PRELIMINARY EVALUATION OF DROP CLOTH SAMPLING EFFICIENCY FOR LYGUS ADULTS IN COTTON

Dale W. Spurgeon
William R. Cooper
USDA, ARS, WICSRU
Shafter, CA

Abstract

The western tarnished plant bug, *Lygus hesperus* Knight, is a key pest of cotton in western production regions. Although the sweep net is the predominant sampling method used for lygus in California cotton, the drop cloth is used in other regions and may be more effective than the sweep net for sampling nymphs. Methodology for mark-release-recapture studies of nymphs has not been developed, but such methods for adult lygus are available. We evaluated the collection efficiency of the standard 1-m drop cloth against marked and released adults to gain insights into the optimal design of subsequent studies of nymphs. Adult lygus, marked to facilitate identification and prevent flight, were released into 1-m sample rows either 1) on the evening before drop cloth samples were collected (PM), or 2) about 1 h before sampling (AM). A completely randomized design was used with two release times and four replications. The experiment was conducted on three dates in Acala cotton, and on two dates in Pima cotton. In Acala cotton, captures of marked bugs were similar among combinations of sample dates and release times. In Pima cotton, captures of marked bugs were lowest on the second sample date and for AM releases. Recovery of marked bugs on the drop cloth was <80% regardless of cotton type. Additional searches indicated some bugs were not dislodged from the sampled plants, or were dislodged onto the surrounding plants or soil. These results illustrate the importance of allowing sufficient time between bug release and sampling, and suggest a need to account for bugs dislodged from the plants but not collected on the drop cloth.

Introduction

The plant bug complex (western tarnished plant bug in the West, tarnished plant bug in the mid-South) is the most important insect pest complex in U.S. cotton (Williams 2009). Lygus management in cotton typically involves the use of insecticides based on nominal thresholds. Improvements in lygus management will require better understanding of currently used sampling methods and of the dynamics of lygus-induced plant injury.

Recent efforts to improve the ability to interpret population estimates of adult lygus collected by the sweep net have involved a mark-release-recapture approach (Spurgeon 2009, Cooper and Spurgeon 2010). In this approach, adult lygus are marked to permit identification and prevent flight. This approach can provide the ability to sample populations of known density provided that released bugs remain in the assigned rows. Cooper and Spurgeon (2010) reasoned that dispersal of marked bugs or losses to predation could be minimized if bugs were released close to the time of sampling. However, their results suggested that releases too close to the time of sampling yielded inflated estimates of sampling efficiency because the released bugs did not adequately redistribute within the plant canopy. In the absence of direct estimates of the retention of released bugs, Spurgeon (2009) and Cooper and Spurgeon (2010) included 1-m "recovery rows" to estimate retention of released bugs. Herein we refer to these 1-m row sections as "retention rows" to avoid confusion during discussions of recovery of marked bugs from the sample rows. Bugs were released into these short row sections at the time of release into the sample rows. After sweepnet collections were made from the sample rows, released bugs were recovered from the 1-m retention rows by visual searches accompanied by plant dismemberment. However, processing of the retention rows is labor intensive, which limits the practical sample size. Methodology to more rapidly recover marked bugs from 1-m retention rows would enhance ongoing sampling studies.

The drop cloth is a sampling method commonly used in cotton production regions of the Mid-South. This method is considered superior to the sweep net for sampling lygus nymphs (Snodgrass 1993, Musser et al. 2007), and its use has been recommended for sampling nymphs and teneral adults in cotton (Willers et al. 1999). If the ground cloth collects all, or nearly all, of the marked adults from short sections of row, its use may negate the need to dissect the plants. In addition, we are interested in obtaining estimates of collection efficiency for lygus nymphs using the drop cloth, but adequate methods of marking nymphs have not been validated. Efforts to obtain corresponding estimates for marked, flightless adults may yield insights that will lead to improved study design when methods for marking nymphs become available. Therefore, our objective was to conduct a preliminary evaluation of the collection efficiency of the drop cloth for marked adult lygus in cotton.

