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Characterization of Intercrop Movement of *Lygus hesperus* between Cotton and Alfalfa

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ABSTRACT

Lygus hesperus Knight (Miridae: Hemiptera), a key pest of cotton in the United States, is a highly polyphagous insect. Upland cotton (*Gossypium hirsutum* L. var. *hirsutum*) and alfalfa (*Medicago sativa* L.) are two major field crop hosts of *Lygus hesperus* in the Texas High Plains. While alfalfa is considered a source of *Lygus* in cotton, *Lygus* intercrop movement behavior has not been fully characterized in cotton-alfalfa systems. Understanding the intercrop movement behavior of *Lygus* may facilitate better decision-making for *Lygus* management in these crops. A series of studies including a mark-release-recapture study and season-long field monitoring of *Lygus* were conducted in the Texas High Plains, USA. Season-long field marking and monitoring of *Lygus* intercrop movement revealed bidirectional *Lygus* movement and confirmed that *Lygus* preferred alfalfa over cotton. Net movement of *Lygus* between cotton and alfalfa was influenced by cotton phenology. A "two-crop/two-marker" field-marking and monitoring approach was successfully applied in characterizing *Lygus* seasonal intercrop movement. This approach can be used to study the effect of various crop management practices on *Lygus* intercrop movement and is applicable to other pests and cropping systems.

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1. Introduction

The "Push and Pull" strategy is an important component of integrated pest management (IPM) (Cook et al., 2007). The strategy of preserving sink habitats (trap crops) and destroying source habitats (alternate hosts) of insect pests is effective in reducing pest populations in field crops. Similarly, maintaining source habitats for predators and parasitoids increases biological control services (Khan & Pickett, 2004). While knowledge of source-sink dynamics of a pest population is valuable in formulating IPM strategies, determining whether a host acts as a source or a sink is challenging, especially when the pest species is highly polyphagous.

Lygus hesperus Knight, the western tarnished plant bug, is a highly polyphagous insect. It can survive and reproduce on a broad range of hosts (Day, 1996; Young, 1986). This species has been reported in 26 unique roadside weed hosts in the Texas High Plains (Parajulee et al., 2003; Parajulee, Shrestha, Barman & Carroll, 2008). Alfalfa is a primary host of *L. hesperus* in the Texas High Plains, particularly during the spring and early summer. Previous studies have demonstrated that *Lygus* prefer alfalfa over cotton and several other weed hosts (Sevacherian & Stern, 1974). Jackson (2003) reported that *L. hesperus* lay significantly more eggs (78%) in alfalfa than cotton. Past studies have also indicated that *Lygus* can move from alfalfa and other weed hosts into cotton (Fleischer, Gaylor & Hue, 1988; Sevacherian & Stern, 1975).

The severity of Lygus infestations in cotton depends upon local source-sink dynamics. For example, dispersal of Lygus populations from alfalfa to adjacent cotton could be encouraged by government-enforced mowing of roadside-growing "source" host species such as alfalfa. However, researchers in California have shown that strip-cutting commercial alfalfa fields prevents the dispersal of *L. hesperus* to cotton (Mueller, Summers & Goodell, 2005). Similarly, an areawide *Lygus* management project in Mississippi has demonstrated that roadside weed management is an effective means of minimizing tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), and bollworms in adjacent cotton. Expanding current knowledge of Lygus source-sink dynamics by quantifying the contribution of roadside-volunteer alfalfa to Lygus infestations in adjacent cotton could benefit Lygus management strategies.

Lygus can lay eggs and complete their life cycle in both cotton and alfalfa. Therefore, it is often confusing to determine whether roadside alfalfa is acting as a source or a sink for a Lygus population in an adjacent cotton field. In some alfalfa fields, large numbers of *Lygus* are found while

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very low numbers are detected in adjacent cotton. It seems logical, for such a scenario, to assume that alfalfa is acting as a sink for *Lygus*, potentially drawing them from cotton. While it is seemingly logical, such a conclusion may not be congruent with reality. Lack of consensus exists among researchers on the role of alfalfa in Lygus severity in adjacent cotton fields. In general, a higher density of *L. hesperus* in alfalfa than cotton might be due to a higher level of preference for oviposition in alfalfa than cotton. Carrière et al. (2006, 2012) reported alfalfa acted as a source of *L. hesperus* to nearby cotton fields; however, others reported alfalfa served as sink and reduced *L. hesperus* infestation in nearby cotton (Stern, Bosch & Leigh, 1964). A scientific approach characterizing the source or sink role of a weed host involves quantifying insect movement throughout the crop-growing season and determining their survival and reproductive success.

It has been reported that *L. hesperus* prefer laying eggs in alfalfa over cotton. If the mortality and survival rates are the same in both crops, then logically, alfalfa would be a source because of higher *L. hesperus* reproduction in this crop. However, the actual rates of reproduction, survival, and mortality of *L. hesperus* in these two hosts growing under actual field situations are not well understood. A source-sink relationship is a dynamic phenomenon, which can be affected by numerous factors, including competitors, predators, intercrop movement, environment, and host phenology. Also, because the realized niche of any organism is an n-dimensional hypervolume, it is inherently affected by many factors simultaneously. By elucidating the role of these factors, a greater understanding of the source-sink relationship between alfalfa and cotton can be characterized and better-informed pest management decisions can be made.

