Biology of *Chauliognathus* spp. (Hentz) (Coleoptera: Cantharidae) in Virginia agroecosystems

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ACADEMIC ABSTRACT

Biology of *Chauliognathus* spp. (Hentz) (Coleoptera: Cantharidae) in Virginia agroecosystems

Katlyn Catron

Chauliognathus pensylvanicus (De Geer) and *C. marginatus* (Fabr.) are two of the most common species of cantharid beetles observed in agroecosystems in Virginia. Despite their widespread abundance and early recognition and description in the 1700s, little is known about their phenology and ecological role in agricultural systems. Immatures have been recorded feeding upon soft-bodied insects such as lepidopteran larvae, and adults frequently visit flowering plants, where they eat nectar and/or pollen. Beyond those observations, relatively little is known about these species, which are conspicuous elements on and around farms, though there is considerable potential for their use as pollinators and predators in a biological pest control context.

I employed a phenological study to determine the period of *C. marginatus* activity in Southwestern Virginia, and found adults active on a variety of flowers from mid-May to late July. On these flowers, they spent a large percentage of their day mating or feeding, with significant differences in the percentage of beetles participating in behaviors based on time of day and beetle sex, but not based on month. The majority of *C. marginatus* observations occurred on narrowleaf mountain mint (*Pycnanthemum tenuifolium* Schrad.), poison hemlock (*Conium maculatum* L.), and wild bergamot (*Monarda fistulosa* L.).

I also used a community science-based approach to evaluate the usage of host plants and flower colors by *C. marginatus* and *C. pensylvanicus*. *Chauliognathus marginatus* utilized white flowers more than expected based on their potential

availability, while *C. pensylvanicus* utilized yellow flowers over all other flower colors and more than expected based on their potential availability. Communities of flowers available to and observed with *C. marginatus* and *C. pensylvanicus* differed significantly at the family and genus levels. Laboratory bioassays supported these conclusions regarding *C. pensylvanicus* color preference, but not *C. marginatus* color preference.

Lastly, because of their susceptibility to nontarget chemical exposure in agroecosystems, I tested the efficacy of both organic and conventional vegetable insecticides against *C. marginatus* adults. The highest mortality was seen in the acetamiprid, flupyridifurone, sulfoxaflor, and imidacloprid treatments, which reached 97-100% mortality at two days after treatment. High mortality (60-97% two days after treatment) was also seen in the organic treatments containing azadirachtins, pyrethrins, and *Chromobacterium subtsugae* strain PRAA4-1T. Mortality in the *Burkholderia* spp. strain A396 and spinosad treatments was not significantly different from that of the controls, however, control mortality was relatively high in both assays.

GENERAL AUDIENCE ABSTRACT

Biology of two common soldier beetles on Virginia farms

Katlyn Catron

Soldier beetles are common but underappreciated members of many ecosystems across the world. Two species are seen especially frequently on or around farms in Virginia: the margined leatherwing and the goldenrod soldier beetle. These beetles are active during different periods of the year (spring and fall, respectively), and adults gather in large numbers on flowering plants and may be beneficial pollinators of some flower species. Their immatures are stealthy and typically crawl along the ground, but may climb into crop plants to eat pest insects. Because of these potential roles in pest management, we studied their yearly activity, preferences in flower types and colors, and their susceptibility to chemical insecticides that are commonly used in vegetable crops.

Margined leatherwing adults were active on wildflowers and flowering weeds from mid-May to late July. While they were on these flowers, they spent most of their day mating and feeding. Most of the observations we made were on narrowleaf mountain mint (a native perennial wildflower), poison hemlock (a toxic biennial invasive weed), and wild bergamot (a native perennial wildflower).

I was interested in determining what flowers and flower colors each soldier beetle was found most frequently on throughout their entire range, so we used community science data collected from iNaturalist to evaluate those questions. Margined leatherwings were found much more often on white flowers than flowers of any color, and more than I expected based on the proportion of white flowers blooming in the spring. Goldenrod soldier beetles were found much more often on yellow flowers, and

more than I expected based on the proportion of yellow flowers that bloom in the fall. The types of flowers blooming for each of these two species were different, as well as the types of flowers they visited. Our results were partially supported with a laboratory experiment where beetles had to choose colored panels to escape an enclosure; the goldenrod soldier beetles chose the yellow panel a great majority of the time, but the margined leatherwings did not choose the white panel as much as we expected. These differences in color and flower preferences are interesting and should be explored further.

Finally, I tested several organic and conventional (non-organic) insecticides against margined leatherwing adult beetles to see how they would react if incidentally exposed in a farm setting. Insecticides of both types caused considerable mortality over two days, though two of the products we tested seemed not to have major negative effects on the beetles. This experiment was difficult because it seems that margined leatherwings are sensitive to being contained to experimental chambers, and many died without being exposed to any insecticide at all.

In summary, these beetles are important, unique, and interesting, and deserve more study, especially regarding their potential role in agricultural pest management. These studies are some of the first to explore the behavior, host plants, and insecticide tolerance of these common soldier beetles, but will hopefully inspire others to look deeper into their biology in the future.

DEDICATION

An advanced degree is a difficult undertaking in the best of times. Add in some major medical problems, a global pandemic, and a pregnancy, and it’s a miracle that I’m even writing this dedication.

This dissertation is dedicated to the students with extenuating circumstances that make graduating seem impossible at times. You can do this.

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I found a wealth of mentors, friends, and family within the student body of the Ent Department that made my time at VT something really special. My heart stays with the "old" ento crew, the new folks who saw me as "old", and especially the Kuharmy. I do and will miss Andy, Rachel, Adam, Sean, Kyle, Kelly, Ary, Tim, Kemper, Mika, Daniel, Brian, Lucas, and, especially, my best bug Kadie. Thanks for keeping grad school fun and helping me be a better person.

Finally, I would not be here if not for the support of my family, Dave and Anita Amos, Julia and David Helveston, and my husband, Sam. Thank you for keeping me on track, helping me make good decisions, and providing alternate text for my discussions that always made me laugh, but also made me better at telling this story. Sam, you made this degree possible with your unending support, constant encouragement, and the persistent reminder that there is more good to life than just bugs. I love you all!

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CHAPTER ONE

Literature Review

Soldier beetles (Coleoptera: Cantharidae) are a reasonably diverse beetle family with >470 described species in 23 genera in North America (Arnett and Thomas 2002, Pelletier and Hébert 2014) and approximately 5100 species in 160 genera globally (Ślipiński et al. 2011). They are found worldwide outside of the Arctic and Antarctic (Lawrence et al. 2018), and preserved specimens have been recovered from amber deposits as old as the Early Cretaceous period, or as old as 145 million years (Poinar and Fanti 2016). Within the superfamily Elateroidea, Cantharidae is part of a monophyletic clade with Lampyridae, the fireflies (Kundrata et al. 2014).

Within the subfamily Chauliognathinae are four genera: *Belotus*, *Chauliognathus*, *Ichthyurus*, and *Trypherus* (Arnett and Thomas 2002). Most *Chauliognathus* species are found in the western United States, in Australia, and in South America, but two species are prevalent in agroecosystems in Virginia: *Chauliognathus pensylvanicus*[1] (De Geer) and *C. marginatus* (Fabr.) (Horn 1867, Fender 1964, Pelletier and Hébert 2014). These beetles have been in the scientific record since the late 1700s; *C. pensylvanicus* was first described as *Telephorus pensylvanicus* by De Geer (1768), and *C. marginatus* was described by Fabricius (1775) as *Cantharis marginata* (Stugard 1931, Miskimen 1966). Though they are abundant in the central and eastern United states and have been described for over 200 years, little attention has been paid to the phenology or biology of members of the *Chauliognathus* genus. In fact, most research has focused solely on the entomopathogenic fungi that utilize *C. pensylvanicus* as a host (e.g. Carner 1980, Wheeler Jr.

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[1] *"pensylvanicus"* is commonly misspelled as *"pennsylvanicus"* in the literature. *"pensylvanicus,"* with a single "n" following the "pe" is the correct spelling (Egler 1941, Williams 2006).

1988, Steinkraus et al. 2017) or the mating preferences of *C. pensylvanicus* females (e.g. Weiner 1974, McCauley and Wade 1978, McLain 1982, 1985).

Cantharids are generally dorsoventrally flattened with leathery elytra, parallel body margins, threadlike antennae, and long legs (Arnett and Thomas 2002). Some superficially resemble adults in the family Lampyridae, but they differ in head shape, eye size, and the lack of a light-emitting organ in Cantharid adults (Arnett and Thomas 2002). Adult *Chauliognathus* spp. are medium-sized (8-15 mm long), conspicuous, orange-yellow beetles with variable black markings on their elytra and pronota (Fig. 1). The head is visible from above and the antennae are long, filiform, and black (Stugard 1931, Miskimen 1966, Pelletier and Hébert 2014). In both species, females are larger than males (Miskimen 1966). Many Cantharids, including *Chauliognathus* spp., have well-developed abdominal and thoracic defensive glands that produce 8*Z*-dihydromatricaria acid (DHMA), a potent antifeedant chemical with a long evolutionary history in this group (Meinwald et al. 1968, Eisner et al. 1981, Poinar et al. 2007, Haritos et al. 2012).

Reported seasonal activity of each species varies depending on the source consulted. Pelletier and Hébert (2004) state that *C. pensylvanicus* is univoltine and adults are active from early June to early October, with peak activity occurring from early August to mid-September. *C. marginatus* adults are active from mid-June to late July in Canada, but seasonal activity and voltinism is unknown in the United States, aside from anecdotal observations of activity in spring (Pelletier and Hébert 2014, Philips et al. 2014). Miskimen (1996) agrees that *C. pensylvanicus* is univoltine and active from August to early October, but states that *C. marginatus* is bivoltine or multivoltine and has a larger peak of activity in late spring/early summer and a smaller, second peak in late September. During their periods of seasonal activity, adults are diurnal, and are active during the heat of the day after dew evaporates from flowers (Williams 2006).

The larval seasonality of the two species is undocumented, but larval natural history has been explored (Riley 1892, Stugard 1931). First instars are pale white and alligator-like, but grow into velvety, black, rufous-headed later instars with the terminal abdominal segment acting as a prehensile "limb" (Stugard 1931, Philips et al. 2014). Larvae have been recorded as nocturnally active (Lundgren et al. 2006, Weber et al. 2008). It is assumed that both species overwinter as larvae and pupate in the warmer months, then eclose into adults at each species' time of peak adult activity (Stugard 1931).

Adults have been found on over 180 species of flowering plants and are believed to feed upon pollen, nectar, and/or other insects, but a definitive summary of their diet composition is yet unknown. (Riley 1892, Balduf 1935, Brown and Brown 1984, Williams 2006, Graham et al. 2012). Though adults may be palynivorous, they have also been considered important pollinators to at least two species of flowering plants (Primack and Silander 1975, Weiherer et al. 2020). Larvae are regarded as generalist predators, but their diet composition is also not definitively known and there is some evidence of phytophagy (Balduf 1935). *Chauliognathus* spp. and related Cantharidae larvae have been shown to feed upon engorged American dog tick larvae (*Dermacentor variabilis* Say), balsam twig aphids (*Mindarus abietinus* Koch), corn earworms (*Helicoverpa zea* Boddie), plum curculio larvae (*Conotrachelus nenuphar* Herbst), apple worm (species not given), Colorado potato beetle eggs (*Leptinotarsa decemlineata* Say), tiger beetle eggs and larvae (*Pseudoxycheila tarsalis* Bates), and other soft-bodied insects and insect eggs. (Riley 1892, Phillips and King 1923, Winburn and Painter 1932, Carroll 1987, Schultz 1994, Berthiaume et al. 2001, Weber et al. 2008, Philips et al. 2014).

Because *Chauliognathus* species are attracted to flowering plants as adults and are generalist predators as larvae, they seem to be ideal insects for manipulation into biological control of insect pests. The success of interplanting flowering plants with crops for pest management is mixed (Landis et al. 2000, Woltz et al. 2012, Veres et al. 2013, Lu et al. 2014, Rusch et al. 2016). However, Traugott (2002) specifically mentioned the potential of cantharid larvae to move from meadows into crop fields because of their high rates of dispersal as both adults and larvae. This potential, combined with the lack of knowledge of basic biology and ecology of these species, presents a unique opportunity to explore the natural history of these insects and test it in an applied context.

This research focused on the three following objectives: 1) to characterize *C. marginatus* natural history and population dynamics in Southwest Virginia agricultural systems, 2) to investigate the host plant use and preferences of *C. marginatus* and *C. pensylvanicus* throughout their native ranges, and 3) to evaluate the responses of *C. marginatus* to common agricultural insecticides.

Figures

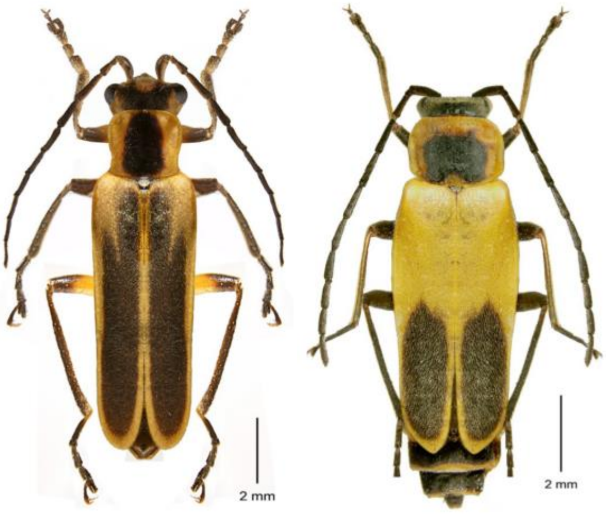

Figure 1. Adult *C. marginatus* (left) and *C. pensylvanicus* (right). Images reprinted with permission from Pelletier and Hébert (2014).

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CHAPTER TWO

Phenology and behavior of C*hauliognathus marginatus* (Fabr.) (Coleoptera: Cantharidae) in Virginia agroecosystems

Abstract

Chauliognathus marginatus (Fabr.) is a frequent visitor of weeds, wildflowers, and disturbed areas in and around agricultural areas throughout eastern North America. Because of its pollination and biological pest management potential, it is important to understand their seasonal activity and behavior, to maintain any benefits they are providing and protect them from unintentional exposure to pest control methods. I studied the phenology of *C. marginatus* for two years (2018-2019) and found that adult beetles are active on a variety of flowers from mid-May to late July. On these flowers, they spent a large percentage of their day mating or feeding, with significant differences in the percentage of beetles participating in behaviors based on time of day and beetle sex, but not based on month. The majority of *C. marginatus* observations occurred on narrowleaf mountain mint (*Pycnanthemum tenuifolium* Schrad.), poison hemlock (*Conium maculatum* L.), and wild bergamot (*Monarda fistulosa* L.). To my knowledge, this is the first study to characterize the phenology or behavior of this common and conspicuous beetle species.

Introduction

Chauliognathus marginatus (Fabr.) is a medium-sized (8-15 mm in length) orange and black beetle that is found in large aggregations on weeds, wildflowers, and various plants (Miskimen 1966, Pelletier and Hébert 2014). Adults are suspected pollinators (Primack and Silander 1975, Weiherer et al. 2020) and larvae have been recorded preying upon pests in sweet corn and other fruit, vegetable, and row crop systems (Riley 1892, Phillips and King 1923, Balduf 1935, Winburn and Painter 1932, Weber 2009). Because these insects are frequent visitors of plants in and around agricultural areas, it is important to understand their seasonal activity and behavior, to help maintain and possibly augment any benefits they are providing and protect them from unintentional exposure to pest control methods.

Almost no literature exists attempting to characterize the phenology of *C. marginatus*. Pelletier and Hébert (2014) provide a general period of adult activity for *C. marginatus* in Canada, from mid-June to early July. Miskimen (1966) suggests that *C. marginatus* are multivoltine in warmer climates throughout North America and bivoltine in the eastern United States, with the first generation appearing in late spring or early summer, and a second generation appearing in September. Even less is written about larval phenology aside from attempts to rear related species in captivity (Stugard 1931, Traugott 2000). Stugard (1931) was able to rear *Chauliognathus pensylvanicus* (De Geer) through five instars through pupation and to adulthood, but concluded that ecological factors had a considerable effect on stadium length and gave no average duration of the larval life stage. Stugard (1931) also attempted to rear *C. marginatus* and found a larval period length of 55 days in captivity. Both species were noted to molt and overwinter in small cells in the soil that they excavated before entering their resting periods.

Similarly, very little research has been published concerning the daily activities and behaviors of *Chauliognathus* spp. beetles, with no published record of any behavioral studies about *C. marginatus*. *Chauliognathus pensylvanicus* is a closely related species that is found in abundance in the late summer and fall in eastern North America (Pelletier and Hébert 2014) and has been used as the study subject of several behavioral investigations of mating and mate choice (McCauley and Wade 1978, Mason 1980, Mccauley 1981, McLain 1982, 1985, Pratt et al. 2017). Rausher and Fowler (1979) studied a population of the congener *C. distinguendus*

(Gorham) in South America and found evidence for aggressive resource guarding between individuals of the same and opposite sexes, which is unusual in insects.

Considering the lack of basic biological knowledge about *C. marginatus*, this study aimed to explore their phenology and daily behavior in agroecosystems in southwest Virginia.

Methods

Phenology - Adults

Phenological sampling of soldier beetles was conducted in 2018 and 2019 at Kentland Farm (37.199985, -80.564797) and Homefield Farm (37.202840, -80.562285) in Whitethorne, VA (Fig. 1). Kentland Farm is a Virginia Tech research facility that contains experimental dairy, row crop, vegetable, tree fruit, berry, forage, and other agricultural research plots. Neighboring Homefield Farm provides organically grown vegetables to Virginia Tech dining services and hosts row crop, wildflower, and vegetable research plots. *Chauliognathus marginatus* adults had been observed aggregating on the mixed wildflowers and in the bramble plots of both Kentland Farm and Homefield Farm in previous growing seasons, so those location types were selected for population monitoring in the 2018 growing season.

