

5-2006

Searching response of a wandering spider to potential cues associated with lepidopteran eggs

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**SEARCHING RESPONSE OF A WANDERING SPIDER TO POTENTIAL CUES
ASSOCIATED WITH LEPIDOPTERAN EGGS**

A Thesis

by

ROSA ISELA RUIZ

Submitted to the Graduate School of the
University of Texas-Pan American
In partial fulfillment of the requirements for the degree of
MASTERS OF SCIENCE

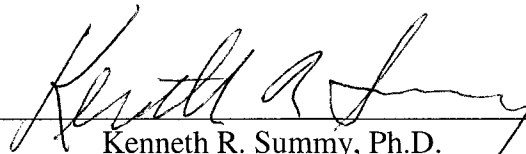
May 2006

Major Subject: Biology

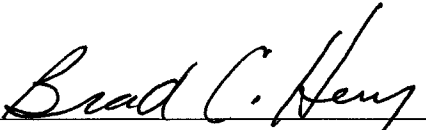
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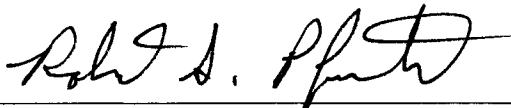
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ABSTRACT

Ruiz, Rosa I., Searching Response of a Wandering Spider to Potential Cues Associated with Lepidopteran Eggs. Master of Science (MS), May, 2006, 30 pp., 4 tables, 6 illustrations, references, 28 titles.

Cheiracanthium inclusum (Hentz), a wandering spider, has been frequently observed feeding on the eggs of *Helicoverpa zea* (Boddie), an important cotton pest. Wandering spiders are known to feed mainly on mobile prey, thus mechanisms used by these spiders to locate sessile prey remain understudied. Vibratory and visual are among the main cues used by spiders to identify prey. However, other sensory modalities such as chemoreception may be used but are poorly understood. This study investigated the types of cues utilized by *C. inclusum* to locate and identify *H. zea* eggs as prey. Results show that *C. inclusum* responds positively to substrates previously exposed to *H. zea* eggs and substrates containing moth scales. These results suggest that *C. inclusum* may be using a combination of contact and chemical cues from eggs and scales to recognize *H. zea* eggs as prey. Further studies identifying key chemical compounds may provide a better understanding on how *C. inclusum* perceives chemical cues and initiates searching behavior.

ACKNOWLEDGMENTS

I would like to thank Dr. Robert Pfannenstiel (USDA Research Entomologist) for his support and guidance while completing this thesis. I wish to thank my major Professor Dr. Kenneth Summy, for all his advice and patience and to the thesis committee, Dr. Brad Henry and Dr. Robert Edwards, for their help in editing the final product. I also would like to thank my dear friends, Marissa Gonzalez and Dora Conner, for their support and help and who always believed in me and did not let me quit during this lengthy process. In addition to my friends, I would like to thank Scott J. Gallagher who came into my life at the right moment and gave me his love and support that inspired me to obtain this goal. Finally, I wish to thank the most important person in my life, my grandmother, who through hard times gave me the strength and perseverance to become the person I am today.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
ACKNOWLEDGMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
INTRODUCTION.....	1
Background.....	2
Objectives.....	7
MATERIALS AND METHODS.....	8
RESULTS.....	11
DISCUSSION.....	21
CONCLUSION.....	24
LITERATURE CITED.....	26
VITA.....	30

LIST OF FIGURES

Figure 1, <i>C. inclusum</i> spiderling feeding on <i>H. zea</i> eggs.....	13
Figure 2, Adult <i>C. inclusum</i> feeding on <i>H. zea</i> eggs.....	14
Figure 3, Response of spiderlings to substrate exposed to <i>H. zea</i> eggs and scales and to scales only. Data are presented as mean percentage of spiderlings responding to substrates \pm SE (means followed by different letters within each group are significantly different, $P<0.05$).....	15
Figure 4, Time-response of <i>C. inclusum</i> to substrates exposed to eggs and scales of <i>H. zea</i> . Data are presented as mean percentage of spiderlings responding to substrates \pm S.....	16
Figure 5, Time-response of <i>C. inclusum</i> to substrates exposed to scales of <i>H. zea</i> . Data are presented as mean percentage of spiderlings responding to substrates \pm SE.....	17
Figure 6, Response of <i>C. inclusum</i> spiderlings to substrate exposed to egg and scale extracts from different solvents.....	20

INTRODUCTION

Spiders are known to be generalist predators that feed on insects and other arthropods. Approximately half of the known families of spiders are non-webbuilders that either “sit and wait” or wander around in search for potential prey (Nentwig 1986). These spiders are commonly known as hunting or wandering spiders. Spiders use a variety of stimuli to locate their prey. Some may capture and eat anything that moves and utilize only visual and vibratory cues. Vision plays a minor role in the behavior of many spiders, with the exception of jumping spiders and a few other hunting spiders; the majority of wandering spiders are active only at night. Vibration appears to be important for most spiders in particular web-building spiders. The web captures a high percentage of flying insects and the slightest vibration alerts the spider, which in turn can discriminate between possible prey and other artifacts caught on it. A few other spiders that “sit and wait” for prey depend on dragging lines of silk placed nearby their retreat to alert them of any potential prey, which may also be considered as a vibration cue (Reviewed in Foelix 1982).

Other spiders may also employ olfactory and chemical cues, which can be utilized individually or in any combination in the prey recognition process, (Jackson and Tarsitano 1993, Persons and Uetz 1996, Punzo and Kukoyi 1997, Clark et al. 2000, Hoefler et al. 2002). Chemical cues have been known to play an important role in courtship communication for many spider species and recent studies have attempted to gain a better understanding of their role in prey recognition as well. For instance, wolf spiders are polyphagous hunting predators known to use chemical cues in courtship

communication and are capable of using similar cues as important components of foraging decisions (Persons and Uetz 1996).