Suppression of roadside weed hosts (potential source of Lygus bugs) using herbicides reduced the level of Lygus infestation in adjacent cotton fields and reduced the application of insecticides in cotton in the Mid-South USA (Abel, Snodgrass & Gore, 2007). However, indiscriminate killing of roadside weeds using herbicide is not permissible in Texas. A common belief of producers and extension specialists in the Texas High Plains is that mowing and/or drying of roadside alfalfa and other weed hosts forces Lygus into adjacent cotton. If this is true, then a well-designed mowing strategy could be developed with the aim of "holding" Lygus in alfalfa and preventing their emigration to cotton. The vulnerability of cotton to Lygus injury changes with cotton phenological stages. It is more critical to manage *L. hesperus* during early boll development stages than in the boll maturation stage in the Texas High Plains (Parajulee, Adhikari, Kerns, Shrestha & Carroll, 2011). It is possible that the timing of alfalfa mowing can be managed to avoid or reduce *L. hesperus* movement during phenological stages of cotton critically vulnerable to Lygus. In addition, the application of biological control agents or pesticides on alfalfa strips prior to alfalfa mowing may reduce *L. hesperus* movement into cotton. A pest management practice that minimizes the movement of pest insects from source habitats into crop fields will reduce the amount of insecticides applied on the crop.

Sweep-net sampling has been used for the indirect assessment of contribution of weed hosts in the infestation of Lygus bugs in adjacent cotton (Cleveland, 1982; Parajulee & Shrestha, 2014). However, sampling *L. hesperus* without specific marking does not demonstrate actual movement between unique hosts. Stern and Mueller (1968) used micronized fluorescent powder to study movement of *L. hesperus*. Physical marking is labor intensive and potentially interferes with insect biology and behavior. Moreover, physical markers should be environmentally safe, scalable, cost-effective, and easy to use (Hagler & Jackson, 2001). Techniques involving insect protein marking and subsequent detection using enzyme-linked immunosorbent assay (ELISA) have been used successfully in studies involving insects such as *Hippodamia convergens* Guérin-Méneville (convergent lady beetle) (Bastola et al., 2016; Hagler, 2004; Hagler & Naranjo, 2004), *Pectinophora gossypiella* (Saunders) (pink bollworm) (Hagler & Miller, 2002), *Cacopsylla pyricola* Foerster (pear psylla) (Jones, Hagler, Brunner, Baker & Wilburn, 2006), *Pieris rapae* L. (cabbage worm) (Schmaedick, Ling, Gonsalves & Shelton, 2001), and thrips species *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande) (Jasrotia & Ben-Yakir, 2006). Thus, it is presumed that this technique may prove satisfactory in evaluating *L. hesperus* intercrop movement in the Texas High Plains.

The objective of this study was to characterize intercrop movement behavior of *L. hesperus* to elucidate cotton-alfalfa source-sink dynamics, with an expectation that information generated would prove useful in *L. hesperus* pest management, specifically with regard to reducing *L. hesperus* movement from roadside alfalfa to adjacent cotton. This study was designed to evaluate *L. hesperus* host selection between alfalfa and cotton, the impact of alfalfa mowing on *L. hesperus* abundance in adjacent cotton, *L. hesperus* host preference and dispersal behavior, and season-long *L. hesperus* intercrop movement between alfalfa and cotton.

2. Materials and Methods

The study was conducted in Lubbock County (33.5779° N, 101.8552° W), Texas, which is located centrally in the Texas High Plains region of the United States. Two field experiments were conducted to characterize intercrop movement behavior of *L. hesperus* between cotton and alfalfa in the Texas High Plains during 2005-2008: 1) *L. hesperus* host preference under field conditions, and 2) Season-long monitoring of *L. hesperus* intercrop movement behavior.

2.1. L. hesperus Host Preference under Field Conditions

Because past field studies revealed that *L. hesperus* preferred alfalfa over cotton, it was hypothesized that more Lygus bugs would move from cotton to alfalfa than from alfalfa to cotton under natural field conditions, provided only the two host choices were available. In order to evaluate this hypothesis, two types of insect marking-recapture studies were conducted near Lubbock, Texas: 1) Mark, release, and recapture (MRR) using laboratory-marked field collected *L. hesperus* adults, and 2) Field marking, mowing, and capture (FMMC), an *in-situ* test of *L. hesperus* intercrop movement.

Mark, Release, and Recapture (MRR). A field experiment with two treatments (alfalfa and cotton) and three blocks was deployed in a strip-block design. A 12-row patch of alfalfa (1.02 m rows running north-to-south), measuring approximately 180 m x 12 m, was planted in the middle of a field and cotton was planted on both sides of alfalfa during the last week of April in 2007. Alfalfa and cotton fields were divided into three blocks measuring approximately 60 m x 12 m each. In August 2007, approximately 4,000 *L. hesperus* adults were collected from a nearby alfalfa field near Idalou, Texas. Active *L. hesperus* adults were externally marked with non-arthropod protein in the laboratory by nebulizing adults with the marker-protein solution for fifteen minutes. An Invacare® Envoy (Model RC1001) nebulizer was used to convert marker protein solutions to an aerosol. A 50% nonfat dry milk (NFDM) solution was used to mark 1,500 *L. hesperus*, while another 1,500 were marked using a 100% egg white (EW) solution. The bovine milk casein from NFDM and chicken egg albumin from EW served as the non-arthropod marker proteins.

EW-marked *L. hesperus* were released onto cotton plants at the center of each block at a rate of 500 adults per block. Similarly, NFDM-marked Lygus were released at the centers of alfalfa blocks at the same rate. Both releases were performed in the evening on the same day of collection. This study was conducted while cotton was in peak bloom, and alfalfa was in its post-blooming stage. Released Lygus adults were recaptured using a "Keep It Simple" or "KIS" sampler at 24- and 96-hours post-release. The KIS sampling device consisted of an Echo® model PB 265 backpack leaf blower (nominal airflow rating: 458 cfm) modified with an insect collecting net. Two KIS samples each covering 30 meters of row were collected from each block in each crop. Lygus samples were killed by freezing, sorted, and eventually stored individually in microcentrifuge tubes at -20°C for further processing via indirect enzyme-linked immunosorbent assay (ELISA). The detailed protocol for ELISA has been described in the *Indirect ELISA* sub-section below.

