Preliminary evaluation of absolute sampling methods for *Lygus*

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Justification and Problem Statement

The western tarnished plant bug is a key pest of cotton in western arid production regions. Populations of lygus bugs are difficult to monitor because adults are active fliers, and nymphs inhabit cryptic habitats and move rapidly when disturbed. Management decisions are complicated by these difficulties. Because the principal management tactic for lygus bugs is chemical pesticides, treatment decisions based on inaccurate sampling data may result in either unnecessary crop loss, or unneeded pesticide applications that my contaminate the environment and induce secondary pests. Considerable effort has been devoted in cotton production regions of the West and Mid-South to evaluate and improve sampling methodology for lygus bugs. However, in recent efforts the criteria for the selection of sampling methods have focused on maximum numbers of bugs collected or apparent precision of population estimates without consideration for the fidelity of those estimates to actual bug populations. Development of efficient and practical absolute or near-absolute sampling methods for one or more stage of lygus would allow more meaningful evaluation and perhaps calibration of relative methods.

Our initial proposal was to evaluate absolute population estimation techniques including caging, whole plant inspections, and plant bagging. However, success in our early efforts at marking, releasing, and recovering adult lygus changed our focus to using this technique to calibrate the sweep net method for adult lygus. The mark-release-recapture technique offers significant advantages over absolute sampling techniques. Among those are the ability to establish bug populations of known density at the crop stages of interest, rather than relying on unpredictable natural populations, and the ability to control the age distribution of the population sampled. Most importantly, the ability to establish lygus populations of known density eliminates the often considerable variation associated with absolute population density estimates. This variation is typically unaccounted for in efforts to calibrate relative sampling methods, but can constitute a substantial source of error in the resulting relationships.

Procedures

Marking and handling of bugs. Marking efforts were intended to meet two criteria, 1) allow unambiguous and rapid identification of released bugs, and 2) eliminate the ability of adult lygus to fly. Previous efforts to similarly mark bugs used Testors paint and were unsuccessful (Wilson et al. 1984). However, Raulston et al. (1998) used fingernail polish to mark and prevent the flight of boll weevil adults in studies to estimate collection efficiency of a pneumatic sampler.

Preliminary efforts to mark bugs involved using a fine paint brush to place a droplet of fingernail polish at the point at which the wings overlapped. Several brands and colors were evaluated, and most were satisfactory. Tests in which small cohorts of bugs (usually 10) were painted with various colors indicated the marking procedure eliminated flight and did not produce significant mortality.

Evaluation of bug retention on plants. Establishment of known bug populations requires that released bugs remain on the desired row sections. Preliminary evaluations of bug movement from plants were conducted using 39-inch sections of row which were enclosed by a wooden frame. Each frame was constructed of 1" × 4" pine lumber, and dimensions were about 57" × 30" (L × W, outside). Four frames were used for each evaluation. Before each evaluation, 39-inch sections of row were selected and isolated by removing adjacent plants for a distance of about 39 inches from each end of each section. Frames were then centered over the row sections. Beds and furrows under the frame were leveled and the base of the frame was sealed with soil. The top edge of each frame was then coated with Tangle-Foot adhesive. Burial of the bottom surface of the frame and coating of the top surface with adhesive was intended to prevent bugs from leaving the plants by walking.

Bug movement from plants was evaluated on 30 May, and 3 and 6 June. Plant height from the soil surface to the mainstem terminal averaged 6.7, 8.2, and 8.2 inches on these respective dates. Corresponding numbers of mainstem true leaves were 7.1, 7.8, and 8.2. Median fruiting phenologies were either 'sub-pinhead' (squares < 2 mm in width, including bracteoles, 30 May), or 'pin-head' (bud enclosed by the bracteoles < 3 mm in diameter, 3 and 6 June). For each test date, 10 marked bugs were released onto the upper leaves of separate plants after 7:00 PM. The following morning (between 9:00 – 10:00 AM), the plants, frame, and surrounding soil surface was examined for marked bugs. Recovered bugs were recorded as captured in the adhesive, recovered dead, or recovered alive. After each test each frame was moved to a new row section.

Determination of sweepnet collection efficiency. Our objective was to determine 1) the proportion of lygus adults present that are collected by the sweepnet, and 2) whether this proportion is consistent enough to be of practical use.

