Vertical Distribution of Threecornered Alfalfa Hopper Nymphs (Homoptera: Membracidae) and Girdles on Soybean[1]

Dale W. Spurgeon and A. J. Mueller

Department of Entomology, University of Arkansas
Fayetteville, AR 72701

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ABSTRACT The vertical distributions of *Spissistilus festinus* (Say) instars, petiole girdles, and main stem girdles on soybean, *Glycine max* (L.) Merrill, were investigated by inspecting randomly selected whole plants. Vertical distribution patterns were characterized by dividing sampled plants into three strata: lower (the lower main stem), middle (the lower half of the canopy), and upper (the upper half of the canopy). Most nymphs were located in lower strata on plants of growth stages V2 through V7 and in middle strata on more developed plants. First and fifth instars tended to be distributed higher than third and fourth instars on plants of growth stage ≥ V10. Most main stem girdles occurred in lower plant strata regardless of plant phenology. Petiole girdles were distributed similarly to nymphs except for a scarcity in lower plant strata. Although plant phenology appeared to affect nymphal vertical distribution few nymphs or girdles were ever located in the upper plant canopy.

KEY WORDS Insecta, *Spissistilus festinus, Glycine max,* vertical distribution.

The threecornered alfalfa hopper, *Spissistilus festinus* (Say), is a pest of soybean, *Glycine max* (L.) Merrill, in the southeastern United States, producing characteristic injuries called girdles. Within-plant distributions of *S. festinus* eggs and main stem girdles in vegetative stage soybeans have been described (Rice and Drees 1985, Daigle et al. 1988), and Mitchell and Newsom (1984) have reported nymphs and girdling sites to move from lower main stems to petioles and lateral branches as plants develop. However, little information is available concerning vertical distributions of nymphs and petiole girdles.

The distributions within soybean canopies of *S. festinus* nymphs and girdles have implications in understanding the ecology of the insect. Benefits to *S. festinus* from girdling have not been demonstrated and the physiological and yield responses of soybean to girdling have not been adequately examined. Meaningful investigation of these phenomena under controlled conditions will require information concerning the within-canopy distributions of nymphs and girdles. Such information could also improve the interpretation of other research findings. The purpose of this study was to define the vertical distribution patterns of *S. festinus* instars and girdles on soybean at different stages of crop development.

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Materials and Methods

Studies in southwest Arkansas soybeans were conducted in 1987 and 1988. Study sites during 1987 were soybean fields ('Leflore' and 'Bragg') planted in 0.91-m rows on 10 May and 0.76-m rows on 5 June, respectively, in Little River County. Data were collected in the 'Leflore' field from plant growth stage V3 (Fehr et al. 1971) (18 June) until stage V12, R2 (21 July) and in the 'Bragg' field from growth stage V2 (23 June) to stage V16, R5 (19 September). A center-pivot irrigated field of 'Centennial' soybean in Lafayette County and a field of 'Bragg' in Little River County, both planted in 0.76-m rows on 15 May, were sampled during 1988. The 'Centennial' field was sampled from growth stage V7 (25 June) to stage V13, R2 (17 July), and the 'Bragg' field was sampled from stage V9 (29 July) to stage V13, R6 (17 September). All soybean varieties examined were typical of the full-season, determinate varieties grown in southern Arkansas. Each study site consisted of a 0.8-ha plot.

Vertical locations of nymphs and girdles were determined by visually inspecting randomly selected whole plants. An individual plant was chosen as the sampling unit to minimize the effects of sampling activities on nymphal location because nymphs move readily when disturbed. Vertical locations were determined by counting main stem nodes, begining with the cotyledonary node as node 0. For this purpose each "node" included the internode directly below it as well as structures arising from it. Nymphs were removed from the plants by hand as they were recorded. When the canopies of adjacent plants overlapped, as many nymphs as possible were recorded and removed before a plant was separated from surrounding foliage. Plant growth stage, and in 1988, the lowest structure-bearing-node (leaf, lateral branch, or raceme), were also recorded for each plant. Plots were sampled at 5 - 8 d intervals except when sampling was prevented by rain or irrigation. On most sample dates 100 plants were inspected in each plot.

Vertical distributions of nymphs and girdles were defined in relation to plant phenology. Nymphal and girdle locations ("nodes") were combined to form lower and upper plant strata on plants ≤ V6, and lower, middle, and upper strata on plants >V6 (Table 1). The lower stratum was defined as the main stem from the soil surface to and including the lowest mean structure-bearing-node among the sample dates in each pooled data set. Thus, the lower stratum contained only some of the lowermost leaves. Approximately one-half of the remaining nodes were assigned to each of the middle and upper strata. The objective of plant strata assignments was to divide the plants into relatively homogeneous regions of available habitat. Because plant growth stage was defined by the number of nodes with expanded leaves, plants generally had one or two more nodes available to *S. festinus* nymphs than were indicated by the growth stage. The ranges of nodes included in upper plant strata were assigned to accommodate the largest plant in each growth stage class.

