CANE DISEASES OF BLACKBERRY: IDENTIFICATION OF CAUSAL AGENTS AND MODIFICATIONS OF MANAGEMENT RECOMMENDATIONS FOR CANE BLIGHT AND ORANGE CANE BLOTCH

by

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(Under the Direction of Jonathan E. Oliver and Phillip M. Brannen)

ABSTRACT

Orange cane blotch (OCB) and cane blight (CB) are two prominent diseases of blackberry in the southeastern United States. OCB disease is characterized by cane cracking and yield reduction, and is caused by the parasitic alga *Cephaleuros virescens* (Cv). To understand the disease cycle and optimize management, field monitoring and chemical control studies were carried out. Results suggested that OCB is a monocyclic disease, that applications of potassium phosphite starting with algal sporulation are effective for disease control, and that in vitro potassium phosphite directly reduces algal growth. *Leptosphaeria coniothyrium,* the causal agent of CB, is thought to be the primary cause of cane dieback. However, field surveys revealed that *Fusarium oxysporum*, *Pestalotiopsis microspora*, *Neofusicoccum kwambonambiense*, *Neofusicoccum parvum*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia theobromae*, and *Colletotrichum siamense* are capable of causing significant blackberry dieback. A fungicide trial revealed that Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG (thiophanate-methyl) provided effective dieback control.

INDEX WORDS: Cane disease, Cane blight, dieback, orange cane blotch, *Cephaleuros virescens,* ProPhyt, potassium phosphite, *Fusarium oxysporum*, *Pestalotiopsis microspora*, *Neofusicoccum kwambonambiense*, *Neofusicoccum parvum*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia theobromae*, *Colletotrichum siamense*

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DEDICATION

To my loving mother, family, and friends who have supported me in all my endeavors.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Blackberry production. *Rubus fructicosis* are a type of caneberry grown in many regions of the world. In the United States, production is concentrated in California, Oregon, and Washington (NARBA 2017). Due to the development of blackberry cultivars with lower chill requirements and higher tolerance to heat, production in the southeastern U.S. has significantly increased. This is largely due to breeding programs in Arkansas, Louisiana, Virginia, and Texas, which, over the last fifty plus years, have bred for desirable traits crucial to production in the southeastern U.S. (Ballington 2016). It is thought that the numerous associated health benefits of blackberries, and their increased availability, has driven the demand for this fruit. In 2014, blackberry production in the United States was valued at $50.1 million, $45.2 million from processed sales and $4.9 million from fresh market sales (Marzolo 2015). According to the USDA, blackberry production and sales have more than tripled in the past ten years (NARBA 2017), and blackberry production in the southeastern U.S. has mirrored this trend.

Blackberry production in Georgia and the Southeast in general has been made possible by new cultivars with lower chilling requirements; however, several challenges still persist. These include high humidity, heat, and the short winters. These climate factors allow many pathogens and pests of blackberry to thrive in the southeastern U.S. According to the 2015 Farm Gate Value Report (Wolfe and Stubbs 2016), Georgia blackberry production was valued at just over seven million dollars, constituting fourteen percent of blackberry production in the United States.

The production of blackberry within Georgia is concentrated in the Southern Coastal Plain region. In blackberry production, farmers desire an extended growing season, and the Coastal Plain region is by far the most appropriate area for achieving an extended market window. Northern Georgia might provide a more suitable environment with relatively less disease and insect pressure, but because of a shorter growing season, potential for freeze damage, more expensive and less available land, and the lack of processing and buying points, production in the northern part of the state has not been significantly developed.

Different types of blackberries are grown in Georgia. Although thornless cultivars are typically preferred, thorny cultivars are also produced. Furthermore, the stature of the blackberry plant can vary, resulting in what are termed 'erect', 'semi-erect', and 'trailing' types; 'erect' and 'semi-erect' cultivars are the preferred choice in large-scale commercial production in Georgia. All blackberries grown in Georgia are trellised. Popular cultivars in Georgia and the Southeast in general include 'Ouachita', 'Navajo', and 'Arapaho'. Currently, breeding programs at the University of Arkansas, North Carolina State University, and the USDA (Poplarville, MS) are actively creating new blackberry cultivars (Ballington 2016). Many of these new cultivars are expected to be even better adapted to the growing conditions of the southeastern U.S.

Major diseases of blackberry. There are many diseases that affect blackberry production. These diseases may simply impact yield or, in other cases, kill the plant – causing huge economic losses. Algal, fungal, oomycete, and viral pathogens, as well as nematodes, cause disease on blackberry. Both thorny and thornless cultivars are susceptible to diseases caused by these pathogens. All parts of the plant can be impacted including fruit, leaves, stems, canes, and roots. The prevalence and impact of these diseases are exacerbated by the high humidity, short winter, and heat of the southeastern U.S.

Common fungal diseases of blackberry include anthracnose, cane and leaf rust, fruit rot, cane blight, blackberry rosette, Botryosphaeria cane canker, powdery mildew, and Phytophthora root rot (Kirkpatrick and Sanders). Fungal diseases are by far the most prevalent. Symptoms typical of these diseases are lesions, stunted growth, shoot dieback, defoliation, and fruit drop. Disease control is achieved with exclusion, cultural management, and regular use of targeted and broad-spectrum fungicides.

Bacterial diseases are less common, but can have equal or greater impact on the crop. Crown gall and hairy root are the most common bacterial diseases of blackberry. These diseases are caused by *Agrobacterium rubi, Agrobacterium tumefaciens,* or *Agrobacterium rhizogenes* (TAMU). Crown and hairy root galls only differ in their location on the plant. All cause localized tumorous growth following bacterial insertion of portions of bacterial DNA into the plant and the subsequent production of undifferentiated cell growth (Kirkpatrick and Sanders). Some galls may form in clusters while others are solitary. Though rare in the southeastern U.S., fire blight, caused by *Erwinia amylovora* (Schilder 2007), is another bacterial disease of blackberry. This disease is easily identified by blackened cane tips and seeping bacterial ooze. There are limited antibiotic or chemical control options for bacterial diseases in blackberry, and exclusion and cultural methods are therefore emphasized for disease management.

Over thirty viral pathogens of blackberry have been classified (Martin et al. 2013). These viruses are vectored by many different organisms such as nematodes, whiteflies, aphids, thrips, and mites (Martin et al. 2013). Many are also transmitted through pollen and seed. Blackberry yellow vein disease is seemingly the most prevalent, but the symptoms caused by many of the viruses infecting blackberry are similar. Symptoms indicative of virus infection of blackberry include intraveinal and interveinal chlorosis, mottling, stunting, leaf curl, ring spot, and fruit drop

(Martin et al. 2013). Control of viruses is achieved primarily through cultural methods, exclusion and removal of infected plants, sanitization of tools, and vector control.

Nematodes can also be an issue, especially in sandy soils like those prevalent in the coastal plain of Georgia. Various species of nematodes can cause problems such as root knot, root swelling, and dwarfed fruiting canes and fruit (TAMU). In addition, dagger nematode (*Xiphinema americanum*) is highly associated with viral transmission (Williamson 2017). For nematodes, there are many recommended chemical and cultural controls including soil fumigation prior to planting in areas known to be infested. Unfortunately, post-plant options have not been found to be effective, and few chemicals or biological control agents are registered for this purpose.

Orange cane blotch (OCB) is caused by the algal species *Cephaleuros virescens*. This alga causes orange blotches on the canes of the plant, and blackberry growers associate its presence with yield loss and plant death. Recent research has shown that this organism negatively impacts the blackberry plant and its fruit production. Studies show a strong negative correlation between increasing disease severity and yield as a result of decreased fruit number (Browne et al. 2019a). This reduction in yield is most likely the cumulative effect of some of the many symptoms of the disease. These include cane girdling and cracking, desiccation of plant tissues, and intercellular and intracellular cell growth among plant cells.

Cane blight (CB) is a fungal disease of blackberry and other *Rubus* spp., caused by *Leptosphaeria coniothyrium* (Jones 2015). In the southeastern United States, CB can have a significant impact on blackberry production. In other areas, it is not typically reported as a major disease and is more prevalent on raspberry (Brannen and Krewer 2005). However, the humid and warm climate of the southeastern U.S. allows for this pathogen to thrive. This is especially true if

the host plants are injured, exposing susceptible tissues for infection. In this case, CB can impose significant economic losses. *L. coniothyrium* can be found worldwide on several hosts other than *Rubus* spp. It has been reported causing symptoms on alternative hosts, largely stem canker of roses and other ornamentals (Brannen and Krewer 2005). It has also been identified on bamboo, strawberry, stone fruits, and blueberries. However, cane blight disease in these crops is not extensively studied.

There are also several other fungi associated with blighted canes that resemble the symptoms caused by *L. coniothyrium*. *Botryosphaeria dothidea*, *Botryosphaeria obtusa*, *Neofusicoccum parvum*, *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, and *Fusarium oxysporum* have all been associated with blackberry cane dieback (Faedda et al. 2018; Maas et al. 1989; Marulanda et al. 2014; Pastrana et al. 2017). These species are known to be pathogenic on a wide array of plant species leading to vast array of symptoms (Gonsalves and Ferreira 1993; Maas et al. 1989; Marulanda et al. 2014; Pastrana et al. 2017; Peres et al. 2005). However, in the Southeast the exact organisms involved in causing blighted canes and cane dieback are not known.

In the southeastern U.S., cane blight and orange cane blotch are significant diseases affecting blackberry production, yet they are poorly understood. The OCB disease cycle, the timing of infection with respect to susceptibility to cane blight, and interactions with other cane pathogens have not been extensively researched. Both of these diseases are among those that can have a significant impact on yield and crop survival in blackberry production. In addition, there may be other cane diseases that have not been identified. Knowledge gained through research on these diseases and the pathogens that cause them could lead to improved disease management practices and reduce the negative economic impact that they have on the industry.

Cane Blight of Blackberry. Cane blight is caused by *Leptosphaeria coniothyrium* of the Ascomycota phylum, Pleosporales order, and Leptosphaeriaceae family. This pathogen may also be referred to as *Paraconiothyrium fuckelii, Kalmusia coniothyrium, Microspharropsis fuckelii,* or *Coniothyrium fuckelii* (Williamson 2017). It reproduces both sexually and asexually. Sexual structures are pseudothecia which produce bitunicate asci, each containing eight pale olive brown ascospores with three septa (Williamson 2017). Asexual structures include pycnidia that produce globose, single-celled conidia. When spores are released, the canes appear to be grayish-silver (Humphreys 1975). Both structures are embedded in the necrotic bark tissues. The disease can be identified in the field by the symptoms induced, and *L. coniothyrium* can be definitively confirmed in a laboratory using morphology or molecular methodology. Incubation of diseased canes in moist chambers will induce both types of sporulation within three to four days (Brannen and Krewer 2005). Microscopic observation of the sporulating structures and spores themselves are sufficient to confirm the presence of the pathogen. With the threat that this pathogen poses to blackberry production in the southeastern U.S., research is needed to better understand its epidemiology and management.

Although CB may not always kill the plant, cane dieback can significantly reduce fruit yield (Jones 2015). Infections are associated with wounds caused by a multitude of factors. One primary location of infection is on primocanes following pruning. Symptoms of CB have been described as dark red to purple lesions with purple borders (Brannen and Krewer 2005). These may form in the summer, fall, and winter following wounding, and may expand and merge, thereby girdling the cane. CB has a direct effect on yield, killing the cane and inhibiting new cane development above the site of infection. Areas above girdled canes become blighted and die. CB infected canes produce weak growth and wilt as fruit develops or begins to develop

(Snover-Clift and Jensen 2012). Pathogen fruiting structures and asexual pycnidia are visible as small black bumps embedded on the diseased or dead cane tissues (Brannen and Krewer 2005).

The disease cycle of CB is largely understood. *L. coniothyrium* overwinters on dead floricanes (if not removed), infected floricanes, or on debris on the ground (Brannen and Krewer 2005). Sexual or asexual spores land on injured blackberry tissues during the summer and fall. They may be spread by wind, rain splash, or insects (Snover-Clift and Jensen 2012). Wounds are important to the pathogen, allowing ingress of mycelia into vascular tissues and initial infection establishment (Williamson 2017). Without a wound, hyphae may still penetrate the epidermis, but it has been observed to take several months to do so (Williamson 2017); therefore, significant disease is not expected to occur without a wound as an entry site. Experiments have also shown that the age of the plant and timing of wounding are important in the success of disease development. The open wounds from the removal of floricanes after harvest are of primary concern; however, injury may also result from extreme weather, other diseases, pruning, field maintenance equipment, insects, trellising structures, or the rubbing together of canes. Rainfall immediately following wounding increases the likelihood of infection (Brannen and Krewer 2005). Disease seems to be most severe when drought stress occurs after infection (Bost 2015). Once the infection becomes established, it spreads through the cane during fall and winter. This causes lesion or canker formation, floricane bud failure, and cane dieback (Brannen and Krewer 2005; UofM 2017).

There are several instances where *L. coniothyrium* has been observed as a secondary pathogen. In a 1930 observation of yellow rust of raspberry in Oregon, *L. coniothyrium* was found to colonize the cankers induced by yellow rust (Zeller 1930). It was found that *L. coniothyrium* enlarged the cankers it colonized, often leading to sunken and split areas on the

cane. The combination of pathogens often killed the raspberry plants. Another study in Scotland described a similar interaction between a raspberry cane midge, *Resseliella theobaldi*, and *L. coniothyrium* (Williamson 1984). Although much is known about the CB disease cycle in blackberry, the epidemiology of the disease is lacking with regard to possible interactions of *L. coniothyrium* with other cane pathogens, such as *C. virescens*. Further understanding the CB disease cycle will allow for the development of more effective management strategies. With other diseases, more comprehensive understanding of disease cycles have led to more precise timing of chemical controls as well as many other disease management practices. These may include targeting a pathogen, host, or vector when it is most susceptible to limit disease.

Cultural controls are a suggested means of limiting infection and have been established for CB. Cultural practices include reducing plant stress, limiting wounding, greater in-row and between-row spacing of plants and thinning plants for increased air flow, installing good drainage systems, reducing weed presence, using drip irrigation instead of overhead irrigation, reducing primary inoculum by removing all crop debris, pinching off herbaceous tissue instead of clipping off, and removing infected canes and old floricanes as soon as possible after harvest (Brannen 2012; Brannen and Krewer 2005; Jones 2015; Snover-Clift and Jensen 2012; Williamson 2017).

Chemical controls for cane blight management are not as well studied. Effective fungicides have been identified, but efficacy, application timing, and targeted spray sites have not been tested extensively. Brannen and Krewer (2005) recommend applying fungicides after pruning each day to protect wounds until they heal. Fungicides with some degree of effectiveness include benomyl, thiophanate-methyl, carbendazim, pyraclostobin and combinations of these (Williamson 2017). In the United Kingdom, spraying before, during, or

immediately after harvest has proven effective against CB. Spraying cut ends after pruning as well as covering the bottom half of all canes are also recommended.

The raspberry cultivar 'Latham' and *Rubus pileatus* species of raspberry have shown tissue resistance to CB (Williamson 2017). Similar resistance testing of blackberry cultivars has not been reported. Collectively, there are knowledge gaps in the understanding of the epidemiology and control of CB in blackberry. Further research in these areas would lead to advancements in controlling this potentially devastating disease.

Orange Cane Blotch of Blackberry. Orange cane blotch (OCB), also known as orange felt disease, is caused by the algal species *Cephaleuros virescens* (Brannen 2012). This parasitic alga thrives in subtropical and tropical climates (Nelson 2008). *C. virescens* has been reported on almost every continent in tropical to subtropical regions and has been identified on at least 287 plant species and cultivars (Holcomb et al. 1998). OCB of blackberry was first reported in the summer of 1997 in Arkansas (Holcomb et al. 1998). The symptoms at the time were noted as cracked stems, discolored sub-bark tissue, and numerous orange, velvet-like lesions on the canes. The coloration of the lesions was discovered to be caused by pigmented sporangiospores and zoosporangia (Holcomb et al. 1998). After the identification of OCB in blackberry, emerging populations of OCB have been recognized throughout the southeastern United States.

The current understanding of algae as plant pathogens is quite limited. Algae are a polyphyletic group, and much of the nomenclature used to describe algae is the same as that used with fungi. Algae, for clarification purposes, are members of a group of photosynthetic organisms in the kingdom Protista that are largely aquatic (Andersen and Lewin 2017). Parasitic algae are known to be filamentous and are in the Trentephlicaeae family (Brooks 2004); however, not all algae in this family are parasitic. Their lifestyles range from free living to

damaging plant parasites; however, all require moisture, either humid air or direct contact with a water source, to survive and reproduce (Brooks 2004). There are six genera of the Trentepohliales order that contain plant parasites. These include *Cephaleuros, Stomatochroon, Phycopeltis, Physolinum, Trentepohlia*, and *Printzina* (Brooks 2004). *Cephaleuros* spp. typically live under the leaf cuticle, and *C. virescens* targets the cane epidermis. *Stomatochroon* spp. target substomatal chambers of leaves; species of *Phycopeltis* and *Physolinum* can target many plant tissues; and species of *Trentepohlia* and *Printzina* are found on both non-living and living mediums (Brooks 2004). Host ranges of these algae are immense. There are over four hundred host plants of *Cephaleuros* spp. alone (Brooks 2004). In some cases, fungi have parasitized these parasitic algal genera to form lichens; however, in these cases, it was found that the alga injures the plant before the fungus colonizes it (Brooks 2004).

When reviewing the anatomy of *Cephaleuros* spp., it becomes apparent how these algae may damage their host. The thallus consists of branched filaments in the form of irregular disks. The thallus can form either below the leaf cuticle or below the epidermis, as in OCB, and it later becomes pigmented orange from carotenoid production (Brooks 2004). Sterile and fertile filaments protrude through the cuticle or bark of the host plant, with the fertile filaments bearing sporangiophores with zoosporangia for reproduction. Haustorial cells have also been observed inside host tissues, suggesting possible nutrient exchange between the two organisms (Nelson 2008). Damage from plant parasitic algae is thought to originate from the reduced photosynthesis due to physical coverage of host cells by the algae, intercellular and intracellular intrusion of algal filaments into plant tissues, and the desiccation of nearby host plant cells; however, there has been little research confirming these hypotheses. The degree of damage observed also varies drastically between host plant species and environment. For example, top-down foliar necrosis

has been observed with *C. parasiticus* on guava, but in other instances there was no observed damage at all (Nelson 2008). Additional research on plant parasitic algae would significantly aid in our understanding of these organisms as a whole, and would likely lead to advancements in the management of diseases such as OCB.