Spiders have demonstrated the ability to react to chemical stimuli when presented with highly concentrated chemical substances. In a study where spiders were subjected to these substances they tended to run away and/or exhibited a more localized reaction such as withdrawing a leg. These observations suggest the presence of an olfactory sense which spiders may use to find a mate and perhaps to recognize prey and possible enemies as well (Foelix 1982).

Spiders have also shown the ability to use chemical stimuli to locate prey (Persons and Uetz 1996, Punzo and Kukoyi 1997, Persons and Rypstra 2000). Foraging and patch-residence time of spiders have been linked to the presence of chemical cues. Prey cues and environmental factors influence foraging decisions of spiders, playing an important role in determining, for instance, how long to stay in a particular foraging patch before moving to another one (patch residence time).

Persons and Uetz (1996) observed the ability of *Schizocosa ocreata* (Araneae: Lycosidae), a wolf spider, to perceive residual chemical cues left by crickets. The time this wolf spider spent foraging on treated substrate was significantly higher than that of blank controls. This study concluded that *S. ocreata* may use chemical cues as a source of information while foraging in addition to other possible cues such as vision or vibration.

Pardosa milvina (Araneae: Lycosidae), another wolf spider, also showed chemosensory abilities when exposed to treated substrate containing chemical cues left behind by crickets. Foraging time spent on treated substrate was also higher on cricket-

treated substrate when compared to untreated substrate, thus supporting the premise that the presence of chemical cues influences foraging decisions of wolf spiders (Hoefler et al. 2002).

Chemical cues associated with recently consumed prey are also used by wolf spiders. In a recent study, *Hogna helluo* (Araneae: Lycosidae), showed preference for cues associated with its most recent prey. This wolf spider had been kept on a diet of *P. milvina*, a smaller wolf spider, and was given the choice of a substrate treated with *P. milvina* or a substrate treated with crickets. *H. helluo* responded positively to chemical cues from substrate treated with the small wolf spider instead of the cricket-treated one, thus demonstrating that it can detect cues associated with its most recently consumed prey (Persons and Rypstra 2000).

The wolf spider *Trochosa parthenus* (Araneae: Lycosidae) and the lynx spider *Oxyopes salticus* (Araneae: Oxyopidae) have also demonstrated their ability to utilize chemical cues associated with prey present in their natural habitat. These two field collected spiders were exposed to a substrate containing odors associated with potential prey insects that are found at the sites where they were originally collected. In this study, the wolf and lynx spiders spent significantly more time on patches containing odors associated with those insects collected in their natural habitat, suggesting that these spiders utilize chemical cues to recognize previously consumed prey (Punzo and Kukoyi 1997).

Many previous studies strongly support the concept of olfactory and chemical cues being utilized by cursorial spiders (Jackson and Tarsitano 1993, Persons and Uetz 1996, Punzo and Kukoyi 1997, Clark et al. 2000, Hoefler et al. 2002). The studies were mainly

based on their patch residence time when exposed to substrate previously exposed to motile prey; however, it is unclear what mechanisms these spiders are using or specifically what types of cues are being used to identify a suitable foraging patch.

In addition to normal motile prey, many of these spiders are known to feed on artificial diets, plant components (Taylor and Foster 1996), and arthropod eggs (Nyffeler et al. 1990, Miliczky and Calkins 2002). Spiders from the families Anyphaenidae, Clubionidae, and Corinnidae, for example, were found feeding on extrafloral nectaries from the Florida castor bean. It appears that pollen can be another source of nutrition that spiders may be using (Taylor and Foster 1996).

It was not until recently that more interest has been focused on spiders feeding on arthropod eggs and the possibility that these generalist predators may be capable of suppressing pest population. Observations of spiders feeding on insect eggs are largely based on cotton fields from North America, South America, and Australia. Additional evidence has been collected from soybeans, tobacco, sugarcane, citrus, forest habitats and laboratory settings. The majority of observations of insect egg predation involved eggs of Lepidoptera in the families Noctuidae, Tortricidae, Lymantriidae, Pyralidae and Sphingidae as well as eggs of Curculionidae in the Coleoptera (Nyffeler et al. 1990).

Cannibalism is often observed in spiders as well. Newly hatched spiderlings of *Cheiracanthium carnifex*, *C. inclusum* (Hentz) and *C. mildei* L. Koch (Araneae: Miturgidae), have been observed to feed on infertile eggs in their egg mass (Peck and Whitcomb 1970, Mansour et al. 1980). Ibarra (1985) determined that laying infertile eggs with the fertile ones increased the growth rate of the spiderlings, allowing them to reach the nymphal stage faster than those without the infertile sibling eggs. Other spiders

that exhibit cannibalistic behavior include species in the families Clubionidae, Pholcidae and Loxoscelidae (Ibarra 1985). Spiders also prey on eggs and spiderlings of different species of spiders. Willey and Adler (1989) reported *C. inclusum* as the primary predator of eggs and emerged spiderlings of *Peucetia viridans* (Hentz) in South Carolina.

There are very few studies on predation by spiders on insect eggs and little or no information available on how wandering spiders manage to locate and recognize sessile prey, such as lepidopteran eggs. It is quite possible that wandering spiders may be using chemical cues to locate and recognize these eggs as prey. For instance, parasitoids have shown the ability to use different chemical cues to recognize sessile hosts (e.g., lepidopteran eggs). Several active chemicals involved in the recognition process have been characterized as volatile kairomones. Kairomones are volatile chemicals that provoke a response beneficial to the receiver but not to the sender of the signal. Kairomones may be classified into four main categories: foraging kairomones (food location), enemy-avoidance kairomones (avoiding natural enemies), sexual kairomones (mating), and aggregation kairomones (attracting both sexes of an organism) (Ruther et al. 2002). The latter authors defined foraging kairomone as “a kairomone used by the benefiting organism in the context of the location of food for the organism itself or its offspring”. These volatile chemicals are used by herbivores, parasitoids, parasites, or fungivores to locate hosts or host plants (Ruther et al. 2002).

