Title: Understanding habitat colonization by Tarnished Plant Bug as basis for developing an attraction-based management system for berry crops

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Abstract:

The goal of this research was to monitor Tarnished Plant Bug (TPB), an important insect pest of numerous agricultural commodities, on different weed and crop hosts during the growing season and evaluate potential factors associated with differential colonization patterns. Specifically we 1) monitored colonization and population dynamics of the TPB in replicated 2 X 2 m plots of different weed or crop species as well as adjacent crop and noncrop habitats over two field seasons in upstate New York and 2) began an assessment of the role of plant volatiles and visual cues in mediating TPB colonization of host plants. We observed marked differences in abundance of TPB among habitats through the season as measured by roughly weekly vacuum samples and captures on yellow sticky cards. Plant phenology, particularly onset of flowering, appeared to play a key role in determining colonization and abundance patterns for both small and large habitats. Although TPB has a very large host range, it feeds on young, rapidly developing tissue, such as flower buds or young fruit. We hypothesized that TPB adults use both visual and volatile cues to locate plants with the appropriate tissue. Significantly more TPB adults were captured on white 13 cm by 18 cm plastic cards coated with stickum than red cards under field conditions. Also in the field, volatiles from flowering *Erigeron canadensis*, an important weed host of TPB, directed to white sticky cards, captured more TPB than filtered air lacking plant volatiles, although the number of total captures was small and the difference was not statistically significant. Information on colonization patterns and the cues used by TPB may be useful in improving monitoring techniques or developing trap crop or attract and kill techniques to suppress TPB populations in agriculturally important crops.

Background and Justification:

Tarnished plant bug *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. 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 generally have broad-spectrum activity and as such, can disrupt natural control of arthropod pests. Thus, 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.

One approach, based on attracting TPB into a favored habitat (trap crop) and out of the strawberry planting, has been shown to have some promise in managing other plant bug species, including the very similar western tarnished plant bug *L. hesperus*. A related approach would involve attracting TPB into traps laced with pesticides. As a foundation for developing a trap crop or other type of attraction-based control system for TPB and strawberries, we need to determine the factors that make a habitat or host plant more or less attractive to TPB adults, especially female adults. Females make colonization and egg-laying decisions that determine, for the most part, distribution patterns of nymphs. TPB has a very large host range, having been reported feeding on over 300 species. Indeed, this is one reason TPB damage in crops is often associated with weedy fields or borders where ample alternative food sources are available. However, TPB feeds on young, rapidly developing tissue, such as flower buds or young fruit. In this sense, TPB is a tissue specialist. The interesting questing is how do adult TPB find host plants that have suitable tissue? Is it a random process or are there traits associated with suitable host tissue? Previous research and anecdotal evidence suggests that plant bugs use both vision and host plant volatiles to locate food plants at the proper phenological stage.

We addressed two specific objectives to explore the process of habitat and host plant colonization by TPB to establish a foundation for developing an attraction based management system for strawberries and potentially other crops:

1) Colonization of alternative habitats: monitor the colonization and population dynamics of TPB in replicated 2 by 2 m plots of different weed or crop species as well as adjacent crop and noncrop habitats.

2) Assessment of visual and olfactory cues: assess the role of plant volatiles and visual cues in mediating TPB colonization of host plants.

Methods

Colonization of alternative habitats

We monitored abundance of adult TPB and nymphs through the 2005 and 2006 field seasons, starting in early spring, in habitats comprised of either weed species or crops. In the fall of 2004 we established 2 by 2 m plots containing one of the following 5 "habitats" known to harbor TPB and to flower at different times during the season: garlic mustard *Alliaria petiolata* (winter annual/biannual flowering in early spring), curly dock *Rumex crispus* (early to mid-season flowering perennial), annual fleabane *Erigeron annuus* (winter annual that flowers in from mid to late-season), alfalfa *Medicago sativa* (early to mid-season flowering perennial crop plant), and mixture of old field weeds (early to late flowering annual and perennial species). The garlic mustard did not flower during 2005 and therefore was treated with round-up herbicide in mid-July in preparation for the 2006 field season. There were two replicates of each of these 2 by 2 m plots. The small plots were located next to or within 0.5 km of a 3 year-old planting of perennial strawberry (mixture of Earliglow and Cavandish), a fall-bearning red-raspberry planting (Heritage), a summer-bearing mixture of red-raspberry cultivars, an apple orchard (mixed varieties), and several small to large old-field habitats. All of these "habitats" included

host tissue suitable for TPB at some period during the field season. In 2006 we repeated this trial with some modifications. In particular, in the place of garlic mustard, we seeded penny-cress *Thlaspi perfoliatum* (early season flowering annual). We also added a new 2 by 2 m plot that was treated with round-up herbicide three times during the season to keep mostly weed-free (bare ground treatment) and sampled in a small planting of blueberries adjacent to the strawberry planting. We did not monitor TPB in the summer bearing raspberries in 2006 nor three of the four old-field habitats.

