized harvestable green fruit. Twelve pepper fruit were injected with the conidial suspension while the remaining six were injected with water. A few peppers had started to ripen and were partially red in color. After the fruit were rinsed in de-ionized water for three minutes, they were dipped in a 10% bleach solution (300 mL of Clorox bleach in 3000 mL of de-ionized water) for 30 seconds and immediately placed in a tub of de-ionized (5000 mL) water for an additional 30 seconds. Fruit were then removed from the tub and allowed to air dry. Plastic containers (Tupperware,

Hartford, CT) were lined with moist paper towels and used as incubation chambers for the detached, inoculated and un-inoculated fruit.

Field Inoculations

Inoculations were conducted for Field Trial 1 and Field Trial 2 on 18 May, 2007 and 21 December, 2007, respectively. Each inoculation was conducted at approximately 2300 h EST, when the dew point was within 3 to 5°C of the ambient temperature, ensuring dew formation and leaf wetness for at least 8 hours. The nighttime temperatures during the inoculations for Field Trial 1 and Field Trial 2 were between 18 and 20°C, and the humidity was between 88 and 92%. The treatments were applied over the top of the plants using a 10 L backpack pump-sprayer (Solo® 435, Detroit, WI) until run-off, ensuring good coverage of the fruit and foliage. For each inoculation, the same inoculum batch was used on all three crops within a span of one hour. The water-sprayed treatments were applied prior to the inoculated treatments using the same backpack pump sprayer containing de-ionized water but with no conidia. For strawberry and tomato plants, both unripe green fruit, and ripened red fruit, occurred in the treated plots. For pepper plants, unripe green fruit ranging from very small to fully-sized fruit, but not red-ripened fruit, occurred in the plots at the time of the inoculations.

Laboratory Inoculation

Using a black Sharpie, circles (approximately 3 cm in diameter) were drawn on each pepper and tomato fruit to identify the wound-inoculation site. On the strawberries, which could not be easily marked, the inoculation site was located on the side of the fruit facing directly upward after placing in the plastic containers. Fruits were inoculated using a 1 cc syringe (25G needle, Becton Dickinson and Co., Rutherford, NJ) containing a conidial suspension of *C. acutatum* (conidial concentration of 10^3 per mL) in de-ionized water. Controls consisted of

similarly treated fruit injected with de-ionized water without conidia. For the wound-treatments, the tip of the syringe was used to penetrate the skin of each fruit and a small amount (approximately 0.01 mL) of either the conidial suspension or de-ionized water without conidia was injected under the skin. In many cases, a small amount of liquid formed a droplet on the surface of the wound. The fruit were allowed to remain in the sealed plastic containers with moist paper towels (100% humidity) at approximately 20°C for 5 days. At 3 and 5 days after inoculation, the fruit were assessed for the development of lesions, and the presence or absence of lesions on each fruit was recorded.

Disease Assessments

For Field Trial 1 and Field Trial 2, fruit in each plot was assessed for anthracnose symptoms at 10 days after inoculation and again 7 days later. The number of lesions were counted in each plot for all three crops and for all three treatments within each crop, inoculated, uninoculated, and the water-treated control. For Laboratory Trial 1, the fruits were assessed for lesions and scored as ‘infected’ or ‘uninfected’ after 3 days for strawberry and pepper, and 5 days for tomato.

Results

Inoculation Field Trials

In both Field Trial 1 and Field Trial 2, moderate to heavy anthracnose symptoms were observed on inoculated pepper fruit within 7 to 10 days after inoculation in the inoculated plots (Fig. 3-2). However, no symptoms were observed in the water-treated or untreated control. In Field Trial 1, there was a mean of 22.7 lesions among fruit in the inoculated pepper plots after 10 days. A mean of 39.3 lesions were recorded in Field Trial 2 (Table 3-2). In both trials, no lesions were found on pepper in either untreated or water-treated control plots.

In the tomato and strawberry plots, no lesions or anthracnose symptoms were observed among fruit in any of the treatment plots at both 10 (Table 3-2) and 17 days after treatment (data not shown). The number of lesions counted in each of the plots at 10 days after inoculation for all treatments in both trials was identical to the number of lesions after 17 days. Although both unripe, green fruit and ripened, red fruit were inoculated in the strawberry and tomato plots, no lesions or anthracnose symptoms were observed on any fruit (Fig. 3-3). These results confirm that the *C. acutatum* isolate recovered from pepper is pathogenic on pepper. However, this isolate was not pathogenic on field-grown strawberry or tomatoes following an artificial inoculation during environmental conditions highly conducive to disease development.

Detached-Fruit Inoculation

In the detached fruit study (Laboratory Trial 1), 12 out of 12 (100%) of the wound-inoculated peppers formed an anthracnose lesion at the site of inoculation within 3 days. No lesions occurred in the six fruit that were injected with de-ionized water. Interestingly, both strawberry and tomato fruit that were wound-inoculated with *C. acutatum* conidia also formed lesions, whereas those fruit injected with de-ionized water did not (Figs. 3-4 and 3-5). The number of fruit that were wound-inoculated and formed lesions was 17 out of 20 (85%) for strawberry, and 10 out of 10 (100%) for tomato. The lesions formed within 3 days on strawberry and pepper (Fig. 3-4), and within 5 days on tomato (Fig. 3-5). The characteristic salmon-colored conidial matrix could be observed within the lesions on the strawberry and pepper fruit (Fig. 3-4), but were initially less obvious on tomato fruit. However, microscopic examination confirmed the presence of conidia on all three types of fruit after 5 days (data not shown) (Fig. 3-5), and large lesions were eventually observed with profuse sporulation after 15 days on tomato and pepper (Fig. 3-6). Although anthracnose symptoms on tomato and strawberry did not occur in

the field inoculations, wound-inoculations on detached fruit did produce lesions characteristic of anthracnose disease (Figs. 3-4, 3-5 and 3-6).

Discussion

Colletotrichum acutatum is a devastating pathogen of many crops and the focal point of a great deal of research in the United States and abroad (Bailey et al., 1992; Correll et al., 2000; Freeman et al., 2000; Freeman et al., 2001; Guerber et al., 2003; Kim et al., 1986; Kim et al., 2002; Kwon and Lee, 2002; Legard, 2000; Park and Yoon, 2003; Park, 2007; Peres et al., 2005; Prusky and Plumbley, 1992; Roberts and Snow, 1990; Zong-Ming et al., 2007). The fact that this fungus is now confirmed as a pathogen of pepper in Florida causing ‘early anthracnose’ only adds to the significance of this pathogen as a continual and emerging threat to pepper crops throughout the world (Black and Wang, 2007; Hadden and Black, 1988). Although *C. gloeosporioides* was already recognized as a ‘ripe-rot’ anthracnose pathogen of pepper in Florida (Alexander and Pernezny, 2003; McGovern and Polston, 1995; Roberts et al., 2001), the addition of *C. acutatum* as a pathogen of pepper brings new implications to pepper growers attempting to manage anthracnose. In addition, it adds new challenges to other potential host crops that grow adjacent to pepper, such as tomato or strawberry, or those rotated as a plant-back crop into harvested pepper fields such as tomato, cucurbits, or even another crop of peppers. Certainly, understanding and comprehending the epidemiology and host range of this pathogen on peppers would lead to more informed decisions concerning crop rotation and other cultural practices.

