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DIEL ACTIVITY PATTERNS AND MICROSPATIAL DISTRIBUTION OF THE HARVESTMAN *PHALANGIUM OPILIO* (OPILIONES, PHALANGIIDAE) IN SOYBEANS

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ABSTRACT. *Phalangium opilio* L. is a polyphagous predator frequently found in agricultural habitats. Although the potential importance of *P. opilio*'s feeding on pests has been recognized, little is known about its activity patterns or its within-plant distribution in crops. We determined diel activity patterns and microspatial distribution in small, fenced arenas in soybean fields. The fenced arenas allowed us to track known numbers of particular size categories of *P. opilio* for each 24 h trial. *Phalangium opilio* were separated into the following categories based on body size and sex: medium-sized nymphs, large-sized nymphs, adult females and adult males. Medium-sized nymphs occupy the bottom and middle portions of plants regardless of time of day; they remain still during the day, but they exhibit leg palpating behavior from 21:00–01:00 h. Large-sized nymphs rest in the bottom and middle portions of plants during the day, but they walk and palpate on the ground from 21:00–01:00 h. Adult females rest in the bottom, middle and top portions of plants during the day, and they walk and palpate on the ground from 21:00–01:00 h. Adult males remain stationary in the bottom, middle and top portions of plants during the day, but they walk on the ground from 21:00–04:00 h.

Keywords: Predator, behavior, microhabitat separation

The microspatial distribution and diel activity patterns of predators in crops affect the prey they encounter, which potentially affects their value in biological control. When different instars of the same species separate their location and activities spatially and temporally, the separation may reduce cannibalism and/or intraspecific competition for resources. All these factors have the potential to affect the population dynamics of arthropod species in agricultural systems. Predatory members of the group Opiliones are sometimes overlooked in crops. One such predator is *Phalangium opilio* L. 1758. We studied *P. opilio*'s microspatial distribution and diel activity because of its potential importance in biological control of soybean pests (Anderson 1996; Pfannenstiel & Yeargan 2002).

Microhabitat separation of different life stages is seen in some Opiliones. For example, *Mitopus morio* (Fabricius 1799) exhibits vertical stratification, with late instars found at high vegetation strata (Adams 1984). Vertical distribution of *P. opilio* is believed to vary among habitats. When sparse shrub cover is present, 88% of *P. opilio* are found in shrubs and brushy vegetation, but with dense cover, the highest percentage of *P. opilio* is found on the ground layer (Edgar 1980). Cloudsley-Thompson (1968) also observed *P. opilio* to primarily inhabit low vegetation or grass and other herbaceous plants, but he stated that the early instars only occur on the ground. Several hypotheses have been presented to explain microhabitat separation in Opiliones. Opilionids may be found on vegetation to eliminate competition with strict ground predators (Halaj & Cady 2000). It also has been hypothesized that the vertical expansion of the distribution of late instars is due to the need for larger prey, more moving space, mating, and/or different temperature and humidity requirements (Sankey 1949; Todd 1949). Not all individuals abandon the ground; Williams (1962) found individuals of the same species and instar both in pitfall traps and on vegetation. Based on those results, Williams hypothesized opilionids may expand their microhabitat distribution without completely abandoning the ground. However, it is possible that Williams' (1962) results reflected diel movement, not microhabitat separation.

Opiliones generally are nocturnal (Sankey 1949; Todd 1949; Phillipson 1960; Williams 1962; Edgar & Yuan 1969). The increase in activity at night may be attributed to decreased light intensity, increased relative humidity and decreased temperatures (Todd 1949). Pfannenstiel & Yeargan (2002), who observed predation events on *Helicoverpa zea* (Boddie 1850) eggs in soybean fields at 3 h intervals during 24 h cycles, found that all observed events of predation by Phalangiidae occurred at night. Although *P. opilio* occasionally is active under diurnal conditions, individuals exhibit 90% of their total activity between 1800–0600 h (Edgar & Yuan 1969; Edgar 1980).

