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Non-Consumptive Effects in *Myzus persicae*: Dispersal and Feeding Behavior in Response to Predation Risk

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by

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For my family and Eric Sibbald, for their love and support

ABSTRACT OF THE THESIS

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Aphids are small phloem sap-feeding pests, which transmit economically damaging plant viruses. Natural enemies are used as an environmentally sustainable method of aphid control, and can have either (1) consumptive effects: the consumption of the prey or (2) non-consumptive effects: the change in behavior, reproduction, or morphology in response to predation risk. Non-consumptive effects can be induced when a prey species perceives a predator. Prey often rely on chemical cues to detect predation risk. Lady beetles, generalist predators of aphids, deposit hydrocarbons while walking. Insects can respond to the chemical cues of predators by altering either their movement or feeding behavior, however, the latter has not yet been explored in aphids. Aphids may alter dispersal and feeding behaviors in response to detection of a predator cue. This non-consumptive effect could enhance or suppress virus transmission. Non-consumptive effects are vastly understudied, and have the potential to be equally important in shaping predator-prey dynamics compared to consumptive effects. In this thesis, I investigate the hypothesis that an important aphid pest (*Myzus persicae*) changes its movement and

feeding behavior in response to hydrocarbons deposited by a key natural enemy, the convergent lady beetle, *Hippodamia convergens*. Using gas-chromatography and mass-spectrometry, I identified the chemical components in the *H. convergens* footprints, which include several straight-chained hydrocarbons: tricosane, pentacosene, pentacosane, heptacosene, heptacosane, and nonacosene. Using dispersal assays, I found that *M. persicae* disperse from areas exposed to *H. convergens* cues similarly to aphids dispersing from areas treated with mineral oil, a plant surface treatment that deters feeding by piercing-sucking insects. However, *M. persicae* did not change its in-leaf feeding behavior when forced to feed on leaves treated with lady beetle "footprints". Based on the results of this thesis, we found that aphids exposed to predation risk will increase their dispersal which has implications for pathogen spread.

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Introduction

Predators can impact prey behavior. The impact of predators occurs directly through consumptive effects, where a predator kills and eats prey (Henery, *et al.*, 2010), or indirectly through non-consumptive effects which is defined as changes in prey behavior, reproduction, or morphology in response to predation risk (Thaler and Griffin, 2008; Peckarsky, *et al.*, 2008). While the vast majority of the work on predator-prey interactions has focused on consumptive effects (Lima, 1998; Preisser, *et al.*, 2005; Thaler and Griffin, 2008; Peckarsky, *et al.*, 2008), non-consumptive effects have been found in a wide array of vertebrate and invertebrate taxa, including in both terrestrial and aquatic insects (For reviews highlighting some of the breadth of taxa, see: Lima and Dill, 1990; Lima, 1998; Tollrian & Harvell, 1999; we have highlighted some examples in Table 1). Despite being vastly understudied, non-consumptive effects may be as important as consumptive effects in shaping prey population dynamics (Preisser *et al.*, 2005).

Non-consumptive effects are induced when the prey perceives predation risk. Detection of risk can occur through multiple mechanisms, including chemical cues (Kats and Dill, 1998). One example of a predator chemical cue inducing a non-consumptive effect is lady beetle footprints (the group of chemicals deposited by lady beetles as they walk), which induce the increased production of alates (winged morphs) in pea aphids (Dixon and Agarwala, 1999).

A significant research gap in non-consumptive effect literature is how sap-feeding insects respond to detected predation risk. Sap feeding insects have limited numbers of

non-consumptive effect studies compared to chewing insects, especially in regard to feeding behavior, according to the meta-analysis by Buchanan *et al.*, 2017 (see Table 2 for greater detail). Sap-feeding insects are important because they are pests to agriculture, as plant pathogen vectors, including those of plant viruses. Understanding how sap-feeding insects' behavior changes in response to predation risk is important because how a vector feeds and disperses can influence virus spread, acquisition, and inoculation (Prado and Tjallingii, 1994; Martin, *et al.*, 1997; Fereres and Moreno, 2009; Finke, 2012; Dader, *et al.*, 2012).

Dispersal is one of the behavioral changes by vectors in response to perceived predation risk which can alter the spread of viruses (Finke, 2012). There is experimental evidence for the predator causing increased vector dispersal and linking it to increased viral spread, without isolating non-consumptive effects from consumptive effects (Table 3). Only a few studies have isolated non-consumptive effects changing vector dispersal. Ninkovic *et al.*, found that the *Rhopalosiphum padi* avoided areas coated with lady beetle "footprints" (2013). Seo *et al*., found that the *Diaphorina citri* avoids setting on plants with lady beetle "footprints" (2018). Kersch-Becker and Thaler found that the *Macrosiphum euphorbiae* increased their dispersal to other plants when exposed to a predator risk (2014).

Feeding behavior of sap-feeding insects have been found to change when these insects are under predation risk (Seo, *et al*., 2018; Tholt, *et al*., 2018). But this has only been investigated very recently in the last couple years. Seo *et al.* showed that predation risk from lady beetle trails can decrease feeding of the Asian citrus psyllid, *Diaphorina*

citri (2018). Predation risk also alters leafhoppers' feeding behavior by delaying and decreasing feeding from the phloem, as well as decreasing salivation before phloem ingestion (Tholt et al 2018). To the best of our knowledge, no study to date that has investigated the in-leaf feeding behavior of aphids under predation risk.

Aphids are important to study because they are a global pest and a major vector of plant viruses. Approximately half of all known insect-vectored plant viruses are vectored by aphids (Hogenhout, *et al.,* 2008). One such aphid, *Myzus persicae*, is known to transmit over 100 plant viruses (Kennedy, *et al.*, 1962). It also is a global pest and a generalist feeder, feeding on hosts from 40 different plant families (CABI, 2019).

Aphids are unique in their ability to transmit a range of virus types ranging in transmission and acquisition times, as well as their retention span in the vector (Hogenhout, *et al*., 2008). These virus types include those that require short probes for transmission and are retained briefly for only a few probes, and others requiring sustained phloem ingestion for transmission and are retained in the vector for their entire lifespan (Prado and Tjallingii, 1994; Martin, *et al*., 1997; Feres and Moreno, 2009). It is important to study the range of feeding behaviors under predation risk because they are associated with the acquisition and transmission of the different viruses types. This will enable us to hypothesize the potential changes in virus transmission, dependent on virus type, caused by predation risk (Finke, 2012; Dader, *et al*., 2012).

In this thesis, I address the research gap with regard to non-consumptive effects and aphids, which are poorly studied from this perspective despite their importance as pests and vectors. To investigate non-consumptive effects on aphid behaviors of

relevance for virus transmission, we employed a system consisting of *M. persicae* as the aphid herbivore and *Hippodamia convergens* "footprints", a known inducer of non-consumptive effects in other sap-feeding insects, as the predator chemical cue. We hypothesized that predator chemical cues will induce non-consumptive effects by causing changes in aphid behavior, specifically dispersal and in-leaf feeding. We predicted that *M. persicae* will increase their dispersal from leaves with lady beetle "footprints" following initial encounters during foraging. This prediction is based on the fact that "footprint" chemical cues can be perceived during the host selection process prior to initiating long-term phloem feeding. Our second prediction is that *M. persicae* will change its feeding behavior when forced to feed on leaves treated with lady beetle "footprints". Specifically, we predict that *M. persicae* will reduce time spent in phloem elements due to an increase in and number of probes and intracellular punctures that occur during host selection. We base the prediction of the reduced feeding (ingestion of and salivation into phloem sap) observed in a study by Tholt *et al.*, (2018) describing leafhoppers' in-leaf feeding behavior under predation risk, as well as the Seo *et al*., (2018) study of the Asian citrus psyllid feeding on leaves with lady beetle "footprints". It has been hypothesized that perceived predation risk on a plant is evaluated as part of host plant quality (Dicke, 2000), and therefore predation risk will lower the host plant quality, leading to more attempts and rejections of the plant.

Methods

Organism Rearing and Treatments

Myzus persicae (Sulzer 1776) (Hemiptera: Aphididae) were raised in colonies on canola, at room temperature (~24℃) and kept under LED lights (OOOLED 42W 4800LM 5000K Daylight White) for 16:8 hour light dark light cycle.The aphid colony used for this study originated from a single female collected in Imperial County, CA (USA) from broccoli (*Brassica olearaceae L.*) and maintained for some time on *B. vulgaris* (Jiménez, *et al.*, 2019). For experiments, *M. persicae* aphids were switched to canola, *Brassica napus*, as a preferred host plant and allowed to reproduce for several generations before experimentation.

We used three-week old canola, *B. napus* cv. Dwarf Essex, for all experiments. Canola is grown in temperate regions for its seeds, which are processed into oil. Plants were grown in a climate controlled room at 23℃, and kept under LED lights for 16:8 hour light dark light cycle. They were planted with 9-16 seeds per pot (10 x 10 x 9 cm) and transplanted to individual pots (8.5 x 8.5 x 10 cm) after one and a half weeks post germination.

Hippodamia convergens (Guerin, 1842) (Coleoptera: Coccinellidae) were used to establish the predation risk treatment, using lady beetle footprints. The treatment was established by having 10 lady beetles, which were starved for 24 hours, walk on a leaf contained by a fluoned clip cage (Fig. 1) for 24 hours. We starved the lady beetles to prevent or at least reduce feces contamination of the "footprints". *H. convergens* were

purchased from both Hirt's Garden (Ohio, USA) and ARBICO Organics (Arizona, USA), according to availability. *H. convergens* were kept together in cages at room temperature (24℃) under LED lights for 16:8 light dark light cycle, and fed a combination of *Aphis gossypii* and *Acyrthosiphon pisum*.

