Diel Patterns of Threecornered Alfalfa Hopper (Homoptera: Membracidae) Nymphs on Soybean[1]

Dale W. Spurgeon and A. J. Mueller

Department of Entomology, University of Arkansas
Fayetteville, AR 72701

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ABSTRACT Diel patterns of intra- and interplant movement, vertical distribution, molting, tending by ants, and attrition of *Spissistilus festinus* (Say) nymphs on soybean (*Glycine max* (L.) Merrill) were investigated by observing nymphs at 4-h intervals in 24-h observation sessions. Both intraplant (defined in relation to main-stem nodes) and interplant movements occurred most often between 0800 and 1200 h and least often between 2400 and 0400 h (CDT). Most (80.9%) intraplant movements covered short distances (<4 nodes). The frequency of intra- and interplant movements usually increased with nymphal age (instar). Periodicity of nymphal movement did not result in periodicity in vertical distribution. Most molts occurred between 0400 and 0800 h (85%) when daily high leaf temperatures were ≥ 31.8°C or between 0800 and 1600 h (86%) when high temperatures were ≤ 30.1°C. The frequency of tending by ants was reduced or absent at 1200 and 1600 h at two sites, but periodicity at other sites was not detected. Differences in tending among *S. festinus* instars were observed but trends were different among sites. Attrition (found dead, killed by predators, or not located) of nymphs was greatest between 0800 and 2000 h, and least between 2400 and 0400 h. Observed predation accounted for only 13% of the total attrition but underestimated actual predation.

KEY WORDS Insecta, *Spissistilus festinus, Glycine max,* diel periodicity.

Many aspects of the biology of the threecornered alfalfa hopper, *Spissistilus festinus* (Say), a pest of soybeans (*Glycine max* (L.) Merrill) in the southern U.S., are poorly known. Information concerning diel patterns is particularly limited. Johnson and Mueller (1990) studied diel patterns in flight activity of adult *S. festinus* in soybeans and alfalfa, but no information on diel patterns of the nymphal stage is currently available.

Many insects exhibit temporal patterns in behavioral or physiological activity in response to exogenous and endogenous factors (Chapman 1971). These patterns represent important components of the life histories, influencing interactions with both biotic and abiotic aspects of the environment. Beyond contributing to an understanding of an insect's basic biology, information concerning diel patterns is often necessary for the meaningful evaluation of other aspects of insect behavior or ecology (Smith 1978). Our objective was to examine intra- and interplant movement, vertical distribution, molting activity, tending by ants, and attrition of *S. festinus* nymphs for diel periodicity and to determine the effects of nymphal age (instar) on these patterns.

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Materials and Methods

Nymphs were observed at 4-h intervals during 24-h observation sessions in Arkansas soybean fields during the summers of 1987 to 1989. Three sessions (17 July, 24 and 26 August) during 1987 were conducted in 'Bragg' soybeans planted in 0.91-m rows in Little River County. During 1988 one session (18 July) was conducted in 'Bragg' soybeans (0.91-m rows) in Little River County, and two sessions (1 and 2 August) were conducted in 'Lee' soybeans (0.97-m rows) in Washington County (University of Arkansas Agricultural Experiment Station). During 1989 three sessions (26, 27, and 28 August) were conducted in 'Centennial' soybeans (0.91-m rows) in Little River County. Also, five sessions (12, 13, 17, 22, and 23 August) were conducted in 'Lee' and three sessions (7, 19, and 20 September) in 'Bragg' soybeans (0.97-m rows) in Washington County.

Twenty-five nymphs (5 of each instar) on separate plants were observed in each session in 1987. Because nymphs were difficult to locate and identify following interplant movements, death, or molting, in 1988 and 1989 they were marked with fluorescent powder (Rocket Red New A-13-N fluorescent pigment, Day-Glo Color Corp., Cleveland, OH) applied with a small paint brush. This pigment has been used in studies of other insects without apparent toxicity or behavioral effects (Turner and Gerhardt 1965, Sheppard et al. 1973). Marked nymphs were highly visible during daylight hours and were easily located at night with the aid of a hand-held fluorescent flash light fitted with a 'black-lite' bulb. Because marked nymphs were easier to locate, 50 nymphs (10 of each instar) were observed in each session in 1988 and 1989.

