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Population Ecology

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

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Ornamental plant production in eastern Virginia nurseries have been greatly impacted by *Systena frontalis* (F.), also known as the red-headed flea beetle. With the advent of *S. frontalis* as a prevalent pest in the past 2 decades, baseline phenology and behavior are currently understudied within Virginia nurseries. This pest is costly to control due to insecticide expenses and loss of saleable plants. In 2021 and 2022, populations of this insect were monitored at 2 commercial nurseries in eastern Virginia in order to better understand their temporal and spatial population dynamics. Patterns that emerged indicated *S. frontalis* could have up to 3 generations in eastern Virginia, with peaks of adult abundance in June, late July, and late August to early September. Phenylethyl alcohol was tested as an adult attractant lure, but it was found to be ineffective under nursery conditions. Diel monitoring demonstrated these adults were most active from 1100 to 1500 h. Severity of defoliation at the leaf level increased linearly with increased density of adults, where 5 individuals defoliated up to 4% of any *Hydrangea paniculata* cv. 'Limelight' leaf in 1 wk under greenhouse conditions. Timing of scouting and insecticide sprays according to the adult activity peaks of the day and across the season may allow reduction in overall insecticide usage.

Key words: diel activity, growing degree day, no-choice assay, ornamental, phenology

Systena frontalis (F.) (Coleoptera: Chrysomelidae), commonly known as the red-headed flea beetle and described in the United States by Riley (1884) and Chittenden (1902), has recently become a major pest in nursery crops in the midwestern and northeastern US regions (Joseph et al. 2021). This species is also called the cranberry flea beetle, since it is a pest in the cranberry production system (Jaffe et al. 2021). This insect is a key economic pest of containerized ornamental plants grown outdoors (Joseph et al. 2021). Despite the economic importance of this pest, there are a few recent studies on its biology (Herrick and Cloyd 2020, Jaffe et al. 2021, Joseph et al. 2021), phenology, and its diel and peak seasonal activities in ornamental crops across affected nurseries, including facilities in Virginia.

Adults of *S. frontalis* defoliate a variety of woody and herbaceous crops, such as grapes (*Vitis vinifera*), cabbage (*Brassica oleracea* var. *capitata*), beets (*Beta vulgaris* subsp. *vulgaris*), potatoes (*Solanum tuberosum*), corn (*Zea mays*), beans (*Phaseolus vulgaris*), clover (*Trifolium* spp.), gooseberries (*Ribes* spp.), mangelwurzels (*Beta vulgaris*), pear (*Pyrus communis*) (Jacques and Peters 1971), cranberry (*Vaccinium macrocarpon*) (Jaffe et al. 2021), and ornamentals, such as sweet spire iteas (*Itea virginica*), hydrangeas (*Hydrangea paniculata*), hollies (*Ilex crenata*), dogwood (*Cornus* spp.), and weigelas (*Weigela* spp.) (Cloyd and Herrick 2018). Damage from this pest is a top concern for Virginia nurseries, since it can increase production costs for infested ornamental plants by rendering them unsellable due to foliar injury (Joseph et al. 2021). Damage inflicted on foliage appears as shot-holes or skeletonization of leaves, often with chlorosis or necrosis of the tissues around the shot-holes depending on the type of plants (Lane and Del Pozo-Valdivia 2021). Hydrangeas, sweet spire iteas, and hollies have been particularly affected by this insect within nurseries (Joseph et al. 2021).

Timing of *S. frontalis* 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, 3 larval instars were observed with time between eclosion and complete larval development being approximately 30 days (Jacques and Peters 1971). This insect overwinters as eggs in the soil and its development time to eclosion is estimated to be around 15 wk at temperatures between 0 and 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 and Cloyd 2020). Generally, larvae are present beginning in late spring (March–April) in northern latitudes (Jaffe et al. 2021). In North Carolina, eggs of this insect have been observed to hatch between 250 and 480 growing degree days (GDD), calculated at base 50 °F (10 °C) (Lauderdale 2017). First-generation adults are expected to emerge from infested nursery pots between 517 and 1,028 GDD_{50}, with second-generation larvae developing at 1,570–1,860 GDD_{50} and second-generation adults emerging at 1,878–2,318 GDD_{50} (Lauderdale 2017). Growing degree-day models could be an important tool for improving the timing of insecticidal applications (Rice et al. 1984, as an example in pear orchards).

Nursery growers in Virginia could spray insecticides up to twice a week to control severe infestations of *S. frontalis* adults (Del Pozo-Valdivia, anecdotal observation). These 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 could be harmful to beneficial insects such as pollinators (Main et al. 2020) predators, parasitoids, and other naturally occurring beneficial insects. Documentation of these non-target effects has resulted in guidelines at the insecticide label level, specifying mitigation practices to avoid such issues. Moreover, continued use of these insecticides may result in the development of resistance in exposed individuals, as summarized by Sparks and Nauen (2015). Reducing predator and parasitoid populations due to exposure to frequent insecticide sprays may lead to secondary pest outbreaks, such as thrips and mites, under different settings (Hajek and Eilenberg 2018), including nurseries. Insecticide testing within cranberry (Guédot and Perry 2015) and ornamental crop systems (Lane and Del Pozo-Valdivia 2022) showed that few insecticides were effective against this pest. Potential options within nurseries include the novel IRAC Group 30 insecticide, isocycloseram (Lane and Del Pozo-Valdivia 2022), which has yet to become commercially available as of 2022.

Chemical control methods like spraying new classes of insecticides could be expensive, and effective options have become less available due to regulatory restrictions (Joseph et al. 2017, Joseph 2019). While it is likely that *S. frontalis* population dynamics may be influenced by geographical location and weather, they may also respond to herbivore-induced plant volatiles (HIPV) (Braasch and Kaplan 2012). Potential cultural control methods, such as the deployment of phenylethyl alcohol (PEA), a HIPV, have been studied to determine attractant or repellent properties within insects (Braasch and Kaplan 2012). PEA, presented as a lure, was distributed across at least 8 m between experimental units, and despite repellency against herbivorous insects, *S. frontalis* was attracted to those PEA lures in soybean experimental plots (Braasch and Kaplan 2012).