We used *Drosophila melanogaster* (Miegen, 1830) (Diptera: Drosophilidae) footprints to test the alternative hypothesis that any change in aphid behavior was not a predator specific response, but rather a response to the plant being primed by being walked upon or that the aphid is responding to the presence of any organism. Drosophila do not interact with aphids, therefore can serve as an insect positive control. We purchased flightless *D. melanogaster* from Tiberline-Fisheries (Illinois, USA) at Petco (Riverside, CA, USA). They were kept at room temperature (24℃) on the artificial medium that they were sold with. The fly "footprint" treatment was established by having 20 starved (for 1 hour) flies walk on a leaf contained by a fluoned clip cage (Fig. 1) for 24 hours. The flies feet were washed of food residue by having the flies walk on the wet filter paper during the starvation period, to prevent contamination of the "footprints".

We used mineral oil, which is a petroleum-based pesticide that is a known feeding and settling deterrent (Powell, 1992; Powell, *et al*., 1998; Buteler and Stadler, 2011), as a positive control. We applied 2% mineral oil (Pest Fighter, Master Nursery, Summit Chemical Co Baltimore, MD, purchased at Parkview Nursery Riverside, CA), diluted with deionized water, to all surfaces of all leaves with a paint brush, and left to dry for 12 to 24 hours.

All organisms used during the treatment application and during the experiments were stored between 24-25 °C under a 16:8 light dark light cycle of LED lights.

Dispersal Bioassay

We measured the rate of aphid dispersal in the presence of a predator cue, lady beetle "footprints", using the behavior bioassay dispersal test described in Mauck et al. (2010). In our test, *M. persicae* aphids started on the treated leaf so that they must contact the treatment before they can access the control leaf, Fig. 2, which is different from most choice tests. The bioassay arenas were composed of 100 x 15 mm diameter petri dish (Fisherbrand, cat. 08-757-12, Fisher Scientific), where two conjoining holes (~17 mm^2) were melted with a heated metal puncture then sanded until edges were smooth. Brown construction paper was glued to the back of the petri dish for a uniform background. Figure 3 shows the test arenas in use. At the beginning of the experiment we placed 20 adult *M. persicae* on a circle of filter paper on the treated leaf. We then recorded the position of the aphids at the following intervals: 15, 30, 45 and 60 minutes; and 3, 6 and 24 hours either on the treated leaf ("start leaf"), control leaf ("other leaf"), on the petri dish ("off"), on the filter paper ("paper"), or marked as could not be found ("missing"). Once all the aphids had left the filter paper, we removed it so that the aphids beneath it could be more easily counted. The plants and choice tests were set up as shown in Figure 4, surrounded by white poster board on four sides to provide a uniform background.

We conducted two series of bioassays, the first were lady beetle footprint treatments and the second were 'clean' fly footprint treatments, with both having a canola

leaf as a negative control and canola leaf coated with 2% mineral oil as a positive control. The predation risk dispersal bioassay was conducted first to test the hypothesis that aphids are able to perceive and respond to lady beetle footprint. Once it was established that they change their dispersal behavior in response to predator presence, we ran the fly "footprint" treatments to test the alternative hypothesis that either the plant was primed by being walked upon or that aphids respond to any organism and that the aphid response is not predator specific.

Footprint Identification

We extracted "footprints", the hydrocarbons that *H. convergens* deposit when they walk, with a variation of the methods from Wheeler and Carde (2014) and Hemptinne *et al.* (2001). All glassware was washed with acetone and hexane, and glass syringes were used to avoid contamination of the samples prior to their use in collection. To collect *H. convergens* footprints, we contained 10 starved *H. convergens* in a glass vial for 24 hours during which time they were allowed to walk on the interior surface. We collected "clean" footprints from flies by having fifteen to twenty *D. melanogaster*, whose feet were washed to remove food residue (by having them walk on wet filter paper for an hour), placed in glass vials for 12 hours before removal. We recovered the hydrocarbons in the footprints by adding 2 mL of hexane into the vials and vortexing it for 5 minutes. All extracts were then concentrated with nitrogen gas to 100 μL.

We used gas chromatography-mass spectrometry (GC-MS) to analyze the composition of the footprints we collected. A GC-MS is an instrument that is composed

of a temperature-controlled oven containing a separatory column which is linked to a mass spectrometer. The sample is injected into the hot inlet of the instrument, where it then enters the column and collects on the forward portion. The oven temperature is gradually ramped as an inert gas (helium) is moved at a constant flow rate through the column. The different components of the sample are eluted off the column and carried to the detector at different time points based on size and polarity. As each component reaches the end of the column it is ionized and goes through a mass spectrometer, which detects the mass and fragments the molecule.

We used a non-polar column (TG-5MS, 28.33 m x 0.250 mm X 0.25 μm film), on Trace 3110 Gas Chromatograph and TSQ Duo Mass Spectrometer (Thermo Fisher Scientific: Waltham, MA) instruments to identify the chemicals in the footprints. The GC temperature program was as follows: injector temperature of 280 °C, transfer line temperature of 280 °C, oven starting temperature of 100 °C with a ramp to 160 °C at a rate of 20 °C/min, followed by a slower ramp of 4 °C/min to 280 °C. Helium was the carrier gas, at a flow rate of 1 ml/min, with spitless mode for 1 minute. We used electron impact ionization to generate mass spectra, with the source at a temperature of 250 °C.

We initially identified the alkanes using the method of Carlson *et al.* (1998), using mass spectrometry/fractionation pattern and a linear retention index, calculated using a C8-C30 alkane standard ladder. A linear retention index is calculated using the following equation:

$$I_x = 100n + 100(t_x - t_n)/(t_{n+1} - t_n)$$

Where I_x is the linear retention index, and n is the number of carbons in the nearest preceding alkane in the ladder. t_x is the retention time of the unknown compound, t_n is the retention time of the preceding alkane in the ladder, and t_{n+1} is the retention time of the nearest proceeding alkane standard in the ladder to the unknown compound.

Alkenes were tentatively identified by their mass, lack of fragmentation pattern but with a lower linear retention index. We confirmed peaks as either alkenes or alkanes by fractionating the samples in a silica silver nitrate column using the following procedure: 300 mg of 10% $AgNO_3$ on silica gel placed into a glass pipette on top of a small ball of glass wool, and baked in an oven for 1.5 hours at 120 °C. The pipette was cooled to room temperature and wetted with hexane. We then placed 100 µL of sample onto the bed of the silica column, and then carefully added two volumes of 100 µL of hexane to move the sample onto the column. The alkanes fraction was eluted with 2x 1 mL of hexane, and alkenes were eluted with 2x 1mL of 5% cyclohexane. Each elution was collected in a separate vial, and rerun through the GC on the same temperature program as stated before.

The resulting peaks were analyzed as stated above, using a combination of linear retention indices and mass spectrometry/fractionation pattern. All alkanes were identified, but alkenes could only be identified to the number of carbons and not the position of the double bond.

In-Leaf Feeding Behavior

We measured the in-leaf feeding behavior of *M. persicae* using an electrical penetration graphing or electropenetrography technique (EPG), the main method since its invention in the 1960's (Walker, 2000). EPG creates an electrical circuit between the plant and the aphid which is completed every time the aphid penetrates the leaf (Tjallingii, 1978). As the aphid feeds, both the changes in resistance and in voltage are measured and recorded, and the resulting waveforms indicate certain behaviors: intracellular punctures, salivation, phloem ingestion, and xylem ingestion (Walker, 2000). The frequency, time and duration of each of these different feeding behaviors can be measured.

We used an 8-Giga DC EPG system to investigate how predator cues alter the in-leaf feeding behavior of aphids (Figure 5). Just before tethering, we collected 15-25 aphids with a paintbrush and placed them in a petri dish, to starve for around 30 minutes as we tethered them. These aphids were raised in cohorts on canola until they were 7-10 days old. To immobilize the aphid during tethering, we placed it on top of a 10 μl pipette tip connected to a gentle vacuum under a dissecting microscope. To tether *M. persicae* adults, we used 12.5μm diameter gold wire and water-based silver glue (recipe in Walker & Medina-Ortega, 2012), which was attached to the aphid's dorsal abdominal tergites (Figure 6). We rotated leaves to expose the abaxial side of the leaf, fixing leaves in place between a piece of foam and a microscope glass slide, held together by a rubber band (Figure 5). We then inserted the plant probes near the base of the canola stem to finish the plant set up in a Faraday cage. We randomly assigned tethered aphids to leaves with the

following treatments: control, lady beetle "footprints", fly "footprints", and coated with 2% mineral oil. Treatments were applied as described in the first section. Probing behavior was recorded for nine hours, but we only analyzed the first eight hours post-placement to allow for aphids to be replaced during the first hour if needed. We used Stylet+D to record the data and Stylet+A to annotate the different waveforms (EPG Systems, www.epgsystems.eu), and EPG-CALC 6.1 to generate the descriptive statistics of the data (Giordanengo, 2014). We focused on the following EPG parameters: time to the first probe, number and total duration of probing events, time spent in the pathway phase (C phase), number of cell punctures (potential drops pd), time to reach the phloem, number and total duration of both salivation and ingestion into and of phloem sap.

Statistical Analysis

All data were analyzed using R (Version 3.6.1) (R Core Team, 2019). We analyzed the choice test data using a generalized linear mixed model with a binary distribution of the number of aphids that made a choice at 24 hours, either the start or the other control canola leaf, and using date as a random effect. For the EPG data (the in-leaf feeding behaviors) we used: (1) a GLM with a gamma distribution, inverse link, for parameters of duration of certain behaviors (2) a negative binomial GLM for the number of times certain behaviors occurred because data was overdispersed, and (3) a Cox Proportional Hazard Model for the time to the first occurrence of certain behaviors. See appendix A for R code and packages used.