Isolate HB05 from pepper produced typical anthracnose symptoms in the field and laboratory. Only in the laboratory, using wounded and detached fruit, did it produce anthracnose-like lesions on strawberry and tomato. These observations cast doubt on host-range reports developed for this or other pathogens based solely on detached fruit assays. One recent example is the result by Black and Wang (2007), where little correlation was found between field

inoculations and laboratory wound-inoculations of anthracnose pathogens on different varieties of pepper fruit. Although certain varieties did express a partial resistance to artificial inoculation of *C. acutatum* in the field, these same varieties provided for no such conclusion when detached fruit were wound-inoculated in the laboratory (Black and Wang, 2007). Perhaps of even more concern, some researchers have drawn conclusions regarding the host range of certain species of *Colletotrichum* by exclusive use of detached-fruit studies (Freeman et al., 1998; Hong and Hwang, 1998; Kim et al., 2001; Manandhar et al., 1995a). Our study challenges the validity of previous reports that used wound-inoculations to determine pathogenicity of different species of *Colletotrichum* on pepper fruit.

Currently in Florida, *C. acutatum* is now well-known as a pathogen of citrus (Brown et al., 1996; Lahey et al., 2004; Peres et al., 2004; Timmer et al., 1998) and strawberry (Harp et al., 2003; Legard, 2000; Peres et al., 2005). The fungus has also been reported as a pathogen on lychee (Davis, 2003), where infection was shown to initiate unnoticed in the flowers and then become symptomatic in the subsequent fruits. Additionally, the fungus is a pathogen of certain ornamental crops, such as flowering dogwood in central and north Florida (Strandberg and Chellemi, 2002), where it causes dogwood anthracnose and is responsible for significant losses and constraints to flowering dogwood production. On pepper, *C. acutatum* infection has not been reported previously in Florida; however, there are recent reports from other states such as Ohio (Lewis-Ivey et al., 2004), Virginia (Marvel et al., 2003) and more recently in Georgia (Chapter 2), where the disease appears to be emerging as a significant threat to peppers and is gaining considerable attention (Correll et al., 2007). This work demonstrates that *C. acutatum* is indeed a pathogen of pepper in Florida and is based for the first time on a large collection of

isolates. Therefore, it has significant implications for growers attempting to manage anthracnose on pepper.

The fact that *C. acutatum* is responsible for the recent outbreaks of early anthracnose in Florida (Harp et al., 2006) has potential implications for other crops where *C. acutatum* is a pathogen, such as citrus and strawberry. Various management strategies have been implemented to facilitate the control of anthracnose diseases on these crops. Tomatoes, an economically important crop in Florida, have not been reported as a host of *C. acutatum* in the U.S. However, other species of *Colletotrichum* that infect strawberry and pepper, such as *C. gloeosporioides*, do infect tomato (Freeman et al., 1998; Hadden, 1989; Lewis-Ivey et al., 2004; Legard, 2000). The most important anthracnose pathogen of tomato, *C. coccodes* (Dillard, 1992; Farley, 1976; Hong and Hwang, 1998; Peres et al., 2002; Tsrer and Johnson, 2000) also is reported as a pathogen of pepper (Hadden, 1989; Harp et al., 2006; Lewis-Ivey et al., 2004; Legard, 2000; Roberts et al., 2001). Therefore, *C. acutatum* on pepper might be a threat to tomato in the U.S., particularly since this species has been recovered from tomato in New Zealand (Guerber et al., 2003). In addition, *C. acutatum* is a well-known pathogen of strawberry, and therefore one could easily speculate that this disease on strawberry could spread to pepper, or vice versa. In this study, a *C. acutatum* isolate recovered from pepper was found to be non-pathogenic in field inoculations on both tomato and strawberry. Since no cross-infection occurred during ideal conditions in the field, infected pepper fields in close proximity to strawberry or tomato probably do not constitute a serious threat to the latter crop.

The same conclusion would not be reached based strictly on a detached-fruit bioassay conducted in the laboratory. In our laboratory experiment, lesions were formed on wound-inoculated fruit of tomato and strawberry from the same isolate of *C. acutatum* recovered from

pepper used in the field tests. Therefore, one must conclude that although *C. acutatum* did not cause anthracnose symptoms when applied to healthy, attached field-grown strawberries or tomatoes, a detached, wounded fruit could become symptomatic under artificial conditions. Perhaps severe wounding and forcible injection of spores is necessary on these fruits for infection to occur, but this is unlikely under field conditions based on two seasons of study. One deficiency in these investigations was the use of only one isolate of *C. acutatum* from pepper for all of the pathogenicity tests. Isolates may vary in ability to infect strawberry and tomato naturally in the field. Studies using more isolates would be a fruitful area for further research but may be limited by the logistics of conducting such large-scale field experiments.

More recently, enhanced molecular techniques beyond the traditional use of ITS sequence data (Chapter 2) have been employed in studies of *Colletotrichum* and have further delineated species boundaries in this taxonomically complex genus (Correll et al., 2007; Correll et al., 2000; Guerber et al., 2003). Although a great deal of work has traditionally been used to identify and distinguish species of *Colletotrichum* based on the conserved ITS region (Adaskaveg and Forster, 2000; Brown et al., 1996; Freeman et al., 2001; Harp et al., 2006; Horowitz et al., 2002; Lewis-Ivey et al., 2004; Mills et al., 1992; Peres et al., 2005; Sreenivasaprasad et al., 1996; Timmer and Brown, 2000), more contemporary approaches have more recently been employed to identify and examine sub-species populations within *C. acutatum* and other species of *Colletotrichum* (Correll et al., 2007; Du et al., 2005; Guerber et al., 2003; Peres et al., 2005). One method analyzes and compares the genetic sequence variation of a 900-bp intron of the glutamine synthetase gene between a diverse collection of *C. acutatum* isolates from different hosts (Guerber et al., 2003; Liu and Correll, 2000). Several ‘sub-species’ populations, or clades, were identified and shown in some examples to correlate with differences in host range within *C.*

acuatum (Correll et al., 2007; Correll et al., 2000; Peres et al., 2005). If these and other genetic differences can be found to correspond directly to differences in host range, then this would support the thought that isolates of *C. acutatum* recovered from pepper are potentially ‘different’ than isolates recovered from strawberry or citrus, and therefore pose less of a threat for cross-infection. Indeed, isolate HB05 from pepper was recently found to be different from strawberry and citrus isolates collected in Florida in Dr. James Correll’s laboratory at the University of Arkansas (Harp and Correll, unpublished). Furthermore, by RFLP analysis, HB05 was found to be most closely related to isolates recovered from pepper in Taiwan (Correll, personal communication), which were grouped into the mtDNA haplotype D3 (Guerber et al., 2003).

This study has demonstrated that a highly virulent isolate of *C. acutatum* recovered from pepper could be used to re-infect pepper plants in the field, but did not infect adjacent strawberry, a known host of *C. acutatum*. Although wound-inoculations did produce disease symptoms on detached fruit, molecular differences between the pathogen recovered from pepper and those recovered from strawberry could be the reason that a pepper isolate could not cause disease on field-grown strawberry fruit. It is likely that genetic differences between *C. acutatum* isolates from different hosts could affect the molecular signaling and recognition needed by a specific pathogen to identify and detect a potential host. If the pathogen does not recognize a substrate as a host, it simply will not undergo the transformations needed to be pathogenic on that host, such as spore germination and / or appressorium formation and development. It appears that the isolates of *C. acutatum* recovered from pepper and used in this study are genetically different than isolates of *C. acutatum* recovered from strawberry or citrus, and therefore pose no threat to nearby strawberry fields or citrus groves. The reverse is also likely true, in that isolates recovered from strawberry or citrus would not cause disease in pepper.