During each session, plants of similar size were selected at about 1-m intervals from two adjacent rows. Each plant was marked with a wire flag (1987) or fluorescent powder (1988-1989), and an upper leaf was numbered with a permanent marker. Occasionally the canopies of adjacent plants interfered with observations of marked plants. Therefore, some leaves and lateral branches of adjacent plants were pruned. Pruning was not sufficient to be detected by casual visual examination. Growth stage (Fehr et al. 1971) and the lowest structure-bearing node (leaf, lateral branch, or raceme) of each marked plant were recorded.

When possible, nymphs already present on marked plants were observed. Otherwise, the desired instars were collected outside the study area. Collected nymphs were placed on marked plants as nearly as possible at the same height and plant structure that they had occupied prior to collection.

Observation sessions started at 1200, 1600, 2000, or 2400 h CDT, depending on the time required to set up each session. Nymphs were left undisturbed for ≥ 4 h before the first observation. Vertical location and plant-part (main-stem, lateral branch, petiole, leaf, bud, raceme) occupied by each nymph, interplant movement, molts, predation, and tending by ants were recorded during most sessions. Interplant movements, molting, and ant tending were not recorded during all sessions in 1987 because some nymphs could not be identified positively. Vertical location was determined by counting main-stem nodes from the cotyledonary node (node 0). For this purpose a 'node' also included the internode directly below it. Nymphs that were not located were recorded as missing. Molted, killed, or missing nymphs were immediately replaced. In addition, temperature of five shaded leaves in the lower canopy were measured at the conclusion of each observation period using a hand-held thermal infrared thermometer (model 112C, Everest Interscience, Inc., Tustin, CA.) Observations in each 4-h period required about 0.5 - 1 h, depending on the number of nymphs moved or missing.

Diel patterns were examined by comparing the frequencies of events occurring between observation periods in contingency tables, using the Chi-square statistic of the SAS procedure PROC FREQ (SAS Institute 1988). When statistical significance ($P \leq 0.05$) was indicated, individual cells were examined to determine their contribution to the Chi-square statistic. When >20 percent of cell expected values were <5 the Chi-square test was considered inappropriate (Cochran 1942) and Fisher's exact test was used (the EXACT option of PROC FREQ). Data from each session were examined for periodicity. Also, most data were examined for heterogeneity within sites and among sites (Zar 1984) and pooled whenever possible. When cell expected values were small, differences within and among sites were examined using Fisher's exact test. When within-site heterogeneity was indicated, sessions were examined for pooling according to daily high leaf temperatures.

Intranode movements were considered trivial so only nymphs that changed vertical location (node) were considered to have moved in analysis of the frequency of intraplant movements. When distances of intraplant movements of instars were examined, tables were collapsed so movements were classified as < 4 nodes or ≥ 4 nodes. For analysis of diel patterns in vertical distribution, data were tested for heterogeneity and pooled into five groups based on similarity of mean plant growth stages (Table 1). Vertical locations also were combined to form lower, middle, and upper plant strata (Table 1). The lower stratum extended from the soil surface to and including the mean lowest structure-bearing node among plants in a growth stage group. About one-half of the remaining nodes were assigned to each middle and upper strata.

Results and Discussion

Periodicity of intraplant movement occurred in 13 of 16 sessions examined (12 sessions, $P < 0.01$; 1 session, $P < 0.05$). Movements were most frequent between 0800 and 1200 h in 10 sessions, between 1200 and 1600 h in one session, and between 2000 and 2400 h in two sessions. However, movement was much more frequent than expected in the first observation period of six sessions, indicating that the time between nymphal establishment and the first observation period was probably insufficient. Heterogeneity testing among the remaining 10 sessions resulted in two groups of sessions pooled according to daily high mean leaf temperatures (7 sessions, 35.7 - 40.3°C; 3 sessions, 23.9-30.1°C). Daily high leaf temperatures were observed at 1600 h in all sessions except 13 August 1989 (30.1°C, 1200 h). Periodicity of intraplant movement was observed in both groups (35.7 - 40.3°C, n = 1575, Chi-square=107.97, df=5, P<0.01; 23.9 - 30.1°C, n = 873, Chi-square = 69.47, df =5, P < 0.01) (Fig. 1). Nymphs moved less frequently in sessions with lower temperatures (movement on 12% of observations) than when temperatures were higher (movement on 18% of observations). Intraplant movements occurred most frequently between 0800 and 1200 h and least frequently between 2400 and 0400 h regardless of temperature.