Results

Dispersal choice test: predation risk - H. convergens footprints

M. persicae responded to the predator treatments by increasing their dispersal (Figure 7, GLMM (binary distribution), N = 75, $F_{2,72}$= 20.896, $p < 0.0001$). The baseline dispersal was an average of 31%, (6.2 aphids out of 20) moving from a control *B. napus* "release" leaf to the untreated "choice" leaf. Relative to this baseline, significantly more aphids, (an average dispersal of 40%, 8 aphids out of 20) dispersed from leaves treated with *H. convergens* chemical footprints, (Tukey method, z = -4.28, $p < 0.0001$) or 2% mineral oil (average dispersal of 49%, 9.8 aphids out of 20) (Tukey method, z = -6.29, $p < 0.0001$). However, dispersal from mineral oil and predation risk treatments did not differ (Tukey method, z = 1.77, $p = 0.18$).

Dispersal choice test: insect positive control - D. melanogaster footprints

M. persicae dispersal varied across treatments (Figure 8, GLMM (binary distribution), N = 51, $F_{2,49}$ = 13.612, $p = 0.0002$). *M. persicae* dispersal from *B. napus* leaves treated with *D. melanogaster* chemical footprints (average of 21.1%, or 4.22 out of 20 aphids) was similar to dispersal from controls (average of 21%, 4.2 out of 20 aphids) (Tukey method, z = -1.15, $p = 0.4802$). As observed for the prior set of behavioral tests, there was increased dispersal of aphids from *B. napus* leaves treated with 2% mineral oil (average of 37%, 7.5 aphids out of 20) relative to control (Tukey method, mineral oil vs control: z = 4.89, $p < 0.001$). However, unlike the prior trials with *H. convergens*, significantly more aphids dispersed from leaves treated with 2% mineral oil relative to

those treated with *D. melanogaster* footprints (Tukey method, mineral oil vs. fly: z = 3.56, $p = 0.0012$).

GC analysis of chemical footprints

H. convergens and *D. melanogaster* footprints are composed of alkanes and alkenes; and some of the alkanes are shared between the two species but differ in their ratios, and the alkenes present in the footprints differ between the two species (Table 4).

In-leaf feeding behavior

Only one in-leaf feeding behavior of *M. persicae* feeding on *B. napus* differed between the treatments (leaves treated with *H. convergens* chemical footprints [predation risk], 2% mineral oil [positive control], *D. melanogaster* chemical footprints [insect positive control], or untreated [negative control]) (Tables 5 and 6). Specifically, the number of probes, i.e the number of times the aphid stylet pierced the leaf with its stylet (Table 5, GLM, negative binomial: $F_{3,81} = 2.812$, $p = 0.0378$), was increased by 13 additional probes on average in the 2% mineral oil treatment (Mean ± SE: 27.6 ± 4.4 number of probes) compared to the control (Mean ± SE: 14.2 ± 2.5 number of probes) (post-hoc Dunnett method, control vs. mineral oil treatment: z = 2.51, $p = 0.0355$). We observed a trend of an increase in the number of probes in the *H. convergens* chemical footprints treatment (Mean ± SE: 26.1 ± 5.2 number of probes) compared to the control (Mean ± SE: 14.2 ± 2.5 number of probes) (post-hoc Dunnett method, control vs. predation risk treatment: z = 2.33, $p = 0.0535$), an average of 12 additional probes. The

D. melanogaster chemical footprints treatment (Mean ± SE: 17.8 ± 4.0 number of probes) did not differ from the control treatment (Mean ± SE: 14.2 ± 2.5 number of probes) in the average number of probes (post-hoc Dunnett method, control vs. predation risk treatment: $z = 0.832$, $p = 0.719$). No statistical outliers were detected.

We also observed a trend of effect of treatment on total time spent probing, i.e time spent with stylet deposited in the leaf (Table 5, GLM, gamma distribution, inverse link: $F_{3,81} = 2.6906$, $p = 0.05167$). Both the *H. convergens* chemical footprints (Mean ± SE: 445.2 ± 8.3 minutes probing, post-hoc Dunnett method, control vs. predator, $z = 2.041$, $p = 0.108$) and 2% mineral oil treatments (Mean ± SE: 444.8 ± 6.3 minutes probing, post-hoc Dunnett method, control vs. mineral oil, $z = 2.068$, $p = 0.1015$) caused 15 minute reduction in the total time *M. periscae* spent probing in comparison to the control (Mean ± SE: 462.7 ± 4.1 minutes probing). The *D. melanogaster* footprint treatment did not differ from the control (Mean ± SE: 462.3 ± 4.3 minutes probing, post-hoc Dunnett method, control vs. predator, $z = 0.049$, $p = 0.9991$), and was nearly identical to the control in the average duration of the total time *M. persicae* spent probing. There were three statistical outliers, two in the predation risk treatment, and one in the mineral oil treatment; which if excluded cause a reduction in F-value and increase in p when looking at the effect of treatment on the total duration of the time *M. persicae* spent in the leaf (GLM, gamma distribution, $F_{3,78} = 2.0468$, $p = 0.1142$). There was a stronger trend of mineral oil treatment causing a reduction in time when compared to the control (Mean ± SE: 444.8 ± 5.1 minutes probing, post-hoc Dunnett method, control vs. mineral oil, $z = 2.161$, $p = 0.0817$). But the predator treatment no longer has a trend in

reduction when compared to the control (Mean ± SE: 455.2 ± 4.9 minutes probing, post-hoc Dunnett method, control vs. predator, z = 1.152, $p = 0.5136$).

There was no change in the time it took *M. persicae* to engage in its first probe, i.e., the time it took to enter the leaf, due to application of footprint or mineral oil treatments (Table 6, Cox Proportional Hazard Model). Time to first probe did not differ vs. controls for the *H. convergens* footprint treatment (Mean ± SE: 33.8 ± 9.6 seconds to first probe, CPH model, control vs. predator: HR =1.378, z = 1.058, $p = 0.29$), the 2% mineral oil treatment (Mean ± SE: 27.6 ± 7.2 seconds to the first probe, CPH model, control vs. mineral oil: HR = 1.581, z = 1.487, $p = 0.137$) or the *D. melanogaster* footprint treatment (Mean ± SE: 51.2 ± 16.0 seconds to the first probe, CPH model, control vs. fly: HR = 1.01, z = 0.032, $p = 0.975$). There were four statistical outliers included in the above analysis, one from each treatment, but their exclusion does not alter the statistical output: *H. convergens* footprint treatment (Mean ± SE: 25.9 ± 5.7 seconds to first probe, CPH model, control vs. predator: HR =1.4215, z = 1.131, $p = 0.258$), 2% mineral oil treatment (Mean ± SE: 21.6 ± 4.1 seconds to the first probe, CPH model, control vs. mineral oil: HR = 1.6556, z = 1.599, $p = 0.11$), and *D. melanogaster* footprint treatment (Mean ± SE: 39.6 ± 11.6 seconds to the first probe, control vs. fly: HR = 0.9861, z = -0.042, $p = 0.966$).

M. persicae did not alter the time it spent in the pathway phase (C phase), i.e. the stylet path prior to phloem contact due to any of the treatments (Table 5, GLM, gamma distribution, inverse link: $F_{3,81} = 0.3961$, $p = 0.7561$). Time to first probe did not differ vs. controls for *H. convergens* footprint treatments (Mean ± SE: 122.9 ± 17.1 minutes in

pathway phase, post-hoc Dunnett method, control vs. predator, z = -1.073, $p = 0.5648$), 2% mineral oil treatments (Mean ± SE: 107.8 ± 12.2 minutes in pathway phase, post-hoc Dunnett method, control vs. mineral oil, z = -0.459, $p = 0.912$), or *D. melanogaster* footprints (Mean ± SE: 109.3 ± 20.4 minutes in pathway phase, post-hoc Dunnett method, control vs. fly, z = -0.519, $p = 0.8875$). There were no statistical outliers detected.

The number of potential drops, i.e. the number of intracellular punctures of the mesophyll cells by the aphid's stylet, was not altered by any of the experimental treatments (Table 5, GLM, negative binomial, inverse link: $F_{3,81} = 0.2457$, $p = 0.8645$). Number of potential drops did not differ vs. controls for the *H. convergens* footprint treatment (Mean ± SE: 103.9 ± 13.3 number of potential drop, post-hoc Dunnett method, control vs. predator, z = 0.582, $p = 0.8583$), 2% mineral oil treatment (Mean ± SE: 110.1 ± 15.5 number of potential drop, post-hoc Dunnett method, control vs. mineral oil, z = 0.840, $p = 0.7139$), or *D. melanogaster* footprint treatment (Mean ± SE: 102.8 ± 15.8 number of potential drop, post-hoc Dunnett method, control vs. fly, z = 0.518, $p = 0.8879$). There were no statistical outliers detected.

The time it took *M. persicae* to reach the phloem was not affected by experimental treatments (Kaplan-Meier, Chisq=1.6, $p = 0.70$, N=81), (Mean ± SE minutes to reach the phloem: control: 78.0 ± 15.5; predator: 66.5 ± 12.3; mineral oil: 55.1 ± 8.0; and fly: 62.8 ± 15.7). There were two statistical outliers, one in control treatment and one in the fly treatment. When these outliers were removed, the data meet the proportionality assumption of the Cox Proportional Hazard Model, but excluding them

did not change the statistical output (Table 6, Cox Proportional Hazard Model): *H. convergens* footprint treatment (Mean ± SE: 66.5 ± 12.4 minutes to reach the phloem, CPH model, control vs. predator: HR = 1.073, z = 0.227, p = 0.821), 2% mineral oil treatment (Mean ± SE: 52.1 ± 8.0 minutes to reach the phloem, CPH model, control vs. mineral oil: HR = 1.405, z = 1.066, p = 0.287), and *D. melanogaster* footprint treatment (Mean ± SE: 51.3 ± 11.2 minutes to reach the phloem, CPH model, control vs. fly: HR = 1.411, z = 1.065, p = 0.287).

