EVALUATION OF INTERCROP MOVEMENT OF *LYGUS* BETWEEN COTTON AND ROADSIDE ALFALFA Ram B. Shrestha Megha N. Parajulee Stanley C. Carroll Anup Bastola Texas AgriLife Research Center Lubbock, Texas

<u>Abstract</u>

A 3-year field survey was conducted to determine the field population dynamics of *Lygus* and insect predators in a cotton-alfalfa farming system. Various field and laboratory studies were conducted to optimize *Lygus* field marking protocols with two proteins [non-fat dairy milk (NFDM) and eggwhite (EW)] and their detection by indirect ELISA technique. The technique developed was evaluated for quantification of intercrop movement and host selection behavior of *Lygus* in alfalfa and cotton in the Texas High Plains. Successful application of indirect ELISA technique for detection of milk casein from NFDM and chicken egg albumin protein from EW has been discussed. Alfalfa sources adjacent to cotton fields acted as both source and sink for the *Lygus* in cotton depending on the phenological stage of cotton. *Lygus* bugs seem to show a two directional movement behavior between cotton and adjacent alfalfa based on the cotton and alfalfa growth conditions. In general, alfalfa was found to harbor more *Lygus* compared to cotton and *Lygus* preferred alfalfa over cotton as a host.

Introduction

One of the popular IPM practices for sustainable insect pest management is to utilize the source-sink dynamics and maintain the sink habitat (trap crops) around the field crop in significant proportion and destroy the source habitat (alternate host or breeding place) of the pest. At the same time we have to maintain the source of beneficial predators and parasitoid populations. Determining the role of a host as a source or sink is a challenging task, especially for the highly polyphagous insects. Lygus is a polyphagous insect which can live on a broad range of hosts (Young 1986, Day 1996). It has been reported from 26 different roadside weed hosts from Texas High Plains (Parajulee et al. 2003). Roadside alfalfa is a primary host of Lygus in the Texas High Plains, especially during the spring and early summer months. It had been shown that Lygus prefers alfalfa over cotton and several other weed hosts (Sevacherian and Stern 1974). L. hesperus laid significantly more eggs (78%) in alfalfa than in cotton (Jackson 2003). Previous research had indicated the possible movement of Lygus from alfalfa and other weed hosts in to the cotton field (Fleischer et al., 1988 Sevacherian and Stern 1975). Thus, quantification of the contribution of roadside alfalfa in intensity of Lygus infestation in adjacent cotton is very important in the development of Lygus management practices. Dispersal of Lygus from alfalfa to adjacent cotton could also be encouraged by mowing by governmental agencies. Researchers in California have shown that strip cutting of commercial alfalfa fields prevents the dispersal of western tarnished plant bugs (Lygus hesperus) to cotton (Mueller et al. 2005). Similarly, an area-wide Lygus management project in Mississippi, funded by USDA, has demonstrated that roadside weed management is an effective means of minimizing tarnished plant bugs (Lygus lineolaris) and bollworms in adjacent cotton.

Because *Lygus* complete their lifecycle in both cotton and alfalfa hosts, it is often confusing to determine whether the roadside alfalfa is acting as a source or sink for a *Lygus* population found in an adjacent cotton field. It has been reported that they prefer laying more eggs in alfalfa compared to that in cotton. If the mortality and survival rates are the same in both crops, alfalfa will then serve as a source because of the higher *Lygus* reproduction in this crop. However, the actual rates of reproduction, survival and mortality of *Lygus* in these two hosts growing under actual field situations are not well understood. A source-sink relationship is a dynamic phenomenon because it may change based on many factors including competitors, predators, movement and migration, and other changes in environmental factors, the growth or phenology of the host or habitat. Also, because the realized niche of any organism is the n-dimensional hyper volume, it is affected by many factors simultaneously. The potential of overall increase in reproductive success of *Lygus* bugs in the presence of alfalfa patches or fields near cotton field needs to be evaluated. In some alfalfa fields, large numbers of *Lygus*. The direct way of determining source-sink function of a particular host is to quantify the bug movement throughout the crop growing season and determine their survival and reproductive success after the host switching.