The specific symptoms and signs of OCB disease are similar to those caused by other parasitic algae, but they do differ. C. *virescens* colonizes the canes of the blackberry and can be identified by the presence of diagnostic yellow-orange lesions. These lesions may be initially confused with blackberry rust, but can easily be distinguished through microscopy. Lesions are typically localized to the lower sections of the cane and become visible in late summer (Brannen 2012). Immature thalli may initially be green in color, but as they mature and start to sporulate, they become pigmented (Nelson 2008). The pathogen may spread through the dispersal of the mobile flagellated zoospores which cover the lesion surface, as well as through the expansion of the filamentous algae into new tissues; however, there has not been extensive research as to these mechanisms. The primary concern is that lesion development can lead to girdling and desiccation of the canes. Girdling occurs when these lesions merge due to their growing diameters. Eventually, these form a large lesion that surrounds the entire cane. Combined with the observed damage to the epidermal and cortex cells, cane death is possible (Chapman and Good 1983). In the areas of merged or large lesions, canes crack, possibly leading to invasion by secondary pathogens. This could further exacerbate disease damage and adds to the complexity of this disease in the field.

The disease cycle of OCB on blackberry is not fully understood. It is thought to be similar to diseases caused by *C. virescens* on other host plants, but little research has been done to verify this on blackberry. Both asexual and sexual reproduction have been observed. Hosts are

inoculated when either thallus fragments, zoospores, or meiozoospores land on susceptible host tissues (Nelson 2008). Some theorize that these structures can directly penetrate plant host tissue and others believe a wound is necessary for infection. Several insects and arachnids that feed on plant tissues have been observed carrying zoosporangia of *Cephaleuros* spp. (Chapman and Good 1983); however, this interaction has not been observed on blackberry, and further research would be needed to verify this mode of transmission. Algal fragments, zoospores, and meiozoospores all give rise to new disc-like algal thalli, with algal filaments colonizing below the cuticle or epidermis of the host plant. Filamentous algae have been seen largely growing intercellularly, but in some cases they may grow intracellularly (Brooks 2004). This has not been confirmed in blackberry and intracellular growth has only been reported in wounded or weak epidermal cells. It is not known if the algae are the cause of the damage done to probed cells, or whether they are simply opportunistic. After establishing themselves, the algae then undergo asexual or sexual reproduction resulting in the production of new propagules for dispersal.

Algal fragments and zoospores are formed by asexual reproduction. Zoospores are released by zoosporangia on sporangiophores from terminal and lateral cells of the algal thallus (Chapman 1976). Zoospores are quadriflagellate, and efficiently travel through films of water (Chapman 1976). They are thought to serve as the primary inoculum for OCB (Chapman 1976; Nelson 2008). Meiozoopores represent sexual reproduction propagules, and their role in the disease cycle of OCB is unknown. They originate from meiozoosporangia from a sporophyte generated by fused gametes that fuse externally near the parent thallus or within a gametangium (Chapman and Good 1983).

These algae overwinter in the lesions they cause, either on fallen or dormant plant tissues (Nelson 2008). Lesions become apparent throughout the summer and continue to expand and

coalesce. Currently, the disease cycle of OCB is thought to be monocyclic based upon the interactions between Cv and its other hosts, but this has not been verified for OCB (Suto and Ohtani 2013). In the case of OCB, the disease cycle is thought to take eight to nine months to complete (Brannen 2012), but this has not been extensively studied.

Limited research has been conducted relative to the management of OCB. Cultural methods have been recommended, but these have not been researched to confirm their utility. Cultural practices recommended to help manage OCB include removal of diseased crop debris, field drainage pipes to reduce humidity and standing water, greater plant spacing, removal of floricanes immediately after harvest, use of drip lines instead of overhead irrigation, intercropping, reducing plant stress, and thinning of blackberry plants (Nelson 2008). Unfortunately, these measures are not sufficient to control OCB in the southeastern United States.

Though several chemical control trials have been conducted, only one chemical, ProPhyt (potassium phosphite; Luxembourg Chemical) was found to suppress OCB development in blackberries (Browne et al. 2019b). Other phosphonate fungicides may also be effective for OCB and other parasitic algal diseases. It is not known exactly how potassium phosphite impacts this alga, but in other cases, the impact can be direct (toxicity) or indirect (turning on the host plant defenses) (Spolti et al. 2015). More research is needed to understand if the control is due to a reduction in size or number of lesions on the canes of infected blackberry. Previously, copper products were recommended, but success in controlling OCB has not been reported in Georgia. This could be because Cv exists largely within the epidermis of the plant cane and is protected from surface applications of copper, which has contact activity only.

JUSTIFICATION AND OBJECTIVES

Orange cane blotch of blackberry. Significant research has been conducted on other parasitic algae and the diseases they cause, but knowledge gaps exist pertaining to OCB on blackberry. Many of these knowledge gaps pertain to the disease cycle and mechanisms of the disease. It is also unclear how potassium phosphite reduces disease severity. Data from previous studies have not shown if the size and or number of algal blotches are reduced. It is also unknown if potassium phosphite is directly toxic to the pathogen or is activating plant defenses. These are critical knowledge gaps to be filled if control measures are to be optimized. This disease is prolific and can cause extensive economic losses. The goals of this work were to develop information that will allow a more effective disease management program. Accordingly, the objectives of this study were as follows:

1) Determine the distinctive features of the orange cane blotch disease cycle on commercial blackberry (Chapter 2).
2) Detail the impact of potassium phosphite (ProPhyt®) on *Cephaleuros virescens* and establish new management recommendations for orange cane blotch disease of blackberry (Chapter 3).

Cane blight and other cane diseases of blackberry inducing cane dieback. As a whole, knowledge is insufficient regarding the epidemiology and management of cane blight and other cane dieback diseases of blackberry, especially in the southeastern United States. Fungicide applications after pruning are recommended, but efficacy trials, timing of applications, and targeted spray sites have not been extensively researched. Cane blight is potentially severe, but occurs sporadically, making it difficult to study and test in the field. Work in both the greenhouse and field would help develop and validate any new management strategies. In this work, fungal

isolates collected from blighted canes in field surveys were used to compare the efficacy of fungicides on intentionally inoculated wounds. Knowledge gained from the results of the experiments conducted in this work should aid in developing new management strategies that can be implemented to reduce the economic impact of cane blight and dieback. The objectives of this study were as follows:

1) Identify organisms associated with cane dieback of commercial blackberry in Georgia and develop options for chemical management (Chapter 4).

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CHAPTER 2

ORANGE CANE BLOTCH DISEASE CYCLE ON CULTIVATED BLACKBERRY (*RUBUS FRUCTICOSIS*) [1]

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ABSTRACT

The high humidity and short mild winters of the southeastern United States are conducive for many plant diseases. Among these is an algal disease of blackberry known as orange cane blotch (OCB) caused by *Cephaleuros virescens* (Cv). Since its discovery on blackberry, its presence has been associated with cane cracking, cane girdling, and yield loss. Research detailing the disease cycle and disease management on blackberry is limited and is largely inferred from the interactions of Cv with its other hosts. To further detail the disease cycle of OCB, diseased canes were examined by photography and microscopy. By combining observations made from photography and microscopy, key events in the disease cycle on blackberry were elucidated as they correspond to the phenology of blackberry. The alga was observed to be active for a majority of the season, only exhibiting dormancy from December through mid-April, concurrently with blackberry. While it appeared that the presence of algal sexual reproductive structures did not coincide with emerged primocanes, asexual reproductive structures were observed in the summer while primocanes were present. All new infections on newly emerged primocanes appeared around mid-summer, indicating a single infection cycle per year for OCB. These findings provide a foundation for further study and the development of targeted management strategies to protect noninfected canes and inhibit OCB progression.

INTRODUCTION

Orange cane blotch (OCB) of blackberry, also known as orange felt disease, is caused by the alga *Cephaleuros virescens* (Cv) (Brannen 2012). This parasitic alga is filamentous in morphology and belongs to the Trentephlicaeae family (Brooks 2004). It thrives in subtropical and tropical climates (Nelson 2008), and it has been identified on 287 plant species and cultivars (Holcomb et al. 1998). OCB was first reported in the summer of 1997 in Arkansas (Holcomb et al. 1998). Since then, emerging epidemics of OCB have been recognized throughout the southeastern United States (Holcomb et al. 1998). Its presence on blackberry is associated with cane cracking (Browne et al. 2019a), cane girdling (Brannen 2012), and subsequent yield loss (Browne et al.).

The disease cycle of OCB on blackberry is not fully understood (Browne et al. 2019b). It is thought to be similar to diseases caused by Cv on other host plants, but little research has been done to verify this on blackberry (Browne et al. 2019b). Infection is believed to occur when either thallus fragments, zoospores, or meiozoospores land on susceptible host tissues. These give rise to new disc-like algal thalli, with algal filaments penetrating below the cuticle or epidermis of the host plant (Nelson 2008). The mode of entry into susceptible host tissues is currently unknown, but these propagules likely either enter through wounds, natural openings like lenticels or stomata, or directly penetrate the host tissue (Chapman and Good 1983). Filamentous algae have been seen largely growing intercellularly, but in some cases they may grow intracellularly (Brooks 2004). This has been confirmed in blackberry, where intracellular growth was observed on occasion. However, it was not known if algal filaments penetrated these cells or entered only after the cell had been compromised (Browne 2017). After infection and colony expansion, Cv undergoes asexual and sexual reproduction resulting in the production of

new propagules (Nelson 2008). Algal fragments and zoospores are formed by asexual reproduction. Zoospores are released by zoosporangia on sporangiophores from terminal and lateral cells of the algal thallus (Chapman 1976; Suto and Ohtani 2009). Zoospores are quadriflagellate and travel efficiently through films of water (Chapman 1976). They are thought to serve as the primary inoculum for Cv (Chapman 1976; Nelson 2008). Quadriflagellate meiozoospores represent sexual reproduction propagules, and their role in the disease cycle of OCB is unknown, but they are assumed to infect similarly to other propagules and produce a typical thallus (Browne 2017; Browne et al. 2019b; Chapman and Good 1983). Meiozoospores are produced by meiosporangia which originate from a zygote resulting from fused biflagellate isogametes. These isogametes are produced within a gametangium and can either fuse within the gametangium or externally near the parent thallus (Brooks et al. 2015). These algae overwinter in the lesions they cause, either on fallen or dormant plant tissues (Nelson 2008). Lesions become apparent throughout the summer and continue to expand and coalesce. Other diseases caused by Cv are monocyclic (Suto and Ohtani 2013), but this has not been verified with OCB on blackberry (Browne et al. 2019b). The OCB disease cycle is thought to take 8 to 9 months to complete (Brannen 2012), but it has not been studied extensively.

In this study, the objective was to validate and further characterize the disease cycle of OCB induced by Cv in commercial blackberry plantings. To accomplish this objective we relied on photographic images and microscopy of diseased canes. Photographic images were used to record the emergence and development of symptoms on the surface of primocanes (first-year canes) and floricanes (second-year canes). Observations from microscopy were used to characterize the algal structures that were present within blackberry epidermal tissue and when they were present. Host phenology was recorded throughout the study through field observations.

Information gathered on OCB from both photographic observations and microscopy were combined to elucidate the disease cycle on blackberry.

MATERIALS AND METHODS

Commercial field sites. For observing season-long progress of OCB on blackberry, three southern Georgia commercial blackberry plantings were used. Each site was selected because of its high incidence of OCB. One planting was located in Lanier County (cv. 'Osage') and two plantings were located in Irwin County (cv. 'Ouachita'). For duration of the observational work, growers were asked not to spray the studied rows or their two neighboring buffer rows with any phosphonates to minimize extraneous impacts on the algal pathogen. Otherwise, management was consistent with commercial blackberry practices observed in southern Georgia.

Confirmation of causal organism. Isolates of Cv were obtained from infected blackberry tissue collected from all three plantings and plated on Bold's basal medium (PhytoTechnology Laboratories, Shawnee Mission, KS) containing 10 ppm of the organic fungicide benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) (Chem Service Inc., West Chester, PA) and 50 ppm of the antibiotic streptomycin (Browne et al. 2019a; Suto and Ohtani 2010). Algal filaments were taken from growing isolates for DNA extraction using 5% Chelex®100 sodium (Sigma-Aldrich, St. Louis, MO). PCR was performed using GRC (Hamby et al. 1988) and PCRB (Medin et al. 1988) primers based upon a modified PCR protocol (Rindi et al. 2009). Specifically, each 20 µl reaction contained 10µM of 1µl GRC (AGGGCAAGTCTGGTGCCA), 1µl PCRB (TGATCCTTCTGCAGGTTCACCTAC), 7 µl molecular grade water, and 10 µl 2x GoTaq® Green Master Mix (Promega, Madison, WI). The PCR protocol consisted of an initial denaturing phase of 95°C for 1 min, followed by 34 cycles

of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min, and an 8 min final extension at 72°C. A BioRad S1000s Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) was used for amplification, and the PCR product was visualized on a 1% agarose gel stained with GelRed Nucleic Acid Stain (Biotium, Fremont, CA) using a BioRad Molecular Image Gel Doc XR+ with Image Lab Software (Bio-Rad Laboratories, Hercules, CA). The PCR product was cleaned using an E.Z.N.A.® Cycle Pure Kit (Omega Bio-tec, Inc., Norcross, GA), checked for quality using a NanoDrop One C spectrophotometer (ThermoFisher Scientific, Waltham, MA), and then sent to Eurofins Genomics (Louisville, KY) for sequencing. Sequences were trimmed, aligned, and edited using Geneious v. 2019.1.3 (Geneious, Auckland, New Zealand). Isolates were confirmed to be Cv by comparing processed sequences to known Cv sequences in the Genbank NCBI database (National Center for Biotechnology Information, Bethesda, MD) using the BLASTn function. Sequences with a greater than 99% query coverage, greater than 98% identity, and an E-value of 0 to the best match sequence in Genbank were considered to belong to the same species.

Photographic observation of diseased canes. Starting in October of 2017, blackberry canes in the three selected plantings exhibiting symptoms of OCB were arbitrarily selected and secured to wooden stakes using cable ties. A 20-cm zone of interest was marked on these wooden stakes. A photograph was taken of this 20-cm zone of each cane every 2 weeks and saved for analysis and comparison over time. The corresponding phenology of blackberry at each photography time point was also recorded. This process was repeated for two full seasons from primocane emergence to floricane removal (Oct 2017 to Jul 2018, Jun 2018 to Jul 2019, and Jul 2019 to Nov 2019). From Oct 2017 through Jul 2018, photos were taken of 40 marked canes in total with 20 at the Lanier County location and 10 at each Irwin County location. From Jun 2018

through Jul 2019 and Jul 2019 through Nov 2019 photos, there were a total of 30 marked canes for each time interval, 10 in Lanier and 10 for each Irwin County location. Blackberry canes were photographed with a Nikon Coolpix B700 camera (Nikon, Tokyo, Japan).

Microscopy of diseased tissue. To observe the progression of OCB within the blackberry tissue and on the epidermal surface, microscopy slides and Z-stack microscopy methods were used to examine samples of diseased tissue. To prepare microscopy slides, a razor blade was used to excise sections of OCB blotches. Sections were placed in a fixative in the field and delivered for processing and staining at the Georgia Electron Microscopy (GEM) facility at the University of Georgia. The fixative and buffer consisted of 2% glutaraldehyde in 0.1M potassium phosphate buffer (pH 7.2). At the microscopy laboratory, at 4°C, samples were rinsed two times in buffer for 15 min each and post-fixed in 1% osmium tetroxide for 2 h. Samples were then rinsed at room temperature for 15 min two times in deionized water and dehydrated using a graded ethanol series 25%, 50%, 75%, 95%, 100%, and 100% followed by two changes in 100% propylene oxide (PO). Samples were infiltrated in PO and Spurrs resin, with 8 h each in 75% PO and 25% Spurrs, 50% PO and 50% Spurrs, 75% PO and 25% Spurrs, 100% Spurrs, and then 100% Spurrs. Samples were polymerized at 60°C for 24 h. One-micron-thick sections were cut using a Diatome diamond knife and a Reichert Ultracut E ultramicrotome. Sections were placed on Superfrost Plus slides and dried on a hot plate. Slides were stained with 1% toluidine blue staining solution for 1 min and viewed with light microscopy. Microscopy sections were made from samples collected monthly from Dec 2017 to Jun 2018. For each month, nine blotch sections were collected for processing, three from each planting in Lanier and Irwin Counties. Processed sections on glass slides were examined and analyzed for each of the sampling periods, and changes over time in algal vegetative and reproductive structures between healthy and

diseased tissue were recorded. Z-stack microscopy, an image processing method which combines images of different focal planes into one homogeneous image, was performed periodically throughout the season. This imaging process captures detailed images of three-dimensional surfaces or objects like the protruding asexual reproductive structures of Cv. Diseased cane samples were collected from the field and surface features were examined using a Leica DVM6 M S Z-stacking Stereoscope (Leica, Wetzlar, Germany). Changes over time in algal vegetative and reproductive structures between healthy and diseased tissue were recorded.

RESULTS

Confirmation of causal organism. DNA sequences of seven algal isolates from the field were generated [accessions: MN637827-MN637833]. Analysis of these sequences indicated >98.95% identity similarity, >99.0% query coverage, and an E-value of 0 to the best match sequence in Genbank of previously identified Cv isolates (accession numbers KM020145, KM020143, DQ399595, or AY220984), confirming that the organism isolated from diseased blackberry plants in these commercial fields was Cv, the causal organism of OCB.

Photographic observation of diseased canes. Biweekly photography (Fig. 2.1) provided information about the disease progress on blackberry. In late June through early July, small red spots, less than 1 mm in size, were first observed on primocanes which had emerged in May (Fig. 2.1A). Red spots expanded into clearly identifiable blotches, 2-3 mm in diameter, by August (Fig. 2.1C). Observed blotches continued to expand though early December at which time blotch expansion ceased as canes entered dormancy (Fig. 2.1G). Blotches observed by December (Fig. 2.1F) could be traced back through photographic evidence as having originated as small red spots as early as late June (Fig. 2.1A) and as late as August (Fig. 2.1C). Concurrent

with cane emergence from dormancy in mid-April, algal spot expansion was observed (Fig. 2.1K). Starting in late May (Fig. 2.1L) through the third week of July (Fig. 2.1N), the blotches on these canes (now floricanes) took on a felt-like texture and appearance. In this study, 100 cane sections were photographed in total, and in all cases, no new lesions were observed after the initial disease development in June through August, indicative of a monocyclic disease cycle.

Microscopy of diseased tissue. Information gathered from microscopy slides included timing and morphological information about Cv development (Fig. 2.2). Initial observations of algal blotches showed algal filaments separating cane epidermal layers (Fig. 2.2B). Space and filament density in-between epidermal cell layers appeared to remain constant during the observed months from December to May. Gametangia were first observed in January (Fig. 2.2C). From January to May, gametes within gametangia became more developed and defined over time. Algal filaments were observed exiting the cane epidermis in April (Fig. 2.2D). Around this time, some gametangia were observed to be collapsed and empty (Fig. 2.2E). In May, nearly all observed gametangia were collapsed and empty. Starting in June, sporangiophores emerged and developed sporangia (Fig. 2.2F). Using Z-Stack microscopy, the felt-like material observed in biweekly photography was confirmed to be asexual sporulating structures: sporangiophores bearing zoosporangia. The dense mats of asexual structures (Fig. 2.2G) as well as single sporangiophores bearing zoosporangia (Fig. 2.2H) were observed from diseased cane samples.

