

## SHORT COMMUNICATION

# THE EFFECTS OF MOISTURE AND HEAT ON THE EFFICACY OF CHEMICAL CUES USED IN PREDATOR DETECTION BY THE WOLF SPIDER *PARDOSA MILVINA* (ARANEAE, LYCOSIDAE)

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**ABSTRACT.** Little is known about how environmental conditions affect the relative efficacy of information present in chemical cues. The wolf spider, *Pardosa milvina*, responds to silk and excreta from a larger species of wolf spider, *Hogna helluo*, with effective antipredator behavior. We investigated whether wetting or heating chemotactile cues of *Hogna helluo* would reduce the amount of antipredator behavior displayed by *Pardosa milvina* relative to unmanipulated cues. *Pardosa milvina* showed less antipredator behavior on chemotactile cues that had been wetted then dried but did not respond differently in the presence of cues that had been heated and then cooled. The results suggest that, in the field, morning dew may degrade some of the cues deposited by *H. helluo* at night and reduce the ability of *P. milvina* to avoid predation. However, typical periods of daily heating of cues may not affect the efficacy of predator detection by *P. milvina*.

**Keywords:** Antipredator behavior, chemotactile cues, moisture, heat

Many animals have evolved behaviors that reduce the risk of predation, often at the cost of lost foraging opportunities (Sih 1980; Stephens & Krebs 1986). To minimize the costs of antipredator behavior, some animals adjust their behavioral response to a predator depending on the relative magnitude of the threat (Kats & Dill 1998; Dicke & Grostal 2001). A variety of cues, including visual information, vibrations, and chemicals, may be used to detect the presence of and threat posed by a predator (Lima & Dill 1990). Chemotactile cues may be especially important for predator detection by the wolf spider *Pardosa milvina* (Hentz 1844) (Araneae, Lycosidae; Persons et al. 2002). This relatively small wolf spider (ca. 20 mg) significantly reduces movement in the presence of silk and excreta from the larger (adult female, ca. 300–800 mg) syntopic wolf spider *Hogna helluo* (Walckenaer 1837) (Araneae, Lycosidae; Persons & Rypstra 2001; Barnes et al. 2002). This reduction in movement results in a lower probability of predation for *P. milvina* (Persons et al. 2002). However, long term exposure to cues can have significant costs, includ-

ing weight loss and lower egg production (Persons et al. 2002). Thus, *P. milvina* finely adjusts its antipredator behavior depending on the size of the predator (Persons & Rypstra 2001), the diet of the predator (Persons et al. 2001), and the length of time since predator cues were deposited (Barnes et al. 2002).

The level of antipredator behaviors exhibited (i.e. reductions in activity) may depend on the reliability of cues present in the environment. Past studies of the response of *P. milvina* to cues of *H. helluo* have been conducted in controlled laboratory environments (Barnes et al. 2002; Persons et al. 2002). Yet, in nature, cues may be exposed to a variety of environmental conditions that may affect the quality of cues and the information they contain about a predator. Even within a single day, conditions may change from cool and wet (e.g. from dew) in the morning, to hot and dry during the middle of the day. Information is needed on how environmental conditions may affect predator cues in order to extrapolate the results of laboratory studies to the natural environment. The purpose of the study was to

examine the impact of two potentially important environmental variables, moisture and heat, on the relative efficacy of chemotactile cues that induce antipredator behavior by *P. milvina*.

All *P. milvina* used as experimental subjects were collected in the soybean fields at the Miami University Ecology Research Center (Oxford, Butler County, Ohio, USA) between June and August 2003. We used field caught adult female *P. milvina* in the experimental trials. None of the females used in the trials had produced an egg sac in the week prior to testing. *Hogna helluo* used for cue collection were adult and late instar immature females, all of which had been either field-caught or lab-reared from populations originating at the Ecology Research Center.

