VARIATION AMONG SAMPLERS USING THE SWEEP NET FOR *LYGUS HESPERUS* ADULTS IN COTTON

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Abstract

The sweep net is a standard sampling method for adults of the western tarnished plant bug, *Lygus hesperus* Knight, in cotton. However, factors that influence the relationship between true population levels and population estimates obtained using the sweep net are poorly documented. Improved understanding of these factors is needed for the development and application of refined treatment thresholds. Recent reports suggest significant among-sampler differences in sweep net-based population estimates of the adult tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). These differences would seem to preclude meaningful comparisons of population estimates collected by different investigators. We used a mark-release-recapture method and the standard sweep net to evaluate among-sampler differences in population estimates of *L. hesperus* adults. Adult lygus, marked with fingernail polish to facilitate identification and prevent flight, were released into 10-m sample rows on the evening before 10-sweep samples were collected the following morning. The experimental design was a randomized complete block with three replications of three treatments (sampler). Separate experiments were conducted in two plantings each of Pima (*Gossypium barbadense* L.) and Acala (*G. hirsutum* L.) cotton. Collections of marked bugs from each study were evaluated for effects of sampler, sample date, and their interaction. Although differences in lygus collections were observed among sample dates in some tests, no differences were detected in the population estimates by different samplers. These results demonstrate that the sweep net technique can be sufficiently standardized to allow direct comparison of population estimates obtained by different samplers.

Introduction

The western tarnished plant bug (*Lygus hesperus* Knight) and other lygus species are key pests of cotton in western arid cotton production regions. The lygus complex has also become increasingly important in other US cotton production regions. The elevated pest status of lygus bugs in much of the U.S. cottonbelt has prompted research to improve management strategies. However, efforts to develop effective lygus management rules are hampered by uncertainty in the interpretation of population estimates provided by commonly used relative sampling methods. Improved knowledge of the relationships between estimates of lygus populations and their true densities would facilitate the design and interpretation of studies of cotton responses to lygus infestation.

Numerous studies of sweep net sampling for lygus have incorporated one or more absolute sampling methods for comparison (Byerly et al. 1978, Ellington et al. 1984, Fleischer et al. 1985, Snodgrass and Scott 1977, Zink and Rosenheim 2004). However, results of those studies are difficult to interpret because of the variation among studies in the methods of sweeping. More recent studies have focused on relative sampling methods, but made no attempt to relate population estimates by those methods to true population densities (Gore and Catchot 2005, Musser et al. 2007, Sharp and Bagwell 2006, Stewart et al. 2006). A notable finding by Musser et al. (2007) was that different samplers using the sweep net in cotton obtained statistically different population estimates of the tarnished plant bug. If that conclusion is valid, is seems unlikely that reliable and widely applicable treatment thresholds could be developed on the basis of samples collected by the sweep net. Furthermore, interpretation of results of research conducted by different investigators or involving multiple samplers would not be straightforward. One potential shortcoming of the study by Musser et al. (2007) is that different samplers obtained estimates of lygus populations in different fields or different sites within fields. Therefore, it seems likely that at least some of the variability attributed to differences among samplers was a reflection of the different lygus population levels sampled.

Spurgeon (2009) developed a mark-release-recapture method for evaluating the sweep net as a sampling method for adult lygus in cotton. This approach permits the investigator to collect samples from known lygus population levels, thereby alleviating many of the problems associated with the collection of absolute population estimates and reducing the heterogeneity among sampled areas. We used these mark-release-recapture methods to evaluate among-sampler differences in collections of adult lygus bugs from both Upland and Pima cotton.

Materials and Methods

We conducted a total of four experiments including two separate plantings of each the Acala variety 'Phytogen 72' and the Pima variety 'Phytogen 800'. The first planting of each variety was in late April, and the second planting was in early June. All plantings used a 40-inch row spacing. For simplicity we refer to these experiments according to planting (experiment 1, Acala or Pima; experiment 2, Acala or Pima). Within each planting, 3–5 sampling areas (blocks) were established. Each block was composed of 12–16 parallel 10-m row sections. The ends of each of these row sections was separated from the remainder of the row by a buffer area (≥1 m) from which the plants were removed.

