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Impacts of commercial biopesticides on crapemyrtle bark scale (Acanthococcus lagerstroemiae) and beneficial insects

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IMPACTS OF COMMERCIAL BIOPESTICIDES ON CRAPEMYRTLE BARK SCALE (*ACANTHOCOCCUS LAGERSTROEMIAE*) AND BENEFICIAL INSECTS

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Abstract

The crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is an important pest of crapemyrtles, *Lagerstromia* spp. (Myrtales: Lythraceae) since its damage results in an unpleasant aesthetic. Current CMBS management methods depend heavily on pesticides which impact on beneficial insects. Biopesticides show potential for pest control, host specificity, and low impact towards non-target organisms. The objectives of my thesis were to determine (1) if biopesticides are effective against CMBS infestations when applied in different seasons, and (2) their effects towards coccinellids known to attack CMBS.

To test the efficacy of selected biopesticides, treatments were delivered to potted plants or full-grown trees infested with CMBS in different seasons. The fungal treatments Ancora® (*Isaria fumosorosea* strain PFR97) and BioCeres® (*Beauveria bassiana* strain ANT-03), and bacterial products Venerate® (*Burkholderia* spp. A396 strain) and Grandevo® (*Chromobacterium subtsugae* PRAA4-1T strain) failed to control CMBS in greenhouse conditions. When BioCeres®, Ancora®, and BotaniGard® (*B. bassiana* strain GHA) were delivered in field settings, BioCeres® significantly increased proportion of dead to total scales on full grown trees in the winter trial; whereas BotaniGard® was more effective during the Fall trial on small potted plants. In the spring trial, biopesticides failed to control CMBS. Temperature impacted product efficacy in the field. According to laboratory studies, the highest fungal germination was achieved at 28°C, and highest germination rates were between 25 and 30°C. Therefore, products containing *B. bassiana* can be a good management tool for CMBS on cooler seasons.

Susceptibility of natural enemies to biopesticides in laboratory conditions was assessed

by submerging the insects in a biopesticide mixture and observing survival over time. BotaniGard® significantly reduced the survival of larvae and adults of both *Hyperaspis bigeminata* and *Chilocorus* spp. by at least 57%. Adults of *Chilocorus* spp. had survival also reduced by 40% when treated with BioCeres®; and Ancora® reduced the survival of *H. bigeminata* larvae by 69%. In field conditions, entomopathogenic fungi spores were collected from live coccinellids, ensuring contact but not pathogenicity. Results of this project provide a basic understanding of the impact of biopesticides for the management of CMBS.

Chapter 1. Background and literature review

1.1. Importance of crapemyrtles

Crapemyrtles, *Lagerstroemiae* spp. (Myrtales: Lythraceae) are a genus of trees native from southeastern Asia and commonly used as ornamentals due to their beautiful flowers, bark, and easy maintenance. Crapemyrtles are highly valuable in the southeastern region of the United States, having an annual nursery plant wholesale value of $66 million (USDA NASS 2017). (Egolf and Andrick 1978, Chappell *et al*. 2012, Gu *et al*. 2014). Crapemyrtles were introduced into United States over 175 years ago, and since then the USDA National Arboretum and private breeding programs have developed a wide variety of cultivars (Chappell *et al.* 2012). Crapemyrtles can be classified as trees and shrubs, have panicles measuring from 15.3cm to 45.8cm that may be composed by hundreds of small florets (Knox 2003).

The genus *Lagerstroemiae* has more than 50 species and *L. indica* L. and *L. fauriei* Koehne are two of the most commonly used species in crapemyrtle hybridization (Wang *et al.* 2011). Their hybrids are some of the most popular varieties on the market (Ye *et al.* 2009, Wang *et al.* 2011, Chappell *et al.* 2012). Tetraploid lines of crapemyrtles have been developed to maximize its varieties and ornamental attributes (Ye *et al.* 2010).

Abiotic and biotic stressors can interfere with crapemyrtle's aesthetic. Abiotic stressors affecting plant vigor and response to other stressors include soil amendment, water availability, nutrients, and light (Islam *et al.* 2018, Kumar and Trivedi 2018, Sestili *et al.* 2018). The most common biotic stressors affecting crapemyrtles in the United States are Japanese beetle (*Popillia japonica* Newman), flea beetle (*Altica* spp.) (Pettis *et al.* 2004), crapemyrtle aphid [*Tinocallis kahawaluokalani* (Kirkaldy)] (Vassiliou and Drees 2008, Herbert *et al.* 2009), powdery mildew

[*Erysiphe lagerstroemia* (West)] (Hagan *et al.* 1998), cercospora leaf-spot (*Cercospora lythracearum* Heald & F. A. Wolf), and the most recently, crapemyrtle bark scale [*Acanthococcus lagerstroemiae* (Kuwana)] (Wang *et al.* 2016).Because of the significant economic value of crapemyrtles, in 2015, the crapemyrtle bark scale (CMBS) was deemed by the Greenhouse Grower magazine as one of the top nine pest reported in the United States (Miller 2015).

1.2. Biology, distribution, and ecology of CMBS

Crapemyrtle bark scale is a member of the family Eriococcidae, which are commonly known as felted scales, characterized by purple or dark red body coloration, and being covered by waxy secretions (Miller and Miller 1993). Native from Asia, the method and timing of arrival to the United States was unknown, but CMBS was first reported in a nursery in Richardson, TX in 2004 (Robbins *et al*. 2014).

Being highly fecund and having multiple generations within a year makes CMBS an aggressive pest. It can take 36 days at 17.5°C and 10 days at 27.5°C for eggs to hatch, 154 days at 20°C to 56 days at 30°C for a nymph turn into male prepupa, and 137 days at 25°C to 68 days at 30°C for a nymph turn into a gravid female (Wang *et al.* 2019b). Crapemyrtle bark scale's eggs are pink and wrapped by a waxy egg-sac secreted by the female which, as in other scale insects, maintains ideal humidity and protects the eggs from predators (Uma-Devi *et al.* 2008). First instars or crawlers emerge from egg-sacs and are responsible from localized dispersal (Gu *et al.* 2014). The immature developmental stages of CMBS are consisted of three nymphal stages and is followed by differentiation between males and females (Wang *et al.* 2016). Male immatures are enclosed by white sacs and undergo from prepupa to pupa stage. Males have one pair of wings, no mouthparts, five pairs of ocelli distributed on dorsal, ventral, and lateral sides

of the head, and two long white filaments at the distal part of the abdomen (Wang *et al.* 2016). Females are wingless, sessile, and once mated, start to produce a waxy coverage and proceed to lay eggs; a single female can lay up to 320 eggs (Wang *et al.* 2016).

Crapemyrtle bark scale has been reported in Louisiana, Arkansas, Oklahoma, Virginia, Tennessee, North Carolina, Mississippi, Alabama, New Mexico, Kansas, and Georgia (EDDMapS 2020). Dispersal of CMBS and other coccoid insects occurs by active or passive movement of crawlers (Hanks and Denno 1998, Gu *et al.* 2014). The crawlers move around for about one to two days, and once settled, will start feeding, and excreting honeydew. Passive movement of CMBS may occur due to wind, animals, or human activities, such as shipping of nurseries stock plants or containerized plants (Gu *et al.* 2014). Winter temperature is a major factor affecting CMBS establishment. Based on laboratory experiments, Wang et al. (2019a) found that CMBS has adaptations to establish up the latitude 43°N, matching the limit where crapemyrtles are cultivated in the U.S.

Crapemyrtle bark scale is not a specialist on crapemyrtles. As reported from different countries in southeast Asia and Hungary, CMBS was able to attack several different species (Hoy 1963, Kozár et al. 2013). In the United States, *A. lagerstroemiae* was inoculated onto a total of 13 plant species and nymph development was observed on five plant species other than *Lagerstroemia* sp., including economical important plants such as *Callicarpa americana* L. (American beautyberry) and *Punica granatum* L. (pomegranate) (Wang *et al*. 2019b). This bark scale colonizes the trunk, branches, stems, and even found on leaves, and fruits with heavy infestations (Wang *et al.* 2016), damaging crapemyrtles directly and indirectly. Direct damage by CMBS is due to its sap-sucking behavior leading to branch dieback and sometimes fewer flowers and stunted growth (Luo *et al*. 2000, Ma 2011). Whereas indirect damage is due to honeydew

excretion, which provides substrate to sooty-mold growth, that is not only unsightly but also decreases the overall the photosynthesis because of reduced exposure of leaves to sunlight (Wang *et al* 2016, Gill 2018) (Figure 1.1).

Figure 1.1. Crapemyrtle bark scale infestation on crapemyrtle trees. A: White waxy filaments from different generations of crapemyrtle bark scale build up on the trunk. B: Sooty mold growing on surrounding plants due to honey-dew drop by crapemyrtle bark scale. Photo by G.M. Franco.

Impacts of CMBS extend to economic and ecological aspects. After the detection of CMBS and its fast expansion, management costs increased (Wang *et al.* 2016, Layton 2019). Ecological impacts due to CMBS presence are also extensive. Bees and other beneficial insects rely on pollen of crapemyrtles in urban areas and late summer (Deyrup *et al.* 2002, Mach and Potter 2018), when pollen from other trees is no longer available (Riddle and Mitzel 2016, Braman and Quick 2018); however, recent studies have shown that neonecotinoids, the systemic insecticides that are being recommended to manage CMBS can translocate to the pollen and

provide enough chemical to exceed the toxicity threshold for bees (Mach *et al.* 2018, Thurmond 2019).

1.3. Methods of control of CMBS

Management of *A. lagerstroemiae* relies on mechanical, chemical, and biological control (Gu *et al.* 2014, Wang *et al.* 2016, Thurmond 2019). To achieve a higher level of pest control, an integration of two or more methods should be considered (Gu *et al.* 2014).

1.3.1. Mechanical control

Mechanical control consists on the manual removal of scales or destroying infested trees. Small scale infestations can be mechanically treated by washing the infested trees, so females and egg masses are removed (Gu *et al*. 2014). However, scales usually settle under bark crevices or on pruning scars, making it harder to access, and when scales are present in large trees, washing the trees may not be feasible (Gu *et al.* 2014). Tree removal is recommended when only a few plants are infested and could serve as inoculum to other plants, or the homeowner is not willing to treat CMBS (Layton 2019).

1.3.2. Chemical control

Systemic and contact insecticides are recommended to manage *A. lagerstroemiae* (Kilpatrick *et al.* 2014). Contact insecticides might not be very effective since CMBS is usually protected with a waxy coverage and is present within bark crevices or pruning scars (Gu *et al.* 2014); although, crawlers and nymphs could be targeted with contact products since they are more exposed to external effects (Vafaie *et al.* 2020). If product delivery is correlated to scale phenology and nymphs are targeted, contact products such as pyriproxyfen, azadirachtin, and horticultural oil can be used to manage CMBS (Chen 2017). Systemic insecticides are usually

from the neonicotinoid class, mostly containing imidacloprid, clothianidin, dinotefuran, and thiamethoxam active compounds, and are delivered to crapemyrtle by soil injection or drench (Gu *et al.* 2014, Thurmond 2019, Layton 2019). Dinotefuran and imidacloprid residues due to soil drench application were tested against the two most common pest on crapemyrtles, crapemyrtle aphid [CMA - *Sarucallis kahawaluokalani* (Kirkaldy)], and CMBS in different seasons (autumn, late winter, and spring) (Thurmond 2019). As result, CMA had increased mortality 24h after treatment with both products during the spring; and there was no treatment effect for CMBS (Thurmond 2019). This shows that residues of imidacloprid and dinotefuran significantly impact CMA but not CMBS within 24h of application.

Soil drench was considered having less non-target pesticide exposure, since it will be directly applied to the soil, and provides better coverage, even on young branches of a tree (Gill *et al.* 1999, Mach *et al.* 2017). However, several studies have shown that plants can translocate insecticides to pollen and nectar, leading to mortality of beneficial insects (Mach *et al.* 2017, Thurmond 2019). When imidacloprid and dinotefuran were soil-applied on broadleaf evergreen tree (*Ilex* x *attenuate* L.) and a deciduous shrub (*Clethra alnifolia* L.) during autumn (post-bloom) and spring (pre-bloom), the insecticide concentration found in both species and timing exceeded the threshold of adversely affect individual and colony-level traits of bees (Mach *et al.* 2017). When similar studies were conducted on crapemyrtles, applying imidacloprid and dinotefuran in different times pre and post-blooming leads to high concentration of chemicals in the pollen creating a potential harm for visiting pollinators (Thurmond 2019).

