# Diversity & Flight Periods of Jewel Beetles (Coleoptera: Buprestidae) in Alabama: A Focus on the *Chrysobothris femorata* Species Complex and a Common Host, Red Maple (*Acer rubrum*)

by

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# Abstract

Flatheaded borers in the *Chrysobothris femorata* species complex are important pests of tree crops throughout North America. These taxonomically unresolved borers are common pests of hardwood trees and shrubs grown in nurseries. The larval stages grow and feed internally on their hosts, which leaves behind tunnels called galleries. Larval damage is difficult to manage, especially when it is virtually undetectable until adults emerge from their hosts. Furthermore, management would benefit from knowledge of specific visual and/or olfactory cues involved in host location and choice. Although borers belonging to the *C. femorata* complex are well established in Alabama, their geographical distributions and flight periods are not well documented. Additionally, there is virtually no information on how effective insecticides like imidacloprid work to protect hosts, as direct toxicity assays have not been conducted on larvae or adults. Because imidacloprid can positively impact plant growth and drought tolerance, it may be protecting hosts from *C. femorata* attacks indirectly. The first objective of this study addresses the distribution and phenology of species in Alabama. The flight activity was monitored using purple traps at three locations (Alpine, Auburn, Mobile) in 2021, 2022, and 2023. Trapping in 2021 yielded only 1 beetle from all sites. In 2022, we collected 8 species of *Chrysobothris* at traps statewide, most of which were recorded in Mobile from May to July. In 2023, 12 species of *Chrysobothris* were collected statewide, with most recorded in Auburn from April to July. The second objective of this study focuses on changes in host physiology in response to the effects of imidacloprid treatment and drought. Twenty four 5-gallon red maple (*Acer rubrum*) trees grouped into 6 replicates were assessed for physiological responses to four treatments (-Drought/-imidacloprid, +Drought/-imidacloprid, -Drought/+imidacloprid, +Drought/+imidacloprid) via nitrogen analysis, maple spider mite inoculation, and spectral reflectance tests. Overall, maple spider mite mortality and fecundity were not significantly impacted by treatments, however, survival of adult mites was significantly reduced at the end of mite trials in +Drought/-imidacloprid plants. Additionally, general mite populations seemed to follow a consistent trend, with significantly more eggs, immatures, and adults on trees treated with imidacloprid, regardless of drought.

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## List of Abbreviations

*C. femorata: Chrysobothris femorata*
*C. chrysoela: Chrysobothris chrysoela*
*C. shawnee: Chrysobothris shawnee*
*C. cribraria: Chrysobothris cribraria*
*C. viridiceps: Chrysobothris viridiceps*
*C. quadriimpressa: Chrysobothris quadriimpressa*
*C. scitula: Chrysobothris scitula*
*C. azurea: Chrysobothris azurea*
*C. rugosiceps: Chrysobothris rugosiceps*

# Chapter 1
# Introduction

## Nursery Production in the United States

In the United States, the nursery production of ornamental, fruit, and nut crops is a highly valuable industry employing hundreds of thousands of workers, as well as generating billions in profit each year (US Census of Agriculture, 2017; US Census of Horticultural Specialties, 2019). In the southeastern United States alone, Georgia, Florida, and North Carolina ranked within the top ten states responsible for 66 percent of the country's horticultural sales (US Census of Horticultural Specialties, 2019). Despite smaller scale production, Alabama boasts a highly productive nursery industry, with over 100 million in annual sales (US Census of Horticultural Specialties, 2019). When factoring in the production of greenhouse plants and sod, Alabama accounted for nearly five percent of the total market value of all sold farm products according to the 2017 US Agricultural census, totaling nearly 300 million in sales.

Though Alabama's agricultural industries rely heavily on manual labor, nurseries producing ornamental plants face special challenges involving insect pest control that can result in even greater labor needs. In order to fulfill demands for aesthetically pleasing, healthy looking plants, growers are often extremely selective about what products can be sold (Sadof & Sclar 2002). Because of this selectivity, even superficial and non-lethal pest damages can result in unmarketability. Despite large scale efforts to prevent aesthetic damages such as pest scouting and preventative insecticide treatments, damages from some insect pests continue to evade control in nurseries throughout the United States. Across the Southeastern United States, a few species of metallic wood boring beetles (family: Buprestidae) have been a recurring problem for the last 40 years in the production of nursery hardwood trees such as red maple (*Acer rubrum*) (Potter et al. 1988).

## Woodboring Pests in the United States

Woodboring life strategies are common across many insect groups, and can be observed in the bark, stems, roots, and new growth of woody plants (Solomon 1995). In North America, woodboring behavior can be found within the orders Coleoptera, Lepidoptera, Hymenoptera, and Diptera, with the majority belonging to Coleoptera (Solomon 1995). Because woodboring insects belonging to these orders are holometabolous, or have true larval stage(s), much of the boring behaviors we see today involve larval instars surviving within hosts . Evidence of arthropod woodboring behavior dates back to the late carboniferous period in the form of galleries within trees and bore holes in fern tissue (Chaloner et al. 1997). Among orders such as Coleoptera and Lepidoptera, woodboring behaviors originate from ancestral traits like the ones described above, but in orders like Diptera, woodboring behaviors are more derived and evolved independently of ancestral insect woodboring behavior (Tooker & Giron 2020). In order for these behaviors to persist and proliferate across different insect orders, it is believed that various selection pressures such as nutritional needs, inter/intraspecies competition, and natural enemies drove selection (Tooker & Giron 2020).

Within the order Coleoptera, hundreds of woodboring species are known, and many belong to economically important families. Well known families include

Cerambycidae (longhorned beetles), Curculionidae (weevils and ambrosia beetles), Bostrichidae (powderpost beetles), and Buprestidae (jewel beetles). Woodboring beetles in the family Buprestidae can be found worldwide, and belong to one of the largest families within the order Coleoptera, which contains approximately 15,500 known species belonging to 775 genera (Bellamy & Nelson 2002). In North America, nearly 170 species in 23 genera can be found (Paiero et al. 2012). In the United States, the vast majority of buprestid diversity is housed within the subfamilies Agrilinae and Buprestinae, including species considered agricultural pests (Paiero et al. 2012). One well-known pest species, the Emerald Ash Borer (*Agrilus planipennis*), is an invasive buprestid that has decimated ash tree (*Fraxinus*) populations across the Midwest after being found in Michigan and Ontario in 2002 (Haack et al. 2002). Native to northeastern Asian regions, it is believed to have been introduced in the mid to late 90's via infested shipping containers (Haack et al. 2002). Since its discovery, large scale trapping efforts intended to intercept and prevent range expansion of the Emerald Ash Borer have occurred, however, its range has expanded into 36 US states, including Alabama (aphis.USDA.gov).

## The *Chrysobothris femorata* Species Complex

In addition to invasive threats, the Flatheaded Appletree Borer (*Chrysobothris femorata*) is a buprestid species native to North America that is known to attack a number of hardwood trees, including red maple (*Acer rubrum* L.). The Flatheaded Appletree Borer poses specific threats to ornamental hardwoods due to their highly polyphagous diets and elusive flight behavior (Klingeman et al. 2015; Solomon 1995).

According to Potter et al. (1988), nurseries have reported up to 30 percent infestation rates of *C. femorata* for hardwood hosts such as *A. rubrum.* In a more recent study, *A. rubrum* cultivars not treated with any insecticides sustained up to 40 percent losses annually (Oliver et al. 2010). Among *A. rubrum* cultivars, Burgundy Belle and October Glory have been found to be most susceptible to *C. femorata* damages in a nursery setting (Seagraves et al. 2013).

Though *C. femorata* has been fairly well documented over the years in the United States, it belongs to a species complex that remains taxonomically unresolved (Wellso & Manley 2007). Collectively, these pest borers are known as the *Chrysobothris femorata* species complex – a group of approximately 12 individuals, including *C. femorata* (Wellso & Manley 2007). In the most recent review done by Wellso & Manley in 2007, six new species were described, including *C. shawnee*, and one species, *C. quadriimpressa*, is resurrected. Despite review, the number of species outlined in various resources might differ, as species like *C. shawnee* or *C. quadriimpressa* might be conserved under the name *C. femorata* or even remain unlisted (See: Paiero et al. 2012). These discrepancies highlight the challenges associated with identification to species level, as key characteristics can be cryptic and difficult to assess.

Adults belonging to the *C. femorata* complex are torpedo shaped and dorsoventrally flattened. They range from 5-20 mm in length, and are mostly mottled brown-gold in color. Like other buprestids, their heads are compressed anterolaterally, with large, kidney bean shaped eyes on either side. Their elytra are highly punctate and appear distinct, with characteristic ridges and irregular depressions that are often metallic. Beneath the elytra, the tergites are a bright metallic blue. Their limbs and body surfaces are also highly punctate, with smoother regions medially on the venter, generally around the coxae. Ventral body surfaces are also metallic, and often reflect bronze-maroon. Larval stages of species belonging to the *C. femorata* complex are much less distinct, and are not identifiable. Referred to as “flatheaded borers”, Their characteristic upper body region is enlarged and flattened, followed by a number of bulging body segments. These larvae are often yellow or cream in color, and have no limb-like structures.

Like other Buprestids, male genitalia is key for the identification of *Chrysobothris* species (Wellso & Manley 2007), since the shape and color is usually unique. In addition, characteristics such as the frons, clypeus, antennomere(s), elytral fovea, tibiae, and even hind coxa are useful for species identification. With female specimens, a heavy reliance on the above morphological characteristics, as well as the appearance of the pygidium, is necessary for proper species identification. For species belonging to the *C. femorata* complex, extra care must be taken during identification due to the overlap of various characteristics that make them appear extremely similar.

## Biology & Phenology

The *C. femorata* species complex is difficult to study due to their cryptic developmental behavior within their hosts. In general, female adult *Chrysobothris* species will lay eggs on the trunk surface of host trees in spring and summer months, usually in cracks and depressions in the bark surface. In general, borers within the *C. femorata* complex are attracted mainly to young trees that are undergoing some form of stress such as drought, sunscald, or pruning, which can make nursery stocks particularly vulnerable to attack (Frank et al. 2013; Potter et al. 1988). Once the eggs hatch, first instar larvae will immediately tunnel underneath the bark, where they form hollow spaces called galleries. Within these gallery spaces, buprestid larvae feed and develop until they are ready to emerge the following year (Frank et al. 2013). This feeding can cause damage to soft woody tissues and phloem, as well as compromise the structural integrity of young trees. Because first instar larval buprestids are invisible to the naked eye, growers with infested trees do not detect damages until adult borers emerge, leaving behind D-shaped exit holes. Because these beetles are univoltine, or take up to one year to complete their development (Burke 1919), damages can go undetected for over a year in nursery hosts. In a nursery setting, even one borer can result in a tree being unmarketable due to aesthetic and structural damages.

