▶ :ILL: 5751943 :Borrower: SUS :ReqDate: 20030416 :NeedBefore: 20030516
:Status: IN PROCESS 20030416 :RecDate: :RenewalReq:
:OCLC: 2245984 :Source: OCLCWEB :DueDate: :NewDueDate:
:Lender: *PGM,EXW,EXW,IAH,VXW ¶
▶ :CALLNO: *Lender's OCLC LDR: 11-25 1985-1999 ¶
▶ :TITLE: Journal of chemical ecology. ¶
▶ :IMPRINT: New York [Plenum Pub. Corp.] ¶
▶ :ARTICLE: Airborne chemical communication in the solf spider ... / Searcy et al. ¶
▶ :VOL: 25 :NO: :DATE: 1999 :PAGES: 2527-eoa ¶
▶ :VERIFIED: OCLC ISSN: 0098-0331 [Format: Serial] ¶
▶ :PATRON: Persons, Matt ¶
▶ :SHIP TO: ILL/Blough Weis Library/Susquehanna University/514 University Ave./Selinsgrove, PA 17870-1050 ¶
▶ :BILL TO: SAME/Copy of request must accompany invoice for payment. FEIN 23-1353385N ¶
▶ :SHIP VIA: Ariel/IDS#173/lib.rt. :MAXCOST: $20.00IFM
:COPYRT COMPLIANCE: CCG ¶
▶ :FAX: (570)372-4310 ARIEL: (NEW NUMBER) 216.87.202.20 Ariel Email: ariel@susqu.edu ¶
▶ :E-MAIL: ritchey@susqu.edu ¶
▶ :BILLING NOTES: Prefer IFM; will accept invoice. ¶
▶ :BORROWING NOTES: PHA#90144000028834. CAI#016916. ISI#016916. British Lib.#51-3001. Reciprocal borrowing welcome. Copyright fees paid to CCC. ¶
▶ :AFFILIATION: Members of ACLCP, SLC,TCLC,LVIS,PALCI. ¶
▶ :LENDING CHARGES: :SHIPPED: :SHIP INSURANCE: ¶
▶ :LENDING RESTRICTIONS: ¶
▶ :LENDING NOTES: ¶
▶ :RETURN TO: ¶
▶ :RETURN VIA: ¶

Journal of Chemical Ecology, Vol. 25, No. 11, 1999

AIRBORNE CHEMICAL COMMUNICATION IN THE WOLF SPIDER *Pardosa milvina*

L. E. SEARCY,[1] A. L. RYPSTRA,[2] and M. H. PERSONS[1,*]

[1]*Department of Zoology, Miami University*
Oxford, Ohio 45056

[2]*Department of Zoology, Miami University*
Hamilton, Ohio 45011

(Received November 23, 1998; accepted June 29, 1999)

Abstract—Most studies involving chemical communication in spiders focus on contact pheromones attached to spider silk. Here we tested if males of the wolf spider *Pardosa milvina* use airborne pheromones to identify, locate, and follow females. Using a two-choice olfactometer, we tested the response of adult male *P. milvina* to a number of potential chemical cues while controlling for concomitant visual and vibratory stimuli. An airborne chemical cue from adult virgin female *P. milvina* elicited a positive taxis response from the male. We also tested adult male responses to penultimate instar female *P. milvina*, one adult male *P. milvina*, and two adult males together. In each case, test males showed no attraction to the stimuli. Additional experiments were run with pitfall traps baited with adult virgin female *P. milvina* as attractants. Again, we controlled for visual and vibratory cues from females. Pitfall traps containing virgin females captured significantly more males than control traps. Collectively, these experiments demonstrate evidence of an airborne sex pheromone in *P. milvina*.

Key Words—*Pardosa milvina*, Lycosidae, airborne, sex pheromone, wolf spider, olfactometer, pitfall traps.

INTRODUCTION

Pheromone-based communication has been extensively studied among many species (Shorey, 1976; Bradbury and Vehrencamp, 1998), while research on chemical communication among spiders has lagged behind (reviewed in Tietjen and Rovner, 1982; Foelix, 1996). Most experimental studies have focused primarily on pheromones deposited on webs (Suter and Renkes, 1984; Suter and

*To whom correspondence should be addressed at: Department of Biology, Susquehanna University, Selinsgrove, Pennsylvania 17870.