Sampling began when the first adult *C. marginatus* beetle was observed on May 28, 2018. From May 29, 2018 to July 19, 2018, samples were taken visually by walking along the margins of mixed wildflower (37.202832, -80.562288; 37.203628, -80.564255; and 37.199937, -80.586230) or bramble plots (37.207245, -80.591505 and 37.202108, -80.563181) at approximately 0.2 m/s. Over a one-minute period, all adult beetles present along the 12m-long transect were counted (Fig. 2). Five counts were taken at each site each week. Count transects did not overlap. Sampling ended when beetles were not observed for two consecutive weeks.

Results from the 2018 season informed sampling in 2019. Sampling began a full month earlier, and anecdotal observations of beetle activity on other plants indicated a wider variety of location types was necessary for adequate population monitoring. From April 29, 2019 to July 24, 2020, visual sampling procedures were repeated in five varieties of plant plots (southern arrowwood shrub [*Viburnum dentatum* L.] [37.200294, -80.563884], mixed wildflower [37.202832, -80.562288; 37.203628, -80.564255; and 37.199937, -80.586230], mixed blackberries and raspberries [*Rubus* spp. L] [37.207245, -80.591505], poison hemlock [*Conium maculatum* L.] [37.199673, -80.564981], and milkweed [*Asclepias* spp. L.] [37.199405, -80.565918]). Sampling ended when beetles were not observed for two consecutive weeks.

Phenology - Larvae

To sample larval phenology, pitfall traps and clear sticky cards were deployed in cucurbit and sweetcorn plots in August of 2018. Pitfall traps were placed at the end of a 1m strip of plastic flashing inserted perpendicular to the ground surface to intercept ground-dwelling insects and direct them into the traps. Sticky cards were baited with egg masses of Colorado potato beetle (*Leptinotarsa decemlineata* Say) or harlequin bug (*Murgantia histrionica* Hahn). A paint stirrer was pounded securely into the ground and a clear sticky card was stapled to the stirrer so that the bottom edge was flush with the soil (Fig. 3).Cards and traps were checked twice weekly for four weeks. Reitter-Winkler samples were taken of the leaf litter/topsoil in potato, corn, and pumpkin plots throughout the 2018 growing season. Additionally, visual searches for larvae were made throughout the 2018 and 2019 growing seasons in a variety of habitats, including vegetable plot margins and wildflower plots at Kentland Farm (37.199985, -80.564797) and Homefield Farm (37.202840, -80.562285), separate wildflower gardens at the YMCA Hale Community Garden in Blacksburg, VA (37.267198, -80.412041), a large pollinator meadow at the Pandapas

Pond Recreational Area outside of Blacksburg, VA (37.281625, -80.474070), and a personal fruit and vegetable garden in Blacksburg, VA (37.233016, -80.490033).

Two grids of pitfall traps were deployed in October of 2019. Nine evenly spaced traps were placed in a 3m × 3m grid in a wildflower plot and in a field of sweetcorn crop residue following harvest four weeks earlier. Traps consisted of a 15.2 cm length of 10.2 cm diameter PVC pipe driven into the soil until flush with ground level. Any soil left inside the pipe was removed to create a cavity. The inner edge of the PVC pipe was lined with rubberized weatherproofing foam to securely hold a standard 8.9 cm diameter disposable plastic cup within. Cups were pricked to prevent water accumulation and filled halfway with loose rocks to provide insects with harborage and discourage predation. Traps were set monthly from October 2019 to February 2020 by emptying debris from the PVC pipe, inserting a plastic cup, and emptying the contents every 12 hours for 48 hours.

Behavior - Adults

Once large aggregations of *C. marginatus* were observed during the adult phenology visual sampling, behavioral observations started. Observations took place on either poison hemlock or in mixed wildflower plots as described in Angelella and O’Rourke (2017). Naturally occurring breaks in plant density were used to define the sampling area, which was limited to 2m long by 0.3 m deep into the plant canopy. Once the sampling area was delineated, the plants were not disturbed in order to facilitate natural behaviors by the beetles.

Beetles were observed for 108 hours throughout the 2018-2019 growing seasons. Half-hourly observations were made and included the time, temperature, cloud cover, rain level, wind level, and number of beetles participating in each of the following activities: mating, feeding, traveling, preening, resting, or fighting. Behavior categories were exclusive, and mating took precedence over all other behaviors. In 2019, beetle sex and plant species hosting the behavior was also recorded.

Behavior - Larvae

Observations of *Chauliognathus* spp. larvae in various plant systems were made throughout the late summer and fall of 2018, 2019, and 2020.

To assess larval behaviors when presented with different prey items, larvae were subjected to choice and no-choice assays in September of 2018. Larvae were collected by hand from wingstem flowers in the pollinator meadow at the Pandapas Pond Recreational Area outside of Blacksburg, VA (37.281625, -80.474070). Once collected, larvae were kept in large, cylindrical, clear acrylic containers (approximately 23 cm tall by 20 cm in diameter) with mesh lids for airflow. Larvae were provided with 6 cm of moistened potting soil, foliage, and several water wicks. No more than 25 larvae were kept in each container at any time to lessen cannibalization pressure.

Choice tests were conducted at night under red light. Larvae were presented with four equally sized food items evenly spaced in the lid of a 15.24 cm glass Petri dish: raw cow liver (animal protein used for rearing in captivity [Stugard 1931]), peanut butter (plant protein), sugarcane aphids (potential prey item in nature; *Melanaphis sacchari* Zehntner), and watermelon (sugar and water source). A single larva was placed in the Petri dish and allowed 5 minutes to make a choice. The assay was concluded after the larva clearly began feeding on the food source (i.e., sunk its mouthparts into the food item and stilled motion). This was repeated with 64 starved *Chauliognathus* spp. larvae and the total number of larvae that chose each food item was compared using Pearson's Chi-squared tests for given probabilities.

No-choice tests were also conducted at night. Twenty starved larvae were placed individually in 10 cm Petri dishes with a thin layer of moist soil covering the bottom. Enumerated prey items (either harlequin bug eggs or sugarcane aphids) were offered to the larvae and then counted after 12 hours. The average number of each prey item eaten per larva was calculated and compared visually.

Data analysis

Degree days were calculated for 2018 and 2019 using the simple average method ([daily maximum temperature plus daily minimum temperature divided by 2] minus the minimum developmental threshold). Temperature data were sourced from public weather records taken at the KBCB Virginia Tech Montgomery Executive Airport weather station in Blacksburg, VA (37.206214, -80.408978). In the absence of a known minimum developmental threshold for *Chauliognathus* spp., the lower threshold was set at 10˚C (a standard or default level for many plants and insect species) and the starting date for each year was set to March 1. *Chauliognathus marginatus* abundance data (in average number of beetles counted per minute of observation) were plotted by degree day for each monitoring site type. Abundance data were averaged per minute of observation by site type to facilitate comparisons between site types with different total numbers of sites. General trends were visually compared by year and by site type.

For ease of analysis and intuitive real-world application of the results, daylong observations were sorted by time period: early morning (6:00am – 8:30am), late morning (9:00am – 11:30am), early afternoon (12:00pm – 2:30pm), late afternoon (3:00pm – 5:30, and evening (6:00pm – 8:30pm). Several nighttime observations were made but were excluded from analysis because of their small sample size and the difficulty determining behaviors and sexes at night. Pearson's Chi-squared tests for given probabilities and Bonferroni-adjusted pairwise nominal tests of independence were used to determine differences in the percentage of beetles participating in each behavior within each time block. Analyses were performed in R Statistical Software v.4.0.2 (R Core Team 2020) using the base and “rcompanion” packages (Mangiafico 2020).

Differences in beetle behavior by sex were analyzed using Pearson's Chi-squared test with Yates' continuity correction and an experiment-wide Bonferroni-corrected p-value of 0.01. Observations of behaviors were summed by plant species and differences were explored with Pearson's Chi-squared test with Yates' continuity correction and an experiment-wide Bonferroni-corrected p-value of 0.01. Analyses were performed in R Statistical Software v.4.0.2 (R Core Team 2020) using the base and “rcompanion” packages (Mangiafico 2020).

Observations of beetle behavior throughout the observation season were plotted to visually compare proportions of beetles engaging in a behavior by observation date.

Results

Phenology - Adults

Chauliognathus marginatus activity in 2018 was noticed in late May, or after approximately 655 cumulative degree days (DD) base 10ºC (Fig. 4). Average number of adult beetles per minute of observation varied, with a general increasing trend, until the 2018 peak of activity was recorded after 1400 DD. After the peak, the number of adult beetles steadily dropped, until none were seen at 1800 DD. In 2019, monitoring began considerably earlier than in 2018, at 250 DD. The first beetles were noticed in mid-May, after 429 DD. Again, a general increasing trend was seen until a peak at 908 DD, and a second, smaller peak at 1265 DD. After this second peak, the number of adult beetles dropped until only one was seen in 20 minutes of observation at 1988 DD.

By examining the 2018 monitoring data by site type, one can see that the majority of beetles were seen at the wildflower sites, with fewer occurring at the bramble sites (Fig. 5). Beetle activity at the bramble sites ended mid-season, at 1274 DD, while activity at the wildflower sites picked up at about this same time.

The first beetles of 2019 were observed a southern arrowwood, bramble, and mixed wildflower sites at 492 DD **(**Fig. 6). Immediately following, a huge spike in beetle counts occurred at the poison hemlock sites starting at 472 DD and peaking at 908 DD. As activity at the poison hemlock sites waned, it increase in the milkweed and wildflower plots, with smaller peaks at 1266 DD.

Phenology - Larvae

No larvae were captured using any of the sampling methods deployed – sticky cards, two types of pitfall traps, or Reitter-Winkler samples. However, many (>200) *Chauliognathus* spp. larvae were observed on plants in blooming wingstem (*Verbesina alternifolia* L.) in the pollinator meadow at Pandapas Pond in September 2018 and in the fruit of strawberry plants in a personal garden in September 2018. Additionally, several hundred *Chauliognathus* spp. larvae were found on the surface and under the husks of sweet corn ears in August and September of 2019 and 2020 (between 2500 – 3500 DD, Fig. 7).

Behavior - Adults

Two hundred and sixteen half-hourly observations of *C. marginatus* adult behaviors were made over 16 days during the 2018 and 2019 growing seasons, which included 6114 total records of beetles engaging in the six behavior activities. Only two instances of fighting were recorded throughout the entire experiment. The majority of observations were of beetles mating or feeding, followed by traveling, resting, preening, and lastly, fighting. Insufficient numbers of observations were made during cloudy, rainy, or windy weather conditions, so those variables were excluded from analysis.

The percentage of beetles observed participating in each behavior varied throughout the day (Fig. 8). In the early morning, observations of resting and traveling occurred significantly more than of feeding, mating, or preening (x^2=406.82, df=5, p<0.001). In the late morning, the primary activity switched to feeding, which was observed significantly more than mating, traveling, resting, or preening (x^2=1116.7, df=5, p<0.001). The early afternoon was dominated by mating and feeding, which were observed significantly more than traveling, resting, or preening (x^2=1126.7, df=5, p<0.001). In the late afternoon, mating was the predominant activity, followed by traveling, feeding, resting, and preening (x^2=1782.1, df=5, p<0.001). Mating occurred significantly more than resting, feeding, traveling, or preening (x^2=669.14, df=5, p<0.001) through the evening and until the end of the observation period.

In 2019, a total of 2999 males and 1601 females were observed. Some sex-based differences in behavior observations were seen (Fig. 9). The proportion of females observed mating was significantly greater than that of males mating (x^2=262.78, df=1, p<0.001). Conversely, the proportion of males observed traveling (x^2=211.99, df=1, p<0.001) and feeding (x^2=16.419, df=1, p<0.001) was significantly greater than females. There were no significant differences in the proportion of males and females resting (x^2=0.45, df=1, p=0.5) or preening (x^2=0.07, df=1, p=0.79).

Beetles were most frequently observed visiting narrowleaf mountain mint (*Pycnanthemum tenuifolium* Schrad.), poison hemlock (*Conium maculatum* L.), and wild bergamot (*Monarda fistulosa*. L.) (Fig. 10). Other flowers visited by *C. marginatus* included purple coneflower (*Echinacea purpurea* [L.] Moench), daisy fleabane (*Erigeron annuus* [L.]

Pers.), beardtongue (*Penstemon* spp. Schmidel), Maximillian sunflower (*Helianthus maximiliani* Schrad.), showy primrose (*Oenothera speciosa* Nutt.), and black-eyed-susan (*Rudbeckia* spp. L.). The majority of observations occurred on narrowleaf mountain mint (48.1%), poison hemlock (29.4%), and wild bergamot (9.6%), with the remaining flower species making up less than 5% of observations, so analyses focused on these three flower species. Some differences in behavior were seen based on flower species (Fig. 11). Significantly more observations of mating occurred on wild bergamot than on narrowleaf mountain mint or poison hemlock (x^2=35.009, df=2, *p*<0.001). Conversely, significantly more observations of traveling (x^2=11.661, df=2, *p*=0.003) and feeding (x^2=11.898, df=2, *p*=0.003) occurred on narrowleaf mountain mint than on wild bergamot. There were no significant differences in the observations resting (x^2=1.551, df=2, *p*=0.4595) or preening (x^2=5.6203, df=2, *p*=0.0602) based on flower species.

The proportion of beetles engaging in each behavior did not change throughout the season (Fig. 12).

Behavior - Larvae

Larvae were observed inserting their mandibles into several plant-provided food sources, including wingstem flowers and strawberry fruits (Supplementary videos 1 and 2).

Of the 64 larvae offered different food items in the choice tests, 4 made no choice, 7 chose aphids, 18 chose watermelon, 17 chose cow liver, and 18 chose peanut butter **(**Fig. 13). There was no statistically significant difference among the number of *Chauliognathus* spp. larvae that chose each food source (x^2=5.7, df=4, *p*=0.125). Larvae offered harlequin bug eggs consumed an average of 7.2 eggs per night, and those offered sugarcane aphids consumed an average of 13.3 aphids per night (Fig. 14).

Discussion

To my knowledge, this is the first concerted effort to record the phenology of *C. marginatus*. I saw no evidence of bivoltinism or multivoltinism (Miskimen 1966), but agree with Pelletier and Hébert (2014) that *C. marginatus* activity begins in the late spring and lasts until mid to late July. *Chauliognathus marginatus* exhibited similar, albeit asynchronous, phenological trends in 2018 and 2019. Activity started, was recorded, and peaked earlier in 2019 than in 2018, even after accounting for temperature differences using DD. However, monitoring in 2018 was limited to only two site types – bramble and wildflower – while 2019 sites included a wider variety of flower types. The first peak in 2019 is largely driven by observations in poison hemlock, a flower that was not known to be an aggregation site in 2018. If the observations made on poison hemlock were removed from the 2019 data, the overall phenological trend would almost exactly mirror that seen in 2018.

Rausher and Fowler (1979) found that, in *Chauliognathus distinguendus*, both males and females aggressively defended nectar-producing inflorescences of *Coccoloba floribunda* (Benth.) (Polygonaceae) in Costa Rica. As discussed in the paper, *C. floribunda* produces nectar for a very short daily window and their experiment took place during a dry season with little other nectar available. The authors concluded that this rare intersexual aggression would likely not be seen in a system without intense resource limitation like that of the *C. floribunda* plant. Because I saw only two aggressive interactions between *C. marginatus* adults during all observations, I suggest that nectar may be a more abundant resource in poison hemlock and narrowleaf mountain mint than it is in *C. floribunda*, and therefore aggression in defense of this resource may not be necessary for these beetles.

Based on studies of the behavior of other coleopterans, I expected to see more resting and preening behavior than exhibited by *C. marginatus*. Preening, or grooming, is a common activity among beetles and ensures their cuticle, including antennae and other appendages, are in clean condition. Valentine (1973) observed several prominent grooming behaviors in cantharid adults, including the double antennal clean, the palpus clean, and the body-mid-hindleg rub, among others. All of these behaviors were observed in *C. marginatus,* but for very brief periods of time and only intermittently throughout the day. Because *Chauliognathus* adults do not interact much with other insects, predators, or any prey items (Williams 2006), perhaps their cuticles remain in generally good condition and they have little need for constant maintenance. In fact, after being gently handled and placed back in a safe location, many *C. marginatus* specimens captured throughout the observation period would immediately and fastidiously groom themselves, as if offended by the disturbance to their normal state.

Similarly, Shepard et al. (2008) found that female tiger beetles spend the majority of their day resting, with brief periods of movement and predation. However, unlike nectar-feeding *C. marginatus* adults, tiger beetles are ambush predators, so the shorter periods of rest seen in *C. marginatus* are logical. Instead of relying upon a few large, protein-based meals like tiger beetles, *C. marginatus* must work throughout the day to obtain enough nutrition from flowers, which would lead to considerably more traveling and feeding than resting.

Differences in *C. marginatus* behavior throughout the day follow logical patterns. Early in the morning, when the temperatures were still cool and the dew had not evaporated from flowering plants, most of the beetles were resting or traveling. Travel during this time was typically limited to slower crawling movements as the beetles still had wet wings and were unable to fly. As the dew evaporated and temperatures warmed, more feeding was observed, as the beetles broke their nightly fast and consumed calories to power the day's activities. By early afternoon, mating and feeding were observed at statistically similar percentages, as males found females to mate and others focused more on nectar gathering. Mating in other *Chauliognathus* species has been shown to last anywhere from 5 to more than 17 hours (Bernstein and Bernstein 1998), which may explain the large proportion of mating observations which occurred during the late afternoon and evening until observations ended.

Populations of *C. pensylvanicus* have been shown to have balanced (1.13♂ :1♀, McCauley and Wade 1978) and male-dominated (1.91♂ : 1♀, Weiner 1974) sex ratios. I found a 1.87:1 ratio of males to females in *C. marginatus*, which falls between the two values in the literature for *C. pensylvanicus*. Females may have been lower in the canopy of the flowers or at ground-level laying eggs. Because more males than females were observed, and males of other *Chauliognathus* species are aggressive initiators of mating (McCauley and Wade 1978, Pratt et al. 2017), males would likely pair with any and all receptive females, leaving few females to engage in other behavior activities. Males, however, would be left unpaired to travel and feed at significantly higher percentages than females.

