

AGE STRUCTURE AND SEX RATIO OF *LYGUS HESPERUS* POPULATIONS IN CALIFORNIA COTTON

Andrew G. Zink and Jay A. Rosenheim
Department of Entomology
University of California
Davis, CA

Abstract

The impact of *Lygus* bugs on cotton yield is poorly understood. Growers have long observed that there is a large amount of unexplained variability between the number of *Lygus* observed in a cotton field and the amount of damage the crop sustains. We have begun to investigate this phenomenon by asking the question: can differences in age structure and sex ratio among *Lygus* populations affect sampling accuracy and/or damage to cotton squares? In 2002 we documented a wide range of age structures (adults versus nymphs) and sex ratios (males versus females) across ten different *Lygus* populations in California. Experiments using "whole cage sampling" allowed us to examine the efficiency of sweep sampling for assessing the absolute age structures and sex ratios of these populations. Results suggest that sweep samples do predict *Lygus* presence, but that they are more efficient in capturing adults versus nymphs. The underestimation of nymphs due to sweep sampling bias presents a problem for *Lygus* management because nymphs feed on squares as much as (or more than) adults. Sweep nets were also more efficient in capturing adult males versus adult females. This underestimation of female adults may pose a similar problem for *Lygus* management because adult female *Lygus* spend more time feeding on squares relative to adult males. Adult female density was positively correlated with egg density, suggesting that the propensity of sweep nets to favor adult males could be an additional problem for predicting future generations of *Lygus* in a field.

Introduction

Lygus has been known as a key pest of cotton since early in the 20th century (reviewed by Leigh and Goodell 1996). The key damage comes from square-feeding: young squares that are attacked by *Lygus* die and abscise from the plant and heavy feeding can severely depress yields (Leigh et al. 1988, Ellsworth 2000). Growers have repeatedly stressed an enigma that is familiar to cotton entomologists: sometimes there are lots of *Lygus* in a field, but little or no crop damage, whereas at other times even a few *Lygus* appear to generate heavy crop damage. This enigma is reflected in scientific studies as well. Some studies have found a very large impact of *Lygus* on yields (e.g. Falcon et al. 1971, Leigh et al. 1988) but other results suggest that comparable densities of *Lygus* have little effect on yields (Falcon et al. 1968, 1971, Gutierrez et al. 1977).

Due to the uncertainty surrounding the impact of *Lygus*, growers are consistently forced to adopt very low, conservative action thresholds even though they realize that they might be spraying unnecessarily. Many growers and PCA's use *Lygus* sweep samples that are based solely on adult numbers to trigger sprays. In general, *Lygus* nymphs are very hard to sample, making them "invisible" from a management perspective (Byerly et al. 1978). This presents a problem because past work has shown that *Lygus* nymphs can inflict damage to squares that is equal to (or greater than) that of adults (Gutierrez et al. 1977, Ellsworth 2000). Therefore it is possible that the disconnect between *Lygus* densities and square damage reflects the inherent difficulty of assessing *Lygus* age structure (i.e. nymphs in addition to adults).

Another aspect of *Lygus* populations that is largely ignored during monitoring is that of sex ratio. Past work suggests that when male and female adults are constrained to feed on a square, females cause greater damage and square shed relative to males (Gutierrez et al. 1977). In addition to square damage, female density affects the overall level of egg deposition in fields which results in the next generation of *Lygus*. Individual *Lygus* females lay about 200 eggs on average, with the potential to lay up to 400 eggs (Strong et al. 1970). These eggs can be expected to hatch after only 6 days at a temperature of 35° C (Butler and Wardecker 1971). If populations vary in sex ratio, the overall number of *Lygus* adults may not represent the true impact of *Lygus* adults in that field. For example, a field may be able to sustain more adults if females are rare or fewer adults if females are in the majority.

