Understanding Red Headed Flea Beetle Biology to Inform Sustainable Pest Management Practices in Virginia Nurseries

Eleanor Lynn Lane

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Sciences

in

Entomology

Alejandro Del Pozo-Valdivia, Chair

Thomas Kuhar, Member

Sally Taylor, Member

December 7, 2022

Virginia Beach, Virginia

Keywords: red headed flea beetle, ornamentals, open-field nurseries, integrated pest management, phenology, defoliation, insecticide bioassays

Understanding Red Headed Flea Beetle Biology to Inform Sustainable Pest Management Practices in Virginia Nurseries

By Eleanor Lane

Abstract: (Academic)

Systena frontalis (F.) (Coleoptera: Chrysomelidae), the red headed flea beetle (RHFB), is a ubiquitous pest of ornamental nursery crops in the eastern United States. Defoliation by adults renders plants unsaleable. Control costs and economic losses from injured plants have become a top concern among Virginia nurseries in the past decade. Current management tactics include spraying insecticides up to three times a week during the adult active season. The frequent use of insecticides poses a risk to the environment, non-target organisms, and evolution of resistance within the targeted beetle population. To develop a sustainable pest management program, more information is needed to understand pest biology, quantify the injury potential, and explore control tactics. Methods to monitor this beetle were used to observe peak activity throughout the growing season as well as the adult daily activity levels. There were three observed generations with population peaks in late June, late July, and late August into early September. Within the scale of one day, adult beetles were found to be diurnal with peaks in activity in the middle of the day. These findings will inform growers of the most efficient times to scout and spray, ultimately reducing insecticide usage. Quantification of feeding damage to individual leaves, the entire plant, and preference between older and newer growth may relate plant injury with specific population densities. The use of phenyl ethyl alcohol as an attractant lure, was explored to bolster pest control. Finally, insecticide bioassays were performed to compare those currently used to others yet untested.

Understanding Red Headed Flea Beetle Biology to Inform Sustainable Pest Management Practices in Virginia Nurseries

By Eleanor Lane

Abstract: (General Audience)

The red headed flea beetle (RHFB) is an important pest of ornamental crops in open-field nurseries along the Eastern United States. Defoliation caused by adult feeding renders plants unsellable. Japanese hollies, hydrangeas, and sweetspire iteas are among the most affected plants. Infested nurseries suffer severe economic losses due to insecticide costs and plant inventory reductions. Growers are spraying insecticides up to three times a week to manage RHFB. Frequent use of insecticides poses risks to the environment non-target organisms. This study will help improve control tactics for this pest and potentially minimize non-target effects by reducing insecticide usage. Understanding when beetles are active throughout the day, and throughout the growing season, may inform timing of scouting and insecticide sprays to maximize effectiveness. With populations highest in late June, late July, and late August, RHFB is estimated to have three generations per year. Adults are most active in the middle of the day and sedentary at night. It was observed how different densities of beetles cause injury to individual leaves and entire plants, and compared feeding preference on older versus newer growth. Chemical control tactics were explored including insecticides labeled and not labeled for RHFB such as iscycloseram. Finally, the effectiveness of phenyl ethyl alcohol as an attractant lure for RHFB was assessed and found ineffective within the open-field nursery ecosystem.

Acknowledgements

It is fitting that I begin these acknowledgements with thanking those who were instrumental to my success and interest in entomology in the first place. To James Wilson, who was my first entomology professor, thank you for making your class a wonderful experience and sparking my interest in what was to me a completely unknown field. To Jeremiah Foley, with whom I assisted in undergraduate research, thank you for your guidance and mentorship. The experience I gained was key in my discovery of entomology as a career path and for that I am eternally grateful.

I would like to thank my advisor, Alejandro Del Pozo, for his guidance and endless patience in helping me with my research. I have grown as a student and a scientist under his direction. Our innumerable meetings about everything from data analysis to field work led to this thesis coming out in one piece. He took a chance by taking me on as a student when I had little prior research experience, but a great passion for entomology and extension. I don't know that he was expecting his first student to be so stubborn, but he worked to mold me into a better researcher and helped me learn the ways of academia. As his first student from beginning to end, he always gave me his full attention and dedication so that I could succeed.

I would also like to thank my other committee members Thomas Kuhar and Sally Taylor for their help in fine tuning my research projects to make sure my work was well-rounded. They have been helpful in editing my papers, providing research advice, and further guidance on navigating graduate school.

My gratitude goes out to the Del Pozo applied ecology lab who were essential in my research coming to completion as well as my sanity staying intact. Julie Brindley, Elidah Sisk, Devin Calpo, Kaylee Armstrong, Joseph Leo, and Mireya Turcios helped with field work and brainstorming ideas as well as creating a welcoming and joyful work atmosphere.

In addition to the people within my own lab, it is essential to mention and thank the other members of the Hampton Roads Agricultural Research and Extension Center such as the farm manager, Rob Holtz, technician Greyson Dockiewicz, station director Jeffrey Derr, and professor emeritus Pete Schultz.

A special thank you goes out to my fellow grad students who suffered along with me and provided moral support over the past years. Sierra Bradley and Mollie Wyatt, you have been irreplaceable friends and supports during this time.

Shannon Bradley gets her own paragraph. I cannot thank her enough for being the best friend and roommate I could ask for. She has been the best person to come back to after long days in the field and in the office. Without our shenanigans, this degree would not be the same. To my other braincell, you have gotten me through some of the hardest times in my life and been there with me to celebrate my accomplishments no matter how small.

Last but not least, I want to thank my family for their support and love throughout my degree. To my parents, Lisa, and Snickers, you have all been there for me and I cannot express my gratitude enough for all you do for me.

Table of Contents:

List of Figures

List of Tables

Chapter 1: Red headed flea beetle in Virginia nurseries

Manuscript published on VCE (ENTO-464) as a pest profile

Introduction

The red headed flea beetle, *Systena frontalis* Coleoptera: Chrysomelidae, (RHFB) is a prominent pest of ornamentals in open-field nurseries. It has been endemic to the eastern United States since its discovery in the 1800's, but has only become an economic concern within the past decade throughout the eastern region.

Damage

The RHFB is mainly a defoliating insect, causing damage to the leaves of many ornamental plants. It may also feed on the flowers, however the damage to foliage is much more prominent and severe. The adult beetles will eat small holes into the leaves, often leaving skeletonized brown spots where they did not eat through the entire leaf (Fig. 1). Minute levels of damage can render a plant unsellable, meaning that any amount of RHFB damage present is unacceptable to nurseries. As few as five beetles per plant will cause noticeable damage to the foliage if left untreated. Just one beetle can damage over a quarter of a leaf in as little as 24 hours. The more beetles present, the more damage will occur, and the more it will cost growers to manage it.

Host plants

RHFB can feed on a wide variety of plant material but have shown considerable preference towards a few key plants. *Hydrangea paniculta, Itea virginica, and Ilex crenata* are some of the most greatly infested and damaged plants in Virginia nurseries. They have also been known to feed on viburnum, dogwood, forsythia, azalea, and at least ten other common They have been a pest on some crops like cranberries and blueberries.

Figure 1. RHFB foliar damage on *Hydrangea. paniculata*. Photo by Eleanor Lane

Figure 2. Top left: RHFB eggs, Top right: Larva RHFB, Bottom left: Adult RHFB (left: male, right: female) Bottom right: Adult RHFB. Photos by Eleanor Lane and Alejandro Del-Pozo

Identification

Adult RHFB are around 1/10-1/4 inch long, males being smaller than the females (Fig. 2). RHFB are named for their reddish

colored head that contrasts their shiny black body. From a distance however, the difference in coloration may be unclear. They have long antennae as well as robust back legs that allow them to jump like fleas. Their larvae live in the soil of containerized plants and can be difficult to find. They are around 1/4 inch long and white in color with a brown head capsule and a hair-like appendage at the end of their body (Fig. 2). The larvae do not cause measurable damage to infested plants. RHFB eggs are also found in the potting soil. The eggs are no larger than the head of a pin and are tan in color (Fig. 2).

Life cycle

Adults have been observed to emerge in late May and are active throughout the summer and into the fall (late October). The adults lay eggs throughout the summer, giving rise to up to three generations in one year. After the adults die off, their eggs remain in the potted soil over the winter. The eggs hatch and larvae are present in the spring. During the summer months when adults are active, they tend to move around more during the day. They will often be found jumping, flying, walking, mating, and eating. At night, the beetles can mostly be found sitting still on the plant leaves; resting, eating, or mating.

Monitoring

Monitoring is key in managing insect pests. Presence of RHFB can be monitored in a few different ways. Damaged leaves are a clear giveaway that there are insect pests present (Fig. 3). Confirmation of RHFB infestation can be made by finding the beetles themselves. During the day when they are active they can often be found by visual scouting. They will be visible on leaves, but sometimes are tucked away towards the base of the leaf near the stem. They hop like a flea or fly away when disturbed. In more densely packed plants or those with darker colored leaves they may be more difficult to see. They can be caught easily by using a sweep net across sections of plants. This helps show how many beetles may be present in a certain area.

Figure 3. Left: Japanese Holly with RHFB damage, Photo by Eleanor Lane. Right: Sweetspire Itea with RHFB damage, Photo by Alejandro Del-Pozo

Management

The most common method of managing adults is insecticide sprays. Currently, nurseries may spray up to twice a week when there are high levels of infestation. There are a variety of insecticide options that are effective at killing off adults. To avoid development of resistance, different mode of actions (or IRAC groups) can be alternated. Effective options include pyrethroids, neonicotinoids, and diamides. To read more about specific active ingredients used to control RHFB, please visit our Pest Management Guide series at https://resources.ext.vt.edu/

Systena frontalis life history and behavior as a native pest of ornamentals

The purpose of this thesis is to understand the biology of red headed flea beetle (RHFB, *Systena frontalis*, F. Coleoptera: Chrysomelidae) to inform sustainable pest management practices under open-field nursery conditions. Generating knowledge on RHFB interactions within its environment and specifically its injury potential, may allow nursery growers to approach management in a targeted manner. Reduction in levels of insecticide usage will enhance sustainability of affected nurseries, and welfare of the workers, non-target organisms, and the environment.

Red headed flea beetle is a native species that has become a major pest in nursery crops and ornamental plants in the midwestern and northeastern U.S. (Herrick and Cloyd 2020). The first published work describing its appearance and its feeding habit was over a century ago (Riley 1884). They are also known as 'cranberry flea beetle' in some regions as they affect cranberries (Dixon and Hillier 2002). As of 2022, it is also a pest of nursery crops and ornamental plants, causing economic losses due to defoliation when present in significant numbers.

The second chapter of this thesis discusses the seasonal life history and daily behavior of the RHFB in commercial nursery conditions. The RHFB has egg, larval, pupal, and adult life stages and timing of their life cycle varies by geographic location. In laboratory studies, three larval instars were observed with time between eclosion and the mature larval stage being 30 days (Jacques and Peters 1971). They overwinter as eggs in the soil of containerized plants where larvae will feed and pupate until adult emergence (Herrick and Cloyd, 2020).

Larvae feed on roots of host plants, but do not inflict significant injury. Larvae are creamy white, around 5-10mm long with a sclerotized head capsule, and a posterior tube-like tubercule with six small anterioventral legs. Adults are black and have a brownish red head, with black filiform antennae about twice as long as the head's width, and robust saltatorial hind legs. Larvae are expected to hatch from the eggs between 250-480 growing degree days in base 50°F or 10℃ (Lauderdale 2017). Generally, larvae are expected to be present beginning in late spring (April). Adults emerge throughout summer and there could be up to three generations per year, however the number of generations is not known in eastern Virginia. While these times have been studied in laboratory settings, existence of multiple generations implies that life cycles during the active season are based on temperature and humidity needs which fluctuate naturally. Two scouting methods (vacuum and visual) are investigated in the second chapter. Viability of phenyl ethyl alcohol as an attractant lure was tested based on previous work proposed by Braasch and Kaplan 2012. Testing lure effectiveness may confirm its use to attract RHFB and potentially pull them away from valuable plants in addition to supplementing monitoring methods.

Further, the second chapter discusses RHFB behavior by detailing the extent of foliar damage. The third chapter explores and specifically examines feeding behavior and percent injury to hydrangeas in choice and non-choice assays. Injury inflicted by adult RHFB feeding on foliage appears as holes or browning of tissue with skeletonization. These polyphagous pests cause unsightly damage that is unacceptable for nurseries who value the high quality of their products. Hydrangeas, iteas, and hollies comprise large portions of ornamental nursery sales and they are among the most affected plants (Herrick and Cloyd 2020). High RHFB infestations cause considerable economic losses to affected nurseries (Cooper and Rephann 2017).

Frequent insecticide sprays are currently the most effective method for growers to control RHFB adults. In the active season, they may spray up to three times a week. The fourth chapter of this thesis assesses current insecticides and compares them to others untested on RHFB adults in ornamentals. Between the losses from unsold crops and the cost of insecticide sprays, these nurseries are working under an unsustainable model. Learning more about the biology of the RHFB can provide insight on improving the efficiency of scouting and subsequently timing of foliar applications, which ultimately will result in reducing spraying of insecticides.

Pest management tactics such as scouting and broadcast insecticide sprays can be supplemented with monitoring information to improve efficacy. Data on when the beetles are most active throughout the season and the day can inform scouting procedures. Combining information from these projects will suggest a holistic framework to improve the IPM program for Virginia nurseries. The research objectives of this thesis are: 1.) monitor the phenology and behavior of RHFB across the nursery landscape, over the course of the active season, and within the span of 24 hours, 2) determine the extent of defoliation and RHFB host preference, and 3) explore alternative chemical alternatives to currently used insecticides.

References

Braasch, J., and I. Kaplan. **2012**. Over what distance are plant volatiles bioactive? Estimating the spatial dimensions of attraction in an arthropod assemblage. Entomol. Exp. Appl. 145: 115–123.

Cooper, W., and T. J. Rephann. **2017**. The economic impact of Virginia's agriculture and forest indusTries.

Dixon, P. L., and N. K. Hillier. **2002**. Insect pests of wild cranberry, Vaccinium macrocarpon, in Newfoundland and Labrador. Phytoprotection. 83: 139–145.

Foye, S., and S. Steffan. **2019**. Two native Wisconsin nematodes represent virulent biocontrol agents in cranberries. Biol. Control. 138: 104042.

Guédot, C., and R. S. Perry. **2015**. Evaluation of Soil and Foliar Applications of Insecticides for the Control of Flea Beetle in Cranberry, 2014: Table 1. Arthropod Manag. Tests. 40: C9.

Herrick, N. J., and R. A. Cloyd. **2020**. Overwintering, Host-Plant Selection, and Insecticide Susceptibility of *Systena frontalis* (Coleoptera: Chrysomelidae): A Major Insect Pest of Nursery Production Systems. J. Econ. Entomol. 113: 2785–2792.

Jacques, R. L., and D. C. Peters. **1971**. Biology of Systena frontalis with Special Reference to Corn. J. Econ. Entomol. 64: 135–138.