Phalangium opilio is known to feed, primarily nocturnally, upon a variety of arthropod pests. In Kentucky, this predator appears to overwinter in the egg stage and undergo three generations per year (Newton & Yeargan 2002), with the second generation being the most relevant to predation in soybean (due to seasonal timing of this annual crop), where it feeds on *H. zea* eggs (Pfannenstiel & Yeargan 2002). Other aspects of its ecology relevant to its role in soybean fields are poorly known, including its diet breadth, its spatial distribution in large fields, its within-plant/epigeal distribution and its diel activity patterns. We investigated the diel activity patterns and microspatial distribution of *P. opilio* for nymphal instars three through seven and both adult sexes.

METHODS

This study was done during the summers of 2001 and 2002 at the University of Kentucky's North Farm near Lexington, KY. In each year, three small plots (1 m of soybean row per plot) were established within a 0.6 ha field of soybeans. The soybean variety used in both years was Asgrow 4702 and planting occurred on 1 May 2001 and 20 May 2002. Each 1 m plot was surrounded by a fence of galvanized sheet metal (20 cm tall, 0.5 m from plants on either side of fence); preliminary studies showed *P. opilio* could not scale this fence. In both years, the entire soybean field was treated at planting with recommended rates of conventional pre-emergence herbicides (alachlor, metribuzin and chlorimuron ethyl) and was subsequently treated once (2002) or twice (2001) with the post-emergence herbicide glyphosate for additional weed control. Post-emergence herbicide treatments occurred several weeks before trials began. No insecticides were applied during either year. Trials were conducted weekly from 23 July–9 September 2001 and from 7–24 August 2002.

Phalangium opilio were collected in the field no more than 5 d prior to the trial dates. Individuals were taken to the laboratory, measured and/or sexed in order to be placed in a category (described below), and provided with food (i.e., *H. zea* eggs and cornmeal/bacon diet) and water; food was removed 24 h prior to the initiation of observations and individuals were marked with a small dot of paint 12 h prior to the initiation of field observations. In the laboratory, individuals were kept in 8.5 × 8.5 cm (diam × ht) containers in incubators at 24 ± 1 °C (15:9 L:D) with high humidity via open water containers on the floor of the incubators. Prior to the initiation of trials, field arenas were checked for naturally occurring opilionids that were removed when found. No opilionid species other than *P. opilio* were encountered in this study. No other potential prey were removed from or added to an arena. Three arenas were monitored on each date. In each arena, three field collected *P. opilio* of the same category marked with pink fluorescent, water-based paint (Apple Barrel Colors, Plaid Enterprises, Inc.) were introduced simultaneously on the ground in the center of an arena 1 h before observations began. There is no accurate morphological indicator for sex or instar in *P. opilio* nymphs; therefore, size categories (hereafter referred to as *P. opilio* categories) based on cephalothorax width were used (after Newton & Yeargan 2002). Small nymphs were less than 1.0 mm, medium nymphs ranged from 1.0–1.5 mm, and large nymphs were greater than 1.5 mm in cephalothorax width. Adults were discriminated from nymphs based on the presence or absence of a genital opening beneath the operculum (Sankey & Savory 1974) and adult males and females were identified based on the presence or absence of the sexually dimorphic horns on the distal segment of male chelicerae (Sankey & Savory 1974).

During the trials, 5 min observations were made at each arena at 20 min intervals for 1 h at approximately: 1200, 1500, 1800, 2100, 0000, 0300 and 0600 h (EDT). A red light filter, which minimized disturbance to the animals, was used to make nocturnal observations and an ultraviolet light was used to locate individuals only if they could not be found with the red light. The ultraviolet light was used for ≈10% of the observations. During each observation, the location of individuals (i.e., on ground or plant; if on plant, bottom, middle, or top third of the plant, and exterior or interior portion of the plant) and the behavior of individuals (i.e., walking, grooming, feeding, stationary, palpating, drinking) were recorded. Exterior and interior portions of the plants were differentiated based on whether or not the view of the observer was obstructed by other plant parts (i.e., if an opilionid was on a plant part that had no other plant part between it and the observer, it was recorded as being on the exterior). For the purposes of this study, palpating behavior is defined as the movement of the sensory legs (i.e., the long second pair) in a slow tapping motion on the surrounding substrate. All behaviors recorded were mutually exclusive, and only the first observation of an individual was recorded within each 5 min observation period.

