MARKED INSECTS RELEASED IN RECAPTURE STUDIES MAY INFLUENCE POPULATION ESTIMATES OF NATIVE INSECTS

Dale W. Spurgeon
USDA, ARS, ALARC
Maricopa, AZ
William Rodney Cooper
USDA, ARS, YARL
Wapato, WA

Abstract

Recent studies of sweepnet collection efficiency for the western tarnished plant bug, *Lygus hesperus* Knight, in cotton have capitalized on the utility of mark-release-recapture methods. During such sampling studies, native lygus are commonly captured along with the insects that were marked and released. In some situations, it would be useful to draw inferences regarding the populations of native insects so long as their behavior is not influenced by the released insects. During a recent study of the influence of *L. hesperus* gender and age class on sweepnet sampling efficiency, we noted a bias in the distributions of captured native lygus adults. Exploratory analyses indicated that, in both Acala and Pima cotton, captures of native adult male lygus were significantly higher where reproductively mature females were released, compared with areas receiving pre-reproductive females, or males of either physiological age class. We presume the native males were attracted to pheromone released by the marked reproductive females, or to some other olfactory attractant associated with those females. These support earlier contentions of a volatile, female released sex attractant in *L. hesperus*, and suggest caution should be exercised in interpreting captures of native insects collected in conjunction with mark-release-recapture studies.

Introduction

Recent efforts to identify factors influencing collection efficiency of the standard sweepnet for adults of the western tarnished plant bug, *Lygus hesperus* Knight, in cotton have utilized mark-release-recapture methods (Spurgeon 2009; Cooper and Spurgeon 2010; Spurgeon and Cooper 2011). During publication of these works, reviewers and journal editors have consistently requested information regarding coincidental captures of native lygus. Although those data and corresponding analyses have been provided, we have been reticent to draw more than tentative conclusions about the native populations because the tested hypotheses were unplanned. In addition, use of the mark-release-recapture method for estimation of native insect population levels requires that several assumptions are satisfied, including 1) released insects mix thoroughly with the native population, and 2) behavior of the marked insects and their susceptibility to capture is the same as for the native insects (Southwood 1978). We have no firm evidence that these assumptions have been met in our studies.

During studies of the influence of adult lygus gender and physiological age class on population estimates obtained by the sweepnet we observed patterns in collections of native lygus. Those patterns appeared to correspond to the class (age, gender) of the marked adults that we released. Herein we report a retrospective examination of the influence of marked and released lygus adults on population estimates of native lygus in Acala and Pima cotton.

Materials and Methods

During the summer of 2011, separate studies were conducted in fields of Pima (PHY805RF, Dow AgroSciences, Indianapolis, IN) and Acala cotton (PHY725RF) on the Shafter Cotton Research Station, Shafter, CA. Both fields were planted to 40-inch rows on raised beds on 26 April. Furrow irrigations were applied as needed, and no insecticides were used during the study period (from pinhead square stage to second (Acala) or third week of bloom (Pima); 14 June to 27 July).

The experimental design was a randomized complete block with 3 replications on each sample date. Experimental blocks were each composed of 20 adjacent rows, each row 33 ft in length and separated from other plants in the same row by an alley 6 ft wide. Because a given row was used only once, more than 3 blocks were established in each field to ensure the number of sample dates was not limited by availability of previously unsampled rows. Experimental treatments (marked and released adult lygus) included the 4 combinations of gender and physiological age class (pre-reproductive, reproductive). Pre-reproductive males lacked seminal vesicles filled with sperm whereas the seminal vesicles of reproductive males were filled or distended with sperm. Likewise, pre-reproductive females lacked oocytes with yolk. Reproductive females contained oocytes with yolk, and most contained eggs. Each study was sampled on 6 dates.

Adult lygus ≤1 day old were obtained from a laboratory colony reared on green beans and raw sunflower seeds. The colony originated from insects collected in local alfalfa fields, and had been under continuous culture for ≤6 generations. All adults were initially held in mixed-sex groups of <300 individuals within 1-gal. plastic rearing buckets containing shredded paper and green beans. Insects assigned to the reproductive treatment were held at about 80°F with a photoperiod of 14:10 (L:D) h until their release 7–10 days later. Adults assigned to the pre-reproductive treatment were held at temperatures ranging from 55 to 75°F with a 14-h photophase. The specific temperature regime for a given cohort was determined by the number of days required to accumulate insects for release and our knowledge of the influence of temperature on adult reproductive development (Spurgeon and Cooper 2012). On the morning before field releases the pre-reproductive insects were moved to an environmental chamber maintained at a temperature of 80°F. One to 3 days before release, adults were marked on the dorsum with fingernail polish so as to prevent flight, as described by Spurgeon (2009) and Cooper and Spurgeon (2010). At the time of marking, insects assigned to the pre-reproductive treatment were separated by gender. This separation was maintained thereafter. A different color of polish was used for each release, and males assigned to either age class were further distinguished by a small dot of white polish near the center of the larger mark.

