## THE DIURNAL ETHOLOGY OF THE SOUTHERN GREEN STINK BUG, NEZARA VIRIDULA (L.), IN COWPEAS

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#### ABSTRACT

A behavioral catalogue of the adult southern green stink bug, *Nezara viridula* (L.), was developed from observations taken in a cowpea field. A total of 23 behaviors are described, and the effects of sex, mating status, time of day, height on the plant, and substrate on these behaviors are quantified. Of the seven most common behaviors, which occupied 97% of the total observation time, sex significantly influenced the duration and/or frequency of kicking, rocking, and mating. Mating status significantly affected the frequency of antennal waving and walking. Time of day had a significant effect on the duration and/or frequency of resting, antennal waving, feeding, kicking, and mating. Height on the plant significantly affected the duration and/or frequency of resting, antennal waving, walking, feeding, kicking, rocking, and mating. Substrate significantly influenced the duration and/or frequency of antennal waving, feeding, kicking, and mating. The proportion of time spent basking on the tops of plants decreased significantly from morning to noon and from noon to afternoon.

Key Words: Hemptera, Pentatomidae, behavior, ethogram, feeding, mating.

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#### INTRODUCTION

The southern green stink bug, Nezara viridula (L.), is a cosmopolitan pest of numerous crops (DeWitt and Godfrey 1972). Despite this pest's economic impact, only a few, isolated behaviors have been examined. Studies of specific behaviors have generally been conducted in the laboratory without the benefit of understanding the field behavior, or ethology, of N. viridula.

As observed by Lockwood and Story (1985), research on the behavior of insect pests has largely been directed at either the behavior of individuals in the laboratory or populations in the field; there has been little consideration of the ethology of the individual in its natural habitat. An understanding of an insect's behavior is fundamental to developing a responsible and effective integrative pest management system. In turn, a behavioral catalogue is the necessary starting point for an understanding of an insect's behavior (Eibl-Eibesfeld 1975; Lehner 1979). The relevance of behavior to the integration of control strategies for N. viridula was recognized by Waite (1980). The present study should be particularly valuable in providing a field context for the interpretation of previous and future behavioral studies of N. viridula.

The purpose of this study was to develop a behavioral catalogue for N. viridula and then to analyze the frequency and duration of behaviors in context of the biology and temporal/spatial ecology of the insect. This work is similar in design

to Lockwood and Story's (1985) study of the ethology of the green stinkbug, Acrosternum hilare (Say). This continuity of methodology allows for comparisons of pentatomid biology and an opportunity for insight with regard to the adaptive functions and the convergent evolution of behavior.

#### MATERIALS AND METHODS

Nezara viridula adults were observed in a cowpea field at the Burden Research Center, Baton Rouge, Louisiana, between September 28 and October 24, 1984. Data were collected using focal animal sampling (Lehner 1979) by a single observer during three time blocks: morning (0700 - 1100 h), noon (1100 - 1500 h), and afternoon (1500 - 1900 h). Eight to 10 individuals of each sex were observed during each time block.

Subjects were located in the field at a distance of several meters to avoid eliciting escape or alarm behavior. The observer slowly approached the insect to a distance of ca. 0.5 m and observed its behavior for a period of 30 min or until the insect flew far enough away such that it could not be located. Each behavior and its duration were recorded, which provided measures of both the frequency and duration of each behavior. The only case in which a behavior was not recorded due to simultaneous occurrence of another behavior was antennal movements, which were not recorded during locomotion. In addition to the duration of each behavior, the subject's location with respect to sunlight, height on the plant (top, middle, or bottom one-third) and substrate (leaf, stem, or pod) was recorded. The mean frequency of a behavior was defined as the number of discrete occurrences of the behavior per hour. The mean duration of behavior was defined as the mean number of minutes the given behavior lasted in all instances in which it occurred. The proportion of time spent in a behavior was calculated by dividing the total time engaged in the behavior by the total observation time. Fagen and Goldman (1977) developed an index of sample coverage,  $0 = 1 - (N_1/I)$ , where 0 is the estimated degree of sample coverage (0 = no coverage, 1 = complete coverage),  $N_1$  is the number of behaviors seen only once, and I is the total number of behaviors seen. This index was used to estimate the degree of completeness of the behavior catalog. For each behavior accounting for more than 0.25% of the total observation time, the effects of sex, mating status, time of day, height on the plant, and substrate, on duration were assessed by analysis of variance and the 1sd post-ANOVA test. The frequency and proportion of time spent in each behavior were analyzed with respect to the aforementioned variables using chisquare tests. Behaviors not meeting the criterion of occupying more than 0.25% of the total observation time were too uncommon to be meaningfully analyzed. The proportions of time spent basking and spent at each height on the plant during each time block were compared using a chi-square test. In all statistical tests, variables were considered significantly different at  $P \leq 0.05$ .

