Sensory Ecology and Behavioral Management of Two North American *Lygus* species

By

Matthew C. Hetherington

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The dissertation is approved by the following members of the Final Oral Committee:
Christelle Guedot, Associate Professor, Entomology
Hiroshi Maeda, Professor, Botany
Shawn Steffan, Associate Professor, Entomology
Amy Trowbridge, Assistant Professor, Forest and Wildlife Ecology

Dedication

I dedicate this work to my family and friends; without their support this dissertation would not exist.

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Chapter 1

INTRODUCTION

The studies reported in this dissertation were conducted on two species, *Lygus hesperus* (Knight) and *Lygus lineolaris* (Palisot de Beauvois), both of which are native to North America. While *L. lineolaris* is present throughout North America, it is the predominate *Lygus* species east of the Rocky Mountains and only represents a small proportion of the *Lygus* species complex in the west. *Lygus hesperus* is found throughout western North America, where it is the most common *Lygus* species. Both species are polyphagous, feeding on hundreds of plant species from dozens of families, and are major pests of strawberry, alfalfa, cotton, and canola, among numerous other fruit, vegetable, oilseed, and fiber crops (Scott, 1977; Snodgrass et al., 1984; Young, 1986). *Lygus* spp. prefer to feed on nitrogen-rich shoot tips, buds, and flowers (Wheeler, 2001), and feeding causes floral bud abortion, the production of inviable seed, and fruit deformation (Handley & Pollard, 1991). *Lygus* bugs overwinter as adults and lay eggs on a variety of broadleaf plants as temperatures warm in the spring. Large populations disperse into agricultural fields as early season hosts senesce (Snodgrass et al., 1984).

Host selection is a multifaceted decision-making process that operates across multiple spatial scales. It involves locating patches where hosts are likely to be encountered (habitat finding), before evaluating hosts within patches (host finding), and eventually accepting a suitable host (host acceptance) (Bell, 1990; Silva & Clarke, 2020). Olfactory and visual cues are largely responsible for habitat and host finding, while gustatory and tactile cues mediate host acceptance (Bruce et al., 2005; Bruce & Pickett, 2011; Carrasco et al., 2015). Specialist herbivores rely on host-specific blends of volatile compounds to identify suitable host plants (Bruce et al, 2005; Bruce and Pickett, 2011). However, as diet breadth increases, it becomes increasingly difficult to

efficiently relate host quality to volatile emissions (Bernays, 2001; Carrasco et al., 2015). Relying on volatiles that are emitted by many host plants or associated with a particular context or plant structure may allow polyphagous herbivores to overcome this challenge (Bernays, 2001; Carrasco et al., 2015; Silva & Clarke, 2020). Studies have recently demonstrated that the polyphagous mirid, *Apolygus lucorum* utilizes floral volatiles to track the succession of flowering hosts across the season (Pan et al., 2013, 2015, 2021) and floral volatiles have been shown to attract *Lygus rugulipennis* in the field (Baroffio et al., 2018; Fountain et al., 2021; Koczor et al., 2012). It is possible that *L. hesperus* and *L. lineolaris* also utilize floral volatiles to locate high quality host plants.

The overarching goal of my research is to better understand the chemical basis of host selection and preferences in *L. hesperus* and *L. lineolaris* and to determine whether this knowledge can be deployed to improve monitoring and management of these pest insects. This dissertation contains six chapters. Chapter 1 is the introduction. Chapters 2 and 3 focus on the chemical basis of host selection in *L. hesperus*. In Chapter 4, I examined the use of a preferred *Lygus* host, *Medicago sativa*, as a trap crop to manage *L. lineolaris* in June-bearing strawberry production. Chapter 5 focused on optimizing the visual parameter of *L. lineolaris* traps and how trap parameters influence beneficial bycatch, while Chapter 6 examined the chemical basis of host preference in *L. lineolaris*.

In Chapter 2, I examined *L. hesperus* attraction to seventeen host species in wind tunnel and Y-tube assays, before comparing the volatile emissions of four species that elicited different levels of attraction. Although *M. sativa* is thought to be the most preferred *Lygus* spp. host (Armstrong & De Azevedo Camelo, 2003; Barman et al., 2010; Esquivel & Mowery, 2007), large populations may develop from successful reproduction over time, high attraction to the host

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plant, or a combination of the two. Determining relative *L. hesperus* to a diverse set of host plants is an important first step to understanding the chemical basis of orientation in *L. hesperus*.

Chapter 3 represented a continuation of the work begun in Chapter 2. Gas chromatography with electroantennographic detection (GC-EAD) was employed to determine which compounds, emitted from the four host plants, elicit antennal depolarization in *L. hesperus*. Y-tube behavioral assays were then conducted to determine which compounds elicited a behavioral response. Finally, compounds that attracted *L. hesperus* in laboratory assays were tested in the field.

Medicago sativa is a preferred *Lygus* spp. host and has been deployed as a trap crop against *L. hesperus* in cotton and strawberry (Godfrey & Leigh, 1994; Sevacherian & Stern, 1975; Swezey et al., 2007), and against *L. rugulipennis* in lettuce and strawberry (Accinelli et al., 2005; Easterbrook & Tooley, 1999). Chapter 4 describes a three-year experiment evaluating the potential of *M. sativa* as a trap crop for *L. lineolaris* in June-bearing strawberry.

White traps are frequently recommended for *L. lineolaris* monitoring. However, strawberry growers mentioned that they were generally ineffective. In Chapter 5, I investigated how the visual parameters of traps influence the capture of *L. lineolaris* and beneficial arthropods.

Chapter 6 expanded on our findings from Chapters 4 and 5. I applied GC-EAD to determine which compounds emitted from strawberry and alfalfa plants elicited antennal depolarization in *L. lineolaris* and assessed whether any antennally-active compounds increased *L. lineolaris* capture on traps. Subsequently, I examined the influence of compound stereochemistry and trap color on *L. lineolaris* trap catch.

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Chapter 2

DIFFERENTIAL ATTRACTION OF *LYGUS HESPERUS* KNIGHT (HEMIPTERA: MIRIDAE) TO HOST PLANTS: A ROLE FOR FLORAL VOLATILES?

ABSTRACT – *Lygus hesperus* (Knight) is a generalist pest of alfalfa, strawberry, cotton, and many other crops in Western North America. Although many plant species are suitable for *L. hesperus* reproduction, offspring survivorship and development rate vary among hosts. In this study, we compared *L. hesperus* attraction to seventeen host species in flight tunnel and Y-tube olfactometer assays. We then compared the volatile profiles from four host species that elicited differential responses from *L. hesperus* females. Overall, the attractiveness to the seventeen host species followed a gradient bounded by *Medicago sativa* and *Erigeron canadensis* as the most attractive hosts and *Capsella bursa-pastoris* and *Plantago major* as the least attractive. When *L. hesperus* females were given a choice between *M. sativa* and every other species in Y-tube assays, *M. sativa* was preferred to *Artemisia vulgaris*, *Fragaria ananassa*, *Hordeum jubatum*, *Plantago major*, and *Capsella bursa-pastoris*, but not the other plant species. The volatile emissions of four host plants examined in this study were found to vary significantly along the attractiveness gradient. The separation of attractive and unattractive species was associated with the emission rate of (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, α-pinene, β-myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, and (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT). *Medicago sativa* tended to emit (*Z*)-3-hexenyl acetate, β-myrcene, (*Z*)-β-ocimene, (*E*)-β-ocimene, and DMNT at higher rates than less attractive species. Our findings suggest that *L. hesperus* utilizes floral volatile emissions to locate suitable host plants. Future studies should examine the potential of floral volatiles to enhance monitoring or mass trapping of this polyphagous insect.

Introduction

Herbivorous insects must discriminate between suitable and unsuitable potential host plants in order to survive and reproduce. Olfactory and visual cues, or combinations thereof, appear to be critical to this decision-making process (Carrasco et al., 2015; Schoonhoven et al., 2005). However, the sensory cues that mediate host selection largely depend on diet breadth. The overwhelming majority of insect herbivores are relatively specialized, requiring hosts of a particular genus (monophagy) or family (oligophagy) to complete their lifecycle, while a small proportion are polyphagous and thus able to develop on hosts from many families (Futuyma & Moreno, 1988; Jaenike, 1990; Janz et al., 2001). While specialist herbivores utilize host-specific blends of plant volatiles to identify suitable feeding or oviposition sites (Bruce et al., 2005; Bruce & Pickett, 2011), mechanisms of host selection by polyphagous insects are less clear. Polyphagous insects frequently exhibit oviposition preferences, but these tend to be less correlated with offspring performance than those exhibited by oligophagous insects (Gripenberg et al., 2010). This may suggest the presence of non-adaptive constraints on decision-making or a weaker selection on oviposition preference in polyphagous insects.

Efficiently relating host quality to volatile emissions becomes increasingly difficult as the diversity of suitable hosts increases (Bernays, 2001; Carrasco et al., 2015). Polyphagous insects may overcome this challenge by narrowing their perceptual range, relying on highly conserved volatiles that are likely to be emitted by many suitable hosts or compounds that are more closely associated with a particular context than a particular host (Bernays, 2001; Bruce et al., 2005; Bruce & Pickett, 2011; Carrasco et al., 2015; Silva & Clarke, 2020). For example, populations of the polyphagous *Apolygus lucorum* (Hemiptera: Miridae) track the succession of flowering plants across the season; a phenomenon mediated by its attraction to common floral volatiles (Pan et

al., 2013, 2015, 2021). The relevance of olfactory cues may also depend on scale. The sequential cues hypothesis proposed by Silva and Clarke (2020) suggests that polyphages rely on common cues to locate areas where suitable hosts are likely to be encountered before ranking individual hosts within a patch. This study seeks to establish behavioral preferences of *Lygus hesperus* Knight (Hemiptera: Miridae) to various host plants and to elucidate the chemical basis of any observed preferences.

Lygus hesperus is a highly polyphagous insect responsible for serious economic damage to alfalfa, strawberry, cotton, and conifer production, among numerous other fruit, vegetable, and seed crops in the western United States. Like many mirids, *Lygus* spp. prefer to feed on nitrogen-rich shoot tips, buds, and flowers (Wheeler, 2001). *Lygus* bugs overwinter as adults and lay eggs on a variety of broadleaf plants as temperatures warm in the spring. Alfalfa is thought to be the most preferred host of *L. hesperus* (Armstrong & De Azevedo Camelo, 2003; Barman et al., 2010) and large populations commonly develop in alfalfa fields and unmanaged surrounding areas before dispersing into other crops (Carrière et al., 2006; Pansa & Tavella, 2009; Snodgrass et al., 1984). Interestingly, Barlow and colleagues (1999) found that *L. hesperus* preferred shepherd's purse (*Capsella bursa-pastoris*) and common groundsel (*Senecio vulgaris*) for feeding over alfalfa in cage trials, and that these weedy hosts were superior for nymphal development and adult survival compared to alfalfa. Visual, gustatory, and olfactory cues may work individually or in combination to mediate behavioral preferences and understanding stimuli that underlie preferences may facilitate the monitoring and management of this pest species.

Because *Lygus* spp. prefer to feed on nutrient-rich reproductive tissues (Wheeler, 2001), it could be expected that they utilize floral volatiles to identify flowering hosts. Indeed, *L. hesperus* populations in alfalfa fields peak at flowering (Barman et al., 2010). Similar to *A.*

lucorum (Pan et al., 2013), *L. lineolaris* appears to follow the succession of flowering hosts (Fleischer & Gaylor, 1987). Moreover, some floral volatiles (i.e., phenylacetaldehyde and (E)-cinnamaldehyde) have been shown to attract *L. rugulipennis* in the field (Koczor et al., 2012) and phenylacetaldehyde increases the number of female *L. rugulipennis* caught in traps baited with pheromone (Baroffio et al., 2018).

Previous studies of host selection in *L. hesperus* revealed that nymphs are attracted to volatiles emitted by flowering and vegetative alfalfa, but that adult females were only attracted to the volatile emissions of alfalfa plants that had been damaged by conspecifics (Blackmer et al., 2004). Feeding damage was associated with significant changes in mono- and sesquiterpene emissions, as well as increased emission of indole and methyl salicylate (Blackmer et al., 2004). The addition of visual cues, in the form of a green LED, enhanced female response to alfalfa volatiles (Blackmer & Cañas, 2005). Female *L. hesperus* are attracted by (*E*)-β-ocimene, (*R*)-(+)-α-pinene, and (*E,E*)-α-farnesene (Williams et al., 2010). As a highly conserved floral volatile (Farré-Armengol et al., 2017) and the main component of the alfalfa floral bouquet (Buttery et al., 1982), (*E*)-β-ocimene is likely to signal the presence of flowers in many *Lygus* hosts.

Although *L. hesperus* feeds and reproduces on numerous plant species, it nevertheless displays clear preferences (Barman et al., 2010). This study examined the chemical basis of host finding in *L. hesperus* by 1) examining *L. hesperus* attraction to seventeen host plant species, before 2) comparing the volatile emissions of four host species that elicited different levels of *L. hesperus* attraction. Understanding the role of olfactory cues as mediators of host finding in *L. hesperus* may facilitate population monitoring or mass trapping during dispersal events.

Methods and Materials

Insects. Lygus hesperus were sourced from a colony maintained at the U.S. Arid Land Agricultural Research Center in Maricopa, AZ. Insects were reared on an artificial diet (Frontier Scientific - Newark, DE) at 27°C and 35% RH under a 14:10 (L:D) photoperiod. Experiments were conducted with 10-20-day old females, as mating typically occurs prior to this period (Strong et al. 1970). Insects were housed individually in mesh-bottomed aspirator vials at 21°C and 45% RH for 16-24 hours prior to testing, allowing subjects to acclimate to testing conditions. During the acclamation period, insects had access to DI water only. Wind tunnel and Y-tube assays assessing *L. hesperus* response to *Medicago sativa, Capsella bursa-pastoris,* and clean air were repeated with the progeny of field-collected *L. hesperus* to confirm that observed results were not the product of long-term lab rearing.

Plants. Seventeen plant species (Table 2-1) were selected based on available literature on *Lygus* host utilization (e.g., Barman et al. 2010; Ramert et al. 2010; Esquivel and Mowery 2007). Seeds were collected from wild specimens of each species native to southern Wisconsin and individual plants were transplanted to a greenhouse at UW-Madison in the fall of 2017. Seeds of non-local species were sourced from the Germplasm Resources Information Network. All species used in assays were grown in 10.16 cm pots with Pro-Mix potting soil in a greenhouse under a 14:10 light cycle. Plants were watered every other day and fertilized weekly. Plants were continuously grown, maintained, and used in experiments between September 2017 and January 2021. Flowering plants were used in all experiments.

Plants used as stimuli in behavioral tests were flowering and standardized based on the relative abundance of reproductive and vegetative structures and overall biomass. When testing relatively small host species (e.g. *Capsella bursa-pastoris* and *Senecio vulgaris*), it was often

necessary to include multiple plants in the stimulus chamber, while excess stems from larger species (e.g. *Artemisia vulgaris* and *Medicago sativa*) were often enclosed in Teflon® bags to prevent the volatiles emissions from contributing to the stimulus (Fluorolab, Dover, NH). The plastic pots and below-ground plant parts of stimulus plants were enclosed in a 3.8 L Teflon® bag, which was sealed with a steel wire, to ensure only volatiles from stems, foliage, and flowers were presented to insects. Plants were then enclosed in a 3.8 L glass container, to which charcoal-filtered, humidified air was introduced at a rate of 2 L/min via Teflon® tubing and allowed to flow into the flight tunnel or olfactometer.

Induction of Upwind Flight. The ability of each of 17 host species to elicit upwind flight in *L. hesperus* was examined in a 152x60x60 cm acrylic flight tunnel (Analytical Research Systems, Gainsville, FL). Assays were conducted in a windowless room between 0900 and 1800 hours at 21°C and 45% RH. Laminar airflow of approximately 0.15 m/s was maintained for all assays. Stimulus plants and test subjects were prepared as described above, and the stimulus airstream was introduced perpendicular to the prevailing wind at a height of 30 cm, to create a turbulent odor plume.

For each host plant species, 30 insects were introduced individually to an 81 cm^2 acrylic disc, which was positioned at a height of 30 cm and 140 cm downwind of the odor source. Each subject was allowed to interact with the stimulus for 10 minutes, during which time, positive anemotaxis was determined to have occurred if an insect left the disc and flew upwind. A single stimulus plant was used for five insects before the arena was cleaned with 70% ethanol and the stimulus plant species was changed. Each day, 5-8 host species were tested depending on the availability of flowering plants of appropriate size.

Orientation in a Y-tube. As sustained directional movement was difficult to observe in the wind tunnel, the response of *L. hesperus* females to each plant species was examined in a Y-tube olfactometer (Internal diameter: 25 mm, interior angle: 50°, length: 25 cm; manufactured in the Chemistry Department at UW-Madison). Due to the number of plant species involved, it was untenable to make every possible comparison, we therefore assessed the ability of each plant species to attract *L. hesperus* compared to either clean air or alfalfa, as alfalfa elicited upwind flight in the highest proportion of insects in the wind tunnel. Stimulus chambers were prepared as previously described and each chamber purged at a rate of 2 L/min for 5 min prior to use as a stimulus.

For each paired choice treatment, 30 insects were individually introduced to the base of the Y-tube and allowed to interact with olfactory stimuli for up to 10 minutes. Insects were determined to have made a choice if they moved halfway up the length of an arm (5 cm beyond the junction). Each Y-tube was washed with Alconox® detergent and rinsed with deionized water and 70% ethanol before drying at 100°C for 30 minutes prior to reuse. The direction from which stimuli were delivered was alternated with each replicate to avoid directional bias that may be introduced by environmental conditions and the olfactometer. On a given day, five randomly selected plant species and *Medicago sativa* were tested with identity of the stimulus species changing after each assay.

Volatile collection and chemical analysis. Behavioral assays revealed an attraction gradient among host species. To investigate the chemical basis of this gradient, the volatile emissions of four plant species, representing different levels of attractiveness: *Capsella bursa-pastoris* (no attraction), *Fragaria ananassa* (low attraction), *Lotus corniculatus* (moderate attraction), and

Medicago sativa sativa (high attraction) were collected and analyzed by gas chromatography-mass spectrometry (GC-MS), and compared amongst each other.

Dynamic headspace extractions were conducted for five flowering individuals of each of the four selected plant species over the course of 3 hrs at approximately 25°C. For each plant species, 10-20 flowers/racemes and 10-20 leaves were enclosed in a 3.8 L Teflon bag, and charcoal-filtered air was introduced at a rate of 300 ml/min and drawn out at the same rate through an adsorbent trap containing 20 mg of Porapak (Sigma-Aldrich, St. Louis, MO). After extraction, plants were cut just below the Teflon bag and placed in paper bags. Plant material was dried at 80°C for 48 hours before being weighed. Traps were eluted with 200 µL of chloroform and the volume of each extract was noted. To serve as an internal standard, 1-bromododecane was added to each sample at a rate of 0.4 ng/µL prior to analysis via GC-MS.

Chemical analysis was conducted using a Thermo Scientific Trace 1300 gas chromatograph coupled to an ISQ series single quadrupole mass spectrometer. The split/splitless injector was operated in splitless mode. The inlet temperature was 250°C and the oven was maintained at 35°C for 2 min before increasing to 150°C at a rate of 5°C/min, then increased at a rate of 20°C/min to a final temperature of 210°C, which was held for 2 minutes for a total run of 30 min. The MS Detector began scanning 35-350 *m/z* after a solvent delay of 5 minutes and continued until the end of the run. An injection volume of 1 µL was used for each sample. Chromatograms were subsequently converted to CDF format and deconvolution was completed in PARADISe (Johnsen et al., 2017). Tentative identification was achieved by comparing mass spectra to the NIST 2008 MS library, and compounds representing at least 1% of the total area of a sample were retained in the analysis. Peak areas were compared to the bromododecane internal

standard which was present at 0.4 ng/µL. Analyte masses were then divided by the dry mass of plant material and length of extraction to provide a final estimate of emission rate in ng/g×h.

Statistical analysis. Statistical analyses were conducted in R (R Core Team 2017). For flight tunnel assays, pairwise comparisons of the proportion of insects initiating flight when exposed to each host plant were made using Fisher's exact test (function: pairwise.fisher.test, package: fmsb, Nakazawa, 2023), Benjamini and Hochberg's (1995) method was applied to control the false discovery rate during multiple testing. A similar strategy was applied to data from Y-tube comparisons against clean air. Fisher's exact test was applied to compare the proportion of insects moving halfway up the length of the stimulus arm in response to each host plant and the false discovery rate was controlled (Benjamini & Hochberg, 1995). The decision to control false discovery rate rather than family-wise error rate stems from the number of pairwise comparisons (Korthauer et al., 2019). Data from Y-tube assays comparing *M. sativa* and other host species directly were subjected to chi-squared tests based on the null hypothesis of random movement based on equal frequencies. Distance-based redundancy analysis (db-RDA) based on Bray-Curtis dissimilarity was applied to compare the overall volatile emissions of each species and visualize differences (overall: package: vegan, function: capscale; pairwise: package: BiodiversityR, function: multiconstrained;(Dixon, 2003; Kindt & Coe, 2005). Permutational MANOVA (PERMANOVA) was then applied to confirm overall differences in the volatile profile (package: vegan, function: adonis), before univariate Kruskal-Wallis tests were applied to investigate differences in the emission of individual compounds. Following a significant Kruskal-Wallis test, Dunn's test (package: FSA, function: dunnTest; Ogle, 2018) was conducted to elucidate species differences.

RESULTS

Induction of upwind movement. The 17 host species examined in this study were not equally likely to elicit upwind flight from *L. hesperus* females in flight tunnel assays. Only 10% of insects took flight when exposed to clean air or the volatile emissions of *C. bursa-pastoris*. Pairwise Fisher's exact test indicated that a significantly lower proportion of *L. hesperus* females responded to *C. bursa-pastoris* than *M. sativa* ($P_{adj} < 0.001$), *E. canadensis* ($P_{adj} < 0.001$), *L. corniculatus* ($P_{adj} < 0.001$), *E. annuus* ($P_{adj} = 0.003$), *M. officinalis* ($P_{adj} = 0.003$), *A. vulgaris* ($P_{adj} = 0.005$), *B. napus* ($P_{adj} = 0.005$), *D. carota* ($P_{adj} = 0.016$), and *S. loeselii* ($P_{adj} = 0.016$; Figure 2-1). *Fragaria ananassa* and *H. jubatum* each elicited upwind flight in 40% of subjects, a marginally significant increase compared to clean air and *C. bursa-pastoris* ($P_{adj} = 0.055$), while insects were as likely to respond to *S. rosmarinus, G. hederacea, S. vulgaris, C. album, and P. major* as clean air or *C. bursa-pastoris* ($P \geq 0.097$). Twice as many insects took flight in response to *M. sativa* compared to *F. ananassa* and *H. jubatum* ($P_{adj} = 0.016$), and *M. sativa* elicited upwind movement in a marginally higher proportion of subjects than *S. loeselii* and *D. carota* ($P_{adj} = 0.055$). No other significant differences were detected among hosts that elicited anemotaxis in a significantly higher proportion of insects than clean air.

Movement towards paired odor choice – Plants vs. blank. Insects tended to respond similarly in Y-tube compared to flight tunnel assays. The lowest proportion of insects moved halfway up the stimulus arm of the olfactometer when exposed to *P. major* (13%) and *C. bursa-pastoris* (23%). *Medicago sativa* ($P_{adj} < 0.001$), *E. canadensis* ($P_{adj} < 0.001$), *B. napus* ($P_{adj} < 0.001$), *M. officinalis* ($P_{adj} < 0.001$), *G. hederacea* ($P_{adj} < 0.001$), *E. annuus* ($P_{adj} < 0.006$), *L. corniculatus* ($P_{adj} < 0.013$), *A. vulgaris* ($P_{adj} < 0.024$), and *D. carota* elicited upwind movement in a significantly higher proportion of subjects than *C. bursa-pastoris* (Figure 2-2), while *S.*

rosmarinus (P_{adj} = 0.008), *S. loeselii* (P_{adj} = 0.015), and *F. ananassa* (P_{adj} = 0.028) only elicited sustained movement in a significantly higher proportion of insects than *P. major* (Figure 2-2). A significantly higher proportion of subjects traveled halfway up the stimulus arm of the olfactometer when exposed to *M. sativa* compared to *D. carota* (P_{adj} = 0.015) and *A. vulgaris* (P_{adj} = 0.028), while a marginally lower proportion of insects responded to *L. corniculatus* (66.7%) than *M. sativa* (93.3%) (P_{adj} = 0.051).

Movement towards paired odor choice – Plants vs. M. sativa. No alternative hosts were preferred to *M. sativa*, but *M. sativa* was preferred to *A. vulgaris* (χ^2 = 4.80, df = 1, P = 0.028), *F. ananassa* (χ^2= 6.53, df= 1, P = 0.011), *H. jubatum* (χ^2 = 8.53, df = 1, P = 0.004), *P. major* (χ^2= 10.80, df= 1, P = 0.001), and *C. bursa-pastoris* (χ^2 = 16.13, df = 1, P < 0.001; Figure 2-3).

Comparison of volatile emissions. Multivariate analysis of host plant volatile profiles revealed that species identity was a significant predictor of volatile emissions (db-RDA: $F_{3,15}$ = 3.26, P = 0.001; PERMANOVA: $F_{3,15}$ = 3.33, P = 0.001). Subsequent pairwise comparisons indicated that differences between *M. sativa* and *C. bursa-pastoris* (Figure 2-4; db-RDA: *M. sativa – C. bursa-pastoris*: $F_{1,9}$ = 6.06, P_{adj} = 0.036) and *M. sativa* and *F. ananassa* (*M. sativa – F. ananassa*: $F_{1,9}$ = 7.17, P_{adj} = 0.048) are primarily responsible for this separation.

The first canonical axis (CAP1) generated via db-RDA explains 30.84% of the variation in the volatile emissions dataset and is the only axis that represents variation that can be distinguished from random ($F_{1,14}$ = 7.34, P = 0.001). By calculating the correlation of each variable with CAP1, it was possible to identify the compounds that drive separation between highly attractive and less attractive host plants. The correlation coefficients of seven compounds, (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), (*Z*)-3-hexenol, (*E*)-β-ocimene, (*Z*)-β-ocimene, β-myrcene, α-pinene, and (*Z*)-3-hexenyl acetate, were greater than 0.5 and no similarly extreme

negative correlations were observed (Table 2-2). Individual Kruskal-Wallis tests determined that DNMT (χ^2= 8.281, df= 3, P = 0.041), (*E*)-β-ocimene (χ^2= 10.435, df= 3, P = 0.015), (*Z*)-β-ocimene (χ^2= 10.476, df= 3, P = 0.015), β-myrcene (χ^2= 8.812, df= 3, P = 0.032), and (*Z*)-3-hexenyl acetate (χ^2= 8.439, df= 3, P = 0.038) were emitted at significantly different rates across species, while Dunn's test confirmed that these compounds were emitted at significantly higher rates in *M. sativa* compared to *F. ananassa* (DMNT [Z = 2.681, P_{adj} = 0.044], β-myrcene [Z = 2.946, P_{adj} = 0.019], and (*Z*)-3-hexenyl acetate [Z = 2.690, P_{adj} = 0.043]), *C. bursa-pastoris* ((*E*)-β-ocimene [Z = 2.688, P_{adj} = 0.043]), or both ((*Z*)-β-ocimene [*Capsella*: Z = 2.877, P_{adj} = 0.024; *Fragaria*: Z = 2.704, P_{adj} = 0.034]).

Discussion

To survive and reproduce, herbivorous insects must identify suitable host plants among complex communities of unsuitable non-host species (Carrasco et al., 2015; Schoonhoven et al., 2005). While specialist herbivores are attracted to host-specific blends of plant volatiles (Bruce et al., 2005; Bruce & Pickett, 2011) and deterred by non-host volatiles (Zhang & Schlyter, 2004), host selection in polyphagous insects is not well-understood (Bernays, 2001; Carrasco et al., 2015; Silva & Clarke, 2020). This study compared the attraction of female *L. hesperus* to a variety of cultivated and non-crop host species to examine the chemical basis of host selection in this polyphagous pest. A shallow attractiveness gradient was observed among host species, with *M. sativa* eliciting the strongest response in all bioassays and few non-attractive hosts, but with few statistical differences among attractive hosts detected. These data suggest that *L. hesperus* may be responding to general cues associated with flowering host plants in most cases, while such cues are greatly reduced or absent in non-attractive hosts. Comparison of the volatile emissions profile of hosts along this gradient revealed that attractive hosts (*M. sativa* and *L. corniculatus*) emitted

β-myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, DMNT, (±)-linalool, (*Z*)-3-hexenyl acetate, and hexyl acetate at significantly higher rates than less attractive hosts (*F. ananassa* and *C. bursa-pastoris*), suggesting that these volatiles may be involved in *L. hesperus* host selection.