Marking Bugs

Lygus adults for release were obtained from a laboratory colony reared on green bean pods and sunflower seeds, or were collected from plots of alfalfa. Lygus adults were harvested from the colony three times weekly. When adequate numbers were available from each cohort, four age classes (2–5, 5–7, 7–9, and 9–12-d-old) were marked and released into each sampled row in equal numbers. When numbers of adults of any age cohort were insufficient to accommodate releases into all rows sampled on a given date, field collected bugs of unknown ages were substituted for those age classes. Overall, about 45% of released bugs originated from the laboratory colony.

Lygus adults, whether they were obtained from the colony or field, were held for ≥24 h before they were marked. To facilitate marking, small aliquots of 5–10 bugs were aspirated into plastic vials and lightly anesthetized with CO_2. Anesthetized bugs were decanted into a Petri plate bottom lined with moist filter paper. Each bug was oriented so a small droplet of fingernail polish could be applied to the dorsum near the posterior of the scutellum. After bugs were marked the Petri plate was closed by a screened lid and set aside until the polish was dried. Marked bugs that were able to separate their wings or that had legs, antennae, or heads coated with polish, were discarded. Remaining marked bugs were held in same-age groups of 200–400 individuals within 1-gal plastic buckets containing shredded paper and fresh green bean pods, and closed with a screened lid. Marked bugs originating from the colony were held in environmental chambers at 24°C with a 14-h photoperiod until they were released. Marked bugs originating from the field were held in the laboratory at room temperature. A different color of mark was used on each sample date within each planting.

Bug Releases and Sampling

Each week, three rows from each of three sampling areas (blocks) in each cotton type were selected for sampling. Sampling rows within each block were selected on the basis of similarity in plant size and the absence of large (≥1-m) skips in the plant stand. Each of three samplers was then randomly assigned to one row in each block. On the evening before sampling, marked bugs were aspirated into 12-dram plastic vials for transport to the field. Each of the 80 labeled vials contained 10 bugs of a given age class, and was closed with a snap-cap lid. Each vial lid was penetrated by a hole (~0.8 cm diameter) which was closed with a rubber stopper. The vials were then sorted so that 10 bugs of each age class (a total of 40 bugs) were assigned to each sample row. Marked bugs in the eight extra vials were used as replacements for bugs that were dead by the time of release. Beginning after 1900 h (PDT), 40 marked bugs were released into each 10-m sample row. Marked bugs were released onto plant terminals and upper leaves, and were spaced as evenly as possible down each row.

Between 0900 and 1000 h on the morning following bug releases, each row was sampled by 10 pendulum sweeps using a standard 38-cm diameter sweep net. One pass of the net through the upper 8–10 inches of the plant canopy constituted a single sweep. Collected bugs were transferred from the sweep net to a sealable plastic bag, which was transferred to the laboratory where both marked and unmarked lygus adults were counted.

Immediately after sampling, five plants from locations evenly spaced down each sampled row were examined to characterize crop development. Measurements recorded included plant height (mainstem length from the soil surface to the terminal), mainstem node number (considering the hypocotyl as node zero and counting to the uppermost expanded leaf), canopy width, and phenological stage (vegetative, sub-pinhead, pinhead, matchhead, and 1/3-grown square, candle, bloom, and boll).

Statistical Analyses

All analyses were conducted using SAS (SAS ver. 9.2, SAS Institute, Cary, NC). Plant development for each cotton type on each sample date was characterized by calculating means for each plant measurement, except phenological

stage for which the median stage was calculated. Collections of both marked and unmarked (native) lygus adults were analyzed separately for each experiment (combination of planting and cotton type) using mixed-model ANOVA (PROC GLIMMIX). Each analysis included fixed effects of sampler, date, and their interaction, and the random effect of block. Where differences among levels of a fixed effect were indicated, means were compared controlling the experiment-wise type I error rate with the ADJUST=SIMULATE option of the LSMEANS statement.

Results and Discussion

Experiment 1 – Acala

Sampling was not initiated until plants averaged 10 nodes and more than 40 cm in height because early square retention was poor. Over the duration of the experiment, average plant heights corresponding to different sample dates ranged from 43.5 cm on 10 June to 83.7 cm on 1 July (Table 1). Canopy width was generally less than plant height. In addition, less than half of the plants had bolls until 8 July (Table 1). The relatively narrow canopy width and extended pre-flower squaring period were likely indicative of poor fruit retention until the last weeks of the experiment. One consequence of poor fruit retention was a concentration of squares in the upper portions of the plants.