In this study we conducted focal observations for nymphs, adult females, and adult males in order to quantify the amount of time spent feeding on reproductive structures (i.e. the relative impact on squares). We then set out to quantify the absolute age structure (adults vs. nymphs) and sex ratio (males versus females) of ten different *Lygus* populations. We combined these data with sweep net samples from the same fields to test the efficiency of sweep samples in predicting the actual numbers of *Lygus* nymphs versus adults (both male and female) in a field.

Materials and Methods

Direct observations of 87 individual *Lygus hesperus* were conducted from June through September, 2001 on upland cotton in the southern San Joaquin Valley, California in Fresno, Kern and Kings counties. Ten commercial fields were chosen for *Lygus* observations on the basis that they supported populations of *L. hesperus* and had not received pesticide applications three weeks prior to observations. Individual *L. hesperus* for observation were chosen by walking slowly through the field inspecting the top portion of the cotton plants for presence of any *Lygus* stage (males, females, and nymphs). The aim was to observe equal numbers of adults versus nymphs at each site and time. Observers worked in teams of two, and attempted to continuously record the behavior of the *Lygus* over the course of an hour. Although the goal was to observe individual insects for an hour, this was not always possible as some individuals were lost in flight. Continuous recordings were made of the position on the plant (leaf, stem, fruiting structure, etc.) and feeding activity. Feeding was recorded if the individual remained still with its beak on the substrate. Adults were collected after observation to determine their sex.

In 2002 we sampled ten fields spanning the San Joaquin Valley (Firebaugh to Bakersfield) and the main plant growth stages during which squares are being set (late June through August). We focused on Acala cotton fields (primarily cultivars Maxxa and Phytogen 72) for our sampling, and chose fields that had not been sprayed in the previous three weeks. The *Lygus* adults in these fields appeared to be almost exclusively *L. hesperus* (versus *L. elisus*) matching estimates of 96-99% *L. hesperus* in other fields (Godfrey 2000). In order to accurately sample the absolute numbers of *Lygus*, we constructed a 4x2x2 meter cage surrounded by insect netting. By carefully carrying this cage high above our heads we were able to drop the cage across two rows of plants at three different points in each field. We chose to use large cages as a sampling device because our experiences with smaller sampling units suggested that they invariably flushed the adult *Lygus* off the plants being sampled before they could be put in place, thus producing major underestimates of the absolute densities of adults in the field. Our large cages appeared to produce minimal disruptions of adults in the field. After the cage had been dropped, all of the adult *Lygus* that were trapped in the cage eventually flew onto the insect netting. Adults were then collected using an aspirator and taken back to the lab for identification of sex. This allowed us to quantify the total number of male and female adults per cage (i.e. eight meters of row).

We randomly chose twenty plants within each cage and conducted whole-plant searches in order to collect all nymphs. Nymphs were collected with aspirators and taken back to the lab where they were identified to developmental stage (1st through 5th instar). We counted the total number of plants in each cage, allowing us to convert the nymph counts into an absolute number of nymphs per meter of row. In each sampled field we also collected 10 samples of 50 sweeps in the same plot where we took the cage samples. The *Lygus* adults and nymphs from these sweep samples were collected and taken to the lab for identification of sex and developmental instar. In each sampled field we also collected one leaf from the third node of 100 plants. These leaves were then examined in the lab for *Lygus* eggs; in previous work we determined that *Lygus* eggs are deposited primarily in the petiole.

Results

For the behavioral observations, we found no differences in the proportion of overall time spent feeding (versus resting or moving) for adults versus nymphs or males versus females. Because it was difficult to observe nymphs feeding (they were often inside the bracts of the square) it is possible that nymphs spend more time feeding relative to adults. There were clear differences in the total amount of time (feeding, resting and moving) spent on reproductive versus vegetative structures. Adult males spent significantly less time on fruiting structures of the plants than did either adult females or nymphs ($\chi^2=12.6$, $p=0.002$; Fig. 1) and more time on vegetative structures such as the tops of leaves. In addition, males spent significantly more time resting (versus moving or feeding) relative to females ($\chi^2=5.1$, $p=0.02$). These results are consistent with the interpretation that nymphs and adult females were primarily engaged in feeding on nutrient-rich plant foods (fruiting structures), whereas males were primarily engaged in searching for receptive females.