As a continued effort to address gaps in the current knowledge of *S. frontalis*, as a key pest of ornamental crops, 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 (a) to monitor the seasonal phenology of *S. frontalis* adults and larvae as well as the presence of adults nearby affected nurseries; (b) to observe diel activities performed by adults and determine timing of activity levels under field conditions; (c) to explore the utility of PEA attractant lures in nursery settings, aiming to create an additional monitoring tool and/or proposing a mass trapping system; and (d) to quantify the feeding potential of adults throughout canopy layers and between varying densities in hydrangea plants under controlled conditions. These objectives provided insight for nurseries in Virginia 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 *S. frontalis* Populations

Each week from May to September of 2021, *S. frontalis* populations were scouted at 2 commercial nurseries in eastern Virginia (Nursery 1, near the town of Smithfield, and Nursery 2, in the City of Suffolk). Visual scouting for the presence of foliar damage occurred at each sampling point and, if no damage was detected, vacuuming did not occur. There were 3 sampling points for each scouted crop. Each sampling point consisted of 3 individual potted plants. A Stihl leaf blower (model SH 56 C-E, Virginia Beach, VA, USA) set to vacuum with a mesh bag over the end was used to suction adults from selected crops and to count their density per plant in *Hydrangea paniculata* cv. 'Limelight', *Itea virginica* cv. 'Little Henry', or *Ilex crenata* cv. 'Bennett's Compacta' blocks of each plant species. These individual blocks were separated at least 30 m apart from one another. All crop management tactics used at each nursery site and year, including fertilization, cultivation, and application of pesticides (herbicides, fungicides, or insecticides) followed commercial standards and were decided and executed by each cooperating grower. The visual scouting was modified by the third week of August 2021 when scouting procedures were updated to those used in 2022.

The same leaf blower was used to sample the number of adults per plant at the same 2 nursery locations weekly from March to December in 2022. A nursery pad at the Hampton Roads Agricultural Research and Extension Center (HRAREC) in the City of Virginia Beach was added as a third location in 2022. Three blocks of 3 sampling points each were collected within each location chosen by moving diagonally across each block in a zig-zag pattern (Buffington and Redak 1998). Blocks were divided in thirds, and sampling points were selected at each third. As of 2021, each sampling point consisted of 3 individual plants. When available, multiple commercial blocks of the same cultivar and planting date were scouted during the same sampling week to increase sample size. To calculate the growing degree days associated with *S. frontalis* phenology events, air temperature was collected from a HOBO data logger (model MX2301A, Onset, Bourne, MA) placed on a representative area of each sampling location, dominated by grasses. The following equation was used to calculate these growing degree days: GDD_{50} = ((*high* temperature (°F) + *low* temperature (°F))/2) – 50 °F (Lauderdale 2017).

In addition to the monitoring of adults, larvae within the potting media mix of containerized hydrangeas were counted weekly from May to October 2022 only at the HRAREC location. The outer 5.1 cm (2 inches) of potting media in 2 hydrangea plants were scraped off the containerized plants weekly and visually examined to document the number of *S. frontalis* larvae contained within pots. These plants were part of the same block of hydrangeas that were examined to collect adult densities, as previously mentioned.

Presence of *S. frontalis* Adults Nearby Affected Nurseries

To determine the prevalence of *S. frontalis* outside affected nurseries in eastern Virginia, 3 scouting trips were conducted at directly adjacent fields planted with soybeans. One farm next to Nursery 1

and 2 were scouted using a 38-cm-diameter sweep net on 25 June 2021, 19 July 2021, and 9 June 2022. Within each soybean field, 30 sweeps per field were collected, and the content was checked for *S. frontalis* adult densities. Additionally, sticky traps were placed at the 2 selected nurseries to monitor the *S. frontalis* adults in the interphase area between ornamental blocks and adjacent soybean fields. Previous studies (Kuhar et al. 2002, Cárcamo et al. 2008) had used sticky cards to collect flea beetles. The traps were comprised of a 3 cm × 3 cm × 1.50 m wood stake with a 20 cm × 14 cm yellow sticky card (double sided, Alpha Scents Inc., Canby, OR) attached 65 cm from the ground and a 30 cm × 15 cm translucent sticky card (clear panel trap, Alpha Scents Inc., Canby, OR, USA) placed 102 cm from the ground. The rationale behind placing those cards was to capture any adult potentially exploiting alternative hosts in that interphase area or to intercept any adult flying away or towards the nurseries. Four equidistant traps were placed on the side of the perimeter of each nursery adjacent to the soybean fields, and 2 additional traps were located by the perimeter of a hydrangea and a holly blocks, respectively, for a total of 6 traps at each nursery location. In 2021 and 2022, these traps were checked weekly for counting adults per card from May to August. Sticky cards were replaced once a month or as needed.

Monitoring *S. frontalis* Diel Activity Under Field Conditions

Visual observations of adult behavior were taken at the HRAREC using *Hydrangea paniculata* cv. 'Limelight' plants that were naturally infested. These behavioral events were classified as (i) flying, (ii) jumping, (iii) walking, (iv) mating, or (v) sedentary. Adults 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 adult performed more than one activity, it was marked as separate events. The observations were made every 2 h over the course of a 24-h period for a total of 12 observation periods within that day. Four sampling points were marked as the observation areas within a block of hydrangeas. Each point was at least 2 m away from the others and was constituted by a grouping of 12 containerized plants. For 1 min, the number of adults performing each activity was observed in the same manner 2 more times for a total of three 1-min intervals per sampling point and per timeslot. Overhead irrigation for the observation areas was suspended during data collection. These visual observations were completed 3 times (12 August 2021, 1 June 2022, and 26 August 2022).

Characterizing Herbivory Using Cage and No-Choice Whole-Plant Assays

Defoliation caused by *S. frontalis*, at the leaf level, was quantified using no-choice assays. Different adult densities (0, 5, and 25 adults per plant) were placed inside a 60 cm × 60 cm × 91 cm mesh cage (butterflyhabitatXL, RestCloud, Zhejiang, China) containing one hydrangea plant. Potted hydrangeas cv. 'Limelight' with no prior foliar damage and no insecticide treatment were selected from the HRAREC nursery stock. There were 4 individually caged plants per adult density and the trial was repeated 3 times in 6 June, 19 August, and 1 September 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 7 days, the cages were removed from the greenhouse and the adults were removed by suction. The percent defoliation was then assessed by choosing 3 leaves from the center of each canopy layer. These canopy layers were selected by dividing the plant in thirds (bottom, middle, and top). Percent defoliation of each leaf was estimated using the visual guidelines proposed by Chong (2021, unpublished data).