Table 1. Pooled soybean growth stage groups and assignments of plant strata.

	Nodes† Assigned to Plant Strata		
Growth Stage Group*	Lower	Middle	Upper‡
V6.8 - V10.6	0 - 2	3 - 5	6 - 12
V12.7	0 - 3	4 - 8	9 - 14
V13.2	0 - 3	4 - 8	9 - 15
V14.4 - V14.7	0 - 3	4 - 9	10 - 17
V17.5	0 - 4	5 - 11	12 - 19

* Range of mean growth stages of pooled observation sessions.
† Nodes are numbered from the cotyledonary node (node O) and include the subtending internode.
‡ Range of nodes accommodates the tallest plant in each growth stage group.

Differences among instars in frequency of intraplant movement were indicated in five sessions ($P < 0.05$) but trends were not always consistent among sessions. No within-site heterogeneity was detected but among-site heterogeneity was indicated ($0.025 > P > 0.01$). Heterogeneity testing among sessions grouped according to daily high mean leaf temperatures resulted in three groups of pooled sessions (> 36°C, 5 sessions; 32 - 36°C, 6 sessions < 32°C, 6 sessions). Older nymphs moved more frequently than younger nymphs when daily high leaf temperatures were > 32°C, except that the frequency of movement of fifth instars was reduced when high temperatures were > 36°C (> 36°C, n = 1010, Chi-square = 13.03, df = 4, $P = 0.01$; 32 - 36°C, n = 1665, Chi-square = 23.41, df = 4, $P < 0.01$) (Fig. 2). Frequency of movement was similar among instars when high temperatures were < 32°C (n = 1747, Chi-square =1.99, df = 4, $P = 0.74$) (Fig. 2).

The longest intraplant distance moved was 12 nodes but only 19.1% of movements were ≥ 4 nodes. Fisher's exact test indicated differences among instars in distances moved ($P < 0.05$) in only two sessions but sample sizes in individual sessions were small ($29 \le n \le 88$). When sessions within sites were examined by Fisher's exact test for differences in frequencies of short (< 4 nodes) and long (≥ 4 nodes) intraplant movements for each instar differences for second instars were indicated within two sites. One session in each site appeared to be anomalous (24 August 1987, 23 August 1989). No differences were found within or among sites when these sessions were excluded, so remaining sessions were pooled. The proportion of intraplant movements ≥ 4 nodes increased with nymphal age from 8% (first instar) to 28% (fifth instar) (n = 783, Chi-square = 33.60, df = 4, $P < 0.01$).

Nymphs moved between plants in 12 of 14 sessions examined but interplant movements were generally few. No differences in periodicity or frequencies of instars moving were detected within or among sites, so all sessions were pooled for both analyses. Interplant movements occurred most frequently between 0800 and 1200 h and least frequently between 2400 and 0400 h (n = 4039, Chi-square = 23.97, df = 5, $P < 0.01$) (Fig. 3), and the frequency of interplant movements approximately doubled with each increasing instar from 2 (0.25% of first instar observations) to 24 (2.96% of fifth instar observations).

