Relationship Between Population Estimates of Cotton Fleahoppers (Hemiptera: Miridae) Obtained by Terminal and Whole Plant Examinations[1]

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Abstract The standard method for assessing cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), abundance in cotton, *Gossypium hirsutum* L., involves direct counts of adults and nymphs on main stem terminals. Although this practice appears to provide adequate estimates for pest management programs, the accuracy and precision of population estimates obtained from terminal sampling, relative to whole plant examinations and potential time-of-day sampling effects, have not been investigated. We examined the distribution of cotton fleahopper adults and nymphs within cotton plants twice a day (0800 - 1130 h and 1300 - 1630 h) in 2007 and 2008 to determine whether the numbers of fleahoppers in the terminal of plants accurately and reliably reflect the numbers of fleahoppers on those plants. Overall, the mean numbers and distribution patterns of fleahoppers observed during the morning and afternoon sampling periods were statistically similar. Consequently, time-of-day sampling effects were not observed. When the numbers of fleahoppers found on plants were regressed on the numbers of fleahoppers observed in the terminal of those plants, the r^2 and coefficient of variation (CV) values for adults were 0.81 and 40, respectively. Corresponding values for nymphs were 0.97 and 22. Based on regression slopes, the terminal accounted for 64% of the adults and 78% of the nymphs observed on plants. Our results suggest fleahopper counts obtained from terminal examinations accurately reflect the numbers of fleahoppers on those plants. However, this sampling practice may not provide the level of precision typically required in population research.

Key Words cotton fleahopper, *Pseudatomoscelis seriatus*, sampling, within-plant distribution

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), has long been recognized as an economically-important pest of cotton, *Gossypium hirsutum* L., in Texas and neighboring states (Reinhard 1926, Metcalf and Flint 1928). Both adults and nymphs damage cotton plants by feeding on the sap of prefloral buds (young "squares") and terminal growth, which can result in excessive fruit loss, abnormal plant growth, and delayed plant maturity (Reinhard 1926, Almand et al. 1976, Ring et al. 1993). In 1999, the cotton fleahopper was considered the most economically damaging insect pest of U.S. cotton, causing an estimated $196 million in costs and losses to U.S. farmers (Williams 2000). In the following year cotton fleahoppers were ranked the 9th most damaging insect pest of cotton, infesting approx. 42% of U.S. cotton acreage (Williams 2001). In 2007, fleahoppers were considered the 4th most damaging insect pest of US cotton and reduced yields by nearly 120,000 bales (Williams 2008). Although the

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economic importance of cotton fleahoppers on a national level has varied over the past decade, fleahoppers have consistently been considered one of the most destructive insect pests of cotton in Texas.

Presently, cotton producers rely primarily on insecticides for control of cotton fleahoppers, typically making one or more applications during the initial 3 or 4 wks of squaring when plants are most susceptible to fleahopper damage (Parker et al. 2008). Although treatment thresholds and effective insecticides exist, reevaluation of the economic impact of cotton fleahoppers has been identified as a top research priority among research and extension entomologists in Texas. Motivating factors include recent demonstrations of the cotton plant's ability to compensate for substantial prebloom square loss (Doederlein et al. 2002, Leser et al. 2004) and considerable reductions in yield losses to heliothines and boll weevils, *Anthonomus grandis* Boheman, resulting from the widespread adoption of *Bacillus thuringiensis* (Bt) Berliner cotton and implementation of a statewide boll weevil eradication program, respectively. Because cotton production has changed substantially during the past decade, reassessment of action thresholds for the cotton fleahopper is deemed critically important. Imperative to the success of this mission is the ability to efficiently and accurately estimate fleahopper population levels in fields.

Several sampling methods have been evaluated and include the use of pneumatic devices (Suh 2008) or shake buckets (Pyke et al. 1980). However, the effectiveness of these tools appears to be limited to a particular range of plant sizes or insect stage. Parajulee et al. (2006) examined the seasonal distribution of cotton fleahoppers on cotton plants and reported that 89% of the total 305 fleahoppers found on plants were located within the upper and middle one-third portion of plants. Consequently, those authors suggested sampling efforts should target the upper and middle strata of plants. Currently, the most common sampling method used by producers, consultants, and extension agents involves direct counts of adults and nymphs on whole plants or main stem terminals. However, examining whole plants becomes increasingly laborious and time-consuming as plants increase in size. Consequently, the terminal examination method is generally preferred. Although this sampling practice appears to provide relative population estimates that are adequate for pest management programs, the accuracy and precision of estimates obtained with this practice, relative to those obtained by whole-plant examinations, have not been investigated. Furthermore, potential time-of-day sampling effects associated with this sampling practice have not been examined.