In addition to cryptic development, the phenology and behavior of the *C. femorata* complex is not comprehensively studied across the southeastern United States. In a study done by Potter et al. (1988), phenological information on *C. femorata* was conducted across three growing seasons, in which adult borers were reared out of infested trees and bolts originating from nurseries around the state of Kentucky. On average, *C. femorata* emergence began after a 412 C degree day accumulation between 1984-1986 (Potter et al. 1988). Additionally, though the calendar dates of first *C. femorata* emergences varied nearly a month between each year, the actual accumulated degree days varied at maximum 56 degree days, or around 2 days (Potter et al. 1988). A more recent publication on buprestid phenology focused on characterizing flight periods of five buprestid subfamilies in North Carolina and Tennessee, which included those in the *C. femorata* complex. By utilizing data from recent collections, as well as specimens from private museum collections, over 15,000 specimens collected between 1901 and 2013 were characterized across both states (Klingeman et al. 2015). Within the genus *Chrysobothris*, collections began around late February, however, degree day information is not provided (Klingeman et al. 2015). Within the *C. femorata* complex, it was noted that these species not only had overlapping phenological activity in each state, but they were also collected from the same counties in each state. This, in addition to the fact that a handful of *C. femorata* complex species have not been able to be distinguished as monophyletic using genetic testing (Hansen et al., 2015), supports the idea that species within the *C. femorata* complex might be interbreeding (Frank et al. 2013; Potter et al. 1988).

In Alabama, little information is available regarding the spatial distribution and phenological activity of the *C. femorata* complex state-wide. Records of *Chrysobothris* species date back to the early 1900's, with the earliest recorded specimen in the Auburn area from April 1909 (Auburn museum collection, unpublished), but these records are fairly outdated, and contain specimen labels with missing date/location information, as well as species identities that are no longer recognized or accurate. Additionally, while the published resources from other southeastern states can be useful for Alabama nursery growers to get an idea of when to expect *C. femorata* activity periods. These resources lack regional specificity and may not be reliable for monitoring borers in Alabama. This lack of available data highlights the need to recharacterize modern species distribution and phenology of the *C. femorata* complex across Alabama through trapping.

### Monitoring *C. femorata* Populations

In addition to labor and expenses, challenges with simple monitoring of *C. femorata* species complex populations makes it difficult to make informed treatment or prevention decisions. Unlike other woodboring beetles such as ambrosia beetles, that are easily attracted using a simple ethanol lure (Oliver & Mannion 2001; Reding et al. 2010), borers in the *C. femorata* species complex are difficult to collect consistently. Attraction of the *C. femorata* species complex is thought to be driven by a combination of visual and olfactory cues, but only a handful of attractants have been extensively studied (Braman et al. 2003; Perkovich et al. 2023). Visually, borers in the *C. femorata* species complex have been found to be most attracted to insect traps that are purple (~400 nm) (Perkovich et al. 2022), which might be due to wavelengths emitted from hosts, or even reflected off of other borers. In addition, traps shaped more similarly to tree trunks were more successful at collecting individuals in the *C. femorata* species complex than simple flat panels (Perkovich et al. 2023), indicating that these borers may be discerning shapes that mimic potential host trees. Though the potential olfactory cues that attract borers in the *C. femorata* species complex remain unclear, higher attack rates on stressed trees may be explained by attraction to volatiles released by trees undergoing stresses. There is limited evidence that herbicides like glyphosate and scythe can increase attacks on trees that have had surrounding weeds treated, however it is unknown if volatiles from weed senescence or specific active ingredients might be involved (Dawadi et al. 2019).

## Methods of Control

Though insecticide treatments are currently the standard for the prevention of attacks from the *C. femorata* complex, developing alternative methods of control that do not rely on chemical application are important for developing more comprehensive, IPM-friendly strategies in nurseries. Currently, no alternative strategies have been widely adopted, but there is evidence supporting the use of cover crops and physical barriers to prevent *C. femorata* attacks. In work done by Dawadi et al. (2019), a winter cover crop consisting of crimson clover and winter wheat grown around the base of host trees resulted in less than 2% tree loss, compared to the 23% tree loss observed in non-cover-cropped hosts over the course of the study. Since borers in the *C. femorata* species complex tend to oviposit on the bottom few feet of tree trunks, Dawadi et al. (2019) hypothesized that a grassy cover crop actually obscures potential oviposition sites, or alters microclimate conditions preferred by *C. femorata* species at the oviposition site. Though this kind of non-insecticide control method was successful here, it was noted that trees grown alongside a grassy cover crop actually grew slower and were smaller overall than the same non cover-cropped trees (Dawadi et al. 2019), which is undesirable for growers and nurseries. Additionally, applying and maintaining a cover crop alongside trees likely requires more labor than a single neonicotinoid insecticide that can work for multiple years. Similar experimental control methods involving physical barriers like trunk guards and fencing are also not currently utilized in large production centers, since labor costs and implementation times are of primary concern.

Because of their endophagous larvae and the difficulty in assessing when a tree is infested, pest control methods for the *C. femorata* species complex rely heavily on preventative insecticide treatments. Neonicotinoid insecticides like imidacloprid (4A) are generally regarded as the most effective method of control, and have even been shown to provide protection from *C. femorata* attacks up to 4 years post-treatment in some cultivars of red maple (Oliver et al. 2010). Despite their effectiveness, neonicotinoid applications must be done prior to the first flight *C. femorata* complex adults (Dawadi et al. 2019; Oliver et al. 2010), which is difficult to estimate correctly from year to year in Alabama in absence of reliable phenological records. Additionally, direct effects of popular systemic treatments like imidacloprid on the *C. femorata* species complex have not been studied on living larvae or adults, making it unclear how these treatments work. Since species in the *C. femorata* complex develop towards the more superficial phloem tissues, larvae already present within hosts prior to treatment will likely not come in contact with large amounts of imidacloprid residues, as is translocated primarily through the xylem (Mota-Sanchez et al. 2009; Sur & Stork 2003; Tanis et al. 2012). *C. femorata* adults are also unlikely to come in direct contact with drench residues due to infrequency and location of treatments. This suggests that there may be some indirect effects of imidacloprid drenches on hosts that alter physical or chemical signature(s) used by *C. femorata* adults to locate and/or choose hosts.

**Potential Impacts of Imidacloprid on Host Trees**

Though indirect effects of imidacloprid drenches may impact host choice by somehow altering host trees, these changes are not currently characterized. In row crops, it is well documented that imidacloprid treatments can both increase plant vigor, as well as increase the tolerance of drought (Oosterhuis et al. 2003; Thielert 2006). Though less documented in woody plants, work done by Chiriboga (2009) found that hybrid poplars reacted similarly to imidacloprid treatments, displaying improved plant vigor regardless of fertilization rate or watering rates. Since borers in the *C. femorata* complex are known to be highly attracted to trees experiencing stress, treatments like imidacloprid that might mitigate drought stress may reduce the attractiveness of tree hosts.

In addition to effects such as stress tolerance, imidacloprid drenches have been shown to alter the chemical physiologies of treated plants, resulting in secondary pest outbreaks. Szczepaniec et al. (2013) noted that imidacloprid applied to cotton, corn, and tomato plants resulted in an alteration of induced plant defenses, resulting in outbreaks of two-spotted spider mites (*Tetranychus urticae*). Across all plants, neonicotinoid treatments resulted in altered expression of genes regulated by jasmonic acid (JA) and salicylic acid (SA), resulting in the suppression of certain defense chemicals. In red maple, it is unknown whether imidacloprid alters defense chemicals, however, evaluating secondary pest outbreaks could indicate possible physiological changes within treated trees. If imidacloprid drenches do impact red maple chemical physiology, outbreaks of introduced spider mite populations may occur due to increased plant nutrition and/or decreased plant defenses. This process is known as hormesis (Calabrese & Baldwin 2002).

**Project Objectives**

The major goals of this project are to characterize the distribution and phenology of the *Chrysobothris femorata* species complex across Alabama, as well as investigate how factors such as drought and imidacloprid application alter the suitability of hosts for the *Chrysobothris femorata* species complex.

**Chapter 2:** Characterize the distribution and phenology of the *Chrysobothris femorata* species complex across Alabama.

(2a) Collect and identify members of the *C. femorata* species complex across Alabama.

(2b) Determine phenological patterns of the C*. femorata* species complex across Alabama.

(2c) Investigate a new visual cues as potential attractants for flatheaded borers.

(2d) Use trap trees to correlate traps captures with tree attacks.

**Chapter 3:** Investigate potential effects of the neonicotinoid insecticide imidacloprid on red maple host trees that may act as indirect control against the *Chrysobothris femorata* species complex.

(3a) Confirm imidacloprid translocation through host tissues.

(3b) Determine potential impacts of drought stress & imidacloprid treatment on host physiological changes via spider mite infestation.

# Chapter 2

## Distribution and Phenology of the *C. femorata* Species Complex in Alabama

### Introduction

The *Chrysobothris femorata* species complex is a group of buprestid beetle pests native to the United States known for causing damages to ornamental trees in both nursery and landscape settings. This species complex is taxonomically unresolved, and approximately 12 species can be found across the United States, with active periods from early spring to mid-summer (Paiero et al. 2012; Wellso & Manley 2007). Larval stages develop within hosts underneath the bark, making it difficult to confirm infestations until adult emergence (Burke 1919; Frank et al. 2013). Phenological records of adult emergences and activity periods is key for timing treatments and preventing repeat attacks in nursery or landscape settings.

In other southeastern states such as Tennessee and North Carolina, extensively published species records are available alongside information regarding phenological behaviors (Klingeman et al. 2015). In Kentucky, species records alongside actual degree day models (Potter et al. 1988) provide clear and detailed information about which species belonging to the *C. femorata* species complex can be found in certain locations at specific times. In contrast, state-wide records of the *C. femorata* species complex in Alabama are inconsistent, and knowledge of which species can be found in different regions is limited. Museum records in Alabama contain a handful of collections made over the last 100 years, but many contain outdated, inaccurate or incomplete information. This lack of available data on the diversity of species and flight periods further complicates management. This highlights the need to recharacterize modern species distribution and phenology of the *C. femorata* species complex across Alabama through trapping.

In addition to basic trapping, investigating potential visual and olfactory cues used by *C. femorata* adults to locate hosts can provide valuable information about which kinds of hosts are most attractive to any particular species. Visually, borers in the *C. femorata* species complex have been found to be most attracted to insect traps that are purple (~400 nm) (Perkovich et al. 2022), which might be due to wavelengths emitted from hosts, or even reflected off of other borers. In addition, traps shaped more similarly to tree trunks were more successful at collecting individuals in the *C. femorata* species complex than simple flat panels (Perkovich et al. 2023), indicating that these borers may be discerning shapes that mimic potential host trees. These types of cues are poorly understood within this species complex, and information that is known is based off of records of few species that are commonly collected. Determining whether these kinds of preferences exist within this species complex could impact how we manage and control for *C. femorata* damages. Here, I discuss the manipulation of the trap type in order to determine whether individuals belonging to the *C. femorata* species complex prefer traps that look more like hosts, and whether a trap corresponding to a specific caliper commonly found in nurseries is preferred over others.