1.3.3. Biological control

Biological control of CMBS can be accomplished using natural enemies such as predators, parasitoids, and entomopathogens. In Asia, CMBS is attacked by six parasitoids from

the family Encyrtidae (Hymenoptera) (Hayat *et al.* 1975, Zeya and Hayat 1993, Jiang and Xu 1998, Zhang and Huang 2001, Wang *et al.* 2014), and six predators from the families Coccinelidae (Coleoptera) and Chrysopidae (Neuroptera) (Jiang and Xu 1998). In Louisiana, at least four ladybeetles (Coleoptera: Coccinelidae) were found on plants containing *A. lagerstroemiae* infestation, *Chilocorus cacti* L., *C. stigma* (Say), *Hyperaspis bigeminata* (Randall), and *Harmonia axyridis* (Pallas) (Wang *et al.* 2016). In addition, parasitoids the presence of *A. lagerstroemiae* parasitoids in Louisiana has been reported, but they were not identified (Wang *et al.* 2016).

Entomopathogens are microorganisms that germinate, infect, and reproduce in an insect's body, leading to its death. The fungi species *Beauveria bassiana* (Balsamo) (Hypocreales: Cordycipitaceae) and *Isaria fumosorosea* Wize (Hypocreales: Clavipitaceae) are recommended for the control of scale insects, or sap-sucking, or soft body insects (van Lenteren *et al.* 2018, Arthurs and Dara 2018) and are commercially available. Furthermore, bacterial organisms such as *Chromobacterium* spp. (Neisseriales: Neisseriaceae) and *Burkholderia* spp. (Burkholderiales: Burkholderiaceae) are recommended to control aphids, psyllids, whiteflies and mealy bugs (Marrone Bio Innovations 2013, Marrone Bio Innovations 2015), hence they can be tested as a method of control for CMBS. Due to the recent arrival of CMBS, there is a lack of information on the effectiveness of commercial entomopathogens to control this pest.

1.4. Biological control of scale insects using entomopathogens

Entomopathogens are widely known to kill many species of insects in the order Hemiptera. The entomopathogenic fungus *Alternaria alternata* (Fr.) Keissler can infect different aphid species, such as *Aphis fabae* Scopoli, *A. gossypii* Glover, *A. pisum* Harris, *Anuraphis nerii* (Boyer de Fonscolombe), *Rhopalosiphum padi* (L.), *Sitobion fragariae* (Walker), and *Uroleucon*

sp. (Christias *et al.* 2001). The entomopathogenic fungi *B. bassiana* and *I. fumosorosea* are recommended to treat ornamental plants to control *Bemisia tabaci* (Gennadius) (Aristizabal *et al.* 2018), a whitefly that causes severe damage in crops (Oliveira *et al.* 2001). However, scale insects are able to produce a waxy cover to protect against predators, dehydration, and pathogens by decreasing the conidia adhesion (Uma-Devi *et al.* 2008). Entomopathogens can infect and kill scale insects by penetrating the wax layer or when insects lack the protective structure such as in nymphs or eggs (Shabana and Ragab 1997). For example, pathogens from the genera *Fusarium*, *Penicillium*, *Purpureocillium*, and *Saracladium* infect nymphs of *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Sharma *et al.* 2018). Different soft scales species (Hemiptera: Coccidae) were reported to be susceptible to different entomopathogenic fungus; such as the guava scale [*Chloropulvinaria psidii* (Borchsenius)] to *Fusarium oxysporum* (Schlencht) (Gopalakrishnan and Narayanan 1989), and the fig wax scale (*Ceroplastes rusci* L.) to *Alternaria infectoria* (Simmons) (Shabana and Ragab 1997).

Crapemyrtle bark scale is from the Eriococcidae family, which is paraphyletic to Coccidae. In addition, according to Afifi (1968), Eriococcidae share several characters with Pseudococcidae and later researchers agued if Pseudococcidae is in fact an Eriococcidae ancestor (Boratynki and Davies 1971, Hodgson 1997). Therefore, since all these families are closely related and several entomopathogens are confirmed to attack mealybugs and soft scales, the pathogenicity against CMBS should be assessed.

1.4.1. Types of entomopathogenic organisms

Entomopathogens can be nematodes, virus, bacteria, or fungi. Entomopathogenic nematodes (EPN) are agents that may be used to control insects in soil habitats or above the ground (Lacey and Georgis 2012). The most studied and successful EPN in biological control of

arthropods are from the families Heterorhabditidae and Steinernematidae, which have symbiosis with bacteria of the genera *Photorhabdus* and *Xenorhabdus,* respectively (Grewal *et al*. 2005a, Lacey *et al*. 2015). These nematodes enter the insect's body throughout natural openings, wounds or even cuticle (Heterorhabditidae). Once inside the insect's body, the symbiotic bacteria releases chemicals that kills the insect making it a suitable place for the nematode continue its life cycle (Salvadori *et al.* 2012). Nematodes can have two to three generations inside one host, then the infective juvenile exits the cadaver to find new hosts (Kaya and Gauler 1993).

Entomopathogenic viruses used on the field are from the family Baculoviridae (Moscardi *et al.* 2011, Lacey *et al.* 2015). Baculoviruses (BV) are used to control insects from the orders Lepidoptera, Coleoptera and Hymenoptera (Ibarra and Del Rincón-Castro 2014). They enter the insect's body throughout ingestion, once inside they start replicating in the cells leading to internal tissue destruction, which cause to dysfunction of the systems and death of the insect (McNeil 2010, Ibarra, and Del Rincón-Castro 2014).

Entomopathogenic bacteria can be from different families and have different modes of action. They can kill insects by acting in the digestive system (Nicolas *et al*. 1990, Bravo *et al*. 2007, Marshall *et al*. 2012, Ruiu 2015), associated with nematodes (Torres-Barragan 2011, Ruiu 2015), producing insecticidal toxins (Waterfield *et al.* 2001), outcompete essential microorganisms, or excreting enzymes that will degrade the hemocoel of insects (Riui 2015). In some cases, the active ingredient produced by these organisms can be isolated and formulated as insecticides (Ruiu 2015).

The bacteria from the class *Betaproteobacteria* contains species that can kill insects by oral toxicity, contact effects, and changing behavior (antifeedant and feeding inhibition) (Martin *et al.* 2007, Cordova-Kreylos *et al.* 2013, Ruiu 2015). These organisms are effective against

insects with different feeding behavior such as Lepidoptera, Coleoptera, and Hemiptera (Martins *et al.* 2007, He *et al.* 2014). Since it is known that these bacteria are efficient against *B. tabaci* and scale insects (Marrone Bio Innovations 2013, Marrone Bio Innovations 2015) these biopesticides should be tested against *A. lagerstroemiae*.

Entomopathogenic fungi is a group that infects both soft and hard body's insects (Lacey *et al.* 2011, Lacey *et al.* 2015). Their mode of action consists in penetrating insect's body by the cuticle, natural openings or any injury (Tanada and Kaya 1993). Once inside, the fungus can colonize the tissues through hyphae and blastospores growth (Vega *et al.* 2009). The immune system of the insect is suppressed by the synthesis of fungal compounds, so the fungus is able to colonize the insect's body (Hajek and Leger 1994). Due to host specificity and availability of species and strains, entomopathogenic fungi can be used as biocontrol agents (McNeil 2011). According to van Lenteren *et al.* (2018), different entomopathogens can be used in biological control for scale insects, such as various strains of *B. bassiana*, *Lecanicillium lecanii* (Zimmermann), *L. muscarium* (Zare & Gams), and *Paecilomyces tenuipes* [now *Isaria tenuipes* (Peck)]. However, only the strains GHA and ANT-03 of *B. bassiana* are available to purchase in the United States. For those reasons, the fungi *B. bassiana* GHA strain, *B. bassiana* ANT-03 strain, *I. fumosorosea* PFR97 strain, and the bacterial organisms *Burkholderia* spp. A396 strain, and *Chromobacterium subtsugae* PRAA4-1T strain should be tested against *A. lagerstroemiae* and the pathogenicity assessed.

1.4.2. History of the use of entomopathogens to control pests

The first effort to study the ability of a fungus to infect an insect was made by Agostino Bassi in 1895 while studying the white muscardine disease on silkworms (Vega *et al.* 2009). The white muscardine disease, caused by *B. bassiana*, is a parasitic fungus that targets several

arthropods leading to their death (Zimmermann 2007). Later on, Pasteur and LeConte inferred that different fungi could be used as a method of insect control (Vega *et al.* 2009). In Russia during the 1880s, *Entomopthora anisopliae* [= *Metarhizium anisopliae* (Metsch)] was being mass-produced to manage the sugar beet weevil (*Asproparthenis punctiventris* Germar) (Vega *et al.* 2009). Between 1911 and 1915, German and Japanese researchers discovered a bacterium that was able to produce a protein with insecticidal activity, the organism was named as *Bacillus thuringiensis* Berliner (Beegle and Yamamoto 1992). Colonies of *B. thuringiensis* were stablished in several countries throughout Europe, Asia, and North America and the metabolites were used to control different pests, such as European corn borer [*Ostrinia nubilalis* (Hubner)] (Beegle and Yamamoto 1992). Efforts to mass produce entomopathogens decreased in the 1940s due to insecticide expansion and war events, but in the 1980s, in reason to pest resistance and concerns on environmental contamination, studies on entomopathogenic organisms were strengthened (Beegle and Yamamoto 1992, Liu *et al.* 2002, Vega *et al.* 2009).

During the 2010s, there was an expansion on the market of biological products because of an increase on pesticide resistance, increase in numbers of successful biological control programs, and stricter pesticide regulations (van Lenteren *et al.* 2018). In a review, Lacey and collaborators (2015) estimated the growth and importance of microbial control, having over 50 entomopathogenic biopesticides current in use for augmentative biological control. According to Marrone (2019), the biopesticide market consists on 5 – 6% of the total pesticide market, with U.S and Europe composing 67% of the global biopesticide sales in 2020. The projected growth of the biopesticide market is unevenly distributed around the globe, being mostly concentrated in the Latin America (Marrone 2019).

1.4.3. Biopesticides

Biopesticides are defined as products derived from natural organisms with pesticidal activity (Glare *et al.* 2012). Microbial pesticides, which is a subcategory of biopesticides, are products that contain pathogenic organisms, such as bacteria, fungi, and viruses that will serve as biological control agents against pests (Arthurs and Dara 2018). Biopesticides are regulated by the Biopesticide Pollution Prevention Division (BPPD) of the U.S. Environmental Protection Agency (EPA) to ensure environmental safety (Braverman *et al*. 2014).

Biopesticides were originally developed to manage pests on specialty crops. However, a wider range of products are being developed to manage pests of agricultural crops including hemipterans such as aphids, thrips, whiteflies, leafminers, and mealybugs (Lacey *et al.* 2015, Arthurs and Dara 2018). To attend the increasing demand of biological products, research is being conducted with the goal to discover a new pest-pathogenic organism. After discovered, microorganisms need to be mass produced in such way that deterioration of favorable traits does not happen (Blackburn *et al.* 2016). To mass produce an entomopathogenic organism, several studies should be proceeded to identify the best conditions. Depending on the pathogen, different compounds are required for better efficacy (Jaronski 2014); as an example, the fungus *Lagenidium giganteum* (Schenk) requires exogenous sterols to produce reproductive structures and extensive research was conducted to better supply the nutrients for this pathogen (Domnas *et al.* 1977, Kerwin and Washino 1983, Maldonado-Blanco *et al.* 2011, Jaronski 2014). Biopesticide formulation interferes on its efficacy on field conditions. Formulations can be dry or liquid, depending on the pathogen, best conditions to increase shelf-life, and lower costs (Jackson *et al.* 2010). Different pathogenic structures can be used in the formulations leading to different period of time until insect mortality (Bernardo *et al.* 2018, Morales-Reyes *et al.* 2018).

Product formulation and carriers are also important aspects to be considered (Schisler *et al.* 2004, Rice 2019).