The total time that *M. persicae* spent salivating (E1) into the phloem did not differ due to experimental treatments (Table 5, GLM, gamma distribution, inverse link: $F_{3,81}$ = 1.1692, p = 0.3267). Relative to controls, *M. persicae* had the same E1 duration in the *H. convergens* footprint treatment (Mean ± SE: 4.2 ± 0.8 minutes spent salivating, post-hoc Dunnett method, control vs. predator, z = -0.317, p = 0.959), 2% mineral oil treatment (Mean ± SE: 5.7 ± 1.0 minutes spent salivating, post-hoc Dunnett method, control vs. mineral oil, z = -1.634, p = 0.2462), and *D. melanogaster* footprint treatment (Mean ± SE: 4.2 ± 0.5 minutes spent salivating, post-hoc Dunnett method, control vs. fly, z = -0.289, p = 0.9661). There were no statistical outliers detected.

The number of times *M. persicae* salivated into the phloem (E1) did not differ due to experimental treatments (Table 5 GLM, negative binomial, inverse link: $F_{3,81}$ = 0.8327, p = 0.4756). Relative to controls, aphid salivation events were similar for *H. convergens* footprint treatments (Mean ± SE: 7.182 ± 1.272 number of salivation events into the phloem, post-hoc Dunnett method, control vs. predator, z = 0.239, p = 0.977), 2% mineral oil treatment (Mean ± SE: 9.7 ± 1.8 number of salivation events into the phloem,

post-hoc Dunnett method, control vs. mineral oil, z = 1.455, p = 0.3339), and *D. melanogaster* footprint treatment (Mean ± SE: 7.9 ± 1.5 number of salivation events into the phloem, post-hoc Dunnett method, control vs. fly, z = 0.616, p = 0.8412). There were no statistical outliers detected.

The total time that *M. persicae* spent ingesting phloem sap (E2) did not differ due to experimental treatments (Table 5, GLM, gamma distribution, inverse link: $F_{3,81}$ = 0.4236, p = 0.7366). Relative to control, aphid phloem sap ingestion were similar for *H. convergens* footprint treatment (Mean ± SE: 309.8 ± 26.2 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. predator, z = 1.016, p = 0.6017), 2% mineral oil treatment (Mean ± SE: 320.6 ± 17.5 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. mineral oil, z = 0.655, p = 0.8209), and *D. melanogaster* footprint treatment (Mean ± SE: 336.3 ± 25.8 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. fly, z = 0.169, p = 0.9888). There were two statistical outliers detected, one in the predator treatment and one in the fly treatment, but their exclusion does not alter the statistical output (GLM, gamma distribution, $F_{3,79}$ = 0.4895, p = 0.6906): *H. convergens* footprint treatment (Mean ± SE: 324.3 ± 22.8 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. predator, z = 0.615, p = 0.8417), 2% mineral oil treatment (Mean ± SE: 320.6 ± 17.5 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. mineral oil, z = 0.75, p = 0.7681), and *D. melanogaster* footprint treatment (Mean ± SE: 351.7 ± 21.8 minutes spent ingesting phloem sap, post-hoc Dunnett method, control vs. fly, z = -0.319, p = 0.9585), and control (Mean ± SE: 342.0 ± 20.3 minutes spent ingesting phloem sap).

The number of times *M. persicae* ingested phloem sap (E2) did not differ by experimental treatments (Table 5, GLM, negative binomial, inverse link: $F_{3,81} = 1.1211$, $p = 0.3389$): *H. convergens* footprint treatment (Mean ± SE: 6.2 ± 1.1 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. predator, z = 0, $p = 1.0$), 2% mineral oil treatment (Mean ± SE: 9.1 ± 1.7 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. mineral oil, z = 1.558, $p = 0.2816$), and *D. melanogaster* footprint treatment (Mean ± SE: 7.4 ± 1.4 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. fly, z = 0.694, $p = 0.7999$). There was one statistical outlier, one in the fly treatment (different from the fly treatment outlier in the total time of ingestion of phloem sap), but its exclusion did not alter the statistical output (GLM, negative binomial, $F_{3,80} = 1.3212$, $p = 0.2654$): *H. convergens* footprint treatment (Mean ± SE: 6.2 ± 1.1 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. predator, z = 0, $p = 1.0$), 2% mineral oil treatment (Mean ± SE: 9.1 ± 1.7 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. mineral oil, z = 1.607, $p = 0.2586$), and *D. melanogaster* footprint treatment (Mean ± SE: 6.3 ± 0.9 number of phloem sap ingestion events, post-hoc Dunnett method, control vs. fly, z = 0.023, $p = 0.9998$).

The time it took *M. persicae* to reach the sustained feeding (defined as ten or more minutes of continuous phloem sap ingestion) was not affected by experimental treatments (Table 6, Cox Proportional Hazard Model): *H. convergens* footprint treatment (Mean ± SE: 112.8 ± 23.4 minutes to reach sustained ingestion, CPH model, control vs. predator: HR = 0.6911, z = -1.199, $p = 0.231$), 2% mineral oil treatment (Mean ± SE:

61.0 ± 8.4 minutes to reach sustained ingestion, CPH model, control vs. mineral oil: HR = 1.356, z = 0.974, $p = 0.330$), and *D. melanogaster* footprint treatment (Mean ± SE: 88.5 ± 21.0 minutes to reach sustained ingestion, CPH model, control vs. fly: HR = 0.8555, z= -0.496, $p = 0.620$). There were four statistical outliers, one from control treatment, two from the fly treatment, and one from the predator treatment, which was the only one out of 85 replicates not to reach sustained ingestion of phloem sap in eight hours. The exclusion of these statistical outliers did not alter the statistical output does not change: *H. convergens* footprint treatment (Mean ± SE: 95.4 ± 16.4 minutes to reach sustained ingestion, CPH model, control vs. predator: HR = 0.6572, z = -1.323, $p = 0.186$), 2% mineral oil treatment (Mean ± SE: 61.0 ± 8.4 minutes to reach sustained ingestion, CPH model, control vs. mineral oil: HR = 1.2747, z = 0.768, $p = 0.442$), *D. melanogaster* footprint treatment (Mean ± SE: 63.3 ± 12.8 minutes to reach sustained ingestion, CPH model, control vs. fly: HR = 1.129, z= 0.327, $p = 0.744$).

Discussion

Non-consumptive effects (NCE) are well documented across a variety of systems (Lima and Dill, 1990; Lima, 1998; Tollrian & Harvell, 1999), but are vastly understudied for sap-feeding insects, especially in regards to feeding behavior (Buchanan, *et al*., 2017; Table 2). Sap-feeding insects are global pests due to their ability to transmit plant pathogens, including plant-viruses. Transmission is a direct result of dispersal and feeding behaviors, which may be influenced by predation risk. Therefore, consideration of NCE of sap-feeders may lead to better management practices, especially with regard

to optimal use of biological controls (Prado and Tjallingii, 1994; Martin, *et al.*, 1997; Fereres and Moreno, 2009; Finke, 2012). One group of vectors, the aphids, is particularly understudied, despite transmitting 50% of all known insect-vectored plant-viruses (Hogenhout, *et al.,* 2008). This thesis tested the hypothesis that aphid in-leaf feeding and dispersal would change in response to predator chemical cues, indicating predation risk.

We found that *Myzus persicae* do increase their dispersal from areas treated with lady beetle "footprints", a known chemical cue indicating predation risk. But *M. periscae* do not change their feeding behavior when forced to feed on a leaf treated with lady beetle "footprints". We tested the alternative hypotheses that the plant was being primed or the aphids would respond to the presence of any other organism by using fly "footprints" as the positive insect control. *M. persicae* did not increase their dispersal or feeding behavior in response to these fly "footprints", therefore we reject the alternative hypotheses. We hypothesize that the different responses to lady beetle "footprints" than fly "footprints" are due to the differences in chemical composition of the footprints which we identified with a GC-MS.

We found that the composition of the lady beetle "footprints" differs from the fly "footprints" (Table 4). There are more components in the fly "footprints" than the lady beetle "footprints", though each has unique components, such as nonacosene in lady beetle "footprints". While some of the alkenes detected were of the same length between the two species, they had different retention indices, indicating different composition (i.e. the position of the double bond is different). Every detected alkane in lady beetle

"footprints" was also present in the fy "footprints": tricosane, pentacosane, and heptacosane.

We found similar components in lady beetle "footprints" as those found by Wheeler and Carde (2014) in diapausing *H. convergens*, but they detected more components (Table 7). All alkanes we detected were also detected by Wheeler and Carde in 2014. Also many of the alkenes of similar lengths were detected by both of us, but Wheeler and Carde identified more components overall, especially dienes of longer length. These differences between their results and ours may be due to differing methods of establishing the footprints, such as a longer period and a greater number of beetles used to deposit footprints. Thus, their methodology may have allowed detection of more components in low abundance. Additionally, use of a wild-collected population of *H. convergens* may differ from the commercially available populations used for our study, because different populations have slightly different cuticular ratios or components (Jallon and David, 1987). Furthermore, the diets differed, as Wheeler and Carde did not feed their lady beetles, while we did. Difference in diet can change the cuticular hydrocarbon composition (Rojas, *et al*., 2005; Fedina, *et al*., 2012). Finally, Wheeler and Carde used *H. convergens* that were diapausing, which may alter their cuticular hydrocarbon profile by altering the amount (Benoit and Delinger, 2007) or composition of cuticular hydrocarbons (Kaneko and Katagiri, 2004).