Strand and Vinson (1982) showed that there is a secretion found on deposited eggs of *Heliothis virescens*, which the parasitoid *Telenomus heliothidis* Ashmead used to locate the eggs. The source of this secretion was the accessory gland of adult female *H. virescens*, which served as an adhesive for the attachment of eggs to a chosen substrate.

It was concluded that proteins present in the accessory gland of *H. virescens* serve as an egg recognition kairomone for the parasitoid *T. heliothidis*. Sources of chemical stimuli may include oviposition-related chemicals, frass, gland secretions, accessory glands, and moth scales (Jones et al. 1973, Strand and Vinson 1982, Noldus and van Lenteren 1985).

The cursorial spider *Habronestes bradleyi* (Zoodariidae) is a specialist predator of the meat ant, *Iridomyrmex purpureus*. These ants are very aggressive toward intruders and alert colony members with an alarm pheromone, 6-methyl-5-hepten-2-one. This alarm pheromone, which is released by a few injured workers, is used as a prey-location kairomone by the spider (Allan et al. 1996). A recent study by Clark et al. (2000), reported the use of olfactory and contact chemical cues to locate and identify ants as prey by *Habrocestum pulex*, a jumping spider. Spiders used in this study responded to the chemical 6-methyl-5-hepten-2-one found in mandibular and anal gland secretions of ants. This chemical appears to be a kairomone, which induces changes in foraging behavior of this particular spider, similar to that of parasitoids.

It has been established that some spiders are capable of perceiving volatiles in the form of kairomones, which affect their foraging behavior when searching for prey. Information available about spiders preying on lepidopteran eggs is limited, and their searching behavior when they encounter sessile prey remains understudied.

In this study, the hypothesis that wandering spiders utilize chemical cues to locate and recognize lepidopteran eggs as prey is explored. *Cheiracanthium inclusum* is a polyphagous, nocturnal wandering spider found in many crop habitats (examples include: apples, citrus, corn, cotton, soybeans and tobacco). Peck and Whitcomb (1970) describe *C. inclusum* as “a pale yellow spider with an average length of about six millimeters. It

has no distinctive markings except the dark brown color of the mouthparts and eye area and a faint, greenish mark on the anterior, dorsal part of the abdomen. Its legs are long and delicate, slightly darker than the body, and without any spines that are readily discernible to the naked eye. The first pair of legs are distinctly longer than the other three pairs.” This spider spends the day hiding in a sac-like retreat and is an active foliage runner at night. *C. inclusum* moves rapidly over foliage and produces a length of silk which is carried by air currents until it catches on another piece of foliage of a nearby plant. Adult females live an average of 70 days, longer than males, which live only 43 days.

Field studies have revealed that *C. inclusum* feeds on lepidopteran eggs of important crop pests, such as *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), commonly known as the cotton bollworm (Nyffeler et al. 1990, Young and Edwards 1990, Ruberson and Greenstone 1998). Laboratory observations have demonstrated that this particular spider can survive and reproduce on a strict diet of *H. zea* eggs (Pfannenstiel, unpublished data), thus making it a suitable subject to determine what types of cues are utilized by wandering spiders to locate and identify lepidopteran eggs as prey. The main goal of this study is to determine the types of cues utilized by *C. inclusum* to locate and identify *H. zea* eggs as prey and to describe searching behavior exhibited when exposed to these cues.

The objectives of this study were to investigate spiderlings searching behavior and their ability to recognize cues associated with *H. zea* eggs and to investigate behavioral responses of *C. inclusum* to potential chemical cues (contact kairomones) involved in the prey recognition process.

Materials and Methods

A laboratory colony of *C. inclusum* was started from females collected in cotton fields at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center in Weslaco, Texas. Collected spiders were reared and mated in individual 150 x 25 mm. (diameter x height) plastic petri dishes at 24°C with 14:10 (L:D) photoperiod. Adults and emerged spiderlings were fed *H. zea* eggs every three days and water was supplied by watering a cotton wick inside the dish during feeding days. Newly emerged spiderlings were isolated and reared in individual 100 x 15 mm. plastic petri dishes. Spiderlings reaching adulthood were mated randomly and placed in individual 150 x 25 mm. plastic petri dishes for oviposition. Newly emerged spiderlings (1-3 d) were isolated and separated in groups of seven in 100 x 15 mm. plastic petri dishes. Groups of spiderlings were conditioned by holding them without food for 24 hours prior to each experiment. A wet cotton wick was supplied to provide moisture during conditioning periods.

The behavioral responses of conditioned *C. inclusum* spiderlings were observed during one hour and the number of spiderlings on a given substrate was counted and recorded every five minutes. Preliminary observations included behaviors characteristic of local-area search such as decreasing speed, increase turning frequency, slow front leg movements, slow body rotation, abdomen inclination, and dragging pedipalps. Because spiderlings exhibiting any of the above mentioned behaviors remain in a treated patch longer we can use this to evaluate searching behavior. Fifteen replicates were done with a corresponding control consisting of a separate plastic petri dish. Wandering spiders are more active at night, therefore, testing was carried out in the late afternoon (after

6:00pm). A laboratory hood was covered with black cloth to prevent any light from coming into the hood during experiments. A flashlight covered with white paper was used during behavioral observations, which did not seem to bother spiderlings throughout the experiments.

Searching responses to gross cues. A no-choice test was designed to study the types of cumulative or gross cues utilized by *C. inclusum* to locate and identify *H. zea* eggs as possible prey. Spiderlings were presented with a given substrate previously exposed to components directly related to *H. zea* eggs or to an untreated control. The searching response of *C. inclusum* to two different treatments was observed in this study. To determine and identify the types of cues utilized by this wandering spider, cardboard containers were lined with green florist paper for *H. zea* adults to oviposit. Before oviposition, areas of the paper containing scales of *H. zea* (from both female and male) were cut covering approximately $\frac{1}{4}$ of a 150 x 25 mm plastic petri dish (37.5 mm. in diameter). After oviposition, *H. zea* eggs were carefully removed from the florist paper and egg-free areas of the paper were cut covering $\frac{1}{4}$ of a 150 x 25mm. plastic petri dish. Conditioned spiderlings (7 per dish) were introduced into a plastic petri dish containing a substrate (florist paper) with scales only or a substrate with scales previously exposed to eggs. Both tests were performed along with their corresponding control, which consisted of a plastic petri dish with florist paper not exposed to either scales or eggs.