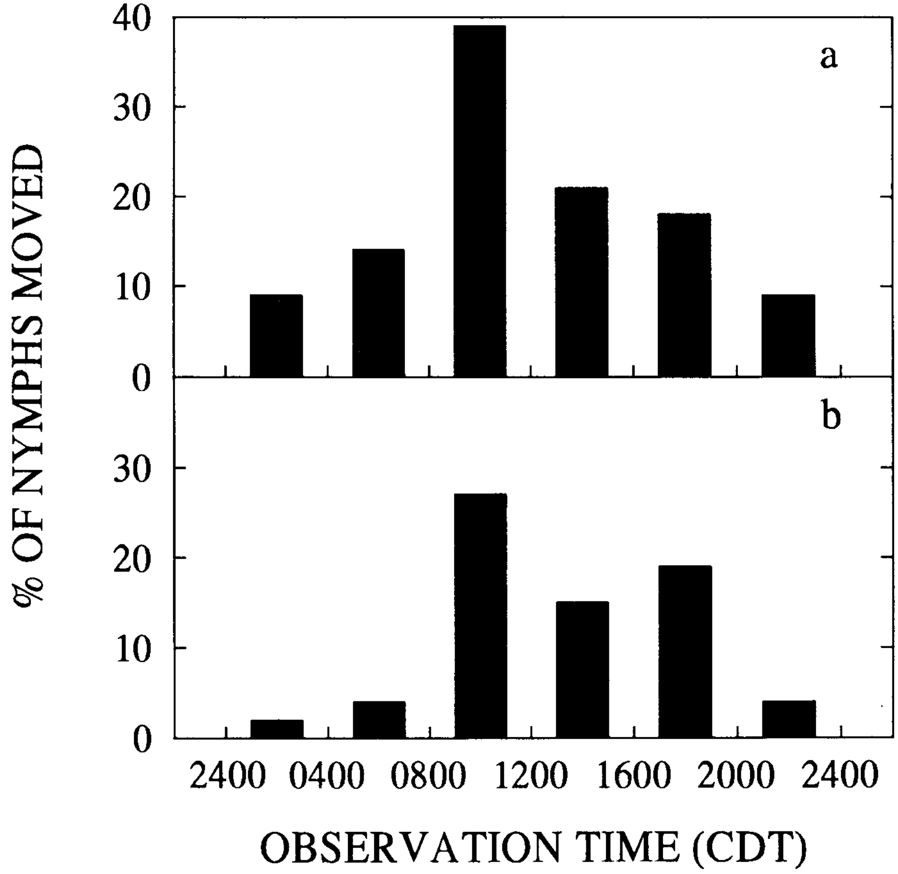

Fig. 1. Diel patterns of intraplant movements of *S. festinus* nymphs on soybean in observation sessions pooled by daily high mean temperature of lower canopy leaves; a) 35.7 - 40.3°C; b) 23.9 - 30.1°C.

Jordan (1952) attributed the production of most plant damage (girdles) to nymphs because of their sedentary nature. Although the frequencies of intra- and interplant movements that we observed appear low (16% and 1% of observations, respectively), observations were made at 4 h intervals so nymphs averaged about 1 movement nymph^{-1} d^{-1}. Furthermore, nymphal development is likely to require about 18 to 25 d according to published development times (Meisch and Randolph 1965, Mitchell and Newsom 1984, Spurgeon and Mack 1990), so nymphs appear to be more active than was previously reported.

Nymphal movement may affect the distribution and number of injuries (girdles) to plants. Although most intraplant movements were < 4 nodes (80.9%) a movement of one node can result in increased plant injury. Also, girdle-producing instars (Moore and Mueller 1976) tend to move farther and more often than younger instars.

Despite the observed patterns in intra- and interplant movements diel changes in vertical distribution of nymphs were observed in only two sessions

