Density-dependent Foraging of Walnut Aphid *Chromaphis juglandicola* by the Parasitoid Wasp *Trioxys pallidus*

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ABSTRACT

The control of walnut aphids by the parasitoid wasp *Trioxys pallidus* is a classical biological control success story. However, since 2004, research has been showing that larger populations of aphids have been surfacing in California's walnut orchards, suggesting that the current methods of biological control are potentially no longer as effective for maintaining populations of walnut aphids below their economic threshold. Therefore, a greater understanding of the relationships between aphid density and patterns of parasitoid predation on aphids is necessary in order to better understand these recent changes in the effectiveness of the biological control agent. Using different patch sizes of the walnut aphid (*Chromaphis juglandicola*) of 5, 15, or 20 individuals, I observed defensive kicking of *C. juglandicola* and foraging behaviors, calculated successful oviposition rates of *T. pallidus*, and the sex ratio of the emerging adult wasps. I found that as the density of aphids increases, the amount of time wasps spend looking for aphids decreases, and number of successful and total oviposition attempts decreases.

KEYWORDS

biological control, predator-prey interactions, behavior, patch size, sex ratio

INTRODUCTION

Biological control is an environmentally friendly alternative approach for reducing damage from undesired pest organisms (Bale et al. 2008). A classical biological control program suppresses an invasive or pest species through the introduction and release of natural enemies from the area of origin of the invader (Hoddle 2004). Parasitoid wasps are often a highly effective and host-specific natural enemy used for control of these insect pests (Mills 2000, Mills et al. 2006). One such agricultural pest in California is the walnut aphid (*Chromaphis juglandicola*), which potentially reaches densities of up to 100 aphids per leaflet without intervention (Sluss 1967). The California walnut industry, which produces roughly 250,000 tons of walnuts per year, (Ramos 1997) has been significantly impacted by the presence of *C. juglandicola* which reduces tree vigor, and size and quality of nuts (Michelbacher and Ortega 1958).

A potential biological control agent for *C. juglandicola* was identified, and the parasitoid wasp *T. pallidus,* was introduced and effectively used to control the population of *C. juglandicola* to such a level that the aphid no longer had an economic impact. After an earlier failed attempt to introduce *T. pallidus* from France, the parasitoid wasp was introduced from Iran in 1968, and over two years, quickly spread throughout California (van den Bosch 1970). By 1970, it had colonized almost all major walnut-growing areas of the state, and parasitized aphids at rates up to 100% (van den Bosch et al. 1970, 1979). The parasitoid's impact on *C. juglandicola* benefitted the walnut industry $0.5 to $1 million annually in the years after its introduction (van den Bosch 1979).

However, in the past several years, some walnut producers have needed to control aphid populations with in-season insecticide applications (Hougardy and Mills 2009). Purchasing pesticides is a financial burden to farmers, and their use contributes to pollution in the surrounding area. Furthermore, research has shown that pesticides that are effective on the walnut aphid are equally toxic towards *T. pallidus* (Purcell and Granett 1984). Though links between aphid density and instances of parasitism have been found, it is still unclear why a system that has long been so successful is suddenly losing its value (Hougardy and Mills 2009).

Density is an important stabilizing factor in host-parasitoid interactions (Chesson and Murdoch 1986) and may be a factor in the walnut aphid/biocontrol system. For example, in a system of western tussock moths, more foraging parasitoids were found on bushes containing a higher density of hosts (Umbanhowar et al. 2003). Aphids also respond to attack differently when in different group sizes (Desneaux et al. 2009), which could compromise the ability of *T. pallidus* to successfully parasitize *C. juglandicola*. Female parasitoids may also change the sex ratios of their offspring based on patch sizes (Strand 1988; Bayram et al. 2004; Chong and Oetting 2006), which may affect proper control of the pest population, although this pattern does not hold true in all systems (Van Dijken et al. 1989; Sagarra et al. 2000, Mahmoudi et al 2010). Changes in sex-ratio may be important with regard to the rate of inbreeding in a population, which could have overall fitness effects for the population. However, it is unclear if host densities affect emerging sex ratio of *T. pallidus,* or if its behavior changes as number of aphids per leaflet changes.

The objective of the study is to determine whether a change in host density affects the foraging behavior of *T. pallidus.* In addition I will examine whether aphid defensive behaviors change in response to the number of aphids in a patch.