Searching response to potential chemical cues. To determine what type of kairomones spiders use to locate and recognize *H. zea* eggs as prey; the response of *C. inclusum* to solvent washes of *H. zea* eggs and scales was observed by a no-choice test

using five different treatments. Spiderlings were presented with a filter paper containing components directly related to *H. zea* eggs and moth scales, correspondingly.

H. zea eggs were washed with solvents of different polarities to extract potential chemical compounds being recognized by *C. inclusum* spiderlings as prey cues. The solvents were chosen according to their decreasing polarities, and included hexane, ethyl acetate, acetone, methanol and water. After allowing *H. zea* to oviposit on florist paper, approximately 300 eggs were collected in a glass vial containing 1ml of solvent. Each solvent used was filtered and placed in an eppendorf tube for testing purposes. The scales of 25 mated female moths were also collected in a separate glass vial and washed overnight with the same group of solvents used for egg washings. Approximately 100 μ l of solvent wash was loaded to a filter paper. The filter paper covered approximately $\frac{1}{4}$ of a 150 x 25 mm plastic petri dish (37.5 mm. in diameter), which was used as testing arena.

Prior to statistical analysis, proportional data were arcsin transformed to stabilize their variance (Zar 1999). Transformed data were then analyzed using a SAS mixed model for repeated measurement analysis to show differences between treatments.

Results

Searching responses to gross cues. *C. inclusum* spiderlings were more frequently observed on substrates previously exposed to *H. zea* eggs and scales than to untreated substrates. In the first test, where substrates were previously exposed to both eggs and scales, the number of spiderlings found on the treated substrates was significantly higher ($F=79.8$; $df=1, 28$; $P<0.0001$) than that of untreated substrates (Fig. 3). The mixed model analysis also allowed the observation of both the time ($F=1.32$; $df=11, 308$; $P=0.21$) and the time by treatment interaction ($F=1.10$; $df=11, 308$; $P=0.36$). Interactions were not significant and it did not affect the number of spiders found on the treated substrate (Fig. 4). In the second test, where substrates were previously exposed to scales only, the number of spiderlings found on substrates was significantly higher ($F=17.59$; $df=1, 28$; $P<0.0002$) than that of untreated substrates (Fig. 3). The time effect observed within treatments ($F=1.87$; $df=11, 308$; $P=0.04$) and the time by treatment interaction varied significantly ($F=2.21$; $df=11, 308$; $P=0.01$) (Fig. 5).

Searching response to potential chemical cues. *C. inclusum* spiderlings were more frequently observed on substrates containing solvent washes of *H. zea* eggs ($P<0.0001$) (Table 1). Similarly, there was a high frequency of spiderlings responding to substrates containing solvent washes of scales ($P<0.0001$) (Table 3). However, the percentage of spiderlings responding to egg solvent washes appeared higher than that of scale solvent washes (Fig. 6). The mixed model analysis also allowed the observation of both the time and the time by treatment interaction in the experiments. The response to eggs solvent washings throughout the testing time varied significantly ($P<0.0001$) among solvents and

solvents and there was indication of a time by treatment interaction ($P < 0.0011$). The effect of time on the percentage of spiderlings recorded on the substrate treated with egg extract obtained from different solvents also varied. Hexane showed significantly higher responses ($P < 0.01$) than any other solvent used to wash *H. zea* eggs (Table 2). The response to scale solvent washings throughout the testing time varied significantly ($P < 0.0005$) among solvents and there was no indication of a time by treatment interaction ($P < 0.085$) (Table 3). The effect of time on the percentage of spiderlings recorded on the substrate treated with scale extract obtained from different solvents was not significant. Spiderlings showed significantly higher responses to hexane washes of scales than any other solvent washes (Table 4). Treated substrates with hexane washes triggered a positive response for *C. inclusum* and distinct foraging behavior was observed.

The attempt to identify specific chemical compounds in hexane solvent washes of eggs and scales was also made. Gas chromatography (GC) techniques required a more specialized method of extraction since no chemical compounds were successfully identified. Several GC injections were made and a variety of methods were tested, however, no positive identification of chemical compounds was obtained when compared to a chromatograph database. Further studies exploring extraction of chemical cues might be helpful in the identification of the involved chemical compounds.

Figure 1. *C. inclusum* spiderling feeding on *H. zea* eggs



Figure 2. Adult *C. inclusum* feeding on *H. zea* eggs



(Picture by R.Pfannenstiel)

Figure 3. Response of spiderlings to substrate exposed to *H. zea* eggs and scales and to scales only. Data are presented as mean percentage of spiderlings responding to substrates \pm SE (means followed by different letters within each group are significantly different, $P < 0.05$).