0098-0331/99/1100-2527$16.00/0

Hirscheimer, 1986; Schulz and Toft, 1993; Coyle and Shear, 1981), draglines (Dondale and Hegdekar, 1973), or the substratum (Richter et al., 1971; Tietjen, 1979). Typically, spiders detect sex pheromones by making physical contact with either another spider, silk from a dragline, or a web. However, there has been evidence of direct airborne chemical communication among some species of web-building spiders. Newly molted female *Nephila clavata* (Araneae, Tetragnathidae) produce a distinct chemical cue after molting. This stimulates males to induce mating when females are still vulnerable. In addition, these males then release similar mimic chemicals to attract prey (Miyashita and Hayashi, 1996). In the Sierra dome spider (*Linyphia litigiosa*) (Linyphiidae), airborne pheromones are so important to mate attraction that, upon locating a virgin female's web, the male wads up the web to prevent further pheromone dispersion (Watson, 1986).

Ground-dwelling wolf spiders (Araneae, Lycosidae) live in low vegetation and leaf litter (Hallander, 1967; Bultman and Uetz, 1982), a habitat that may restrict visual or vibratory communication (Scheffer et al., 1996). Experiments on pheromone use in lycosids has focused mainly on pheromones associated with spider silk (i.e., contact pheromones) (Hegdekar and Dondale, 1969; Richter et al., 1971; Sarinana et al., 1971; Dondale and Hegdekar, 1973). These studies show that male lycosids, including *Pardosa* spp., respond to contact pheromones, but show no clear evidence for male orientation based on airborne sex pheromone detection. Hegdekar and Dondale (1969) concluded that olfactory stimuli are not likely to be involved in the sexual behavior of male lycosids. However, Richter et al. (1971) demonstrated that olfaction does at least play a role in stimulating courtship in the wolf spider *Pardosa amentata* and that females with sealed spinnerets (therefore, no silk production) elicit a search response from the male. Furthermore, Tietjen (1979) found either orthokinetic or orientation responses in two *Schizocosa* spp. to airborne stimuli from hidden females.

In the present study, we examined the use of an airborne pheromone in the thin-legged wolf spider *Pardosa milvina* (Araneae, Lycosidae). We presumed that, if present, the pheromone would most likely be sex-related, so we specifically investigated whether males detect and respond to a chemical released by females. We presumed that conspecifics that were not mature virgin females would elicit no attraction response from males. In both laboratory and field studies, we observed the responses of males to a chemical released into the air by adult virgin females, and we contrasted these responses with those from any possible airborne stimuli from juvenile and male conspecifics.

METHODS AND MATERIALS

Study Species. *Pardosa milvina* are relatively small wolf spiders (females weigh approximately 20 mg) that actively search for prey as they move across

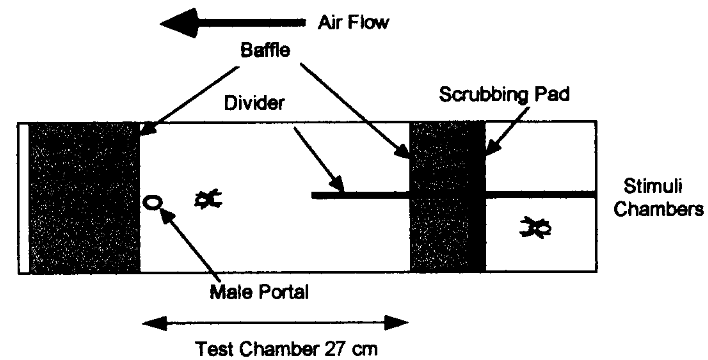

FIG. 1. Diagram (top view) of the two-choice olfactometer used to test male *Pardosa milvina* for positive taxis response.

the ground and low vegetation. *Pardosa milvina* used in these experiments were collected in soybean fields at Miami University Ecology Research Center (ERC) (Butler County, Ohio). Juvenile spiders were collected and reared in the laboratory (two to three weeks) until maturity to ensure virgin mating status of males and females. Males and immature *P. milvina* used in tests were caught three to four days prior to experimentation and were released after testing. All animals were fed three fruit flies (*Drosophila melanogaster*) 24 hr prior to their experimental trial to control for hunger. Spiders in the lab were kept in separate vials (8 cm × 3 cm diam.), provided with water, and kept on a 12L : 12D photoperiod. Adult female *P. milvina* are difficult to distinguish from other *Pardosa* species while alive. Therefore, we verified the species of all females used in trials by preserving and examining the external genitalia of each individual after completion of experiments.