For each sampling date we used four population levels (10, 20, 40, and 60 bugs / 33 row ft) individually established on sections of row. Each population level was replicated twice on each date using a completely randomized design. The only deviation from this design was on 8 July, when 72 bugs were inadvertently released into a row assigned to the 60 bugs/row treatment. The study was established in a plot of Pima cotton ('Phytogen 800') 48 rows wide by about 300 ft long and planted to 40-inch rows. The field was characterized by a marked difference in soil type near the southern margin which resulted in much smaller plants compared to plants in the remainder of the field. This difference was exploited to allow evaluation in plants of similar fruiting phenology but different plant height and canopy development during similar time periods. A tier of eight study rows was established on each end of the plot. The tier on the northern end of the field began about 50 ft from the northern field margin. The tier on the southern end began about 30 ft from the southern margin. Both tiers extended 33 ft toward the field center. The outermost row of each study area marked the 6th row from the field margin. In each study area, eight rows were designated for bug releases and sampling, with each sample row separated by four buffer rows. Each sample row was 33 ft in length, and a buffer area of about 3 ft was established at each end of each row by removing plants. On each subsequent sample date, the entire tier of sample rows was shifted one row farther from the field margin. Also, on each sample date, two of the frames used in the evaluations of bug movement from plants were established between the tier of sample rows and the northern (or southern) field

margin. Plants for enclosure in the frames were selected based on their similarity to those in the sample rows, and the frames were placed using the same procedures previously described. Frames were moved to new locations for each sample date.

Consecutive samplings alternated between northern and southern tiers of rows, beginning with the northern tier, until the plant canopy was nearly closed in the northern tier (8 July). After 8 July, only the southern tier was used. Also, beginning on 23 July, sample rows in the southern tier were shifted 20 rows farther from the western field margin to avoid large differences in plant height within the sample tier. At that time, the number of buffer rows separating sample rows was reduced from four to three.

On each sample date except 8 July a total of 280 marked bugs were used. A total of 260 bugs were released into sample rows as previously described for the frames, but making an effort to distribute the bugs as evenly as possible down the row. Ten additional bugs were released into each of the frames to provide an estimate of availability of bugs for sampling on each date. Sampling was conducted on 12 dates (10, 20, 24, and 27 June; 1, 3, 8, 11, 16, 23, and 30 July; 6 August). Wild bugs collected from alfalfa were used on 30 July and 8 August. These bugs were collected 3-4 days before release.

Bugs were released into sample rows and frames after 7:00 PM on the evening before sampling. Samples were collected between 9:15 and 9:45 AM the following morning. Each row was sampled by taking 10 sweeps with a standard 15-inch sweepnet. Pendulum sweeps were used, with one pass of the net across one row constituting a sweep. All samples were collected by the same person, and the time to collect each sample was recorded to provide a measure of consistency of walking speed down the row. Concurrent with sample collection, plants within the frames were examined for marked bugs, and to collect plant data.

Linear regression was used to examine the relationship between population levels of marked bugs and numbers of bugs recovered by the sweep net for each sample date. For these calculations, we assumed each pass of the sweepnet sampled 15 inches of row, resulting in a total of 12.5 ft of sampled row per 10 sweeps. The expected number of bugs collected, assuming 100% collection efficiency (number of bugs released × 12.5 ft/33 ft), was used as the independent variable, and the number of bugs collected by the sweepnet was used as the dependent variable. The regression equations for all sample dates were examined for common slopes. Based on these analyses, regressions from the various dates were pooled into two groups, each described by a common regression equation.

Our sample rows were designed to accommodate 10-sweep samples because of the logistical constraints imposed by the availability of bugs and the labor associated with marking. However, a 10-sweep sample is smaller than that used in many research or monitoring programs. To examine the influence of sample size on model adequacy, duplicate 10-sweep samples within a sample date were pooled to make a single 20-sweep sample for each combination of population level and sample date. The relationship between numbers of bugs collected and expected numbers of bugs was examined using linear regressions as for the 10-sweep samples.

Results and Discussion

Evaluation of bug retention on plants. Preliminary studies indicated most marked bugs placed on plants within the frames remained on the plants, although it was apparent that many bugs did not remain on the plant on which they were originally placed. Recovery of live bugs from the frames ranged from 70 to 100%, and averaged 92.5%. Three (2.5%) of the 120 released bugs were found dead, and 2 (1.7%) were captured in the adhesive on the top edge of the frame base. Four released bugs (3.3%) were not accounted for.

Determination of sweepnet collection efficiency. Sampling studies to evaluate the collection efficiency of the sweepnet were initiated on 10 June when the median stage of fruit development was matchhead square. Plant populations averaged about 47,500 plants/acre on the northern field end and 43,300 plants/acre on the southern end. Sampling continued until canopy closure (8 July, early bloom, northern sampling tier) or cut-out (6 August, southern sampling tier; Table 1).