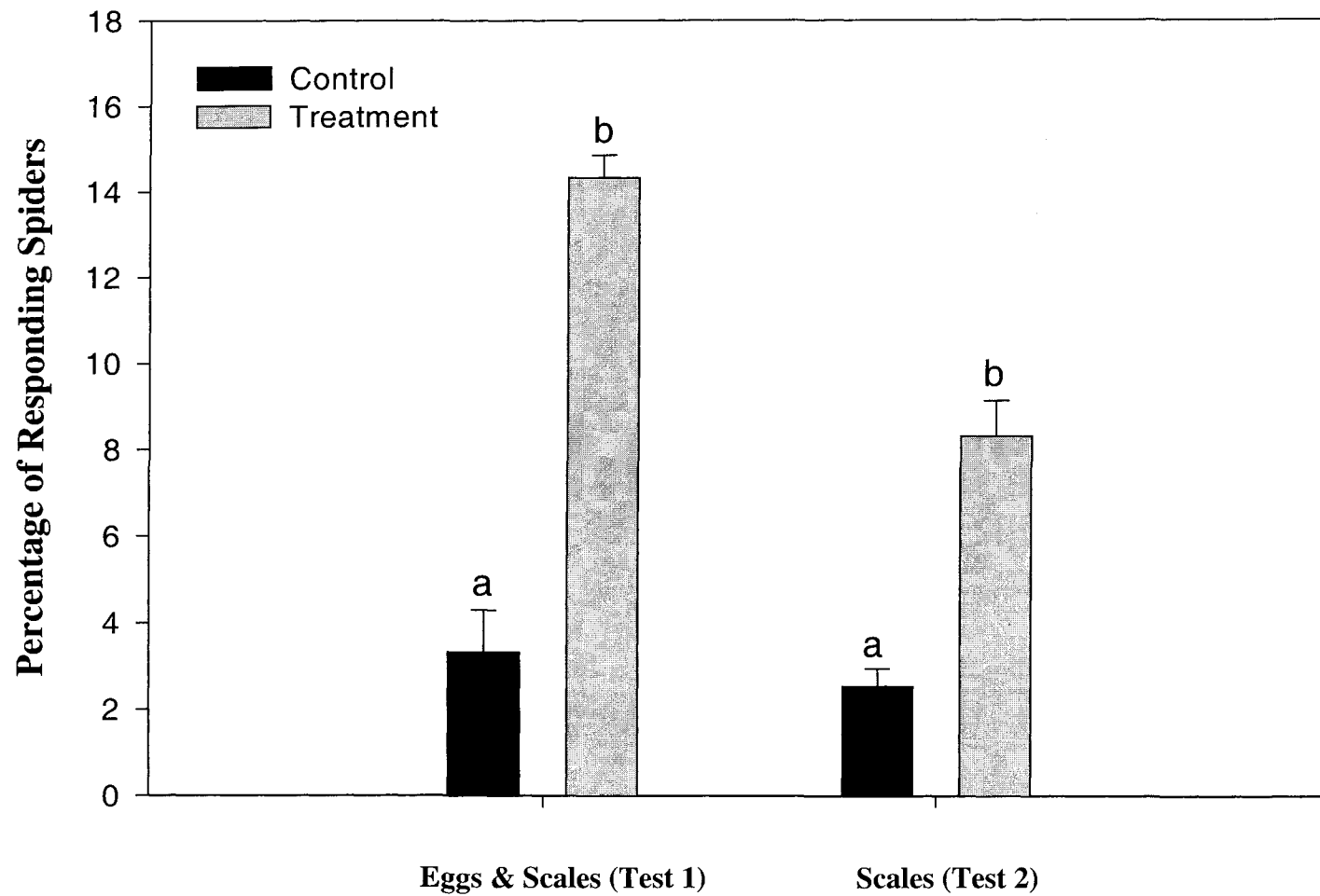


Figure 4. Time-response of *C. inclusum* spiderlings to substrates exposed to eggs and scales of *H. zea*. Data are presented as mean percentage of spiderlings responding to substrates \pm SE.