Medicago sativa and *E. canadensis* consistently elicited the strongest response from *L. hesperus* females. *Medicago sativa* supports large populations of *Lygus* spp. throughout much of the year (Armstrong and De Azevedo Camelo 2003; Barman et al. 2010; Esquivel and Mowery 2007), and previous studies have demonstrated *Lygus* spp. attraction to the volatile emissions of *M. sativa* (Blackmer et al., 2004; Blackmer & Cañas, 2005; Ondiaka et al., 2016; Rämert et al., 2010). While we observed that *L. hesperus* was more attracted to *M. sativa* than *C. bursa-pastoris* and *S. vulgaris,* in a previous study *L. hesperus* preferentially utilized the latter species in multiple choice cage trials (Barlow et al., 1999). Moreover, adult longevity and nymphal survivorship increases when *L. hesperus* utilizes *C. bursa-pastoris* and *S. vulgaris* for feeding and oviposition (Barlow et al. 1999). This discrepancy between attraction over distance and utilization in cage trials may stem from the scale over which the interaction takes place and the cues that mediate attraction and acceptance (Bell, 1990). *Lygus hesperus* females may be more likely to follow chemical cues upwind toward *M. sativa* than *C. bursa-pastoris* or *S. vulgaris*, but post-contact or short-range cues may encourage dispersal onto alternative more suitable hosts, if available.

Plant traits are shaped by interactions with both mutualists and antagonists (Brody & Mitchell, 1997; Gómez et al., 2015; Kessler et al., 2019; Ohashi & Yahara, 2009). While pollinator attraction generally benefits plants, herbivores may also exploit these communication channels (Andrews et al., 2007; Brody & Mitchell, 1997; Cornell & Hawkins, 2003; Gómez et al., 2015). As a primarily self-fertilizing plant, cryptic flowers are unlikely to reduce the reproductive output of *C. bursa-pastoris* (Hintz et al., 2006), whereas *M. sativa* relies on insect pollination and must

therefore remain apparent to pollinators and herbivores (Haedo et al., 2022). Differential floral chemical apparency may explain the pattern of attractiveness observed in this study. *Lygus* spp. and other mirids prefer to feed on nitrogen-rich meristematic and reproductive tissues (Wheeler, 2001) and are thought to track the succession of flowering hosts across the year (Fleischer & Gaylor, 1987; Pan et al., 2013). Floral volatiles may mediate *L. hesperus* attraction to patches of flowering plants, as has been documented in *Lygus rugulipennis* (Koczor et al. 2012; Baroffio et al. 2018) and *Apolygus lucorum* (Pan et al., 2015).

Analysis of the volatile emissions profile of four species sampled along the attractiveness gradient revealed significant differences between the most attractive host, *M. sativa*, and the low-attraction species, *F. ananassa* and *C. bursa-pastoris*. The volatile emissions of *L. corniculatus* were highly variable, and it was significantly different from other species. This variability may explain the inconsistencies in *L. hesperus* attraction to *L. corniculatus* between flight tunnel and Y-tube assays, although *L. rugulipennis* behavior has previously been shown to vary between Y-tube and flight tunnel assays (Frati et al., 2008). Overall patterns of volatile emissions appear to reflect patterns of *L. hesperus* attraction, suggesting that differential volatile emissions are responsible for differential attraction of *L. hesperus*. (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, hexyl acetate, α-pinene, β-myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, DMNT, and (±)-linalool were the main drivers of separation. Previous studies have demonstrated that *L. hesperus* attraction to *M. sativa* and *L. rugulipennis* attraction to *Vicia faba* increase with conspecific damage (Blackmer et al., 2004; Blackmer & Cañas, 2005; Frati et al., 2008), and that *Lygus* spp. feeding increases emission of α-pinene, β-myrcene, (*E*)-β-ocimene, (*Z*)-β-ocimene, and hexyl acetate, in addition to (*E*)-β-caryophyllene and methyl salicylate (Blackmer et al., 2004; Frati et al., 2009; Rodriguez-Saona et al., 2002). Adult female *L. hesperus* attraction to (*E*)-β-ocimene and (*R*)-α-pinene has

previously been reported, while no attraction to β-myrcene or (±)-linalool has been observed, and (*Z*)-3-hexenol and (*S*)-α-pinene deterred females (Williams et al., 2010). Our chemical analysis suggests that major components of the *M. sativa* floral bouquet (Buttery et al., 1982) underlie differentiation between attractive and non-attractive host species and supports the hypothesis that floral volatiles mediate *L. hesperus* host selection.

This study leaves room for subsequent examination of the antennal and behavioral activity of plant extracts and individual compounds under laboratory and field conditions. Clarifying the role of plant volatiles in *L. hesperus* orientation will facilitate the development of more sustainable monitoring and management strategies for this devastating pest. Moreover, such experiments will contribute to resolving longstanding questions about the nature of host selection in polyphagous insects.

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Species	**Family**
Artemisia vulgaris	Asteraceae
Brassica napus	Brassicaceae
Capsella bursa-pastoris	Brassicaceae
Chenopodium album	Amaranthaceae
Erigeron canadiensis	Asteraceae
Dacus carota	Apiaceae
Erigeron annuus	Asteraceae
Fragaria ananassa	Rosaceae
Glechoma hederacea	Lamiaceae
Hordeum jubatum	Poaceae
Lotus corniculatus	Fabaceae
Medicago sativa	Fabaceae
Melilotus officinalis	Fabaceae
Plantago major	Plantaginaceae
Salvia Rosmarinus	Lamiaceae
Senecio vulgaris	Asteraceae
Sisymbrium loeselii	Brassicaceae

Table 2-1: *Lygus* spp. hosts compared in behavioral assays

Compound	RI	*Capsella*	*Fragaria*	*Lotus*	*Medicago*	X^2	p value	Loadings CAP1
(*Z*)-3-Hexen-1-ol	866	32.29 ± 1.31	38.58 ± 4.36	237.69 ± 61.56	129.55 ± 24.11	4.621	0.202	0.697
α-Pinene	951	1.00 ± 0.34	0.20 ± 0.05	2.22 ± 0.59	11.68 ± 3.24	6.298	0.098	0.539
Benzaldehyde	932	2.17 ± 0.48	0.56 ± 0.12	3.86 ± 1.51	1.21 ± 0.21	3.142	0.370	0.318
β-Myrcene	965	0.47 ± 0.09 ab	0.23 ± 0.06 a	1.37 ± 0.51 ab	9.07 ± 2.3 b	8.812	0.032	0.557
(*Z*)-3-Hexenyl acetate	985	6.26 ± 1.73 ab	3.92 ± 1.13 a	54.98 ± 25.09 ab	405.3 ± 136.04 b	8.439	0.038	0.519
Hexyl acetate	990	0.09 ± 0.02 ab	0.02 ± 0 a	0.65 ± 0.31 ab	6.69 ± 2.82 b	8.546	0.036	0.429
Limonene	1004	2.63 ± 0.7	8.25 ± 2.33	178.85 ± 64.31	11.2 ± 3.86	4.742	0.192	0.432
2-Ethylhexanol	1005	25.01 ± 6.87	1.00 ± 0.35	3.19 ± 1	1.15 ± 0.37	2.384	0.497	-0.102
(*Z*)-β-Ocimene	1012	0.02 ± 0.01 a	0.05 ± 0.02 a	0.77 ± 0.22 ab	8.43 ± 2.5 b	10.476	0.015	0.594
(*E*)-β-Ocimene	1022	0.05 ± 0.02 a	0.63 ± 0.19 ab	35.11 ± 10.56 ab	187.44 ± 54.46 b	10.435	0.015	0.639
(*E*)-4,8-Dimethylnona-1,3,7-triene	1089	0.2 ± 0.06 ab	0.02 ± 0 a	3.71 ± 1.45 ab	5.66 ± 1.02 b	8.281	0.041	0.747
Linalool	1073	1.22 ± 0.43 ab	0.08 ± 0.03 a	0.01 ± 0 a	2.14 ± 0.36 b	9.155	0.027	0.356
2-Ethylhexyl acetate	1123	8.7 ± 2.66	0.3 ± 0.09	0.91 ± 0.26	0.44 ± 0.09	1.920	0.589	-0.093
Methyl salicylate	1163	0.03 ± 0.01	1.58 ± 0.58	0.67 ± 0.19	0.04 ± 0	6.854	0.077	-0.092
Decanal	1173	4.68 ± 0.63	1.49 ± 0.31	20.58 ± 6.82	1.55 ± 0.3	5.242	0.155	0.398
Ethyl isobutyrate	1245	1.45 ± 0.48	0.17 ± 0.06	0.29 ± 0.14	0.07 ± 0.02	2.306	0.512	-0.083
2-ethylhexyl butyrate	1285	3.23 ± 1.1	0.03 ± 0.01	0.07 ± 0.03	0.02 ± 0	2.830	0.419	-0.117
Tetradecane	1357	2.11 ± 0.86	0.03 ± 0	0.71 ± 0.2	0.08 ± 0.01	6.132	0.105	-0.005
Isocaryophyllene	1373	0.56 ± 0.23	NA	0.35 ± 0.16	0.03 ± 0	6.640	0.084	0.052
Unknown sesquiterpene	1377	1.05 ± 0.28	0.35 ± 0.03	4.71 ± 1.38	0.28 ± 0.01	3.893	0.273	0.397
(*E*)-Geranyl acetone	1405	0.45 ± 0.11	0.46 ± 0.09	1.99 ± 0.65	0.2 ± 0.05	1.163	0.762	0.342
Unknown sesquiterpene	1449	1.14 ± 0.22	0.52 ± 0.05	5.73 ± 1.54	0.51 ± 0.03	2.562	0.464	0.446
α-Muurolene	1453	0.11 ± 0.02	0.63 ± 0.11	1.83 ± 0.53	0.05 ± 0	6.140	0.105	0.329
α-Farnesene	1457	0.42 ± 0.13	0.5 ± 0.16	1.58 ± 0.43	0.16 ± 0.01	1.121	0.772	0.331

Mean amount (+/- SEM) (unit?) of chemical emission of individual compounds from four hosts collected over 2hrs… Also need to spell out RI in a legend

Table 2-2: ~~Comparison~~ of volatile emissions profiles for four *L. hesperus* hosts eliciting different levels of attraction. X^2 and p-values refer to Kruskal-Wallis test, different letters denote significant differences between hosts based on Dunn's test at $\alpha = 0.05$.

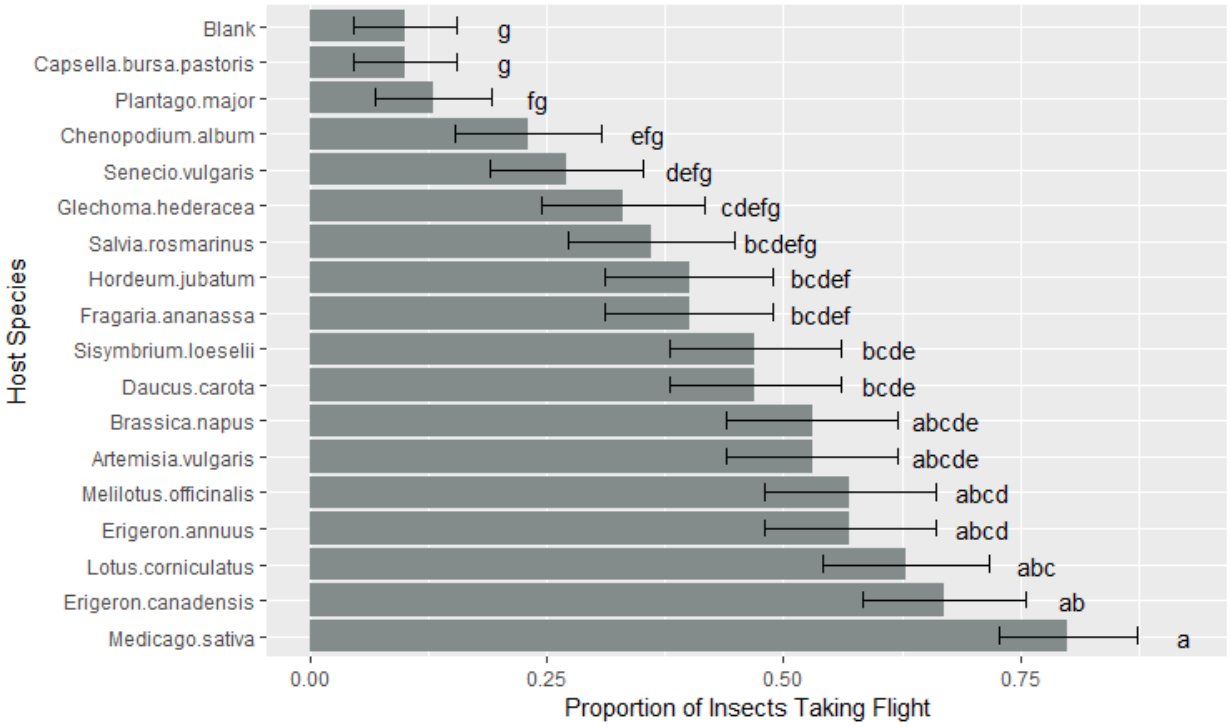


Figure 2-1: Proportion of *L. hesperus* adult females initiating flight when presented with each host species. Pairwise comparisons were made with Fisher's exact test, applying Benjamini and Hochberg's (1995) method to control the false discovery rate. Letters indicate significant ($P_{adj} < 0.05$) or marginally significant ($0.075 > P_{adj} > 0.05$) differences among host species.

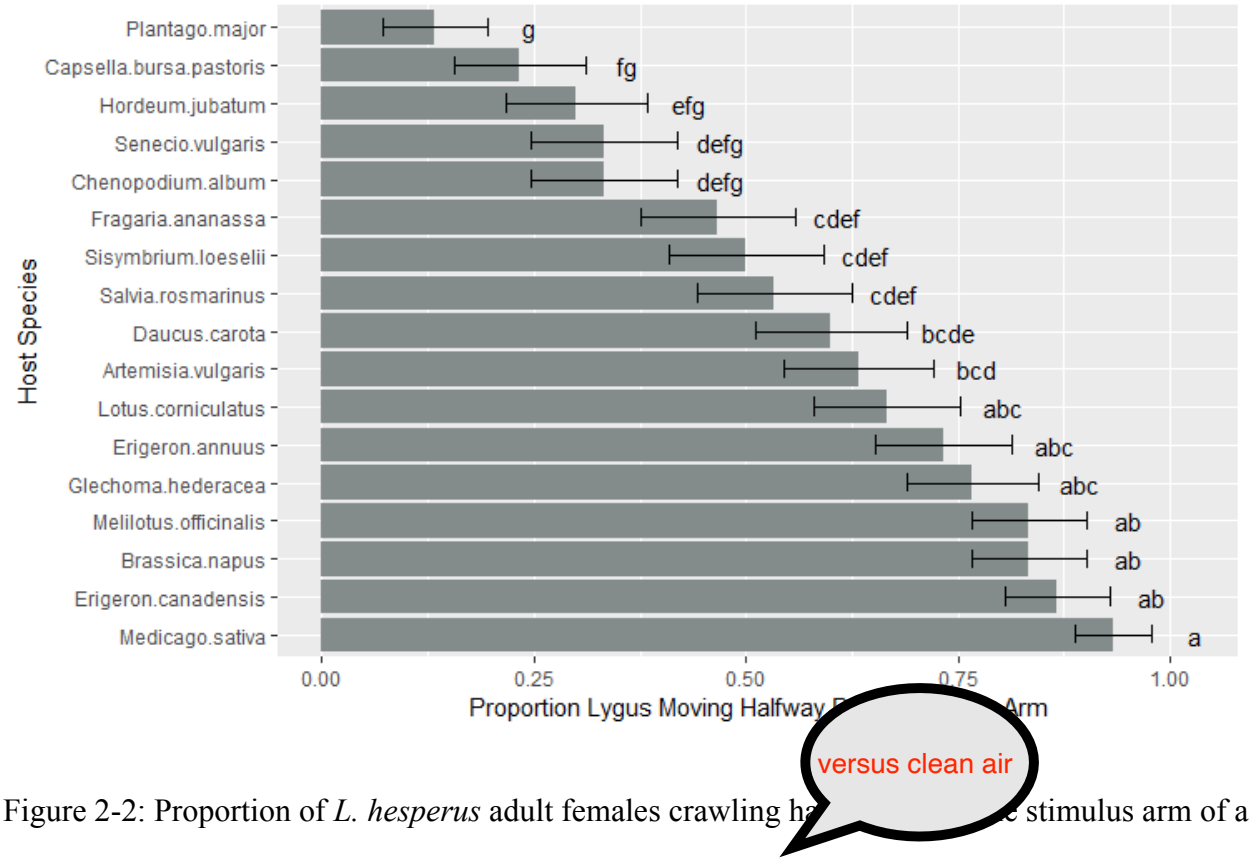
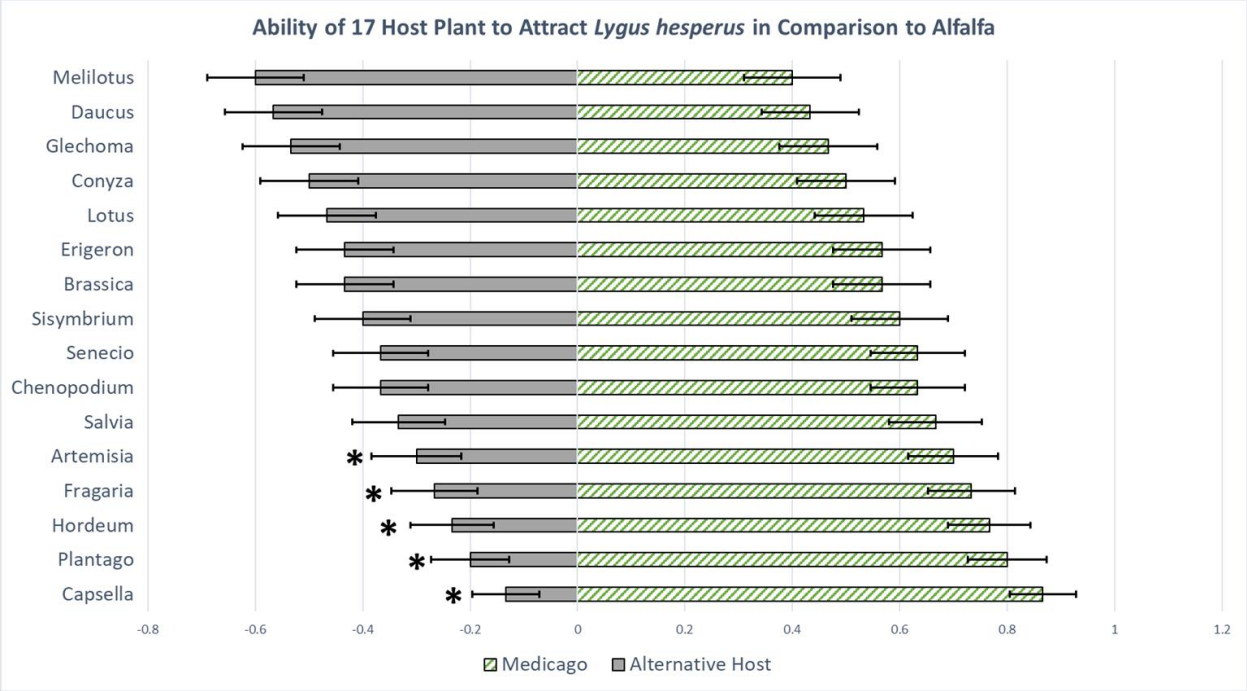


Figure 2-2: Proportion of *L. hesperus* adult females crawling ha stimulus arm of a Y-tube olfactometer when presented with each host species. Pairwise comparisons were made with Fisher's exact test, applying Benjamini and Hochberg's (1995) method to control the false discovery rate. Letters indicate significant ($P_{adj} < 0.05$) or marginally significant ($0.075 > P_{adj} > 0.05$) differences among host species.



each host species vs. M. sativa

Figure 2-3: Proportion of insects responding to ~~*Medicago sativa* or each alternative host~~ in Y-tube olfactometer assays. Chi-square tests were applied to identify deviations from the null hypothesis of equal frequencies, asterisks indicate significant deviations.

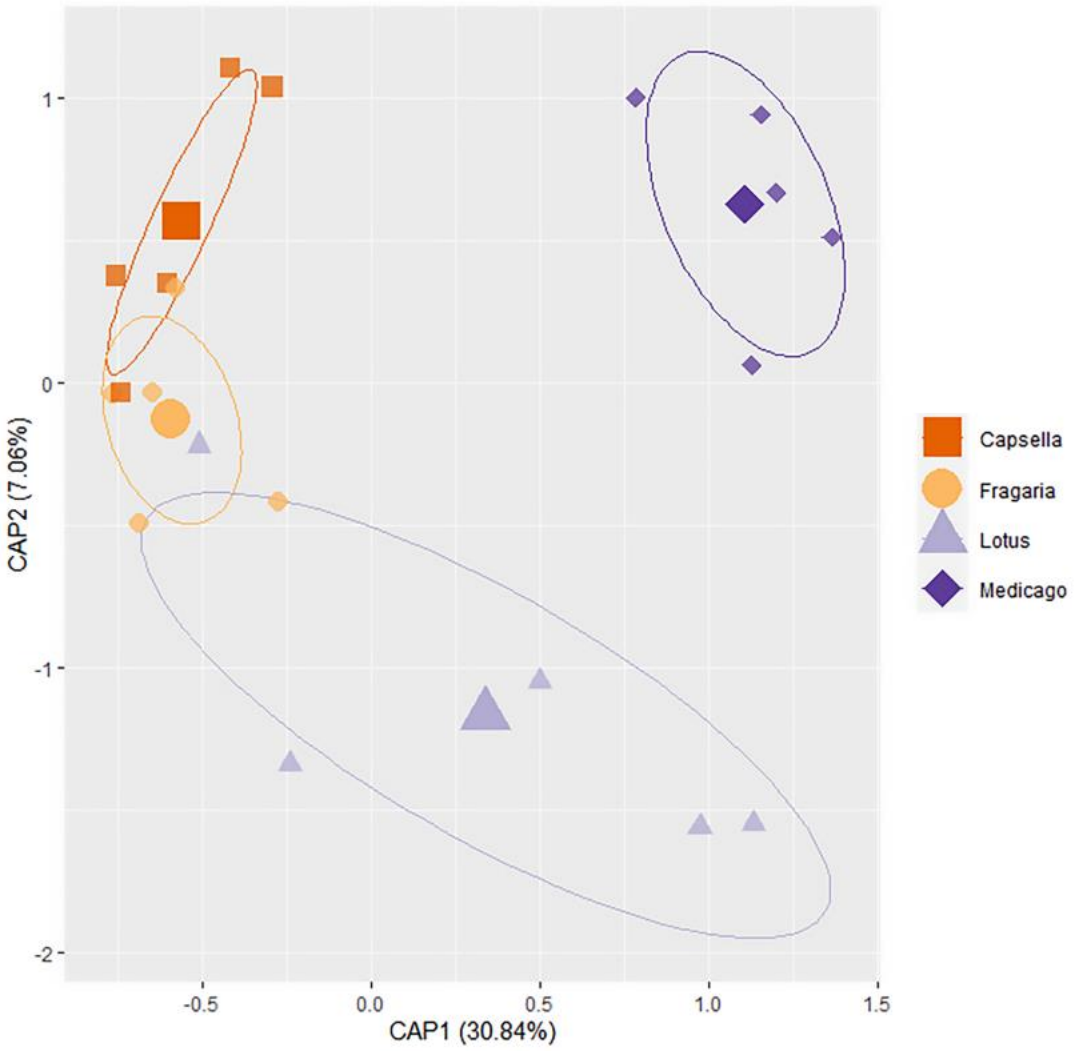


Figure 2-4: Distance-based redundancy analysis ordination based on the volatile emissions of four *L. hesperus* hosts that elicited different levels of attraction in behavioral assays.

Chapter 3

ELECTROPHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF *Lygus hesperus* KNIGHT (HEMIPTERA: MIRIDAE) TO HOST PLANT VOLATILES

ABSTRACT – *Lygus hesperus* Knight is a polyphagous pest of major concern to numerous cropping systems across western North America. Despite its wide diet breadth, *L. hesperus* exhibits well-documented host preferences. This study utilized electrophysiological and behavioral assays to examine *L. hesperus* attraction to host plant volatiles. Insect antennae were exposed to volatile extracts from four host plants previously shown to elicit varying degrees of attraction, leading to the identification of 17 compounds that elicited antennal depolarization. In Y-tube olfactometer assays, females showed no preference for six of the tested compounds, were attracted to six of the compounds, and avoided five of the compounds when challenged against clean air. Females exhibited a significant preference for an equal-parts blend of the six attractive compounds over clean air, and no preference was observed between the attractant blend and alfalfa. Subsequently, we examined *L. hesperus* attraction to each compound individually and an equal-parts blend of five attractive compounds in the field, first in strawberry in Spring and again in alfalfa in summer. In both field settings, neither the individual compounds nor the blend increase *L. hesperus* capture rate compared to control traps. Low attraction in the field may be due to a masking effect of background volatiles, the need to further refine dispensers or trap parameters, or the need for a more complete blend. These data emphasize the difficulty of translating attraction in the laboratory to field efficacy.

Introduction

Herbivorous insects rely on olfactory and visual cues to locate suitable host plants in complex environments (Carrasco et al., 2015; Louis M. Schoonhoven, 2005). Most insect herbivores can utilize a small number of related host plants (Futuyma & Moreno, 1988; Jaenike, 1990; Janz et al., 2001). Such specialists use host-specific blends of volatile organic compounds to identify suitable hosts (Bruce et al., 2005; Bruce & Pickett, 2011), and may simultaneously be deterred by the presence of non-host volatiles (Q. H. Zhang & Schlyter, 2004). Patterns of information use in herbivorous insects with wide diet-breadth are less well-understood (Carrasco et al., 2015), but performance-preference relationships appear to be weaker in polyphagous insects (Gripenberg et al., 2010). This may be due to weaker selection on host choice or constraints on the efficient processing of information from many potential host species (Grippenberg et al., 2010). The generalization of olfactory receptors (Carrasco et al., 2015), reliance on cues shared across many hosts (Bernays, 2001), utilization of context- or habitat-dependent cues (Silva & Clarke, 2020), or a combination of these may allow polyphagous insects to overcome challenges associated with the abundance of chemical information in the landscape.

Lygus hesperus Knight (Hemiptera: Miridae) is a polyphagous herbivore species that causes significant economic damage to numerous fruit, vegetable, and seed crops in western North America. *Lygus hesperus* overwinters as adults and lays eggs on a variety of broadleaf plants as temperatures warm in the spring. As spring hosts senesce or are harvested, *L. hesperus* populations disperse into nearby crops (Carrière et al., 2006; Pansa & Tavella, 2009; Snodgrass et al., 1984). Despite decades of investigation, effective early detection strategies for *L. hesperus* remain elusive. Pheromone components have been discovered for several *Lygus* species (Byers

et al., 2013; Fountain et al., 2014; Innocenzi et al., 2004a; Q. H. Zhang et al., 2007; T. Zhang et al., 2021), but only pheromone trapping studies targeting *L. rugulipennis* (Baroffio et al., 2018a; Fountain et al., 2014; Innocenzi et al., 2005) and, more recently, *L. lineolaris* (George et al., 2023; Parys & Hall, 2017) have demonstrated consistent success. Host plants may represent another source of behaviorally active semiochemicals. As *Lygus* spp. prefer to feed on plant reproductive structures (Wheeler, 2001) and may track the succession of flowering plants (Fleischer & Gaylor, 1987), floral volatiles represent promisingappealing candidates for investigation.

