Recruiting lady beetles using olfactory and visual cues for the biocontrol of *Acanthococcus lagerstroemiae* (Hemiptera: Eriococcidae)

by

Oluwatomi Daniel Ibiyemi

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 12, 2020

Keywords: *Acanthococcus lagerstroemiae*, coccinellids, urban landscape entomology, Herbivore-Induced Plant Volatiles, visual attractants

Approved by

David W. Held, Chair, Professor, Department of Entomology and Plant Pathology
Katelyn A. Kesheimer, Assistant Professor, Department of Entomology and Plant Pathology
Alana L. Jacobson, Associate Professor, Department of Entomology and Plant Pathology

Abstract

Acanthacoccus lagerstroemiae (crapemyrtle bark scale, CMBS) is an exotic scale insect that feeds on the sap of crapemyrtle trees as its primary host. Heavy Infestations of CMBS leads to reduced flowering and sooty mold growth on the leaves and branches. This reduces the aesthetic value of crapemyrtle trees in urban landscapes. Lady beetles (Coleoptera: Coccinellidae) are generalist predators and they have been observed feeding on CMBS. Several laboratory and field studies have demonstrated the attraction of lady beetles to olfactory and visual cues. In order to achieve biocontrol of CMBS and reduce dependence on chemical control methods, we evaluated responses of lady beetles to olfactory lures and yellow visual attractants on infested landscape trees. Significantly more lady beetles were recruited to unbaited (Control) infested trees and infested trees baited with a combination of Predalure and Limonene compared to trees baited with limonene alone. Similarly, yellow rectangular panels placed 1m above the base of an infested tree also recruited up to twofold more lady beetles than control trees. Significant reduction in CMBS was observed on infested trees with yellow rectangular panels in tree's canopy. Yellow rectangular panels are more likely to recruit lady beetles in an urban landscape than olfactory lures. Management of CMBS is currently achieved using systemic insecticides. This study provides a new tactic to recruit lady beetles for biological control of CMBS, an advancement toward integrated management of this exotic pest.

Acknowledgments

I would like to thank my major professor, Dr. David Held, for his support and guidance during my master's program. Also, a huge thanks to my committee members Dr. Katelyn Kesheimer and Dr. Alana Jacobson, for their advice and critical review of this thesis. I would also like to thank my fellow lab members, especially Elijah Carroll, Amanda Dukes, Bryant Byrd, and Kayla Sullins for helping with my project. A big thank you to Dr. Charles Ray for his advice and identification of some of my coccinellid species. I would also like to thank Jeremy Pickens at the Ornamental Horticulture Research Center in Mobile, AL, for the generous donations of trees used in some of my studies. A big thank you to Dr. H. Fadamiro for letting me use equipment in his laboratory during my project. Finally, I appreciate my parents and siblings, and Fola Sade for their support and encouragement throughout my degree.

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CMBS	Crapemyrtle Bark Scale
MeSA	Methyl Salicylate
CM	Crapemyrtle
HIPV	Herbivore-induced plant volatile

Chapter 1: Introduction and Literature Review

1.1 Crapemyrtle

Crapemyrtle belongs to the genus *Lagerstroemia*- a group of small to medium-sized deciduous shrubs or trees native to southeast China, Korea, Japan, Oceania, and Australia (Chappell et al. 2012). Crapemyrtle trees are not true myrtles (Myrtaceae); their name is derived from the crepe-like petals of its flowers and its foliage's resemblance to true myrtles (*Myrtus* sp.) (Chappell et al. 2012). Since its introduction to the U.S. over 175 years ago, *Lagerstroemia* sp. has become widely grown and cultivated across the southeastern U.S as an ornamental landscape plant for its long colorful bloom period, attractive exfoliating bark, vibrant fall foliage, and tolerance to drought and heat (Chappell et al. 2012, Wang et al. 2019). Over time, various breeding programs have crossed *Lagerstroemia indica* (L.) Pers. with other *Lagerstroemia* species to produce hybrids with desirable traits such as varied flower color, large flower panicle and disease tolerance (Chappell et al. 2012). Some of these hybrids which have been bred and frequently used in the landscape include 'Natchez,' 'Apalachee,' 'Basham's Party Pink,' 'Caddo, 'Fantasy,' 'Osage' and 'Tuscarora,' and are all desired for their resistance against powdery mildew [*Erysiphe australiana* (McAlpine) Braun & Takamatsu (Erysiphales: Erysiphaceae)] (Chappell et al. 2012). Crapemyrtle is well-adapted to the southeastern U.S and can tolerate temperatures from USDA Hardiness Zones 6 to 10 (− 23.3 °C to − 1.1 °C) (Dirr 1990). Flowering begins in May in Zones 8 and 9 (− 12.12 °C to − 6.7 °C and − 6.7 °C to − 1.1 °C) and continues sporadically in the deep south until the first frost (Chappell et al. 2012). The flowers can range in color from shades of white, red, pink, and purple (Dirr 1990). *Lagerstroemia* sp. grows best at sites with ample sunlight, good airflow, and well-drained clay or clay-loam soil with a slightly acidic pH of 5.0-6.5. Crapemyrtles not receiving adequate sunlight may experience stunted growth and be susceptible to foliar pathogens (Chappell et al. 2012).

Crapemyrtle is considered a low maintenance plant. Once established in a landscape, it requires little to no fertilization or irrigation and minimal but appropriate pruning (Chappell et al. 2012, Gu et al. 2014). This exotic plant species also has relatively few herbivore pests within the expanded range in the U.S. Crapemyrtle aphid *T. kahawaluokalani*, Japanese beetle [*Popillia japonica* (Newman) (Coleoptera: Scarabaeidae)], flea beetles (mainly *Altica* sp.) (Coleoptera: Chrysomelidae), and granulate ambrosia beetle [*Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae)] are the most notable insect pests other than Crapemyrtle bark scale that infest crapemyrtle (Chappell et al. 2012). The primary diseases of crapemyrtle are powdery mildew caused by the fungus *Erysiphe australiana* (=lagerstroemiae), and Cercospora leaf spot caused by *Pseudocercospora lythracearum* (Heald & Wolf) Liu & Guo (Capnodiales: Mycosphaerellaceae) (Chappell et al. 2012). Activities of these pests and diseases can be managed on crapemyrtle trees using environmentally friendly insecticides such as insecticidal soaps or horticultural oils, and implementing proper landscape designs (Knox 2003, Chappell et al. 2012, Gu et al. 2014).

1.2 Crapemyrtle Bark Scale

Acanthococcus lagerstroemiae is an invasive scale insect pest threatening the landscape value and production of crapemyrtle trees in the U.S (Gu et al. 2014). The means by which CMBS became introduced to the United States may never be known, but it was first reported at a nursery in Richardson, TX (Dallas County), in 2004 (Gu et al. 2014). Ever since its first sighting, it has spread to 13 other states from New Mexico to Delaware (EDDMapS 2020). *Acanthococcus lagerstroemiae* (Fig. 1) feeds on the phloem of its host and, as a result, produces copious amounts of honeydew that support the growth of black sooty mold (Fig. 2) (Gu et al. 2014, Wang et al. 2016). CMBS damage symptoms may include reduced flower size, branch dieback, aesthetic damage (due to black sooty mold colonization), and in rare cases, death of small potted plants (Gu et al. 2014, Wang et al. 2016). Production of crape myrtle in the nursery industry is estimated to be an annual wholesale value of $66 million (USDA-NASS 2014). Tree inventories across significant cities in southeastern U.S. reveal that crapemyrtle is among the most common ornamental and landscape trees planted in this region (USDA-NASS 2014, Borden et al. 2018). The value to horticultural production and the ubiquitous presence of crapemyrtle trees in southern landscapes provided the optimal conditions for the introduced CMBS to quickly become an economically important pest.

1.2.1 Taxonomy, Distribution, and Dispersal

Acanthococcus lagerstroemiae (Hemiptera: Eriococcidae) (Crapemyrtle bark scale; CMBS) was previously considered in the genus *Eriococcus*; however, this group was reviewed and placed, after much deliberation among systematists, in the genus *Acanthococcus* alongside 345 other scale insect species (Kozár et al. 2013, Borden et al. 2018). CMBS is native to Asia, with populations commonly distributed in China, Japan, South Korea, and India (Kozar et al. 2013, Wang et al. 2016). This insect has been reportedly encountered outside its native region, including England and the U.S. It is suspected that CMBS utilizes several strategies for short or long-range dispersal; morphological and behavioral adaptations of coccoids indicate some of these strategies.

Passive dispersal via wind can carry first instars anywhere from a few meters to several kilometers away from the host plant upon which they emerge (Hanks and Denno 1998). Miller and Denno (1977) hypothesized that the long legs, antennae, and lateral setae relative to pseudococcids' small body size facilitate their aerial dispersal. Furthermore, wandering behavior has been recorded in a coccid (*Pulvinaria mesembryanthemi*) without access to a suitable feeding site (Washburn and Frankie 1985). Washburn and Frankie (1985) also noted that crawlers from several genera in Coccidae would actively seek wind dispersal, orientating themselves downwind with forelegs and antennae outstretched. Gu et al. (2014) further suggest that birds might transport CMBS crawlers.

1.2.2 Description and Biology

Acanthococcus lagerstroemiae appear as white patches on trunks, branches, and twigs of their host plant (Fig. 1) (Borden et al. 2018). Adult females lay eggs between May and September under a white felt-like covering secreted over its body (Wang et al. 2016). *Acanthococcus lagerstroemiae* females are highly fecund. Up to 300 pink eggs may be laid within the ovisac, after which the females begin to shrink and eventually die. The eggs (approximately 0.35 mm long and 0.15 mm wide) are incubated beneath the ovisac until they hatch into first instar nymphs, also known as crawlers (Gu et al. 2014, Layton 2015, Borden et al. 2018). The nymphs (crawlers) upon hatching will disperse throughout the host plant and eventually settle on trunks, branches, and twigs, where they begin feeding on the phloem (Gu et al. 2014, Wang et al. 2016). When crawlers were monitored using sticky tapes, up to four peaks of crawler activity were recorded in March, May, June, and October in College Station, TX, and up to three peaks recorded in March, May, and August in Little Rock, AR (Gu et al. 2014, Merchant et al. 2014). This finding suggests that crawlers may be encountered in the spring and fall of each year in the southeastern region of the U.S. CMBS crawlers are mobile and are the dispersal life stage of CMBS since adult females are immobile and adult males are non-feeding. Reports suggest that other instars become sessile after the first molt (Robbins et al. 2014). However, laboratory and field observations showed that other nymphs could relocate to new feeding sites on the host after each molt (Wang et al. 2016, *personal* *observations*). From recorded observations, CMBS nymphs are approximately 0.5 mm long and 0.15 mm wide (Wang et al. 2016).

