State-dependent sampling bias in insects: implications for monitoring western tarnished plant bugs

Andrew G. Zink* & Jay A. Rosenheim
Department of Entomology, University of California Davis, Davis, CA 95616, USA
Accepted: 5 August 2004

Key words: Lygus hesperus, Hemiptera, Miridae, stage structure, sex ratio, sampling bias, cotton

Abstract
Insect populations vary in the proportion of individuals exhibiting a particular ‘state’ (e.g., developmental stage, sex, egg load, or nutritional status). Because an insect’s developmental state often determines both its size and its behavior, it is likely that this will also affect the probability of being sampled. We propose that a comprehensive approach to pest management must consider the interaction between the structure of a pest population (i.e., the relative number of individuals in each state) and any state-dependent sampling bias. To illustrate the usefulness of this method we sampled populations of the western tarnished pest bug, Lygus hesperus Knight (Heteroptera: Miridae), in cotton fields. Our sampling technique utilized large cages to measure the absolute densities of each L. hesperus stage and adult sex within a population. This technique allowed us to document a wide range of absolute stage structures and sex ratios across 10 L. hesperus populations in California. Using a combination of cage samples and sweep net samples, we quantified the state-dependent sampling bias by calculating the efficiency of sweep sampling as a function of L. hesperus developmental stage and sex. We found that the efficiency of sweep nets increased steadily with each successive developmental stage (i.e., nymphal instar) of L. hesperus. We also found that sweep nets are slightly more efficient in capturing male vs. female L. hesperus adults. Since other studies have documented that the stage and sex of L. hesperus can affect feeding impact on cotton flower buds, our results suggest that accurate predictions of Lygus damage will need to incorporate stage and sex-dependent sampling biases.

Introduction
Populations of insect pests vary in the proportion of individuals falling into different states, where ‘state’ is either a fixed variable (e.g., genotype, sex, or species) or a dynamic variable (e.g., developmental stage, age, or egg load) affecting an individual’s phenotype. Because an insect’s state can affect its size and foraging behavior, it is important to recognize that each insect state is susceptible to a uniquely inherent sampling bias. For example, sweep sampling can overestimate or underestimate the component species in an insect community relative to absolute counts of species numbers (Larson et al., 1999). In some pest species, smaller developmental stages are more cryptic than adults, making nymphs more difficult to collect with sweep nets (Byerly et al., 1978; Fleischer et al., 1985). In contrast, the adults of other pest species are more cryptic than nymphs, making the adults more difficult to collect in sweep nets (Browdie et al., 1992). In this paper we focus on relative sampling bias across the developmental stages and sex of the western tarnished pest bug, Lygus hesperus Knight (Hemiptera: Miridae) in cotton fields of California, USA.

Lygus has been known as a key pest of cotton since early in the 20th century, and although Lygus bugs are present at relatively low densities in cotton, they have been thought to cause large reductions in yields (Leigh et al., 1988; Ellsworth, 2000). Lygus adults and nymphs feed on developing cotton flower buds (‘squares’), injecting pectin-digesting salivary enzymes that cause cotton plants to actively abscise squares (Strong & Kruitwagen, 1968; Strong, 1970; Addicott, 1982). Abscission from feeding insects is economically important, primarily during the early part of the cotton plant’s reproductive period, with later buds and fruit being shed mainly due to physical stresses (Stewart & Sterling, 1988, 1989). In California, where L. hesperus comprises the vast majority of Lygus individuals, growers have repeatedly stressed the difficulties in connecting Lygus densities in a field with damage to...
cotton squares. This disconnection is reflected in scientific studies that have found a large negative impact of *L. hesperus* on yields (e.g., Falcon et al., 1971; Leigh et al., 1988) as well as little effect of *L. hesperus* on yields (Falcon et al., 1968, 1971; Gutierrez et al., 1977).

Past experiments suggest that *L. hesperus* nymphs inflict damage to squares that is equal to (and possibly greater than) that of adults (Gutierrez et al., 1977; Ellsworth, 2000). In addition, when male and female adults are constrained to feed on a cotton square, females cause greater damage and square shed relative to males (Gutierrez et al., 1977). Field-based behavioral observations have also suggested that nymphs and adult females spend more time on squares than do adult males (Rosenheim et al. in press). The sex ratio of *Lygus* populations may therefore influence the overall amount of crop damage. Sex ratio is also expected to have a direct influence on the rate of egg deposition in cotton fields. *Lygus hesperus* females have the potential to lay up to 400 eggs over their lifetimes. Because *Lygus* eggs hatch after 1 week, female densities within a field should correlate with the short-term recruitment of young nymphs (Leigh, 1963; Strong et al., 1970; Butler & Wardecker, 1971). Therefore, in addition to measuring the overall density of *L. hesperus* in a field, an accurate knowledge of both the stage structure and the sex ratio of the population appears to be necessary in order to be able project damage to cotton squares.