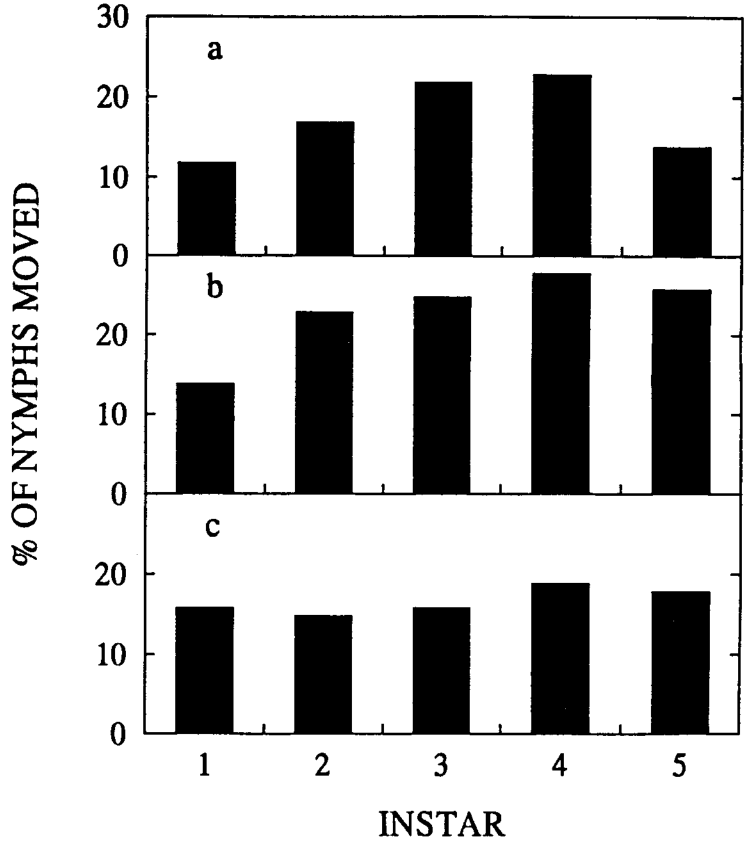

Fig. 2. Intraplant movements of *S. festinus* instars on soybean in observation sessions pooled by daily high mean temperatures of lower canopy leaves; a) >36°C; b) 32 - 36°C; c) <32°C.

(24 August 1987, $n = 144$, Chi-square = 19.02, df = 10, $P = 0.02$; 12 August 1989, $n = 291$, Chi-square = 65.87, df = 10, $P < 0.01$). Trends observed on 12 August 1989 did not resemble a diel pattern but appeared to be caused simply by nymphs moving from their original location. A similar trend was not observed in the following session (13 August 1989). No differences among sessions within growth stage classes were detected when 12 August 1989 was excluded. No periodicity in vertical distribution was detected in any growth stage class (Table 2). The method of plant strata assignment may have reduced the sensitivity of the analysis; however, small net changes in vertical distribution were probably observed because most nymphs moved short distances and nymphs often moved in opposite directions during a given observation interval.

Periodicity of molting was observed ($P < 0.05$) in 10 of the 15 sessions in which molting occurred. Few nymphs (1-7) molted in sessions where periodicity was not indicated. Most molts occurred between 0400 and 0800 h (73-100%) in

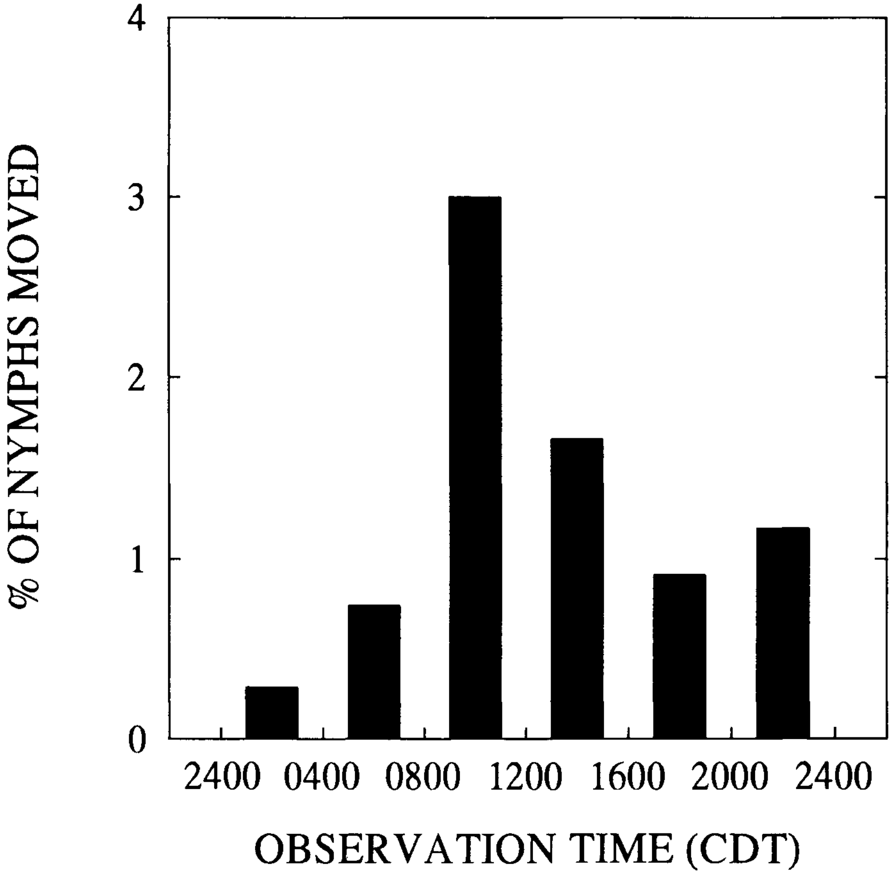

Fig. 3. Diel pattern of interplant movements of *S. festinus* nymphs on soybean.