Narrowleaf mountain mint is a native perennial forb that grows in clusters and blooms with copious small, white flowers in corymbs over an extended period through the summer (Sheahan 2012). It is a high-value floral resource (Tooker et al. 2006), receiving more bee visitors than expected given its floral area (Russo et al. 2013) and hosting more beneficial arthropods than almost any others in a study of Wisconsin-native flowers (Bennett and Gratton 2013). Wild bergamot is a native perennial herb that grows singly or in small patches and blooms with clusters of small, tubular, purple flowers (glomerules) throughout the summer (Anderson 2000). It is also a valuable nectar source, providing high-sugar nectar throughout the day and quickly replenishing that nectar after being depleted by insect visitors (Cruden et al. 1984). Conversely, poison hemlock is an introduced species from Eurasia and has been described as highly toxic in all parts (Goeden and Ricker 1982). It is a very tall biennial weed that grows in large, dense clusters in disturbed areas and blooms in the form of umbels with many small, white flowers (Vetter 2004). Several studies have evaluated the few polyphagous insects that feed on poison hemlock, but none have specifically investigated its potential as a nectar source for insects (Goeden and Ricker 1982, Berenbaum and Harrison 1994, Vetter 2004).

Of all the observations of *C. marginatus* on flowers, the vast majority (87%) occurred on plants that bloom in clusters of very small individual flowers. The remaining 13% of observations occurred mostly on flowers that bloom in heads or single flowers, including purple coneflower, daisy fleabane, Maximillian sunflower, showy primrose, and black-eyed-susan, or that bloom in clusters of larger flowers, like beardtongues. The availability of copious amounts of nectar in a small space may have attracted and retained *C. marginatus* to these plants in particular. McLain (1984) found the greatest abundances of *C. pensylvanicus* adults on flowers with large, broad, clusters of multiple flowers, concluding that the amount of horizontal surface area these inflorescences present provided both food and habitat for greater numbers of beetles. This is likely another reason for the great percentage of beetles I saw on narrowleaf mountain mint and poison hemlock.

In addition to nectar availability and floral anatomy, abundance of plants may have influenced observations of *C. marginatus*. In the poison hemlock site type, poison hemlock was the only aggregation of flowering plant available to the beetles in that area. Similarly, narrowleaf mountain mint was the most abundant type of flowering plant available to *C. marginatus* in the wildflower plots while it was blooming. Wild bergamot was present in the wildflower plots in some abundance, but not in the density that narrowleaf mountain mint was found. Trends seen in the phenology monitoring of these beetles may help explain their flower usage, as well. In 2018, the early spike of activity on poison hemlock was followed with a later spike on wildflowers. Six species of South American *Chauliognathus* were shown to aggregate in large numbers based on the availability of floral resources, moving collectively when flower quality declined seasonally or due to dryness (Machado and Mellender De Araújo 2001). *C. pensylvanicus* has also been noted to aggregate upon different flower species as floral resources change (Williams 2006). It seems that *C. marginatus* also follows this trend, and moves *en masse* once floral resources are depleted or as seasonal changes in plant abundance occur.

This mass movement would also explain the lack of differences seen in beetle behaviors by plant species. There were no significant differences in the percentages of beetles observed in any behavior on narrowleaf mountain mint or poison hemlock – both plants that hosted large aggregations of beetles at different points in the season. Beetles were observed mating at significantly higher percentages on wild bergamot than narrowleaf mountain mint or poison hemlock, and at significantly lower percentages on wild bergamot traveling or feeding than the other two examined plants. Wild bergamot tended to be an "island" of excellent floral resources surrounded by less enticing plants in both this and Cruden et al. (1984). Perhaps, if males found the wild bergamot flowers before females did, they opportunistically initiated mating whenever a female happened upon a flower, increasing the percentage of mating pairs versus traveling or feeding beetles.

The lack of seasonality in *C. marginatus* behavior suggests that these beetles participate in all behaviors throughout their adult season. There are no similar studies of *Chauliognathus* behavior with which to compare these results, but I would expect a similar trend to be seen in related species. Future studies confirming this suspicion would be useful in *C. marginatus* and other *Chauliognathus* species.

While phenological data could not be collected on larval *Chauliognathus* spp., the anecdotal observations of larvae in wildflowers, strawberries, and sweet corn are worth discussing. Almost all previous mentions of cantharid larvae characterize them as ground-dwelling, nocturnal predators (Riley 1892, Stugard 1931, Fender 1973, Traugott 2002, Lundgren et al. 2006, Williams 2006, Weber et al. 2008, Weber 2009). Also, Phillips and King (1923) noted that *Chauliognathus* spp. larvae follow corn earworm (*Helicoverpa zea* Boddie) larvae into ears of corn to prey upon them, but the time of this activity was unmentioned. Berthiaume et al. (2001) also documented the cantharid *Podabrus rugosulus* (LeConte) preying upon balsam twig aphids (*Mindarus abietinus* Koch) in the canopy of balsam fir Christmas trees (*Abies balsamea* L.) during unspecified daylight hours. The *Chauliognathus* spp. larvae observed in this study were present in the wingstem canopy for no readily apparent reason; there were no insect prey items visible for them to consume, whereas they may have been consuming insects or plant matter while in the corn ear. This is, to our knowledge, the first record of *Chauliognathu*s spp. larval activity in the canopy of wildflowers or strawberries during the day and one of the first records of their activity within ears of corn (Philips and King 1923).

This work examined the seasonality and behavior of *C. marginatus* adults in Virginia agroecosystems. Because these beetles are found in disturbed areas, weedy margins, wildflowers, and vegetable crops (McLain 1984, Williams 2006, Pelletier and Hébert 2014), it is important for us to understand their presence and role in these systems to protect them from non-target impacts of pest management such as insecticide applications (Catron and Kuhar 2020, 2021). Being able to predict their seasonal activity and behavior throughout the day helps growers make informed pest management decisions with fewer non-target effects. Future studies should focus on finer-resolution phenological monitoring in a wider diversity of agroecosystems.

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Author Contributions

Katlyn Catron: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization, Project administration **Daniel Wilczek:** Investigation **Chris McCullough:** Formal analysis, Visualization **Thomas Kuhar:** Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Figures and Tables

Figure 1: *Chauliognathus marginatus* adult sample sites at Homefield Farm and Kentland Farm, Whitethorne VA.

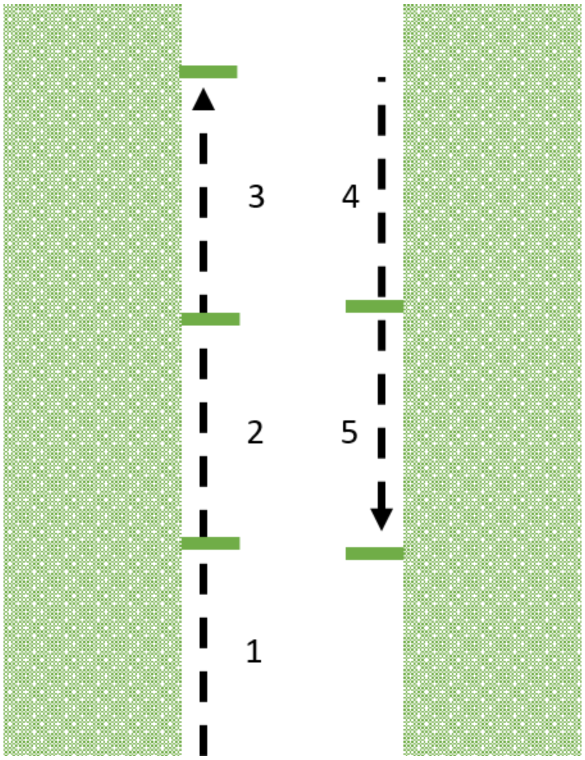

Figure 2: Example wildflower plot transects used for adult *Chauliognathus marginatus* visual sampling. Each marked transect was walked at a pace of approximately 0.2m/s for 1 minute over a distance of about 12m per sample. Five transects were sampled per site per week.

Figure 3: Sticky trap setup for sampling *Chauliognathus* spp. larvae in sweetcorn and cucurbit plots. A paint stirrer was pounded securely into the ground and a clear sticky card was stapled to the stirrer so that the bottom edge was flush with the soil. Traps were baited with egg masses of Colorado potato beetle (*Leptinotarsa decemlineata* Say) or harlequin bug (*Murgantia histrionica* Hahn).

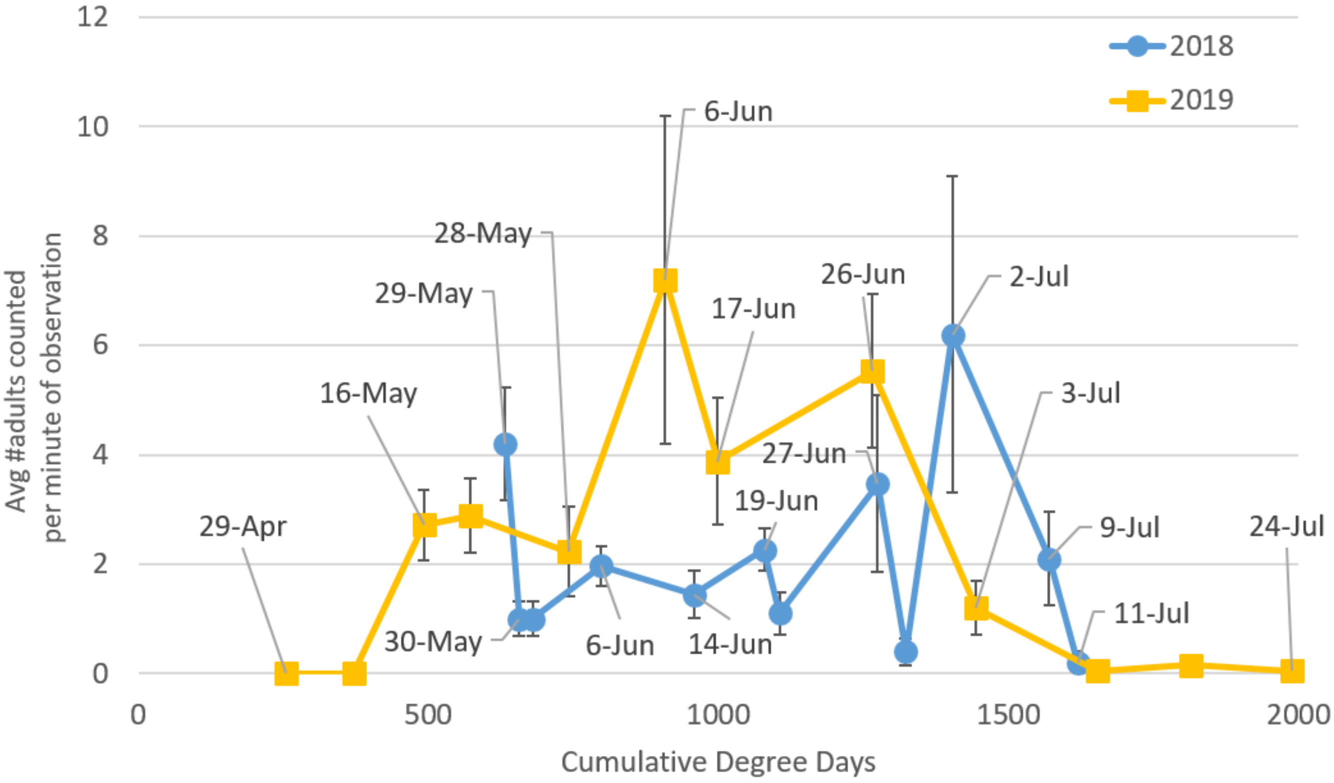

Figure 4: Average number of adult *Chauliognathus marginatus* beetles per minute of observation through the 2018 and 2019 observation periods, plotted by cumulative degree days (base 10ºC). Error bars represent standard error of the mean.

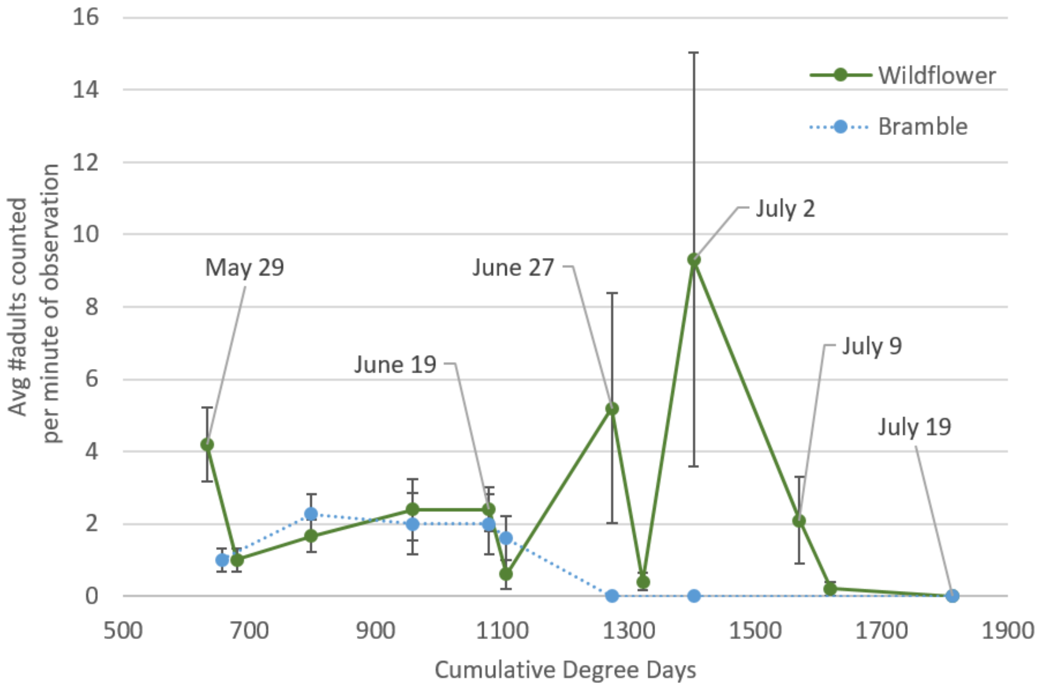

Figure 5: Average number of adult *Chauliognathus marginatus* beetles per minute of observation in wildflower and bramble sties from 633 to 1813 cumulative degree days (base 10ºC) in 2018. Error bars represent standard error of the mean.

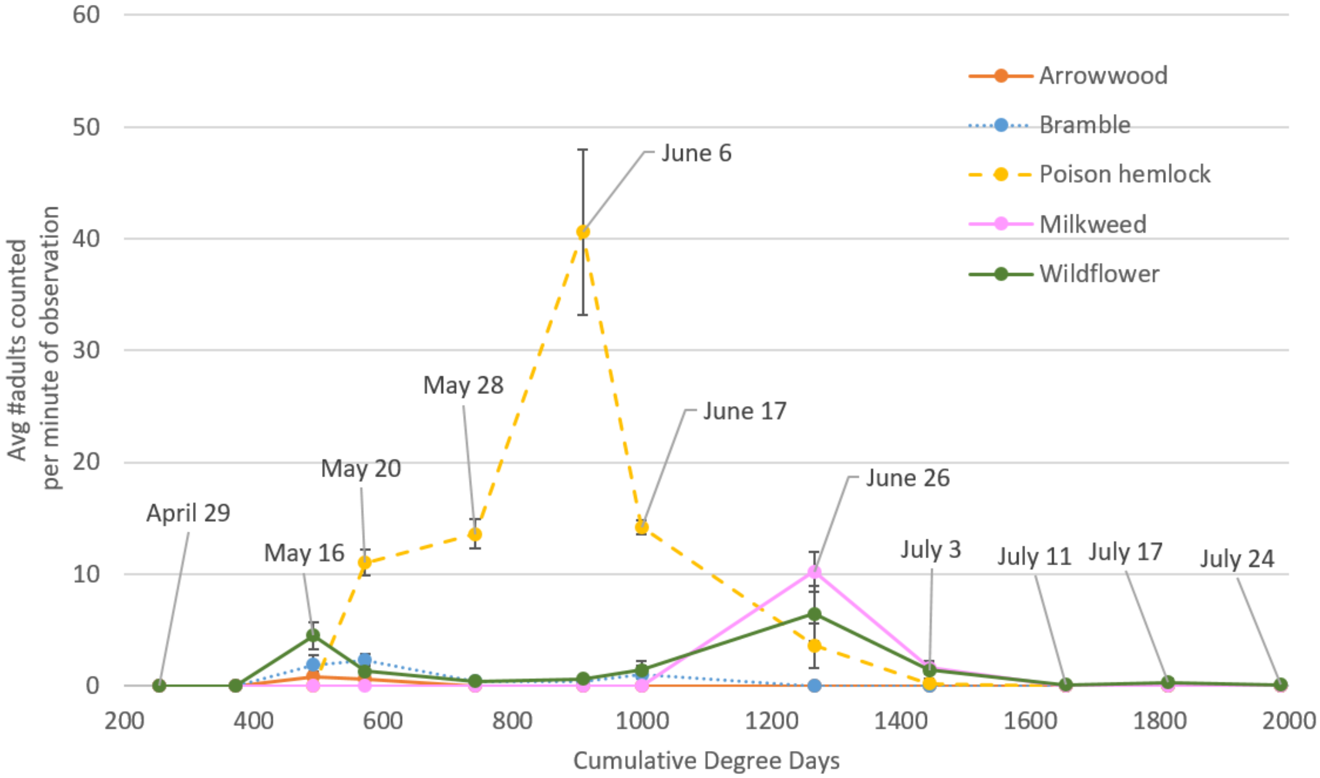

Figure 6: Average number of adult *Chauliognathus marginatus* beetles per minute of observation in arrowwood, bramble, poison hemlock, milkweed, and wildflower sites from 254 to 1988 cumulative degree days (base 10ºC) in 2019. Error bars represent standard error of the mean.