Late on the afternoon of the day of release, the insects were aspirated into plastic vials for transport to the field. Beginning after 1900 h (PDT), marked adults, segregated by treatment (gender and age class), were released into their assigned row sections. A total of 50 marked adults was released onto upper leaves of plants in each row, taking care to disperse the released insects as evenly as possible down the row. Between 0900–1000 h on the morning following releases, each row was sampled with 10 pendulum sweeps using a standard 15-inch diameter sweepnet. Collected insects transferred to sealed plastic bags were transported to the laboratory where they were separated into four groups (marked and unmarked males and females) and counted. Marked insects were dissected to verify assigned age classes (data reported elsewhere).

In each experiment, counts of native (unmarked) adult lygus were compared among treatments and sample dates using mixed-model ANOVA (PROC GLIMMIX; SAS Institute 2008). Counts of native males and females were analyzed separately. In each analysis fixed effects were treatment (combination of gender and age class of released insects), sample date, and their interaction. Block was included as a random effect. Mean captures of native adults were compared among levels of each main effect using the SIMULATE option of the LSMEANS statement unless the treatment by block interaction was significant. When this interaction was significant, simple effects of treatment within sample date, and of sample date within each treatment were examined using the SLICE option of the LSMEANS statement.

Results and Discussion

Response of Native Females

Numbers of native adult female *L. hesperus* that were captured from Acala cotton by the sweepnet were relatively consistent among sample dates and experimental treatments (rows containing released adults of specified gender and age class; Figure 1). Neither of the ANOVA main effects (sample date, $F = 1.30$; df = 5, 46.4; $P = 0.28$; treatment, $F = 1.69$; df = 3, 45.4; $P = 0.18$), nor their interaction ($F = 0.84$; df = 15, 45.4; $P = 0.63$), indicated that the released lygus adults influenced female adults of the native population.

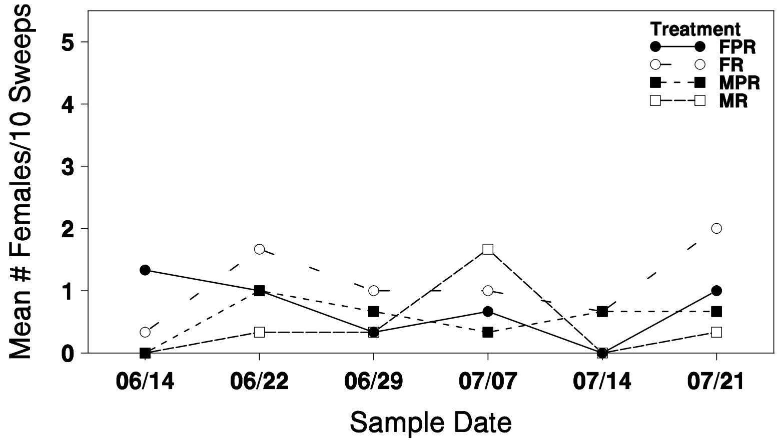

Figure 1. Mean numbers of native adult female *L. hesperus* captured by sweepnet from rows of Acala cotton in which marked adults of specified gender and physiological age class were previously released (gender and age class combinations were: FPR, pre-reproductive females; FR, reproductive females; MPR, pre-reproductive males; MR, reproductive males).

Analyses of sweepnet captures in Pima cotton failed to indicate an influence of released adults on numbers of native females ($F = 1.53$; df = 3, 45.7; $P = 0.22$). In contrast to the results in Acala cotton, the main effect of sample date was significant ($F = 4.75$; df = 5, 44.5; $P < 0.01$) despite generally lower numbers of native lygus in the Pima cotton (Figure 2). However, interpretation of both main effect tests was conditional on levels of the other effect because of a significant date by treatment interaction ($F = 2.90$; df = 15, 45.7; $P < 0.01$). Examination of simple effects of treatment within each sample date indicated more native females were captured in the rows containing released reproductive females compared with other treatments on 23 June ($P < 0.01$, Figure 2). Significant differences among the experimental treatments were not observed on other dates. Examination of the effect of sample date within treatments indicated no significant differences except for rows containing released reproductive females. Captures of native adult female lygus within rows containing released reproductive females were higher on 23 June than on other sample dates ($P < 0.01$, Figure 2).