Disease Cycle Timeline. While disease monitoring was conducted in the field, blackberry phenological stages were recorded. Blackberries were observed breaking dormancy in April. Primocanes began to emerge in May. On floricanes, fruit maturation was observed from mid-June through mid-July. Following the harvest of matured fruit by the producer, blackberry floricanes began to senescence naturally until they were pruned out. In December, canes entered

dormancy. Information gathered from photographs of diseased canes and microscopic examination of diseased tissue was overlaid to create a disease progress timeline as it corresponded to blackberry phenology (Fig. 2.3). After combining the findings on OCB and recorded timing of blackberry phenological stages, associations between the two could be made. The OCB pathogen and blackberry phenology largely coincided, as each was observed to exit and enter dormancy concurrently in April and December, respectively (Fig. 2.3). Gametangia were observed to be collapsed and empty largely prior to blackberry primocane emergence in May. By contrast, zoosporangia were present during and after primocane emergence and fruit maturation in June and July (Fig. 2.3).

DISCUSSION

Based upon our observations, OCB was confirmed to be a monocyclic disease on blackberry in southern Georgia, having one infection cycle per year occurring in the summer. The timing of the appearance of both sexual and asexual reproductive structures of Cv were described in detail. The findings suggested that zoospores likely represent the primary source of initial primocane infection on blackberry, reinforcing a prior suggestion regarding the importance of asexual reproduction (Chapman 1976; Nelson 2008). During the period in which gametangia were observed to have emptied their contents (presumably biflagellate isogametes), few primocanes had emerged, making it doubtful that sexual propagules play a major role in spread to primocanes. By contrast, primocanes were present for the entirety of the observed asexual reproductive period. In addition, evidence collected from the study suggests that OCB management could potentially be improved by timing control measures to when key algal reproductive structures are present.

Photographic observation of diseased canes revealed many characteristics of the OCB disease cycle. The disease cycle of OCB had not been clarified to date (Browne et al. 2019b). It was observed that algal development coincided with plant developmental stages. Cv entered and exited dormancy concurrently with blackberry and exhibited the most vigor during the summer months. The OCB disease cycle was theorized to be monocyclic (Browne et al. 2019b; Suto and Ohtani 2013) and this study revealed several characteristics that support this premise. All blotches observed in December could be traced back through photographic evidence as having originated as small red spots in late June to August. Also, all observed sporulation occurred during the period from late May to early July. Previously, it had been theorized that initial infections may be overlooked considering immature thalli consist of a few cells (Browne et al. 2019a) and this appears to be likely based upon our observations. The hematochrome pigment that gives the algae its orange color is also limited during the early life stages of Cv (Chapman and Good 1983). We observed that algal blotches start as small red spots, but these are not clearly distinguishable as algal blotches until late August (~2 months after initial symptoms) when their size and pigmentation is much more defined.

Results of microscopic examination validated observations made from field photography and provided more detailed histological evidence of the timing of algal expansion after dormancy and asexual sporulation. Microscopic examination of cross-sections also revealed there may be some algal activity during the dormant season despite the lack of significant blotch expansion during this time. Specifically, gametangia were not evident in samples collected in December, but they were observed in January samples. Based on microscopic observations of diseased tissue, it is theorized that biflagellate isogametes are released from April to late May. Meiosporangia bearing meiozoospores were not observed, suggesting isogametes were the

primary pathway of any successful sexual reproduction. However, it should be noted that we did not directly observe isogametes exiting gametangia, rather an increasing proportion of gametangia were observed to be empty and collapsed throughout this period suggesting the release of their contents. Structures observed parallelled those detailed in studies about Trentepholiales and *Cephaleuros* spp. (Brooks et al. 2015; Chapman 1980; Rindi and Guiry 2002; Suto and Ohtani 2013); however, in those studies, individual zoospores, isogametes, and/or meiozoospores were observed.

Although both sexual and asexual reproductive structures were observed in our study, our observations suggest that it is likely that sexual spores play a minor role in infection of new primocanes, as they appear to be released largely before the emergence of primocanes. In other studies of *Cephaleuros* spp., sexual processes have been further characterized. In those studies, the sexual isogametes of Cv never conjugated but germinated directly like asexual zoospores to form a new thallus (Suto and Ohtani 2013). This was confirmed first in other species within Trentepohliales, *Printzina* spp. and *Trentepohlia* spp. (Rindi and Guiry 2002). They termed these isogamete propagules "biflagellate swarmers" from "presumptive gametangia". Brooks et al. (2015) and Suto and Ohtani (2013) suggest this same terminology for other *Cephaleuros* spp. Our study was not able to characterize these isogamete propagules specifically, and they may have germinated directly. We observed gametangia presumably bearing biflagellate swarmers (biflagellate isogametes) and empty gametangia, whereas meiosporangia bearing meiozoospores were never observed. Additional work will be needed to further characterize the identity of these propagules within gametangia and to determine what role they are playing in the disease cycle on blackberry. Nonetheless, the fact that observed gametangia had already apparently emptied their

contents prior to primocane emergence suggests that these propagules may be of minor importance.

The biweekly imaging and microscopy results support previous hypotheses and define the disease cycle of Cv on blackberry. The timing of algal growth, sexual and asexual reproduction, and dispersal have now been detailed on a monthly timeline. Gathered details ultimately expose potential management opportunities. Because a detailed disease cycle was not available previously, developing practical spray programs has been difficult (Browne et al. 2019a). This work strongly supports the potential for current chemical management options to be targeted to when they may be more efficacious, such as during production of asexual spores and infection of primocanes. Evidence suggests OCB is a monocyclic disease, meaning there is one infection cycle per season. Targeted management strategies within this period may reduce disease severity on uninfected primocanes by hindering disease development on already diseased canes or provide protection of emerging primocanes. Phosphonates have been found to be effective in reducing OCB severity (Browne et al. 2019b). The current chemical management recommendation is to spray these phosphonates when symptoms on new primocanes are apparent, in late summer, until cane dormancy. However, small red spots later determined to be the start of algal blotches were first spotted in the middle of the asexual reproductive period in late June. Therefore, it may be advantageous to treat canes with applications of phosphonates prior to and during algal sporulation. Doing so may provide cane protection or inhibit algal sporulation.

Based upon the evidence presented here, future management studies should be carried forward. It would be beneficial to further investigate ProPhyt (potassium phosphite) application timing and resulting efficacy against OCB development on infected canes and spread to new

primocanes. This product was found to be effective in reducing OCB disease severity (Browne et al. 2019b). ProPhyt is known to have direct toxic effects on numerous pathogens, but it also induces systemic host resistance through priming plant defenses (Spolti et al. 2015). Determining the exact effect of ProPhyt on algal blotches would aid in maximizing its efficacy. Lastly, given that the asexual sporulation period observed here extended past harvest, studies focusing on post-harvest floricane removal and the resulting impact on OCB dissemination to neighboring uninfected primocanes may be merited. This would help quantify the benefit of recommended cultural management practices and further define the infective period of OCB.

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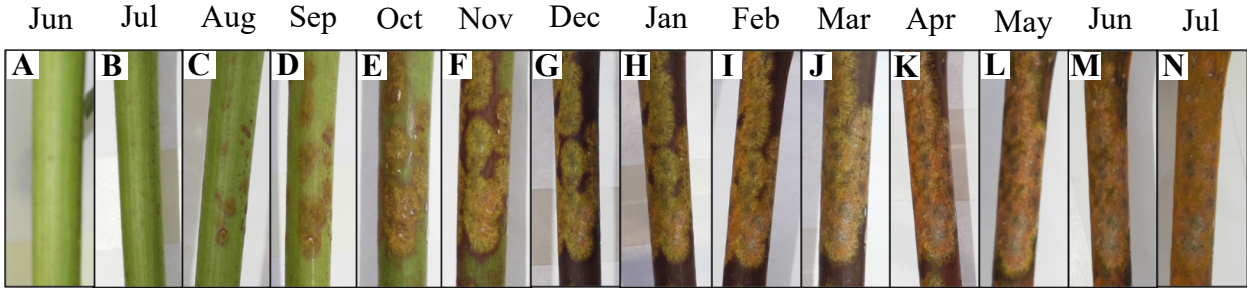

Figure 2.1. Monthly algal blotch development, cropped images representing 8 cm of a single infected cane taken every two weeks. (A-G) months June through December 2018, (H-N) months January through July 2019.

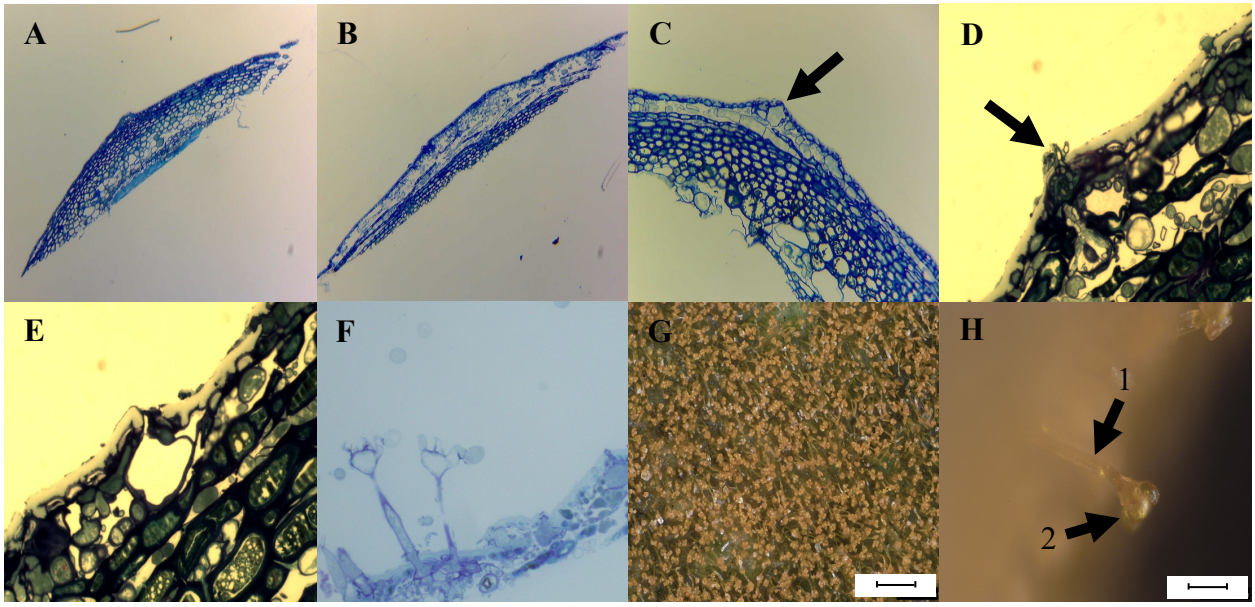

Figure 2.2. Microscopic imaging of OCB disease on floricanes. (A) Healthy blackberry epidermal tissue, (B) diseased epidermal tissue with algal filaments separating epidermal cells, (C) large sexual reproductive cells known as gametangia give rise to isogametes in January, (D) algal filaments observed protruding from cane epidermis in April, (E) empty gametangia observed in April to late May, (F) asexual fruiting structures consisting of sporangiophores bearing zoosporangia containing flagellated zoospores observed in late May to late July, (G) dense mats of sporangiophores bearing zoosporangia, and (H) a single sporangiophore (1) bearing two to four zoosporangia (2). Magnification = 100X, 100X, 200X, 400X, 400X, 400X for A, B, C, D, E, and F respectively. Scale bar = 250 µm and 50 µm for G and H, respectively.

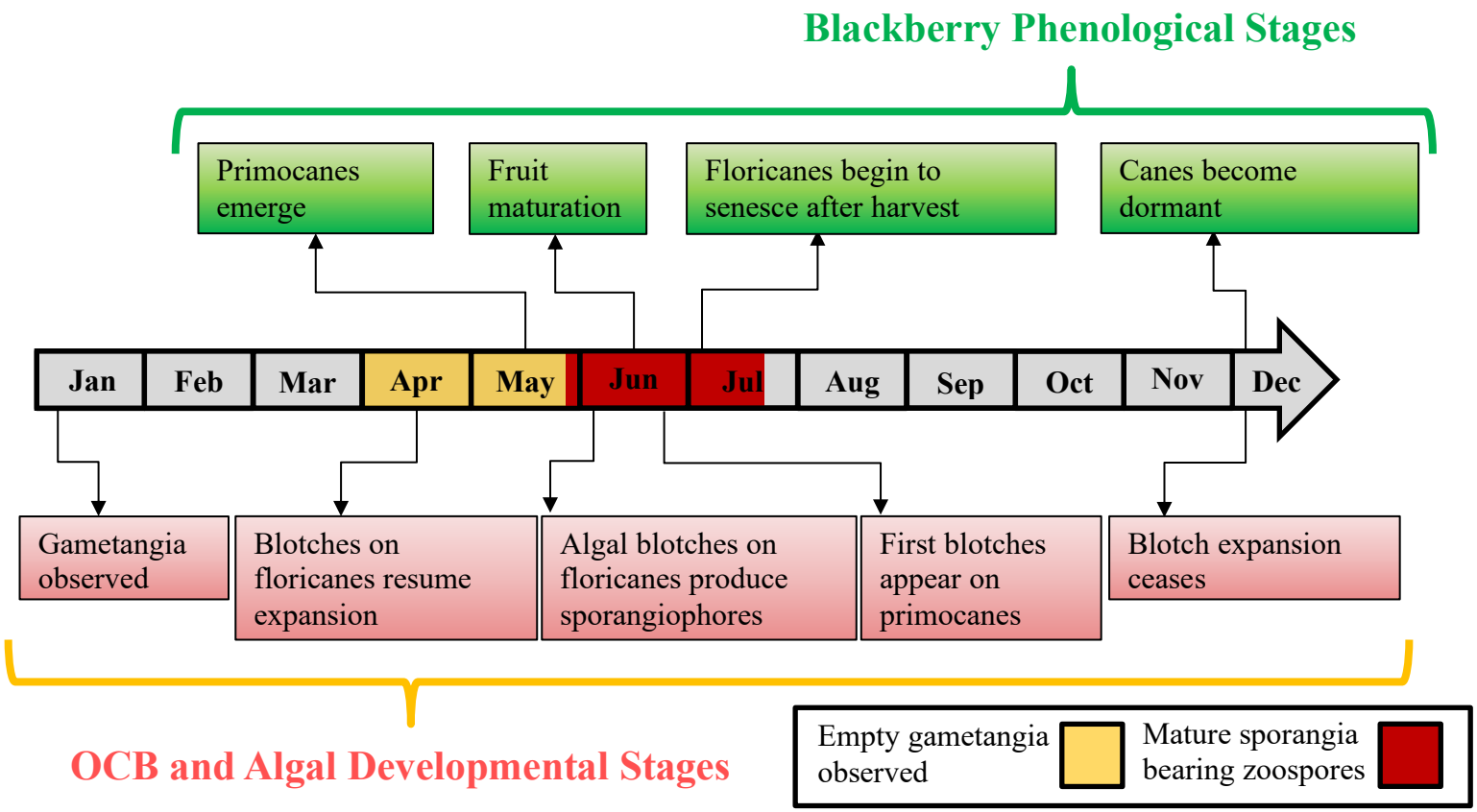

Figure 2.3. OCB and algal developmental stages as they correspond to blackberry phenology.

CHAPTER 3

THE IMPACT OF POTASSIUM PHOSPHITE ON *CEPHALEUROS VIRESCEN*S AND MANAGEMENT OF ORANGE CANE BLOTCH DISEASE OF BLACKBERRY (*RUBUS FRUCTICOSIS*) [2]

[2]Will H. Hemphill, Phillip M. Brannen, Katherine L. Stevenson, and Jonathan E. Oliver. To be submitted to: *Plant Disease.*

ABSTRACT

Orange cane blotch (OCB), also known as orange felt disease, is a disease of blackberry induced by the parasitic algal species *Cephaleuros virescens* (Cv). This disease has been of increasing concern in blackberry production in the southeastern United States since its first report in Arkansas in 1997. The presence of OCB is associated with blackberry cane cracking, cane girdling, and yield loss. Pesticide efficacy studies in recent years have determined that potassium phosphite (ProPhyt®) reduces OCB disease severity. However, the specifics of how disease severity is reduced by potassium phosphite and optimal spray timings for its use have not been detailed previously. To better understand the impact of potassium phosphite on OCB, field trials and an in vitro study were conducted. Data collected from the field showed that applications of potassium phosphite significantly reduced algal blotch number and size. Moreover, additional potassium phosphite applications in spring had no effect on floricane disease severity over applications of potassium phosphite made the previous summer and fall. In addition, results suggested that starting potassium phosphite applications during the algal sporulation period would allow for effective OCB management. In vitro studies revealed that potassium phosphite is directly toxic to Cv, reducing the growth of Cv by significantly reducing final algal colony area. Together, these field and in vitro studies detail the impact of potassium phosphite on OCB, and aid in the development of more efficacious ways to reduce OCB disease severity.

INTRODUCTION

Many pathogens and pests thrive in the high humidity, heat, and short winters in the southeastern U.S., and the management of some diseases has proven challenging to the region's blackberry producers. First reported in Arkansas in 1997 (Holcomb et al. 1998), orange cane blotch (OCB), has become more prevalent in southeastern blackberry production (Brannen 2012). OCB, also known as orange felt disease, is caused by the algal species *Cephaleuros virescens* (Cv), and this alga has been identified on at least 287 plant species and cultivars (Holcomb et al. 1998).

OCB is known to cause yield loss by reducing fruit number per cane (Browne et al.) and also causes cane cracking (Browne et al. 2019a) and cane girdling (Brannen 2012). The open wounds caused by OCB may also serve as an entry site for other opportunistic and deadly blackberry pathogens (Brannen 2012). OCB is easily identified by the orange pigmented velvet-like sporulating lesions or blotches produced from algal thalli. Parasitic algae, like Cv, are known to utilize host nutrients and moisture as well as potentially secreting secondary metabolites toxic to its host (Joubert and Rijkenbe 1971). The interactions between Cv and blackberry are not fully understood (Browne et al. 2019b); however, recent studies have further characterized the OCB disease cycle (Chapter 2) and have confirmed OCB as a monocyclic disease, with only one infection period per year on blackberry. The alga overwinters on blackberry primocanes and sporulates the next summer, thereby providing inoculum for infections of newly emerged primocanes.

Limited research has been conducted on management of this disease. Cultural practices have been recommended, including removal of diseased crop debris, installation of field drainage pipes to reduce humidity and standing water, greater plant spacing, removal of floricanes

immediately after harvest, use of drip lines instead of overhead irrigation, intercropping, reducing plant stress, and thinning of blackberry plants (Nelson 2008). However, the utility of these practices has not been confirmed. These measures alone are not sufficient to control OCB in the southeastern United States.