Joseph, S. V., J. H. Chong, B. Campbell, B. Kunkel, D. Lauderdale, S. Jones, S. Gill, Y. Chen, P. Schultz, D. Held, F. Hale, A. Dale, E. Vafaie, W. Hudson, D. Gilrein, and A. Del Pozo-Valdivia. **2021**. Current Pest Status and Management Practices for Systena frontalis (Coleoptera: Chrysomelidae) in Ornamental Plants in the Eastern United States: An Online Survey. J. Integr. Pest Manag. 12: 17–18.

Lauderdale, D. 2017. Red-headed Flea Beetle Biology and Management. Nurs. Landsc. Notes.

33–35.

Lauderdale, D. 2021. Red-Headed Flea Beetle Management in Container Nursery Production.

Main, A. R., M. L. Hladik, E. B. Webb, K. W. Goyne, and D. Mengel. **2020**. Beyond neonicotinoids-Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. Sci. Total Environ. 742.

Riley, C. V. 1884. Reports of Observations and Experiments in the Practical Work of the Division. Washington.

Sparks, T. C., and R. Nauen. **2015**. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121: 122–128.

Chapter 2: Temporal and spatial factors influencing *Systena frontalis* (Coleoptera: Chrysomelidae) phenology and behavior in Virginia nurseries

Manuscript submitted to Environmental Entomology 12/01/2022

Abstract

Ornamentals in eastern Virginia nurseries have been greatly impacted by red headed flea beetle (RHFB), *Systena frontalis* F. With the advent of the RHFB as a prevalent pest in the past two decades, baseline behavior and phenology are currently understudied within Virginia open-field nurseries. The RHFB is costly to control due to insecticide expenses and loss of saleable plants. In 2021 and 2022, RHFB populations were monitored at two commercial nurseries in eastern Virginia in order to better understand their temporal and spatial population dynamics. Patterns that emerged indicated RHFB populations have three generations in eastern Virginia, with peak s of adults in late June, July, and August. Phenylethyl alcohol attractant lures were tested as potential expedient monitoring but found to be ineffective as such. On an individual scale, the study of RHFB behavior throughout the day and amongst individual plants demonstrated their damage potential and activity patterns. Diel monitoring demonstrated RHFBs are most active from 1100-1500. Sedentary beetles, including those feeding, were found at all times of the day and throughout all levels of plant canopy evenly. Percent defoliation increased linearly with increased density of beetles, where five beetles defoliated up to 4% of a hydrangea plant in one week. Timing of scouting and insecticide sprays according to the RHFB activity peaks of the day and across the season may allow reduction in overall insecticide usage.

Key words

Ornamentals, growing degree days, phenology, diel activity, non-choice assays

Introduction

The red headed flea beetle, RHFB, *Systena frontalis* is a species long familiar to the United States (Riley 1884) (Chittenden 1902), and has recently become a major pest in nursery crops in the midwestern and northeastern U.S. regions (Herrick and Cloyd 2020). This species is also called 'cranberry flea beetle' since it is a pest in that system (Jaffe et al. 2021). *S. frontalis* is also a key economic pest of ornamental plants grown outdoors (Joseph et al. 2021). Although it was first described many decades ago, there are few recent studies on its biology (Herrick and Cloyd 2020, Jaffe et al. 2021, Joseph et al. 2021). In terms of commercial nursery operations, this pest is not well understood. There have been no studies observing daily activity levels or peak seasonal activity for this insect in ornamentals or any crop system in Virginia.

Adult RHFBs defoliate a variety of woody and herbaceous crops such as grapes, cabbage, beets, potatoes, corn, beans, clover, gooseberries, mangelwurzels, and pear leaves (Jacques and Peters 1971) and ornamentals such as *Itea* sp., *Hydrangea* sp., *Cornus* sp. (dogwood), and *Weigela* sp. (Cloyd and Herrick 2018). Damage from RHFB is a top concern for Virginia nurseries and can reduce crop yield and increase production costs for infested ornamental plants by rendering those unsellable due to foliar injury (Joseph et al. 2021). Damage inflicted on foliage appears as shot-holes or skeletonization in leaves, often with a browning or whitening of the tissue around the hole depending on the type of plant (Lane and Del Pozo-Valdivia 2021). Hydrangeas, Iteas, and Ilex have been particularly affected within open-field nurseries (Herrick and Cloyd 2020). Timing of the RHFB life cycle varies by geographic location. In Iowa, one generation was observed with adults emerging in late July (Jaffe et al. 2021). In laboratory studies, three larval instars were observed with time between eclosion and the mature larval stage being 30 days

(Jacques and Peters 1971). Red headed flea beetle overwinters as eggs in soil and the development time to eclosion is estimated to be around 15 weeks at temperatures between 0 to 5°C, and time from egg hatch to adult emergence was around 40 days at 20°C (Jaffe et al. 2021). Larvae feed on root hairs and pupate without causing detectable damage to the infested plant until adult emergence (Herrick & Cloyd, 2020). Generally, larvae are present beginning in late spring (March to April). In North Carolina, RHFB eggs have been observed to hatch between 250-480 growing degree days, calculated at base 50°F (10°C). First generation adults have emerged from infested nursery pots between 517 to 1028 GDD_{50}, second generation larvae at 1570 to 1860 GDD_{50}, and second-generation adults at 1878 to 2318 GDD_{50} (Lauderdale 2017). These GDD models are important for timing of insecticidal sprays (Rice et al. 1984).

Nursery growers in Virginia are spraying insecticides up to three times a week to control RHFB adults. Growers rely on applications of either pyrethroids, neonicotinoids (Joseph et al. 2021), or organophosphates (Lane and Del Pozo-Valdivia 2022) to reduce populations of this pest. The aforementioned insecticides are harmful to beneficial insects such as pollinators (Main et al. 2020), leading to Environmental Protection Agency guidelines specifying mitigation practices to avoid such issues. Moreover, continued use of these insecticides may result in the development of resistance in these beetles (Sparks and Nauen 2015), in addition to negatively impacting non-target organisms, such as predators, parasitoids, and other naturally occurring beneficial insects. Reducing beneficial insect populations can cause secondary pest outbreaks, such as thrips and mites, under nursery settings (Hajek and Eilenberg 2018). Insecticide testing within cranberry crop systems (Guédot and Perry 2015) and on hydrangea leaves (Lane and Del Pozo-Valdivia 2022) show that few chemicals are effective. Potential options within nurseries include the novel

IRAC Group 30 insecticide, isocycloseram, which has yet to become commercially available as of 2022. Insight into RHFB biology is essential to improve the efficiency of any proposed control tactic.

Chemical control methods like spraying new classes of insecticides could be expensive, and effective options have become less available due to regulatory restrictions. While it is evident that RHFB population dynamics could be influenced by geographical location and weather, they may also respond to herbivore induced plant volatiles (HIPV) (Braasch and Kaplan 2012). Cultural control methods such as synthetic deployment of phenyl ethyl alcohol (PEA), a HIPV, have been studied to determine attractant or repellent properties within insects (Braasch and Kaplan 2012). Phenylethyl alcohol, presented as a lure, was distributed across at least 8m between experimental units, and despite the naturally repellent effect on herbivorous insects, the RHFB was attracted to those units (Braasch and Kaplan 2012). Previous studies had only collected samples on sticky cards, but vacuum collection of samples (Buffington and Redak 1998), could ensure capture of a broader range of insects for evaluation of lure efficacy.

As a continued effort to address gaps in current knowledge of the RHFB, observations and experiments regarding phenology, biology, and behavior were conducted within the context of ornamental production in eastern Virginia nurseries. The goals for this project were 1.) to monitor the seasonal phenology of adults and larvae as well as their movement throughout the landscape; 2.) to observe daily activities performed by RHFB adults to determine timing of activity levels in field conditions; 3.) to explore further control options PEA attractant lures were investigated within open-field nursery settings, aiming to create an additional monitoring tool

and a mass trapping system; and 4.) to quantify the feeding potential of adults throughout canopy layers and between varying densities in Hydrangea plants. These objectives provide insight for open field nurseries that are vastly different than other systems which have been studied thus far, such as cranberries (Jaffe et al. 2021), or nurseries in North Carolina (Lauderdale 2017).

Materials and Methods

Seasonal monitoring of RHFB populations

Each week from May to September of 2021, RHFB populations were scouted at two commercial nurseries in eastern Virginia (Nursery 1, near the town of Smithfield; and Nursery 2, in the City of Suffolk), or at the HRAREC (Hampton Roads Agricultural Research and Extension Center in the City of Virginia Beach). A Stihl leaf blower (model SH 56 C-E, Virginia Beach, VA) set to vacuum with a mesh bag over the end was used to suction beetles from crops to count number of beetles per plant in nursery blocks containing *Hydrangea paniculata, Itea virginica,* or *Ilex crenata*. Each sampling point consisted of three individual plants. Visual scouting for presence of foliar damage occurred at each sampling point and if no damage was detected, vacuuming did not occur. This was modified by the third week of August when scouting procedures were updated to those used in 2022.

The same leaf blower was used to sample the number of beetles per plant at the same three locations weekly from March to September in 2022. Three blocks of three sampling points each were collected with each location chosen by moving diagonally across the commercial blocks in a zig-zag pattern (Buffington and Redak 1998). A visual estimation of percent defoliation of each vacuumed plant was also recorded at each sampling point. To calculate the growing degree days, associated with RHFB phenology events, air temperature was collected from a HOBO data

logger (model MX2301A, Onset, Bourne, MA). These loggers were placed on a representative area of the nursery, dominated by grasses. The following equation was used to calculate these growing degree days (GDD_{50}): $\frac{(Temp\ high + Temp\ low)}{2} - 50°F$ (Lauderdale 2017). In addition to the monitoring of adults, larvae within the potting soil mix of containerized hydrangeas were counted weekly from May to September 2022. The outer two inches of soil in two hydrangeas from the HRAREC block were scraped off the containerized plant weekly and examined for presence of RHFB larvae.

Movement of RHFB across the landscape

To determine if RHFB were moving between nurseries in eastern Virginia and adjacent soybean, cotton, and peanut fields, several scouting trips were conducted. One farm next to each nursery was scouted using a 38 cm- diameter sweep net on 25 June 2021, 19 July 2021, and 9 June 2022. Within each farm, 30 sweeps per crop were collected, and the content was checked for RHFB presence. Additionally, sticky traps were placed at these two nurseries to monitor the potential movement of RHFB adults between the nurseries and the adjacent farms. The traps were comprised of a 3 × 3 cm × 1.50 m wood stake with a 20 × 14 cm yellow sticky card (double sided, Alpha Scents Inc., Canby, OR) attached 65 cm from the ground and a 30 × 15 cm translucent sticky card (clear panel trap, Alpha Scents Inc., Canby, OR) placed 102 cm from the ground. Four traps were placed around the perimeter of each nursery and two traps within the nursery for a total of six traps at each location. In 2021 and 2022, these traps were checked weekly for the presence of RHFB adults from May to August and sticky cards replaced once a month or as needed.

Monitoring RHFB Daily Activity

Visual observations of RHFB adults were taken at the HRAREC, using hydrangeas that were naturally infested. These behavioral events were: 1) flying, 2) jumping, 3) walking 4) mating, or 5) sedentary. Beetles observed as sedentary included those standing stationary as well as those who were feeding, since differentiating them would have required closer observation that could have interfered with the individuals under field conditions. If one beetle performed more than one activity, it was marked as separate events. The observations were made every two hours over the course of a 24-hour period for a total of 12 observation periods within that day. Four flags marked the observation areas. Each flag was at least 2 meters away from the others and was within a grouping of 12 containerized plants. For one minute, the number of beetles and their activities were observed in the same manner two more times for a total of three one-minute intervals per flag per timeslot. Overhead irrigation for the observation areas was prevented during data collection. These visual observations were completed three times (12 August 2021, 1 June 2022, and 26 August 2022).

Characterizing herbivory using cage and non-choice assays

Foliar damage caused by RHFB was quantified using non-choice assays. Different population densities (0, 5, and 25 beetles per plant) were placed inside a 60 × 60 × 91 cm mesh cage (butterflyhabitatXL, RestCloud Zhejiang, China) with one hydrangea. Potted Hydrangea cv. 'Limelight' with no prior foliar damage were selected from the HRAREC nursery stock. There were four caged plants per treatment and the trial was conducted three times in 2022. The caged experimental units were housed in a plastic-covered greenhouse (temperature ranging from 30 to 35°C, RH ~65% and a 14:10 L:D regime) with irrigation and fertilization following commercial standards. After seven days, the cages were removed from the greenhouse and the beetles were suctioned. The foliar damage was then assessed by choosing three representative leaves from

each canopy layer. These canopy layers were selected by dividing the plant in thirds (bottom, middle, and top). We noted the percent damage of each leaf using visual estimation guidelines (Chong 2021).

The use of phenylethyl alcohol (PEA) as an adult attractant

Several traps were placed within collaborating nurseries to determine the efficacy of PEA as an adult attractant lure. Experimental treatments included 1) yellow sticky card with no PEA lure, 2) translucent sticky card with no lure, 3) yellow sticky cards with PEA lure, 4) translucent sticky card with lure. Traps were placed 10m apart within blocks and at 5m from the edge of selected commercial blocks. The design included a wooden stake placed amongst crops most affected by RHFB (Hydrangea, Ilex, and Itea), affixed with a sticky card placed with the top at the height of the canopy of the surrounding crops. Lures of PEA 99% (Acros organics, Geel, Belgium) were constructed by placing 3mL of PEA within a 10mL plastic vial and stuffing in a cotton wick. The wick design allowed for dissemination of PEA (Braasch and Kaplan 2012).

Four experimental blocks containing each treatment were placed within Ilex commercial blocks at aforementioned Nursery 1. This trial was repeated in July 2021, June 2022, and July 2022. Due to spatial restrictions, only treatments 1 and 3 were placed within Hydrangea and Itea commercial blocks at Nursery 1 as well as Hydrangeas at the HRAREC. One week and two weeks after deployment of traps, sticky cards were checked for RHFB captures and the four plants most closely surrounding the trap were vacuumed and adults counted. After each data collection, treatments within each block were rerandomized as part of a randomized complete block design.

Data Analysis

Seasonal monitoring data were analyzed comparing number of beetles per plant to sampling week between 2021 and 2022 using a t-test, since data for each year were used as independent samples. Further analyses were conducted within the 2022 data set. Data was analyzed using linear mixed models (Proc mixed; SAS v9.4, Cary, NC). An ANOVA was performed on each crop (Hydrangea, Ilex, and Itea) within each location (Nursery 1, Nursery 2, and HRAREC) for those with enough data points. The response variable was the average number of RHFB found per plant. Fixed effects were crop type and location. Random effects were block and replication within each date. The Kenward-Roger correction method was used to calculate degrees of freedom (Kenward and Roger 1997). A mean separation using Tukey's HSD test at $\alpha = 0.05$ was performed to determine the differences in beetles per plant and by sampling week. The density of RHFB per week demonstrated the peaks of activity over the sampling periods of monitoring. Peaks and lows in population density were then compared and associated to an accumulation of growing degree days.