We monitored adult TPB using yellow sticky cards attached to stakes in each 2 X 2 m plot and in two locations within each of the adjacent habitats. Cards were replaced roughly each week, starting on 12 May through 22 September in 2005 and 28 April through 4 August in 2006. Nymphs and adult TPB were also sampled in each plot and two locations in the larger habitats once per week using a bug vacuum. Samples were returned to the laboratory, chilled and plant bugs enumerated. After counting, bugs were returned to sample plots from which they were collected. Vacuum sampling started on 1 June and continued roughly once per week until 22 September in 2005. In 2006 we started on 5 May and continued once per week until 12 August. Monitoring in 2006 was cut short because insecticide drift from the apple orchard on 10 August killed most TPB adults and many nymphs in most habitats. Hence, data from 12 August 2006 were not included in analyses.

In addition to sampling for TPB, we monitored plant phenology/flowering status. In 2005 this involved recording when plants started to flower and fruit in both single species plots and mixed weed species plots. For the 2 by 2 m plots of old field weeds we also recorded plant species identify and time of flowering. In addition to recording the same plant data as in 2005, in 2006 we monitored four 0.25 m^2 quadrats for each of three transects through the large old-field habitat adjacent to the 2 by 2 m plots, recording species identity, phenology, and % cover each month during the field season (10 May, 14 June, 10 July, 9 August). This provided us with a quantitative assessment of weedy species, many of which are recorded food sources for TPB, growing in the area.

Assessment of visual and olfactory cues.

TPB are thought to be attracted from a distance to the visual display of flowering plants. In particular, white and yellow flowers are thought to be attractive. We tested for differential attraction to white and red (control) using 13 cm by 18 cm pieces of corrugated plastic, coated with stickum, and placed in an old field during July and early August of 2006. Five cards of each color were attached to wooden stakes about 1.5 meters above the ground and monitored for adult TPB.

To assess host volatiles, we conducted field trials comparing the number of TPB adults captured on white sticky cards (13 cm by 18 cm) that were bathed in a slow stream of charcoal-filtered air (1.5L/min) that was either passed through an empty plastic Kapak bag or a bag surrounding a flowering *E. canadensis*, a highly preferred host plant for TPB. Sticky cards were attached to bamboo stakes at about 1m above the ground and checked for TPB after 3 days per trial. A total of three trials were conducted in August 2006 with 2 replicates of each treatment per trial.

Results

Colonization of alternative habitats

Plant phenology

We were successful in establishing single-species of plants that flowered in the 2 by 2 m plots in both 2005 and 2006 with the exception of the garlic mustard plot in 2005 (see above). Plant surveys of the different habitats, particularly the small plots of old field habitat, revealed the presence of a few to many flowering species at any given time during the entire season (Table 1). Briefly, early-flowering plant species included *Erysimum repandum*, *Capsella bursa-pastoris* (shepherd's purse), *Lamium purpuretum* (purple dead nettle), *Thalaspi perfoliatum* (penny cress), *Stellaria media* (common chickweed), *Arabadopsis thaliana* (mouse-eared cress), *Veronica arvensis* (corn speedwell), and *Taraxacum offinale* (dandelion). Plants flowering mid May to mid-June included cultivated strawberry, apples, blueberries, alfalfa and the weed species *Rumex crispus* (curly dock), *Trifolium* repens (white clover), Anthemis *arvensis* (corn chamomile), *Veronica perrgrina* (purslane speedwell), *Cardaria draba* (heart-podded hoary cress), *Lepidium campestris* (field pepper grass), and *Polygonum persicaria* (red-shank smart weed). In June and into July summer-bearing raspberry began to bloom along with the weeds *Erigeron annuus* (common fleabane), *Chenopodium album* (lambs quarters), *Senecio vulgaris* (common groundsel), *Oxalis stricta* (yellow wood sorrel), *Polygonum aviculare* (prostrate knotweed), *Amaranthus powellii* (green pigweed), and *Medicago lupulina* (black medic). In Late July to August fall-bearing raspberry began to bloom along with weedy species such as *Erigeron canadensis* (horseweed fleabane), *Daucus carota* (queen anne's lace), and several species of *Solidago* (goldenrod).