L. hesperus movement between crops was quantified based upon positive or negative to marker protein in ELISA. Lygus adults collected from alfalfa testing positive for EW protein were recorded as Lygus having moved from cotton to alfalfa. Similarly, all Lygus adults collected from cotton testing positive for NFDM protein were recorded as Lygus having moved from alfalfa to cotton. Net *L. hesperus* movement into cotton for each block was calculated by subtracting the number of emigrant Lygus bugs (those having moved from cotton to alfalfa) from the number of immigrant Lygus bugs (those having moved from alfalfa to cotton).

Field Marking, Mowing, and Capture (FMMC). Because the MRR study demonstrated the physical, "unidirectional" movement of Lygus bugs between alfalfa and cotton and the numbers of Lygus bugs marked and recaptured in the MRR study were too small to represent natural intercrop movement of Lygus bugs, a subsequent study using FMMC was conducted. A split-plot randomized block experiment with three blocks was designed. The main plot factors were two cotton growth stages: blooming and post-blooming (boll development). The subplot treatments were two hosts (cotton versus alfalfa). In July 2007, six field sites were selected in Lubbock County, Texas. Each site consisted of a long patch of blooming roadside alfalfa (>60 m in length) adjacent to a cotton field. Three sites were with blooming cotton and three sites with cotton at post-blooming stage. Sites were approximately 3 km apart. Each site represented an experimental block.

Alfalfa was sampled using a standard sweep-net (40-cm diameter) prior to the experiment to verify presence of *L. hesperus*. Thirty-meter long x 12 m wide alfalfa plots were marked using colored flags. Alfalfa plots received two high-volume spray applications of 10% NFDM with the intention of thoroughly drenching the alfalfa plants with the protein marker. Following alfalfa marking, the natural population of *L. hesperus* were allowed to forage for 24 hours, after which the alfalfa was mowed to a height of 12 cm with a tractor-mounted mower. A portion of the alfalfa plot was not sprayed and left uncut, hereinafter referred to as 'unmowed', to provide migrating Lygus with unmowed alfalfa as a host choice along with the adjacent cotton. The Lygus population was then allowed to forage, roam, and settle in its preferred host (unmowed patch of alfalfa versus cotton). Then Lygus adults were collected using a KIS sampler at 24 h and 96 h after mowing the alfalfa. While only one KIS sample, covering 30 m of row, was collected from unsprayed and unmowed alfalfa, four samples were collected from adjacent cotton (5^{th}, 10^{th}, 20^{th}, and 40^{th} rows, counting outward from the road into the field). More samples were collected from cotton to ensure that a sufficient number of marked Lygus would be collected for analysis by ELISA because Lygus population density is typically low in Texas High Plains cotton. Collected Lygus samples were killed by freezing, sorted, and stored individually in microcentrifuge tubes at -20°C for further processing via indirect ELISA.

Adult *L. hesperus* emigration from mowed alfalfa was determined by detecting NFDM marker protein adherence to Lygus via indirect ELISA. All Lygus adults collected from cotton or undisturbed alfalfa testing positive for NFDM protein were recorded as Lygus having emigrated from mowed alfalfa (where NFDM solution was originally applied). *L. hesperus* emigration from mowed alfalfa to cotton and to unmowed alfalfa was thus quantified.

2.2. Season-long Monitoring of L. hesperus Intercrop Movement

The intercrop movement of *L. hesperus* between cotton and alfalfa was monitored for seven weeks each in 2008 and 2009 cotton growing seasons. Field experiments were conducted at the Texas A&M AgriLife Research and Extension Center farm near Lubbock, Texas. *L. hesperus* intercrop movement was determined by field-marking of natural populations of Lygus adults in alfalfa and adjacent cotton field using two protein markers, capturing the adults using a KIS sampler, and detecting protein markers using indirect ELISA.

A field experiment was deployed in a randomized block design with two host crop treatments (cotton and alfalfa) and three blocks. A 12-row patch of alfalfa (measuring 180 m x 12 m) was planted in advance (30 April 2007) to establish an acceptable crop hosting a natural Lygus population. The alfalfa plot was adjoined bilaterally by cotton (cultivar FM 9063 B2F, Bayer Crop Science). Cotton was planted on 19 May 2008 and 22 May 2009. Alfalfa and cotton plots were divided latitudinally into three blocks measuring 60 m x 12 m each. Alfalfa blocks were arranged in a single long patch while cotton blocks were randomly assigned at either the north or south side of the alfalfa to facilitate crop-specific irrigation and cultivation requirements and weekly spraying of crop-specific marker protein.

Six weeks after cotton planting, the weekly spray applications of 10% EW marker solution in alfalfa and 10% NFDM marker solution in cotton were made for a period of seven consecutive weeks (from the initiation of cotton squaring to cotton boll maturation). KIS samples (covering 30 m x 1.02 m crop area) were collected from alfalfa and adjoining cotton fields 24 h after each field marking. In 2008, four KIS samples per week were collected from random locations within each block from each host for a period of seven weeks. In 2009, three samples were collected weekly from each block. Lygus adults collected by KIS sampling were killed by freezing and stored individually in microcentrifuge tubes at -20°C for further processing via indirect ELISA.

L. hesperus intercrop movement was determined based on the detection of externally applied insect protein markers in ELISA. Based on the ELISA results, Lygus adults were categorized into "immigrant," "resident," "roaming," and "visitor" groups. Lygus bugs from one crop host testing positive for only a protein marker applied in another host were categorized as "immigrants." Similarly, collected Lygus testing positive only for the protein marker applied to the collection source host were categorized as "residents." Lygus bugs testing positive for both protein markers were recorded as "roaming" insects. Lygus testing negative for both protein markers were recorded as "visitors," having migrated from a totally different source host outside these two crop hosts. Emigrant (outgoing) Lygus for alfalfa were considered as immigrant (incoming) Lygus for cotton and vice versa. For each host, net 24 h Lygus influx was calculated for each subplot by subtracting the average number of immigrant specimens from the number of emigrant specimens.