Each trial consisted of three individuals observed in a field arena for 24 h. In 2001, the following number of trials were conducted: small-sized nymphs ($n = 1$), medium-sized nymphs ($n = 6$), large-sized nymphs ($n = 4$), adult females ($n = 5$), and adult males ($n = 5$). In 2002, the following number of trials were conducted: medium-sized nymphs ($n = 1$), large-sized nymphs ($n = 3$), adult females ($n = 2$), and adult males ($n = 2$). Small nymphs were excluded from the study after the first trial due to the difficulty in seeing the nymphs, due in part to their tendency to hide in tiny crevices. The combined years yielded seven trial dates for all *P. opilio* categories excluding small. After each trial date, all individuals were removed from the arenas and nymphs were reared in the laboratory until maturity for positive identification to species. Individuals were not always found at the time of observations, but all individuals were recovered at the end of each trial. Voucher specimens were placed in the arthropod collection of the Department of Entomology at the University of Kentucky.

Statistical analysis.—In order to analyze the microspatial distribution and behaviors for the different nymphal and adult *P. opilio* categories, proportions were calculated for each set of three individuals during each 1 h observation period. There was a maximum of nine observations per arena per hour (i.e., three individuals times three visits = the denominator for calculating proportions). These proportions reflected the frequency of observations of *P. opilio* at a particular place (ground, bottom of plant, middle of plant, top of plant) or engaged in a particular behavior (stationary, walking, feeding, palpating, drinking).

Category X time interactions were examined to determine if different *P. opilio* categories (medium-sized nymphs, large-sized nymphs, adult males and adult females) were at different locations at different times of the day and if they exhibited different behaviors. Because the ANOVA assumptions of homogeneity of variances and normality of the variables were not met, even after several transformations, we used profile analysis, which can be performed with both parametric and non-parametric statistics to test for the category X time interactions (Ende 1993). To compute a profile analysis the response variable (i.e., percentage of time per hour) was subtracted between repeated measures and tested for differences among *P. opilio* categories on the resulting variable. The new variables did not meet the ANOVA assumptions; therefore, the non-parametric Kruskal-Wallis ANOVA (Siegel & Castellan 1988) was used to test for differences among *P. opilio* categories with the new (subtracted) variables. There were seven time periods. Six new variables were calculated for the profile analysis performed on each location and behavior. Each variable was derived by subtracting the response variable (i.e., percentage of time per hour) of every time period from the response at one arbitrarily selected time period (i.e., 00:00 h). Thus, the first variable for profile analysis was 00:00 minus 03:00 h, the second was 00:00 minus 06:00 h, and so on. This allowed interactions to be detected and showed which time periods had a larger change in location or behavior. Therefore, we tested for significance of six variables, four locations and five