We found comparable components in fly footprints as did Jallon and David, who identified the cuticular hydrocarbons of several species of fruit flies, including from three distinct populations of *D. melanogaster* (1987) (Table 8). The results of our study and

theirs identified some of the same alkenes and alkanes like, tricosene, tricosane, pentacosene, pentacosane, heptacosene and heptacosane. However, there were some differences between our results and theirs. We found shorter alkanes in our results, while Jallon and David identified some branched alkanes that we did not detect. These differences between their study and ours may be due to Jallon and David (1987) use of only 7-day-old virgins adults, and hydrocarbon profiles can change over time with age (Kuo, *et al.*, 2012) and mating status (Everaerts, *et al*., 2010). The difference may also be due to the fact that Jallon and David studied cuticular hydrocarbons rather than footprints because flies produce a specialized adhesive substance secreted by pulvilli, the pads on between the tarsal claws (Bauchhenß, 1979). Additionally, our collections of fly footprints may have contained hydrocarbons from eggs and trace chemicals from the diet. Although we took efforts to remove the trace chemicals from diet before experiments by having flies walk on wet filter paper for an hour to wash their feet.

Our dispersal bioassay results are congruent with other studies of aphid behavior under predation risk. We found that *M. persicae* increased their dispersal in response to *H. convergens* chemical "footprints" treatment" and to 2% mineral oil treatment when compared to the baseline dispersal of the control (Fig A). This indicates that predation risk applied in this experiment is as effective a repellent as a known feeding deterrent of aphids, a stylet blocker. This is likely a predator specific response because fly "footprints" did not increase aphid dispersal. Therefore, we can reject the alternative hypotheses that the plant was being primed when being walked upon, or that the response of increased dispersal was not a predator specific response, but one to any organism.

Our results are congruent with other studies that found that aphids do increase their avoidance or dispersal. Ninkovic et. al found that bird cherry oat aphid avoids areas treated with lady beetle "footprints", but did not use plant cues, instead applying the treatment to the surface of petri dish (2013). Kersch-Becker and Thaler (2015) found that the potato aphid increases dispersal between plants in response to risk predators (manipulated so they are non-lethal) on low and medium resistance plants, demonstrating that predator disturbance facilitates aphid dispersal through non-consumptive effects. Non-consumptive effects of predators have also been shown to cause increased aphid dispersal even when both consumptive and non-consumptive effects are present. Roitberg and Meyers measured increased aphid dispersal on plants in arenas in the presence of fully functional lady beetles (1978).

Increased dispersal of vectors may lead to increased virus spread (Madden, *et al.*, 2000; Dader, *et al*., 2012; Shaw, *et al*., 2017). In the Roitberg and Meyers paper, they also measured the spread of the Bean Yellow Mosaic Virus (BYMV), which increased in the presence of predators (1978). Though Dader, *et al.*, found that parasitoids caused *A. gossypii* aphids to disperse more, and linked it to greater Cucumber Mosaic Virus spread using the Spatial Analysis by Distance Indices, SADIE method (2012). Theoretical papers have supported this experimental evidence of increased vector dispersal leading to greater virus spread as most plant viruses are obligately transmitted and spread by their vectors (Madden, *et al.*, 2000; Hull, 2002; Shaw, *et al*., 2017).

We only tested the dispersal of apterous (wingless) aphids at a small scale. Therefore, this is only the beginning of a series of studies needed to test non-consumptive

effects in isolation on aphid dispersal and experimentally link that dispersal with virus spread. Both apterous and alates (wingless and winged aphids) must be tested since each have different categories of dispersal.

Apterous aphids account for the majority of aphid dispersal, which occurs within a plant because of their lack of wings, or between neighboring plants. In contrast, alate aphids account for long-distance dispersal and the founding individual in crop fields. We hypothesize that alates and apterous aphids may have different responses because they differ in their fecundity; alates produce fewer offspring because more resources are spent on wing and flight muscle production (Awmack and Leather, 2007) and differ in the sensitivity of their taste receptors (Pettersson, *et al*., 2007).

The results of our aphid in-leaf feeding experiments differed from previous studies looking at how sap-feeders change their feeding behavior under predation risk (Seo, *et al*., 2018; Tholt, *et al*., 2018). We found no change in any of the measured parameters of in-leaf feeding behavior in response to predator cues. The in-leaf feeding behaviors we measured included the time to the first probe, the number of probes, the total time spent in the pathway phase, the number of potential drops, time to reach the phloem, total time spent salivating into the phloem, and total time spent ingesting the phloem sap. Because we relied on live lady beetles to walk to establish the "footprints", we cannot guarantee uniform distribution on the leaf. Our results may have been significant if we had used a whole predator to establish predation risk rather than isolating a chemical cue, like Tholt *et al*. (2018) did in their leafhopper feeding behavior under predation risk study. Tholt *et al.* (2018) found that leafhoppers are half as likely to

reach the phloem when exposed to a predator, and for those that do, they have reduced salivation into the phloem. We propose one difference in Tholt and our results may be due to the perceived level of predation risk. Some have shown that prey can modulate their response based on the perceived level of risk (Lima, 1998; Wiackowski, & Staronska, 1999; Lima and Steury, 2005). Seo, *et al*. (2018) also found reduced feeding of psyllids in response to lady beetle "footprints" when they measured the number of honeydew droplets produced as a proxy for the amount of sap ingested. Their experimental set up allowed the psyllids to "escape" the leaf discs treated with lady beetle "footprints" to untreated agar, unlike the aphids in our experiment which were tethered and unable to move off the treated area. This difference in experimental design may account for changes in feeding.

How a vector feeds will determine the acquisition and inoculation of a plant virus (Prado and Tjallingii, 1994; Martin, *et al.*, 1997; Fereres and Moreno, 2009; Finke, 2012). This is because there are different types of viruses, grouped on how they are acquired and transmitted (Hogenhout, *et al*., 2008). Aphids transmit plant viruses in a non-persistent and persistent manner (Hogenhout, *et al.*, 2008). Non-persistent plant viruses are acquired and inoculated by short probes of the stylet into mesophyll or epidermal cells (and associated sampling of cell contents) (Martin, *et al.,* 1997; Wang & Ghabrial, 2002). The virus particles attach to the aphid's stylet and are then transferred to a new host plant when the aphids feed in a similar manner (Martin, *et al.*, 1997). This transfer must occur within minutes to hours, with diminishing probability of transmission, between host plants (Hogenhout, *et al.*, 2008). In contrast, persistent viruses

are acquired and inoculated when the aphid has long feeding bouts in the phloem, typically for hours or days (Mauck, *et al.*, 2012; Hogenhout, *et al.*, 2008). Persistent viruses must pass through the midgut and travel through the hemolymph to the salivary glands, before the aphid can inoculate a new plant with the virus (Hogenhout, *et al.*, 2008). Using EPG, different feeding behaviors have been linked to acquisition and transmission of an insect-vectored plant virus, both for persistent and non-persistent viruses (Martin, et al., 1997; Prado & Tjallingii, 1994). For persistent viruses, ingestion in the sieve element results in virus acquisition and salivation into the sieve element for inoculation (Prado & Tjallingii, 1994).

Feeding behavior was not changed by the lady beetle "footprint" treatment. Thus, we can predict that predator chemical cues will not change persistent virus spread through this manner. Non-persistent virus transmission and acquisition occurs when the stylet is in the interior of the cells, called potential drops, and happens during the first subphase and third subphase respectively (Martin et al., 1997). This behavior also did not change due to treatment, and therefore we can hypothesize that the transmission of non-persistent viruses will not change either when aphids are exposed to predator chemical cues.

This is the first study, to our knowledge, that investigates the in-leaf feeding behavior of aphids under predation risk, which is a research gap because aphids transmit 50% of known insect-vectored plant viruses, and transmit both persistent and non-persistent viruses. This is a research gap that needs to be understood, and this is only at the start. Further studies need to be conducted that investigate multiple predator cues

and different aphid species. First, it is necessary to see if a whole predator, which might be perceived by the aphid as higher risk, may elicit changes in feeding behavior. For any modeling or more accurate predictions to be made, the distance an aphid will disperse when they encounter a predator or a predator chemical cue should be experimentally determined. Finally, experimentally linking vector dispersal and virus spread, under predation is needed since most studies have not isolated non-consumptive effects and investigated virus spread experimentally.

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Table 1: These are selected examples that describe some of the breadth of taxa where non-consumptive effects have been studied.

Study	Habitat	Predator Type	Prey Type	Description
Tigreros, *et al.,* 2017	Terrestrial	Stink Bugs (Pentatomidae)	Leaf Beetle (Chrysomelidae)	*L. decemlineata* females exposed to the presence of *P. maculiventris* produce more non-viable eggs to facilitate sibling cannibalism in their offspring.
Fischer, *et al.*, 2012	Aquatic	Backswimmers (Notonectidae)	Mosquitos (Culicidae)	*C. pipiens* have reduced development rates, smaller adult sizes and lower fecundity when they are raised as larvae in the presence of their predator *N. sellata*
Breviglieri and Romero, 2016	Terrestrial	Artificial Snakes	Tanagers (Birds) (Thraupidae)	Artificial snakes reduce fruit collection from the tree *M. nigra* by *T. seledon.*
Petrusek *et al*., 2009	Aquatic	Tadpole Shrimp (Triopidae)	Water Fleas (Cladocera)	*D. atkinsoni* induce anti-predator trait, called the "crown of thorns" in response to the chemical cues from its predator *T. cancriformis*, which increases its survival in the presence of predators.
Shelly and Werner, 1990	Aquatic	Darner (Dragonfly) (Aeshnidae)	True Toad (Bufonidae)	Tadpoles of the species *A. americanus* (formally known as and named in this paper as *B. americanus*) reduce its metamorphosis size, likely due to changes in behavior such as reduced feeding, when raised with *A. junius*.

Table 2: Comparison of the number of experiments in different categories of non-consumptive effects between terrestrial piercing-sucking, sap-sucking and chewing insect prey based on the data that Buchanan, *et al.*, 2017 meta-analysis compiled. The different categories are: activity (i.e. migration from and immigration to resource, speed, movement, or preference for preferred host), fecundity (i.e. number of eggs or offspring produced), feeding (i.e. time spent feeding, feeding rate (amount/time), or assimilation efficiency), and growth (i.e. mass, size, or growth rate). In the highlighted row, feeding is well studied comparatively compared to other non-consumptive effects categories in chewing insect prey, but there are no studies of changes in feeding behavior in piercing-sucking, sap-feeding prey.