We found a broad range of age structure in *Lygus* populations: nymphs ranged from 18% to 71% of the individuals collected across all three cages in a given field (Fig. 2). We calculated the percent nymphs for each cage (three per field) and found significant differences in age structure among fields (ANOVA, $F=3.6$, $p=0.009$). This suggests that adult density alone cannot accurately reflect (or predict) *Lygus* presence in a particular field. Sweep net samples were significantly positively correlated with absolute numbers of *Lygus* for each age class (1st through 5th instar and adults). Our results revealed that for each adult collected per 50 sweeps there is one adult foraging in 2.8 meters of row. Sweeps were less successful at capturing nymphs but were incrementally more successful with more advanced instars (Fig. 3). For example, first instars were collected at less than 1/10th the rate of adults, whereas fifth instars were almost equivalent to adults. Therefore the magnitude of the bias generated by sweep nets is directly dependent on the absolute age structure of the nymph population.

The absolute sex ratio of the total adults collected in the cages was also highly variable across fields (Fig. 4). We calculated the percent females for each cage (three per field) and found significant differences in sex ratio among fields (ANOVA, $F=4.2$, $p=0.004$). These differences were statistically significant even when eliminating a field (Poplar) in which there were

only four adults collected ($F=2.5$, $p=0.05$). Sweep nets appear to be biased toward collecting males over females for this same subset of nine fields (Fig. 5). This bias against detecting females has direct implications for *Lygus* management because the density of *Lygus* eggs per leaf (i.e. the future generation) is positively correlated with the number of adult females in a field ($r=0.78$, $p=0.01$; Fig. 6). Therefore assessing female numbers is important for predicting impact to squares (Fig. 1) as well as the production of future generations of *Lygus* through oviposition.

Discussion

Our large cage sampling regime suggests that cotton fields exhibit variable numbers of *Lygus* nymphs relative to adults. Therefore the number of *Lygus* adults collected in sweep samples is not a reliable measure of overall *Lygus* presence because some fields contain many nymphs and few adults (and vice versa). However, even if nymphs are accurately counted in sweep nets there is an inherent bias against collecting smaller instars. Therefore any true measure of nymph densities will need to multiply each instar by a factor that is proportional to its sampling bias. The general rule of multiplying nymphs by a factor of 2 is only appropriate for third or fourth instars; this rule will underestimate nymphs if a population primarily consists of first and second instars. It is important to note that because we spent more time than a typical field scout searching for nymphs in our sweep nets, it is possible that the number of nymphs is further underestimated in most field counts.

The sweep net bias against nymphs is consistent with previous findings that adults tend to feed higher on the plant and on more exposed surfaces such as leaves and bolls (Wilson et al. 1984). Our behavioral data suggest that males spend more time on exposed vegetative structures (such as leaf surfaces) possibly increasing the probability of their capture relative to adult females. Byerly et al. (1978) found that *Lygus* adults were 3.5 times more likely than nymphs to be collected in sweep nets. However these researchers combined all instars and derived this estimate from a single field. There are other reasons to suggest that the whole-plant-bag-sampling method of Byerly et al. (1978) is less accurate than our own measures of absolute *Lygus* density. We used large cages to capture adults because we were concerned that the closure of individual bags would cause resident adults to fly away before capture. In addition, we used whole-plant searches for nymphs instead of the vacuum suctioning technique of Byerly et al., which allowed us to look for nymphs inside and around more concealed areas (such as the squares).

It is possible that *Lygus* nymphs and adults are inflicting damage at different spatial scales. At the level of the field, nymphs have been found to be more clustered in space relative to adults (Sevacherian and Stern 1972). Ellsworth (2000) found that nymph density, and not adult density, is the best predictor of damage to plants and decrease in yields. These results could be explained by the relatively low mobility of nymphs relative to adults. Because adults move through a sampled area fairly quickly, their relative impact depends 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 management of adults is more appropriate at a landscape scale (across several fields and crops; see Goodell 2000) whereas the management of nymphs is more appropriate at the scale of a particular field. Even if adults are moving quickly through a field, females are depositing the eggs that result in the next generation. Therefore monitoring of adults will need to incorporate sex ratio and sex-dependent sweep net bias. Because females appear to cause more damage to squares relative to males, future investigations of the relative impact of nymphs versus adults will also need to account for differences in sex ratio across populations.