While useful, use of trapping to collect data is often non-specific and only provides approximate windows of activity. Apart from rearing species from infested trees, there is limited information on particular host associations relative to seasonal flight periods or abundance in traps.

## Materials & Methods

### ***Seasonal phenology and species recruited to traps***

Trapping was conducted from 2021 – 2023 in Alabama at three locations representing different regions or habitats. These were a commercial tree nursery in Alpine AL (33.38258°N, 86.34145°W), a suburban wooded area and powerline cut at the Kreher Preserve and Nature Center in Auburn, AL (32.66240°N, 85.48704°W), and the Ornamental Horticulture Research Station (30.70245°N, 88.14770°W) which is adjacent to a large park and botanical gardens in Mobile, AL. Traps were fabricated from purple plastic panels coated with glue (Pestick™, Gempler's, Janesville, WI) on each side using a putty knife.

In 2021, single panels were attached to the ends of 1.52 m pieces of 0.95 cm rebar using zip ties, and coated with pestick™ once in place. Ten traps were placed at each site, roughly 1.2 m above the ground and 5 m apart. Traps were assessed weekly from 22 February to 23 August. Traps were cleaned using a putty knife and glue was re-applied every couple of weeks, or when bycatch covered 50-75% of the trap surface. In the field, buprestid adults were collected from panels using soft forceps and placed in vials filled with histoclear (VWR, Radnor, PA) for transport to the lab. In the lab, samples were cleaned and stored in 100% ethanol before being identified using Paiero et al.

(2012), as well as online key resources. Date collected, location, and trap number was recorded for each sample.

In 2022, purple prism traps (Braman et al. 2003; Perkovich et al. 2023) were used which provided more surface area than a single panel trap. Single panels had a surface area of 929.03 $cm^2$, and prism traps had a surface area of 2787.10 $cm^2$. Three of the panels used for trapping in 2021 were affixed at the edges with zip ties to form Prism shaped traps. Prism traps were attached to rebar using zip ties, and coated with pestick™ on all three outward facing sides. Ten traps were placed at each site, roughly 1.2 m above the ground and 5 m apart. Traps were assessed weekly from 24 February to 27 October, and were cleaned and glue re-applied using the same protocol as in 2021. Buprestid adults were collected and processed as in 2021 and date, locality and trap number were recorded for each sample.

In 2023, panel or prism traps were replaced with purple pole traps (see next section). Ten pole traps were placed at each site, and coated with pestick™ by spreading it along the surface from top to bottom wearing nitrile gloves. Pole traps were fitted over 1.52 m pieces of 2.54 cm rebar, spaced 5 m apart. Traps were assessed from 23 February to 30 October, and cleaned and glue re-applied as needed. To clean the poles, a tourniquet of thick twisted mason line (Ace Hardware, Auburn, AL) was slid from top to bottom, removing the glue, which was then discarded. Buprestid adults were collected and processed as in 2021 and date, locality and trap number were recorded for each sample. Because pole trap surface areas differ from panel trap surface areas, no direct comparisons are made between trap types.

### *Assessing diameter of pole traps as a visual cue*

In spring 2022, traps were made from 36, 1.52 m pieces of PVC pipe ranging in diameter from 1.27 cm, 1.91 cm, 2.54 cm, 3.81 cm, 5.08 cm, 7.62 cm diameters. Surface areas of pole traps ranged from 608.98 cm to 3729.93 cm. To use the same purple color as the panels, a panel was taken to a local paint store where a custom purple paint mix was made ("Pretty Purple Primer": N448-4x-1 gallon; s1-0x-0, w1-0x-0, m1-6x-12, b1-2x-8; "Pretty Purple Paint": N448-4x-1 gallon; s1-0x-0, w1-1x-16, m1-6x-12, b1-2x-8). Poles were painted with one coat of primer and two coats of purple paint at least two weeks before use.

In the field at the Mobile, AL site, Traps were grouped into six clusters made of rows of three, with one of each size pole randomly placed per cluster, and coated with pestick using nitrile gloves. Traps were assessed every other week from 2 June to 25 August, and cleaned as previously described. Buprestid adults were collected and processed as previously described. Date, location, replicate, and trap diameter were recorded for each sample.

### *Evaluation of trap trees to connect activity with tree attacks*

In 2023, an experiment was conducted to expose a susceptible host tree to attack within an array of pole traps. In 2023, red maple trees (*Acer rubrum*, October Glory), roughly 4 m tall in 15 gallon pots were obtained from a commercial nursery then transported to Ornamental Horticulture Research Station. To increase attractiveness of trees to *C. femorata* attacks, a 1 m$^2$ existing vegetation ground was sprayed with Scythe herbicide (57% Pelargonic acid, 3% related fatty acids, Dow AgroSciences, Indianapolis, IN). A single potted tree was placed in the center of the square and maintained using drip irrigation. Every 2 weeks from 23 March to 4 October, a tree was placed on the herbicide-treated square in between the set of 10 pole traps. After exposure, the trees were moved to a protected location, the pots labeled with the exposure period, and maintained on drip irrigation. On 4 October, all trees were clipped at the base of the trunk using large shears, the branches were removed, then the trunk was further cut into 1 m sections. In the lab, those sections were placed into rearing boxes labeled with the exposure dates and held in a non-climate controlled store room. Boxes were checked weekly for emergence of buprestid adults from January to June 2024 (the expected emergence period).

### *Statistical Analyses*

All statistical analyses were conducted in R, and figures were created using ggplot2 and Microsoft Excel. One- and three-way ANOVAs were conducted on the pole trap data (Figure 7).

## Results

### *Seasonal phenology and species recruited to traps*

Trapping from 2021 yielded only one specimen from the Mobile site. In 2022, a total of 8 species totaling 79 specimens were found across all sites, with a majority of species found at the Mobile site (Table 1). In 2023, a total of 12 species totaling 344 specimens were found across all sites, with a majority found at the Auburn site (Table 2).

In both years, collections of some species spanned multiple sites, whereas other species were found only at one location (Tables 1 & 2).

**Table 1:** All *Chrysobothris* species collected across Alabama in 2022.

| **Alpine, AL: Commercial Nursery** | **Total Collected** |
|---|---|
| *Chrysobothris adelpha* | 1 |
| *Chrysobothris azurea* * | 1 |
| *Chrysobothris caddo* (2022) | 1 |
| *Chrysobothris femorata* | 2 |
| *Chrysobothris quadriimpressa* Δ | 1 |
| *Chrysobothris shawnee* Δ | 2 |
| **Total** | **8** |
| **Auburn, AL: Kreher Preserve** | **Total Collected** |
| *Chrysobothris quadriimpressa* Δ | 1 |
| *Chrysobothris shawnee* Δ ~ | 2 |
| **Total** | **3** |
| **Mobile, AL: Ornamental Hort. Research Center** | **Total Collected** |
| *Chrysobothris adelpha* | 4 |
| *Chrysobothris caddo* (2022) | 1 |
| *Chrysobothris chrysoela* * | 14 |
| *Chrysobothris cribraria* * | 8 |
| *Chrysobothris femorata* | 1 |
| *Chrysobothris quadriimpressa* Δ | 9 |
| *Chrysobothris shawnee* Δ ~ | 31 |
| **Total** | **68** |

** denotes a species found at only one site, Δ denotes a species found at all sites, ~ denotes the species most commonly collected at each site.* (2022) *denotes a species found only in 2022.*

**Table 2:** All *Chrysobothris* species collected across Alabama in 2023.

| **Alpine, AL: Commercial Nursery** | **Total Collected** |
|---|---|
| *Chrysobothris azurea* | 1 |
| *Chrysobothris cribraria Δ* | 20 |
| *Chrysobothris femorata Δ* | 5 |
| *Chrysobothris harrisi ** (2023) | 1 |
| *Chrysobothris quadriimpressa Δ~* | 33 |
| *Chrysobothris scitula ** (2023) | 2 |
| *Chrysobothris shawnee Δ* | 20 |
| *Chrysobothris viridiceps* (2023) | 1 |
| **Total** | **83** |
| **Auburn, AL: Kreher Preserve** | **Total Collected** |
| *Chrysobothris adelpha ** | 2 |
| *Chrysobothris azurea* | 5 |
| *Chrysobothris cribraria Δ~* | 141 |
| *Chrysobothris femorata Δ* | 6 |
| *Chrysobothris quadriimpressa Δ* | 51 |
| *Chrysobothris rugosiceps ** (2023) | 5 |
| *Chrysobothris sexsignata ** (2023) | 1 |
| *Chrysobothris shawnee Δ* | 20 |
| *Chrysobothris viridiceps* (2023) | 1 |
| **Total** | **232** |
| **Mobile, AL: Ornamental Hort. Research Center** | **Total Collected** |
| *Chrysobothris chrysoela ** | 7 |
| *Chrysobothris cribraria Δ* | 5 |
| *Chrysobothris femorata Δ* | 2 |
| *Chrysobothris quadriimpressa Δ* | 7 |
| *Chrysobothris shawnee Δ~* | 8 |
| **Total** | **29** |

** denotes a species found at only one site, Δ denotes a species found at all sites, ~ denotes the species most commonly collected at each site.* (2023) *denotes a species found only in 2023.*

In 2022, collections spanned from April to September, with peaks from May to July (Figure 1D). At the Alpine, AL location, species were found between April and June, with a small peak in June (Figure 1A). At the Auburn, AL location, few individuals were collected in April and June (Figure 1B). At the Mobile, AL location, Species were collected from April to August, with peaks in May and July (Figure 1C).