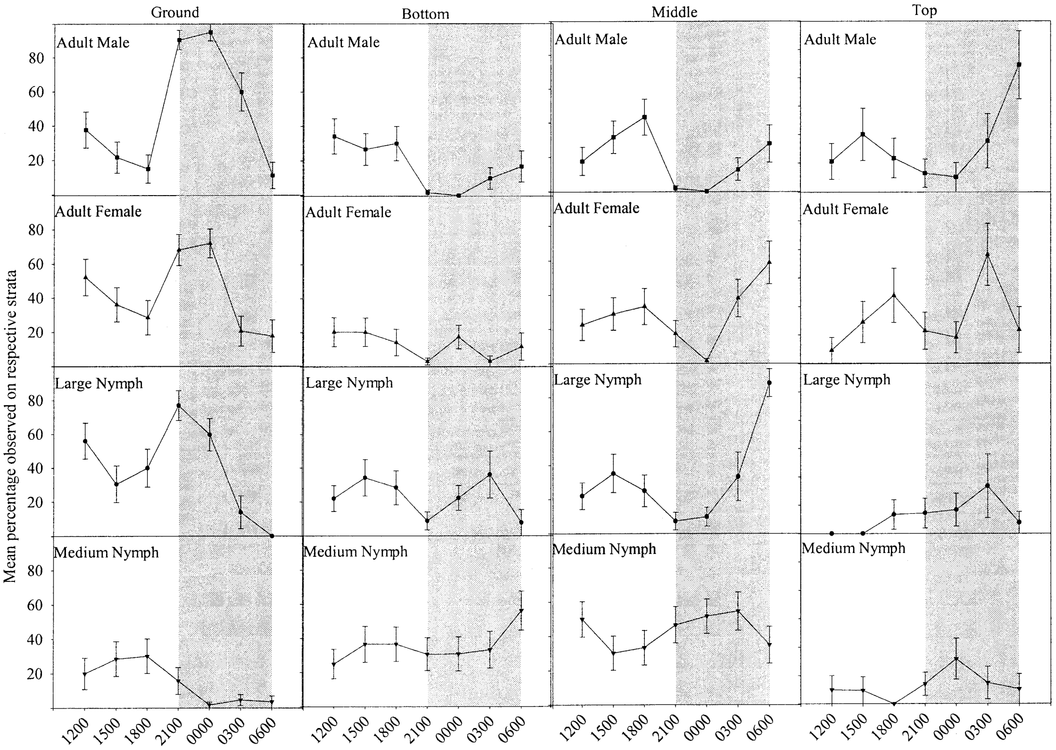

Figure 1.—Percentage of observations (mean ± SE) for all *Phalangium opilio* categories on the ground, in the bottom, in the middle, and in the top portions of soybean plants during diurnal (not shaded) and nocturnal (shaded) hours.

behaviors, for a total of 6 × (4+5) = 54 tests. Because significance of the overall category X time interaction could not be tested as it is done in repeated measures ANOVA, the criterion to decide whether there was an overall significant interaction for each category and location or behavior was whether a combined probability test (Fisher's meta-analysis; Sokal & Rohlf 1995) across the six tests was significant. The resulting value is compared with the chi-square distribution with twice as many degrees of freedom as number of tests performed (in this case 2 × 6 = 12 d.f.).

To test if, at different heights, the different *P. opilio* categories were more likely located on the exterior or in the interior part of the plant, a logistic regression analysis was used. The response variable was interior or exterior on the plant, which were coded as 0 and 1 respectively. For this analysis the data for all time periods were pooled and the frequency at which individuals were on the interior or exterior of the plant at every height was included in the model by using the FREQ statement in the SAS LOGISTIC procedure (Allison 1999). For graphical purposes, the percentage of time per hour in which individuals were exterior at every height was averaged across individuals. A significant category X height interaction in the logistic regression indicates that there is spatial segregation in relation to exterior and interior portions of the plant and that it is different at different heights.

RESULTS

There was a significant category X time interaction for all vertical strata (i.e., ground, bottom, middle and top) indicating that different *P. opilio* categories were at different strata at different times (Fig. 1; Table 1). All *P. opilio* categories, excluding medium-sized nymphs (which remained on the plants), moved to the ground at nightfall, 21:00 h, where they remained until 03:00 h. Males were on the ground significantly more than the females, and large nymphs and males remained on the ground in the 03:00 h when the