The factors underlying host selection and preferences in this highly polyphagous species remain largely unresolved. The response of female *L. hesperus* to a wide range of host species revealed a shallow gradient of attraction, where most plant species were not different from each other (Hetherington et al., unpublished data). Interestingly, *Capsella bursa-pastoris*, an early-season *Lygus* spp. host (Barlow et al., 1999; Snodgrass et al., 1984) that *L. hesperus* preferentially utilizes over *M. sativa* (Barlow et al., 1999), consistently failed to attract *L. hesperus* females (Hetherington et al., unpublished data). *Lygus hesperus* reproductive output is significantly higher on *C. bursa-pastoris* than *M. sativa* (Barlow et al., 1999) so preferential utilization of *C. bursa-pastoris* is beneficial. Differences in floral chemical apparency may be one explanation for the discrepancy between long-range attractiveness and utilization. As a primarily self-fertilizing plant, cryptic flowers are unlikely to reduce the reproductive output of *C. bursa-pastoris* (Hintz et al., 2006), whereas *M. sativa* depends on insect pollination and must remain apparent to pollinators and herbivores (Haedo et al., 2022).

Lygus hesperus is known to respond to host plant volatiles (Blackmer et al., 2004a; Blackmer & Cañas, 2005; Williams et al., 2010). While nymphs are attracted to vegetative and

flowering *M. sativa* with and without damage from conspecifics, adult females only responded to the volatile emissions of *M. sativa* plants that had been previously damaged by conspecifics (Blackmer et al., 2004). *Lygus hesperus* females are more generally responsive when plant volatiles are paired with visual stimuli (Blackmer & Cañas, 2005). Male *L. hesperus* are less responsive to plant volatiles than females and nymphs, exhibiting attraction only to vegetative alfalfa in combination with conspecifics when paired with a visual stimulus (Blackmer et al., 2004b; Blackmer & Cañas, 2005). The search for different resources may underlie sex differences in *L. hesperus* responses to host plant volatiles (Bell, 1990). Males primarily searching for mates fits well with the observation that they only responded to host plants when conspecifics were present (Blackmer & Cañas, 2005). Females, on the other hand, are likely searching for suitable oviposition sites on which emergingand nymphs mayrequire hosts to complete their development, explaining stronger attraction to host plant volatiles.

Lygus spp. tend to be highly sensitive to green leaf volatiles (GLVs), particularly alcohols and butyrate esters, and moderately sensitive to terpenoids (Chinta et al., 1994; Feng et al., 2022; Frati et al., 2008; Williams et al., 2010). (*E*)-β-ocimene, (*R*)-(+)-α-pinene, and (*E,E*)-α-farnesene have been shown to attract female *L. hesperus* in laboratory assays (Williams et al., 2010). As the emission of (*E*)-β-ocimene and (*E,E*)-α-farnesene from *M. sativa* increase with *L. hesperus* damage (Blackmer et al., 2004), these compounds may mediate the increased *L. hesperus* female attraction to conspecific-damaged plants. *L. hesperus* males were deterred by (*E*)-2-hexenyl acetate, (±)-linalool, (*E,E*)-α-farnesene, and methyl salicylate, while females were deterred by (*S*)-(-)-α-pinene, methyl salicylate, and (*Z*)-3-hexenol (Williams et al., 2010). Deterrence of males by (*E*)-2-hexenyl acetate, (±)-linalool, and (*E,E*)-α-farnesene, which are neutral or attractive to females may contribute to the differential response of male and female *L. hesperus*

to *M. sativa* volatiles (Blackmer & Cañas, 2005; Blackmer et al., 2004). Methyl salicylate is often up-regulated in response to herbivory and may attract predators and parasitoids (Hare, 2011; Mallinger et al., 2011). This compound may convey information about competition, host quality, or predation risk, and similar responses across sex may therefore be expected.

Lures containing phenylacetaldehyde attract *L. rugulipennis* (Koczor et al., 2012) and increase female attraction to pheromone-baited traps (Baroffio et al., 2018). Yet, phenylacetaldehyde failed to attract *L. hesperus* in *Lesquerella* fields (Blackmer & Byers, 2009) and adding sunflower volatiles to *L. lineolaris* pheromone traps reduced captures of *L. lineolaris* compared to pheromone traps alone (Chouinard-Thuly et al., 2020). As host plant volatiles attract both male and female insects, they are an appealing tool for pest monitoring and management, but the development and successful deployment of these tools is challenging (Suckling, 2016). A disadvantage of plant volatile lures is their abundance in the background odorscape of agricultural fields (Cai et al., 2017), background odor may mask attractive lures if similar compounds are present (Cai et al., 2017; Riffell et al., 2014).

We previously compared the volatile emissions of four species eliciting different levels of attraction:, *C. bursa-pastoris*, *Fragaria ananassa*, *Lotus corniculatus*, and *M. sativa*. TRevealing that the more attractive species (*M. sativa*, *L. corniculatus*) emitted several compounds at higher rates than less attractive hosts (*C. bursa-pastoris*, *F. ananassa*), but of these compounds only (*E*)-β-ocimene has been demonstrated to attract *L. hesperus* (Hetherington et al. unpublished data). This study set out to better elucidate the chemical basis of host selection in *L. hesperus* by 1) applying gas chromatography with electroantennographic detection (GC-EAD) to identify plant volatiles that elicit antennal depolarization in *L. hesperus*, 2) assessing the response of adult female *L. hesperus* to antennally -active volatiles in Y-tube olfactometer assays, and 3)

evaluating the potential of attractive compounds to enhance *L. hesperus* trap capture in the field.

Materials and Methods

Insects. Insect eggs were sourced from a colony maintained at the U.S. Arid Land Agricultural Research Center in Maricopa, AZ. Insects were reared on an artificial diet (Frontier Scientific - Newark, DE) at 27°C and 35% RH under a 14:10 (L:D) photoperiod. Both electrophysiological and behavioral experiments were conducted with 10- to 20-day- old *L. hesperus* females, as mating typically occurs prior to this period (Strong, 1970).

Dynamic Headspace Extraction of Host Plant Volatiles. We examined the response of *L. hesperus* antennae to volatile emissions collected from four known host plants, i.e., *Capsella bursa-pastoris, Fragaria ananassa*, *Lotus corniculatus*, and *Medicago sativa*, that were shown previously to cover a range of attractiveness to *L. hesperus* (Hetherington et al. unpublished data). Dynamic headspace extractions were conducted for five flowering individuals of each of the four plant species. Headspace extractions took place for 3 hrs at approximately 25°C. For each plant, 10-20 flowers/racemes and 10-20 leaves were enclosed in a 3.8 L Teflon pail liner (Welch Flurocarbon - Dover, NH). The bag was sealed with a steel wire and charcoal-filtered air (Sigma-Aldrich - St. Louis, MO) was introduced at a rate of 300 ml/min. Simultaneously, a vacuum pump (Robinair - Warren, MI) drew air out of the bag through an adsorbent trap containing 20 mg of Porapak (Sigma-Aldrich - St. Louis, MO) at 300 ml/min. After 3 hrs, each trap was eluted with 200 µL of chloroform (Sigma-Aldrich - St. Louis, MO).

Chemical analysis. Plant extracts were concurrently analyzed on a Thermo Scientific Trace 1300 gas chromatograph (GC) coupled to an ISQ series single quadrupole mass spectrometer (MS;

Thermo Fisher Scientific - Waltham, MA) and an Agilent 7890B GC with the output split between a flame ionization detector (FID) and an electroantennogram detector (EAD; Syntech - Buchenbach, Germany) in a 1:2 FID:EAD ratio. In both cases, the split/splitless injector was operated in splitless mode. The inlet temperature was 250°C and the oven was maintained at 35°C for 2 min before increasing to 150°C at a rate of 5°C/min, then increased at a rate of 20°C/min to a final temperature of 210°C, which was held for 2 min for a total run of 30 min. An injection volume of 1 µL was used for each host plant extract. The MS Detector began scanning 35-350 *m/z* after a solvent delay of 5 min and continued until the end of the run. A continuous stream of charcoal-filtered, humidified air (20 mL/s) carried eluting compounds to the antennal detector. Antennae were mounted on two glass capillary electrodes filled with biological saline solution (Malo et al., 2004). To prepare the EAD, insects were chilled for 5 minutes, the tip of the right antenna was cut using sharp microdissection scissors (VWR - Radnor, PA). To ensure proper contact of the antennae with the recording electrode, gentle pressure was applied to the insect abdomen. A bubble of hemolymph appearing at the tip of the antenna indicated that the antenna was not pinched during cutting. Insects were subsequently decapitated, and the head was mounted on the indifferent electrode and the tip of the antenna connected to the recording electrode. Output traces were exported from GC-EAD software (Syntech - Buchenbach, Germany) in ASCII format and analyzed with an automated system described by Slone and Sullivan (2007). Briefly, three algorithms were applied to identify deflections with characteristics associated with olfactory stimulation. In this study, the “Additive method” which filters depolarizations based on deflection and wavelength by adding the amplitudes of the initial negative deflection and the corresponding positive deflection during repolarization was used to define EAD-active peaks (Slone & Sullivan, 2007). This algorithm was selected because Slone

and Sullivan (2007) reported that it identified the most true olfactory responses with only a modest number of false-positives. Retention indices were calculated for peaks associated with antennal depolarization based on a C8-C20 alkane series, allowing peaks to be matched across the two GCs. Tentative identification was achieved by comparing mass spectra obtained from the GC-MS to the NIST 2008 MS library and analyte identities of the chemical compounds reported in Table 3-1 were confirmed via comparison to authentic standards (Sigma-Aldrich - St. Louis, MO).

Insect response to plant volatiles. Behavioral assays were conducted in a glass Y-tube olfactometer (Internal diameter: 25 mm, interior angle: 50°, stem length: 15 cm, arm length 10 cm manufactured in the Chemistry Department at UW-Madison). Charcoal-filtered, humidified air was introduced to two 2 L glass jars via Teflon tubing. During each assay one jar contained an empty vial and cotton wick (control) and the other contained a vial, cotton wick, and 500 mg of a test chemical (stimulus). From each jar, the air flowed through Teflon tubing to the respective arms of the Y-tube and was maintained at 2 L/min via an inline flow meter (Aalborg Instruments, Orangeburg, New York). Before each assay, the stimulus and control jars were purged for 5 min.

Insects were housed individually in mesh-bottomed aspirator vials at 21°C and 45% RH for 16-24 hours prior to testing, allowing subjects to acclimate to testing conditions. During the acclimation period, insects had access to distilled water only. Prior to testing, insects were kept in an adjacent room to limit exposure to stimulus chemicals. At the beginning of each trial, the top of an aspirator vial was removed, and the vial attached to the base of the Y-tube. Insects were allowed to interact with olfactory stimuli for up to 10 min, beyond which the assay was terminated. An insect was determined to have made a choice if it moved halfway up one of the

arms of the olfactometer (5 cm beyond the junction), after which the trial was terminated. The direction from which stimuli were delivered was alternated with each trial to avoid directional bias that may be introduced by environmental conditions and/or the olfactometer. After each use, Y-tubes were washed with Alconox® detergent and rinsed with deionized water and 70% ethanol, then dried at 100°C for 30 min (Thelco Model 17 Lab Oven - Precision Scientific, Chicago, IL). For each of the 17 bioactive compounds, trials continued until 30 insects made a choice within the allotted time.

Following the evaluation of individual compounds two additional rounds of trials were conducted assessing the attractiveness of an equal parts blend of all attractive compounds against clean air and alfalfa plants.

Field assessment of attractive volatiles. Six compounds (3-methylbutanol, 1-(*R*)-α-pinene, hexyl butyrate, ocimene, (±)-linalool, and (E)-4,8-Dimethyl-1,3,7-nonatriene [DMNT]) and an equal-parts blend of these compounds were found to attract *L. hesperus* females in laboratory Y-tube assays. Only five of the six compounds were available in sufficient quantities to assess in the field, and DMNT was omitted in field assays. A set of field experiments waswere conducted in strawberry and alfalfa fields to examine the ability of the five compounds to increase *L. hesperus* capture rates against different backgrounds. Lures consisted of 4 ml polypropylene vial (Nalgene - Rochester, NY) containing a 1 cm length cotton dental wick (Dynarex - Orangeburg, NY). A 3 mm hole was drilled through the vial lid to regulate evaporation of each compound from the lures. Each lure was loaded with 500 mg of dichloromethane (control), and 500 mg of neat material of 3-methylbutanol, 1-(*R*)-α-pinene, hexyl butyrate, ocimene, (±)-linalool, or an equal parts blend of all compounds. All compounds were sourced from Sigma-Aldrich (St. Louis, MO). Lures were stored at -20°C prior to use.

Lygus hesperus attraction to synthetic compounds was tested in commercial strawberry fields in California and alfalfa fields in Idaho. Sampling in strawberry was conducted over two three-week periods, between May 2nd and May 17th, 2022, and between May 31st and June 13th, 2022, at five farms in Watsonville and Salinas CA. Alfalfa field trials were conducted in Malad City, ID between June 19th and July 3rd, 2022, and replicated in Kimberly, ID between August 9th and August 30th, 2022.

Strawberry trials compared an equal parts blend of all attractive compounds to a negative control. White sticky traps were placed in the tenth row of a strawberry field. Insects were removed weekly and *L. hesperus* populations in the strawberry field were assessed by collecting vacuum samples from the third and tenth rows. Lures were replaced every three weeks.

In alfalfa fields, white sticky traps (Great Lakes IPM) were baited with lures containing 500 mg of either dichloromethane (control), 3-methylbutanol, 1-(*R*)-α-pinene, hexyl butyrate, ocimene, (±)-linalool, or an equal parts blend of all compounds. Lures were arranged in randomized complete blocks (n = 10) along the perimeter of a (381m × 516.6m) alfalfa field in Malad City, ID. The blocks were arranged on the Northern (n = 4) and Southern (n = 6) perimeter of the field, as the prevailing wind direction varied between morning and evening at this site. Lures and traps were replaced weekly, and sampling took place over two weeks. To assess background levels of *Lygus* spp. populations near each block, five sweeps along each block were taken weekly with a 38 cm diameter sweep net (Manufacturer). This experiment was replicated at the Kimberly Research and Extension Center in Kimberly, ID in August of 2022. Seven randomized complete blocks were sampled weekly for three weeks, with blocks arranged on the updownwind (w, Western) perimeter of alfalfa fields ranging from 0.85 to 2.23 hectares and one block per field.

All traps were retrieved weekly from the field, covered in cellophane, and brought back to the laboratory where *Lygus* were counted as adults or nymphs. Adults were sexed and males were identified to species, while females were only identified to genus, as females cannot be reliably identified to species based on morphological features (Mueller et al., 2003). All alfalfa samples were processed at the University of Wisconsin – Madison and the strawberry samples at a Discoll's facility in Watsonville, CA.

Statistical analysis. Classification of antennally-active peaks was conducted algorithmically using a macro-enabled Microsoft Excel spreadsheet developed by Slone and Sullivan (2007). Statistical tests for data from utilized behavioral and field assays were performed using R version 4.1.3 (R core team 2022). The behavioral Y-tube count data were subjected to independent chi-square tests to compare insect responses to the null hypothesis of random movement based on equal frequencies. Chi-square tests were performed using the chisq.test function from the "stats" package in R. For the field assessments, three linear mixed effects models were applied to examine the relationships between lure identity and the capture rate of males, females, and *Lygus* overall. In each case, capture rate (male *L. hesperus*, female *Lygus* spp., or total *Lygus* spp./trap/week) was the response variable and lure identity was included as a categorical predictor variable. A random effect term consisting of Block within Date within Site (1|Site/Date/Block) was included to account for non-independence in the data. We decided *a priori* to compare each lure with the negative control rather than consider all potential comparisons as the goal of this experiment was to determine whether individual lures increase capture rate compared to standard traps without lures. Models were fitted using the lme4 package (Bates et al., 2015). The package lmerTest (Kuznetsova et al., 2017) was used to assess overall model outputs and the emmeans package (Searle et al., 2023) was employed for post-hoc testing.

A square root transformation was applied to count data to improve homogeneity of variance and normality prior to analysis.

Results

Antennal response to plant volatiles. A total of seventeen compounds consistently elicited antennal depolarizations in *L. hesperus* females (Table 3-1). Antennally-active compounds were most common in the alfalfa headspace extract (13 compounds) and were identified as 3-methylbutanol, 2-phenylethanol, (Z)-3-hexenol, α-pinene, sulcatone, (Z)-3-hexenyl acetate, (E)-β-ocimene, linalool, DMNT, hexyl acetate, hexyl butyrate, (Z)-3-hexenyl butyrate, and hexyl tiglate. *Capsella bursa-pastoris* emitted the fewest antennally-active compounds (6 compounds), of which three were not detected in *M. sativa*, i.e., 2-ethylhexanol, phenylacetaldehyde, and 2-ethylhexyl acetate. Nine compounds in headspace extracts of *F. ananassa* elicited antennal depolarizations and all were shared with *M. sativa* and/or *C. bursa-pastoris.* Headspace extracts from *L. corniculatus* contained twelve compounds that elicited antennal depolarizations one of which, (*E*)-cinnamaldehyde, was unique.

Behavioral response to plant volatiles. Female *L. hesperus* challenged with each of the 17 antennally active compounds vs. clean air exhibited significant or marginally significant preferences to six of these compounds in Y-tube choice assays (Figure 3-1A). The attractive compounds were 3-methylbutanol ($X^2 = 10.80$, $P = 0.001$), hexyl butyrate ($X^2 = 8.53$, $P = 0.004$), 1-*R*-α-pinene ($X^2 = 6.53$, $P = 0.011$), ocimene ($X^2 = 4.80$, $P = 0.029$), while a marginally significant preference was observed for ±-linalool ($X^2 = 3.33$, $P = 0.068$), and DMNT ($X^2 = 3.33$, $P = 0.068$). Additionally, a significant preference for clean air was observed over the stimulus when the stimulus was (Z)-3-hexenyl butyrate ($X^2 = 13.33$, $P < 0.001$), (Z)-3-hexenyl acetate ($X^2 = 8.53$, $P = 0.004$), (Z)-3-hexenol ($X^2 = 6.53$, $P = 0.011$), 2-ethylhexanol ($X^2 = 4.80$, $P = 0.029$),

and 2-ethylhexyl acetate ($X^2 = 3.33$, $P = 0.068$). No preferences were observed when (E)-cinnamaldehyde ($X^2 = 2.13$, $P = 0.144$), phenylacetaldehyde ($X^2 = 0.53$, $P = 0.465$), hexyl tiglate ($X^2 = 0$, $P = 1$), hexyl acetate ($X^2 = 0.13$, $P = 0.715$), 2-phenylethanol ($X^2 = 0.53$, $P = 0.465$), or sulcatone ($X^2 = 1.2$, $P = 0.273$) were used as stimuli vs. clean air.

When combining the six attractive compounds 3-methylbutanol, hexyl butyrate, 1-*R*-α-pinene, ocimene, (±)-linalool, and DMNT into an equal-parts blend, the blend was significantly more attractive than clean air (Figure 3-1B; $X^2 = 4.80$, $P = 0.029$), but when compared against flowering *M. sativa* plants, no preference was observed between the blend and *M. sativa* (Figure 3-1B; $X^2 = 0.53$, $P = 0.465$).

Field assessment of attractive volatiles. Due to the lack of commercial supply for DMNT, the field experiment tested the blend of the other five behaviorally attractive compounds in California strawberry fields and this blend yielded no clear results. No *Lygus* spp. were collected on traps baited with the blend and only one *Lygus* female was captured on the control trap. This is likely driven by low *Lygus* populations throughout the experiment. A total of 36 and 46 *Lygus* bugs were collected from the control and treatment strawberry plots, respectively, over six weeks of collecting, with 48.8% of adult *Lygus* collected on the final sampling date. *Lygus* spp. densities in strawberry averaged 0.6 ± 0.145 and 0.767 ± 0.169 insects/vacuum collection in the treatment and control plots, respectively.

The field experiment testing each of the five behaviorally attractive compounds individually and a blend of the five compounds near alfalfa fields showed that no lure tested (neither individual compounds nor the blend) increased *Lygus* spp. capture rate compared to a control (Figure 3-2A; $F_{6,237.4} = 1.163$, $P = 0.327$), nor was a significant effect of lure observed for female (Figure 3-2B; $F_{6,238.3} = 0.297$, $P = 0.938$) or male (Figure 3-2C; $F_{6,273.1} = 1.784$, $P =$

0.103) capture rates. Examination of the sweep net samples confirmed that *Lygus* populations were present in the alfalfa fields throughout both sampling periods, with weekly mean densities ranging from 0.842 ± 0.225 to 5.28 ± 0.631 *Lygus* spp. per sweep. Of the *Lygus* spp. collected from the field, 44.99% were female. Females were underrepresented in the trap capture, comprising between 21.7-32.4% of total *Lygus* spp. trap captures.

Discussion

Lygus hesperus is a highly mobile, multivoltine, polyphagous herbivore that represents a major challenge to many crop production systems in Western North America. This study sought to identify semiochemicals associated with host preference in *L. hesperus*. We identified 17 compounds from four host plants that consistently elicited antennal depolarizations, six of which were attractive in Y-tube olfactometer assays. An equal-parts blend of these six compounds was as attractive as flowering *M. sativa* in laboratory assays. However, neither individually-attractive compounds nor the blend were attractive to *L. hesperus* in the field.

Here we identified antennally-active compounds detected in the volatile emissions of four hosts showing varying degrees of *L. hesperus* attraction and suitability as reproductive hosts *C. bursa-pastoris, F. ananassa, L. corniculatus,* and *M. sativa.* Most of the compounds identified were GLVs (i.e. (Z)-3-hexenol, (Z)-3-hexenyl acetate, hexyl acetate, hexyl butyrate, (*Z*)-3-hexenyl butyrate, hexyl tiglate) or terpenoids (i.e, α-pinene, (*E*)-β-ocimene, (±)-linalool, and DMNT), with a few other alcohols (i.e. 3-methylbutanol, 2-phenylethanol, 2-ethylhexanol), aldehydes (i.e. (*E*)-cinnamaldehyde, phenylacetaldehyde), ketones (i.e. sulcatone), and an acetate (i.e. 2-ethylhexyl acetate) also present. *Lygus* spp. tend to have high antennal sensitivity to GLVs and moderate sensitivity to terpenoids (Chinta et al., 1994; Williams et al., 2010), and here we confirm *L. hesperus* antennal sensitivity to (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene,

and (±)-linalool (Williams et al., 2010). Hexyl butyrate is thought to be an important component of the *L. hesperus* pheromone blend (Byers et al. 2013), and previous studies have demonstrated antennal sensitivity to this compound in *L. lineolaris* (Chinta et al. 1994), *L. rugulipennis* (Innocenzi et al., 2004b), and *L. pratensis* (Zhang et al., 2021). *Lygus lineolaris* is also sensitive to hexyl acetate, α-pinene, and phenylacetaldehyde (Chinta et al., 1994), while (*Z*)-3-hexenyl butyrate has been shown to elicit antennal responses in *L. pratensis* (Feng et al., 2022). By comparing the chemical profiles of hosts of varying attractiveness, we documented the antennal responses of *L. hesperus* to 3-methylbutanol, 2-phenylethanol, sulcatone, (*E*)-cinnamaldehyde, DMNT, hexyl tiglate, 2-ethylhexanol, and 2-ethylhexyl acetate.

Lygus hesperus females were attracted to six of the seventeen antennally-active compounds (ocimene, (*R*)-α-pinene, DMNT, 3-methylbutanol, (±)-linalool, and hexyl butyrate) reported in this study. We observed significant preference for ocimene and (*R*)-α-pinene over clean air, as has been previously reported (Williams et al., 2010). Although a mixture of ocimene isomers was used in behavioral tests, we expect *L. hesperus* females responded to (*E*)-β-ocimene, as it was the only compound associated with antennal depolarization when standards were used to confirm antennal sensitivity (unpublished data). Additionally, we report attraction to DMNT, hexyl butyrate, and 3-methylbutanol. DMNT is constitutively emitted from flowering and vegetative alfalfa (Blackmer et al. 2004) and is a major driver of the separation between attractive and unattractive plant species among 17 host plants (Hetherington et al. unpublished data). The compound 3-methylbutanol is a component of *M. sativa* floral scent (Buttery et al., 1982) and while not previously reported to attract *Lygus* spp., traps baited with 2-phenylethanol, 2-methylbutanol, and 3-methylbutanol caught moderate numbers of *Lygus* spp.(Davis & Landolt, 2013). Female *L. hesperus* also exhibited marginal attraction to (±)-linalool, whereas a previous

study found this compound failed to attract female and deterred male *L. hesperus* (Williams et al., 2010). This discrepancy may be driven by the nutritional state of test subjects (Gadenne et al., 2016). Starvation tends to increase responsiveness to food-associated cues (Bell, 1990; Edgecomb et al., 1994; Martel et al., 2009; Wäckers, 1994) and sensitivity to cues associated with oviposition sites increases as eggs develop (Crnjar et al., 1990). Lastly, Hexyl butyrate is a component of the metathoracic gland secretions of *L. hesperus* and has been implicated in female-female attraction in *L. rugulipennis* (Frati et al., 2008, 2009; Glinwood et al., 2003).

Five compounds ((*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenyl butyrate, 2-ethylhexenol, and 2-ethylhexyl acetate) deterred female *L. hesperus* in laboratory assays. (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, and (*Z*)-3-hexenyl butyrate are GLVs and frequently associated with plant tissue damage and facilitate foraging in a variety of predators and parasitoids (Ameye et al., 2018; Matsui & Engelberth, 2022; Shiojiri et al., 2006). Accordingly, these compounds may convey the presence of competition or danger. While *L. hesperus* females are more likely to orient toward conspecific-damaged plants, *Lygus* spp. feeding is not associated with increased emission of GLVs from *V. faba* or *M. sativa* (Blackmer et al., 2004; Frati et al., 2009). The deterrent effect of (*Z*)-3-hexenol has previously been reported for *L. hesperus*, while previous tests of (Z)-3-hexenyl acetate showed no behavioral effect in females (Williams et al. 2010). The compounds 2-ethylhexanol and 2-ethylhexyl acetate have been reported in association with pathogen and aphid infestation (De Lacy Costello et al., 2001; Fernando et al., 2005; Nakamura & Hatanaka, 2002; Pareja et al., 2012) and may therefore convey information about host quality, competition, or induced plant defenses.

Herbivorous insects rely on olfactory cues to locate suitable host plants interspersed among non-hosts (Carrasco et al., 2015; Schoonhoven et al., 2005). Although attraction in many

specialist herbivores depends on the co-detection of multiple chemical compounds (Bruce et al., 2005; Bruce & Pickett, 2011), individual compounds may be sufficient to elicit responses from polyphagous insects, such as *A. lucorum* and *L. rugulipennis* in the field (Baroffio et al., 2018b; Koczor et al., 2012; Pan et al., 2015). However, more complex blends tend to attract more herbivores (Szendrei & Rodriguez-Saona, 2010). While the individual compounds and the blend of these compounds were attractive to female *L. hesperus* in Y-tube assays, they did not attract *Lygus* spp. to traps in the field. The discrepancy between laboratory and field results may be driven by several factors, including the exclusion of DMNT from field tests, the influence of the background odorscape (Cai et al., 2017; Schröder & Hilker, 2008), differences in the physiological state of field-active and lab-tested individuals (Bell, 1990; Martel et al., 2009; Wäckers, 1994), the use of low-purity or suboptimal release of synthetic volatiles, or a combination of the above.

Frequently, while some individual compounds are critical for insect attraction, others may only increase attraction and are not essential for a blend to be attractive (Thöming & Knudsen, 2014). Here, the 6-component blend identified with electrophysiology and behavioral assays contained DMNT, which was not included in the field trials due to the lack of commercial availability at the time and the prohibitive cost of synthesis. It is possible that DMNT is fundamental to the attractiveness of *M. sativa*. However, contexts that change *L. hesperus* attraction to *M. sativa* (i.e. flowering, conspecific damage) are not associated with changes in DMNT emission (Blackmer et al. 2004) and attraction to this compound in behavioral assays was marginal, suggesting DMNT is likely not an essential component of an attractive blend.