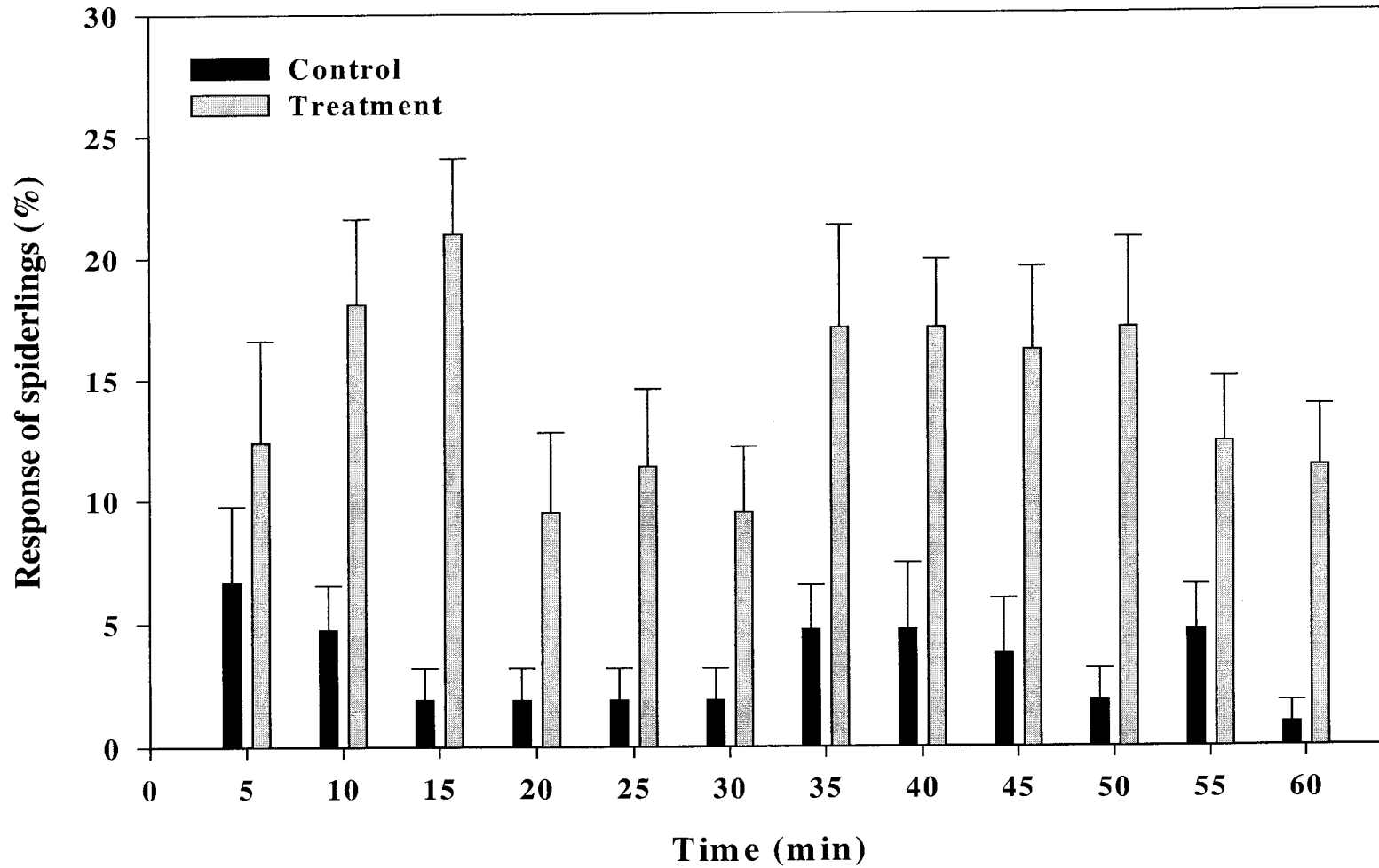


Figure 5. Time-response of *C. inclusum* spiderlings to substrates exposed to scales of *H. zea*. Data are presented as mean percentage of spiderlings responding to substrates \pm SE.

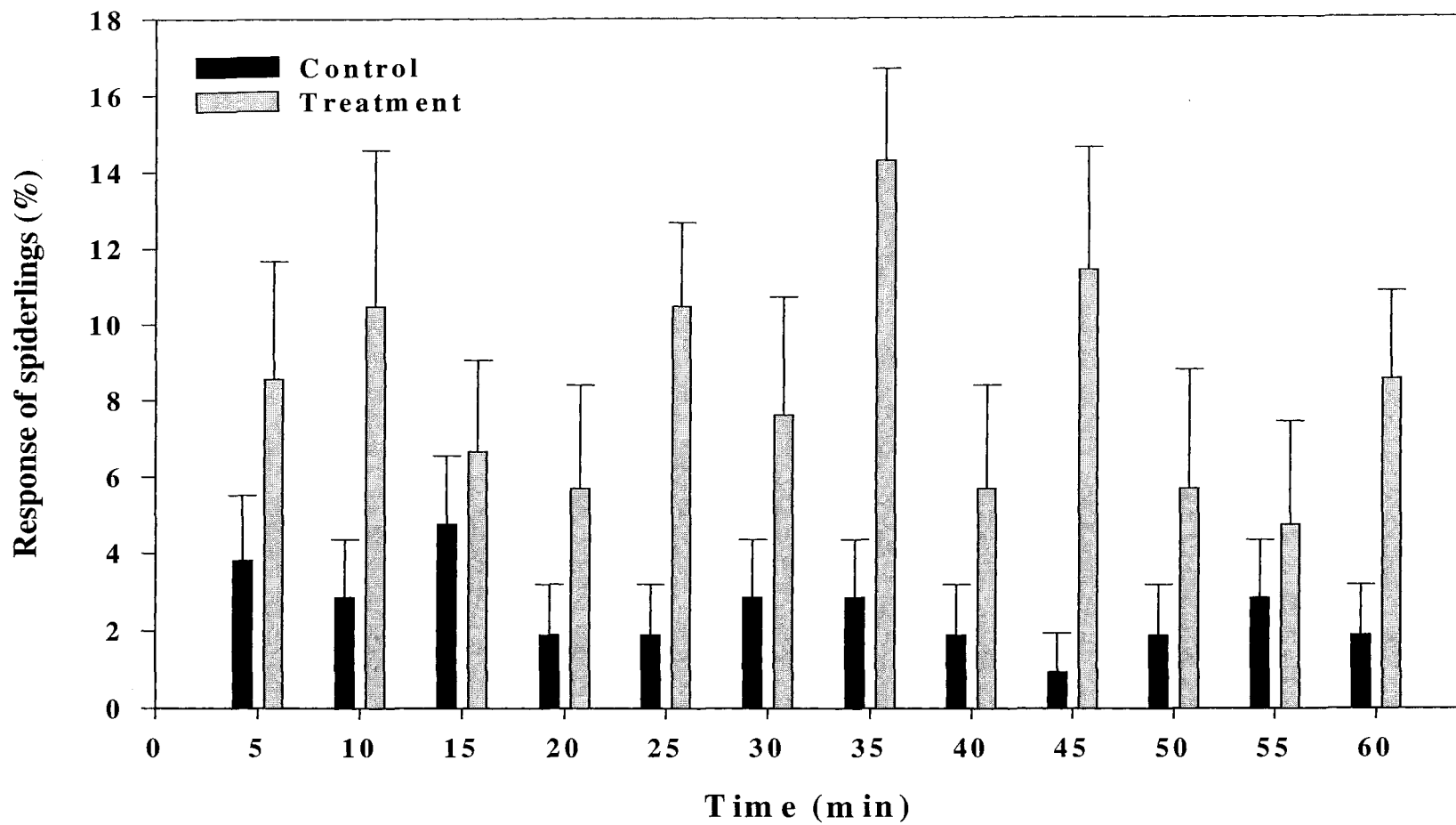


Table 1 Mixed model analysis of the effect of solvent treatment and observation time on the response of spiderlings to substrates treated with egg washes

Factor	Numerator df	Denominator df	F-value	P-value
Treatment	5	84	25.93	< 0.0001
Time	11	924	6.66	0.0001
Treatment × Time	55	924	1.72	0.0011

Table 2 Effect of time on the percentage of spiderlings recorded on the substrate treated with egg extract obtained from different solvents

Treatment	Intercept		Linear term		Quadratic term	
	Parameter	SE	Parameter	SE	Parameter	SE
Control	4.81 ^{**}	0.98	-0.51 ^{ns}	0.35	0.02 ^{ns}	0.03
Water	0.11 ^{ns}	2.14	0.47 ^{ns}	0.76	-0.04 ^{ns}	0.06
Methanol	6.86 ^{**}	2.14	-1.27 ^{ns}	0.76	0.07 ^{ns}	0.06
Acetone	2.60 ^{ns}	2.14	-0.51 ^{ns}	0.76	0.03 ^{ns}	0.06
Ethyl Acetate	9.67 ^{**}	2.14	-0.17 ^{ns}	0.76	-0.02 ^{ns}	0.06
Hexane	21.70 ^{**}	2.14	-3.69 ^{**}	0.76	0.17 ^{**}	0.06

ns = non significant, * = significant (P < 0.05) and ** = highly significant (P < 0.01)