The performances of daily activities were compared across time of the day using an ANOVA where the response variable was the average number of RHFB performing each activity at each time. Time of day was a fixed effect. Random effects included the block and repetition within each sampling date. For individual activities, number of sedentary beetles was $\log_{10}$ transformed to comply with the normality assumption. Back-transformed data were plotted and presented in the results.

An ANOVA was used for analysis of caged non-choice assays as well as the PEA attractant lure data. Canopy layer and number of beetles were considered fixed effects and were compared using percent foliar damage as a response variable. Block was a random effect. Means were separated by Tukey's HSD test at $\alpha = 0.05$. Both average number of adult beetles vacuumed and

those collected by sticky card (response variable) from the PEA experiments were compared using ANOVA to treatments including lure and no lure, and card type (yellow and translucent) as fixed effects across all crops. Random effects were block and repetition within each sampling date.

Results

Seasonal monitoring of RHFB populations

Red headed flea beetle adults were present on all sample dates across the experiment (Fig. 1). In the 2021 growing season across all crops, the highest number of beetles per plant was found in the third week of June (1.23 ± 0.33) and in the fourth week of July (0.83 ± 0.17). No beetles were found in the second week of June as well as the first and second weeks of July (Fig. 1). There was no significant difference in beetles per plant and by sampling weeks from 2021 and 2022 ($t = 1.01$; $df = 28$; $P = 0.3228$). Because the two years were not significantly different, further analysis by crop and location was conducted on 2022 data.

HRAREC sampling location

There was a significant difference between beetles per plant within Hydrangeas by sampling week ($F = 12.22$; $df = 23, 46$; $P < 0.0001$). The highest beetle numbers in Hydrangeas were in the fifth week in June (4.33 ± 0.19; 2470.05 GDD_{50}) and the lowest numbers were found at second week in June (0.44 ± 0.29; 1870.55 GDD_{50}) (Fig. 2). There was also a significant difference between beetle numbers within Iteas by sampling week ($F = 3.10$; $df = 18, 38$; $P = 0.0016$). The peak of beetle numbers was in the third week in August (2.22 ± 0.55; 4238.75 GDD_{50}) and the lowest at in the third week of May (0.17 ± 0.09; 1263.55 GDD_{50}). There was not enough data from Ilex crops to determine if there were differences between sampling weeks. Additionally,

larvae within the potting soil of containerized Hydrangeas were found to have higher numbers around the first week of May (6.00 larvae per pot) and the second week of August (4.50 larvae per pot) whereas numbers were lower in early June, early July, and early September (Fig. 3).

Nursery 1 sampling location

There was a significant difference between number of beetles per plant within Hydrangeas by sampling week ($F = 12.93$; $df = 19, 40$; $P < 0.0001$). The highest beetle numbers were found at the second week of September (1.26 ± 0.04; 4743.72 GDD_{50}) and the lowest in the first week of June (0.00; 1546.57 GDD_{50}) and the first week of July (0.00; 2397.29 GDD_{50}). Overall, beetle numbers were lower in the first half of the season (from May to mid-July) and increased in the second half (July to September; Fig. 3). There was a significant difference between number of beetles within Iteas by sampling week ($F = 16.16$; $df = 17, 34$; $P < 0.0001$). The highest number of beetles were found during the first week of September (1.72 ± 0.20; 4548.71 GDD_{50}) (Fig. 3). Throughout the season there were several weeks with no beetles, (fourth week of June, 1962.25 GDD_{50}; third week of July, 2871.16 GDD_{50}; and second week of August, 3657.24 GDD_{50}), but there was an overall increase in beetle numbers later in the season around September (Fig. 3). Additionally, there was a significant difference between beetle numbers within Ilex crops by sampling week ($F = 10.04$; $df = 22, 46$; $P < 0.0001$). The highest beetle numbers were found in the first week of September (1.78 ± 0.45; 4548.71 GDD_{50}) as well as in the fifth week of June (1.48 ± 0.26; 2190.04 GDD_{50}), while the lowest was in the second week of August (0.07 ± 0.04; 3657.24 GDD_{50}) (Fig. 3).

Nursery 2 sampling location

Number of beetles per plant across all sampling weeks and crops were very low, therefore there was not enough data to perform an analysis. Although most of the season had no beetles, there were higher numbers in all crops towards the beginning of September.

Movement of RHFB across the landscape

A total of 450 sweep samples across two years and two locations scouting for RHFB yielded no beetles when monitoring adjacent soybean fields to nursery locations. Once at the nursery, a total of 624 sticky card data collection events around the perimeter of the properties caught no beetles in either 2021 or 2022. However, a total of 208 sticky card collection events at traps within each nursery commercial block caught a total of 10 beetles.

Monitoring RHFB daily activity

Activities performed by RHFB adults at each collection date were found to be similar, so data were pooled for analysis ($F = 0.16$; $df = 11, 144$; $P = 0.9900$). Across all collection dates, number of RHFB adults executing different activities characterized as flying, jumping, walking, mating, and being sedentary varied throughout hours of the day ($F = 6.10$; $df = 44, 118$; $P < 0.0001$). The number of beetles observed flying across plant structures varied over time ($F = 7.11$; $df = 11, 130$; $P < 0.0001$). The highest number of beetles performing this activity was found at 1100 (0.56 ± 0.15), and none were found actively flying at 0100, 0300, 0700, 0900, and 2300 (Fig. 4). Differences were also observed among the number of beetles jumping across multiple leaves over time ($F = 4.02$; $df = 11, 132$; $P < 0.0001$). The highest number of beetles jumping was found at 1300 (0.28 ± 0.07), and none were found jumping at 0100, 0300, 0500, 2100, and 2300 (Fig. 4). Once moving on a plant, number of beetles observed walking was different over time ($F = 10.46$; $df = 11, 121$; $P < 0.0001$). The highest number of beetles performing this activity was

found at 1300 (1.47 ± 0.34), and the lowest was at 0300 (0.03 ± 0.03) (Fig. 4). The number of beetles recorded in a mating position with limited movement across a plant structure, was different over time ($F = 2.17$; $df = 11, 121$; $P = 0.0200$). The highest number of beetles mating was found at 1300 (0.39 ± 0.39), 1700 (0.39 ± 0.39), and 1900 (0.39 ± 0.24) and none were found mating at 0100, 0300, 0500, 0700, 0900, and 1100 (Fig. 4). Additionally, the number of sedentary beetles observed on plant tissue was also different over time ($F = 4.88$; $df = 11, 121$; $P < 0.0001$). The highest number of sedentary beetles (not actively moving) was found at 1900 (5.64 ± 1.06), and the lowest was at 1100 (1.72 ± 0.34) and 1300 (1.72 ± 0.22) (Fig. 4).

Characterizing herbivory using cage and non-choice assays

There was no difference amongst collection dates when comparing damage to number of beetles ($F = 0.02$; $df = 2, 6$; $P = 0.9815$). Therefore, the data was pooled together for further analysis. In fact, there was a significant difference in percent damage between different numbers of beetles across all trials ($F = 255.57$; $df = 2, 88$; $P <0.0001$). Percent defoliation was square root transformed for analysis. Plants infested with 0 beetles presented 0.36 ± 0.28 % damaged, those with 5 beetles were 2.73 ± 0.32 % damaged and those with 25 beetles were 6.59 ± 0.09 % damaged (Fig. 5). The interaction between number of beetles and damage to each plant canopy layer was not significant ($F = 0.86$; $df = 4, 88$; $P = 0.4940$). Damage inflicted by the beetles to each layer (bottom, middle, and top) within one plant was found to be similar ($F = 0.52$; $df = 2, 88$; $P = 0.5953$). The bottom layer had 3.06 ± 0.23 % damage, the middle layer had 3.52 ± 0.11 % damage, and the top layer had 3.11 ± 0.21 % damage.

The use of PEA as an adult attractant

Hydrangeas at Nursery 1 had almost no beetles present, and no analysis was performed for that location. Iteas at Nursery 1 had low numbers and showed no significant difference between adults captures on sticky cards between lure and no lure ($F = 1.00$; df =1, 6; $P = 0.3559$). Hollies had higher numbers of captured beetles on cards across all sampling dates, and analyses were performed for each replication. For replication 1, conducted in July 2021 at Nursery 1, there was no significant interaction between lure and card type treatments ($F = 1.02$; df = 1, 25; $P = 0.3231$). Furthermore, there was no significant difference on beetles captured between card type ($F = 1.02$; df =1, 25; $P = 0.3231$) or presence of lure ($F = 0.01$; df = 1, 25; $P = 0.9117$). The second replication, conducted in June 2022 at Nursery 1, showed the same findings with lure and card type interaction ($F = 0.44$; df = 1, 25; $P = 0.5146$), presence of lure ($F = 0.91$; df = 1, 25; $P = 0.3487$) and card type ($F = 0.91$; df = 1, 25; $P = 0.3487$). The third replication, conducted in July 2022 at Nursery 1, also documented the same findings with lure and card type interaction ($F = 0.85$; df = 1, 28; $P = 0.3631$), presence of lure ($F = 1.59$; df =1, 28; $P = 0.2178$) and card type ($F = 3.74$; df = 1, 28; $P = 0.0634$). Beetle captures in the hydrangeas at the HRAREC showed no significant difference based on presence of lure and using yellow sticky cards only ($F = 0.27$; df = 1, 3; $P = 0.6376$).

When the number of RHFB vacuumed at plants surrounding the trap was used as the metric, results for difference amongst treatments was similar to those by card collection. Hydrangeas at Nursery 1 had too few beetles collected for an analysis to be performed. Iteas at Nursery 1 showed no significant difference on adults vacuumed near traps based on presence of lure ($F = 1.00$; df = 1, 6; $P = 0.3559$). Hollies at Nursery 1 (Table 1) for the first replication, conducted in July 2021, showed no significant interaction between lure and card type treatments ($F = 3.34$; df

=1, 25; P = 0.0796). No significant effect was detected for presence of lure (F = 1.30; df = 1, 25; P = 0.2642), or for card type (F = 1.88; df = 1, 25; P = 0.1826) on beetle captures by vacuum. The second replication, conducted in June 2022, showed a significant interaction between lure and card type (F = 26.03; df = 1, 28; P <0.0001). The highest number of beetles was found at traps with no lure and a yellow sticky card. However, this was similar to the number found by the trap with a lure and a translucent sticky card. The third replication, conducted in July 2022, showed no significant interaction between lure and card type (F = 0.07; df = 1, 28; P = 0.7962). There was no significant difference and furthermore no biological difference based on presence of lure (F = 1.70; df = 1, 28; P = 0.2030), or sticky card type (F = 0.07; df = 1, 28; P = 0.7962) in this third replication (Fig.7). Hydrangeas at the HRAREC showed a significant difference based on presence of lure (F = 27; df = 1, 3; P = 0.0138). There were slightly fewer beetles collected from plants by the lures than there were near the traps without lures.

Discussion

This study on phenology and biology of the RHFB begins to fill in knowledge gaps of this pest of ornamentals in Virginia nurseries that will enable the improvement of pest management tactics. On a spatial scale, adult RHFB appeared to come from within the nursery rather than surrounding areas. On a temporal scale, we discovered RHFB adult populations fluctuate throughout the active season with peaks in populations occurring in late June, July, and August. At this level, phenylethyl alcohol (PEA) was found to be ineffective in attracting RHFB adults as a potential lure. Scaling in further within open-field nurseries, adult behavior exhibited higher activity levels during the day between 1100 and 1500 and were more sedentary after dark from 1700 to 0500. Sedentary beetles can inflict severe foliar damage within days. Feeding is

distributed evenly throughout layers of the affected plant canopy and increases linearly with the number of beetles. Documenting RHFB biology and behavior on multiple scales has demonstrated its clear potential as a key pest in the nursery setting.

RHFB population phenology varies across states where they have been studied in ornamentals such as North Carolina, Indiana, Georgia, Kansas, Alabama, and Wisconsin. They are present in other crop systems (cranberries and soybeans) in northern states such as Maine, North Dakota, South Dakota, Michigan, and Massachusetts but these are not related to the system of concern in Virginia. In Virginia (Lane and Del Pozo-Valdivia 2021), multiple generations are present each year in open-field nurseries. First generation adults were found at the HRAREC beginning in the first week of May at 896 GDD_{50}, Nursery 1 at 1523 GDD_{50} (1st week June) and Nursery 2 at 1560 GDD_{50} (1st week June). This differs from nurseries in North Carolina where first-generation adults were found from 517 to 1028 GDD_{50} while second-generation adults appeared between 1878 to 2318 GDD (Lauderdale 2017). A third generation has been observed on the Eastern Shore of Virginia and is estimated to appear in September through October (Brian Kunkel, *personal observation*). Untreated crops at the HRAREC exhibited peaks in populations of first-generation adults in hydrangeas around 1149 (4th week May), second generation at 2470 (5th week June, and third generation at 4178 (3rd week Aug.), all of which are earlier than NC populations. Treated crops at Nursery 1 exhibited first generation adult peak at 2136 GDD (4th week June), second generation adult peak around 3155 GDD (4th week July) and third generation adult peak around 4485 GDD (1st week Sept.). The nurseries used for scouting located in eastern Virginia in the Tidewater region have a vastly different climate than other states of previous study i.e., the temperatures in this coastal region can be warmer and relative humidity is higher.

Red headed flea beetle thrives in lower temperatures with a higher egg hatch rate (Jaffe et al. 2021), suggesting that development may slowed by warmth as demonstrated in our studies. Each of the population peaks is close to 1000 GDD apart from each other, implying that generations take around that many GDD and around one month to go from egg to adult during the active season. This corroborates findings that this process can occur in around 40 days at 20 C (720 GDD_{50} in laboratory settings (Jaffe et al. 2021). From the last adults found in 2021 at 3628 GDD to the first at 896 GDD of 2022, there was a total of 1497 GDD using January 1 as the biofix date. This is close to the differences in GDD between in-season generations. Larvae within the potted soil of containerized hydrangeas showed higher numbers during weeks with lower adult numbers in the active season. These numbers were not strictly inverses of each other, but demonstrate that, as expected, there are fewer larvae when adult populations are high and more larvae when adult populations are low.

The direct scouting of adults and larvae requires frequent observations resulting in time and labor constraints for affected growers. The deployment of a chemical lure was tested as a potential adult attractant. Phenylethyl alcohol (PEA) was found to attract RHFB adults in soybean fields (Braasch and Kaplan 2012). Using this concept to apply to ornamentals in open-field nurseries was unsuccessful. There were no attractant or repellent effects of the lure on RHFB adults within Ilex, Itea, or Hydrangea blocks. Differences in ecosystems likely contributed to this discrepancy. Soybeans are shorter in stature and are planted into the ground in rows close together whereas ornamentals in the nurseries of interest are separated into their own containers that are placed on a semipermeable mat. In addition to the layout differences, pruning is a component of ornamental maintenance and may have affected the distribution of HIPVs that could interact with

the PEA. Although both systems are treated with insecticides, the maximum label rates are different based on the crop system. Soybeans as food crops tend to have lower label rates for insecticides which may have prevented interference with PEA in that system. Further investigation would be required to determine if presence of insecticides within a system could influence attractant effects of PEA on RHFB adults.