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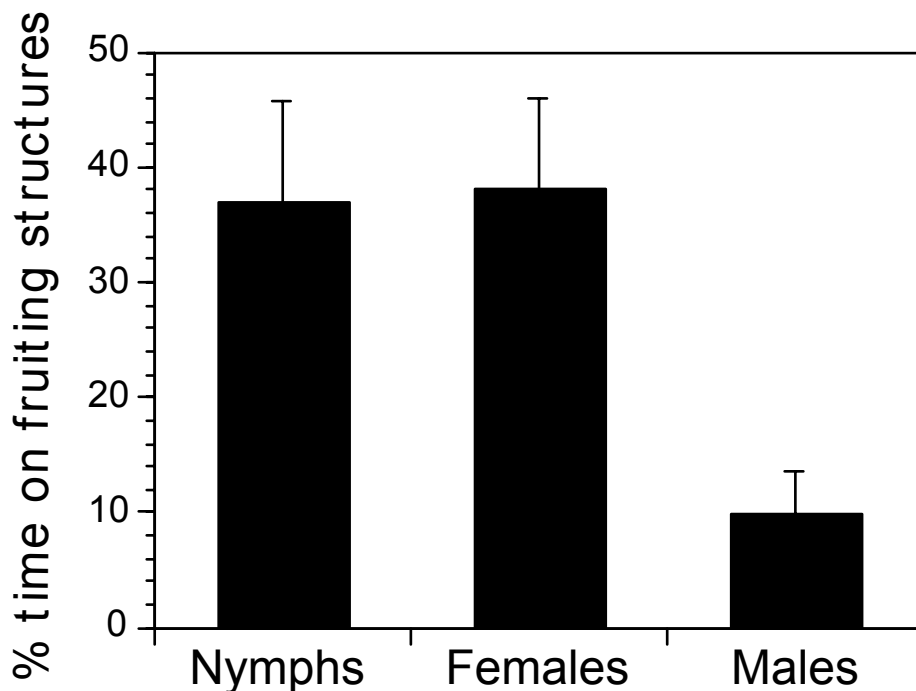


Figure 1. The proportion of total time spent on fruiting structures (square, flower, boll) versus vegetative structures for nymphs, adult females and adult males of *Lygus hesperus*. While there is no difference between nymphs and females, males spend significantly less time on fruiting structures relative to nymphs and females.