### **RESULTS AND DISCUSSION**

The sample coverage was 0.83 for both females and males, indicating relatively complete coverage. Twenty-three behaviors were documented and classified into eight categories: resting, mating, feeding, locomotion, grooming, positional orientation, componential movements, and excretion. Table 1 shows the proportion of the time

n of time spent in each behavioral category by N. viridula with respect to sex, mating status, time of day,	
by I	
ategory ]	
Table 1. Proportion of time spent in each behavioral ca	height on plant, and substrate.

						10 %	% of total time spent	spent					
Behavioral		Sex	Mating	Mating status	Ĺ	Time of day	day	H	Height on plant	plant	S	Substrate	<i>.</i>
category	Male	Male Female	Yes	No	Morning	Noon	Morning Noon Afternoon	Top	Middle	Top Middle Bottom	Leaf	Stem	Pod
Resting	88 a	44 b	1	65	38 a	41 a	62 a	3 <b>4 a</b>	20 a	30 a	66 a	31 b	34 b
Mating	7 a	12 a	63	ł	21 a	29 a	11 a	54 a	17 a	25 a	21 ab	35 a	d 6
Feeding	3 a	34 b	30 a	23 a	27 a	21 a	21 a	6а	36 a	36 a	5 a	16 a	52 b
Locomotion	2 a	8 a	6 a	10 a	12 a	8 a	5 а	5а	24 a	8 a	7 a	16 a	4 a
Grooming	0 a	1 a	0 a	1 a	1 a	0 a	0 а	1 a	1 a	0 a	1 a	0 а	0 a
Positional													
orientation	0 a	0 a	1 a	0 a	0 a	1 a	0 a	0 a	1 a	0 a	0 a	0 a	0 a
Componential													
movements	0 a	1 a	0 a	1 a	1 a	0 a	1 a	0 a	1 a	1 a	0 a	l a	1 a
Excretion	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 а	0 a	0 a	0 a

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occupied with respect to sex, mating status, time of day, height on plant, and substrate for each category of behavior. The mean durations and frequencies of behaviors which occupied more than 0.25% of the total observation time are shown with respect to sex, mating status, time of day, height on plant and substrate in Tables 2 and 3, respectively. The behaviors characterized in these two tables accounted for 99% of the total observation time.

Resting was characterized by a state of complete immobility of all body parts of an insect which was not mating, during which the ventral surface was held close to the substrate. Resting was clearly the most common behavior in males but involved less than half of the total observation time of females. This difference was made up by the disproportionate amount of time that females spent feeding, as will be discussed later. Resting was also the most common behavior found in field studies of *A. hilare* (Lockwood and Story 1985). Corpuz (1969) observed that during hot weather, *N. viridula* rests on leaves at midday; if mating is included as resting in the present study, our results would agree with these earlier observations.

Mating included the following six behaviors which were seen while the insects were in copulo. Kicking was characterized by an individual rapidly stroking, with its hind legs, the ventrolateral abdomen of its parnter. Rocking involved an individual raising and lowering its abdomen by straightening and bending its hind two pairs of legs. Rocking and kicking were seen only in males. Arching was a rarely observed behavior in which the tips of the abdomens of a mating pair were raised together to an acute angle and held in this position for several seconds. As opposed to rocking, this behavior could not be attributed to the action of either individual alone. Mating females exhibited another rarely observed behavior in which the hind pair of legs were lifted so that the tarsi were held above the male's abdomen for several seconds to over a minute. Butting was seen in a single male which struck its head several times against a mated female. Finally, it should be noted that mating was defined as the condition in which an individual remained motionless while in copulo. Given this definition, mating durations and frequencies do not indicate the length of time a pair remained in copulo or the rate at which stink bugs changed mating parnters. No observations were made of stink bugs beginning or ending copulation.

