EFFECT OF ROADSIDE ALFALFA MOWING ON *LYGUS HESPERUS* ABUNDANCE IN ADJACENT COTTON: A FIELD MARKING STUDY

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Abstract

Quantification of *Lygus hesperus* intercrop movement between volunteer roadside alfalfa and adjacent cotton is not possible by indirect sampling methods such as sweep net or drop-cloth. In 2007, a field marking and capture study was conducted at three selected sites in Lubbock County, Texas to quantify *L. hesperus* insect movement from roadside alfalfa into neighboring cotton and to characterize *in situ L. hesperus* insect host preference behavior. Roadside alfalfa marking was accomplished by spraying the plants with a 10% chicken egg albumin protein solution (25 gal/acre target rate) 24 hours prior to mowing. Subsequently, adult *Lygus* insect samples were collected from adjacent cotton and undisturbed alfalfa by sweep sampling 24 hours after alfalfa mowing. *L. hesperus* insect movement was inferred by marker protein detection in the laboratory from collected *Lygus* insect specimens via direct Enzyme-Linked Immunosorbent Assay (ELISA). Forced to relocate due to alfalfa unsuitability resulting from mowing, a significantly greater percentage of marked and relocated *Lygus* insects was subsequently detected in undisturbed alfalfa than in cotton. Specifically in the alfalfa, 86.7% of the marked and relocated *Lygus* insects were found when mowing was performed during cotton blooming period, and 87.2% during boll maturation period. Only a slightly greater percentage of *Lygus* moved to cotton when mowing was performed at cotton blooming stage (13.3%) versus during boll maturation (12.8%), indicating that cotton flowering and boll development stages were equally attractive to dispersing *Lygus.* This study revealed that undisturbed alfalfa is likely to attract and retain more *L. hesperus* insects than neighboring cotton when nearby alfalfa is mowed. As a possible cultural control practice in similar landscape configurations, leaving a narrow strip of undisturbed roadside alfalfa when mowing may reduce *L. hesperus* movement into cotton.

Introduction

Lygus hesperus is a polyphagous insect capable of surviving in a broad range of hosts (Young 1986, Day 1996). Its presence has been reported in 26 unique roadside weed hosts in the Texas High Plains (Parajulee et al. 2003). Roadside alfalfa is a primary host of *Lygus* insects in the Texas High Plains, particularly during spring and early summer months. It has been shown that *Lygus* bugs prefer alfalfa as a host versus cotton and several other weed hosts (Sevacherian and Stern 1974). One study reported that *L. hesperus* laid 78% more eggs in alfalfa than in cotton (Jackson 2003). Furthermore, previous studies have indicated *Lygus* emigration from alfalfa and other weed hosts into cotton (Fleischer et al. 1988, Sevacherian and Stern 1975).

Since the development of recommended *Lygus* management practices depends upon source-sink dynamics, quantification of net *Lygus* insect emigration and immigration in adjacent volunteer roadside alfalfa and cotton fields is important. *Lygus* population dispersal from alfalfa to adjacent cotton might inadvertently be encouraged by roadside mowing performed by government agencies. Researchers in California have shown that strip-cutting commercial alfalfa fields prevent the dispersal of western tarnished plant bugs (*Lygus hesperus*) to cotton (Mueller et al. 2005)**.** Similarly, an area-wide *Lygus* management project in Mississippi has demonstrated that roadside weed management is an effective means of minimizing tarnished plant bugs (*Lygus lineolaris*) in adjacent cotton (Abel et al. 2007).

Because *Lygus* insects can complete their lifecycle in cotton and alfalfa, understanding the source-sink dynamics of the two habitats is difficult. In our region, it has been reported that *Lygus* females prefer laying eggs in alfalfa than in cotton as evidenced by a much greater number of immature *Lygus* in alfalfa versus cotton (Carroll et al. 2005, Barman et al. 2006). If the survival rates are the same in both crops, then logically, alfalfa is a source because of higher *Lygus* reproduction in this crop. However, the actual rates of reproduction and survival of *Lygus* harbored by these two habitats under actual field situations are not well understood. A source-sink relationship is a dynamic phenomenon, because it fluctuates based upon numerous factors, including competitors, predators, movement and migration, changes in environmental factors, and habitat phenology. Also, because the realized niche of any organism is an n-dimensional hypervolume, it is inherently affected by many factors simultaneously.