Vertical distributions of instars on each sample date were compared in contingency tables and, to avoid problems associated with small cell expected values, comparisons also were made within pooled plant growth stage classes (Table 1). Analyses were conducted using either the Chi-square statistic of the SAS procedure PROC FREQ, or an extension of Fisher's exact test (the EXACT option of PROC FREQ) (SAS Institute 1988). Where statistical significance by

Table 1. Assignments of strata to soybean plant canopies of pooled growth stage classes.

Growth Stage Class*	Nodes† Assigned to Plant Strata		
	Lower	Middle	Upper‡
V2.2 - V3.2	0 - 1	–	2 - 5
V5.1	0 - 1	–	2 - 7
V6.5 - V7.2	0 - 1	2 - 4	5 - 10
V8.0 - V8.9	0 - 1	2 - 5	6 - 13
V10.2 - V11.6	0 - 2	3 - 7	8 - 15
V12.1 - V12.7	0 - 3	4 - 8	9 - 17
V13.4 - V15.9	0 - 3	4 - 8	9 - 21

* Range of mean growth stages of pooled sample dates.

† Nodes are numbered from the cotyledonary node (node 0) and include the subtending internode.

‡ Range of nodes accommodates the tallest plant in each growth stage class; 1 -2 nodes occur above the highest node used to define growth stage.

the Chi-square test was indicated, individual cells were examined to determine their contributions to the Chi-square statistic.

Results and Discussion

Vertical distributions of nymphs among plant strata changed with plant phenology. Most nymphs on plants <V8 occurred in lower strata even though these strata represented relatively small portions of the plants (Table 2). On plants ≥V8, most nymphs occurred in middle strata and fewest nymphs occurred in upper strata, except for growth stages >V13 when nymphal occurrence in upper and lower strata were similar (Table 2). The observed shift away from lower plant strata as the dominant nymphal location began shortly after growth stage V5. Our observations are consistent with those reported by Rice and Drees (1985), who found that nymphs switch from main stems to petioles at about growth stage V5. Mitchell and Newsom (1984) also reported changes in nymphal location with plant phenology and related them to increasing thickness and woodiness of main stems, resulting in the movement of nymphs to petioles of actively photosynthesizing leaves. Regardless of plant phenology, we never observed <20% of the nymphs to be associated with lower plant strata. Many of these nymphs were tended by ants and excreted honeydew, indicating that they were successfully feeding.

Differences among instars in vertical distribution could be legitimately examined on only three of 28 sample dates according to the criteria of Cochran (1942) (80 percent of cell expected values >5 and the remainder >1). When the more liberal criteria of Slakter (1965) were used (all cell expected values >1) only three additional dates could be examined. Significant differences among instars in vertical distribution were indicated on three sample dates (Table 3),

Table 2. Vertical distributions of *S. festinus* nymphs in strata of soybean plants of pooled growth stage classes.

		% of Nymphs in Plant Strata		
Growth Stage Class*	*n*†	Lower	Middle	Upper
V2.2 - V3.2	310	62	– ‡	38
V5.1	267	66	– ‡	34
V6.5 - V7.2	358	47	38	15
V8.0 - V8.9	215	27	62	11
V10.2 - V11.6	233	29	54	17
V12.1 - V12.7	739	25	61	14
V13.4 - V15.9	124	20	55	25

* Range of mean growth stages of pooled sample dates.
† Total number of nymphs observed.
‡ Only lower and upper plant strata were assigned.

when plants were of growth stage V10 or greater. In most cases, more first instars than expected were observed in upper strata and (or) fewer first instars than expected were observed in lower strata. Opposite trends were observed for third instars.

The small cell expected values observed in most tests resulted from the large number of nymphs required for a valid test. This number was increased when nymphal population age distributions and distributions of nymphs among plant strata were uneven. The nymphal population levels we observed were often high; sampling studies conducted concurrently (Spurgeon and Mueller 1991) indicated that the treatment threshold suggested by Sparks and Boethel (1987) was exceeded on six dates . However, age- and vertical distributions of nymphal populations were almost always very biased. Low power resulting from small cell counts can be overcome by collapsing the contingency table (Steel and Torrie 1980) or by pooling homogeneous data sets (Zar 1984). Bailey et al. (1970), Mitchell and Newsom (1984), and Rice and Drees (1985) previously reported that plant phenology influences the location of oviposition sites, girdles, and nymphs. Thus, pooling of sample dates according to similarities in plant phenology was the most acceptable strategy.

Small cell expected values on the individual sample dates precluded a proper test of heterogeneity among pooled sample dates. Alternatively, differences in vertical distribution of each instar among sample dates in pooled growth stage classes were examined using Fisher's exact test (SAS Institute 1988).