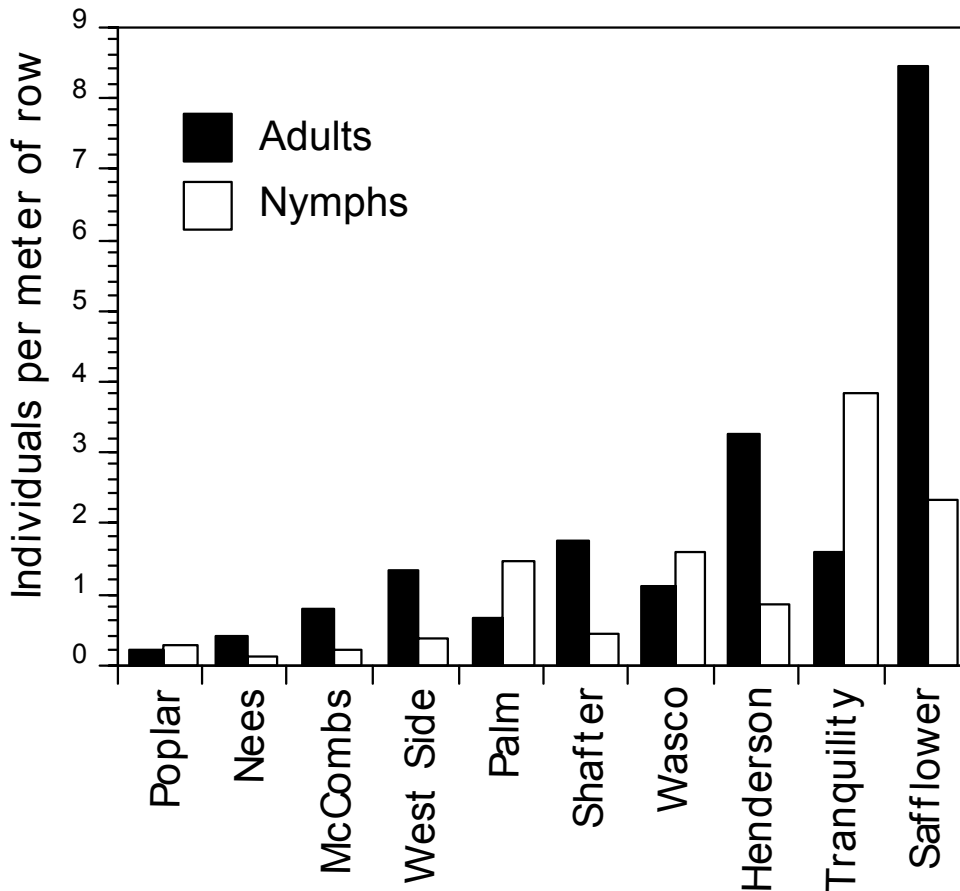


Figure 2. The absolute numbers of nymphal vs. adult *Lygus hesperus* as determined from whole-cage sampling. Fields vary in relative proportion of adults vs. nymphs and adults alone are an unreliable indicator of total numbers of *Lygus* in a field.

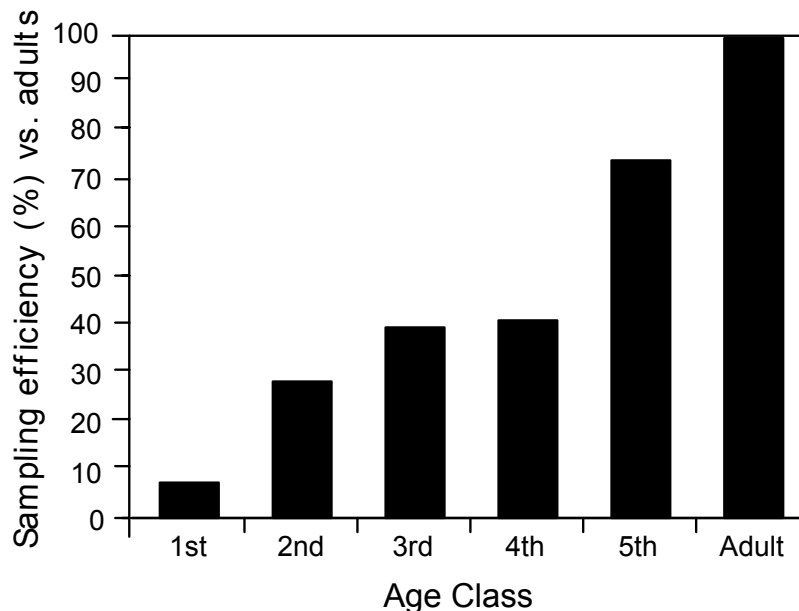


Figure 3. The relative efficiency of sweep nets for collecting *Lygus* of each age class. This figure shows that smaller nymphs are systematically underestimated in fields. A first instar nymph is more than 10 times more difficult to collect than an adult. In contrast, a fifth instar nymph is collected with almost the same probability as an adult. Here one adult per 50 sweeps reflects an absolute number of one adult per 2.8 meters of row.