Hypothesis 1: The parasitoid, *T. pallidus*, will spend the majority of its time searching for hosts within the larger patches, and will attempt to oviposit significantly more in larger patch sizes.

Alternative Hypothesis 1: The parasitoid will spend more time searching for and ovipositing in individual or small groups of hosts.

Hypothesis 2: As the number of aphids in per leaflet (i.e. in a patch) increases, aphid defensive behaviors will change.

Alternative Hypothesis 2: The number of aphids in a patch will have no effect on aphid defensive behaviors.

METHODS

Insect Preparation

I collected individuals of *T. pallidus* from lab colonies using an aspirator, and in sex ratios from 1:1 (male:female) to 1:3. I placed them in vials with a thin streak of 50:50 honey and water solution for 24-48 hours at 21.5 °C. No more than 6 individuals were in kept in each vial at a time. The time spent in vials allowed females to mate, an important step for the offspring sex ratio portion of this study, because unmated females are only capable of producing female eggs, while unmated ones can only produce male eggs.

Experimental Setup

I created micro-arenas in which 5, 15, or 20 3rd-instar aphids (identified by wing buds that are visible under dissecting microscope) were transferred from a lab colony using a small pesticide free paintbrush to the center of an individual walnut leaf in a petri dish. The walnut leaf was placed upside-down, so veins on the underside were exposed as a food source and taped on all edges
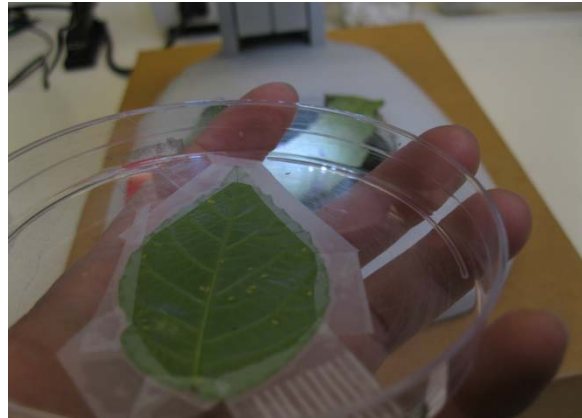
Fig. 1. Experimental Arena. A walnut leaf, taken from a sapling less than an hour prior to the experiment, was taped face-down on a plastic petri dish, and filled with aphids.

to prevent aphids from crawling underneath the leaf and out of view of the recording device. (See *Fig. 1*.) A drop of 50:50 honey and water solution was placed on the leaf, midway between the central vein and the edge of the leaf as a nectar resource for the duration of the experiment. One female wasp was introduced at the side of the dish at the beginning of each experiment. I recorded activity in the arena using a Sony HDR-CX700V video camera.

Data Collection

I recorded the following landmark behaviors to the nearest second: (1) time to first oviposition attempt, (2) durations of *T. pallidus* stationary movement for over 30 seconds, and (3) time of last oviposition attempt if more than 30 seconds before the end of the recording period. The time prior to first oviposition and times spent stationary during the 30 minutes recorded for each trial were subtracted out of the total time to find time *T. pallidus* spent in the arena. I tallied occurrences of the following behaviors: (1) successful oviposition (ovipositor insertion greater than 3 seconds and not ending as a result of aphid defensive behaviors), (2) interrupted oviposition (all other sting attempts), and (3) kicking, an aphid defensive behavior (as defined by Desneux et al. 2009). I also recorded the number of aphids approached during the recording period, which included all aphids contacted by the wasp by antenna or ovipositor.

Statistical Analysis

I used a generalized linear model (glm), a form of linear regression that corrects for non-normal data. I analyzed 4 response variables: successful oviposition, interrupted sting, aphid kick, and aphid approach. Successful oviposition is total number of completed ovipositions divided by total number of aphids included in that trial. Interrupted oviposition is total number of ovipositions divided by total number of aphids in that trial. Aphid kick is also expressed as a ratio: I totaled aphid kicks observed while a wasp was within 4 mm. of the aphid, including while attempting oviposition. Aphid approach totals the number of aphids approached by *T. pallidus* during the 30-minute duration of the experiment. Each aphid was only counted once, even if it was approached multiple times.

RESULTS

There was a general trend of wasp foraging behaviors and aphid responses decreasing as aphid density increased.