Although multiple trials over a 3-year period have been conducted to evaluate various chemical controls, only one chemical, ProPhyt® (potassium phosphite; Luxembourg-Pamol, Houston, TX) was found to suppress OCB development in blackberries (Browne et al. 2019b). It is assumed that similar phosphonate fungicides may also suppress OCB and other parasitic algal diseases. The exact mechanism of action of potassium phosphite on OCB is unknown, but with other plant diseases, the impact of potassium phosphite can be direct (toxicity) or indirect (by turning on the host plant defenses) (Spolti et al. 2015). The impact of potassium phosphite is most often associated with the inhibition of pathogen growth (Achary et al. 2017), but in the case of OCB, it is unknown if the observed decrease in disease severity is due to a reduction in size or a reduction in number of blotches on the canes of infected blackberry. More research is needed to understand how the pesticide is impacting Cv. Previously, copper fungicides have been recommended for control of OCB, having been successful on other algal diseases (Huq et al. 2010). However, copper applications did not provide effective OCB control during previous trials in Georgia (Browne et al. 2019b), possibly because Cv on blackberry exists largely within the epidermis of the plant cane where it is protected from surface applications of protectant materials. Collectively, there are knowledge gaps in the management of OCB, and further research is needed to detail potential application timings and the impact of successful chemistries on OCB. The interactions between Cv and blackberry are not fully understood, but recent

findings detailing the disease cycle of OCB can now be utilized to establish an optimized chemical management strategy (Browne 2017; Chapter 2).

The objectives of this study were to determine the impact of potassium phosphite (ProPhyt®) on OCB of blackberry and to improve management strategies using this product. Three potassium phosphite field trials and an in vitro potassium phosphite study were used to accomplish these objectives. An initial field trial was used to determine the impact of potassium phosphite on disease severity through the evaluation of its effect on size and number of algal blotches. Utilizing the latest information on the disease cycle, two additional field trials were conducted to evaluate the impact of seasonal potassium phosphite applications on disease severity on floricanes and primocanes, respectively. In one trial, an early application treatment focused on protecting emerging primocanes through the end of algal sporulation, while a late application treatment focused on applications beginning with algal sporulation and continuing until cane dormancy. Collectively, to determine the impact of potassium phosphite and to establish a more effective OCB management program, these field studies utilized recent findings on OCB chemical control evaluations and an enhanced understanding of the OCB disease cycle. In vitro trials were also conducted to evaluate the direct impact of potassium phosphite on Cv.

MATERIALS AND METHODS

Commercial blackberry sites for field work. Field experiments were conducted at three commercial blackberry plantings in southern Georgia with a history of OCB. One planting was located in Lanier County (cv. ‘Osage’) and two plantings were located in Irwin County (cv. ‘Ouachita’). The presence of Cv was previously confirmed in these fields (Chapter 2). Each row of plants used in potassium phosphite spray trials was flanked by a buffer row on each side. For

all three trials, the growers were asked not to spray the studied rows or their two neighboring buffer rows with any phosphonates to minimize extraneous impacts on the algal pathogen. Otherwise, management was consistent with commercial blackberry practices observed in southern Georgia.

Impact of potassium phosphite on disease severity in the field. Field trials were carried out from June through November 2018 at three sites, one in Lanier and two in Irwin Counties. Treatments consisted of potassium phosphite applications and an untreated control. ProPhyt® (potassium phosphite; Luxembourg-Pamol, Houston, TX) was applied at a rate of 4.7 liters/ha using a Roundup PRO® backpack sprayer and 0.15 GPM narrow 30° flat-fan nozzle (Monsanto, St. Louis, MO) until runoff (equivalent to 467.7 liters water/ha) on 12 Jun, 3 Jul, 24 Jul, 14 Aug, 4 Sept, 25 Sept, 16 Oct, and 6 Nov. Treatments were applied in a randomized complete block design with four (Lanier and Irwin County site one) or six (Irwin County site two) replications. Blocks of five treated and five untreated control plants were randomly assigned within a row of blackberries at all three sites. An unsprayed plant was used as a buffer between each treatment and block. The center three plants of each five-plant plot were used for disease severity assessment. Disease severity on blackberry primocanes was assessed on 19 Nov. Two different metrics were used to assess disease severity: the total number of algal blotches divided by the total number of canes and ten random algal blotch sizes (mm) across multiple canes in each plot. Disease severity was assessed in the cane area that extended from the crown to 76 cm in height. Statistical analysis was performed by comparing the treatments using analysis of variance (ANOVA) followed by Tukey's honest significance difference test (HSD) using the package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria).

Impact of spring applications on floricanes. To assess the potential for additional efficacy of spring applications of potassium phosphite to floricanes, trials were conducted from June 2018 to May 2019 at all three locations. Treatments were as follows: 1) no fungicide applied in 2018 or 2019 (a true nontreated control); 2) fungicide applied in 2018 only (summer and fall); 3) fungicide applied in 2018 (summer and fall) and spring 2019; and 4) fungicide applied only in spring 2019. Potassium phosphite was applied at a rate of 4.7 liters/ha (sprayed until runoff using a backpack sprayer). In 2018, potassium phosphite and a nontreated control were applied to plots consisting of five plants each. In order to develop the treatments for 2019, half of each treated and untreated plot from 2018, respectively, received potassium phosphite applications (two of five plants per plot). Therefore, treatments were applied using a split plot design, with 2018 treatments (summer and fall; with or without potassium phoshite) being the main plots and 2019 treatments (spring; with or without potassium phosphite) being the subplots, randomly assigned. The first two and last two plants in each block of five treated and five untreated control plants from the 2018 trial were used for each subplot, and buffer plants separated each subplot. This trial was carried out at all three sites, with four blocks at the Lanier and Irwin County site 1 and six in Irwin County site 2. The 2018 treatments with potassium phosphite were applied on 12 Jun, 3 Jul, 24 Jul, 14 Aug, 4 Sept, 25 Sept, 16 Oct, and 6 Nov. Spring 2019 applications were made on 17 Apr, 8 May, and 29 May. Disease severity was assessed as previously described on blackberry floricanes on 3 and 5 Jun, in Lanier and Irwin Counties, respectively. Statistical analysis was performed by comparing the treatments using analysis of variance (ANOVA) followed by Tukey's honest significance difference test (HSD) using the package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria).

Impact of spring and summer applications on primocanes. In order to better assess the impact of initiating applications of potassium phosphite at primocane emergence on disease development, additional trials were conducted from April 2019 (primocane emergence) through October 2019 at all three locations. Treatments consisted of potassium phosphite sprayed at three periods throughout the year and a nontreated control. The three timings included early-, late-, and long-season applications. Early-season applications targeted OCB from primocane emergence and thereafter for six sprays, one every 3 weeks on 17 Apr, 8 May, 29 May, 19 June, 10 July, and 31 July. Late-season applications targeted OCB just after sporulation and thereafter for six sprays, one every 3 weeks on 10 July, 31 July, 21 Aug, 11 Sept, 2 Oct, and 23 Oct. Long-season applications started with early-season applications and ended with late-season applications for ten sprays total, one every 3 weeks on 17 Apr, 8 May, 29 May, 19 June, 10 July, 31 July, 21 Aug, 11 Sept, 2 Oct, and 23 Oct. Potassium phosphite was applied at a rate of 4.7 liters/ha until runoff using a backpack sprayer (equivalent to 467.7 liters water/ha), as previously described. Treatments were applied in a randomized complete block design. Blocks consisted of five plants for each treatment period and the nonsprayed control. In each of the three plantings, treatments were randomly assigned within a row of blackberries. All plots and blocks were separated by a nonsprayed buffer plant. The neighboring plants to the buffers were left out of measurements. Therefore, the center three plants from each plot were used for data collection. In Lanier County, four blocks of treated and control plants were selected within a row of blackberries. In the first Irwin County planting (site 1), three blocks in one row were evaluated. In the second Irwin County planting (site 2), four blocks in one row were evaluated. On 30 and 31 Oct, in Irwin and Lanier Counties respectively, disease severity was assessed as described previously. Statistical analysis was performed by comparing the treatments using analysis of

variance (ANOVA) followed by Tukey's honest significance difference test (HSD) using the package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria).

In vitro fungicide efficacy trial. Two in vitro experiments with ProPhyt® (potassium phosphite) were repeated using a previously confirmed Cv culture [MN637833] isolated from field-grown blackberry (Chapter 2). Cultures of this isolate were expanded onto new Bold's basal medium (BBM) plates (Suto and Ohtani 2010), overlaid with 10 mL of Bold's basal broth (BBB) (Chapter 2; Appendix A), and incubated at 27°C for 1 month before starting the in vitro trial. After one month, four of the most rapidly growing pure cultures were selected for use in the trial. Each of the four starting culture plates was used as a source of inoculum for one replicate of all treatments, and accordingly each treatment was replicated four times. Replicates consisted of a single plate with one starting cluster of algal filaments. Similarly sized clusters of algal filaments were removed from the grown-out cultures, partially implanted in 25 ml of nonamended or amended with ProPhyt® BBM, and overlaid with the respectively amended 10 ml of BBB. Treatments included a nonamended control and amended BBM and BBB with 1%, 0.1%, 0.01%, 0.001%, 0.0001%, and 0.00001% of ProPhyt® (54.5% potassium phosphite or 34.30% phosphorous acid equivalent by volume). In the event that pH had an impact on algal growth, two additional control treatments were included consisting of medium adjusted to pH 6.035 and pH 6.15, to match the pH of 1% and 0.1% ProPhyt® amended BBM and BBB, respectively. Sulfuric acid (0.1%) was used to adjust pH. Plates were placed under 12-hour cool fluorescent light, and a blank agar plate was placed on top of all treatment stacks to protect from direct light. The approximate area (mm^2) of the algal colony was marked and recorded every 2 weeks and final chlorophyll content (a and b) and weight (g) of algal colonies were recorded 8 weeks after planting. For all measurements, the original colony as well as emerged satellite

colonies were assessed. Using the recorded colony area over time, cumulative area under the growth progress curve (AUGPC) was calculated for each isolate. To estimate the algal biomass, each algal culture was dried and weighed. As an indirect measure of algal colony health, chlorophyll content was assessed. This was accomplished by first air drying the algal contents of each plate for 2 h in pre-weighed weigh boats under a laminar flow hood and then submerging dried algal colonies from each plate in 5 ml of 95% EtOH at 4°C for 24 h. Extractions were used to measure and calculate Chlorophyll a, b, and a + b (C_{a+b}) according to the methods of Wintermans and DeMots (1965) using a NanoDrop One C spectrophotometer (ThermoFisher Scientific, Waltham, MA) at absorbance wavelengths of 665 nm, 654 nm, and 649 nm. Final algal area was used to calculate final chlorophyll content per area using C_{a+b} values. To estimate the algal biomass, each algal culture was also dried and weighed following chlorophyll measurements. After 8 weeks, average AUGPC, weight, and chlorophyll content were compared versus untreated control using analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test using the package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria).

RESULTS

Impact of potassium phosphite on disease severity in the field. In this trial, potassium phosphite had a significant impact on OCB severity. Blotches were smaller and fewer in number on blackberry primocanes treated with potassium phosphite relative to the untreated control (Table 3.1). The reduction in number of algal blotches per cane was 77.4%, 91.3%, and 95% in Lanier, Irwin site 1 and Irwin site 2, respectively (Table 3.1). Algal blotch diameter was reduced

by 14.3%, 42.9%, and 24.0% compared to the nontreated control in Lanier, Irwin site 1, and Irwin site 2, respectively (Table 3.1).

Impact of spring applications on floricanes. Compared to the nontreated control, only potassium phosphite applications made in the summer/fall of 2018 or the summer/fall of 2018 plus the spring of 2019 resulted in statistically significant reductions in number of blotches per cane (all three locations) and blotch diameter (all three locations) (Table 3.2). The addition of potassium phosphite in the spring of 2019 did not improve disease control over that provided by the summer/fall 2018 applications (Table 3.2). At the Lanier site, the mean number of algal blotches per cane was significantly reduced, as compared to the nontreated control, by 82.8% for the summer/fall 2018 plus spring 2019 applications and 77.0% for the summer/fall 2018 only applications of potassium phosphite (Table 3.2). The number of algal blotches per cane at Irwin site 1 was significantly reduced by 88.1% with summer/fall 2018 plus spring 2019 and 82.4% with the summer/fall 2018 applications of potassium phosphite compared to the nontreated control (Table 3.2). At Irwin site 2, applications in summer/fall 2018 and spring 2019 significantly reduced the mean number of algal blotches per cane by 88.1% and summer/fall 2018 applications reduced the number of blotches per cane by 82.1% compared to the nontreated control (Table 3.2). At the Lanier site mean algal blotch diameter was significantly reduced by 16.7% with summer/fall 2018 plus spring 2019 applications and 4.16% with summer/fall 2018 applications compared to the nontreated control (Table 3.2). Mean algal blotch diameter at Irwin site 1 was significantly reduced by 34.5% with summer/fall 2018 plus spring 2019 and 31.0% with summer/fall 2018 applications compared to the untreated control (Table 3.2). At Irwin site 2, average blotch diameter showed a 20.0% reduction in average algal blotch diameter with

summer/fall 2018 plus spring 2019 applications and a 10% reduction with summer/fall 2018 applications as compared to the nontreated control (Table 3.2).

Impact of spring and summer applications on primocanes. All application intervals significantly reduced algal blotch number, and the late-season and long-season applications significantly reduced algal blotch size (Table 3.3). At all three locations, the applications significantly reduced the mean number of algal blotches per cane, compared to the nontreated control (Table 3.3). At the Lanier site, the number of algal blotches per cane was significantly reduced by 66.4%, 75.1%, and 58.4% with early-, late-, and long-season applications, respectively, as compared to the nontreated control (Table 3.3). At Irwin site 1, there was a significant reduction in the mean number of algal blotches per cane as compared to the nontreated control: 76.2%, 87.13%, and 94.45% with long-season applications with early-, late-, and long-season applications, respectively. As compared to the nontreated control, Irwin site 2 exhibited a significant 93.1% reduction in the mean number of algal blotches with early-season applications, a 94.0% reduction with late-season applications, and 98.58% reduction with long-season applications (Table 3.3). In all three sites, blotch numbers were lower on the plants receiving the late-season applications versus those receiving the early-season applications; however, this difference was not statistically significant. At Irwin site 1 and 2, fewer blotches were observed on the plants receiving the long-season applications versus plants receiving the early-season or late-season applications only (Table 3.3), but this difference was not statistically significant. As Irwin site 2 did not have enough algal blotches to appropriately assess mean blotch size on treated plants, blotch diameter was assessed for the Lanier site and Irwin site 1 only. For the Lanier site, late-season and long-season applications resulted in statistically lower mean blotch sizes compared to the nontreated control while early-season and late-season

applications did not statistically differ from one another (Table 3.3). Blotch sizes on Lanier plants receiving early-season applications did not significantly differ from blotch sizes on those receiving the nontreated control (Table 3.3). Algal blotch size was significantly reduced by 13.3% with late-season and 21.6% with long-season season applications compared to the nontreated control (Table 3.3). At Irwin site 1, all three application intervals significantly reduced mean algal blotch size compared to the nontreated control, but blotch sizes were significantly reduced following late-season and long-season applications versus the early-season applications (Table 3.3). Early-season applications reduced mean blotch size by 18.0%, late-season applications reduced blotch size by 53.7%, and long-season applications reduced mean blotch size by 48.9% compared to the nontreated control (Table 3.3).

In vitro fungicide efficacy trial. The 1% ProPhyt® treatment significantly reduced AUGPC as compared to the unamended control (Table 3.4; Fig. 3.1). The mean AUGPC of algae on medium amended with 1% ProPhyt® was also significantly less than on medium at the same pH (6.035), but without ProPhyt® (Table 3.4). In trial 1 only, the mean AUGPC on medium at pH 6.035 without ProPhyt® was significantly higher compared to all other treatments, including the nonamended control. The mean AUGPCs on medium amended with 0.1% ProPhyt®, the pH 6.15 control without potassium phosphite, and the nonamended control did not significantly differ from one another (Table 3.4). In trial 1, algal biomass was significantly higher on medium at pH 6.035 than all the other treatments and nonamended control; other treatments did not differ significantly from each other (Table 3.4). Colonies on amended plates treated with 1% ProPhyt® had a lower final algal biomass mean weight as compared to all other treatments and controls, but this result was not statistically significant. In trial 2, algal biomass on medium at pH 6.035 was higher than all other treatments except for the nonamended control (Table 3.4). In trial 2,

algal biomass on medium amended with 0.01%, 0.1%, and 1% ProPhyt® was significantly lower compared to the nonamended control (Table 3.4), and algal biomass on medium amended with 1% or 0.1% ProPhyt® was significantly less than all other treatments as well as the nonamended control (Table 3.4). In both trials, chlorophyll content of algal colonies differed significantly among treatments; however, no treatment resulted in chlorophyll content that statistically differed from the nonamended control (Table 3.4).

DISCUSSION

The impact of potassium phosphite on Cv and OCB of blackberry was assessed in three field trials and an in vitro study. An initial trial indicated that potassium phosphite significantly reduced disease severity by reducing both the number and size of algal blotches, while subsequent trials (based upon newly detailed OCB disease cycle information) indicated effective timings for fungicide applications. These trials indicated that additional applications of potassium phosphite in the spring did not have a significant impact on the size or number of algal blotches on floricanes, and that the most effective application period for reducing OCB severity on primocanes coincided with algal sporulation and the period following sporulation. In vitro studies with Cv and potassium phosphite suggested that potassium phosphite has the ability to negatively impact algal growth. This indicated that potassium phosphite has a direct toxic effect on Cv.

In previous studies, Browne et al. (2019a) determined that potassium phosphite significantly reduced OCB disease severity; however, this was based upon visual estimation of percent cane coverage over time, so the manner by which potassium phosphite reduced severity was not determined directly. From our initial 2018 field trial, we determined that potassium

phosphite reduces OCB severity by reducing both blotch size and number. In terms of the impact on total diseased area on affected canes, the reduction in blotch number observed here is more influential than the reduction in blotch size. This result suggests that potassium phosphite does slow algal growth after infection, but has a more significant role in preventing new infections. This information can be paired with new knowledge on the disease cycle of OCB to apply targeted sprays and potentially increase the efficacy of potassium phosphite applications (Chapter 2).

In our second field trial, we determined that spring applications of potassium phosphite to blackberry floricanes (post dormancy) have no significant impact on algal blotch size and number. This supports previous disease cycle studies indicating that new infections of second year floricanes are few or do not appear to occur (Chapter 2). This result also provides further evidence that the ability of potassium phosphite to prevent infection is likely more significant than limiting algal growth post infection on overall disease severity. Given recent studies showing that Cv sporulates from late May to late July (Chapter 2), it is not surprising that the impact of spring sprays on floricanes would be minimal. Disease severity of primocanes was not evaluated in this trial, and it is more likely that potassium phosphite applications in mid-May would reduce infection of newly emerging primocanes rather than have a noticeable impact on floricane disease.