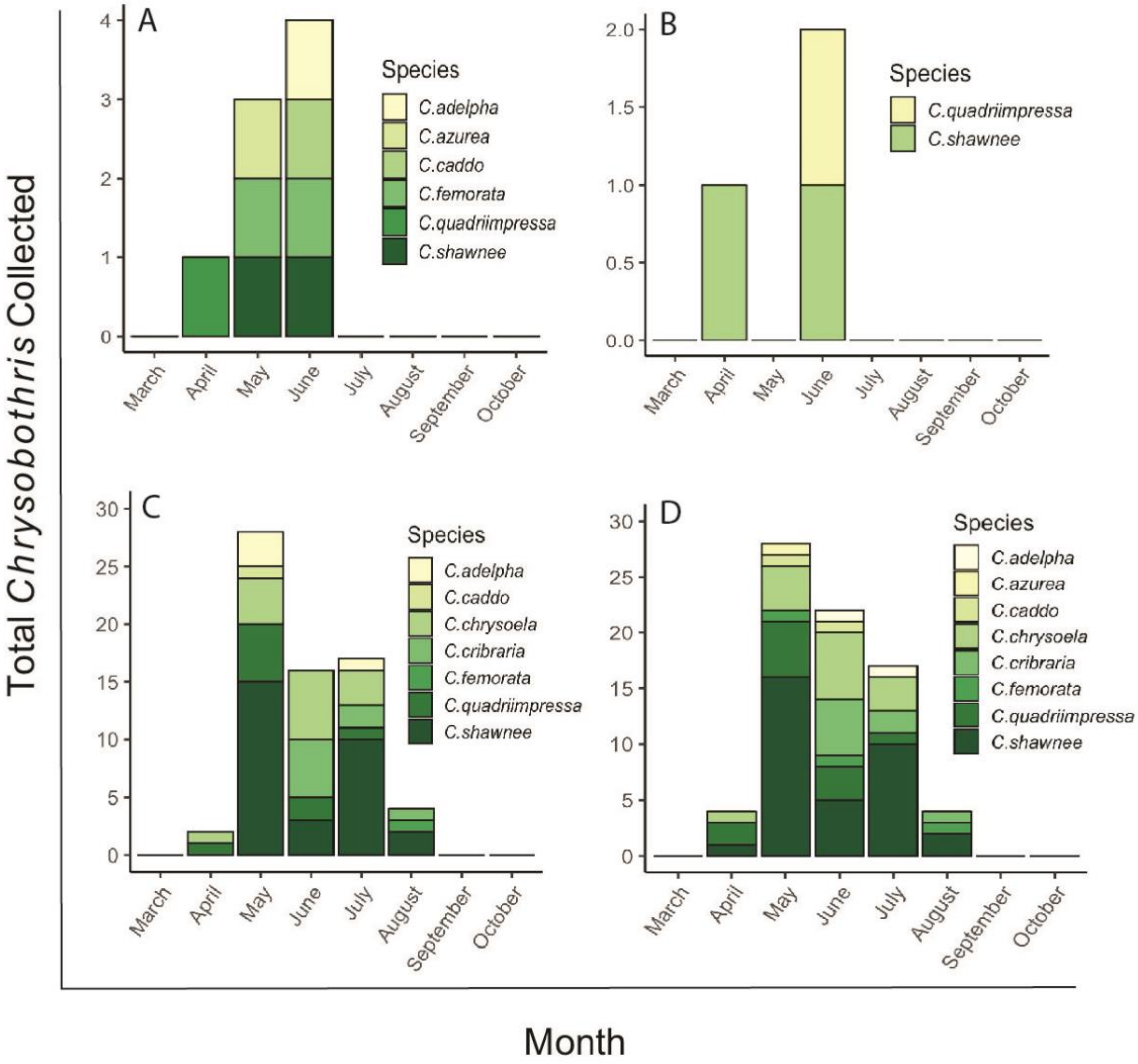

**Figure 1:** Seasonality of adult *Chrysobothris* spp. collections on traps during 2022 (March to October) at three locations: (A) Alpine, AL at a commercial nursery (B) Auburn, AL at the Kreher Preserve and Nature Center (C) Mobile, AL at the Ornamental Horticultural Research Station (D) Combined collections across all sites.

In 2023, collections spanned from April to October, with peaks from April to June (Figure 2D). At the Alpine, AL location, species were found between April and August, with peaks in May and June (Figure 2A). At the Auburn, AL location, species were found between April and October, with a large peak in April and zero collections in July (Figure 2B). At the Mobile, AL location, species were found between April and August, with peaks between May and July (Figure 2C).

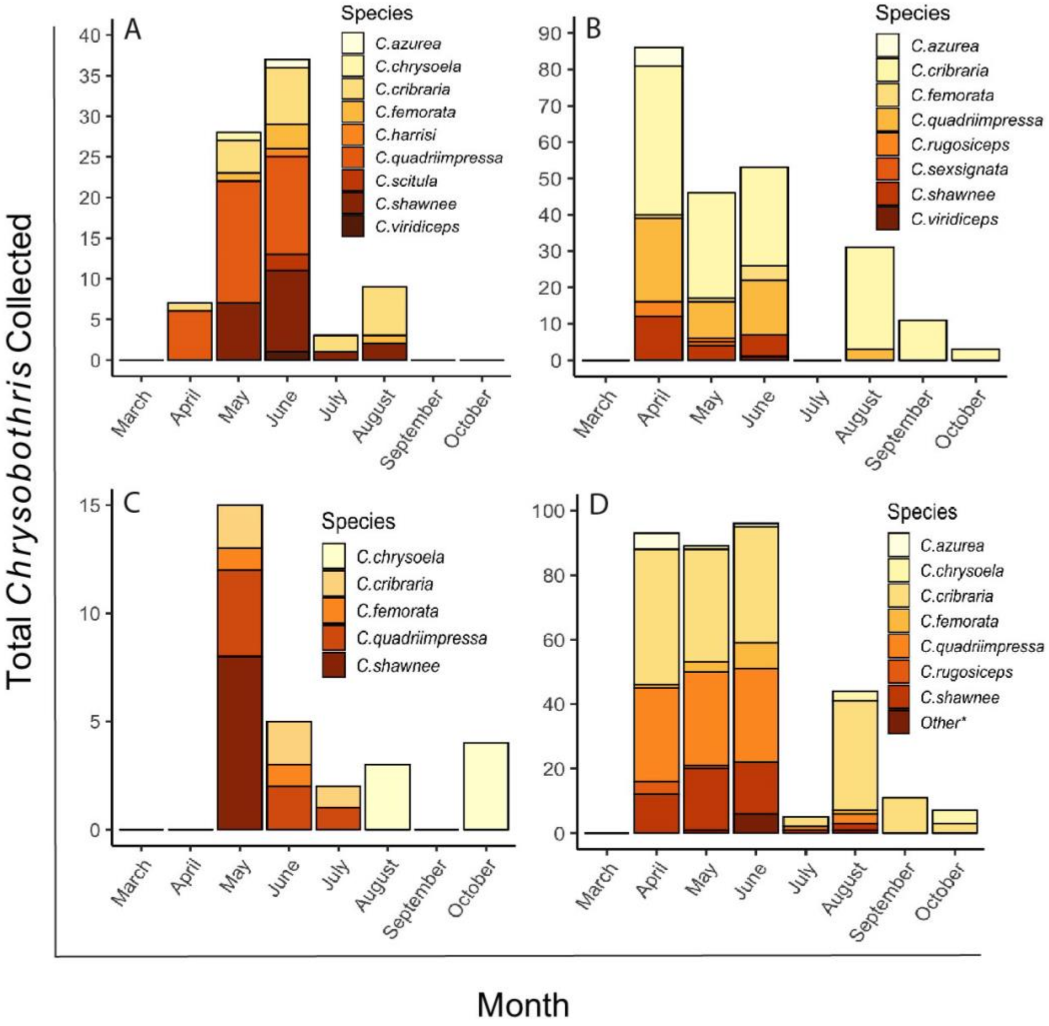

**Figure 2:** Seasonality of adult *Chrysobothris* spp. collections on traps during 2023 (March to October) at three locations: (A) Alpine, AL at a commercial nursery (B) Auburn, AL at the Kreher Preserve and Nature Center (C) Mobile, AL at the Ornamental Horticultural Research Station (D) Combined collections across all sites.

In 2022, *C. shawnee* and *C. chrysoela* were collected most frequently in Mobile, with active periods ranging from late April/early May to early August (Figure 3). *C. shawnee* was collected more frequently than *C. chrysoela*. Overall, collections of *C. shawnee* were female biased, with males and females emerging within similar windows (Figure 4).

