Title: The Use of Semiochemicals by Tarnished Plant Bug *Lygus lineolaris*

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Summary

The overall goal of this research was to develop new approaches to monitoring and managing Tarnished Plant Bug (TPB), an important pest of strawberries and many other crops, through an improved understanding of its use of semiochemicals to find mates and host plants. We proposed to 1) Evaluate potential components of the TPB sex pheromone under realistic field conditions and 2) Initiate a study to identify attractive volatile compounds from a highly preferred weed host and test with virgin females and potential components of sex pheromone. We used funnel traps baited with either live, virgin females or different combinations of putative pheromone components and/or extracts of volatiles collected from *Erigeron annuus* or extracts from virgin females confined to traps in the field to test for attractiveness under field conditions. We also assessed antagonistic properties of putative pheromone components by placing single compounds beneath traps baited with virgin females. In addition, we attempted to test the relative role of visual and plant volatiles in host colonization.

A total of 11 field experiments were conducted during the season using funnel traps, starting on 19 June and continuing until 3 September. Putative pheromone compounds in various combinations over this time period captured 5 male TPB while virgin females captured over 100 males. When placed beneath a trap baited with a live virgin female, 4-oxo-E-(2)-hexenal or its derivative appeared to enhance captures, while the other principal components (hexyl butyrate, and E-2-hexenyl butyrate) appeared to be antagonistic. An extract from the volatiles of flowering *E. annuus* alone or when added to the derivative of 4-oxo-E-(2)-hexenal did not result in any captures of TPB in live traps. When the plant extract was added with a virgin female we captured 4 males compared to 16 in traps baited with only the female. In late summer we used a battery, pump and filters to collect volatiles produced by virgin female TPB in the field. When placed in a live trap, the extracts from TPB captured 3 males while live virgin females captured 65 males. We conducted a choice test where we presented a group of TPB to chrysanthemum, a host plant of TPB, with or without its yellow flowers and with or without yellow cards or volatile extract from the flowers. Most TPB flew to top of the screen house and made no choice. Of the 9 TPB that moved to a plant, three colonized mums with flowers removed, but with yellow cards and flower extract added, three colonized chrysanthemums with flowers removed but yellow cards, one colonized flowering chrysanthemums (positive control) and two colonized chrysanthemums with flowers removed and nothing added (negative control). Although observation of colonization events were small, 7 out of 9 events were directed at plants with yellow color (cards or flowers) compared to 4 out of 9 events to flower odors.

Introduction

Tarnished plant bug (TPB) *Lygus lineolaris* is a key arthropod pest of strawberries and many other crop plants grown in North America. Both adult and immature TPB use their piercing and sucking mouthparts to feed on young, actively growing plant tissue, including developing strawberry fruit. In strawberries, this feeding activity kills achenes and leads to misshapened or catfaced fruit that is not marketable as fresh fruit. Given the high value of strawberry fruit, the threshold for damage from TPB is quite low and insecticide control measures are often required. In addition to expense, the insecticides used for control of plant bugs generally have broad-spectrum activity and as such, can disrupt natural control of arthropod pests. Therefore, there is a need and interest in developing alternative approaches to their management including narrow-spectrum insecticides, biological control, host plant resistance, and cultural control.

Female TPB produce a sex pheromone that is highly attractive to males in the field and there has been considerable interest in identifying the chemical constituents of this pheromone to be exploited for management purposes. Several volatile compounds have been identified from female TPB in the laboratory that are candidate pheromone constituents, although to date, activity in the field has not been demonstrated.

Because of the importance of TPB for strawberry growers and the potential benefits of a having a synthetic version of the TPB pheromone, we initiated a collaborative project with Dr. Aijun Zhang, a chemical ecologist with the USDA ARS with several years of experience working specifically on the TPB pheromone and many more years successfully identifying pheromones of other related pest species. Using electrophysiological and analytical chemistry techniques, Dr. Zhang and others have identified several compounds that produce strong antennal responses in male TPB and are found in greater abundance in females than males, suggesting potential roles in the sex pheromone. We used funnel traps (traps that capture TPB without killing them) to begin testing the attractiveness of some of these compounds under realistic field conditions.

We started initial testing of three candidate compounds: 4-oxo-E-(2)-hexenal, hexyl butyrate, and E-2-hexenyl butyrate. One of these, 4-oxo-E-(2)-hexenal, is fairly unstable and deteriorates after a short time period (hours to days) to non active compounds. Hence, Dr. Zhang developed a derivative of 4-oxo-E-(2)-hexenal that slowly changes to 4-oxo-E-(2)-hexenal with the idea that this would produce roughly the correct concentration being released from the emitter (rubber septa) over several days.