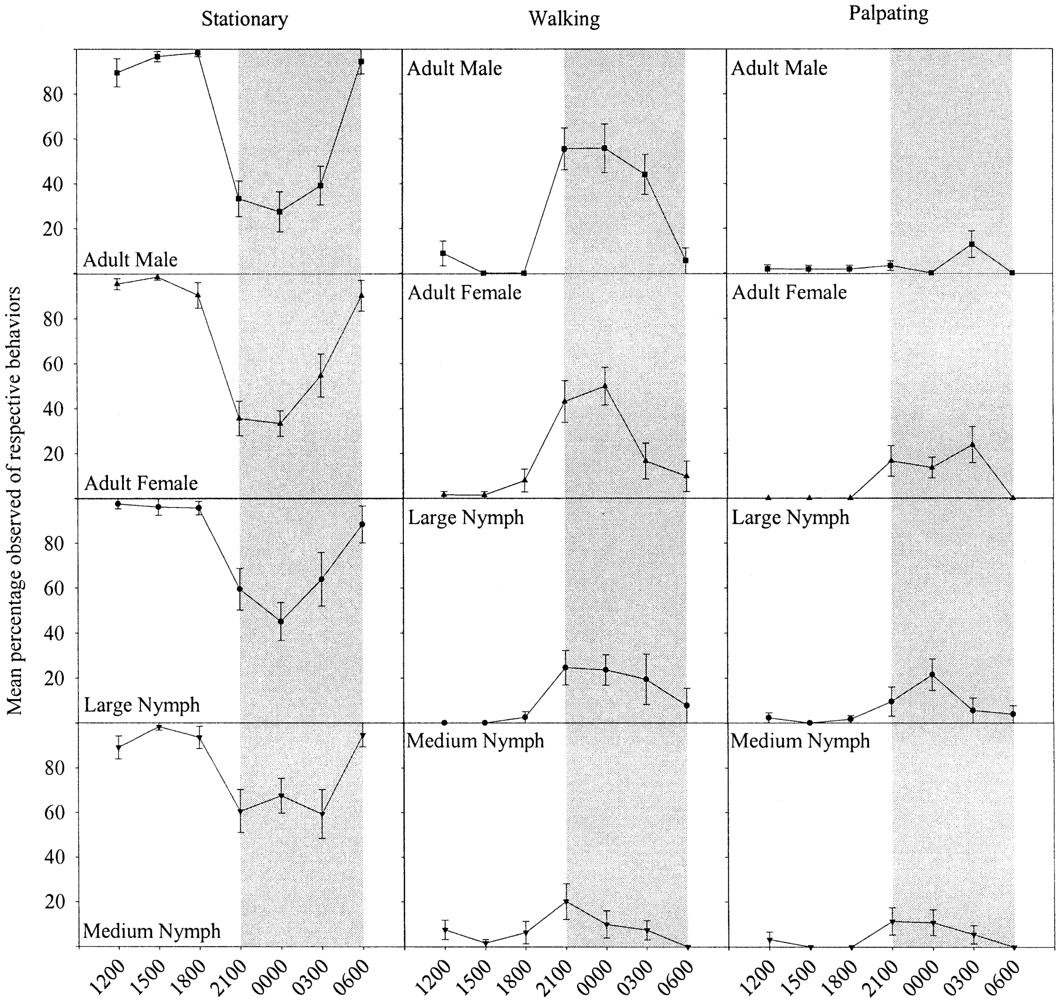

Figure 2.—Percentage of observations (mean ± SE) for all *Phalangium opilio* categories that were stationary, walking, and palpating in soybean plots during diurnal (not shaded) and nocturnal (shaded) hours.

females and large nymphs returned to the plants (Fig. 1). For the majority of the day all *P. opilio* categories were found on the plants. During this time, all *P. opilio* categories were recorded in the bottom and middle portions of the plants; however, small percentages of males and females were recorded in the top portion of the plants during the 15:00 and 18:00 h, respectively. Medium-sized nymphs remained on the bottom and middle portions of the plants at night. Early in the morning there was an increase in large nymph and female observations in the middle portions of the plants. A substantial percentage of the females was found on the top portion of the plants only during 03:00 h, followed by males at 06:00 h.

There also were significant category X time interactions for the following behaviors: stationary, walking, and leg palpating (Fig. 2; Table 1). Drinking, feeding and grooming were excluded because there were only a few recorded observations. Only a single case of predation was observed, namely a large immature individual feeding on an immature leafhopper (Hemiptera, Cicadellidae) on a soybean plant. The majority of all *P. opilio* categories were stationary during the day but stationary behavior was less common in the nocturnal hours. Virtually no walking behav-

Table 1.—Results from the Kruskal-Wallis ANOVA by ranks for the overall response of size and gender categories (average across time) of *Phalangium opilio* to heights on soybean plants and behaviors. Palpating = leg palpating.