Movement of RHFB throughout the landscape was unclear regarding their source and if they traveled in or out of the nurseries themselves. RHFB are present in soybeans (Anonymous 2022) in other states, and farms surrounding the nurseries of interest grow soybeans, cotton, and peanuts. Based on the lack of beetles present in farms nearby, there was no evidence for their movement into the nursery from elsewhere. Sticky card traps along the perimeter of the nurseries found no beetles, which also indicates the beetles were not moving in or out of the nursery. This landscape dispersal was of concern as the beetles are polyphagous and known to defoliate many crops besides those found at nurseries and farms in eastern VA (Herrick and Cloyd 2020, Lane and Del Pozo-Valdivia 2021).

After clarifying the RHFB locations and seasonal timing, the next step to understanding their behavior was to focus on their activities throughout the day. In corn, another species of leaf beetle has been found to be more active during the night (Miwa and Meinke 2015), compared to the increase in motionless beetles during the day. The activities motionless and feeding were found to be opposite for the RHFB in ornamentals during their peak times. In this study, the category of sedentary included those who were feeding and resting as the activities were indistinguishable in the field. RHFB were found to be more sedentary (motionless and feeding

combined) between 1900 and 0700 and more active during the day with peaks in activity between 1100 and 1500. Over the course of 24 hours, these beetle activities were observed and fell into two main categories, active or sedentary. Flying, jumping, walking, and mating were categorized as active. Growers could use the data on when beetles are moving around plants and active so that they may adjust their spray schedules accordingly. Visual scouting of the beetles would be most accurate if performed during the same time of the day. Higher numbers of beetles active during the day makes them easier to visually scout since they are expected to be moving around. Visual scouting may therefore be most representative of populations if consistently performed between the hours of 1100 and 1500.

Caged hydrangeas infested with adult RHFB for one week represents a longer range of time that an affected nursery may go without spraying. Although the seasonal activity data shows fewer than five beetles per plant, the relation between density of beetles and damage was studied in a controlled setting. As the number of beetles increased so did the damage to the plant. One repetition of control treatments with zero beetles infested suffered a small percent damage to the plant because a beetle had emerged from the soil during the study (Fig. 5). Removal and count of beetles post treatment showed that additional beetles did not emerge in other experimental units. Related studies corroborate this relationship and indicate that linear regressions can determine damage and furthermore be used to target when insecticide treatments are to be sprayed (Dreistadt and Dahlsten 1989). Density of beetles per plant explained a large proportion of percent damage inflicted in this study ($R^2 = 0.6908$). The regression equation, leaf damage = $0.8964 + 0.2332 \times$ (number of beetles per plant), represents damage inflicted by one beetle on one plant over seven days. Growers have no tolerance for damage to their crops, therefore this

equation could be used to model the damage they may expect to observe based on beetle density. This controlled assay was conducted on hydrangeas not treated with any insecticides, so it is possible that actual damage levels may be lower at the commercial nurseries. Regardless, this equation aims to present the maximum damage per beetle per plant over one week. In addition to percent defoliation based on beetle density, the distribution of that damage throughout the canopy was also observed. Each layer of the canopy (bottom, middle, and top) suffered similar levels of defoliation which means that the beetle feeding was distributed evenly across the plant. With the beetles likely being located evenly across the plant, this means that visual scouting from the tops of plants, which are the most easily visible parts, will be accurate as other layers. This observation could also play into insecticide spraying practices just as timing of the day could. Based on intra-plant RHFB distribution, insecticides will likely only reach a third of the beetles if the droplets only cover the top third of the plant. Getting an even penetration of the spray application throughout the plants is important in ensuring coverage to affect all beetles.

Virginia commercial nurseries have proved to be an ecosystem with differences in RHFB phenology that, now investigated, can provide affected growers with information to improve IPM methods. Their population lows in early June, mid-July, and mid-August indicate weeks where growers can expect to need fewer insecticide applications and see fewer beetles. The differences in seasonal activity peaks between coastal Virginia and other states suggest factors in emergence may go beyond the growing degree day accumulation concept. The RHFB populations are confirmed to remain within the confines of the nursery rather than infiltrating to or from surrounding farmland. At the individual plant scale, in the night hours from 1700-0500, beetles were found to be sedentary, resting, or feeding while the sun was down. Their activity

levels increased in the heat and light of the day from 1100 to 1500. Crops of interest are subjected to severe defoliation in short periods of time where such damage affects the entire plant evenly and increases with higher RHFB density. Further investigation into timing of feeding as well as in-field spray application trials would clarify the relationship between activity levels in the day such as feeding, and effectiveness of differently timed sprays. The multiscale monitoring and observations from this project lead to a further understanding of the RHFB as a pest of ornamentals in Virginia nurseries with the goal of improving IPM strategies in such context. Reduction in levels of insecticide usage, currently a main component of RHFB management, will enhance sustainability of nurseries, and welfare of the workers, non-target organisms, and the environment.

Acknowledgements

We would like to acknowledge members of the Virginia Tech applied ecology lab who contributed to the process of data collection, including Julie Brindley, Elidah Sisk, Devin Calpo, Shannon Bradley, Joseph Leo, Mireya Turcios, and Kaylee Armstrong. Drs. Thomas Kuhar, Sally Taylor, and Peter Schultz provided edits to this manuscript. We thank the growers who graciously let us use their nurseries as field sites. We would also like to thank the funding sources for this project; SARE for graduate student grant number GS2 1-245 and the Virginia Tech Hatch Project number VA-160164.

References

Braasch, J., and I. Kaplan. **2012**. Over what distance are plant volatiles bioactive? Estimating the spatial dimensions of attraction in an arthropod assemblage. Entomol. Exp. Appl. 145: 115–123.

Buffington, M. L., and R. A. Redak. **1998**. A comparison of vacuum sampling versus sweep-netting for arthropod biodiversity measurements in California coastal sage scrub. J. Insect Conserv. 2: 99–106.

Chittenden, F. 1902. Notes on flea beetles, pp. 111–113. *In* Some Insects Injurious to Vegetable Crops. USDA Division of Agricultural Entomology, Washington, DC. 117 pp.

Chong, J. 2021. Visual Scale for Percent Defoliation in Hydrangea. Clemson University, Florence, SC. 8 pp.

Cloyd, R. A., and N. J. Herrick. **2018**. Don't get foiled by the flea beetle. Nursery Management Magazine. September 2018 issue. 70 pp.

Dreistadt, S. H., and D. L. Dahlsten. **1989**. Density–Damage Relationship and Presence–Absence Sampling of the Elm Leaf Beetle (Coleoptera: Chrysomelidae) in Northern California. Environ. Entomol. 18: 849–853.

Guédot, C., and R. S. Perry. **2015**. Evaluation of Soil and Foliar Applications of Insecticides for the Control of Flea Beetle in Cranberry, 2014: Table 1. Arthropod Manag. Tests. 40: C9.

Hajek, A., and J. Eilenberg. **2018**. Conserving Natural Enemies: Reducing Effects of Pesticides on Natural Enemies, p. 87. *In* Natural Enemies. Cambridge University Press, Cambridge, UK.

Herrick, N. J., and R. A. Cloyd. **2020**. Overwintering, Host-Plant Selection, and Insecticide Susceptibility of *Systena frontalis* (Coleoptera: Chrysomelidae): A Major Insect Pest of

Nursery Production Systems. J. Econ. Entomol. 113: 2785–2792.

Jacques, R. L., and D. C. Peters. **1971**. Biology of *Systena frontalis* with Special Reference to Corn. J. Econ. Entomol. 64: 135–138.

Jaffe, B. D., S. Rink, and C. Guédot. **2021**. Life History and Damage by *Systena frontalis* F. (Coleoptera: Chrysomelidae) on *Vaccinium macrocarpon* Ait. J. Insect Sci. 21.

Joseph, S. V., J. H. Chong, B. Campbell, B. Kunkel, D. Lauderdale, S. Jones, S. Gill, Y. Chen, P. Schultz, D. Held, F. Hale, A. Dale, E. Vafaie, W. Hudson, D. Gilrein, and A. Del Pozo-Valdivia. **2021**. Current Pest Status and Management Practices for *Systena frontalis* (Coleoptera: Chrysomelidae) in Ornamental Plants in the Eastern United States: An Online Survey. J. Integr. Pest Manag. 12: 17–18.

Lane, E., and A. Del Pozo-Valdivia. **2021**. Red Headed Flea Beetle in Virginia Nurseries. Virginia Cooperative Extension. ENTO 464NP. Blacksburg, VA.

Lane, E., and A. Del Pozo-Valdivia. **2022**. Bioassays comparing different insecticides against *Systena frontalis* adults on Hydrangea paniculata, 2022. Arthropod Manag. Tests. 47:1

Lauderdale, D. 2017. Red-headed Flea Beetle Biology and Management. Nursery & Landscape Notes. Winter 2017 issue. 33-35 pp.

Main, A. R., M. L. Hladik, E. B. Webb, K. W. Goyne, and D. Mengel. **2020**. Beyond neonicotinoids-Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. Sci. Total Environ. 742.

Miwa, K., and L. J. Meinke. **2015**. Diel Patterns of *Colaspis brunnea* and *Colaspis crinicornis* (Coleoptera: Chrysomelidae) in Southeastern Nebraska. Environ. Entomol. 44: 1553–1561.

NDSU. **2022**. Red-Headed Flea Beetle in Soybean, Corn and Wheat. NDSU Agric. Ext. (https://www.ndsu.edu/agriculture/ag-hub/ag-topics/crop-production/diseases-insects-and-

weeds/insects/red-headed-flea-beetle-soybean).

Rice, R. E., C. V Weakley, and A. A. Jonesl. **1984**. Using Degree-Days to Determine Optimum Spray Timing for the Oriental Fruit Moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 77: 698–700.

Riley, C. V. 1884. Reports of Observations and Experiments in the Practical Work of the Division. U.S. Department of Agriculture, Washington, DC. 50 pp.

Sparks, T. C., and R. Nauen. **2015**. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121: 122–128.

Figures

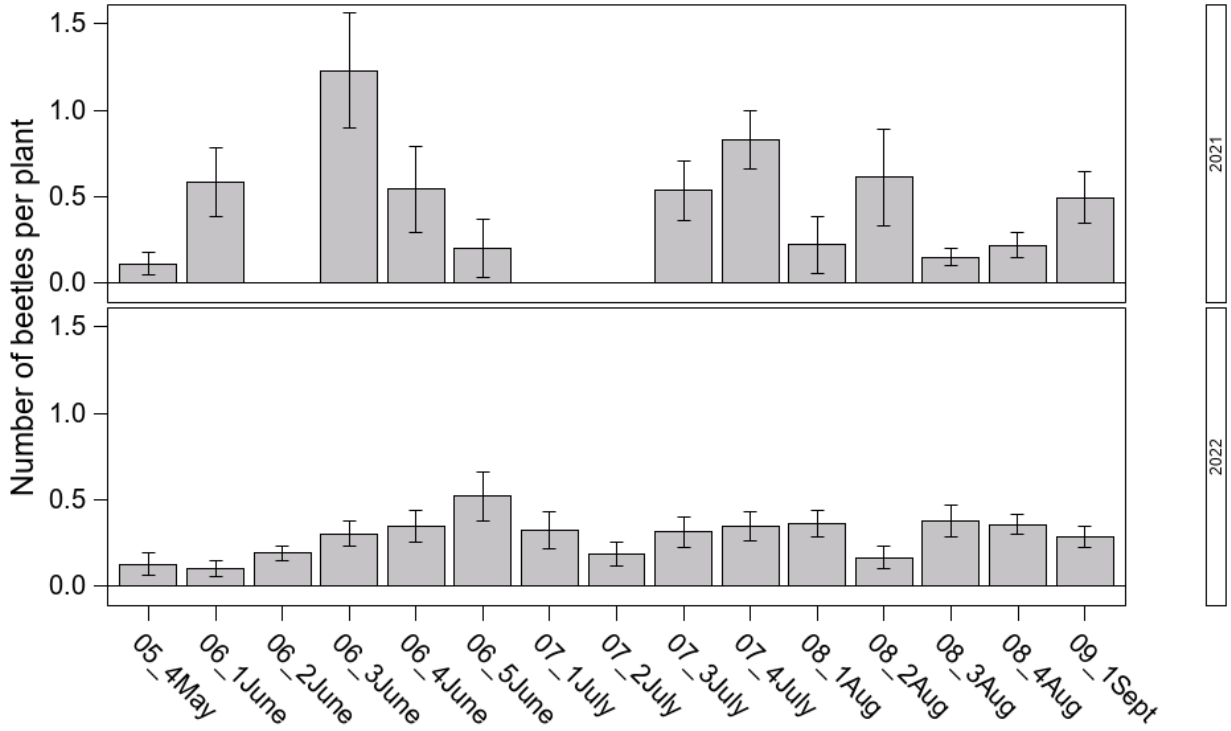

Figure 1. Average number of beetles ± SE vacuumed per plant across all crops (hydrangeas, iteas, and ilex) and locations (Nursery 1, Nursery 2, and HRAREC) in eastern Virginia, organized by sampling date (X-axis). Rows present data by each year. Each sampling week is denoted using "number month _number of week month name."

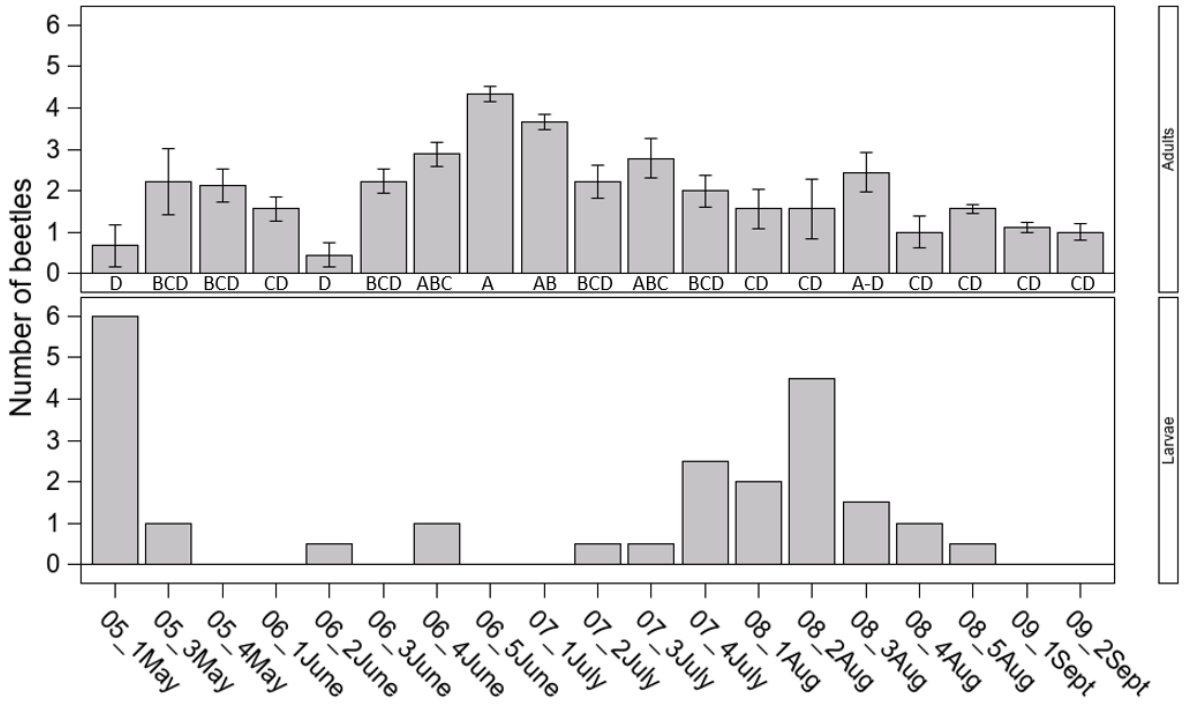

Figure 2. Average number of beetles per plant found in hydrangeas at the HRAREC. Rows denote life stage (adults captured by vacuum and larvae found in potting soil of containerized plants) by week (X-axis). Each sampling week is denoted using "number month _number of week_ month name." Letters under the bars denote separation of means where those with a common letter are not significantly different.