Although these three compounds are present in females and not in males, this does not necessarily imply all of them are important as pheromone constituents. Indeed some may even act as antagonists. Thus, some of our trails were focused on testing this potential role as antagonists. Another factor that might be important is the presence of volatiles from good plant host such as the weed *Erigeron annus* during flowering or its close relatives. Food odors have been found to be important co-attractants with pheromones in other herbivorous insects. An important component of all these trials related to pheromones is the time of day or night when TPB females produce the pheromone. Hence, one of our trials involved quantifying capture of males in traps baited with virgin females during the 24 hour cycle.

In our previous research we found that although TPB feeds on many, many different host plant species and hence can be considered an extreme host generalist, it actually is quite sensitive to host phenology, specializing on plant during fruiting or

flowering. Mechanistically, however, we were uncertain how TPB finds hosts at the correct phenology. We hypothesized they use either visual cues or olfactory cues or both. Hence, we report on a small behavioral experiment to assess the relative role of visual and olfactory cues in colonizing host plants.

Methods

Method for live-trapping TPB

A non-sticky trap constructed from 2 L plastic soft drink bottles and funnels from boll weevil traps was used to assess attractiveness of virgin females, candidate pheromones, or plant odors. Traps are designed such that the attractant is placed in the center of the trap. TPB from the field enter the trap through the inverted funnels on either end and become trapped in the interior chamber. Virgin females are placed in a small, screen cage, along with pieces of green bean for food. Candidate compounds are added to rubber septa and also placed inside the small, screened cage with green beans. A cage baited with green beans alone is used as a negative control.

Circadian cycle of TPB pheromone production

Live traps baited with either two virgin female TPB (one 3 days old and the other 8 to 9 days old) plus a piece of green been or only green beans were placed in a weedy field on a farm managed by NYSAES in Geneva, NY on the early morning of 2 July 2007. Traps were checked for captured TPB approximately every two hours until approximately 8 pm on 3 July 2007.

Potential antagonism of putative pheromone components

To test potential antagonism of the four putative pheromone components, we deployed live traps baited with two to three virgin female TPB (ages 5 to 12 days old) in the weedy field at NYSAES for several trials during the summer of 2007 (2 July, 5 July, 18 July, 26 July). Rubber septa, loaded with either hexane (control), 4-oxo-E-(2)-hexenal (I), derivative of 4-oxo-E-(2)-hexenal (Ia), hexyl butyrate (II), or E-2-hexenyl butyrate (III), were attached to the outside of the bottom of the trap with a pin. Number of captured TPB in funnel traps and sex were recorded over a 2 to 4 day period, depending on the trial. Traps were usually checked at least twice per day.

Testing putative pheromone constituents and food odors

We conducted a number of trials using live traps in the field to assess attractiveness of different combinations and concentrations of putative pheromone constituents. In all trials we included a positive control in the form of 2 or 3 live virgin females (age 4 to 12 days old), along with green beans as a food source and a negative control in the form of green beans only. Trials ran from two to 7 days in length, with fresh beans provided to live females at least every other day. Initial experiments compared different combinations of the four candidate compounds but later experiments

focused more on 4-oxo-E-(2)-hexenal and its derivative. In one experiment (7 July – 9 July), we included volatiles collected from flowering *E. anuus* with beans, or in combination with a live virgin female TPB or with 4-oxo-E-(2)-hexenal or its derivative. Number and sex of captured TPB were recorded during the experiment. We usually checked traps at least twice per day.

Assessment of visual and olfactory cues

We used the garden perennial plant chrysanthemum, *Dendranthema x grandiflorum* in an experiment to examine relative role of vision and olfaction in host colonization by TPB. TPB feeds on chrysanthemum and is considered a pest. We obtained potted chrysanthemum with an abundance of yellow flowers from a local distributor in July 2007. We assigned plants to one of five treatments: flowers left intact (positive control), 2) flowers removed with scissors (negative control), 3) flowers removed, 6 yellow, non-sticky cards (roughly 6 cm square) positioned just above foliate with wire stakes, 4) flowers removed, methylene chloride extract from charcoal filter collection of chrysanthemum flower volatiles loaded in rubber septa, 2 per plant, and 5) flowers removed, cards and extract added. Plants were arranged in three groups, with one rep for each of the five treatments per group, in a large screen house (4m X 6m X 3m) on the NYSAES campus. The day before a trial, we collected adult TPB from the field and placed in a plastic container with wet cotton for moisture but no food (100 to 150 per trial). On the day of a trial, the TPB were placed in the center of the three groups and the lid removed. The container was placed on a bed of ice for approximately 5 minutes to chill TPB before release. Two to three observers, sitting in the back of the screen house, recorded where TPB went as they exited the container. In particular, TPB were recorded as either flying to a plant, in which case the treatment was recorded, flying to the side of the screen house, or flying to the top of the house. Observations were made for 1 to 2 hours after release. Three trials were conducted (18 July, 19 July and 31 July), with all trials conducted in a similar manner.