Table 1. Bar chart showing observed flowering periods for weeds in the two 2x2m plots. Shaded bars span the dates, indicated by check marks, when flowering was observed.

	2x2m	Flowering Dates in 2006										
Plant species	**Plot**	13-Apr	5-May	10-May	17-May	24-May	7-Jun	14-Jun	10-Jul	27-Jul	9-Aug	5-Sep
Amaranthus powellii	A								P	P	P	P
Anagallis arvensis	A&B						P	P	P	P		
Anthemis arvensis	A&B						P	P	P	P		
Capsella bursa-pastoris	A&B	P	P	P	P	P	P	P	P			
Chenopodium album	A&B								P	P	P	P
Daucus carota	B									P	P	P
Erigeron acris	A&B									P	P	P
Erigeron annuus	A&B							P	P	P	P	P
Erigeron strigosus	B									P	P	P
Erysimum repandum	A&B	P	P	P	P	P	P	P	P			
Gallium aparine	B						P					
Lamium amplexicaule	A&B	P			P		P					
Lamium purpureum	A&B	P	P	P	P	P		P				
Lobelia inflata	B											P
Malva neglecta	A							P	P	P	P	P
Medicago lupulina	A								P	P		
Oxalis stricta	A&B						P	P	P	P	P	P
Polygonum aviculare	B							P	P	P	P	P
Polygonum convolvulus	A&B									P	P	P
Polygonum persicaria	B						P		P	P	P	P
Portulaca oleracea	B									P	P	P
Stellaria media	B				P	P						
Taraxacum officinale	A&B		P	P	P	P	P	P	P		P	P
Trifolium repens	A&B					P	P	P	P	P	P	P
Veronica arvensis	B				P	P	P	P				
Veronica peregrina	A					P						

Tarnished plant bug populations

2005 Growing Season. TPB overwinters as adults and activity in the spring was noted as early as late April. In order to assess the relationship between plant phenology and TPB in single-species habitats it is useful to understand the background seasonal pattern of TPB population dynamics in weedy habitats that contain flowering host plants throughout the growing season. Figure 1A shows the abundance of TPB adults and nymphs in weedy habitats in 2005 based on vacuum sampling. The pattern based on sticky cards is similar, although more variable (data not shown). Since vacuum sampling did not start until 1 June we likely missed the overwintered adults. However, nymphs show three distinct peaks indicating three generations during the season.

Since population size of TPB varied significantly in the weedy habitats through the season it was difficult to detect patterns of colonization in single species plots. Therefore, we computed a relative measure of abundance in the 2m by 2m plots corrected for this background population using the following formula.

$$\text{RelAbundance} = ((X_i - X_{weeds})/X_{weeds}) + 1.0.$$

Where X_i is abundance for a specific sample point for a given date and X_{weeds} is the mean value from mixed weed plots for a given sample date. RelAbundance is 0 when no TPB are captured in a plot, 1 when abundance is similar to the background population, and greater than 1 when abundance is greater than background.

The pattern of colonization in 2 by 2m single-species plots was quite distinct from the weedy plots (compare Fig 1A with Figs 1B-D for curly dock, alfalfa and annual fleabane, respectively). There was a clear spike early in the season for curly dock, early and middle parts of the season for alfalfa, and the middle to end of the season for fleabane. Although not an exact match, peaks in these small, single-species plots occurred during or shortly following the onset of flowering. For the earlier-flowering species (curly dock and alfalfa), the populations declined after flowering even as they were increasing in fleabane. The alfalfa was not cut in 2005 and therefore, tended to go to seed in July and August. Thus, flowering appears to be an important determinant of TPB colonization and egg-laying. To test this another way, we compared mean abundance of TPB from all the sampled habitats as a function of whether flowering was occurring in the habitat at four time periods during the season (spring- 5/12-6/1, early summer- 6/1-7/8, mid-summer- 7/8-8/25, late summer- 8/25-9/22) for TPB adults and nymphs from vacuum samples and adults from sticky card samples (Table 1). With the exception of vacuumed adults in spring, there was a consistent pattern of more TPB in habitats that were flowering compared to habitats that were not and many times these differences were statistically significant, especially for nymph samples.