While courtship was not observed, other aspects of mating can be compared with previous accounts of N. viridula reproductive behavior. Harris and Todd (1980a) reported that in studies of caged insects, 45% of the time is spent in copulation, which is greater than the proportion of time observed in our study. They also reported a much greater frequency of butting than was observed in the present study. The high frequency of mating and butting reported by Harris and Todd (1980a) may have been a function of ideal sex ratios, favorable environmental conditions, and high population densities in their observation cages. The kicking behavior described in our study was tentatively termed "reinforcement" by Harris and Todd (1980a); we refer to this behavior as kicking as this label describes the observed action and it does not imply a function when none has been demonstrated. Similar behaviors have been reported in four other pentatomids, Brachymena sulcata Van D. (Ruckes 1938), B. quadripustulata (F.) (Gamboa and Alcock 1973), Murgantia histrionica (Hahn) (Lanigan and Barrows 1977), and Acrosternum marginatum (Palisot de Beauvois) (Harris and Todd 1980a). Kicking may be more frequent under field conditions than Harris and Todd (1980a) suggest in their observations of caged insects. The commonly observed rocking behavior of mated

of behaviors occupying more than 0.25% of the time of N. viridula with respect to sex, mating status, time of	
Table 2. Duration of behaviors occupying more than 0.25% of the time (	day, height on plant, and substrate.

						n L	Duration (x, sec)	eu)					
	ŝ	Sex	Mating status	status	f	Time of day	day	H.	Height on plant	plant	S	Substrate	0
Behavior	Male	Female	Yes	No	Morning	Noon	Morning Noon Afternoon	Top	Middle	Middle Bottom	Leaf	Leaf Stem	$\mathbf{Pod}$
Rest	88 a	80 a	ļ	88	93 a	52 b	119 a	59 a	119 b	134 b	103 a	73 a	78 a
Antennal wave	2 a	2 a	2 a	2 а	2 a	з а	2 a	2 a	2 а	2 а	2 a	2 a	2 a
Walk	18 a	18 a	23 a	18 a	21 a	16 a	19 a	15 a	23 a	24 a	15 a	27 a	11 a
Feed	102 a	110 a	119 a	94 a	103 ab	72 a	133 b	90 a	163 b	t	273 a	69 b	111 a
Kick	2 a	2а	2	ł	2 a	3 a	3 a	2 a	2 а	I	2 a	2 a	2 a
Rock	5 a	3 a	ņ	I	3 a	10 a	7 a	5 a	27 a	ļ	2 a	8 8	4 a
Mate	40 a	125 b	41	I	55 a	18 b	64 a	24 a	106 b	327 c	157 a	31 b	22 b
Leg rub	2 a	9 a	3 а	9а	3 a	10 a	14 a	3 <b>a</b>	14 a	10 a	10 a	9 a	2 a
Duck	2 a	2 a	2 a	2 a	2 a	2 a	2 а	2 a	2 a	I	2 a	2 a	2 a

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Table 3. Frequencies of behaviors occupying more than 0.25% of the time of N. viridula with respect to sex, mating status, time of day, height on plant, and substrate.

						Frequei	Frequency (bouts per hour)*	per hour)*					
		Sex	Mating status	status	T	Time of day	lay	Hei	Height on plant	ant	S	Substrate	
Behavior	Male	Female	Yes	No	Morning	Noon	Morning Noon Afternoon	$\operatorname{Top}$		Middle Bottom	Leaf	Stem	$\mathbf{Pod}$
$\operatorname{Rest}$	15 a	20 a	I	27	26 a	16 a	11 a	18 a	48 b	9 a	23 a	15 a	15 a
Antennal													
wave	7 a	10 a	3 a	11 b	13 a	6 p	7 b	10 ab	21 a	3 b	8 a	9 a	9 a
Walk	16 a	16 a	10 a	20 a	26 a	14 a	9 a	18 ab	36 a	4 L	16 a	21 a	13 a
Feed	6а	11 a	10 a	9а	13 a	7 b	6 b	14 a	8 8	I	1 a	9 p	17 c
Kick	28 a	0 p	45	I	27 a	13 ab	4 b	24 a	1 b	1	3 a	28 b	11 a
Rock	8 a	q 0	13	ł	4 a	6а	1 a	7 a	1 b	I	0 a	5 a	6 a
Mate	33 a	4 b	60	I	13 a	22 a	12 a	30 a	5 b	6 b	5 а	37 b	14 a
Leg rub	24 a	19 a	44 a	18 a	64 a	14 b	3 b	29 a	21 a	22 a	13 a	29 a	5 а
Duck	76 a	80 a	109 a	92 a	119 a	68 a	49 a	115 a	109 a	I	12 a	63 a	85 a
* Behavioral parameters fol	arameters fo	ollowed by	different le	tters diffe	ər significantı	y (P = 0).	llowed by different letters differ significantly ( $P = 0.05$ ) according to the chi-square test	to the chi-se	quare test.				