The 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 better strategy of mowing

could be developed to hold *Lygus* in alfalfa and prevent their movement to cotton. Time of alfalfa mowing can be changed so the *Lygus* forced movement at critical phonological stage of cotton (small boll development stage) will be reduced. If possible, biological control agents or chemical pesticides can be applied to alfalfa stripes before alfalfa mowing the alfalfa so the movement of *Lygus* into cotton field due to mowing will be reduced. However, these approaches remove beneficial predators (e.g., lady beetles, big-eyed bugs, pirate bugs, and spiders) and parasitoids from the system thereby reducing the natural biological control of pests in cotton. Therefore, a management plan that minimizes *Lygus* movement from roadside alfalfa and also conserves natural enemies and reduces the pesticide load in cotton production system should be developed.

Sweep sampling of *Lygus* from different host plants has been done to quantify the *Lygus* population and estimate the movement of *Lygus* from those hosts to cotton but sampling *Lygus* without specific marking does not show the actual movement of the insect between the host plants. Stern and Mueller (1968) used micronized fluorescent dust for *Lygus hesperus* movement study. Using this kind of physical marking procedure is more labor intensive and may interfere with insect biology and behavior. Moreover, the marking should be environmentally safe, cost-effective, and easy to use (Hagler and Jackson 2001). Protein marking and its detection by ELISA (Enzyme linked Immune Sorbent Assay) technique have been successfully used in various insects such as convergent lady beetle (Hagler 2004, Hagler et al. 2004), pink bollworm (Hagler et al. 2002), pear psylla (Jones et al. 2006), cabbage bagworm (Schmaedick et al. 2001), and thrips (Jasrotia et al. 2006). Thus, we hypothesize that this technique will be equally satisfactory in determining *Lygus* intercrop movement under Texas High Plain climatic conditions.

Our primary objective was to quantify the role of roadside weed management, particularly the mowing of alfalfa, on *Lygus* population dynamics in adjacent cotton. The overall goal of this project is to develop a management plan to reduce *Lygus* movement from roadside alfalfa to adjacent cotton and conserve natural enemies in cotton. Specific objectives of this project were to:

- 1. Quantify the population dynamics of Lygus in roadside alfalfa and adjacent cotton.
- 2. Evaluate the impact of alfalfa mowing time on Lygus numbers in adjacent cotton fields.
- 3. Evaluate the impact of alfalfa mowing heights (1-2" versus 5-6" from the soil surface) on *Lygus* numbers in adjacent cotton fields.
- 4. Evaluate the host preference and Lygus dispersal behavior by mark, release and recapture techniques.

Materials and Methods

Six different Texas High Plains field studies were conducted in 2007 to evaluate the intercrop movement behavior of *Lygus* in locations where cotton and alfalfa were growing in adjacent to each other. The studies were a) Field population dynamics of *Lygus* and insect predators, b) *Lygus* intercrop movement at different cotton phenological stages, c) *Lygus* intercrop movement and alfalfa mowing height, d) Alfalfa field marking concentration, e) Mark, release and recapture for *Lygus* host selection behavior, and f) *Lygus* foraging time in field and cage studies.

A. Field Population Dynamics of Lygus

This study was conducted in Lubbock County, Texas for three years (2005 to 2007). Four study sites located 3-5 miles apart were selected and represented the replications for the study. In June of 2005, one 400-ft. long stretch of relatively pure roadside alfalfa growing adjacent to cotton fields was identified at each of the three sites. The roadside alfalfa and adjacent cotton at each site was sub divided into 4 plots. Each study site had 4 roadside alfalfa strips (100 ft. x 20 sq ft) alongside 4 adjacent cotton plots (100 x 100 sq ft). In 2006 and 2007, the sites remained same with the exception of one site which was moved less than one mile in order to use a better stand of roadside alfalfa.

In 2005, one hundred sweeps were taken from each plot, with a total of 1200 sweeps in alfalfa and 1200 sweeps in adjacent cotton plots taken for 5 times during the cotton growing season (20th and 28th July, 4th and 11th August and 12th September). In 2006, *Lygus* and predators were sampled from all 3 sites for 11 weeks from May to September. In 2007, cotton at the 3 study sites was planted during the first week of June and *Lygus* sampling was initiated on June 19 when the cotton was in the cotyledon stage and sampling was done on a total of six dates (June 19, June 29, July 12, August 6 and 29, and September 22).