2.3. Indirect ELISA

An indirect enzyme-linked immunosorbent assay was performed for each sample to detect protein marker adhered on *L. hesperus* body. Antigen samples were prepared by incubating a Lygus sample in 300 µl of 1X Tris-Buffered Saline (TBS, 2.92 g NaCl + 2.42 g Tris + 1000 ml distilled water) in 2 ml microcentrifuge tubes at 4°C for 12 hours. Then, 80 µl of the antigen solution from each sample was added into a well of microtiter plate (Falcon 96 well Assay plate, VWR#62406-321) along with the same volume of known positive samples (n = 3) and negative samples (n = 8) and TBS control (n = 5). The 10% solution of NFDM or EW was used as positive control, *L. hesperus* without marker protein incubated in TBS as negative control, and pure TBS buffer without Lygus was used as TBS control. Then, the microtiter plate filled with antigen was incubated for an hour for binding antigen protein on the wall of microtiter plate well. The plates for testing NFDM were incubated at 27°C while the plates for testing EW were incubated at 37°C throughout this assay. After an hour of incubation, the plates filled with antigen were washed three times with Phosphate-Buffered Saline with Tween 20 (PBST). We used 2X PBST (i.e. 16.0 g NaCl + 2.28 g Na_2HPO_4 dibasic + 0.40 g KPO_4 monobasic + 0.40 g KCl + 999 ml distilled water +1 ml Tween 20) for washing plates and testing NFDM and 5X PBST (i.e., 40.0 g NaCl + 5.70 g Na_2HPO_4 dibasic + 0.60 g KPO_4 monobasic + 0.40 g KCl + 997.5 ml distilled water + 2.5 ml Twin 20) for washing plates and testing EW. After washing the excess unbound antigen, the inner surface of wells of microtiter plates not occupied with antigen was blocked by adding 180 µl of blocker protein and incubating for one hour for blocking the surface of the plate not covered by antigen. PBS with 1% Bovine Serum Albumin (BSA, Sigma-Aldrich # P3688) was used as blocker protein for testing EW, whereas 25% Egg white (All Whites, 100% Liquid Egg Whites, Crystal Farms, Walmart) diluted in 1X TBS was used for testing NFDM. The plates were again washed three times with 2X PBST to remove excess unbound blocker protein.

Immediately after washing excess blocker protein, wells were filled with 80 µl of primary antibody and incubated for 1 hour for binding primary antibody with the antigen protein. The primary antibody for testing NFDM was 1:2000 dilution of anti-bovine casein antibody produced in sheep (Biodesign International, #K20025) in blocker solution (25% egg white in 1X TBS). However, the primary antibody for testing EW was 1:8000 dilution of anti-chicken egg albumin antibody produced in rabbit (Sigma #C6534) in blocker solution (1% PBS-BSA plus Silwet @ 1.3 µl per ml). The plate filled with primary antibody was then washed three times with 5X PBST to remove excess unbound primary antibodies.

After removing excess unbound primary antibody, wells were filled with 80 µl of secondary antibody and incubated for one hour for binding secondary antibody with chain of antigen and primary antibody. The secondary antibody used for testing NFDM was 1:4000 dilution of anti-sheep IgG-peroxidase produced in donkey (Sigma #A3415) in blocker solution (25% egg white in 1X TBS). However, the secondary antibody for testing EW was 1:2000 dilution of anti-rabbit IgG-peroxidase produced in goat (Sigma #R2004) in blocker solution (1% PBS-BSA plus Silwet @ 1.3 µl per ml). Both secondary antibodies were conjugated with Sigma Horseradish Peroxidase enzyme. Then, excess and unbound secondary antibody was removed by washing three times with 5X PBST.

After washing excess unbound secondary antibodies, the wells were filled with 80 µl of the one component 3, 3', 5, 5'-Tetramethylbenzidine substrate (#TMBW-0100-04, BioFX Laboratory, Inc.) and allowed to complete reaction in room temperature. This reaction produced blue-colored reaction product. Following ten minutes of reaction time, the reaction was halted using 50 µl of TMB Stop solution (650 nm Stop reagent for TMB Microwell Substrates, BioFX laboratory, #LBSP), after which spectroscopy was performed on the microtiter plate, with absorbance readings taken at a light wavelength of 650 nm using a Stat Fax 3200 plate reader (Awareness Technology, Inc., FL).

Absorbance values or optical density (OD) data for each Lygus sample were then compared with a threshold OD value. The threshold OD value was calculated as the mean plus three times the standard deviation of the OD values for eight known negative samples tested on the same plate. The test sample was categorized as positive for the protein marker when the absorbance value (OD) of the test sample was equal to or greater than the threshold value. The samples with OD less than threshold value were categorized as negative for the tested protein marker.

2.4. Data Analysis

Data were analyzed with analysis of variance (ANOVA) using PROC MIXED procedure in SAS (SAS Institute, 2003). Means were separated using LSMEANS procedure at α=0.05. For the ANOVA of number of emigrant adults in the MRR study, the fixed effects included blocks, hours after release, host crop, and their interactions. The interaction between block and hours after release was a random factor. Two-sample one-tailed t-tests (PROC T-TEST, SAS Institute, 2003) were used separately for each phenological stage of cotton to test the effect of forced movement of Lygus adults from marked-and-mowed alfalfa to nearby undisturbed alfalfa versus adjacent cotton field. The effect of cotton crop phenology on Lygus intercrop movement behavior was determined by grouping the data from seasonal monitoring study into three cotton phenological stage categories: 1) cotton squaring (first, second, and third sampling weeks), 2) cotton blooming (fourth and fifth sampling weeks), and 3) cotton boll maturation (sixth and seventh sampling weeks). Data from each phenological stage category were averaged and the effect of cotton phenology on movement behavior (emigration, immigration, and net movement) was analyzed. The relationship between Lygus abundance in cotton and the number of immigrants from alfalfa was evaluated via correlation and regression analyses of the two-year combined data.