eight sessions and between 0800 and 1200 h (80%) or 1200 and 1600 h (50%) in the remainder. Daily high mean leaf temperatures were ≥ 32°C in sessions in which molting was most frequent between 0400 and 0800 h and ≤ 30.1°C in the other sessions, so sessions were divided into two groups according to daily high leaf temperatures (31.8 - 40.0°C, 10 sessions; 23.9 - 30.1°C, 5 sessions). Fisher's exact test detected no differences among sessions within groups but heterogeneity testing indicated differences between groups. Periodicity of molting was indicated in both groups (31.8 - 40.0°C, n = 2720, Chi-square = 297.07, df = 5, $P < 0.01$; 23.9 - 30.1°C, n = 1461, Chi-square = 38.78, df = 5, $P < 0.01$) (Fig 4). Molts occurred later in the day and were less synchronized in the group of sessions with lower temperatures than in the group with higher temperatures.

Mechanisms responsible for the apparent effects of temperature on synchrony and periodicity of molting were not identified. However, high-temperature inhibition of development has been reported for some instars (Spurgeon and Mack 1990) and may have influenced periodicity of molting when temperatures were high.

Table 2. Chi-square analyses of diel variations in vertical distribution of *S. festinus* nymphs on soybean.

Growth Stage Group	% of Nymphs in Plant Strata*			*n*	Chi-Square	df	*P*
	Lower	Middle	Upper				
V6.8 - V10.6	42 - 54	36 - 42	9 - 16	715	6.59	10	0.76
V12.7	29 - 42	57 - 71	0 - 1	586	-†	-†	0.64
V13.2	25 - 28	59 - 61	13 - 14	871	0.40	10	1.00
V14.4 - V14.7	21 - 36	58 - 72	5 - 7	840	15.09	10	0.13
V17.5	23 - 27	63 - 67	10 - 11	1165	1.34	10	1.00

* Range from observations at six times day.
† Fisher's exact test.

Ant tending of *S. festinus* nymphs was observed in 12 observation sessions and periodicity of tending was detected in four of them (24 and 26 August 1987, 1 and 2 August 1988). Tending activity in these sessions was reduced or absent at 1200 and 1600 h. In addition, tending was not observed at 0800 h on 1 August 1988 but occurred frequently (23% of nymphs) at that time in the following session. Ants apparently had not located the nymphs by the first observation (0800 h) on 1 August 1988 so this session was excluded from further analyses. No other differences were detected among sessions within sites. Periodicity of tending was detected in only two sites ('Bragg', 24 and 26 August 1987, *n* = 286 , Chi-square = 57.71, df = 5, *P* < 0.01; 'Lee', 2 August 1988, *n* = 269, *P* < 0.01, Fisher's exact test). Tending occurred least often at 1200 and 1600 h (0-5% of nymphs) and most often at 0400 and 0800 h (18-23%) on 2 August 1988 and 0400 h (56%) at the 'Bragg' site.

Differences in frequency of tending were also observed among instars in 9 sessions. Within-site heterogeneity was detected in only one site ('Lee', 12, 13, 17, 22, and 23 August 1989). No differences among instars were detected on 12 or 22 August. On 13 August second and third instars were most frequently tended (27 and 33%, respectively) and first, fourth, and fifth instars were least often tended (10 - 15%) (*n* = 287, Chi-square = 14.52, df = 4, *P* < 0.01). On 17 and 23 August third and fourth instars were most frequently tended (25-34%) and first, second, and fifth instars were least often tended (7-19%) (17 August, *n* = 296, Chi-square = 17.20, df = 4, P < 0.01; 23 August, *n* = 295, Chi-square = 17.29, df = 4, P < 0.01). Causes of these inconsistencies could not be determined. Differences among instars were detected in three other sites (Table 3). Third and fourth instars were usually tended more often than other instars and first instars were least often tended, but trends for other instars were inconsistent among sites. Nickerson et al. (1977) reported that *S. festinus* nymphs were tended by five ant species in a study in Florida soybeans. Ant species were not identified in our study, and presence of different species at different sites or more than one species at a site may have obscured periodicity and differences among instars in tending at some sites.

