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SHORT COMMUNICATION

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Efficiency of volatile baited sticky traps for the Tarnished Plant Bug (*Lygus lineolaris*) in strawberry fields

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Abstract

Insecticides have adverse effects on human health and the environment. Thus, the development of non-chemical replacement to manage insect pests is urgent. An alternative is to bait sticky traps with attractive insect sex pheromones or plant volatiles. In Quebec, the tarnished plant bug (*Lygus lineolaris)* is a major insect pest. We tested the efficiency of sex pheromones (mixture of hexyl butyrate (HB), (E)-2-hexenyl butyrate (E2HB) and (E)-4-oxo-2-hexenal (KA)) and sunflower (*Helianthus annuus*) volatiles (pinene, sabinene and phenylacetaldehyde) as sticky trap baits for the tarnished plant bug in strawberry fields of the Laurentians in Southern Québec, Canada. The pheromones decreased the number of tarnished plant bug caught in the traps compared to a control. The sunflower volatile did not have any effect on the number of individuals caught in the traps. Different *Lygus* species use HB, E2HB and KA in different ratios for sexual signals and alarm signals and for species recognition. GC-MS analysis of the sex pheromone bait revealed that the ratios between the three main compounds did not match the intended ratio for the *L. lineolaris* species. This mismatch probably explains our results. Individuals were not attracted to sunflower volatiles. Our results point to the difficulty of manufacturing and using sex pheromones as baits. Future work should assess the effect of several pheromone ratios.

KEYWORDS

chemical communication, pest control, pheromones, plant volatiles

1 | INTRODUCTION

The primary approach to insect pest management is the application of insecticides. However, many insect species are resistant to insecticides (Hawkins, Bass, Dixon, & Neve, 2019). The use of these substances also raises more and more concerns for human health and ecosystem functioning (Aktar, Sengupta, & Chowdhury, 2009). A promising alternative to the application of insecticides is to use attractive volatiles, such as sex pheromones or plant volatiles, to bait sticky traps. Volatiles act to increase the capture success of the traps (Parys & Hall, 2017). Using such traps offers green and species-specific alternatives to insecticides.

In North America, the tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) is a major insect pest that impairs the development of fruits and vegetables through its feeding behaviour (Layton, 2000; Mailloux & Bostanian, 1988). In Quebec, Canada, the tarnished plant bug affects strawberry cultures. Their density is controlled by the application of neonicotinoid and pyrethroid insecticides (CRAAQ, 2014).

Previous work has tested sticky traps baited with sex pheromones and plant volatiles in other cultures outside of Canada for *Lygus lineolaris* and other closely related species (Byers, Fefer, & Levi-Zada, 2013; Fountain et al., 2014; Parys & Hall, 2017). However, the efficiency of volatile baits in increasing trap capture success for tarnished plant bugs in local strawberry cultures is unknown. We quantified the efficiency of two volatile baits (sunflower (*Helianthus annuus* L.), volatile and sex pheromones) for the capture of male and female *L. lineolaris* in strawberry fields in Quebec, Canada. Based on previous work in a closely related species (Blackmer, Rodriguez-Saona, Byers, Shope, & Smith, 2004; Ondiaka et al., 2016), we predicted the sunflower volatile would increase the number of captures for both sexes. We also predicted that sex pheromones would increase the number of captures for males. In addition, we quantified the bycatch of honey bees (*Apis mellifera* L.) in the traps and chemically analysed the contents of the sex pheromone bait.

2 | METHODS

2.1 | Experimental procedures

We deployed sticky traps baited with (a) a sex pheromone, (b) a sunflower volatile, (c) their combination or (d) a control (four treatments) in 15 commercial strawberry fields of different varieties in the Laurentians. Tarnished plant bugs use pheromones made of a mixture of hexyl butyrate (HB), (E)-2-hexenyl butyrate (E2HB) and (E)-4-oxo-2-hexenal (KA) in a 4:10:7 ratio for sexual communication (as identified by Byers et al., 2013). Our sex pheromone treatment consisted of custom ordered polymer disc (diameter of 38mm) soaked in the pheromone blend of 4:10:7 ratio of HB:E2HB:KA and wrapped in a semi-permeable plastic (50 mm × 50 mm; AlphaScent, Syracuse, New York distributed by Solida, St-Ferréol-Les-Neiges, Québec). The sunflower volatile, containing 48.9% pinene, 34.2% sabinene and 16.9% phenylacetaldehyde, was used as an attractant for both sexes (Ondiaka et al., 2016) and delivered from a semi-permeable 1.5 ml Eppendorf tube (AlphaScent). Control traps were not baited with any volatile.