Lygus bugs have become a major concern in the Texas High Plains during recent years. Alfalfa is an important *Lygus* host because, in the Texas High Plains, it can support continuous *Lygus* populations, although overwintering populations in the region are small. *Lygus* populations develop during late spring and early summer, and one presumption of crop managers and producers is that *Lygus* populations in volunteer roadside alfalfa disperse to nearby cotton fields during summer. The significance of roadside alfalfa's contribution to *Lygus* populations in adjacent cotton is not well understood. The objective of this study was to determine the effect of roadside alfalfa mowing on *Lygus* insect abundances in adjacent cotton fields.

Materials and Methods

The study was conducted during 2007 in Lubbock County, Texas. Three sites were selected at one-mile minimum intervals. A site consisted of a ≥200-ft long, ~90% pure stand of volunteer alfalfa situated between a roadway and an irrigated cotton field. Each site was inspected to ensure sufficient *Lygus* population presence in alfalfa. At each site, a protein marker consisting of 10% chicken egg albumin solution (25 gal/acre target rate) was applied, via a high volume spot-sprayer, to a flagged, 100-ft section of volunteer roadside alfalfa. Twenty-four hours following marker application, marked alfalfa was mowed to a height of 3-5 inches with the intention of inducing *Lygus* movement to either the adjacent cotton field, or nearby undisturbed alfalfa. Sections of undisturbed alfalfa measuring 16 inches X 100 ft and adjacent cotton row (40-inch row spacing) X 100 ft were sampled using a "Keep It Simple Sampler" (KISS; Beerwinkle et al. 1997). Specimens were analyzed for marker protein using indirect ELISA (Fig. 1).

ELISA Procedure

Antigen samples were prepared by incubating one *Lygus* bug in 200 µl of 1x Tris-Buffered Saline (TBS) at 4°C for 12 hours. The antigen solution was loaded into a microtiter plate also containing known positives and negatives, and then incubated at 37°C. The plate was then washed three times with 2x phosphate-buffered saline with Tween 20 (PBST), and then blocked with 180 µl of blocker protein for one hour. Next, the plate was washed three times with 2x PBST, then incubated for one hour using 80 µl of primary antibody solution. Then, the plate was washed three times with 5x PBST, and incubated for another hour using 80 µl of secondary antibody solution. Once more, the plate was washed three times, only this time using 5x PBST, after which 80 µl of substrate (3,3',5,5'-Tetramethylbenzidine (TMB) was added to each well. The microtiter plate was incubated at room temperature for five minutes with frequent shaking. Finally, the reaction was stopped using 50 µl of 650 nm liquid stop solution (BioFX, laboratory). Absorbance was measured at a wavelength of 650 nm using a Stat Fax® 3200 Microplate Reader (Awareness Technology, Inc., FL) (Fig. 1).

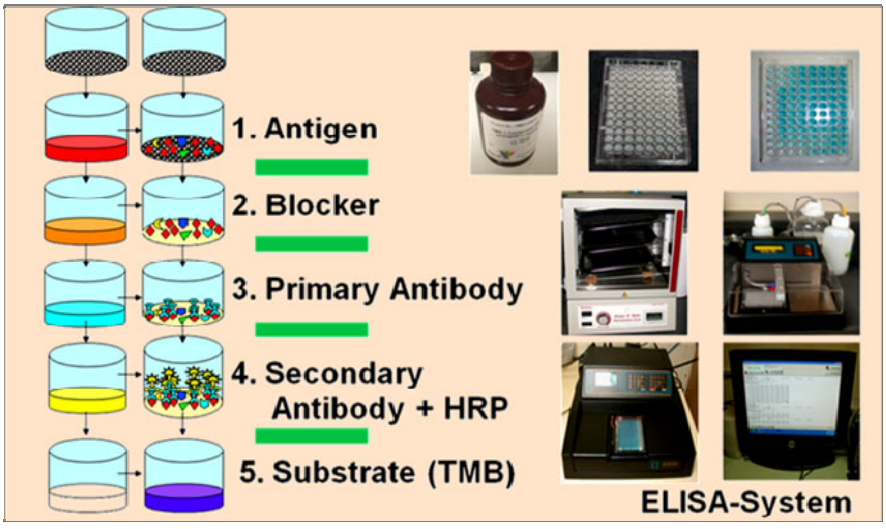

Figure 1. Diagram of indirect ELISA procedure and pictures of equipment used.