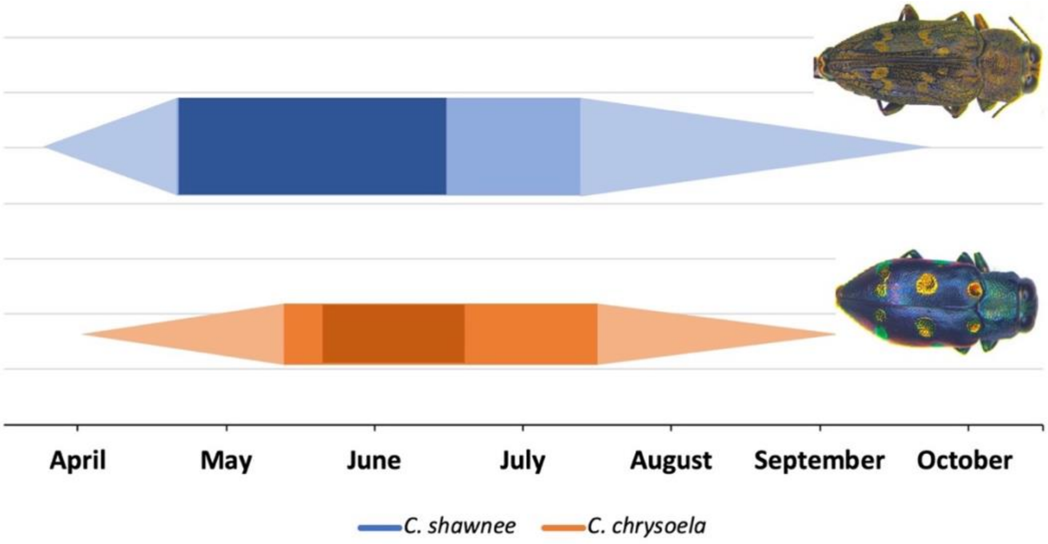

**Figure 3:** Observed flight periods for the most commonly collected species in Mobile, AL in 2022.

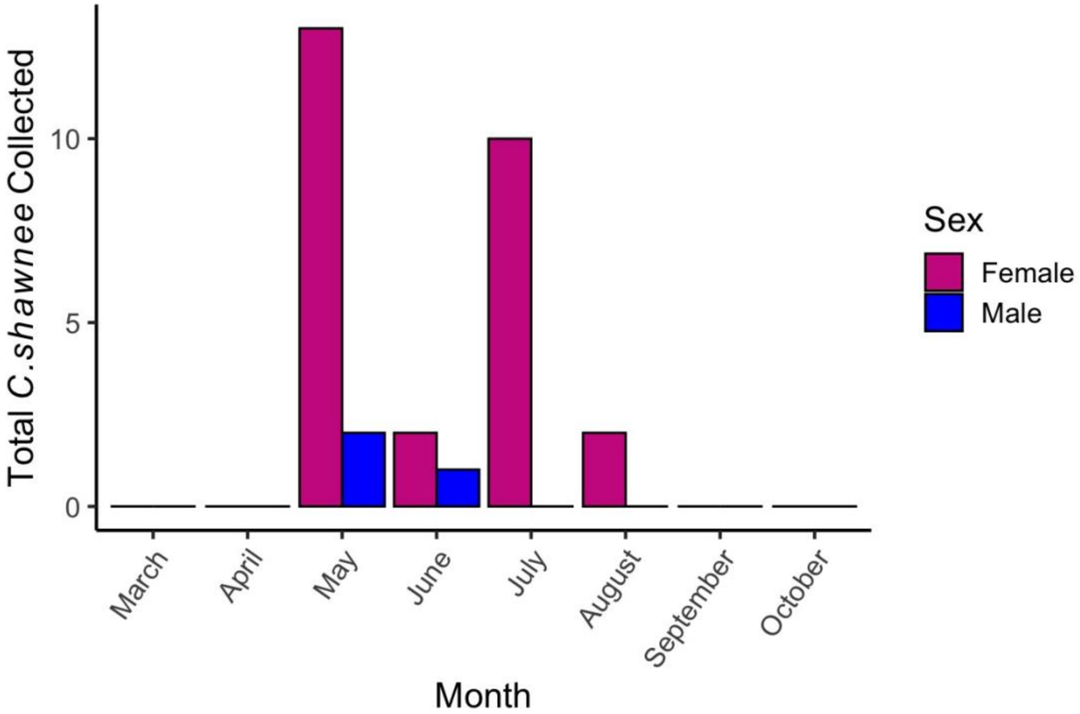

**Figure 4:** Total number of *C. shawnee* males and females collected monthly in Mobile in 2022.

In 2023, *C. shawnee, C. cribraria, and C. quadriimpressa* were collected most frequently in Auburn, with active periods ranging from early/mid-April to late September/October (Figure 5). *C. cribraria* and *C. quadriimpressa* were collected more frequently than *C. shawnee*. Overall, collections of *C. cribraria* were slightly male biased (Figure 6A), whereas collections of *C. quadriimpressa* were female biased (Figure 6B), with males and females emerging within similar windows (Figure 6). The activity periods of *C. shawnee* differed between years and across sites (Figures 3 & 5).

Across both collection years, more females were collected than males. In 2022, 57 females were collected, and 22 males were collected overall. In 2023, 195 females were collected, and 150 males were collected overall.

25

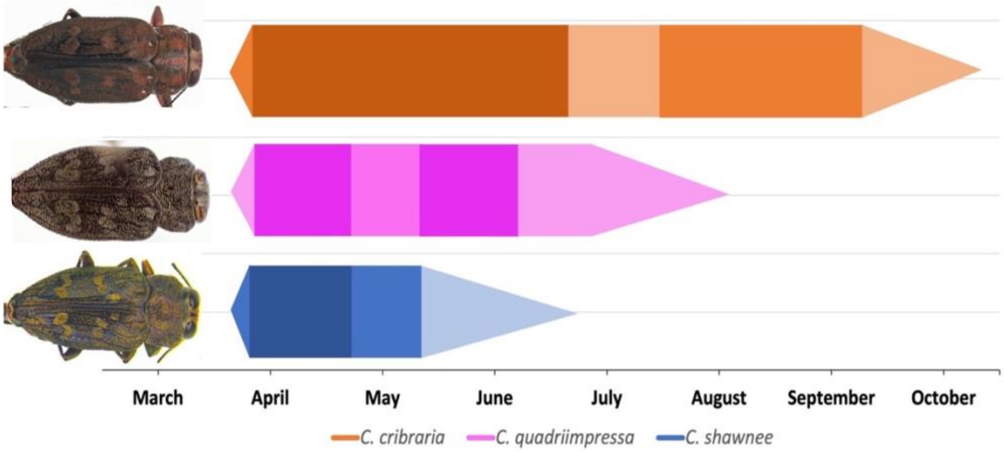

**Figure 5:** Observed flight periods for the most commonly collected species in Auburn, AL in 2023.

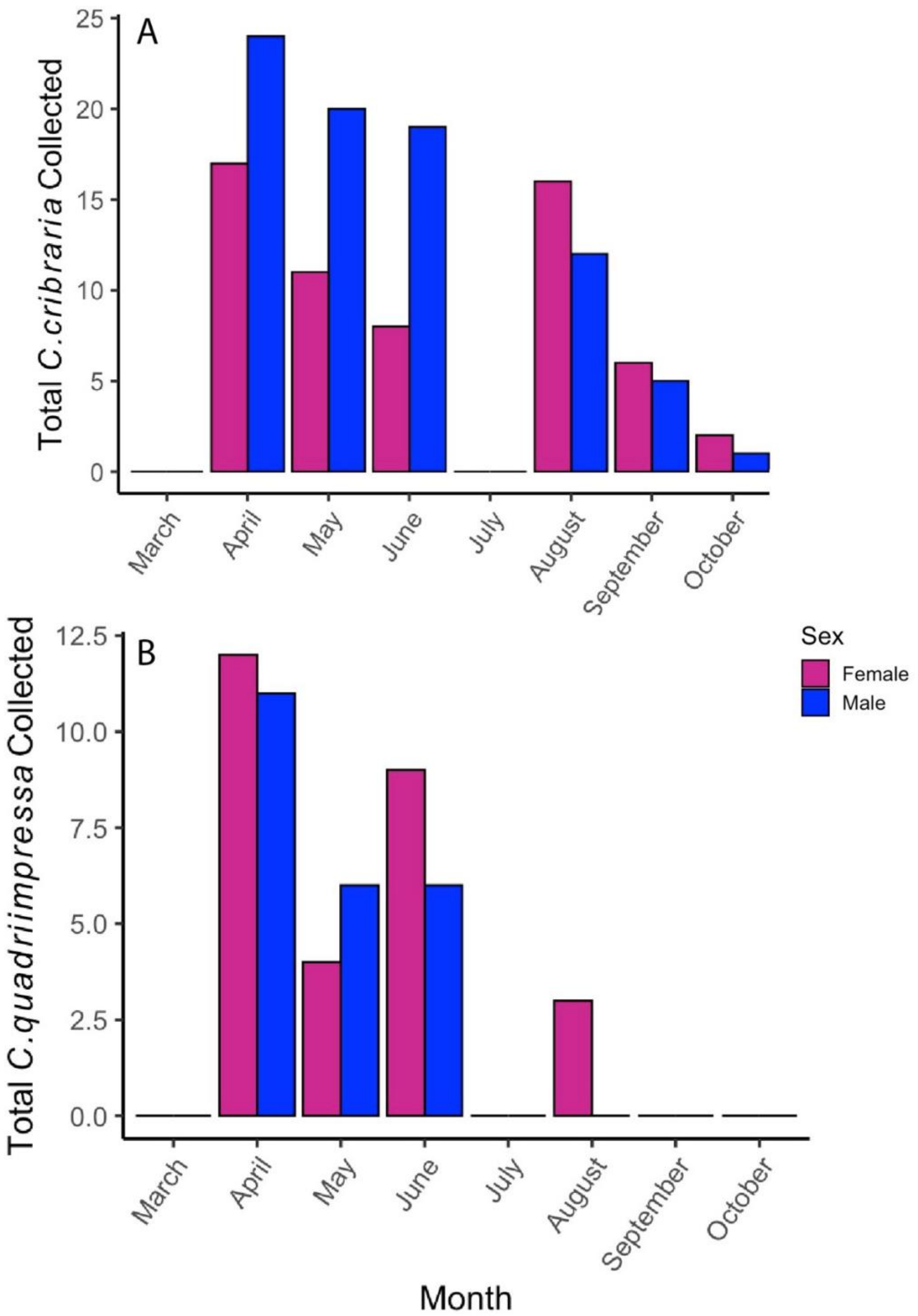

**Figure 6**: Total number of (A) *C. cribraria* males and females and (B) *C. quadriimpressa* males and females collected monthly in auburn in 2023.

### *Assessing diameter of pole traps as a visual cue*

In 2022, 38 total specimens were collected from pole traps in Mobile. Significantly more females than males were collected from pole traps ($p<0.005$, DF=40),(±95% CL; ±0.4296), with 28 of the 38 collected specimens being female. A one-way ANOVA found no significant differences in the total collections from the six different pole trap sizes ($p>0.05$) (Figure 7). A three-way ANOVA (month x species x trap size) found no significant differences in total collections, and found no significant trends based on month or species collected ($p>0.05$).

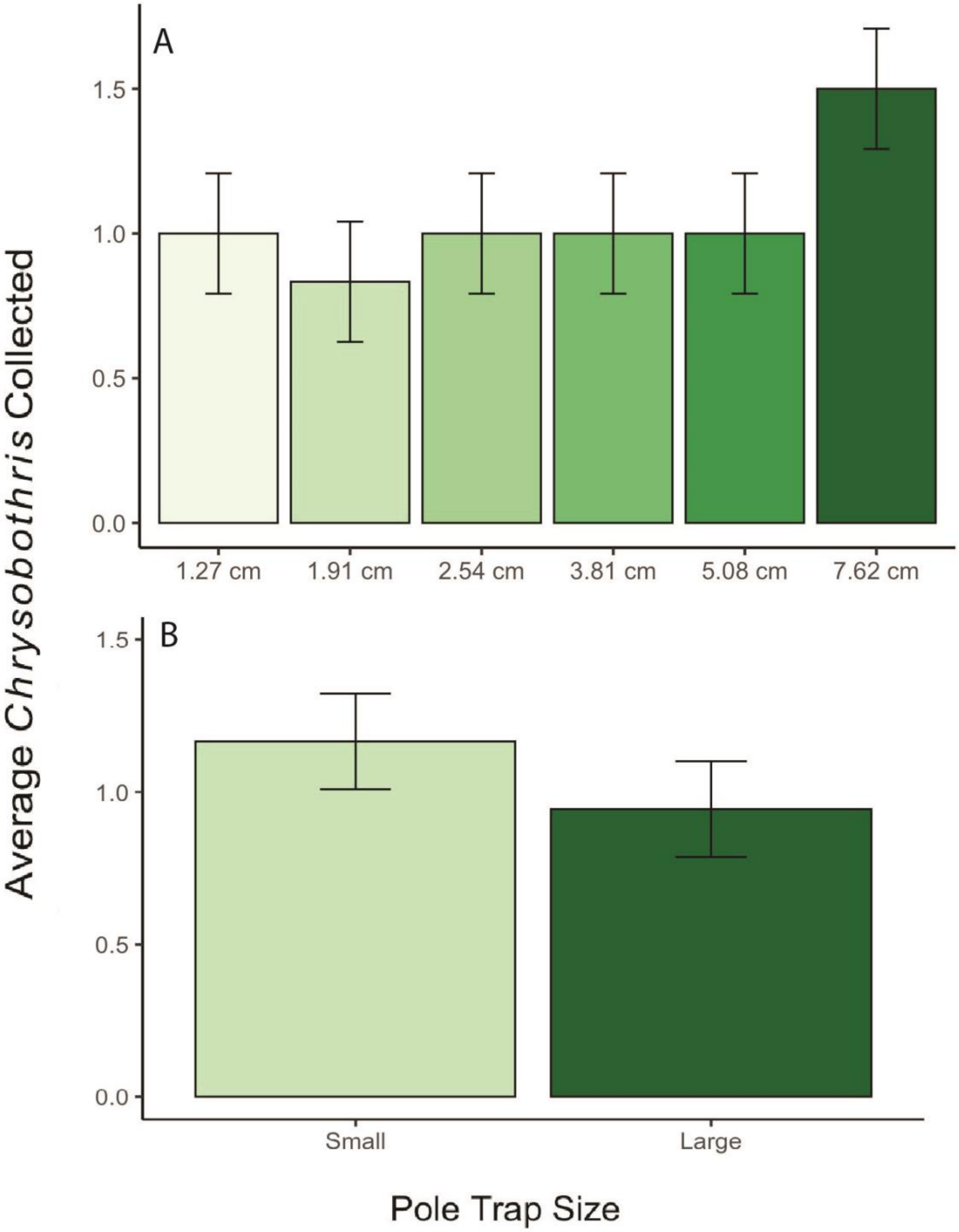

**Figure 7:** Average collected *Chrysobothris* from (A) all pole trap sizes in Mobile between June and August of 2022 (B) pole trap sizes designated as “small” (1.27 cm – 2.54 cm) and “large” (3.81 cm – 7.62 cm).