3. Results and Discussion

3.1. L. hesperus Host Preference in Field Condition

Data generated from the two-year MRR and FMMC studies were used to quantify the host preference and intercrop movement of *L. hesperus* between alfalfa and cotton as well as to assess the effectiveness of the protein marking technique in monitoring Lygus intercrop movement under natural field conditions.

Mark, Release, and Recapture. Analysis of variance of MRR data revealed significant effect of host crop (df = 1, 6; F = 13.53; P = 0.01) and there was no significant interaction (df = 2, 6; F = 0.94; P = 0.45) between host crop and time on the movement of marked *L. hesperus* adults. A total of 187 *L. hesperus* adults were captured in 540-m row of KIS sampling in cotton and alfalfa, of which 33% (62 adults) were from the group of marked-and-released *L. hesperus* adults. Lygus released in alfalfa were found in approximately equal amount in both alfalfa (24 resident adults) and cotton (21 immigrant adults) after a 24 h foraging period (Figure 1). This indicates that at the cotton blooming stage, Lygus adults moved from alfalfa to cotton. However, Lygus released in cotton were primarily

recaptured in cotton (13 resident adults), while a few moved to alfalfa (4 immigrant adults) (Figure 1). A significantly higher number of immigrant adults were found in cotton than alfalfa (P<0.05; Table 1). The bidirectional movement of *L. hesperus* occurred between cotton and alfalfa during cotton blooming; however, the net movement was from alfalfa to cotton (17 adults from alfalfa to cotton) (Table 1). On average, more *L. hesperus*, including unmarked "visitor" insects, were captured in cotton than in alfalfa (Figure 1). This was true at both 24 h and 96 h after insect release. This was likely due to host quality because cotton was blooming while the adjacent alfalfa was senescing.

Table 1. Average (± SE) number of unidirectionally relocated protein-marked and released *L. hesperus* adults between alfalfa and adjacent cotton based on enzyme-linked immunosorbent assay of adults captured in samples covering 60-m of row per sample unit (n=3) in a mark-release-recapture study.

Foraging Time	Immigrant *Lygus* in cotton	Immigrant *Lygus* in alfalfa	Net Movement from alfalfa to cotton
24 h	7.0 ± 4.5 A	1.3 ± 0.7 B	5.7 ± 5.2
96 h	10.0 ± 2.6 A	2.0 ± 1.0 B	8.0 ± 3.6
Average	8.5 ± 1.5 A	1.7 ± 0.3 B	6.8 ± 1.2

Means followed by different uppercase letters were significantly different (P<0.05) between cotton and alfalfa within the same foraging time.

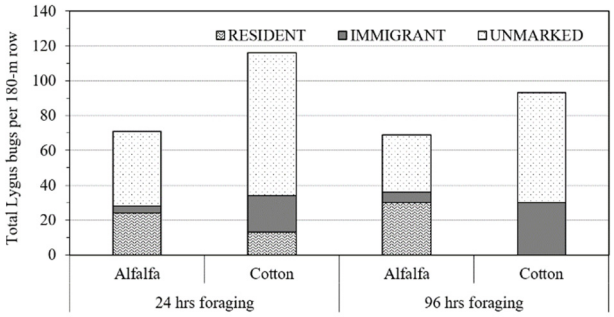

Figure 1. Categories of *L. hesperus* collected from blooming cotton and post-blooming alfalfa in mark-release-recapture study, Lubbock, Texas, 2008.

Of the total 3,000 *L. hesperus* adults released, only 62 (2%) were recaptured. Such a small percentage recovery could have resulted from rapid Lygus dispersal, or high mortality of marked insects caused by physical injury inflicted by sweep-net and aspirator use during collection. While Lygus mortality following field collection can be minimized by rearing them temporarily in a controlled environment and by specifically selecting healthy, uninjured insects for marking and release, this was not done in this study because laboratory rearing of field-collected insects using a food source and climate parameters to which they are unaccustomed could alter their host selection behavior.

Field Marking, Mowing, and Capture. In FMMC, roadside alfalfa was sprayed with NFDM marker solution. Twenty-four hours after marker application, the alfalfa was mowed resulting in most *L. hesperus* adults being forced to move and choose adjacent cotton or undisturbed alfalfa. When the roadside alfalfa was mowed, a significantly higher number of marked *L. hesperus* relocated to adjacent undisturbed alfalfa (85% at cotton blooming stage; 87% at cotton boll maturation stage) than to cotton (15% at cotton blooming; 13% at cotton boll maturation) (Table 2). It was anticipated that cotton phenology would reveal a more significant impact on *L. hesperus* movement into cotton from mowed alfalfa; however, this was not the case. In both phenological stages of cotton, fewer adults moved to cotton than to undisturbed alfalfa. However, due to possible attraction to abundant floral nectar, it was expected that more Lygus would migrate to cotton during blooming than during boll maturation. The number of migrant Lygus at cotton blooming stage and boll development stage cannot be compared directly because of the difference in Lygus densities between these two crop phenological stages. The total number of Lygus captured in alfalfa at cotton blooming stage was 4.7 times higher than at boll maturation stage. Similarly, the total number of Lygus captured in a cotton field at blooming stage was 3.8 times higher than cotton boll maturation stage (Figure 2). Previously published results have indicated a general decline in *L. hesperus* population during the time when cotton was typically maturing (Parajulee & Shrestha, 2014), and our data from FMMC study supported this observation (Figure 2). These observations made in FMMC study encouraged development of a new hypothesis regarding cotton-alfalfa source-sink dynamics with respect to *L. hesperus*. Thus, a season-long study was designed to test the effect of cotton phenology on the intercrop movement of *L. hesperus* between alfalfa and cotton.

Table 2. Average (± SE) number of immigrant *L. hesperus* adults found in cotton and undisturbed alfalfa (per KIS sample covering 30-m of row) 24 h after mowing of the adjacent protein-marked alfalfa.