Table 1. Sample dates and corresponding plant measurements in evaluation of sweepnet collection efficiency.

Date	Field end	Plant height (in.)	No. nodes	Median fruiting stage
10 June	North	9	9.2	matchhead square
20 June	South	9	10	matchhead square
24 June	North	16.5	14.3	one-third grown square
27 June	South	11.8	13.3	one-third grown square
1 July	North	21.8	15.7	candle
3 July	South	15.5	14.3	one-third grown square
8 July	North	26.6	18.2	bloom
11 July	South	18.5	16.2	candle
16 July	South	21	16.2	boll
23 July	South	20.8	17.5	boll
30 July	South	20.6	17.3	boll
6 August	South	20.2	17.2	boll

Based on the recovery of bugs from the frames, about 86.2% (207 of 240) of released bugs were available for capture at the time of sampling. Only one bug (0.4%) was recovered from the adhesive on the frames, and 13 (5.4%) were recovered dead. Most bugs recovered dead were partially eaten, and predation by both nabids and ants was observed. Only 7.9% of released bugs were not accounted for. Recovery of live bugs from frames on individual sample dates ranged from 70% (16 July and 6 August) to 100% (1 and 3 July). Recovery was ≥80% on nine of the 12 sample dates, so we made no effort to adjust population levels released in sample rows to account for mortality.

Sampling times were very consistent, ranging from 6.4 to 7.4 seconds per 10 sweeps. Sampling times on the two sample dates with the largest averages (8 July, 7.4 sec; 16 July, 7.3 sec) were recorded by a different individual than on other dates (range from 6.4 to 6.8 sec). This suggested

the differences in sampling times observed were more dependent on the person recording times than on variation in actual times to collect the samples.

Regression equations for individual sampling dates were combined into two groups. A sharp decrease in the regression slopes occurred at a plant height of roughly 20 or 21 inches, with some overlap in plant height between the two groups. Analysis of the regressions corresponding to the first group (mean plant height from 9 to 20.8 inches) indicated a common slope adequately described the pooled data ($P = 0.996$). The resulting regression equation was $y = -0.195 + 0.226x$, where y is the number of bugs collected in 10 sweeps, and x is the expected number of bugs per 12.5 ft of row (the area sampled by 10 sweeps). This model explained 56.5% of the variability in the data. A no-intercept model fitted to these data indicated that collection efficiency of the sweepnet was about 21.4%.

The pooled data for sample dates with the generally larger plants yielded a regression model of $y = 0.165 + 0.067x$, with x and y defined as above. However, the model only explained 21.4% of the variation observed in the data. The corresponding no-intercept model for these data indicated that sweepnet collection efficiency on these sample dates was only about 7.6%.

Analyses of sample data pooled for population levels within dates (20-sweep samples) resulted in the same groupings of regressions as for the 10-sweep samples. The regression model for the first group, representing generally smaller plants, was $y = -0.390 + 0.226x$. This regression explained 75.1% of variation in the data. The corresponding no-intercept model indicated a sweepnet collection efficiency of 21.4%. The model for the second group of plants, which were generally larger, was $y = -0.026 + 0.081x$, explaining 39.2% of variation in the data. Based on the no-intercept model, collection efficiency of the sweepnet in the larger plants was 8.0%.

The results of our study illustrate the potential usefulness of the mark-release-recapture method for sampling studies of lygus adults. Based on our results, sweepnet sampling during the morning hours provides predicted population estimates that are sufficiently accurate to be useful in research and monitoring efforts, especially on plants less than 20 inches in height. However, the relationship between sweepnet-based population estimates and actual lygus population levels becomes more variable with increasing plant development. The factors responsible for the sharp decline in sweepnet collection efficiency later in the season are not fully understood. Additional research will be needed to identify and quantify these factors.

Few contemporary sampling studies of lygus utilize absolute population estimates because of the labor involved and the perceived inadequacies of these methods. Our mark-release-recapture technique can be easily adapted to unambiguously define factors such as time of day effects, plant size and development, varietal differences, bug age and physiological status, and the variation among individual samplers.

References

Raulston, J. R., D. W. Spurgeon and A. N. Sparks, Jr. 1998. Influence of fruit on sampling and control of adult boll weevils in cotton. Southwest. Entomol. 23: 1-10.

Wilson, L. T., T. F. Leigh, D. Gonzalez and C. Foristiere. 1984. Distribution of *Lygus hesperus* (Knight) (Miridae: Hemiptera) on cotton. J. Econ. Entomol. 77: 1313-1319.