The Use of PEA in the Field as an Adult Attractant

Several traps were placed in Nursery 1 and at the HRAREC to determine the efficacy of PEA as an adult attractant. Experimental treatments included (a) yellow sticky card with no PEA lure, (b) translucent sticky card with no lure, (c) yellow sticky cards with PEA lure, and (d) translucent sticky card with lure. Traps were placed 10 m apart within blocks and at 5 m from the edge of selected commercial blocks. The design included a 1.5-m wooden stake placed among plants of the most affected crops by this pest including hydrangeas, hollies, and sweet spire itea, affixed with a sticky card placed with the top at the height of the canopy of the surrounding crops. Lures of PEA at the 99% concentration (Acros organics, Geel, Belgium) were constructed by placing 3 ml of PEA within a 100-ml 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 commercial blocks of holly plants cv. 'Bennett's Compacta' at aforementioned Nursery 1. This trial was repeated in July 2021, June 2022, and July 2022. Due to the smaller areas, only treatments 1 and 3 were placed within hydrangea cv. 'Limelight' and sweet spire itea cv. 'Little Henry' commercial blocks at Nursery 1 as well as hydrangeas at the HRAREC. One week and 2 wk after deployment of traps, sticky cards were checked for adult captures and the 4 plants closest to a trap were vacuumed and adults counted. Vacuum collection of samples (Buffington and Redak 1998) could ensure the capture of a broader range of insects for evaluation of the efficacy of PEA as a lure. After each data collection, treatments within each block were rerandomized as part of a randomized complete block design.

Data Analysis

Adult densities of *S. frontalis* per plant collected weekly were analyzed separately by each year, since collection method slightly varied. Repeated-measure ANOVAs were calculated using linear mixed models (PROC MIXED; SAS 9.4, Cary, NC, USA). An average of adults per plant across locations and crops was calculated for each sampling point at each collection week. This average was the response variable, where collection week was a fixed and repeated effect. Sampling points were the random effect and were also used as subjects, with compound symmetry (CS) selected as a covariance structure (CS was the selection for all repeated-measure ANOVAs). Then, using data from 2022 as the most complete set, repeated-measure ANOVAs were individually calculated for each crop (hydrangea, hollies, and sweet spire iteas) within each location (Nursery 1, Nursery 2, and HRAREC). The response variable was the average number of *S. frontalis* adults found per plant. The fixed and repeated effect was sampling week. Sampling points were the random effect and were also considered as subjects. A final repeated-measure ANOVA was calculated using number of larvae per plant (only at HRAREC) as a response variable and sampling month as the fixed and repeated effect. Sampling week was used as a random effect, where sampling points were the subjects. Additionally, peaks and lows in *S. frontalis* population density per plant were associated with an accumulation of growing degree days in base 50 °F.

The frequency of performances of daily activities were compared across time of the day using a repeated-measure ANOVA, where the

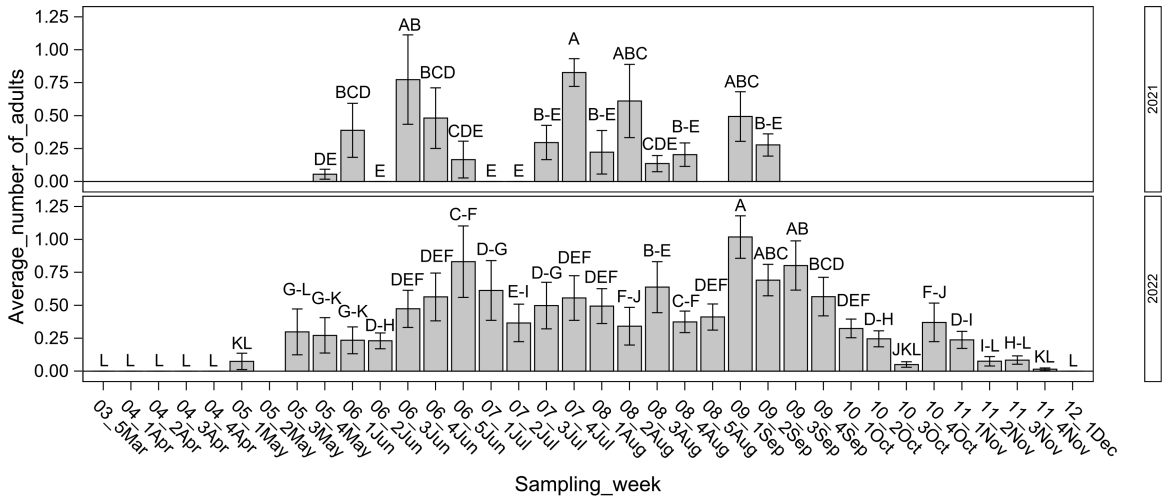

Fig. 1. Average number of *Systena frontalis* adults ± SE vacuumed per plant across all crops (hydrangeas, sweet spire iteas, and hollies) and locations (Nursery 1, Nursery 2, and HRAREC) in eastern Virginia, organized by sampling date (X-axis). Rows present data by year. Sampling week is denoted using "number month _number of week" and "month name." Mean separations were performed by each year using Tukey's HSD test. Bars sharing the same letters are not significantly different ($\alpha = 0.05$).

response variable was the average number of adults performing each activity at each time slot. Time of day was a fixed and repeated effect. Sampling points within each sampling date were considered as random effect and subjects.

One-way and 2-way ANOVAs were used for analysis of caged no-choice assays and PEA attractant lure data, respectively. Canopy layer and density of adults were considered fixed effects and were compared using percent foliar damage as a response variable. Replication ($r = 4$) was a random effect. Additionally, a regression was performed (PROC REG, SAS 9.4) using the leaf percent defoliation as the dependent variable and densities of adults (0, 5, and 25) as the independent variable. For the PEA lure trial, total number of adults collected by sticky cards were calculated by adding densities recorded in the first and second weeks after treatment deployment. An average of adults vacuumed per plant was calculated between those 2 collection weeks. Total adults per card and average adults vacuumed per plant were the response variables for the PEA trials. Separate ANOVAs were calculated, where treatments including lure and no lure, and card type (yellow and translucent) were included as fixed effects at each location and replications was considered as a random effect.