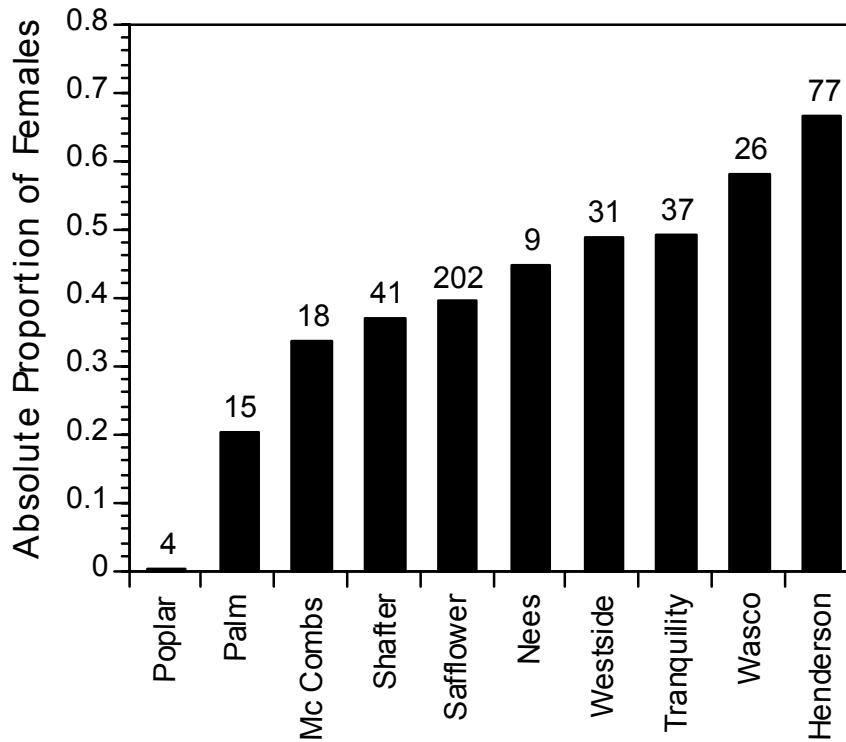


Figure 4. Proportion of adults (collected in large cages) that are females across fields with the total number of adults collected in each field above the bars. Note that fields are highly variable in overall *Lygus* sex-ratio.