However, it is interesting that pepper isolates will still form lesions on strawberry and tomato with wound-inoculation of detached fruit. Perhaps wounding the fruit allows the pathogen to bypass the molecular recognition needed for ingress, and once inside the cuticle the fungus can continue to germinate, grow, and produce conidia. This is not unlike the fungus growing on artificial media, where appressoria and other pathogenic structures are typically not produced, but the fungus continues to grow and reproduce. Certainly, more research is needed to draw further conclusions on ability for isolates of the same species to infect different hosts, both by molecular characterization and epidemiology studies (Peres et al., 2005).

For now, Florida growers can feel fairly confident that an epidemic of pepper anthracnose in a given season is not necessarily a significant threat to nearby tomato or strawberry crops. Certain species of *Colletotrichum*, such as *C. gloeosporioides*, are known for their wide host range and potential devastation to many crops (O'Connell et al., 2000; Sutton, 1992). Although the recent discovery of *C. acutatum* on pepper and the implications of this disease on younger, underdeveloped fruit is a grave concern, it seems likely that this species is selective to bell and chili pepper, and does not likely threaten other valuable crops that are hosts of *C. acutatum* strains. Even so, the knowledge of the inoculum source on pepper and the ability of this pathogen to maintain a viable inoculum reservoir between pepper crops would be of great significance to assist in the management of this disease. This study hopes to provide a firm starting point to evaluate the epidemiology of pepper anthracnose caused by *C. acutatum* and should contribute to the understanding and management of this disease now and into the future.

Table 3-1. Plot size and planting conditions for each crop evaluated in Field Trial 1 and Field Trial 2.

Trial / crop	Plot size(m) ^w	No. of plants ^x	Row spacing(m) ^y	Plant spacing(cm) ^z
Field Trial 1				
Tomatoes	3.0 x 1.5	8	1.5	45.7
Strawberries	3.7 x 1.5	12	1.5	30.5
Peppers	3.0 x 1.5	8	1.5	45.7
Field Trial 2				
Tomatoes	6.1 x 1.5	15	1.5	61.0
Strawberries	9.1 x 1.5	15	1.5	45.7
Peppers	4.6 x 1.5	8	1.5	61.0

^wLength x width (m). All crops were transplanted on raised beds covered in white plastic mulch with drip irrigation. ^xThe number of plants within each plot. Tomatoes and peppers were planted on raised beds in a single row, while strawberries were planted in a double row. ^yRaised beds were 30 cm tall, 1.5 m wide, and 1.5 m apart. ^zCentimeters between plants within a row. For strawberries, the plants are 61.0 cm apart along the row and between the double row plants.

Table 3-2. Mean number of lesions per plot on pepper, strawberry and tomato fruit in inoculated, un-inoculated, and water-sprayed treatments for Field Trial 1 and Field Trial 2 at 10 days following an artificial inoculation with *Colletotrichum acutatum*. The inoculation occurred only in the ‘inoculated’ treatments. No infection occurred on either strawberry or tomato fruit in any of the treatments.

Trial / crop	No. of lesions		
	Inoculated ^x treatment	Un-inoculated ^y treatment	Water-sprayed ^z treatment
Field Trial 1			
Peppers	22.7 a	0.0	0.0
Strawberries	0.0 b	0.0	0.0
Tomatoes	0.0 b	0.0	0.0
Field Trial 2			
Peppers	39.3 a	0.0	0.0
Strawberries	0.0 b	0.0	0.0
Tomatoes	0.0 b	0.0	0.0

^x Pepper, strawberry, and tomato plots were inoculated with a conidial suspension (2.5×10^5) of *C. acutatum*, and the number of lesions per plot were counted for each crop. ^y Each crop in this treatment did not receive any inoculation or application of water. ^z Each crop in this treatment was sprayed with de-ionized water but without the addition of conidia.

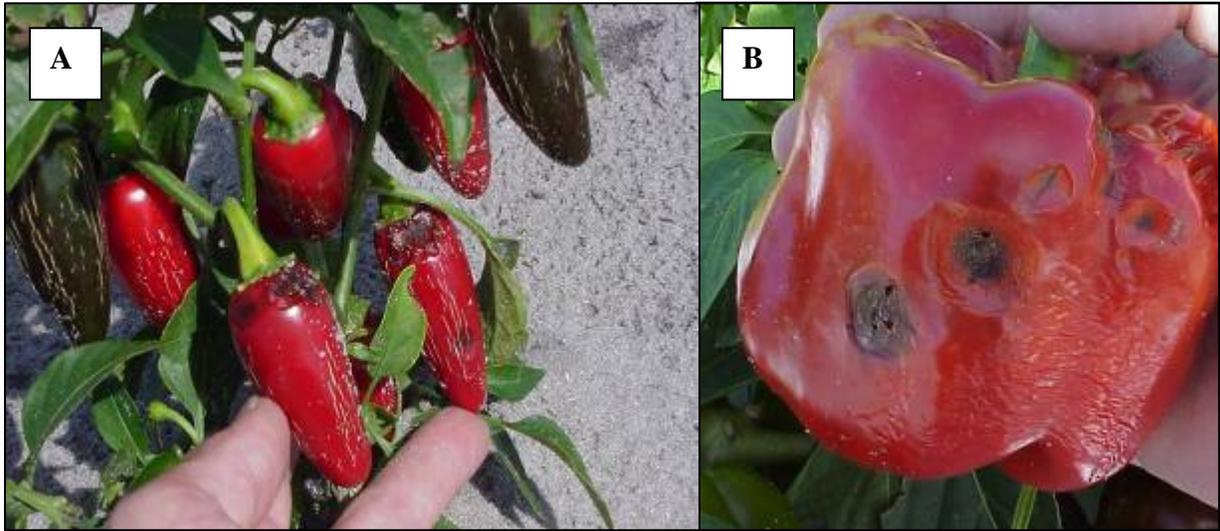


Figure 3-1. Ripened jalapeno (A) and bell pepper (B) with anthracnose lesions caused by *Colletotrichum gloeosporioides*. Once ripened, the pepper fruit become susceptible to infection by *C. gloeosporioides* and symptoms occur at once throughout the entire field.



Figure 3-2. Unripe bell pepper with anthracnose symptoms caused by *Colletotrichum acutatum*. Unlike anthracnose symptoms which appear on strictly ripened, red fruit caused by *C. gloeosporioides*, *C. acutatum* can cause symptoms on unripe, green pepper fruit, including very young fruit (right).

Un-inoculated

Inoculated



Figure 3-3. Strawberry, tomato, and pepper plants from un-inoculated (left) and inoculated plots (right) in Field Trial 1. Only the pepper fruit in the inoculated plots developed lesions observed within 7 to 10 days after the artificial inoculation with *Colletotrichum acutatum* (pictured above at 14 days after inoculation). In the strawberry and tomato plots, both green unripe fruit, and red-ripened fruit were inoculated, but no lesions formed up to 21 days after the inoculation.