	Number of Experiments based on Prey Feeding Mode	
NCE Category	**Piercing-Sucking, Sap-Feeding Prey**	**Chewing Prey**
Activity	6	5
Fecundity	3	4
Feeding	0	19
Growth	1	5

Table 3: Here are some studies that link natural enemies with changes in viral spread. Dader *et al*., link vector spread induced by predator presence with increased viral spread (2012).

Study	Natural Enemy Type	Increased Virus Spread	Increased Vector Dispersal	Description
Roitberg and Meyers, 1978	Predator	Yes	Yes	Found that the aphid *A. pisum* increases their dispersal when in the presence of the predator lady beetle *C. california* and there was greater virus spread of the virus Bean Yellow Mosaic Virus in the predator treatments.
Dader, *et al*., 2012	Parasitoid	Dependent on virus type and time	Yes	Found that *A. gossypii* does increase its dispersal in the presence of the parasitoid *A. colemani*, and linked this dispersal through the SADIE method to the spread of the non-persistent virus Cucumber Mosaic Virus, in the short time period of 2 days.The parasitoid reduced the spread in the long term of the persistent virus Cucurbit aphid-borne yellows virus (discussion of non-persistent and persistent viruses can be found in the discussion of this thesis)
Long and Finke, 2015	Predator	No	Yes	While predators reduced the prevalence of the virus Cereal yellow dwarf virus strain RPV (persistent virus), predators led to greater interplant movement of aphid *R. padi*. This study did not study the link between aphid movement and virus spread.

Figure 1: The fluon-coated clip cage used to contain the insects on the leaf and ensure the establishment of the footprints. It is composed of a hair clip (Walmart 6 pack of Goody Hair clips), the bottom of a small petri dish, and a small plastic cup with a 4 cm diameter at the top and 4 cm tall, then fluon coated on the inside to prevent insects from climbing. During preliminary experiments, we observed 90% of the lady beetles to be on the leaf using this method, with the remaining 10% on top of their compatriots rather than the leaf. Of these, 25% were observed in motion at two-minute intervals during the first hour.

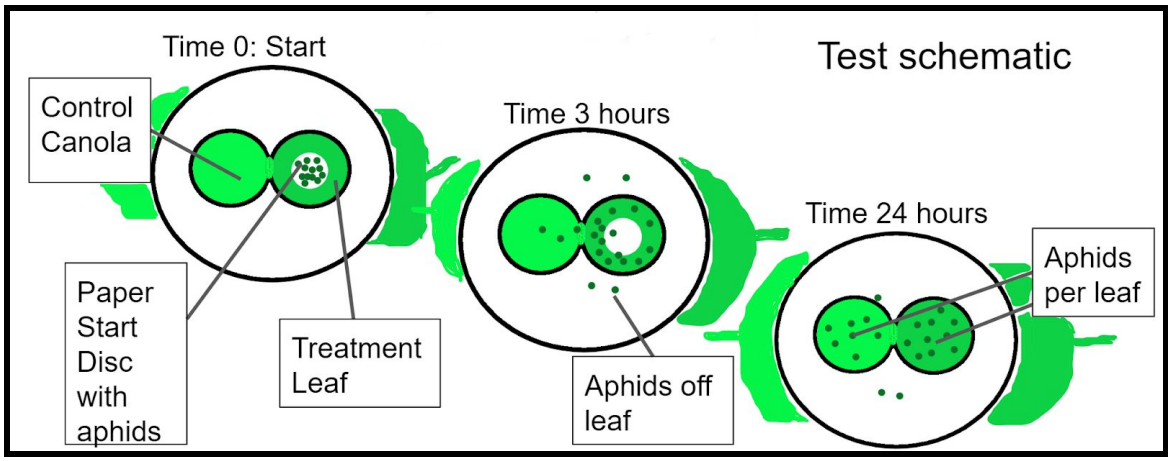

Figure 2: Schematic of the bioassay dispersal test. A filter paper disc with 20 adult *M. persicae* was placed on the treated leaf so the aphids had to contact the treatment when they left the disc, before they could move to the control leaf. The treatments are (1) control canola, (2) lady beetle footprints, (3) 'clean' fly footprints, and (4) mineral oil. The aphids on each leaf were counted at the following intervals: 15, 30, 45 and 60 minutes; and 3, 6 and 24 hours.

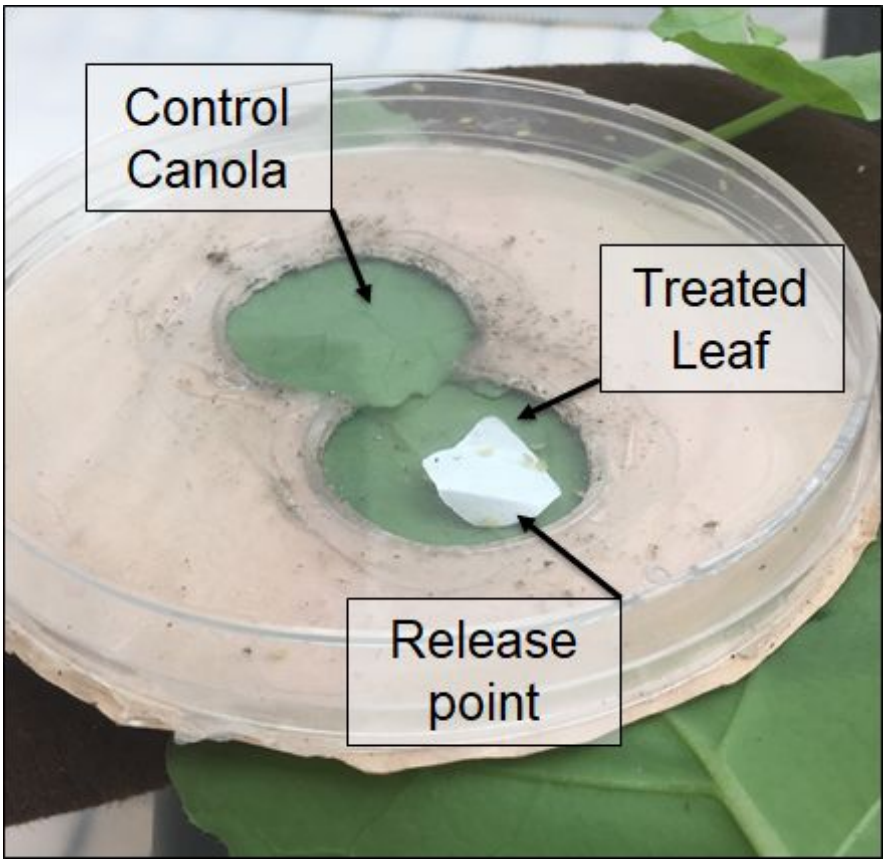

Figure 3: Petri dish dispersal bioassay arenas

Figure 4: Two leaves from each plant were used, and one going to its own choice test arena. The choice test experiments were enclosed by white poster board to create a uniform background.

Figure 5: 8-channel EPG set up with canola plants.

Figure 6: Tethered adult *M. persicae* aphid.

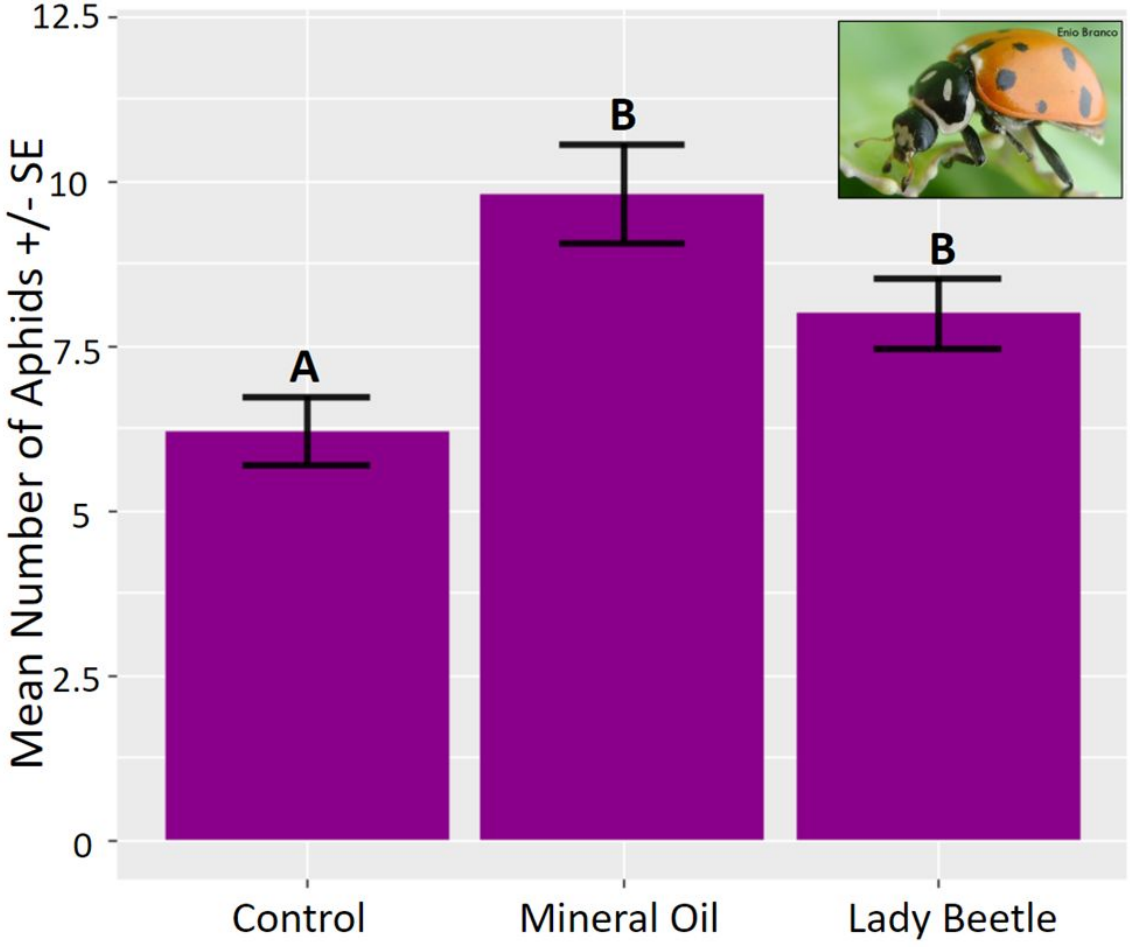

Figure 7: Dispersal of *M. persicae* in response to *H. convergens* footprints. After 24 hours, significantly more aphids have dispersed from both footprint-treated leaves and mineral oil treated leaves relative to untreated leaves. Different letters indicate significance level of $p < 0.0001$.