After going through three nymphal stages, most nymphs will continue development into sessile adult females. Others will complete an extra pre-pupa and pupa stage to emerge as alate adult males (Wang et al. 2016). Adult CMBS females are pink and wingless; they are paedomorphic (resembling immature stages), likewise immobile, continuously feeding on their host's sap (Wang et al. 2016, Borden et al. 2018). On the other hand, male CMBS are pink, winged, and have two long filaments at the tip of their abdomen; however, they are non-feeding. Males have no mouthparts, therefore they only mate for a few days before they die (Wang et al. 2016, Borden et al. 2018).

Antecedent literature concludes that, from the native range of *A. lagerstroemiae*, there are two to four generations per year in Asia (Gu et al. 2014, Wang et al. 2016). In the U.S, CMBS is predicted to have more than two generations in USDA Hardiness Zone 8 (− 12.12 °C to − 6.7 °C) (Wang et al. 2019a), and up to four generations are expected in Zones 9 and 10 (− 6.7 °C to 4.4 °C) (Gu et al. 2014).

1.2.3 Economic Damage and Host Range

Due to the feeding activity of CMBS, honeydew secretions are produced in large amounts (Fig. 3), which leads to the growth and accumulation of sooty mold on trunks and leaves of the crape myrtle tree, thereby reducing its aesthetic value (Jiang and Xu 1998, Ma 2011, Gu et al. 2014). The unsightly sooty mold growth caused by CMBS is also associated with infestations of the crape myrtle aphid on crapemyrtle trees. CMBS presence and activity can be devastating as they may contribute to the loss of vigor or death of crapemyrtle trees (Robbins et al. 2014). Crapemyrtle trees infested with the CMBS have their trunks and bark covered with sooty mold, affecting the plant's photosynthetic ability. Branch diebacks, reduction in flower bloom, and reduction in plant growth and vigor have also been reported as symptoms of CMBS infested trees (Luo et al. 2000, Ma 2011, Gu et al. 2014).

Crape myrtle bark scale is a polyphagous herbivore. In its native region, it attacks plants from 17 genera in 14 families that are of significant economic and ecological importance, including pomegranate (*Punica granatum*), Korean boxwood (*Buxus microphylla*), Chinese hackberry (*Celtis sinensis*), Japanese persimmon (*Diospyros kaki*), border privet (*Ligustrum obtusifolium*), and brambles (*Rubus* sp.) (Wang et al. 2016, Borden et al. 2018). In the U.S., reported hosts of *A. lagerstroemiae* are crapemyrtle (Gu et al. 2014), American beautyberry (*Callicarpa americana* L.), and most recently in Virginia, St. John's wort (*Hypericum kalmianum*) (Schultz and Szalanski 2019).

1.2.4 Current Management

Acanthococcus lagerstroemiae populations are currently managed using chemical and mechanical methods in the U.S., while biocontrol tactics are being studied and developed (Wang et al. 2016). Contact insecticides have proven ineffective in achieving control of *A. lagerstroemiae* owing to its feeding behavior under bark crevices and secretion of protective waxy threads over its body (Gu et al. 2014). Mechanical removal methods involving brushing the trunk of an infested tree with a mild dishwashing solution or using high-pressure water may reduce CMBS numbers and sooty mold colonization (Gu et al. 2014). Neonicotinoids such as imidacloprid, thiamethoxam, and dinotefuran are effective chemical control options mainly when applied via soil drenches or soil injection (Gu et al. 2014). Neonicotinoid insecticides provide significant long-term reduction of CMBS crawlers, and two applications of bifenthrin over 17 days can provide a short-term decrease of crawler abundance (Vafaie and Knight 2017). The application rates of pesticide utilized for ornamental plants are quite higher than for field crops (Krischik et al. 2015). These high rates allow translocation into nectar and pollen of ornamental crops which are often hazardous to visiting pollinators and thus remain a concern (Cowles and Eitzer 2017) .

1.3 Lady Beetles as Biocontrol Agents of *Acanthococcus lagerstroemiae*

Lady beetles (Coleoptera: Coccinellidae) have been used as biocontrol agents for over a century and are regarded as essential predators of scale insects, aphids, and whiteflies (Obrycki and Kring 1998) Several authors have documented the successful implementation of lady beetles in conservation, augmentation, and classical /importation biocontrol programs (Caltagirone and Doutt 1989, Frank et al. 1992, Dreistadt and Hagen 1994). There are several natural enemies of *A. Lagerstroemiae* listed in its native region, including parasitoids from two hymenopteran families (Aphelinidae and Encyrtidae) and nine genera, and predators from four families (Anthocoridae, Chrysopidae, Coccinellidae, and Cybocephalidae) and at least seven genera (Wang et al. 2016). In the United States, several lady beetles have been reported feeding on CMBS, including *Chilocorus cacti* L., *Chilocorus stigma* (Say), *Hyperaspis bigeminata* (Randall), *Harmonia axyridis* (Pallas) (Fig. 4), and *Hyperaspis lateralis* (Mulsant) (Wang et al. 2016). Field and laboratory studies confirmed that *C. cacti* and *H. bigeminata* could successfully develop on CMBS hosts, with a single 4th instar *C. cacti* being able to consume 400 CMBS eggs in a day (Wang et al. 2016). *H. axyridis* are well established in many parts of the United States (Adedipe and Park 2010) and have also been reported to feed on CMBS, however, the extent of their predation on CMBS are yet to be evaluated.

It is reported that their control services may occur too late in the season to prevent aesthetic damage. The delay in control is because lady beetles may not appear on infested trees until CMBS populations have been established (Gu et al. 2014, *personal observations*). Therefore, conservation or augmentation biocontrol methods that may recruit and increase coccinellid abundance on infested trees early in the season should be evaluated for its effectiveness against CMBS.

1.3.1 Recruiting Lady Beetles via Semiochemical and Visual Cues

Predators such as coccinellids use both semiochemical and visual cues to locate their prey, and several studies have investigated the recruitment and attraction of coccinellids to these cues. Upon herbivory, plants release volatile organic compounds into the atmosphere to communicate with neighboring plants and attract natural enemies to control the herbivore (Dudareva et al. 2006, Held 2020). These volatiles, known as herbivore-induced plant volatiles (HIPV), are potent attractants of natural enemies and can lure natural enemies to reduce pest populations below damaging levels (Kaplan 2012). For instance, the infestation of lima bean leaves by spider mites triggers HIPV's release, attracting predatory mites that prey on the spider mites (Dicke et al. 1990, Takabayashi and Dicke 1996). Several compounds have been identified in HIPV blends from at least 23 plant species attacked by herbivores, including cotton damaged by *Helicoverpa armigera* Hübner (Yu et al. 2008). Some of the HIPV compounds induced by insect herbivores and released by a wide range of plants include the terpene 3,7-dimethyl-1, 3,6-octatriene, the methylene terpene 4,8-dimethyl-1,3,7-nonatriene, the ester Z-3-hexenyl acetate, methyl salicylate (MeSA), and limonene (Dicke et al. 1990, Paré and Tumlinson 1996).

Volatile profiles and compounds emitted by infested plants have been identified and evaluated for their attractant properties in coccinellids (Zhu and Park 2005a, Khan et al. 2008, Yu et al. 2008, Alhmedi et al. 2010, Cai et al. 2020). For instance, Zhu and Park (2005) observed lady beetle aggregations in soybean fields infested with the soybean aphid, *Aphis glycines* Matsumura, and reported that this response was mediated by HIPV's. The authors compared the volatile profiles of aphid-infested and aphid-free soybeans, followed by coupled gas-chromatography-electroantennographic detection (GC-EAD) analyses for aphid-infested plants. They found that aphid feeding induces the emission of methyl salicylate (MeSA) and lady beetles show a strong electroantennographic response to the compound, inferring a possible attraction. Studies investigating the attraction of coccinellids to semiochemicals have evaluated single HIPV compounds (James and Grasswitz 2005, Zhu and Park 2005a, Alhmedi et al. 2010) rather than a complex blend of compounds that are likely to be more effective (Kaplan 2012). For example, although the spider mite destroyer *Stethorum punctum picipes* (Casey), did not show attraction to MeSA or benzaldehyde when used alone, however, they were attracted to a mixture of methyl salicylate, *cis*-3-hexen-1-ol, and benzaldehyde (Pettersson 2012).

Irrespective of the promising benefits of HIPV's, Kaplan (2012) raised issues regarding the volatile compound to be targeted and used, the compound's actual release rate to attract the desired natural enemy, and non-target effects. Recruiting and retaining natural enemies for pest control using HIPV's is a complex process and seems yet to be fully understood (Kaplan 2012). While looking through antecedent works of literature, most studies (James and Grasswitz 2005, Mallinger et al. 2011, Gadino et al. 2012) investigated the attraction of entomophagous arthropods, utilizing sticky cards to report natural enemy capture. This raises questions as it is unknown if the captured individuals were present to forage prey or simply bycatch (Kaplan 2012). Since lady beetles seem to be the primary natural enemies associated with CMBS, it is crucial to evaluate the use of HIPV as a tactic to promote the biological control of *A. lagerstroemiae*.

Insects rely on several visual cues to locate their host, prey, or oviposition sites, and some of these cues have been employed to manipulate insect populations for achieving pest management. Several studies have investigated color as a visual cue, and the behavioral response of different insect taxa to different types of color has been reported (Walker 1974, Campbell and Hanula 2007, Rodriguez-Saona et al. 2011, Broughton and Harrison 2012, Kemp and Cottrell 2015). When various single-color traps were placed in pecan and peach orchards, predators, particularly coccinellids, showed greater recruitment and response to yellow traps (Kemp and Cottrell 2015). Several authors have investigated the attraction of coccinellids to various colors and it has been asserted that they show an excellent preference for yellow objects and traps (Braman et al. 2003, Adedipe and Park 2010, Kemp and Cottrell 2015). In a laboratory assay to evaluate adult *Harmonia axyridis* Pallas, color preference, six different colored rectangular cardboard papers coated with Tanglefoot, were randomly arranged on a plexiglass frame on an experimental box. They recorded numbers of lady beetles found on each colors after 30 min and found that *H. axyridis* significantly prefered yellow compared to other colors (Adedipe and Park 2010). Their findings were consistent with the results of Mondor and Warren (2000). They showed that *H. axyridis* made significantly more visits and spent more time on yellow-colored pillars than green pillars. These findings suggest that lady beetles may be recruited for biocontrol purposes into a landscape using yellow objects and traps (Mensah 1997, Adedipe and Park 2010, Gadino et al. 2012, Rodriguez-Saona 2011, Kemp and Cottrell 2015). Most of these studies utilized rectangular yellow sticky traps ranging in surface area from 104 to 1800 cm^2 and suspended them in the air to capture lady beetles (Kemp and Cottrell 2015), but several of these studies didn't evaluate if attraction leads to increased predation.

Very few studies have investigated the interactive effect of HIPV's and color in recruiting natural enemies for biocontrol purposes (Rodriguez-Saona et al. 2011, Kemp and Cottrell 2015). Rodriguez-Saona et al. (2011) found that syrphid flies were attracted to MeSA, however, the number of syrphid flies significantly increased by up to four-fold when yellow and white traps were baited with MeSA. Considering the importance of visual and olfactory cues in prey location by predators, evaluating both single and interactive effects of these cues in attracting coccinellids for the conservation biocontrol of *A. lagerstroemiae* is therefore considered imperative.