Proved efficacy in novelty pests may encourage the use of these products (Glare *et al.* 2012). When Ndereyimana and collaborators (2019) tested the commercially available products derived from *M. anisopliae* and *B. bassiana* against *Tuta absoluta* (Meyrick), in laboratory conditions, all the tested biopesticides were pathogenic and led to higher insect mortality. Even though there are several biopesticides in the market to manage insects in the order Hemiptera, there are no records in the literature of entomopathogenic organisms on CMBS. Therefore, our objective was to test available biopesticides in the United States to manage CMBS.

1.4.4. Interactions between entomopathogens and beneficial insects

Interactions between biological control agents can be beneficial or detrimental. A beneficial relation occurs when the pest control is enhanced by the presence of different organisms. For example, Mohammed and Hatcher (2017) found better control of the green peach aphid [*Myzus persicae* (Sulzer)] by the combination of *Lecanicillium muscarium* (Zare and Gams) and the parasitoid *Aphidius colemani* (Viereck). Also, the integration of the fungus *I. fumosorosea* and the ladybeetle *Thalassa montezumae* (Mulsant) was beneficial against the control of the croton green scale *Phalacrococcus howertoni* (Hodges and Hodgson), since the ladybeetle helped dispersing the fungal spores (Barahona *et al*. 2018).

Detrimental effects, in the other hand, can occur when pest control is reduced by negative interactions along biological control agents. Smith and Krischik (2000) found that BotaniGard® (*B. bassiana* GHA strain) decreased the survival of the *Cryptolaemus montrouzieri* Mulsant ladybeetle, but not *Hippodamia convergens* (Guerin-Meneville), *Coleomegilla maculata*

(DeGeer) and *Harmonia axyridis* Pallas. When *M. anisopliae* spores were directly sprayed on *H. convergens* ladybeetles, significant higher mortality and fungal growth was observed (Ginsberg *et al.* 2002). Also, when immatures *Coccinella septempunctata* L. were fed with aphids that were infected by *Neozygites fresenii* (Nowakowski), they had longer developmental time, higher mortality, and became adults with lower fitness, which can alter the predator's ability to control aphid population (Simelane *et al.* 2007). However, there are no studies on the impacts of the fungi *Beauveria bassiana* GHA strain, *B. bassiana* ANT-03 strain, *I fumosorosea* PFR97 strain on natural enemies that are regularly present in Louisiana, such as *H. bigeminata* and *Chilocorus* sp. Therefore, the objective of this study was to measure the possible impacts of biopesticide application towards natural enemies commonly associated with CMBS in laboratory and field conditions.

Chapter 2. Mortality of the crapemyrtle bark scale (Hemiptera: Eriococcidae) by commercial biopesticides under greenhouse and field conditions

2.1. Introduction

Crapemyrtles, *Lagerstroemia* sp. (Myrtales: Lythraceae) are ornamental trees native to Asia and one of the most important ornamental plants in the southeastern United States, having an annual plant wholesale value of $66 million (USDA NASS 2017). Crapemyrtles are valued because of its distinct bark, leaf coloration, abundant flowers, and easy maintenance (Egolf and Andrick 1978, Chappell *et al*. 2012, Gu *et al*. 2014, Riddle and Mizell 2016). The crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae) is native to Asia, was first detected at a nursery in Texas (Robbins *et al.* 2014), and has spread to 11 other states in the United States (EDDMAPS 2020). CMBS colonizes the trunk, branches, leaves, and fruits (Wang *et al.* 2016), leading to branch dieback, sooty mold growth due to honeydew excretion, and reduced number of flowers (Luo *et al*. 2000, Ma 2011, Wang *et al* 2016, Gill 2018). CMBS is known to attack 13 plant species (Hoy 1963, Kozár *et al*. 2013), and in the United States, it was able to develop on six species including American beautyberry (*Calicarpa americana* L.), pomegranate (*Punica granatum* L.), and crapemyrtles (Wang *et al*. 2019b).

Crapemyrtle bark scale management currently relies on the use of contact and systemic insecticides; however, the first may not be effective because scales are protected by waxy cover or host plant structures (Kilpatrick *et al.* 2014, Gu *et al.* 2014). Systemic insecticides are delivered to the trees by soil drench, once the roots absorb the chemical it is translocated in the plant's vessels reaching leaves and pollen (Gu *et al.* 2014, Layton 2019, Thurmond 2019). In

recent studies, insecticide residues containing imidacloprid and dinotefuran did not increase CMBS mortality within 24 hours after application and led to toxic concentrations of chemicals in the pollen months after application, which can be harmful to pollinators (Thurmond 2019). Crapemyrtles are an important source of pollen to beneficial insects in urban landscapes (Riddle and Mizell 2016, Braman and Quick 2018, Mach and Potter 2018); therefore, other methods of control of CMBS should be evaluated to increase scale mortality and decrease impacts towards beneficial insects.

Mechanical control including washing trees and destroying infested plant material have been proposed; however, these tactics may not be effective against large infestations (Gu *et al.* 2014). Biological control of CMBS has been documented in Asia and in the United States (Wang *et al.* 2016). In Asia, six parasitoids from the family Encyrtidae (Hymenoptera) (Hayat *et al.* 1975, Zeya and Hayat 1993, Jiang and Xu 1998, Zhang and Huang 2001, Wang *et al.* 2014) and six predators from the families Coccinellidae (Coleoptera) and Chrysopidae (Neuroptera) were found on CMBS infestations (Jiang and Xu 1998) reducing the pest population. In the United States, the coccinellids *Chilocorus* sp., *Hyperaspis bigeminata* (Randall), *H. lateralis* (Mulsant), *Scymnus* sp., and *Harmonia axyridis* (Pallas) were found preying on CMBS and one unidentified parasitoid (Wang *et al.* 2016, Vafaie *et al*. 2020). Local natural enemies may control CMBS under local conditions; however, frequent pest outbreaks suggest the need for more tactics.

Entomopathogens are microorganisms such as fungi, bacteria, or viruses that infect arthropods (Arthurs and Dara 2018, Marrone 2019). Entomopathogenic fungi and bacteria have been formulated into biopesticides and have shown effectiveness against soft body and/or sap-sucking insects (Jurat-Fuentes and Jackson 2012, Van Lenteren *et al.* 2018), being able to infect pests through the cuticle, natural openings, and/or any injury (Tanada and Kaya 1993). Products

evaluated for scale insects have shown mixed efficacy for pest control. For example, Xiao and collaborators (2016) treated cycad aulacaspis scale (*Aulacaspis yasumatsui* Takagi) with *Beauveria bassiana* (Balsamo) GHA strain and saw an 80% scale reduction on infested fronds; however, it did not prevent the scale from spreading to new fronds. Concentrations of the pathogen *Isaria fumosorosea* Wize strain PFR97 were tested against *A. yasumatsui*; resulting in a significant reduction in the LT_{50} with the highest concentration of blastospores (5.4×10^7) when compared to other concentrations (Castillo *et al*. 2011).

Due to the demand of environmentally-friendly approaches to pest control (Bale *et al.* 2008) and the commercial availability of products in the United States, the goal of this study was to evaluate the efficacy of biopesticides towards CMBS infestations. The specific objectives of this research were to (1) determine if biopesticides can interfere in CMBS's life cycle; (2) assess CMBS mortality by biopesticides in the field; (3) confirm that the selected entomopathogens can infect CMBS; and (4) understand how temperature impacts biopesticide effectiveness. To accomplish these objectives, laboratory, greenhouse, and field trials were conducted and scale mortality was assessed. Optimal temperature for pathogen growth was evaluated in the laboratory and correlated with product effectiveness in the field.

2.2. Material and Methods

2.2.1. Biopesticide treatments

Three entomopathogenic fungi: *Beauveria bassiana* GHA strain (BotaniGard® ES, 0.008 L/L) (BioWorks 2016), *B. bassiana* ANT-03 strain (BioCeres® WP, 6.0 g/L) (BioSafe Systems 2016), and *Isaria fumosorosea* strain PFR97 (Ancora®, 2.1 g/L) (OHP 2017), and two bacteria: *Burkholderia* sp. A396 strain (Venerate® XC, 0.02 g/L) (Marrone Bio Innovations 2015) and

Chromobacterium subtsugae PRAA4-1T strain (Grandevo® WGD, 7.35 g/L) (Marrone Bio Innovations 2013) were tested against CMBS. The doses for each treatment and solution preparation methods were according the recommendation for scales, aphids, whiteflies or soft bodied insects or communication with a product manufacture.

2.2.2. Greenhouse trial

The objective of this experiment was to evaluate the impacts of bacterial and fungal biopesticides on CMBS development from nymph to adult stage and compare the development ratio (final total number of adults: initial number of nymphs) with water control under greenhouse conditions. The treatments used in this experiment were two fungal biopesticides: *B bassiana* ANT-03 strain and *I fumosorosea* PFR-97 strain; and two bacterial biopesticides: *Burkholderia* spp. A396 strain and *C. subtsugae* PRAA4-1T strain. To infest CMBS on potted plants, crapemyrtle segments naturally infested with CMBS nymphs were collected from Louisiana State University Hammond Research Station (30°30’9.9”N, 90°22’23.05”W). Segments were inspected in the lab for other pests than CMBS or natural enemies, cut into 5cm segments, and attached to the plants (1m tall) in 1L pots using a twist tie. After a couple days, the nymphs molted and moved from the old branch to the new sapling.

Two months after inoculation, crapemyrtle potted plants were inspected for scale population size. A 5-cm-long segment containing at least 25 scales was selected (Figure A.1), the number of CMBS nymphs was recorded, and CMBS adults were removed. A 3-cm-long sticky tape was placed on each side of the segment to prevent entrance of new nymphs. Sticky tapes were replaced every week to ensure efficacy.

To deliver the products, plants were sprayed with the respective treatments using a CO_2 backpack sprayer with a TeeJet® (Springfield, Illinois) tip 11001V3 at 30psi. The application time was calibrated on an equal sized plant that was not used in product trials. Products were sprayed until the entire plant was wet but not dripping. To avoid contamination between treatments, plants were sprayed outside the greenhouse, left to air dry for 15 minutes, and moved back to the greenhouse. Each treatment was applied to five potted plants (repetition). Scale development determined by molting into adults was recorded weekly, and any new female or male was counted and carefully removed. Temperature data was collected using a HOBO Pendant® data loggers (Onset Computer Corporation, Bourne, MA) set to record data every 30 minutes. The percent development was analyzed using PROC GLIMMIX ($\alpha = 0.05$) using a Gaussian distribution in SAS (SAS Institute 2016) which included treatment as a fixed effect and replication as a random effect.

2.2.3. Mature trees field trial

The objectives of this study were to (1) evaluate the ratio of dead and total CMBS after scale infestation was treated with selected biopesticides and compared with water control, and (2) evaluate if tested pathogens were able to infect CMBS. A field plot containing 23 trees was located at the campus of Louisiana State University in Baton Rouge (30°24’25.2”N, 91°10’40.8”W) (Figure A.2). Twenty irrigated, ≈5-m-tall, crapemyrtles ‘Natchez’ variety were selected according to scale presence. Trees were assigned to treatments in a complete randomized design and sprayed with three fungal biopesticides (*B. bassiana* GHA strain, *B. bassiana* ANT-03 strain, and *I. fumosorosea* PFR97 strain) and water as control, using a CO_2 pressurized backpack sprayer using the tip TeeJet® 11001V3 at 30psi. The sprayer was calibrated to make the area that scales were present wet, but not drip. This experiment was

conducted twice, once during winter (February 2019) and other during spring (May 2019), with five repetitions (tree) per treatment. Temperature data were recorded every 30 minutes using a HOBO Pendant® data loggers protected by a case of anti-radiation protection solar. To confirm biopesticide viability, before spraying the trees, treatments were sprayed onto 6-cm-diameter Petri dishes containing Sabouraud's media.

During the winter (February 2019) trial, scales were removed daily from a randomly selected area on the main trunk using a fine tweezer for a week (Figure A.3). Whereas during the spring (May 2019) trial, four 10-cm-long branches were haphazardly trimmed from trees every other day for a two-week period. During the spring trial, treatments were delivered twice (20 and 27 of May, 2019). The death of the scales, in both trials, was checked in the laboratory by observing insect leg movement and response to touch (live scales shrink when gently touched with forceps). The proportion of dead to total scales per tree was analyzed using PROC GLIMMIX ($\alpha = 0.05$) using a binomial distribution with SAS (SAS Institute 2016). The proportion of dead and total number of scales was averaged across sampling days, treatment was included as a fixed effect and replication and day as a random effect.