Table 1. Summary of GLM analysis.

Model Name	Coefficient	Std. Error	t Value	Pr (>|z|)
Intercept	15.493	2.316	6.691	2.83 e-06
Successful Oviposition	-2.479	1.519	-1.632	0.12
Intercept	14.646	2.25	6.509	4.04E-06
Interrupted Sting	-5.538	4.435	-1.249	0.228
Intercept	16.043	2.151	7.458	6.55E-07
Aphid Kick	-2.239	1.056	-2.12	0.0482
Intercept	20.728	4.086	5.073	7.93E-05
Aphid Approach	-12.041	5.643	-2.134	0.0469

Successful vs. Interrupted Oviposition

Boxplots were used to summarize data (Fig. 2). Mean proportion of aphids approached was 0.80(±0.12) at 5 aphids per leaflet, 0.68(±0.10) for 15 aphids, and 0.53 (±0.13) for 20 aphids. From the total number of aphids the mean number of interrupted stings per aphid was 0.48(±0.21) at 5 aphids per leaflet, 1.17(±0.65) at 15 aphids, and 0.75(±0.30) for 20 aphids. Average proportion of successful ovipositions per aphid was 1.57(±0.41) at 5 aphids per leaflet, 1.17(±0.65) for 15 aphids, and 0.76(±0.30) for 20 aphids.

These summaries of the data both reflect a trend of decreasing foraging efficiency by *T. pallidus* as density of *C. juglandicola* increases. Also following this trend is mean proportion of aphids approached: 0.80(±0.24) at 5 aphids per leaflet, 0.68(±0.19) for 15 aphids, and 0.53(±0.25) for 20 aphids. But not only does number of aphids approach decrease as aphid density increases - average total time spent in the arena decreases as well - at 5 aphids time spent in the arena averaged 1486.63(±190.94) seconds, 1579.50 (±143.48) seconds at 15 aphids, and 1501.833(±90.06) seconds when there were 20 aphids per leaflet.

Occurrences of Defense

In most instances, when an aphid is first approached by *T. pallidus,* the aphid first kicks a few times, prompting the wasp to circle to the front of the aphid, where the wasp uses its antennae to situate itself by the head of the aphid, and oviposits at least once without any resistance from the aphid. I found that as density of aphids increased, occurrences of defensive kicking by aphids decreased. (Fig. 2) Mean number of aphid kicks per aphid was 2.25(±0.80) at 5 aphids per leaflet, 1.369(±0.39) for 15 aphids, and (0.91±0.52) at 20 aphids.