Materials and Methods

The experiments were conducted in a field of Acala (Phytogen 72, Dow AgroSciences, Indianapolis, IN) and a field of Pima cotton (Phytogen 800), both planted to 40-inch rows on 6 May, 2010 at the Shafter Cotton Research Station, Shafter, CA. In each field, tiers of parallel 1-m sections of row were delineated and separated from other plants in the same rows by removing plants for about 1-m on each end of the sample row section. Each tier consisted of eight 1-m sample rows. Adjacent sample rows within a tier were separated by two buffer rows, resulting in a sampling area 22 rows in width. Experimental treatments (time of bug release, PM, release on the evening before sampling; AM, release about 1 h before sampling) were randomly assigned to 1-m sample rows within tiers. A separate tier was used on each sampling date (9, 16, and 23 July in Acala; 16 and 23 July in Pima).

Source and Marking of Insects

Adult *Lygus hesperus* for release as adults were reared from late-instar nymphs collected from alfalfa. Field-collected nymphs were maintained on green bean pods and raw sunflower seeds, whereas the resulting adults were maintained on green beans only. At least 24 h after adult eclosion, adult bugs were marked with fingernail polish. To facilitate marking, small aliquots of adults (5–10) were aspirated into plastic vials with screened lids, where they were lightly anesthetized with CO_2. The anesthetized bugs were decanted into the bottom of a 100 × 15-mm Petri plate lined with moistened filter paper. Then a small droplet of fingernail polish was placed on the dorsum near the posterior tip of the scutellum to cement the wings together and thereby prevent flight. In addition, males received a second (white) mark near the center of the original mark so that gender could be distinguished. Marked bugs with polish on their eyes, antennae, or legs were discarded. After marking, bugs were held in mixed-gender groups within an environmental chamber at either 26.6 or 28°C with a photoperiod of 14:10 (L:D) h.

Bug Releases and Sampling

Marked adult lygus (3 ♂♂, 3 ♀♀) ranging from 4 to 9 d old were released onto upper leaves of the designated 1-m sample rows either after 1900 h on the evening before sampling (PM treatment) or after 0800 h on the morning of sampling (AM treatment). Sampling was conducted between 0900 and 1000 h. The drop cloth used was 1 × 1 m, constructed of black fabric, and reinforced on two opposing sides by wooden dowels. During sampling, one of the dowels was placed as closely as possible to the bases of the plants to be sampled, and the plants were shaken vigorously (10-15 sec) over the cloth. It was not practical to shake the entire 1-m section of row at once. Therefore, ½ to ⅓ of the plants were shaken at one time, beginning with the plants closest to the sampler and taking care to minimize disturbance to the remaining plants. Marked adults found on the drop cloth were aspirated into a vial and their numbers were recorded. Immediately following the drop cloth sample, the canopy width, plant height, number of mainstem nodes (beginning with the hypocotyl as node zero), and fruiting phenology were recorded from three plants in each sample row section. After the plant measurements and when <100% of the marked bugs were recovered on the drop cloth, the plants were individually cut at the soil line, shaken over the drop cloth, and the bracteoles of any fruit large enough to conceal a marked bug were opened in an effort to locate remaining marked bugs. In addition, the soil surface and foliage of adjacent plants were visually examined for the presence of marked bugs. The numbers of marked adults recovered during plant measurement, or from cut plants, the soil surface, or adjacent plants, were recorded separately from those recovered on the drop cloth.

Statistical Analyses

Plant size and development was characterized in each cotton type on each sampling date by calculating the mean plant height, canopy width, and numbers of mainstem nodes. In addition, the ratio of plant height to canopy width was calculated from these means. The stage of fruiting development was characterized as a median based on the stage of the most developed square on each plant.