Analysis of variance did not indicate differences among samplers in the numbers of marked lygus that were recovered by the sweep net (F = 0.44; df = 2, 32.3; P = 0.65). The absence of a significant sampler by date interaction (F = 1.27; df = 10, 32.3; P = 0.29) indicated the lack of differences among samplers was consistent across sample dates. However, the numbers of marked lygus that were recovered differed among sample dates (F = 3.86; df = 5, 24.72; P = 0.01). The mean of counts of marked bugs was higher on 10 June than on any other sample date except 25 June (Table 1). The higher numbers of marked bugs collected on 10 June was likely a consequence of the relatively small plant size, compared with other dates. In contrast, numbers of unmarked lygus recovered by the sweep net did not vary significantly among samplers (F = 0.97; df = 2, 36; P = 0.39) or sample dates (F = 0.68; df = 5, 36; P = 0.64; Table 1), and no sampler by date interaction was observed (F = 0.42; df = 10, 36; P = 0.93).

Table 1. Means of plant parameters (growth stages are medians) and of numbers of marked and unmarked adult *Lygus hesperus* collected per 10-sweep samples from a late-April planting of Acala cotton.

Date	Plant Height	No. Nodes	Canopy Width	Growth Stage	Marked Bugs*	Unmarked Bugs
10 June	43.5	10.2	35.3	matchhead square	5.7a	2.6
17 June	52.4	11.7	46.1	matchhead square	3.4b	2.2
25 June	72.0	14.0	59.8	1/3-grown square	3.5ab	2.1
1 July	83.7	15.9	67.7	1/3-grown square	3.2b	2.2
8 July	58.3	15.4	43.6	boll	3.0b	1.7
22 July	80.4	18.9	74.2	boll	2.9b	3.1

*Means in a column followed by the same letter are not significantly different (P > 0.05, SIMULATE option of the LSMEANS statement in SAS)

Experiment 1 – Pima

As for the first planting of Acala cotton, sampling was not initiated in the first planting of Pima cotton until plants averaged nearly 10 mainstem nodes in development (Table 2). Although canopy width in the Pima planting was generally similar to plant height, the Pima planting also exhibited poor early fruit retention and a concentration of squares in the upper canopy. Because the Pima variety we used matures more slowly than the Acala variety, and both plantings exhibited similarly poor early fruit retention, plants in the Pima planting were generally less developed than those in the Acala planting. In fact, most plants in the Pima planting did not have 1/3-grown squares or bolls until 1 July and 22 July, respectively.

Numbers of marked adult lygus were similar among samplers (F = 0.48; df = 2, 36; P = 0.48), but varied significantly among sample dates (F = 4.14; df = 5, 36; P < 0.01). The non-significant sampler by date interaction (F = 1.02; df = 10, 36; P = 0.44) indicated differences observed among dates were consistent among samplers. Numbers of marked lygus that were recovered in 10-sweep samples were higher on 10 and 17 June than on 8 July (Table 2). No differences among other dates were demonstrated. The general tendency was for numbers of recovered bugs to decrease with increasing plant size until about 1 July.

Sample counts of native lygus also did not differ among samplers (F = 1.35; df = 2, 36; P = 0.27). Although collections of unmarked bugs varied among sample dates (F = 7.35; df = 5, 36; P < 0.01), the pattern of variation with date was different from that observed for marked bugs (Table 2). For native lygus, the numbers collected were significantly higher on the last sample date (22 July) compared with earlier dates. As for the marked bugs, the sampler by date interaction (F = 0.76; df = 10, 36; P = 0.66) indicated the temporal patterns in collections of native lygus were similar among samplers.

Table 2. Means of plant parameters (growth stages are medians) and of numbers of marked and unmarked adult *Lygus hesperus* collected per 10-sweep samples from a late-April planting of Pima cotton.