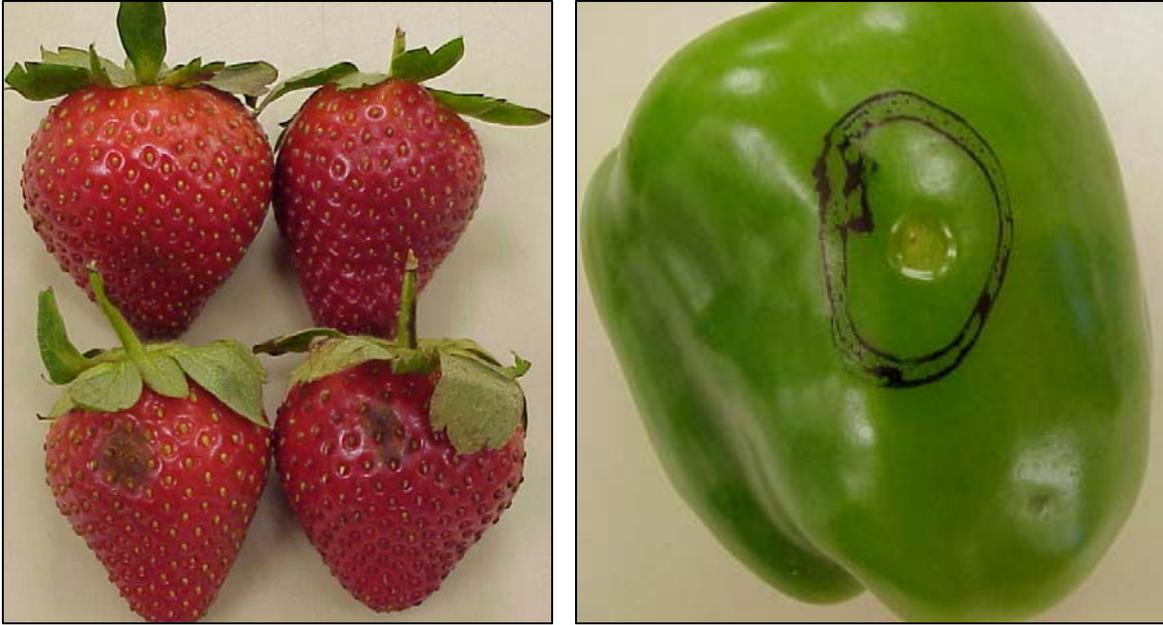


Figure 3-4. Detached, wound-inoculated fruit of strawberry (left) and pepper fruit (right) three days after inoculation. At left, the top two strawberry fruit were injected with de-ionized water while the bottom two were wound-inoculated with a spore suspension of *Colletotrichum acutatum*, isolate HB05 recovered from pepper. At right, detached pepper fruit was wound-inoculated as a positive control. Conidia were observed within the lesions on strawberry and pepper after only 3 days following the wound-inoculation.

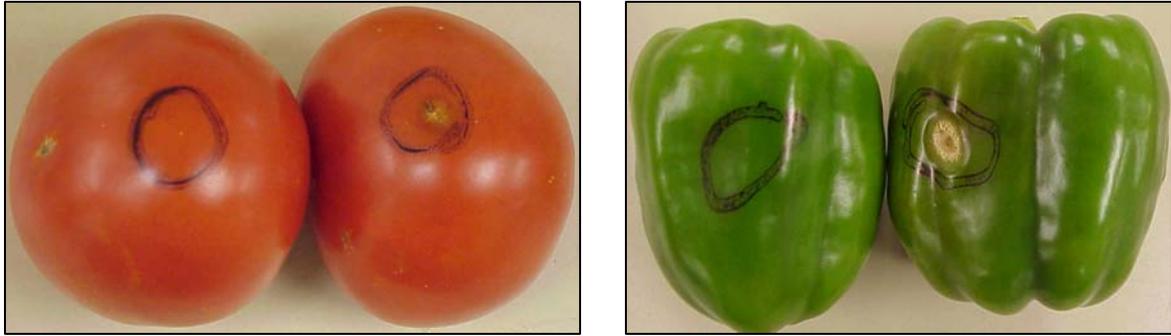


Figure 3-5. Detached, wound-injected fruit of tomato (left) and pepper fruit (right) five days after the inoculation with a conidial suspension of *Colletotrichum acutatum*. In each photograph, the fruit on the left was injected with de-ionized water, and the fruit on the right wound-inoculated with a conidial suspension of *C. acutatum*, isolate HB05 recovered from pepper. Five days after the inoculation, lesions on the inoculated tomato fruit became apparent and conidia were recovered from the lesion.



Figure 3-6. Detached, wound-inoculated tomato (top) and tomato and pepper (bottom) 12 days after inoculation with a conidial suspension of *Colletotrichum acutatum*. Profuse sporulation, observed as a salmon-colored conidial matrix exuding from the lesion, was apparent on both types of fruit.

CHAPTER 4 CHEMICAL CONTROL OF PEPPER ANTHRACNOSE

Introduction

Pepper anthracnose is a potentially devastating disease of pepper in most regions where pepper is grown (Alexander and Pernezny, 2003; Hadden and Black, 1998; Kwon and Lee, 2002). Although traditionally thought of as a ‘ripe-rot’ disease, anthracnose caused by *Colletotrichum acutatum* has been recently recovered from immature, green pepper fruit in Ohio (Ivey, et al., 2004), Louisiana (Haden, 1998), Virginia (Marvel et al., 2003), Georgia, and Florida (This dissertation, chapter 2). Therefore, unlike the ‘ripe rot’ phase of anthracnose typically caused by *C. gloeosporioides* or *C. coccodes* (Alexander and Pernezny, 2003; Lewis-Ivey et al., 2004; Roberts et al., 2001) anthracnose disease caused by *C. acutatum* would warrant additional chemical control measures in order to harvest healthy, fully-sized green fruit. Although a few fungicides are labeled for pepper anthracnose control, no data evaluating efficacy of these products in Florida for control of ‘early anthracnose’ caused by *C. acutatum* are currently available.

At present, most pepper fields throughout Florida undergo a pesticide spray program which often includes both insecticides and fungicides. Typically, these products are tank-mixed and used to target insect pests, such as the pepper weevil, and diseases such as Phytophthora blight, powdery mildew, frog-eye leaf spot, and bacterial spot. Anthracnose disease, caused by *C. gloeosporioides*, or *C. coccodes*, is traditionally considered a ripe-rot disease occurring only on ripened, red pepper and is not targeted with pesticides unless red pepper is the intended harvested product. For pepper harvested green, which includes the majority of the acreage in Florida (Maynard et al., 2003), anthracnose caused by *C. gloeosporioides* is typically not considered a disease needing special chemical treatment. However, now that *C. acutatum* has been confirmed

to infect immature green pepper fruit in Florida, pepper growers need to include this disease as a target in their pest management program.

Currently, there are five commonly used fungicides labeled for anthracnose of pepper in Florida. These include azoxystrobin (Quadris), famoxadone plus cymoxanil (Tanos), pyraclostrobin (Cabrio), maneb, and copper hydroxide (Kocide or Champ). The first three fungicides contain an active ingredient of the strobilurin class of chemistry, and are effective against a broad range of pathogens on a large number of crops. The remaining two pesticides are usually applied as a tank mix on pepper for control of bacterial spot. However, when applied separately, copper or maneb can be used for control of anthracnose or other fungal diseases, such as *Phytophthora* blight.