Because population research generally requires more accurate and precise population estimates than pest management programs (Pedigo 2002), additional information is needed before this sampling practice can be recommended for population research. The objective of our study was to examine the distribution of cotton fleahoppers on cotton plants during different periods of the day to determine whether the numbers of fleahoppers in the terminal of plants accurately and reliably reflect the numbers of fleahoppers on those plants.

Materials and Methods

The distribution of cotton fleahopper adults and nymphs on cotton plants was examined in 3 commercial fields in 2007 and in 4 fields in 2008. Fields were distributed among Burleson and Robertson counties, TX, and were planted using conventional practices and cotton varieties for that area. Sampling was confined to a 0.5-ha area in each field divided into 25 equal-sized plots (15 rows × 12 m long) in a 5-by-5 arrangement.

Each field was sampled 3 days a week (MWF) during the initial 3 or 4 wks of squaring unless production practices (e.g., pesticide application) or rainfall prevented sampling. All fields were sampled in the morning (0800 - 1130 h CDT) and again in the afternoon (1300 - 1630 h CDT) to reveal potential time-of-day sampling effects. On each sampling occasion, 1 row within the center 7 rows of each plot was selected and 2 plants within the chosen rows were directly examined for fleahoppers (50 plants per field). The numbers of adults and nymphs observed within and below the "terminal" of each plant were recorded, and respective totals in each field were used as model inputs in subsequent analyses. Initially, when plants possessed ≤ 7 nodes, the terminal was defined as the portion of the plant consisting of the terminal bud and top 2 nodes with fully-expanded leaves. As plants increased in size and development (plants with ≥ 8 nodes), the terminal bud and top 4 nodes with fully-expanded leaves constituted the terminal of plants. Given the growth pattern of cotton plants, the terminal consisted of the top 2 - 8 cm portion of plants. All fields were sampled in the same order each day to minimize potential time-of-day sampling variation, and a different row was selected on each sampling date until all rows had been sampled at least once. Thereafter, the sampling row order was repeated until the experiment was concluded. Additionally, plant height, node count, and fruiting profile were assessed weekly on 50 plants in each field to provide supporting information.

Statistical analyses. Data for adults and nymphs were analyzed separately using a two-way mixed-model analysis of variance (PROC MIXED, SAS Institute 2007). Because current treatment thresholds are based on the combined abundance of adults and nymphs, a third analysis was performed with counts of adults and nymphs combined. In each analysis, fixed effects in the model contained terms for sampling period (morning, afternoon), location of fleahopper (terminal, below terminal), and their interaction. Random effects included year, field nested within year [field(year)], and sample date nested within field [sample date(field)]. Corrected denominator degrees of freedom were obtained using the Kenward-Roger adjustment (DDFM=KR option of the MODEL statement). Estimates of least-square means and corresponding standard errors were obtained using the LSMEANS statement, and differences among levels of fixed effects were identified using the ADJUST=TUKEY option of the LSMEANS statement.

The relationship between the total numbers of fleahoppers observed on plants (dependent variable) and in the terminal portion of those plants (independent variable) was assessed using PROC REG (SAS Institute 2007). Similar to the mixed-model analysis, counts of adults and nymphs were combined as well as analyzed separately. In each case, data were pooled across years, fields, and sampling periods to examine the respective relationships under a range of fleahopper densities, plant sizes, and environmental conditions. The R option of the model statement and residual-by-predicted plots were used to diagnose nonlinearity or nonconstant error variance. The STB and CLB options of the MODEL statement were used to obtain standardized parameter estimates and associated 95% confidence limits.