ANOVAs of the numbers of marked bugs recovered on the drop cloth *per se*, and of the total numbers of marked bugs recovered (drop cloth + soil, cut plant, and adjacent plants), were conducted separately for each cotton type using the SAS GLIMMIX procedure (SAS Institute 2002). Each analysis included terms for sample date, release time, and their interaction. The original counts were not transformed based on residual and quantile-quantile plots. When the sample date by release time interaction suggested the influence of release time may be dependent on sample date, simple effects of both sample date and release time were examined using the SLICE option of PROC GLIMMIX.

Results and Discussion

Early square set was poor in both cotton types because of poor growing conditions and a naturally-occurring lygus infestation. This poor fruit set explains the extended period over which fruiting phenology was restricted to the squaring stages (Table 1). In general, the Pima plants possessed larger leaves, longer petioles, and more lateral branches than the Acala plants. These differences in plant architecture provided the Pima plants with a broader canopy and a lower height: width ratio than the Acala plants (Table 1).

Table 1. Means of plant parameters (fruiting stages are medians) by sample date during an evaluation of efficiency of collection of marked and released adult *Lygus hesperus* by the drop cloth.

Cotton type	Sample date	Plant height (in.)	Canopy width (in.)	H/W ratio	Nodes	Fruiting stage[1]
Acala	9 July	15.5	14	1.1	11.7	matchhead
	16 July	21	20	1.0	14.2	third-grown
	23 July	27.5	25.5	1.1	15.6	third-grown
Pima	16 July	21	24.5	0.9	14.1	third-grown
	23 July	25	30	0.8	15.7	third-grown

[1]Matchhead, square diameter ≥3 mm but < 6 mm; third-grown, square diameter >6 mm.

Analyses of the numbers of marked adult lygus collected on the drop cloth *per se* from the Acala cotton did not indicate significant effects of sample date ($F = 0.30$; df = 2, 18; $P = 0.74$), release time ($F = 1.54$; df = 1, 18; $P = 0.23$), or their interaction ($F = 0.90$; df = 2, 18; $P = 0.42$; Table 2). Overall recovery of marked adults within sample dates ranged from 69% on 23 July to 75% on 16 July. Recovery of bugs released on the evening before sampling (4.6 ± 0.28; 76%) was nominally, but not statistically, higher than for bugs released one hour before sampling (4.1 ± 0.28; 68%).

Table 2. Mean numbers of marked and released *Lygus hesperus* adults collected per 1-m drop cloth sample and % recovery, Acala cotton.

Sample date	Release time[1]	Mean no. bugs collected/m (SE)	% recovery
9 July	PM	4.5 (0.49)	75
	AM	4.2 (0.49)	70
16 July	PM	4.5 (0.49)	75
	AM	4.5 (0.49)	75
23 July	PM	4.8 (0.49)	80
	AM	3.5 (0.49)	58

[1]PM, releases after 1900 h; AM, releases at 0800 h.

Analyses of the total number of marked adults collected on the drop cloth plus those subsequently recovered from the ground, severed plants, or adjacent foliage in Acala cotton, also did not indicate significant influences of sample date ($F = 0.14$; df = 2, 18; $P = 0.87$) or release time ($F = 0.05$; df = 1, 18; $P = 0.83$; Table 3). In addition, the date by release time interaction ($F = 0.33$; df = 2, 18; $P = 0.72$) was not significant, indicating interpretation of the main effects was straightforward. Overall, recovery of marked bugs ranged from 83 to 87% among dates, and from 85 to 86% between release times.