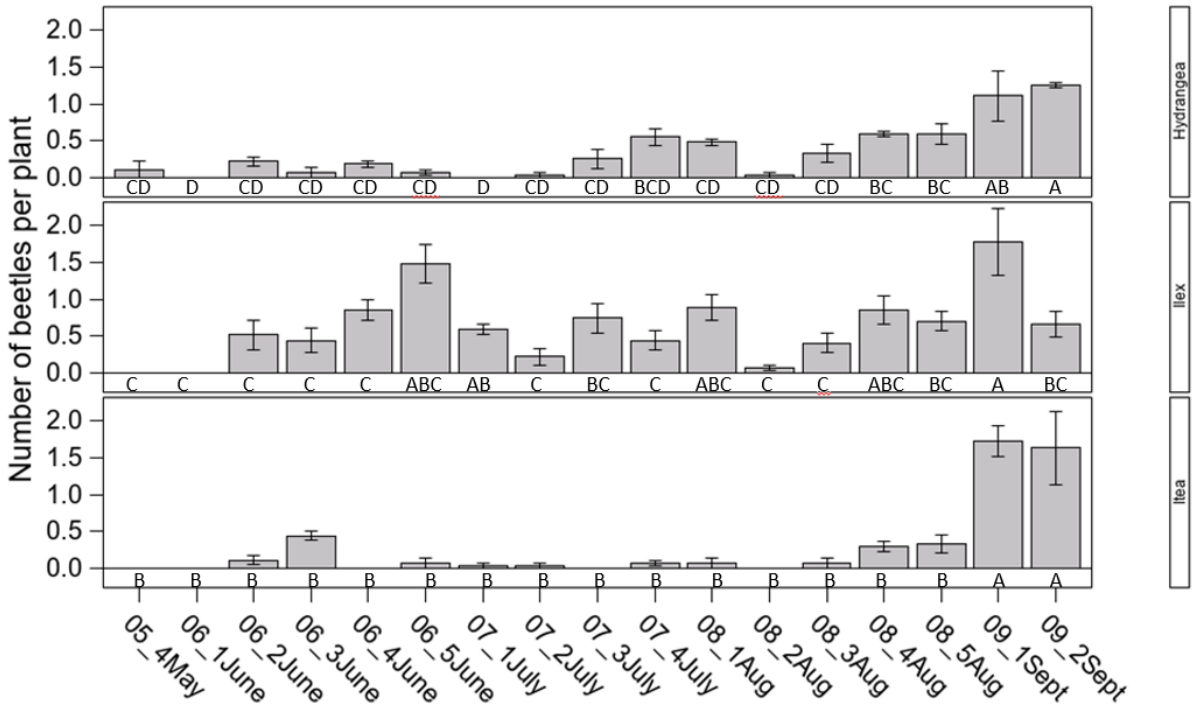

Figure 3. Average number of beetles ± SE per plant collected within Nursery 1 in eastern Virginia. Rows denote different crops (hydrangea, ilex, and itea). Each sampling week is denoted using "number month _number of week_ month name." Letters under the bars denote separation of means where those with a common letter are not significantly different.

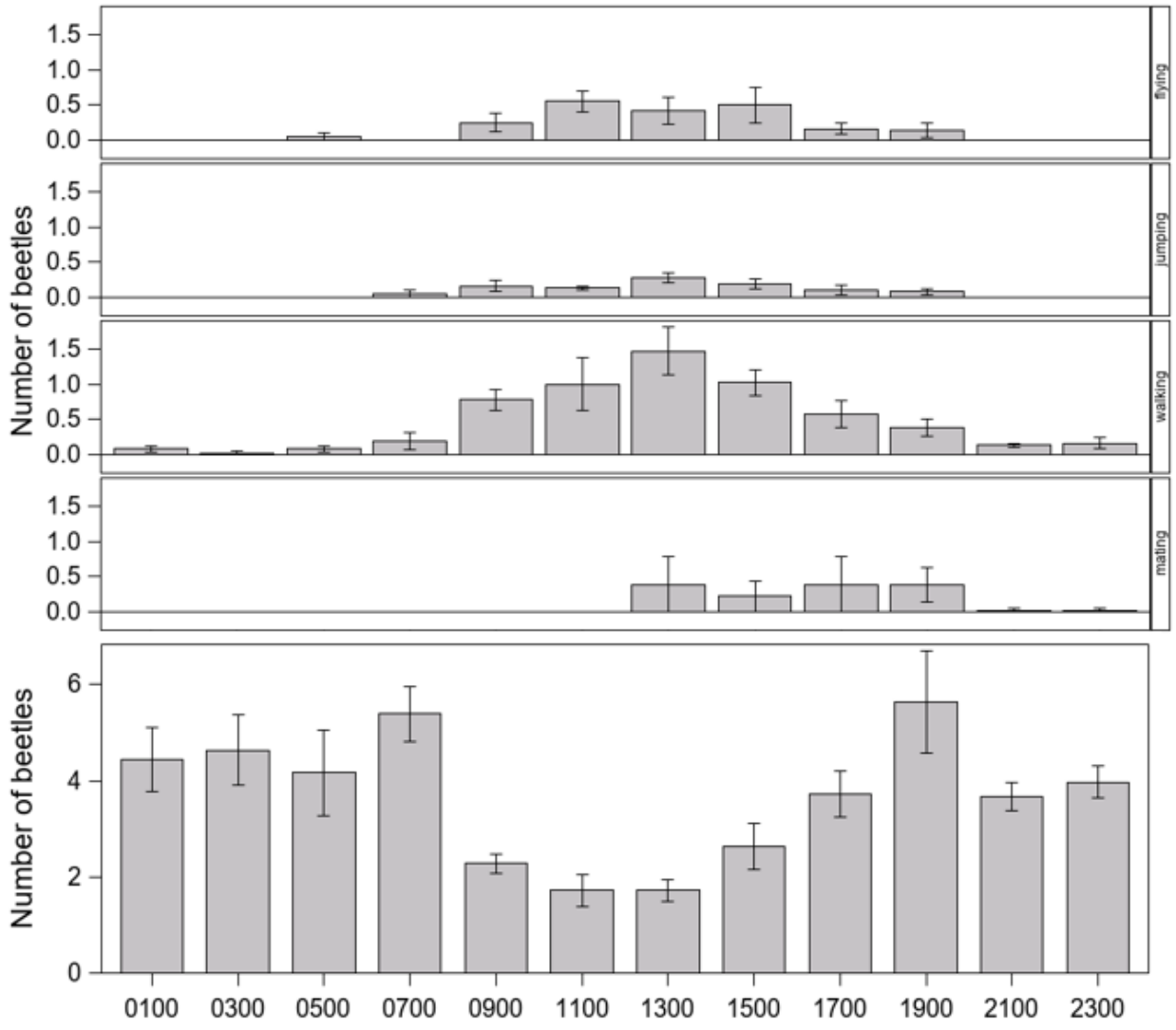

Figure 4. Number of beetles ± SE per observation area performing different activities, from the top row with high movement activity (flying), to the bottom row presenting densities on sedentary/ lower movement activities at different times of the day (X-axis).

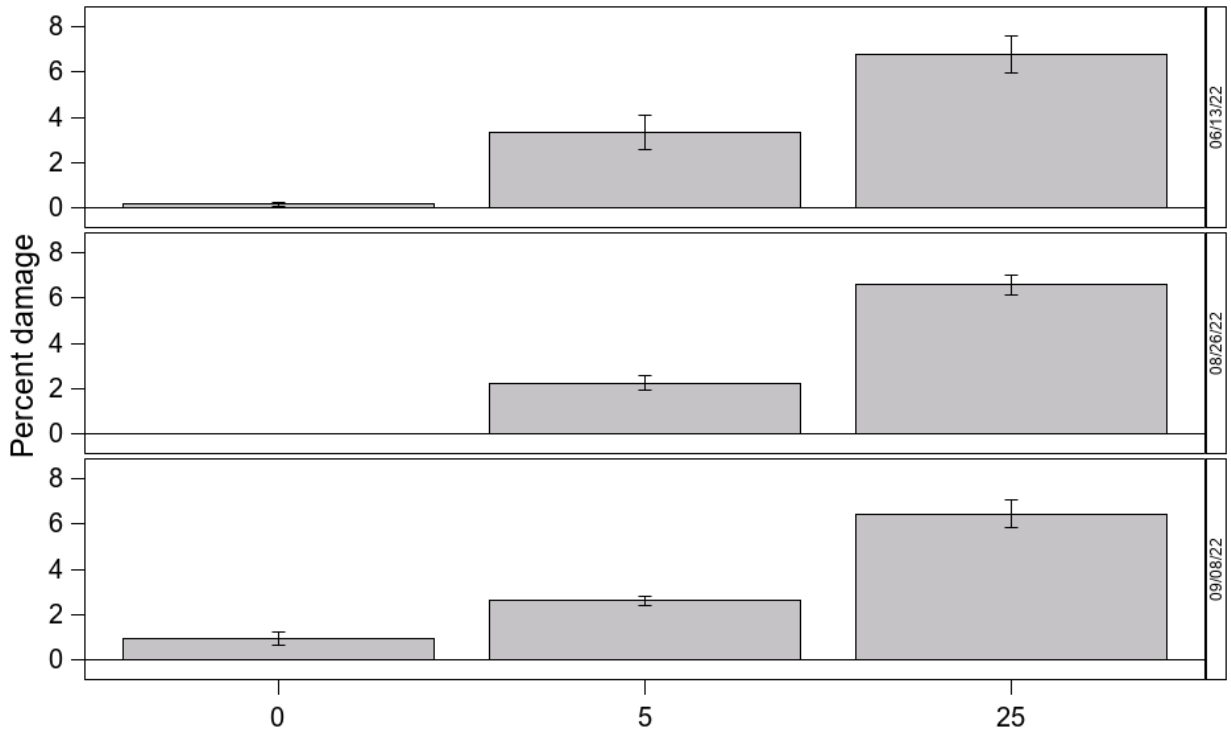

Figure 5. Percent canopy damage ± SE based on number of RHFB adults infesting each experimental plant (X-axis). Data shows the average between all layers and replications within each trial. Rows denote sampling dates for separate trial.

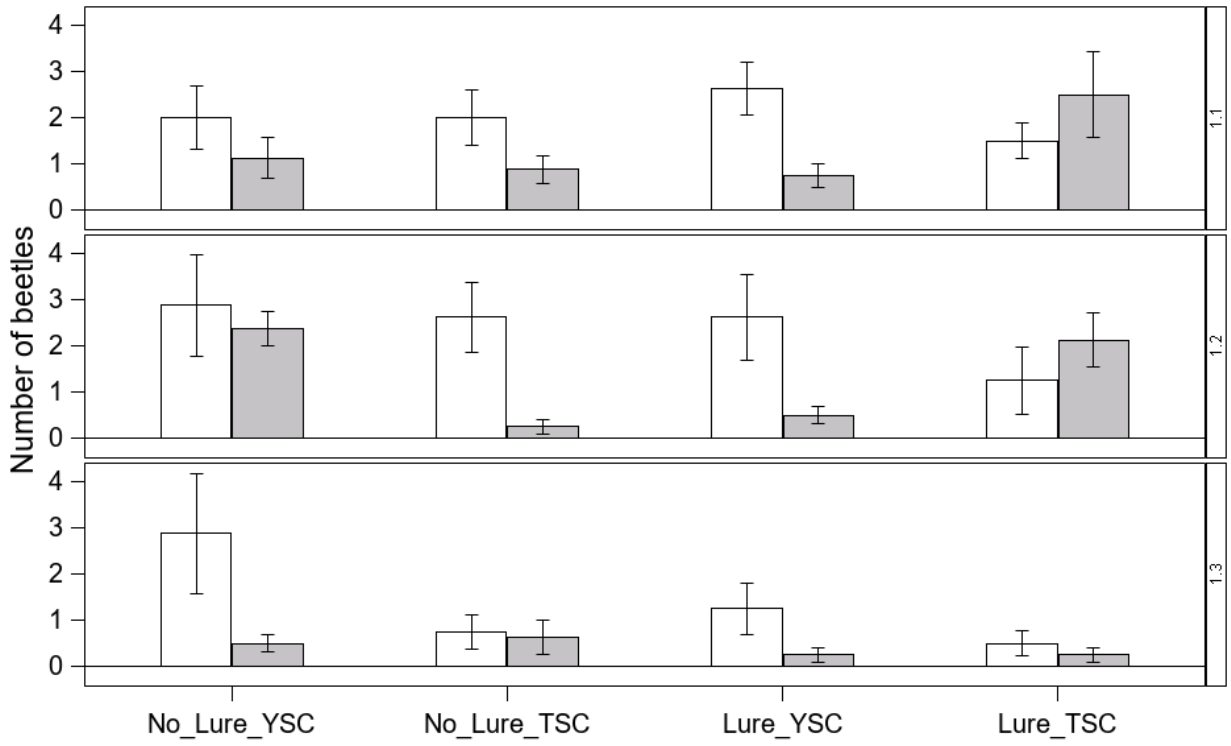

Figure 6. Average ± SE number of beetles collected from vacuum samples (grey bars) and on sticky cards (white bars) within Ilex blocks at Nursery location 1 when using PEA and sticky cards. X-axis showing treatment 1 = no lure, yellow sticky card (ysc); 2 = no lure, translucent sticky card (tsc); 3 = lure, ysc; 4 = lure, tsc. Rows denote data from each replication (1.1-1.3).