Table 3 Mixed model analysis of the effect of solvent treatment and observation time on the response of spiderlings to substrates treated with scale washes

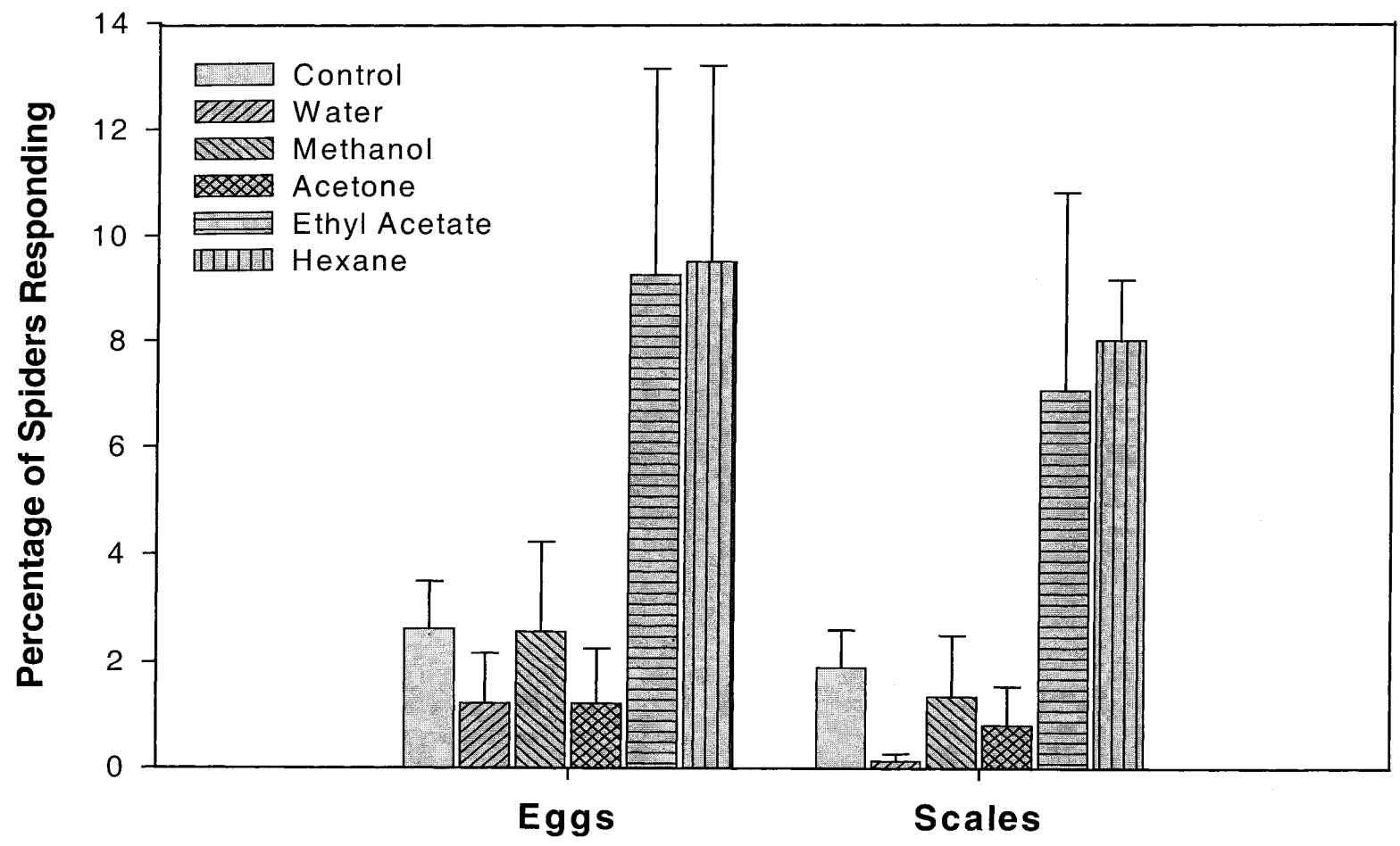
Factor	Numerator df	Denominator df	F-value	P-value
Treatment	5	84	25.92	< 0.0001
Time	11	924	3.06	0.0005
Treatment × Time	55	924	1.28	0.085

Table 4 Effect of time on the percentage of spiderlings recorded on the substrate treated with scale extract obtained from different solvents

Treatment	Intercept		Linear term		Quadratic term	
	Parameter	SE	Parameter	SE	Parameter	SE
Control	4.16 ^{**}	0.87	-0.56 ^{ns}	0.31	0.02 ^{ns}	0.02
Water	0.66 ^{ns}	1.89	-0.15 ^{ns}	0.67	0.01 ^{ns}	0.05
Methanol	3.29 ^{ns}	1.89	-0.60 ^{ns}	0.67	0.03 ^{ns}	0.05
Acetone	1.19 ^{ns}	1.89	-0.37 ^{ns}	0.67	0.04 ^{ns}	0.05
Ethyl Acetate	5.08 ^{**}	1.89	0.65 ^{ns}	0.67	-0.07 ^{ns}	0.05
Hexane	14.35 ^{**}	1.89	-2.08 ^{**}	0.67	0.11 [*]	0.05

ns = non significant, * = significant (P < 0.05) and ** = highly significant (P < 0.01)

Figure 6. Response of *C. inclusum* spiderlings to substrate exposed to egg and scale extracts from different solvents. Data are presented as mean percentage of spiderlings responding to substrates \pm SE.