The *Lygus* sampling protocols were similar in 2005 and 2006. However, in 2007, we switched the insect sampling technique from sweep net sampling to using a KIS (Keep It Simple) sampler. The KIS sampler was locally made from an Echo[®] model PB 265 backpack leaf blower (air volume rated at 458 cfm) and an insect collecting net. With a KIS

sampler, we were able to sample cotton during an earlier stage of cotton (2-3 leaf stage)) and also able to collect more insect samples which were comparatively clean and easy to process. We sampled the same number of row-ft distances crop row distances (100 ft/plot in both cotton and alfalfa) with both the sweep net and KIS sampler. Insects collected by sweeping or KIS sampling were brought to the laboratory in Ziploc[®] plastic bags, freeze-killed, processed, and their numbers were recorded.

B. Lygus Intercrop Movement at Cotton Blooming Stages

Effect of roadside alfalfa mowing time on the intercrop movement behavior of Lygus was tested for two different cotton growing stages which included the blooming stage and the cotton boll development (post blooming stage). On July 12, 2007, four sites each located more than 2 miles apart were selected in Lubbock County. The cotton and adjacent roadside alfalfa (>200 feet strip) at each site were both in the flowering stage. The alfalfa strips were sampled with a sweep net before field marking and the mowing treatment to insure the presence of Lygus in these patches. The 100 ft long plots were flagged in the middle of these all patches for marking and mowing treatment. These alfalfa sections were sprayed with a 5% NFDM solution applied at high volume. After marking Lygus were allowed to forage for 24 hours and then marked alfalfa was moved at a 3-6 in, moving height with a tractor mounted mover. After moving the plots, the Lygus bugs were allowed to move around freely for 24 hours and settle down in their host of choice (i.e., mowed alfalfa, nearest cotton field, non-mowed alfalfa patches) in 24 hr. After the 24 hr host-selection period, Lygus were sampled with a KIS sampler in 100 ft distances in non-mowed alfalfa, mowed alfalfa stubble and various number of rows into the nearby cotton field (rows 5, 10, 20 and 40 rows). The samples were placed into Ziploc[®] plastic bags and brought to the laboratory for further processing by ELISA assay. Similarly, three more sites were selected in Lubbock County near Idalou, Texas on 23rd July 2007. At those sites, cotton was in the boll development stage (post blooming stages) and alfalfa was in the blooming stage. We followed similar field marking; mowing and sampling procedure as in above mentioned first site except the field marker protein used was a 5% EW solution.

C. Alfalfa Mowing Height and Lygus Intercrop Movement

A long strip of alfalfa (700 x 40 ft) was planted in the middle of a cotton field on the Texas AgriLife Research farm at Lubbock on April 30, 20077. The alfalfa plot was divided into 3 blocks of 210 ft long plots and each block was further divided into 3 subplots (70 x 40 ft plots). When the adjacent cotton was in full bloom, three mowing treatments (mowing alfalfa at 6-in. height, at 2-in. height and not mowed control) were randomly assigned to each block. Twenty-four hours before and after application of the treatments, Lygus and predators were sampled by sweep sampling (50 sweeps per plot). All three 2-in. mowing height treatment plots of alfalfa were sprayed with chicken albumin marker (5% solution of egg white) and all three 6-in. mowing treatment plots of alfalfa were sprayed with bovine casein marker (5% solution of non fat dairy milk) at the rate of 10 gallon per plot. After field marking 24 hrs of exposure time was given to the Lygus and other predators to pick up the marker proteins from the alfalfa plants by physical contact. The alfalfa plots were then mowed with a tractor mounted mower for the 6-in. height treatment but hand-held Toro[®] "weedeaters" were used to mow the 2-in. height mowed plots. After 24 hrs, all alfalfa plots and the cotton field adjacent to each alfalfa plot were sampled with a sweep net. Cotton fields on both sides of alfalfa plots were sampled. Cotton field samples were taken from 5. 10, 20 and 30 rows of cotton counted from the border between cotton and alfalfa field to evaluate how far the Lygus and predators can move into the cotton field. The sweep samples were brought to the laboratory to be freeze-killed and processed by counting the Lygus and predators. The insects were saved individually in micro centrifuge tubes in -20 °C for further processing by Indirect ELISA.