Both species were maintained in covered plastic cups (*P. milvina*: 5 cm high  $\times$  8 cm wide, *H. helluo*: 8 cm high  $\times$  12 cm wide) with a moist peat moss substrate in the laboratory on a 13:11 light:dark cycle at approximately 25 °C and 70% humidity. Both *P. milvina* and *H. helluo* were maintained on a diet of two appropriately sized domestic cricket nymphs (*Acheta domesticus*) once a week. The predators to be used as a source of chemotactile stimuli, *H. helluo*, were fed to satiation with eight juvenile crickets in the 24 hours preceding cue collection. This served to equalize the potential volume of silk and feces deposited on the substrate.

We collected predator silk and excreta cues on white filter paper (18.5 cm diameter) housed in covered, round plastic containers (20 cm in diameter  $\times$  8 cm high). Chambers were swabbed with alcohol and allowed to dry before we added the filter paper. A cotton dental wick saturated with double-distilled water was taped to the inside of each container lid to prevent spider desiccation. A single *H. helluo* was housed in each chamber for a minimum of 24 h (i.e., preceding the first trial run on a given day). Because the level of *P. milvina* response to *H. helluo* cues declines with cue age (Barnes et al. 2002), we did not remove the stimulus spider from the cue collection chamber until we were ready to treat the cue-laden filter paper with water or heat two hours before each trial.

The testing arena consisted of the same container type used for cue collection, except that the lid was removed to allow video recording of each trial from above. For each trial, we lined one side of the arena with unmanipulated *H. helluo* cues (control) and the other side with *H. helluo* cues that had been exposed to one of our experimental treatments. Before handling filter paper, and between handling filter paper with unmanipulated cues and experimentally-manipulated cues, we cleaned our hands by washing them with soap and water and then sterilizing them with alcohol. Experimental vs. control sides were alternated between trials during each experiment (i.e., the left side of the arena was designated as the

control in approximately 50% of the trials and vice versa). Arenas were swabbed with alcohol and allowed to dry between trials. Individual *P. milvina* were introduced to the center of the test chamber under a clear glass vial (2.5 cm in diameter  $\times$  6.5 cm high) on a small round circle of filter paper (diameter = 4.5 cm) which had not been exposed to predator cues. After an acclimation period of two minutes, we removed the glass vial and recorded *P. milvina* behavior remotely using a video camera.

We conducted trials from 25 August–5 September 2003, between 0930 h and 1630 h. The behavior of *P. milvina* was recorded from another room to minimize human disturbance during the trials. The video camera was mounted 1 m above the test chamber and the area was illuminated with fluorescent lighting; room temperature was ca. 25 °C. We quantified locomotor activity of the experimental *P. milvina* using an automated digital data collection system (Videomex-V, Columbus Instruments) connected to a Sony© Hi8 video camera. The system recorded spider movements on each side of the arena for one-minute intervals throughout each 30 min trial. We compared the following parameters between treatment and control sides of the arenas: distance traveled, time spent resting, time walking, residence time on each side of the arena, and time spent in non-forward movement (e.g. leg movements or turning). We discarded data from several animals that failed to move more than 100 cm during the 30 minute trial because these individuals may not have had sufficient experience sampling both sides of the arena. Typical distances traveled by *P. milvina* for 30 min in equivalent test arenas range from 300–1000 cm (Persons et al. 2001). We summed data over the 30 min trial and used paired t-tests to compare movement behaviors on the cue and control sides of the arena.

In our first experiment, *P. milvina* were given a choice between filter paper with *H. helluo* cues that had been saturated with water then allowed to dry for two hours (experimental treatment) vs. filter paper with cues collected from the same spider, not treated with water but allowed to age for the same two hour period (control treatment). Two hours before each trial, we removed the stimulus spider from the cue collection chamber, cut the filter paper in half with scissors, and wet the experimental section with 1.5 mL double-distilled water, dripped evenly across the cue-laden surface. Both experimental and control treatments ( $n = 14$ ) were left open to the air (at room temperature ca. 22.5 °C; humidity ca. 60%) to allow the wet side to dry for 2 hours.