Cotton phenology	Alfalfa	Cotton
Blooming	17.33 ± 8.99 A	3.00 ± 2.67 B
Boll development	11.33 ± 5.89 A	1.72 ± 0.43 B
Average	14.33 ± 4.99 A	2.36 ± 1.24 B

Means within each row followed by different uppercase letters are significantly different (one-tailed t-test; α=0.1).

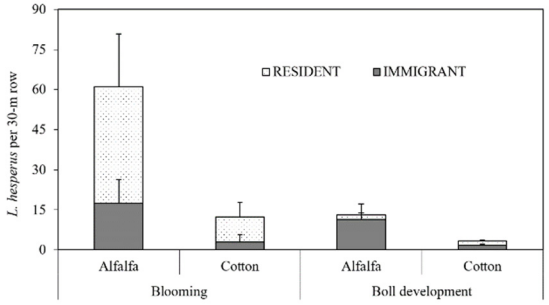

Figure 2. Prevalence of immigrant and resident *L. hesperus* adults in cotton versus alfalfa following the mowing of protein-marked adjacent alfalfa during the two phenological stages of cotton, Lubbock, Texas, 2008.

3.2. Season-long Monitoring of Lygus Intercrop Movement

Lygus Abundance. A total of 294 KIS samples were collected (147 from cotton and 147 from alfalfa) over 2008 and 2009. From these samples, a total of 1,273 adult *L. hesperus* were retrieved (580 in 2008 and 693 in 2009). There was no significant difference (df = 1, 2.17; F = 9.26, P =

0.084) in average seasonal Lygus abundance between 2008 (5.69 ± 0.85 bugs per sample) and 2009 (7.07 ± 0.66 bugs per sample). Numerically higher *L. hesperus* abundance in 2009 could be explained by a longer "window" for Lygus colonization in alfalfa. The alfalfa crop was one year older in 2009 than in 2008, and thus may have been better established and of generally higher quality. Host crop significantly affected the abundance of Lygus (df = 1, 25.2; $F = 43.51$; $P = <0.0001$), with 82% of the insects (1,111 bugs) found in alfalfa versus 13% in cotton (162 bugs). Lygus abundance varied significantly among the sampling weeks (df = 6, 18.8; $F = 5.35$; $P = 0.0023$). In 2008, significantly more bugs (24.5 ± 7.5 per KIS sample) were found in the second sampling week (the week of 20 July 2008) in alfalfa than in other weeks, but in 2009, the peak (20.56 ± 3.08 bugs per KIS sample) occurred during the sixth sampling week (the week of 20 August 2009). In cotton, average *L. hesperus* abundance was always relatively low (<3.5 bugs per KIS sample) and remained statistically similar across sampling weeks and among cotton phenological stages. Barman, Parajulee, and Carroll (2010) also demonstrated a lower rate of colonization of *L. hesperus* in cotton compared to that in alfalfa in a multi-host choice field study.

Temporal Dynamics of Intercrop Movement of *L. hesperus*. Bidirectional *L. hesperus* intercrop movement between alfalfa and cotton was evaluated using a "two fields/two markers" approach. Based on the results of ELISA performed on Lygus bugs retrieved via KIS sampling, all collected Lygus bugs were categorized as residents, immigrants, roamers, or visitors. All data are presented in terms of *number per ha* (Figure 3). Over two years, 162 Lygus bugs were retrieved from cotton. In 2008, 64% of bugs retrieved from cotton were verified as having at some point inhabited marked alfalfa. In 2009, this increased to 96%. These data clearly indicate that alfalfa had a Lygus source effect upon adjacent cotton.

Prior to this study, no satisfactory technique for quantification of actual net intercrop movement of a population of small insects during a specified duration had been developed. The "two fields/two markers" approach used in conjunction with ELISA for determination of insect origin is capable of clearly demonstrating both the direction and net balance of Lygus intercrop movement, following a specific foraging or roaming period (Hagler & Naranjo, 2004). However, this capability is limited to what could be described as a "snapshot" of the net balance and interpreted direction of movement at the time of sampling.

Because it is within the realm of possibility, and even probable, that *L. hesperus* moved back and forth between cotton and alfalfa during each foraging period (between marking and retrieval), the technique used is incapable of clearly characterizing the true dynamic, temporal fluctuation of *L. hesperus* intercrop movement. This aspect of the study is somewhat analogous to the difference between a photograph and a motion picture. The possibility that marked insects may have made "test flights," or temporarily changed hosts during the short foraging period, cannot be fully accounted for with the methods used. Despite this possibility, such an accounting of temporal movement fluctuation is not necessary in order to ascertain the vector and net balance of bidirectional Lygus intercrop movement, or more importantly, the net influx of Lygus into cotton from alfalfa. Given this limitation, and with no credible scientific rationale for doing so, no distinction was made between potential movement transience or permanence.

FMMC was the obvious technique of choice for a season-long intercrop movement study. It was selected for its effectiveness, efficiency, and practicality. MRR is commonly used in movement and migration studies (Hagler & Jones, 2010), but it is not feasible for use in a large-scale season-long intercrop movement study. The primary disadvantage of MRR is its usual small marked-recapture rate (2% with *L. hesperus*, as was discovered during the MRR study). Exposure to a laboratory environment, mass-rearing, handling, and marker application are all factors of MRR use which may interfere considerably with natural insect behavior.

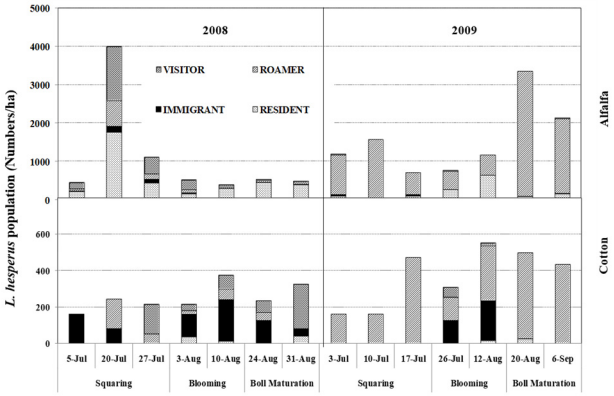

Figure 3. Temporal dynamics of *L. hesperus* immigrant, resident, visitor, and roamer populations in alfalfa and cotton, Lubbock, Texas, 2008-2009.