D. Mark-Release-Recapture Study to Examine Lygus Host Selection Behavior

On August 9, 2007, about 3,000 live *Lygus* adults were collected from a nearby alfalfa field and brought in laboratory. The alive and active *Lygus* were externally marked by nebulizing for 15 minutes with a marker protein solution. A 50% NFDM solution was used to mark 1500 *Lygus* and rest 1500 were marked with 100% EW solution. The EW marked *Lygus* were release in 3 different locations spaced 100 ft apart in the 10th row of a cotton field at the rate of 500 adults per release point. The NFDM marked *Lygus* were released at 3 different locations 100 ft apart in the middle of the alfalfa field. Both releases were done on the same day during the night period. The cotton was in the full bloom stage but alfalfa was in the post bloom stage when the *Lygus* were released. *Lygus* were recaptured using a KIS sampler 48 and 96 h after the field releases (August 11th and 14th). *Lygus* samples were brought to the laboratory, killed by freezing and stored individually at -20 °C for further processing by ELISA.

ELISA Assay

Indirect ELISA assay was performed for each sample to determine whether the *Lygus* picked up the marker protein or not. The antigen samples were prepared by incubation of *Lygus* in 300 ul of 1X Tris-buffered saline (TBS) at 4 °C for 12 hr with frequent vortexing. The antigene solution was loaded in wells of microtiter plates along with positive and negative samples and incubated at 37 °C for EW and at 27 °C for the NFDM test. The plate was then washed with 2x Phosphate-buffered saline with tween 20 (PBST) 3 times and blocked with 180 µl of blocker protein for 1 hr. The plate was again washed with 2x PBST 3 times and incubated with 80 µl of primary antibody for 1 hr. The plate was again washed with 5x PBST 3 times and 80 µl of substrate (Tetramethyl benzidine - TBM) was added to each well. The absorbance reading was taken at 650 nm using Stat Fax 3200 plate reader (Awareness Technology Inc, FL) after a 10 minute reaction time or reaction was stopped with 50 µl of stopping solution.

Data Analysis

Both adult and nymph data of *Lygus* from one sample were combined together and all predator species data from one sample were combined into total predator data and analyzed using GLM (SAS 2005). The total *Lygus* and total predator data from different cotton fields and adjacent alfalfa fields were also analyzed using correlation and regression analysis. The seasonal total of each species and their percentages were calculated for the species composition of predators from cotton and alfalfa. The absorbance value for each *Lygus* sample was compared with the absorbance value of 8 known negative samples and interpreted as positive only when the absorbance value of the sample is equal or more than the mean absorbance value of the negative samples plus 3 times standard deviation of those negative samples. When the value was smaller than that value samples were said to be negative. The percentage of the positive samples out of the total sample tested for each treatment was calculated and analyzed using GLM (SAS 2005).

Results and Discussion

A. Field Population Dynamics of Lygus

Totals of 74, 159 and 84 samples were collected from alfalfa and cotton fields at the 4 sites in 2005, 2006 and 2007, respectively. One sample constitutes the insects collected in 100 sweeps or a KIS sample from a 100 row-ft distance. The seasonal average number per sample of both *Lygus* and predators were significantly low in 2005 and 2006, and high in 2007 (P=0.1) both in the alfalfa and cotton field (Figure1). During 2005 and 2006, relatively low insect activity was observed in the Lubbock possibly due to long periods of little rainfall and less vegetation while 2007 turned out to be a comparatively high insect activity year primarily due to timely rains, better crops and more vegetation. The high number of predators in cotton (150/sample) was mainly due to aggregation of convergent lady beetles in the sample plots due to an early outbreak of cotton aphids. In 2005 and 2006 there were very few aphids observed in the test plots and hence the convergent lady beetle numbers were also low. With this 3-year study, we were able to compare the *Lygus* and predator population dynamics under low and high population density levels.

Lygus abundance was found to be higher in alfalfa plots as compare to adjacent cotton in all three years (Figure 1). The higher number of *Lygus* in alfalfa might be due to: 1) *Lygus* may have higher reproduction and survival rate in alfalfa as compare to cotton, 2) alfalfa may be a more preferred host for adult *Lygus* and it is attracting more number of adults *Lygus* from all other hosts and acting as a sink habitat, or 3) *Lygus* mortality in cotton field could be high due to predators or other biological, cultural, or physical factors resulting in *Lygus* populations developing very slowly in cotton. We have analyzed all three possibilities with various studies. First we evaluated the reproductive success by comparing the immature nymph population in these two hosts. There was a significantly higher (P=0.1) seasonal average number of *Lygus* nymph in alfalfa (0.36, 0.66 and 61/sample in 2005, 2006 and 2007, respectively) as compared to that in cotton (0.01, 0.00 and 1.75/sample in 2005, 2006 and 2007 respectively) in all three years.