Chapter 2: Olfactory and visual attractants recruit lady beetles for biological control of crapemyrtle bark scale

2.1 Introduction

Semiochemicals emitted by plants in response to herbivory (i.e., Herbivore-Induced Plant Volatiles; HIPV) are essential in tri-trophic interactions, eliciting a top-down control of herbivores via recruitment of natural enemies (Zhu and Park 2005, Alhmedi et al. 2010, Gadino et al. 2012). More than 13 different crop plants reportedly release the phenolic compound methyl salicylate (MeSA), that has been reported to attract natural enemies (Zhu and Park 2005b, James 2006, Rodriguez-Saona et al. 2011, Gadino et al. 2012, Kemp and Cottrell 2015). In a soybean field study, up to threefold more seven-spotted lady beetle *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) were attracted and trapped on yellow sticky cards baited with 100mg of MeSA compared to yellow sticky traps with no lure. In the same study, multicolored Asian lady beetle *Harmonia axyridis* Pallas, another coccinellid, showed no significant attraction to MeSA compared to a control (Zhu and Park 2005), suggesting that attraction may not be universal among related species. In a two-year study, coccinellid counts in vineyards (with spider mite destroyer *Stethorus sp*. Weise and *C. septempunctata* as dominant species) were significantly higher, reaching up to twofold more on MeSA-baited yellow sticky cards compared with sticky cards with no lure (Gadino et al. 2012). Another HIPV that has been evaluated for the recruitment of coccinellids is the monoterpene limonene (Alhmedi et al. 2010, Kemp and Cottrell 2015). Yellow pan traps in the field baited with 100 µl of limonene captured significantly greater numbers of *H. axyridis* compared to unbaited control plots (Alhmedi et al. 2010). Previous studies evaluating the attraction of coccinellids to MeSA and limonene baited traps were done in agro-ecosystems such as soybean fields, pecan orchards, vineyards and chicory fields (Zhu and Park 2005, Alhmedi et al. 2010, Gadino et al. 2012, Kemp and Cottrell 2015) and to our knowledge, just one recent study evaluated these HIPV's in an urban landscape (Graham et al. 2020). Azalea (*Rhododendron* spp.) bushes established in urban landscapes were baited with sticky traps that had three different HIPV blends; methyl salicylate (MS) + acetic acid (AA) + 2 phenylethanol (2-PE) (MS blend); acetophenone + AA + 2-PE (AP blend); phenylacetaldehyde (PAA)+ MS + AA (PAA blend). A significantly greater number of the mycophagous coccinellid *Psyllobora* sp. were captured on PAA blend baited sticky cards, however no groups of predators or parasitoids were different between baited and unbaited azalea bushes (Graham et al. 2020).

Insects rely on several visual cues to locate their host, prey, or oviposition sites. Color alone can be a significant attractant to natural enemies like coccinellids. For example, when various single-color traps were placed in pecan and peach orchards, there was significantly greater recruitment of predators, mainly coccinellids, to yellow traps (Kemp and Cottrell 2015). This preference for yellow objects and traps has been supported across many different studies (Mensah 1997, Adedipe and Park 2010, Gadino et al. 2012, Rodriguez-Saona et al. 2011, Kemp and Cottrell 2015). However, very few studies have evaluated the interactive effect of HIPV's and color in recruiting natural enemies for biocontrol purposes. When the interactive effects of HIPV's and color in natural enemy attraction was evaluated in cranberry fields, the number of syrphid flies significantly increased by up to four-fold when yellow and white traps were baited with MeSA compared to other colors baited with the same HIPV (Rodriguez-Saona et al. 2020).

Acanthococcus lagerstroemiae is an exotic felt scale that originates from East Asia (Wang et al. 2016). In the United States, it is found primarily on crapemyrtle trees, a popular and widely cultivated shrub, and tree in the southeastern U.S (Chappell et al. 2012, USDA NASS 2014). *Acanthococcus lagerstroemiae* produces large amounts of honeydew, which may result in accumulations of sooty mold fungi on the trunk, branches, and twigs (Gu et al. 2014). Heavy infestations of the bark scale and associated honeydew and sooty mold, can render these trees, planted for aesthetic value, as aesthetically displeasing in the landscape (Borden et al. 2018). *Acanthococcus lagerstroemiae* infestations are currently managed via chemical and mechanical methods (Wang et al. 2016). Soil applications of systemic neonicotinoids such as dinotefuran and imidacloprid are the most effective (Gu et al. 2014, Wang et al. 2016, Vafaie and Knight 2017). However, unfortunately, when neonicotinoids are systemically applied as soil drenches or soil injections, they are usually translocated into pollen and nectar of ornamental plants, posing a significant threat to visiting pollinators and natural enemies (Wang and Diaz 2016, Mach et al. 2018, Thurmond 2019). Therefore, alternative control methods that are environmentally friendly and more sustainable, such as biological control, should be considered and evaluated.

In the United States, lady beetles (Coleoptera: Coccinellidae) have been reported feeding on *A. lagerstroemiae* (Fig. 4) (Wang et al. 2016). Based on previous studies, the abundance of predatory coccinellids can be manipulated with visual and olfactory cues. This study aimed to evaluate both single and interactive effects of these cues in attracting coccinellids for the conservation biological control of *A. lagerstroemiae* populations on trees in urban landscapes.

2.2 Materials and Methods

2.2.1 Semiochemical Experiments

2.2.1.1 Potted Trees Baited with Semiochemical Lures.

This experiment was conducted on an open field at EV Smith Research Center, Shorter, AL, USA (32.442015, -85.897341). The crapemyrtle trees were placed on the open field and staked to the ground using wooden stakes and string to keep them upright. Two trials, June 21 to July 2 and July

22 to July 31, 2019, were conducted with the same trees and treatments. Potted (3-gallon trade containers) crapemyrtle trees (*Lagerstroemia* sp. "Natchez") with an average height of 1.4 m, were used in this study. The trees were left in place for 9-10 days and sampled and watered daily. Two volatile lures, methyl salicyclate (Predalure, 5 g load/lure; 90-day lure; average release rate ~35 mg/day at 30 °C constant in the lab; Agbio, West Minster, CO, USA) and D-Limonene (CAS Reg. Number-5989-27-5) were used in this study. The methyl salicylate-based lure is purported to recruit some species of lady beetles (Gadino et al. 2012, Rodriguez-Saona et al. 2020). Limonene lures were prepared as described by Cottrell and Horton (2011). One hundred µL of limonene was pipetted into white rubber sleeve stoppers/septa (Ace Glass, Inc.), allowed to be absorbed at room temperature, and kept in a mason jar with a screw top lid in a -20°C freezer until used in the trial. Limonene lures release at an average rate of 0.08g per day at 30 °C constant temperature (see Appendix Table 1). Limonene lures were replaced every day of each trial, and one MeSA lure was used for each trial. Samples were taken between 0800 and 1000 hours CST daily, with temperature ranging from 29 to 34 °C during sampling days.

The experimental design was a randomized complete block with trees randomly assigned to a control (unbaited) group and three treatment groups. The treatments include Predalure (P), limonene (L), and a combination of Predalure and limonene (P+L) and had four replicates in each trial. As in previous studies, the trees were placed 10 m apart from one another (Kemp and Cottrell 2015) . Lures were hung directly on trees (Fig 5, Fig. 6), with each tree having one lure each, and P+L baited trees contained one Predalure sachet and one limonene septa. Trees were left in place from June 21 to July 2 (Trial 1) or July 22 to July 31 (Trial 2). Pre-treatment lady beetle counts were taken on all trees. Lady beetles were sampled using the branch beating method using a beat stick and a sweep net. Four branches, one in each cardinal direction, were beaten five times (20 beats per tree) into the net. The contents were emptied into a plastic zipper-top bag, placed in an ice cooler, and transported to the laboratory for sorting and identification. Natural enemies in each sample (Coccinellidae, lacewings) were identified, data recorded, and voucher specimens filed in the Auburn University Biodiversity Learning Center, Auburn, AL., USA. Other natural enemies observed were in low numbers and were not included in the analysis. The mortality of CMBS associated with the treatments was determined in each trial. Two branches were randomly selected on each tree and marked at a length of 15 cm. Live settled adult CMBS were counted in marked sections and recorded before the lures were applied and again at each trial's end. CMBS mortality was recorded as percentage mortality.

2.2.1.2 Experiments with Olfactory Lures on Landscape Trees

Experiments to evaluate olfactory lures were conducted from April to June 2020 using established landscape crapemyrtle trees in Huntsville, AL. Some experiments were conducted simultaneously with one another, and others were designed based on the outcome of previous experiments. This experiment evaluated the response of lady beetles to commercially-available lures. Trees in this experiment were infested with *A. lagerstroemiae* in residential and commercial landscapes before experiments were conducted. The trees in the residential landscapes were established under tall pine trees with grass and pine straw as the primary ground cover (Fig. 7). Tree heights ranged from 4 to 5 m, with approximately 11 m between trees at this site. The commercial landscape trees were near parking areas with no overstory and impervious surfaces as the primary ground cover. The tree heights ranged from 3 to 5 m, with approximately 13 m between trees at this site.

Two commercially-available volatile lures, Predalure and D-limonene, were used. Predalure (Agbio, West Minster, CO, USA) used in this experiment had a 30-day field life and released an average of 35 mg per day at 30 °C (see Appendix Table 4). D-limonene (Alpha Scents Inc., West

Linn, OR) lures have an average release rate of 0.183g per day at 30 °C constant temperature (see Appendix Table 2). Lures were replaced one month after deployment of experimental trees. The experimental design was a randomized complete block with either a control (unbaited) group or three treatment groups; Predalure (P), limonene (L), or a combination of Predalure and limonene (P+L). Treatments were assigned to each of the four replicates using initial populations of CMBS abundance. Lures, one per tree, were hung (Fig. 10) in an approximate consistent position with the P+L treatment having one of each type of lures.

Populations of CMBS initially were assessed by visually inspecting 15 cm marked off portion of four randomly selected branches and counting all settled crawlers and live female adults. Since we expect density-dependent predation, the initial populations (before lures were deployed) were used to assign trees to replicates with a similar number CMBS. Similarly, before the application of lures, lady beetles were sampled using the branch beating method. Four branches, one in each cardinal direction, were beaten five times (twenty beats per tree) into a sweep net. The contents were emptied into a plastic zipper-top bag (Fig. 11), placed in an ice cooler, and transported to the lab for sorting and identification. These sampling procedures for CMBS and lady beetle were repeated at one week, one month, and two months after lure deployment, and data were recorded for all Coccinellidae.

2.2.1.3 Constant vs Pulsed D-Limonene Lures

In previous experiments, we noticed the initial recruitment of lady beetles after lure deployment, followed by a waning of recruitment over time. This experiment was developed to evaluate a constant versus pulsed release of D-limonene. This experiment was conducted in the same commercial and residential landscapes, as previously described, from July to September 2020.