Date	Plant Height	No. Nodes	Canopy Width	Growth Stage	Marked Bugs*	Unmarked Bugs*
10 June	30.6	9.8	30.3	pinhead square	6.0a	2.0b
17 June	30.8	10.7	34.3	pinhead square	5.2a	1.7b
25 June	37.4	11.6	38.0	matchhead square	4.9ab	1.0b
1 July	42.4	13.4	44.5	1/3-grown square	3.6ab	2.0b
8 July	49.3	14.2	40.6	1/3-grown square	2.3b	1.2b
22 July	61.5	18.2	53.8	boll	3.7ab	4.7a

*Means in a column followed by the same letter are not significantly different (P > 0.05, SIMULATE option of the LSMEANS statement in SAS)

Experiment 2 – Acala

Conditions at the time of the second planting of Acala cotton facilitated vigorous vegetative growth. The first pinhead squares were generally observed at mainstem nodes 8 and 9, and early square retention was relatively low. Average plant heights during the study period ranged from about 37 to 60 cm. Canopy widths were considerably less than plant heights (Table 3), resulting in an erect and narrow plant canopy structure with few lower fruiting branches. As in the first experiment, fruit tended to be concentrated in the upper portions of the plant canopy on most sample dates.

No differences were observed among samplers in the mean numbers of marked lygus collected per 10-sweep sample (F = 0.69; df = 2, 24; P = 0.51). Absence of a significant sampler by date interaction (F = 1.12; df = 6. 24; P = 0.38) indicated the lack of differences among samplers was consistent across sample dates. In contrast with the first experiment, no temporal pattern in the collection of marked lygus was detected (F = 2.26; df = 3, 24; P = 0.11; Table 3). Analyses of collections of native lygus also indicated no significant influence of sampler (F = 1.39; df = 2, 22; P = 0.27) and no significant sampler by date interaction (F = 0.29; df = 6, 22; P = 0.93). However, a temporal pattern in the collection of native lygus was detected (F = 5.30; df = 3, 22; P < 0.01; Table 3). The numbers of native lygus collected per 10-sweep sample were lowest in the first and last sample date, and peaked on 12 August.

Table 3. Means of plant parameters (growth stages are medians) and of numbers of marked and unmarked adult *Lygus hesperus* collected per 10-sweep samples from an early-June planting of Acala cotton.

Date	Plant Height	No. Nodes	Canopy Width	Growth Stage	Marked Bugs	Unmarked Bugs*
29 July	36.9	10.4	26.0	matchhead square	4.7	0.3b
5 August	49.2	12.3	38.3	1/3-grown square	4.0	1.0ab
12 August	57.3	13.8	37.6	1/3-grown square	2.7	1.6a
19 August	59.2	14.0	40.7	boll	3.0	0.4b

*Means in a column followed by the same letter are not significantly different (P > 0.05, SIMULATE option of the LSMEANS statement in SAS)

Experiment 2 – Pima

Fruiting in the second planting of Pima was also delayed, and the median phenological stage of development did not advance beyond 1/3-grown square during the study (Table 4). Although plant heights were comparable to those in the Acala cotton, canopy widths were generally greater and the canopy structure was less erect.

No differences among samplers were observed in the numbers of marked lygus collected by the sweep net (F = 0.88; df = 2, 22; P = 0.43), and this lack of differences was consistent across sample dates (sampler by date interaction, F

= 0.80; df = 6, 22; P = 0.58). Differences in the numbers of marked lygus collected were observed among sample dates (F = 3.80; df = 3, 22; P = 0.02). More marked bugs were collected during the 5 August sampling than on 12 August (Table 4), whereas counts on other dates were intermediate to these extremes. Collections of native lygus also failed to indicate significant effects of sampler (F = 0.20; df = 2, 24; P = 0.82) or a sampler by date interaction (F = 0.87; df = 6, 24; P = 0.53). However, in contrast to marked bugs, no differences in the mean counts of native bugs were observed among sample dates (F = 1.12; df = 3, 24; P = 0.36; Table 4).

Table 4. Means of plant parameters (growth stages are medians) and of numbers of marked and unmarked adult *Lygus hesperus* collected per 10-sweep samples from an early-June planting of Pima cotton.

Date	Plant Height	No. Nodes	Canopy Width	Growth Stage	Marked Bugs*	Unmarked Bugs
29 July	36.6	11.1	35.1	matchhead square	3.6ab	1.0
5 August	45.0	12.2	39.5	1/3-grown square	4.6a	0.4
12 August	58.2	15.2	48.3	1/3-grown square	1.9b	0.4
19 August	56.2	14.8	45.4	1/3-grown square	3.2ab	0.7

*Means in a column followed by the same letter are not significantly different (P > 0.05, SIMULATE option of the LSMEANS statement in SAS)

Summary

We evaluated the influence of sampler on counts of *Lygus hesperus* adults collected using the standard sweep net in both Acala and Pima cottons representing a variety of plant sizes and stages of development. At no time did we observe any statistical evidence of a sampler effect. This finding clearly indicates that if care is taken, different samplers can use the sweep net to obtain equivalent estimates of adult lygus populations.