In this study, six fungicides and one ‘systemic-acquired resistant’ (SAR) pesticide were evaluated for efficacy against artificially inoculated *C. acutatum* on pepper plants in Florida. The isolate used for the artificial inoculation (HB05) was originally recovered from an anthracnose lesion on an unripe, green pepper fruit in 2004 from Hendry, Co., Florida, and was among those responsible for a severe epidemic in a grower’s field that caused nearly 50% reduction in yield (Harp, personal observation). HB05 was used to artificially inoculate pepper plants treated with various fungicides to evaluate the efficacy of these products against pepper anthracnose. The evaluation of pesticides against this potentially devastating new disease in Florida will assist growers in developing successful management strategies to control this disease and minimize the potentially severe economic losses that could result. The purpose of this study was to determine the efficacy of seven pesticides against pepper anthracnose in Florida and to provide insight into chemical management recommendations necessary to optimize control of this disease.

Materials and Methods

Fungicide Field Trials

Fungicide field trials were conducted during three different pepper-growing seasons in Florida, fall 2006 (Fungicide Field Trial 1), spring 2007 (Fungicide Field Trial 2), and fall 2007 (Fungicide Field Trial 3). All three trials were conducted at the Syngenta Vero Beach Research Center, Indian River Co., Florida.

Pepper Plants

Seven-week-old pepper ('Revolution') transplants were purchased from Speedling nursery in Sun City, Florida, and transplanted in single rows on raised beds under white plastic mulch (1.5 m centers) with drip irrigation. Pre-plant pesticides, mefenoxam (Ridomil Gold SL, 1.2 L / ha), PCNB (Terraclor Super X, 7.1 L / ha), and diazinon (Diazinon AG500, 2.4 L / ha) were broadcast-incorporated into the soil for soil-borne fungi and insect control, respectively. Approximately 168 kg / ha of fertilizer (10-10-10) was also applied to the soil prior to planting with two drip-line injections of fertilizer conducted approximately six weeks apart after planting. A rotational spray program of spinosad (Spintor, 0.44 L / ha), emamectin benzoate (Proclaim, 0.29 L / ha), and lambda-cyhalothrin (Warrior, 0.29 L / ha) were applied on a 7 to 14-day interval for insect control. The planting dates for Fungicide Field Trial 1, 2, and 3 were 06 October, 2006, 06 March, 2007, and 15 October, 2007, respectively. In Fungicide Field Trial 1 and 2, the row spacing was 1.5 m, and the plant spacing was 45.7 cm with 10 plants per plot (plot size 4.6 x 1.5 m). In Fungicide Field Trial 3, the row spacing was 1.5 m, and the plant spacing was 60.9 cm with 6 plants per plot (plot size 3.7 x 1.5 m). Plot sizes and relevant planting details for each fungicide field trial are shown in Table 4-1.

Inoculum Production

Isolate HB05 was recovered from pepper as previously described (Chapter 2) and grown for seven days on PDA under continuous lighting at 20°C. Conidia were harvested by rinsing cultures with de-ionized water and filtering through three layers of cheesecloth to remove mycelia. Conidial concentration was adjusted to 2.5×10^5 conidia per mL in 5 L of de-ionized water using a hemacytometer.

Fungicide Treatments

In all three trials, treatments consisted of an untreated check, azoxystrobin (Quadris 250SC, 1.02 L / ha), famoxadone plus cymoxanil (Tanos 50WG, 0.56 kg / ha), mancozeb (Manzate 75WG, 1.68 kg / ha), acibenzolar-S-methyl (Actigard 50WG, 0.05 kg / ha), copper hydroxide (Kocide 2000 53.8DF, 2.24 kg / ha), and fludioxanil plus cyprodinil (Switch 62.5WG, 0.84 kg / ha). In Fungicide Field Trial 3, difenoconazole (Inspire 250 EC, 0.51 L / ha) was included. Each trial consisted of either three (Fungicide Field Trial 1 and 3) or four (Fungicide Field Trial 2) weekly applications beginning at late flowering to early fruit set (fruit size 25 to 50% of harvestable size).

Fungicide Applications

Applications were conducted using a back-pack CO₂ sprayer with a hand-made spray-boom containing three nozzles (Tee-Jet hollow-cone size 8) at 40.6 cm spacing. The two end nozzles dropped 10.1 cm below center and pointed inward at a 45° angle. The spray pressure was adjusted to 2.1×10^5 pascals, and all applications were conducted weekly at 7 to 10-day intervals using a spray volume of 325 L / ha. In Fungicide Field Trial 1 and 2, seven treatments were included, while Fungicide Field Trial 3 contained eight treatments (see ‘Fungicide Treatments’). For Fungicide Field Trial 1, the application dates were 29 November, 2006, 06 December, 2006, and 13 December, 2006. For Fungicide Field Trial 2, the application dates

were 04 May, 2007, 11 May, 2007, 18 May, 2007, and 25 May, 2007, and for Fungicide Field Trial 3, the application dates were 14 December, 2007, 21 December, 2007, and 30 December, 2007. No rainfall occurred within 4 hours after any of the applications, except for in Field Trial 3, where rainfall did occur within 2 hours after the second application (21 December, 2007). Within the treatment plots, the primary pepper (first pepper formed on primary inflorescence) was picked within one day prior to the first application in all three trials to allow for improved development and growth of the remaining secondary peppers.

Artificial Inoculation

An artificial inoculation was conducted for each trial within one day following the second (Fungicide Field Trial 1 and 3) or third (Fungicide Field Trial 2) application. For Fungicide Field Trial 1, 2, and 3 the inoculations took place during the evening (approximately 2300 hr) on 07 December, 2006, 18 May, 2007, and 21 December, 2007, respectively. Night-time was chosen to ensure adequate conditions for infection, either during or just prior to dew formation under the same conditions as described in Chapter 3. Inoculum was applied to the plants using a back-pack pump sprayer (Solo® 425 pump sprayer, Detroit, MI) until run-off, ensuring good coverage of fruit and foliage. Inoculum was applied to each pepper plant in all plots, and in some cases, plants in between the marked plots. A total of approximately 5 L of inoculum was used for each fungicide field trial. For all three inoculations, at least 8 hours of leaf wetness was obtained on the night of the inoculation.

Disease Assessments

Assessments were made by harvesting fully-sized, green pepper fruit and evaluating each fruit for lesions. The number of fruit with lesions was recorded for each plot, along with the number of healthy fruit. However, due to the nature of this disease upon artificial inoculation,

many of the younger fruit and flowers became severely infected and either fell off or aborted. Therefore, a large number of fruit that would have been counted as ‘infected’ were never developed and so were not counted in the assessments. For this reason, the amount of healthy fruit recovered per plot represented a better indication of treatment performance and was used as the primary assessment for all trials. For Fungicide Field Trial 1, only one harvest was conducted (28 December, 2006), while two harvests were conducted for Fungicide Field Trial 3 (06 January, 2008 and 17 January, 2008) and three harvests for Fungicide Field Trial 2 (25 May, 2007, 31 May, 2007, and 07 June, 2007).