Figure 7: *Chauliognathus* larva in *Verbesina alternifolia*, *Zea mays*, and *Fragraria* sp., photos by Katlyn Catron, Sept 2018-Sept 2020.

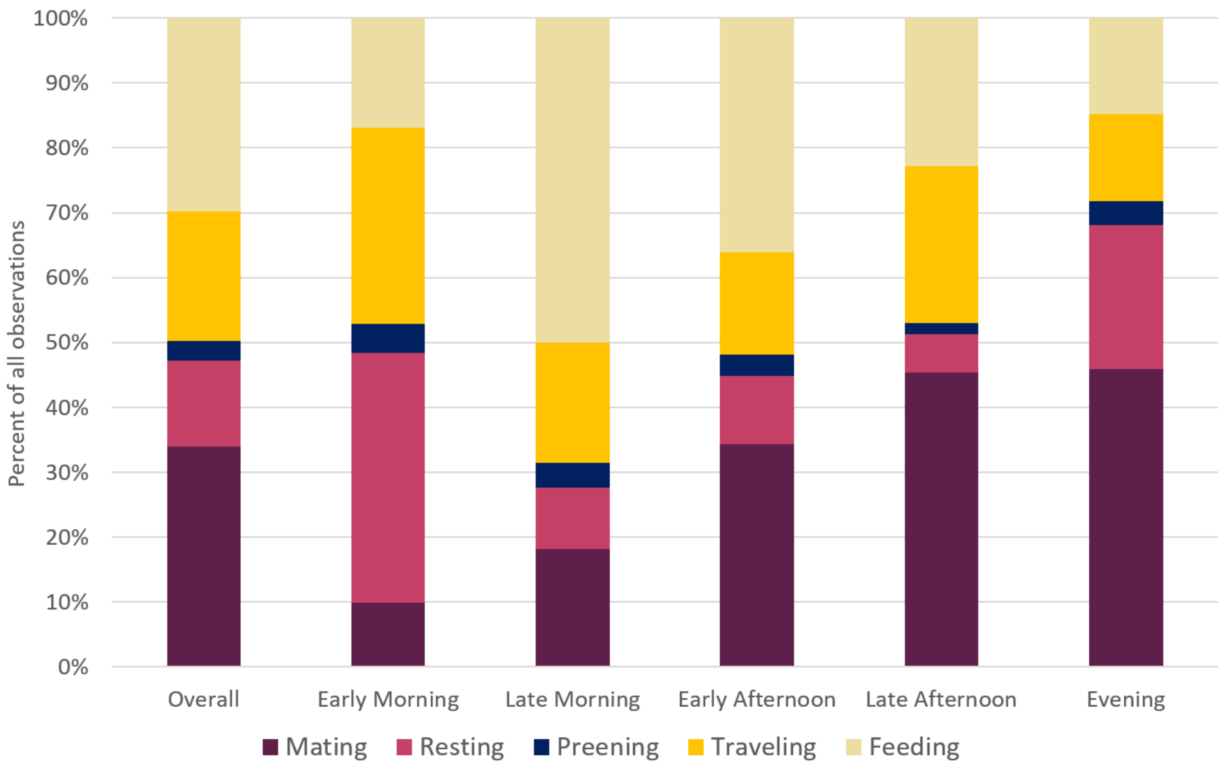

Figure 8: Percentage of *Chauliognathus marginatus* adults observed engaging in mating, resting, preening, traveling, and feeding overall and in five blocks of time throughout the day in 2018 and 2019.

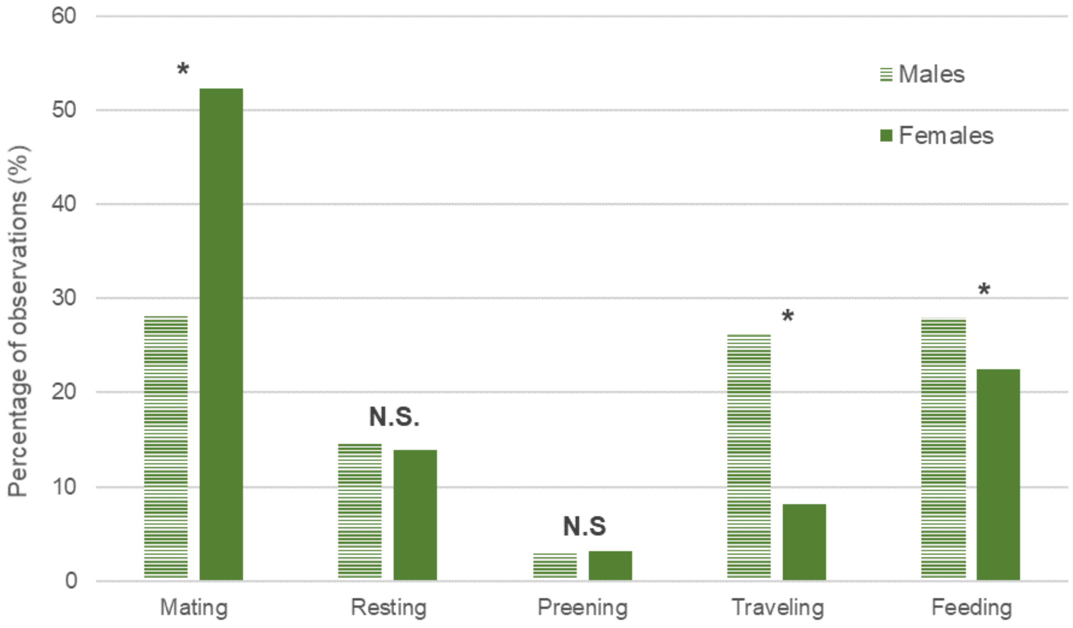

Figure 9: Comparisons of the percentage of male and female *Chauliognathus marginatus* observations mating, resting, preening, traveling, and feeding. Bar pairs headed with “*” are significantly different using an experiment-wide *p*-value of 0.01 with Bonferroni-corrected pairwise nominal tests of independence. “N.S.” indicates no significant difference.

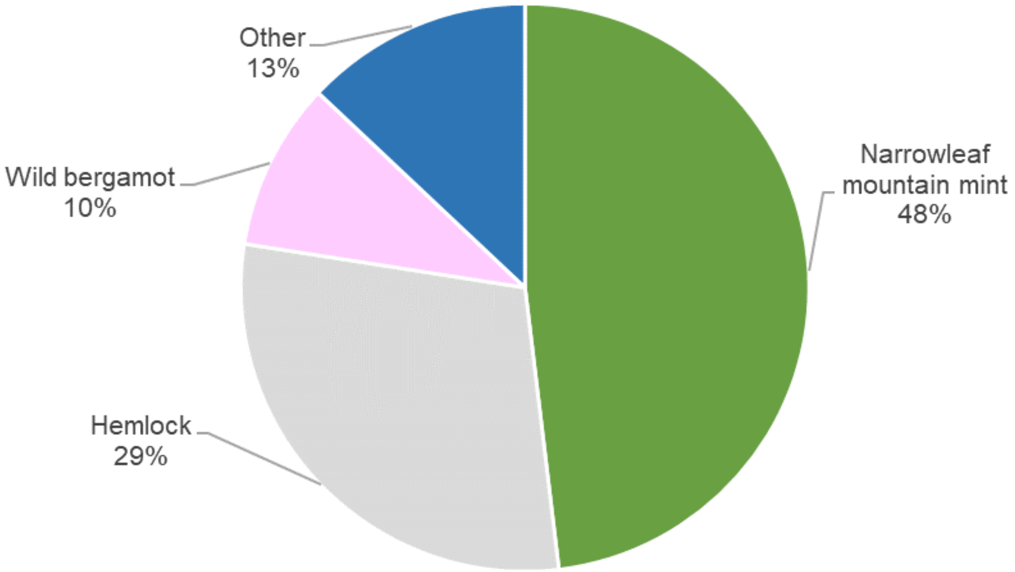

Figure 10: Percentage of *Chauliognathus marginatus* observations made on narrowleaf mountain mint (*Pycnanthemum tenuifolium*), poison hemlock (*Conium maculatum*), wild bergamot (*Monarda fistulosa*), and other flowers in 2019.

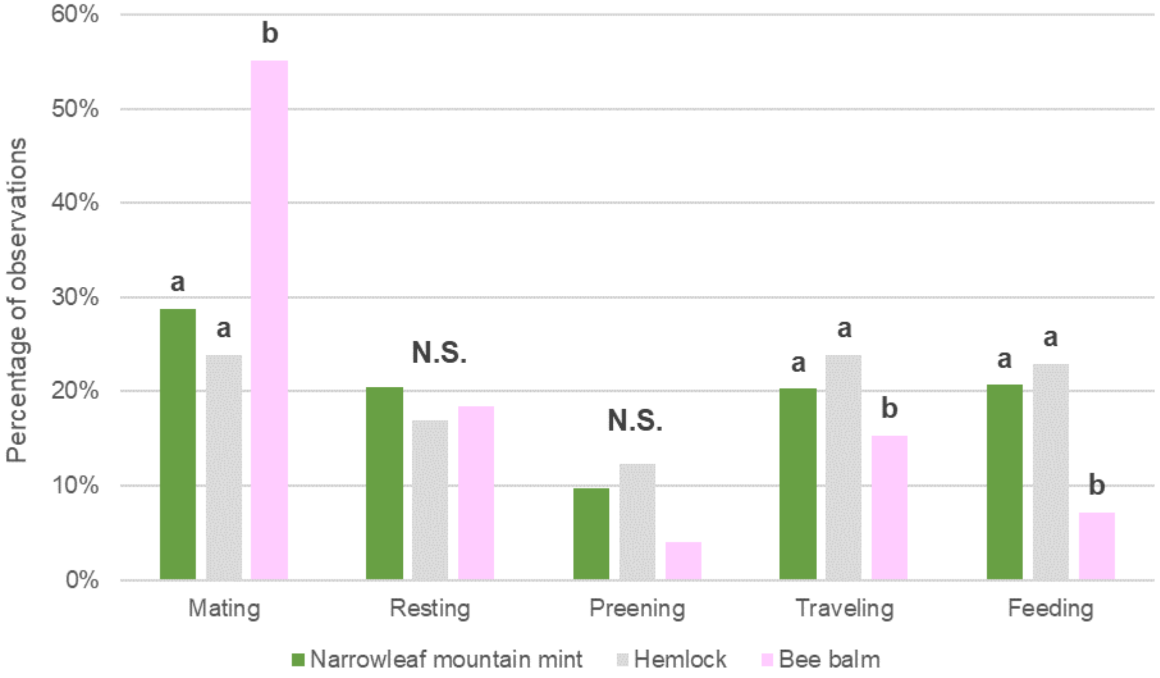

Figure 11: Comparisons of the percentage of *Chauliognathus marginatus* observations occurring on narrowleaf mountain mint (*Pycnanthemum tenuifolium*), poison hemlock (*Conium maculatum*), and wild bergamot (*Monarda fistulosa*). Bar trios headed with different letters are significantly different using an experiment-wide *p*-value of 0.01 with Bonferroni-corrected pairwise nominal tests of independence. “N.S.” indicates no significant difference.

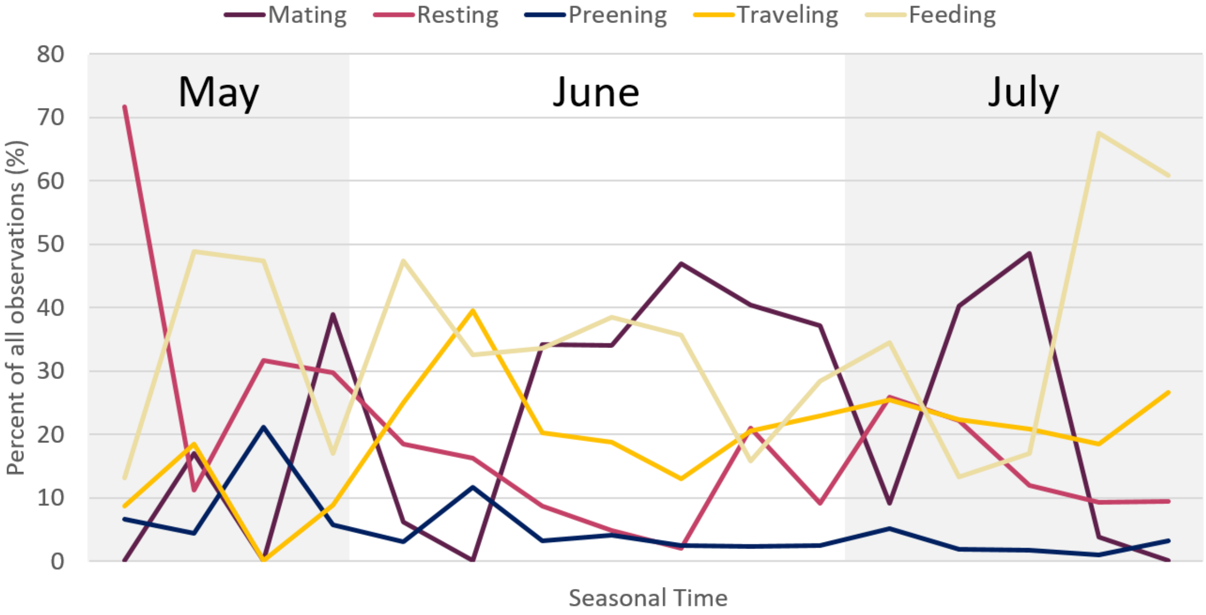

Figure 12: Proportion of *Chauliognathus marginatus* behavior observations over seasonal time.

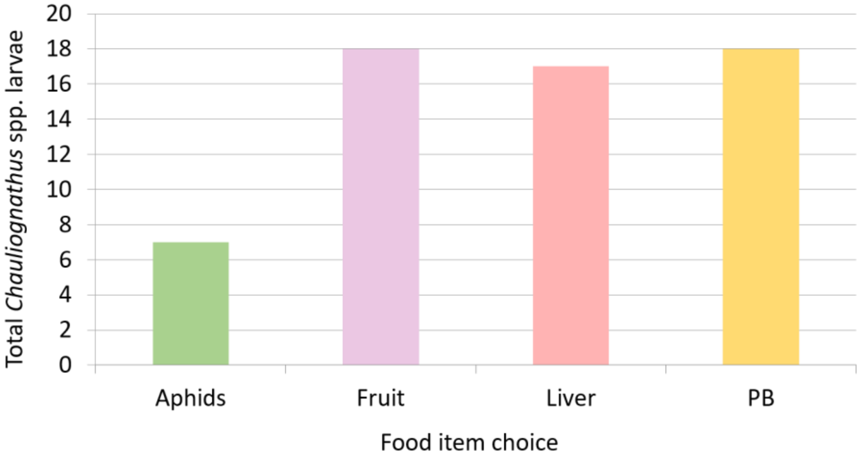

Figure 13: Food item choices made by 60 *Chauliognathus* spp. larvae in a nocturnal bioassay. Food items included sugarcane aphids (*Melanaphis sacchari* Zehntner), watermelon (fruit), cow liver (liver), and peanut butter (PB) in equal amounts. Four larvae (not shown) did not make a food selection within the 5-minute assay window. There were no significant differences in the number of larvae which chose each food item (x2=5.7, df=4, p=0.125).

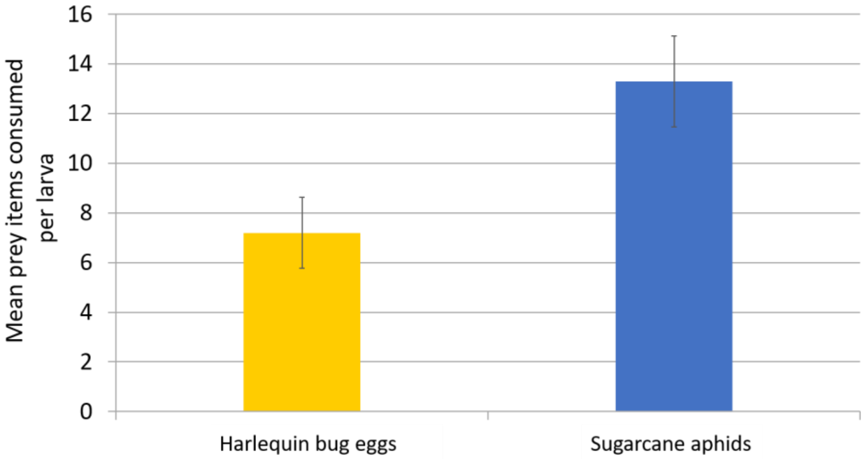

Figure 14: Mean number of prey items (harlequin bug [*Murgantia histrionica* Hahn] eggs or sugarcane aphids [*Melanaphis sacchari* Zehntner]) consumed in a 12-hour overnight period by a single *Chauliognathus* spp. larva. Error bars represent standard error of the mean. Total n=20 larvae.

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CHAPTER THREE

Analysis of host plant availability and use by two species of soldier beetles (Coleoptera: Cantharidae) using community-gathered digital specimens

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Abstract

Digital specimens collected by community members are a largely untapped data source of entomological data. By mining data from photo observations uploaded to online community data repositories, researchers can utilize this wealth of information to address questions about predator-prey interactions, phenotypic variation within species, plant-pollinator interactions, and a host of other topics. We mined data from photo observations on the community science website iNaturalist to investigate patterns in host plant availability and use by two species of soldier beetles, *Chauliognathus marginatus* (Fabr.) and *Chauliognathus pensylvanicus* (De Geer). *Chauliognathus marginatus* utilized white flowers more than expected based on their potential availability, while *C. pensylvanicus* utilized yellow flowers over all other flower colors and more than expected based on their potential availability. Communities of flowers available to and observed with *C. marginatus* and *C. pensylvanicus* differed significantly at the family and genus levels. Finally, laboratory color choice bioassays supported the overall conclusions drawn regarding *C. pensylvanicus* color preference, but were not consistent with those regarding *C. marginatus* color preference. To our knowledge, this is the first study to utilize a dataset mined and curated from community science photo observations to address species-specific behavior and ecology questions. With appropriate study design and analytical methods, these types of data

could prove invaluable in future investigations of a variety of entomological and ecological questions.