Our second experiment consisted of a choice test between *H. helluo* cue-laden filter paper that had been heated to 40 °C, then cooled to room temperature (experimental treatment) vs. filter paper with cues collected from the same spider, not heated but

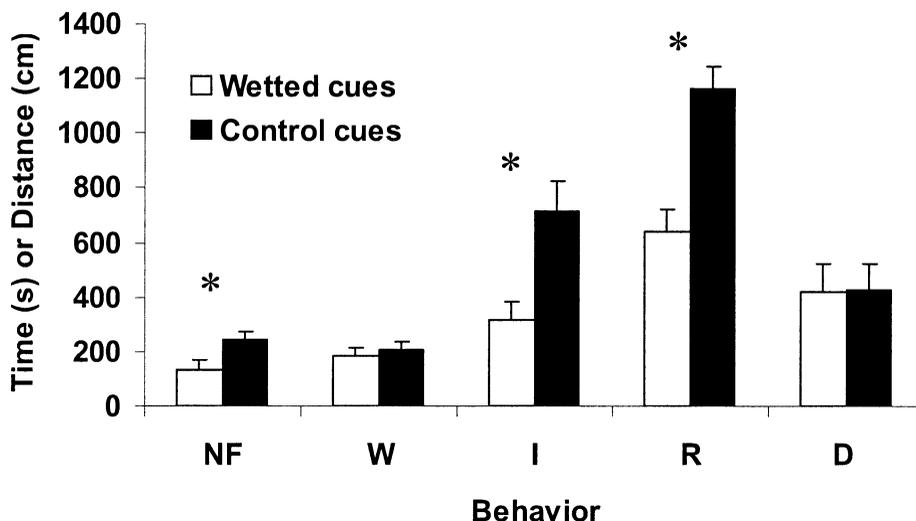


Figure 1.—Comparisons of the behavior of *P. milvina* *H. helluo* cues that were wetted then dried. Behaviors are denoted as follows: NF = time in non-forward movement, W = time walking, I = time immobile, R = residence time (on half of arena), D = Distance traveled. \* indicates  $P < 0.05$ .

allowed to age for the same two hour period (control treatment). Mean daily soil surface temperatures range between 20 and 30 °C from June–August in corn and soybean fields, where *P. milvina* and *H. helluo* are found in high abundance (NSIDC 2002). Occasionally, temperatures may rise above 40 °C for brief periods. However, a temperature of 40 °C was chosen for this study to evaluate if typical periods of heating during the summer would be sufficient to degrade the information contained in *H. helluo* cues. We removed the stimulus *H. helluo* from each chamber and divided the filter paper; the experimental half was placed in a drying oven preheated to 40 °C for 1.5 h, while the control half aged at room temperature (ca. 22.5 °C; humidity ca. 60%). The experimental filter paper was kept covered during heating to minimize the effect of the drying oven on the water content of the cue-laden paper. Each control filter paper was kept covered while the corresponding paper in the experimental treatment was in the oven. Containers with both treatments were left open to the air at room temperature during the 30 minute cooling period.

Wetting then drying the predator cues had a significant effect on the movements of *P. milvina* (Fig. 1). Spiders spent less time in non-forward movement (e.g. turning and appendage movements) on the wetted side of the arena than the control side ( $df = 8$ ,  $t = 4.40$ ,  $P = 0.002$ , Fig. 1). In addition, *P. milvina* spent significantly less time immobile ( $df = 8$ ,  $t = 2.58$ ,  $P = 0.03$ ) and had a lower residence time ( $df = 8$ ,  $t = 3.09$ ,  $P = 0.02$ ) on the treatment side of the arena that had previously been wet (Fig. 1). There was no effect of wetting the cues on time

spent walking ( $df = 8$ ,  $t = 1.37$ ,  $P = 0.21$ ) or distance traveled ( $df = 8$ ,  $t = 0.23$ ,  $P = 0.83$ ).