Discussion

The no-choice experiments showed that *C. inclusum* spiderlings have the ability to perceive cues left by ovipositing *H. zea* moths. Spiderlings were more frequently observed on substrates with residues from eggs and scales giving the indication that there may be a chemical being perceived by these spiders. The presence of scales and the essence of egg residues on the treated substrate seemed to induce a prey searching behavior on *C. inclusum* spiderlings. These wandering spiders showed a tendency to actively explore a great part of the testing arena before finding the substrate. A decrease in speed immediately after coming into contact with a treated substrate was frequently observed. *C. inclusum* evidently detects prey cues and begins local-area search as characterized by significantly slowing down and investigating the surroundings with its front legs. The forelegs typically moved slowly, feeling the substrate, perhaps in an attempt to locate food. After spiderlings engaged in this initial searching behavior, most of them tended to stay on the treated substrate. Slow rotation of the spider's body along with continuous and rapid pedipalp movements were often observed. In addition, *C. inclusum* frequently dragged its pedipalps over treated substrates, an indication of egg residues being sensed or recognized, since pedipalps have been previously associated with taste. A final foraging behavior characterized by this wandering spider was the upward inclination of the abdomen toward the treated substrate (Fig. 1). Such behavior had been previously observed on adult spiders before and during feeding on lepidopteran eggs (Fig. 2).

Spiderlings exhibited a more explicit searching behavior when presented with egg-scale treated substrates than substrates containing scales only. In the first test, the substrate presented to spiderlings had been exposed to both moth eggs and scales, with the eggs carefully removed. A higher response from spiderlings to this treatment was observed when compared to substrates that were exposed to scales only, indicating that although cues from both eggs and scales appear to be used by spiderlings, eggs and their components play a more important role in the prey recognition process.

During the third and fourth tests, *C. inclusum* exhibited a similar searching behavior when exposed to substrates treated with hexane washes of both eggs and scales. Although some egg and scale solvent washes showed a significantly higher response than the controls, there was a higher percentage response to egg washes than to scale washes, suggesting that chemical cues perceived by spiderlings might be stronger on moth eggs than scales. The responses of spiderlings varied with time among treatments. The amount of solvent containing extracted compounds placed on filter paper was subject to evaporation and a decrease of strength throughout the testing period. Since data collected did not show a specific trend, response differences due to time are attributed to a decline on solvent strength during the experiments. Spiderlings may also be responding to more than one compound associated with *H. zea* eggs and scales. Different solvents may only extract a portion of these compounds and spiderlings searching responses may degrade rapidly in the absence of the full suite of cues.

Spiderlings showed a significantly higher response to hexane washings of both eggs and scales. Hexane is a non-polar solvent, which seemed to extract chemical cues from moth eggs and scales much better than the rest of the tested solvents. Non-polar solvents

tend to extract chemical compounds better from hydrophobic structures, like scales and eggs, than polar solvents. Scales possess hydrophobic properties that protect moths from polar solvents such as water and similarly, eggs are not significantly affected by polar solvents. Hexane is highly volatile and when it evaporated, presumably left nothing but chemical cues associated with moth eggs and scales. Spiderlings showed particular foraging behavior such as reduction of speed and dragging of pedipalps. Although there was a significant response to hexane washings, the behavior of spiderlings was not as explicit as when the actual gross cues were present. *Cheiracanthium inclusum* behavior suggests that the chemical cues presented in these experiments are utilized as contact kairomones, rather than volatile kairomones.

Spiderlings seemed to recognize chemical cues only when in contact with the treated area. It is unlikely that spiderlings would be able to recognize prey chemical volatiles from greater distances before getting close and in actual contact with these chemical cues. Sources of chemical stimuli may also include other oviposition related chemicals, frass, gland secretions, and accessory glands (Jones et al. 1973, Strand and Vinson 1982, Noldus and van Lenteren 1985) that aid in recognition of lepidopteran eggs as prey. Although there was no indication that *C. inclusum* used olfaction to locate *H. zea* eggs as prey, there are other examples of wandering spiders that exhibit this behavior. The cursorial spider *Habronestes bradleyi* (Zoodariidae), for example, is a specialist predator of the meat ant, *Iridomyrmex purpureus*. These aggressive ants release an alarm pheromone, 6-methyl-5-hepten-2-one, when injured. This alarm pheromone, in contrast, is used as a prey-location kairomone, which induces changes in foraging behavior of this particular spider, similar to that of parasitoids (Allan et al. 1996).

CONCLUSION

Wandering spiders rarely sit and wait for prey to appear; instead, these spiders are known to be active hunters constantly searching for prey. Searching behavior varies among wandering spiders and within prey types. It has been established that visual cues seem unlikely to be used by *C. inclusum* because the spider hunts primarily at night and vision plays only a minor role in most spiders' behavior (Foelix 1982). Detection of prey cues through contact chemoreception is a more likely modality for location of eggs. A combination of cues from *H. zea* scales and eggs might be needed by the spiderlings to successfully locate eggs and recognize them as prey. These no-choice experiments indicated that *C. inclusum* spiderlings are capable of perceiving chemical cues in the form of contact kairomones from *H. zea* eggs and scales. These contact kairomones have an effect on the spiderlings' behavior and tend to initiate foraging activities.

It is unclear what chemicals might be detected by spiderlings, as these experiments have focused primarily on the recognition and use of scales and eggs as cues on treated substrate to recognize sessile prey. These observations are part of the groundwork for studies supporting the use of chemical cues by wandering spiders; additional studies are needed to identify such chemicals. Strand and Vinson (1982) showed that there is a secretion found on deposited eggs of *Heliothis virescens* F., which the parasitoid *Telenomus heliothidis* Ashmead uses to locate the eggs. The source of this secretion was the accessory gland of adult female *H. virescens*, which serves as an adhesive for the attachment of eggs to a chosen substrate. It was concluded that proteins present in the accessory gland of *H. virescens* serve as an egg recognition kairomone for the parasitoid

T. heliothidis. In this study, accessory glands were not tested but it is likely that spiderlings are using the same kairomone or similar egg components to identify lepidopteran eggs as prey.

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