Insect counts and percent defoliation were $\log_{10}$- and square-rooted transformed, respectively, to comply with the normality assumption. The Kenward–Roger correction method was used to calculate degrees of freedom (Kenward and Roger 1997). Mean separations, post-ANOVA, were calculated using the Tukey's HSD test, with $\alpha = 0.05$. Back-transformed data were plotted and presented in the results.

Results

Seasonal Monitoring of *S. frontalis* Populations

There were 186 vacuum samples collected in 2021 to scout for *S. frontalis* adults in 558 potted plants across locations and crops. Sample size was increased in 2022, with a total of 1,959 vacuum samples, scouting 5,877 potted plants. Adults were present on almost all sampling dates across locations and crops (Fig. 1). In 2021, high numbers of adults per plant were found during the middle of June, last week of July, and the first week of September (Fig. 1). Similarly, high number of adults per plant in 2022 were documented during the last week of July and the first week of September. However, the first peak of adult activity during 2022 was registered in late June (Fig. 1).

HRAREC sampling location

There was a significant difference between density of adults per plant within hydrangeas by sampling week in 2022 ($F = 19.43$; df = 31, 62; $P < 0.0001$). The highest adult numbers in hydrangeas were in the fifth week in June (2,470.05 GDD_{50}) and the lowest numbers were found during March, April, and the last 2 weeks of October and November (Fig. 2). There was also a significant difference between adult numbers within sweet spire iteas by sampling week ($F = 7.58$; df = 26, 52; $P < 0.0001$). The peak of adult numbers was in the third week in August (4,238.75 GDD_{50}) and the lowest numbers in May, October, and November (data not shown). There was not enough data from holly crops to determine whether there were differences between sampling weeks. Additionally, larva densities within the potting media of containerized hydrangeas were different among the months of scouting ($F = 2.77$; df = 5, 34; $P = 0.0333$), with higher larvae densities in October (4.50 ± 0.50) and May (2.33 ± 1.74), compared to low numbers in June (0.38 ± 0.26) and July (0.88 ± 0.40) (Fig. 2).

Nursery 1 sampling location

There was a significant difference between number of adults per plant within hydrangeas by sampling week in 2022 ($F = 12.62$; df = 30, 60; $P < 0.0001$). The highest adult numbers were found at the second week of September (4,743.72 GDD_{50}), followed by densities in late July and late September, and the lowest numbers of adults during late March, April, early May, early July, mid-August, November, and early December (Fig. 3). Similarly, there was a significant difference between number of adults within sweet spire iteas by sampling week ($F = 19.70$; df = 28, 56; $P < 0.0001$). The highest number of adults were found during the fourth week of September (5,033.89 GDD_{50}) (Fig. 3). Throughout the season there were several weeks with low to no adults in May, early June, late June, July August, late October, November, and early December (Fig. 3). Additionally, there

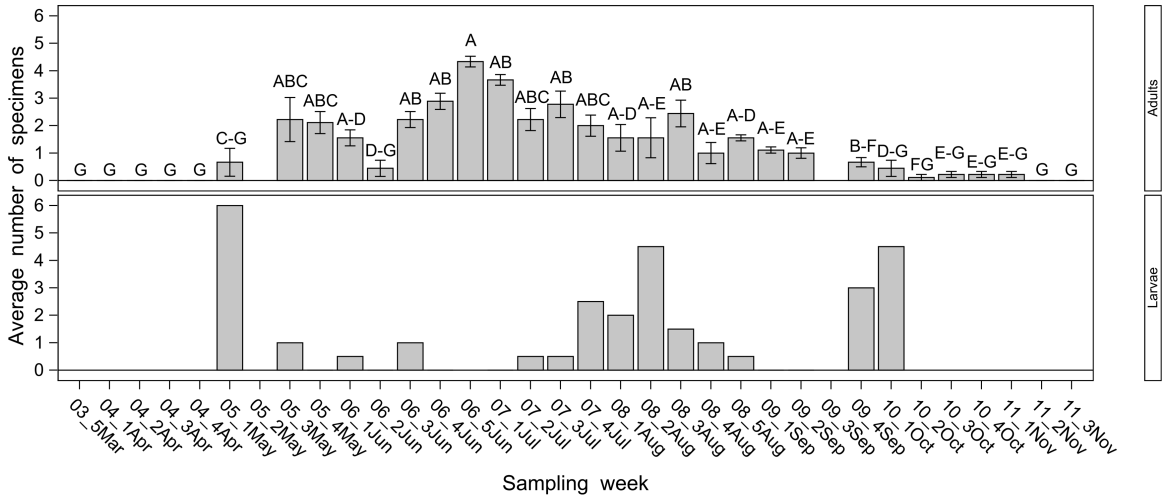

Fig. 2. Average number of *Systena frontalis* adults per plant found in hydrangeas at the HRAREC. Rows denote life stage (adults captured by vacuum and larvae found in potting media of containerized plants) scouted by week (X-axis). Each sampling week is denoted using "number month _number of week" and "month name." Mean separation was performed using Tukey's HSD test. Bars sharing the same letters are not significantly different ($\alpha = 0.05$).