In contrast to the effects of water on cue efficacy, heating then cooling the predator cues had no effect on the movement of *P. milvina* (Fig. 2). There were no differences in non-forward movement ( $df = 12$ ,  $t = 1.55$ ,  $P = 0.15$ ), time spent walking ( $df = 12$ ,  $t = 0.03$ ,  $P = 0.98$ ), time immobile ( $df = 12$ ,  $t = 1.21$ ,  $P = 0.25$ ), residence time ( $df = 12$ ,  $t = 1.36$ ,  $P = 0.20$ ) and distance traveled ( $df = 12$ ,  $t = 0.79$ ,  $P = 0.44$ ) of *P. milvina* between previously heated and control predator cues.

Previous studies have shown that *P. milvina* responds to *H. helluo* silk and excreta with greater time spent immobile and greater residence time on cue substrates relative to controls (Persons et al. 2001; Barnes et al. 2002). Immobility has been shown to be an effective means of reducing predation risk from *H. helluo*, which may hunt using visual and/or vibratory cues (Persons et al. 2002). Thus the increase in activity we observed on the side of the arena that had been treated with water suggests that the cues deposited by *H. helluo* are significantly less effective in producing anti-predator behavior in *P. milvina*. Likewise, the greater amount of time that the spiders spent immobile on the control side of the arena where there were more effective chemical cues likely resulted in the counterintuitive observation they actually had longer residence times on the side of the arena where they perceived greater risk. We suspect that the greater time spent in non-forward movement (e.g. turning and appendage movements) in the presence of cues may constitute directional sampling of predator

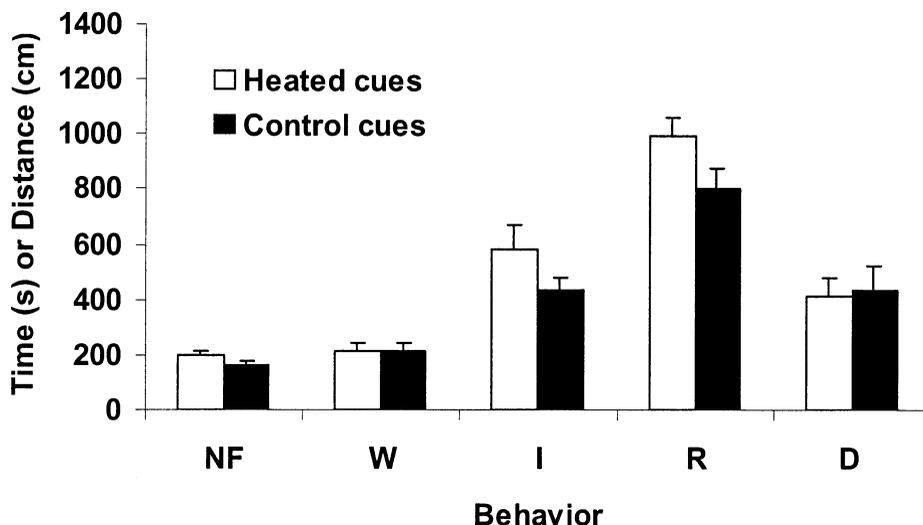


Figure 2.—Comparisons of behavior of *P. milvina* on *H. helluo* cues that were heated then cooled. Behaviors are denoted as follows: NF = time in non-forward movement, W = time walking, I = time immobile, R = residence time (on half of arena), D = Distance traveled.

cues and visual searching for nearby predators. Thus, less time in non-forward movement and less time immobile, and lower residence time on the previously wet substrate relative to control *H. helluo* cues, may indicate that water either reduces or completely eliminates the efficacy of the chemical cues used in predator detection by *P. milvina*.