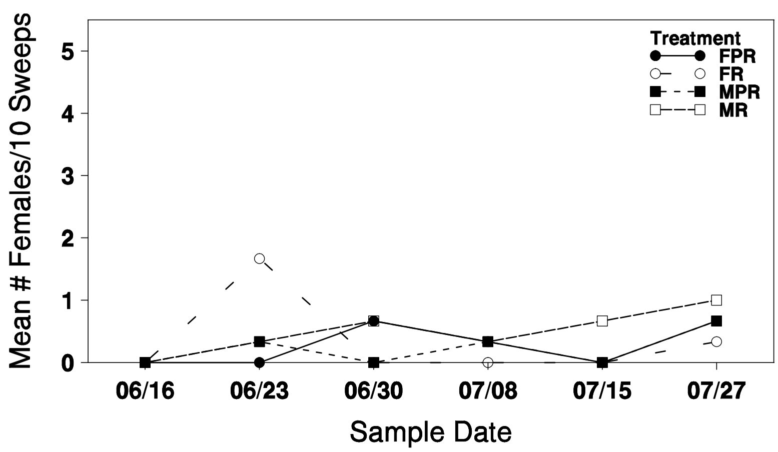

Figure 2. Mean numbers of native adult female *L. hesperus* captured by sweepnet from rows of Pima cotton in which marked adults of specified gender and physiological age class were previously released (gender and age class combinations were: FPR, pre-reproductive females; FR, reproductive females; MPR, pre-reproductive males; MR, reproductive males).

Numbers of captured native females estimated in the two studies (Acala and Pima) differed among the experimental treatments on only one of the combined 12 sample dates. Therefore, it seems likely the single difference observed was attributed to sampling error in estimating the numbers of native females. Overall, the evidence from our experiments is insufficient to conclude that population levels of native female adult lygus were influenced by the adults that we marked and released.

Response of Native Males

In contrast to results corresponding to native females, sweepnet captures of native male lygus in the Acala cotton clearly indicated an effect of the marked and released insects. Highest numbers of native male lygus were consistently captured in rows containing released reproductive females ($F = 10.65$; df = 3, 43.2; $P < 0.01$; Figure 3). No differences in captures of native males were indicated among the remaining treatments of released lygus representing combinations of adult gender and age class. Captures of native male lygus also varied among sample dates, as numbers of native males captured on 22 June were higher than those on 14 or 29 June, or 14 July ($F = 5.77$; df = 5, 22.4; $P < 0.01$, Figure 3). Absence of a significant interaction between sample date and experimental treatment ($F = 0.73$; df = 15, 43.2; $P = 0.74$) indicated the influence of marked and released reproductive females on the population of native adult males was consistent among sample dates.

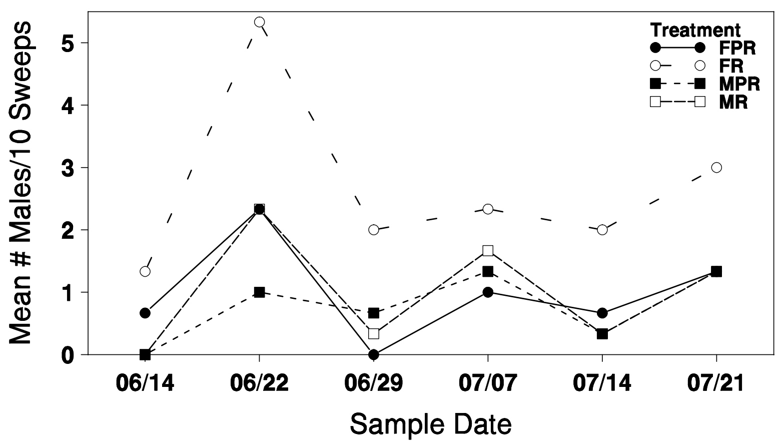

Figure 3. Mean numbers of native adult male *L. hesperus* captured by sweepnet from rows of Acala cotton in which marked adults of specified gender and physiological age class were previously released (gender and age class combinations were: FPR, pre-reproductive females; FR, reproductive females; MPR, pre-reproductive males; MR, reproductive males).