In order to accurately assess the structure of *L. hesperus* populations, however, it is first necessary to account for the variation among insect states in their probability of being sampled. For example, it is generally thought that *L. hesperus* nymphs are very hard to sample using the traditional sweep-net sampling technique, making them ‘invisible’ from a management perspective (Byerly et al., 1978). This has led to a general method of multiplying sweep net sampled *Lygus* nymphs by two (Sevacherian & Stern, 1972). In addition, because the male adults spend more time on the more exposed areas of the plant, it is possible that sampling techniques are also sex-biased (Rosenheim et al. in press). Although it is clear that sweep nets miss a large number of *L. hesperus* individuals in a row of plants, it has been difficult to quantify both the overall amount of bias and the relative degree of bias for each nymphal instar or adult sex (Race, 1960; Ellington et al., 1984). Ideally, these comparisons must be expressed using a common basis, such as individuals per meter of row, as was done for a comparison between visual and sweep net sampling of *L. lineolaris* (Flescher et al., 1985).

**Materials and methods**

During the 2002 growing season (late June through August) we sampled nine upland cotton fields, *Gossypium hirsutum* L. (primarily cultivars Maxxa and Phytogen 72), and one Pima field, *Gossypium barbadense* L., across the San Joaquin Valley of California, USA from Firebaugh (latitude 36.8°N; longitude 120.2°W) to Bakersfield (latitude 35.5°N; longitude 119.0°W). Fields were only sampled for 1 day, and the sampling date was designated as early (28–30 June), middle (16–19 July), or late (5–7 August), corresponding to a mean plant stage of 15.1 (0.7) nodes, 19.0 (0.5) nodes, or 19.6 (0.5) nodes, respectively. We restricted our sampling to fields that were indicated by preliminary sampling to have at least one *Lygus* adult per 50 sweeps. In addition, we intentionally chose fields that had not been sprayed with insecticides over the previous 3 weeks. To sample the absolute numbers of *Lygus* adults, we constructed a 4 × 2 × 2 m steel cage surrounded by insect netting. We carefully carried this cage into the field to be sampled, holding it well above the canopy of the cotton plants, and then rapidly dropped it over two rows of plants (enclosing a total of 8 m of row) to capture all adults present. We chose to use large cages as a sampling device because our experiences with smaller cages suggested that they invariably flushed the adult *Lygus* off the plants being sampled. In contrast, our large cages appeared to produce a minimal disruption to the adults across the entire area sampled. After the cage had been dropped, all of the *Lygus* that were trapped in the cage eventually flew onto the insect netting (plants were gently shaken before the cage was removed to flush out any final adults). Adults were then collected using an aspirator, allowing us to quantify the total number of adults per cage. Adults were sexed in the laboratory, and the species of the males were identified (*L. hesperus* vs. *L. elisus*) using the key of Mueller et al. (2003).

In each sampling cage (three per field) we randomly removed 20 whole cotton plants on which we conducted whole-plant searches for *Lygus* nymphs. Nymphs were collected with aspirators and taken back to the lab where their developmental stage was identified (1st through 5th instar). By counting the total number of plants in each cage (across the 8 m of row) we were able to convert the nymph counts into an absolute number of nymphs per meter of row. Finally, in each field we collected 10 samples of 50 sweeps each. These samples were spatially interspersed with the locations of the three cage samples. The *Lygus* nymphs from these sweep samples were collected and taken to the laboratory for identification to instar. Adults were scored for sex and adult males were identified by species. Comparisons of stage structure (percentage of all *Lygus* that were nymphs) and sex ratio (percentage of *Lygus* adults that were female) were made for the three cages across all 10 fields sampled. We used an ANCOVA to examine differences among fields in the percentage of...
nymphs and females per cage, with field nested within time of season (early, middle or late) as a covariate. Linear regressions were used across all fields, to estimate the slope (and standard error) of the relationship between absolute densities of Lygus and sweep netted Lygus for each stage and sex.