Two attempts were made to confirm pathogenicity of dead scales in the laboratory. The first attempt was during the winter trial using Scanning Electron Microscopy (SEM). The SEM pictures were taken to observe the surface of the scale and look for signs of pathogenic infection (Mauchline *et al.* 2011). To take the SEM pictures, scales were collected from the field and kept in a formaldehyde solution (50mL Ethanol 95%, 5mL Glacial Acetic Acid, 10mL Formaldehyde 37-40%, 35mL water). Sample preparation consisted of a series of specimen dehydration using ethanol, first at 70%, then two times at 100%, followed by mixture at 1:1 ratio of 100% ethanol and Hexamethyldisilazane (HMDS), and finally HMDS only. In each step, the sample was

placed on a shaker for 20 minutes, and before adding the next product, the liquid phase was carefully removed using a micropipette. Upon completion of these steps, the samples were left to air dry overnight. The insects were placed with their back facing up on a metal stub using a sticky carbon disc and coated with gold (2.5ηm). SEM pictures were taken using a camera model JEOL JSM-6610LV SEM (Peabody, MA).

The second attempt to confirm pathogenicity of dead scales was made during the spring trial. To assess fungal infection, the pathogen recovery process consisted of collecting scales that were considered dead in the spring trial, dead scales were placed inside of microcentrifuge tube, capped with moist cotton ball, and kept in a growth chamber (25°C and 14:10 L:D photoperiod) to induce fungal growth. Once pathogen growth was observed, scales were imprinted on Sabouraud's media with cetyltrimethylammonium bromide (CTAB) as an agent for selective media (Posadas *et al*. 2012). Dishes were monitored daily and when microbial growth was observed, it was identified to species using morphology (Zimmermann 2007, 2008).

2.2.4. Potted plants field trial

The objective of this study was to test the impact of fungal biopesticides on the ratio of dead and totals CMBS individuals on potted plants under field conditions. CMBS-infested crapemyrtles (1m tall) in 1L pots were randomly placed on a lawn outside the LSU Ben Hur greenhouse complex (30°21'39.6"N, 91°10'26.4"W) during fall 2019 (October 2019). The treatments *B. bassiana* GHA strain, *B. bassiana* ANT-03 strain, *I. fumosorosea* PFR97 strain and water were delivered as described in section 2.2.2. Scale mortality was assessed by haphazardly sampling a 5-cm-long branch before treatment and 3, 6, 9, 12, 18, and 21 days after the first treatment. Products were reapplied six days after the first treatment to evaluate if it would increase scale mortality (24 and 30 of October, 2019). Collected branches were brought to the

laboratory where they were examined for scales. Scales were considered dead when no response to touch with a forceps or absence of leg movements was observed. Temperature was recorded using a HOBO Pendant® data logger set to collect data every 30 minutes and protected by a case of anti-radiation protection solar case. The proportion of dead scales per day was analyzed using PROC GLIMMIX ($\alpha = 0.05$) using a binomial distribution with SAS (SAS Institute 2016). The proportion of dead to total number of scales was averaged across sampling days, treatment was included as a fixed effect and replication and day as a random effect.

2.2.5. Spore germination assays

The objectives of this study were to determine the best temperature range for each fungal entomopathogen and to build germination curves. Due to the importance of temperature on spore germination (Hiromori *et al.* 2004, Alali *et al.* 2019, Seid *et al.* 2019), a temperature-dependent spore germination experiment was conducted in growth chambers. The entomopathogens *B. bassiana* GHA strain, *B. bassiana* ANT-03 strain, and *I. fumosorosea* PFR97 strain formulated into biopesticides were tested according to the methodology described by Seid *et al.* (2019) and diluted to 10^6 spores/mL. Each biopesticide mixture was prepared according to label recommendation and number of spores per mL was assessed using a hemacytometer (Scientific Instruments, Buffalo, NY, USA). Subsequently, 100µL of the biopesticide mixture was spread onto a 9-cm-diameter Petri dish containing Sabouraud's media using a spatula and placed in a growth chamber (Thermo Fisher Scientific, Marietta, OH). The growth chambers were set at 8, 12, 20, 28, and 32°C and 0:24h L:D photoperiod. Pictures of the dishes were taken 24h after treatment and spore germination percentage was assessed by counting at least 100 spores in three different places haphazardly selected in the Petri dish. Germination was confirmed if the germination tube was twice as long as the spore diameter (Alali *et al*. 2019). The experiment was

setup in a completely randomized design where dishes with one different pathogen in each were distributed to different temperature treatments. This experiment was conducted twice (January 2020 and February 2020). The effect of temperature on spore germination was compared using PROC GLIMMIX (SAS Institute 2016), followed by Tukey-Kramer for means separation with temperature and pathogen as a fixed effect and replication as a random effect. Germination curves were built using JMP® Pro 13.0.0 (SAS Institute Inc., Cary, NC, 2016). To decide which type of model would best fit to the results, the parameters R^2, SSE, MSE, and RMSE, and the Aikake's information criterion (AICc) and Bayesian information criterion (BIC) were evaluated.

2.3. Results

2.3.1. Greenhouse trial

The average temperature during the study was 28.9°C with maximum of 36.6°C and minimum of 24.3°C. After seven weeks, only 46.2 ± 15.61% (SE) of the scale nymphs developed into adults when potted plants were treated with *B. bassiana* ANT-03 strain. When water was delivered as a treatment, 83.7 ± 15.47% of the nymphs developed to adult stage; however, there was no difference among treatments (p= 0.4430).

2.3.2. Mature trees field trial

Average ambient temperature for the winter trial was 17.5°C (maximum of 27.8°C and minimum of 4.6°C); and for spring trial was 28.5°C (maximum of 36.1°C and minimum of 19.9°C). In the winter trial, there was a significant treatment effect (p= 0.0119). There was a significant increase in the proportion of dead to total scales on trees treated with *B. bassiana* ANT-03 strain (46.5 ± 4.47%) when compared to water control (26.1 ± 3.79%) (p= 0.0209). The

proportion of dead to total scales in other biopesticide treatments were similar to water control (p= 0.0733).

During the spring trial, there was no difference in the proportion of dead to total scales among treatments (p= 0.2186). When scales were treated with water, a proportion of dead to total scales of 34.6 ± 6.14% was observed, which was statistically similar to *B. bassiana* ANT-03 strain (p= 0.7249), *B. bassiana* GHA strain (p= 0.0606), and *I. fumosorosea* PFR97 strain (p= 0.9909), 39.8 ± 7.05%, 46.5 ± 8.23%, and 32.8 ± 5.84%, respectively.

Scanning electron microscopy pictures from CMBS in the winter trial showed few likely fungal structures but no characteristic spore shape (Figure 2.1). This could have been due to sample preparation.

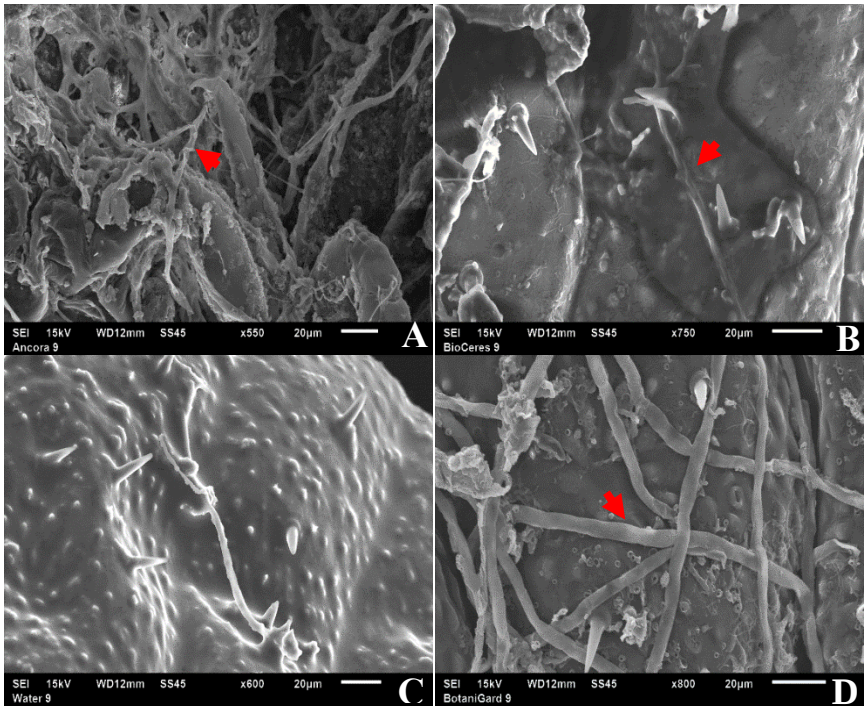

Figure 2.1. Surface of the cuticle of CMBS collected from winter trial. Likely to be fungal structures pointed with red arrow in pictures A, B, and D (treatments *I. fumosorosea* PFR97 strain, *B. bassiana* ANT-03 strain, and *B. bassiana* GHA strain, respectively). Note clean surface of picture C (water treatment).

Beauveria bassiana was commonly found infecting scales treated with the biopesticides in the spring trial (Figure A.4), but not on scales collected from the water control.

2.3.3. Potted plants field trial

There was a significant effect of treatments (p= 0.0008). Plants treated with *B. bassiana* GHA strain had a significant increase in the proportion between dead and total number of scales compared to water control (p= 0.0014) and other biopesticides (p= 0.0009). The other biopesticides had similar ratio as water control (p= 0.5952). *Beauveria bassiana* GHA strain provided significantly higher scale mortality (63.3 ± 15.49%) than *I. fumosorosea* strain PFR97 (21.6 ± 5.34), *B. bassiana* strain ANT-03 (18.7 ± 4.62%), and water (27.2 ± 6.70%).

2.3.4. Temperature-dependent spore germination

There was a significant effect of pathogen germination by temperature (p= <0.0001). All entomopathogens had significantly higher germination at 28°C (Table 2.1). *Beauveria bassiana* ANT-03 strain had germination of 66.8 ± 5.98%, *B. bassiana* GHA strain, 68.3 ± 5.15%, and *I. fumosorosea* PFR97, 85.6 ± 3.64% (Table 2.1). At 20°C, *B. bassiana* GHA and *I. fumosorosea* PFR97 had similar germination rate (p= 0.14); however, it was significantly higher than *B. bassiana* ANT-03 (p< 0.001). For 8°C (p= 1.0000), 12°C (p= 0.3471), and 32°C (p= 0.9961), there was no significant difference in percent germination among treatments. A model curve was built to describe the different fungal species germination depending on temperature (Figure 2.2). Germination for all three entomopathogens had its greatest value between 25 and 30°C, followed by a reduction after 32 °C. For *I. fumosorosea* PFR97 strain and *B. bassiana* GHA strain, cubic regressions were the best fit, R^2= 0.7141 and R^2= 0.8088, respectively. The best fit for *B. bassiana* ANT-03 strain was a quartic regression curve with R^2= 0.7819.

Table 2.1. Fungal percentage germination as affected by pathogen and incubation temperature

Temperature (°C)	Product	Pathogen	Germination (mean ± SE)	
8	BioCeres®	*B. bassiana* ANT-03	13.7 ± 3.77	D
8	BotaniGard®	*B. bassiana* GHA	11.3 ± 3.24	D
8	Ancora®	*I. fumosorosea* PFR97	11.9 ± 4.31	D
12	BioCeres®	*B. bassiana* ANT-03	13.9 ± 2.51	D
12	BotaniGard®	*B. bassiana* GHA	9.9 ± 2.30	D
12	Ancora®	*I. fumosorosea* PFR97	27.4 ± 5.67	CD
20	BioCeres®	*B. bassiana* ANT-03	18.8 ± 1.66	D
20	BotaniGard®	*B. bassiana* GHA	56.6 ± 5.23	B
20	Ancora®	*I. fumosorosea* PFR97	57.6 ± 6.58	B
28	BioCeres®	*B. bassiana* ANT-03	66.8 ± 5.98	AB
28	BotaniGard®	*B. bassiana* GHA	68.3 ± 5.15	AB
28	Ancora®	*I. fumosorosea* PFR97	85.6 ± 3.64	A
32	BioCeres®	*B. bassiana* ANT-03	51.1 ± 6.08	B
32	BotaniGard®	*B. bassiana* GHA	49.7 ± 4.62	BC
32	Ancora®	*I. fumosorosea* PFR97	57.7 ± 8.06	B

Similar letters in the same column are not statistically different by Tukey-Kramer Grouping with alpha= 0.05.