Introduction

Community science (CS), or the collection of data by non-expert volunteers, has a long history of use in governmental, academic, and private research (Audubon 2018)[2]. Community member-collected data have been used by the National Audubon Society (Sullivan et al. 2009), the Bill and Melinda Gates Foundation (Longbottom et al. 2018), the US Environmental Protection Agency, US National Oceanic and Atmospheric Administration and even the US National Weather Service, which has solicited weather data from non-scientist citizens since the start of their Cooperative Weather Observer program in 1890 (Freitag et al. 2016). The majority of papers using CS data assess the biodiversity of distinct geographic regions (Prudic et al. 2017, Li et al. 2019, Zambrano et al. 2019); report new, introduced, or endangered taxa in locations that were previously unreported (Hiller and Haelewaters 2019, Jones et al. 2019, Liebgold et al. 2019); or describe the movement or phenology of specific taxa through space and time (Goldstein et al. 2018, Longbottom et al. 2018). However, considerable potential exists for the use of these data to answer a wealth of other questions.

Just as preserved physical specimens provide research-relevant metadata such as collection date, location, environment, and associated species, digital specimens (e.g. photo observations on CS repository websites) can also serve as a source of data for further analysis. Photos uploaded to CS databases, such as eBird and iNaturalist, include not only observation date and location, but also all of the information contained within the photo. Data that can be extracted from these photos are limited only by photo quality and research question. Examples

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[2] Please see the referenced Audubon Society press release for a brief discussion about the terms “citizen science” vs. “community science”

include several types of animal interactions, for instance, predator-prey interactions in insects. Many iNaturalist photos show predatory arthropods consuming prey. Data mined from these photos could be analyzed to address questions about predator-prey interactions or patterns of predation across geographic ranges. Similarly, many iNaturalist and other CS photo observations show birds consuming arthropod prey. These types of photos have been analyzed to quantify the diet of Eastern bluebirds (*Sialia sialis* L.). This new dietary data has been used to encourage planting of native species to support arthropods that feed native birds (Kennedy 2019). Finally, up to 60% of the iNaturalist observations of bees show the insects visiting flowers (Gazdic and Groom 2019). This wealth of untapped information regarding plant-pollinator interactions could be analyzed to determine frequently visited flower taxa, spatial or temporal trends in flower visitations, prevalence of native vs. introduced flowers in pollinator visits, and countless other pertinent research topics.

While CS has a long history worldwide, iNaturalist (http://www.inaturalist.org) is a relatively new entity in crowd-sourced data generation. It began as a joint master's thesis project of students at the University of California Berkeley, and is now a joint initiative of the California Academy of Sciences and the National Geographic Society (Seltzer 2019). iNaturalist is a website where users upload photo, video, or audio observations of plants, fungi, or animals in nature. Observations can be uploaded directly to the iNaturalist website or by using the iNaturalist app. Once uploaded, observations are open to the public, and both the uploader and other users may suggest an identification for the organism in the observation. Additionally, the iNaturalist app itself uses artificial intelligence to suggest a likely identification based on the user's geographic region and the time of year (Horn et al. 2018). If an identification consensus is reached by 2/3 of commenting users and several other conditions are met (including a clear photo/video/audio record, geographic coordinates, and observation date) the observation is determined to be "research grade".

Because iNaturalist is a repository of observations made without specific hypotheses in mind, it is an example of surveillance monitoring rather than targeted monitoring, the latter being composed of observations made based on a priori hypotheses or conceptual models (Dickinson et al. 2010). This is unlike many other CS datasets in which data are collected by citizens but at the direction of a researcher attempting to answer a specific question (e.g., citizens sending in samples of plants from their backyards, or taking photos of specific interactions between animals). The inherent complexity of analyzing these data means that interdisciplinary collaboration is required (Dickinson et al. 2010). Research teams using iNaturalist data to answer questions should include not only biologists or ecologists, but statisticians, computer scientists, geospatial ecologists, applied mathematicians, and/or others, to ensure that proper techniques are being applied and the data are being appropriately analyzed.

To test the potential of digital specimen data for use in entomological studies, we investigated host plant affiliation in two species of beetles in the family Cantharidae: *Chauliognathus marginatus* (Fabr.) and *Chauliognathus pensylvanicus* (De Geer). These *Chauliognathus* species are common in eastern North America and are frequently seen on wildflowers, weeds, and other flowering plants (Williams 2006, Pelletier and Hébert 2014). *Chauliognathus marginatus* adults are active during the spring and early summer, while *C. pensylvanicus* adults are active during the late summer and early fall (Pelletier and Hébert 2014). They are medium-sized (8-15 mm), aposematically-colored beetles with various orange, yellow, and black markings on their bodies (Fig. 1) (Fender 1964, Pelletier and Hébert 2014). We chose these beetles for this study because of their distinctive appearances, diurnal activity, association with flowering plants, and relative popularity among gardeners, Extension professionals, and non-scientist naturalists (e.g. Mahr 2009, Raupp 2014, Cloyd 2015, Dietz 2018). These qualities make *Chauliognathus* spp. likely to be photographed by community members interested in either insects or wildflowers, which would increase the number and variety of observations submitted to a CS data repository site like iNaturalist.

Our goals were to: 1) test the use of digital voucher data in an entomological context; 2) evaluate differences among flower colors, families, or genera potentially available to *C. marginatus* and *C. pensylvanicus*; and 3) determine differences in flower utilization by *C. marginatus* and *C. pensylvanicus* according to flower color and community. We hypothesized that the flowers potentially available to each beetle species would differ in color and community composition, and that beetles would utilize flowers of different colors, families, and genera.

Methods

Data sources

All observation data were sourced from iNaturalist. We used only research-grade observations: those confirmed by a consensus of at least two-thirds of iNaturalist user identifications. We organized observations of *C. marginatus* and *C. pensylvanicus* using the "Projects" function within iNaturalist and then downloaded them using the "Export Observations" feature. Observations of flowers blooming during periods of *C. marginatus* and *C. pensylvanicus* activity were downloaded using the "Export Observations" feature and the search parameters listed below.

Flowers observed with C. marginatus *and* C. pensylvanicus

We downloaded all research-grade iNaturalist photo observations of *C. marginatus* and *C. pensylvanicus* from ca. 2000 to December 2019. Each photo was viewed to determine if it showed a beetle on a flower (*C. marginatus* n=1224, *C. pensylvanicus* n=3184). The primary petal color of that flower was classified as either brown, green, orange, red, blue, purple, pink, white, or yellow. When possible, flowers were then identified to plant family (*C. marginatus* n=1041, *C. pensylvanicus* n=3033) or genus (*C. marginatus* n=678, *C. pensylvanicus* n=1987) by the two authors with prior flower identification experience (J. Wagner and D. Hennen, Fig. 2).

Flowers potentially available to C. marginatus *and* C. pensylvanicus

The dataset of flowers potentially available to *C. marginatus* and *C. pensylvanicus* was composed of research-grade iNaturalist photo observations of flowering plants in the orders Asterales, Fabales, Lamiales, Myrtales, Malvales, Ericales, Brassicales, Piperales, Rosales, Ranunculales, Laurales, Solanales, Arecales, Caryophyllales, Proteales, and Sapindales. Flower observations were limited to those occurring in the same range of iNaturalist observations of *C. marginatus* and *C. pensylvanicus*, and their geographic range according to Pelletier and Hébert (2014) which includes the following American States, Canadian provinces, and Mexican states: North Dakota, South Dakota, Nebraska, Kansas, Colorado, Oklahoma, Texas, Minnesota, Iowa, Missouri, Arkansas, Mississippi, Louisiana, Wisconsin, Illinois, Indiana, Kentucky, Tennessee, Alabama, Michigan, Ohio, West Virginia, Québec, Maine, Vermont, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, Pennsylvania, New Jersey, Washington, DC, Virginia, North Carolina, South Carolina, Georgia, Florida, Nova Scotia, New Brunswick, Prince Edward Island, Ontario, and Nuevo León, MX. We took a completely randomized subset of 5000 photos taken during the periods of seasonal activity for each species in 2018, then eliminated photos that did not include a blooming flower. This subset was used to determine the flowers "potentially available" to each species (*C. marginatus* n=1348, *C. pensylvanicus* n=717).

We then classified flower color as either brown, green, orange, red, blue, purple, pink, white, or yellow (Fig. 3).

Adult C. marginatus *and* C. pensylvanicus *color choice bioassays*

To provide a real-life experiment to compare to trends observed in the digital voucher data, *C. marginatus* and *C. pensylvanicus* adults were allowed to select colored panels in a color choice bioassay. We collected *C. marginatus* by hand from white poison hemlock flowers (*Conium maculatum* L.) at Kentland Farm, Whitethorne VA (37.199690, -80.564945), and *C. pensylvanicus* from yellow wingstem flowers (*Verbesena alternifolia* L.) at Pandapas Pond Recreational Area, (37.281418, -80.474092), in their respective periods of activity in 2020. Prior to the assays, approximately 100 adult beetles per cage were starved for 24 hours in 30cm by 30cm pop-up rearing and observation cages (BioQuip Products, Inc., Rancho Dominguez, CA) in a sheltered outdoor location exposed to ambient light and temperature. We provided adults with non-flowering foliage for shelter and a cotton water wick for moisture. The pentagonal assay arena was composed of 15cm by 20cm panels of each red, white, yellow, green, and blue corrugated plastic panels (Coroplast, Vanceburg, KY) joined using clear plastic packaging tape. The arena floor (387cm^2) was constructed using brown corrugated cardboard. We removed groups of ca. 10 sexed or unsexed beetles from the pop-up cages, placed in a 50mL polypropylene conical tube (Falcon Tubes, Corning Life Sciences, Tewksbury, MA), and chilled for 2 minutes at 4°C. We then released the beetles into the center of the assay arena and observed them until each had climbed a colored wall of the assay arena to escape or until 5 minutes had elapsed. Each adult was only tested once and was then released. We tested a total of 167 *C. marginatus* (males n=88, females n=79) and 107 *C. pensylvanicus* adults (males n=57, females n=50).

Data analysis

We totaled the number of flowers of each color associated with *C. marginatus* and *C. pensylvanicus* and then calculate percentages. Percentage of flower families and genera associated with each species were calculated out of the total number of flowers that could be identified to family and genus, respectively. Similarly, percentages of flower color, flower families, and flower genera potentially available to *C. marginatus* and *C. pensylvanicus* were also calculated. Percentages of flower color were compared by *Chauliognathus* species (*C. marginatus* vs *C. pensylvanicus*), by season (spring vs fall), and by utilization (observed vs potentially available) using contingency tests (either Pearson's Chi-square test with Yates' continuity correction or Fisher's exact test depending on sample sizes) and post-hoc pairwise tests of independence with Bonferroni corrections. Analyses were carried out in R version 4.0.3 (R Core Team 2020) using the "stats" and "rcompanion" packages (Mangiafico 2020).

Because data collection of observed and potentially available flower families and genera occurred singly, with one photo taken per discrete unit of time, and not over multiple balanced, comparable, discrete sampling units (e.g., a once-weekly sampling day), those counts were compiled into summary vectors that could not be analyzed via typical community ecology analysis methods (Fig. 4A). To convert the actual count data into a form that could be analyzed, the count vectors were reshaped in R to form matrices of the single observations (Fig. 4B). These matrices were resampled using bootstrapping (sample size = 50, observations = 500) to produce matrices composed of hypothetical sampling data based on the distribution of the original data. (Fig. 4C). This data manipulation was carried out in R using the "base" package (R Core Team 2020). Once sufficient hypothetical data were generated (total n=200000 observations in 4000 discrete samples), tests of location and dispersion were used to analyze differences in community composition and spread between beetle species, potential flower availability, flower families, and flower genera. To determine differences in community composition, permanova tests were performed in R using the "vegan" package and *adonis* function (Oksanen et al. 2019). Cluster dispersion was analyzed using the "vegan" package and the *vegdist* and *betadisper* functions (Oksanen et al. 2019). Statistically significant differences in dispersion were detected with ANOVA, using the *anova* function in the "stats" package (R Core Team 2020). Differences in flower community cluster location and dispersion were visualized using principal coordinates analysis with the "ape" package and *pcoa* function (Paradis and Schliep 2019). All dissimilarity indices were calculated using the Bray-Curtis distance metric (Bray and Curtis 1957).

The percentages of male and female beetles that selected each color plastic panel were calculated for each beetle species. Differences between sex and species were found using contingency tests (either Pearson's Chi-square test with Yates' continuity correction or Fisher's exact test depending on sample sizes) and post-hoc pairwise tests of independence with Bonferroni corrections and an experimentwide *p*-value of 0.05.

Results

Flowers observed with C. marginatus *and* C. pensylvanicus

Of the 1224 iNaturalist observations of *C. marginatus* that occurred on a flower, 62.3% were on white, 14.3% on pink, 13.7% on yellow, 6.7% on purple, 1.3% on green, 1.0% on orange, and <1.0% on blue, brown, or red flowers (Fig. 5). In 85% of the *C. marginatus* observations that occurred on a flower, we could identify the flower to family. *Chauliognathus marginatus* was photographed visiting flowers in 42 families, with Asteraceae (42.3%), Apiaceae (19.4%), and Apocynaceae (17.0%) the most frequently observed. In 55% of the *C. marginatus* observations that occurred on a flower, we were able to identify the flower to genus.

Chauliognathus marginatus was photographed visiting flowers in 66 genera, with *Achillea* (15.9%), *Daucus* (15.9%), *Asclepias* (13.9%), and *Apocynum* (8.3%) the most frequently observed.

Of the 3184 iNaturalist observations of *C. pensylvanicus* that occurred on a flower, 53.4% were on yellow, 28.0% on white, 8.4% on purple, 7.7% on pink, 1.4% on orange, and less than 1.0% on red, blue, brown, or green flowers (Fig. 5). In 95% of the *C. pensylvanicus* observations that occurred on a flower, we could identify the flower to family. *Chauliognathus pensylvanicus* was photographed on 33 families, with Asteraceae (81.0%) representing the majority of observations. In 62% of the *C. pensylvanicus* observations that occurred on a flower, we could identify the flower to genus. *Chauliognathus pensylvanicus* was photographed on 76 genera, with *Solidago* (47.7%) and *Daucus* (9.9%) the most numerous.

Chauliognathus marginatus were observed significantly more than *C. pensylvanicus* on pink (x^2=43.437, df=1, p<0.001) and white (x^2=442.52, df=1, p<0.001) flowers. *Chauliognathus pensylvanicus* were observed significantly more than *C. marginatus* on yellow (x^2=568.06, df=1, p<0.001) flowers. There were no significant differences in the observations of *C. marginatus* and *C. pensylvanicus* on brown, green, purple, blue, orange, or red flowers. The communities of flowers observed with *C. marginatus* and *C. pensylvanicus* had significantly different centroid locations and dispersions at both the family and genus level (Fig. 6, Tables 1 and 2). This suggests that the communities observed with *C. marginatus* and *C. pensylvanicus* were composed of different families and genera, and that there were differences in the proportion of observations occurring on individual taxa in the flower community.

Flowers potentially available to C. marginatus *and* C. pensylvanicus

Of the 1348 flowers blooming during the period of *C. marginatus* activity in 2018 (April 1 to July 15) included in this analysis, 31.2% were white, 23.3% yellow, 17.0% purple, 16.9% pink, 6.8% red, 2.8% orange, 1.4% blue, 0.3% brown, and 0.2% green (Fig. 5). Flowers in 56 families and 319 genera were available to *C. marginatus*, with flowers in Asteraceae (21.4%), Fabaceae (12.9%), and Lamiaceae (8.9%) the most numerous families, and with no single genus composing more than 3% of the sample pool.

Of the 717 flowers blooming during the 2018 period of *C. pensylvanicus* activity (August 1 to September 30) included in this analysis, 29.6% were white, 25.7% yellow, 23.0% purple, 8.2% pink, 4.7% orange, 4.5% blue, 3.8% red, 0.4% brown, and 0.1% green (Fig. 5). Flowers in 44 families and 212 genera were available to *C. pensylvanicus*, with flowers in Asteraceae (39.7%), Fabaceae (8.6%), and Lamiaceae (5.3%) the most numerous families, and *Impatiens* (4.0%), *Lobelia* (3.9%), and *Solidago* (3.1%) the most numerous genera.

Significantly more pink flowers were blooming during the period of *C. marginatus* activity than *C. pensylvanicus* activity ($x^2=28.782$, df=1, $p<0.001$). Significantly more purple ($x^2=10.616$, df=1, $p<0.001$) and blue ($x^2=16.872$, df=1, $p<0.001$) flowers were potentially available to *C. pensylvanicus* than *C. marginatus*. There were no other significant differences in the proportion of brown, green, orange, red, white, or yellow flowers potentially available to the two species. The communities of flowers potentially available to *C. marginatus* and *C. pensylvanicus* had significantly different centroid locations and dispersions at both the family and genus level (Fig. 6, Table 1, Table 2). Again, this indicates that the communities of flowers potentially available to *C. marginatus* and *C. pensylvanicus* were different, and the distribution of individual taxa within those communities also differed.

Flower utilization by C. marginatus *and* C. pensylvanicus

By using the color distribution of flowers available to each species in 2018 as the expected color frequencies, we were able to determine whether the beetles were utilizing flowers of each color more or less than expected based on what flowers were blooming during their respective periods of activity. *Chauliognathus marginatus* utilized white (x^2=247.32, df=1, p<0.001) and green (p=0.002) flowers significantly more than expected based on the availability of those colors. Conversely, *C. marginatus* utilized yellow (x^2=37.941, df=1, p<0.001), purple (x^2=62.922, df=1, p<0.001), red (x^2=73.589, df=1, p<0.001), orange (x^2=10.432, df=1, p=0.001), and blue (p<0.001) flowers significantly less than expected. There were no significant differences in the utilization of brown or pink flowers (Fig. 7A). The communities of flowers observed with and available to *C. marginatus* had significantly different centroid locations and dispersions at both the family and genus level (Fig. 6, Table 1, Table 2). This indicates that *C. marginatus* utilized a very different community of flowers than the community made by the representative sample of what was blooming at the time.