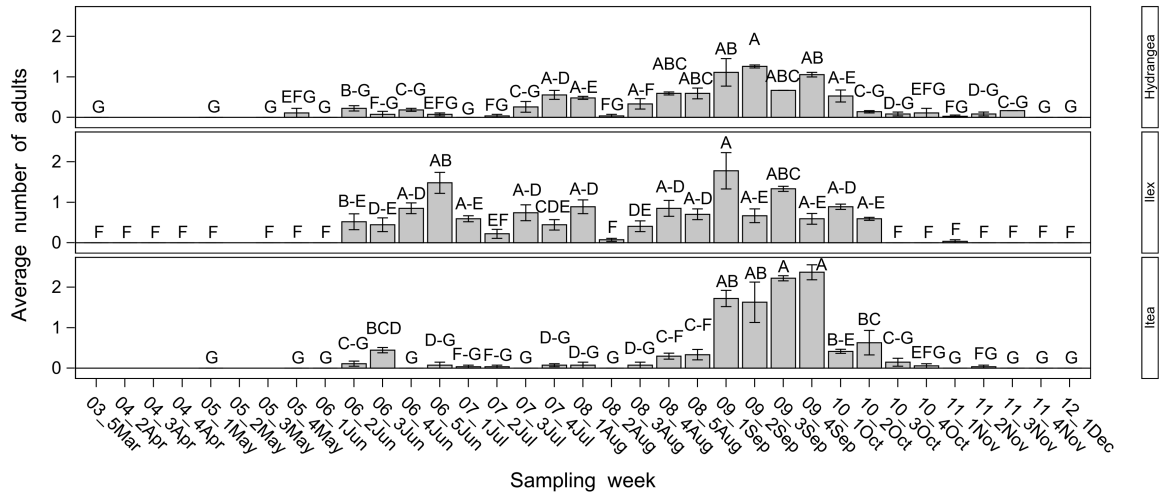

Fig. 3. Average number of *Systena frontalis* adults ± SE per plant collected within Nursery 1 in eastern Virginia. Rows denote different crops (hydrangeas—*Hydrangea*, hollies—*Ilex*, and sweet spire iteas—*Itea*). Sampling week is denoted using "number month _number of week" and "month name." Mean separations were performed by each crop using Tukey's HSD test. Bars sharing the same letters are not significantly different ($\alpha = 0.05$).

was a significant difference between adult numbers within hollies by sampling week ($F = 33.11$; df = 33, 66; $P < 0.0001$). The highest adult numbers were found in the first week of September (1.78 ± 0.45; 4,548.71 GDD_{50}) as well as in the fifth week of June, while the lowest were recorded in late March, April, May, early June, mid-August, late October, November, and early December (Fig. 3).

Nursery 2 sampling location

Number of beetles per plant across all sampling weeks and crops in 2022 were very low; therefore, there were 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 (data not shown).

Presence of *S. frontalis* Adults Nearby Affected Nurseries

A total of 450 sweep-net samples across 2 yr and 2 locations scouting for this insect yielded no adults when monitoring adjacent soybean fields to affected nursery locations. Once at the nursery, a total of 624 sticky card data collection events around the perimeter of the properties caught no adults in either 2021 or 2022. However, a total of 208 sticky card collection events at traps by the perimeter of nursery commercial block caught a total of 10 adults between the 2 yr, 2 crops, and the 2 locations.

Monitoring *S. frontalis* Diel Activity Under Field Conditions

Frequency of each activities performed by adults at each collection date was found to be similar, so data were pooled for analysis ($F = 0.16$; df = 11, 144; $P = 0.9900$). Across all collection dates, numbers of adults executing different activities characterized as flying, jumping, walking, mating, and being sedentary varied throughout hours of the day ($F = 5.60$; df = 11, 88; $P < 0.0001$) (Fig. 4). Significantly more beetles were flying at 1100 h and none flew at 0100, 0300, 0700, 0900, and 2300 h ($F = 12.00$, df = 11, 88, $P < 0.0001$) (Fig. 4).

Differences were also observed among the numbers of adults jumping across multiple leaves over time ($F = 5.20$; df = 11, 88; $P < 0.0001$). The highest number of adults jumping was found at 1300 h, and none were found jumping at 0100, 0300, 0500, 2100, and 2300 h (Fig. 4). Once moving on a plant, number of beetles observed walking was different over time ($F = 17.14$; df = 11, 88; $P < 0.0001$), with the peak for this activity registered at 1300 h and the lowest frequency at 0300 h. (Fig. 4). The number of adults recorded in a mating position with limited movement across a plant structure was different over time ($F = 3.64$; df = 11, 88; $P = 0.0003$), having the higher counts at 1300, 1700, and 1900 h, and no mating pairs found at 0100, 0300, 0500, 0700, 0900, and 1100 h (Fig. 4). Additionally, the number of sedentary beetles observed on plant tissue was also different over time ($F = 19.31$; df = 11, 88; $P < 0.0001$). The highest number of sedentary adults (not actively moving) was found at 1900 h, and the lowest was at 1100 and 1300 h (Fig. 4).

Characterizing Herbivory Using Cage and No-Choice Whole-Plant Assays

There was no difference amongst collection dates when comparing percent defoliation from the different adult densities ($F = 0.02$; df = 2, 6; $P = 0.9815$). Therefore, the data were pooled together for further analysis. In fact, there was a significant difference in percent defoliation among different adult densities across all repetitions ($F = 255.57$; df = 2, 88; $P < 0.0001$). Plants infested with zero adults recorded the lowest percent defoliation at the leaf level, followed by plants with adult densities of 5 and 25, respectively (Fig. 5). One repetition of caged plants with zero beetles infested suffered a small percent defoliation because an adult emerged from the potting media during the study (Fig. 5). The interaction between adult densities and location of the damage at each plant canopy layer was not significant ($F = 0.86$; df = 4, 88; $P = 0.4940$). Percent defoliation inflicted by adults to leaves at each canopy's layer (bottom, middle, and top) within one plant was found to be similar ($F = 0.52$; df = 2, 88; $P = 0.5953$). Percent defoliation of leaves ranges from 3.06% to 3.11% of the area damaged across canopy layers.

Density of *S. frontalis* adults per plant explained a large proportion of the leaf percent defoliation inflicted in this study ($R^2 = 0.6908$). The regression equation, leaf percent defoliation = 0.8964 + 0.2332 × (number of adults per plant), represents damage inflicted at the leaf level by a selected adult density across one plant over 7 days.