Only four of the 35 analyses conducted indicated significant differences ($P < 0.05$) in the distribution of an individual instar among pooled sample dates. Differences were not detected for any instar in more than one growth stage class and in three of these analyses the number of nymphs was extremely low (<3 nymphs per cell). Pooling heterogeneous samples would result in an increase in the variablility of the distributions of individual instars and a loss of power in the test of pooled counts. Because no consistent differences across growth stage classes occurred for any instar, data were analyzed for pooled growth stage classes.

Table 3. Chi-square analyses of differences among *S. festinus* instars in vertical distribution among soybean plant strata.

Field	Plant Stratum	Instar, *n* (%) 1	2	3	4	5	Chi-square	df	*P*
'Bragg'	upper	32 (36)	17 (29)	19 (37)	10 (40)	2 (50)	1.61*	4	0.807
7 July 1987	lower	56 (64)	41 (71)	33 (63)	15 (60)	2 (50)			
V5.1									
'Bragg'	upper	17 (23)	5 (14)	3 (10)	3 (15)	2 (20)	13.82†	8	0.086
14 July 1987	middle	34 (47)	11 (31)	10 (33)	11 (55)	6 (60)			
V6.9	lower	22 (30)	20 (56)	17 (57)	6 (30)	2 (20)			
'Centennial'	upper	18 (36)	3 (14)	1 (7)	2 (22)	1 (14)	24.25†	8	0.002
5 July 1988	middle	27 (54)	12 (57)	6 (40)	1 (11)	3 (43)			
V10.2	lower	5 (10)	6 (29)	8 (53)	6 (67)	3 (43)			
'Bragg'	upper	3 (9)	5 (14)	0	4 (24)	3 (19)	21.04†	8	0.007
2 September 1988	middle	25 (78)	21 (60)	13 (45)	7 (41)	10 (62)			
V12.6 R5	lower	4(12)	9 (26)	16 (55)	6 (35)	3 (19)			
'Bragg'	upper	3(11)	7 (17)	5 (12)	15 (31)	15 (43)	19.00*	8	0.015
10 September 1988	middle	20 (71)	26 (63)	23 (55)	24 (49)	13 (37)			
V12.5 R5	lower	5 (18)	8 (20)	14 (33)	10 (20)	7 (20)			
'Bragg'	upper	5 (50)	4 (17)	4 (11)	8 (27)	22 (34)	15.22*	8	0.055
17 September 1988	middle	5 (50)	18 (75)	23 (62)	16 (53)	32 (49)			
V12.7 R6	lower	0	2 (8)	10 (27)	6 (20)	11 (17)			

* Satisfies the criteria of Cochran (1942) for cell expected values.

† Satisfies the criteria of Slakter (1965) for cell expected values.

Plant phenology influenced the differences in vertical distribution patterns among instars. Of the seven plant growth stage classes examined, differences in distribution patterns among instars were detected only in those with mean growth stages >V10 (Table 4). Where differences were found, first and fifth instars tended to occur higher (more frequently in upper strata and (or) less frequently in lower strata) than third and fourth instars. Second instars were intermediate in their distribution.

The relatively higher distribution of first instars observed on larger plants can be explained by the oviposition habits of adults. Both Daigle et al. (1988) and Rice and Drees (1985) reported that a large proportion of the eggs occurred at heights corresponding to our upper strata at all plant stages, but few occurred at heights corresponding to our lower strata on larger plants. Differences in distribution among other instars are not as easily explained, but it is possible that microclimate and host quality were involved. Instars differ in sensitivity of development rate to high temperatures (Spurgeon and Mack 1990), thus temperature gradients may differentially affect the distributions of different instars.

Table 4. Chi-square analyses of differences in vertical distributions on soybean of *S. festinus* instars in plant strata of pooled growth stage classes.