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males and, less often, females appear to be the same as the tipping behavior associated with sound production in N. viridula (Harris 1980) and other pentatomids (Leston 1954; Jordan 1958). Rocking also appears to be similar to more rapid movements during mating in M. histrionica (Lanigan and Barrows 1977) and B. quadripustulata (Gamboa and Alcock 1973). The rarely observed behavior of arching resembles the raised position which occurs at the start of copulation in N. viridula (Mitchell and Mau 1969) and B. quadripustulata (Gamboa and Alcock 1973), although in our study this behavior was not restricted to the first few minutes of mating. Female leg lifting has not been previously reported in N. viridula but resembles the behavior of male B. quadripustulata during mating (Gamboa and Alcock 1973). This behavior of N. viridula also bears some resemblance to the posture assumed by leaf footed bugs, e.g. Leptoglossus phyllopus (L.), when they are approached in the field (Lockwood, unpublished observations).

Locomotion was characterized by behaviors which changed the location of the entire insect. Locomotory behaviors in *N. viridula* were more common than in *A. hilare* (Lockwood and Story 1985). In *N. viridula*, flights were extremely variable in length, covering from 15 cm to over 20 m, with a single individual often making a series of short, ca. 1 m, flights. The movements observed in this study indicate a greater gross mobility than that suggested by the net dispersal estimates of Nishida (1966). Our findings support earlier observations that mating pairs are more sluggish and that while they may move about and feed, they do not fly (Drake 1920; Mitchell and Mau 1969). However, *N. viridula* was not more sluggish in the morning as suggested by Jones (1918). In Kaufman's (1966) studies of *Caliden dregii* Germer, activity was least in the morning and afternoon, the opposite of what we found for *N. viridula*.

Dropping was an unusual form or locomotion in which an insect released hold of the substrate and fell into the plant. This behavior was seen only once, when a female was competing with a wasp for access to a small pod. The dropping behavior of disturbed *N. viridula* has been previously reported (Drake 1920; Zeck 1933; Mitchell and Mau 1969) and also occurs in *Euschistus conspersus* Uhler (Alcock 1971). In our experiences with hand collecting, once one's hands are contaminated with the defensive secretion, individuals will drop and then run before they are even contacted. Thus, dropping appears to be an adaptive response to the defensive secretion which functions as an alarm or disperal pheromone, to physical contact, or to sudden movements of the substrate.

Feeding was characterized by stylet extension, in which the mouth parts were unfolded away from the body and feeding proper, in which the stylet pierced the substrate. Only occasionally did feeding, or stylet insertion, not follow stylet extension. Feeding was the longest behavior in duration and occupied a much greater portion of females' time than males'. Females often fed while *in copulo* and feeding while mating was more frequent and longer in duration than otherwise. Females probably require greater amounts of nutrients for reproduction than do males. Indirect support for this hypothesis is provided by our earlier work which found that A. *hilare* feeds very rarely when mating did not occur (Lockwood and Story 1985). Feeding during copulation is also common in M. *histrionica* (Lanigan and Barrows 1977). The temporal pattern of feeding in N. viridula was similar to that described by Corpuz (1969), with feeding being most common in the morning and least common during midday. Feeding is most pronounced in the afternoon in E. conspersus (Alcock 1971), the opposite of what we observed for N. virdula. Interestingly, excretion occupied less time in actively feeding *N. viridula* than it does in essentially nonfeeding *A. hilare* (Lockwood and Story 1985).

Grooming was defined as those behaviors which involved the movement of one body part against another, usually in rapid succession. Antennal grooming involved the insect placing the proximal end of the tibiae around the base of an antenna and pulling the tarsal segments down over the antenna two to five times. Abdominal grooming consisted of four to eight rapid, longitudinal strokes of the ventral abdomen with the hind legs. This behavior was usually followed by tarsal grooming in which the hind tarsi and tibiae were rapidly rubbed together. Tarsal grooming occasionally occurred without being preceded by abdominal grooming, but grooming resembled antennal grooming in form, the base of the stylet was grasped between the front tarsi and the tarsi drawn over the mouthparts three to five times. Tarsal grooming was the most common form of grooming followed by antennal, abdominal and stylet grooming.

As in the study of *A. hilare* (Lockwood and Story 1985), grooming behaviors were relatively uncommon. *Nezara viridula* exhibited essentially the same grooming behaviors as *A. hilare* with the exception of stylet grooming. While antennal, stylet, and perhaps tarsal grooming seemed to have sanitary functions, abdominal grooming remained without an obvious function. The behavior of kicking while *in copulo* was similar to both abdominal grooming and hind leg tarsal grooming which frequently followed abdominal grooming. If, as Harris (personal communication) suggests, there are ventral abdominal exocrine glands, these grooming and kicking behaviors may function in contacting exocrine sources on either the individual or its mate.