**Results**

The results of species identifications from cage samples revealed that eight of 10 fields were comprised entirely of *L. hesperus*. Six of 16 males in the McCombs field and one of five males in the Poplar field were identified as *Lygus elisus*. Across all 10 fields, 97% of the 253 collected males were identified as *L. hesperus*. We found a broad range of absolute stage structures across the Lygus populations we sampled. Nymphs comprised from 32% to 90% of the *Lygus* population across all fields sampled (Figure 1). There were significant differences among sites in the proportion of individuals found to be nymphs using a model that nested field within time of season (ANCOVA; $F_{7,30} = 5.3, P = 0.002$). The same model revealed that season was a marginally significant factor affecting stage structure (ANCOVA; $F_{2,30} = 3.4, P = 0.054$). Fields late in the season (first week of August) showed a trend toward more nymphs, but fields in which nymphs far outnumbered adults were found during each of the time periods sampled (Figure 1). In addition, and somewhat remarkably, there was no significant correlation between the number of adults in a field per meter and the number of nymphs per meter in that field ($r = 0.311, P = 0.381, n = 10$). Taken together, these results suggest that adult density alone does not accurately reflect the overall *L. hesperus* population of a particular field.

When we examined a particular developmental instar of *L. hesperus*, we also found significant differences among sites in the number of individuals found per cage. This was true for 1st instars (ANCOVA; $F_{7,30} = 7.4, P = 0.0002$), 2nd instars (ANCOVA; $F_{7,30} = 5.1, P = 0.002$), 4th instars (ANCOVA; $F_{7,30} = 2.8, P = 0.03$), and 5th instars (ANCOVA; $F_{7,30} = 3.7, P = 0.01$) but not 3rd instars (ANCOVA; $F_{7,30} = 1.8, P = 0.15$). In all five cases, the time of season was not a significant factor. These differences in the densities of a particular nymphal instar are also reflected in a comparison of the four fields with the greatest number of *Lygus* nymphs (Figure 2). This suggests that the absolute (and relative) densities of a particular instar
such as the larger, more visible 4th and 5th instars) do not necessarily predict overall absolute (and relative) nymph density.

Sweep net samples taken from the 10 fields were positively correlated with absolute numbers of *L. hesperus* for each developmental stage (1st through 5th instar and adults). These relationships were always linear, with slopes generally significant and intercepts not different from zero (Table 1). Our results revealed that for each adult collected in a 50 sweep sample there is approximately one adult foraging every 3 meters of row. Sweeps were less successful at capturing nymphs, relative to adults, but were progressively more successful with more advanced instars (Figure 3). For example, first instars were collected at less than 1/10th the rate of adults, whereas fourth and fifth instars were more nearly equivalent to adults. Therefore, the magnitude of bias against collecting nymphs in sweep nets was directly dependent on the stage structure of the nymph population.

The sex ratio of the adults collected in the cages was also highly variable across fields (Figure 4). However, there were no differences among fields in the proportion of adults that were females using a nested design within time of season (ANCOVA; *F*<sub>7,30</sub> = 1.8, *P* = 0.141). However there were significant differences in the proportion of adults that were female when focusing on seasonal changes, with fewer females in the early and late season (ANCOVA; *F*<sub>2,30</sub> = 5.0, *P* = 0.017). Sweep net samples were significantly positively correlated with absolute numbers of male and female adult *L. hesperus*. For both sexes, the relationship was linear, with slopes significant and intercepts not different from zero (Table 1). When comparing the proportion of female (vs. male) adults collected in sweep nets against those collected in cages, one population (Poplar) was excluded as only four adults (all of them males) were collected across all three cages. The remaining nine fields, with one exception, showed a consistent trend toward male bias in sweep nets. Using a paired t-test, there was a marginally significant difference between the proportion of females collected in sweep nets vs. in the large cage samples (paired-*t* = 2.1, *P* = 0.07, *n* = 9).

### Table 1

Linear regressions, across fields, relating the number of *Lygus hesperus* of a particular stage caught in sweep nets (independent variable; individuals per 50 sweeps) to the number of *L. hesperus* of the same stage found in a sample taken within large field cages (dependent variable; individuals per meter of row). The slope (*b*) is an appropriate multiplier for converting state-dependent sweep samples to individuals per meter (*Si*).