We also observed temporal patterns in lygus collections corresponding to sample date. In the first experiment, counts of marked lygus adults tended to be highest on the earliest sample dates, which corresponded to the smallest and least developed plants. This observation is consistent with the report of Spurgeon (2009), who documented a plant-size effect on the collection efficiency of the sweep net. However, temporal patterns in lygus collections during the second experiment deviated somewhat from this pattern. During the second experiment, we regularly observed predation by assassin bugs (Hemiptera: Reduviidae) on both marked and unmarked lygus (unpublished data). Therefore, predation in the second experiment seems the most likely explanation for the observed differences in temporal population patterns between the two studies.

Relatively high population levels of native (unmarked) lygus bugs were present during most samplings of both studies. As for the marked bugs, we observed no evidence of among-sampler differences in counts of native bugs, although differences in the numbers of native lygus were observed among sample dates in two of the tests. Whether or not differences in counts of native bugs were observed among dates, the observed temporal patterns were generally inconsistent with the patterns observed for marked bugs. High levels of predation in the second experiment may have influenced the observed temporal patterns of counts of marked bugs, rendering comparisons with such patterns of native bugs irrelevant. However, retention of marked adults in the first experiment was generally high (≥79%, unpublished data). In that experiment, counts of marked lygus tended to decrease with increasing plant development in the first weeks of the study, while counts of native lygus either did not change (Acala) or exhibited a pattern different from that of marked bugs (Pima). These observations suggest that changes in sweep net sampling efficiency corresponding to increasing plant development may have obscured the true temporal patterns of the native lygus population. This finding may be of critical importance to ecological studies and to efforts to develop improved treatment thresholds, and warrants continued study to elucidate the plant-based factors influencing collection efficiency of lygus adults by the sweep net.

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References

Byerly, K.F., A.P. Guiterrez, R.E. Jones, and R.F. Luck. 1978. A comparison of sampling methods for some arthropod populations in cotton. Hilgardia 46:257–282.

Ellington, J., K. Kiser, G. Ferguson, and M. Cardenas. 1984. A comparison of sweep net, absolute, and insectavac sampling methods in cotton ecosystems. J. Econ. Entomol. 77:599–605.

Fleischer, S.J., M.J. Gaylor, and J.V. Edelson. 1985. Estimating absolute density from relative sampling of *Lygus lineolaris* (Heteroptera: Miridae) and selected predators in early to mid-season cotton. Environ. Entomol. 14:709–717.

Gore, J., and A. Catchot. 2005. Tarnished plant bug sampling and management in the Mississippi Delta. p. 1234–1238. *In* Proc. Beltwide Cotton Conf., New Orleans, LA. 4–7 Jan. 2005. Natl. Cotton Counc. Am., Memphis, TN.

Musser, F., S. Stewart, R. Bagwell, G. Lorenz, A. Catchot, E. Burris, D. Cook, J. Robbins, J. Greene, G. Studebaker, and J. Gore. 2007. Comparison of direct and indirect sampling methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. J. Econ. Entomol. 100:1916–1923.

Sharp, J.W., and R.D. Bagwell. 2006. Evaluation of multiple bug sampling techniques for effectiveness and efficiency. p. 954–956. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 3–6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN.

Snodgrass, G.L., and W.P. Scott. 1997. A conversion factor for correcting numbers of adult tarnished plant bugs (Heteroptera: Miridae) captured with a sweep net in cotton. Southwest. Entomol. 22:189–193.

Spurgeon, D.W. 2009. Using marked *Lygus hesperus* (Knight) (Hemiptera: Miridae) adults to evaluate sweep net sampling in cotton. J. Cotton Sci. 13:196–205.

Stewart, S.D., G. Studebaker, G.M. Lorenz, F. Musser, A. Catchot, J. Greene, and R.D. Bagwell. 2006. Update on regional tarnished plant bug sampling in the Mid-South. p. 1591–1596. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 3–6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN.

Zink, A.G., and J.A. Rosenheim. 2004. State-dependent sampling bias in insects: implications for monitoring western tarnished plant bugs. Entomol. Exp. Appl. 113:117-123.