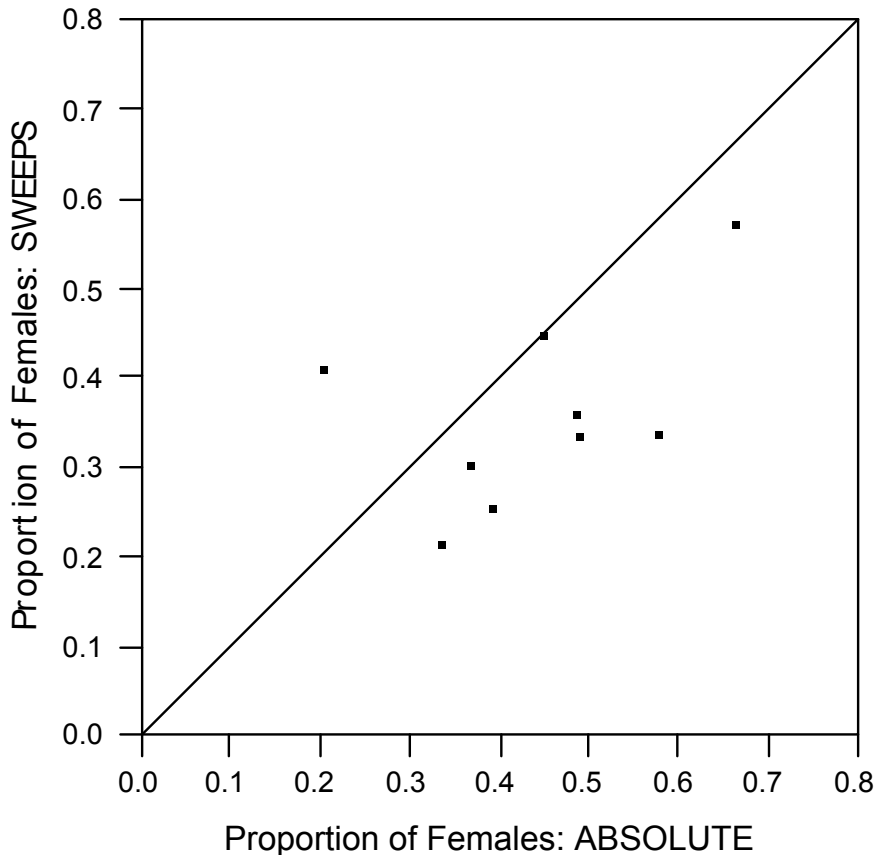


Figure 5. The proportion of females collected in sweep nets versus the absolute numbers in the large-cage samples for each field. Sweep sampling underestimates female number in the majority of fields.

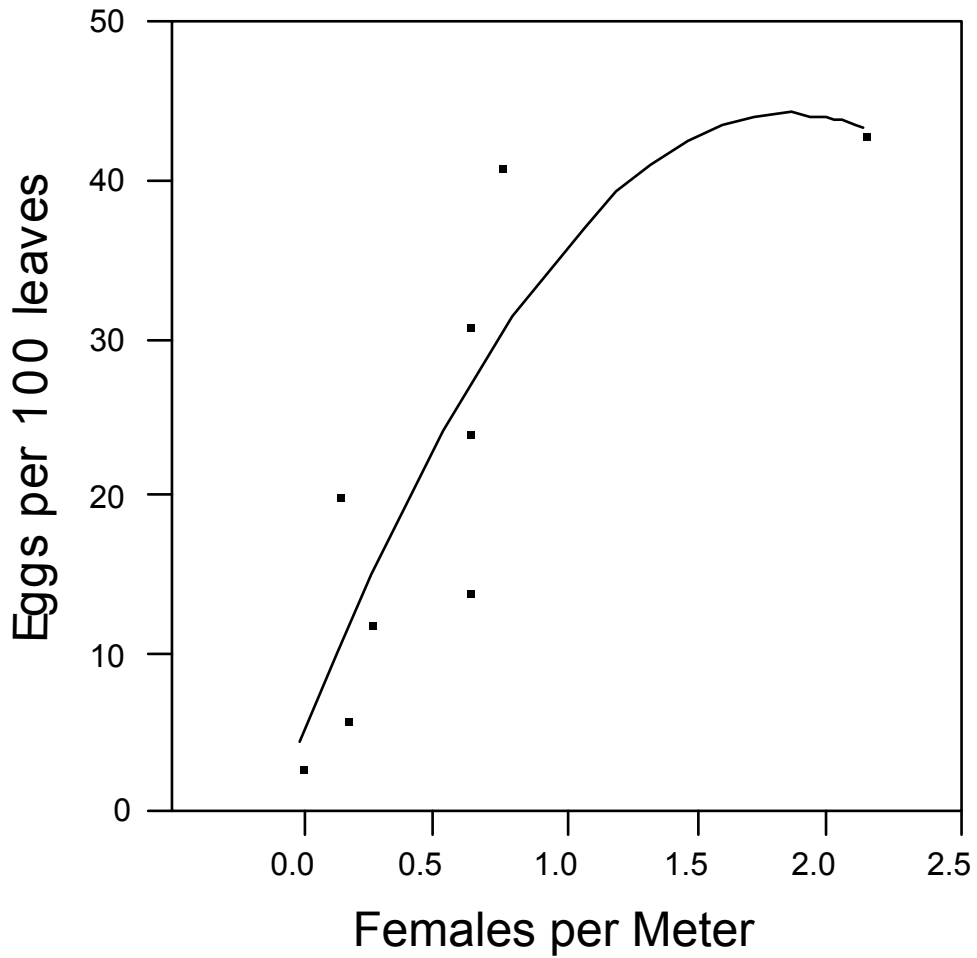


Figure 6. Across fields, greater female density results in a greater number of eggs laid on plants. These eggs provide an estimate for the size of the next generation of *Lygus*.