Results and Discussion

In both years of the field study, sampling was initiated and terminated when plants averaged 5 - 6 and 10 - 12 nodes, respectively. The average height of plants sampled in 2007 ranged from 11 - 54 cm and from 10 - 47 cm in 2008. A range of fleahopper population densities was observed (Fig. 1), and densities on most sample dates exceeded the currently recommended action threshold of 10 - 15 fleahoppers per 100 terminals

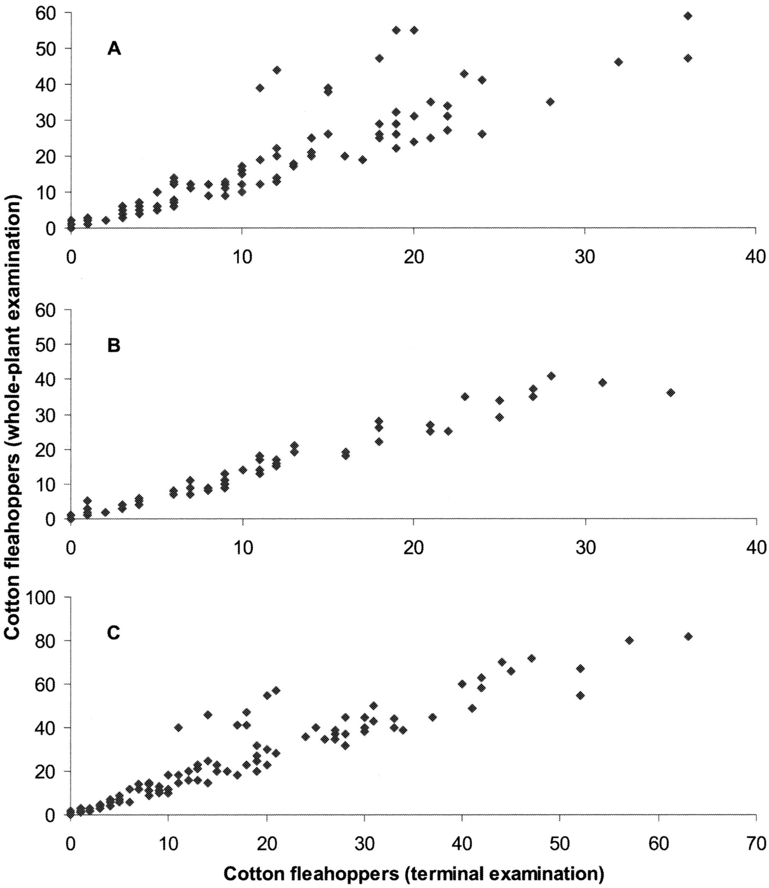

Fig. 1. Relationship between the numbers of cotton fleahoppers obtained from whole-plant and terminal examination of 50 cotton plants during the initial three or four weeks of prefloral bud production in 2007 and 2008; A) adults, B) nymphs, and C) adults and nymphs combined.

(Parker et al. 2008). Overall, the mean ± SE numbers of fleahoppers observed per 50 plants per sample date in 2007 (adults, 22 ± 2; nymphs 12 ± 2) were considerably higher than those observed in 2008 (adults, 10 ± 1; nymphs, 5 ± 1). However, similar population trends were observed between years. In general, population levels of adults in fields peaked between the second and third week of squaring, and substantial numbers of nymphs (> 10 nymphs per 50 plants) were not observed until this time.

Overall, the mean numbers of adult fleahoppers observed on plants during the morning and afternoon sampling periods were statistically similar ($F = 2.58$; df = 1, 173; $P = 0.110$). This was also the case for nymphs ($F = 0.05$; df = 1, 179; $P = 0.823$) and for combined counts of adults and nymphs ($F = 1.45$; df = 1, 175; $P = 0.230$). However, differences were detected between the numbers of fleahoppers observed within and below the terminal of plants (adult, $F = 32.99$, df = 1, 168; $P < 0.001$;

nymph, $F = 51.58$; df = 1, 173; $P < 0.001$; combined, $F = 59.56$; df = 1,169; $P < 0.001$). In each case, significantly more fleahoppers were observed within the terminal than below the terminal of plants (Table 1). The location-by-sampling period interaction was not significant for adults ($F = 1.05$; df = 1, 168; $P = 0.306$), nymphs ($F = 0.03$; df = 1, 173; $P = 0.853$), or combined counts of adults and nymphs ($F = 0.57$; df = 1, 169; $P = 0.452$), indicating the respective densities of fleahoppers within and below the terminal of plants remained consistent between the morning and afternoon sampling periods. These findings indicate the time of day when plants are sampled (from 0800 - 1630 h) should have minimal influence on fleahopper population estimates, regardless of whether estimates are based on terminal or whole-plant examinations.