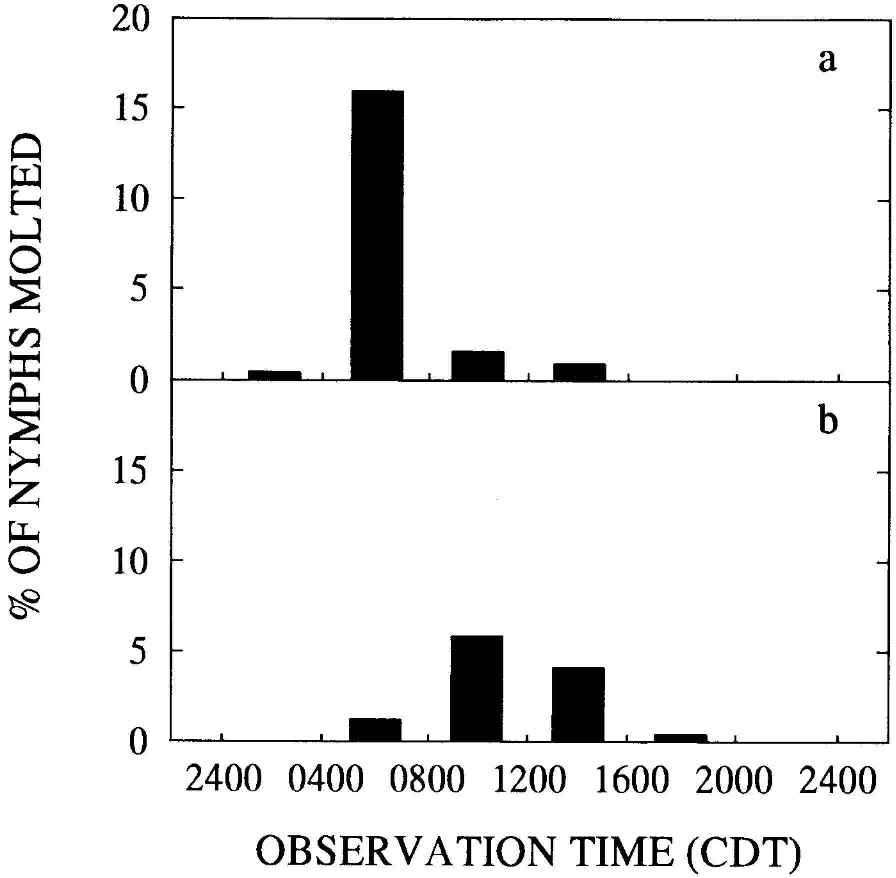

Fig. 4. Diel patterns of *S. festinus* molting on soybean in observation sessions pooled by daily high mean temperatures of lower canopy leaves; a) 31.8 - 40.0°C; b) 23.9 - 30.1°C.

A total of 181 nymphs was recorded as missing (including those found dead or killed by predators). Overall, the frequency of missing instars ranged from 30 (fifth instar, 3% of observations) to 46 (first instar, 5% of observations) but no differences among instars were detected ($n = 4649$, Chi-square = 4.72, df = 4, $P = 0.32$).

Fisher's exact test indicated periodicity of nymphal attrition in only two sessions (17 July 1987, $n = 150$, $P < 0.01$; 7 September 1989, $n = 300$, $P = 0.02$) and attrition was greatest between 1600 and 2000 h in both. However, the small number of missing nymphs in individual sessions (2-23 nymphs) probably resulted in low power in most tests. Fisher's exact test failed to detect differences among sessions within sites and no heterogeneity was detected among sites so all sessions were pooled. Nymphal attrition was greatest between 1600 and 2000 h and least between 2400 and 0400 h ($n = 4649$, Chi-square = 28.56, df = 5, $P < 0.01$) (Fig. 5). Attrition also was high between 0800 and 1600 h.

Table 3. Differences among *S. festinus* instars on soybean in frequency of tending by ants at three sites.