The same commercial D-limonene lures from the previous experiment were used again. These were part of the constant release treatment since they release 0.183 g per day at 30 °C constant temperature (see Appendix Table 2) and would not expire during one month of deployment. A second lure (Alpha Scents Inc., West Linn, OR) was purchased to release D-limonene at an average of 0.033g per day at 30°C constant temperature (see Appendix Table 3). The lure with the lower release rate was intended to stop releasing after 1 wk in the field (see Appendix 1). Both lures were replaced one month after deployment on their respective trees, providing a pulse of D-limonene on one set of trees compared to a constant release on other trees.

The experimental design was a randomized complete block with trees assigned to either a control (no lure) group, two, D-Limonene treatment, constant release (C.R.), or pulsed release (P.R.), each with four replicates. Treatments were assigned to each of the four replicates using initial populations of CMBS abundance. Lures, one per tree, were hung in an approximately consistent position in tree's canopy. Populations of CMBS and lady beetles were initially assessed, as previously described. These sampling procedures for CMBS and lady beetle were repeated at one month and two months after lure deployment, and data were recorded for all Coccinellidae.

2.2.2 Experiments with Visual Attractants

A landscape trial was conducted from July to September 2020 to evaluate yellow panels and traps to recruit lady beetles to trees infested with *A. lagerstroemiae*. This experiment was conducted using established crapemyrtle trees with no overstory in Huntsville, AL. Tree heights ranged from 3 to 4 m, with an average distance of approximately 8 m between each study tree.

To evaluate the effect of trap position on lady beetle recruitment, yellow pyramid traps (Fig.13) were compared to rectangular panels placed at different positions on study trees. Cross-vane, pyramidal traps (Tedders and Wood 1994) made of fiberboard, 1.22 m tall, 54 cm wide at the base of each panel tapering to 6 cm at the top were purchased (Great Lakes IPM Inc., Vestaburg, MI). When used in this study, the insect collection container typically placed at the top of the trap was removed since the aim was to recruit but not trap lady beetles. Each cross-vane panel was first painted with white latex primer (Kilz 2 Latex, interior/exterior, Masterchem Industries, Imperial, MO), allowed to dry, and then painted with two coats of yellow latex paint (3006-1B, Dandelion Chain, Valspar season flex, Exterior Semigloss, Tint Base 4, 105-2.5, 113-5Y18.5, 115-0.5, 214-6Y26.5, The Valspar Corporation, Wheeling, IL). Rectangular panels were black and white corrugated tree protectors (A.M. Leonard Horticultural Tool and Supply Co., Piqua, OH) cut into rectangular sizes of 60 x 30 cm^2 and painted with white latex primer (Kilz 2 Latex, interior/exterior, Masterchem Industries), allowed to dry. Each then received two coats of yellow latex paint (3006-1B, Dandelion Chain, Valspar season flex, Exterior Semigloss. Tint Base 4, 105-2.5, 113-5Y18.5, 115-0.5, 214-6Y26.5, The Valspar Corporation, Wheeling, IL). Three rectangular panels have a similar surface area to one pyramid (approximately 5,665 cm^2). At 1 m above the trees' base, the traps were strapped using cable ties (Utilitech®, Wilkesboro, NC) while panels hung in the tree's canopy (Fig. 14) were held in place using a transparent shower curtain hook (Mainstays™, Bentonville, AR).

The experimental design used was a randomized complete block with trees blocked based on initial CMBS abundance, as previously described. Study trees were assigned to either a control group (Infested tree without a yellow pyramid) or one of three treatment groups; yellow pyramid placed 1 m above the tree's base (YP1), three yellow rectangular panels placed 1 m above the tree's base (Fig. 15) (YR1), and three yellow rectangular panels hung in tree's canopy (Y). Each visual treatment and control were replicated with four separate trees. Populations of CMBS and lady beetles were initially assessed as previously described for the semiochemical experiments. These sampling procedures for CMBS and lady beetle were repeated at one month and two months after lure deployment, and data were recorded for all Coccinellidae.

2.2.3 Interactive Effects of Visual and Olfactory Lures in the Landscape

A landscape trial was conducted from March to May 2020 to evaluate the combined effects of visual and olfactory lures using established crapemyrtle trees infested with *Acanthococcus lagerstroemiae*. The trees used in this experiment were the same ones used for the experiments with visual attractants. Cross-vane, pyramidal traps (Tedders and Wood 1994) painted yellow, and without the insect collection containers were used. These yellow pyramids were strapped to the tree trunk at 1 m above the ground using cable ties (Utilitech®). Two volatile lures, Predalure (30-day lure; release rate ~35 mg per day at 30 °C constant temperature; Agbio) and D-limonene lures (Alpha Scents Inc.; release rate of 0.183g per day at 30 °C constant temperature) were used. Lures were deployed then replaced one month after deployment.

The experimental design was a randomized complete block with trees blocked based on an initial CMBS abundance sampled as previously described. Study trees were assigned to either a control group (Infested tree but no visual or olfactory attractants) or three treatment groups; yellow pyramid baited with Predalure (Fig. 16) (Y+P), yellow pyramid baited with limonene (Y+L), or a yellow pyramid with no lure (Y). Each treatment group and control group were replicated four times.

Pre-treatment lady beetle counts were taken on all trees using the branch beating method using a beat stick and a sweep net (Fig. 17). Four branches, one in each cardinal direction, were beaten five times (20 beats per tree) into the net. The contents were emptied into a plastic zipper-top bag, placed in an ice cooler, and transported to the lab for sorting and identification. Coccinellidae in each sample was identified, and data recorded. After the initial samples, lady beetles and CMBS populations were re-sampled at one week, one month, and two months after initial deployment. CMBS populations were re-sampled at one week and one month after initial deployment.

2.2.4 Statistical Analyses

Recruited lady beetles and other natural enemies, and CMBS mortality in EV Smith trials were analyzed separately. All data from both trials were submitted to a univariate repeated-measures analysis of variance (ANOVA; Statistix 10, Analytical Software, Tallahassee, FL). When a significant effect was detected ($P < 0.05$), means were separated using Least Significant Difference (LSD).

Native lady beetles, exotic lady beetles and the combination of all coccinellid species recruited were analyzed separately in all landscape trials. Recruited lady beetles were analyzed using a univariate repeated-measures analysis of variance (ANOVA; Statistix 10, Analytical Software, Tallahassee, FL) when effects of commercially-available lures, constant vs. pulsed D-limonene release, position of panel or pyramid in the tree, and visual × olfactory interactions on lady beetle recruitment were tested. Similarly, associated effects of treatments on CMBS populations were also tested using a univariate repeated-measures ANOVA in all landscape studies. When a significant effect was detected ($P < 0.05$), means were separated using Least Significant Difference (LSD).

2.3 Results

The exotic species of lady beetles recruited in this study include *Harmonia axyridis* Pallas and *Coccinella septempunctata* Linnaeus*,* while the native species include *Hyperaspis bigeminata* Randall, *Chilocorus stigma* Say, *Coleomegilla maculata* De Geer, *Scymnus* sp. Kugelan, *Hippodamia convergens* Guerin-Meneville*,* and *Cycloneda munda* Say.

2.3.1.1 Potted Trees Baited with Semiochemical Lures

There was no significant treatment effect in numbers of lady beetle recruited on potted crapemyrtles baited with olfactory lures compared to non-baited control crapemyrtle trees during June 2019 (F=0.09; df=3, 12; *P*=0.9631) and July 2019 trials (F= 0.97; df= 3, 12; *P*=0.4257). Similarly, sampling date had a significant effect on numbers of lady beetles recruited in June (F=4.85; df=11, 33; *P*<0.05) and July trials (Fig. 18) (F=6.12; df=11, 33; *P*<0.05). Lady beetles recruited on P+L baited trees were numerically higher in July 2019 trial but were not significant (Table 1). Lures did not affect other natural enemy recruitment in either the June (F=1.82; df=3, 12; *P*=0.1970) or July trial (F=0.00; df=3, 12; *P*=1.000) (Table 1). The mortality of CMBS was high on control trees (80-84% across both trials; Table 1; Fig. 19). No significant differences were observed in CMBS mortality on baited trees compared to control in either trial (F= 0.31, df= 3, 12 P= 0.8196).

2.3.1.2 Experiments with Olfactory Lures on Landscape Trees

A total of 105 lady beetles were captured in this study. Of the total number captured, 85% were *H. axyridis*, 12% were *H. bigeminata,* 2% were *C. septempunctata* and 1% were the *Scymnus* sp.

Altogether, exotic lady beetles dominated lady beetle recruitment irrespective of time and treatment. When the response of coccinellids to commercially-available lures was tested, control (non-baited) trees and P+L baited trees significantly recruited more lady beetles (all species combined) than L baited trees (Table 2). Significantly more exotic lady beetles were also recruited on unbaited, and P+L baited trees (Table 3). Furthermore, a timing effect was observed with significantly more lady beetles (all species combined) recruited two months after lure deployment (June 2020) than at initial pre-treatment sampling time (April 2020) (Table 2). Similarly, a significant timing effect was also observed in recruitment of native lady beetles, at two months (June 2020) after lure deployment than at other sampling times (Table 3). When the reduction of CMBS associated with lure was tested, no significant treatment or time effect was observed on baited trees compared to control trees (Table 4).

2.3.1.3

Constant vs. Pulsed d-Limonene

A total of 135 lady beetles were captured in this study. Of which, 20% were the exotic *H. axyridis* and 80% were the native *H. bigeminata*. Limonene lures (Constant-release, pulsed vs release, and an unbaited control) on infested trees did not significantly increase lady beetle recruitment compared to trees without a lure (all species combined; Table 2). However, more lady beetles were recruited at one month after lure deployment (August 2020) irrespective of treatments (all species combined; Table 2). Furthermore, a time effect was observed in the recruitment of exotic and native lady beetle species. Significantly more exotic species were present before lure deployment (July 2020) and at one month after lure deployment (August 2020) (Table 3). Similarly, more native ladybeetle species were recruited one month after lure deployment than at other sampling times (Table 3). No significant treatment effect was observed with CMBS reduction associated with treatment, but sampling date (time) was significant (Table 4).

2.3.2 Experiments with Visual Attractants

A total of 100 lady beetles were captured in this study. Of the total number captured, 56% were *H. bigeminata*, 43% were *H. axyridis*, and 1% were the *Scymnus* sp. Panel position on the tree significantly affected the recruitment of lady beetles (all species combined; Table 2), with significantly more lady beetles, up to two-fold, recruited to trees with yellow rectangular panels at 1 m above the tree's base compared to control trees and yellow rectangular panels hung in tree's canopy (Fig. 20 A). Exotic lady beetle recruitment was not affected by panel positions (Fig. 20 B; Table 3); however, more exotic species were present before treatment deployment (July 2020) compared to other sampling times (Fig. 20 C; Table 3). In contrast, panel positions affected native lady beetle capture with more significant recruitment on trees with yellow rectangular panels at 1m of the tree's base compared to other treatment groups (Fig. 20 B; Table 3). Sampling time was significant with more native coccinellids recruited at one month after trap deployment (August 2020) than before deployment (July 2020) (Table 3). The abundance of CMBS was significantly lower on trees with yellow rectangular panels hung in canopy than control trees and trees with yellow pyramid traps at 1m above the tree's base (Table 4).