The Use of PEA in the Field as an Adult Attractant

Hydrangeas at Nursery 1 were not infested by this insect, therefore, no statistical analysis was performed. Adult captures on sticky cards placed in sweet spire iteas at Nursery 1 were not different between lure presence and no lure ($F = 1.00$; df =1, 6; $P = 0.3559$). Hollies had higher numbers of captured adults at Nursery 1 on cards across all sampling dates, and analyses were performed for each repetition. For repetition 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 in adult captured between card type ($F = 1.02$; df = 1, 25; $P = 0.3231$) and the presence of lure ($F = 0.01$; df = 1, 25; $P = 0.9117$). The second and third

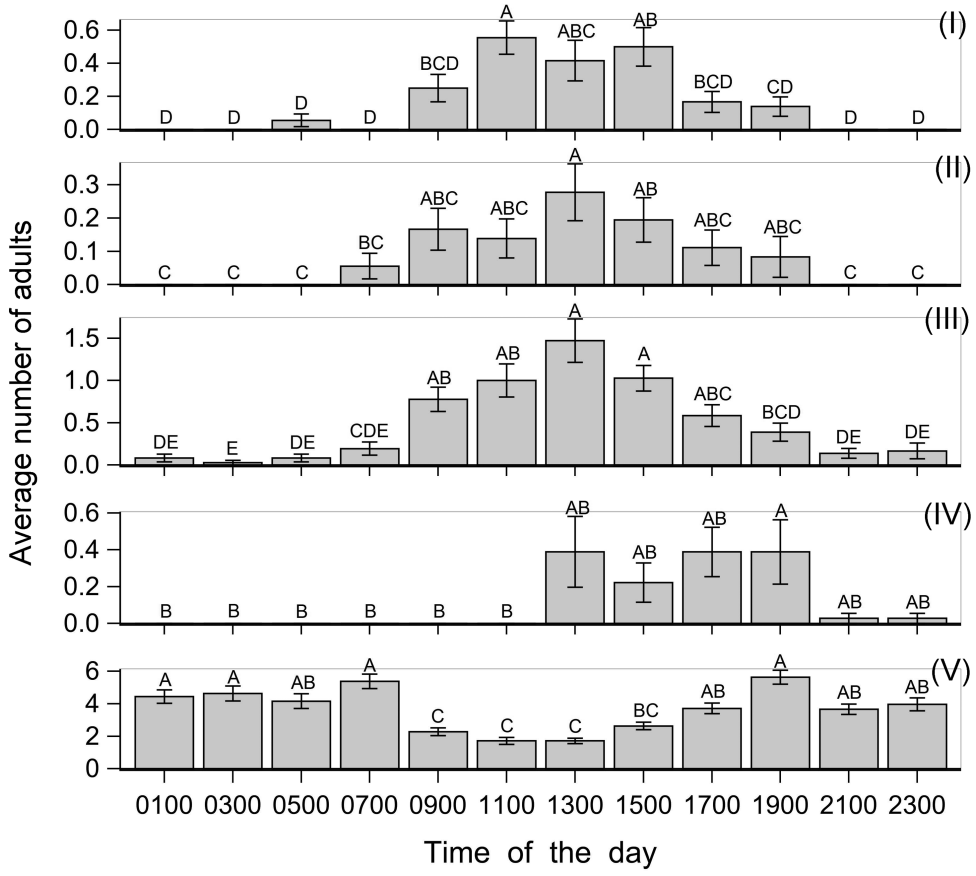

Fig. 4. Average number of *Systena frontalis* adults ± SE per observation area performing different activities, from the top row: (I) flying, (II) jumping, (III) walking, (IV) mating, and (V) sedentary, with lower movement activities at the bottom row, across different times of the day (X-axis). Mean separations were performed by each activity using Tukey's HSD test. Bars sharing the same letters are not significantly different ($\alpha = 0.05$).

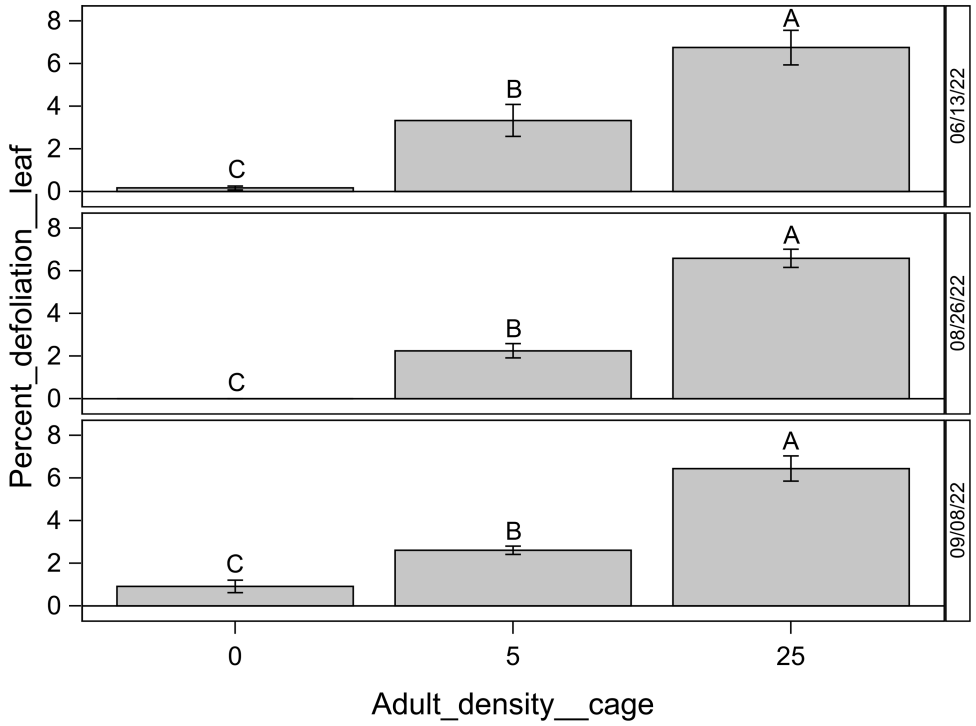

Fig. 5. Percent defoliation at the leaf level ± SE for *Hydrangea paniculata* cv 'Limelight' based on densities of *Systena frontalis* adults infesting each experimental plant (X-axis). Data show the average among all canopy layers within each trial. Rows denote sampling dates for separate trial. Mean separations were performed by each trial using Tukey's HSD test. Bars sharing the same letters are not significantly different ($\alpha = 0.05$).

repetitions showed the same findings with lure and card type interaction (second: $F = 0.44$; df = 1, 25; $P = 0.5146$; third: $F = 0.85$; df = 1, 28; $P = 0.3631$), the presence of lure (second: $F = 0.91$; df = 1, 25; $P = 0.3487$; third: $F = 1.59$; df =1, 28; $P = 0.2178$) and card type (second: $F = 0.91$; df = 1, 25; $P = 0.3487$; third: $F = 3.74$; df = 1, 28; $P = 0.0634$). Adult captures in the hydrangeas at the HRAREC showed no significant difference based on the presence of lure and using yellow sticky cards only ($F = 0.27$; df = 1, 3; $P = 0.6376$).