	Instars Tended								
	1	2	3	4	5				
Site	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	*n*	Chi-square	df	*P*
Bragg 1987	6 (11)	8 (14)	20 (35)	26 (45)	21 (36)	286	25.17	4	<0.01
Lee 1988	1 (1)	11 (10)	14 (13)	23 (21)	4 (4)	555	31.76	4	<0.01
Bragg 1989	2 (2)	19 (16)	47 (40)	30 (25)	1 (1)	586	89.06	4	<0.01

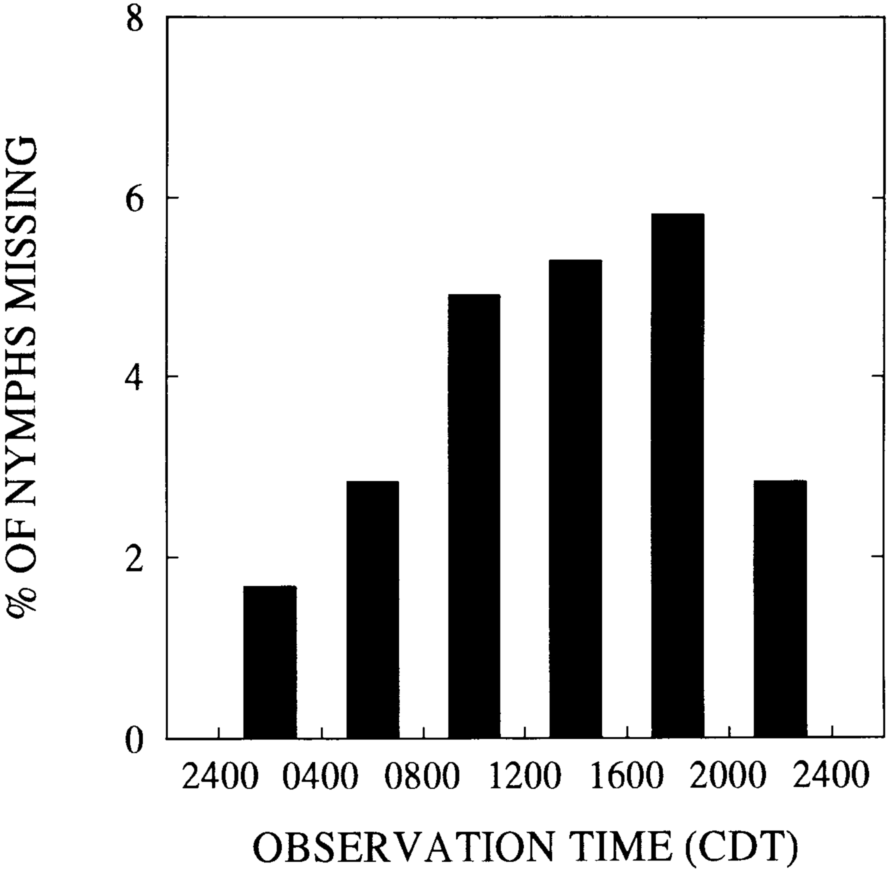

Fig. 5. Diel pattern of nymphal attrition of *S. festinus* on soybean.

Predation was observed only 24 times (13% of missing nymphs). Observed predators included nabids (11), *Geocoris* spp. (6), lacewing larvae (3), spiders (2), a coccinellid larva (1), and an *Orius* spp. adult (1). Observations of predation were too few for meaningful tests of periodicity or differences among instars in individual sessions or sites. Overall, the periodicity of observed predation could not be distinguished from that of other missing nymphs (n = 181, P = 0.37, Fisher's exact test). Neither were differences among instars detected (n = 4492, P = 0.14, Fisher's exact test). The low frequency of observed predation and the mixture of predator species may have precluded adequate power in these tests.

Predation was often difficult to observe because nabids tended to drop the nymphs and escape and *Geocoris* tended to flee with the nymphs. Also, handling times of predators for *S. festinus* nymphs are unknown but are probably much shorter than the time between observations. Thus, predation was probably grossly underestimated. Graham (1938) suggested that natural enemies play a minor role in *S. festinus* population regulation. Jordan (1952) indicated that few natural enemies of *S. festinus* have been reported, and that the only one of possible importance in his study was a Strepsipteran parasite. Our study did not adequately evaluate the impact of predators on *S. festinus* populations but our observations suggest that interactions between *S. festinus* and predators on soybean should be investigated.

Our data demonstrate the occurrence of several diel patterns of *S. festinus* nymphs. The data offer no information on the mechanisms controlling these patterns but could be valuable in planning studies in which time-of-day effects are a concern, such as sampling or behavioral studies. Investigation of diel patterns of other behaviors, such as feeding or girdling, and of the controlling mechanisms would contribute greatly to a more comprehensive understanding of the interactions between *S. festinus* and its environment in soybeans.

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