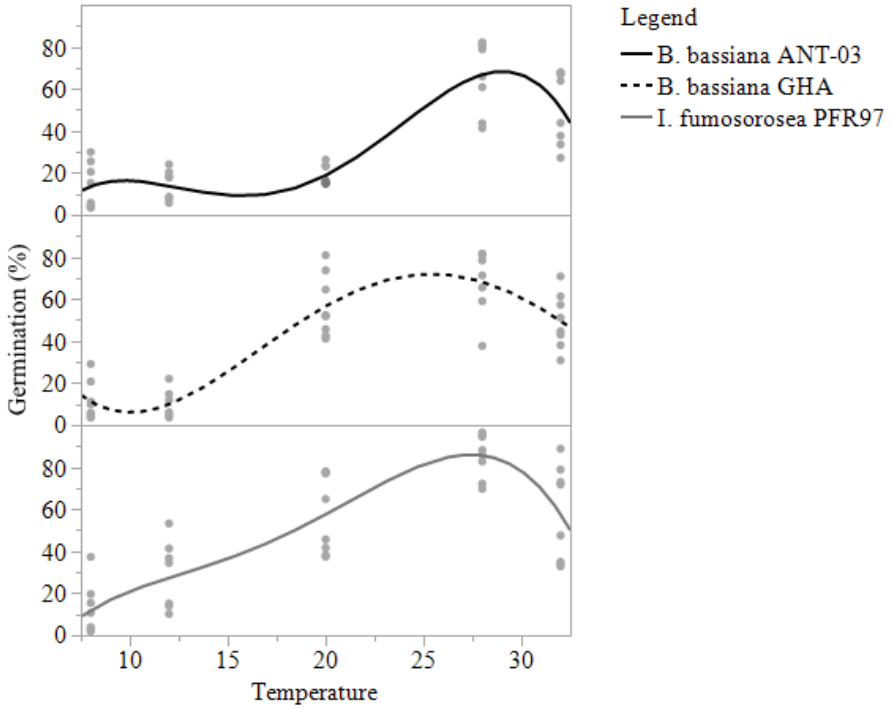

Figure 2.2. Predicted temperature-dependent germination curve for *Isaria fumosorosea* PFR97 strain, *Beauveria bassiana* ANT03 strain, and *B. bassiana* GHA strain.

2.4. Discussion

Results from experiments showed that biopesticides can significantly increase CMBS mortality when compared to water control. However, product coverage and temperature may have influenced on scale mortality. Crapemyrtle bark scale development from nymphs to adults was not impacted when treated with both bacterial (*Burkholderia* spp. A396 strain and *C. subtsugae* PRAA4-1T strain) and fungal biopesticides (*B. bassiana* ANT-03 strain and *I. fumosorosea* PFR97 strain) in greenhouse conditions (Table 2.1). In other studies, the bacterial biopesticides *Burkholderia* spp. A396 strain and *C. subtsugae* PRAA4-1T strain have also not reduced CMBS (Vafaie 2019a) or other insect populations Vafaie and Rydzak 2017, Shannag and Capinera 2018). *Beauveria bassiana* ANT-03 strain was recently tested against madeira mealybug (*Phenacoccus madeirensis* Green) and it did not decrease the pest populations (Vafaie 2019b). However, different strains of *B. bassiana* and *I. fumosorosea* have shown efficacy towards scale insects in laboratory (Castillo *et al.* 2011), greenhouse (Barahona *et al.* 2018), and field conditions (Xiao *et al.* 2016). Although, I suspect that extreme high temperatures in the greenhouse affected spore germination leading to lower impact on scale development from nymphs to adults. A similar pattern was also observed in the spring trial and by other authors when evaluating temperature-dependent fungal infection (Walstad *et al*. 1970, Alali *et al*. 2019).

When the biopesticides *B. bassiana* ANT-03 strain, *B. bassiana* GHA strain, and *I. fumosorosea* PFR97 strain were tested under field conditions to manage CMBS infestations, the results were season dependent. During the winter trial, *B. bassiana* ANT-03 strain significantly increased scale mortality compared to the water control; however, during the spring trial there was no treatment effect on scale mortality when compared to water control. A putative explanation is that product coverage and temperature affected CMBS mortality since nymphs

were observed moving to the main trunk during the winter, and to upper branches during spring and summer (G.M. Franco personal observation). Therefore, there was probably more contact between scale and biopesticides during the winter trial, leading to greater scale mortality. The mode of action of entomopathogenic fungi is through contact of fungal spore and cuticle of the insect (Tanada and Kaya 1993), similar to a contact insecticide. Although contact insecticides are deemed less effective against CMBS (Gu *et al*. 2014), if applied when scales are more susceptible to external factors, such as the crawler stage, it may help increase treatment efficacy (Quesada and Sadof 2017, Quesada *et al.* 2018, Vafaie *et al.* 2020).

Ambient temperature is likely a major factor influencing biopesticide efficacy against CMBS. High temperatures may have inhibited spore germination during the spring trial (maximum temperature= 36.1°C) and the greenhouse experiment (maximum temperature= 36.6°C). Alali *et al*. (2019) mentioned the how temperature can limit pathogen efficacy and how temperature-resistant strains of *B. bassiana* should be studied. However, to enhance efficacy of currently available strains, application timing can be improved. In Louisiana, the temperatures in late spring (May) to early fall (September) are high and may be detrimental for entomopathogenic organisms. Hence, these products should be considered as management techniques during the cooler months.

During the fall trial on potted plants, a three-fold increase on CMBS mortality was achieved with the treatment *B. bassiana* GHA strain compared to water control I suspect that such efficacy was due to a combination of temperature and product coverage. During the fall trial, lower temperatures were observed, but they were not extreme (lower= -3.7°C) and followed by a temperature increment (10.1°C). As investigated by Hiromori *et al.* (2004), if fungal spores are exposed to lower temperatures followed by a temperature increment, germination is delayed

but not impacted. When germination of spores of *B. bassiana* GHA strain were tested at 12°C, the germination was 9.9 ± 2.30%, however, if the temperature raised to 20°C, the germination was significantly higher, reaching 56.6 ± 5.23%. During the fall trial product coverage was enhanced due to plant size, since infested plants were only 1m tall, thus improving the contact between scales and pathogens.

Entomopathogenic organisms have their optimum temperature for development (Wang *et al.* 2002). In laboratory trials, 28°C seemed to be the best for *I. fumosorosea* PFR97 strain, *B. bassiana* GHA strain, and *B. bassiana* ANT-03 strain. When spore germination was assessed at 32°C, significant lower germination was observed for *I. fumosorosea*, but not for both *B. bassiana* strains. However, I did not assess fungal growth at different temperatures, but in personal observations, the size of the germination tube was smaller at 32°C when compared to 20 and 28°C. This information will help estimating best timing for product application in field conditions enhancing its efficacy. Abdulhai *et al.* (2010) reported that *B. bassiana* isolates had optimum growth between 20°C and 25°C and no growth was observed at 35°C, which are in agreement with my findings in the greenhouse and spring trials. Lower temperatures may have also impacted spore germination. Studies conducted by Seid *et al.*(2018) have shown that *B. bassiana* strains that were collected in arctic regions had greater percentage of germination in colder temperatures than when compared to tropical strains. In the temperature-dependent germination study, all tested mycoinsecticides had higher germination at 28°C. When germination curves were built, maximum predicted germination for tested pathogens were between 25 and 30°C (Figure 2.2), followed by a rapid germination drop. Similar results were found when Parker *et al.* (2003) tested the growth of fungi in the genera *Beauveria*, *Paecilomyces,* and *Verticillium* under different temperature regimes.

In summary, commercial strains of *B. bassiana* can be a good management tool against CMBS. However, temperature and product coverage should be carefully adjusted to increase product efficacy. Temperatures higher than 32°C may disrupt spore germination decreasing product efficacy. Low temperatures also decreased spore germination; although, if a temperature increment occurs, spores may germinate and increase pest mortality. Product coverage may be enhanced by correlating application timing, CMBS phenology, and tree size. When the coverage improves, biopesticides may significantly increase pest mortality. The ability to successfully infect and kill CMBS combined with understanding of scale phenology (Vafaie *et al.* 2020), suggests that *B. bassiana* formulated into biopesticides has potential as an IPM tool to manage CMBS. The results of this study provide a baseline for potential use of biopesticides against CMBS; however, further studies on other *B. bassiana* strains and formulation testing should be conducted.

Chapter 3. Interactions among crapemyrtle bark scale, *Acanthococcus lagerstroemiae* (Kuwana) predators and commercial biopesticides

3.1. Introduction

Crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae), are economically important ornamental trees in United States. Native to Asia, crapemyrtles are well known for desirable ornamental traits such as colorful and abundant flowers, colorful trunk, and easy maintenance (Egolf and Andrick 1978, Chappell *et al*. 2012, Riddle and Mizell 2016). In 2004, the crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae) was detected in a tree nursery in Texas (Robbins *et al.* 2014). CMBS is also native to Asia, but the timing and method of arrival to United States are unknown. Direct CMBS damage to crapemyrtles is due to sap-sucking behavior which could lead to branch die-back and less inflorescence (Gu *et al*. 2014, Vafaie *et al.* 2020); and indirect damage is due to sooty-mold growth which reduces the aesthetic of crapemyrtles and surrounding plants (Luo *et al*. 2000, Ma 2011, Wang *et al* 2016, Gill 2018).

The host range of CMBS includes at least 13 different species in several plant families (Hoy 1963, Kozár *et al*. 2013) in Hungary and Asia. A population in the United States was able to develop in six plant species including *Callicarpa americana* L. (American beautyberry) and *Punica granatum* L. (pomegranate) (Wang *et al*. 2019b). CMBS has been found in 12 states in the United States (EDDMAPS 2020) and thermal ecology studies demonstrated that it can establish in the same range as crapemyrtles (Wang *et al.* 2019a). CMBS dispersal over short distances results from on active crawler movement, or over longer distances due to wind, animals, and shipping of infested plants (Gu *et al.* 2014, Layton 2019). Management techniques

include mechanical, biological, and chemical control (Gu *et al.* 2014, Kilpatrick *et al.* 2014, Wang *et al.* 2016, Layton 2019). Mechanical control consists of washing or brushing the scales and sooty mold off, or destroying infested trees (Gu *et al.* 2014, Layton 2019); however, it can be impractical for large infestations or tall trees. Chemical control is the primary management tool for CMBS in the United States (Gu *et al.* 2014, Kilpatrick 2014, Thurmond 2019); however, its efficacy and safety is questionable. Translocation of imidacloprid and dinotefuran to pollen was assessed; as result, these neonicotinoids were found in high concentrations exceeding mortality thresholds in pollen samples (Thurmond 2019).

Biological control of CMBS occurs due to action of predators and parasitoids (Wang *et al.* 2016). In Asia, *A. lagerstroemiae* is attacked by six parasitoids (Hymenoptera: Encyrtidae) (Hayat *et al.* 1975, Zeya and Hayat 1993, Zhang and Huang 2001, Wang *et al.* 2014) and six predators (Coleoptera and Neuroptera) (Jiang and Xu 1998). In the United States, coccinellids are the most common natural enemies of CMBS (Wang *et al.* 2016). Interestingly, native coccinellids preying on CMBS include *Chilocorus cacti* L., *C. stigma* (Say), *Hyperaspis lateralis* Mulsant, and *H. bigeminata* (Randall) (Wang *et al.* 2016). A similar scenario has been described by Hernández-González and Cruz-Rodríguez (2018), when *C. cacti* was reported as an important biological control agent of the non-native wild cochineal (*Dactylopius opuntiae* Cockrell) in Mexico. Coccinellids could be major predators of eggs, crawlers, and nymphs of CMBS (Wang *et al.* 2016), as they have been documented as key predators of other scale pests of grapes (Rakimov *et al.* 2015), citrus (Liang *et al.* 2010), greenhouse crops (Ellis *et al.* 2001, Lucas *et al.* 2004), and croton (Barahona *et al.* 2018). Even though predators are able to decrease scale numbers, other methods of control are necessary to reduce pest densities below economic thresholds (Gu *et al.* 2014, Layton 2019).