To examine the potential impact of spring and fall sprays on primocane disease and determine the most effective application period for potassium phosphite, an additional field trial was carried out. In this trial, an early application window targeted primocane protection, making applications from primocane emergence until the end of OCB sporulation in late July. The late-season applications targeted OCB from early July, right after sporulation initiation until late

October, a month before cane dormancy. The long-season application treatment covered both the early-season and late-season application windows. This trial revealed that partial season applications of potassium phosphite may be as effective as full/long-season applications. In addition, late-season applications starting with the algal sporulation period have the ability to reduce initial infection of primocanes as well as reduce algal blotch expansion. An optimal application timing to maximize the efficacy of potassium phosphite would most likely involve beginning applications just prior to algal asexual sporulation (approximately mid-May) and continuing applications at the maximum label rate every three weeks for six total applications. Such an application interval would allow for potassium phosphite to protect primocanes from initial infection and also inhibit growth of the algae after infection.

In vitro studies using a Cv isolate indicated that potassium phosphite had a direct toxic effect on algal growth in the absence of any plant defense activation effect that potassium phosphite may elicit in the field. While Cv exposed to potassium phosphite was able to maintain chlorophyll content at all levels of potassium phosphite exposure, growth was severely inhibited on medium containing 1% ProPhyt® (which is the same concentration applied in the field). Of note, the acidic pH of potassium phosphite was found not to be the driving force behind the growth inhibition effect of 1% ProPhyt® as algal colonies on medium at the same pH (6.035) but without ProPhyt® were significantly larger compared to colonies on medium amended with 1% ProPhyt® (in trials 1 & 2) and the nonamended control plates (in trial 1 only). This further confirms that at high concentrations, potassium phosphite has a direct toxic effect on Cv. The phosphite anion is truly systemic in plants moving both acropetally and basipetally (Cohen and Coffey 1986), but the exposure level of subdermal algae is not known. Any protruding algal filaments or sporulating bodies would be exposed to the full application rate, eliciting a negative

toxic effect on algal growth. The phosphite anion in potassium phosphite is known to be toxic to many species of fungi, oomycetes, and some bacteria by inhibiting several cellular processes; ultimately interfering with phosphorylation to inhibit pathogen metabolism and growth (Achary et al. 2017). The toxicity of phosphite to algae has not been researched as extensively, though on microalgae, a unicellular algal group, phosphite was previously found to be non-toxic nor metabolized (Loera-Quezada et al. 2015). In plants, the effects of phosphite are still debated, with some research suggesting that phosphite is biostimulatory and other research showing that it either has no effect or can be harmful if phosphate availability is low (Achary et al. 2017; Thao and Yamakawa 2009). The plant defense systems activated by phosphite are more defined through research (Achary et al. 2017), and phosphite is known to lead to the production of phytoalexins and pathogen-related proteins that may act activate neighboring cell defenses or act on the pathogen directly. However, as Cv primarily resides intercellularly within epidermal cells, it is not known how this impacts Cv. Phosphite can be paired with potassium (ProPhyt®), ammonium, sodium, and aluminum (Achary et al. 2017), and these components may also be responsible for toxicity or potential biostimulation to algae. More research is needed to clarify whether the direct toxicity of potassium phosphite to Cv observed in our study is due the potassium or phosphite components of the product.

Future studies evaluating various rates of potassium phosphite for effective disease management could help increase the efficacy of applications and/or reduce the amount of product needed for OCB management. The evaluation of other phosphonate fungicides would verify the efficacy of this fungicide class on OCB. Additional in vitro studies to evaluate the impact of the components of potassium phosphite on Cv would help determine what specifically is responsible for the negative impact of the fungicide on Cv observed here. In addition, potential

induction of systemic resistance by potassium phosphite, and its role in OCB control should be further investigated. Now that the period of algal sporulation has been shown to be an effective target for potassium phosphite applications, application trials with other materials during this period may lead to expanded chemical management options.

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Table 3.1. Impact of potassium phosphite applications on the severity of orange cane blotch disease during the 2018 field trial at three commercial blackberry sites.

	Mean Number of Blotches/Cane[y]			Mean Blotch Diameter (mm)[z]		
Treatment and rate/ha	Lanier	Irwin 1	Irwin 2	Lanier	Irwin 1	Irwin 2
Nontreated control	92.7 a	58.5 a	10.4 a	6.3 a	6.3 a	5.0 a
ProPhyt® (4.7 liters/ha)	20.9 b	5.1 b	0.5 b	5.4 b	3.6 b	3.8 b

[y]Numbers of blotches per cane within 76 cm from the ground. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

[z]Mean blotch diameter determined based upon the diameter of ~30 arbitrarily selected blotches per plot. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 3.2. Impact of potassium phosphite applications on orange change blotch disease severity on blackberry floricanes.

	Mean Number of Blotches/Cane[y]			Mean Blotch Diameter (mm)[z]		
Application timings[x]	Lanier	Irwin 1	Irwin 2	Lanier	Irwin 1	Irwin 2
Nontreated control	212.8 a	120.5 a	44.2 a	7.2 a	8.4 a	6.0 a
Spring 2019	176.0 a	146.1 a	32.6 a	7.9 a	7.4 a	5.9 a
Summer/Fall 2018	48.9 b	21.2 b	7.9 b	6.9 b	5.8 b	5.4 b
Summer/Fall 2018 + Spring 2019	36.59 b	14.3 b	6.25 b	6.0b	5.5b	4.8b

[x]Applications in 2018 were made on 12 Jun, 3 Jul, 24 Jul, 14 Aug, 4 Sept, 25 Sept, 16 Oct, and 6 Nov. Applications in 2019 were made from primocane emergence to harvest on 17 Apr, 8 May, and 29 May. ProPhyt® was applied at a rate of 4.7 liters/ha to treatment plots.

[y]Numbers of blotches per cane within 76 cm from the ground. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

[z]Mean blotch diameter determined based upon the diameter of ~30 arbitrarily selected algal blotches per plot. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 3.3. Impact of potassium phosphite application timings on the severity of orange cane blotch disease on blackberry primocanes.

	Blotch Number/Cane[y]			Blotch Diameter (mm)[z]	
Application timings[x]	Lanier	Irwin 1	Irwin 2	Lanier	Irwin 1
Nontreated control	20.45 a	21.83 a	16.89 a	5.13 a	4.99 a
Early-season	6.88 b	5.20 b	1.17 b	4.75 ab	4.09 b
Late-season	5.10 b	2.81 b	1.01 b	4.45 bc	2.31 c
Long-season	8.15 b	1.21 b	0.24 b	4.02 5c	2.55 c

[x]Early-season applications occurred on 17 Apr, 8 May, 29 May, 19 June, 10 July, and 31 July. Late-season applications occurred on 10 July, 31 July, 21 Aug, 11 Sept, 2 Oct, and 23 Oct. Long-season applications included early-season and late-season applications for ten sprays total. ProPhyt® was applied at a rate of 4.7 liters/ha to all treatment plots.

[y]Numbers of blotches per cane within 76 cm from the ground. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

[z]Mean blotch diameter determined based upon the diameter of ~30 arbitrarily selected algal blotches per plot. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 3.4. Impact of ProPhyt® on *Cephaleuros virescens* in vitro.

Treatment	AUGPC [x]		Weight (g)[y]		Chlorophyll Content C_{a+b} (g/mm^2)[z]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1% ProPhyt	1235.5 c	425.25 b	0.002 b	0.001 d	0.031 ab	0.013 b
0.1% ProPhyt	5484.5 b	2875.3 a	0.003 b	0.002 d	0.027 ab	0.025 ab
0.01% ProPhyt	5215.0 b	3720.5 a	0.003 b	0.003 c	0.035 ab	0.018 ab
0.001% ProPhyt	4362.8 b	3607.6 a	0.007 ab	0.004 bc	0.031 ab	0.021 ab
0.0001% ProPhyt	4070.5 b	3615.5 a	0.004 b	0.004 bc	0.026 b	0.016 b
0.00001% ProPhyt	3514.0 b	4339.1 a	0.003 b	0.004 bc	0.044 a	0.019 ab
pH 6.15	4917.5 b	4157.1 a	0.008 ab	0.004 bc	0.027 b	0.020 ab
pH 6.035	8527.8 a	3382.8 a	0.013 a	0.006 a	0.025 b	0.032 a
Unamended control	4586.8 b	4213.1 a	0.004 b	0.005 ab	0.041 ab	0.024 ab

[x]Mean area under the growth progress curve (AUGPC) of algal colonies for each corresponding treatment in mm^2. Means in each column followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

[y]Average dry weight of algal colonies for each corresponding treatment in grams. Means in each column followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

[z]Mean chlorophyll a + chlorophyll b content per unit area (mm^2) of algal colonies for each corresponding treatment, means in each column followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

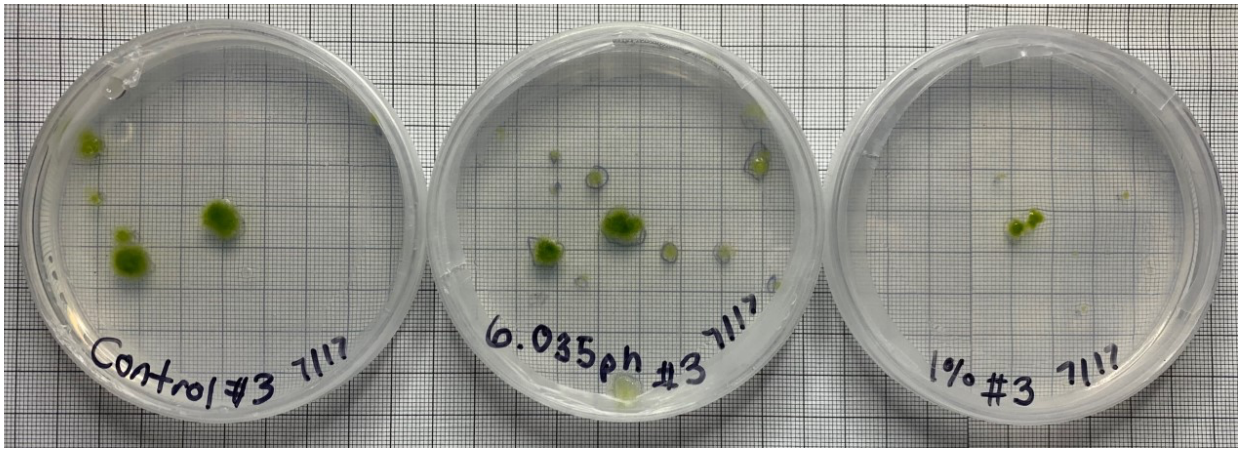

Figure 3.1. Comparison of algal colonies treated with ProPhyt® versus controls. Growth of colonies of *Cephaleuros virescens* on nonamended medium (left), on nonamended medium at pH 6.035 (middle), and on medium amended with 1% ProPhyt, at pH 6.035 (right).

CHAPTER 4

IDENTIFICATION OF ORGANISMS ASSOCIATED WITH CANE DIEBACK OF CULTIVATED BLACKBERRY (*RUBUS FRUCTICOSIS*) IN GEORGIA AND OPTIONS FOR CHEMICAL MANAGEMENT[3]

[3]Will H. Hemphill, Phillip M. Brannen, Marin T. Brewer, and Jonathan E. Oliver. To be submitted to: *Plant Disease.*

ABSTRACT

The climate in the southeastern United States is conducive for many diseases of blackberry and can pose a significant challenge to blackberry production. Cane dieback or blight has been of recent and increasing concern. Its presence is most often associated with *Leptosphaeria coniothyrium* the causal agent of cane blight disease of blackberry. However, cane dieback may also be associated with several members of the Botryosphaeriaceae family, *Colletotrichum* spp., and *Fusarium oxysporum*. The exact organisms involved in causing blighted blackberry canes in the southeast and their options for chemical management are relatively unknown and understudied. A series of field surveys, pathogenicity trials, and a chemical control study were conducted to better understand potential causal agents of blighted canes and their chemical management. Based on the results of these studies, fungal isolates from *Fusarium oxysporum*, *Pestalotiopsis microspora*, *Neofusicoccum kwambonambiense*, *Neofusicoccum parvum*, *Lasiodiplodia pseudotheobromae*, *Lasiodiplodia theobromae*, and *Colletotrichum siamense* were determined to cause cane dieback on inoculated cane terminals. Incognito 85WDG (thiophanate-methyl), Switch 62.5WG (cyprodinil & fludioxonil), Pristine (pyraclostrobin & boscalid), and Abound (azoxystrobin) can provide a significant reduction in cane dieback with some of these pathogens. While there was no single chemistry that provided the greatest reduction in cane dieback, Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG (thiophanate-methyl) provided the greatest broad-spectrum reduction of cane dieback among screened isolates.

INTRODUCTION

In the United States, the majority of blackberry production is concentrated in Oregon, Washington, and California (NARBA 2017); however, the development of blackberry cultivars with lower chill requirements and higher tolerance to heat has led to a significant increase in production in the southeastern U.S. (Ballington 2016). High humidity, heat, and short winters in this region allow many pathogens and pests of blackberry to thrive and the management of some of these diseases has proven challenging to commercial blackberry producers. Many diseases affect blackberry production, impacting yield or leading to plant death, resulting in huge economic losses. Both thorny and thornless cultivars are susceptible to infection by numerous pathogens, and all parts of the plant can be impacted including fruit, leaves, stems, canes, and roots. Fungal organisms are by far the most prevalent cause of disease in blackberry. Symptoms typical of these fungal diseases are lesions, stunted growth, shoot dieback, defoliation, and fruit drop. Cane dieback or blight has been a challenging issue to manage in southeastern production sites, and its presence has previously been associated with *Leptosphaeria coniothyrium* (Brannen and Krewer 2005).

In the southeastern U.S., cane blight induced by *L. coniothyrium* can have a significant impact on blackberry production. Outside of the Southeast, it has not typically been reported as a major disease of blackberry and is more often a problem on raspberry (Brannen and Krewer 2005). Nonetheless, if the host plants are injured, exposing susceptible tissues for infection, cane blight can become a major issue. *L. coniothyrium* (syn. *Paraconiothyrium fuckelii*) (Williamson 2017), causes symptoms that include dark red to purple lesions with purple borders (Brannen and Krewer 2005). These may form in the summer, fall, and winter following wounding, and may expand and merge to girdle the cane. Cane blight has a direct effect on yield, since it can kill the

cane and inhibit new cane development above the site of infection via cane girdling. Fruiting canes with a cane blight infection produce weak growth and wilt as fruit develops or begins to ripen (Snover-Clift and Jensen 2012). The open wounds from the removal of floricanes after harvest and open wounds from the "tipping" of primocanes are of primary concern for cane blight infection; however, injury may also derive from the weather, other diseases, pruning, field maintenance equipment, insects, trellising structures, or the rubbing together of canes, especially thorny ones. Rainfall immediately following wounding increases the likelihood of infection of the vascular tissues of the plant (Brannen and Krewer 2005).

Although much is known about the cane blight disease cycle in blackberry, the etiology of the disease is lacking with regard to possible interactions of *L. coniothyrium* with other cane pathogens. For example, *L. coniothyrium* has been observed as a secondary pathogen colonizing cankers induced by yellow rust of raspberry where it enlarged the cankers it colonized, resulting in sunken and split areas on the cane and subsequent plant death (Zeller 1930). In another study in Scotland a similar interaction between *Resseliella theobaldi* and *L. coniothyrium* was observed (Williamson 1984).

The specific organisms involved in causing blighted canes in the southeastern U.S., and options for their chemical management, are relatively unknown and understudied. Cane dieback is also sometimes associated with members of the Botryosphaeriaceae family, *Colletotrichum* spp., and *Fusarium oxysporum* (Faedda et al. 2018; Maas et al. 1989; Marulanda et al. 2014; Pastrana et al. 2017). *Botryosphaeria dothidea*, *Botryosphaeria obtusa*, and *Neofusicoccum parvum* are all members of the Botryosphaeriaceae family and have been reported to cause disease on blackberry (Faedda et al. 2018; Maas et al. 1989). Botryosphaeriaceae species can cause a vast array of symptoms on their hosts, but are most frequently associated with causing

cane cankers and complete plant collapse on blackberry (Maas et al. 1989). *Colletotrichum* spp. are known to be pathogenic on a range of crops and *C. gloeosporioides* and *C. acutatum* are known to cause anthracnose of blackberry (Marulanda et al. 2014; Peres et al. 2005), which is characterized by cane lesions, cane dieback, and fruit rot. *Fusarium oxysporum* has recently been identified on blackberries in Mexico and California, where it is associated with yield loss, chlorosis, wilting, and cane death (Gonsalves and Ferreira 1993; Pastrana et al. 2017).

Management of dieback and blight diseases of blackberry generally rely on a combination of cultural and chemical controls. Cultural controls recommended for blight management include reducing plant stress, increasing airflow through the plant canopy, limiting wounding, and reducing primary source inoculum by removing all crop debris (Brannen and Krewer 2005; Jones 2015; Snover-Clift and Jensen 2012; Williamson 2017). Chemical controls are not as well studied with cane blight, and much of the knowledge regarding management comes from studies in Scotland on raspberry. Efficacy trials addressing application timing are limited. Brannen and Krewer (2005) recommend applying fungicides each day after pruning to protect wounds until they heal. Promising fungicides to reduce disease severity include benomyl, thiophanate-methyl, carbendazim, pyraclostobin and combinations of these (Williamson 2017). In the United Kingdom, spraying before, during, or immediately after harvest has proven effective against cane blight on raspberry (Williamson 2017). Researchers also recommend spraying cut ends after pruning as well as covering the bottom half of all canes. Collectively, there are knowledge gaps in the understanding of the etiology and control of fungal organisms causing dieback in blackberry. Further research in these areas would lead to advancements in controlling these potentially devastating diseases of blackberry.

The objectives of this study were to determine the prevalence of fungal pathogens associated with dieback of blackberry canes, determine isolate pathogenicity, and identify effective chemical controls. Field surveys were conducted to identify the fungal organisms present on diseased primocanes and floricanes in commercial blackberry plantings. Pathogenicity studies were performed to fulfill Koch's postulates and determine which of these organisms were causal agents of disease. Fungicide efficacy studies were carried out to determine effective chemical controls. Collectively, these studies identified fungal pathogens capable of causing disease on blackberry canes in southeastern blackberry production and determined potential management recommendations.