Offspring Sex Ratio

I found a higher ratio of females to males in offspring of wasps foraging in arenas where there were only 5 aphids per leaflet as compared to those with 15 aphids, but when the number of aphids was increased to 20, the ratio of female to male is even higher than the offspring from the 5-aphid treatmens (Fig. 2). Therefore, there appears to be no correlation between aphid density and wasp offspring sex ratio.
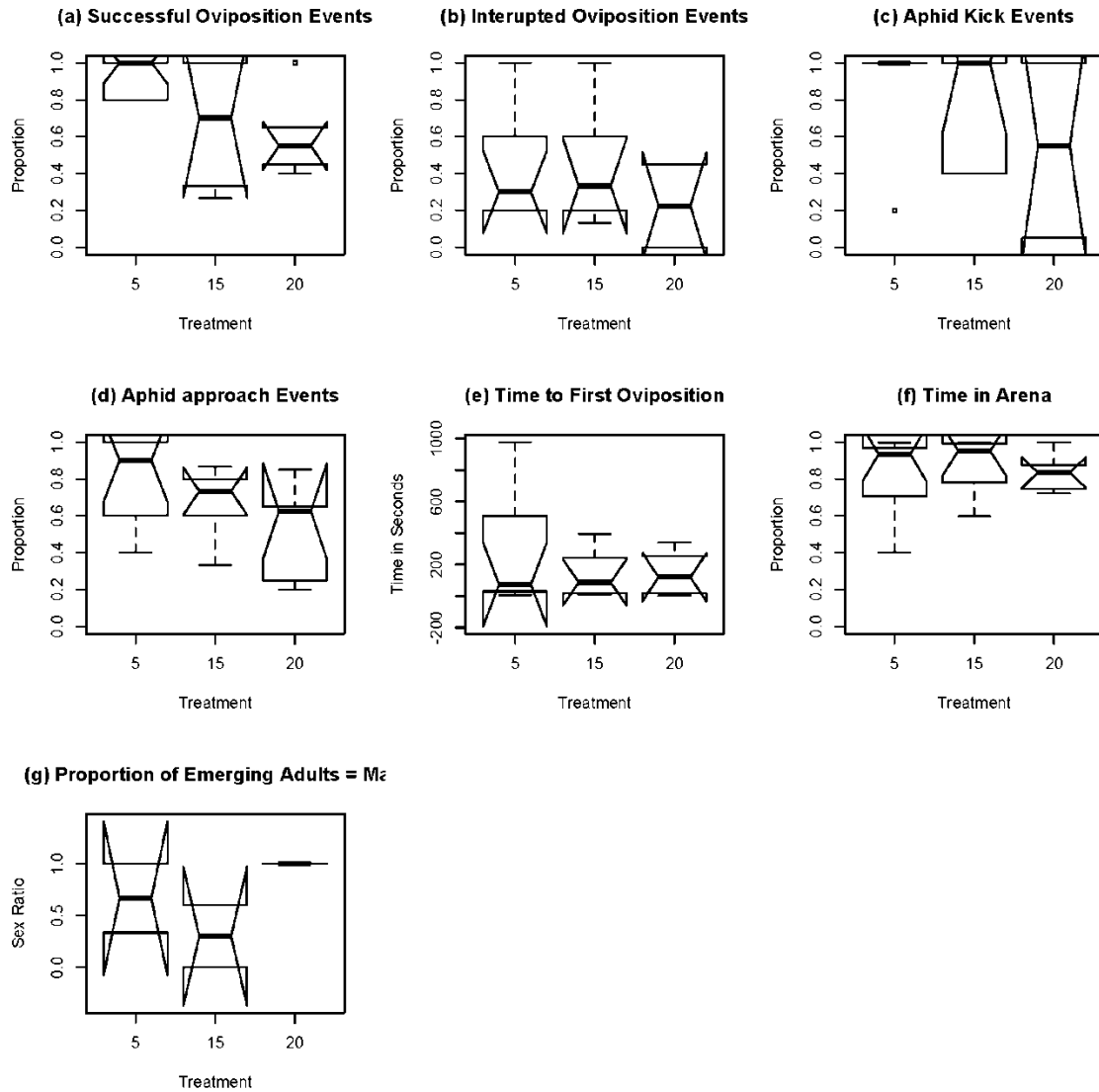
Fig.2 Data Summary. All boxplots are notched to indicate significance of difference. If there is vertical overlap in the coerner notches, the differences are not significant. If there is no overlap, the differences are significant and can be treated as a trend. For all graphs, the horizontal axis represents the different categories in aphid density: 5, 15, and 20. In (a)-(d), the vertical axis represents the number of times the response variable was encountered. In (a), the response was successful ovipositions, which decrease as aphid density increases. In (b), interrupted ovipositions also decrease as aphid density increases. In (c), aphid defensive kicks decrease as aphid density increases, and in (d) we find that a smaller fraction of the aphids in the arena were apprached during the trials for higher densities of aphids. In (e), I compared amount of time from the start of the trial to the first oviposition attempt. The vertical axis shows time in seconds. (f)

DISCUSSION

The effects of aphid density on foraging behaviors of *Trioxys pallidus* will provide insight into why the walnut aphid population has recently resurged in California orchards. Using behavioral studies of aphid defenses and wasp foraging behaviors, I found that as aphid density increases, total ovipositions and successful ovipositions per aphid decreases, as do interrupted oviposition attempts. In addition, aphid defense events also decrease per aphid as the density of aphids increases.

These behaviors are not consistent with field study data, where *T. pallidus* was able to control a larger population of walnut aphids, and was less successful with smaller numbers of aphids. However, they do reflect Optimal Foraging Theory, which argues that one of the most prominent predator behaviors is specialized feeding on the more profitable prey type (Křivan and Sikder 1997). In a case where prey is not the limiting factor in how many offspring a predator can produce, the predator is more selective in choosing which prey to attack. In this case, egg clutch may be the limiting factor (Minkenberg et al. 1992), which can lead to *T. pallidus* not attempting to oviposit as much as possible in 20 different aphids, but rather to oviposit more times in the most promising hosts to ensure healthy, viable offspring.