The potential for background plant volatiles to influence the efficacy of kairomone lures cannot be ignored. As each of the compounds tested in the field trial are emitted by *M. sativa*, it

seems plausible that the lures were masked by the background emissions of the alfalfa fields, as Cai and colleagues (2017) observed in tea plantations. While most compounds tested in this study were relatively pure, blends of isomers can affect the attractiveness of a lure, as some isomers yield differential behavioral responses, as the enantiospecific response of *L. hesperus* to α-pinene demonstrates (Williams et al., 2010). This could be the case with linalool, where female cabbage looper *Trichoplusia ni* are attracted by (*S*)-(+)-linalool and deterred by (*R*)-(-)-linalool, while racemic linalool elicits intermediate responses (Heath et al., 1992). The ocimene lure also contained a mixture of several isomers. (*E*)-β-ocimene was the only isomer to elicit antennal depolarization when synthetic compounds were used to confirm antennal activity, so other ocimene isomers interfering with attraction seems unlikely. However, impurities dilute the active compound, and this may exacerbate the masking effect of background odor.

This study sought to elucidate the host plant volatiles associated with host preference in *L. hesperus*. We identified several new antennally-active compounds and observed *L. hesperus* attraction to a subset of these in laboratory bioassays, but these failed to increase trap captures of *Lygus* in the field. While the alfalfa-derived compounds failed to increase *L. hesperus* capture rates compared to controls in alfalfa fields, subsequent experiments should be conducted to optimize the release of these compounds and evaluate them in other cropping systems. This study provides new insights into *L. hesperus* chemical ecology and emphasizes difficulties associated with the deployment of kairomone lures to facilitate pest monitoring.

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Compound	*C. bursa-pastoris*	*F. ananassa*	*L. corniculatus*	*M. sativa*
(*Z*)-3-hexenyl butyrate	Not Detected	Not Detected	Not Detected	3
(*Z*)-3-hexenyl acetate	2	3	4	5
(*Z*)-3-hexenol	4	4	5	5
2-ethylhexanol	4	Not Detected	0	Not Detected
2-ethylhexyl acetate	3	Not Detected	Not Detected	Not Detected
Sulcatone	Not Detected	2	1	3
2-phenylethanol	Not Detected	3	2	2
Hexyl acetate	Not Detected	2	4	2
Hexyl tiglate	Not Detected	Not Detected	Not Detected	3
Phenylacetaldehyde	2	2	3	Not Detected
(*E*)-cinnamaldehyde	Not Detected	Not Detected	3	Not Detected
DMNT	Not Detected	Not Detected	2	3
(±)-linalool	1	3	2	3
(*E*)-β-ocimene	Not Detected	3	4	5
(*R*)-α-pinene	0	2	3	4
Hexyl butyrate	Not Detected	Not Detected	Not Detected	4
3-methylbutanol	Not Detected	Not Detected	3	3

Table 3-1: Frequency of compounds eliciting antennal depolarization in female *L. hesperus* antennae (n = 5). “Not Detected” indicates that a particular compound was not present in any samples tested, while “0” indicates that the compound was detected in at least one extract and no extracts elicited antennal depolarization.

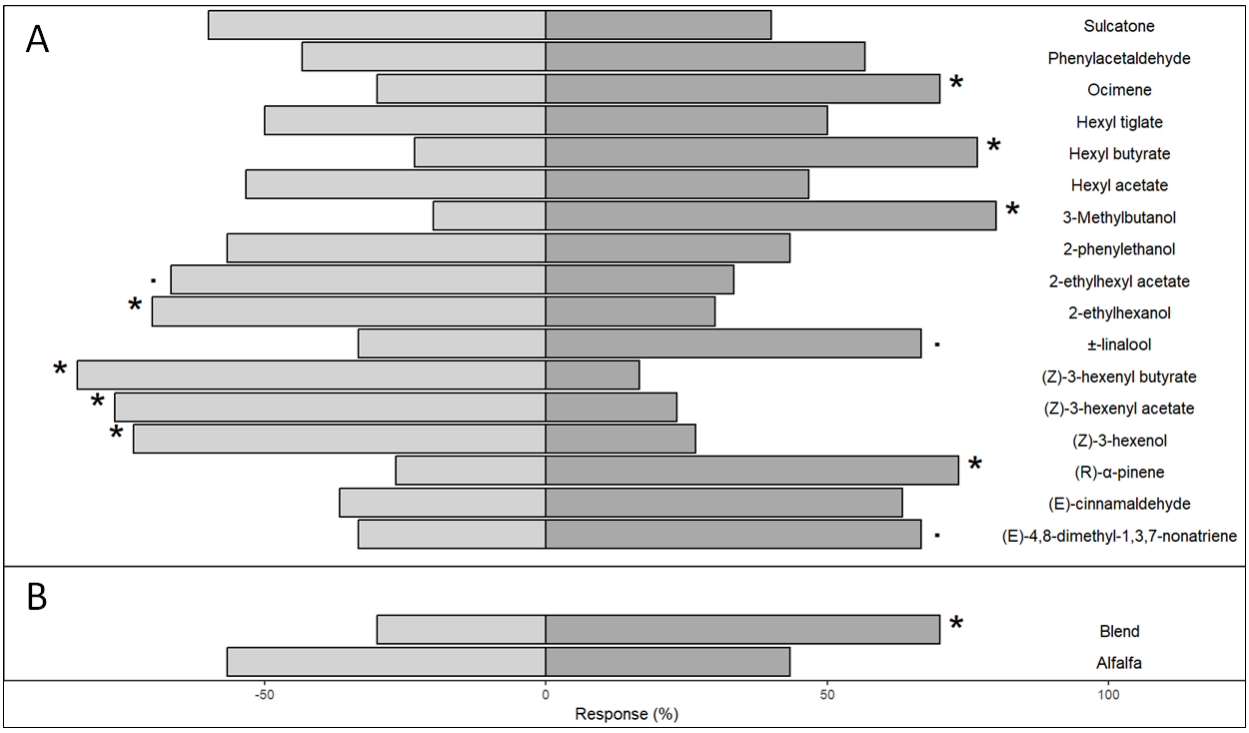

Figure 3-1: Proportion of female *L. hesperus* moving halfway up Y-tube arm. "*" denotes significant deviation from the null hypothesis of equal frequencies at $\alpha = 0.05$, "·" indicates marginally significant deviation, $0.05 < p < 0.07$. A) Comparison of each antennally-active plant volatile to clean air. B) Comparison of blend to clean air and the volatile emissions of flowering *M. sativa.*

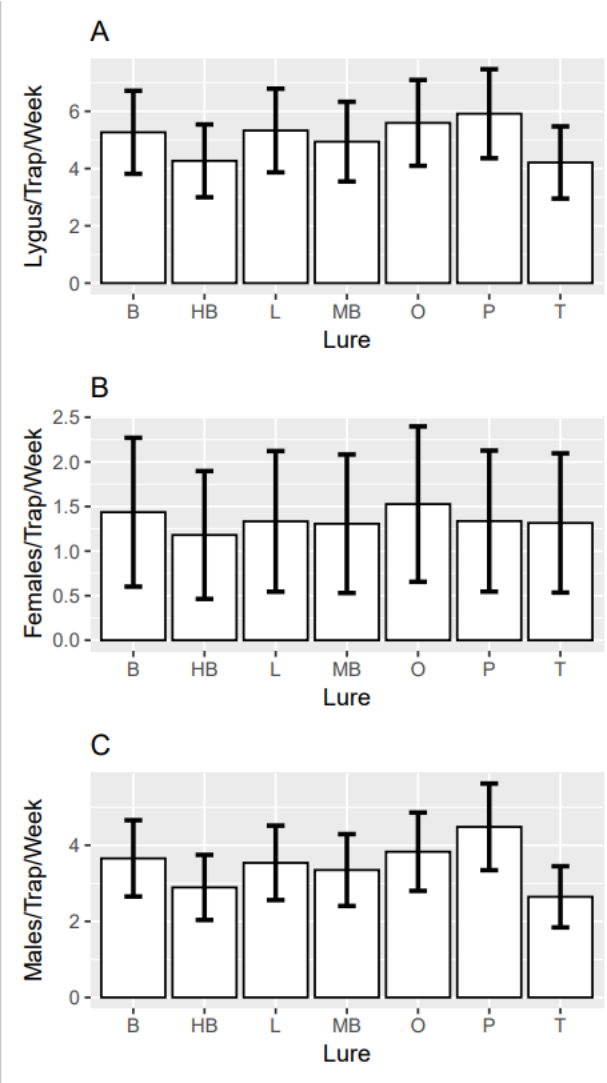

Figure 3-2: Back-transformed, bias adjusted estimated marginal mean *Lygus* capture rate ± SE. B = blank, HB = hexyl butyrate, L = (±)-linalool, O = ocimene, P = (*R*)-α-pinene, T = equal parts blend. A) Total *Lygus* spp. B) Female *Lygus* spp. C) Male *Lygus hesperus*. "*" denotes significant difference from blank at $\alpha = 0.05$.

Chapter 4

ALFALFA PERIMETER STRIPS REDUCE *Lygus lineolaris* PALISOT DE BEAUVOIS (HEMIPTERA: MIRIDAE) POPULATIONS IN JUNE-BEARING STRAWBERRY FIELDS

ABSTRACT – *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) is the primary insect pest of strawberry in eastern and central North America. Strategies to minimize *L. lineolaris* colonization of strawberry at bloom and peak susceptibility without impacting pollinator health must be developed. To this end, we examined the potential of alfalfa perimeter strips to reduce *L. lineolaris* populations in June-bearing strawberry fields. Over a three-year experiment, *L. lineolaris* densities and beneficial arthropod abundance were monitored on three commercial strawberry farms where alfalfa as a trap crop was established near strawberry plots. Alfalfa perimeter strips were found to concentrate *L. lineolaris* populations and led to a 36% reduction in *L. lineolaris* densities in adjacent strawberry plots compared to controls. When a protein immunomark-capture experiment was conducted to examine the extent of movement between the alfalfa strips and adjacent strawberry plot, it was determined that approximately 3 times as many *L. lineolaris* migrated from strawberry to alfalfa than vice versa. Moreover, adult females were overrepresented among immigrants to alfalfa, suggesting that alfalfa may be a preferred oviposition site for *L. lineolaris* females. While the presence of alfalfa perimeter strips influenced the beneficial arthropod community in experimental plots overall, most effects were limited to the alfalfa strip itself, with little spillover into adjacent strawberry plots. These data suggest that preferential utilization of alfalfa by *L. lineolaris*, rather than biological control underlies the observed population reductions and that alfalfa perimeter strips act as a trap crop in June-bearing strawberries.

Introduction

Lygus lineolaris Palisot de Beauvois (Hemiptera: Miridae) is a highly polyphagous pest in North America (George et al., 2021; Snodgrass et al., 1984; Young, 1986). More than half of the crop species grown in the United States are listed as *L. lineolaris* host plants, alongside hundreds of uncultivated species (Dumont & Provost, 2022; Esquivel & Mowery, 2007; George et al., 2023; Capinera, 2001; Young, 1986). *Lygus* spp. overwinter as adults and utilize weedy hosts early in the season (Barman et al., 2010; Easterbrook, 1997; Esquivel & Mowery, 2007; Fye, 1980; Snodgrass et al., 1984), before dispersing into cultivated fields as early season hosts senesce. Alfalfa (*Medicago sativa*) is the primary crop host of *L. lineolaris* (Esquivel & Mowery, 2007), and supports large *L. lineolaris* populations throughout its range (Day, 1996b; Matos & Obrycki, 2004; Wold & Hutchison, 2003). Mass dispersal of *Lygus* spp. into more susceptible crops is thought to occur when alfalfa fields are harvested. Alfalfa management has been shown to influence *Lygus rugulipennis* colonization of strawberry (Pansa & Tavella, 2009) and strip harvesting or retaining a border of alfalfa during harvesting has been suggested as strategy to reduce *Lygus hesperus* dispersal into cotton (Godfrey & Leigh, 1994; Mueller et al., 2005; Summers, 1976). However, the impact of *Lygus* spp. dispersal from alfalfa fields varies. While alfalfa forage and seed fields act as a source of *L. hesperus* in cotton (Carrière et al., 2006), neither *L. lineolaris* populations in canola fields (Cárcamo et al., 2003) nor *L. hesperus* populations in bean fields (Stoltz & Mcneal, 1982) are affected by the harvesting of adjacent alfalfa fields. The importance of alfalfa harvest on *Lygus* spp. populations in nearby crops likely depends on a variety of factors, including host quality and the timing of harvest.

Lygus lineolaris is the primary insect pest of strawberries in the north-central (Matos & Obrycki, 2004; Rose et al., 1996) and northeastern United States (Rhainds et al., 2001), where

annual strawberry production exceeds 18,000 tonnes (Samtani et al., 2019; USDA, 2012). Strawberries are highly susceptible to *Lygus* spp. damage from flowering through fruit development, with susceptibility decreasing as fruit matures (Handley & Pollard, 1991). *Lygus* spp. feed on the achene and/or receptacle of developing fruit (Allen & Gaede, 1963; Handley & Pollard, 1991), leading to apical seediness and "cat-facing", reducing fruit size, quality, and marketability (Schaefers, 1980). Strawberry producers typically rely on insecticide applications to manage *L. lineolaris* populations (Rhainds et al., 2001). As strawberries are most susceptible to *L. lineolaris* damage during and shortly after bloom, balancing crop management and pollinator protection can be difficult. Non-chemical management strategies including the use of tractor-mounted vacuums (Pickel et al., 1994; Vincent & Lachance, 1993), reflective mulches (Rhainds et al., 2001), and the identification of resistant cultivars (Handley & Pollard, 1991) have failed to achieve sufficient control. Hymenopteran and Dipteran parasitoids attack *L. lineolaris* eggs, nymphs, and adults throughout its range, but the introduced parasitoid, *Peristenus digoneutus* Loan (Hymenoptera: Braconidae), is more efficient than native nymphal parasitoids (Clancy & Pierce, 1966; Day, 1996a, 2005). After *P. digoneutis* establishment, *L. lineolaris* populations in alfalfa fell by 75% (Day, 1996a) and mean parasitism rates of 19.7% have been reported on New York strawberry farms, with rates reaching 70% (Tilmon & Hoffmann, 2003). While *P. digoneutis* has become established throughout the northeastern US since being introduced in the 1980s and its westward expansion continues, it is not yet present in the north-central US (Day et al., 2008)

Trap cropping, whereby preferred plant species are planted alongside the primary crop to divert, intercept, and retain pest insects (Shelton & Badenes-Perez, 2006) is another management strategy that has shown promise against *L. hesperus* in California (Godfrey & Leigh, 1994;

Sevacherian & Stern, 1975; Stern, n.d.; Swezey et al., 2007), *L. rugulipennis* in Europe (Accinelli et al., 2005; Ondiaka et al., 2016), *L. pratensis* in China (Wang et al., 2021; Zhang et al., 2020), and *L. lineolaris* in Canada (Dumont & Provost, 2019, 2022). Trap cropping depends on the target pest exhibiting a behavioral preference for the trap species over the primary crop. As alfalfa is considered a preferred *Lygus* spp. host, it has been deployed against *L. hesperus* (Swezey et al., 2007; Godfrey and Leigh, 1994; Sevacherian and Stern, 1974; Stern 1966) and *L. rugulipennis* (Accinelli et al., 2005; Easterbrook & Tooley, 1999). Often supplemental steps, such as vacuuming (Swezey et al., 2007), strip cutting (Godfrey and Leigh, 1994), or insecticide applications (Zhang et al., 2020; Weng et al., 2020; Dumont et al., 2019; Accinelli et al., 2005), are required to manage *Lygus* spp. populations and prevent dispersal from the trap crop into the primary crop.

In the north-central United States, the first harvest of alfalfa typically occurs when strawberry plants are most susceptible to *L. lineolaris*, making the potential for mass movement of *L. lineolaris* particularly problematic. Since strip cutting or leaving a border row have been suggested as harvest strategies that retain *Lygus* spp. in alfalfa fields (Godfrey & Leigh, 1994; Mueller et al., 2005; Summers, 1976), we hypothesized that perimeter strips of alfalfa around strawberry fields will likewise arrest dispersing *L. lineolaris* and retain them through the short period of susceptibility. We therefore conducted a three-year field experiment to examine 1) the impact of alfalfa perimeter strips on *L. lineolaris* populations in June-bearing strawberry, 2) the extent of *L. lineolaris* movement between alfalfa strips and strawberry fields, and 3) the effect of incorporating alfalfa perimeter strips on beneficial arthropods in a strawberry agroecosystem.

Materials and Methods

L. lineolaris host preference

Laboratory cage trials were conducted in 2019 to assess *L. lineolaris* preferences for alfalfa and strawberry. Thirty adult *L. lineolaris* were released into a 30 x 30 cm mesh cage containing one flowering strawberry and alfalfa plant. Insects were allowed to interact with plants over 24 hrs, after which plants were bagged and insects were counted. This experiment was replicated 8 times over a two-week period. The number of insects settling on each plant species was compared with a paired t-test.

Site Selection

Trials were conducted between 2020 and 2022 at three commercial strawberry farms in Southern Wisconsin. All farms employed a perennial, matted-row production system, as is typical of strawberry production in the north-central United States (Samtani et al., 2019). The strawberry cultivars varied across farm and field, and included ‘Wendy’, ‘Annapolis’, ‘Honeoye’, and ‘Jewel’. Strawberry fields were watered and fertilized at the growers’ discretion. Bifenthrin was applied to the strawberries at two farms in 2020 and 2022 to manage thrips, these applications were applied to both control and experimental plots and data was collected normally. Fields that had been in production for at least three years and thus were nearing the end of the production cycle were selected to minimize unforeseen negative impacts of the trap cropping strategy, such as increases in *L. lineolaris* pressure.

Experimental Design

Strawberry fields were divided into paired 0.1 hectare experimental and control plots, which were separated by a 0.05 hectare buffer zone (Figure 4-1). The distance between blocks

was at least 20 m. At each experimental plot, 1 m-wide x 20-40 m-long strips of alfalfa were established along both sides of each experimental plot, running parallel to the strawberry rows, approximately 1 m from the first strawberry row. Alfalfa perimeter strips were established by tilling the soil and transplanting greenhouse-started alfalfa plants in a single row every 0.33 m along the entire length, and then seeding the entire strip at a rate of 13 kg/ha alfalfa seeds (manufacturer). Control plots retained the standard turf border. This design was replicated five times in 2020, six times in 2021, and four times in 2022. Alfalfa strips were initially established at all sites in the spring of 2020 and were established at two new sites in 2021, as some of the 2020 fields were rotated out of strawberry production. Second or third year alfalfa strips were then present alongside all experimental plots in 2022.

Insect Sampling

Insects were sampled passively, with clear sticky traps (Alpha Scents Inc., Canby, OR, USA) and pitfall traps (Dart Container Corporation, Mason, Michigan, USA), and actively with 38 cm diameter sweep nets (Oakfield Apparatus, Oakfield, WI, USA). Traps were located in the field perimeter (either alfalfa strip or turf border) and in the second and tenth strawberry rows from the field perimeter.Trapping was conducted over seven days every other week from the last week of May through the end of strawberry harvest in 2020 and 2021. Sticky traps consisted of 15.25 cm squares hung from garden stakes at a height of 1 m. Traps were removed after seven days, wrapped in cellophane, and taken to the lab for insect identification. Pitfall traps consisted of a 240 ml solo cup containing drowning solution consisting of 15% food-grade propylene glycol in water with a small amount of unscented dish soap (Colgate-Palmolive, New York, NY, USA) to reduce the surface tension of the liquid. Each trap cup was placed such that the lip of the cup was flush with the surrounding ground. After seven days, the contents of the

trap were filtered through a disposable paint strainer with a 226 μm mesh size (Trimaco Inc, Elk Grove, IL) and the drowning solution was discarded. Samples were stored in 70% ethanol until identification. In each plot, a set of 20 sweeps was taken from the centermost 10 m of the field perimeter, each of the first three strawberry rows, and the 10th strawberry row on each side of the field weekly, beginning the last week of May through the end of strawberry season in 2020, 2021, and 2022. Sweep net samples were stored in paper bags and stored at -20°C until processed upon returning to the lab.

Lygus species and beneficial arthropods were identified and counted under a stereomicroscope (Olympus XZS10, Olympus Life Science, Waltham, MA, USA). Non-insect arthropods were identified to order. Beneficial insects in the pitfall traps and sweep net samples were identified to family with the exception of the minute parasitic wasps. Because arthropods collected on the sticky traps were frequently badly damaged, we used the subsection Calyptratae to replace family-level identification for the Anthomyiidae, Calliphoridae, and Tachinidae, bees were identified to Anthophila, and other Apocrita were identified as large wasps, minute wasps, or ants.

L. lineolaris movement

In 2022, a protein immunomark-capture study (Hagler, 2019) was conducted to determine the extent of *L. lineolaris* movement between the alfalfa trap crop and adjacent strawberry plants. This experiment was replicated at two of the three farms, as *L. lineolaris* populations at one farm were too low in 2022 to assess movement. A 12.5% egg white solution was applied to the alfalfa perimeter strips at a rate of 1 L/5 m, while a 100% solution of non-fat milk was applied to the first three strawberry rows at the same rate. Protein markers were applied to four plots on June 5th and reapplied on June 19th using dedicated gas-powered backpack

sprayers to prevent contamination. Before the second protein application, ten leaves were randomly collected from each strawberry and alfalfa row to which a protein marker was applied to verify that markers were no longer present. *Lygus* spp. were collected 36 hrs after each application on June 6th and June 20th, following the sweep net sampling protocol described previously. To prevent the risk of contamination via sweep nets, all collection nets were machine-washed before use and only used once. Prior to beginning the mark-capture experiment, insects serving as negative controls were collected from an alfalfa field at the West Madison Agricultural Research Station in Madison, WI. Insects were then transferred to bags and placed on dry ice. Upon returning to the lab, bags were stored in a freezer -20°C until sorting.

Lygus lineolaris were sorted and identified as nymphs, adult males, or adult females and transferred to individual 1.5 mL microcentrifuge tubes with a clean toothpick. Anti-chicken ovalbumin and anti-bovine casein enzyme-linked immunosorbent assays (ELISAs) were performed to determine whether individuals moved between the alfalfa and strawberry rows over the 36 hours between application and sample collection. Briefly, all insects were soaked in TBS at 4°C overnight and 80 ul of rinse was added to each well of an ELISA plate (medisorp coating, manufacturer) and allowed to incubate at room temperature for 2 hrs before the rinse was discarded and wells were washed five times with 300 ul of PBS-Tween. 300 ul of 50% soy milk solution was added to each well and incubated for 30 min, after which wells were washed twice with 300 ul of PBS-Tween. 50 ul of primary antibody solution, either 1:2000 rabbit anti-casein (manufacturer) or 1:4000 rabbit anti-ovalbumin in 50% soy milk solution with 1.3 ug/ml silwet L-77 (Helena Chemical, Memphis, TN, USA) was added to each well and allowed to incubate for 1 hr before the antibody solution was discarded and wells were washed five times with 300 ul PBS-Tween. 50 ul of goat anti-rabbit-HRP 1:10000 in 50% soy milk with 1.3 ug/ml silwet L-77

before the antibody solution was discarded and wells were washed five times with 300 ul PBS-Tween. 50 ul of TMB solution was then added and allowed to incubate for 10 minutes before the reaction was stopped with 50 ul of TMB stop solution. Optical density was determined at 450 nm on an ELISA plate reader.

One column of each 96-well ELISA plate was dedicated to negative control samples. For each plate, mean (± SD) absorbance values were calculated for the negative controls, and *L. lineolaris* were considered positive for a particular marker if the absorbance was three standard deviations above the negative control mean (Buczkowski & Bennett, 2007; Jasrotia & Ben-Yakir, 2006). Rinses from all *L. lineolaris* were subjected to both ELISAs. The proportion of individuals collected from strawberry rows (casein-marked) testing positive for casein and individuals collected from alfalfa (ovalbumin-marked) testing positive for ovalbumin were used to assess the efficiency of marker application, while casein-marked individuals collected from alfalfa and ovalbumin-marked individuals collected in strawberry were assumed to have moved between plants.

Statistical analysis

All statistical analyses were performed using R version 4.1.3 (R core team 2022). Linear mixed models were fitted using the "lme4" package (Bates et al., 2015) and the package "lmerTest" (Kuznetsova et al., 2017) was employed to assess overall model outputs. The package "emmeans" (Searle et al., 2022) was utilized to compare geometric means after statistically significant effects were identified. Chi-square tests were conducted using the "chisq.test" function in the package "stats", employing the package chisq.posthoc.test (Beasley & Schumacher, 1995) to conduct post-hoc comparisons based on the residuals of a chi-squared test.

Multivariate analysis of beneficial arthropod datasets were conducted using the package "vegan" (Dixon, 2003).

Effect of alfalfa perimeter plantings on *L. lineolaris* density

Lygus lineolaris densities collected on the same day from opposite sides of each plot were averaged, and a linear mixed model was fit to examine the relationship between *L. lineolaris* density (insects/sweep) and the presence of alfalfa strips, row of collection, week of collection, and all interactions of alfalfa, row, and week. The nested random effects term consisted of week within field within farm within year (1|Year/Farm/Field/Week). To improve homogeneity of variance and normality, data were analyzed following a log(n+0.1) transformation. Geometric means were then compared across levels of all significant effects, using Tukey's HSD test to control family-wise error rate during multiple comparisons.

Lygus lineolaris movement between alfalfa and strawberry

A chi-squared test was applied to compare the proportion of immigrants collected from strawberry and alfalfa based on the null hypothesis of equal frequencies. Subsequently, the frequency of adult males, adult females, and nymphs among immigrant *L. lineolaris* were compared between strawberry, alfalfa, and the overall population to determine whether the direction of *L. lineolaris* movement varied across sex and lifestage.

Effect of alfalfa perimeter plantings on beneficial arthropods

Beneficial arthropod counts from sticky cards, sweep net samples, and pitfall traps collected on the same day from opposite sides of each plot were averaged and Bray-Curtis distance matrices were constructed for each dataset using the function 'vegdist'. Distance-based

redundancy analyses (db-RDA) were then applied to examine the relationship between each distance matrix and the presence of alfalfa perimeter strips, the row from which samples were collected, and the interaction of perimeter strips and row. A conditional variable was included to partition out the variance associated with year, field, and week. Following a significant db-RDA, the correlation of each taxon with the significant canonical axes was examined to determine which taxa underlie observed differences in bycatch community composition. Distance-based multivariate methods can confound effects of location and dispersion (Warton et al., 2012), we therefore applied the "betadisper" function to conduct multivariate analogs of Levene's test for homogeneity of variances for each significant predictor variable.

Following significant multivariate analyses, individual univariate linear mixed-effects models were fitted to examine the relationship between the abundance of the most commonly encountered taxa, the presence of alfalfa perimeter strips, the row from which samples were collected, and their interactions. The nested random effects term for these models consisted of week within field within year (1|Year/Field/Week). To preserve statistical power when making multiple comparisons, univariate tests were restricted to the ten most common taxa in each data set or all taxa present in at least 1% of samples, if fewer than ten taxa were present in 1% of samples. Bonferroni correction was applied to control family-wise error rate during multiple testing. Geometric means were then compared across levels of all significant effects, using Tukey's HSD test to control family-wise error rate during multiple comparisons.

Results

Host preference

After 24 hrs of exposure to flowering alfalfa and strawberry plants, 102.70% more *L. lineolaris* were collected from alfalfa compared to strawberry (t= 3.46, df= 7, P = 0.011; Figure 4-2). This indicates a preference for alfalfa over strawberry.

Effect of alfalfa perimeter plantings on *L. lineolaris* density

Lygus lineolaris density was significantly influenced by the presence of alfalfa perimeter strips ($F_{1,443.52}$ = 20.81, P < 0.001), the row from which insects were collected ($F_{4,62.85}$ = 3.96, P = 0.006), and the week of collection ($F_{7,374.86}$ = 38.39, P < 0.001). Additionally, the effect of alfalfa perimeter strips depended on the row from which insects were collected ($F_{4,443.76}$ = 26.63; P < 0.001). When averaged across row and week, *L. lineolaris* densities in strawberry fields with alfalfa perimeter plantings were 36.77% lower than that of control fields, and similarly *L. lineolaris* in the alfalfa strips were 90.16% greater than in the control field borders (Figure 4-3).