2.3.3 Interactive Effects of Visual and Olfactory Lures in the Landscape

A total of 155 lady beetles were captured in this study. Of the total number captured, 57% were *H. bigeminata,* 35% were *H.axyridis*, 2% were *C. stigma,* and 6% were *Scymnus* sp. When a visual and olfactory lure were present, there were no significant interactive effects on lady beetle recruitment (all species combined; Table 2). Similarly, there was no significant treatment effect in the recruitment of exotic and native lady beetle species (Table 3). However, irrespective of treatments, sampling date had a significant effect on lady beetle recruitment, with significantly more exotic lady beetles recruited two months (May 2020; Fig. 21 B) after treatments than at other sampling times (Table 3). Similarly, more exotic lady beetles were recruited at one week after treatment (April 2020) than other sampling times. More native lady beetles were also recruited initially before treatment (March 2020) than one month (April 2020) and two months (May 2020) after treatment (Table 3). There was no significant time and treatment effect observed on the abundance on CMBS (Table 4).

2.4 Discussion

Lady beetles play an essential role in the biocontrol of herbivorous insect pests such as aphids, whiteflies, and scale insects (Obrycki and Kring 1998). Therefore, recruiting and conserving them for their biocontrol services is important. This study evaluated lady beetles' response to semiochemical and visual attractants on CMBS infested trees. Limonene and Predalure (containing methyl salicylate) were ineffective in recruiting additional lady beetles when potted or landscape infested crape myrtle trees were baited with lures. Several studies have documented the attraction of coccinellids to HIPV's in fields (James 2003, Alhmedi et al. 2010, Kaplan 2012), however, failure to recruit coccinellids using these HIPV's are not uncommon (Salamanca et al. 2018, Rodriguez-Saona et al. 2020). Our results are consistent with a study that observed no significant difference in lady beetle capture on Predalure-treated and untreated plots in a soybean field (Mallinger et al. 2011). It is suggested that MeSA may suppress release of certain HIPV's dependent on Jasmonic Acid (JA) pathways and reduce attractiveness of certain natural enemies to infested plants (Turlings and Erb 2018). If coccinellids rely on volatiles from the JA pathway to locate pests on infested trees, this may explain why lady beetles were not recruited on HIPV baited trees in our study. Several studies have documented coccinellids' attraction to methyl salicylate and limonene lures in agroecosystems. Our literature review suggests these experiments in ornamental landscape may be one of the few to evaluate these lures for biological control (Zhu and Park 2005a, Adedipe and Park 2010, Alhmedi et al. 2010, Graham et al. 2020, Rodriguez-Saona et al. 2020). Our study only reported a significant difference in recruitment on landscape trees baited with a combination of Predalure and limonene (P+L) compared to trees baited with Predalure (P) or limonene (L) alone. However, P+L trees did not recruit more lady beetles than control (non-baited) trees on landscape infested crape myrtle trees. When Graham et al. (2020) evaluated natural enemy recruited in azalea bushes using HIPV's, their methyl salicylate blend did not significantly recruit coccinellids associated with CMBS predation. However, azalea bushes with phenylacetaldehyde blend, which also contained methyl salicylate, significantly recruited more fungus-eating coccinellid compared to non-baited bushes. It is currently unknown if Predalure or Limonene used singly have any repelling effects; however, it is suggested that blends of HIPV's are likely to be more effective in recruiting natural enemies than single HIPV compounds (Kaplan 2012).

Furthermore, it has been documented that different coccinellid species may respond differently to a particular HIPV (Zhu and Park 2005a). For example, adult *Coccinella septempunctata* were significantly attracted to MeSA baited sticky cards; however, there were no differences in the capture of *H. axyridis* on MeSA-baited cards compared to control groups in the same study (Zhu and Park 2005a). The majority of lady beetle species recruited at this study site were the exotic species, *H. axyridis*. It is suggested that these exotic species were not attracted to our deployed lures and were only recruited based on CMBS availability.

Similarly, the constant or pulsed release of D- Limonene did not affect lady beetle recruitment on landscape infested trees. Our results were inconsistent with Alhmedi et al. (2010) findings, who identified limonene as a potential kairomone of *Harmonia axyridis*. The discrepancy in findings of this current study and that of Alhmedi's may be attributed to geographical variation in the distribution of lady beetles, different lady beetle capture methods, and varying release rates of limonene lure. Alhmedi et al. (2010) conducted their experiment in an agroecosystem (Chicory crop field) close to a wooded area, which may serve as a reservoir/ bank for lady beetles. They collected lady beetles using yellow water pan traps with controlled-release dispensers containing 100 ul of limonene. In our study, the trees were in a landscape characterized by car parking areas, no overstory, and impervious surfaces as the major ground cover. Landscape components such as these can influence the distribution of insect populations present in such landscape (Held 2020). Additional conservation biocontrol methods such as providing alternative food and floral sources, beetle banks, and companion planting that could recruit and retain natural enemies in an urban landscape should be considered (Rebek et al. 2005). We did not observe a significant reduction in the population of CMBS despite the presence of lady beetles on landscape trees with olfactory lures. It is worth noting that the trees used in this current study also had infestations of crape myrtle aphids, and it may be possible that lady beetles were feeding on crape myrtle aphids than on CMBS. However, to our knowledge, no study has investigated the feeding preference of coccinellids when presented with crapemyrtle aphids or CMBS.

Visual cues play an essential role in the host-seeking activity of predaceous coccinellids (Adedipe and Park 2010, Kemp and Cottrell 2015). The potential of recruiting coccinellids for their biocontrol services via yellow objects have been investigated and documented in several studies (Mensah 1997, Braman et al. 2003, Kemp and Cottrell 2015, Cottrell 2017). It is suggested that the attraction and aggregation of lady beetles in the field would mostly be accomplished using a yellow object as opposed to a semiochemical (Kemp and Cottrell 2015). The interactive effects of HIPV's and visual cues (colors) on natural enemy recruitment have also been investigated (Rodriguez-Saona et al. 2020). When yellow pyramid traps were used alone or in synergy with semiochemical lures, there was no significant difference in the number of lady beetles found on control trees compared with other treatments. Consistent with our results, Kemp and Cottrell (2015) baited yellow pyramid traps with Predalure or limonene in pecan orchards and found no interaction of trap color with lures in capturing lady beetles in 2012 and 2013. However, they documented that trap color did affect trap capture, with yellow pyramid traps capturing more lady beetles than other trap colors utilized. One of the factors responsible for the disparity in our results may be attributed to geographical variations in lady beetles' distribution and density due to the limited structural complexity on our urban study sites. It is also been reported that available CMBS infestations could negatively impact recruitment to traps as it has been shown that yellow objects will not lure lady beetles away from available food (Hoddle et al. 2013, Kemp and Cottrell 2015).

This current study showed that trees with yellow rectangular panels at 1 m above the tree's base recruited more lady beetles than other trap positions. It is worthy to note that the lady beetle species recruited to the yellow rectangular panels were comprised predominately of the native species *Hyperaspis bigeminata* with very few exotic species recruited. Kemp and Cottrell (2015) captured more significant exotic species (particularly *H. axyridis*) on yellow pyramid traps than native coccinellid species when sampled for twelve months. However, *H. axyridis* in yellow pyramid trap capture declined up to three fold in August compared to trap capture in June while native lady beetle capture increased by three fold in August compared to June (Kemp and Cottrell 2015). It is possible that the timing of recruitment may have influenced the capture of native lady beetles over exotic species in our study.

Acanthococcus lagerstroemia is currently being managed using chemical insecticides like neonicotinoids, which may be harmful to visiting pollinators and other beneficial insects. This study suggests a sustainable alternative control method by using lady beetles as potential biocontrol agents capable of reducing CMBS populations. Overall, recruiting lady beetles on landscape infested trees via semiochemicals seems to be of little efficacy. Future studies should be directed towards evaluating the right blend of HIPV's and the actual release rate of the choice compounds that will effectively recruit the desired biocontrol agent. Yellow rectangular panels, however, attract some coccinellid species, particularly native ones. Modification of this attractive visual object to enhance lady beetle recruitment for biocontrol of insect pests should also be considered. The structural complexity of urban landscapes (majorly with impervious surfaces) may influence the distribution and density of coccinellids. Therefore, local improvements (overwintering sites, alternative food and floral sources, and more vegetation cover) to enhance natural enemies' distribution and density in urban landscapes may improve the efficacy of lady beetle against CMBS.

Table 1: Number of lady beetles and other natural enemies recruited to infested potted crapemytrle trees and subsequent mortality of CMBS when baited with either Limonene, Predalure, a combination of Predalure and Limonene, or no lures in two separate trials.

Treatment	**Lady beetle abundance (mean±SEM)**		**[a]Other natural enemies (mean±SEM)**		**CMBS Mortality (mean %)**	
	June	**July**	**June**	**July**	**June**	**July**
Control	15.5±3.571	34.7±12.31	6.75±1.37	5.50±0.65	83.85	80.79
Limonene	18.25±4.03	31.25±4.44	11.25±1.49	5.50±1.32	91.58	83.24
Predalure	17.00±3.32	23.75±2.66	7.75±1.80	5.50±2.36	89.92	84.50
Predalure+Limonene	17.75±2.40	41.50±6.79	11.25±2.17	5.50±0.65	95.83	87.80

[a] other natural enemies were Araneae and Hemerobiidae (brown lacewings)

Table 2: Mean (±SEM) of lady beetles (all species combined) recruited to CMBS-infested trees in an urban landscape, 2020.

Experiment	Treatment	No of lady beetles (mean ± S.E.)	ANOVA for Repeated Measures		
			Treatment	Time	Time x Treatment
Olfactory lure	Control	2.88 ± 0.75a	F= 3.77, df=3, 12, *P*=0.0408*	F= 4.11, df=3, 36, *P*=0.0132*	F= 0.94, df=3, 36, *P*=0.5048
	Limonene	0.44 ± 0.18b			
	Predalure	1.50 ± 0.37ab			
	Predalure+Limonene	2.31 ± 0.61a			
Constant vs Pulsed	Control	4.01 ± 1.45a	F= 0.06, df=2, 9, *P*=0.9437	F= 7.99, df=2, 18, *P*=0.0033*	F= 0.07, df=2, 18, *P*=0.9912
	Pulsed Limonene	3.67 ± 1.84a			
	Constant Limonene	3.50 ± 1.40a			
Trap positions	Control	1.58 ± 0.43b	F= 4.00, df=3, 12, *P*=0.0346*	F= 0.75, df=2, 24, *P*=0.4818	F= 1.41, df=2, 24, *P*=0.2519
	YR1	3.58 ± 0.83a			
	Y	1.17 ± 0.32b			
	YP1	2.00 ± 0.48ab			
Trap x Lure	Control	1.69 ± 0.44a	F= 1.43, df=3, 12, *P*=0.2833	F= 8.08, df=3, 36, *P*=0.0003*	F= 1.51, df=3, 36, *P*=0.1818
	Y+L	2.13 ± 0.46a			
	Y+P	3.56 ± 0.58a			
	Y	3.56 ± 0.88a			

* indicates a significant difference at $P<0.05$

Means with different letters are significantly different (Means separated by LSD $P < 0.05$).