	Ground		Bottom		Middle		Top	
Time periods	H	*p*	H	*p*	H	*p*	H	*p*
0000 minus 0300 h	5.738	0.125	5.564	0.135	5.738	0.125	8.287	0.041
0000 minus 0600 h	37.052	<0.001	9.396	0.025	12.908	0.005	13.782	0.003
0000 minus 1200 h	16.835	0.001	7.745	0.052	2.883	0.410	3.418	0.332
0000 minus 1500 h	27.552	<0.001	4.358	0.225	13.443	0.004	6.985	0.072
0000 minus 1800 h	33.475	<0.001	5.872	0.118	12.923	0.005	7.069	0.070
0000 minus 2100 h	1.308	0.727	4.811	0.186	4.047	0.256	2.491	0.477
Combined test		<0.001		0.006		<0.001		0.001

	Stationary		Walking		Feeding		Palpating		Drinking	
Time periods	H	*p*	H	*p*	H	*p*	H	*p*	H	*p*
0000 minus 0300 h	5.509	0.138	6.691	0.083	8.326	0.040	11.239	0.011	1.166	0.761
0000 minus 0600 h	10.667	0.014	15.569	0.001	7.822	0.050	8.889	0.031	1.911	0.591
0000 minus 1200 h	10.008	0.019	18.396	<0.001	8.854	0.031	10.568	0.014	1.911	0.591
0000 minus 1500 h	12.740	0.005	19.336	<0.001	7.965	0.047	12.290	0.007	1.911	0.591
0000 minus 1800 h	8.951	0.030	18.334	<0.001	8.854	0.031	10.218	0.017	1.911	0.591
0000 minus 2100 h	0.932	0.818	1.096	0.778	4.179	0.243	6.406	0.094	0.538	0.911
Combined test		0.000		<0.001		0.000		<0.001		0.960

ior was exhibited during the day, but with nightfall there was a large increase in walking behavior for males and females and a small increase for nymphs. Males and females exhibited walking behavior from nightfall through the midnight hour, and males continued walking through the early morning, 03:00 h, while female walking decreased. None of the *P. opilio* categories exhibited leg palpating during the day. Large nymphs showed a peak in leg palpating during the midnight hour, and medium nymphs exhibited a small amount of leg palpating from nightfall through the midnight hour. Females exhibited leg palpating at night, and males showed a small peak in leg palpating at 03:00 h but not at any other time.

The percentage of *P. opilio* categories found on the exterior, as opposed to the interior, of plants varied with height on the plant and *P. opilio* category. Some *P. opilio* categories tended to be on the exterior or on the interior of the plant at a relatively different rate from each other at different heights. The full logistic regression model was highly significant ($\chi^2_{11} = 157.3$, $P < 0.001$). Both the category ($\chi^2_3 = 21.2$, $P < 0.001$) and the height ($\chi^2_2 = 31.8$, $P < 0.001$) effects were significant, as well as the interaction term between them ($\chi^2_6 = 27.2$, $P < 0.001$). In the bottom portion of the plants, all *P. opilio* categories tended to stay in the interior of the plant. In the middle portion of the plant, some individuals were found on the exterior, with the notable exception of large nymphs, which were almost exclusively found in the interior. Except for adult males, individuals of all other categories were found on the exterior more frequently in the top portion of the plant than in either of the other two plant strata (Fig. 3).

DISCUSSION

Nymphal *P. opilio* were somewhat more restricted than adult *P. opilio* in their microspatial distribution. Although distribution of adult males and females changed over the diel cycle, they were found on the ground and in all plant strata. Large nymphs, however, were seldom found in the top portion of plants. Medium nymphs appeared to be even more restricted, primarily occurring on the middle and lower portions of plants. The restricted distribution of nymphs may reduce encounters with adult *P. opilio*.