Lygus lineolaris movement

The mark-capture experiment revealed that *L. lineolaris* were moving between the strawberry field and the alfalfa perimeter strip. Thirty-six hours after marker application, x *L. lineolaris* were collected from marked plots, with y collected from alfalfa and z collected from marked strawberry rows. Marker coverage averaged 76% in the alfalfa and 80% in the strawberry. Immigrants represented 18.06% of the *L. lineolaris* collected from the alfalfa strip, and 5% of *L. lineolaris* collected from strawberry (Figure 4-4A; x^2= 6.20, df= 1, P = 0.013). Moreover, 73.68% of the immigrants collected from the alfalfa strips were female adults, a

significantly higher proportion than expected based on the ratio of females to males to nymphs (73.68:26.32:0) (Figure 4-4B; x^2= 23.5, df = 2, P < 0.001), while the immigrant population in strawberry (14.29:28.57:57.14) reflected the overall population (25:29.92:45.08) (x^2 = 0.54, df = 2, P = 0.7635).

Effect on beneficial arthropods

Sweep net samples:

The beneficial bycatch of the sweep net samples were dominated by a few taxa (Table x). The Anthomyiidae represented 19.83% of all collected beneficial arthropods, while minute parasitic wasps (16.69%), Dolichopodidae (11.61%), Syrphidae (9.85%), Araneae (8.58%), Rhagionidae (7.35%), Braconidae (6.92%), Coccinellidae (3.85%), Anthocoridae (2.81%), and Opiliones (2.67%) were also common in sweep net samples. The remaining 26 families accounted for 10.89% of sweep net collected beneficial arthropods, and included Nabidae, Formicidae, Halictidae, Tachinidae, Empididae, Ichneumonidae, Proctotrupidae, Andrenidae, Apidae, Chrysopidae, Reduviidae, Calliphoridae, Pompilidae, Sphecidae, Carabidae, Muscidae, Asilidae, Fanniidae, Sarcophagidae, Megachilidae, Vespidae, Mutillidae, Geocoridae, Chrysididae, Scoliidae, Conopidae, and Mantispidae.

Distance-based redundancy analysis indicated that the presence of alfalfa perimeter plantings had a significant influence on the community sampled by sweep nets ($F_{1,258}$ = 2.37, P = 0.002), as did the row from which insects were collected ($F_{4,258}$ = 3.33, P = 0.001) and the week of collection ($F_{5,258}$ = 10.72, P = 0.001). Within experimental and control plots, arthropods tended to exhibit an edge-biased distribution, with 36.44% collected from the perimeter, 21.75%, 17.09%, and 14.84% collected from strawberry rows 1, 2, and 3, respectively, and only 9.87%

collected from the 10th strawberry row. Overall, 57.4% of all beneficial arthropods were collected from experimental plots. The impact of alfalfa depended on the week of sample collection ($F_{5,258} = 1.36$, $P = 0.019$), but not on the row sampled ($F_{4,258} = 1.15$, $P = 0.179$). Likewise, the effect of row varied by week ($F_{20,258} = 1.20$, $P = 0.01$), but no three-way interactions were detected (Alfalfa:Row:Week: $F_{20,258} = 0.87$, $P = 0.958$). Both the Alfalfa:Week and Row:Week interactions are largely due to low arthropod abundance early in the first two weeks of sampling, with weeks 1 and 2 accounting for 3.77% and 8.51% of beneficial arthropods collected over this period, while, on average, 21.93% of beneficial arthropods were collected in each of weeks 3 through 6. These differences may stem from differences in centroid location or the dispersion of the data. Distance-based multivariate methods can confound effects of location and dispersion (Warton et al., 2012), it is therefore necessary to determine whether differences in dispersion may be influencing our results. Dispersion was observed to be similar across row ($F_{4,318} = 0.898$, $P = 0.466$) and treatment ($F_{1,321} = 3.279$, $P = 0.071$), but varied across week ($F_{5,317} = 5.84$, $P < 0.001$). The beneficial arthropod data collected for week 3 was significantly less variable than that of weeks 1, 2, 5, and 6 (Supplemental table 4-2). However, separation among week appears to be largely driven by differences between the first two weeks of collection and later weeks (Supplemental figure 4-1), indicating that true differences in location are present in the data.

Subsequent univariate tests revealed that the Rhagionidae, were, on average, 891.31% more abundant in trap cropped plots regardless of row (Table 4-1; Alfalfa - $F_{1,281} = 14.79$, $P_{adj} < 0.001$; Alfalfa:Row – $F_{4,281} = 2.28$, $P_{adj} = 0.613$), while the Anthomyiidae, Braconidae, and Coccinellidae tended to be more abundant in the alfalfa strips, but similar in experimental and control strawberry rows (Table 4-1; Anthomyiidae: Alfalfa - $F_{1,281} = 2.86$, $P_{adj} = 0.921$;

Alfalfa:Row – $F_{4,281} = 3.73$, $P_{adj} = 0.056$; Braconidae: Alfalfa - $F_{1,281} = 7.80$, $P_{adj} = 0.035$; Alfalfa:Row – $F_{4,281} = 6.37$, $P_{adj} < 0.001$; Coccinellidae: Alfalfa - $F_{1,280} = 10.45$, $P_{adj} = 0.014$; Alfalfa:Row – $F_{4,280} = 5.68$, $P_{adj} = 0.002$). Neither alfalfa perimeter plantings nor row was found to influence the abundance of Araneae, Opiliones, or Anthocoridae in sweep net samples (Table 4-1). All of the most common beneficial arthropod taxa except for Araneae ($F_{4,280} = 3.28$, $P_{adj} = 0.12$) and Rhagionidae ($F_{4,281} = 1.63$, $P_{adj} = 1$) were more abundant at the edge of the field than in the tenth strawberry row (Table 4-1).

Sticky Cards:

The Calyptratae, minute parasitic wasps, and Dolichopodidae were the dominant beneficial arthropods captured on clear sticky traps, representing 44.54%, 27.61%, and 14.68% of total captures, respectively. Distance-based redundancy analysis indicated that row ($F_{2,125} = 2.73$, P= 0.005) influenced the beneficial arthropod community captured on clear sticky traps, while the week of collection had a marginal effect ($F_{2, 125} = 1.90$, P = 0.057), but the presence of alfalfa perimeter plantings did not affect the community of beneficial arthropods collected on sticky traps ($F_{1,125} = 1.47$, P = 0.194). Analysis of the dispersion of each variable revealed that neither the effect of alfalfa perimeter plantings nor the effect of row could be attributed to differential dispersion (Alfalfa: $F_{1,154} = 2.19$, P = 0.141; Row: $F_{2,153} = 0.67$, P = 0.513). However, the effect of week may be due to dispersion, alone, or a difference in both dispersion and location (F2,153 = 3.78, P = 0.025).

Univariate tests on the most common morpho-taxa revealed that sticky traps collected, on average, 30.39%, 78.47%, and 372.69% more spiders (Table 4-2; $F_{1,125} = 15.32$, $P_{adj} = 0.002$), large wasps (Table 4-2; $F_{1,125} = 10.07$ $P_{adj} = 0.019$), and bees (Table 4-2; $F_{1,125} = 10.15$ $P_{adj} =$

0.018), respectively, in trap cropped plots than controls (Table 4-2). The presence of alfalfa perimeter plantings did not influence capture of minute parasitic wasps, Calyptratae, Dolichopodidae, or Syrphidae (Table 4-2). Row significantly affected the capture of minute parasitic wasps (Table 4-2)

Pitfall Traps:

Myriapoda were the most common beneficial arthropod captured in pitfall traps, representing 31.57% of total captures, while minute parasitic wasps, Araneae, and Opiliones were also common, representing 19.8%, 16.02%, and 14.03% of captured beneficial arthropods, respectively (Table 4-3). Distance-based redundancy analysis indicated that the community of beneficial arthropods sampled by pitfall traps was not influenced by the presence of alfalfa perimeter plantings ($F_{1,126} = 1.61$, $P = 0.183$), row ($F_{2, 126} = 1.18$, $P = 0.292$), or week ($F_{2,126} = 0.96$, $P = 0.444$).

Discussion

Lygus lineolaris is the primary insect pest of strawberries in the north-central United States (Matos and Obrycki, 2004; Rose et al., 1996), as small populations can dramatically reduce marketability of fruit. Large populations of *L. lineolaris* accumulate in alfalfa fields in spring (Wold and Hutchison, 2003), and may disperse from alfalfa fields during harvest in late May, when strawberries are most susceptible to damage. Here, *L. lineolaris* densities were significantly greater in alfalfa perimeter strips than adjacent strawberry fields and *L. lineolaris* were significantly less abundant within strawberry fields in the alfalfa trap cropped plots than the paired controls. As alfalfa perimeter strips had a limited impact on the community of beneficial arthropods in adjacent strawberry fields, biological control is unlikely to account for differences

in *L. lineolaris* abundace. Indeed, when movement between alfalfa strips and strawberry edge rows was quantified, it became apparent that not only were *L. lineolaris* moving primarily from the strawberry into the alfalfa, but that emigrants from strawberry were overwhelmingly adult females, while emigrants from alfalfa were reflective of the overall sex and life stage composition. These data suggest that alfalfa acts as a trap crop for *L. lineolaris* in June-bearing strawberry, but additional data on the incidence of *L. lineolaris* damage is required to determine whether this strategy also reduces damage.

When presented with a binary choice in laboratory cage trials, twice as many *L. lineolaris* adults settled on alfalfa compared to strawberry. Olfactory cues may underlie this behavior. A previous study in our lab compared *L. hesperus* attraction to alfalfa and strawberry in Y-tube olfactometer assays, finding that *L. hesperus* females were significantly attracted to alfalfa headspace volatiles (Hetherington et al. unpublished data). In that study, we also observed that (±)-linalool was emitted by alfalfa at higher rates than strawberry (Hetherington et al. unpublished data), which we subsequently determined attracts *L. lineolaris* in the field (Hetherington et al., unpublished data). Differential emission of (±)-linalool may contribute to the observed preference for alfalfa, although other plant volatiles may also influence *L. lineolaris* attraction. (*E*)-β-ocimene and (*R*)-α-pinene attract *L. hesperus* females in laboratory assays (Williams et al., 2010; Hetherington et al., unpublished data) and elicit antennal responses from *L. lineolaris* (Hetherington et al., unpublished data). While *L. lineolaris* were not attracted to these compounds in the field when presented individually, they may represent important components of a multi-compound blend (Thöming & Knudsen, 2014; Hetherington et al., unpublished data).

Various trap crops have been deployed to manage *Lygus* spp., including alfalfa for *L. hesperus* in California (Godfrey & Leigh, 1994; Sevacherian & Stern, 1975; Swezey et al., 2007), sunflowers, alfalfa, and white mustard against *L. rugulipennis* in Europe (Accinelli et al., 2005; Easterbrook and Tooley, 1999; Ondiaka et al., 2016), sunflower and safflower against *L. pratensis* in China (Wang et al., 2021; Zhang et al., 2020), and white mustard, buckwheat, and muellin against *L. lineolaris* in Canada (Dumont & Provost, 2019, 2022). Fewer than 10% of the trap crop systems reviewed by Shelton and Badenes-Perez (2006) provide adequate control of an insect pest without supplemental management of the pest population and previous studies examining trap cropping against *Lygus* spp. have demonstrated the need for supplemental inputs (Dumont and Provost, 2019; Swezey et al., 2007; Accinelli et al., 2005, Easterbrook and Tooley, 1999). Swezey and colleagues (2007) observed that alfalfa trap crops without supplemental vacuuming increased *L. hesperus* damage in the strawberry row adjacent to alfalfa compared to the grower standard and vacuumed trap crops, demonstrating the risk that a trap crop may cease to be a sink for pests and instead become a source of infestation. This risk may be somewhat lower in June-bearing strawberries than in day-neutral and everbearing varieties examined in previous studies, as the period of susceptibility is much shorter and begins earlier, when *L. lineolaris* populations tend to be relatively low.

Over the 36 hours between marker application and insect collection, three times as many *L. lineolaris* moved from the strawberry rows into the alfalfa as moved from alfalfa into strawberry, indicating that alfalfa perimeter strips attract *L. lineolaris* out of strawberry rather than simply reducing initial colonization. Despite representing only 25% of *L. lineolaris* collected during the movement experiment, adult females constituted 73.68% of emigrants from strawberry. This is further evidence for a female preference for alfalfa over strawberry and is

consistent with observations that *L. hesperus* females, but not males, are attracted to alfalfa volatile emissions (Blackmer et al., 2004; Blackmer & Cañas, 2005; Hetherington et al., unpublished data). The high attraction of females, and therefore eggs, away from the primary crop suggests that alfalfa strips may be useful as a component as a push-pull management system. Fountain and colleagues (2021) recently developed a push-pull system that reduced *L. rugulipennis* damage to strawberries by approximately 80% in organic field trials. Their system combined *L. ruguilpennis* sex pheromone lures and phenylacetaldehyde as the pull, and hexyl butyrate emitters as the push (Fountain et al. 2021). Reflective mulches may be another potential deterrent in a push-pull system. Rhainds and colleagues (2001) found that reflective mulches significantly reduced the number of *L. lineolaris* nymphs in small plots of day-neutral and June-bearing strawberries. Moreover, a lower proportion of June-bearing fruits were damaged by *L. lineolaris* when reflective mulches were present, though this may reflect increased productivity more than reduced feeding as the number of damaged fruits per hectare was similar between the reflective mulch and control treatments (Rhainds et al. 2001).

Beneficial arthropod taxa tended to be more abundant in plots with alfalfa perimeter plantings, however such increases were typically restricted to the alfalfa itself. Easterbrook and Tooley (1999) similarly observed that natural enemies were abundant in trap crops but did not spillover into strawberry. Although *L. lineolaris* predation or parasitism may be increased in the alfalfa strips, as has been documented for *L. hesperus* in trap cropped strawberry fields (Hagler et al., 2018; Swezey et al., 2014). Minute wasps, Braconidae, and Coccinellidae were all significantly more abundant in the alfalfa strip than strawberry rows or control plots. However, most braconids in our collections were members of subfamily Aphidiinae, not the euphorine wasps that parastize *Lygus* spp. (M.C. Hetherington, personal observation) and Coccinellidae

were recently shown to consume *L. hesperus* at relatively low rates (Hagler et al., 2020). These taxa were likely targeting aphids, which were abundant in the alfalfa strip (M.C. Hetherington, personal observation). The abundance of Dolichopodidae and Rhagionidae in sweep net samples was significantly higher in experimental strawberry rows compared to controls, although dolichopodids were captured at similar rates on sticky traps and rhagionids were below the threshold to warrant univariate testing. Although wildflower borders increased the abundance of ground-dwelling predators in strawberry in New York (McCabe et al., 2017), we saw no differences among the most common ground-dwelling arthropods detected in this study. Overall, these data suggest that incorporating alfalfa perimeter strips into strawberry field design has limited effects on the beneficial arthropod community. Additional research could be conducted to determine whether predation or parasitism of *L. lineolaris* is enhanced in alfalfa perimeter strips.

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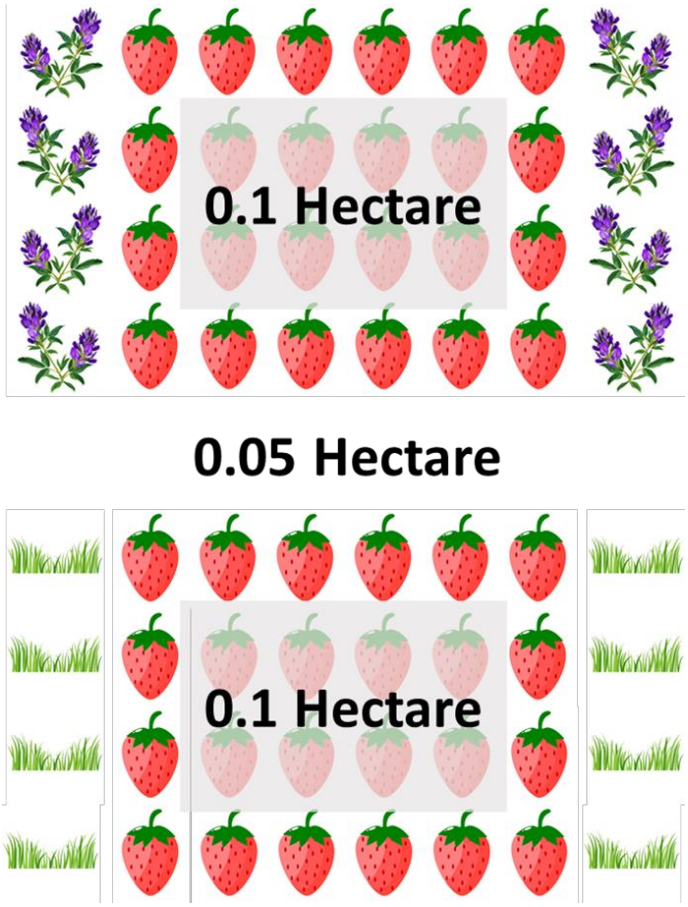
Figure 4-1: Diagram of plot layout. Experimental plots received 1 m-wide perimeter plantings along each side of the field, while paired control plots retained the standard turf field border.



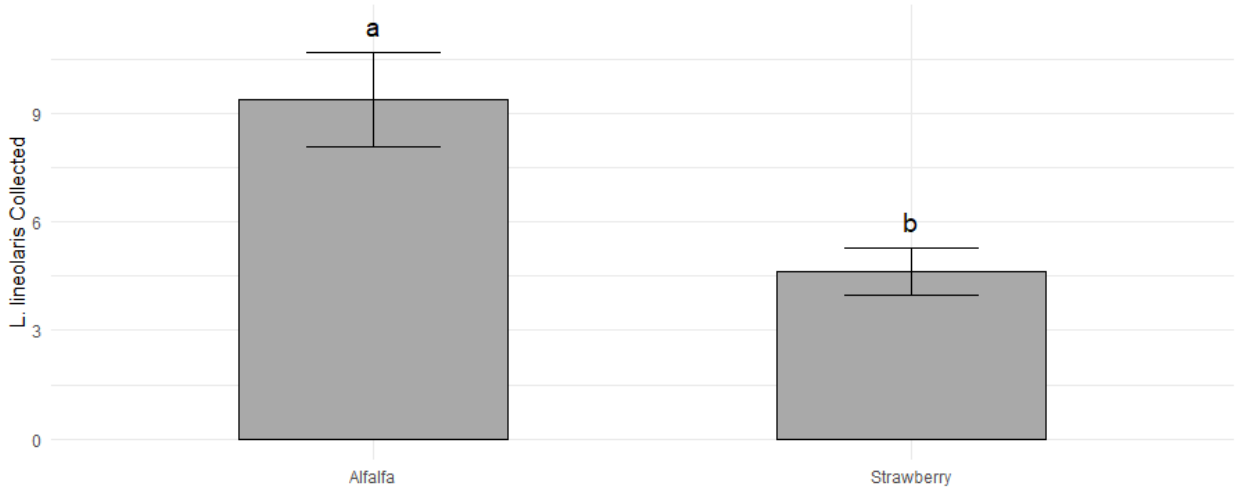


Figure 4-2: Mean ± SE number of *L. lineolaris* adults collected from flowering alfalfa or strawberry plants after 24 hrs of exposure. Different letters denote significant differences at $\alpha = 0.05$.

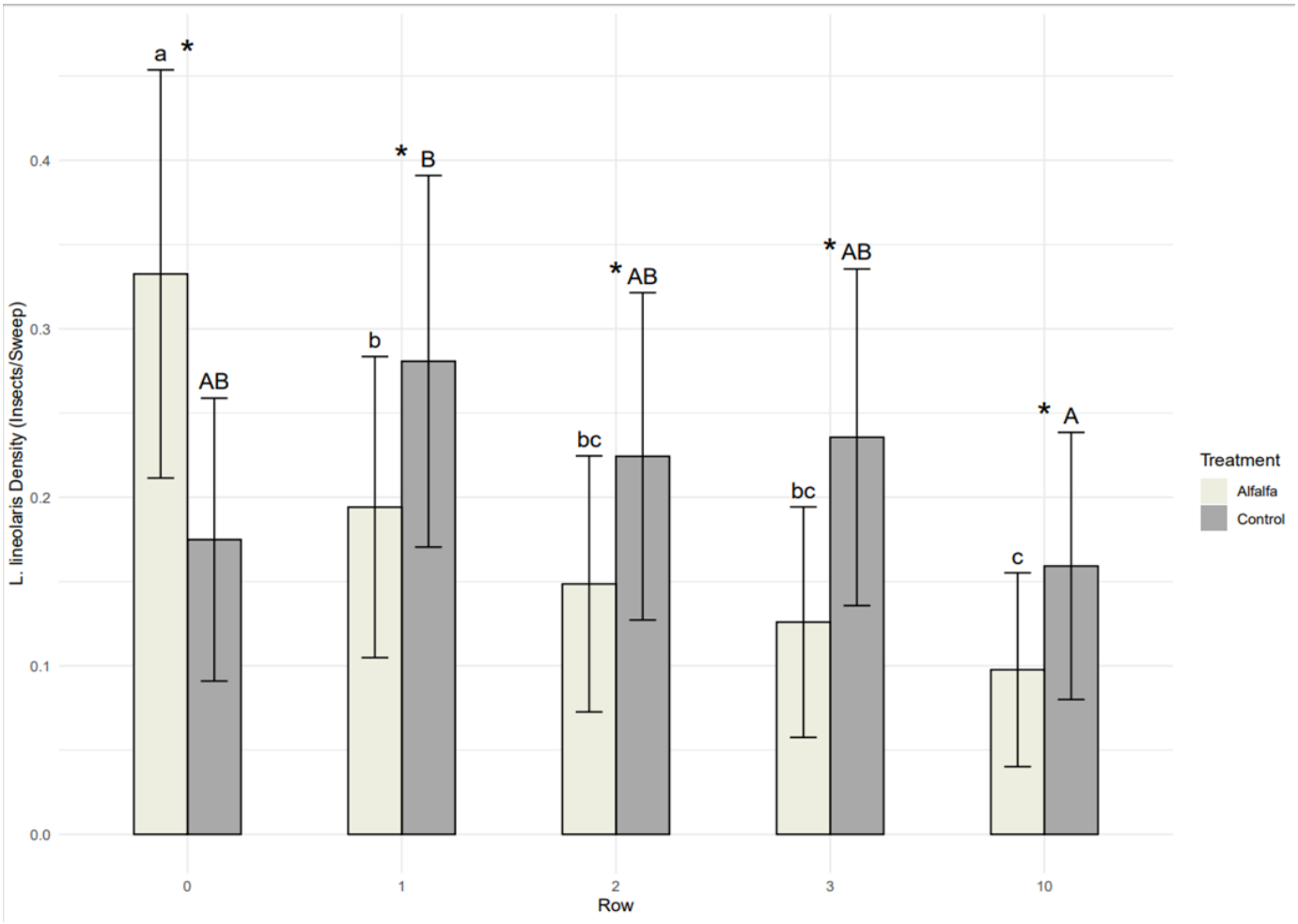


Figure 4-3: Back-transformed, bias-adjusted geometric means ± standard error of *L. lineolaris* density in trap cropped and control plots. Asterisks denote significant differences between alfalfa trap cropped and control plots at $\alpha = 0.05$. Letters denote significant differences between rows at $\alpha = 0.05$.

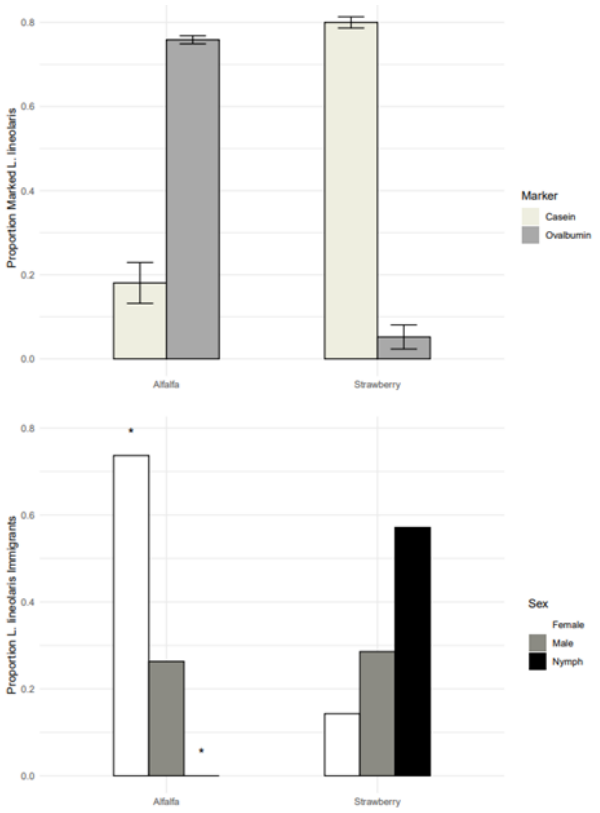


Figure 4-4: A) Mean ± standard error proportion of *L. lineolaris* testing positive for protein immunomarkers 36 hours after marker application. Ovalbumin and casein were applied to alfalfa and strawberry, respectively, and provide a measure of marker coverage. Casein-marked individuals in the alfalfa and ovalbumin-marked individuals in the strawberry represent immigrants. B) Sex and life-stage information of insects moving from strawberry to alfalfa, alfalfa to strawberry, and overall. Data indicate that females moved from strawberry to alfalfa at higher rates than expected, while fewer nymphs than expected moved into the alfalfa.

Taxon	Perimeter		Strawberry 1		Strawberry 2		Strawberry 3		Strawberry 10	
	Alfalfa	Control	Alfalfa	Control	Alfalfa	Control	Alfalfa	Control	Alfalfa	Control
Anthomyiidae	**5.61 ± 1.3 A**	**2.88 ± 0.85 b**	**2.14 ± 0.48 b**	**2.48 ± 0.88 b**	**1.96 ± 0.37 b**	**1.98 ± 0.42 B**	**1.92 ± 0.38 B**	**1.69 ± 0.3 b**	**1.36 ± 0.26 b**	**1.8 ± 0.51 b**
Minute Wasps	**5.44 ± 1.16 A**	**2.67 ± 0.55 b**	**1.53 ± 0.42 bc**	**1.53 ± 0.43 bc**	**1.17 ± 0.33 bc**	**0.82 ± 0.2 C**	**0.55 ± 0.15 C**	**1.04 ± 0.26 c**	**0.79 ± 0.2 c**	**0.92 ± 0.19 bc**
Dolichopodidae	**2.35 ± 0.69 Aa**	**1.25 ± 0.38 Ab**	**2.14 ± 0.63 Aa**	**1.24 ± 0.35 Ab**	**1.72 ± 0.58 Aa**	**1.15 ± 0.33 Ab**	**1.18 ± 0.33 Aa**	**0.91 ± 0.31 Ab**	**0.73 ± 0.22 Ba**	**0.92 ± 0.24 Bb**
Syrphidae	**2.09 ± 0.66 AB**	**1.02 ± 0.24 AB**	**1.61 ± 0.42 A**	**1.98 ± 0.63 A**	**1.01 ± 0.34 BC**	**1.06 ± 0.35 BC**	**0.89 ± 0.34 BC**	**1.08 ± 0.39 BC**	**0.17 ± 0.06 C**	**0.43 ± 0.18 C**
Araneae	1.69 ± 0.34	1.34 ± 0.25	1.29 ± 0.33	0.75 ± 0.12	0.96 ± 0.21	0.91 ± 0.18	0.53 ± 0.08	1.33 ± 0.29	0.63 ± 0.16	0.99 ± 0.22
Rhagionidae	**1.68 ± 0.8 A**	**0.07 ± 0.05 b**	**1.76 ± 0.87 a**	**0.14 ± 0.09 b**	**2.32 ± 1.02 a**	**0.84 ± 0.5 B**	**1.35 ± 0.84 A**	**0.29 ± 0.14 b**	**0.12 ± 0.09 a**	**0.3 ± 0.17 b**
Braconidae	**3.83 ± 0.69 A**	**1.29 ± 0.28 B**	**0.54 ± 0.12 bc**	**0.53 ± 0.11 bc**	**0.48 ± 0.12 c**	**0.36 ± 0.11 C**	**0.3 ± 0.1 C**	**0.26 ± 0.09 c**	**0.24 ± 0.06 c**	**0.32 ± 0.08 c**
Coccinellidae	**1.86 ± 0.45 A**	**0.35 ± 0.1 B**	**0.47 ± 0.16 b**	**0.25 ± 0.06 b**	**0.16 ± 0.07 b**	**0.07 ± 0.04 B**	**0.2 ± 0.09 B**	**0.29 ± 0.14 b**	**0.22 ± 0.1 b**	**0.1 ± 0.04 b**
Anthocoridae	**0.83 ± 0.2 A**	**0.92 ± 0.41 A**	**0.21 ± 0.1 B**	**0.33 ± 0.12 B**	**0.14 ± 0.05 B**	**0.2 ± 0.06 B**	**0.09 ± 0.07 B**	**0.27 ± 0.15 B**	**0.06 ± 0.03 B**	**0.13 ± 0.05 B**
Opiliones	0.31 ± 0.1	0.09 ± 0.05	0.38 ± 0.12	0.59 ± 0.18	0.34 ± 0.14	0.17 ± 0.08	0.58 ± 0.2	0.23 ± 0.1	0.16 ± 0.05	0.28 ± 0.16

Table 4-1: Mean ± SE of the most common beneficial arthropods collected via sweep net samples in strawberry plots with and without alfalfa perimeter plantings and test statistics for univariate predictors. Bold text indicates a significant effect of alfalfa perimeter plantings and/or row on the abundance of a taxon at $\alpha = 0.05$. Capitalized letters denote significant differences between rows only, while lower case letters indicate significant differences between treatment and control plots.