Chauliognathus pensylvanicus utilized yellow flowers (x^2=179.1, df=1, p<0.001) significantly more than expected, and purple (x^2=126.46, df=1, p<0.001), orange (x^2=30.054, df=1, p<0.001), blue (x^2=98.184, df=1, p<0.001), and red (x^2=40.466, df=1, p<0.001) flowers significantly less than expected based on their availability. There were no significant differences in the utilization of white, pink, brown, or green flowers (Fig. 7B). The communities of flowers observed with and available to *C. pensylvanicus* had significantly different centroid locations and dispersions at both the family and genus level (Fig. 6, Table 1, Table 2). Similar to *C. marginatus*, *C. pensylvanicus* utilized a different community of flowers than what we would have expected based on the community of flowers blooming during its period of activity.

Color choice bioassays

Chauliognathus marginatus females showed no significant preference for any of the five color panels, with blue selected at the lowest frequency of 13.0% and red at the highest of 25.0% (Fig. 8A). *Chauliognathus marginatus* males selected the red panel (39.8%) significantly more than the yellow (17.0%), blue (8.0%), or white (8.0%), but not significantly more than the green (27.3%) panel **(**Fig. 8B**).** *Chauliognathus pensylvanicus* females showed a stark preference for the yellow panel (76.0%), which was chosen significantly more frequently than the red (12.0%), white (6.0%), blue (4.0%), or green (2.0%) panels (Fig. 8C). *Chauliognathus pensylvanicus* males showed a similar preference, with yellow (59.6%) selected significantly more frequently than red (39.0%); red, however, was selected significantly more frequently than the blue (5.3%), white (5.3%), or green (1.8%) panels (Fig. 8D).

Discussion

Field observations led us to hypothesize that *C. marginatus* would be found more frequently on white flowers and *C. pensylvanicus* on yellow flowers, and that this trend would be due to differences in the proportion of white and yellow flowers blooming seasonally. Except for pink, purple, and blue, the distribution of flower colors available to *C. marginatus* and *C. pensylvanicus* were not significantly different. The proportion of white, yellow, orange, red, green, and brown flowers available to the two species was roughly equivalent. However, the colors of flowers actually observed with each of these soldier beetle species showed significant differences, especially in white and yellow flowers. Over 60% of the flowers observed with *C. marginatus* were white, and over 50% of the flowers observed with *C. pensylvanicus* were yellow. Both species were observed with their preferred color at almost twice the frequency of that color’s potential availability to them. These data show that the beetles are indeed found more frequently with flowers of different colors, but not because more flowers of their preferred colors are blooming seasonally. This conclusion must be made with qualifications, however, because we did not match photos of potentially available flowers to *C. marginatus* or *C. pensylvanicus* microclimates or habitats, and therefore the flowers included may not have actually been available to all or even any *Chaulioganthus* spp. beetles. Matching the photos and including a larger sample size of potentially available flowers would help strengthen this conclusion.

Results of the color choice bioassay provide some support that the beetles' color preferences are not due to flower color availability. The strong yellow preference of *C. pensylvanicus* adults was expected, as these beetles are very commonly found feeding on *Solidago* spp., which are yellow, to the extent that goldenrod is the namesake of one of the beetle's common names, goldenrod soldier beetle (Williams 2006). However, the lack of a strong color preference in *C. marginatus* was completely unexpected and did not follow the results of the flower color analysis. We hypothesized that *C. marginatus* would choose the white panel most frequently, but instead, males chose the red panel and females chose all panels with no statistically significant differences. The red panel choice is especially surprising when compared to the distribution of colors observed with *C. marginatus* in the wild, where less than 1% of observations occurred on red flowers. This unexpected result should perhaps be explored further; it may be due to ultraviolet reflectance, which our color classification did not account for (Van Der Kooi et al. 2021). These color choice bioassays were limited by the color availability of plastic sheeting. In future experiments, all colors observed with the beetles in the wild should be included in the assay arena setup and flower colors should be compared to plastic sheeting colors using UV spectrophotometer readings.

Chauliognathus pensylvanicus and others in the *Chauliognathus* genus are frequently recorded on composites, flowering plants in Asteraceae, which was the family observed most often with both *C. marginatus* and *C. pensylvanicus* in this study (McLain 1984, Williams 2006, Pérez-Hernández 2018). While composites were the most numerically abundant family available to both beetle species, they were utilized at a far higher proportion than their availability. Indeed, composites were the most utilized family of flowers in both species of *Chauliognathus* beetles, with the remaining community of flower families composed of much smaller proportions.

Differences in the communities of flowers available to and utilized by *C. marginatus* and *C. pensylvanicus* may, in part, be explained by the proportion of composites versus the other families composing the pools. The vast majority (over 80%) of flower families photographed with *C. pensylvanicus* were composites, compared to about 40% of the available flower pool that was made of composites. In the remaining 32 families photographed with *C. pensylvanicus*, only four composed greater than 1% of the total observations, compared to the 16 of the 44 families available to *C. pensylvanicus* that made up greater than 1% of the sample pool. Based on this work and others, *C. pensylvanicus* showed a clear affinity for flowers in the Asteraceae family.

In contrast, though composites still made up a great proportion of the flowers photographed with *C. marginatus* (42.3%), they utilized a diversity of flowers from other families more frequently than *C. pensylvanicus*. Of the 42 families photographed with *C. marginatus*, 10 of those composed more than 1% of the total observations. *Chauliognathus marginatus* utilized composites as well, but relied on a more diverse variety of flower families than did *C. pensylvanicus*.

Differences in flower communities at the genus level were less intuitive to interpret. The sheer numbers of genera included made it difficult to pinpoint which genera may be driving the patterns exhibited. However, based on the results of the principal coordinates analysis, it is still clear that the genus-level communities of flowers observed with *C. marginatus* and *C.*

pensylvanicus differ in both composition and distribution. However, it seems that, unlike in the family-level analysis, the communities observed with and available to *C. pensylvanicus* showed wider distributions than that of *C. marginatus.* This is unexpected based on the high proportion of observations of *C. pensylvanicus* that occurred on flowers in the *Solidago* genus (48%).

This investigation warrants further study and would be greatly aided with a larger pool of flowers available to both beetle species. Additionally, floral anatomy should be taken into consideration. McLain (1984) found the greatest aggregations of *C. pensylvanicus* on composites that bloomed in clusters of small individual inflorescences and suggested that those flowers had the greatest horizontal surface area on which the beetles could feed, mate, and rest. Multiple inflorescences also provide copious amounts of nectar in a small space, which could signal optimal feeding habitat to these beetles regardless of color (Williams 2006). Other resources, such as space, food, shelter, and mates, may be influencing *C. marginatus* and *C. pensylvanicus* as much or more than color.

To our knowledge, this is the first study to utilize data mined from digital voucher specimen photos on a CS repository website to address species-specific behavior and ecology questions. By extracting and curating these data ourselves, we avoided many of the data quality pitfalls several others have discussed as possible reasons to avoid analyzing CS data, including incorrect identifications, overrepresentation of flashier or more “photogenic” individuals, and underrepresentation of organisms users frequently encounter (Dickinson et al. 2010, Pimm et al. 2015, Prudic et al. 2018). We argue that, because this study was designed carefully and with this exact dataset in mind, it is no more or less biased than a study based on scientist-collected survey data (Royle et al. 2007, Boakes et al. 2010). Using this study as a framework, the authors encourage other scientists to use the wealth of data available in CS repository websites to address similar questions throughout the entomological world.

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Author Contributions

Katlyn Catron: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization, Project administration **Derek Hennen:** Investigation, Writing – Review & Editing **Jennier Wagner**: Investigation, Writing – Review & Editing **Bryan Brown**: Software, Validation, Formal analysis **Thomas Kuhar:** Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Figures and Tables

Figure 1: *Chauliognathus marginatus* (left) and *C. pensylvanicus* (right) adults. Photos by Katlyn Catron.

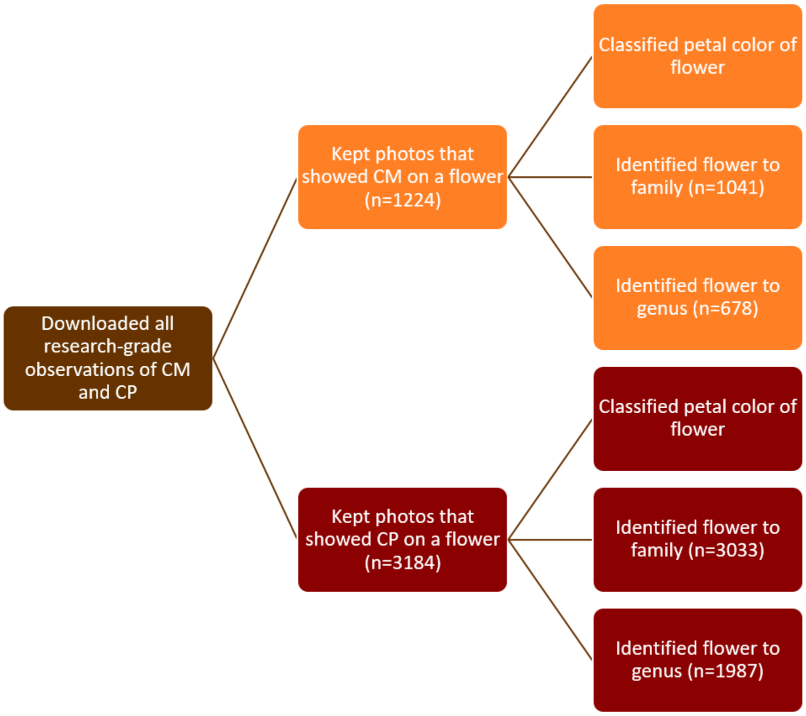


Figure 2: Flow chart of data collection protocol for data set of flowers observed with *Chauliognathus marginatus* (CM) and *C. pensylvanicus* (CP).

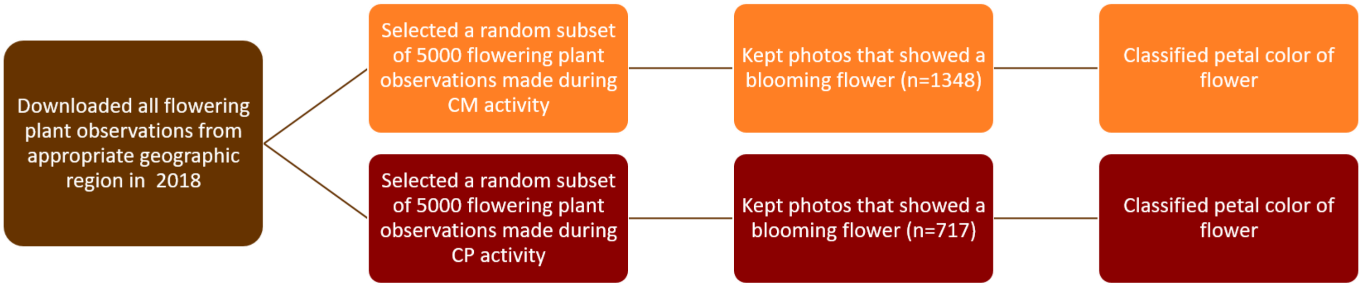
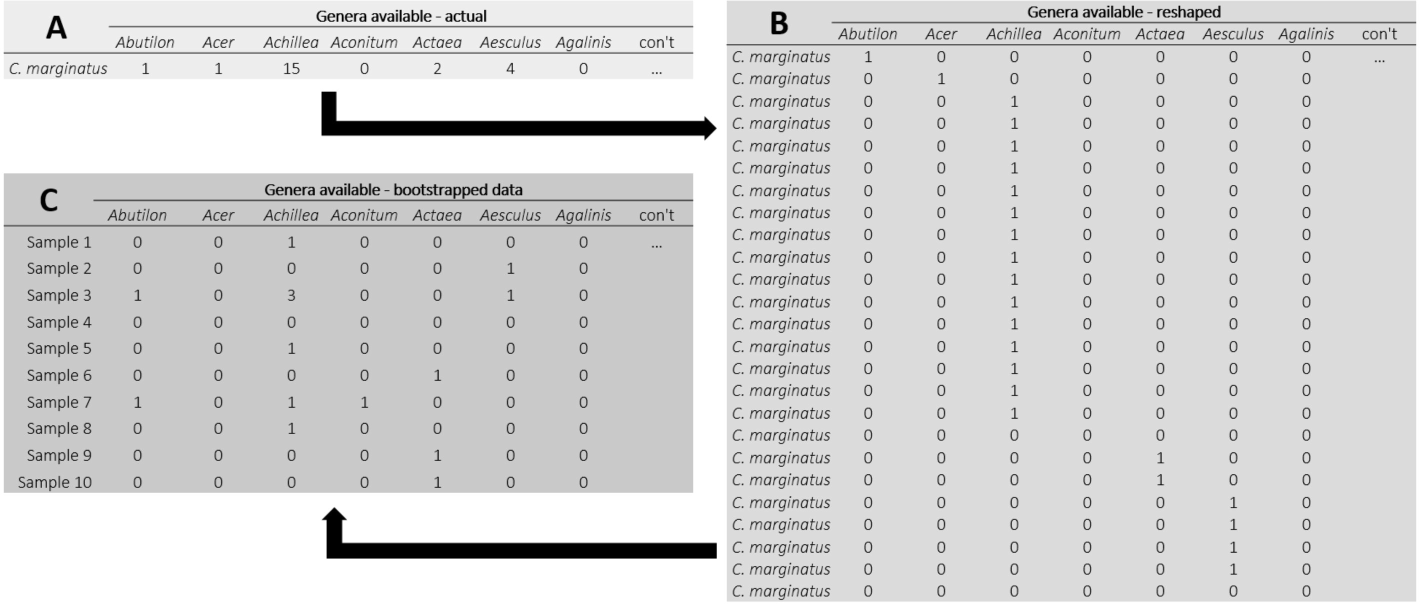


Figure 3: Flow chart of data collection protocol for data set of flowers potentially available to *Chauliognathus marginatus* (CM) and *C. pensylvanicus* (CP).



A

Genera available - actual

	Abutilon	*Acer*	*Achillea*	*Aconitum*	*Actaea*	*Aesculus*	*Agalinis*	con't
C. marginatus	1	1	15	0	2	4	0	...

B

Genera available - reshaped

	Abutilon	*Acer*	*Achillea*	*Aconitum*	*Actaea*	*Aesculus*	*Agalinis*	con't
C. marginatus	1	0	0	0	0	0	0	...
C. marginatus	0	1	0	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	1	0	0	0	0	
C. marginatus	0	0	0	0	0	0	0	
C. marginatus	0	0	0	0	1	0	0	
C. marginatus	0	0	0	0	1	0	0	
C. marginatus	0	0	0	0	0	1	0	
C. marginatus	0	0	0	0	0	1	0	
C. marginatus	0	0	0	0	0	1	0	
C. marginatus	0	0	0	0	0	1	0	
C. marginatus	0	0	0	0	0	0	0	

C

Genera available - bootstrapped data

	Abutilon	*Acer*	*Achillea*	*Aconitum*	*Actaea*	*Aesculus*	*Agalinis*	con't
Sample 1	0	0	1	0	0	0	0	...
Sample 2	0	0	0	0	0	1	0	
Sample 3	1	0	3	0	0	1	0	
Sample 4	0	0	0	0	0	0	0	
Sample 5	0	0	1	0	0	0	0	
Sample 6	0	0	0	0	1	0	0	
Sample 7	1	0	1	1	0	0	0	
Sample 8	0	0	1	0	0	0	0	
Sample 9	0	0	0	0	1	0	0	
Sample 10	0	0	0	0	1	0	0	

Figure 4: Example workflow of **A.** original vector of beetle count data as collected in Fig. 2 and Fig. 3 **B.** matrix of single observations reshaped from original vector data, and **C.** bootstrapped sample matrix of hypothetical data matching original distribution.

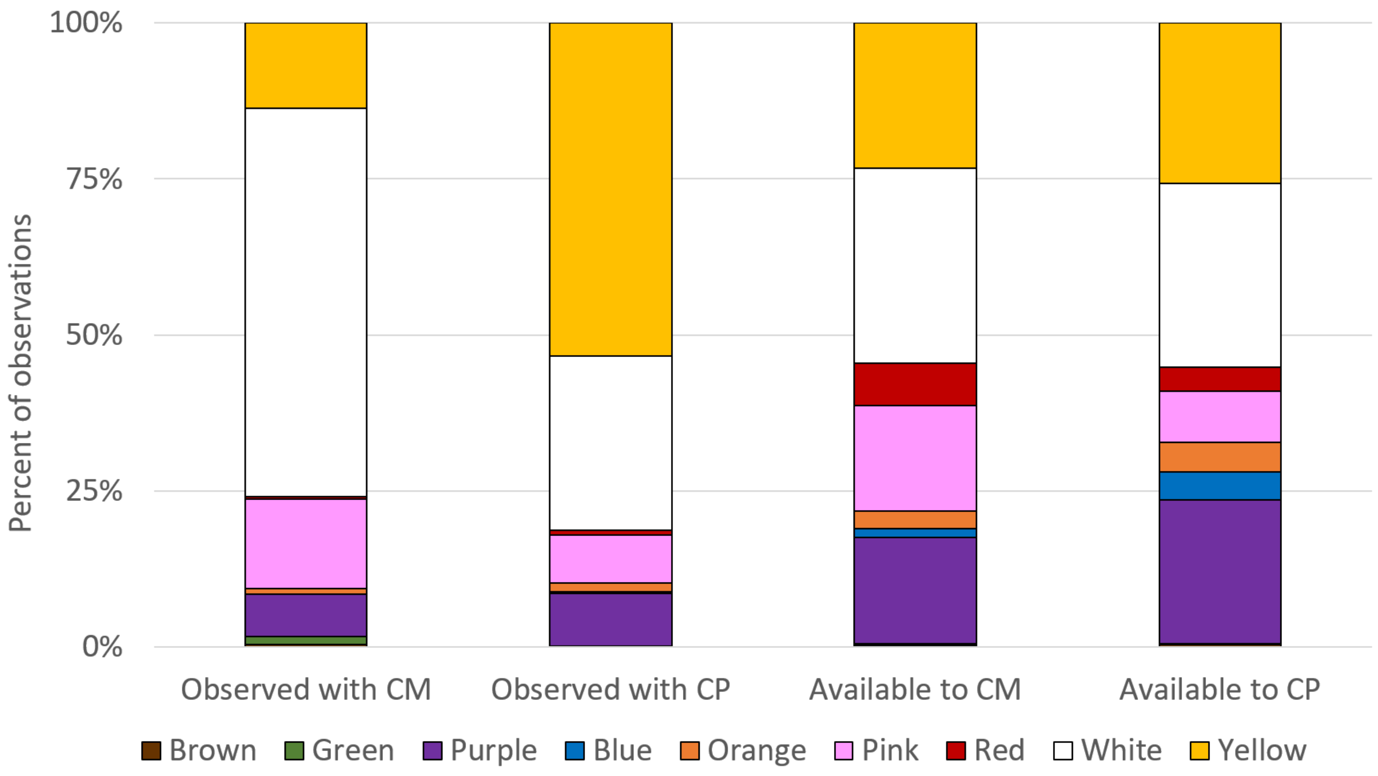


Figure 5: Color of flowers on iNaturalist observed with *Chauliognathus marginatus* (CM) from 2004-2019, observed with *Chauliognathus pensylvanicus* (CP) from 2000-2019, blooming during CM periods of adult activity in 2018, and blooming during CP periods of activity in 2018.