Chapter 3: Feeding potential of *Systena frontalis* adults on hydrangea leaf tissue

Manuscript submitted to Journal of Insect Science 12/14/2022

Abstract

Systena frontalis, red headed flea beetle (RHFB, Coleoptera: Chrysomelidae) is a defoliating pest of a variety of crop systems such as ornamentals and food crops. Defoliation caused by RHFB renders ornamental nursery plants, such as hydrangeas, unsaleable. In Virginia, RHFB has become a major pest at commercial nurseries, and their feeding potential has not been quantified. In this study, we determined the extent of their damage to individual leaves and host preference between leaf ages. The rate of herbivory on mature and young Hydrangea leaves was measured over 24 and 48 hours and between different numbers of beetles. A single beetle can cause up to 10% damage to a young leaf or 5% to a whole mature leaf in 24 hours. Without choice, there was a higher percent damage to young leaves. When size of leaves was controlled by cut-out mature leaves, area damaged was found to be the same between young and mature leaves. Adult RHFB feeding between mature or young tissue was further investigated by choice assays on a full caged plant and within a containerized system. In these choice assays, RHFB adults inflicted higher percent damage on mature leaves in both caged plant assays and containerized direct choice assays. The choice assays were more similar to field conditions than the non-choice. This demonstrates that RHFB show a preference for mature tissue over young tissue within Hydrangeas.

Key words: ornamentals feeding behavior, plant insects, insect-plant interactions, biology

Introduction

The red headed flea beetle (*Systena frontalis* F., RHFB, Coleoptera: Chrysomelidae) is a defoliating pest of ornamentals in open-field Virginia nurseries (Lane & Del Pozo-Valdivia 2021) as well as other crop systems such as soybeans (NDSU 2022) and cranberries (Jaffe et al. 2021). Adult feeding results in leaves with shot holes and skeletonization that are unsightly and ornamentals that are unsaleable (Lane & Del Pozo-Valdivia 2021). As a polyphagous insect, they feed on a wide variety of economically important nursery crops (Joseph et al. 2021). The threat they pose to open-field nurseries in eastern Virginia is clear and the extent has not been fully quantified. Beetle density, in relation to defoliation, has been studied in cranberries and in hydrangeas. Higher numbers of RHFB will cause more damage in a positive linear fashion on hydrangeas (Lane & Del Pozo-Valdivia 2022), and cranberries (Jaffe et al. 2021).

There have not been studies determining feeding preferences of RHFB on hydrangeas or other ornamentals. Injury to cranberries is inflicted on leaves closer to the apical end of uprights (younger growth) and less so the further away from the apical end (older growth) (Jaffe et al. 2021). While this has been shown in a laboratory setting, choice assays, as well as percent defoliation, amongst tissue ages have not been quantified. Polyphagous lepidopterans such as some geometrids and erebids show preferences for mature leaves when given a choice (Cates 1980) and this concept may apply to the RHFB since they are also polyphagous. Beetles within the same family as RHFB (Chrysomelidae) have been found to feed on young leaves in choice assays (Ernest 1989).

There are several methods of quantifying insect herbivory, including visual inspection, surface area meter, and picture-based software for leaf area. LeafByte is a mobile application which has been utilized in lieu of other more complex and time-consuming software. It can be

used in the field since it has a user friendly interface and maintains a high level of accuracy compared to other methods of measuring herbivory (Getman-Pickering et al. 2020). Based on the merits and applicability of this mobile-based method, it was used in this study to supplement the measurements taken by a surface area meter. Visual assessment of percent defoliation is one of the methods to measure herbivory that requires no instrumentation or removal of damaged tissue (Johnson et al. 2016). It is accurate and precise when performed by trained individuals (Johnson et al. 2016). Visual assessment for herbivory quantification benefits from using standardized references and scales. For instance, the reference for percent defoliation in hydrangeas proposed by Chong (2021), could be an accurate method to be used in entire plant injury estimations.

Thes investigation of feeding potential will provide insight into the biology of the RHFB. The objectives of this study are: 1) determining feeding preference between tissue ages on a non-choice and whole plant setting; 2) calculating feeding potential within a Petri dish non-choice assay based on tissue age, number of beetles, and time of exposure; and 3) determining feeding preference in laboratory choice assays based on tissue age and beetle sex.

Materials and Methods

Individual leaf petri dish non-choice assays

RHFB adults were vacuum collected from hydrangeas var. ‘Limelight’, grown under open-field conditions at the Hampton Roads Agricultural Research and Extension Center (HRAREC) in Virginia Beach, VA between May and August 2021 and 2022. After collection from the field, the beetles were placed in a ventilated bucket temporarily and their respective Two circular pieces of moistened coffee filter paper lined 100mm Petri dishes for hydration (Ernest 1989). Four dishes of each treatment were prepared for one, three, and five beetles at

each 24- and 48-hours exposure time. They were kept in a growth chamber (Percival, model I-30BLL, Perry, IA) for 24 hours to ensure controlled conditions while starving. The growth chamber was set to 26°C and 14:10 Light: Dark regime. The next day, 24 new growth Hydrangea leaves var. 'Limelight' and 24 older growth leaves of a similar size with no damage were collected from the HRAREC. An additional 24 older growth leaves were collected and 9cm^2 squares were cut out to be used in comparisons of experimental area to standardize the sizes between tissue type. The surface area of the undamaged leaves was measured three times using a LI-COR LI-3100C area meter (LI-COR Environmental, Lincoln, NE). The leaves were placed in their respective Petri dishes and wrapped in parafilm to prevent desiccation. At the end of the respective time periods (24 h and 48 h), the beetles were suctioned out of the petri dishes and the leaves were removed for analysis. The injured areas of the leaf that had not been entirely eaten through were removed using forceps under a stereoscope. The surface areas were measured again three times using the LI-3100C area meter and the LeafByte mobile application (Getman-Pickering et al. 2020). Whole leaves, both mature and young growth, were repeated four times and the square mature leaves were repeated three times. Data were analyzed using linear mixed models (Proc mixed; SAS v9.4, Cary, NC). The response variables were both percent foliar injury calculated from the LiCor and from LeafByte, as well as area (cm^2) consumed. Fixed effects were tissue age, number of beetles, length of exposure, and their respective interactions. Replication (r = 4) was included as random effects. Degrees of freedom were calculated using the Kenward-Roger correction method (Kenward & Roger 1997). Mean separation post-ANOVA was calculated by using Tukey's HSD at $\alpha = 0.05$. Percent injury on hydrangea leaves calculated by Li-Cor as well as the LeafByte mobile application was $\log_{10}$ transformed. Percent defoliation data were back-transformed and presented as mean ± standard error.

Old versus new growth preference whole plant choice assays

Four hydrangeas var. Limelight were chosen from the HRAREC for this experiment. Unsexed RHFB were collected from infested hydrangeas at the HRAREC on June 6, August 18, and September 1, 2022 and 25 were placed in each 60 × 60 × 91 cm mesh cage (RestCloud Zhejiang, China). The plants were left for seven days in a plastic greenhouse (temperature between 30 to 35°C, RH ~65% and a 14:10 L:D regime) with irrigation and fertilization following commercial standards. After beetles were removed, 10 young leaves and 20 mature leaves were chosen from across all layers of the plant to be assessed. The percent damage was visually estimated using the Clemson University guidelines (Chong 2021). This was repeated three times in 2022. Percent injured difference between old and new growth were compared using a T-test (Proc ttest; SAS).

Laboratory containerized choice assays

Adult RHFB were collected from hydrangeas at the HRAREC and starved for 24 hours. Groupings of 2-3 leaves of both young tissue and mature tissue were clipped from untreated Limelight hydrangeas. A 14.5 cm length by 7.5 cm diameter tube acrylic tube had a 5mm hole drilled through one side halfway down the length of it. Leaves with stem attached and poked through a piece of parafilm were placed at the opposite ends of the tube (Fig. 1). Parafilm was secured to keep the leaves in place and seal the tube. Ends of the stems were surrounded by moistened cotton balls to prevent desiccation. One RHFB was placed into the tube using the drilled hole halfway between the leaves and then was sealed with another piece of parafilm. Type

of tissue was randomly assigned to each end of the tube. All replicates were placed in a growth chamber for 24 hours with 26°C and 14:10 L:D ratio. Acrylic tubes were placed in a freezer to kill the beetles so their sex could be determined. In each experimental unit, the leaves were observed to determine if injury had been inflicted to either or both tissue types. Ten experimental units were run at a time and the percent injury as well as sex of the beetles was recorded. This trial was repeated five times, and the proportions of sex and tissue type preference were used as response variables to perform linear mixed models (Proc mixed; SAS). Fixed effects were sex of the beetle and age of tissue available. A t-test (Proc ttest; SAS) was performed comparing proportion of beetles feeding on mature and those feeding on young tissue within each replicate (r = 5). Tissue preference and proportion of sex were log10 transformed for their analysis. Defoliation data were back-transformed and presented as mean ± standard error.

Results

Individual leaf petri dish non-choice assays

Feeding potential expressed as percent injured

All individual factors were significant in their effect on the percent leaf consumed; including age of tissue ($F = 58.96$; $df = 1, 161$; $P < 0.0001$), number of beetles ($F = 61.09$; $df = 1, 161$: $P < 0.0001$), and time exposed ($F = 33.45$; $df = 1, 162$; $P < 0.0001$) (Fig. 2). Age of tissue, number of beetles, and time exposed did not interact with percent injured or consumed ($F = 0.43$; $df = 2, 161$; $P = 0.6492$). There were no interactions between age of tissue and number of beetles ($F = 0.20$; $df = 2, 161$; $P = 0.8188$), or number of beetles and time exposed ($F = 0.51$; $df = 2,$

162; P = 0.6023), and age of tissue and time exposed (F = 3.23; df = 1, 161; P = 0.0743). Notably, one beetle in one day can consume 10.88 ± 3.44 % of a young leaf and 4.61 ± 0.80 % of a mature leaf.

When LeafByte data were analyzed, there were no interactions between age of tissue and number of beetles (F = 0.04; df = 2, 164; P = 0.9625) or age of tissue and time exposed (F = 0.38; df = 1, 164; P = 0.5385). Time exposed interacted with the number of beetles to affect the percent injured or consumed (F = 6.40; df = 2, 164; P = 0.0021) where five beetles in 48 h had the highest consumption rate (57.06 ± 6.39 %). Age of tissue also affected the percent of leaf injured or consumed (F = 71.11, df = 1, 164; P < 0.0001), where young tissue was usually the most affected (9.32 ± 1.35 % per beetle in 24 h and 14.19 ± 2.05 % per beetle in 48 h).

Feeding potential expressed as area (cm^2)

Age of tissue did not affect the area consumed (F = 1.43; df = 1, 130; P = 0.2333). Both the number of beetles (F = 38.84; df = 2, 130; P < 0.0001), and the time leaves were exposed (F = 27.09, df = 1, 130; P < 0.0001) significantly influenced the area injured or consumed, resulting in higher feeding injury from five beetles in 48 hours (3.13 ± 0.78 cm^2 young tissue and 1.87 ± 0.66 cm^2 mature tissue). No interactions of factors were found between age of tissue and number of beetles (F = 2.95; df = 2, 130; P = 0.0558), number of beetles and time exposed (F = 1.01; df = 2, 130; P = 0.3677), or age of tissue and time exposed (F = 1.01; df = 2, 130; P = 0.3677).

Caged hydrangea choice assays

Percent defoliation to leaves was significantly different between tissue ages ($t = 13.69$; $df = 22$; $P < 0.0001$). There was a preference for mature leaves which suffered 6.93 ± 0.31 % feeding injury over young leaves which suffered 1.81 ± 0.21 % (Fig. 3).

Laboratory containerized choice assays

In laboratory contained choice assays, beetles showed a difference in which tissue age they preferred ($t = 3.86$; $df = 18$; $P = 0.0012$) (Fig. 4). Seventy percent of beetles chose to feed on mature leaf clusters rather than young leaves. One beetle showed no preference and fed on both tissue ages equally. Sex of beetles did not influence preference ($F = 0.94$; $df = 1, 15$; $P = 0.3474$).

Discussion

Virginia nurseries suffer economic losses caused by RHFB foliar feeding on ornamentals such as hydrangeas, sweetspire Iteas, and hollies. To understand the feeding biology of RHFB on hydrangeas, the injury that they inflict on mature and young leaves was evaluated under controlled conditions. In the laboratory, non-choice assays showed that individual young leaves had a higher percent consumed than mature whole leaves. The whole young leaves did not have more area consumed than square cut standard mature leaves. This indicated higher percent defoliation on young tissue and that the same area was consumed across tissue ages. Once expanded to the whole plant scale assays, mature leaves suffered a higher percent injury, contrasting the findings of the individual leaf assays. To document if a preference was occurring, a two-way containerized choice assay was conducted which confirmed findings at the whole plant scale. Mature leaves were repeatedly chosen over the young leaves within these assays. The

corroboration of the two-way choice assays and the whole plant assays shows that RHFB will feed more on mature leaves when given a choice. The discrepancy between the choice and non-choice assays shows that RHFB will feed on any available tissue.

In the individual leaf petri dish assays, we expected to document higher percent and amount of area fed on in 48 hours rather than 24, in young leaves rather than mature, and in five beetles rather than three or one. We expected the most injury would be a young leaf over 48 hours with five beetles and the least would be a mature leaf over 24 hours with one beetle. These hypotheses were found to be correct within the confines of this non-choice experiment. Young leaves were smaller and injury was expected to be higher compared to the larger whole leaf. Area damaged was expected to be different between the leaf ages since other studies have shown selection in favor of different leaf ages. Specialist leaf beetles have been shown to selectively feed on young leaves in choice assays (Ernest 1989). Because RHFB are in the same family, a similar trend was expected. However, RHFB are polyphagous (Joseph et al. 2021), therefore, the opposite occurs as they followed the patterns of other polyphagous insects (Cates 1980). The polyphagous insects caused more damage to mature tissue because they were avoiding the more highly concentrated toxins within the younger leaves (Cates 1980). The expectation of RHFB feeding potential depended on the host plant physiology, as woody perennials have higher levels of tannins and phenolic compounds in younger leaves compared to mature leaves (Feeny 1976). Therefore, it is hypothesized that hydrangeas would be similar and RHFB would follow the same pattern as the polyphagous insects and avoid feeding on the younger tissue.

Across all treatments and replications of these individual leaf assays, only two individual leaves were 100% consumed or injured. The injury to these leaves was a mixture of skeletonization and shot holes. Insects who skeletonize plant leaves feed on the mesophyll and

leave the epidermis intact (Hochuli 2001). Severe skeletonization results in shriveled leaves without complete removal of plant tissue. Therefore, growers would not scout for these missing leaves but instead for highly injured leaves. When infestation levels are high and foliage is greatly consumed, RHFB have been observed to feed on flower petals, when attacked ornamentals are left untreated (Lane, personal observation). As growers tend to prune away flowers under commercial nursery conditions, flower feeding is not of consequence.

Whole leaves were used for the petri dish non-choice assays to maintain the similarity to field conditions, where adults are exposed to this kind of tissue. Mature leaves were cut down to a smaller standardized size to more accurately compare the area damaged in young and mature tissue. These leaf artifacts were not representative of field conditions but created a more controlled assay, in terms of area presented to RHFB adults. Cutting and exposing edges may have affected distribution of any volatiles but was necessary to elicit the response comparing the same area prone to damage. By using both area meter and mobile application, a more accurate conclusion can be made about the defoliation potential of the RHFB on individual leaves. The resulting area damaged from these standardized leaves did not differ between mature and young tissue. The higher percent damage to younger leaves indicated a potential preference to be explored further. From these non-choice assays, there was no clear conclusion as to which tissue type the adult RHFB preferred.