Correlation and regression analyses of verified total *L. hesperus* cotton influx (cotton-collected immigrants plus cotton-collected roamers) and total cotton-collected *L. hesperus* revealed a highly positive relationship (r = 0.98; n = 35; $P = 0.0001$; Figure 4a). One reason for combining cotton immigrants and roamers into the category of verified total *L. hesperus* cotton influx was the strong relationship between the number of roamers and the total number of *L. hesperus* collected (Figure 4a). The relationship between immigrant only and the total bugs collected was weak (Figure 4a). Regardless of the weakness or strength of these relationships, Lygus immigrants and roamers collected from cotton tested positive for EW protein, proving definitively that these insects had, at some point during the foraging period, inhabited EW-marked alfalfa. Examining immigrants alone does not address this critical fact and the circumstances of such habitation or origination, while interesting, and possibly explainable by the simultaneous presence of NFDM protein, are biologically irrelevant. The total number of *L. hesperus* found in cotton and total Lygus cotton influx shared a similar pattern (Figure 4b) until the last week of sampling in 2008. In 2009, their patterns were nearly identical. The pattern divergence in 2008 could have been due to a sudden flush of new adult emergence during the final week of sampling.

Analysis of variance of *L. hesperus* influx of both crops revealed significant differences in the pattern of *L. hesperus* intercrop movement between the two years (df = 1,3.32; $F = 194.51$; $P = 0.0005$), between two hosts (df = 1,24.6; $F = 39.08$; $P = <.0001$), and among the cotton phenological stages (df = 1,8; $F = 22.12$; $P = 0.0006$) and sampling weeks (df = 6,14; $F = 12.31$; $P = <0.0001$). The difference in the *L. hesperus* intercrop movement patterns in 2008 and 2009 was likely due to differences in alfalfa and cotton crop development because of differential rainfall between the two years. The 2009 cotton growing season was marked by greater rainfall, improving cotton and alfalfa crop growth and quality. As a result, *L. hesperus* densities were higher in both crops in 2009, except for one sample date in alfalfa in 2008 (Figure 5). We hypothesized that *L. hesperus* intercrop movement might have been affected by Lygus density in the source habitat (alfalfa), but correlation (r = 0.14; n = 42; $P = <0.36$)

and regression (R^2 = 0.02; n=42; P = <0.36) analyses failed to reveal any significant relationship between alfalfa Lygus density and cotton Lygus influx.

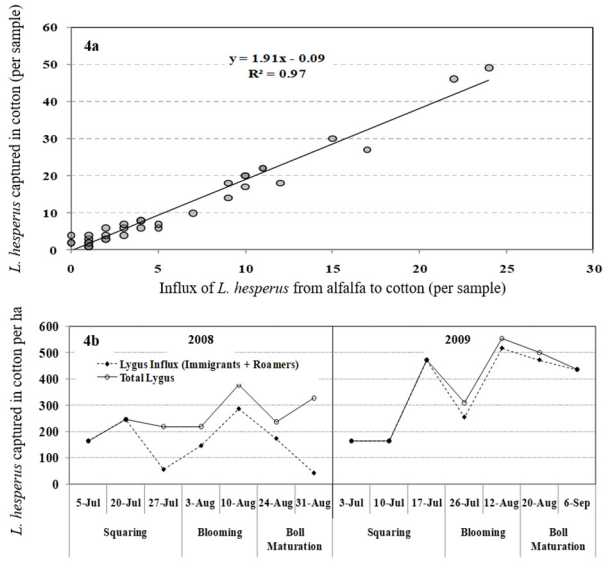

Figure 4. a) Relationship between total *L. hesperus* found in cotton and total *L. hesperus* influx from alfalfa to cotton, b) Weekly pattern of total *L. hesperus* and *L. hesperus* influx in cotton, Lubbock, Texas, 2008-2009.

During the first five weeks after cotton planting, *L. hesperus* were not detected in cotton. *L. hesperus* is typically a late-season pest of cotton in the Texas High Plains (Parajulee, Hakeem & Carroll, 2015). *L. hesperus* began to move into cotton from alfalfa once cotton began squaring. Until mid-July, all Lygus found in cotton (100%) were verified as having inhabited marked alfalfa (Figure 5). As the *L. hesperus* population increased in cotton, influx from alfalfa decreased. This was likely a dilution effect resulting from the emergence of new Lygus adults in cotton and influx of Lygus "visitors" from sources other than the protein-marked alfalfa.

Protein-marked alfalfa contributed significantly to *L. hesperus* population growth in adjacent cotton throughout the growing season. Net *L. hesperus* intercrop movement with respect to cotton in the cotton-alfalfa system was calculated by subtracting Lygus cotton influx (EW-marked *L. hesperus* captured in cotton) from Lygus cotton outflux (NFDM-marked bugs captured in alfalfa). Year (df = 1, 2; F = 199.41; P = 0.0050) and cotton phenology (df = 2, 32; f= 9.71; p= 0.0005) affected average *L. hesperus* net movement significantly (Figure 5). In 2009, average *L. hesperus* net movement was significantly lower (df = 2, 16; f= 10.82; p= 0.001) during cotton blooming (113 bugs per ha outflux) than during squaring (893 bugs per ha outflux) or boll maturation (2,161 bugs per ha outflux). In 2008, average *L. hesperus* net movement was significantly higher (df = 2, 16; F = 3.64; P = 0.05) during cotton blooming (161 bugs per ha influx) and boll maturation (70 bugs per ha influx) than during squaring (286 bugs per ha outflux). The influx-outflux disparity during cotton boll maturation between years may be partly explained by a slight sampling date incongruence between the two study years. Sampling was conducted slightly later in 2009, into the month of September, and inclusion of this later sampling date, which occurred during a typically pivotal period of crop senescence with regard to *L. hesperus* abundance, in the chronological categorization of boll maturation, may have influenced this disparity.