Within each field, we deployed 12 sticky traps. We placed four traps at the centre of the field, four traps at the border of field and four traps in a non-managed area. Traps were placed randomly and set 15 m apart. All homemade traps consisted of a white sticky pad made of white non-uv reflecting cardboard and glue (Tanglefoot® glue; 14 cm × 26 cm) mounted on a metal or bamboo stick set at the lowest height possible given the terrain, but at a minimum height of 30 cm and at a maximum height of 50 cm. We folded the pad in the middle to produce a roof for the olfactory treatment attached underneath (Figure 1). We changed the sticky pads and the olfactory bait every two weeks and counted the number of male and female tarnished plant bug. In total, we installed 270 traps of each treatment during the summer, between 28 May and 30 September 2018.

2.2 | Chemical analyses

We determined the volatile content of the pheromone discs with a gas chromatograph (Agilent 7890A) coupled with a mass analyzer (Agilent 5975C; henceforth GC-MS). The column was a HP-5-MS (non-polar, 30 m length, 250 µm internal diameter, 0.25 µm coating), and the carrier gas was helium (1 ml/min). Temperature programmed was as follows: 60°C initial, 20°C/min ramp to 130°C (standing 5 min), ramp 5°C/min to 160°C, finally ramp to 300°C and standing 3 min for cleaning. A calibration curve was established with > 97% pure compounds. HB was purchased from Sigma Aldrich, E2HB was prepared via acylation of E-2-hexenol with butyric anhydride and pyridine, and KA was prepared following Fountain et al. (2014). We extracted the content of three unused disc (~1.1 g) with methylene chloride (40 ml) for six hours. We filtered the solution and added ethyl benzoate (6 mg) as the internal standard and methylene chloride for a final volume of 50 ml. We analysed this solution by GC-MS and determined the concentration with the calibration curve.

2.3 | Statistical analyses

We used generalized linear mixed models to model the number of bugs caught in sticky traps from June 25th to September 17th as a function of the sticky trap location, the type of bait (control, sunflower volatile, sex pheromone or both volatiles), the sex of individuals trapped ("male" or "female") and all two-way interactions between the fixed factors. The model used a Poisson error distribution. We included the field and observation-level effects as random effects to correct for pseudoreplication and overdispersion, respectively (Harrison, 2015).

FIGURE 1 Picture of the olfactory bait sticky trap. The olfactory treatment was placed under the white sticky trap and mounted on a bamboo or metal rod

We used R version 3.5.1 with the packages lme4, ggplot2 and emmeans (Bates, Maechler, Bolker, & Walker, 2015; R Core Team, 2015; Lenth, 2018; Wickham, 2009). We also used a generalized mixed model to analyse the number of honey bees captured in our traps as a function of the baiting treatment (Poisson error distribution). This model also included the field and observation as random effects.

3 | RESULTS

We found no tarnished plant bug larvae in our sticky traps. The number of individuals caught in the traps did not vary with the zone and traps caught equal numbers of males and females (Table S1). We thus fitted a model with only treatment as a fixed effect to evaluate the main effect of the experimental treatments on the number of captures. According to this simplified model, traps baited with sex pheromones captured significantly fewer individuals than unbaited traps (Poisson GLMM difference estimate ± *SEM*= −0.69 ± 0.14, $p < .001$; Figure 2). Traps baited with sunflower volatiles captured as many individuals as controls (GLMM difference estimate ± *SEM*= −0.15 ± 0.13; $p = .58$). Traps baited with a mix of sex pheromones and sunflower volatiles captured significantly fewer individuals than our controls (GLMM difference estimate ± *SEM*= −0.75 ± 0.14, $p < .001$).

We caught significantly more bees in the sunflower (GLMM difference estimate ± *SEM*=0.22 ± 0.075, $p < .01$) and in the sunflower with pheromones treatment (GLMM difference estimate ± *SEM*=0.38 ± 0.074, $p < .001$) than in the control, but similar numbers in the pheromones treatment than in the control (GLMM difference estimate ± *SEM*= −0.056 ± 0.076, $p > .1$).