Entomopathogenic fungi have been evaluated as a method to manage scale pests; as *Lecanicillium lecanii* (Zimmerman) Zare and Gams against *Coccus viridis* (Green), *Beauveria bassiana* (Balsamo) against *Aulacaspis yasumatsu* Takagi, and *Alternaria infectoria* (Simmons) against *Ceroplastes rusci* (Shabana and Ragab 1997, Castillo *et al.* 2011, Rosado *et al.* 2014, Xiao *et al* 2016). Currently, several species of entomopathogenic organisms are available in the United States market (van Lenteren *et al*. 2016). However, the type of interaction between entomopathogens and coccinellids is system dependent. Synergetic interactions occur where an increase in pest mortality is observed when both predator and biopesticides are used. This trend was reported between the coccinellid *Thalassa montezumae* Mulsant and the entomopathogenic fungus *Isaria fumosorosea* Wize (Barahona *et al.* 2018), and the entomopathogenic fungus *L. muscarium* with the coccinellid *Adalia bipunctata* L (Mohammed 2018). Antagonistic interactions reduced efficacy of biological control species is observed, as reported by Smith and Krischik (2000) while testing impacts of *B. bassiana* GHA strain against four coccinellid species.

In the CMBS system, commercial biopesticides may be a viable option to reduce scale populations. However, we do not know how biopesticides will interact with the native predators present on crapemyrtle trees. Therefore, the objectives of this study were to (1) survey common natural enemies of CMBS in Louisiana; (2) evaluate direct impacts of biopesticides on different life stages of CMBS natural enemies; and (3) investigate possible interactions between entomopathogens and natural enemies under field conditions. The susceptibility of the coccinellids to biopesticides was tested under laboratory conditions by submerging insects in biopesticide mixture and recording survivorship through time. Interactions in the field were observed by collecting coccinellids after biopesticide treatments were delivered to CMBS

infestations and attempting to recover fungal spores. Results of these studies are discussed in the context of incorporating biopesticides in the management of CMBS.

3.2. Materials and Methods

Three entomopathogenic fungi formulated into biopesticides were used in the trials: *Beauveria bassiana* GHA strain (BotaniGard® ES, 0.008 L/L) (BioWorks 2016), *B. bassiana* ANT-03 strain (BioCeres® WP, 6.0 g/L) (BioSafe Systems 2016), and *Isaria fumosorosea* strain PFR97 (Ancora®, 2.1 g/L) (OHP 2017). These biopesticides were chosen due to efficacy in previous studies against sap-sucking, soft bodied, or scale insects (Xiao *et al.* 2016, Barahona *et al.* 2018). The results of the treatments were compared with water as control. The method of preparation of each biopesticide followed to label for soft body, sap-sucking or scale insects, or communication with product consultant.

3.2.1. Natural enemy field surveys

The objective of this study was to assess the diversity and abundance of natural enemies of the crapemyrtle bark scale in several cities in Louisiana. Crapemyrtle trees were located in Baton Rouge [(30°25'01.4"N, 91°11'02.7"W) (30°26'24.3"N, 91°09'10.3"W); (30°26'55.4"N, 91°08'29.8"W)], Houma [(29°35'38.1"N, 90°43'33.5"W); (29°35'52.7"N, 90°43'22.3"W); (29°35'51.2"N, 90°42'46.1"W)], and Shreveport [(32°33’25.24”N, 93°46’56.3”W); (34°24’3.76”N, 93°46’46.12”W); (32°29’52.82”N, 93°43’10.83”W)]. Sites were composed of crapemyrtles in gardens or parking lots in urban areas, exposed to full sun, and surrounded by asphalt, turf grass, or unmanaged area. In each city, three sites were chosen according to the presence of CMBS and four trees were haphazardly chosen at each site. For each tree, four branches of 30 cm each were removed and placed in a ZipLoc® bag and identified according to

site and tree number. This sample collection was conducted three times within a year including summer and fall of 2018 and spring 2019. Once in the lab, each sample was carefully examined for natural enemies and scales, if natural enemies were found, they were kept for future identification. CMBS nymphs, male pupae, and adult females were identified according to Wang *et al*. (2016) and counted. The population of natural enemies and scale were compared among sites and sampling day, and the most common natural enemies' species were used in further experiments.

3.2.2. Coccinellid survival assays

The objective of this experiment was to assess the impact of biopesticides on the survival of the most commonly found natural enemies of CMBS in Louisiana. Natural enemies were collected in Shreveport, LA, due to their higher abundance in 2019. The predators *H. bigeminata*, *Chilocorus cacti,* and *C. stigma* were collected using aspirators, and placed inside 500 mL plastic containers containing crapemyrtle branches infested with CMBS and closed with lid containing holes for ventilation. Upon arrival at the laboratory in the Entomology Department at Louisiana State University, the containers with insects were placed in a growth chamber (Thermo Fisher Scientific, Marietta, OH, USA) at 25°C and 14:10h L:D photoperiod, and experiments were started the following day. Coccinellids were identified to species and separated in different containers with CMBS infested branches. Since there were not enough individuals of *C. cacti* and *C. stigma* to ensure enough repetitions per species, individuals of both species were combined.

The biopesticides containing *B. bassiana* GHA strain (BotaniGard® ES), *B. bassiana* ANT-03 strain (BioCeres® WP), and *I. fumosorosea* PFR97 strain (Ancora®) were prepared at a rate of 0.008 L/L, 6.0 g/L, and 2.1 g/L, respectively, and constantly stirred to ensure

homogeneity. The susceptibility test was conducted following description by Cottrell and Shapiro-Ilan (2008), where coccinellids were directly exposed to entomopathogens and survival was observed. Coccinellids were divided in groups of 25 in a large sampling tube (50mL) and placed in a fridge (5 ± 1°C) for 30 minutes to reduce insect activity and improve product delivery. Groups of five insects from the same species and life stage were placed into a medium sampling tube (14mL), the treatment was pipetted into the tube, coccinellids and treatment were gently agitated for 5 seconds to make sure the treatment was in contact with the insects. The treatment was pipetted out, and coccinellids were placed onto a clean filter paper so the remaining droplets were removed from the insect's body. Once insect activity was recovered, each coccinellid was placed in a small Petri dish (6-cm-diameter) with a moist sterile cotton ball. All dishes were grouped in a plastic container and placed in a growth chamber at 25°C and 14:10h L:D. Survival of coccinellids was observed every 12 h during a 14-day period. Coccinellids were considered dead if no movement was observed and legs were relaxed beside its body. The experiment was replicated using predators available during three collection times during April, May, and September 2019. The survival of the coccinellids were analyzed using Kaplan-Meier curves and survival in hours was compared two-on-two using Wilcoxon Chi-square tests with 0.05 significance.

3.2.3. Field trial

The objective of this experiment was to evaluate if coccinellids in the field would get in contact with entomopathogens and carry spores attached to their bodies. To achieve this goal, an experiment was conducted using crapemyrtle trees located at Louisiana State University, Baton Rouge campus (30°24'25.2"N, 91°10'40.8"W). The trees were located in a garden, irrigated, and surrounded by turf grass. The most common natural enemies observed at this site were the

coccinellids *H. bigeminata, C. cacti, C. stigma,* and *H. axyridis*. Twenty crapemyrtles 'Natchez' variety were treated with the biopesticides *B. bassiana* GHA strain (BotaniGard® ES , 0.008 L/L), *I. fumosorosea* PFR97 strain (Ancora®, 2.1 g/L), and *B. bassiana* ANT-03 strain (BioCeres®, WP, 6.0 g/L) and water as control. Products were sprayed with a CO_2 pressurized backpack sprayer with a TeeJet® (Springfield, Illinois) tip 11001V3 at 30psi. Products were delivered during the morning of 20 and 27 of May 2019 and the sprayer was calibrated to deliver enough treatment to make the tree wet, but not drip. Scouting for coccinellids happened every other day for a week period in May 2019, trees were observed for five minutes, coccinellids were collected using an aspirator, placed in a 14 mL sampling tube (one coccinellid per tube), and identified according to tree number. In the laboratory coccinellids were identified to species and checked for fungal spores along the body. To check for fungal spores, each insect was placed onto a 6-cm-diameter Petri dish containing Sabouraud's media with cetyltrimethylammonium bromide (CTAB) as an agent to make the media more selective (Posadas *et al*. 2012), and left roam for 3 minutes, subsequently, insects were removed and dishes were sealed with parafilm. When fungal growth was observed on the dishes, it was identified to species using morphological characters (Zimmermann 2007, 2008).

3.3. Results

3.3.1. Natural enemy field surveys

The most common natural enemies on CMBS infested trees were the coccinellids *Chilocorus cacti, C. stigma*, *Hyperaspis bigeminata*, and *Harmonia axyridis* (Coleoptera: Coccinellidae). Other predators recovered include *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Chrysoperla* sp. (Neuroptera: Chrysopidae) (Table 3.1). However, the most abundant were two species of coccinellids from the genus *Chilocorus,* which together made

approximately 36% of the total number of natural enemies, and *H. bigeminata,* which made 55% of the total number of collected natural enemies.

Table 3.1. Abundance and diversity of natural enemies on CMBS infested crapemyrtles collected in Shreveport, Baton Rouge, and Houma, Louisiana during 2018 and 2019. Numbers show the number total individuals summed per city in each sampling day.

City	Season	*Chilocorus* spp.	*Harmonia axyridis*	*Hyperaspis bigeminata*	*Chrysoperla* sp.	*Orius insidiosus*
Shreveport	Summer 2018	0	1	0	0	0
Shreveport	Fall 2018	6	1	2	2	0
Shreveport	Spring 2019	73	0	145	0	0
Houma	Summer 2018	0	6	2	0	2
Houma	Fall 2018	4	1	0	0	0
Houma	Spring 2019	2	0	1	0	0
Baton Rouge	Summer 2018	5	0	0	5	4
Baton Rouge	Fall 2018	2	1	0	2	0
Baton Rouge	Spring 2019	5	0	0	0	0
Total	-	97	10	149	9	6

Chilocorus spp. comprises both *C. cacti* and *C. stigma*.

3.3.2. Coccinellid survival assays

Coccinellids used in the susceptibility trials were collected on different days to add more replicates to the study. The survival of coccinellid adults treated with water control was different among sampling dates. *Hyperaspis bigeminata* adults had similar survival when collected on May 16th and September 27th (p= 0.0665). However, when adults of *Chilocorus* spp. were collected in Shreveport on September 27th, the survival was significantly longer than those collected on May 15th (p= 0.0044) and April 19th (Chi-square= 8.5183; p= 0.0035). All other

combinations of sampling dates were not significantly different (Chi-square= 1.5766; *p*= 0.2092) (Figure 3.1).

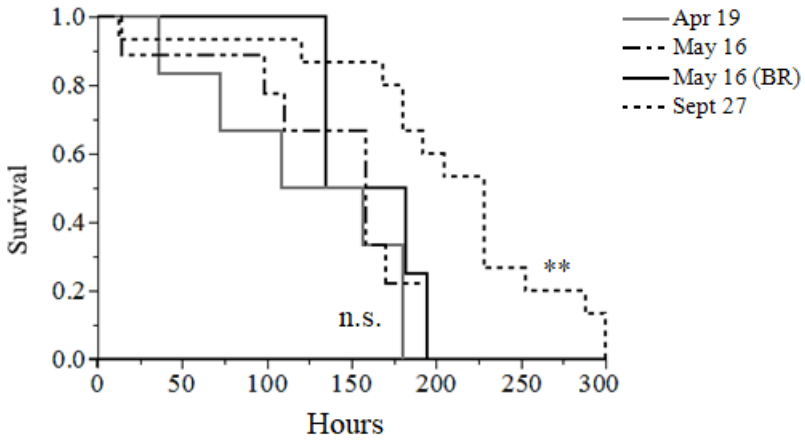

Figure 3.1. Kaplan-Meier survival curves of *Chilocorus* spp. adults treated with water when collected on May 16th in Baton Rouge, and Shreveport, April 19th, and September 27th. Comparisons with ** are statistically different by Wilcoxon test 0.05 significance.