The differing results between percent damaged on whole leaves and area damaged on cut-out mature leaves, led to a larger scale experiment to elucidate factors influencing this behavior of adult RHFBs. Percent damage to mature or young leaves on the scale of an entire plant was observed and found to contradict with data generated from the petri dish individual leaf studies. The RHFBs inflicted higher percent damage to the mature leaves when given the

choice on a caged whole plant. This corresponds with the hypothesis based on other polyphagous insect feeding behavior (Cates 1980). As a polyphagous insect, the RHFB did not behave as other specific leaf beetles on feeding patterns at the plant level (Ernest 1989). An important distinction is the host plant difference with this study compared to others. The only studies of feeding preference in RHFB were on cranberry foliage and showed more damage closer to the apical ends (younger growth) (Jaffe et al. 2021).

Since there were contradictions among the petri dish non-choice assays with whole and cut leaves as well as the choice caged plant assays, an additional experiment was set up to elucidate these opposite results. Two-way containerized choice assays found RHFB tend to feed on mature leaves more often than the young ones. This corroborates the findings of the whole plant caged assays. Because these two experiments had the same conclusions, it is hypothesized that RHFB will likely feed more often on mature leaves under field conditions. The findings of the petri dish assays indicate that they will be able to consume a higher percentage of the younger leaf due to its smaller size when compared to whole mature leaves. The area consumed was similar between the mature cut-out leaves and young leaves. In the two-way choice assays the mature leaves were trimmed to ensure beetles had comparable amounts of leaf and distance to choose from. Even with this trimming, adults still fed on mature leaves. This result emphasized that physical damage on leaves might not deter RHFB from feeding on this type of tissue.

The combined findings from each experiment demonstrate that RHFB inflict higher percent damage to mature leaves when given the choice, and likely under field conditions, but will consume any type of tissue when restricted on leaf availability. This conclusion suggests RHFB preference towards mature leaves in Hydrangeas. The mechanism behind these choices is

still unclear, and the experiments in this study were not set up to address that question. As previously mentioned, secondary compounds may be more concentrated in younger tissue leading to deterrence of RHFB from this type of tissue. Further studies could elaborate on this behavior by identifying compounds from young and mature Hydrangea leaves that might have some activity on RHFB adults. RHFB responses to the different tissue types could be analyzed using an electroantennogram to determine if chemo sensing affects their choice with respect to tissue type. Although further investigation into causation is needed for this behavior, this study demonstrates a preference for mature leaves over young leaves in Hydrangeas.

References

Cates RG. 1980. Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. Oecologia 46: 22–31.

Chong J. 2021. Visual Scale for Percent Defoliation in Hydrangea. Clemson University, Florence, SC

Ernest KA. 1989. Insect herbivory on a tropical understory tree: effects of leaf age and habitat. Biotropica 21: 194.

Feeny P. 1976. Plant apparency and chemical defense, pp. 1–40 *In* Biochemical Interaction Between Plants and Insects. Plenum Press, New York City, New York.

Getman-Pickering ZL, Campbell A, Aflitto N, Grele A, Davis JK, Ugine TA. 2020. LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. Methods in Ecology and Evolution 11: 215–221.

Hochuli DF. 2001. Insect herbivory and ontogeny: how do growth and development influence feeding behaviour, morphology and host use? Austral Ecology 26: 563–570.

Jaffe BD, Rink S, Guédot C. 2021. Life History and Damage by *Systena frontalis* F. (Coleoptera: Chrysomelidae) on *Vaccinium macrocarpon*. Journal of Insect Science 21: 1–8.

Johnson MTJ, Bertrand JA, Turcotte MM. 2016. Precision and accuracy in quantifying herbivory. Ecological Entomology 41: 112–121.

Joseph S V., Chong JH, Campbell B, Kunkel B, Lauderdale D, Jones S, Gill S, Chen Y, Schultz P, Held D, Hale F, Dale A, Vafaie E, Hudson W, Gilrein D, Del Pozo-Valdivia A. 2021. Current pest status and management practices for systena frontalis (coleoptera: chrysomelidae) in ornamental plants in the eastern united states: an online survey. Journal of Integrated Pest Management 12: 17–18.

Kenward MG, Roger JH. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53: 983–997.

Lane E, Del Pozo-Valdivia A. 2021. Red Headed Flea Beetle in Virginia Nurseries. Virginia Cooperative Extension. Virginia beach, Virginia. ENTO-464.

Lane E, Del Pozo-Valdivia A. 2022. Understanding red headed flea beetle biology to inform sustainable pest management practices in Virginia nurseries. Virginia Tech. Virginia Beach, Virginia. 73 pp.

NDSU. 2022. Red-Headed Flea Beetle in Soybean, Corn and Wheat. NDSU Agriculture and Extension. Fargo, ND.

Figure captions

Fig. 1. Laboratory containerized choice assay design. Acrylic tube shown with hole drilled though one side of the tube in the center as well as mature and young tissue leaves at each end. Stems of the leaves were kept hydrated by moistened cotton balls resting on plastic cups.

Fig. 2. Percent damage ± SE measured by Li-Cor area meter by time (in hours, rows) and number of beetles (columns). Grey bars represent whole leaf assays and white bars represent cut leaf squares. There were no cut leaf squares for young leaves and therefore no bars are present for those sections.

Fig. 3. Percent Hydrangea leaf damage ± SE to mature and young tissue (X-axis) in caged choice assays, using whole Hydrangea plants.

Fig. 4. Proportion of red headed flea beetles that fed on different Hydrangea tissue ages (X-axis). Rows denote sex.

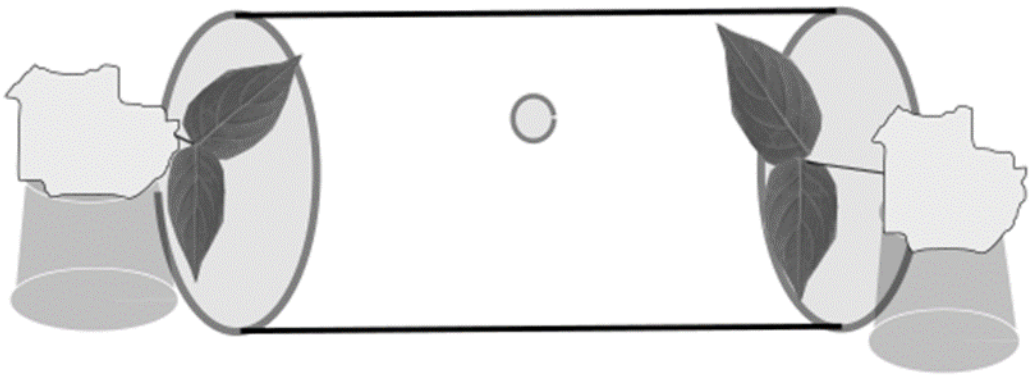

Fig. 1. Laboratory containerized choice assay design. Acrylic tube shown with hole drilled though one side of the tube in the center as well as mature and young tissue leaves at each end. Stems of the leaves were kept hydrated by moistened cotton balls resting on plastic cups.

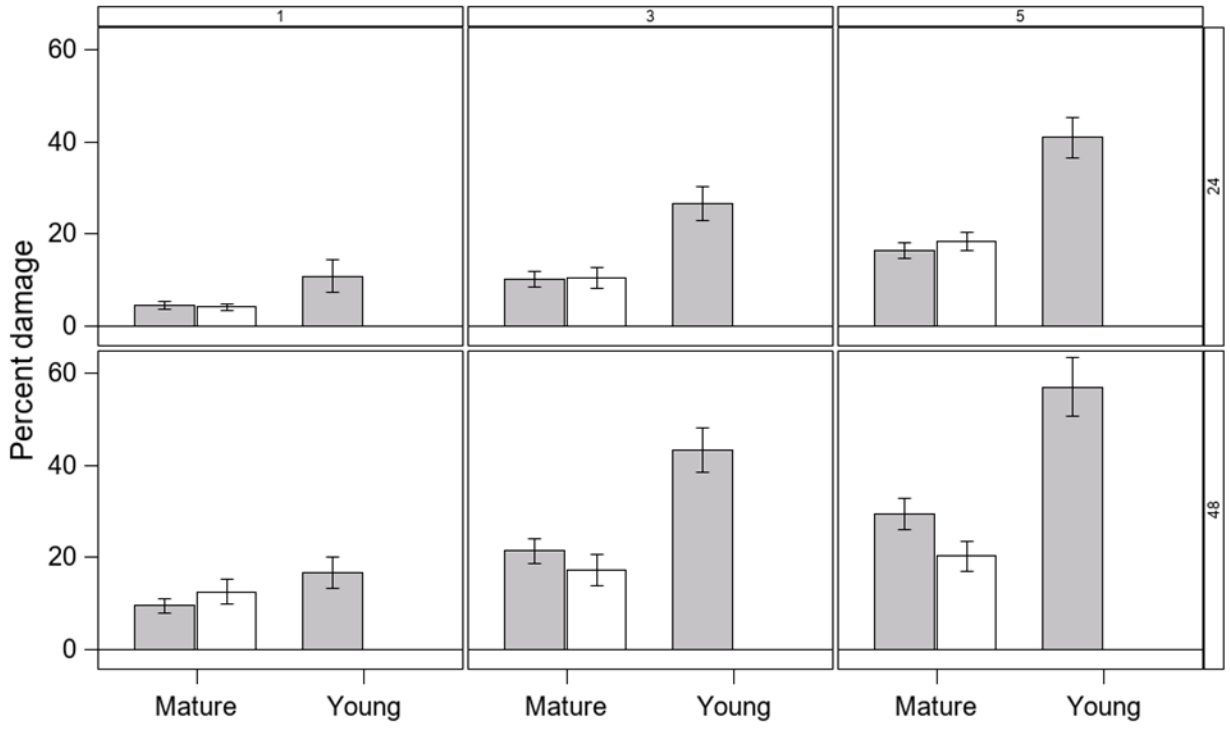

Fig. 2. Percent damage ± SE measured by Li-Cor area meter by time (in hours, rows) and number of beetles (columns). Grey bars represent whole leaf assays and white bars represent cut leaf squares. There were no cut leaf squares for young leaves and therefore no bars are present for those sections.

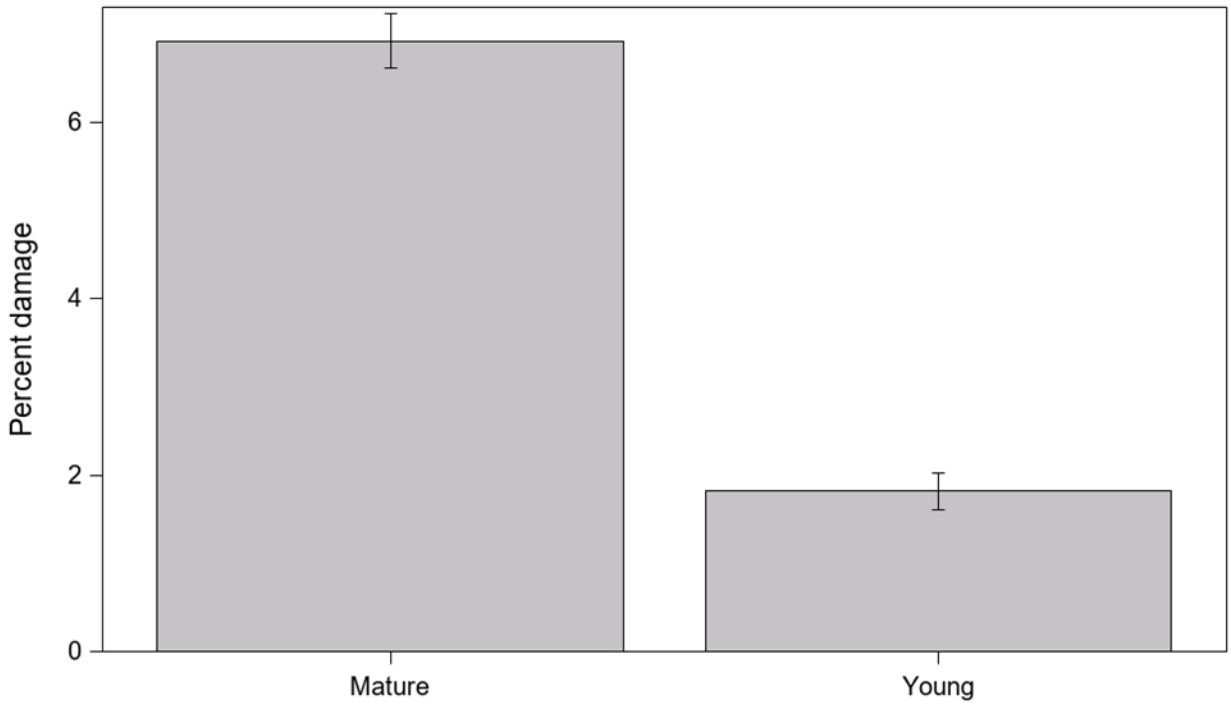

Fig. 3. Percent Hydrangea leaf damage ± SE to mature and young tissue (X-axis) in caged choice assays, using whole Hydrangea plants.

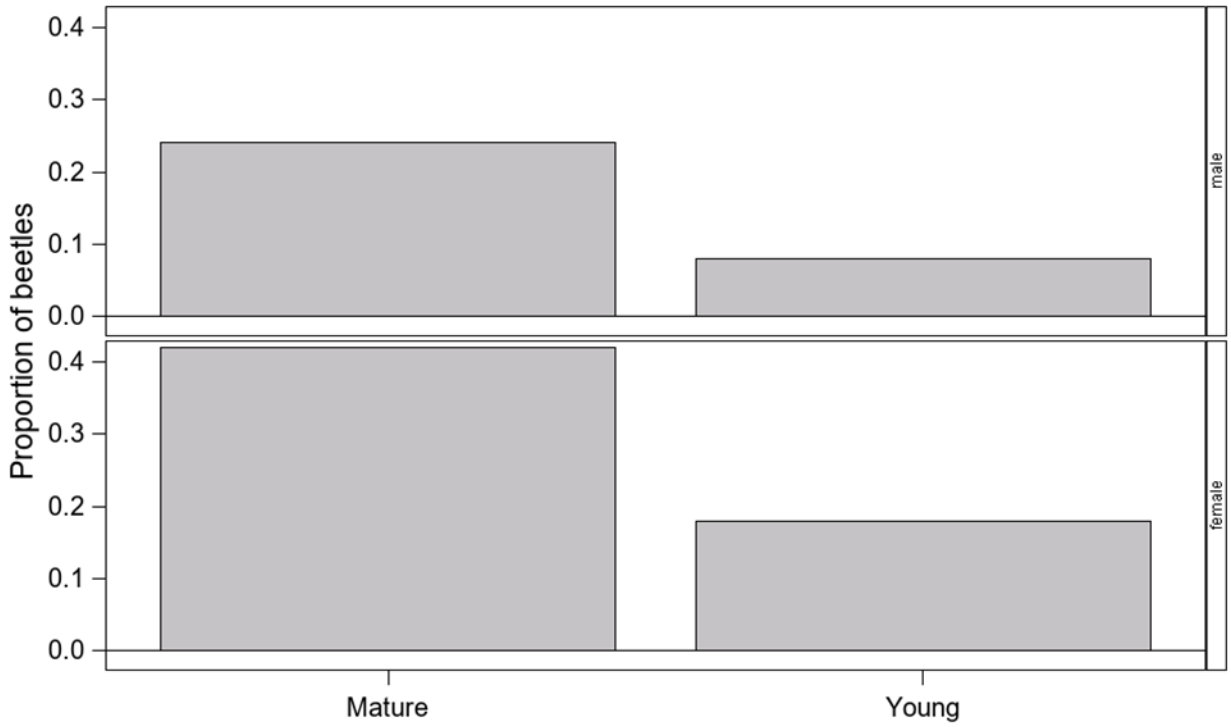

Fig. 4. Proportion of red headed flea beetles that fed on different Hydrangea tissue ages (X-axis). Rows denote sex.