Data Analysis

The absorbance values for each *Lygus* sample were compared with the absorbance values of eight known negative control samples, and were interpreted as positive only when the absorbance values of the samples equaled or exceeded the mean absorbance value of the negative samples plus three times the standard deviation of the negative control samples. When the resulting value was smaller, the specimen was judged negative. *Lygus* samples deemed positive for marker protein reactivity had moved from marked and mowed alfalfa to unmarked hosts. The total number of *Lygus* bugs detected from unmarked habitats was calculated. Data were analyzed with Analysis of Variance (ANOVA). Means were separated using the PROC GLM procedure (SAS Institute 2008).

Results and Discussion

A total of 264 adult *Lygus* were collected from 600 row-ft of cotton and 600 ft of alfalfa. Five times more *Lygus* were found in alfalfa (222 adults) than in adjacent cotton (42 adults). This suggests that as a host, alfalfa is preferred over cotton. Out of 264 total *Lygus* insects analyzed, 99 were found positive for the chicken egg albumin protein marker. These were considered as *Lygus* which emigrated from marked and mowed alfalfa. Significantly (DF=1, 4; F=7.5, P=0.05) more *Lygus* insects (14.3 *Lygus* insects/100 row-ft) emigrated from mowed alfalfa to adjacent undisturbed alfalfa than to cotton (2.3 *Lygus* insects/100 row-ft), regardless of cotton phenological stage (Fig. 2). Cotton phenological stage had no significant effect (DF=1, 2; F=1.04, P=0.42) on *Lygus* movement from marked and mowed roadside alfalfa plots. Despite this, more marker-positive *Lygus* insects per sample date were captured during cotton blooming, an average of 10.2 adult *Lygus* from alfalfa and cotton combined, than during boll development period of time with an average of 6.5.

***Lygus* movement during cotton blooming**

A total of 216 *Lygus* adults were collected from 300 row-ft of cotton and 300 ft of alfalfa during cotton blooming and analyzed by ELISA. Among those, 28%, or 60 insects, tested positive for chicken egg albumin, indicating their origination in marked and mowed alfalfa. Among those, 6.7 times more *Lygus* insects, or 86.7% at a level of 17.3 insects per 100 ft, were attracted by blooming alfalfa than by cotton, which attracted 13.3% at a level of 2.6 insects per 100 row-ft. In alfalfa, 28.4% of *Lygus* insects were found to have immigrated from marked and mowed alfalfa, whereas in cotton, 32.0% originated from marked and mowed alfalfa (Fig. 3).

***Lygus* movement during boll development**

A total of 48 *Lygus* adults were collected from 300 row-ft of cotton and 300 ft of alfalfa during boll development and analyzed by ELISA. Among those, 81%, or 39 insects, tested positive for chicken egg albumin, indicating origination in marked and mowed alfalfa. Among those, seven times more *Lygus* insects, or 87.2% at a level of 11.3 insects per 100 ft, were attracted by alfalfa than by cotton, which attracted 12.8% at a level of 1.6 insects per 100 row-ft. In alfalfa, 87.2% of *Lygus* insects were found to have immigrated from marked and mowed alfalfa, whereas in cotton, 55.6% came from marked and mowed alfalfa (Fig. 4).

Cotton phenology affected the abundance of *Lygus*, with 4.5 times more *Lygus* insects observed during cotton blooming than during boll development stage. This was mainly because both alfalfa and cotton became less favorable as hosts due to alfalfa senescence resulting from low moisture conditions and cotton boll enlargement and hardening through maturation. This study showed that *Lygus* insects prefer dwelling in alfalfa over cotton, thus it may be possible to retain a majority of affected *Lygus* in alfalfa by leaving a narrow unmowed strip of alfalfa or by adjusting roadside alfalfa mowing height to a slightly higher level, either of which would retain foliage and canopy shelter suitable for habitation. Results clearly indicate that a portion of the *Lygus* population inhabiting roadside volunteer alfalfa can be relocated to nearby cotton fields when alfalfa is mowed. Characterization of bidirectional *Lygus* movement dynamics between roadside alfalfa and cotton is necessary to better plan for minimization of *Lygus* movement into cotton from adjacent roadside alfalfa. Thus, the effect of alfalfa mowing height and the seasonal dynamics of net *Lygus* intercrop movement between alfalfa and cotton should be determined.