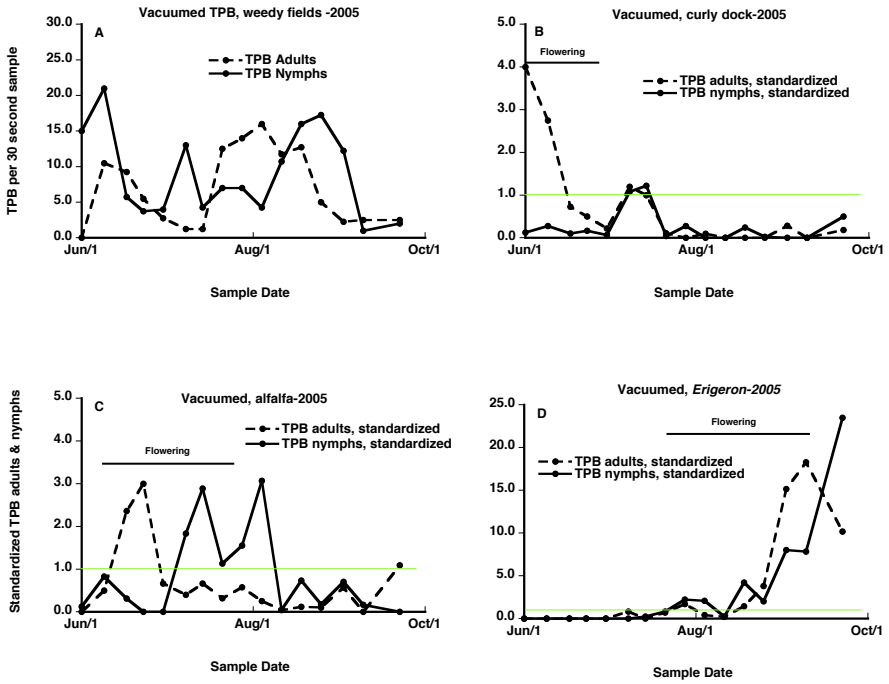

Figure 1. Phenology of tarnished plant bug adults and nymphs in different habitats during 2005 field season based on 30 second vacuum samples. A) Weed fields, absolute numbers shown. B) Curly dock, standardized values. C) Alfalfa, standardized values. D) Erigeron, standardized values.

Table 2. Abundance of TPB adults and nymphs measured with 30-second vacuum samples and adults on yellow sticky cards for flowering and nonflowering habitats at four time periods (spring- 5/12-6/1, early summer- 6/1-7/8, mid-summer- 7/8-8/25, late summer- 8/25-9/22) in 2005. Samples collected roughly every week. Means that were statistically different at $P < 0.05$ level indicated in bold.

Time	Adults-vacuum			Nymphs-vacuum			Adults, sticky cards		
	Flws	Nonfls	P-value	Flws	Nonfls	P-value	Flws	Nonfls	P-value
Spring	0.1	0.2	0.700	**10.1**	**0.9**	**0.001**	**1.2**	**0.4**	**0.02**
Early-summer	**3.2**	**0.3**	**0.039**	**6.1**	**0.5**	**0.002**	2.6	1.3	0.08
Mid-summer	**8.2**	**1.4**	**0.010**	**10.8**	**2.7**	**0.01**	**5.6**	**2.7**	**0.002**
Late-summer	6.2	0.6	0.192	6.6	0.9	0.15	4.0	6.9	0.09

2006 Growing Season. We began sampling for TPB in habitats somewhat earlier in 2006 compared to 2005. Abundance of TPB in the small and large mixed weed plots tended to be lower in 2006 than 2005 for reasons that are not fully understood (Fig 2A

compared to Fig 1A). We collected very few adults, although peaks in nymph populations again suggested 3 generations. We had originally planned to sample plots to the end of September in 2006. However, populations crashed in all plots near the apple orchard on the Aug 12 sample due to insecticide drift from the apple orchard (data not shown for this date).