A significant relationship was detected between the total numbers of adults observed on plants and the numbers of adults observed within the terminal portion of those plants ($F = 453.37$; df = 1, 107; $P < 0.001$). This also was the case for nymphs ($F = 4{,}089.10$; df = 1, 107; $P < 0.001$) and for combined counts of adults and nymphs ($F = 887.98$; df = 1, 107; $P < 0.001$). In all 3 models, r^2 values were ≥ 0.80 with the highest value observed for nymphs (Table 2). Based on the regression slopes (Table 2), the terminal accounted for approx. 64% of the adults and 78% of the nymphs found on plants. When counts of adults and nymphs were combined, the terminal accounted for 74% of the fleahoppers observed on plants.

In regards to the 95% CL of the slopes, the narrowest range was observed for nymphs, followed by the model with combined counts of adults and nymphs (Table 2). Based on the 95% CL of the slopes, the terminal accounted for 59 - 71% of the adults and 75 - 82% of the nymphs found on plants. The model for nymphs also possessed the lowest coefficient of variation (CV), whereas the highest CV was observed for adults (Table 2). These findings suggest sampling the terminal portion of plants provides more accurate and reliable population estimates of nymphs than adults. Although lower CV values are indicative of greater of precision, acceptable values depend on the level of

Table 1. LSMean ± SE numbers of cotton fleahoppers observed within and below the terminal of 50 cotton plants sampled at two time periods of the day during the initial three to four weeks of prefloral bud production in 2007 and 2008.

Time (CDT)	Stage	Terminal *	Below terminal *
0800 - 1130 h	Adult	9.1 ± 2.55a	5.8 ± 2.55b
	Nymph	6.0 ± 1.61a	1.7 ± 1.61b
	Adult + nymph	15.0 ± 3.84a	7.3 ± 3.84b
1300 - 1630 h	Adult	10.9 ± 2.58a	6.2 ± 2.58b
	Nymph	6.2 ± 1.65a	1.8 ± 1.65b
	Adult + nymph	17.2 ± 3.89a	7.9 ± 3.89b

Within a row, least-square means followed by different letters are significantly different ($\alpha = 0.05$; Tukey-Kramer test).

* The terminal bud and the top two nodes with fully expanded leaves constituted the terminal on plants with ≤7 nodes. On plants with ≥ 8 nodes, the terminal consisted of the terminal bud and the top four nodes with fully expanded leaves

Table 2. Regression parameters relating numbers of cotton fleahoppers found on 50 cotton plants to numbers of cotton fleahoppers observed within the terminal of those plants during the initial three or four weeks of prefloral bud production in 2007 and 2008.

Stage	n	Slope Mean ± SE	Slope (95% CL)	Intercept Mean ± SE	Intercept (95% CL)	r^2	CV
Adult	109	1.55 ± 0.07	1.41 - 1.70	0.43 ± 0.94	–1.43 - 2.28	0.81	40
Nymph	109	1.28 ± 0.02	1.24 - 1.32	0.00 ± 0.21	–0.42 - 0.41	0.97	22
Combined	109	1.35 ± 0.05	1.26 - 1.44	2.00 ± 0.98	0.06 - 3.95	0.89	29

precision required. Pedigo (2002) suggested sampling techniques that provided relative variation values near 25 were adequate for pest management programs, but lower values were needed in population research. Based on these criteria, our results indicate terminal examinations provide population estimates that are adequate for pest management programs. However, this sampling practice may not provide the level of precision typically required in population research. Despite this limitation, practical alternatives are not available or have not been extensively evaluated. Consequently, additional factors such as sampling costs and the required level of precision should be considered on a case-by-case basis before discounting this sampling procedure in population research.

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