When the number of adults vacuumed at plants surrounding the trap was used as the response variable, results for difference amongst treatments were similar to those by card collection. Hydrangeas at Nursery 1 had too few adults collected for an analysis to be performed. Sweet spire iteas at Nursery 1 showed no significant difference in adults vacuumed near traps based on presence of lure ($F = 1.00$; df = 1, 6; $P = 0.3559$). Hollies at Nursery 1 for the first repetition 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 the presence of lure ($F = 1.30$; df = 1, 25; $P = 0.2642$) or card type ($F = 1.88$; df = 1, 25; $P = 0.1826$) on adult captures by vacuum.

Results from the second repetition showed a significant interaction between lure and card type ($F = 26.03$; df = 1, 28; $P < 0.0001$). The highest number of adults was found from plants nearby 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. Data from the third repetition showed no significant interaction between lure and card type ($F = 0.07$; df = 1, 28; $P = 0.7962$). There was no significant difference in adults vacuumed based on the 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. 6). Hydrangeas at the HRAREC showed a significant difference based on the presence of lure ($F = 27$; df = 1, 3; $P = 0.0138$). There were slightly fewer adults collected from plants by the lures than there were near the traps without lures.

Discussion

Information on the biology and phenology of *S. frontalis* from these studies will contribute to improving the management of this insect as a pest of ornamentals in Virginia and beyond. On a spatial scale, *S. frontalis* adults were not present inside nearby soybean fields and the perimeter of affected nurseries, in comparison to *S. frontalis* infestations occurring inside those same nurseries. On a temporal scale, adult populations fluctuate throughout the sampling season with density peaks occurring in June, July, and September, and generating significant damage in preferred hosts. At the nursery level, PEA was found to be ineffective in attracting adults. Scaling in further within infested plants, adult behavior exhibited higher activity levels during the day between 1100 and 1500 h and were more sedentary after dark from 1700 to 0500 h. Adult feeding damage was distributed evenly throughout layers of hydrangea canopies and increased with the adult densities present per plant.

Population phenology of *S. frontalis* adults varies across states where they have been studied in ornamental plant systems such as North Carolina, Indiana, Georgia, Kansas, Alabama, and Wisconsin (Herrick and Cloyd 2020, Joseph et al. 2021). They are present and damaging other crop systems (cranberries and soybeans) in northern states, such as Maine, North Dakota, South Dakota, Michigan, and Massachusetts (Jaffe et al 2021, NDSU 2022). Information from these studies also documented that multiple generations could be present each year in nurseries in Virginia. Even though additional data is needed for a robust GDD, first-generation adults were found in 2021 at the HRAREC

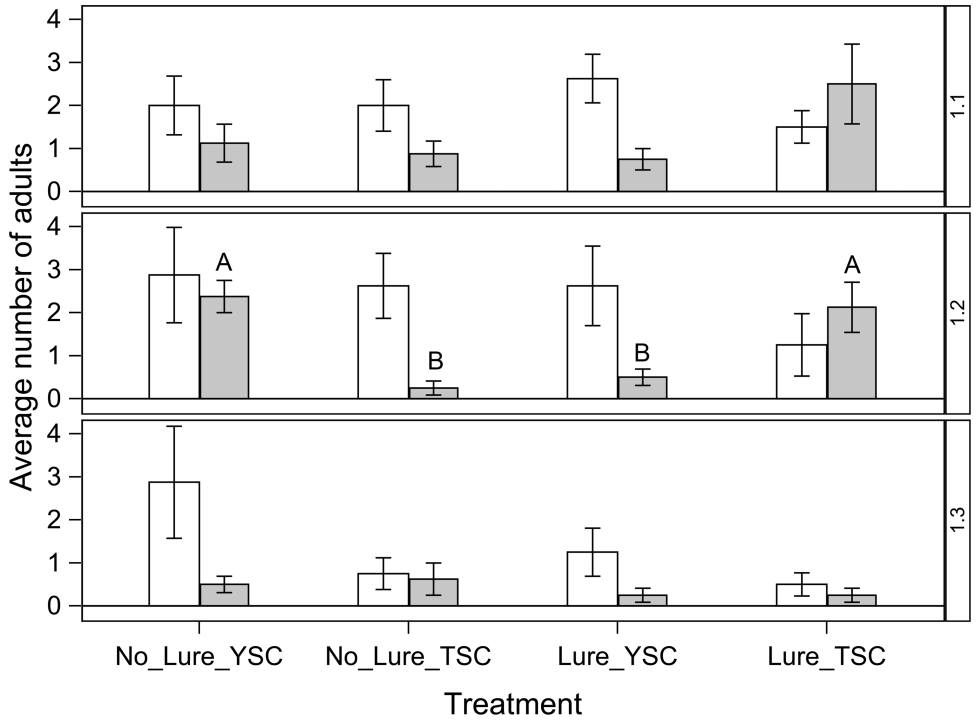

Fig. 6. Average number of *Systena frontalis* adults ± SE collected from vacuum samples (gray bars) and on sticky cards (white bars) within hollies *Ilex crenata* cv. 'Bennett's Compacta' blocks at Nursery location 1 when using PEA as a potential attractant lure. Treatments are denoted on the X-axis: (1) no PEA lure with yellow sticky card (YSC); (2) no PEA lure, translucent sticky card (TSC); (3) PEA lure, with YSC; and (4) PEA lure, with TSC. Rows denote data from each repetition (1.1 done in July 2021, 1.2 in June 2022, and 1.3 in July 2022). Mean separations were performed by each repetition using Tukey's HSD test. Bars sharing the same letters, or bars with no letters are not significantly different ($\alpha = 0.05$).

during the first week of May at 896 GDD_{50}, at Nursery 1, first week of June at 1,523 GDD_{50} and Nursery 2, and first week of June at 1,560 GDD_{50}. This differs from data collected at nurseries in North Carolina where first-generation adults were found from 517 to 1,028 GDD_{50} while second-generation adults appeared between 1,878 and 2,318 GDD_{50} (Lauderdale 2017). A third generation has been observed on the Eastern Shore of Virginia and is estimated to appear in September through October (B. Kunkel, personal observation). Untreated crops at the HRAREC in 2021 exhibited peaks in populations of adults in hydrangeas around 1,149 GDD_{50}, 2,470 GDD_{50}, and 4,178 GDD_{50}, all of which were earlier than populations collected in North Carolina back in 2017. Treated hydrangeas at Nursery 1 in 2022 exhibited the first adult peak at 2,136 GDD_{50}, second adult peak around 3,155 GDD_{50}, and a third adult peak around 4,743 GDD_{50}. The nurseries, part of this study, were located in eastern Virginia in the Tidewater region. This region has a vastly different climate than other states of previous study, that is, the temperatures in this coastal region can be warmer and relative humidity is higher. Difference in weather might explain the difference in timing of adult emergence and peak activity in Virginia when compared to other states.