Taxon	Perimeter		Strawberry 2		Strawberry 10	
	Alfalfa	Control	Alfalfa	Control	Alfalfa	Control
Calyptratae	46.63 ± 6.3	33.5 ± 2.74	43.52 ± 7.06	33.33 ± 4.04	34.98 ± 3.57	43.36 ± 5.28
Minute Wasps	**30.38 ± 4.02 A**	**24.77 ± 2.71 A**	**19.17 ± 2.05 B**	**16.18 ± 1.61 B**	**19.44 ± 1.21 A**	**36.33 ± 12.85 A**
Dolichopodidae	8.35 ± 2.25	12.65 ± 2.24	15.37 ± 3.36	16.96 ± 2.62	13.52 ± 1.86	10.56 ± 1.51
Large Wasps	**5.9 ± 1.19 a**	**3.07 ± 0.75 b**	**4.5 ± 1.39 a**	**2.15 ± 0.37 b**	**3 ± 0.73 a**	**2.24 ± 0.65 b**
Araneae	**2.19 ± 0.26 a**	**1.65 ± 0.21 b**	**2.62 ± 0.35 a**	**2.02 ± 0.29 b**	**2.42 ± 0.36 a**	**1.88 ± 0.28 b**
Syrphidae	**1.94 ± 0.35 ab**	**1.85 ± 0.27 ab**	**2.19 ± 0.33 ab**	**1.71 ± 0.31 ab**	**1.27 ± 0.28 b**	**2.42 ± 0.27 a**
Bees	**0.85 ± 0.33 a**	**0.65 ± 0.21 b**	**2.85 ± 1.06 a**	**0.52 ± 0.14 b**	**3.77 ± 1.47 a**	**0.51 ± 0.13 b**

Table 4-2: Mean ± standard error of the most common beneficial arthropods collected on clear sticky cards in strawberry plots with and without alfalfa perimeter plantings. Bold text indicates a significant effect of alfalfa perimeter plantings and/or row on the abundance of a taxon at $\alpha = 0.05$, or a marginally significant difference $0.075 > P > 0.05$. Capitalized letters denote significant differences between rows only, while lower case letters indicate significant differences between treatment and control plots at $\alpha = 0.05$.

	Perimeter		Strawberry Row 2		Strawberry Row 10	
Taxon	Alfalfa	Control	Alfalfa	Control	Alfalfa	Control
Myriapoda	1.21 ± 0.44	0.48 ± 0.15	0.6 ± 0.23	1.46 ± 0.62	1.13 ± 0.73	1.15 ± 0.47
Minute Wasps	4.2 ± 1.23	3.4 ± 1.07	5.75 ± 1.13	6.56 ± 1.15	6.48 ± 1.19	5.56 ± 1.16
Araneae	3 ± 0.32	2.73 ± 0.43	2.65 ± 0.37	4.87 ± 1.37	2.13 ± 0.34	2.9 ± 0.7
Opiliones	2.94 ± 0.39	2.15 ± 0.34	3.08 ± 0.47	2.4 ± 0.42	2.69 ± 0.54	2.63 ± 0.51
Formicidae	0.67 ± 0.39	0.5 ± 0.14	0.65 ± 0.2	1.23 ± 0.34	0.44 ± 0.12	1.15 ± 0.22
Carabidae	3.57 ± 1.01	3.37 ± 0.7	3.88 ± 0.66	3.12 ± 0.46	3.27 ± 0.59	3.17 ± 0.52
Anthomyiidae	0.05 ± 0	0.04 ± 0.03	0.02 ± 0.02	0.02 ± 0.02	0.1 ± 0.06	0.1 ± 0.05
Staphylinidae	0 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0 ± 0	0.02 ± 0.02

Table 4-3: Mean ± standard error of the most common beneficial arthropods collected in pitfall traps in strawberry plots with and without alfalfa perimeter plantings. Bold text indicates a significant effect of alfalfa perimeter plantings and/or row on the abundance of a taxon at $\alpha = 0.05$. Capitalized letters denote significant differences between rows only, while lower case letters indicate significant differences between treatment and control plots.

Chapter 5

EFFECT OF TRAP COLOR ON CAPTURE RATE OF *LYGUS LINEOLARIS* PALISOT DE BEAUVOIS (HEMIPTERA: MIRIDAE) AND NON-TARGET ARTHROPODS

ABSTRACT – *Lygus lineolaris* (Hemiptera: Miridae) is a highly polyphagous pest of economic importance in North America. Effective management of this species depends on the ability to monitor populations as they move between crop and non-crop hosts. White traps are recommended for monitoring *L. lineolaris* populations, but studies have suggested yellow and pink traps are more effective. As there is uncertainty around the optimal visual parameters of *Lygus* traps, we conducted an experiment to examine the effect of trap color on *L. lineolaris* capture rate. The accumulation of beneficial arthropods can affect the efficacy and sustainability of a monitoring strategy, so capture rate of beneficial arthropods was compared across trap color. Complete, randomized blocks of blue, clear, red, white, and yellow sticky traps were set up along the edge of an alfalfa field for seven days. This experiment was replicated twice in late-July and late-August 2021. Red traps were most effective for capturing *L. lineolaris* but also captured Coccinellidae at a significantly higher rate than all other colors and captured parasitic wasps and Opiliones at higher rates than white traps. Despite relatively high capture rates of some natural enemies, red traps were significantly more selective than all other colors, with *L. lineolaris* representing 7.90% of identified arthropods, compared to 2.76%, 2.24%, 1.26%, and 1.47% for blue, clear, white, and yellow traps, respectively. While this study demonstrates the efficacy of red traps for monitoring *L. lineolaris* in alfalfa fields, whether these findings translate to other cropping systems remains to be addressed.

Introduction

Insects rely on information from multiple sensory channels to successfully navigate complex environments (Campbell and Borden 2009; Prokopy and Owens 1983; Pyke et al. 1977). Olfactory stimuli are often thought to mediate long-range orientation, while visual cues affect behavior over shorter ranges (Prokopy and Owens, 1983). Both sensory channels can be and are frequently exploited to monitor pest populations in agricultural settings. Effective pest monitoring strategies are central to integrated pest management, as growers cannot make informed management decisions without pest population estimates. In an effort to improve *Lygus lineolaris* [(Palisot de Beauvois); Hemiptera: Miridae] monitoring recommendations, we investigated the effect of trap color on the capture rate of *L. lineolaris* and beneficial arthropods.

Lygus lineolaris (Palisot de Beauvois; Hemiptera: Miridae) is a highly polyphagous insect species of major economic importance in eastern and central North America. This insect is known to utilize at least 300 plant species, representing 55 families (George et al. 2021, Esquivel and Mowery 2007; Young 1986). *Lygus* species overwinter as adults and utilize weedy hosts early in the season (Barman et al. 2010; Esquivel and Mowery 2007, Easterbrook 1997; Snodgrass et al. 1984; Fye 1980). As early season hosts senesce, large populations of *L. lineolaris* disperse into the environment (Snodgrass et al. 1984) and onto cultivated crops. Managing this insect requires effectively monitoring populations as they disperse into agricultural fields. Vacuum sampling (Rancourt et al. 2000; Vincent and Lachance 1993; Zalom et al. 1993), sweep net sampling (Snodgrass 1993), sticky traps (Legrand and Los 2003; Wold and Hutchison 2003; Prokopy et al. 1979), pan traps (Landis and Fox 1972), and beat sheets (Rancourt et al. 2000; Snodgrass 1993; Mailloux and Bostanian 1988) have been employed to

assess *Lygus* populations and the optimal strategy varies by crop. Passive strategies (trapping) for monitoring pest populations are less labor-intensive than active sampling methods (vacuum, sweep net, beat sheet) and therefore may be more likely to be adopted. To this end, several studies have examined the olfactory cues that mediate host finding and mate detection in *Lygus* species. Extracts of the metathoracic gland of *Lygus* species contain hexyl butyrate, hexenyl butyrate, 4-oxo-hexenal, and (E)-2-hexenal (Zhang et al. 2007; Wardle et al. 2003). Some studies have demonstrated *Lygus* attraction to these compounds (Parys and Hall 2017; Fountain et al. 2014), but such results are not consistently observed (Innocenzi et al. 2004; Chouinard-Thuly et al. 2020). Recent studies in Europe have found that floral volatiles enhance capture of *L. rugulipennis* (Baroffio et al. 2018, Koczor et al. 2012), while the inclusion of sunflower floral volatiles alongside pheromone lures reduced capture of *L. lineolaris* in Quebec (Chouinard-Thuly et al. 2020). Whm van Tol and colleagues (2022) recently found that light traps were significantly more effective than pheromone traps for monitoring *L. rugulipennis* in greenhouses, emphasizing the importance of visual cues in *Lygus* orientation. Early work looking at visual traps for monitoring *L. lineolaris* populations in apple orchards determined that non-UV reflecting white, yellow, and clear rectangles were more effective than blue, dark red, silver, orange, green, and black traps (Prokopy et al. 1979). As white traps were also useful for monitoring the European apple sawfly (*Hoplocampa testudinea*), they were recommended. Over time such traps became the standard for monitoring *L. lineolaris* populations in many crops. More recent studies demonstrated that pink traps were more efficient than white for monitoring *L. lineolaris* in peach orchards (Legrand and Los 2003), while yellow traps are superior in strawberry and alfalfa fields (Wold and Hutchison 2003). Context may underlie the differential efficacy of colored traps, as the visual background and therefore the detectability of a cue may

vary across cropping system. Studies of other *Lygus* species have determined that blue traps are more efficient than yellow for monitoring *L. rugulipennis* in Europe (Holopainen et al. 2001), while green traps captured more *L. hesperus* than red or black traps (Blackmer et al. 2008).

Visual and olfactory stimuli also underlie the foraging behavior of pollinators and natural enemies, and pest monitoring traps frequently capture beneficial insects (Spears et al. 2016; Spears and Ramirez 2015). Efficient removal of beneficial insects may negatively affect local populations and, perhaps, the ecosystem services these organisms provide. The eggs and nymphs of *Lygus* spp. are parasitized by several wasps, including *Anaphes* spp. (Hymenoptera: Mymaridae), *Leiophron* spp. (Hymenoptera: Braconidae), *Peristenus* spp. (Hymenoptera: Braconidae), and *Telenomus* spp. (Hymenoptera: Platygastridae), while *Phasia* spp. (Diptera: Tachinidae) are known to parasitize adults (Day 2005; Day 1996; Graham et al. 1986; Clancy and Pierce 1966). Generalist predators including minute pirate bugs (Hemiptera: Anthocoridae), big-eyed bugs (Hemiptera: Geocoridae), damsel bugs (Hemiptera: Nabidae), green lacewings (Neuroptera: Chrysopidae), and spiders (Araneae) are known to feed on *Lygus* spp. Approximately 18% of insects and spiders examined in a recent gut content analysis study tested positive for *L. hesperus* remains (Hagler et al. 2018). While the impact of beneficial insect collection in pest monitoring traps on ecosystem services remains unknown (Grocock and Evenden 2020; Meagher and Mitchell 1999), such bycatch may also impede pest monitoring efforts by increasing the time required to process traps and reducing trap longevity (Holthouse et al. 2021; Spears et al. 2021; Spears et al. 2016; Spears and Ramirez 2015; Cha et al. 2015; Weber and Ferro 1991). Pest monitoring traps should therefore optimize selectivity, maximizing the capture of target pests, while minimizing beneficial bycatch. Substantial variation exists in the attraction of beneficial arthropods to various colors. Blue traps were recently found to attract

a greater diversity of wild bees than alternative colors, while red traps attracted the fewest bee species (Acharya et al. 2022; Stephen and Rao 2005). Pollinating hoverflies (Diptera: Syrphidae) and predatory *Orius* spp. are also attracted to blue traps (Furihata et al. 2019; Chen et al. 2004; Ohno and Takemoto 1997), while lady beetles (Coleoptera: Coccinellidae) appear to be most attracted to yellow traps (Kemp and Cottrell 2015; Rodriguez-Saona et al. 2012). Both blue and yellow traps are effective for monitoring minute parasitic wasps, although yellow traps tend to capture wasps in greater numbers (Holthouse et al. 2021, Rodriguez-Saona et al. 2012). While we may expect blue and yellow traps capture *Lygus* species more efficiently than other colors (Wold and Hutchison 2003; Holopainen et al. 2001), they may also have greater non-target effects.

Visual cues are clearly important for effectively monitoring *Lygus* populations, but uncertainty about optimal trap design remains. Field experiments were therefore conducted in July and August of 2021 to 1) compare the capture rate of *L. lineolaris* across colored sticky traps; 2) compare the abundance of beneficial arthropods, i.e., Anthocoridae, Araneae, Coccinellidae, Dolichopodidae, Opiliones, Syrphidae, Tachinidae, and parasitic wasps across colored sticky traps; and 3) compare the selectivity for *L. lineolaris* across trap color. In doing so, we expect to inform future efforts to improve *L. lineolaris* monitoring.

Materials and Methods

Trap Description

This study evaluated *L. lineolaris* attraction to commercially-available traps of different colors (Table 5-1). Red, white, and yellow traps were purchased from Great Lakes IPM (Vestaburg, MI), clear traps were purchased from Alpha Scents Inc. (Canby, OR), and blue traps were sourced from Arbico Organics (Oro Valley, AZ). The dimensions of available traps varied slightly, so sections of blue, clear, red, and yellow traps were removed such that 400 cm^2 of sticky surface was present on each trap. Colorimetric features were examined using a computer program (Byers 2006). Digital photographs of each trap were taken with a 50-megapixel cell phone camera between 1130 and 1230 h. A 500x500 pixel square in the center of each photograph was analyzed to determine the RGB attributes of each trap (Table 5-1). The RGB values were then used to determine hue, saturation, and brightness (Byers 2006).

Experimental Design

Trapping was conducted at the West Madison Agricultural Research Station (Madison, WI). Each block consisted of one linear array of blue, clear, red, white, and yellow sticky traps arranged along the perimeter of a 3.44-hectare alfalfa hay field. Garden stakes were driven into the ground at a slight angle and traps were hung over the alfalfa such that the bottom of each trap was just above the alfalfa canopy. Within each block, traps were spaced 2 m apart and blocks were separated by 10 m. Each block of traps was replicated ten times in a randomized complete block design during each sampling event. Samples were collected over two one-week

periods immediately before the third and fourth alfalfa harvest, in late July and late August of 2021.

Traps were removed after each seven-day sampling period and wrapped with cellophane in the field. *Lygus* species and beneficial arthropods were visually identified and counted under a stereomicroscope (Olympus XZS10). Non-insect arthropods were identified to order, while beneficial insects were identified to family when possible, the principal exception being parasitic wasps. Bycatch was initially compared at the level of order, as this level of taxonomic resolution was consistent for identifiable insects, parasitic wasps, and non-insect arthropods. As pollinator bycatch was limited, subsequent univariate analyses focused on natural enemies. Anthocoridae, Coccinellidae, Dolichopodidae, and Syrphidae were the predominant predatory insect taxa observed in this study, while parasitic wasps and Tachinidae were also commonly collected. Many families of natural enemies were not collected consistently enough for meaningful univariate statistical analyses to be performed, namely the Asilidae, Empididae, Rhagionidae, Nabidae, Reduviidae, Carabidae, and Chrysopidae.

Statistical Analysis

All statistical analyses were conducted in R version 4.1.3 (R Core Team, year). Linear mixed-effects models were fitted using the lme4 package (Bates et al. 2015) and the package emmeans (Lenth 2022) was employed to compare predefined groups. The package vegan (Oksanen 2022) was used to analyze beneficial bycatch data.

Lygus lineolaris capture rate

A linear mixed model was fit to examine the relationship between *L. lineolaris* capture rate (insects/trap/week) and trap color. Square root transformation was applied to *L. lineolaris* capture rate to improve homogeneity of variance and normality of the response variable. Trap color was the sole predictor in our model and the random effect term consisted of block nested within sampling date. After color was determined to significantly influence *L. lineolaris* capture rate, the estimated marginal means of each color were compared. Sidak's method was employed to control family-wise error rate during multiple comparisons testing.

Bycatch Composition

Beneficial arthropods were identified to order and counted. Counts for each sample were converted to relative abundances using the 'decostand' function in vegan. A Bray-Curtis distance matrix was then calculated from the relative abundance data using the 'vegdist' function. Distance-based redundancy analysis (db-RDA) was then applied to examine the relationship between the distance matrix and the categorical predictor variables: trap color, sampling date, and block. As permutational variants of linear models cannot currently include random effects, it was necessary to include sampling date and block as fixed effects. Following a significant db-RDA, the correlation of each taxon with the significant canonical axes was examined to determine which taxa underlie observed differences in bycatch community composition.

Effect on natural enemy taxa

Univariate linear mixed effects models were applied to examine the relationship between the capture rates of parasitic wasps, anthocorids, coccinellids, dolichopodids, syrphids, and tachinids and trap color. As with the *L. lineolaris* models, trap color was the only predictor

variable and the random effect term consisted of block nested within sampling date and follow-up analyses were as described for *L. lineolaris*.

Results

Lygus lineolaris capture rate and trap selectivity

Trap color significantly influenced *L. lineolaris* capture rates (Figure 5-1; $F_{4,74.47} = 20.33$, $P < 0.001$). *Lygus* capture rates were highest for red sticky cards, averaging 167%, 295%, 733%, and 313% more *L. lineolaris* per week than blue, clear, white, and yellow traps, respectively ($\mu_{Red} = 7.48$; Blue: $\mu = 2.81$, $\beta = 1.40 \pm 0.21$, DF = 74, t ratio = 6.53, $P_{adj} < 0.001$; Clear: $\mu = 1.89$, $\beta = 1.74 \pm 0.24$, DF = 74, t ratio = 7.24, $P_{adj} < 0.001$; White: $\mu = 0.90$, $\beta = 1.78 \pm 0.24$, DF = 74, t ratio = 7.47, $P_{adj} < 0.001$; Yellow: $\mu = 1.81$, $\beta = 1.24 \pm 0.21$, DF = 74, t ratio = 5.82, $P_{adj} < 0.001$). The blue and yellow traps, respectively, captured more *L. lineolaris* than white traps, averaging 213% and 102% more *Lygus* per week, respectively (Blue: $\beta = 0.65 \pm 0.182$, DF = 74, t ratio = 3.56, $P_{adj} = 0.007$; Yellow: $\beta = 0.80 \pm 0.24$, DF = 74, t ratio = 3.40, $P_{adj} = 0.011$), while clear and white traps captured *L. lineolaris* at similar rates ($\beta = 0.30 \pm 0.21$, DF = 74, t ratio = 1.430, $P_{adj} = 0.819$). There was no statistical difference in *Lygus* capture rate between blue, yellow, and clear sticky cards ($\mu_{Blue} = 2.81$, $\mu_{Clear} = 1.89$, $\mu_{Yellow} = 1.81$; Blue-Clear: $\beta = 0.08 \pm 0.24$, df= 74, t ratio = 0.324, $P_{adj} = 1.00$; Blue-Yellow: $\beta = 0.11 \pm 0.15$, df = 74, t ratio = 0.77, $P_{adj} = 0.997$; Clear-Yellow: $\beta = 0.23 \pm 0.19$, df = 74, t ratio = -1.243, $P_{adj} = 0.914$).

The proportion of identified arthropods (*L. lineolaris* + beneficials) that were *L. lineolaris* varied significantly with trap color (Figure 5-1: B; $F_{4,75.6} = 17.39$, $P < 0.001$). *Lygus lineolaris* represented a significantly higher proportion of identified arthropods on red traps than

on all other colors. On average, *L. lineolaris* comprised 7.90% of identified arthropods on red traps compared to 2.76%, 2.24%, 1.26%, and 1.47% for blue, clear, white, and yellow traps, respectively (Blue: $\beta = 0.16 \pm 0.023$, DF = 75, t-ratio: 6.74, $P_{adj} < 0.001$; Clear: $\beta = 0.17 \pm 0.026$, DF = 75, t-ratio: 6.80, $P_{adj} < 0.001$; White: $\beta = 0.16 \pm 0.026$, DF = 75, t-ratio: 6.22, $P_{adj} < 0.001$; Yellow: $\beta = 0.15 \pm 0.023$, DF = 75, t-ratio: 6.38, $P_{adj} < 0.001$). No significant differences were observed in the capture proportion of *L. lineolaris* across blue, clear, white, or yellow traps.

Bycatch Composition

Distance-based redundancy analysis revealed that trap color and sampling date significantly affected the order-level assembly of arthropods sampled during the study (Figure 5-2: A, B, C; Color: $F_{4,80} = 11.02$, P = 0.001; Date: $F_{1,80} = 39.06$, P = 0.001), while the effect of block was found to be marginal ($F_{9,80} = 1.49$, P = 0.058). Moreover, the effect of color depended on sampling date (Color:Date: $F_{4,80} = 3.78$, P = 0.001). The first canonical axis (CAP1) was highly correlated with the capture rates of Diptera (r(97) = 0.96) and Hymenoptera (r(97) = -0.95), and the shift associated with sampling date occurs along CAP1. The 95% confidence ellipses shift right along the CAP1 axis between July and August (Figure 5-2: B, C), driven by a 407% increase in the capture rate of Diptera and a 35.2% decrease in the capture rate of Hymenoptera between July and August. As flies began to dominate the bycatch on clear, blue, and yellow traps, the community sampled by these traps became more similar, driving the interaction of sampling date and trap color. The community of beneficial arthropods on red and white traps was similar across sampling period (Figure 5-2: A, B, and C). The second canonical axis was strongly correlated with non-target Hemiptera (r(97) = -0.98).

Trap color significantly influenced capture rate of parasitic wasps (Figure 5-2: D; $F_{4,75.53} = 16.34$, $P < 0.001$) and Opiliones (Figure 5-2: E; $F_{4,75.3} = 15.06$, $P < 0.001$), but not Araneae (Figure 5-2: F; $F_{4,93} = 1.56$, $P = 0.191$). At the family level, we observed that color influenced the capture rate of Anthocoridae (Figure 5-2: G; $F_{4,75.4} = 18.60$, $P < 0.001$), Coccinellidae (Figure 5-2: H; $F_{4,84.0} = 9.56$, $P < 0.001$), Dolichopodidae (Figure 5-2: I; $F_{4,75.4} = 13.63$, $P < 0.001$), Syrphidae (Figure 5-2: J; $F_{4,75.5} = 24.73$, $P < 0.001$), and Tachinidae (Figure 5-2: K; $F_{4,75.1} = 5.35$, $P < 0.001$).

Parasitic wasps (Figure 5-2: D) were captured on yellow traps at significantly higher rates than blue, clear, and white traps ($\mu_{Yellow} = 84.6$; Blue: $\mu = 46.0$, $\beta = 0.344 \pm 0.047$, DF = 75, t-ratio = 7.25, $P_{adj} < 0.001$; Clear: $\mu = 36.3$, $\beta = 0.437 \pm 0.062$, DF = 75, t-ratio = 4.31, $P_{adj} < 0.001$; White: $\mu = 36.1$, $\beta = 0.438$ 0.084, DF = 75, t-ratio = 5.22, $P_{adj} < 0.001$), but not red (Red: $\mu = 59.0$, $\beta = 0.14 \pm 0.089$, DF = 75, t-ratio = 1.57, $P_{adj} = 0.722$). while blue and red traps captured significantly more parasitic wasps than white traps ($\mu_{Blue} = 46.0$; White: $\mu = 36.1$, $\beta = 0.372 \pm 0.114$, DF = 75, t-ratio = 3.28, $P_{adj} = 0.016$; $\mu_{Red} = 59.0$; White: $\mu = 36.1$, $\beta = 0.830 \pm 0.193$, DF = 75, t-ratio = 4.31, $P_{adj} < 0.001$).

Clear and Yellow traps captured Opiliones at significantly higher rates than blue and white traps (Clear-Blue: $\beta = 0.97 \pm 0.26$, df = 75, t-ratio = 3.70, $P_{adj} = 0.004$; Clear-White: $\beta = 1.75 \pm 0.23$, df = 75, t-ratio = 7.51, $P_{adj} < 0.001$; Yellow-Blue: $\beta = 0.85 \pm 0.17$, df = 75, t-ratio = 5.18, $P_{adj} < 0.001$; Yellow-White: $\beta = 1.68 \pm 0.26$, df = 75, t-ratio = 6.45, $P_{adj} < 0.001$). Red traps were observed to capture Opiliones at marginally lower rates than clear and yellow traps (Clear-Red: $\beta = 0.75 \pm 0.27$, df = 75, t-ratio = 2.822, $P_{adj} = 0.060$; Yellow-Red: $\beta = 0.68 \pm 0.24$, df = 75, t-ratio = 2.851, $P_{adj} = 0.055$). Capture rates on blue and red traps were significantly

higher than white (Blue-White: $\beta = 0.81 \pm 0.20$, df = 75, t-ratio = 3.99, $P_{adj} = 0.002$; Red-White: $\beta = 0.98 \pm 0.27$, df = 75, t-ratio = 3.72, $P_{adj} = 0.004$). No significant difference was observed between either clear and yellow or blue and red traps.

Blue traps captured anthocorids at significantly higher rates than clear, red, and white traps (Figure 5-2: G; $\mu_{Blue} = 21.49$; Clear: $\mu = 7.45$, $\beta = 1.49 \pm 0.31$, DF = 75, t-ratio = 4.88, $P_{adj} < 0.001$; Red: $\mu = 8.81$, $\beta = 1.07 \pm 0.28$, DF = 75, t-ratio = 3.87, $P_{adj} < 0.001$; White: $\mu = 5.16$, $\beta = 1.94 \pm 0.24$, DF = 75, t-ratio = 8.24, $P_{adj} < 0.001$) but not yellow ($\mu_{Blue} = 21.49$; Yellow: $\mu = 14.54$, $\beta = 0.40 \pm 0.19$, DF = 75, t-ratio = 2.09, $P_{adj} = 0.34$). Yellow traps collected anthocorids at significantly higher rates than clear, red, and white traps ($\mu_{Yellow} = 14.54$; Clear: $\mu = 7.45$, $\beta = 1.51 \pm 0.24$, DF = 75, t-ratio = 6.377, $P_{adj} < 0.001$; Red: $\mu = 8.81$, $\beta = 1.09 \pm 0.28$, DF = 75, t-ratio = 3.93, $P_{adj} = 0.002$; White: $\mu = 5.16$, $\beta = 1.96 \pm 0.31$, DF = 75, t-ratio = 6.44, $P_{adj} < 0.001$) and no difference was observed between clear, red and white traps.