Results & Discussion

Circadian cycle of TPB pheromone production

Over roughly a 48-hour period we captured a total of 22 males in traps baited with virgin females plus beans and no TPB in traps with beans only. Captures tended to occur in the morning to mid-afternoon and not through the night suggesting TPB females typically call during the day (Figure 1). 14 of the 22 (64%) were captured between 7:30am and 12:30 pm while the remainder was captured between 2:00pm and 5:30pm.

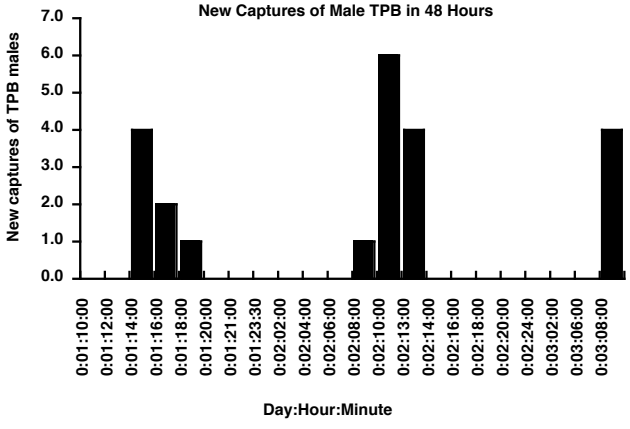

Figure 1. Field trap captures of male TPB over a 48 hour period in early July 2007 (2 July to 4 July) in live traps baited with virgin female TPB.

Potential antagonism of putative pheromone components

The capture of male TPB in funnel traps appeared to be influenced by the compound attached to the outside bottom of the trap. Specifically, females plus the derivative compound (Ia) captured significantly more males than females with 4-oxo-E-(2)-hexenal (I), hexyl butyrate (II), or E-2-hexenyl butyrate (III) ($X^2 = 31.9$, $P < 0.001$). Traps with only virgin females captured an intermediate number (Table 1). These results suggest that 4-oxo-E-(2)-hexenal, hexyl butyrate, and E-2-hexenyl butyrate can act as antagonists to the true sex pheromone being produced by live, virgin females. It is also possible that the derivative somehow enhances the attractiveness of the real female, although more work is necessary.

Table 1. Number of male TPB captured in traps baited with live, virgin female TPB and with different single compounds loaded in rubber septa attached to the outside, bottom of the traps.

Treatment	# male captures
Female plus beans	16
Female , beans, comp I	8
Female, beans, comp Ia	30
Female, beans, comp II	6
Female, beans, comp III	6

Testing putative pheromone constituents and food odors

For these experiments we tested the attractiveness of various combinations of volatiles with virgin females. In all trials we captured some males in traps baited with virgin females but in most trials we did not capture any males it traps baited with putative pheromone components or plant volatiles. Four experiments warrant further examination. The trial conducted from 7 July to 9 July 2007 compared virgin females with 4-oxo-E-(2)-hexenal (I) or its derivative and the volatile extract from the plant host *E. anuus* in various combinations. We captured 16 males with females only, 19 when females were combined with the derivative, 4 when females were combined with the plant volatile. We caught no TPB in traps with the plant volatile or the plant volatile in combination with 4-oxo-E-(2)-hexenal or its derivative.

In a second experiment conducted from1 August to 8 August we compared captures in traps baited with virgin females with different concentrations of the derivative of 4-oxo-E-(2)-hexenal (100μg, 60μg, 30μg, 10μg, 1μg, 0.5μg). In the trial we captured 12 males with the virgin female lures and only 1 male in a trap baited with 60μg of the derivative. We did not capture any males in the other traps.