MATERIALS AND METHODS

Fungal isolation and storage. Over a 2-year period, blighted cane samples were collected from commercial blackberry production sites with a history of cane blight and cane dieback in Atkinson, Berrien, Colquitt, Dougherty, Irwin, Lanier, Oglethorpe, and Pierce counties in Georgia. Cane subsections were surface sterilized by first submerging in 95% ethanol for five seconds then passing the sample through an ethanol flame. Cane subsections were then cut longitudinally using a sterile blade and placed onto acidified quarter strength potato dextrose agar (AqPDA), with one half facing up and the other facing down. Agar was acidified using 184 µl lactic acid (85% w/w) per liter. The cane sections were incubated at 27°C and monitored for fungal growth. Each emerging fungal colony was isolated onto AqPDA and then identified by molecular characterization. For long-term storage, each fungal isolate was transferred to AqPDA plates with sterilized filter paper pieces. After the fungi colonized the filter paper, the pieces were removed and placed in sterilized coin envelopes. Samples were allowed to dry in a laminar

flow hood overnight. The envelopes were then sealed and placed in a plastic container with desiccant pellets in a -20°C freezer for long-term storage.

Isolate identification. Fungal hyphae were collected from growing isolates for DNA extraction using 5% Chelex 100 sodium (Sigma-Aldrich, St. Louis, MO) heated for 30 min at 95°C. PCR was performed using a BioRad S1000s Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) with 10 µM ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers to amplify the highly conserved ITS1 and ITS2 sequences flanking the 5.8S rDNA region (Martin and Rygiewicz 2005; White et al. 1990). Each 20 µl reaction contained 1 µl of ITS1, 1 µl ITS4, 7 µl molecular grade water, and 10 µl 2x GoTaq® Green Master Mix (Promega, Madison, WI) (White et al. 1990). Cycling parameters included an initial activation step at 95°C for 5 min; 35 cycles of a denaturation step at 95°C for 30 s, an annealing step at 55°C for 30 s, an extension step at 72°C for 30 s; and a final extension step at 72°C for 30 s. The resulting PCR product was visualized in a 1% agarose gel stained with GelRed Nucleic Acid Stain (Biotium, Fremont, CA) using a BioRad Molecular Image Gel Doc XR+ with Image Lab Software (Bio-Rad Laboratories, Hercules, CA). An E.Z.N.A. Cycle Pure Kit (Omega Bio-tec, Inc., Norcross, GA) was used to clean PCR products prior to sequencing by Eurofins Genomics (Louisville, KY). Sequences were trimmed, aligned, and manually edited using Geneious v. 2019.1.3 (Geneious, Auckland, New Zealand). Processed sequences were compared to sequences in the Genbank NCBI database (National Center for Biotechnology Information, Bethesda, MD) using the BLASTn function. Sequences with a greater than 98% identity, greater than 99% query coverage, and an E-value of 0 to the best match sequence in Genbank were considered to belong to the same species.

Plants and growing conditions. For greenhouse pathogenicity studies, 50 blackberry transplants cv. 'Ouachita' from North American Plants, Inc. (Lafayette, OR) were planted in December 2017 in 7.6-liter containers with Miracle-Gro® Moisture Control® Potting Mix. Staked and pruned plants were watered 1-3 times daily as needed and fertilized with Osmocote® Plus 15-9-12 slow release (The Scotts Company, Marysville, OH) at a rate of 0.24 liters / 7.6 liters every 6 months. Greenhouse temperatures were maintained at 26°C during the day and 22°C at night. In April 2019, plants were transferred outside to 29.1-liter pots and treated as the outdoor plants described below. For outdoor studies, 60 additional blackberry plants cv. 'Ouachita' were obtained from North American Plants, Inc. (Lafayette, OR) in February 2018 and were maintained in the greenhouse until May 2018. The blackberries were then transplanted into Classic 2800 29.1-liter containers (Nursery Supplies Inc., Chambersburg, PA) with Sta-Green® Potting Mix (Sims Bark Co., Muscle Shoals, AL). Plants were fertilized every 6 months with Osmocote® Plus 15-9-12 slow release (The Scotts Company, Marysville, OH) at a rate of 0.91 liters / 29.1-liter pot. For added moisture control, a thin layer of pine bark was used to topdress outdoor blackberries. The blackberries were trellised with jumbo tomato cages and pruned. Plants received overhead sprinkler irrigation for a total of 45 minutes daily during the spring, summer, and fall, and every other day during the winter.

Plant inoculation procedure. Plant inoculations for all pathogenicity trials and the fungicide efficacy trial utilized the same procedure. Prior to inoculation, fungal isolates were first grown from -20 °C stocks on AqPDA at 23°C for 1 week under 12 h fluorescent light. Agar plugs containing fungal mycelium were removed from the growing edge of the colony using a sterilized 7-mm diameter cork borer. Agar plugs containing mycelium were placed in a 1.5-mL microcentrifuge tube wrapped in white electrical tape. Microcentrifuge tubes containing the agar

plugs were placed onto freshly cut blackberry canes and secured with parafilm. Inoculated cane terminals were flagged with color-coded flagging tape depending on the isolate used, and microcentrifuge tubes and parafilm were removed 10 days after initial inoculation.

Pathogenicity trials. Six trials were carried out in order to determine pathogenicity of collected isolates. In total, 36 isolates from 34 unique species were tested, and isolates found to cause significant cane dieback as compared to the nontreated control were carried over to the next trial with untested isolates. Because of the quantity of isolates and their differing collection dates, six pathogenicity studies were carried out over a year. Trials were conducted in Summer 2018, Fall 2018, Winter 2019, Summer 2019, and Fall 2019. The Summer 2018 (Trial 1), Fall 2018 (Trial 2), Summer 2019 (Trials 4 & 5), and Fall 2019 (Trial 6) trials were conducted on outdoor container blackberries while the Winter 2019 (Trial 3) trial was conducted on container blackberries in the greenhouse.

A randomized complete block design was utilized for each pathogenicity trial; within each block, treatments consisted of fungal isolates of interest randomly inoculated to cut canes as described above, and inoculations were replicated three times for each isolate; three control treatments were applied in each trial and consisted of an uninoculated uncut plant, a cut-only plant, and cane terminals that were cut and covered with an inoculation tube containing an agar plug only (no fungal isolate). Inoculations were made using the previously described procedure. Cane dieback (in mm) was measured at 10 days post-inoculation (after the inoculation tubes were removed) and weekly thereafter for 5 to 8 weeks.

Upon the conclusion of each trial, the cumulative dieback over time (AUDPC) was calculated for each isolate and compared to the respective uninoculated controls using analysis of variance (ANOVA) followed by Tukey's honest significance difference test (HSD) using the

package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria). Isolates shown to cause significant dieback were re-isolated from colonized tissue and their identity was confirmed using morphological characteristics and sequencing of the ITS region, as described previously.

Fungicide efficacy trial. Fungal isolates found to cause significant cane dieback as compared to the nontreated control in pathogenicity trials 1, 2, or 3 were used for plant inoculations in a fungicide efficacy trial. These isolates included *F. oxysporum*, *Pestalotiopsis microspora*, *Neofusicoccum kwambonambiense*, *Lasiodiplodia theobromae*, and *N. parvum*. All plants utilized in the efficacy trial received one inoculation with each of these five fungal isolates and two controls treatments; control were a cut only cane and cane terminals that were cut and covered with a microcentifuge tube containing a sterile agar plug. The trial was carried out on outdoor potted blackberry plants as a randomized complete block design. The trial consisted of four blocks with fungicidal treatments applied randomly to plants within each block. Experimental units consisted of one plant inoculated as described above. Plants within blocks represented whole plots and inoculated shoots represented subplots. Treatments included an nontreated control, azoxystrobin (1.13 liters/ha)(Abound, Syngenta, Greensboro, NC), pyraclostrobin & boscalid (1611.22 g/ha)(Pristine, BASF, Florham Park, NJ), myclobutanil (260.16 g/ha)(Rally 40WSP, Corteva, Wilmington, DE), propiconazole (0.44 liters/ha)(Tilt, Syngenta, Greensboro, NC), captan (4.68 liters/ha)(Captan Gold 4L, ADAMA, Raleigh, NC), cyprodinil & fludioxonil (980.74 g/ha)(Switch 62.5WG, Syngenta, Greensboro, NC), thiophanate-methyl (896.68 g/ha)(Incognito 85WDG, ADAMA, Raleigh, NC), and potassium phosphite (4.68 liters/ha)(ProPhyt, Luxembourg-Pamol, Houston, TX).

Plant canes were marked, cut, and then treated with respective fungicides 24 h before inoculations using an R&D CO_2 sprayer (Research & Demonstration Sprayers, Opelousas, LA)

at 275.79 kPa with a 11002VS spray tip (TeeJet, Louisville, KY). Treatments were applied until runoff with a backpack sprayer (equivalent to 467.7 liters/ha). Plants were inoculated with fungal isolates 24 h after fungicide treatment. Microcentrifuge tubes were removed 10 days after inoculation. Cane dieback (in mm) was measured on a weekly basis for 4 weeks after removal of the inoculation tubes.

Cane dieback was assessed by calculating cumulative dieback over time using an area under the disease progress curve (AUDPC) for each isolate. AUDPC values were compared to the AUDPC of noninoculated cane terminals using analysis of variance (ANOVA) followed by least significant difference test (LSD) in R (R v. 3.4.2, The R Foundation, Vienna, Austria). Fungicide efficacy was assessed by comparing the AUDPC values of each individual isolate among fungicide treatments within a block using analysis of variance (ANOVA) followed by least significant difference test (LSD) using the package agricolae in R (R v. 3.4.2, The R Foundation, Vienna, Austria).

RESULTS

Isolate identification. In total, 126 fungal isolates were identified based upon ITS sequencing [accessions: MN718863-MN718988]. These 126 isolates represent 34 different species (Table 4.1). Most isolates belonged to the Pestalotiopsidaceae family (34 isolates or 27%), Botryosphaeriaceae family (28 isolates or 22%), or Nectriaceae family (21 isolates or 17%) (Table 4.2). Species found within the Pestalotiopsidaceae family included *Neopestalotiopsis clavispora* (17 isolates) and *P. microspora* (17 isolates) (Table 4.2). Species found within the Botryosphaeriaceae family included *Diplodia seriata* (10 isolates), *L. theobromae* (7 isolates), *Lasiodiplodia pseudotheobromae* (4 isolate), *N. kwambonambiense* (3

isolates), *N. parvum* (3 isolates), and *B. dothidea* (1 isolate) (Table 4.2). Species found within the Nectriaceae family included six species of *Fusarium*, the most frequently identified being *F. oxysporum* (9 isolates) and *Fusarium equiseti* (6 isolates) (Table 4.2). Other species found in significant quantities were *Sphaeronaemella fragariae* (10 isolates) and members of the Glomerellaceae family, *C. gloeosporioides* (5 isolates) and *Colletotrichum siamense* (2 isolates) (Table 4.2). Among the 126 isolates, *L. coniothyrium* was only identified once from a single site (Table 4.1).

Pathogenicity trials. Isolates of *F. oxysporum*, *P. microspora*, *N. kwambonambiense*, *N. parvum*, *L. pseudotheobromae, L. theobromae*, and *C. siamense* were determined to cause significant cane dieback in at least one trial (Table 4.3). Isolates that caused significant dieback exhibited between 20 and 80 mm of dieback (Figure 4.1; Table 4.3), whereas uninoculated, cut canes generally showed minimal dieback (<2 mm). The dieback caused by *F. oxysporum* in trial 1, by week 12, had reached the crown and initiated total plant collapse (Figure 4.1); however, this same isolate of *F. oxysporum* in trials 2, 3, and 4 did not cause significant cane dieback (Table 4.3). All isolates observed to cause significant dieback in pathogenicity trials were successfully reisolated from inoculated blackberry canes 5 weeks post-inoculation. These included one isolate each of all seven species observed to cause significant dieback including *F. oxysporum*, *N. kwambonambiense*, *N. parvum*, *L. pseudotheobromae, L. theobromae*, *P. microspora*, and *C. siamense*. Morphological and sequencing results were identical between the isolates used to inoculate cane terminals and those re-isolated from cane terminals 5 weeks later.

Fungicide efficacy trial. *Neofusicoccum kwambonambiense*, *L. theobromae*, *N. parvum*, and *P. microspora* induced significant cane dieback compared to the noninoculated controls (Table 4.4) in the fungicide efficacy trial, whereas *F. oxysporum* did not cause significant cane

dieback. On cane terminals inoculated with *N. kwambonambiense*, there was a significant reduction in cane dieback with the Pristine, Incognito 85WDG, and Switch 62.5WG treatments (Table 4.4). On canes inoculated with *L. theobromae*, there was a significant reduction in cane dieback with the Incognito 85WDG, Switch 62.5WG, Pristine, and Abound treatments (Table 4.4). For *P. microspora*, no fungicide provided a statistically significant reduction in cane dieback (Table 4.4); however, Switch 62.5WG provided the greatest numerical reduction followed by Tilt and Incognito 85WDG (Table 4.4). On cane terminals inoculated with *N. parvum*, no fungicide provided a statistically significant reduction in cane dieback (Table 4.4); however, Incognito 85WDG provided the greatest numerical reduction, followed by Pristine and Switch 62.5WG (Table 4.4).

DISCUSSION

The identification of organisms associated with blackberry cane dieback and options for their chemical management were determined through a combination of field surveys, pathogenicity trials, and a chemical control study. The field survey showed a great diversity of organisms present on blighted blackberry canes. Many of the obtained isolates were re-occurring across a multitude of production sites. Pathogenicity studies showed that *Fusarium oxysporum*, *P. microspora*, *N. kwambonambiense*, *N. parvum*, *L. pseudotheobromae, L. theobromae*, and *C. siamense* are causal agents of dieback on blackberry. Evidence from the fungicide efficacy study suggested that several fungicides including Incognito 85WDG (thiophanate-methyl), Switch 62.5WG (cyprodinil & fludioxonil), Pristine (pyraclostrobin+boscalid), and Abound (azoxystrobin) could potentially be used to manage disease induced by these fungi. Preventative applications of both Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG

(thiophanate-methyl) after pruning may provide the most broad-spectrum protection from the four tested fungal isolates found to cause significant cane dieback in blackberry plantings.

In total, surveys of blighted blackberry canes in blackberry production sites revealed 34 unique fungal species representing 19 families. Among the identified species were several fungi known to be the causal agents of blackberry diseases: *B. dothidea*, *N. parvum*, *L. coniothyrium*, *F. oxysporum*, and *C. siamense* (Brannen and Krewer 2005; Faedda et al. 2018; Marulanda et al. 2014; Pastrana et al. 2017). Although these are known causal agents of disease on blackberry, they are understudied and chemical options and strategies for blackberry disease management are relatively unknown. There is little to no information on the relationships of the other dieback causing organisms identified with blackberry. Many of the other identified organisms are known to be either general decomposers like *Bahusakala longispora* (Tokumasu and Tubaki 1983), or pathogens of other plant species such as *Leptosphaeria spegazzinii* (syn. *Epicoccum sorghinum*) (Pak et al. 2017), *Cladosporium cladosporioides* (Gubler et al. 1999), and *Curvularia clavata* (Mandokhot and Chaudhary 1972).

Pathogenicity studies and fulfillment of Koch's postulates indicated that isolates of *F. oxysporum*, *P. microspora*, *N. kwambonambiense*, *N. parvum*, *L. pseudotheobromae, L. theobromae*, and *C. siamense* caused cane dieback of blackberry following wounding. This result is in agreement with previous reports of the pathogenicity of *F. oxysporum* and *N. parvum* on blackberry (Faedda et al. 2018; Pastrana et al. 2017; Williamson 2017). *Fusarium oxysporum* has recently been reported causing disease in numerous North American blackberry plantings (Pastrana et al. 2017) and *N. parvum* was recently reported as causing stem canker and cane girdling on blackberry in Italy (Faedda et al. 2018).

Leptosphaeria coniothyrium did not induce significant cane dieback in our pathogenicity study. The inoculations did result in a small amount of cane dieback, however, and the isolate was successfully resiolated from tested canes. Only one isolate of *L. coniothyrium* was successfully obtained during field surveys, and it is possible that virulence among isolates of *L. coniothyrium* may vary. The growth of this *L. coniothyrium* isolate was generally very slow in culture and this may signify why cane dieback was not significant during the duration of pathogenicity trials. Cane dieback may be more significant if the isolate were allowed to overwinter on inoculated canes and carry over to the next growing season when floricanes are stressed from crop load and environmental stresses including humidity and heat. This would be more similar to the natural disease cycle of cane blight.

The five other isolates that caused significant cane dieback in this study, *P. microspora*, *N. kwambonambiense*, *L. pseudotheobromae, L. theobromae*, and *C. siamense* had not been previously reported on blackberry. They have, however, been previously reported as pathogens of other plant species. *Pestalotiopsis microspora* has been reported as the causal agent of leaf spot of oil palm (Shen et al. 2014) and Hidcote (Zhang et al. 2010) as well as black spot of pecan (Shi et al. 2015). This fungus belongs to the endophytic fungal genus known to include weak or opportunistic pathogens, and species within this genus can be quite problematic in tropical areas, primarily causing leaf blight and spots, root and stem dieback, and fruit rots of many economic and ornamental crops (Maharachchikumbura et al. 2011). It is suspected that these *Pestalotiopsis* spp. may be symptomless until the host plant is stressed, and in our pathogenicity trials we wounded the plant, potentially facilitating pathogen entry and allowing this fungus to cause disease. In commercial blackberry production sites, blackberries are frequently wounded during pruning or through other means.

Neofusicoccum kwambonambiense, *L. pseudotheobromae*, and *L. theobromae* are members of the Botryosphaeriaceae family. *Neofusicoccum kwambonambiense* is associated with diseases such as post-harvest rot and mummification of strawberry (Lopes et al. 2014), branch cankers and dieback in several African tree species (Jami et al. 2014), and vine dieback in grape (Abreo et al. 2013). *L. pseudotheobromae* is known for its ability to induce fruit rot and dieback of mango (Ismail et al. 2012; Munirah et al. 2017), stem dieback of English walnut (Li et al. 2016), fruit rot of lemon (Awan et al. 2016), and trunk canker of *Acacia mangium* (Castro-Medina et al. 2014). *L. theobromae* is another pathogen with a broad host range, causing cankers and plant collapse in crops such as grape, cotton, ornamentals, citrus, palm, tea, timber, as well as fruits and vegetables (Punithalingam 1980; Urbez-Torres et al. 2008). Members of the Botryosphaeriaceae family including *B. dothidea* and *B. obtusa* have been reported previously on blackberry. As with *Pestalotiopsis* spp., many members of the Botryosphaeriaceae are also believed to be largely endophytic and may only become plant pathogens when their hosts are stressed (Jami et al. 2014; Maas et al. 1989).