Red traps collected more Coccinellidae than all other colors (Figure 5-2: H; $\mu_{Red} = 5.18$; Blue: $\mu = 1.286$, $\beta = 1.37 \pm 0.24$, DF = 84, t-ratio = 5.75, $P_{adj} < 0.001$; Clear: $\mu = 1.69$, $\beta = 1.20 \pm 0.27$, DF = 84, t-ratio = 4.53, $P_{adj} < 0.001$; White: $\mu = 1.06$, $\beta = 1.17 \pm 0.27$, DF = 84, t-ratio = 4.40, $P_{adj} < 0.001$; Yellow: $\mu = 0.79$, $\beta = 1.31 \pm 0.24$, DF = 84, t-ratio = 5.50, $P_{adj} < 0.001$).

Yellow traps captured Dolichopodidae at significantly higher rates than blue, clear, and red traps (Figure 5-2: I; $\mu_{Yellow} = 2.84$; Blue: $\mu = 1.16$, $\beta = 1.29 \pm 0.38$, DF = 75, t-ratio = 3.36, $P_{adj} = 0.012$; Clear: $\mu = 1.21$, $\beta = 1.27 \pm 0.40$, DF = 75, t-ratio = 3.17, $P_{adj} = 0.022$; Red: $\mu = 0.52$, $\beta = 1.45 \pm 0.44$, DF = 75, t-ratio = 3.28, $P_{adj} = 0.016$), but not white. Blue, clear, red, and white traps captured dolichopodids at similar rates.

Blue and yellow traps captured Syrphidae at the highest rates (Figure 5-2: J). Blue traps captured significantly more syrphids than red and white traps (μ_{Blue} = 2.68; Red: μ = 0.003, β = 1.37 ± 0.19, DF = 75, t-ratio = 7.30, $P_{adj} < 0.001$; White: μ = 0.02, β = 1.28 ± 0.16, DF = 75, t-ratio = 8.04, $P_{adj} < 0.001$). The capture rate of syrphids on blue traps was marginally higher than on clear traps (μ_{Blue} = 2.68; Clear: μ = 0.75, β = 0.57 ± 0.21, DF = 75, t-ratio = 2.76, P_{adj} = 0.070). Yellow traps averaged significantly higher capture rates than red and white traps (μ_{Yellow} = 2.21; Red: μ = 0.003, β = 1.44 ± 0.24, DF = 48, t-ratio = 6.07, $P_{adj} < 0.001$; White: μ = 0.02, β = 1.34 ± 0.23, DF = 48, t-ratio = 5.79, $P_{adj} < 0.001$), and a marginally significant increase compared to clear traps (μ_{Yellow} = 2.21; Clear: μ = 0.75, β = 0.62 ± 0.23, DF = 48, t-ratio = 2.69, P_{adj} = 0.070).

Tachinidae were captured at the highest rates on blue and yellow traps (Figure 5-2: K). Yellow traps captured tachinids at significantly higher rates than clear, red, and white traps (Yellow-Clear: β = 1.42 ± 0.42, df = 75, t-ratio = 3.41, P_{adj} = 0.011; Yellow-Red: β = 1.79 ± 0.49, df = 75, t-ratio = 3.65, P_{adj} = 0.005; Yellow-White: β = 1.81 ± 0.54, df = 75, t-ratio = 3.37, P_{adj} = 0.012), while blue traps captured tachinids at higher rates than red and white traps (Blue-Red: β = 1.62 ± 0.49, df = 75, t-ratio = 3.31, P_{adj} = 0.014; Blue-White: β = 1.64 ± 0.42, df = 75, t-ratio = 3.94, P_{adj} = 0.002). No differences in tachinid capture rate were observed among clear, red, and white traps, nor was there a difference between blue and clear traps.

Discussion

This study examined the attraction of *L. lineolaris* to blue, clear, red, white, and yellow traps. Our results suggest that red traps collect *L. lineolaris* at higher rates than the other colors examined in this study. Moreover, the relatively high *L. lineolaris* capture rate combined with

modest non-target impacts observed for red traps led these traps to be more selective than alternative colors. Future studies should examine the relationship between *L. lineolaris* capture rate and damage and translate these findings into more precise and actionable recommendations.

As Wold and Hutchison (2003), we observed that white sticky traps are less efficient than readily available alternatives for monitoring *L. lineolaris* populations in alfalfa fields, findings that contrast with early findings from apple orchards (Prokopy et al. 1978). We observed that red traps captured *L. lineolaris* at the highest rate. Attraction to red traps has been documented in the Cicadellidae, Psyllidae, and Aphidae (Sétamou et al. 2014; Rodriguez-Saona et al. 2012; Straw et al. 2011), but previous studies found red traps to be relatively unattractive to *L. lineolaris* (Prokopy et al. 1978) and *L. hesperus* (Blackmer et al. 2008; Landis and Fox 1972). Although few studies have evaluated spectral sensitivity among Hemiptera (van der Kooi et al. 2021; Döring and Chittka 2007), few insects possess photoreceptors that are maximally sensitive to red wavelengths (620-700 nm; van der Kooi 2021; Briscoe and Chittka 2001). However, the green-sensitive photoreceptors present in most insects exhibit modest sensitivity up to approximately 650 nm (Chittka and Waser 1997), wavelengths well within the red spectrum. Differences in photoreceptor sensitivity to light in the green and red ranges likely lead red wavelengths to be perceived as low-intensity green (Chittka and Waser 1997). It is reasonable to expect that such areas of low-intensity would stand out against the high-intensity background of an alfalfa field. Such color contrasts have been shown to be important in the behavior of *Drosophila suzukii* foraging for berries among foliage (Little et al. 2018) and employing contrasts in trap design improves capture of this pest (Little et al. 2019; Basoalto et al. 2013). This could explain the discrepancy observed between our results and those of Prokopy et al. (1978). Indeed, the shade

of red tested in their study was meant to emulate the spectral reflectance of bark and their traps may have contrasted poorly with a background of foliage and branches compared to the traps used herein.

Like herbivores, beneficial insects rely on visual cues to navigate in complex environments. Ideally, pest monitoring strategies are designed to efficiently target pests while minimizing beneficial by-catch. Reducing the abundance of non-target arthropods on traps is expected to facilitate processing traps quickly and increase trap longevity, thereby reducing the cost of implementing a monitoring strategy (Holthouse et al. 2021; Cha et al. 2015; Weber and Ferro 1991). Trap color is well-known to influence capture rate of non-target arthropods (Spears et al. 2016; Kemp and Cottrell 2015; Jiuxuan et al. 2013; Mori and Evenden 2013; Rodriguez-Saona et al. 2012) and the attractiveness of a color will vary across taxa (Vrdoljak and Samways 2012; Campbell and Hanula 2007; Chittka and Thomson 2001; Disney et al. 1982).

In this study, we observed that red and white sticky traps sampled a similar community of non-target arthropods, but that red traps collected Coccinellidae, Opiliones, and parasitic wasps at higher rates than white traps. Contrary to our observations, multiple studies have demonstrated yellow to be highly attractive to coccinellids (Kemp and Cottrell 2015; Rodriguez-Saona et al. 2012). However, Jiuxuan and colleagues (2013) reported that *Coccinella septempunctata* is most responsive to monochromatic light at UV and red wavelengths. Additionally, *C. septempunctata* are known to consume more red color morph aphids when foraging against a green background, while more green aphids are consumed when foraging in a red arena (Harmon et al. 1998), suggesting that contrasting visual cues may facilitate foraging. *Harmonia axyridis*, on the other hand, has been observed to consume more red color morph aphids than green aphids regardless

of foraging area (Harmon et al. 1998), while *Propylea dissecta* has been shown to preferentially oviposit on red surfaces (Mishra 2003) which may suggest innate preferences in these species. Coccinellidae clearly utilize red visual cues, but there remains much to be understood about the role of long-wavelength light in lady beetle behavior.

High beneficial insect bycatch on blue and yellow sticky traps is consistent with previous studies. Yellow sticky cards captured parasitic wasps at significantly higher rates than blue, clear, and white traps, while red traps captured parasitic wasps at similar rates to yellow. This pattern of parasitic wasp captures is very similar to Rodriguez-Saona and colleagues' (2012) observations from cranberry bogs, where red and green traps captured parasitic wasps at intermediate rates compared to yellow and blue. Anthocoridae capture rates were similar between blue and yellow traps, with relatively few anthocorids captured on clear, red, or white traps. Trapping in cranberry bogs found anthocorids were most abundant on yellow and white traps, and present in low numbers on blue sticky traps (Rodriguez-Saona et al. 2012). White traps may be the most effective color for monitoring *Orius niger*, while blue traps were the least effective for capturing this species (Atakan and Bayram 2011). However, Furihata and associates (2019) observed that blue and white traps capture *Orius* spp. at higher rates than yellow. The variable relationships between anthocorid capture rate and trap color across study systems suggests that color preference in this family is somewhat species-specific. We observed similar syrphid and tachinid capture rates between blue and yellow traps. While yellow traps have been used to monitor syrphids in the field (Hesler 2016), blue traps typically capture higher numbers (Rodriguez-Saona et al. 2012, Chen et al. 2004). Dolichopodids were captured at the highest rate on yellow traps. Few studies have examined the relationship between dolichopodid capture rate and trap color, but Hoback and colleagues (1999) observed that yellow traps captured

dolichopodids at significantly higher rates than blue traps, and yellow pan traps have been successful for sampling dolichopodids in natural environments (Grichanov and Khruleva, 2018). While the degree to which beneficial arthropod bycatch affects ecosystem services remains unknown (Grocock and Evenden 2020; Meagher and Mitchell 1999), reductions in parasitic wasp and anthocorid populations may feedback on *Lygus* biocontrol, as these taxa were the primary *Lygus* natural enemies observed in this study (Hagler et al. 2018; Clancy and Pierce 1966). However, as relatively small insects, the presence of these natural enemies does not impact trap longevity or processing to the same extent as larger bycatch.

White sticky cards are frequently recommended to monitor *L. lineolaris* populations. Our findings suggest that, although red sticky traps collect some non-target taxa (i.e., Coccinellidae, Opiliones, parasitic wasps) at higher rates, they captured 733% more *Lygus* in alfalfa than white sticky traps. While blue and yellow traps also increased *Lygus* capture compared to white, these traps were both less effective and less selective than red traps, indicating that red traps should generally be favored for monitoring *L. lineolaris* near alfalfa fields. Alfalfa fields have a dense green canopy that presents a uniform background against which red traps contrast, and it remains to be seen how well the red traps will perform in other systems. *Lygus* visual ecology must be more thoroughly studied to understand the cues drive *Lygus* visual attraction and how these cues vary across cropping systems. Such knowledge would allow *Lygus* monitoring recommendations based on specific crop attributes to be made.

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Table 5-1: Manufacturer and color characteristics of color traps tested. Color characteristics were based on digital photographs taken with a cell phone camera and RGB and HSB values were extracted using software developed by Byers (2006).

Trap Color	Manufacturer	Red	Green	Blue	Hue	Saturation	Brightness
Blue	Agrisense	52	162	243	0.5701	0.785	0.949
Clear	Alpha Scents	NA	NA	NA	NA	NA	NA
Red	Trece	213	70	59	0.0119	0.723	0.835
White	Great Lakes IPM	231	228	222	0.1041	0.034	0.901
Yellow	Agrisense	202	180	0	0.1485	1.0	0.792

Figure 5-1: A) Back-transformed, bias-adjusted estimated marginal mean ± SE capture rate of *Lygus lineolaris* on blue, clear, red, white, and yellow sticky traps. B) Proportion of identified arthropods that were *L. lineolaris.* Identified arthropods consist of beneficial arthropods (Anthocoridae, Andrenidae, Apidae, Araneae, Asilidae, Carabidae, Chrysopidae, Coccinellidae, Dolichopodidae, Empididae, Megachilidae, Nabidae, Odanata, Opiliones, parasitic wasps, Reduviidae, Rhagionidae, Syrphidae, and Tachinidae) and *L. lineolaris*, the relative capture proportion therefore provides a measure of trap selectivity with respect to beneficial arthropods. Different letters denote significant differences between groups at $P < 0.05$. Trapping was conducted in July and August of 2021 in alfalfa fields at the West Madison Agricultural Research Station in Madison, WI.

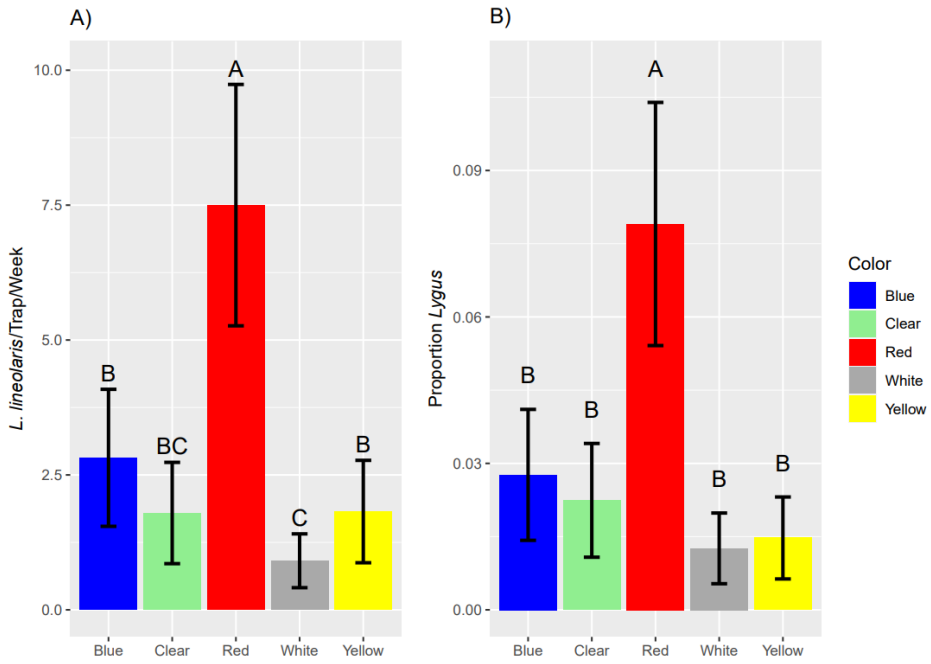
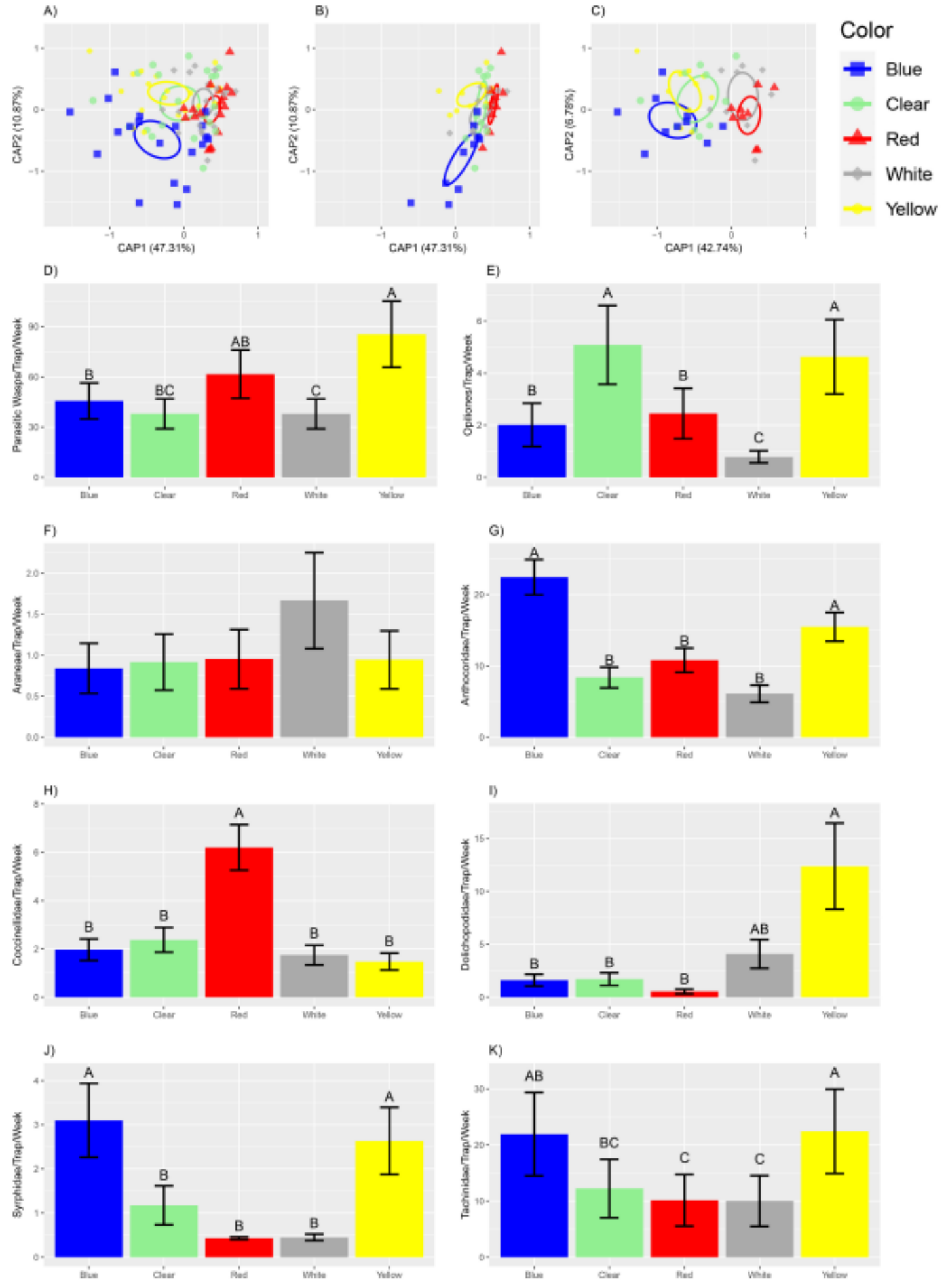


Figure 5-2: A) Ordination derived from distance-based redundancy analysis of order-level arthropod community reveals clear separation based on trap color. B-C) Ordination separated by sampling period to illustrate the interaction between color and sampling. D-K) Back-transformed, bias-adjusted estimated marginal mean ± SE for predominate natural enemy taxa. Different letters denote significant differences between colors at $P < 0.05$. Trapping was conducted in July and August of 2021 at West Madison Agricultural Research Station in Madison, WI.


A)
B)
C)
Color
Blue
Clear
Red
White
Yellow
CAP2 (10.87%)
CAP1 (47.31%)
CAP2 (6.78%)
CAP1 (42.74%)
D)
Parasitic Wasps/Trap/Week
E)
Opiliones/Trap/Week
F)
Araneae/Trap/Week
G)
Anthocoridae/Trap/Week
H)
Coccinellidae/Trap/Week
I)
Dolichopodidae/Trap/Week
J)
Syrphidae/Trap/Week
K)
Tachinidae/Trap/Week

Chapter 6

ENANTIOSPECIFIC ATTRACTION OF *Lygus lineolaris* PALISOT DE BEAUVOIS (HEMIPTERA: MIRIDAE) TO A UBIQUITOUS FLORAL VOLATILE IN THE FIELD

Abstract – *Lygus lineolaris* Palisot de Beauvois is a polyphagous pest throughout North America. Monitoring this pest as it moves between crop and non-crop hosts remains challenging and a lack of effective monitoring tools complicates management of this insect. While the pheromone blend of this species is known, studies of pheromone lures yield inconsistent results in the field. Herbivorous insects utilize olfactory cues to orient on suitable host plants, and such cues may be used to facilitate monitoring. In this study, we examined the electrophysiological and behavioral responses of *L. lineolaris* to volatile emissions of two crop hosts: alfalfa and strawberry. Gas chromatography with electroantennographic detection was applied to identify antennally-active compounds in headspace extracts of flowering alfalfa and strawberry plants, before responses to individual compounds were examined in the field. Five compounds were found to consistently elicit antennal depolarizations in adult *L. lineolaris* and, of these, only (±)-linalool was observed to increase *L. lineolaris* capture rate in the field. A subsequent experiment was conducted to examine the influence of visual cues and stereochemistry on capture rate. The presence of (±)-linalool lures significantly increased *L. lineolaris* capture rates compared to traps baited with (-)-linalool and controls, indicating that (+)-linalool is critical for *L. lineolaris* attraction to (±)-linalool. However, lures did not increase capture rates on white traps, emphasizing the importance of visual cues in *L. lineolaris* monitoring. This study demonstrates that *L. lineolaris* is attracted to (±)-linalool in the field, and that attraction depends on both the presence of (+)-linalool and appropriate visual cues. Future studies should investigate the

response of *L. lineolaris* to stereochemically-pure (+)-linalool and the potential of this compound to be paired with pheromones or other host volatiles to improve *L. lineolaris* monitoring.

Introduction

Herbivorous insects must locate suitable hosts to survive and reproduce and olfactory and visual cues, or combinations thereof, are critical to host location (Carrasco et al. 2015; Schoonhoven et al. 2005). The sensory cues that mediate host selection largely depend on diet breadth. The overwhelming majority of insect herbivores are relatively specialized, requiring hosts of a particular genus (monophagy) or family (oligophagy) to complete their life cycle, while a small proportion are polyphagous and thus able to develop on hosts from many families (Janz et al. 2001; Jaenike 1990; Futuyma and Moreno 1988; Scott 1986). While the sensory ecology governing host selection in specialist insect herbivores is well-established (Bruce et al., 2005; Bruce & Pickett, 2011; Carrasco et al., 2015), wide diet breadth increases the diversity of potentially important signals in the environment, which may complicate efficient decision-making (Bernays, 2001). Polyphagous herbivores may overcome challenges associated with an overabundance of signals through a reduction in perceptual range or information specificity. This may be achieved through reliance on compounds shared among many hosts (Bernays, 2001; Carrasco et al., 2015; Silva & Clarke, 2020), reduced specificity of odorant receptors (Bohbot & Dickens, 2012; Carrasco et al., 2015), focusing on habitat- or context-dependent cues (Carrasco et al., 2015; Silva & Clarke, 2020), or a combination of strategies.

Lygus lineolaris [(Palisot de Beauvois); Hemiptera: Miridae] is a highly polyphagous insect, known to feed on at least 300 plant species from 55 familes. Native to North America, *L. lineolaris* consistently causes economic damage to numerous fruit, vegetable, and oilseed crops

(Esquivel & Mowery, 2007; George et al., 2021; Young, 1986). *Lygus* species overwinter as adults and utilize weedy hosts early in the season (Barman et al., 2010; Easterbrook, 1997; Esquivel & Mowery, 2007; Fye, 1980; Snodgrass et al., 1984), and then disperse into cultivated crops as early season hosts senesce. Monitoring populations as they disperse into agricultural fields is critical to effective management of this detrimental pest.

Host plant volatile emissions represent an alternative source of potentially attractive semiochemicals and may increase capture of both male and female insects (Ibeas et al., 2007). Mirids preferentially feed on nutrient-rich reproductive tissues (Wheeler, 2001) and *L. lineolaris* may track the succession of flowering hosts in the landscape (Fleischer & Gaylor, 1987). Floral volatiles may be an efficient signal of preferred resources in a habitat, and mediate *L. lineolaris* attraction to flowering hosts. This phenomenon was recently demonstrated in another mirid, *Apolygus lucorum* (Pan et al., 2015) and *Lygus* spp. attraction to floral volatiles is well-established. Lures containing the floral volatiles enhance *L. rugulipennis* trap catch (Koczor et al., 2012) and, importantly, increase the capture of female *L. rugulipennis* in pheromone traps (Baroffio et al., 2018). Flowering *Medicago sativa* plants are more attractive to *L,ygus hesperus* Knight than vegetative plants in laboratory assays (Blackmer et al., 2004) and individual components of the *M. sativa* floral bouquet, (*E*)-β-ocimene and (*E*,*E*)-α-farnesene attract female *L. hesperus* in the laboratory (Williams et al. 2010). However, combining sunflower floral volatiles with pheromone lures reduced *L. lineolaris* capture rate, emphasizing the complex nature of these interactions and our incomplete understanding of the factors that mediate *Lygus* spp. orientation in the field (Chouinard-Thuly et al., 2020).

Lures containing attractive semiochemicals are frequently deployed to improve insect monitoring (Ibeas et al., 2007). Female-released sex pheromones have been investigated as one

means to facilitate *Lygus* spp. monitoring. Hexyl butyrate, (*E*)-2-hexenyl butyrate, and 4-oxo-hexenal have been identified in *Lygus* spp. metathoracic gland extracts in varying ratios and appear to be responsible for mate location and reproductive isolation among *Lygus* spp. (Byers et al., 2013; Fountain et al., 2014; Wardle et al., 2003; Q. H. Zhang et al., 2007; T. Zhang et al., 2021). Pheromones have been successfully deployed to monitor *Lygus rugulipennis* Poppius in Europe (Fountain et al., 2014; Innocenzi et al., 2005) and *L. rugulipennis* pheromone lures are now commercially available. While pheromone trapping of *L. lineolaris* shows promise (George et al., 2023; Parys & Hall, 2017), results can be inconsistent (Chouinard-Thuly et al., 2020).

A key principle of integrated pest management is that management decisions are based on pest populations and the likelihood of economic damage (Barzman et al., 2015; Deguine et al., 2021). However, actively monitoring pest populations at the field level is labor-intensive, which may hinder IPM implementation (Ehler, 2006). Optimizing strategies to passively monitor pest populations may reduce time and labor costs for growers and facilitate the implementation of IPM strategies.

This study examined the role of floral volatiles *L. lineolaris* attractants. We conducted electrophysiological assays to 1) identify compounds in headspace extracts of flowering *M. sativa* and *F. ananassa* that consistently elicit antennal depolarization in *L. lineolaris*. Subsequently field experiments were conducted to 2) determine the ability of antennally-active plant volatiles to enhance *L. lineolaris* trap catch, and 3) examine the influence of stereochemistry and trap color on observed patterns of *L. lineolaris* attraction. We expect these data will inform future experiments on *L. lineolaris* chemical ecology and facilitate the use of kairomones in *L. lineolaris* monitoring.

Materials and Methods

Plants

Medicago sativa and *Fragaria ananassa* var "Mara Des Bois" plants were grown in 15.5 cm pots with Pro-Mix potting soil in a greenhouse under a 14:10 light cycle in a 18.6 m^2 room in a greenhouse at the University of Wisconsin – Madison. Temperatures were maintained at 23°C during the winter months and were not controlled during the summer. Plants were watered every 1-2 days and fertilizer (type NPK and manufacturer) was applied weekly. *Medicago sativa* was grown from seed (source) and *F. ananassa* was grown from bare root plants (Burpee, Warminster, PA). Flowering plants were used in all experiments.

Headspace extractions

Floral volatile emissions were collected from five individual plants of each species via dynamic headspace extraction at approximately 23°C over the course of 3 hrs. For each plant, ten leaves and either ten flowers (*F. ananassa*) or ten racemes (*M. sativa*) were enclosed in a 3.8 L Teflon® pail liner (Welch FluoroLab, Dover, NH), which was sealed around the base of the plant with a steel wire. Charcoal-filtered air was introduced to the bag at a rate of 300 ml/min and drawn out through a trap containing 20 mg of Porapak adsorbent (Sigma-Aldrich, St. Louis, MO). Traps were eluted with 200 µL HPLC-grade chloroform (Sigma-Aldrich, St. Louis, MO) containing 1-bromododecane (Sigma-Aldrich, St. Louis, MO) as an internal standard at 0.4 ng/µL.

Chemical analysis GC-EAD/MS

Extracts were concurrently analyzed on a Thermo Scientific Trace 1300 gas chromatograph (GC) coupled to an ISQ series single quadrupole mass spectrometer (MS; Thermo Fisher Scientific) and an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA)

with the output split between a flame ionization detector (FID) and an electroantennogram detector (EAD; Syntech, Buchenbach, Germany) in a 1:2 FID:EAD ratio. In both analyses, the split/splitless injector was operated in splitless mode and used the same temperature program: the inlet temperature was set at 250°C and the oven maintained at 35°C for 2 min before increasing to 150°C at a rate of 5°C/min, then increased at a rate of 20°C/min to a final temperature of 210°C, which was held for 2 minutes for a total run time of 30 min. An injection volume of 1 µL was used when conducting GC-MS analyses of plant extracts and 2 µL were injected for GC-EAD analyses. The MS Detector began scanning 35-350 *m/z* after a solvent delay of 5 min and continued until the end of the run. A continuous stream of charcoal-filtered, humidified air (speed) carried eluting compounds to the antennal detector. Antennae were mounted on glass capillary electrodes filled with biological saline (Moto et al. 2004). To prepare the EAD, insects were chilled for 5 min before the tip of the right antenna was cut using sharp microdissection scissors (VWR, Radnor, PA). Gentle pressure was then applied to the insect abdomen to elicit the release a bubble of hemolymph at the tip of the antenna to confirm that the antenna would make good electrical contact. Insects were subsequently decapitated and the head mounted on the indifferent electrode and the right antenna connected to the recording electrode.