Adults of *S. frontalis* are expected to thrive in lower temperatures with a higher egg hatch rate (Jaffe et al. 2021), suggesting that development may have been slowed by warmer conditions as demonstrated in our studies. Each of the adult population peaks was documented close to 1,000 GDD_{50} apart from each other, implying that generations may take around that many GDD_{50} to develop from egg to adult during the sampling season. Additionally, larvae within the potted media of containerized hydrangeas showed higher numbers during weeks with lower adult numbers in the active season. These numbers were not strictly inversed of each other, but demonstrate that there were fewer larvae when adult populations were high and more larvae when adult populations were low, and potentially indicate the reduced overlapping life stages for this insect at any given time.

Scouting of *S. frontalis* 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. PEA was found to attract *S. frontalis* adults in soybean fields (Braasch and Kaplan 2012). This study found that PEA failed to act as lure for *S. frontalis* adults under nursery conditions. There were no attractant or repellent effects of the PEA on adults within hollies, sweet spire iteas, or hydrangeas at experimental blocks in this study. Population densities of *S. frontalis* in soybeans and ornamental plant systems are likely quite different. Additionally, differences in plant canopy architecture and cropping patterns might have 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. Further investigation would be required to determine whether the presence of other HIPVs within a crop system could influence the attractant effects of PEA on *S. frontalis* adults.

Movement of *S. frontalis* adults throughout the landscape remains unclear regarding their source and if they could travel in or out of the nurseries themselves. Adults of this insect have been reported to be present in soybeans and cotton (NDSU 2022) in other states, causing defoliation. Data from this study showed that no

adults were found during 2 consecutive years in soybean fields adjacent to nurseries heavily infested by *S. frontalis* in eastern Virginia. However, the absence of adults in surrounding fields does not necessarily translate into the lack of movement from fields to nurseries. Additionally, sticky card traps along the perimeter of these nurseries found no adults, which indicated the adults were not present at the interphase between nurseries and those adjacent row crop fields. The landscape movement of this insect could play a fundamental role on management strategies, since adults are polyphagous and known to defoliate many crops besides those found at nurseries (Herrick and Cloyd 2020, Lane and Del Pozo-Valdivia 2021). Future studies using mark and recapture techniques might provide insight into the actual movement of these adults between infested nurseries and surrounding row crops fields. Information on adult flight capacity can also be complemented by flight mill studies.

In corn, *Colaspis brunnea* (F.) (Coleoptera: Chrysomelidae), another species of leaf beetle, was found to be more active during the night (Miwa and Meinke 2015), compared to the increase in motionless adults during the day. The activities characterized as motionless and feeding in *C. brunnea* were found to be opposite for *S. frontalis* in hydrangea plants. In this study, the category of sedentary included those who were feeding and resting as the activities were indistinguishable in the field. Adults of *S. frontalis* were found to be sedentary (motionless and feeding combined) between 1900 and 0700 h and more active during the day with peaks in activity between 1100 and 1500 h. Nursery growers could use the data on when adults are expected to be moving around plants and active so that they may adjust their spray schedules accordingly. Visual scouting of *S. frontalis* adults would be most representative of populations if consistently performed between the hours of 1100 and 1500 h, since they are expected to be moving during that time period. Following the same logic, exposure to insecticides for this insect might be also influenced by the timing of the insecticide application during the day.

Although the seasonal activity data showed fewer than 5 adults per plant across sampling dates and crops, the relationship between density of *S. frontalis* adults and leaf percent defoliation was studied in a controlled setting. As the number of adults increased so did the percent defoliation of leaves. Related studies have also corroborated this linear relationship between adult densities and percent defoliation (Dreistadt and Dahlsten 1989). Growers have no tolerance for any defoliation to their crops; therefore, the proposed equation (leaf defoliation = 0.8964 + 0.2332 × number of adults per plant) could be used to model the expected leaf defoliation based on a recorded adult density in the field. In addition, each layer of the canopy (bottom, middle, and top) suffered similar levels of defoliation which could be interpreted that adult feeding was likely to be distributed evenly across the plant. With the adults potentially being located evenly across the plant, it could be proposed that visual scouting from the top of plants, which are the most easily visible parts, would be as accurate as scouting other layers. This observation could also play into insecticide spraying practices just as timing of the day could. Based on *S. frontalis* intraplant 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 would be important in ensuring coverage to affect all adults evenly distributed within the plant canopy.

Virginia commercial nurseries have been facing *S. frontalis* as a major pest for ornamental crops, including hydrangeas, sweet spire iteas, and hollies (Lane and Del Pozo-Valdivia 2021). In comparison with other crops and states, this study has presented the differences in this insect phenology that, now investigated, could provide affected growers with information to improve management strategies. Their population lows in early July, late August, and early September may suggest periods of time when nursery growers could expect to modify the frequency of control interventions for this pest, in association with field scouting. 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. Once in the landscape, *S. frontalis* was consistently present in nurseries and absent at the nearby soybean fields in eastern Virginia. Adults of this insect were also absent at the interphase between affected nurseries and those surrounding fields. 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 *S. frontalis* adult 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 and effectiveness of differently timed sprays. The multiscale monitoring and observations from this project lead to a further understanding of *S. frontalis* as a pest of ornamentals in Virginia nurseries with the goal of improving IPM programs in such context. Reduction in levels of insecticide usage, currently a main component of *S. frontalis* management, will enhance sustainability of nurseries, and welfare of the workers, nontarget organisms, and the environment.

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