<table>
<thead>
<tr>
<th><em>Lygus</em> stage/sex</th>
<th>R-square</th>
<th>Slope (b) estimate</th>
<th>SE of slope</th>
<th>P-value slope</th>
<th>P-value intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st instar</td>
<td>0.332</td>
<td>1.738</td>
<td>0.872</td>
<td>0.0813</td>
<td>0.576</td>
</tr>
<tr>
<td>2nd instar</td>
<td>0.548</td>
<td>0.702</td>
<td>0.225</td>
<td>0.0144</td>
<td>0.208</td>
</tr>
<tr>
<td>3rd instar</td>
<td>0.554</td>
<td>0.501</td>
<td>0.159</td>
<td>0.0136</td>
<td>0.206</td>
</tr>
<tr>
<td>4th instar</td>
<td>0.450</td>
<td>0.390</td>
<td>0.152</td>
<td>0.0337</td>
<td>0.388</td>
</tr>
<tr>
<td>5th instar</td>
<td>0.542</td>
<td>0.260</td>
<td>0.084</td>
<td>0.0152</td>
<td>0.541</td>
</tr>
<tr>
<td>Adult (M, F)</td>
<td>0.974</td>
<td>0.345</td>
<td>0.020</td>
<td>&lt; 0.0001</td>
<td>0.568</td>
</tr>
<tr>
<td>Males</td>
<td>0.966</td>
<td>0.325</td>
<td>0.022</td>
<td>&lt; 0.0001</td>
<td>0.760</td>
</tr>
<tr>
<td>Females</td>
<td>0.951</td>
<td>0.490</td>
<td>0.039</td>
<td>&lt; 0.0001</td>
<td>0.343</td>
</tr>
</tbody>
</table>
Discussion

The results of this study suggest that a state-dependent approach to pest management is very appropriate for the western tarnished plant bug, *L. hesperus*. Independent of relative sampling bias, our absolute sampling revealed that stage structure and sex ratio are highly variable across *Lygus* populations. Other work suggests that these field-level differences in *Lygus* nymph-to-adult ratios can persist over an entire growing season (A.G. Zink and J.A. Rosenheim, unpubl.). These differences appear to result from the suppression of nymphs, but not adults, by generalist predators, whose densities vary significantly across fields. If *Lygus* populations do indeed vary in the relative numbers of individuals of different states, then state-dependent sampling bias can misrepresent overall pest densities. For example, our results reveal that the number of *Lygus* adults collected in sweep samples is not necessarily a reliable measure of overall *Lygus* presence. Absolute samples revealed that some cotton fields contain many nymphs and few adults; in such fields, sweep net sampling may produce a significant underestimate of overall *Lygus* densities, because sweep nets undersample nymphs. Conversely, other fields contain many adults and few nymphs; in such fields, sweep net sampling may produce an overestimate of overall *Lygus* densities. The same is true for fields that vary in sex ratio. Undersampling of females will underestimate the presence of *Lygus* when the sex ratio is female biased, and overestimate it when the sex ratio is male biased.

In light of the results of our study, any true measure of the overall densities of *Lygus* in a cotton field will need to multiply each nymphal instar and adult sex by a factor that is proportional to its relative sampling bias. For example, our data suggest that the general rule of multiplying nymphs by a factor of two (to equalize with adults) is only appropriate for 2nd or 3rd instars, and that this rule will underestimate nymphs if a population primarily consists of 1st instars (or overestimate densities if the population is made up of many 4th and 5th instars). Behavioral data suggest that these sampling biases in *Lygus* are largely due to the feeding location of the different stages and sexes. Adult male *Lygus* spend more time than females on exposed vegetative structures such as leaf surfaces (Rosenheim et al., 2004). This finding is likely to explain our finding that adult males are 50% more likely to be captured in sweep nets relative to adult females. The sweep net bias that we observed against *L. hesperus* nymphs is also consistent with previous findings that adults tend to feed higher on the plant and on more exposed surfaces of it (such as leaves and bolls; Wilson et al., 1984). Similar observations have been made in *L. lineolaris*, where adults were more common on vegetative structures and nymphs more common on fruiting structures (Snodgrass, 1998).