## Discussion

The *Chrysobothris femorata* species complex is poorly documented in Alabama, and these data represent the first known multi-year survey of *Chrysobothris* species across the state. Between 2021 and 2023, the total number of collections made across the state varied widely, from just one beetle collected in 2021 to over 300 individuals collected in 2023. Additionally, the site responsible for the majority of collections in 2022 and 2023 differed. In 2022, Mobile, AL produced the largest number of collections, whereas Auburn, AL produced the least. In 2023, this pattern reversed, with Auburn producing the largest number of collections and Mobile producing the least.

Little information is known about what might cause such inconsistent yearly patterns of emergence, however, biological and/or environmental factors likely have a large impact on the phenology of the *Chrysobothris femorata* species complex in Alabama. Like other buprestid beetles, individuals belonging to the *C. femorata* species complex are considered to be univoltine, however, evidence for 'semivoltinism', or developmental cycles lasting longer than one year, has been observed in some *Chrysobothris* species found in the United States (Burke 1919). This type of voltinism could result in staggered emergences that are larger in some years than in others. This may also result in more concentrated emergences in different locations from one year to the next.

In addition to voltinism, abiotic environmental factors such as temperature and precipitation likely impact the phenological patterns of *Chrysobothris* species from year to year. In Mobile, average temperatures were higher in 2022 than in 2021 (AWIS, unpublished), and average precipitation was lower in 2022 than in 2021 (AWIS, unpublished), which may have impacted the emergence patterns seen in 2022 and 2023. Surprisingly, less collections were made in 2023 after a drier and hotter season the year before. Since these beetles are attracted to stressed trees, more emergences in the season after hotter, drier weather would not be unexpected.

Though extensive collection records have been organized and published for some southeastern states like North Carolina and Tennessee (Klingeman et al. 2015), these records rely heavily on calendar date for the characterization of active periods. Due to fluctuations in temperature and precipitation from year to year, relative emergence dates likely will not occur on the same dates each year. In work done by Potter et al. (1988), degree day models for *Chrysobothris femorata* were constructed over multiple years of collection in Kentucky, which allowed for much more precise characterization of emergence activity. The degree day model developed found that *C. femorata* emergences occurred at a degree day accumulation around 412° C from 1984 to 1986 (Potter et al. 1988). To date, this study is likely the only published work to develop degree day models for a species belonging to the *C. femorata* species complex. Because the data presented here is strictly collections based upon calendar date, it is more difficult to make precise predictions about emergences from year to year.

In addition to inconsistent emergences across collection years, the collection of particular species at each trapping site was highly variable between 2022 and 2023. Certain species were consistently captured across all trapping sites in both 2022 and 2023, but many of the collected species were found only at one site, during one year, or in very small quantities. Some species, including *C. adelpha*, *C. azurea*, *C. harrisi*, *C. rugosiceps, C. sexsignata* are considered more uncommon or even rare to collect in the southeast (Paiero et al. 2012). Due to the low overall collections of these species in 2022 and 2023, as well as the use of different trap types between years, drawing conclusions about the activity of these species in specific regions of Alabama is difficult.

Species collected more frequently and consistently can provide more detailed information about the activity of the *C. femorata* species complex across the state. Interestingly, *C. cribraria,* a species considered to be a fairly uncommon (Paiero et al. 2012), was found across all sites in 2023, and was the most frequently collected species at the Auburn location – with 50 more collections than the other 8 species combined (Table 2). The high numbers of this species in one location may be explained by the number of host plants in the areas surrounding areas of the traps. At the Auburn site, traps were moved in 2023 to a different powerline cut within the same area at the Kreher preserve due to the previous location's proximity to the road. *C. cribraria* is known as one of the only *Chrysobothris* spp. that use pine (*Pinus*) as a host. Although the 2023 trap location was less than 350 meters from the 2022 trap location, it was in a more heavily wooded area bordered by large stands of coniferous trees. This, in addition to using pole traps instead of panel traps, could have contributed to the overwhelming collection of this particular species at the Auburn location in 2023.

Alongside *C. cribraria*, species collected more consistently at specific sites include *C. shawnee, C. quadriimpressa*, and *C. chrysoela,* allowing for a closer look to be taken at the activity periods of these species at two locations (Figures 3 & 5): Mobile, AL in 2022 and Auburn, AL in 2023. In particular, *C. shawnee* was found consistently at all sites in 2022 and 2023, and could be found for most of the trapping season. It is possible that species like *C. shawnee* may pose the greatest threat to producers depending on host range, however, current trapping practices likely do not account for abundance of *C. femorata* species complex in any particular area. Overall, males and females of the most commonly collected species from Mobile in 2022 and Auburn in 2023 emerged within similar windows (Figures 4 & 6), indicating that males and females are active around the same times. Since only a handful of species were found in enough abundance to observe patterns between sexes, it is unclear whether other species from this complex have similar emergence patterns.

Despite being the most effective way to monitor the *C. femorata* species complex, passive trapping likely results in skewed estimates of regional activity. Notably, it has been documented that traps most successful for trapping *Chrysobothris* species seem to attract more females than males (Perkovich et al. 2022, 2023). In addition to a female bias, Perkovich et al. (2023) discusses a specific trap shape that collected more *Chrysobothris* than others – a narrow and tall plastic panel trap that might mimic host trees in the environment. Overall, the collection data from all Alabama sites in 2022 and 2023 are female biased regardless of the trap type, however, collections taken from the tree-mimicking pole traps yielded significantly more females than males. This supports the idea that visual cues related to color and shape are likely important factors involved in host choice. Though a specific pole trap size did not perform significantly better than the others, a low number of beetles were collected during this experiment. Overall, pole traps likely appear more similar to hosts than panel traps, which could make them a more effective trapping option regardless of size. Pole traps may also be a useful IPM tool in nursery settings, since large collections of females on traps would reduce the number of females actively ovipositing on trees.

Trapping in the state of Alabama has provided valuable insight into *Chrysobothris* species distribution across three regions of the state, as well as characterized some general phenological patterns that may be useful for managing pest species. Continuing to trap over the next few years could further strengthen connections between specific species, locations, and times of year, as well as capture more clearly whether multi-year emergence patterns are occurring due to semivoltinism. In addition to trapping, local weather station data in conjunction with the phenological periods of more consistently trapped species can be used to develop degree day models, which would greatly improve accuracy in predicting emergences and flight periods. Finally, continuing to trap with pole style traps that mimic hosts is important to more clearly understand whether any size preferences may exist, as collections during the 2022 experiment were low overall. It is also important to consider the idea that male *Chrysobothris* focus more specifically on other cues, such as female appearance or sound, rather than actual appearances of hosts, which females likely use to locate oviposition sites. Future work focused on further characterizing the *C. femorata* species complex in Alabama will help inform prevention and management strategies for producers in the ornamental industry.

## Chapter 3

## Drought and Defense: Effects of Imidacloprid and Water Deficit on Red Maple Trees

### Introduction

The ornamental industry in the United States generates billions of dollars annually (US Census of Agriculture, 2017; US Census of Horticultural Specialties, 2019), with some of the top producers in the country belonging to the Southeast. Pest management is a large and important part of nursery production, and it is critical to maintain the aesthetic standards of ornamental products required for marketability (Sadof & Sclar 2002). In ornamental trees like red maple, pests like the flatheaded appletree borer (*Chrysobothris femorata*) belonging to the *Chrysobothris femorata* species complex can cause internal damages during development that can negatively impact hosts. This damage causes not only aesthetic issues such as scarring, warping, and dieback (Frank et al. 2013), but also structural deformities that can make establishment into a landscape unsuccessful.

Though the *C. femorata* species complex has been credited for up to 40% losses in some susceptible red maple cultivars, like October Glory (Oliver et al. 2010), preventative management strategies are limited. Cultural control methods, such as cover cropping, have been shown to be nearly 100% effective at preventing *C. femorata* attacks in potted red maple (Dawadi et al. 2019). Despite its success, this strategy resulted in reduced tree size due to nutrient competition, which is undesirable for ornamental producers. Similarly, physical trunk barriers exhibited some success in the prevention of *C. femorata* attacks, however, these barriers can be costly and time consuming to implement, and can cause overheating and damage to bark surfaces without routine monitoring.

Overall, neonicotinoid insecticide treatments are the preferred management method against attacks from the *C. femorata* species complex due to both cost effectiveness and labor requirements. Systemic insecticides like imidacloprid are highly successful at preventing losses to *C. femorata* in nurseries, and can provide protection to treated trees for up to four years (Oliver et al. 2010).

Despite their ability to reduce damage in the field, it is not explicitly known how imidacloprid prevents attacks. Assessing larvae within hosts is done through destructive sampling, and attempts to rear larvae and adults in lab settings have been largely unsuccessful. Because of these challenges, direct toxicity studies have not been conducted on any life stage. Drenches with imidacloprid move primarily through xylem during translocation (Mota-Sanchez et al. 2009; Sur & Stork 2003; Tanis et al. 2012), while larval *C. femorata* species feed just underneath the bark on phloem tissues that do not receive as much active ingredient.

Indirect effects of systemic insecticides on plants documented in other crops may influence damage from *C. femorata* in maples. Two leading hypotheses focus on how imidacloprid may alter host defenses, or increase plant vigor, especially under stress (Szczepaniec et al. 2013). In multiple crop species, imidacloprid can improve plant vigor or plant tolerance to stressors such as drought (Oosterhuis et al. 2003; Thielert 2006), and there is limited evidence supporting these trends in woody plants (Chiriboga 2009). This mitigation of drought may also be related to altered spectral signatures in treated trees. If imidacloprid treated trees experiencing drought provide spectral signatures similar to trees that are well watered, this could indicate potential changes to how borers in the *C. femorata* species complex perceive them visually. Since the *C. femorata* species complex is highly attracted to trees experiencing stressors such as drought, increased drought tolerance and/or plant vigor resulting from imidacloprid treatments might make trees less attractive hosts.