Phalangium opilio became active with the onset of nightfall (21:00 h). At this time all *P. opilio*, excluding the medium-sized nymphs, moved to the ground. Medium-sized nymphs might suffer higher predation risks

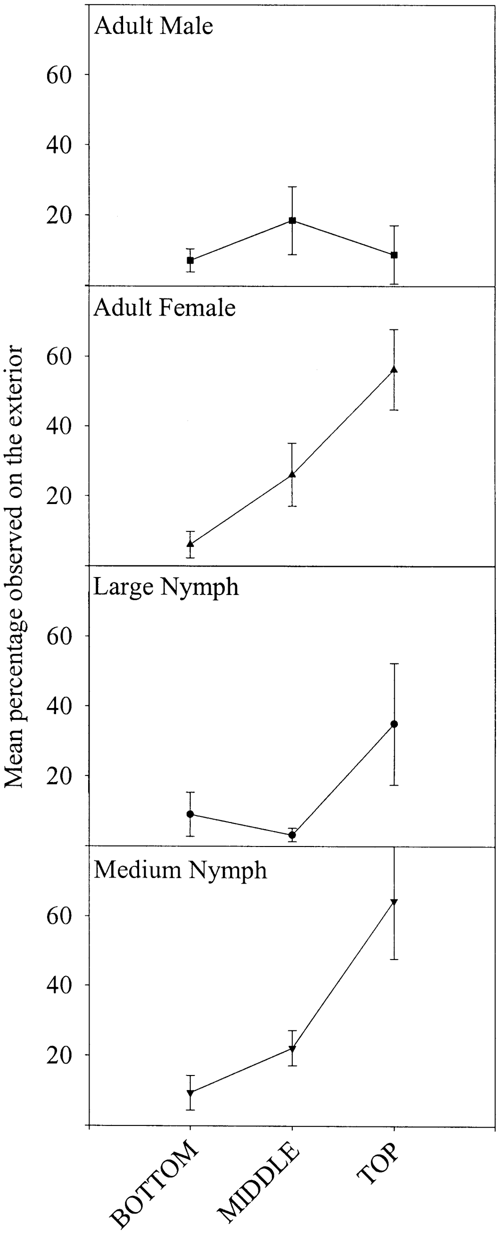

Figure 3.—Percentage of observations (mean ± SE) for all *Phalangium opilio* categories that were on the exterior of soybean plants as opposed to the interior of the plants at different heights.

from other nocturnal ground predators, as well as larger *P. opilio,* which may account for their tendency to remain in the vegetation. While on the ground, *P. opilio* remains active. Males spent most of their time walking and little time palpating. Females also spent considerable time walking on the ground but also spent time palpating. Nymphs spent less time walking than adults, with medium nymphs walking the least of all *P. opilio* categories. Adult females and nymphs engaged in palpating behavior more frequently than did adult males.

If palpating is a foraging strategy in this species, it is not surprising that females and nymphs were observed palpating more than males. Adult females need nutrients to invest in reproduction, and nymphs need nutrients for development, while adult males presumably need less nutrients. It might be expected that adult males would spend more time walking in search of female mates. Females lay eggs in the soil, so the marked tendency for males to spend more time walking on the ground (Fig. 1) may increase their likelihood of encountering females there.

Since *P. opilio* is known to feed on *H. zea* eggs in soybean fields (Anderson 1996; Newton & Yeargan 2001; Pfannenstiel & Yeargan 2002), it is worthwhile to consider the microspatial distribution of *P. opilio* compared to that of *H. zea* eggs in soybean. Terry et al. (1987) observed that *H. zea* oviposited throughout the vertical strata of soybean plants, with approximately 70% of the eggs being laid on main-stem leaves. Hillhouse & Pitre (1976) reported that the upper and middle thirds of the soybean plants were preferred for oviposition compared to the lower third. The microspatial distribution of *P. opilio* does overlap with the distribution of *H. zea* eggs.

Knowledge of the distribution and behavior of *P. opilio* is important in assessing its potential impact on arthropod pests in soybean systems. Its stage-specific activity patterns and distributions may affect reproductive opportunities, intraspecific competition, cannibalism, and potential encounters with prey species.

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