Results

Disease Assessments

Within 7 days after the inoculation, lesions began to appear on pepper fruit in the untreated check plots for all three fungicide field trials. In most of the treated plots, both healthy and infected fruit were observed (Fig. 4-1). Harvests were made when the majority of fruit was fully-sized and comparable in size and shape to green bell peppers harvested commercially. Fruit were not allowed to ripen or change color prior to picking. For each plot, fully-sized green fruit were harvested and the number of fruit with lesions was counted along with fruit that were free of symptoms. In most cases, the number of infected fruit and overall disease incidence could not be properly assessed since many of the infected flowers and smaller fruit aborted prior to harvest (Fig. 4-2), especially in the untreated check plots. Nearly all of the undersized, developing fruit in the untreated plots contained lesions and never grew to harvestable size before rotting and falling off of the plant (Fig. 4-2). Therefore, many of these smaller fruit never developed and could not, of course, be scored as ‘infected’. As a result, the number of infected fruit per plot did not provide an adequate indication of disease severity for these plots, and for many of the treated plots. For that reason, the number of healthy fruit per plot was chosen as the

primary indicator of treatment performance, and is what is reported in the results. In the untreated check plots, nearly every fully-sized, harvestable fruit showed lesions. All fungicide treatments significantly reduced the amount of infected fruit, both harvestable and developing, in all three fungicide field trials in comparison to the untreated check plots. In each trial, the number of healthy fruit harvested per plot reflected the degree of efficacy for each of the fungicide treatments.

Fungicide Field Trial 1

In this trial, pepper plants were of unusually low vigor prior to conducting the fungicide treatments and inoculation. The reason for the low vigor in this trial was not determined, and the amount of harvestable peppers per plot – infected or uninfected – was low. Regardless, applications were conducted and both healthy and infected fruit were harvested 15 days after the last application. In the untreated check plots, a mean of 0.0 healthy fruit were harvested (Table 4-2), with a mean of 12 blemished fruit per plot. Many fruit became infected prior to developing into a fully-sized harvestable pepper fruit, and dropped prematurely (Fig. 4-2). Three treatments, azoxystrobin (Quadris), mancozeb (Manzate), and fludioxanil plus cyprodinil (Switch) provided the highest amount of healthy, harvestable fruit with mean numbers of 7.0, 8.0, and 8.3, respectively. The treatments with the least amount of harvestable, healthy fruit were copper hydroxide (Kocide 2000), acibenzolar-S-methyl (Actigard), and famoxadone plus cymoxanil (Tanos) with 3.0, 3.3, and 5.7 healthy fruit, respectively (Table 4-2). Copper hydroxide and acibenzolar-S-methyl provided significantly less control than azoxystrobin, mancozeb, or fludioxanil plus cyprodinil ($P < 0.05$). Further harvests from this trial were not possible, due to the reduced vigor of these plants, and the low amount of fruit that developed.

Fungicide Field Trial 2

In this trial, plants were much more vigorous than in Fungicide Field Trial 1, and three harvests were collected at 1, 6, and 13 days after the last application. The total number of healthy fruit collected from all three harvests was analyzed by ANOVA ($P < 0.05$) and means were separated by Fisher's Protected LSD (Table 4-2). In this trial, the treatments with the highest amount of healthy fruit were mancozeb (Manzate), azoxystrobin (Quadris), copper hydroxide (Kocide 2000), and fludioxanil plus cyprodinil (Switch) with 31.3, 30.3, 29.0, and 26.0, healthy fruit, respectively. No significant difference in healthy fruit was found among these three treatments. The treatments associated with the least amount of healthy, uninfected fruit were the untreated check plot, acibenzolar-S-methyl (Actigard), and famoxadone plus cymoxanil (Tanos) with 3.8, 16.0, and 17.0 healthy fruit, respectively (Table 4-2). There was a clear significant difference between the three best treatments, the three least effective treatments, and the untreated check plots (Table 4-2).

Fungicide Field Trial 3

Moderate to good vigor occurred in this pepper trial, and two harvests were obtained at 7 and 17 days after the last application. The total number of healthy fruit collected from both harvests was determined and significantly analyzed by ANOVA ($P < 0.05$) and means were separated by Fisher's Protected LSD (Table 4-2). In this trial, the treatments with the highest amount of healthy fruit were azoxystrobin (Quadris), difenoconazole (Inspire), famoxadone plus cymoxanil (Tanos), fludioxanil plus cyprodinil (Switch), acibenzolar-S-methyl (Actigard) and mancozeb (Manzate) with 33.3, 29.0, 24.3, 23.5, 23.0, and 20.8 healthy fruit, respectively. Azoxystrobin provided the best control and was significantly superior to all other treatments except for difenoconazole (Table 4-2; Fig. 4-3). The treatments that provided the least amount of healthy, uninfected fruit were the untreated check plot and copper hydroxide (Kocide 2000),

with 6.3, and 15.8 healthy fruit, respectively (Table 4-2). Copper hydroxide, which provided notably better control in the second trial, was among the least effective treatment in this trial. In addition, mancozeb, the other non-systemic fungicide evaluated in the trial, also provided slightly less control in this trial in comparison to the other fungicides in the other two trials.

Discussion

Fungicide field trials were conducted to evaluate various commercial fungicides for efficacy against pepper anthracnose in Florida. For this study, the isolate used to artificially inoculate the fungicide plots was an isolate of *Colletotrichum acutatum* recovered from Florida and therefore offered an opportunity to evaluate an anthracnose epidemic that might occur in Florida pepper fields. The fact that all fungicides evaluated in these trials provided moderate to good control in the presence of a severe epidemic leads us to conclude that fungicidal sprays can be an important tool in the integrated management of this disease in Florida. In some reports, this disease is purportedly difficult to control chemically (Hadden and Black, 1988; Kwon and Lee, 2002; Lewis-Ivey et al., 2004). The results reported herein clearly identify fungicides that can be used effectively under significant pepper anthracnose epidemics.

The fungicides evaluated in this study represent a fairly broad range of chemistries, mode of actions, and costs. Two of the nine fungicides tested are strobilurins, azoxystrobin and famoxadone, and these compounds are known to be highly active against certain species of *Colletotrichum*, including *C. acutatum* (Peres et al., 2005). Tanos, the fungicide brand that contains famoxadone, also contains cymoxanil, an oomycete fungicide with known activity against late blight and downy mildews. This component of Tanos has no activity on anthracnose but is added to widen the activity of Tanos to include the oomycetes, a significantly large and important group of plant pathogens. According to the label, Tanos is required to be applied in mixture with another fungicide containing an alternate mode of action, such as mancozeb,

chlorothalonil, or a copper-containing fungicide. According to the results obtained from these trials, where famoxadone and cymoxanil provided moderate to good control, the addition of mancozeb to this mixture might have significantly improved efficacy. With applications of straight mancozeb, good to excellent control was obtained in all three trials. Considering the price of application and spectrum of disease control, mancozeb clearly provides a cost-effective option that provides reasonable efficacy. Unfortunately, it is not currently labeled for use on pepper in Florida. Copper hydroxide, which also provided fair to good control in two of the three trials, might also increase the efficacy of famoxadone and cymoxanil (Tanos) and therefore serve as a possible tank-mix combination. In one of the two trials where copper hydroxide was less effective (Trial 3), rainfall occurred within two hours following the second application, and may have contributed to chemical “wash-off” and reduced efficacy. In this trial, the artificial inoculation was conducted on the evening of this application, so the need for a systemic or rainfast product at this application was probably crucial. The other strobilurin fungicide, azoxystrobin (Quadris), provided outstanding control in all three trials and overall provided the highest amount of harvestable fruit of any treatment (Table 4-2). Certainly, the use of azoxystrobin should be considered by any grower faced with heavy anthracnose disease pressure, and prudence dictates that azoxystrobin be mixed or alternated with other fungicides with an alternate mode of action to reduce resistance development, as directed by the label.