Imidacloprid applications to woody plants are also linked to secondary pest outbreaks of arthropods like spider mites (in multiple different crop species including tomato, corn, and cotton and trees (Ford et al. 2010; Szczepaniec et al. 2013). A leading hypothesis supported by research in herbaceous plants, is that imidacloprid can suppress certain phytochemical defenses. Specifically, neonicotinoid treatments can alter expression of genes regulated by jasmonic acid (JA) and salicylic acid (SA) (Ford et al. 2010). In summary, it is not possible to assess direct toxicity of imidacloprid on borer larvae.  Indirect effects of imidacloprid may contribute to increased susceptibility to leaf feeding or stem boring species, especially under drought conditions. In this study, red maples are subjected to drought and imidacloprid treatments and then assessed for physiological changes through spectral analysis, changes in leaf phytochemistry, or reproduction or survival of spider mites.

## Methods

### ***Plant source and treatments*.**

Experiments were conducted with potted red maples (*Acer rubrum*) growing in 5-gallon pots obtained from a commercial nursery. For experiments in 2023 and 2024, sets of 24 potted red maples in 5-gallon pots were arranged in a RCB with six replicates containing four treatments, two levels of imidacloprid and two levels or irrigation (water stress): No imidacloprid w/regular watering, imidacloprid w/regular watering, no imidacloprid w/drought stress, imidacloprid w/drought stress. For the 2024 experiment, the same four treatments were used. Imidacloprid (Imidacloprid 2F, 21.8% AI, QualiPro, Pasadena, TX) was applied on 5 September 2023 at a rate of 6 mL per inch of tree height (adapted from label instructions for shrub drenches) in 1 liter of water. Treatments were drenched slowly into pots to minimize runoff. In 2024, imidacloprid was applied on 5 April at the same rate using the methods as previously described.

On 15 May 2024, watering regimens were determined from four representative potted maples. Pots were watered until runoff and left for one hour before container capacity weight (kg) was obtained using Ohaus Ranger Count 3000 Counting Scale. After a 24 hour dry down period, pots were weighed again in order to obtain pot dry weight (kg). Dry weights were subtracted from container capacity weights and converted into grams, where 1 gram is equal to 1 milliliter of water. The total volume (mL) required for each tree was averaged to produce the total water amount needed for well-watered trees daily. Trees undergoing drought stress received 25% of the well-watered amount. Water was measured out in a 500 mL graduated cylinder and poured directly into pots once daily.

### ***Imidacloprid ELISA assay***

On 19 September 2023 (two weeks post treatment), leaf samples representative of the upper, middle, and lower canopy were collected from each tree and kept on dry ice until stored at -80 °C. On 3 October (four weeks post treatment), another set of leaves were collected and stored the same as above. In February 2024, stored leaf samples were removed from the freezer and extracted using an extraction protocol supplied with the ELISA kit (Creative Diagnostics, Shirley, NY). Leaf samples were first crushed straight out of the -80 °C freezer, then 1 gram of material was added to a vial with methanol. After agitation and centrifugation, filtrate was extracted moved into new vials. After extraction, filtered samples were stored at -18 °C. In April 2024, extracted samples were diluted and plated with duplicates into prepared 96 well plates according to protocol supplied with the ELISA kit. Plates were read within 15 minutes of the stop solution being added at 450nm using a plate reader (Fisherbrand™ accuSkan™ FC Filter-Based Microplate Photometer, Hampton, NH).

### ***Response of spider mites to treatments***

In June 2024, naturally occurring populations of maple spider mites (*Oligonychus aceris*) were noted on the trees. This experiment assessed mite survival and oviposition. Enclosures made from 5 cm diameter tape rings (painter's tape, Tape Providers, Hialeah, FL). One tape enclosure was added to a random leaf on each tree on 7 June, and coated with a thin layer of pestick™ (Gempler's) using a paintbrush. Spider mite females were collected from untreated maple trees under a microscope and 10 females were transferred into each enclosure on a leaf. After three days, leaves with enclosures were collected and brought into the lab, where the total number of living mites, dead mites, and eggs were counted. This process was repeated. In addition to mite enclosure counts, one random leaf was pulled from each tree and brought into the lab to assess total adults, immatures, and eggs under a microscope. On each leaf, two 5 cm circles were marked and all life stages of mites were counted within to assess populations across treatments. This process was repeated on 14 June and 25 June.

In addition to mite enclosure counts, one random leaf was pulled from each tree on 5 July and brought into the lab to assess total adults, immatures, and eggs under a microscope. On each leaf, two 5 cm circles were marked, and all life stages of mites were counted within to assess populations across treatments. This process was repeated once more on 9 July.

### *Statistical Analyses*

All statistical analyses were conducted in R, and figures were created using ggplot2 and Microsoft Excel. Linear regression models with interaction terms (watering treatment x insecticide treatment) were used to analyze imidacloprid concentrations and mite counts.

## Results

### *Imidacloprid ELISA assay*

A Standard curve used to determine concentrations of imidacloprid from leaf samples was constructed using 6 known concentration standards provided with the ELISA kit (Figure 8).

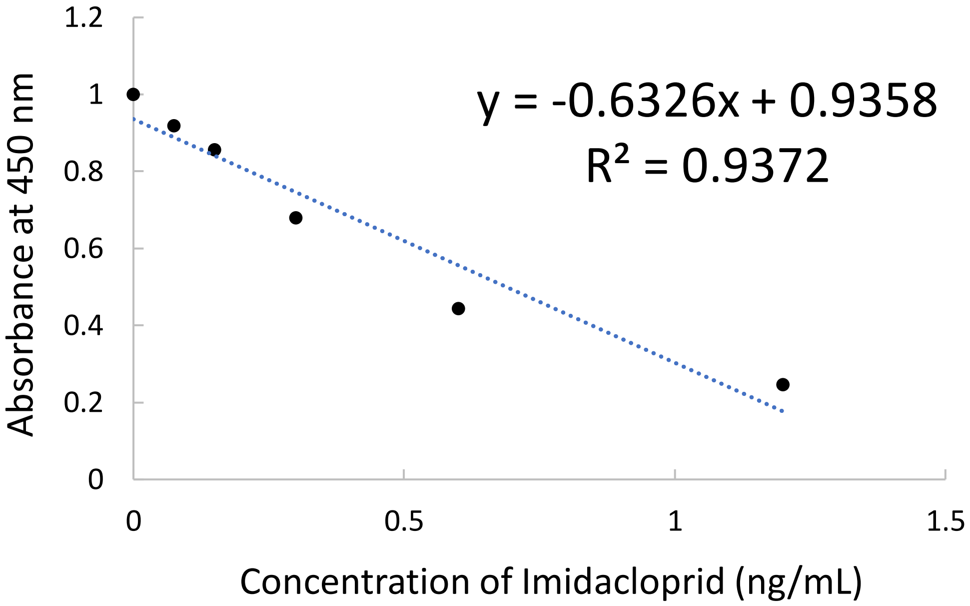

**Figure 8:** Standard Curve used to determine imidacloprid concentrations in leaf tissue.

There were no significant differences between treatments and imidacloprid residues in red maple leaf tissues ($p>0.05$). Treated red maple leaf tissue from 2 weeks post treatment had concentrations of imidacloprid between 25 and 30 mcg/mL (Figure 9A). Treated red maple leaf tissue from 4 weeks post treatment had concentrations of imidacloprid between 15 and 35 mcg/mL (Figure 9B). Untreated red maple leaf tissue from both sample sets consistently tested above the lower threshold limit for imidacloprid residues (Figure 9).

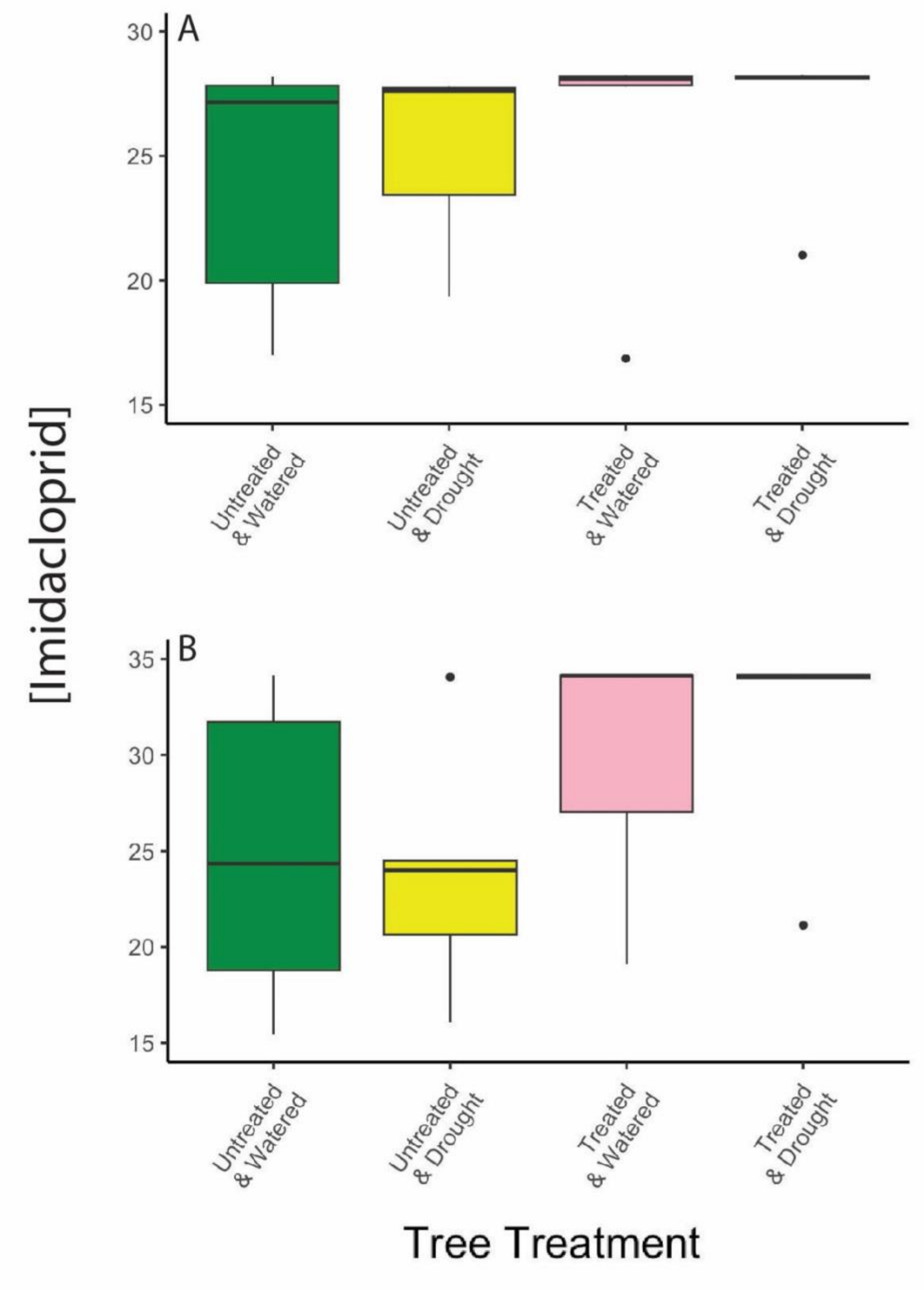

**Figure 9:** Concentrations of imidacloprid (mcg/mL) found in treated and untreated red maple leaf tissues from the 2023 tree experiment (A) two weeks post treatment, and (B) four weeks post treatment.