Defensive Behaviors

As aphid density increased, instances of kicking decreased. Differences in field studies of attack behavior of two parasitoid wasps and defense by their host could explain higher parasitism by wasp (*Aphidius matricariae*) than aphid *Diuraphis noxia* (De Farias and Hopper 1999). Although some *D. noxia* showed defensive behaviors when attacked, aphid defense did not reduce parasitism by either wasp species. The data I collected shows that while aphid defense does change as density changes, the proportion of successful oviposition to interrupted ovipositions is the same, so aphid defense does not impact outcome for this study system either.

Effects of Density on Reproduction

Oviposition success rates decreased as aphid density increased, contrasting with the hypothesis of a positive correlation between parasitism rate and host density (Walde and Murdoch 1988). However, since parasitism rates for small scale systems of *Mallophora ruficauda* increase as density decreases, (Castelo and Corley 2010) it is possible that the trend we observed is only true on a smaller scale, and in a field study, these discrepancies would not be present.

Offspring Sex Ratio

An increase in aphid density did not result in a consistent change in offspring sex ratio. Parasitoid wasps can produce eggs in a precise sex ratio to maximize fitness in local mate competition. The current theory is that they lay male and female eggs in a certain order, regulated by genetic control (Wajnberg 1993), which is not contradicted by my findings.

The offspring sex ratio may also be determined by host quality at the time of oviposition. A different parasitoid, *L. mirzai* adjusts offspring sex ratio when ovipositing in growing stages of the host depending on the host size to best contribute to progeny fitness without knowing about the future host quality (Pandey and Singh 1999). Although the genetics and host size could contribute to the outcome of offspring sex ratio, it is important to remember that there was always some difference in host size and some small changes in genetics, so we can assume that these changes are equally distributed across trials.

External factors

I observed a number of outliers in the number of attempted ovipositions at each density of aphids, so I must consider other factors that could contribute to this change in wasp behavior. Findings from a model based on egg load and host availability show that

insects adjust their foraging and oviposition behavior in response to their perceived risk of having a limited number of eggs. (Minkenberg et al. 1992)

Limitations

Although this study explained behavioral changes that occur in *T. pallidus* in response to changes in aphid density, a laboratory study cannot explain all the factors that impact foraging behaviors of parasitoid wasps. The sample size was somewhat limited (only 6 or 8 trials for each density size), so the data is not statistically robust enough to provide an answer to what effect aphid density has on the predator-prey relationship of *T. pallidus* and *C. juglandicola.*

There are also other external factors, such as competition from other aphid predators, predators of *Trioxys pallidus,* nectar resources, and more outlined by Mills in a 2009 paper, that can impact success of parasitism. These factors need to be considered in a more in-depth study to determine which are leading to a less effective biocontrol system in California's walnut orchards.

FUTURE DIRECTIONS

The findings of this study are simply an indicator for what behavioral trends may occur on a per-leaflet basis. The trends we observed do not reflect historical trends on a landscape scale. *T. pallidus* was found to have a lesser impact on smaller populations of *C. juglandicola*, however, this study observed the opposite trend (Mills 2006). Because of the difference in successes in a lab situation and in the field, I plan to find what factors led to these incongruities with a field study, to include potential external factors of behavioral change.

One important consideration is the habitat in which these foraging and oviposition behaviors take place. Normally, *T pallidus* would be searching a landscape-scale area for *C. juglandicola*, rather than having the aphid presented within visual range, without any shelter. Another concern is the presence of predators of both parasitoid and host in walnut

orchards, and the responses *T. pallidus* and *C. juglandicola* have developed to interact with those organisms.

BROADER IMPLICATIONS

Because increase in aphid density decreases the number of successful ovipositions, we know that the rise in aphid population is not simply *T. pallidus* responding to large numbers of aphids. From here, we know how important density-dependent behavior is in population dynamics of this study system, and why biocontrol in this system isn't as effective as it used to be. From this study, it appears that there is a sharp decline in efficiency of *T. pallidus*' foraging when aphids are at a density of over 15 per leaflet. Therefore, in order to maintain control over aphid populations in walnut orchards, I would recommend that walnut growers employ other methods in conjunction with *T. pallidus*-facilitated biocontrol when aphid populations reach that 15-aphid-per-leaflet threshold.

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