Table 3: Mean (±SEM) of native and exotic lady beetles recruited to CMBS-infested trees in an urban landscape, 2020.

Experiment	Treatment	Exotic spp. (mean ± S.E.)	Native spp. (mean ± S.E.)	ANOVA Results	
				Treatment	Time
Olfactory lure	Control	2.00 ± 0.55a	0.38 ± 0.15a	F= 3.81, df=3, 12, *P*=0.0396*[1]	F= 2.56, df=3, 36, *P*=0.0705[1]
	Limonene	0.44 ± 0.18b	0a	F= 1.68, df=3, 12, *P*=0.2244[2]	F= 4.79, df=3, 36, *P*=0.0065*[2]
	Predalure	1.31 ± 0.33ab	0.19 ± 0.10a		
	Predalure+Limonene	1.94 ± 0.55a	0.38 ± 0.15a		
Constant vs Pulsed	Control	0.92 ± 0.29a	3.17 ± 1.43a	F= 0.94, df=2, 9, *P*=0.4257	F= 8.84, df=2, 18, *P*=0.0021*
	1-week Limonene	0.50 ± 0.26a	3.17 ± 1.90a	F= 0.05, df=2, 9, *P*=0.9522	F= 8.05, df=2, 18, *P*=0.0032*
	1-month Limonene	0.83 ± 0.27a	2.67 ± 1.31a		
Trap positions	Control	0.92 ± 0.34a	0.67 ± 0.25b	F= 0.55, df=3, 12, *P*=0.6583	F= 10.61, df=2, 24, *P*=0.0005*
	YR1	1.00 ± 0.39a	2.58 ± 0.97a	F= 3.88, df=3, 12, *P*=0.0377*	F= 3.94, df=2, 24, *P*=0.0331*
	Y	0.50 ± 0.19a	0.67 ± 0.26b		
	YP1	1.25 ± 0.43a	0.75 ± 0.31b		
Trap x Lure	Control	0.75 ± 0.38a	0.94 ± 0.31a	F= 0.05, df=3, 12, *P*=0.9846	F= 21.52, df=3, 36, *P*<0.0001*
	Y+L	0.81 ± 0.47a	1.31 ± 0.31a	F= 2.22, df=3, 12, *P*=0.1390	F= 17.98, df=3, 36, *P*<0.0001*
	Y+P	0.94 ± 0.38a	2.63 ± 0.65a		
	Y	0.88 ± 0.45a	2.69 ± 0.83a		

* indicates a significant difference at *P*<0.05

Superscript number (1) indicates that in all experiments, the top column under ANOVA results represents Exotic coccinellids, while (2) indicates that the bottom column represents ANOVA results for Native coccinellids.

Means with different letters are significantly different (Means separated by LSD P < 0.05).

Table 4: Mean (±SEM) abundance of CMBS on infested trees in an urban landscape, 2020

Experiment	Treatment	CMBS abundance (mean ± S.E.)	ANOVA Results	
			Treatment	Time
Olfactory lure	Control	69.75 ± 12.68a	F= 0.08, df=3, 12, *P*=0.9679	F= 2.35, df=2, 24, *P*=0.1170
	Limonene	67.25 ± 16.25a		
	Predalure	82.33 ± 12.12a		
	Predalure+Limonene	78.75 ± 19.41a		
Constant vs Pulsed	Control	140.75 ± 37.33a	F= 0.19, df=2, 9, *P*=0.8263	F= 8.84, df=2, 18, *P*=0.0066*
	1-week Limonene	130.25 ± 30.48a		
	1-month Limonene	104.50 ± 25.22a		
Trap positions	Control	42.00 ± 6.82b	F= 3.51, df=3, 12, *P*=0.0494*	F= 0.21, df=2, 24, *P*=0.8143
	YR1	32.92 ± 5.23ab		
	Y	23.25 ± 3.46a		
	YP1	36.67 ± 7.70b		
Trap x Lure	Control	33.50 ± 4.30a	F= 0.62, df=3, 12, *P*=0.6157	F= 1.66, df=2, 24, *P*=0.2104
	Y+L	44.83 ± 9.35a		
	Y+P	51.08 ± 9.63a		
	Y	55.83 ± 9.16a		

* indicates a significant difference at $P<0.05$

Means with different letters are significantly different (LSD $P < 0.05$).

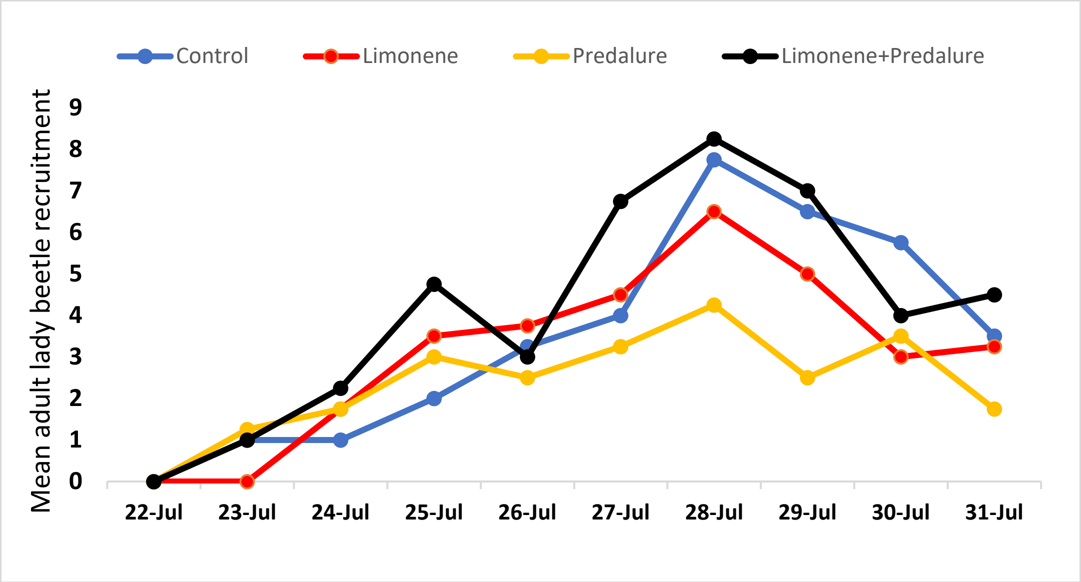
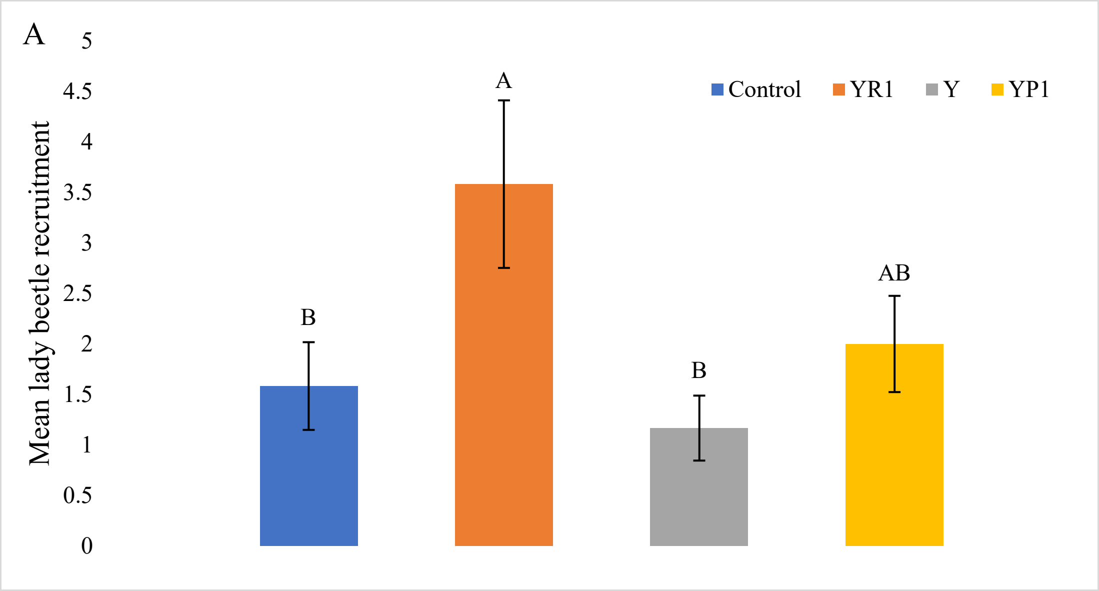
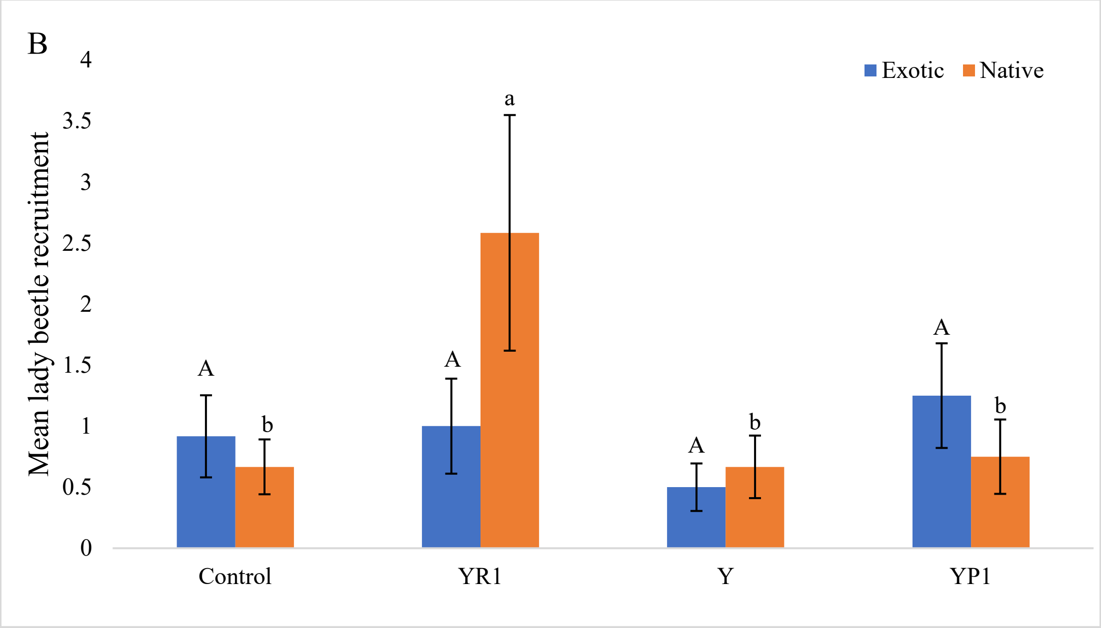


Fig 18. Mean recruitment of lady beetles on infested potted crapemyrtle trees from July 22 to July 31, 2019.