### *Response of spider mites to treatments*

In the mite transfer experiment, no significant differences in the total number of live mites, dead mites, or eggs were found between watering treatment and insecticide treatment ($p>0.05$). No significant interactions between watering treatment and insecticide treatment were found (Figure 9).

In the random mite counts, no significant interactions between watering treatment and insecticide treatment were found. Significantly more adults were found on insecticide treated trees than non-treated trees, regardless of drought ($p<0.05$, df=45),(±95% CL; ±20.82). Significantly more immatures were found on insecticide treated trees than non-treated trees, regardless of drought ($p<0.05$, df=45),(±95% CL; ±10.96). Significantly more eggs were found on insecticide treated trees than non-treated trees, regardless of drought ($p<0.001$, df=45),(±95% CL; ±66.73).

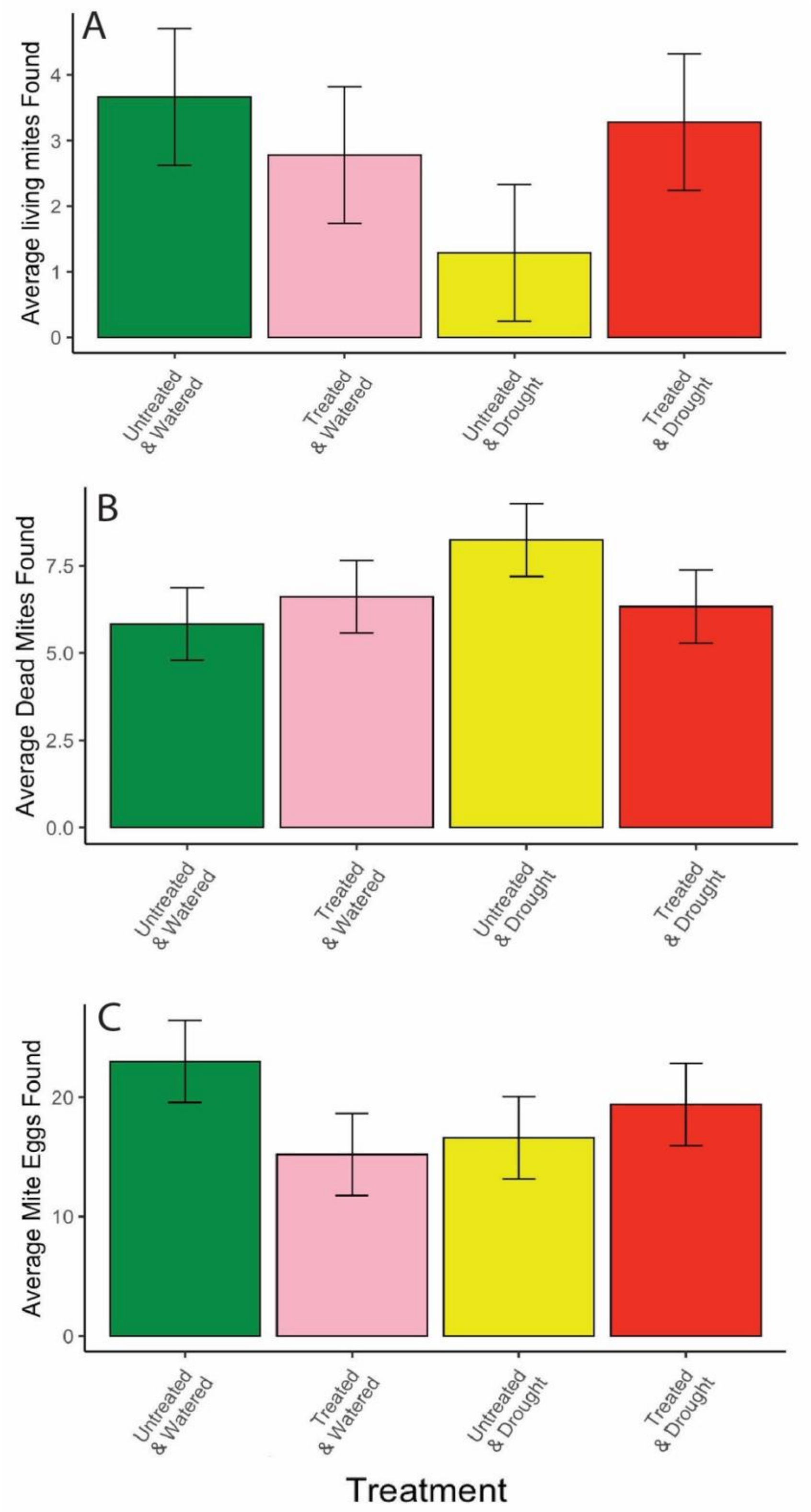

**Figure 10:** Average number of (A) living mites (B) dead mites & (C) mite eggs by treatment found within leaf enclosures three days post transfer in June 2024.

## Discussion

In order to better understand how systemic insecticide treatments like imidacloprid impact host suitability of red maples for the *C. femorata* species complex, the work presented here focuses on characterizing indirect responses of red maple to imidacloprid treatments, as well as responses to stressors like drought.

One of the most important factors that ensures the success of imidacloprid drenches against the *C. femorata* complex is the application timing. Because imidacloprid drenches move through plant tissues from the roots to foliage, it is important that treatments are applied early enough to allow for sufficient translocation time. By monitoring foliar concentrations of imidacloprid from treated trees two and four weeks after treatment, translocation can be confirmed, and an idea of about how long it takes imidacloprid to move through 5 gallon potted woody trees can be gained.

While the results from the imidacloprid ELISA kits show clear concentrations of imidacloprid in treated leaf tissues, considerable residues were also detected within the leaves of untreated trees (Figure 9). Much of the detected residues in untreated leaf tissue exceed the low detection limit from the standard curve, and even approach similar concentration levels found in treated leaf tissue. Since these trees were donated from a wholesale nursery, it is possible that all trees had been treated with some kind of imidacloprid drench, since residues can remain active within hosts for up to four years after treatment (Oliver et al 2010). Additionally, contamination of samples during the extraction and plating process could have also occurred. Overall, the possibility of residual carryover and contamination is low, as precautions to minimize contact between samples were taken throughout the treatment process, as well as the during extraction and plating process. Since this kit was likely intended to detect trace amounts of imidacloprid in samples, the concentrations found in leaf samples may be too large to analyze using the provided standard curve values (Figure 8). Readjusting samples with a different dilution factor might mitigate this issue. Since this assay was done on the tree set from 2023, an imidacloprid ELISA kit will be run on the trees from 2024 as well. For future work focused on treatments using imidacloprid drenches, it will be of utmost importance to locate experimental trees that would not have been treated prior, as well as take extra care to keep non-treated foliage away from treated foliage during processing.

Though imidacloprid translocation from 2023 samples could not be properly characterized, the mite trials from 2024 yielded interesting results. Finding no significant interaction between water treatment and insecticide treatment was interesting, since it was expected that drought responses would vary based on imidacloprid treatments. Additionally, average spider mite mortality and fecundity were not found to be significantly impacted by any tree treatments during the mite enclosure experiments. Still, the average number of living mites found after the mite enclosure experiments appeared lowest in the untreated drought treatments (Figure 10A). Similarly, the average number of dead mites found after the mite enclosure experiments appeared highest in the untreated drought treatments (Figure 10B). This suppressed average survivorship and elevated average death seen only in untreated drought enclosures could indicate increased sensitivity of mites to drought stress when not mitigated with imidacloprid treatments. In other words, drought treated trees also treated with imidacloprid may have fewer overall deaths and greater survival than drought treated trees without imidacloprid due to imidacloprid-induced drought tolerance.

Similar to the enclosure experiments, no significant interaction between water treatment and imidacloprid treatment were found in random population counts. Instead, the total numbers of mite adults, immatures, and eggs were significantly larger on plants treated with imidacloprid, regardless of drought treatment. Finding increased population sizes due to imidacloprid treatment is consistent with the types of physiological changes within a host that trigger hormesis. This response is consistent with the findings in Chiriboga (2009) and Szczepaniec et al. (2013), though overall populations on imidacloprid treated trees did not experience percentages of growth relative to untreated trees as large as findings from these works.

Overall, indirect impacts of imidacloprid related to secondary pest outbreaks were clearly found during overall population sampling of mites on red maple hosts in 2024. Some evidence was found suggesting that imidacloprid treatments reduced the intensity of drought impacts on mite populations, however, this trend was not statistically significant.

Since imidacloprid ELISA samples were taken from the 2023 set of trees, it is unclear whether the same responses would be found in the 2024 set of trees that were challenged with mites. For the 2024 leaf samples, determining whether a different dilution factor can be used to adjust sample concentrations to the provided standards, in addition to being mindful of contamination during the extraction process, will improve the accuracy of the ELISA results.

As overall average mite data from the enclosure treatments was low compared to data from random population counts, some factors related to the setup and longevity of the mite enclosure experiments may have impacted their overall success. The painter's tape circles occasionally became un-stuck from the leaf surface, resulting in small gaps and pockets along veins in the leaves that left potential openings for mites to move in and out of the enclosure. The painted on pestick™ border also seemed to run slightly in the heat of the greenhouse, resulting in patches of the leaf surface dying. Finally, since spider mite populations did not explode within the three day window, a larger starting sample size or longer experiemental duration may have improved results.

## Conclusion

The *Chrysobothris femorata* species complex is understudied in Alabama, and continues to pose a threat to ornamental and landscape trees across the southeastern United States. By improving our knowledge of species distribution and phenological activity across the state, more effective management and prevention strategies may be developed. Additionally, more clearly characterizing indirect impacts of imidacloprid treatments on host tree suitability might shed light on how certain treatments are effective against *C. femorata* by altering host appearance or triggering secondary pest outbreaks. Overall, continued collections in Alabama would allow for more comprehensive species profiles to be constructed. Projects of key importance include using commonly collected species records from 2022 and 2023 to develop specific degree day models for the different trapping sites across Alabama.

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