Chapter 4: Chemical control of *Systena frontalis* adults on ornamentals

Introduction

Chemical control is the most widely used form of pest management against the red headed flea beetle (RHFB, *Systena* frontalis, Coleoptera: Chrysomelidae) (Joseph et al. 2021). Insecticide bioassays have not been performed on RHFB in the context of ornamental plants in Virginia. Those performed on adults in other systems have tested acetamiprid, chlorantranilipole/rynaxapyr, clothianidin, imidacloprid, and spinetoram as broadcast applications on cranberry (Guédot and Perry 2015). Treatment for larvae via drench experiments have used azadirachtin, chlorpyrifos, cyclantraniliprole, tolfenpyrad, and acephate (Lauderdale 2021). Growers in eastern Virginia, commonly use the insecticides: carbaryl (Group 1A), acephate (Group 1B), permethrin (Group 3A), and imidacloprid (Group 4A). Depending on the number of beetles observed, and plant injury, insecticides are broadcast as frequently as three times a week.

Nursery growers across the southeastern United States rely pyrethroid and neonicotinoid applications to manage RHFB, which have been found to be harmful to beneficial insects such as pollinators (Main et al. 2020). Regulatory measures may result from overuse as well as evolution of resistance in target populations (Sparks and Nauen 2015). The risk of harm to non-target organisms, such as predators, parasitoids, and other beneficial insects also increases. Rotation of insecticide modes of action is part of the resistance management program for a target pest. While growers will rotate the Insecticide Resistance Action Committee (IRAC) modes of actions, it is critical for them to be using the most effective insecticide within those classes. Several insecticides may be taken off the market in the future. To prevent overuse of such insecticides, and identify effective insecticides to avoid repeated applications, it is imperative to test

alternatives. Different modes of action were tested using the insecticides: tolfenpyrad (Group 21A), chlorantraniliprole (Group 28), cyantraniliprole (Group 28), and isocycloseram (Group 30). Laboratory non-choice bioassays using maximum label rates of different insecticides were performed to attain this goal.

Bioassays comparing different insecticides against *Systena frontalis* adults on *Hydrangea paniculata*, 2022

(Manuscript published by *Arthropod Management Tests* 2022, 47: tsac129)

The objective of this study was to compare the most common insecticides used by nursery growers to spray for control of *Systena frontalis*, often referred to as the red headed flea beetle (RHFB), against others that have yet to be tested against this pest. In discussions with growers in eastern Virginia, commonly used insecticides included carbaryl, acephate, permethrin, and imidacloprid.

RHFB adults and mature tissue leaves were collected from untreated potted hydrangeas grown at the Hampton Roads Agricultural Research and Extension Center (HRAREC) in Virginia Beach, VA. Discs were cut out of the leaves to fit inside 59mL plastic cups. Insecticide solutions were prepared in which the leaf discs would be dipped. Solutions of each insecticide were comprised of 200mL of tap water and the respective, indicated amount of insecticide. Maximum label rates for use in ornamentals against leaf beetles were selected (see Table 1). The rates given in the labels were scaled down from the per 100 gallons in which they were given. The insecticides were then measured using a micropipette or scale and stirred into the water under a fume hood. After mixing, ten leaf discs were submerged into the solution for one minute.

Bacteriological agar was prepared and about 10mL was poured into each plastic cup and left to solidify. Meanwhile, the leaf discs were removed from the insecticide solution and set to dry briefly on a paper towel. When the agar was almost solid, leaf discs were placed into their respective cups so that the leaf would set in place in the agar and prevent leaf desiccation. After all the treatments had been completed, a set of ten leaves dipped only in distilled water was used as an untreated check.

The RHFB adults were collected at the HRAREC on 29 August 2021, 26 June 2022, 11 July 2022, 25 July 2022, 08 August 2022, then placed individually in each cup and sealed with a lid. The experimental units were then placed in a growth chamber. Conditions in the chamber were set at 26℃, 40% RH and 14:10 L:D. After 24 hours, the units were temporarily removed from the growth chamber to record mortality data. If the mortality was not clearly visible, the lid was opened and the beetle poked with a pin. Beetles with impaired motor coordination such as inability to walk or move without falling over, were marked as moribund. The pin was sterilized with 70% ethanol between uses to avoid contamination. Mortality was checked additionally at 48 hours, 72 hours, 96 hours, and 7 days (168 hours) after treatment placement. Each treatment had ten replicates and was repeated three times for a total of 30 beetles tested. A one-way ANOVA was performed using SAS; and means separated post-ANOVA by Tukey's HSD test with $\alpha = 0.05$.

The mortality at maximum label rate is recorded in Table 1. The insecticides with the highest adult mortality across all days were Sevin and ISM-555. The lowest mortality overall was amongst those treated with Acelepryn. Apta had low mortality for the 48 hours and slightly increased at 72, 96, and 168 hours without more than half of the adults dead. Mainspring had no mortality until 96 hours and slightly increased at 168 hours after treatment. Merit and Orthene

had similar mortality at 24 hour after treatment and mortality continued to increase with Orthene being more lethal by 168 hours after treatment. Permethrin had high mortality across all checkup time frames, similarly to ISM-555 and Sevin.[1]

[1] This research was supported in part by industry gifts of pesticides and research funding.

Table 1. Mortality rates of *Systena frontalis* adults at each checkup time frame after being exposed to experimental insecticides under controlled conditions.

Treatment	Active ingredient	IRAC group	Rate/ 100 gal (fl. oz or oz)	Mortality rates				
				24h[a]	48h[a]	72h[a]	96h[a]	168h[a]
Acelepryn	Chlorantraniliprole	28	16	0.0333c	0.0333c	0.0333c	0.1333bc	0.2000cd
Apta	Tolfenpyrad	21A	27	0.2333b	0.2333b	0.3000b	0.3333b	0.4667bc
ISM-555	Isocycloseram	30	3.08	0.9000a	0.9667a	1.0000a	1.0000a	1.0000a
Mainspring	Cyantraniliprole	28	8.11	0.0000c	0.0000c	0.0000c	0.2667b	0.5667b
Untreated check	N/A	N/A	--	0.0000c	0.0000c	0.0000c	0.0000c	0.0333d
df				4, 145	4, 145	4, 145	4, 145	4, 145
F				71.43	102.16	110.08	40.72	29.16
P				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Merit	Imidacloprid	4A	1.66	0.3667b	0.5667c	0.5333b	0.6667b	0.8000b
Orthene	Acephate	1B	16	0.6500b	0.3000bc	0.2000ab	0.1000ab	0.0500ab
Permethrin	Permethrin	3A	8	0.7333a	0.8333ab	0.8333a	0.8667ab	0.9000ab
Sevin	Carbaryl	1A	32	0.9667a	1.0000a	1.0000a	1.0000a	1.0000a
Untreated check	N/A	N/A	--	0.0000c	0.0000d	0.0000c	0.0000c	0.0333c
df				4, 135	4, 135	4, 135	4, 135	4, 135
F				30.61	36.32	36.29	53.60	67.09
P				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Means within columns followed by a common letter are not significantly different

Discussion

The insecticides with the highest mortality on RHFB across all days were carbaryl (Sevin) and isocycloseram (ISM-555). The lowest mortality was amongst those treated with Acelepryn. Based on these findings, it can be concluded that under laboratory settings, carbaryl and isocycloseram are most effective against RHFB. Currently used insecticides have contact and systemic modes of action where RHFB are killed in short periods of time. Alternatives tested such as diamides, (chlorantraniliprole and cyantraniliprole) require ingestion for mortality, resulting in feeding from adults before they are killed. Prevention of foliar injury is paramount to growers meaning that insecticides working in this manner are not optimal. While these experiments demonstrated that the currently used insecticides were more effective overall than those previously untested, this provides more information for future decisions on insecticide selection. Isocycloseram is unavailable in the United States as of 2022, but with the data showing its high efficacy, it has potential for use in ornamentals against RHFB adults in the future. Further drench and larvae targeted trials are beyond the scope of this experiment. Biological control has begun to be explored and two nematodes (*Oscheius onirici* Torini (Rhabditida: Rhabditidae) and *Heterorhabditis georgiana* Nguyen (Rhabditida: Rhabditidae)) have potential for use to control RHFB despite only having been studied amongst cranberry systems (Foye and Steffan 2019).

References

Guédot C, Perry RS. 2015. Evaluation of Soil and Foliar Applications of Insecticides for the Control of Flea Beetle in Cranberry, 2014: Table 1. Arthropod Management Tests 40: C9.

Joseph S V, Chong J-H, Campbell B, Kunkel B, Lauderdale D, Jones S, Gill S, Chen Y, Schultz P, Held D, Hale F, Dale A, Vafaie E, Hudson W, Gilrein D, Del Pozo-Valdivia A. 2021. Current Pest Status and Management Practices for Systena frontalis (Coleoptera: Chrysomelidae) in Ornamental Plants in the Eastern United States: An Online Survey. Journal of Integrated Pest Management 12: 1–10.

Lauderdale D. 2017. Red-headed Flea Beetle Biology and Management. Nursery & Landscape Notes: 33–35.

Main AR, Hladik ML, Webb EB, Goyne KW, Mengel D. 2020. Beyond neonicotinoids-Wild pollinators are exposed to a range of pesticides while foraging in agroecosystems. Science of the Total Environment 742.

Sparks TC, Nauen R. 2015. IRAC: Mode of action classification and insecticide resistance management. Pesticide Biochemistry and Physiology 121: 122–128.

Chapter 5: Overall Conclusions

Population peaks for adults occured in late June, July, and August. It is likely there are three generations in Virginia nurseries between May and September. Growers can expect to document highs and lows in populations throughout the season based on our findings, starting in late May to early September. For growers, visual scouting is quick, requires no specialized equipment, and can be learned easily. To enhance efficacy, look for beetles between 1100 and 1500. One may underestimate the number of beetles present by searching for beetles early in the morning or late in the day towards evening. RHFB are most active during the middle of the day and are therefore easier to visually track and count.

After scouting, growers can use the data gathered on feeding injury to determine how much defoliation they may expect to see on their crops. The regression equation presented in Chapter 2, damage = 0.8964 + 0.2332 × (number of beetles per plant), can be used to calculate defoliation per plant per week. It is also expected that injury will be distributed evenly throughout the plant canopy. Thorough penetration of insecticide is essential for adequate control. Only a third of beetles will be in contact with the applied toxicant if only the top layer is reached. Individual plant data demonstrates RHFB prefer mature growth over young tissue. It is unclear why this preference exists for more mature leaves. Further studies would be required to determine factors influencing preference. Young whole leaves are smaller in surface area than mature whole leaves; thus, RHFB consumed a higher percent of the young leaves in non-choice assays. Notably, one beetle can consume over 10% of a young leaf in 24 hours under laboratory conditions. While growers have been aware that RHFB can cause severe defoliation, bioassays performed documented RHFB damage potential across multiple adult densities and length of exposure. There is currently no established economic injury level, and growers can use data

presented in Chapters 2 and 3 to inform pest management tactics.

Growers need information to control RHFB effectively. Insecticide bioassays performed in this study tested the most common insecticides currently used by growers at commercial nurseries against others that have not been tested in the system. The most effective option found was isocycloseram (IRAC Group 30). While it is not yet available to growers, if it becomes so, they will be able to use it and keep RHFBs at manageable levels. Chemical control is currently a major part of IPM programs in several systems including ornamentals. The use of insecticides is widespread but with the information presented in this thesis, usage may be reduced.

Reduction of insecticide usage and adopting alternative control options such as biological control would be beneficial to associated ecosystems and to growers as their current control methods are unsustainable. Regulations on chemical usage and concerns about target population resistance merit the exploration of alternatives. Future research is required to explore the efficacy of such solutions in open-field nurseries. The research conducted across all Chapters shows ways to improve scouting and to reduce frequency of insecticide applications. Enhanced scouting procedures and data on RHFB behavior in eastern Virginia provided by this thesis will improve management of RHFB in nurseries and inform further studies.

References

Braasch, J., and I. Kaplan. 2012. Over what distance are plant volatiles bioactive? Estimating the spatial dimensions of attraction in an arthropod assemblage. Entomol. Exp. Appl. 145: 115–123.

Buffington, M. L., and R. A. Redak. 1998. A comparison of vacuum sampling versus sweep-netting for arthropod biodiversity measurements in California coastal sage scrub. J. Insect Conserv. 2: 99–106.

Chong, J. 2021. Visual Scale for Percent Defoliation in Hydrangea. Clemson University, Florence, SC. 8 pp.

Cooper, W., and T. J. Rephann. 2017. The economic impact of Virginia's agriculture and forest industries. University of Virginia. 71 pp.

Gregg, P. C., A. P. Del Socorro, and P. J. Landolt. 2018. Advances in attract and kill for agricultural pests: beyond pheromones. Annu. Rev. Entomol. 63: 453–470.

Hajek, A., and J. Eilenberg. 2018. Conserving Natural Enemies: Reducing Effects of Pesticides on Natural Enemies, p. 87. In Nat. Enemies.

Herrick, N. J., and R. A. Cloyd. 2020. Overwintering, Host-Plant Selection, and Insecticide Susceptibility of Systena frontalis (Coleoptera: Chrysomelidae): A Major Insect Pest of Nursery Production Systems. J. Econ. Entomol. 113: 2785–2792.

Jacques, R. L., and D. C. Peters. 1971. Biology of *Systena frontalis* with Special Reference to Corn. J. Econ. Entomol. 64: 135–138.

Jaffe, B. D., S. Rink, and C. Guédot. 2021. Life History and Damage by *Systena frontalis* F.

(Coleoptera: Chrysomelidae) on *Vaccinium macrocarpon* Ait. J. Insect Sci. 21: 1-8.

Joseph, S. V, W. Hudson, and B. Kunkel. 2018. Red-headed Flea Beetle: An Ornamental Nursery Pest.

Lauderdale, D. 2017. Red-headed Flea Beetle Biology and Management. Nurs. Landsc. Notes. 33–35.

Riley, C. V. 1884. Reports of Observations and Experiments in the Practical Work of the Division. U.S. Department of Agriculture, Washington, DC. 50 pp.