A
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Mean lady beetle recruitment
Control
YR1
Y
YP1
B
A
B
AB


B
4
3.5
3
2.5
2
1.5
1
0.5
0
Mean lady beetle recruitment
Exotic
Native
A
b
A
a
A
b
A
b
Control
YR1
Y
YP1

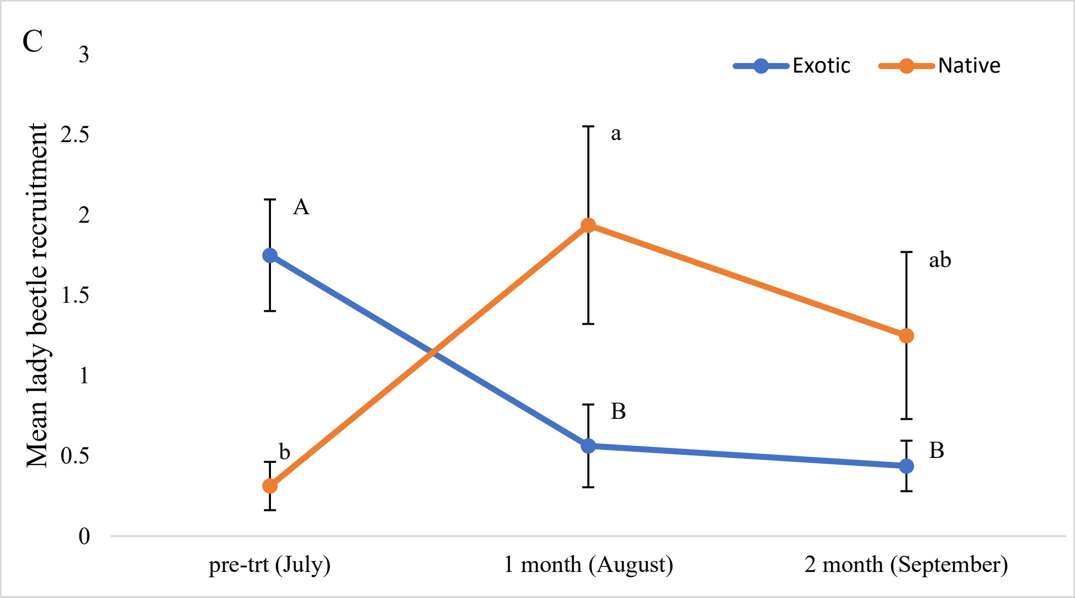
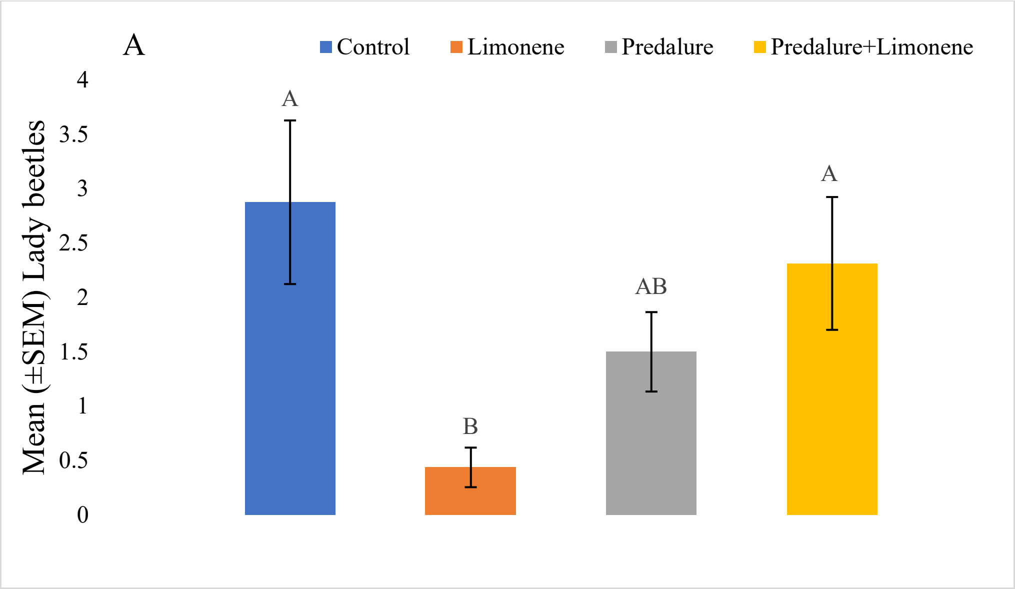


Fig. 20 Mean (± S.E.) adult (A) All species of lady beetles (B) Native and exotic lady beetles recruited when no trap, yellow pyramid trap 1m above tree base (YP1), yellow rectangular panels 1 m above tree base (YR1) or yellow rectangular panels hung in canopy (Y) were installed on trees (C) Native and exotic lady beetles recruited over time, irrespective of treatments. For each chart, different letters above columns indicate a significant difference ($P< 0.05$) among treatments (A and B) or time (C). Means were separated using Least Significant Difference (LSD).



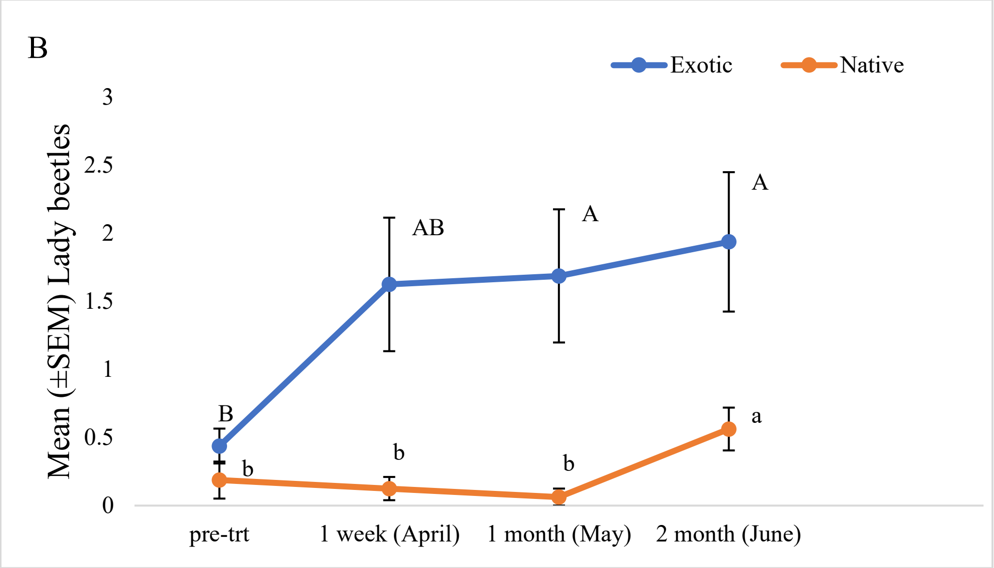


Fig. 21. Mean (± SEM) number of adult lady beetles; (A) All species of lady beetles recruited when trees were baited with Predalure, limonene, Predalure + limonene or baited with no lure. (B) Native and exotic lady beetles recruited over time, irrespective of treatments. For each chart, different letters above columns indicate a significant difference ($P< 0.05$) among treatments or time. Means were separated using Fisher's Protected Least Significant Difference (LSD)

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Appendix 1

Release rates for lures used in all experimental trials.

Limonene lures in rubber septa

Limonene lures (Fig. 8) were prepared as described by Cottrell and Horton (2011). The rubber sleeve septum's initial weight was taken and recorded on an analytical balance before loading limonene to account for the amount of limonene dispensed in each septum. One hundred µL of limonene was pipetted into white rubber sleeve stoppers/septa (Ace Glass, Inc.), and weight was also recorded. Seven different rubber septa were used in this experiment to determine the release rate of D-Limonene released over a 24-hr period. The rubber septa loaded with limonene were kept in a growth chamber set at a constant temperature of 30ºC, and their weight was taken after 24 hours. The amount of limonene released over 24 hours was determined by subtracting the rubber septum's weight after 24 hours from the initial weight of limonene-loaded rubber septum.

An average of 0.088g of limonene was dispensed in rubber septa, and over 24 hours, an average of 0.082g of limonene was released at a constant temperature of 30ºC (Appendix table 1)

Appendix Table 1. Lure released per 24 hours in a growth chamber at a constant temperature of 30ºC

N	Rubber septum(g)	Rubber septum + Limonene (g)	Amount of limonene dispensed (g)	Limonene released after 24 hrs (g)
1	1.235	1.323	0.088	0.083
2	1.228	1.316	0.088	0.080
3	1.237	1.323	0.086	0.080
4	1.232	1.319	0.087	0.078
5	1.25	1.338	0.088	0.085
6	1.242	1.330	0.088	0.085
Average	**1.237**	**1.325**	**0.088**	**0.082**

Limonene lures (one-month field life)

D-Limonene lures (Fig. 9) were purchased from Alpha Scents Inc., West Linn, OR. The initial weight of the limonene lure was taken and recorded. Two lures were placed in a growth chamber set at a constant temperature of 30ºC, and their weight was taken after 24 hours for a period of 7-days to determine the amount of limonene released. Over seven days, an average of 0.1839g of limonene was released per day from the lures.

Appendix Table 2. Lure released per 24 hours over 7 days in a growth chamber at a constant temperature of 30ºC

Days	**Lure 1 (g)**	**Lure 2 (g)**	**lure 1 released (g)**	**lure 2 released (g)**	**Avg. released (g)**
0	4.186	4.056	------	-------	-------
1	3.971	3.833	0.215	0.223	0.219
2	3.784	3.642	0.187	0.191	0.189
3	3.601	3.47	0.183	0.172	0.1775
4	3.423	3.282	0.178	0.188	0.183
5	3.242	3.101	0.181	0.181	0.181
6	3.069	2.929	0.171	0.172	0.1715
7	2.901	2.761	0.165	0.168	0.1665
Avg(g/ 7d)			**0.1829**	**0.1850**	**0.1839**

Limonene lures (one- week field life)

D-Limonene lures (Fig. 12) were purchased from Alpha Scents Inc., West Linn, OR. The initial weight of the limonene lure was taken and recorded. Two lures were placed in a growth chamber set at a constant temperature of 30ºC, and their weight was taken after 24 hours for a period of 7-days to determine the amount of limonene released. Over seven days, an average of 0.033g of limonene was released per day from the lures.