Other fungicides currently not labeled for pepper anthracnose in Florida that provided good to outstanding control in our tests were fludioxanil plus cyprodinil (Switch) and difenoconazole (Inspire). Switch, which is a premix of fludioxanil and cyprodinil, is commonly used in Florida for control of *Botrytis* blight and anthracnose on strawberry; it is not surprising that it provided good control for anthracnose on pepper. Both ingredients in this fungicide have activity on *C.*

acutatum, so this might help explain the increased efficacy of this product. In addition, this product and would have the extra benefit on pepper of controlling Botrytis blight. On strawberry, fludioxanil plus cyprodinil (Switch) is among the most effective fungicide combinations for anthracnose caused by *C. acutatum* (Harp, unpublished) as well as Botrytis blight (Dr. Jim Merteley, University of Florida, personal communication). Difenoconazole (Inspire) is a new fungicide from Syngenta Crop Protection that will be labeled for many leaf spot diseases, including early blight of tomato. Based on the results from one trial, this product certainly looks promising for control of pepper anthracnose should it ever achieve EPA registration for use on pepper.

One other product evaluated in this trial was acibenzolar-S-methyl (Actigard). This product is not a typical fungicide, but works by activating the SAR (systemic acquired resistance) pathway in plants. In short, acibenzolar-S-methyl activates various resistance genes (*R* genes) in plant cells that work to generate resistance proteins (R proteins) that help fight off attack by intruding pathogens. The mechanisms involved can be quite complex; however, it represents a fairly well-studied system in molecular plant pathology and is the focus of a great deal of academic research. In this study, artificial inoculations were conducted at either seven (Fungicide Trial 1 and Fungicide Trial 3) or 14 (Fungicide Trial 2) days after the first application in order to allow time for the SAR pathway to become activated and resistance expressed. If the artificial inoculation had been on the evening of the first application, acibenzolar-S-methyl would probably not have had sufficient time to activate the plant-resistance pathway and therefore probably would have failed to provide acceptable control. Although this treatment was not among the most effective of those tested, it did provide significantly improved control over the untreated check plots, and therefore could be quite useful in a program for integrated

anthracnose control. Acibenzolar-S-methyl is not currently labeled on pepper, but is labeled on tomato in Florida for control of bacterial spot, caused by *Xanthomonas* sp. The results presented in this study certainly show promise for this product on pepper and further research would be needed to evaluate rates, application timings, and the spectrum of disease control on pepper.

Based on the results from all three trials, the most effective fungicides tested were azoxystrobin (Quadris), fludioxanil plus cyprodinil (Switch), and mancozeb (Manzate). Copper hydroxide (Kocide 2000), provided very good control in the second trial, but provided less control in the first and third trial. As with mancozeb, it is likely that copper hydroxide is susceptible to wash-off and adverse environmental conditions common for strictly protectant fungicides. Difenoconazole (Inspire) also provided outstanding control equivalent to azoxystrobin, but was only tested in one of three trials. In all trials, famoxadone plus cymoxanil (Tanos) and acibenzolar-S-methyl (Actigard) treatments appeared to have more disease than some of the other products and therefore a lower amount of harvestable, healthy fruit. This was especially clear in Fungicide Field Trial 2, where both treatments provided significantly less uninfected, healthy fruit than all other chemical treatments. However, considering the heavy disease pressure that occurred in this trial as a result of the artificial inoculation, it could be argued that all treatments provided acceptable control of this disease and could be successfully used in a chemical management program aimed at controlling pepper anthracnose.

Although this study looked strictly at efficacy of these products applied alone, in reality a good fungicide program would consist of mixtures and alternations using products that contain different modes of action with perhaps a wider spectrum of target pathogens. Such a program would address resistance management concerns while providing optimal protection from an array of diseases at reasonable cost. A program such as azoxystrobin (Quadris) alternated with

mancozeb and / or fludioxanil plus cyprodinil (Switch) (if Botrytis blight is also present) would be ideal. If famoxadone plus cymoxanil (Tanos) is used, it should be mixed with copper hydroxide or maneb, as recommended on the label, and alternated with either azoxystrobin (Quadris) or fludioxanil plus cyprodinil (Switch). The active component in Tanos (famoxadone) is cross-resistant to azoxystrobin, so proper rotation and tank-mixing should be practiced for resistant-management purposes. Acibenzolar-S-methyl (Actigard) should not be used alone under heavy disease pressure, but it is likely that Actigard would contribute to efficacy in a fungicide program and perhaps would be effective under light disease pressure.

The purpose of this work was to evaluate various labeled fungicides, such as azoxystrobin, copper hydroxide, and famoxadone plus cymoxanil, for efficacy against a pepper anthracnose epidemic that might occur in pepper fields in Florida. In addition, some unlabeled fungicides were evaluated, since some of these could become labeled in the future. The overall intended aim of this study was to determine if chemical control of this potentially devastating disease was possible, and it appears from these results that acceptable control could be obtained from the use of fungicides. Certainly, further work is necessary to evaluate fungicide programs, i.e., alternations, timings, cost, etc., that could be implemented if pepper anthracnose continues as a significant problem in the future. This work should provide a starting point to determine the optimal chemistries and spray programs necessary for this disease and assist pepper growers in their effort to manage 'early anthracnose' of pepper.

Table 4-1. Plot size and planting conditions for peppers in the fungicide field trials. All crops were transplanted on raised beds covered in white plastic mulch with drip irrigation.

	Fungicide Field Trial 1	Fungicide Field Trial 2	Fungicide Field Trial 3
Plot size (m) ^w	4.6 x 1.5	4.6 x 1.5	3.7 x 1.5
No. plants/plot ^x	10	10	6
Row spacing (m) ^y	1.5	1.5	1.5
Plant spacing (cm) ^z	45.7	45.7	61.0

^wLength x width (m). ^xThe number of plants within each plot. Peppers were planted on raised beds in a single row. ^yRaised beds were 30 cm tall, 1.5 m wide, and 1.5 m apart. ^zCentimeters between plants within a row.

Table 4-2. Effect of fungicides on marketable yield of pepper artificially inoculated with *Colletotrichum acutatum* in three trials conducted in Florida in 2006 and 2007.