Colletotrichum siamense is known to cause anthracnose on several fruit crops including blueberry and peach (Hu et al. 2015), coffee berry (Prihastuti et al. 2009), strawberry (Capobiango et al. 2016), chili pepper (Sharma and Shenoy 2013), and Mandarin orange (Cheng et al. 2013). *Colletotrichum siamense* is also known to be among the 28 phylogenetic species that make up the *C. gloeosporioides* species complex (Sharma and Shenoy 2013; Weir et al. 2012). This complex is responsible for cane, leaf, and fruit disease of many plant species (Freeman et al. 1998). Both *C gloeosporioides* and *C. acutatum* are well-known to cause anthracnose of blackberry (Marulanda et al. 2014). Like *Pestalotiopsis* spp. and members of the Botryosphaeriaceae family, *Colletotrichum* species are prevalent and cause a multitude of plant

diseases worldwide; they are believed to survive on their host asymptomatically as an epiphyte or endophyte (Cannon et al. 2012; James et al. 2014).

In the spring and summer, blackberry floricanes are frequently stressed by heat, drought, excess rainfall, other pathogens, insects, mites, and crop load. Blackberries are also often routinely pruned and wounded during commercial production. These wounds and pruning cuts provide direct access for opportunistic fungi, and members of the Botryosphaeriaceae family, *Pestalotiopsis* species, and *Colletotrichum* species are likely capable of causing cane dieback on the wounded canes, especially when accompanied by stressed conditions in the field.

It is important to note that *L. coniothyrium* was only isolated once from a total of 126 isolates gathered over two and a half years from 8 blackberry sites across southern and northeastern Georgia. This organism was thought to be the primary pathogen associated with blackberry cane disease, and it has been observed in association with substantial dieback of blackberry plants following tropical storms and hurricanes in the past (P. Brannen, personal communication). However, the findings in this study suggest other organisms may be playing a significant if not more substantial role in observed cane dieback than *L. coniothyrium* in southern Georgia.

This study, in part, supports previous claims regarding the efficacy of fungicides for cane disease management. Effective fungicides suggested by previous research include benomyl, thiophanate-methyl, carbendazim, pyraclostobin and combinations of these chemistries (Williamson 2017). Our study suggests that Incognito 85WDG (thiophanate-methyl), Switch 62.5WG (cyprodinil & fludioxonil), Pristine (pyraclostrobin & boscalid), or Abound (azoxystrobin) can provide a statistically significant reduction in cane dieback. Although no single chemistry consistently provided the greatest reduction in cane dieback for all tested

isolates in this study, these four fungicides caused statistically significant reductions in cane dieback and were consistently the most efficacious products against all tested isolates.

When applying these fungicides preventively in a production site, it is beneficial to know the disease history of the planting to ensure the best fungicide selection for protection of newly pruned canes. If the disease history of the planting is not known, Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG (thiophanate-methyl) provided a reduction in cane dieback for all four tested isolates found to cause significant dieback as compared to the nontreated control. For *N. kwambonambiense* and *L. theobromae*, they provided a statistically significant reduction of cane dieback, while cane dieback of *P. microspora* and *N. parvum* inoculated plants was also reduced numerically by Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG (thiophanate-methyl).

For these pathogens, many of which infect through wounds, the most logical sequence to prevent disease following wounding events is to spray as soon as possible after wounding. In the efficacy trial presented here, we first cut, sprayed, and then inoculated cane terminals with tested isolates. Our results ultimately suggest that preventative applications of either Switch 62.5WG (cyprodinil & fludioxonil) or Incognito 85WDG (thiophanate-methyl) after pruning blackberry plantings may provide the most broad-spectrum protection from fungal cane dieback. Further work in the field is needed to validate these findings.

In addition, it would be beneficial to further characterize the prevalence of fungal isolates as well as the conditions necessary for infection of blackberry. This would allow for the further development of cultural and chemical practices to minimize the impact of these disease-causing organisms on blackberry production in the Southeast. In addition to identifying the organisms and effective chemistries, optimal fungicide spray timings could be evaluated in future studies.

Fungicide applications within a few hours of pruning or repeated applications after inoculation may be more effective. Studies evaluating the use of tank mixes of fungicides with different modes of action may also be useful for developing enhanced procedures for management of dieback-causing organisms.

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Table 4.1. Identified isolates recovered from diseased blackberry canes collected in Georgia.

Proposed ID (%)[w]	Isolate ID	Collection Location[x]	Closest Accession Number[y]	Accession Number
Amanita tenuifolia (98.4%)	71	Atkinson	LC206520.1	MN718928
Amanita tenuifolia (98.4%)	107	Pierce	GU053924.1	MN718963
Bahusakala longispora (99.8%)	69	Atkinson	NR_159628.1	MN718926
Botryosphaeria dothidea (100%)	102	Pierce	JX275786.1	MN718958
Cladosporium cladosporioides (100%)	128	Dougherty	MK813962.1	MN718984
Cladosporium cladosporioides (100%)	132	Dougherty	MK813962.1	MN718988
Cladosporium cladosporioides (100%)	103	Pierce	MK813962.1	MN718959
Cladosporium cladosporioides (100%)	105	Pierce	MK813962.1	MN718961
Colletotrichum gloeosporioides (100%)	39	Lanier	KX227593.1	MN718900
Colletotrichum gloeosporioides (100%)	100	Pierce	KJ719315.1	MN718956
Colletotrichum gloeosporioides (98.1%)	124	Dougherty	AB470881.1	MN718980
Colletotrichum gloeosporioides (98.6%)	37	Lanier	KX227593.1	MN718898
Colletotrichum gloeosporioides (99.8%)	10	Lanier	KX227593.1	MN718872
Colletotrichum siamense (100%)	83	Atkinson	KP703372.1	MN718940
Colletotrichum siamense (99.8%)	73	Atkinson	KP703372.1	MN718930
Curvularia clavata (100%)	118	Dougherty	KX610320.1	MN718974
Curvularia clavata (100%)	123	Dougherty	KX022493.1	MN718979
Curvularia clavata (100%)	130	Dougherty	KX022493.1	MN718986
Diaporthe hongkongensis (98.8%)	24	Oglethorpe	KY433556.1	MN718886
Diplodia seriata (100%)	74	Atkinson	MH384924.1	MN718931
Diplodia seriata (100%)	19	Oglethorpe	MH384924.1	MN718881
Diplodia seriata (100%)	23	Oglethorpe	MH384924.1	MN718885
Diplodia seriata (100%)	27	Oglethorpe	MG015750.1	MN718889
Diplodia seriata (100%)	29	Oglethorpe	MG015750.1	MN718890
Diplodia seriata (100%)	33	Oglethorpe	MG015750.1	MN718894
Diplodia seriata (99.6%)	78	Atkinson	KT440899.1	MN718935
Diplodia seriata (99.6%)	2	Irwin	MH384924.1	MN718864
Diplodia seriata (99.8%)	120	Dougherty	MH384924.1	MN718976
Diplodia seriata (99.8%)	25	Oglethorpe	MH384924.1	MN718887
Epicoccum sorghinum[z] (99.8%)	126	Dougherty	MN420978.1	MN718982
Fusarium armeniacum (99.6%)	77	Atkinson	KJ737378.2	MN718934
Fusarium armeniacum (99.6%)	114	Pierce	GQ505462.1	MN718970
Fusarium equiseti (100%)	67	Atkinson	MK212925.1	MN718924
Fusarium equiseti (100%)	68	Atkinson	MK212925.1	MN718925
Fusarium equiseti (100%)	86	Colquitt	MF471699.1	MN718943
Fusarium equiseti (100%)	16	Oglethorpe	MK212925.1	MN718878
Fusarium equiseti (100%)	93	Pierce	MF471699.1	MN718949
Fusarium equiseti (100%)	115	Pierce	MK212925.1	MN718971
Fusarium langsethiae (98.9%)	76	Atkinson	MG274309.1	MN718933
Fusarium oxysporum (100%)	75	Atkinson	MK074845.1	MN718932
Fusarium oxysporum (100%)	4	Barrien	MK074845.1	MN718866
Fusarium oxysporum (100%)	3	Irwin	MK074845.1	MN718865
Fusarium oxysporum (100%)	5	Lanier	MK074845.1	MN718867
Fusarium oxysporum (100%)	42	Lanier	MK074845.1	MN718903
Fusarium oxysporum (100%)	50	Lanier	MK074845.1	MN718911
Fusarium oxysporum (100%)	51	Lanier	MK074845.1	MN718912

Fusarium oxysporum (100%)	53	Lanier	MK074845.1	MN718914
Fusarium oxysporum (100%)	20	Oglethorpe	MG836253.1	MN718882
Fusarium palustre (99.4%)	14	Oglethorpe	MH864236.1	MN718876
Fusarium proliferatum (100%)	6	Irwin	MK372368.1	MN718868
Fusarium proliferatum (100%)	11	Lanier	MK372368.1	MN718873
Gilbertella persicaria (100%)	21	Oglethorpe	KC683539.1	MN718883
Gilbertella persicaria (100%)	26	Oglethorpe	KC683539.1	MN718888
Lasiodiplodia pseudotheobromae (100%)	58	Barrien	LC074359.1	MN718918
Lasiodiplodia pseudotheobromae (100%)	131	Dougherty	MN046825.1	MN718987
Lasiodiplodia pseudotheobromae (100%)	48	Lanier	MN046825.1	MN718909
Lasiodiplodia pseudotheobromae (99.8%)	116	Pierce	MN046825.1	MN718972
Lasiodiplodia theobromae (100%)	70	Atkinson	MN335222.1	MN718927
Lasiodiplodia theobromae (100%)	62	Barrien	MN335222.1	MN718922
Lasiodiplodia theobromae (100%)	127	Dougherty	MN335222.1	MN718983
Lasiodiplodia theobromae (100%)	43	Lanier	MN335222.1	MN718904
Lasiodiplodia theobromae (100%)	30	Oglethorpe	MN335222.1	MN718891
Lasiodiplodia theobromae (100%)	104	Pierce	MN335222.1	MN718960
Lasiodiplodia theobromae (99.2%)	35	Lanier	MN335222.1	MN718896
Leptosphaeria coniothyrium (100%)	110	Pierce	JN017200.1	MN718966
Mycosphaerella pyrolae[z] (98.7%)	112	Pierce	AF312010.1	MN718968
Mycosphaerella pyrolae[z] (99.0%)	90	Pierce	AF312010.1	MN718946
Neofusicoccum kwambonambiense (100%)	22	Oglethorpe	KU997386.1	MN718884
Neofusicoccum kwambonambiense (100%)	32	Oglethorpe	KU997386.1	MN718893
Neofusicoccum kwambonambiense (100%)	106	Pierce	KU997386.1	MN718962
Neofusicoccum parvum (100%)	45	Lanier	EU860378.1	MN718906
Neofusicoccum parvum (100%)	46	Lanier	EU860378.1	MN718907
Neofusicoccum parvum (99.5%)	95	Pierce	EU860378.1	MN718951
Neopestalotiopsis clavispora (100%)	79	Atkinson	EU342214.1	MN718936
Neopestalotiopsis clavispora (100%)	81	Atkinson	EU342214.1	MN718938
Neopestalotiopsis clavispora (100%)	54	Barrien	GQ415344.1	MN718915
Neopestalotiopsis clavispora (100%)	87	Colquitt	EU342214.1	MN718944
Neopestalotiopsis clavispora (100%)	44	Lanier	EU342214.1	MN718905
Neopestalotiopsis clavispora (100%)	49	Lanier	KY606543.1	MN718910
Neopestalotiopsis clavispora (99.6%)	89	Colquitt	AY924263.1	MN718945
Neopestalotiopsis clavispora (99.8%)	82	Atkinson	EU342214.1	MN718939
Neopestalotiopsis clavispora (99.8%)	13	Lanier	EU342214.1	MN718875
Neopestalotiopsis clavispora[z] (100%)	60	Barrien	EU342214.1	MN718920
Neopestalotiopsis clavispora[z] (100%)	12	Irwin	EU342214.1	MN718874
Neopestalotiopsis clavispora[z] (100%)	34	Lanier	EU342214.1	MN718895
Neopestalotiopsis clavispora[z] (100%)	36	Lanier	EU342214.1	MN718897
Neopestalotiopsis clavispora[z] (100%)	38	Lanier	EU342214.1	MN718899
Neopestalotiopsis clavispora[z] (100%)	40	Lanier	EU342214.1	MN718901
Neopestalotiopsis clavispora[z] (99.6%)	84	Colquitt	EU342214.1	MN718941
Neopestalotiopsis clavispora[z] (99.8%)	18	Oglethorpe	EU342214.1	MN718880
Neurospora calospora (99.6%)	119	Dougherty	MH856849.1	MN718975
Neurospora calospora (99.6%)	129	Dougherty	MH856849.1	MN718985
Neurospora dictyophora (99.6%)	92	Pierce	MH862539.1	MN718948
Pestalotiopsis microspora (100%)	66	Atkinson	MK120574.4	MN718923
Pestalotiopsis microspora (100%)	72	Atkinson	MK801280.1	MN718929
Pestalotiopsis microspora (100%)	80	Atkinson	MK801280.1	MN718937
Pestalotiopsis microspora (100%)	55	Barrien	MK801280.1	MN718916

Pestalotiopsis microspora (100%)	57	Barrien	MK801280.1	MN718917
Pestalotiopsis microspora (100%)	59	Barrien	MK801280.1	MN718919
Pestalotiopsis microspora (100%)	61	Barrien	MH094237.1	MN718921
Pestalotiopsis microspora (100%)	121	Dougherty	MK801280.1	MN718977
Pestalotiopsis microspora (100%)	122	Dougherty	MK801280.1	MN718978
Pestalotiopsis microspora (100%)	8	Irwin	MK801280.1	MN718870
Pestalotiopsis microspora (100%)	7	Lanier	MK801280.1	MN718869
Pestalotiopsis microspora (100%)	52	Lanier	MK801280.1	MN718913
Pestalotiopsis microspora (100%)	15	Oglethorpe	MK801280.1	MN718877
Pestalotiopsis microspora (100%)	17	Oglethorpe	MH094237.1	MN718879
Pestalotiopsis microspora (100%)	96	Pierce	MK801280.1	MN718952
Pestalotiopsis microspora (100%)	109	Pierce	MK801280.1	MN718965
Pestalotiopsis microspora (100%)	117	Pierce	MK801280.1	MN718973
Phoma herbarum (99.8%)	9	Lanier	KJ767079.1	MN718871
Pleurostoma richardsiae (100%)	41	Lanier	MH010954.1	MN718902
Schizophyllum commune (99.5%)	47	Lanier	MH539647.1	MN718908
Sordaria conoidea[z] (99.5%)	98	Pierce	MG098252.1	MN718954
Sordaria humana[z] (99.5%)	111	Pierce	EU918705.1	MN718967
Sphaeronaemella fragariae (99.2%)	94	Pierce	HM854852.1	MN718950
Sphaeronaemella fragariae (99.2%)	97	Pierce	HM854852.1	MN718953
Sphaeronaemella fragariae (99.2%)	99	Pierce	HM854852.1	MN718955
Sphaeronaemella fragariae (99.3%)	85	Colquitt	HM854852.1	MN718942
Sphaeronaemella fragariae (99.3%)	31	Oglethorpe	HM854852.1	MN718892
Sphaeronaemella fragariae (99.4%)	125	Dougherty	HM854852.1	MN718981
Sphaeronaemella fragariae (99.4%)	91	Pierce	HM854852.1	MN718947
Sphaeronaemella fragariae (99.4%)	101	Pierce	HM854852.1	MN718957
Sphaeronaemella fragariae (99.4%)	108	Pierce	HM854852.1	MN718964
Sphaeronaemella fragariae (99.4%)	113	Pierce	HM854852.1	MN718969
Sporidiobolus pararoseus (99.7%)	1	Lanier	KP346983.1.1	MN718863

[w]Using the BLASTn function in the Genbank NCBI database, sequences with a greater than 98% identity, greater than 99% query coverage, and an E-value of 0 to the best match sequence in Genbank were considered to belong to the same species. Proposed ID percent is relative to the most similar accession number in Genbank.

[x]Georgia county that the respective isolate was collected from

[y]The top hit generated by the BLASTn function in the Genbank NCBI database for the respective isolate ID

[z]Isolates were renamed, *Pestalotiopsis clavispora* isolates to *Neopestalotiopsis clavispora*, *Leptosphaeria spegazzinii* to *Epicoccum sorghinum*, *Phyllosticta pyrolae* to *Mycosphaerella pyrolae*, *Asordaria conoidea* to *Sordaria conoidea*, *and Asordaria humana to Sordaria humana* based on the current accepted name in Index Fungorum and MycoBank databases.

Table 4.2. Summary of the diversity of fungal species isolated from blighted blackberry canes.

Family (total count)	Species	Counts
Pestalotiopsidaceae (34)	*Neopestalotiopsis clavispora*[z]	17
	Pestalotiopsis microspora	17
Botryosphaeriaceae (28)	*Diplodia seriata*	10
	Lasiodiplodia theobromae	7
	Lasiodiplodia pseudotheobromae	4
	Neofusicoccum kwambonambiense	3
	Neofusicoccum parvum	3
	Botryosphaeria dothidea	1
Nectriaceae (21)	*Fusarium oxysporum*	9
	Fusarium equiseti	6
	Fusarium proliferatum	2
	Fusarium armeniacum	2
	Fusarium langsethiae	1
	Fusarium palustre	1
Microascales incertae sedis (10)	*Sphaeronaemella fragariae*	10
Glomerellaceae (7)	*Colletotrichum gloeosporioides*	5
	Colletotrichum siamense	2
Cladosporiaceae (4)	*Cladosporium cladosporioides*	4
Pleosporaceae (3)	*Curvularia clavata*	3
Sordariaceae (3)	*Neurospora calospora*	2
	Neurospora dictyophora	1
Amanitaceae (2)	*Amanita tenuifolia*	2
Gilbertellaceae (2)	*Gilbertella persicaria*	2
Leptosphaeriaceae (2)	*Epicoccum sorghinum*[z]	1
	Leptosphaeria coniothyrium	1
Mycosphaerellaceae (2)	*Mycosphaerella pyrolae*[z]	2
Sporomiaceae (2)	*Sordaria conoidea*[z]	1
	Sordaria humana[z]	1
Diaporthaceae (1)	*Diaporthe hongkongensis*	1
Didymellaceae (1)	*Phoma herbarum*	1
Dothideomycetes Incertae sedis (1)	*Bahusakala longispora*	1
Pleurostomataceae (1)	*Pleurostoma richardsiae*	1
Schizophyllaceae (1)	*Schizophyllum commune*	1
Sporobolomycetaceae (1)	*Sporidiobolus pararoseus*	1

[z]Isolates were renamed, *Pestalotiopsis clavispora* isolates to *Neopestalotiopsis clavispora*, *Leptosphaeria spegazzinii* to *Epicoccum sorghinum*, *Phyllosticta pyrolae* to *Mycosphaerella pyrolae*, *Asordaria conoidea* to *Sordaria conoidea*, *and Asordaria humana to Sordaria humana* based on the current accepted name in Index Fungorum and MycoBank databases.

Table 4.3. Cumulative dieback observed in the six pathogenicity trials by isolate.