Captures of native male lygus in the Pima experiment also exhibited differences corresponding to the gender and age class of released insects ($F = 29.42$; df = 3, 44.8; $P < 0.01$), but not corresponding to sample date ($F = 2.17$; df = 5, 33.1; $P = 0.08$). However, the interaction between treatment and sample date was also significant ($F = 2.65$; df = 15, 44.8; $P < 0.01$), indicating the influence of the released insects varied among sample dates. Further examination of the interaction indicated captures of native males were higher from the rows with released reproductive females compared with other treatments on all sample dates except 16 and 23 June, when no differences among the treatments were observed (Figure 4). Likewise, differences among sample dates in captures of native males were observed only for the experimental treatment represented by released reproductive females. In the rows with released reproductive females, captures of native male adults were higher on 27 July than other dates except 30 June, and captures of males on 30 June were higher than on other dates except for 8 July (Figure 4). Fewer native males were captured on 16 and 23 June than on any other sample date.

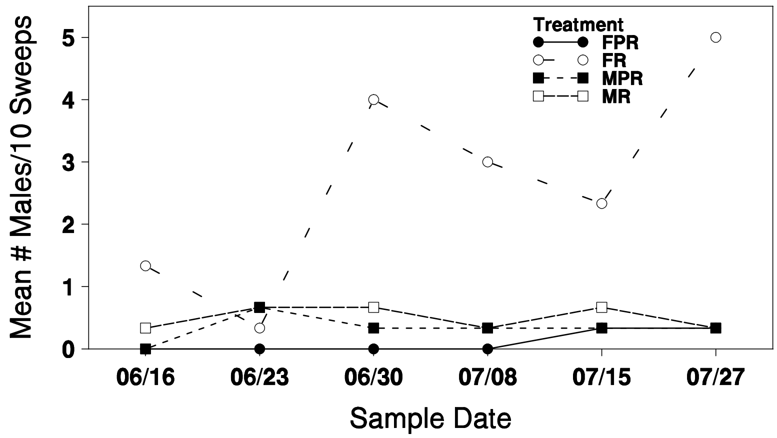

Figure 4. Mean numbers of native adult male *L. hesperus* captured by sweepnet from rows of Pima cotton in which marked adults of specified gender and physiological age class were previously released (gender and age class combinations were: FPR, pre-reproductive females; FR, reproductive females; MPR, pre-reproductive males; MR, reproductive males).

In contrast to the results for native female adult lygus, captures of native males reflected a pronounced influence of marked and released reproductive adult females. This influence was statistically demonstrated on 10 of the 12 combined sample dates. Failure to demonstrate this effect on the first two sample dates in the Pima experiment was likely a result of the generally low resident population levels of native lygus on those dates.

Summary

Results of our studies clearly indicate an influence of marked and released adults of *L. hesperus* on the native lygus population. Strong et al. (1970) reported evidence of a female-released sex pheromone in *L. hesperus*. Our findings that higher numbers of native male lygus were associated with rows containing released reproductive female lygus are consistent with this earlier report. Our results, however, do not indicate the spatial scale over which this attractiveness may operate. Regardless, inferences regarding the native insects in this study, without accounting for the attractiveness of the reproductive females, would have been based on biased population estimates and sex ratios. It may be appropriate to extend the implications of our findings to other field studies (e.g., yield response, host preference, or dispersal) featuring population augmentation by released insects. In those cases, estimates indicating male-biased sex ratios in the native population may provide evidence of unwanted recruitment of native male lygus adults into the experiments. Regardless of the mechanism responsible for our results, it is apparent that conclusions regarding native insect populations estimated concurrent with studies involving released insects should be drawn with considerable caution unless there is evidence of no effect of the released insects.

Acknowledgements

The research reported herein was supported in part by the California State Support Committee, Cotton Incorporated Project No. 11-856CA, Biotic Factors Influencing Sweep Net Efficiency for Western Tarnished Plant Bug Adults. Mention of trade names or commercial products in this article 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.

SAS Institute. 2008. SAS release 9.2 ed. SAS Institute, Cary, NC.

Southwood, T.R.E. 1978. Ecological Methods with Particular Reference to the Study of Insects. 2nd ed. Chapman and Hall, New York, NY.

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

Spurgeon, D. W. and W. R. Cooper. 2011. Among-sampler variation in sweep net samples of adult *Lygus hesperus* (Hemiptera: Miridae) in cotton. J. Econ. Entomol. 104: 685-692.

Spurgeon, D. W. and W. R. Cooper. 2012. Temperature-dependent reproductive development of *Lygus hesperus* (Hemiptera: Miridae). Environ. Entomol. 41: 941-949.

Strong, F. E., J. A. Sheldahl, P. R. Hughes and E.M.K. Hussein. 1970. Reproductive biology of Lygus hesperus Knight. Hilgardia 40: 105-147.