Summary

Once mowed, cut alfalfa gave up more *Lygus* insects to adjacent, undisturbed alfalfa than to cotton. Only a slightly greater percentage of *Lygus* moved to cotton when mowing was performed at cotton blooming stage (13.3%) versus during boll maturation (12.8%), indicating that the roadside alfalfa can be a source of *Lygus* to both blooming and boll maturing adjacent cotton. In similar landscapes configured with proximate cotton and volunteer roadside

alfalfa, mowing of roadside alfalfa such that a narrow unmowed alfalfa strip would be preserved would likely be advisable when the goal is reduction of *L. hesperus* movement into cotton. Quantification of insect movement would be useful in developing a model for ascertaining insect dispersion and intercrop movement. *In situ* insect protein marking and subsequent ELISA detection has the potential to facilitate such research; however, marker concentration is a critical consideration impacting effectiveness.

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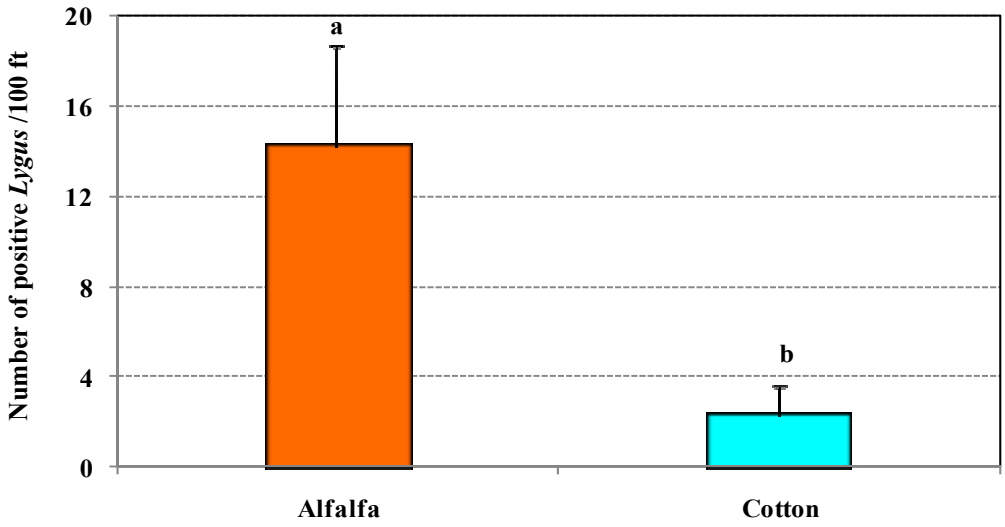

Figure 2. Average number of detected *Lygus* (n=3 sites) emigrating from marked and mowed alfalfa to adjacent cotton and undisturbed alfalfa. Different letters shown atop bars indicate a significantly different number of *Lygus* insects having moved into the two hosts (α= 0.1 level, LSD test).