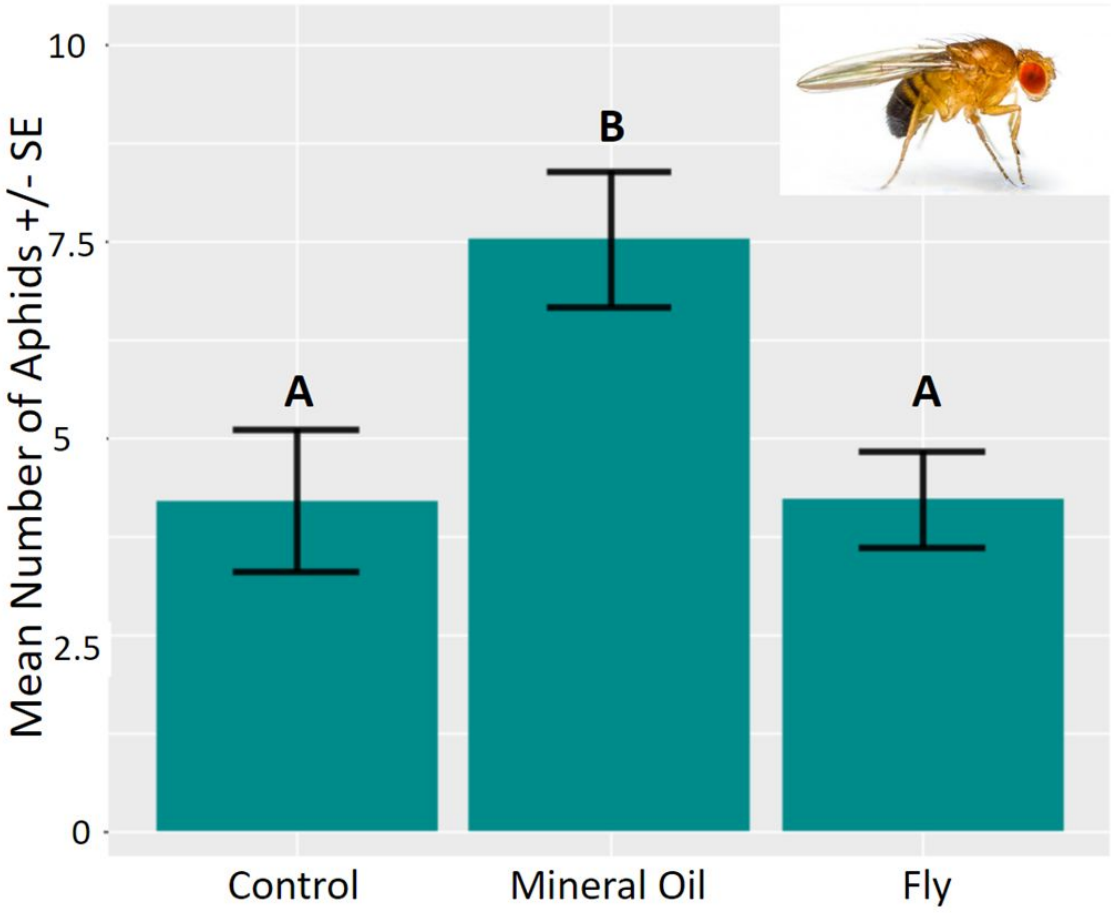

Figure 8: Dispersal of *M. persicae* in response to *D. melanogaster* footprints. *M. persicae* do not have increased dispersal from leaves with *D. melanogaster* footprints compared to the control Different letters indicate significance, $p < 0.01$.

Table 4: The chemical components of the chemical "footprints" of both *Hippodamia convergens* and *Drosophila melanogaster*.

Chemical Identification & Properties				Percent Abundance	
Chemical name	**Chemical Formula**	**KI**	**Mass (m/z)**	***H. convergens* Footprints**	***D. melanogaster* Footprints**
Heneicosane	C21H44	2100	296		1.49%
Docosene	C22H44	2180	308		0.74%
Docosane	C22H46	2200	310.2		0.56%
Tricosene (1)	C23H46	2274	322		3.78%
Tricosene (2)	C23H46	2282	322		40.0%
Tricosene (3)	C23H46	2292	322		4.81%
Tricosane	C23H48	2298	324	13.0%	8.04%
Pentacosadiene	C25H48	2463	348		2.90%
Pentacosene (1)	C25H50	2474	350		1.45%
Pentacosene (2)	C25H50	2483	350		20.16%
Pentacosene (3)	C25H50	2485	350	7.81%	
Pentacosene (4)	C25H50	2492	350		1.37%
Pentacosane	C25H52	2499	352	9.67%	1.62%
Heptacosene (1)	C27H54	2663	377		11.21%
Heptacosene (2)	C27H54	2678	378	34.5%	
Heptacosane	C27H56	2699	380	10.1%	0.45%
Unknown		2863	294		3.43%
Nonacosene	C29H58	2875	406	25.0%	

Legend	Unique *D. melanogaster*	Similar Length Alkenes
	Unique *H. convergens*	Shared Alkanes

Table 5: Feeding behavior variables (mean ± standard error (p-value of comparison to control treatment)) of *M. persicae* on *B. napus* in response to *H. convergens* chemical footprints (Predator FP), and 2% Mineral Oil, and flightless *D. melanogaster* footprints (Fly FP). Italicized means and standard errors indicate trends (p-values between 0.05 and 0.15) and bolded means and standard errors indicate significant results (p-values < 0.05). Total time of different behaviors were analyzed using a GLM, Gamma distribution, with an inverse link. The number of events for different behaviors were all over dispersed, and therefore a negative binomial GLM was used to analyze this data.

Feeding Behavior	**Control** (n=22)	**Predator FP** (n=22)	**Mineral Oil** (n=21)	**Fly FP** (n=20)	**F Statistic**
Total Time of Probing Behavior (minutes)*	462.7 ± 4.140	*445.2 ± 8.340* (*p* = 0.108)	444.8 ± 6.275 (*p* = 0.1015)	462.3 ± 4.326 (*p* = 0.9991)	$F_{3,81}$= 2.6906 (*p* = 0.05167)
Number of Probes (#)	14.23 ± 2.517	26.18 ± 5.216 (*p* = 0.0535)	**27.62 ± 4.427 (*p* = 0.0355)**	17.80 ± 4.031 (*p* = 0.719)	**$F_{3,81}$= 2.812 (*p* = 0.0378)**
Total Time of Pathway Phases (C) (minutes)	97.71 ± 15.99	122.9 ± 17.06 (*p* = 0.5648)	107.8 ± 12.24 (*p* = 0.912)	109.3 ± 20.37 (*p* = 0.8875)	$F_{3,81}$= 0.3961 (*p* = 0.7561)
Number of Potential Drops (Intracellular punctures) (#)	91.55 ± 16.29	103.9 ± 13.34 (*p* = 0.8583)	110.1 ± 15.45 (*p* = 0.7139)	102.75 ± 15.8 (*p* = 0.8879)	$F_{3,81}$= 0.2457 (*p* = 0.8645)
Total Time of Salivation into Phloem Sap (E1) (minutes)	3.889 ± 0.581	4.181 ± 0.779 (*p* = 0.959)	5.728 ± 1.028 (*p* = 0.2462)	4.161 ± 0.5433 (*p* = 0.9661)	$F_{3,81}$= 1.1692 (*p* = 0.3267)
Number of Salivation Events (E1) (#)	6.773 ± 1.227	7.182 ± 1.272 (*p* = 0.0977)	9.667 ± 1.843 (*p* = 0.3339)	7.900 ± 1.480 (*p* = 0.8412)	$F_{3,81}$= 0.8327 (*p* = 0.4756)
Total Time of Ingestion of Phloem Sap (E2) (minutes)*	342.0 ± 20.28	309.8 ± 26.20 (*p* = 0.6017)	320.6 ± 17.54 (*p* = 0.8209)	336.3 ± 25.80 (*p* = 0.9888)	$F_{3,81}$= 0.4236 (*p* = 0.7366)
Number of Ingestion Events (E2) (#)*	6.227 ± 1.149	6.227 ± 1.063 (*p* = 1.0)	9.095 ± 1.722 (*p* = 0.2816)	7.400 ± 1.437 (*p* = 0.7999)	$F_{3,81}$= 1.1211 (*p* = 0.3389)

* Indicates that there were statistical outliers, which were not removed for analysis included in this statistical analysis. The inclusion or exclusion of these statistical outliers did not alter significance below p-values < 0.05. Results of these statistical tests with the statistical outliers removed will be reported in the body of the text to be thorough.