Since there was a significant difference between sampling days, only the survival assessed with the coccinellids *Chilocorus* spp. collected on September 27th, 2019 was used for the analysis of survival. The survival of larvae of *Chilocorus* spp. (n=13) decreased by 84% when treated with *B. bassiana* GHA strain compared to water control (*p*= 0.0209) and *I. fumosorosea* PFR97 strain (*p*= 0.0209). There was no difference in survival among the treatments with water, *B. bassiana* ANT-03 strain, and *I. fumosorosea* PFR97 strain (*p*=0.1698) (Figure 3.2). Adults of *Chilocorus* spp. (n= 60) had survival reduced by 40% and 57% when treated with *B. bassiana* ANT-03 strain (*p*= 0.0112) and *B. bassiana* GHA strain (*p*< 0.0001), respectively, compared to water. The survival of *Chilocorus* spp. adults was not affected when treated with *I. fumosorosea* PFR97 strain (*p*= 0.7548) (Figure 3.2).

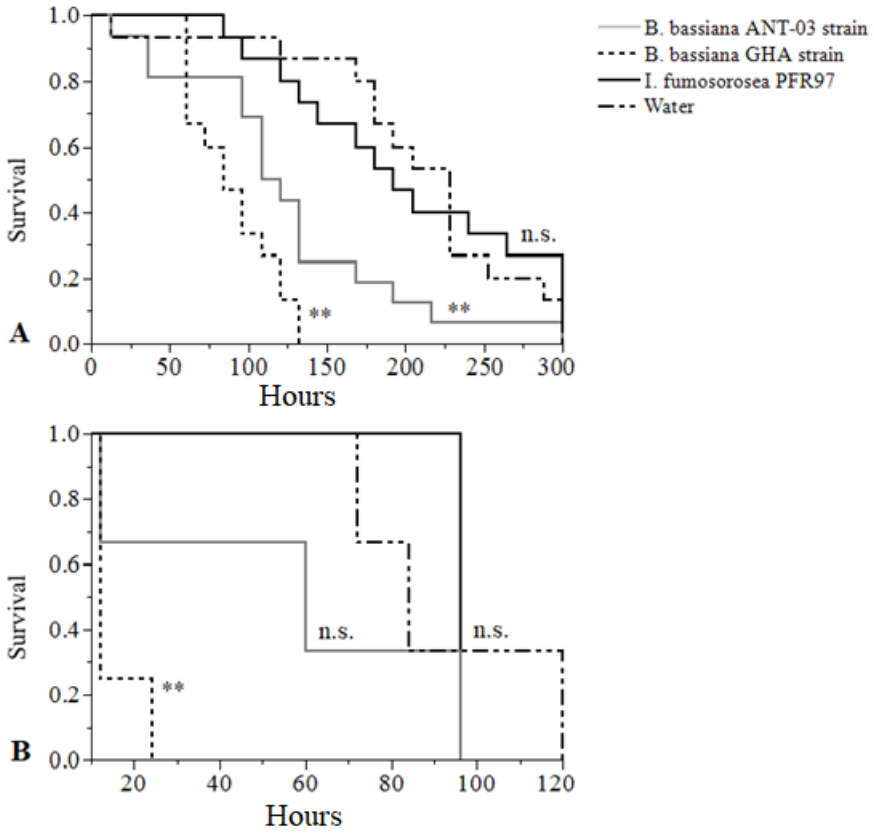

Figure 3.2. Kaplan-Meier survival curves of *Chilocorus* spp. of adults (A) and larvae (B) when treated with *Isaria fumosorosea* PFR97 strain, *Beauveria bassiana* ANT-03 strain and *B. bassiana* GHA strain. Comparisons with ** are statistically different from water control by Wilcoxon test 0.05 significance.

The survival of *H. bigeminata* larvae (n= 40) was reduced by 69% and 93% when treated with *I. fumosorosea* PFR97 strain (p= 0.0078) and *B. bassiana* GHA strain (Chi-square= 12.67; p= 0.0004), respectively, compared to water (Figure 3.5). The survival of *H. bigeminata* larvae was not affected by *B. bassiana* ANT-03 strain (p= 0.8192). The survival of *H. bigeminata* adults (n= 29) was reduced by 84% when treated with *B. bassiana* GHA strain compared to water (p= 0.0035). The survival of *H. bigeminata* adults was not impacted when treated with *B. bassiana* ANT-03 strain and *I. fumosorosea* PFR97 strain (Chi-square= 1.39; p= 0.2386).

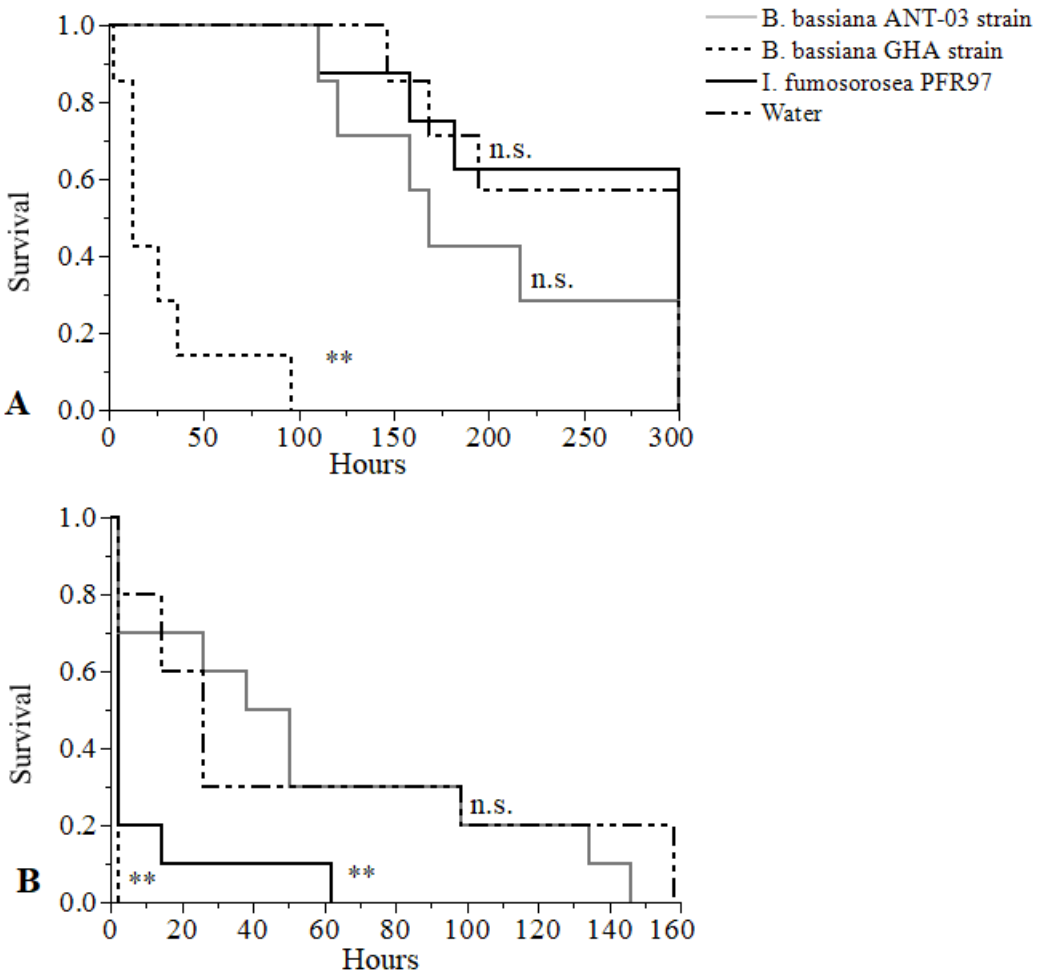

Figure 3.3. Kaplan-Meier survival curves of *Hyperaspis bigeminata* adults (A) and larvae (B) when treated with *Isaria fumosorosea* PFR97 strain, *Beauveria bassiana* ANT-03 strain and *B. bassiana* GHA strain. Comparisons with ** are statistically different from water control by Wilcoxon test 0.05 significance.

3.3.3. Field trial

When coccinellids were collected from trees treated with biopesticides, the majority had spores of entomopathogenic fungi attached to their bodies. The most common entomopathogen was *B. bassiana* (Table 3.2). When coccinellids were collected from trees treated with *B. bassiana* GHA strain and *B. bassiana* ANT-03 strain, only spores of *B. bassiana* were found

attached to their bodies; whereas when coccinellids were collected from trees treated with water and *I. fumosorosea* PFR97, both pathogens were recovered from their bodies (Table 3.2).

Table 3.2. Number of coccinellids collected from trees treated with the biopesticides *Beauveria bassiana* GHA strain, *Beauveria bassiana* ANT-03 strain, and *Isaria fumosorosea* PFR97 strain and spores of pathogens recovered from the surface of the coccinellids' bodies.

	Number of individuals with			% Pathogen recovered	
Pathogen	*B. bassiana*	*I. fumosorosea*	No pathogens	*B. bassiana*	*I. fumosorosea*
None	4	1	4	44	11
B. bassiana GHA strain	9	0	0	100	0
B. bassiana ANT03 strain	3	0	1	75	0
I. fumosorosea PFR97 strain	3	1	2	50	17

The natural enemies collected were from the species: *Hyperaspis bigeminata*, *Chilocorus cacti*, *C. stigma*, and *Harmonia axyridis*.

3.4. Discussion

Natural biological control of CMBS occurs due to the action of native and non-native predator activity (Wang *et al.* 2016). In our survey, we identified the coccinellids *C. cacti*, *C. stigma*, and *H. bigeminata* as the most abundant predators of CMBS in Shreveport, Houma, and Baton Rouge, Louisiana; with the sites in Shreveport having the most abundant natural enemies in Spring 2019. The sampled sites in Shreveport consist on unmanaged areas surrounded by weeds, which may have provided shelter and helped increasing the number of natural enemies. The coccinellids *C. cacti*, *C. stigma*, and *H. bigeminata* are reported to be predators of scale insects (Hodek and Honek 2009), justifying therefore its greater numbers. In personal observations, I have seen large aggregations of coccinellids and most egg-sacs nearby had

predation signs. Despite this abundance and localized impact, predator activity should work in integration with other methods of control to enhance CMBS management (Layton 2019).

Commercial biopesticides are known to infect and control scale insects in different systems (Rosado *et al.* 2014, Xiao *et al* 2016, Barahona *et al.* 2018) and homeowners or landscape managers may be able to use them to manage CMBS. The integration of both biological control agents is desirable and was evaluated in this study. Entomopathogenic fungi formulated into biopesticides reduced survival of different life stages of the most common predators of CMBS. *Beauveria bassiana* GHA strain reduced by at least 57% the survival of both larvae and adults from both genera of coccinellids. Similarly, reduction on adult longevity of the coccinellid *Coleomegilla maculata* (DeGeer) was recorded when treated with *B. bassiana* GHA strain under laboratory conditions (Smith and Krichik 2000). The biopesticides containing *B. bassiana* ANT-03 strain and *I. fumosorosea* PFR97 strain significantly reduced survival of *Chilocorus* spp. adults by 40% and *H. bigeminata* larvae by 69%, respectively. No other studies were found testing the impacts of *B. bassiana* ANT-03 strain towards coccinellids. When *I. fumosorosea* PFR97 (PFR-97®) was tested against the adults and larvae of the coccinellid *Thalassa montezumae* Mulsant, there was no negative impact on survival (Barahona *et al.* 2018).

Despite coccinellids being susceptible to biopesticides in laboratory conditions, this might result in a lack of compatibility. When the fungi *Lecanicillium muscarinum* (Petch) was applied to manage *Aphis fabae* Scopoli population, it impacted negatively the longevity and behavior of the coccinellid *Adalia bipunctata* L.; however, the integration of both biological control agents led to higher aphid mortality (Mohammed 2018). Laboratory trials may overestimate the susceptibility of an insect to an entomopathogen when compared to field

settings (Hajek *et al*. 1995, Roy and Cottrell 2008); since environmental conditions and contact between insect and entomopathogen might not always be ideal for fungal infection.