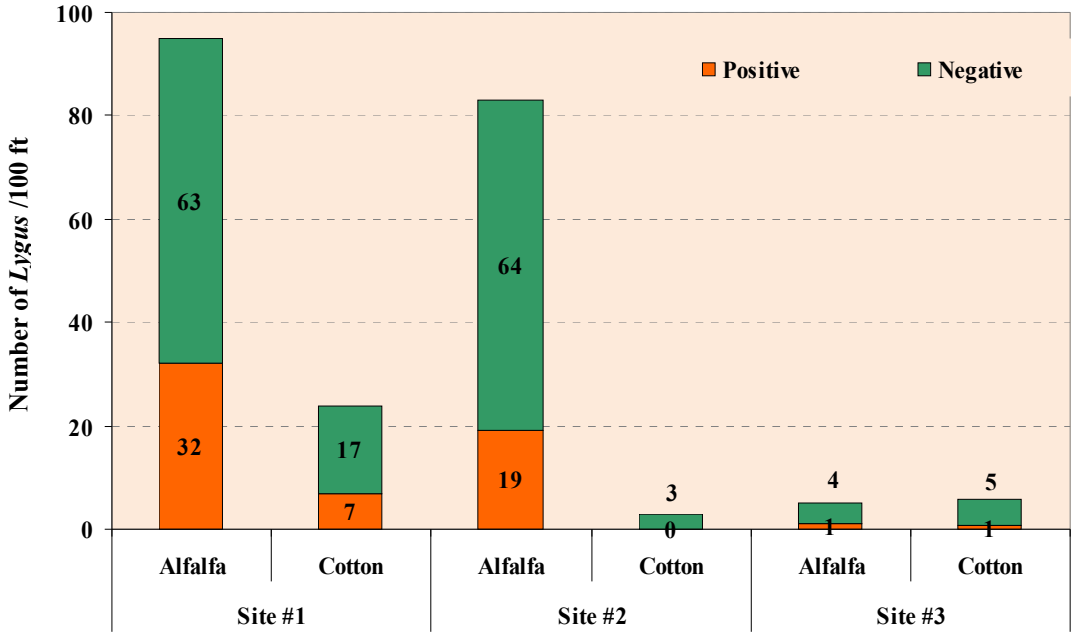

Figure 3. Number of marked/unmarked *Lygus* retrieved from cotton and undisturbed alfalfa adjacent to the marked and mowed alfalfa during cotton blooming. The numbers atop bars show the actual number of *Lygus* insects collected and analyzed by ELISA. *Lygus* insects testing positive for chicken egg albumin originated in marked and mowed alfalfa, while *Lygus* testing negative were residents or recent immigrants.

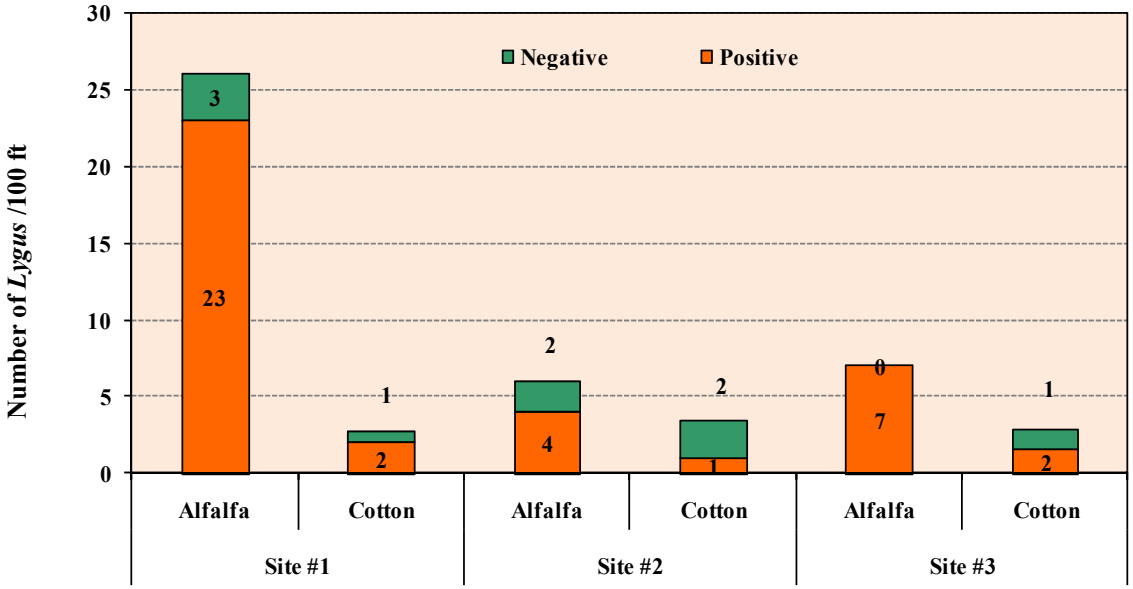

Figure 4. Number of marked/unmarked *Lygus* insects retrieved from alfalfa and cotton adjacent to marked and mowed alfalfa during cotton boll development. Note: numbers atop bars show the actual number of *Lygus* insects collected and analyzed by ELISA. *Lygus* insects testing positive for chicken egg albumin originated in marked and mowed alfalfa, while *Lygus* testing negative were residents or recent immigrants.