Olfactometer Design. Laboratory trials were held in a two choice linear olfactometer (Figure 1). One end was connected to a fan that pulled air at 50–80 cm/min, thus creating a one-way airflow system. Screens and baffling were used on both ends of the test chamber to maintain even flow of the air and prevent contact between stimulus and test spiders.

The potential source of chemical stimulus was randomly assigned for each trial to one side of the divided tube in the upwind end. The stimulus spider was allowed to acclimate in the olfactometer for 5 min prior to introduction of the test male. We then covered the test chamber with a metal sleeve to eliminate external visual cues presented to the test male as well as to eliminate any possible visual detection of the female. An adult male was then placed into the test chamber by a portal in the downwind end. After 10 min, the sleeve was removed, and the location of the male spider noted. The tube system and screening were rinsed before every trial with 70% ethanol to remove any silk or pheromones deposited by the spiders and then allowed to dry.

Behavioral Assay. Adult males were tested in the olfactometer with adult virgin female *P. milvina* (N = 17) and with immature penultimate instar female *P. milvina* (N = 13). In addition, males were tested with one adult male as a stimulus (N = 15), and with two adult males together in a stimulus chamber (N = 15). No test or stimulus spider was used more than once. Control trials were held with no stimulus in either chamber (N = 10).

Male *P. milvina* were found in three places within the olfactometer. If the male was found on the screen divider that separated him from the potential stimulus, we recorded it as a positive choice. If the male was found on the screen divider near the empty chamber, we recorded it as a negative choice. If the male was located in any other place within the olfactometer, we determined that no choice was made. We tested for nonrandom male choice using a χ^2 goodness-of-fit test.

Field Trials. The field trials were conducted in the soybean plots of the Ecology Research Center in July 1997, when *P. milvina* populations were at an observably high density and reproductive peak. Pitfall traps baited with female *P. milvina* in a screen-topped vial (10 cm × 3 cm diam.) were placed into pitfall cups (14 cm deep × 11 cm diam.). The cups were buried such that their lips were flush with the soil surface. This set-up allowed airborne chemicals produced by the females to diffuse out, but eliminated visual and vibratory communication. Traps were run with adult virgin females (N = 17) or empty vials (controls; N = 8) for a 9-hr period (08:00–15:00 hr). Traps were set at randomly determined locations separated by 20–65 m. At the end of each trial, the numbers of male *P. milvina* captured were recorded. We used an unpaired t test to compare the number of males in female-baited traps vs. the number in empty control traps.

RESULTS

Olfactometer Tests. The olfactometer results show that males chose unmated females significantly more often than an empty chamber (χ^2 = 8.94, df = 2, P = 0.011) (Table 1). Of the males tested, 65% chose the female stimulus chamber over the empty chamber. Although male *P. milvina* responded to immature females in a nonrandom way (χ^2 = 11.2, df = 2, P = 0.003), they showed a lack of attraction to the stimulus (Table 1). Seventy-seven percent did not make a choice. The male tested with one male (χ^2 = 3.6, df = 2, P = 0.165) and two-male trials (χ^2 = 10.8, df = 2, P = 0.004) showed results similar to the immature trials (Table 1). The results differed from random, but no attraction to the stimulus was shown. In the single male trials, 53% chose not to make a choice, with only 13% choosing the male chamber. The two-male trials had 73% no choice responses, while only 13% chose the chamber holding the two males.