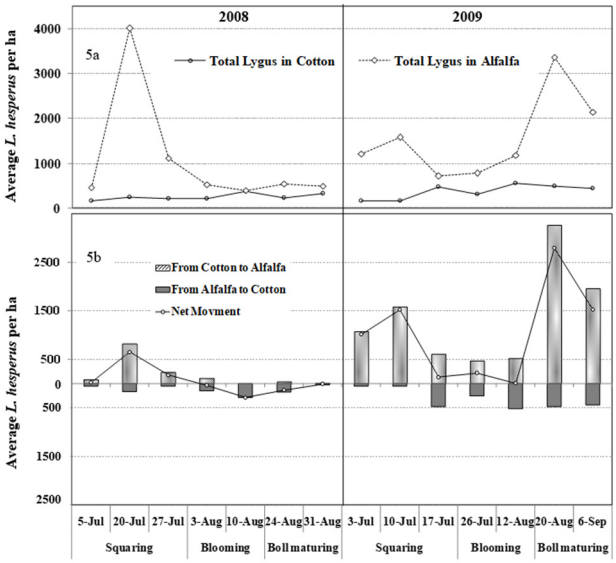

Figure 5. a) Weekly average *L. hesperus* abundance in cotton and alfalfa, b) Net *L. hesperus* intercrop movement between alfalfa and adjacent cotton, Lubbock, Texas, 2008-2009.

It is somewhat puzzling to have observed net movement favoring alfalfa while simultaneously observing increases in EW-marked *L. hesperus* retention and population in cotton (Figure 5). While *L. hesperus* retention in cotton was used as a component in net intercrop movement calculation, the data suggested that net intercrop movement and actual *L. hesperus* population change were weakly related. Actual Lygus population change in cotton is affected more by reproduction success (birth rate), developmental time, and mortality due to natural enemies. A single calculation of net *L. hesperus* intercrop movement, or an intercrop movement "snapshot" obtained on a single sampling date, in the context of this study, indicates only the instantaneous directional flow of insect intercrop movement at the time of sampling. It is the confluence of all snapshots which reveal patterns in the direction of net intercrop movement. Some interesting patterns revealed by this study were the relationships between net *L. hesperus* intercrop movement and *L. hesperus* population densities in cotton. When net *L. hesperus* movement favored cotton, there were strong positive relationships between net *L. hesperus* movement and average *L. hesperus* abundance in cotton. Average *L. hesperus* abundance in cotton also related strongly to net intercrop movement favoring alfalfa, but when net movement exceeded ~2,600 bugs/ha, *L. hesperus* density in cotton decreased drastically.

4. Conclusion

When both habitats are available in proximity, the *L. hesperus* intercrop movement data showed that alfalfa is a more preferred host than cotton for *L. hesperus* colonization (Barman et al., 2010). Despite this preference, alfalfa may dynamically confer both source and sink effects, with respect to *L. hesperus*, depending on crop phenology and host quality (Chen &

Parajulee, 2010; Parajulee et al., 2011; Parajulee et al., 2015). During cotton blooming, net *L. hesperus* intercrop movement between cotton and alfalfa favored cotton. This was true even without forced relocation of *L. hesperus* due to alfalfa mowing. Forced relocation of *L. hesperus* from alfalfa, induced by mowing, resulted in net *L. hesperus* intercrop movement favoring cotton through boll maturation.

During spring and early summer months, alfalfa is more suitable to *Lygus* spp. and it is preferred over cotton as a host (Barman et al., 2010; Chen & Parajulee, 2010; Stern et al., 1964). Carriere et al. (2006) found that a forage alfalfa field located within approximately 114 m distance from a cotton field acted as a source of *L. hesperus* in the Arizona cotton agroecosystem. They found a strong positive correlation between *L. hesperus* abundance in alfalfa and a *L. hesperus* population in nearby cotton. Large populations can develop in an alfalfa field and eventually may move from alfalfa to cotton, especially when alfalfa is harvested (Graham, Jackson & Debolt, 1986). While this phenomenon has been reported, it has never been specifically quantified and characterized. MRR study detected "unidirectional" movement of marked insects from the point of release to the point of sampling. FMMC study allowed us to mark and recapture a large number of *L. hesperus* in field settings. The results obtained from MRR and FMMC did not provide a complete picture of intercrop movement of *L. hesperus*; however, they provided strong evidence confirming the effectiveness of the marking and detection technique. A detailed study of bidirectional *L. hesperus* intercrop movement between alfalfa and cotton in natural field settings will increase our understanding of the cotton-alfalfa source-sink relationships.

Insect intercrop movement behavior is a complex phenomenon affected by biological and ecological factors and dependent upon both the insect and the host habitat. Quantification of insect movement is necessary in developing a model determining insect dispersion and insect intercrop movement. Field-marking using protein markers and subsequent marker detection via indirect ELISA is a potential method for temporal and directional insect intercrop movement quantification. This technique proved superior to traditional surveying techniques in elucidating *L. hesperus* source-sink dynamics in a cotton-alfalfa system. A key limitation of this approach is difficulty in predicting actual insect pest population changes in a field crop due to the process of bidirectional intercrop movement. As an example, higher net insect pest intercrop movement does not necessarily equate to increased damage in the affected host crop. Further studies involving this technique should examine the effect of *L. hesperus* intercrop movement on *L. hesperus* reproductive success in cotton and resulting cotton crop damage (Chen & Parajulee, 2010). Because insect intercrop movement can be influenced by environmental factors, host quality, and crop management practices, a mathematical model, derived from detailed evaluation of these factors, should be developed to predict insect pest intercrop movement behavior. Such a model could then be integrated with the tools available to growers and researchers for ecologically intensive pest management in cotton.

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