The very low number of *Lygus* nymphs found in cotton might be due to the sampling procedure we used sweep or KIS sampling can not capture most of the nymphs hiding inside the bracts of cotton squares and flowers. Thus some other method of quantifying nymph populations; such as visual sampling or whole plant destructive sampling needs to be used for more accurate estimates of immature population. Even though the methods are not very efficient, the number differences are so high we can conclude that the *Lygus* nymph populations are significantly higher in alfalfa as compared to cotton. Our data support the finding of Jackson (2003) where he reported that *Lygus* lay more eggs in alfalfa as

compared to cotton. Not only the lower fecundity but also possible higher mortality of immature *Lygus* in cotton may be another factor for a smaller population in cotton. To check this hypothesis we conducted a pilot laboratory study to identify key predators and their predation rate. The life table and fecundity study is being conducted in our laboratory to evaluate the effect of these two hosts in the reproductive success of the *Lygus*.



Figure 1. Population dynamics of *Lygus* in roadside alfalfa and adjacent cotton fields at Lubbock, TX in 2005-2007 (average of 4 samples from 4 replications or sites).

B. Lygus Intercrop Movement at Cotton Blooming Stages

Carriere et al. (2006) found that a forage alfalfa field located within a distance of 375 ft from a cotton field acted as a source of *Lygus hesperus* in the Arizona cotton agroecosystem. They found a strong positive correlation between *Lygus* abundance in the alfalfa field and the *Lygus* population in the nearby cotton field. In the spring and early summer, alfalfa is more suitable to *Lygus* spp. and preferred over cotton (Stern et al. 1968, Stewart and Jackson 2003). Large populations can develop in alfalfa, and some adults move to cotton, especially when alfalfa is harvested (Graham et al. 1986). When *Lygus* were forced to move out of alfalfa due to mowing, a significantly high number of *Lygus* moved to nearby non mowed alfalfa (69% at cotton blooming stage; 83% in cotton boll maturation stage) compared with that moved into cotton at both the cotton blooming stage and boll maturation stage (31% at cotton blooming stage; 17% in cotton boll maturation stage). Though we used two different marker proteins we found that a similar number of *Lygus* moved into cotton during cotton blooming stage and cotton maturation stage, but a numerically larger number of *Lygus* moved into cotton at the blooming stage (31%) as compared to that in the cotton boll maturation stage (17%) (Figure 2).



Figure. 2. Inter crop movement behavior of *Lygus* between roadside alfalfa and adjacent cotton at the cotton blooming stage. Results of ELISA of *Lygus* captured in mowed and not mowed roadside alfalfa and adjacent cotton 48 hr after alfalfa field marking with NFDM and 24 hr after alfalfa mowing. Lubbock, Texas, 2007. Note: The numbers in the bar are the average number of *Lygus* per 100 ft KIS sample.

B. Alfalfa Mowing Height and Lygus Intercrop Movement

Effect of road side alfalfa mowing height was mimicked by growing 12 rows of alfalfa in the middle of a cotton field and the effect of two mowing heights of alfalfa was evaluated by mowing alfalfa at 6-in. and 2-in. height after field marking by different protein markers (EW in 2-in. mowing plot and NFDM in 6-in. mowing plot). Lygus could not be retrieved by vacuum sampling in the 2-in. mowed plots but an average of 20 Lygus per 100 ft was found in 6-in. mowed plots 24 hours after mowing. Mowing alfalfa at a height of 2-in. forced all Lygus to moved out of these alfalfa plots or killed during alfalfa mowing procedure. Some of the Lygus from the 2-in. mowed alfalfa plots likely died during the alfalfa mowing operation while some EW marked Lygus moved and were found in nearby alfalfa that was not mowed and to the nearby cotton field. A significantly higher number of Lygus adults were found to have moved into alfalfa that was not mowed as compared with nearby cotton. The movement bias toward non mowed alfalfa might be due to the alfalfa being a preferable host and the post blooming cotton being a less preferable host. Lygus biology, host selection and feeding behaviors need to be done to confirm the host preference between alfalfa and cotton in their different life stages. Even after a 6-in. mowing, alfalfa could attract and host larger populations of Lygus than cotton. The 6-in. mowed alfalfa had few green leaves after mowing therefore it could host some Lygus. There were no significant difference between adult Lygus numbers moving out from 2-in. mowed and 6-in. mowed alfalfa plots and went into not mowed alfalfa or cotton field (Figure 3). Lygus adults were found to have dispersed across the cotton to the 40th row (120 feet distance into cotton field from the alfalfa source in 24 hr). This information may be useful in predicting and modeling the dispersal behavior of Lygus into cotton field from alternative habitats.