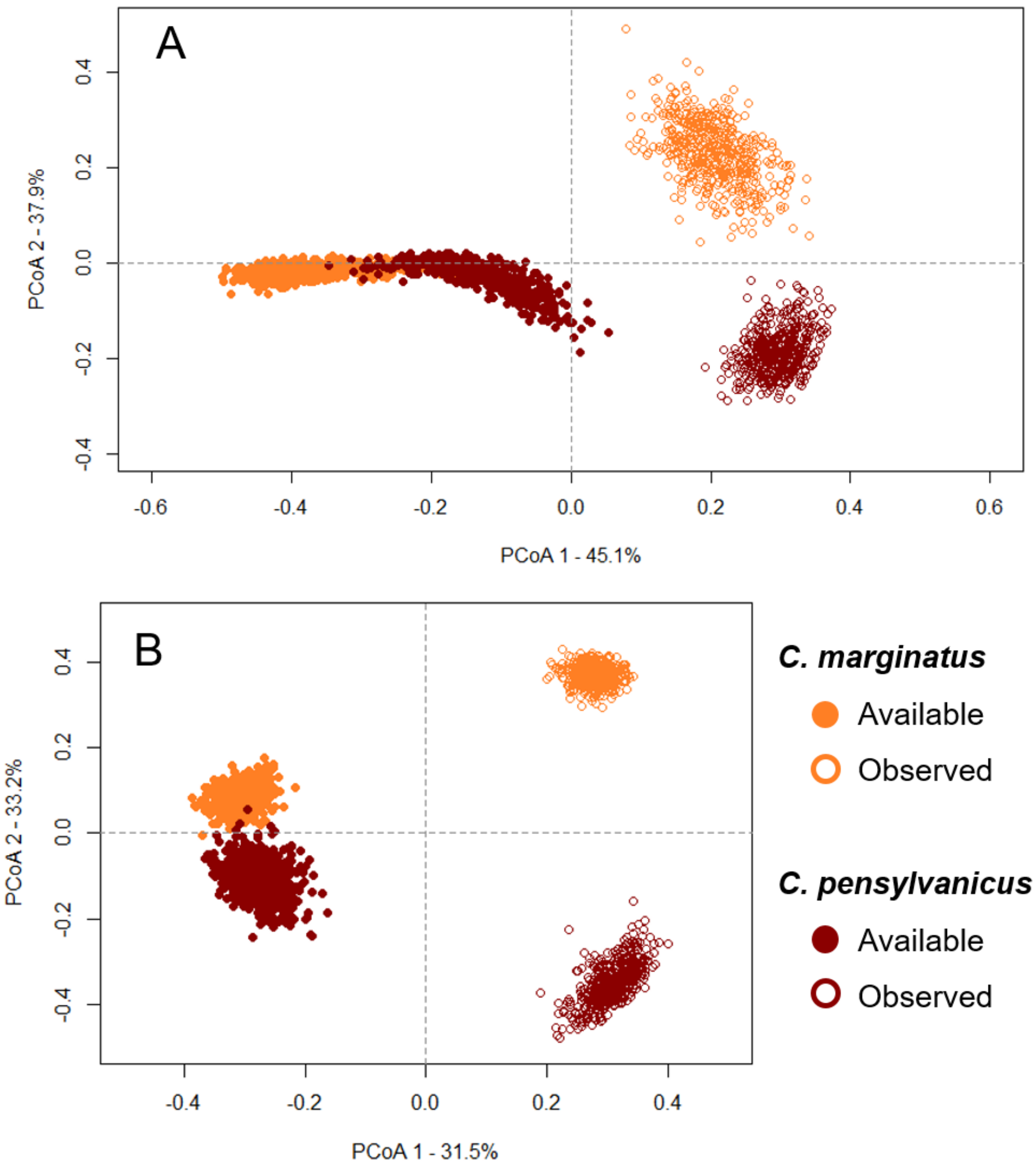


Figure 6: Principal coordinates analysis of flowers observed with (open circles) and available to (closed circles) *Chauliognathus marginatus* (orange) and *C. pensylvanicus* (maroon) at the **A.** family level and **B.** genus level. Axes are labeled with the amount of variation explained by each.

Table 1: Family-level differences in the communities of flowers observed with and available to *Chauliognathus marginatus* and *C. pensylvanicus*. Location test: permanova, dispersion test: beta dispersion.

Comparison				Results			
Species	**Flower type**	**Species**	**Flower type**	**Test**	***df***	***F*-value**	***p*-value**
C. marginatus	Observed (n=500)	*C. pensylvanicus*	Observed (n=500)	Location	1	2463.9	0.001
				Dispersion	1	1306.3	<2.2E-16
C. marginatus	Available (n=500)	*C. pensylvanicus*	Available (n=500)	Location	1	613.01	0.001
				Dispersion	1	253.42	<2.2E-16
C. marginatus	Observed (n=500)	*C. marginatus*	Available (n=500)	Location	1	2107.1	0.001
				Dispersion	1	1305.8	<2.2E-16
C. pensylvanicus	Observed (n=500)	*C. pensylvanicus*	Available (n=500)	Location	1	2314.2	0.001
				Dispersion	1	3776.1	<2.2E-16

Table 2: Genus-level differences in the communities of flowers observed with and available to *Chauliognathus marginatus* and *C. pensylvanicus*. Location test: permanova, dispersion test: beta dispersion.

Comparison				Results			
Species	**Flower type**	**Species**	**Flower type**	**Test**	***df***	***F*-value**	***p*-value**
C. marginatus	Observed (n=500)	*C. pensylvanicus*	Observed (n=500)	Location	1	2500.2	0.001
				Dispersion	1	254.99	<2.2E-16
C. marginatus	Available (n=500)	*C. pensylvanicus*	Available (n=500)	Location	1	273.12	0.001
				Dispersion	1	537.25	<2.2E-16
C. marginatus	Observed (n=500)	*C. marginatus*	Available (n=500)	Location	1	764.46	0.001
				Dispersion	1	15034	<2.2E-16
C. pensylvanicus	Observed (n=500)	*C. pensylvanicus*	Available (n=500)	Location	1	915.37	0.001
				Dispersion	1	13314	<2.2E-16

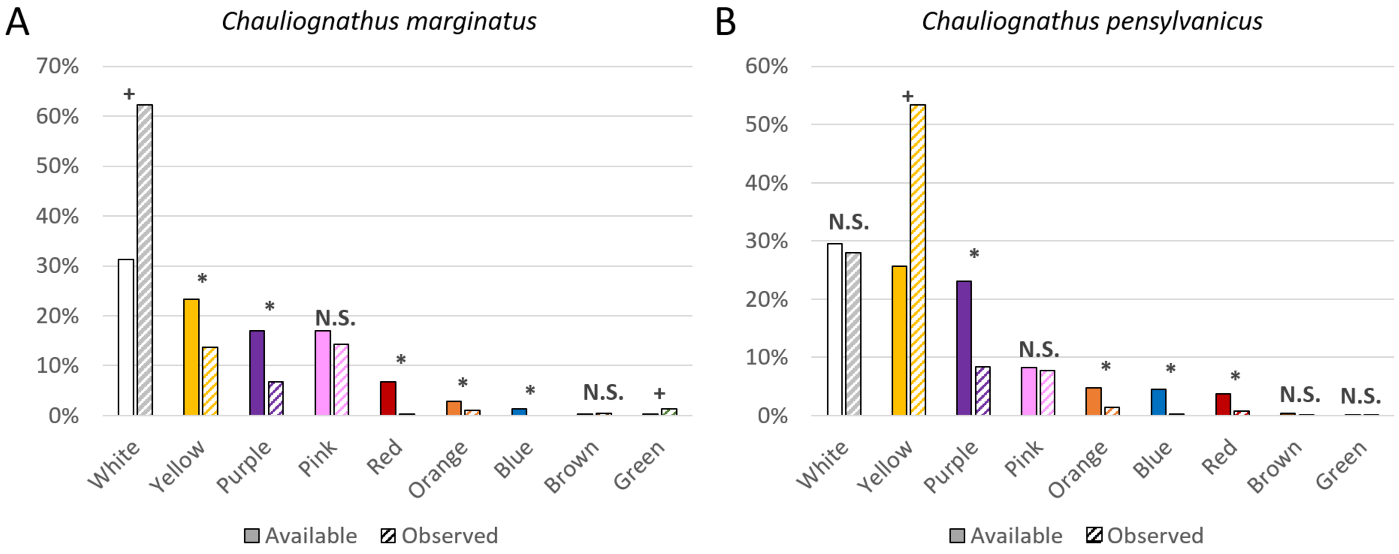


Figure 7: Utilization of flowers by *Chauliognathus marginatus* (**A**) and *C. pensylvanicus* (**B**) based on the colors of flowers available to them and the colors of flowers on which they were observed. Bar pairs headed with asterisks (*) indicate significantly more flowers of that color available than observed with the beetles. Bar pairs headed with plus signs (+) indicate significantly more flowers of that color observed with the beetles than available to them. Bar pairs headed with NS indicate no significant differences. All comparisons were made using Pearson’s Chi-square test with Yates’ continuity correction or Fisher’s exact test and post-hoc pairwise tests of independence with Bonferroni corrections.

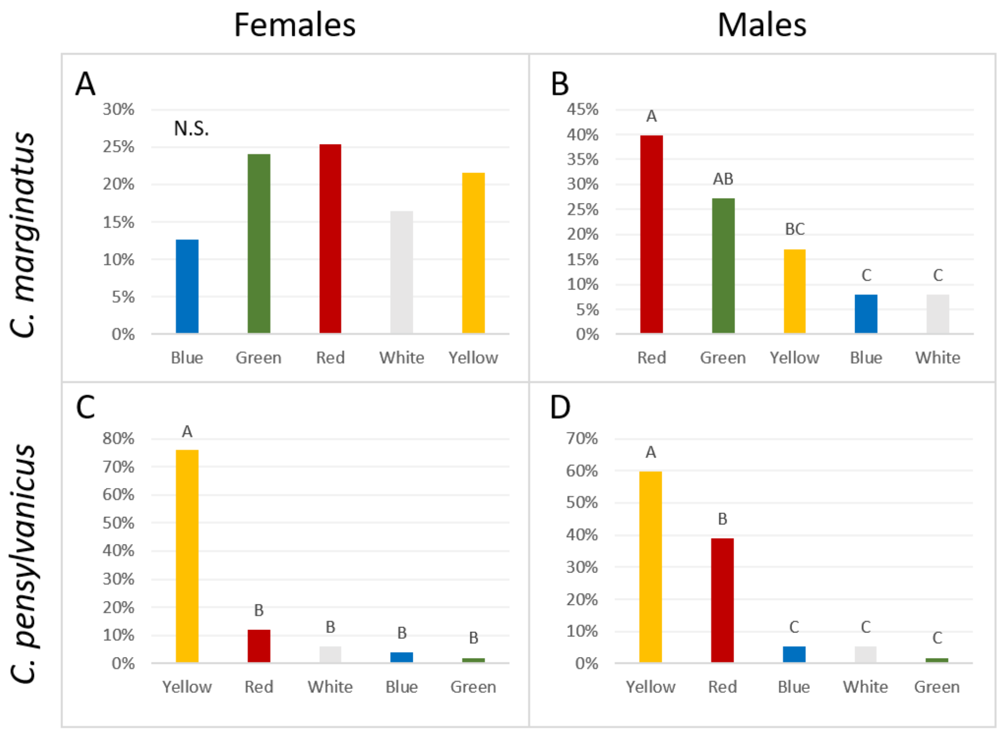


Figure 8: Percentage of beetles observed on different color panels in behavioral choice tests of: **A**. *Chauliognathus marginatus* females; **B**. *C. marginatus* males; **C.** *C. pensylvanicus* females; and **D**. *C. pensylvanicus* males. Bars headed by different letters indicate significant differences determined with Pearson's Chi-square test with Yates' continuity correction or Fisher's exact test and an experiment-wide Bonferroni-corrected *p*-value of 0.05. N.S. indicates no significant difference.

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CHAPTER FOUR

Effects of common vegetable insecticides on *Chauliognathus marginatus* adults

Part I: Introduction

Soldier beetles (Coleoptera: Cantharidae) are commonly-observed insects in agroecosystems, often visiting crops and surrounding vegetation when in bloom (Balduf 1935, McLain 1984, Williams 2006, Graham et al. 2012, Pelletier and Hébert 2014). Unfortunately, when frequenting crops such as fruit or vegetables, which are frequently treated with insecticides to protect them from economic loss due to pests (USDA-NASS 2007), the beetles are susceptible to nontarget chemical exposure.

A wealth of literature exists on non-target and sublethal effects of insecticides on beneficial insects (Croft and Brown 1975). Commonly researched insects and arthropods include pollinators, such as honeybees and bumblebees, predators, such as predatory mites and coccinellids, and parasitoids, such as aphelinid wasps (Michaud and Grant 2003, Desneux et al. 2007, Mužinić and Želježić 2018, Siviter and Muth 2020). Though present in a variety of agricultural systems, beetles in the family Cantharidae have not been well studied regarding their responses to the insecticides used in those systems. There is some information in the literature on this topic. Using a treated filter paper bioassay, Fernandes et al. (2016) showed that a South American cantharid species, *Chauliognathus flavipes* (Fabr.) was negatively impacted by low concentrations of the organophosphates chlorpyrifos and acephate, the pyrethroids deltamethrin and bifenthrin, and the neonicotinoids imidacloprid and thiamethoxam; effects of exposure included reduced feeding, repellency, reduced fecundity, and death. At typical field rate concentrations, the diamide chlorantraniliprole had no toxic effects on *C. flavipes* (Fernandes et al. 2016). Greener and Candy (1994) found that field-relevant rates of the beetle-targeting biological insecticide *Bacillus thuringiensis* var. *tenebrionis* (*Btt*) caused significant mortality in *Chauliognathus lugubris* (Fabr.), an Australian cantharid. Conversely, Beveridge and Elek (1999) found no reduction of predation, fecundity, or longevity in *C. lugubris* due to *Btt*, despite using a considerably higher (~3x) rate of the same product.

While the aforementioned studies provide information on the susceptibility of cantharids to some insecticides, newer, narrower-spectrum insecticides may have a place in preserving agricultural arthropod communities while still providing adequate pest control (Beveridge and Elek 1999, Castle and Prabhaker 2011). For instance, the neonicotinoid acetamiprid has been shown to be 100 times less toxic to honey bees than other neonicotinoids (Zhu et al. 2015). Insecticides such as flupyradifurone and sulfoxaflor have been shown to serve the same pest control niche as neonicotinoids, but with reduced nontarget impacts (Sparks et al. 2013, Bayer 2015, Nauen et al. 2015). In addition, the spinosad has been a versatile tool that has provided excellent control of pests such as lepidopteran larvae and thrips, while minimizing nontarget impacts because of its rapid degradation in sunlight (Studebaker and Kring 2003, Chapman et al. 2009, Biondi et al. 2012). Information on the toxicity of these insecticides on cantharid beetles and many other natural enemies is lacking.

Therefore, I evaluated the toxic effects of acetamiprid, flupyridifurone, sulfoxaflor, spinosad, and imidacloprid on adults of *Chauliognathus marginatus*, a common cantharid found on the foliage or fruit of vegetables and other crops in Virginia and throughout the U.S. In a second experiment, I evaluated several commonly-used OMRI-certified insecticides in organic production to determine lethal effects against *C. marginatus*.

Part II: 2019 Assays

Modified from Catron, K.A. and T.P. Kuhar. 2020. Effects of Insecticide Treatments on Soldier Beetle Adults, 2019. Arthropod Management Tests: 45(1) doi: 10.1093/amt/tsz094

Laboratory bioassays were used to evaluate the effects of several common vegetable insecticide formulations on adult *Chauliognathus marginatus*, commonly referred to as margined leatherwing beetles. *C. marginatus* is a frequent visitor and possible pollinator of several flowering vegetable plants in the eastern United States that may be exposed to insecticide formulations during normal activity.