Growth Stage Class*	Plant Stratum	Instar, *n* (%)					Chi-square	df	*P*
		1	2	3	4	5			
V2.2 - V3.2	upper	96 (38)	15 (33)	5 (71)	1 (50)	–	3.89	3†	0.27
	lower	160 (62)	30 (67)	2 (29)	1 (50)	–			
V5.1	upper	41 (35)	17 (27)	20 (34)	10 (40)	2 (50)	2.09	4	0.72
	lower	77 (65)	45 (73)	38 (66)	15 (60)	2 (50)			
V6.5 - V7.2	upper	33 (19)	12 (13)	4 (8)	4 (13)	2 (17)	13.62	8	0.09
	middle	70 (40)	27 (29)	17 (35)	14 (45)	7 (58)			
	lower	71 (41)	53 (58)	28 (57)	13 (42)	3 (25)			
V8.0 - V8.9	upper	12 (17)	3 (5)	2 (6)	3 (12)	3 (13)	13.39	8	0.10
	middle	45 (65)	41 (65)	17 (50)	16 (62)	15 (65)			
	lower	12 (17)	19 (30)	15 (44)	7 (27)	5 (22)			
V10.2 - V11.6	upper	29 (25)	4 (8)	1 (4)	4 (18)	2 (10)	36.75	8	< 0.01
	middle	69 (60)	29 (60)	13 (46)	5 (23)	9 (45)			
	lower	17 (15)	15 (31)	14 (50)	13 (59)	9 (45)			
V12.1 - V12.7	upper	23 (13)	22 (13)	8 (5)	19 (17)	30 (25)	53.87	8	< 0.01
	middle	135 (75)	106 (61)	86 (56)	57 (52)	68 (56)			
	lower	22 (12)	47 (27)	59 (39)	33 (30)	24 (20)			
V13.4 - V15.9	upper	9 (26)	2 (9)	3 (17)	7 (27)	10 (45)	16.24	8	0.04
	middle	23 (66)	16 (70)	11 (61)	10 (38)	8 (36)			
	lower	3 (9)	5 (22)	4 (22)	9 (35)	4 (18)			

* Range of mean growth stages of pooled sample dates.

† Degrees of freedom are reduced by the absence of fifth instars from the table.

Most main stem girdles occurred in lower strata regardless of plant phenology (Table 5). In all growth stage classes, main stem girdles above the third internode were few and relatively evenly distributed. Rice and Drees (1985) reported that 88 to 93% of main stem girdles occurred on the hypocotyl and lowest internode. Our data were similar except for slightly higher numbers of girdles at the second internode.

Occurrence of petiole girdles in growth stage classes <V6 was similar in upper and lower plant strata (Table 6). The high incidence of petiole girdles in lower strata was due to the high frequency of girdling of unifoliolate petioles, many of which began to senesce and abscise by growth stage V7. The bulk of petiole girdles occurred in middle strata of plant growth stage classes >V6. The proportion of petiole girdles occurring in upper strata exceeded 25% only when growth stage was >V13. Except for the conspicuous scarcity of petiole girdles in lower plant strata, vertical distributions of petiole girdles were similar to those of nymphs, with most occurring near the bottom of the plant canopy.

Similarities between the distributions of girdles and nymphs were not compared statistically because petiole girdles are persistent and remain until abscission of the petiole. Consequently, a given girdle could have been recorded on more than one sample date. No acceleration of the senscence or abscission

Table 5. Vertical distributions of main stem girdles in strata of soybean plants of pooled growth stage classes.

		% of Main Stem Girdles in Plant Strata		
Growth Stage Class*	*n*†	Lower	Middle	Upper
V2.2 - V3.2	66	91	– ‡	9
V5.1	91	87	– ‡	13
V6.5 - V7.2	115	77	22	1
V8.0 - V8.9	102	76	24	0
V10.2 - V11.6	210	91	9	0
V12.1 - V12.7	251	87	7	6
V13.4 - V15.9	173	86	8	6

* Range of mean growth stages of pooled sample dates.
† Total number of main stem girdles observed.
‡ Only lower and upper plant strata were assigned.

processes due to girdling was noted, and many of the lowermost leaves in the later growth stage classes were girdled. Thus, the distributions of petiole girdles may have had little relation to the activities of nymphs present at a given time, particularly on older plants. Also, the contributions of adult girdling activities could not be separated from those of nymphs.

Table 6. Vertical distributions of petiole girdles in strata of soybean plants of pooled growth stage classes.

		% of Petiole Girdles in Plant Strata		
Growth Stage Class*	*n*†	Lower	Middle	Upper
V.2 - V3.2	30	43	– ‡	57
V5.1	115	47	– ‡	53
V6.5 - V7.2	244	30	61	9
V8.0 - V8.9	253	9	83	8
V10.2 - V11.6	616	15	75	10
V12.1 - V12.7	707	3	73	24
V13.4 - V15.9	266	6	58	36

* Range of mean growth stages of pooled sample dates.
† Total number of petiole girdles observed.
‡ Only lower and upper plant strata were assigned.

Consideration of the vertical distributions of nymphs is important in conducting sampling and control programs. Spurgeon and Mueller (1991) reported that ground-cloth methods of sampling *S. festinus* were inefficient and suffered from marked age-class bias. These factors may have been influenced by the vertical distributions of the different instars, as Mueller (1980) had previously reported that the ground-cloth was inadequate unless nymphs were relatively high on the plants. Our results also suggest that chemical control of *S. festinus* nymphs on soybean may be improved by directing treatment to the regions of the canopy where most nymphs are located. Investigation of the effects of insecticide placement on efficacy of control of *S. festinus* nymphs would be warranted.

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