We computed relative abundance estimates of single species plots relative to background populations as was done in 2005 (Figs 2B-F). Although variable, we again observed TPB phenology in the 2 by 2m plots that did not mimic the weed plots but rather corresponded to flowering times. This was more true for TPB nymphs than adults. Penny cress was the earliest flowering species in the 2 by 2m plots and this was reflected in nymph populations. Curly dock flowered next and we observed an associated peak in adults and to some extent, nymphs. However, we also observed a second, larger peak, relative to background populations, a couple of weeks after flowering. At this time curly dock sets and fills large seeds and perhaps this was attractive to adults. Populations in curly dock declined to zero by the third week of July. By cutting the alfalfa plot once in July we were able to extend flowering later into the season in comparison to 2005 where we did not cut the alfalfa. Adult TPB was present very early in alfalfa (May), before flowering, and we believe these were overwintered adults. These early colonizers did not appear to lay eggs, however, since we did not observe nymphs until June. Interestingly, we found a similar pattern for adults in fleabane where overwintered adults were present in May but we did not observe nymphs until late June when the plants began to flower. Annual fleabane is reported to be a good host for TPB early in the spring in the leafy rosette stage, prior to flowering. Finally, we captured very few TPB in the bare ground plots throughout the season. This was true for vacuum samples (Fig. 2F) and sticky cards (data not shown).

Overall, the abundance of TPB was greater in habitats with flowers than habitats without, especially for nymphs and for summer populations of both nymphs and adults (Table 3). In early spring, adult populations were low but abundance was actually higher in nonflowering habitats compared to flowering habitats. This probably reflects habitat colonization patterns of overwintered adults prior to egg-laying. Note that nymph abundance in early spring was greater in flowering habitats than nonflowering habitats, although the difference was not significant.

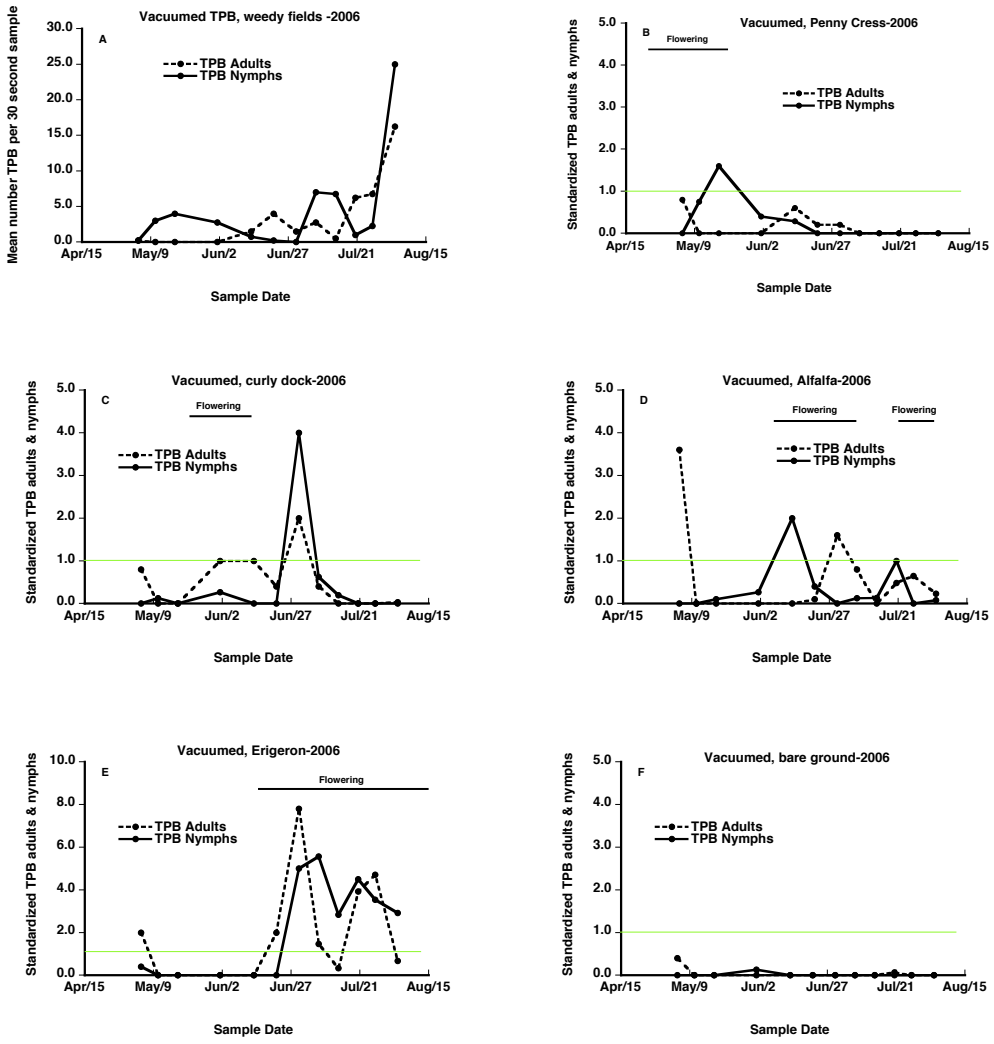

Figure 2. Phenology of tarnished plant bug adults and nymphs in different habitats during 2006 field season based on 30 second vacuum samples. A) Weed fields, absolute numbers shown. B) Penny cress, standardized values. C) Curly dock, standardized values. D) Alfalfa, standardized values. E) Erigeron, standardized values. F) Bare ground, standardized values.