Appendix Table 3. Lure released per 24 hours over 7 days in a growth chamber at a constant temperature of 30ºC

Days	**Lure 1 (g)**	**Lure 2 (g)**	**lure 1 released (g)**	**lure 2 released (g)**	**Avg. released (g)**
0	0.616	0.656	------	-------	-------
1	0.578	0.613	0.038	0.043	0.0405
2	0.543	0.581	0.035	0.032	0.0335
3	0.51	0.543	0.033	0.038	0.0355
4	0.482	0.506	0.028	0.037	0.0325
5	0.453	0.472	0.029	0.034	0.0315
6	0.427	0.447	0.026	0.025	0.0255
7	0.399	0.415	0.028	0.032	0.03
Avg(g/ 7d)			**0.0310**	**0.0344**	**0.0327**

Predalure (Methyl salicylate 30 days field life)

Predalure sachets were purchased from Agbio, West Minster, CO, USA. Two Predalure sachets were used in this experiment. The initial weight of Predalure sachets was taken and recorded. The sachets were placed in a growth chamber set at a constant temperature of 30ºC, and their weight was taken after 24 hours for a period of 7-days to determine the amount of methyl salicylate released. Over seven days, an average of 0.0358g of limonene was released per day from the lures.

Appendix Table 4. Lure released per 24 hours over 7 days in a growth chamber at a constant temperature of 30ºC

Days	**Lure 1 (g)**	**Lure 2 (g)**	**lure 1 released (g)**	**lure 2 released (g)**	**Avg. released (g)**
0	7.229	6.684	------	-------	-------
1	7.188	6.646	0.041	0.038	0.0395
2	7.149	6.606	0.039	0.04	0.0395
3	7.113	6.568	0.036	0.038	0.037
4	7.079	6.532	0.034	0.036	0.035
5	7.043	6.498	0.036	0.034	0.035
6	7.008	6.467	0.035	0.031	0.033
7	6.974	6.438	0.034	0.029	0.0315
Avg(g/ 7d)			**0.0364**	**0.035143**	**0.0358**

Appendix 2: Images

Figure 1. *Acanthococcus lagerstroemiae* on crapemyrtle tree.

Figure 2. Infested crapemyrtle tree covered with honeydew and sooty mold.

Figure 3. Honeydew exudates from CMBS.

Figure 4. *Harmonia axyridis* feeding on *Acanthococccus lagerstroemiae* on an infested twig under the microscope

Figure 5. Infested potted crapemyrtle plant with Predalure

Figure 6. Baiting an infested potted crapemyrtle plant with Predalure.

Figure 7. CMBS infested trees in a residential landscape.

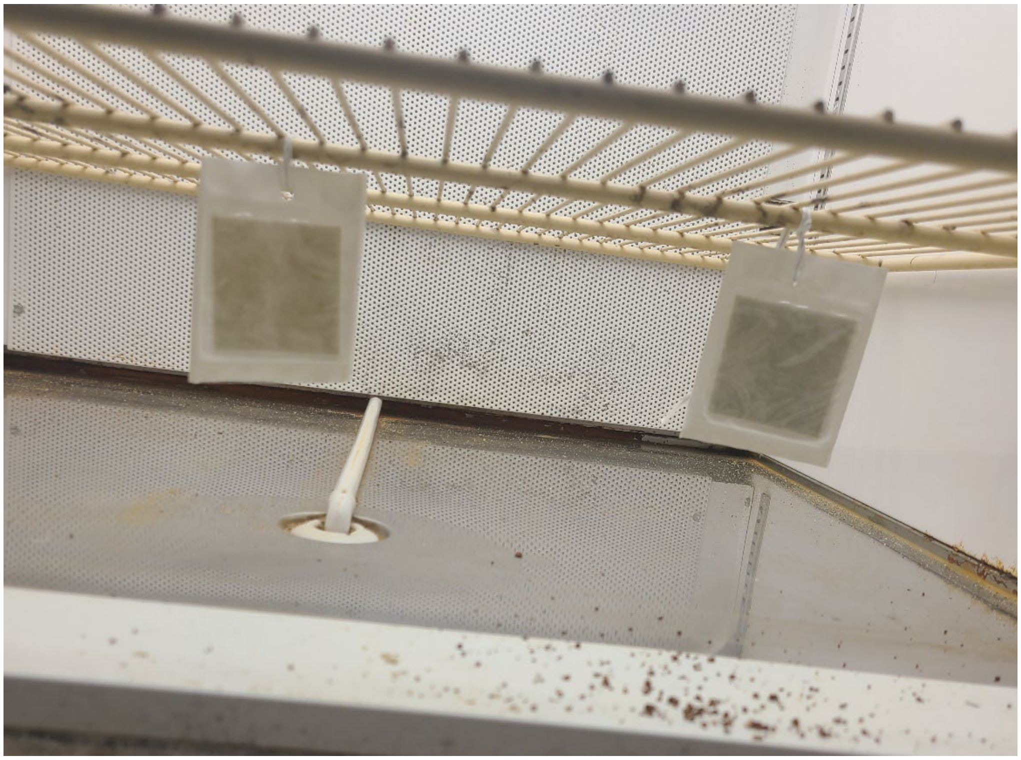

Figure 8. Predalure (30-days field life) in a growth chamber

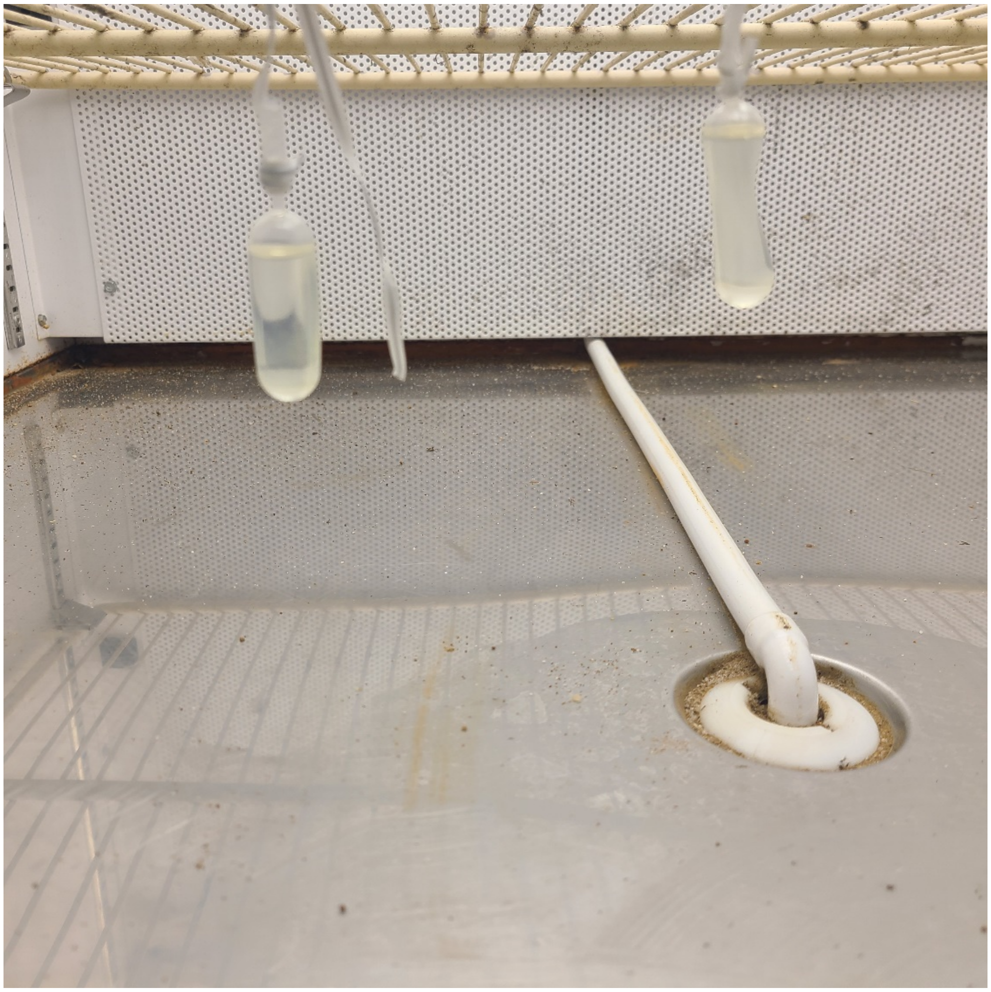

Figure 9. Limonene lures (One-month field life) in a growth chamber

Figure 10. Baiting a CMBS infested tree with an olfactory lure.

Figure 11. Lady beetles in Zipper-top bag

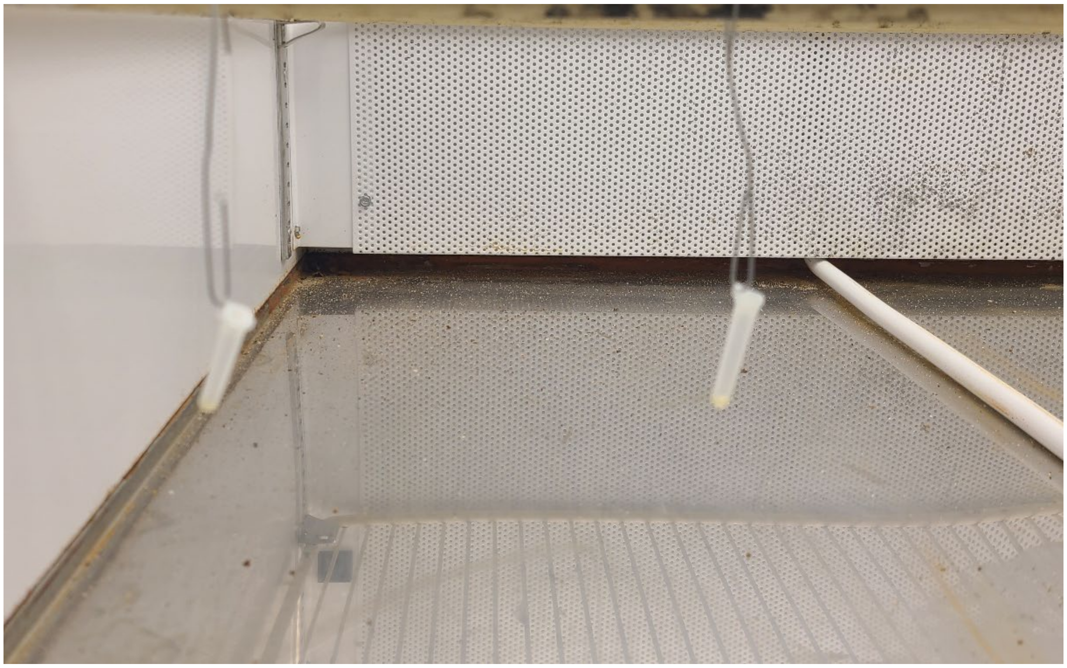

Figure 12. Limonene lures (One-week field life) in a growth chamber

Figure 13. Yellow pyramid traps attached to CMBS infested trees.

Figure 14. Yellow rectangular panels hung in the tree’s canopy.

Figure 15. Yellow rectangular panels attached at 1m above tree’s base.

Figure 16. Yellow pyramid trap baited with Predalure attached to a CMBS infested tree.

Figure 17. Branch-beating an infested tree.

Figure 19 CMBS reduction on twig