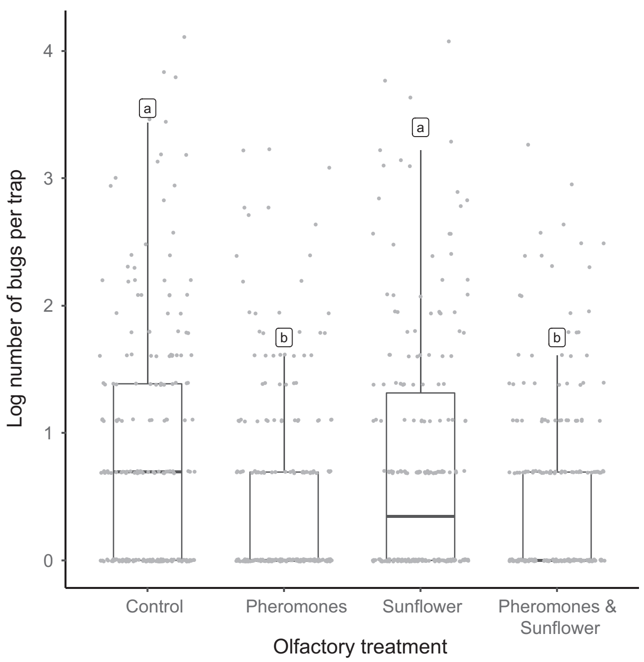

FIGURE 2 Median and interquartile range of the log number of adult tarnished plant bugs per trap, depending on the olfactory treatment. The letters denote significant differences between treatments at $p < .05$

We found the average ± *SD* volatile content of a pheromone disc to be 4.4 ± 0.7 mg HB, 13.8 ± 3.4 mg E2HB and 2.5 ± 0.5 mg KA, in a ratio of 3:10:2.

4 | DISCUSSION

In this study, we evaluated the efficiency of sex pheromones and sunflower volatiles as baits in sticky traps to target tarnished plant bugs in strawberry fields. We found that the sunflower volatile did not have any effect on the efficiency of the sticky trap. Sunflower volatile might not be attractive to *L. lineolaris* or not preferred over strawberries. Previous work demonstrated that the western tarnished plant bug (*Lygus hesperus* Knight) and the European tarnished plant bug (*Lygus rugulipennis* Popp.) are attracted to volatiles emitted by plants that contain the same compounds as we offered (Blackmer et al., 2004; Ondiaka et al., 2016). *L. lineolaris* can discriminate between several host plants (Curtis & McCoy, 1964); thus, it is possible that individuals prefer to feed on strawberry plants when available. We did not test the content of the sunflower volatile, and it is thus also possible that the content was not what was intended, though all components of the mixture should be attractive (Ondiaka et al., 2016). Moreover, the traps baited with sunflower attracted more bees than the controls, suggesting an attractive effect in this species. Non-specific attractant such as sunflower volatiles should be used with caution as they can be harmful if they attract more pollinators than pests.

Contrary to our predictions, we found that sex pheromones decreased the capture success of the sticky traps. In a 4:10:7 (2HB:HB:KA) ratio, its compounds should act as an attractant. The discs we used in this experiment instead contained a 3:10:2 ratio. The low abundance of KA makes our results consistent with previous work. A high abundance of KA is essential for attraction in *L. lineolaris* (Byers et al., 2013; Wardle, Borden, Pierce, & Gries, 2003; Zhang et al., 2007). The effects of pheromones might also depend on the release intensity. The commercial polymer disc could potentially deliver the mixture at high intensities, thereby acting as a repulsive. If this is the case, alternative diffusers like microcapillaries could be more effective than polymer discs (Byers et al., 2013; Innocenzi, Hall, Cross, & Hesketh, 2005). Our results support the idea that for *L. lineolaris*, the presence of KA in high ratio relative to HB is crucial for the attractive effect of sex pheromones.

Our results also point to the technical challenges of producing and using pheromones as baits. The tarnished plant bug uses the same chemical compounds but mixed in different ratios and released at different intensities to produce alarm and sexual signals compared to its related species (Byers et al., 2013; Wardle et al., 2003). Since attraction is not modulated by the presence of a single compound, it is imperative to maintain the ratio during the release of the bait. In our field experiment, no volatile improved the efficiency of the sticky traps relative to controls. For the sunflower volatile, it is likely that is not favoured in a field of strawberries. The failure of the pheromone bait is probably due to a mismatch between the ratio of the three compounds emitted from the bait and from females.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS CONTRIBUTION

FD, P-OM and CP conceived and supervised the field experiment. ML ran and organized the field experiment. DC and OQS ran and interpreted the chemical analyses of the pheromones. LC-T analysed the field data and wrote the manuscript. All authors contributed to the final version of the manuscript and approved publication.

DATA AVAILABILITY STATEMENT

Our data are available in the Appendices S1 and S2 of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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