Analysis of bug collections from the Pima cotton indicated significant effects of both sample date ($F = 17.29$; df = 1, 12; $P < 0.01$) and release time ($F = 7.00$; df = 1, 12; $P = 0.02$). Although the date by release time interaction ($F = 3.57$; df = 1, 12; $P = 0.08$) was not significant at $\alpha = 0.05$, the P-value was low enough to warrant examination of simple effects. Comparisons of counts from the combinations of release time and sample date indicated the numbers of marked bugs collected on the drop cloth were similar between the two release times on the first sample date ($t = -0.53$, df = 12, $P = 0.60$), but the counts were different on the second date ($t = -3.21$, df = 12, $P < 0.01$; Table 4). Comparisons between sample dates by release time indicated collections of bugs released the evening before sampling (PM) were similar on the two sample dates ($t = 1.60$, df = 12, $P = 0.13$). However, collections of bugs

released an hour before sampling (AM) were higher on the first sample date than on the second date ($t = 4.28$, df = 12, $P < 0.01$ Table 4).

Table 3. Mean numbers of marked and released *Lygus hesperus* adults collected from 1-m sample rows of Acala cotton using the drop cloth and plant and soil surface inspections, and % recovery.

Sample date	Release time[1]	Mean no. bugs collected/m (SE)	% recovery
9 July	PM	5.2 (0.47)	87
	AM	5.2 (0.47)	87
16 July	PM	5 (0.47)	83
	AM	5.2 (0.47)	87
23 July	PM	5.2 (0.47)	87
	AM	4.8 (0.47)	80

[1]PM, releases after 1900 h; AM, releases at 0800 h.

Table 4. Mean numbers of marked and released *Lygus hesperus* adults collected per 1-m drop cloth sample and % recovery, Pima cotton.

Sample date	Release time[1]	Mean no. bugs collected/m (SE)	% recovery
16 July	PM	4.5 (0.33)	75
	AM	4.2 (0.33)	70
23 July	PM	3.8 (0.33)	63
	AM	2.2 (0.33)	37

[1]PM, releases after 1900 h; AM, releases at 0800 h.

In Pima cotton the sample date by release time interaction for bugs collected on the drop cloth plus those subsequently recovered from the ground, severed plants, or adjacent foliage ($F = 0.33$; df = 2, 18; $P = 0.72$) was not significant (Table 5). Examination of the model main effects indicated a larger number of marked bugs were recovered on the first sample date (4.8 ± 0.20, 79% recovery) than on the second date (3.6 ± 0.20, 60% recovery) ($F = 16.20$; df = 1, 12; $P < 0.01$). Also, the total number of marked adults that were recovered was higher when they were released on the evening before sampling (4.5 ± 0.2, 75% recovery) than when they were released an hour before sampling (3.9 ± 0.2, 65% recovery) ($F = 5.00$; df = 1, 12; $P < 0.05$).

Table 5. Mean numbers of marked and released *Lygus hesperus* adults collected from 1-m sample rows of Pima cotton using the drop cloth and plant and soil surface inspections, and % recovery.

Sample date	Release time[1]	Mean no. bugs collected/m (SE)	% recovery
16 July	PM	5.0 (0.28)	83
	AM	4.5 (0.28)	75
23 July	PM	4.0 (0.28)	67
	AM	3.2 (0.28)	53

[1]PM, releases after 1900 h; AM, releases at 0800 h.

Cooper and Spurgeon (2010) evaluated the influence of release time on capture of marked lygus adults by the sweep net and found generally higher captures associated with releases close to the time of sampling. These higher captures were attributed to insufficient time for the released bugs to redistribute within the plant canopy before sampling occurred. Where differences were observed in our preliminary study, they were the opposite of those observed by Cooper and Spurgeon (2010). A key to explaining this discrepancy is our observation that many of the marked adults, ultimately recovered but not found on the drop cloth, were recovered from the ground or plants in the adjacent row. We surmise that this happened because the recently released bugs had not become established on the plants and were therefore too easily dislodged. This tendency became more pronounced in the Pima cotton as the

plants became larger and more developed. In addition, increased plant size and development in the Pima cotton was associated with decreased recovery of marked and released bugs. Absence of a similar trend in the Acala cotton can probably be attributed to its narrower canopy and reduced branching, compared with the Pima cotton. These observations may warrant measures to account for insects dislodged from the plants but not captured on the drop cloth in later studies of sampling efficiency for lygus nymphs.