	AUDPC[yz]					
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 6**
Diplodia seriata (2)	422.3					
Pestalotiopsis microspora (7)	447.4					
Colletotrichum gloeosporioides (10)	616.0					
Fusarium proliferatum (6)	638.8					
Sporidiobolus pararoseus (1)	679.0					
Neopestalotiopsis clavispora (13)	825.4					
Phoma herbarum (9)	893.7					
Neopestalotiopsis clavispora (12)	926.3					
Fusarium oxysporum (3)	2326.3 **	424.8	66.5	223.5		
Lasiodiplodia theobromae (30)		1643.7**	218.0	969.5**		
Pestalotiopsis microspora (17)		1400.0**	847.0**	128.6		
Neofusicoccum parvum (45)		1611.1**	973.8**	795.6**		
Neofusicoccum kwambonambiense (22)		1951.8**	1226.5**	861.5**		
Fusarium palustre (14)		434.4	364.0			
Pleurostoma richardsiae (41)		391.3				
Gilbertella persicaria (21)		209.2				
Diplodia seriata (23)		184.2				
Fusarium equiseti (16)		151.7				
Schizophyllum commune (47)		129.1				
Diaporthe hongkongensis (24)		114.7				
Fusarium oxysporum (20)		104.9				
Sphaeronaemella fragariae (31)			691.6			
Neopestalotiopsis clavispora (18)			509.0			
Colletotrichum siamense (83)					1556.1**	
Fusarium langsethiae (76)					713.1	
Fusarium equiseti (67)					395.3	
Fusarium oxysporum (50)					175.2	
Lasiodiplodia pseudotheobromae (131)						2194.6**
Mycosphaerella pyrolae (112)						694.9
Coniothyrium fuckelii (110)						585.8
Fusarium armeniacum (114)						477.1
Curvularia clavate (118)						334.6
Botryosphaeria dothidea (102)						315.2
Fusarium equiseti (93)						191.8
Epicoccum sorghinum (126)						127.4
Cladosporium cladosporioides (103)						92.4
Control: Cut No Tube	290.5	51.7	47.4	27.8	84.8	143.5
Control: Cut Agar & Tube	633.5	153.4	47.8	20.8	64.4	76.2

[y]AUDPC area under the disease progress curve for each of the corresponding trials
[z]Isolate AUDPC values followed by "**" were statistically greater than the control (α=0.05)

Table 4.4. Cumulative dieback observed in the fungicide efficacy trail by isolate and fungicide treatment.

Fungicide Treatment[y]	*Lasiodiplodia theobromae* (30) [z]	*Neofusicoccum kwambonambiense* (22) [z]	*Neofusicoccum parvum* (45) [z]	*Pestalotiopsis microspora* (17) [z]	*Fusarium oxysporum* (3) [z]	Cut With Blank Agar Plug[z]	Cut Only[z]
Abound	808.50b	1267.9abc	537.3ab	1032.5a	168.9	18.7a	21.0ab
Pristine	448.0b	445.7c	491.2ab	541.3a	424.7	35.0a	23.3ab
Rally 40WSP	1906.3ab	1050. 0abc	639.3ab	702.3a	214.7	23.3a	42.0a
Tilt	1044.8ab	1661.6abc	1721.1a	251.1a	139.1	23.7a	20.1b
Captan Gold 4L	1121.8ab	2096.5ab	1231.1ab	742.0a	56.9	26.3a	19.3b
Switch 62.5WG	381.5b	578.4bc	505.8ab	189.0a	34.1	21.9a	20.1b
Incognito 85WDG	241.5b	488.3c	377.1b	398.1a	57.8	33.3a	32.4ab
ProPhyt	995.8ab	1293.3abc	1554.0ab	404.3a	144.4	64.8a	18.7b
Control	2514.8a	2245.3a	960.8ab	587.1a	28.0	72.6a	24.5ab

[y]Fungicide treatment used.

[z]Area under the disease progress curve for each treatment. Means in each column followed by the same letter are not significantly different according to Fisher's least significant difference test (LSD)($\alpha = 0.05$).

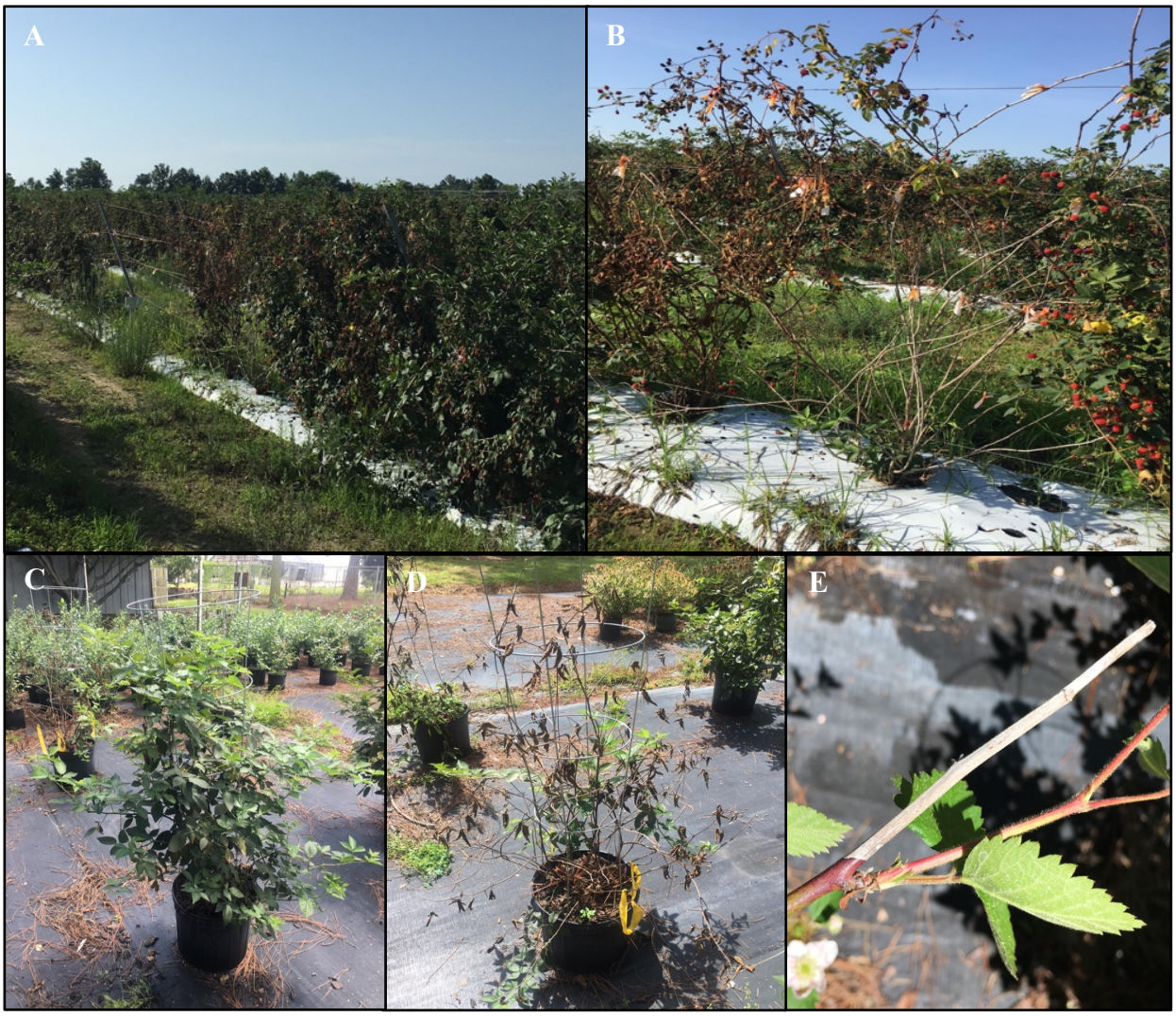

Figure 4.1. Dieback of blackberry. (A) Diseased blackberry plants among healthy blackberry, (B) cane tip dieback to crown, (C)) healthy blackberry, (D) collapse induced by *F. oxysporum* 12 weeks post-inoculation in trial 1, and (E) cane dieback from tip.

CHAPTER 5

CONCLUSIONS

Orange cane blotch (OCB) and cane blight (CB), along with cane dieback diseases, are important yet relatively understudied diseases of blackberry in the southeastern United States. To further understand these diseases, several field and laboratory studies were conducted. To further understand the epidemiology of OCB, field monitoring and microscopy of diseased canes were used to create a detailed disease cycle timeline as it corresponded to blackberry phenology. To further understand how potassium phosphite impacts OCB and *Cephaleuros virescens*, field trials assessing algal blotches after applications of potassium phosphite and in vitro assays were utilized. To determine the most efficacious application period of potassium phosphite for managing OCB, two additional field trials were carried out utilizing the newly developed disease cycle timeline to test potential targeted sprays. To better understand what fungi were associated with blighted canes, numerous field surveys and pathogenicity trials were conducted. A fungicide efficacy trial was performed with isolates found to be pathogenic from field surveys to determine effective chemical management options.

Disease cycle monitoring of OCB revealed many details of the disease cycle on blackberry. OCB was found to be a monocyclic disease having one period of infection through spring and summer. All algal blotches observed on emerged primocanes in the fall could also be traced to a single time period in late summer. Growth of algal blotches coincided with blackberry plant developmental stages. *C. virescens* entered and exited dormancy with blackberry.

Reproductive structures of *C. virescens* were also in line with blackberry fruit set. Ultimately, disease cycle monitoring exposed potential targets for potassium phosphite applications.

Several key observations were revealed from testing how potassium phosphite impacts OCB and *C. virescens*. Field applications of potassium phosphite reduced disease severity by primarily reducing algal blotch number on primocanes. This reduction likely represents the ability of this chemistry to reduce initial infection. Applications of potassium phosphite also significantly, yet more modestly, reduced algal blotch size. This parameter represents the ability of this product to limit the growth of *C. virescens* post-infection. In vitro assay results with *C. virescens* and potassium phosphite also supported this latter finding. Potassium phosphite was observed to reduce total area of algal colonies at the maximum label rate of 1%, indicating a direct toxic effect on *C. virescens*. It is unclear how potassium phosphite may be limiting algal infection of primocanes, although activation of plant defenses may be playing a role. Together, these studies detail the impact of potassium phosphite on OCB and *C. virescens*, providing information that can be used to deploy this chemistry most effectively.

Information gathered from disease cycle monitoring and from studies detailing the impact of potassium phosphite on *C. virescens* and OCB were combined to plan and carry out two field trials evaluating targeted sprays. In these trials, it was observed that when making additional applications to floricanes that may or may not have been treated the previous season as primocanes, disease severity was not impacted on the already infected canes. Disease reduction was evident for blackberry canes that were treated the previous season. Furthermore, assessing the impact of early applications of potassium phosphite that focused on protection of emerging primocanes through algal sporulation, late applications focusing on applications from algal sporulation until dormancy, and season long applications combining the two treatment periods

revealed that partial season applications may be as effective as season long applications and that potassium phosphite applications starting with algal sporulation were the most effective. Applications during this period were able to both limit the number and size of algal blotches. This reaffirms the ability of potassium phosphite to impact the alga and disease by limiting initial infection and the growth of algal blotches after infection.

Isolation of organisms associated with diseased canes exhibiting cane dieback revealed that *Leptosphaeria coniothyrium*, the causal organism of cane blight and reportedly largest contributor to cane dieback in the southeast, may be playing a smaller role in observed dieback in blackberry plantings than previously theorized. Out of 126 fungal isolates recovered from diseased canes at numerous production sites across Georgia over multiple seasons, only one isolate was identified as *L. coniothyrium*. Upon testing in pathogenicity trials, *L. coniothyrium* was successfully reisolated from inoculated cane terminals, signifying successful inoculation; however, it did not cause significant cane dieback. *Colletotrichum siamense*, *Fusarium oxysporum*, *Lasiodiplodia theobromae, Lasiodiplodia pseudotheobromae*, *Neofusicoccum kwambonambiense*, *Neofusicoccum parvum*, and *Pestalotiopsis microspora* caused significant cane dieback in at least one trial. *Fusarium oxysporum* and *Neofusicoccum parvum* were reconfirmed to cause significant cane dieback while the others had never been previously reported to cause cane dieback on blackberry.

The fungicide efficacy study carried out with a subset of these recovered isolates revealed potential pesticides capable of reducing cane dieback caused by these pathogens. *Colletotrichum siamense* and *Lasiodiplodia pseudotheobromae* were not included in the fungicide efficacy study because they were isolated and found to be pathogenic after the fungicide efficacy trial was completed. No single chemistry provided the most reduction in disease severity for all tested

isolates. For each isolate used in the fungicide efficacy trial, dieback was reduced by at least one tested fungicide. Results suggest that in order to best manage cane decline, it may be necessary to identify the organism or organisms present in the diseased planting of interest. However, Switch 62.5WG (cyprodinil & fludioxonil) and Incognito 85WDG (thiophanate-methyl) provided a reduction of cane dieback for all tested isolates that caused significant cane dieback. Therefore, if the causal organism is not known, these chemistries would provide the broadest spectrum of disease severity reduction. Results from this trial supported, in part, previous claims of efficacious chemistries.

Observations made from OCB as well as CB and cane dieback studies provided new knowledge regarding the biology and management of these important yet understudied plant pathogens of cultivated blackberry. There are still unknowns pertaining to these diseases. Testing with various rates of potassium phosphite as well as other pesticides, including other phosphonates, should be further conducted now that the OCB disease cycle has been detailed. This would aid in maximizing the longevity of potassium phosphite and management of OCB. Further studies aimed at clarifying the role of potassium phosphite toxicity to *C. virescens* and potential plant defense activation would also aid in maximizing the use of this chemistry. Cane dieback of blackberry should be studied further by continuing surveys, isolations, and fungicide efficacy trials with newly identified isolates found in association with diseased canes exhibiting cane dieback. Further testing of *L. coniothyrium* isolates should be evaluated to further characterize the role of this organism in cane disease in the Southeast. The findings in this work should be paired with current cultural management practices of both OCB and cane dieback diseases in order to achieve maximum disease management in commercial blackberry plantings.

APPENDIX A

GROWING *CEPHALEUROS VIRESCENS* IN VITRO

Cephaleuros virescens (Cv) was isolated using techniques adapted from both Suto and Ohtani (2010) and Browne et al. (2019). First, sections of orange cane blotch diseased blackberry canes with sporulating Cv were collected from the field. Canes were thoroughly rinsed with deionized water, then placed under a laminar flow hood for Cv isolation. Successful isolations were made by cutting away a thin slice of epidermal tissue containing protruding asexual structures, zoosporangia on sporangiophores, or by scraping off the asexual structures exclusively. Once sampled from the cane, these samples were briefly dipped in 70% EtOH (< 1 sec) and then immediately submerged in sterile deionized water. Samples were then placed onto Bold's basal medium (PhytoTechnology Labratories, Shawnee Mission, KS) containing 10 ppm of the active ingredient in the organic fungicide benomyl ((methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate) (Chem Service, West Chester, PA), 50 ppm of the antibiotic streptomycin, and 1 ml/l of 0.1% aqueous sulfuric acid solution (Suto and Ohtani, 2010; Browne et al. 2019b). All cultures were then placed under 12-h cool fluorescent light to grow for at least 1 month. Once the samples turned green and began to grow filaments (~1 month later), these filaments were used for further isolations using another adjusted protocol. Clusters of algal filaments from the previous cultures were removed. Approximately the lower 1/3 of the filaments were partially implanted in Bold's basal solid media (25 ml), then submerged in Bold's basal broth (10 ml) (using 100 mm × 15 mm petri plates). The newly plated samples were

stacked in stacks of four under the 12-h cool fluorescent light at 27°C. A blank agar plate was placed on top to protect the top isolate from direct light.

Slow algal growth and contamination are the biggest challenges when culturing Cv. The addition of sulfuric acid, benomyl, and streptomycin to the medium and broth help to reduce contamination, but it is also essential to sanitize isolation tools and work under a laminar flow hood. Continually monitor all isolate plates and immediately remove any with contamination. Common contaminants include various fungi, bacteria, and unicellular non-filamentous algae. Mites are also a major issue. Submerging algal isolates in broth reduced all contamination issues. In addition, the plates remained hydrated, eliminating the need for replating; satellite colonies formed around the original transfer site, and the rate of growth of the algae was also much more rapid. Four-month old algal cultures submerged in broth were comparable to isolates grown without submersion for 9 months (Figure A.1). In submerged cultures, the colonies first grew up to the surface of the broth and then spread laterally forming a floating elliptical thallus. The base where the filaments were first placed served as an anchor in the medium. Satellite colonies formed from detached filaments from the floating portion of the thalli or from runners that grew through the media at the anchor point. All new satellite colonies developed a single attachment point to the media with the majority of the thallus floating on the broth surface. Non-submerged thalli expanded radially with elongated algal filaments along the surface of the media. Their maximum size was limited compared to the submerged isolates. From previous in vitro studies, evidence also suggests that lowering the pH past the Bold's basal recommended pH of 6.6 to pH 6.035 or lower will also significantly increase the rate of algal growth. In conclusion, submerging the algal isolates in broth helped to both limit contaminants and almost doubled the algal growth rate.

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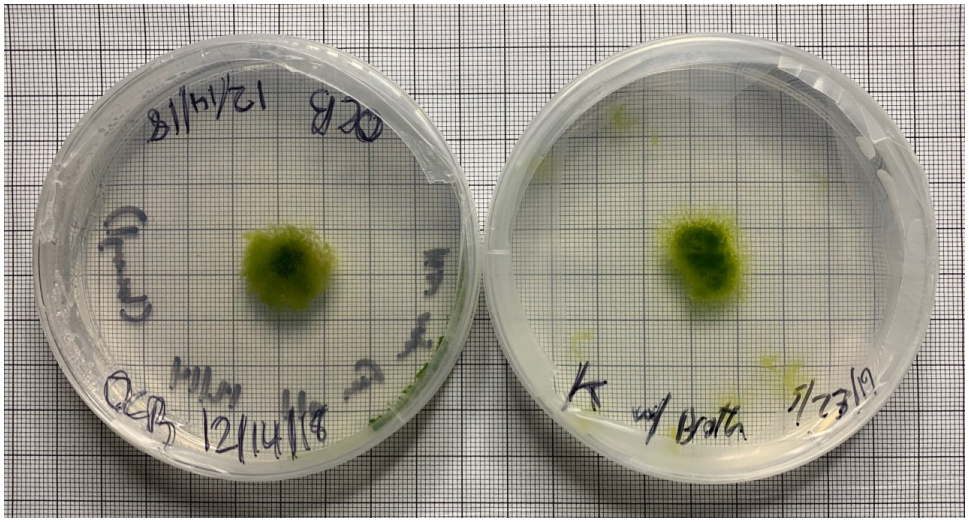

Figure A.1. Comparison of in vitro growth of *Cephaleuros virescens*. Non-submerged with Bold's basal broth 9 months after isolation (left) and submerged with 10 ml of Bold's basal broth 4 months after isolation (right).