Figure 3. Effect of alfalfa mowing heights on movement behavior of *Lygus* in cotton and alfalfa system in Texas High Plains. ELISA procedures was conducted on *Lygus* captured in alfalfa and adjacent cotton field 48 hr after field marking with EW and 24 hr after alfalfa mowing at Lubbock in 2007.

C. Mark-Release-Recapture Study to Examine Lygus Host Selection Behavior

The intercrop movement behavior of insects depends on many factors and the host preference of an insect is one of the crucial factors. The host selection behavior of adult *Lygus* in natural field situation in a two crop choice (cotton and alfalfa) both at the blooming stage was tested by marking field collected adult *Lygus* with protein markers in the laboratory and the field releasing them into the middle of two crops. *Lygus* were allowed to select their preferred host for 24 hr and recaptured and analyzed by indirect ELISA. Out of 3000 adult *Lygus* released, only 62 (0.02%) were recaptured. A total of 187 adult *Lygus* were captured by KIS sampling (900 ft of cotton and 900 ft of alfalfa) out of which 33% were from marked and released *Lygus*. *Lygus* released in the middle of the alfalfa field were found in about the same number (24 in alfalfa and 21 in cotton field) in both cotton and alfalfa field after 24 hr of foraging time. It indicates that at the cotton blooming stage *Lygus* do move from alfalfa to cotton. The *Lygus* released in the middle of the cotton and 4 from alfalfa) (Figure 4). This study suggests that *Lygus* move in both directions between alfalfa and cotton at the cotton blooming stage but the net movement from alfalfa to cotton was high (4 into alfalfa and 21 into cotton so net movement from alfalfa to cotton field was 17). This kind of marking releasing can detect only one directional movement so separate study should be design to detect the back and forth movement to evaluate the back and forth movement behavior of *Lygus* in a natural setting.



Figure 4. Host preference of *Lygus* as determined by mark release recapture study in a cotton and alfalfa system in Texas. Results are from ELISA testing of *Lygus* captured in alfalfa and an adjacent cotton field 48 hr after external marking and releasing into the fields. Note: Red bars represent the *Lygus* marked with EW released in middle of alfalfa field, blue bars represent *Lygus* marked with NFDM and released in middle of cotton field and green bar represent *Lygus* without any marker naturally occurred in cotton and alfalfa field.

Conclusions

Lygus movement data from this study clearly suggest that alfalfa is a more preferred host than cotton for *Lygus* colonization when both habitats are available side-by-side. However, alfalfa may serve as both source and sink for *Lygus* depending on the crop stage and plant quality. At the cotton blooming stage, the net movement of *Lygus* from alfalfa to cotton was positive under natural field conditions even without forcing the movement by mowing the alfalfa. However, a forced movement from alfalfa resulted in net positive movement of *Lygus* from alfalfa to cotton through the boll development stage. The 6-in. height mowed alfalfa retained a significant number of *Lygus* in alfalfa after mowing, resulting into a lower number of *Lygus* forced to move into cotton field. Therefore, frequent mowing of alfalfa at 6" height may be beneficial for *Lygus* management in cotton fields that are next to roadside alfalfa. For field marking studies, the NFDM should be applied at >6% concentration if no adjuvant is applied, whereas a 6% solution can be sufficient for EW. The inter crop movement behavior of an insect is a complex phenomenon that depends on biological and ecological factors affecting both the insect and the crop habitat. Quantification of insect. Field marking with protein markers and their detection by ELISA assay is one of the potential methods of quantification of the movement and dispersal but the efficacy of the system depends on the selection of crop and insect specific markers and marking concentrations.

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