TABLE 1. MALE RESPONSE TO POTENTIAL STIMULI IN TWO-CHOICE OLFACTOMETER

Male Response	Virgin female (N = 17)	Immature female *P. milvina* (N = 13)	1 Male (N = 15)	2 males (N = 15)
Positive choice	11	2	2	2
Negative choice	1	1	5	2
No choice	5	10	8	11
χ^2	8.94	11.2	3.6	10.8
df	2	2	2	2

Field Trials. The field trials showed that pitfall traps baited with unmated females caught significantly more male *P. milvina* than control traps (t = −2.420, df = 19, P = 0.0257). Female baited traps captured a mean of 1.692 ± 0.263 males, whereas control traps caught an average of 0.750 ± 0.250 males.

DISCUSSION

Male *P. milvina* clearly respond to an airborne chemical cue. Under laboratory conditions, male *P. milvina* are attracted to adult virgin female *P. milvina* via an airborne pheromone, while immature females and other adult males elicit no attraction response from the male. Males exhibit a nonrandom response to juveniles and males by showing no choice significantly more often than expected by chance. This suggests that a cue from these latter stimulus sources may inhibit movement. Pitfall traps containing unmated females caught significantly more males, which indicates that an airborne sex pheromone produced by females is an effective form of communication under field conditions.

Richter et al. (1971) found olfactory cues in *Pardosa amentata* to play only a minor role in sexual communication relative to silk-associated contact pheromones. Tietjen (1979) found that males of two *Schizocosa* spp. of wolf spider were affected by female airborne pheromones. In contrast to these and the present studies, other olfaction testing on lycosids, including *Pardosa* spp., has shown either no attraction response of males to females or ambiguous results. *Lycosa rabida* and *Lycosa punctulata* showed no response when tested for detection of possible airborne pheromones (Tietjen, 1979). In experiments conducted by Hegdekar and Dondale (1969), two *Schizocosa* spp. and two *Pardosa* spp. (excluding *P. milvina*) showed positive, although identical reactions of males to contact pheromones from mated and unmated females. However, Hegdekar and Dondale (1969) found no evidence of an olfactory pheromone in any of the four species tested.

For a number of reasons, *Pardosa milvina* males may rely more heavily on an airborne sex pheromone for communication relative to any other species of wolf spider so far studied. First, *P. milvina* are widely ranging spiders and do not exhibit a sit-and-wait foraging strategy typical of most species of wolf spider, including other *Pardosa* spp. (Walker et al., 1999; Ford, 1978). Greater activity may make locating females through silk draglines more difficult and time consuming for males than through airborne pheromones. Second, greater movement of females would likely make draglines more costly to produce. Furthermore, since male *Pardosa* spp. commonly cover more distance than females in daily travel (Hallander, 1967), sole production of this pheromone by females is likely, although female response to airborne pheromones from other females or males was not examined in our study.

For our experiments, *P. milvina* were collected in soybean fields with open ground and little vegetation. Richter (1970) found a correlation between habitat structure of *Pardosa* spp. and the quantity of draglines produced. He found that species collected from open habitats and short vegetation tended to produce smaller quantities of silk as they moved through the environment than species from taller, more dense vegetation. Based on his findings, our population of *P. milvina* should produce less silk, which would increase the benefits of using an airborne pheromone in conjunction with a contact sex pheromone to facilitate communication between the sexes. Although laboratory observations indicate that *P. milvina* females do not produce large amounts of silk, further studies on *P. milvina* need to be conducted in these areas to understand the relative importance of contact versus airborne pheromones.

Male *P. milvina* showed a significant lack of response (neither positive nor negative) in the presence of an airborne chemical from immature females as well as adult males, and this effect increased when two males were used. Ayyagari and Tietjen (1986) found that male *Schizocosa ocreata* produce a male contact pheromone that inhibits male courtship and induces immobility in other males. Such a pheromone is sufficient to prevent courtship behavior when presented in high concentrations even in the presence of female contact sex pheromones. Our results suggest that an airborne inhibitory pheromone is produced by male and possibly subadult female *P. milvina*.

Acknowledgment—This project was funded by the Summer Scholars Program of Miami University Office for the Advancement of Scholarship & Teaching. We thank R. Stander for use of the olfactometer, S. Walker for the statistical analysis, and R. Balfour, S. Marshall, and M. Thomann for help with experiments. Further financial support was provided by National Science Foundation grant DEB-9527710.