To adjust for biases in sweep net sampling, the appropriate multiplier is also influenced by the tenacity of the collector, with an extreme being collectors that ignore certain states entirely (such as all nymphs or particular instars). At the other extreme, we spent more time in our study than a typical field scout searching for nymphs in our sweep nets. It is therefore possible that the number of nymphs is further underestimated in most field counts where there is not enough time to take samples back to the lab. Field counts of sweep samples are generally less accurate than laboratory counts and show increasing disparity as the size of insect sweep samples increase (Fleischer & Allen, 1982). This variability is reflected in other studies of sampling bias in *Lygus*. In results similar to ours, Byerly et al. (1978) found that *L. hesperus* adults were 3.5-fold more likely than nymphs to be collected in sweep nets. However, these researchers combined all instars and derived their estimates from a single field. By using large cages to capture adults, we were able to avoid sampling methods that would cause resident adults to fly away before capture (such as the closure of individual bags; Leigh et al., 1970; Byerly et al., 1978). Furthermore, the use of whole-plant searches for nymphs instead of vacuum suctioning techniques (e.g., Byerly et al., 1978; Ellington et al., 1984) allowed us to look for nymphs inside and around more concealed areas (such as the squares).

The importance of any state-dependent sampling bias will, necessarily, depend on the relative impact of each insect state on the crop of interest. In other work we have found that 4th–5th instars of *L. hesperus* have a larger impact than adults on square damage and abscission (A.G. Zink and J.A. Rosenheim, unpubl.) and these results match other studies focusing on cotton yield (Ellsworth, 2000; Ellsworth & Barkley, 2002). If 4th–5th instars are producing more damage than adults, this underscores the importance of counting nymphs and sorting them according to nymphal instar. For example, if we compare two fields with equal numbers of adults, Tranquility (with many 4th–5th instar nymphs) and Westside (with very few nymphs), it is easy to see how ignoring all nymphs (or even particular instars) could cause one to underestimate damage to plants in the Tranquility field. The current practice of multiplying nymphs by two is likely to be appropriate for the relative magnitude of damage that 4th–5th instars inflict, but not necessarily for their sampling bias (which is comparable to that of adults). In contrast, we should be multiplying 1st–3rd instars by constants according to their relative sampling bias but also devaluing these instars if their per-capita impact on the host plant is negligible. In addition, male adults are about
50% more likely to be captured relative to female adults, but may show a lower impact on square damage (Gutierrez et al., 1977; Rosenheim et al. 2004). Therefore male adults should be devalued for both their sampling bias and for their impact on the plant. Future work will be needed to identify the relative impact of feeding by each nymphal instar and adult sex, through experimental trials.

In addition to state-dependent feeding behavior and impact on the host plant, it is likely that Lygus state affects patterns of dispersal and aggregation within a field. This can affect the efficacy and interpretation of monitoring techniques. Ellsworth’s (2000) and Ellsworth & Barkley’s (2002) findings that nymph density, and not adult density, is the best predictor of decrease in cotton yields could be explained by the relatively low mobility of nymphs relative to adults. Because adults may move through a sampled area fairly quickly, their relative impact depends more on the turnover rate for adults (i.e., whether there is a predictable stream of adults moving through that sampled area). One possibility is that the management of adults is more appropriate at a landscape scale (across several fields and crops), whereas the management of nymphs is more appropriate at the scale of a particular field. Even if adults are moving quickly through a field, however, females are depositing the eggs that result in the next generation. Therefore, the accurate monitoring of Lygus adults will need to incorporate sex ratio and sex-dependent sweep net bias. It is likely that, for both Lygus stage and sex, individual differences in feeding behavior can be scaled up to population densities that incorporate sampling bias. Understanding the interactions between Lygus population structure and state-dependent sampling bias and feeding behaviors should help to resolve the enigma surrounding the impact of Lygus in cotton fields.

Acknowledgements

We thank Benessa Espino, Ann Hendrickson, and Megan Williams for assistance in the field and laboratory. Gail Langellotto, Jason Harmon, Sarina Jepson, Christine Armer, Ken Spence, and John Haloin provided helpful suggestions and comments on earlier versions of this manuscript. This research was supported by the California State Support Board of Cotton Incorporated (USA) and the University of California Statewide Integrated Pest Management Project.

References

Mueller SC, Summers CG & Goodell PB (2003) A Field Key to the Most Common Lygus Species Found in Agronomic Crops of the Central San Joaquin Valley of California. Publication 8104,
University of California Agricultural and Natural Resources, CA.