Field Evaluation of Potential Pheromone Lures for *Lygus lineolaris* (Hemiptera: Miridae) in the Mid-South

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Abstract

Plant bugs (Hemiptera: Miridae) are phytophagous pests of cultivated plants around the world. In the mid-South region of the United States, *Lygus lineolaris* (Palisot de Beauvois) is a primary pest of cotton, and causes economic damage. Previously published research about the volatiles produced by members of the genus *Lygus*, and other closely related groups, indicated that they produce blends of hexyl butyrate, (*E*)-2-hexenyl butyrate, and (*E*)-4-oxo-2-hexenal. Varying ratios of the three compounds were loaded into pipette tips, and screened in combination with non-UV white sticky cards for attractiveness to field populations of *L. lineolaris* in Mississippi. Field screening indicated that a lure expressing a ratio of 4:10:7 was the most effective at collecting *L. lineolaris*, and collected similar numbers of individuals to those reported in other studies using traps baited with live virgin insects over a similar period of time. Availability of a synthetic pheromone usable in the climate of the mid-South will enable broader scale landscape level monitoring for populations of *L. lineolaris* before movement into cotton fields and resulting economic damage.

Key words: tarnished plant bug, Miridae, pheromone, sticky trap

Members of the genus *Lygus* and other closely related mirid species are phytophagous pests throughout the world (Schwartz and Foottit 1998). In particular *Lygus lineolaris* (Palisot de Beauvois), *Lygus hesperus* Knight, and *Lygus elisus* Van Duzee are polyphagous pests of agricultural crops across North America. The western region of the United States has a pest complex consisting of all three species of *Lygus*. The tarnished plant bug, *L. lineolaris*, is a primary pest of cotton across the mid-South, and the only species of *Lygus* present that causes economic damage. In the Delta region of Mississippi, cotton fields receive an average of six insecticide applications directed at controlling tarnished plant bugs (Williams 2015).

Recent work on *Lygus* spp. and other closely related mirids has identified a variety of species-specific attractive chemical blends (e.g. Byers et al. 2013, Fountain et al. 2014, Yang et al. 2015). These species utilize hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal in varying ratios. These three compounds have also been extracted from metathoracic scent glands of *L. lineolaris* collected from the mid-South, and hexyl butyrate and (E)-2-hexenyl butyrate were shown to interfere with attraction to sex pheromones at physiologically relevant doses, but failed to be more attractive to males than virgin females in a blend (Zhang et al. 2007). Byers et al. (2013) screened a variety of ratios of the three compounds in southern AZ, where three *Lygus* pest species are present, and measured attractiveness of the various blends. Although one of Byer's ratio

blends attracted *L. lineolaris*, the landscape composition, climate, and pest complex of the southwestern desert differs significantly from that of the mid-South. Therefore, four potential blends recently published by Byers et al. (2013) and a commercially available lure for mirids available in the United Kingdom were screened in the Mississippi Delta.

Materials and Methods

Four sampling sites were established during the summer of 2014 for field screening of the pheromone blends. Two of the sites were located on research farms belonging to the USDA-ARS' Southern Insect Management Research Unit and the Delta Research and Extension Center at Mississippi State University, both located in Stoneville, MS. The other two sites were located on Heathman Plantation, a commercial farm located just west of Indianola, MS. Although the research farms were planted in a wide variety of crops, the sites at Heathman were placed at the interface between large fields of commercially planted corn (*Zea mays* L.) and soybeans [*Glycine max* (L.)]. At each site, six sampling locations a minimum of 500 m apart were marked with a 1 m green garden stake.

Blends of hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal were formulated in pipette tips as described by Fountain

et al. (2014). These release this blend for several weeks under field conditions (Fountain et al. 2014). Lures were color coded to eliminate confusion in the field and ensure accuracy in deployment; color labels are used here for clarity. The lures contained ratios of the three compounds as follows: 10:1:7 (silver), 10:0:7 (red), 4:10:7 (blue), and 0:10:7 (green). The former two were reported to be attractive to *L. hesperus* and the latter two to *L. lineolaris* by Byers et al. (2013). A fifth lure (UK) tested was a commercially formulated product sold in the United Kingdom for monitoring *L. rugulipennis* (Agralan Ltd., Swindon Wilts, UK) and contained a 10:0.3:2 blend.

Lures were used with non-UV white sticky traps (Great Lakes IPM, Vestaburg, MI), as they are commercially available and recommended for L. lineolaris monitoring in orchards (Prokopy et al. 1979). A horizontally placed pheromone lure (or no lure for control) and sticky trap were clipped to the top of a garden stake with a binder clip ~ 1 m above the ground. Lures were renewed every other week. Sticky traps were collected weekly, replaced with a new card, and lures shifted by one location at each site to minimize potential site-specific issues or interactions. Sticky traps were covered in clear plastic upon removal from the field to reduce specimen damage and returned to the lab for analysis. The total number of L. lineolaris on each trap was counted, insects were removed from the trap using citrus oil, and individuals were sexed. Statistical analyses were performed using a mixed model in Proc Glimmix, including a random statement of location (date) and position (location), with a Poisson distribution (SAS 9.4). All count data had an additional 0.1 added due to the number of traps that collected no insects, and means were separated using least square means. Difference was considered significant at $\alpha < 0.05$.

Results and Discussion

Of the total insects collected, very few female *L. lineolaris* (n = 11) were caught on the traps. There were no significant differences in the number of females among collection dates, lure types, or in the

date*lure interaction. The majority of the insects collected were male, similar to previously published studies on *Lygus*. Males showed significant differences in attraction to different lures (df = 5, 108; F = 10.4; P < 0.0001) but no significant differences between collection dates or in the interaction of date*lure were observed. The total number of insects collected also indicated significant differences between lures (df = 5, 108; F = 10.26; P < 0.0001), but again no significant differences between collection dates or in the interaction of date*lure were observed. Results showed that the blue lure (4:10:7) resulted in the highest trap captures, while the green lure (0:10:7) the second most, followed by the other blends that did not vary significantly from the control (Fig. 1).

Byers et al. (2013) showed that ratios of hexyl butyrate to (E)-2hexenyl butyrate were essential, as heterospecific Lygus males were not attracted to blends designed for other species. Additionally, previous research by Zhang et al. (2007) indicated that these same compounds that act as a lure, can act as a mating deterrent to male L. lineolaris at other concentrations and ratios. Field screening of potential lures here in the mid-South indicated that the blue lure (4:10:7) was the most effective at collecting L. lineolaris, and collected similar numbers of individuals to traps baited with live virgin insects over a similar period of time in other studies conducted in this geographic area (Scott and Snodgrass 2000, Zhang et al. 2007). These results confirm those of (Byers et al. 2013) using more practical dispensers. While the baiting of traps with virgin insects produces similar collection numbers, it is time and labor intensive, requiring food placed in enclosures to be replenished frequently and insects to be replaced as they age. These factors make it impractical for large scale monitoring efforts across the landscape. The availability of a synthetic pheromone that works in the climate of the mid-South will enable landscape level sampling without human variability. Further research is needed to relate these lures to field populations for use in monitoring and potentially to explore possibilities of mating disruption as components of L. lineolaris monitoring.



Fig. 1. Weekly means of the total number of *L. lineolaris* collected (left) and males alone (right) using each lure type in conjunction with a non-UV white sticky trap. Letters above the bars represent significant differences among lure types based on least square means [lures contained hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal in ratios of 10:1:7 (silver), 10:0:7 (red), 4:10:7 (blue), 0:10:7 (green) and 10:0.3:2 (UK)].

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