Materials and methods

Treatments included Sivanto (10 fl oz/acre; AI: flupyradifurone), Closer (4.5 fl oz/acre; AI: sulfoxaflor), Admire Pro (1.3 fl oz/acre; AI: imidacloprid), Assail 30 SG (3 oz/acre; AI: acetamiprid), Blackhawk (3.3 oz/acre; AI: spinosad), and a water-only control. On 10 Jun, adult *C. marginatus* beetles and umbels were collected by hand from wild poison hemlock (*Conium maculatum* L.) at Virginia Tech's Kentland Research Farm in Whitethorne, Virginia. Three 10 cm-long poison hemlock umbels were dipped into 500 mL of an insecticide solution prepared at the per-acre field rate of formula, dried for approximately 30 minutes under a fume hood, inserted into a cube of water-saturated floral foam, and placed upright in a 475 mL deli cup. This was repeated for five replicates of each treatment. Nine beetles were confined in each deli cup and held at laboratory ambient light and temperature. At one and two DAT, beetle mortality was determined by prodding each insect and observing for movement. Percentage mortality data were analyzed with ANOVA and means separated with Tukey's HSD.

Results

Chauliognathus marginatus mortality in all treatments was higher than 20%, but Sivanto, Closer, Admire Pro, and Assail caused significantly higher mortality than Blackhawk or the water-only control (Fig. 1). There was no significant difference between mortality in the water-only control and the Blackhawk treatment.

Part III: 2020 Assays

Modified from Catron, K.A. and T.P. Kuhar. 2021. Effects of Organic Insecticide Treatments on Soldier Beetle Adults, 2020. Arthropod Management Tests: 46(1)
tsab031, https://doi.org/10.1093/amt/tsab031

Laboratory bioassays were used to evaluate the effects of several naturally-derived organic insecticide formulations on adult *C. marginatus*.

Treatments included Azera (32 fl oz/acre; AIs: azadirachtin and pyrethrins), Grandevo CG (2.1 lb/acre; AI: *Chromobacterium subtsugae* Martin), PyGanic EC 5.0_{II} (10 fl oz/acre; AI: pyrethrins), Venerate CG (3.2 qt/acre; AI: *Burkholderia* spp. strain A396), and a water-only check. The insecticides are all OMRI-certified, indicating that they are approved for use in organic growing conditions (OMRI 2021). Azera is a product with two active ingredients: azadirachtin and pyrethrins. Azadirachtin is an active ingredient in its own class and IRAC group UN, because it has an unknown or uncertain mode of action (IRAC International MoA Working Group 2020). It is an extract from plants in the genus *Azadirachta* (A. Juss), also called neem trees, and has been shown to have insecticidal and antifeedant activity against insects (Morgan 2009). Pyrethrins are an active ingredient in both Azera and in PyGanic. Pyrethrins are also plant-derived insecticides, made from extracts of flowers of *Chrysanthemum cinerariaefolium* (L., Schleier III and Peterson 2011). They are sodium channel modulators in their own class and the IRAC group 3A (IRAC International MoA Working Group 2020). Pyrethrins have the same mode of action as synthetically derived DDT and pyrethroids, so resistance is an issue (Schleier III and Peterson 2011). However, use of pyrethrins continues because of their status as a naturally derived product and their OMRI certification (OMRI 2021). Both azadirachtin and pyrethrins effect a broad range of insects, including beneficials and pollinators, and can enter the insect body in a variety of ways, including after eating contaminated leaves or pollen, or via contact with the cuticle (Schleier III and Peterson 2011, Mužinić and Želježić 2018). Grandevo is a product with the active ingredient *Chromobacterium subtsugae* strain PRAA4-1T, a soil bacterium with insecticidal properties against *Leptinotarsa decemlineata* (Say) and other insects, including other beetles, lepidopteran larvae, and hemipterans (Martin et al. 2007). Bacterial agents such as *C. subtsugae* are currently ungrouped in the IRAC classification and have a non-specific mode of action listed as "bacterial agents (non-*Bt*) of unknown or uncertain MOA" (IRAC International MoA Working Group 2020).

Materials and methods

On 13 Jul, adult *C. marginatus* beetles and flower sprigs were collected by hand from wild poison hemlock (*Conium maculatum*) at Virginia Tech's Kentland Research Farm in Whitethorne, Virginia. One 10 cm-long poison hemlock umbel was dipped into 500 mL of an insecticide solution that matched a spray tank concentration of the recommended per-acre field rate assuming 30 gallon per acre output, dried for approximately 30 minutes in ambient, sheltered outdoor conditions, inserted into a cube of water-saturated floral foam, and placed upright in a 475 mL deli cup. This was repeated for fifteen replicates of each treatment. A mean of eight beetles were confined in each deli cup and held at ambient, sheltered outdoor light and temperature. At one and two DAT, beetle mortality was determined by prodding each insect and observing for movement. Mortality data were analyzed with Pearson's chi-squared test and means separated with Bonferroni-corrected pairwise nominal tests of independence.

Results

Chauliognathus marginatus mortality in all treatments was higher than 10%, but Azera, Grandevo, and PyGanic caused significantly higher mortality than Venerate or the water-only check (Fig. 2). There was no significant difference between mortality in the water-only check and the Venerate treatment at one or two DAT.

Part IV: Discussion

Among the conventional insecticides that were evaluated, acetamiprid, flupyridifurone, sulfoxaflor, and imidacloprid all were quite toxic to *C. marginatus* adults, having significantly higher mortality than spinosad. This confirms other studies that have shown spinosad to be minimally or not toxic to arthropod predators (Elzen 2001, Studebaker and Kring 2003). Spinosyns are OMRI-certified and derived from naturally-occurring soil bacteria (Dripps et al. 2011). Insects can be exposed to lethal or sublethal doses of spinosad by eating treated plant tissues or contacting the insecticide with their cuticles, but spinosad is not a very long-lived insecticide in the field and has less effect on beneficial insects and bees when they are exposed to dried residues (Dripps et al. 2011). Because *C. marginatus* beetles were exposed to dried spinosad residues through contact and by eating treated pollen, the low mortality seen in this assay is reasonable.

The highest mortality was seen in the acetamiprid, flupyridifurone, sulfoxaflor, and imidacloprid treatments, and reached 97-100% mortality at 2 DAT. All four of these conventional (non-OMRI certified) products have active ingredients that use the nicotinic acetylcholine receptor (*n*AChR) competitive modulator mode of action (IRAC International MoA Working Group 2020). These nerve poisons force the insect’s nervous system to continuously fire, causing overload, paralysis, and death. Imidacloprid (Admire Pro), and acetamiprid (Assail) are neonicotinoid insecticides in IRAC group 4A (IRAC International MoA Working Group 2020). Neonicotinoids are commonly used because they have low mammalian toxicity and provide control of a wide spectrum of pests at low application rates (Nauen and Jeschke 2011). However, concerns about overuse and insecticide resistance have led to the development of novel *n*AChR competitive modulators that act slightly differently on nicotinic receptors, like sulfoximines and butenolides (Sparks et al. 2013, Nauen et al. 2015). Sulfoxaflor (Closer) is a sulfoximine insecticide in IRAC group 4C and flupyradifurone (Sivanto), is a butenolide in IRAC group 4D (IRAC International MoA Working Group 2020). Though they work in different enough ways to allow for insecticide resistance management and are specified for piercing-sucking pests, these four *n*AChR competitive modulators still have broad enough efficacy to cause mortality in other exposed insects, as was seen in this bioassay.

Of the OMRI-certified products tested in this bioassay, Azera (azadirachtins plus pyrethrins) had the highest mortality, followed by PyGanic (pyrethrins), and Grandevo (*Chromobacterium subtsugae* strain PRAA4-1T). Azera caused significantly greater mortality than PyGanic at 2 DAT, but not 1 DAT, and PyGanic caused significantly greater mortality than Grandevo at 1 DAT, but not 2 DAT. Azera has a low concentration of pyrethrins at 1.4%, compared to PyGanic, with a concentration of pyrethrins at 5.0%, but has the addition of 1.2% azadirachtin. This combination of AIs seems to have a greater mortality effect over time than the single AI found in PyGanic, even at a higher concentration. Grandevo, a bacterial product, has been shown to cause mortality on other plant-feeding beetles (Koivunen et al. 2009). Because insects in this study were restricted to feeding upon treated plant matter, it is plausible that they would suffer mortality as well.

Finally, mortality in the Venerate treatment was not significantly different from control mortality. Venerate contains *Burkholderia* spp. strain A396, another bacterial agent (non-*Bt*) of unknown or uncertain mode of action, and is also OMRI-certified (IRAC International MoA

Working Group 2020). *Burkholderia* spp. strain A396 has been shown to be less effective in controlling aphids and mealybugs than *C. subtsugae*, the bacterial agent in Grandevo, with *Burkholderia* spp. strain A396 mortalities generally remaining under 50% (Shannag and Capinera 2018). Shannag and Capinera's (2018) results align with ours, in that Grandevo caused significantly greater *C. marginatus* mortality than Venerate.

Control mortality was high in both the 2019 and 2020 trials, and also in other bioassays that were attempted but not included in this chapter. In general, *C. marginatus* seems a difficult beetle to try to maintain in captivity, and was sensitive to the slightest insecticide or even soap residues on the bioassay materials. Every precaution was taken to use brand new or meticulously cleaned equipment to capture and handle the insects, but control mortality still ranged from 15.2% at the lowest to 40.2% at the highest. Mortality was higher in the control treatment than in the Venerate treatment in 2020 at both 1 and 2 DAT, and standard error was considerably more variable in the control treatments than in most insecticide treatments. This indicates that mortality in the control varied considerably by replicate, and more replicates per treatment are necessary. With more replicates, hopefully control mortality would be less variable and lower overall, which would make comparisons among treatments more meaningful.

Sublethal effects, such as repellency, feeding inhibition, and reduced fecundity, have been shown as effect of pyrethrins, neonicotinoids, and azadirachtin in a variety of insects (Desneux et al. 2007, Biondi et al. 2012, Fernandes et al. 2016). However, because adults were contained in deli cups for the duration of the assay and the only food source was treated poison hemlock sprigs, significant sublethal effects are unlikely to have been noticed. If adults were repelled or their feeding was inhibited over the 48 hours, they would have likely either died of starvation or survived without ill effect. In field conditions, beetles that were irritated or repelled by the treatments used here could simply fly away before consuming treated plant material. For this reason, a more field-realistic assay would be to expose the beetles to a treated plant for a short (<15 minute) period of time before relocating them to a clean, insecticide-free deli cup with an untreated food source. By doing so, we could assess the results of a more likely exposure time on these beetles and also assess sublethal effects that were missed in this assay.

While several improvements could be made to increase field relevance and accuracy, these bioassays were a promising first look at the effects of several insecticides on *C. marginatus*. These beetles were highly sensitive to the butenolide, sulfoximine, pyrethrins, neonicotinoids, azadirachtin, and one bacterial agent (*C. subtsugae*) tested against them, but were less sensitive to the spinosyn or the second bacterial agent (*Burkholderia* spp. strain A396). Because of their frequent presence in vegetable and other agricultural systems, *C. marginatus* is likely to contact these and other insecticides in nature, and further insecticide assays should be conducted.

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Author Contributions

Katlyn Catron: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization, Project administration **Thomas Kuhar:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Figures

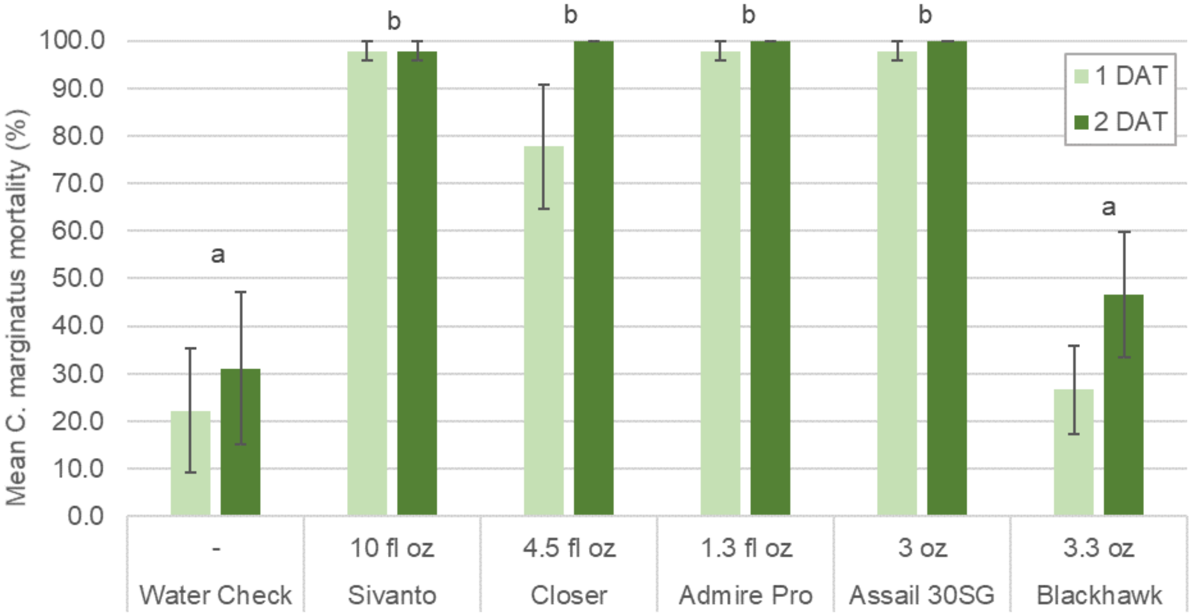

Figure 1: *Chauliognathus marginatus* mortality one and two days after exposure to five insecticide treatments and a water-only check. Product rate per acre is shown above product name. Error bars represent standard error of the mean. Bar pairs headed by the same letter are not significantly different; *P*>0.05, Tukey's HSD.

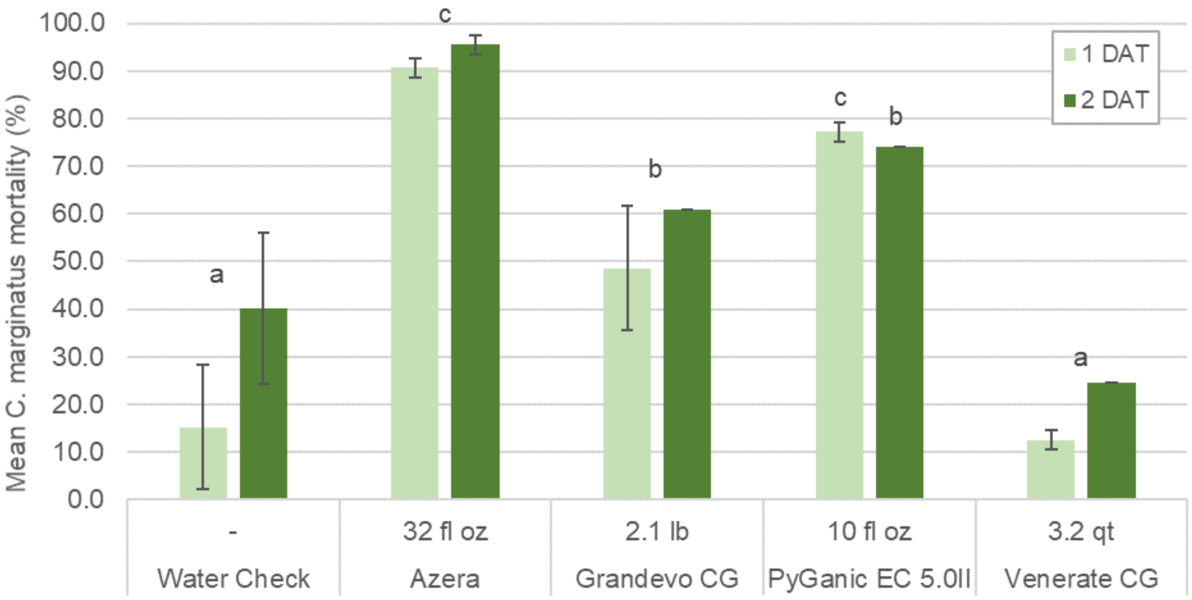

Figure 2: *Chauliognathus marginatus* mortality one and two days after exposure to four insecticide treatments and a water-only check. Product rate per acre is shown above product name. Error bars represent standard error of the mean. Bars headed by the same letter are not significantly different; *P*>0.05, Bonferroni-corrected pairwise nominal tests of independence.

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CHAPTER FIVE

Conclusions

This research focused on the biology of *Chauliognathus* spp. (Hentz) (Coleoptera: Cantharidae) in Virginia agroecosystems. Prior to this work, very little was known about their behavior, host plant preferences, or insecticide susceptibility. By focusing on basic questions about their biology, I have laid the foundation for more applied work on their ecological roles in agroecosystems in Virginia and throughout their native ranges.

I found that *C. marginatus* adults are active from mid-May to late July in Virginia, and they tended to aggregate on plants with large inflorescences with high nectar availability or production. They spent a lot of time mating and feeding, but these behaviors were variable throughout the course of the day and by beetle sex. I also found that community science data is an excellent resource for the analysis of plant-insect interactions as long as the research question and analytical methods are appropriate. Our analysis showed that not only do *C. marginatus* and *C. pensylvanicus* generally utilize flowers of different colors, but they also utilize a different and diverse set of flowering plants. Finally, I found that some conventional and organic insecticides cause significant mortality in *C. marginatus* adults, but not all of the insecticides I tested did.

I began this project with what I thought was a simple question: “Do soldier beetles have a role in the battle against agricultural pests?” While I was unable to answer that question fully in this dissertation, I have identified the next steps in approaching an eventual answer. Larvae are cryptic and surprisingly difficult to monitor, so I was unable to study their phenology and that knowledge gap remains. Determining when larvae are active and how that activity aligns with crop production would be crucial in predicting their effects on agricultural pest management. Similarly, using molecular gut content analysis to determine the diets of larvae in the field would definitively show whether these insects are consuming pests, crop plants, or both. While *C. pensylvanicus* is better represented in the literature, some fundamental research on its behavior and biology is still missing, and could provide an excellent point of comparison for what I found in *C. marginatus*. I do not know if *Chauliognathus* spp. will prove to be beneficial, neutral, or negative insects in agroecosystems, but I have laid the groundwork for others to keep working towards that answer.