In the field trials, entomopathogens' spores were recovered from coccinellids collected from trees treated with biopesticides. *Beauveria bassiana* was recovered from coccinellids despite which treatment was delivered to the tree. Whereas *I. fumosorosea* was only recovered from trees treated with water and *I. fumosorosea*. Although there were twice as much *B. bassiana* treated trees in the field (both GHA and ANT-03 strains) compared to *I. fumosorosea* PFR97, there were over twice as much *B. bassiana* recovery from coccinellids. DNA sequencing could be used to elucidate which entomopathogen and strain was present on the coccinellids. This difference in recovery may have been due to spore attachment to coccinellid's body (Holder and Keyhani 2005). We do not know if these spores present on the coccinellids will kill them, because the laboratory conditions were favorable for spore infection. When the coccinellid *T. montezumae* was used in integration with *I. fumosorosea* to manage the green croton scale, *Phalacrococcus howertini* Hodges and Hodgson, the coccinellid was able to disperse fungal spores leading to greater scale infection (Barahona *et al*. 2018). In the CMBS system, it is not known if the spores collected by the coccinellids would be able to be efficiently delivered to other scale infestations leading to scale mortality. Therefore, further investigations on colony forming units (CFUs) transported by natural enemies should be done and compared to the number that should be delivered to efficiently increase scale mortality.

In conclusion, the coccinellids *H. bigeminata*, *C. stigma*, and *C. cacti* were the most common predators of CMBS in Louisiana. Susceptibility tests in the laboratory showed that *B. bassiana* GHA strain promoted a greater reduction in the survival of the coccinellids compared to *B. bassiana* ANT-03 and *I. fumosorosea* PFR97. Under field conditions, the common

predators of CMBS when in contact with entomopathogens, especially *B. bassiana*, can carry spores on their bodies. However, it is unknown if the coccinellids are dispersing or being killed by the sprayed entomopathogens. Therefore, further studies are needed to evaluate the interaction between biopesticides and coccinellids under field conditions.

Summary and Conclusions

Crapemyrtles, *Lagerstroemia* spp. (Myrtales: Lythraceae) are important ornamental trees in the southeastern United States because of abundant and colorful flowers, bark coloration, and easy maintenance. Crapemyrtle bark scale (CMBS), *Acanthococcus lagerstroemiae* (Kuwana) (Hemiptera: Eriococcidae), is native to Asia and was first detected in United States in 2004. Since its arrival, CMBS has significantly increased management costs of crapemyrtles, leading to increased use of pesticides and concerns on non-target effects on beneficial organisms. The objective of my thesis was to test the efficacy of commercial biopesticides towards CMBS and assess possible non-target effects on natural enemies.

The second chapter of this thesis aimed to evaluate if entomopathogenic bacteria and fungi formulated into biopesticides were able to impact CMBS development and cause pathogenicity. In greenhouse experiments, the bacterial products *Burkholderia* spp. A396 strain (Venerate®) and *Chromobacterium subtsugae* PRAA4-1T strain (Grandevo®), and fungal products *Beauveria bassiana* ANT-03 strain (BioCeres®) and *Isaria fumosorosea* PFR97 (Ancora®) did not impact scale development when compared to water treatment (p= 0.4895). Field trials have shown that the biopesticides *I. fumosorosea* PFR97, *B. bassiana* ANT-03 strain, and *B. bassiana* GHA strain (BotaniGard®) were are able to successfully cause scale pathogenicity; however, *B. bassiana* strains have shown greater efficacy. *Beauveria bassiana* ANT-03 strain was able to kill 46.5 ± 4.47% of the scale population in the winter 2019 trial; and *B. bassiana* GHA strain reduced 63.3 ± 15.49% of the initial population in the fall 2019 trial. This study concludes that entomopathogenic fungi, formulated into biopesticides may significantly increase scale mortality; however, further studies are needed to explore other strains

of *B. bassiana* as potential biological control agents and how different formulations impact on scale mortality

Product coverage and temperature were key factors affecting product efficacy. Since the tested biopesticides act by contact with the pest, infestations that are more exposed or easier to target will have greater mortality. In the winter trial, scale populations were aggregated on tree trunk, therefore it was easier to ensure contact between CMBS and biopesticides. In the fall trial, small potted plants infested with CMBS were used which made product coverage easier. To evaluate the effect of temperature on product efficacy, temperature dependent spore germination trials were conducted to evaluate optimum temperature for fungal growth. The results indicate that the optimum temperature for *B. bassiana* GHA strain, *B. bassiana* ANT-03 strain, and *I. fumosorosea* PFR97 strain is 28°C. Correlating the timing of the scales (phenology) when they are more exposed and optimum temperatures could lead to greater pest control with biopesticides. Further studies are needed to evaluate which life stage (eggs, crawlers, nymphs, or adults) are most susceptible to biopesticides. With these data, it would be possible to correlate with scale phenology and a greater efficacy would be achieved.

The third chapter of this thesis aimed to study the interactions of biopesticides and natural enemies on CMBS in Louisiana. Specifically, the objectives were to identify the most common natural enemies associated with CMBS infested trees; test susceptibility of natural enemies in the laboratory; and understand possible interactions between natural enemies and biopesticides in field settings. I found that the coccinellids *Chilocorus cacti* L., *C. stigma* (Say) and *Hyperaspis bigeminata* (Randall) were most commonly associated with CMBS infestations in Shreveport, Houma, and Baton Rouge and were mostly abundant during spring 2019. When susceptibility was assessed in laboratory, it was observed that biopesticides reduce the survival, especially *B.*

bassiana GHA strain (reduced by at least 57% the longevity of the tested organisms). *Beauveria bassiana* ANT-03 strain reduced the life span of *Chilocorus* spp. adults by 40% and *H. bigeminata* larvae had its longevity reduced by 69% when treated with *I. fumosorosea* PFR97. In field studies, delivered entomopathogens were recovered from live coccinellids; however, it is still unclear how biopesticides and predators will interact in field conditions. This chapter concludes that the integration between coccinellids and biopesticides is promising; however, since further investigation is needed to evaluate possible negative effects, biopesticides should be avoided when coccinellids are abundant. Future studies on colony forming units (CFUs) acquired by coccinellids and CFUs necessary to kill CMBS should be conducted and correlated to estimate possible synergy between biological control agents.

Appendix

Figure A.1. Example of five-centimeter segment used in the scale development experiment in greenhouse conditions. Scales were counted and other life-stages besides nymphs were carefully removed. Photo by J.R. Johnston III.

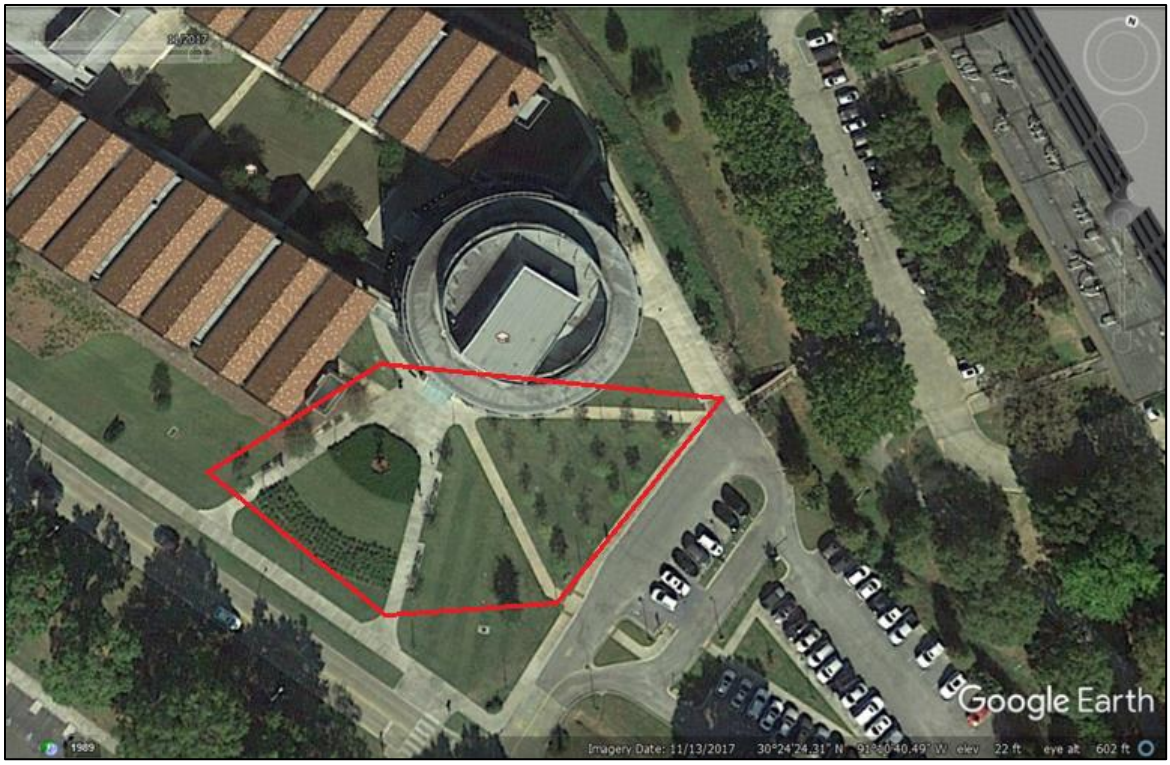

Figure A.2. Satellite picture from Louisiana State University site. Note the area marked with red polygon representing the location of the crapemyrtles used in the study. Photo by Google Earth Pro version 7.3.2.5776.

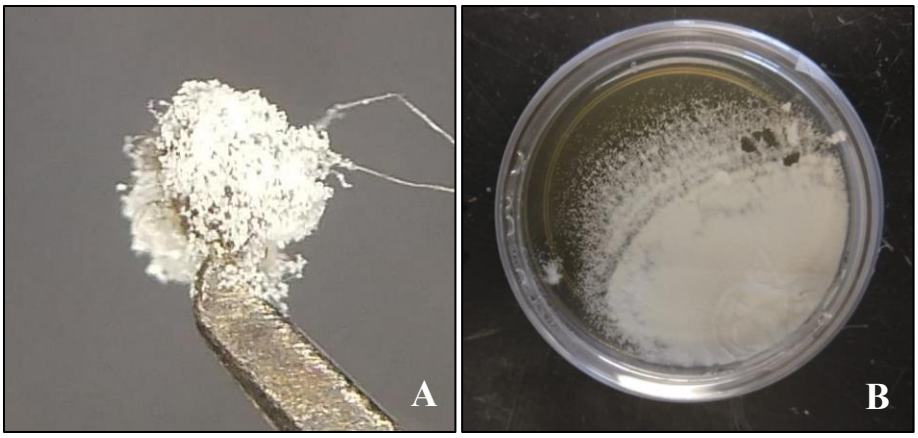

Figure A.3. Pathogenicity confirmation steps. A: CMBS clearly presenting fungal structures growing out of its body. B: Petri dish containing entomopathogenic fungi culture on Sabouraud's media. Photo credits: G.M. Franco.

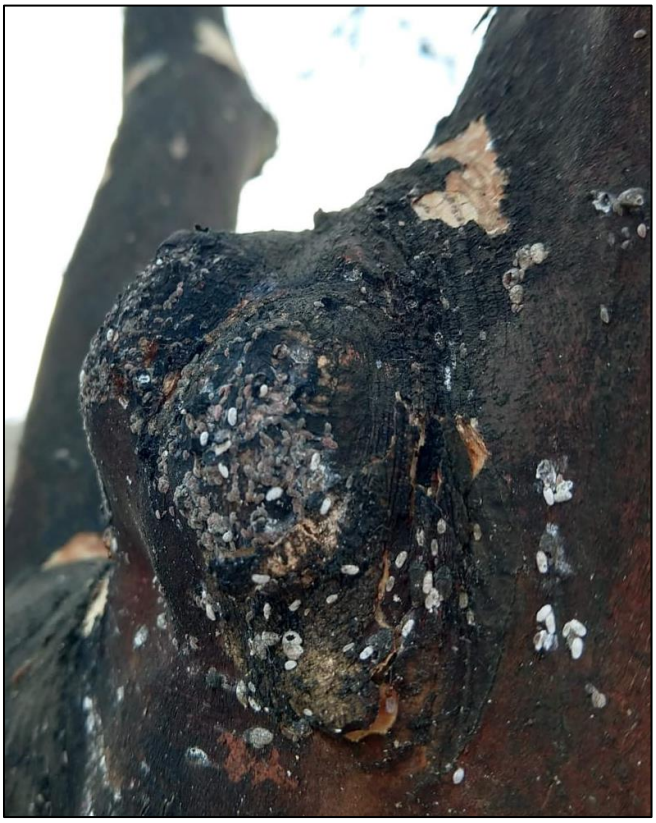

Figure A.4. Crapemyrtle bark scale infestation prior to winter trial in January 2019. Note nymphs aggregated on pruning scar during overwintering period. Photo credit: G. M. Franco.

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Vita

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