Traces were exported from GC-EAD software (Syntech, Buchenbach, Germany) in ASCII format and analyzed with an automated system described by Slone and Sullivan (2007). Briefly, an exponential zeroing filter was applied to the raw EAD data, converting the Gaussian signals to sigmoid deflections, before an exponential average (Lyons, 1997) with a weighting factor of 0.15 was applied to the zeroed data to remove spurious noise spikes. Another exponential averaging function with a weighting factor of 0.001 was used to calculate the baseline of the filtered EAD data. Here, three algorithms were applied to identify deflections

with characteristics associated with olfactory stimulation and the "Additive method" was selected to define EAD-active peaks (Slone and Sullivan, 2007). Retention indices were calculated for peaks associated with antennal depolarization based on a C8-C20 alkane series, allowing peaks to be matched across the two GCs.

Chromatograms generated through GC-MS analysis were exported in CDF format and deconvolution was completed in PARADISe (Johnsen et al. 2017). Tentative identification of analytes was achieved by comparing mass spectra obtained from the GC-MS to the NIST 2008 MS library. The identities of compounds associated with antennal depolarization were confirmed via comparison of retention indexes and antennal responses to authentic standards (Sigma-Aldrich, St. Louis, MO).

***Lygus lineolaris* attraction to antennally-active plant volatiles**

Field experiments were conducted to compare *L. lineolaris* capture rate between traps baited with each of the five antennally-active host plant volatiles identified previously and a negative control. Each block consisted of a linear array of six red sticky traps (Trécé Pherocon SWD STKY adhesive traps; Great Lakes IPM, Vestaburg, MI), as previous experiments conducted in our lab (Chapter 5) and others (George et al., 2023) revealed that these traps capture *L. lineolaris* at significantly higher rates than other colored traps. Each trap was baited with a lure containing 500 mg of either (Z)-3-hexenol, (R)-α-pinene, Ocimene, (±)-linalool, sulcatone, or no stimulus (control). Three blocks were arranged along the Southern (upwind) perimeter of a 3.44 hectare alfalfa hay field at the West Madison Agricultural Research Station (Madison, WI). Garden stakes were placed at a slight angle and traps were hung over the alfalfa, such that the bottom of each trap was just above the alfalfa canopy. Traps were separated by 10 m within each block and blocks were separated by 20 m. Each block was replicated three times

in a randomized complete block design during each sampling period. Traps and lures were changed weekly over two four-week sampling periods beginning one week after the first and second harvest of alfalfa, June 6 – July 4 and July 11 - August 8, 2022. After removal, traps were wrapped in cellophane and returned to the lab, where *L. lineolaris* were identified and counted under a stereomicroscope (Olympus XZS10). Sweep net samples were taken weekly during each sampling period to assess *L. lineolaris* population in the alfalfa field near each block. Ten sweeps were taken with a 45 cm sweep net (Oakfield apparatus, Oakfield, WI) along the length of each block, stored in paper bags, and frozen upon returning to the lab. *Lygus lineolaris* were identified as juveniles or adults and adults were sexed, numbers of juveniles, adult males, and adult females were recorded.

Influence of trap color and stereochemistry on *L. lineolaris* attraction to ±-linalool

An experiment was conducted to examine the enantiospecificity of *L. lineolaris* attraction to ±-linalool and whether this response depends on trap color. Each block consisted of a linear array of three red and three white sticky traps (Great Lakes IPM, MI, USA). Traps were baited with lures containing 500 mg of either (±)-linalool, (-)-linalool, or no stimulus (control) such that each lure was associated with one trap of each color in each block. (+)-linalool was not tested due to the lack of commercial availability. Nine blocks were arranged along the Southern perimeters of two alfalfa hay fields that were separated by 100 m (6.34 ha field, n = 5; 5.48 ha field, n = 4) at the Arlington Agricultural Research Station (Arlington, WI). Trapping was conducted over two weeks, beginning two weeks after the third alfalfa harvest, between August 9-23, 2022. Traps were processed as described above. Sweep net samples were taken weekly during each sampling period to assess *L. lineolaris* population in the alfalfa field near each block as described above. The traps varied in surface area with 406.45 cm^2 and 522.58 cm^2 of sticky

surface for the white and red traps, respectively, thus, *L. lineolaris* capture rates were divided by surface area and are reported as insects/cm^2/week.

Statistical analysis

All statistical analyses were performed using R version 4.1.3 (R core team 2022). Linear mixed models were fitted using the lme4 package (Bates et al. 2015) and the package emmeans (Lenth 2022) was employed to compare predefined groups. The package lmerTest (Kuznetsova et al. 2017) was used to assess overall model outputs.

Data from both experiments were square root transformed to improve residual normality and homoscedasticity. Linear mixed models were fit to examine the relationship between *L. lineolaris* capture rate and predictor variables. In the first experiment, lure identity was the sole predictor in our model, sampling date and block were included as crossed random effects. It was decided *a priori* that comparisons should only be made between each treatment and the negative control, as the goal of this experiment was to determine whether the presence of antennally-active compounds increases *L. lineolaris* capture rate relative to control traps rather than compare attraction between active compounds. In the second experiment, lure identity, trap color, and their interaction were included as predictor variables, while sampling date and block were again included as crossed random effects. All pairwise comparisons were expected to be relevant for experiment 2, so no *a priori* contrasts were defined. The Holm-Bonferroni method was employed to control family-wise error rate during post-hoc multiple comparisons testing.

Results

GC-EAD/MS

Five compounds present in the headspace extracts collected from flowering *M. sativa* and *F. ananassa* plants elicited consistent antennal depolarization in *L. lineolaris* (Figure 6-1). These compounds were α-pinene, (*E*)-β-ocimene, (±)-linalool, sulcatone, and (*Z*)-3-hexenol. Antennae from male and female *L. lineolaris* exhibited variable responses to these compounds. Sulcatone and (*E*)-β-ocimene failed to elicit antennal responses from male *L. lineolaris*, while (±)-linalool elicited inconsistent responses from females.

Most compounds were present in extracts collected from both *F. ananassa* and *M. sativa* ; however, α-pinene was not detected from the volatile emissions of strawberry plants (α-pinene: $F_{1,8} = 7.76$, P = 0.024; Figure 6-2). *Medicago sativa* emitted (*E*)-β-ocimene at significantly higher rates than *F. ananassa*, averaging 1554% more (*E*)-β-ocimene per mg of dry plant material (Ocimene: $F_{1,8} = 14.02$, P = 0.0057; Figure 6-2). No difference was detected in the emission rate of (*Z*)-3-hexenol ($F_{1,8} = 1.614$, P = 0.24), sulcatone ($F_{1,8} = 0.759$, P = 0.409) and (±)-linalool ($F_{1,8} = 0.379$, P = 0.555) between the two plant species.

Response to host plant volatiles in the field. Mean field densities of *L. lineolaris* adults ranged from 0 to 6.73 insects per sweep, with adult densities peaking in weeks three and seven. The presence of (±)-linalool increased *L. lineolaris* capture rates by 85% compared to control traps (Figure 6-3A), the only significant change in capture rate observed in this experiment (β = 0.714 ± 0.25, df = 128, *t*-ratio = 2.422, $P_{adj} = 0.0421$). While numerical increases in capture rate were observed for the other chemical lures, none were significantly different from the control ((*Z*)-3-hexenol: β = 0.060 ± 0.27, df = 128, *t*-ratio = 0.022, $P_{adj} = 1$; ocimene: β = 0.148 ± 0.23, df = 128, *t*-ratio = 0.641, $P_{adj} = 1$; (*R*)-α-pinene: β = 0.338 ± 0.21, df = 128, *t*-ratio = 1.615, $P_{adj} = 0.544$, sulcatone: β = 0.231 ± 0.19, df = 128, *t*-ratio = 1.244, $P_{adj} = 1$).

Influence of trap color and stereochemistry on L. lineolaris attraction. Field densities of adult *L. lineolaris* ranged from 0.4 to 2.1 insects per sweep over the course of this experiment, with a mean density of 0.89 adults per sweep. Both trap color and lure identity significantly influenced overall *L. lineolaris* capture rate (Color: $F_{2, 93} = 128.11$, $P < 0.001$; Lure: $F_{2, 93} = 6.85$, $P = 0.001$), and trap color was determined to influence lure efficacy (Color:Lure: $F_{2, 93} = 3.20$, $P = 0.0378$). Comparison of geometric means revealed that red traps captured *L. lineolaris* at significantly higher rates than white traps, regardless of lure (Figure 6-3B, Table 2), with 412%, 422%, and 753% increases in mean *L. lineolaris* capture rate observed for control, (-)-linalool, and (±)-linalool traps, respectively.

Red traps with (±)-linalool lures captured *L. lineolaris* at significantly higher rates than other red traps, averaging 112% and 108% more *L. lineolaris* than traps baited with (-)-linalool and control traps, respectively (Figure 6-3B, Table 6-1). Red control traps captured *L. lineolaris* at similar rates as those baited with (-)-linalool. Interestingly, similar differences in *L. lineolaris* capture rate were not observed on white traps (Figure 6-3B, Table 6-1), emphasizing the importance of visual and olfactory cues in *L. lineolaris* trapping.

Discussion

Lygus lineolaris is highly mobile, polyphagous insect pest, known to feed on at least 130 economically-important fruit, vegetable, forage, fiber, and oilseed crops (George et al., 2021; Esquivel and Mowry, 2007; Young, 1986). Monitoring *L. lineolaris* as populations move between crop and non-crop hosts is critical to effectively managing this species. This study investigated whether floral volatiles may be deployed as kairomone lures to facilitate *L. lineolaris* monitoring. We determined that α-pinene, (*E*)-β-ocimene, (±)-linalool, sulcatone, and (*Z*)-3-hexenol consistently elicit antennal depolarization in *L. lineolaris* antennae and that (±)-

linalool increases trap catch of *L. lineolaris*. LSubsequent experimentation revealed that lures containing (-)-linalool failed to increase *L. lineolaris* capture compared to control, implying that (+)-linalool is essentialcritical to the observed increase. Moreover, red traps increased trap captures compared to white traps and the visual qualities of the trap and the red color synergised the attractiveness were critical to the efficacy of the (±)-linalool lures. These findings will facilitate the continued optimization of *L. lineolaris* monitoring strategies and emphasize the importance of multimodal cues in *L. lineolaris* orientation.

Olfaction is not the only sensory modality involved in insect orientation, and trap visual parameters are often important for optimizing insect attraction. Visual cues are known to be important to *Lygus* spp. orientation. Green LEDs significantly enhance *L. hesperus* responses to host plant volatiles in laboratory assays (Blackmer & Cañas, 2005), and LED light traps were recently reported to capture 20-30 times as many *L. rugulipennis* as pheromone traps in chrysanthemum greenhouses (van Tol et al., 2022). Red traps were recently demonstrated to capture *L. lineolaris* at significantly higher rates than blue, white, or yellow traps (George et al. 2023). A similar effect of trap color was observed in this study, where unbaited red traps captured five times as many *L. lineolaris* as unbaited white traps. Moreover, (±)-linalool only enhanced *L. lineolaris* capture rates on red traps, emphasizing the importance of both visual and olfactory cues. A recent study of mirid opsins reported that long-wavelength opsins, those sensitive to wavelengths greater than 500 nm, have undergone duplication in this family, while the blue-sensitive opsins have been lost (Xu et al., 2021). The mirid visual system being tuned toward longer wavelengths may explain the efficacy of red and green traps for monitoring *Lygus* spp. (Blackmer et al., 2008; George et al., 2023). Future studies should evaluate *L. lineolaris* sensitivity

to different wavelengths of light to further optimize visual parameters of traps for this species, as was recently done for *Drosophila suzukii* (Little et al., 2019).

Insect herbivores utilize olfactory cues to identify suitable host plants for feeding or oviposition in complex environments (Carrasco et al., 2015; Schoonhoven et al., 2005). As these compounds are frequently utilized by both male and female insects, they represent appealing targets for deployment in monitoring or mass trapping regimes (Ibeas et al., 2007). While this is the first study to demonstrate *L. lineolaris* attraction to (±)-linalool, floral volatiles have been shown to enhance capture of *L. rugulipennis* (Baroffio et al., 2018; Koczor et al., 2012) and attract *L.ygus hesperus* in laboratory assays (Williams et al., 2010; Blackmer et al., 2004). *Lygus lineolaris* is thought to track the succession of flowering (Fleischer & Gaylor, 1987), as it, and mirids more broadly, prefer to feed on nutrient-rich meristematic and reproductive tissues (Wheeler, 2001). Floral volatiles may therefore be an effective habitat-finding cue, relaying information about plant phenology and allowing *L. lineolaris* to identify habitats where reproductive tissues are available, as has been reported for *Apolygus lucorum* (Pan et al., 2015). Although we found that lures containing only (±)-linalool were sufficient to increase *L. lineolaris* capture on red traps, additional research is required to optimize a kairomone lure for this pest. Our data suggest that (+)-linalool is responsible for *L. lineolaris* attraction to the racemic blend, but this remains to be definitively proven. Multicomponent blends are typically more effective for insect monitoring than single compounds (Szendrei & Rodriguez-Saona, 2010), and future studies should consider the addition of other floral volatiles. (*E*)-β-ocimene, (*R*)-α-pinene, and (*E,E*)-α-farnesene have previously been shown to elicit upwind movement in female *L. hesperus* (Williams et al., 2010), and may be good candidates for further study. Additionally, including

(±)- or (+)-linalool lures alongside pheromone blends may be a way to increase the capture of female *L. lineolaris* as has been demonstrated for *L. rugulipennis* (Baroffio et al., 2018).

Enantiospecific responses represent opportunities to examine both the information conveyed by semiochemicals and the structural and functional basis of specificity in the insect olfactory system. As distinct biosynthetic pathways give rise to (+)- and (-)-linalool and the two linalool synthase genes often exhibit distinct spatial and temporal gene expression patterns (Raguso, 2016; Raguso & Pichersky, 1999), the two enantiomers may convey distinct information. Enantiospecific responses to linalool have been extensively studied in *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae), showing thata system in which female responses depend on stereochemistry and males exhibit a generalized response to both enantiomers. Linalool enantiomers activate adjacent, female-specific glomeruli in the *M. sexta* antennal lobe, leading to enantiospecific behavioral responses (Raguso, 2016; Reisenman et al., 2004, 2010, 2013). The gene responsible for (-)-linalool synthesis in tomato, LeMTS1, is expressed in glandular trichomes and up-regulated in response to herbivory (Van Schie et al., 2007). The presence of (-)-linalool in the bouquet of jimsonweed or tomato indicates herbivory and reduces hawkmoth oviposition, while oviposition increases in the presence of (+)-linalool (Raguso, 2016; Reisenman et al., 2010, 2013). Few studies have examined the stereochemistry of linalool emitted from *Lygus* spp. hosts and it is therefore unknown whether (+)-linalool is more likely to convey the presence of flowers or herbivory to *L. lineolaris*. Conspecific damage increases female attraction to *M. sativa* and *Vicia faba* in *L. hesperus* and *L. rugulipennis*, respectively, but is not associated with increased linalool emission (Blackmer et al., 2004; Frati et al., 2008), which may favor the floral signal hypothesis. However, *L. rubrosignatus* damage does increase linalool emission from *Erigeron annuus* and *Glossypium hirsutum* (Halloran et al., 2013), it may

therefore be reasonable to expect responses to *Lygus* damage are dependent on the host plant and *Lygus* species involved. More thorough examination of the enantiospecific attraction of *L. lineolaris* to linalool may lead to deeper understanding of how chemical information guides decision-making in *Lygus* spp. and polyphagous insects, more broadly.

With a few exceptions, our data agree with previous studies of *L. lineolaris* antennal sensitivity. Chinta and colleagues (1994) reported *L. lineolaris* sensitivity to (±)-linalool, α-pinene, (*E*)-β-ocimene, and (*Z*)-3-hexenol and antennal responses to these compounds have also been reported for *L. hesperus* (Williams et al., 2010). However, sulcatone, which is best known as a mediator of mosquito host location, has not previously been reported to elicit antennal responses from *Lygus* spp. Additionally, *L. lineolaris* antennae have previously been shown to exhibit similar responses to (±)- and (-)-linalool, implying a similar number of neurons depolarizing in response to these stimuli (Chinta et al., 1994). This suggests the presence of a generalized linalool receptor in *L. lineolaris*, as the reduced concentration of (-)-linalool or increased concentration of (+)-linalool in the racemate, compared to pure (-)-linalool, would be expected to impact the number of neurons firing if enantiospecific receptors were present. However, the observation that (±)-, but not (-)-linalool increases *L. lineolaris* trap capture is incompatible with this interpretation. An alternative explanation is that a receptor that can interact with both enantiomers but is more sensitive to (+)-linalool exists, as has been reported in *Mamestra brassicae* (Ulland et al., 2006) and *Anopheles gambiae* (Huff & Pitts, 2019). It may be reasonable to expect that the 100 µg stimulus load employed by Chinta and colleagues (1994) in their initial EAG experiment provided sufficient (*R*)-(-)-linalool to stimulate all (*S*)-(+)-linalool receptors despite their relatively low sensitivity. Although, we cannot fully understand the ecological or physiological basis of enantiospecific behavior in *L. lineolaris* based on currently

available data, ongoing efforts to annotate the *L. lineolaris* genome are expected to provide additional insight into the chemosensory repertoire of *L. lineolaris* and may allow a deeper understanding of this interaction and *L. lineolaris* olfaction more broadly.

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Effect	DF	*F*	P

		Lure	**2, 93**	**6.854**	**<0.001**	
		Color	**1, 93**	**128.111**	**<0.001**	
		Lure:Color	**2, 93**	**3.201**	**0.0378**	
Pairwise Contrasts						
Color	Lure		|β| ± SE	DF	|*t*-ratio|	P_{adj}
Red	**(±)-linalool**	**Blank**	**0.0881 ± 0.0157**	**93**	**5.606**	**<0.001**
	(±)-linalool	**(-)-linalool**	**0.0428 ± 0.0157**	**93**	**2.726**	**0.0383**
	Blank	(-)-linalool	0.0219 ± 0.0131	93	1.667	0.2968
White	(±)-linalool	Blank	0.0346 ± 0.0157	93	2.204	0.1199
	(±)-linalool	(-)-linalool	0.0101 ± 0.0157	93	0.64	0.5239
	Blank	(-)-linalool	0.0213 ± 0.0131	93	1.624	0.2968
Lure	Color		|β| ± SE	DF	|*t*-ratio|	P_{adj}
Blank	**Red**	**White**	**0.0708 ± 0.0168**	**93**	**4.201**	**<0.001**
(±)-linalool	**Red**	**White**	**0.1242 ± 0.0168**	**93**	**7.373**	**<0.001**
(-)-linalool	**Red**	**White**	**0.0713 ± 0.0168**	**93**	**4.235**	**<0.001**
	Red – Lure	White – Lure	|β| ± SE	DF	|*t*-ratio|	P_{adj}
	Blank	**(±)-linalool**	**0.0595 ± 0.0145**	**93**	**4.107**	**<0.001**
	Blank	**(-)-linalool**	**0.0728 ± 0.0116**	**93**	**6.245**	**<0.001**
	(±)-linalool	**Blank**	**0.1822 ± 0.0168**	**93**	**10.813**	**<0.001**
	(±)-linalool	**(-)-linalool**	**0.1375 ± 0.0145**	**93**	**9.493**	**<0.001**
	(-)-linalool	**Blank**	**0.116 ± 0.0145**	**93**	**8.01**	**<0.001**
	(-)-linalool	**(±)-linalool**	**0.1047 ± 0.0168**	**93**	**6.216**	**<0.001**

Table 6-1: Summary of statistics from omnibus test and pairwise comparison of geometric means from experiment 2. Bolded text indicates statistical significance at $\alpha = 0.05$.

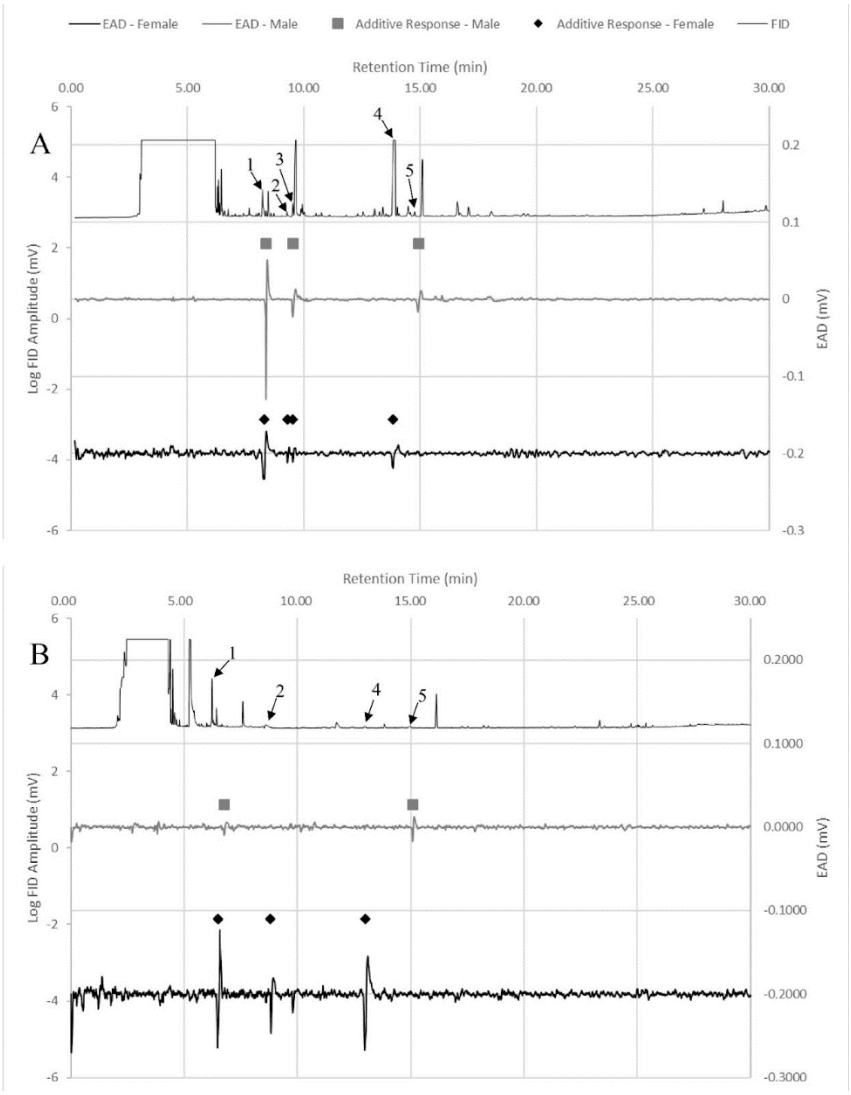
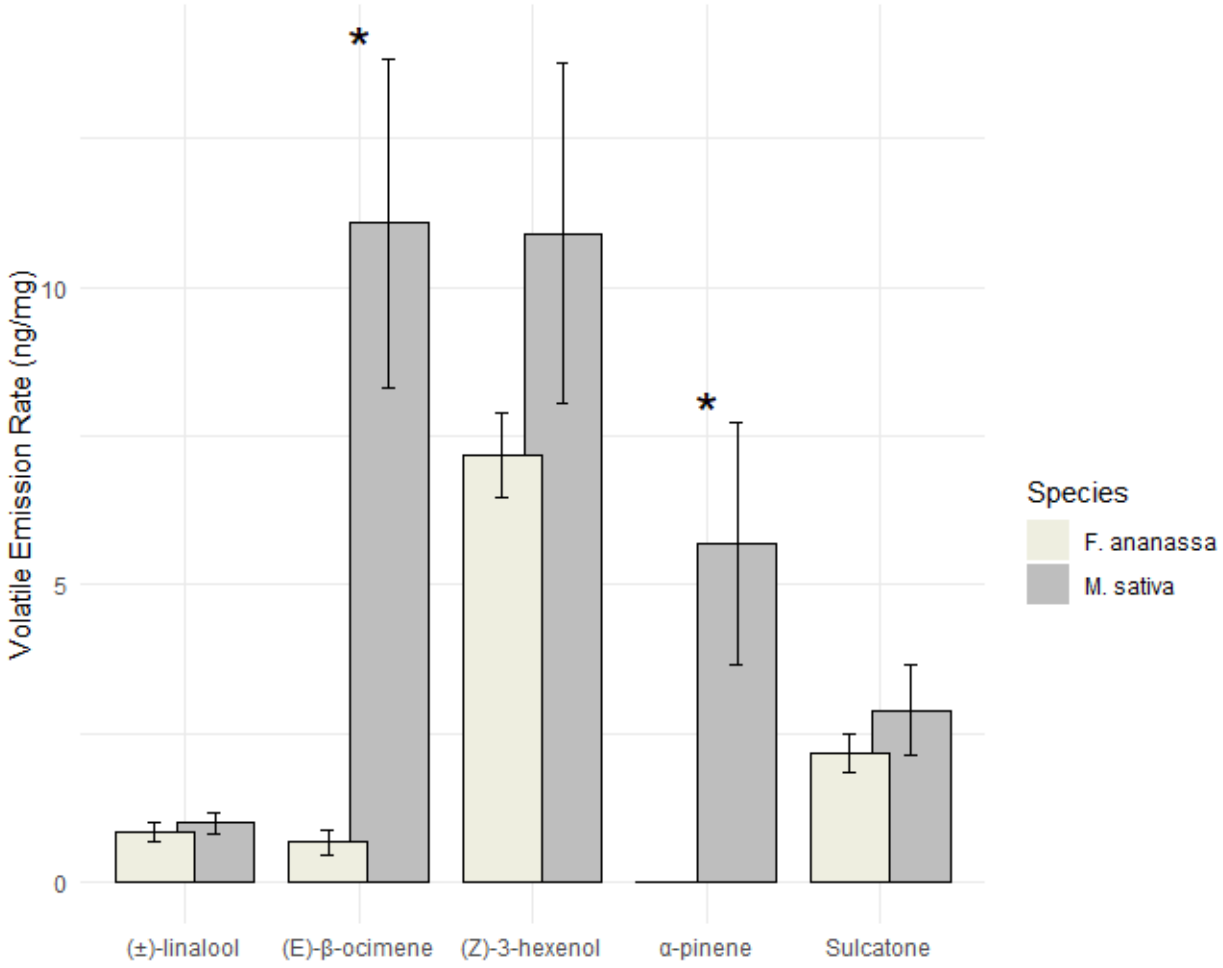


Figure 6-1: A) Representative trace of *M. sativa* headspace extract FID and representative EAD traces for male (top) and female (bottom) *L. lineolaris*. B) Representative trace of *F. ananassa* headspace extract FID and representative EAD traces for male (top) and female (bottom) *L. lineolaris*. In both cases, FID and EAD traces have been smoothed and filtered. To facilitate viewing multiple EAD traces on the same graph, female traces have been shifted -0.2 mV. Markers indicate peaks that satisfy Slone and Sullivan's (2007) additive response criteria. These peaks have been identified as: 1) (*Z*)-3-hexenol, 2) Sulcatone, 3) α-pinene, 4) (*E*)-β-ocimene, and 5) (±)-linalool.



Mean ± SE emission rate of antennally-active floral volatiles from *M. sativa* and *F. ananassa*. Asterisks denote significant differences in volatile emission at $\alpha = 0.05$.

Figure 6-3: A) Geometric mean ± SE *L. lineolaris* weekly capture rate. Asterisks denote significant difference from blank at α = 0.05. B) Geometric mean ± SE *L. lineolaris* weekly capture rate per cm^2 trappable area. Different letters denote significant differences between groups at α = 0.05.