Positional orientation was characterized by a change in position of the body without a change in location of the insect. Positional orientation behaviors included ducking, jerking, and shifting. Ducking, the most common positional orientation behavior, was characterized by a series of very rapid vertical movements often accompanied with quick, lateral thrusts without the insect releasing hold of the substrate. Ducking was seen exclusively during contact or near contact with heterospecific insects, primarily wasps and bees. Although no predation was observed, N. viridula is taken as prey by some wasps (Drake 1920). Ducking would seem particularly well suited for avoiding parasitization by Trichopoda pennipes (F.). No contacts between this parasite and N. viridula were seen although T. pennipes were present in the cowpea field. Jerking was a rarely observed behavior which resembled ducking but included only the rapid changes on horizontal orientation through sudden lateral movements. Jerking was not associated with interspecific contact. Shifting consisted of the insect slowly changing its horizontal orientation by rotating its body within a plane without releasing hold of the substrate. Shifting may have accomplished sound production as did rocking. While shifting was not associated with conspecific contacts and did not involve distinct abdominal tipping, the behaviors associated with sound production in N. viridula are apparently quite subtle and have not been fully documented (Harris 1980). Shifting and jerking in N. viridula appear similar to swaying and jerking behaviors associated with courtship in several other pentatomids (Kaufman 1966; Alcock 1971; Gamboa and Alcock 1973; Lanigan and Barrows 1977).

Componential movements were defined as those activities in which an insect moved a portion of its body without changing its overall orientation or location. These behaviors included antennal waving and wing buzzing. Antennal waving consisted of slow, alternate sweeps of the antennae through an arc of ca.  $45^{\circ}$ . Wing buzzing was seen in three males, in which the hemelytra were lifted, followed by a rapid and audible beating of the hind wings. This behavior did not lead to or precede flight. Both componential movements may have been related to pheromonal communication as suggested for A. hilare (Lockwood and Story 1985). Since antennal waving was much more frequent in unmated individuals at the time of day in which walking was most frequent and resting was shortest in duration, there is inferential evidence that it was related to searching for mates. Mitchell and Mau (1969) reported that the antennae are necessary for mating in N. viridula, at a time previous to courtship. Antennal movements are also associated with mate location and courtship in several other pentatomids (Alcock 1971; Tstowaryk 1971; Gamboa and Alcock 1973; Lanigan and Barrows 1977). Although antennal waving in N. viridula was most common in the morning, Harris and Todd (1980b) found that the greatest attraction of this species to its aggregation pheromone occurs just before dusk, and Alcock (1971) observed that E. conspersus mate almost exclusively in the late afternoon. Wing buzzing has been observed in our laboratory during colony maintenance and it may function in the dissemination of pheromone from the dorsal abdominal glands (Aldrich et al. 1979). Wing raising has been observed in M. histrionica females attempting to avoid male copulatory attempts (Lanigan and Barrows 1977). Wing buzzing has been inferred to have a defensive function in other stink bugs as well (Callan 1944; Alcock 1971).

Basking behavior was defined as any behavior which occurred in direct sunlight. In the morning, 82% of the time was spent basking and 91% of the time was spent at the top of the plant. These proportions were significantly greater than the 76% of the time spent basking and the 72% of the time spent at the top of the plant in the noon time block. In turn, these proportions were significantly greater than the 63% of the time spent basking and the 54% of the time spent at the top of the plant during the afternoon. Thus, *N. viridula* spent a decreasing amount of time in the direct sunlight and at the top of plants as the day progressed, although during all time blocks more than half of the available time was spent in sunlight and at the top of the plant.

Basking was more common in N. viridula, which spent more than half of its time basking at all times of day, than in A. hilare, which spent the majority of its time basking only in the morning (Lockwood and Story 1985). Basking during the morning has also been reported in E. consperus and Chlorochroa sayi Stal (Alcock 1971). An earlier study of basking in N. viridula (Waite 1980) examined the distribution of the insects on the plants. Our findings indicated that the propensity for the top of the plant decreased throughout the day as reported by Waite (1980). However, in our study more than half of the time was spent in the top of the plant at all times, while in Waite's (1980) work there was a virtual abandonment of the top of the canopy after 1100 h. This difference is probably due to our study being conducted on cowpea in which the pods are primarily located near the top of the plant, while Waite's (1980) observations were made in soybean and in which the pods are well below the leaf canopy. As suggested by Waite (1980) the basking of N. viridula in the morning allows for tactical timing of insecticide applications. Moreover, treatment thresholds for N. viridula populations should take into account basking behavior since population estimates arrived at through sweeping the canopy or by direct observation may vary considerably throughout the day.

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