REFERENCES

AYYAGARI, L. R., and TIETJEN, W. J. 1986. Preliminary isolation of male-inhibitory pheromone of the spider Schizocosa ocreata (Araneae, Lycosidae). *J. Chem. Ecol.* 13:237–245.

BRADBURY, J. W., and VEHRENCAMP, S. L. 1998. Principles of Animal Communication. Sinauer Associates, Sunderland, Massachusetts, 882 pp.

BULTMAN, T. L., and UETZ, G. W. 1982. Abundance and community structure of forest floor spiders following litter manipulation. *Oecologia* 55:34–41.

COYLE, F. A., and SHEAR, W. A. 1981. Observations on the natural history of *Sphodros abboti* and *Sphodros rufipes* (Araneae, Atypidae), with evidence for a contact sex pheromone. *J. Arachnol.* 9:317–326.

DONDALE, C. D., and HEGDEKAR, B. M. 1973. The contact sex pheromone of *Pardosa lapidicina* Emerton (Araneida: Lycosidae). *Can. J. Zool.* 51:400–401.

FOELIX, R. F. 1996. Biology of Spiders. Harvard University Press, Cambridge, Massachusetts, 306 pp.

FORD, M. J. 1978. Locomotory activity and the predation strategy of the wolf-spider *Pardosa amentata* (Clerck) (Lycosidae). *Anim. Behav.* 26:31–35.

HALLANDER, H. 1967. Range and movements of the wolf spiders *Pardosa chelata* (O. F. Miller) and *P. pullata* (Clerck). *Oikos* 18:360–364.

HEGDEKAR, B. M., and DONDALE, C. D. 1969. A contact sex pheromone and some response parameters is lycosid spiders. *Can. J. Zool.* 47:1–4.

MIYASHITA, T., and HAYASHI, H. 1996. Volatile chemical cue elicits mating behavior of cohabiting males of *Nephila clavata* (Araneae, Tetragnathidae). *J. Arachnol.* 24:9–15.

RICHTER, C. J. J. 1970. Relation between habitat structure and development of the glandulae ampullaceae in eight wolf spider species (*Pardosa*, Araneae, Lycosidae). *Oecologia* 5:185–199.

RICHTER, C. J. J., STOLTING, H. C. J., and VLIJM, L. 1971. Silk production in adult females of the wolf spider *Pardosa amentata* (Lycosidae, Araneae). *J. Zool. London* 165:285–290.

SARINANA, F. O., KITTREDGE, J. S., and LOWRIE, D. C. 1971. A preliminary investigation of the sex pheromone of *Pardosa ramulosa*. *Notes Arachnol. Southwest.* 2:9–11.

SCHEFFER, S. J., UETZ, G. W., and STRATTON, G. E. 1996. Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Araneae: Lycosidae). *Behav. Ecol. Sociobiol.* 38:17–23.

SCHULZ, S., and TOFT, S. 1993. Identification of a sex pheromone from a spider. *Science* 260:1635–1637.

SHOREY, H. H. 1976. Animal Communication by Pheromones. Academic Press, New York, 167 pp.

SUTER, R. B., and HIRSCHEIMER, A. J. 1986. Multiple web-borne pheromones in a spider *Frontinella pyramitela* (Araneae, Linyphiidae). *Anim. Behav.* 34:748–753.

SUTER, R. B., and RENKES, G. 1984. The courtship of *Frontinella pyramitela* (Araneae, Linyphiidae): patterns, vibrations and functions. *J. Arachnol.* 12:37–54.

TIETJEN, W. J. 1979. Tests for olfactory communication in four species of wolf spiders (Araneae, Lycosidae). *J. Arachnol.* 6:197–206.

TIETJEN, W. J., and ROVNER, J. S. 1982. Chemical communication in lycosids and other spiders, pp. 249–279, *in* P. N. Witt and J. S. Rovner (eds.). Spider Communication. Mechanisms and Ecological Significance. Princeton University Press, Princeton, New Jersey.

WALKER, S. E., MARSHALL, S. D., RYPSTRA, A. L., and TAYLOR, D. H. 1999. The effects of hunger on locomotor behaviour in two species of wolf spider (Araneae, Lycosidae). *Anim. Behav.* In press.

WATSON, P. J. 1986. Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa* (Araneae, Linyphiidae). *Science* 223:219–221.