The effects of water on predator cue efficacy may have important implications for *P. milvina*. *Hogna helluo* are primarily nocturnal and may deposit dense accumulations of silk and excreta around the entrance to their burrows, where they spend much of their time during the day (Walker et al. 1999a; b). Morning dew may then degrade much of the cues that were deposited at night and limit the ability of diurnal *P. milvina* to avoid or reduce movement in the proximity of *H. helluo* burrows. Periods after brief rainfall may also be dangerous to *P. milvina*. In addition to degrading predator cues, there is evidence that water degrades female sex pheromones, which may decrease the ability of males to find and mate with females (Dondale & Hegdekar 1973). Thus, the frequency of rainfall in a region may have implications for predator-prey interactions among *P. milvina* and *H. helluo*.

A temperature of 40 °C appeared to have no effect on the efficacy of *H. helluo* chemical cues. Lack of an effect of heating may be because the chemical cues in silk and excreta are tolerant of high temperatures, or because the heating period of the experiment was too short or of too low of a temperature to create a detectable difference. Further studies are needed to determine if longer periods of heating or higher temperatures, such as those experienced on some sunny summer after-

noons on barren ground, where cues may be exposed to short periods (ca. 1–2 hours) of temperatures in excess of 40 °C, affect the efficacy of *H. helluo* chemical cues.

It is not known what chemical, group of chemicals or tactile information in the silk or excreta of *H. helluo* is responsible for eliciting antipredator behaviors in *P. milvina*. However, the results of this study suggest that the cue responsible for changes in behavior by *P. milvina* may degrade in the presence of water. Further studies of the properties of predator cues may aid in identifying the specific cue responsible for eliciting antipredator behavior in *H. helluo* silk and excreta.

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#### LITERATURE CITED

Barnes, M.C., M.H. Persons & A.L. Rypstra. 2002. The effect of predator chemical cue age on an-

- tipredator behavior in the wolf spider *Pardosa milvina* (Araneae: Lycosidae). *Journal of Insect Behavior* 15:269–281.
- Dicke, M. & P. Grostal. 2001. Chemical detection of natural enemies by arthropods: an ecological perspective. *Annual Review of Ecology and Systematics* 32:1–23.
- Dondale, C.D. & B.M. Hegdekar. 1973. The contact sex pheromone of *Pardosa lapidicina* Emerton (Araneida: Lycosidae). *Canadian Journal of Zoology* 51:400–401.
- Kats, L.B. & L.M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394.
- Lima, S.L. & L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68: 619–640.
- NSIDC (National Snow and Ice Data Center). 2002. SMEX tower-based radiometric surface temperature, Walnut Creek, Iowa. <http://nsidc.org/daac/> Accessed: March 3, 2004.
- Persons, M.H. & A.L. Rypstra. 2001. Wolf spiders show graded antipredator behavior in the presence of chemical cues from different sized predators. *Journal of Chemical Ecology* 27:2493–2504.
- Persons, M.H., S.E. Walker, A.L. Rypstra & S.D. Marshall. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae, Lycosidae). *Animal Behaviour* 61:43–51.
- Persons, M.H., S.E. Walker & A.L. Rypstra. 2002. Fitness costs and benefits of antipredator behavior mediated by chemotactile cues in the wolf spider *Pardosa milvina* (Araneae, Lycosidae). *Behavioral Ecology* 13:386–392.
- Sih, A. 1980. Optimal behavior: can foragers balance two conflicting demands? *Science* 210: 1041–1043.
- Stephens, D.W., & J.R. Krebs. 1986. *Foraging Theory*. Princeton University Press, Princeton, NJ.
- Walker, S.E., S.D. Marshall, A.L. Rypstra & D.H. Taylor. 1999a. The effects of hunger on locomotory behaviour in two species of wolf spider (Araneae, Lycosidae). *Animal Behaviour* 58: 515–520.
- Walker, S.E., S.D. Marshall & A.L. Rypstra. 1999b. The effect of feeding history on retreat construction in the wolf spider *Hogna helluo* (Araneae, Lycosidae). *Journal of Arachnology* 27:689–691.

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