In a third experiment, we examined the attractiveness of various combinations of the putative pheromone compounds compared to live, virgin females, using 60μg of the derivative in all lures and combinations of the other compounds loaded at either 10μg per septa or 1μg. The trial was conducted from 9 August to 22 August. We captured 30 males in traps with females and 4 in traps with the derivative plus other compounds at a total of 1μg (1 in trap with 4-oxo-E-(2)-hexenal, 2 in trap with 4-oxo-E-(2)-hexenal and E-2-hexenyl, and 1 with 4-oxo-E-(2)-hexenal, hexyl butyrate, and E-2-hexenyl butyrate.

We did not catch any males in traps with the derivative plus 10μg of the other compounds. For the first time we caught two in a trap just containing green beans (1 of unknown sex and 1 male). For one trap, there was a large *E. canadensis*, a highly preferred host, growing into the funnel that probably explains the capture.

In the fourth experiment we compared live, virgin females with an extract collected from virgin females, and combinations of the derivative of 4-oxo-E-(2)-hexenal at 60μg and other components at different amounts (30ng, 300ng, and 900ng). We also included a fourth compound found in volatile collections of TPB (Z-3-hexenyl butyrate). This trial was conducted between 28 August and 3 September. We captured 65 males in trap with females, 3 in traps with extract from virgin females and none in the other traps baited with putative pheromone components.

Assessment of visual and olfactory cues

The vast majority of TPB left the release point and flew either to the top or sides of the screen house. Only 9 insects flew to one of the plants (Table 2). With this few observations, it is impossible to draw any definitive conclusions. The only possibly interesting result is that 7 TPB colonized a plant with color (either artificial or real) while 4 colonized plants with odor. We hope to repeat a version of this experiment again in 2008 but conducted outdoors where we expect bugs will behave more normally.

Table 2. Number of TPB observed colonizing chrysanthemum in different conditions under screen house conditions. Results of three trails summarized

Treatment	# Colonizing TPB
Chrysanthemum	1
Chrysanthemum, no flowers	2
Chrysanthemum, no flowers, yellow cards	3
Chrysanthemum, no flowers, flower volatiles	0
Chrysanthemum, no flowers, yellow cards + volatiles	3

Conclusions

A major goal of this research was to make progress in identifying the sex pheromone of TPB. As others have shown, female TPB clearly produce a sex pheromone. Funnel traps baited with virgin females captured many male TPB during the course of the field season. They never captured any females. Hence, it seems reasonable that volatile compounds collected from virgin females would include the pheromone constituents. Synthetic versions of three compounds collected from virgin female TPB (4-oxo-E-(2)-hexenal, hexyl butyrate, and E-2-hexenyl butyrate) but not present in males, along with a derivative of 4-oxo-E-(2)-hexenal, were tested in various combinations in the field without much success in attracting and capturing males, especially in comparison to virgin females. Part of the problem may be that some of these compounds are acting as antagonists rather than attractants. We found support for antagonistic

influence for at least two of the compounds (hexyl butyrate, and E-2-hexenyl butyrate). It's interesting that of the 5 males captured using synthetic lures, four were captured with the derivative plus low amounts (lower than normally used) of the other putative pheromone components. This suggests that at higher release rates some compounds can act as antagonists but at lower release rates they may improve attraction.

Clearly we still have a ways to go in identifying the sex pheromone of TPB. The fact that the derivative was somewhat attractive suggests we are on the right track. It is possible that the derivative is not converting to 4-oxo-E-(2)-hexenal at the appropriate rate to mimic live females. Indeed, we collected volatiles coming from a rubber septa containing the derivative and found very little evidence of 4-oxo-E-(2)-hexenal suggesting the conversion rate is slow. We are currently working on ways to increase conversion rate. It is also possible that we are still missing an important pheromone component. The fact that we were able to collect volatiles from virgin females in the field and obtain some activity in the field suggests that this approach is worth pursuing.

We had hypothesized that host plant volatiles may be important co-attractants with sex pheromone in TPB. Our results are limited, but do not support this hypothesis. Under the field conditions we worked in there was abundance of weed hosts and volatiles of weed hosts in the area. Apparently adding host volatiles with virgin females in our traps was inconsequential in this environment. This is an issue we probably need to visit again once the pheromone has been identified and synthesized.

Finally, we attempted to gain more insights into the relative role of vision and olfaction in host colonization by TPB. The experimental approach we used was flawed and hence, no definitive conclusions can be drawn. The underlying premise of the experiment seems appropriate, but we need to find a different setting where the TPB behave normally.

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