Treatment ^u	Number of Marketable Fruit ^v		
	Field Trial 1 ^w	Field Trial 2 ^x	Field Trial 3 ^y
Untreated	0.0 d ^z	3.8 c	6.3 d
Azoxystrobin	7.0 ab	30.3 a	33.3 a
Famoxadone plus cymoxanil	5.7 abc	17.0 b	24.3 bc
Mancozeb	8.0 a	31.3 a	20.8 bc
Acibenzolar-S-methyl	3.3 bcd	16.0 b	23.0 bc
Copper hydroxide	3.0 cd	29.0 a	15.8 c
Fludioxanil plus cyprodinil	8.3 a	26.0 a	23.5 bc
Difenoconazole	NT	NT	29.0 ab
LSD	3.76	8.47	8.61

^u Treatments were applied three (Fungicide Trial 1 and Fungicide Trial 3) or four (Fungicide Trial 2) times on a 7 to 10-day interval. ^v Number of total marketable fruit from all harvests per trial that were fully-sized, green fruit with no anthracnose lesions. ^w Field Trial 1 was conducted during the fall growing season, 2006, and the data represent the total number of marketable, healthy fruit from one harvest. ^x Field Trial 2 was conducted during the spring growing season, 2007, and the data represent the total number of marketable, healthy fruit from three harvests. ^y Field Trial 3 was conducted during the fall growing season, 2007, and the data represent the total number of marketable fruit from two harvests. ^z In each column, numbers followed by a different letter are significantly different ($P < 0.05$) by Fisher's Protected LSD.



Figure 4-1. Fully sized, harvestable fruit from the treated pepper plots inoculated with *Colletotrichum acutatum*. In each fungicide field trial, marketable peppers were found in treated plots that contained anthracnose lesions (left) or were healthy (right). Each fully-sized, marketable pepper, with or without lesions, was harvested and counted in each plot.



Figure 4-2. Heavy infection of the flowers and newly-formed fruit as a result of the artificial inoculation by *Colletotrichum acutatum*. In the untreated plots and many of the treated plots, the flowers (top left) and the newly-formed fruit became severely infected and usually aborted. Therefore, these fruit never obtained harvestable size and were not counted as 'infected' fruit during the assessments. As a result, the number of infected, harvestable fruit per plot was not a good measure of disease levels in a particular treatment plot. Instead, the number of healthy fruit obtained was the best representation of treatment efficacy for a particular treatment plot.



Figure 4-3. Harvested pepper from treated (left) and untreated (right) pepper plots in Fungicide Field Trial 3. The peppers on the left were picked from plot 102 (azoxystrobin, 1.02 L / ha) and the peppers on the right were harvested from plot 101 (Untreated). In this trial, azoxystrobin provided the least amount of infected peppers and the highest amount of marketable, healthy peppers.

CHAPTER 5 SUMMARY AND DISCUSSION

Colletotrichum acutatum caused anthracnose lesions on pepper fruit recovered from four pepper fields in Florida. The lesions formed on unripe, green fruit (Fig. 5-1), which was different than the well-described ‘ripe-rot’ anthracnose that reportedly occurred on ripened, red fruit (Alexander and Pernezny, 2003; Roberts et al., 2001). In this study, a collection of 50 isolates was recovered from pepper anthracnose lesions on pepper fruit from younger fields that contained unripe, green fruit, as well as mature fields that contained ripened, red fruit. Using ITS species-specific primers (Lewis-Ivey et al., 2004; Sreenivasaprasad et al., 1996), the isolates recovered from unripe, green pepper fruit were identified as *C. acutatum*, and the isolates recovered from the ripened, red fruit were *C. gloeosporioides*. This is the first report of *C. acutatum* as an anthracnose pathogen of pepper in Florida.

The implications of a new anthracnose disease on pepper caused by *C. acutatum* could be quite significant. The disease is an aggressive pathogen of pepper in Asia, and has recently been reported in the U.S. (Lewis-Ivey et al., 2004; Marvel et al., 2003). It causes destructive, sunken lesions on developing and fully-sized pepper fruit that essentially destroy the fruit and significantly decrease marketable yields. Clearly, new management strategies would be necessary to control this disease in fields where *C. acutatum* becomes well-established. Considering the impact of this disease on pepper in comparison to the ‘ripe-rot’ form of anthracnose, we propose the name ‘early anthracnose’ for anthracnose of pepper caused by *C. acutatum*.

In Florida, *C. acutatum* is an important pathogen of strawberry, citrus, dogwood, and lychee. In strawberry, anthracnose outbreaks caused by *C. acutatum* are extremely destructive and were responsible for heavy losses. Chemical control is currently the best means to control

strawberry anthracnose. Now that the pathogen has been found on pepper, further research leading to new management strategies is needed for successful control of this potentially destructive disease.

Field pathogenicity studies demonstrated that an isolate of *C. acutatum* recovered from pepper was not directly pathogenic on field-grown strawberries or tomatoes, both reported hosts of *C. acutatum*. This provides some sense of assurance that anthracnose epidemics in pepper fields pose little threat to nearby strawberry or tomato fields. However, in the laboratory using wounded, detached fruit, anthracnose lesions could be induced on both strawberry and tomato by injecting conidia into the surface of the fruit. This demonstrates that using detached, wounded fruit is probably not a good indication of pathogenicity for *Colletotrichum* pathogens on fruit, and challenges the validity of reports that have used detached fruit studies to evaluate host range of *Colletotrichum* spp.

Fortunately, chemical control measures do hold promise for managing pepper ‘early anthracnose’ in Florida. Fungicide field trials conducted over three seasons in east-central Florida have demonstrated that good efficacy can be obtained from labeled fungicides, even under heavy disease pressure. In addition, various unlabeled fungicides provided acceptable control and could be management options in the future pending registration by the EPA. All fungicides tested provided significantly improved control over the untreated check plots and could be used successfully in a chemical control program. Results provide hope to pepper growers that preventive chemical control options exist and could be implemented to control ‘early anthracnose.’

Anthracnose disease on pepper in Florida caused by *C. acutatum*, or ‘early anthracnose’, adds a new challenge for pepper growers throughout the state. The potentially destructive

disease can spread quickly and cause heavy losses if not managed and controlled. Further research on early anthracnose is needed in Florida to continue efforts to understand this disease and apply successful management strategies.



Figure 5-1. Pepper anthracnose isolate on unripe, green bell pepper caused by *Colletotrichum acutatum* in Florida.

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BIOGRAPHICAL SKETCH

Tyler L. Harp, son of Jack D. Harp of Vero Beach, Florida, and Janice L. Pingel of Edgemont, Arkansas, was born in 1970 in Saint Louis, Missouri. At 12 years old, Tyler moved from Saint Louis to Mountain Home, Arkansas, where he attended Jr. High and High School. He graduated from the University of Arkansas, Fayetteville, where he worked on staff as a Research Specialist from 1994 to 1998, with a bachelor's degree in biology and a Master of Science degree in plant pathology. During his time in Fayetteville, Tyler met and married his wife, Cheryl L., and had three children, Caleb Machin, Jordan Paisley, and Canaan Cole.

Shortly after obtaining his M.S. degree in 1998, Tyler became employed with Zeneca Agricultural Products in the Experimental Biology Department as a Research Scientist in Richmond, California. In 2001, Tyler accepted a relocation to work for Syngenta Crop Protection in the Biological Research and Development Department as R&D Scientist at the Vero Beach Research Center in Vero Beach, Florida, where he remained for over seven years. In 2004, Tyler enrolled as a Ph.D. graduate student at the University of Florida, Gainesville, in the Plant Pathology Department. He graduated with a Ph.D. in plant pathology in 2008 and continues his employment with Syngenta Crop Protection. Shortly after finishing his degree, Tyler accepted an 'International Assignment' to work at the Syngenta headquarters in Basel, Switzerland.

Tyler has maintained an active membership in the American Phytopathological Society (APS) since 1994, and continues to dedicate his time and interests to the study and application of plant pathology.