Table 3. Abundance of TPB adults and nymphs measured with 30-second vacuum samples and adults on yellow sticky cards for flowering and nonflowering habitats at four time periods (early spring- 4/28-5/18, spring- 5/18-6/14, early summer- 6/148-7/14, mid-summer- 7/17-8/4) in 2006. Samples collected roughly every week. Means that were statistically different at $P < 0.05$ level indicated in bold.

Time	**Adults-vacuum**			**Nymphs-vacuum**			**Adults, sticky cards**		
	Flws	**Nonfls**	**P-value**	**Flws**	**Nonfls**	**P-value**	**Flws**	**Nonfls**	**P-value**
Early Spring	**0.1**	**0.7**	**0.03**	1.5	0.1	0.06	**0.1**	**0.6**	**0.01**
Spring	0.8	0.3	0.15	**1.6**	**0.4**	**0.001**	0.1	0.02	0.08
Early-summer	**3.8**	**0.5**	**0.004**	**6.4**	**0.4**	**0.02**	**1.6**	**0.4**	**0.01**
Mid-summer	**8.3**	**0.1**	**0.003**	**9.6**	**0.1**	**0.005**	**2.9**	**0.7**	**0.001**

Assessment of visual and olfactory cues

Visual cues

Capture of TPB on sticky cards in the field depended on the color of the sticky card. White cards captured a total of 27 TPB while similar sized red cards only captured 7. This was significantly different than what would be expected if cards were equally attractive ($X^2 = 11.8$, $P < 0.05$, 1 df). This result suggests TPB can use visual cues, such as white flower color, to find host plants in flowering. However, more specific manipulative experiments need to be conducted to better assess the capabilities of adult TPB to locate flowering plants based on visual cues independent or in combination with olfactory cues.

Olfactory cues

We captured relatively few TPB on sticky cards during the three trials to test the attractiveness of volatiles from flowering *E. canadensis*. Hence, it is difficult to draw any definitive conclusions. Of the total of 14 TPB captured, 9 were captured on sticky cards being bathed with *E. canadensis* volatiles (64%) and 5 were captured on control sticky cards being bathed with clear air (36%). Clearly more work is required, but the pattern suggests that TPB responded to olfactory cues even in a weedy field that contained hundreds of flowering plants.

Conclusions

Conclusions from monitoring TPB abundance in these different habitats are consistent with general observations that TPB damage in crops such as strawberries and peaches is positively correlated with weedy conditions. It is somewhat surprising, however, that TPB appeared to be able to track flowering phenology even in small 2 by 2m plots suggesting that TPB is attuned to host plant condition, and in particular, flowering condition. How do they find host plants at the correct phenology? It seems there are two possible hypotheses. First, TPB adults, being mobile, explore the environment in a more or less random fashion, but once they encounter a suitable host

plant, they stay longer and also lay eggs. The second is that they use cues to direct their search to suitable host plants and once there, remain longer, on average, and lay eggs. Our observation that significantly more TPB were captured on white compared to red sticky cards implies that visual cues are used at a distance by adult TPB. Similarly, we captured more TPB on white sticky cards bathed with the odors of flowering *E. canadensis* than on white sticky cards bathed by filtered air lacking host plant odors, although we caught very few TPB in these trials and therefore, definitive conclusions can not be drawn. Moreover, we have no information on the relative importance of visual and olfactory cues. In the future, we plan to examine these questions under more controlled laboratory conditions.

In summary our results indicate that 1) colonization of specific host plants is not random but appears to coincide with flowering status, 2) this occurs at a small scale indicating a good ability to discriminate, and 3) either visual and/or olfactory cues may play a role in orientation behavior. Our results indicate, despite being amazingly polyphagous, that TPB shows considerable selectivity, apparently searching for plants with flowers and young fruit. An improved knowledge of the colonization process and the cues used by TPB to find suitable hosts may lead to the development of new approaches to managing TPB in strawberries and other crops.

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