Table 6: The time (minutes) to the first feeding behavior events (mean ± standard error, HR (Hazard Ratio), z, and *p*) of *M. persicae* on *B. napus* in response to *H. convergens* chemical footprints (Predator FP), and 2% Mineral Oil, and flightless *D. melanogaster* footprints (Fly FP). Italicized means and standard errors indicate trends (*p* between 0.05 and 0.15) and bolded means and standard errors indicate significant results ($p < 0.05$). The data was analyzed using a Cox Proportional Hazard Model, a type of survival curve, which can be applied to an event.

Feeding Behavior	**Control** (n=22)	**Predator FP** (n=22)	**Mineral Oil** (n=21)	**Fly FP** (n=20)
Time to First Probe (sec) *	42.18 ± 8.91	33.78 ± 9.566 HR =1.378 z ratio = 1.058 *p* = 0.29	27.60 ± 7.168 HR = 1.581 z ratio = 1.487 *p* = 0.137	51.24 ± 16.00 HR = 1.01 z ratio = 0.032 *p* = 0.975
Time to Reach Phloem (min)**	69.34 ± 13.5	66.53 ± 12.35 HR = 1.073 z ratio = 0.227 *p* = 0.821	52.12 ± 7.95 HR = 1.405 z ratio = 1.066 *p* = 0.287	51.29 ± 11.22 HR = 1.411 z ratio = 1.065 *p* = 0.287
Time to Reach Sustained Ingestion (>10 min) of Phloem Sap (sE2) (min)*	82.28 ± 15.6	112.81 ± 23.43 HR = 0.6911 z ratio = -1.199 *p* = 0.231	61.02 ± 8.40 HR = 1.356 z ratio = 0.974 *p* = 0.330	88.51± 20.95 HR = 0.8555 z ratio= -0.496 *p* = 0.620

* Indicates that there were statistical outliers, which were not removed for analysis included in this statistical analysis. The inclusion or exclusion of these statistical outliers did not alter significance below *p*-values < 0.05. Results of these statistical tests with the statistical outliers removed will be reported in the body of the text to be thorough. But since the outlier identity was inconsistent among related behavior parameters (meaning that say replicate number 15, 37, and 97 were statistical outliers for behavior A, were not outliers for a related behavior B), were the most representative statistical analysis due to their was no justification to exclude any of the replicates.

** Indicates that the 2 statistical outliers were removed from this test because with the inclusion of the statistical outliers, the assumption of proportionality was violated. A Kaplan-Meier test was also run with the outliers included, and the effect of treatment on the time it took to reach the phloem was non-significant (p=0.7)

Table 7: The comparison to our results of the chemical components of the chemical "footprints" *Hippodamia convergens* to that of the "footprints" identified by Wheeler and Carde (2014), from that of diapausing *H. convergens*. When there are multiple alkenes of the same length (but different position of the double bonds), the number of different alkenes are listed in order in parentheses, next to the chemical name, in order of Percent Abundance columns from left to right. Then the percent abundance is given for each different alkene of the same length are listed in order of increasing retention times.

Chemical Identification & Properties		**Percent Abundance**	
Chemical name	**Chemical Formula**	**Wheeler & Carde, 2014**	**Norris, *et al***
Tricosane	C23H48	6.20%	13.0 %
Tetracosane	C24H50	0.16%	
Pentacosene (1, **3**)	C25H50	2.61%*	7.81%
Pentacosane	C25H52	3.05%	9.67%
Hexacosane	C26H54	0.26%	
Heptacosene (2, 1)	C27H54	0.31%, 10.3%*	34.5%
Heptacosane	C27H56	4.0%	10.01%
Octacosene	C28H56	0.63%	
Nonacosadiene	C29H56	1.69%**	
Nonacosene (2, 1)	C29H58	5.38%, 18.9%	25%
Triacontadiene	C30H58	0.39%	
Hentriacontadiene	C31H60	13.6%	
Hentriacontene	C31H62	10.5%**	
Dotriacontadiene	C32H62	0.39%	
Tritriacontadiene (2)	C33H64	6.87%, 13.1%	
Pentatriacontadiene (2)	C35H54	1.04%, 0.70%	

*Alkene longer retention time than the alkane of the same length
**Diene longer retention time than the alkenes of the same length

Legend	Unique Wheeler & Carde	Similar Length Alkenes & Dienes
		Shared Alkanes

Table 8: The comparison to our results of the chemical components of the chemical footprints *Drosophila melanogaster* to that of the cuticular hydrocarbons identified by Jallon and David (1987), from the Canton-S laboratory strain of *D. melanogaster* (see paper for specifics). Percent abundance is given for each component with a percent except for the "+" which indicates trace amounts detected by Jallon and David (1987). When there are multiple alkenes of the same length (but different position of the double bonds due to different peaks), the number of different alkenes are listed in order in parentheses, next to the chemical name, in order of Percent Abundance columns from left to right. Then the percent abundance is given for each different alkenes of the same length are listed in order of increasing retention times. Table continued on the next page, legend for the table is on the next page too.

Chemical Identification & Properties		**Percent Abundance**		
		Jallon and David, 1987 Cuticular Hydrocarbons		**Norris *et al.* Footprints**
Chemical name	**Chemical Formula**	**Male**	**Female**	**Male and Female**
Heneicosane	C21H44			1.49%
Docosene	C22H44			0.74%
Docosane	C22H46			0.56%
Branched Tricosane	Unspecified		+	
Tricosene (3, 2, 3)	C23H46	4%, 47.7%, 2.8%	+, 3.5%	3.78%, 40.0%, 4.81%
Tricosane	C23H48	10.5%	5.7%	8.04%
Pentacosadiene	C25H48		2.4%	2.90%
Branched Pentacosane	Unspecified	7.2	3.1%	
Pentacosene (2, 3, 3)	C25H50	+, 10.8%	7.1%, 7.1%, 0.5%	1.45%, 20.16%, 1.37%
Pentacosane	C25H52	2.2%	5.2%	1.62%
Heptacosadiene			28.0%	
Branched Heptacosene	Unspecified	7.9%	12.2%	
Heptacosene (1, 1, 1)	C27H54	+	4.7%	11.21%

Table 8 continued: The comparison to our results of the chemical components of the chemical footprints *Drosophila melanogaster* to that of the cuticular hydrocarbons identified by Jallon and David (1987).

Chemical Identification & Properties		**Percent Abundance**		
		Jallon and David, 1987 Cuticular Hydrocarbons		**Norris *et al.* Footprints**
Chemical name	**Chemical Formula**	**Male**	**Female**	**Male and Female**
Heptacosane	C27H56	1.4%	3.0%	0.45%
Nonacosadiene			12.8%	
Branched Nonacosane	Unspecified	5.4%	4.8%	
Unknown (mass 294 m/z)				3.43%

Legend	Unique Norris, *et al.*	Similar Length Alkenes
	Unique Jallon & David	Shared Alkanes

Appendix A

We used the lme4 package (version 1.1-12, Bates *et al.*, 2019) to calculate the generalized mixed model for the dispersal bioassays using the following code: *glmer(dependent variable~independent variable + (|random variable), data=data, family = binomial)*. The command *anova(model, test="F")* from the stats package (R Core Team, 2019) was used to get the F statistic to get the effect of treatment on the dispersal of *M. persicae*. Finally, we conducted post hoc tests using *summary(glht(model, mcp(Treatment="Tukey")))* from the multcomp package (version 1.4-10, Hothorn, *et al.*, 2019).

We also used the lme4 package (version 1.1-12, Bates *et al.*, 2019) to run the generalized linear models using *glm(dependent variable~independent variable, data = data, family = Gamma(link="inverse")* for the parameters of duration for different feeding behaviors. Initially, we altered the previous code used to calculate the number of events using a poisson distribution and a log link, but according to the output of the overdispersion test: *dispersiontest(model, trafo = 1)*; from the AER package (version 1.2-7, Kleiber and Zeileis, 2019), all parameters were overdispersed, necessitating the use of a negative binomial GLM. We used the MASS package (version 7.3 - 51.4, Ripley *et al.*, 2019) to conduct the negative binomial GLM using *glm.nb(dependent variable~independent variable, data=data, link=log)*. Both the gamma distributed GLM and the negative binomial models were run through *anova(model, test="F")* from the stats package (version 3.6.1, R Core Team, 2019), to get the F statistic for the effect of treatment on the different behavior parameters. Then the emmeans package (version

1.4.2, Lenth et al., 2019) was used to calculate the post hoc test for the GLM of the duration data and the negative binomials of the number of times, specifically using *emmeans(model, spec = trt.vs.ctrl)* for the Dunnett method.

The Survival package (version 3.1.6, Therneau & Lumley, 2019) was used to run the survival tests of the "time to" behavior parameters of the EPG data including the cox proportional hazard model: *coxph(Surv(dependent variable Time, dependent variable Status) ~ independent variable, data = data)* and the Kaplan-Meier: *survfit(Surv(dependent variable Time, dependent Status) ~ independent variable, type="kaplan-meier", conf.type="log", data=data)*.

All of the parameters from the EPG data were checked for statistical outliers using the LMERConvenienceFunctions package (version 2.10, Tremblay and Ransijn, 2015) using *romr.fnc(model, data, trim = 2.5)*. Statistical outliers were not removed because there was no *a priori* justification to do so on an individual level; everything was grown or raised in the same conditions, and the plants were within the same age range and healthy. In addition, not all behavioral parameters of the same individuals had the outliers, and if they did, the outliers were often different individuals for related behaviors. Therefore results are reported with and without the outliers to be thorough and transparent, but the tables and discussion will focus on the analysis with the outliers included.

We also used the plyr package (version 1.8.4: Wickham, 2016) to calculate the means and standard error for the parameters using *ddply(data, . (independent variable), summarize, new variable name=mean(response variable), new variable*

name=se(response variable)). The ggplot2 package (version 3.2.1, Wickham *et al.*, 2019), was used to generate graphs.