Snodgrass (1993) reported the drop cloth collected lygus nymphs from cotton with an average efficiency of 46%, although he suggested that some of the nymphs released onto plants may have been lost to predation before sampling occurred. In addition, Snodgrass (1993) estimated, using linear regressions, a capture efficiency for nymphs of 51% from plants smaller than 31 inches in height. For larger plants the collection efficiency was reduced. In comparison, our estimate of collection efficiency for adult lygus in Acala cotton, and for the first sample date in Pima cotton, was about 75%. However, the decrease in drop cloth collection efficiency that we observed with increasing plant size and development in the Pima cotton occurred at a lower plant height than was observed by Snodgrass (1993) in Upland cotton. Wilson et al. (1984), Snodgrass (1998), and Rosenheim et al. (2004) reported different within-plant distributions or plant part associations for lygus nymphs and adults. Therefore, the differences between our results and those of Snodgrass (1993) could be attributed to these behavioral differences. Alternatively, or in addition, these differences may have been caused by differences in plant growth, development, and canopy architecture between studies. Regardless, even after inspection of the ground and adjacent plants, the collection efficiency we observed in this preliminary study was not high enough to justify substituting the drop cloth for detailed plant inspections in sampling studies using marked and released adult lygus.

Summary

We conducted a preliminary evaluation of the effectiveness of the standard 1-m drop cloth for recovering marked and released lygus adults from cotton. Over the narrow range of plant sizes and phenological stages that we examined, recovery of marked and released lygus adults was about 75% in Acala cotton. In Pima cotton, recovery of marked adults was reduced with increased plant size and development, and was lower for adults released close to the time of sampling compared with those released during the evening before samples were collected the next morning. The different responses in Acala and Pima cotton are attributed to differences in canopy widths and canopy architecture. Our results also indicate that adults released onto cotton plants may be too easily dislodged from the plants when the time of release is too close to the time of sampling. Collectively, our results suggest the drop cloth is not a suitable substitute for intensive whole plant inspections where one requires accurate counts of the numbers of marked adult lygus resident on plants. Furthermore, in future studies of collection efficiency of the drop cloth for nymphs, some effort should be devoted to account for insects that are dislodged from the sampled plants but not ultimately collected on the drop cloth.

Acknowledgements

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References

Cooper, W.R., and D.W. Spurgeon. 2010. Sweep net recapture of marked *Lygus hesperus* Knight (Hemiptera: Miridae) adults after different release times in cotton. J. Cotton Sci. 14: 131-137.

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.

Rosenheim, J.A., R.E. Goeriz, and E.F. Thacher. 2004. Omnivore or herbivore? Field observations of foraging by *Lygus hesperus* (Hemiptera: Miridae). Environ. Entomol. 33: 1362-1370.

SAS Institute. 2002. The SAS System for Windows, ver. 9.2. SAS Institute, Cary, NC.

Snodgrass, G.L. 1993. Estimating absolute density of nymphs of *Lygus lineolaris* (Heteroptera: Miridae) in cotton using drop cloth and sweep-net sampling methods. J. Econ. Entomol. 86: 1116-1123.

Snodgrass, G.L. 1998. Distribution of the tarnished plant bug (Heteroptera: Miridae) within cotton plants. Environ. Entomol. 27: 1089-1093.

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.

Willers, J.L., M.R. Seal, and R.G. Luttrell. 1999. Remote sensing, line-intercept sampling for tarnished plant bugs (Heteroptera: Miridae) in mid-south cotton. J. Cotton Sci. 3: 160-170.

Williams, M.R., 2009. Cotton insect losses 2008. p. 897-940. *In* Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

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.