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PESTICIDES AFFECT THE MATING BEHAVIOR OF *RABIDOSA RABIDA* (ARANEAE, LYCOSIDAE)

William James Tietjen: Department of Biology, Bellarmine University, Louisville, KY 40205 USA. E-mail: btietjen@bellarmine.edu

ABSTRACT. The effects of exposure to a single sublethal dose of the pesticide malathion on the courtship and mating behavior of the lycosid, *Rabidosa rabida* (Walckenaer 1837) is explored. Animals were tested in combinations where one or both sexes were exposed to the insecticide. The data indicate that while there was no effect on the patterning of courtship behavior, control males initiated courtship more rapidly than dosed animals. Mating behavior was severely disrupted and resulted in most dosed males being killed by females without achieving copulation.

Keywords: Malathion, courtship, reproductive behavior, insecticide

Many insecticides kill both target and non-target species by affecting specific sites within the nervous system. Since an animal's behavior is governed by interactions among nerve cells, it is not surprising that even low doses of pesticides can influence behavior. While most research on sublethal doses of pesticides has concentrated on economically-important insects (Haynes 1988), few studies have assayed the effects on this aspect of spider biology. This is surprising given that spiders are known to reduce and help regulate insect pests (Luczak 1979; Mansour et al. 1980; Winfield et al. 1992; Riechert 1998, 1999; Maloney et al. 2003).

Many insecticides used to control insect pests may also affect spider populations either directly (through death) or indirectly (changes in behavior or physiology). Given the likely importance of spiders in insect control, researchers have evaluated pesticide toxicity for various spider species. In one major study, the susceptibility of several spiders to 30 pesticides was tested (Mansour & Nentwig 1988). Toxicity ranged from no mortality (from biological compounds, herbicides, fungicides) to medium mortality (from pyrethrins, organophosphates, catbamates), and high mortality (from cyclo compounds). Other studies have suggested that many insecticides have little effect on spider population densities (Riechert & Lockely 1984; Hilburn & Jennings 1988; VanDenBerg et al. 1990) and have led IPM researchers to rate the environmental risk of many pesticides to beneficial arthropods, including spiders, as low (Higley & Wintersteen 1992). Other research, however, has shown that even sublethal doses of insecticides adversely affect insect (Haynes 1988) and arachnid species (Chu et al.1976; Chu et.al. 1977; Samu & Vollrath 1992).

As many insecticides affect insect reproduction to one degree or another (for example, mate location, courtship and oviposition behaviors; Haynes 1988) the present study was undertaken to investigate the effects of malathion on the mating behavior of the lycosid, *Rabidosa rabida* (Walckenaer 1837). *Rabidosa rabida* was chosen for this study because of the author's familiarity with its courtship display (Tietjen & Rovner 1982) and because of its presence in agricultural settings (e.g., Halaj et al. 2000). All tests were run using adult *R. rabida* that were laboratory-raised from antepenultimate animals captured from a grassland habitat owned by the author in Shelby County, Kentucky (USA; Location near lat. 38.21°N, long. 85.23°W). This field had not been sprayed with insecticides for at least 10 years. Spiders were tested only once and all animals were virgin. In the laboratory, spiders were housed in a manner similar to that described in Tietjen 1979. Trials were run during the 1997–2001 field seasons while analyses were performed in 2003. Data were analyzed using Stata (Stata Corporation, College Station, TX, USA).

Adult *R. rabida* were exposed to malathion or water in an 8.5 cm dia glass Petri dish with a filter paper substratum that covered the bottom of the dish. Ten μl of 10^{-5} (vol %) malathion (dosed spiders) or 10 μl of distilled water (control spiders) was applied to the center of the filter paper. The appropriate concentration was determined by exposing groups of *R. rabida* males and females ($n = 10$ for each treatment) to concentrations from 10^{-3}–10^{-8} (vol %) malathion. Animals exposed to 10^{-3} and 10^{-4} malathion exhibited obvious behavioral anomalies such as difficulty in locomotion. At an exposure of 10^{-5} no such behavioral anomalies were apparent. Experimental animals were exposed to either malathion or water for 24 hr and then were tested for potential behavioral anomalies 24 hr post-exposure. This dosage technique simulates spiders walking on surfaces that have been sprayed with pesticide. No animals died when exposed to either malathion or water. For all tests I alternated between dosed and control treatments.

In the first experimental series, the effect of malathion on male courtship behavior was tested. The responses of dosed males ($n = 40$) was compared to that of control males ($n = 40$). Males were first tested for response to female pheromone by measuring latency to chemoexploratory behavior and by comparing the courtship pattern in dosed and control animals (Tietjen 1979; Tietjen & Rovner 1982).

To obtain pheromone samples, silk was collected by housing females in a standard glass Petri dish with a filter paper substratum for 24 hr. The test arena (45 cm diam.) was equipped with a paper substratum and the filter paper disk (8.5 cm diam. with a female's silk) was placed in the center. A male was then introduced near the edge of the arena and his behavior was videotaped for 10 min. Between trials, the arenas were washed with water, then 70% ethanol and allowed to air dry. The paper substratum and filter paper with silk were replaced between runs.

The responses of dosed males to a substrate with female pheromone differed from that of control males. The latency for discovering the pheromone cue and beginning palpal chemoexploratory behavior was longer for control males (6.5 min ± 6.29 SD) than for dosed males (2.6 min ± 5.57 SD; $P < 0.007$, Mann-Whitney Test). This result can be explained by the elevated activity of dosed males, which allowed them to contact the silk stimulus sooner than control animals (Tietjen, unpublished data). Once control males contacted and explored the pheromone source they switched to courtship behavior faster than dosed males (control: 1.1 min ± 1.97 SD; dosed: 1.8 min ± 1.73 SD; $P < 0.01$, Mann-Whitney Test).

In order to compare courtship behavior, videos of the male's courtship behavior were digitized at 10 frames per second and examined using a proprietary computer program. First-leg tapping and palpal rotation sequences were analyzed by examining the time between palpal rotations, the time between first leg taps, and the overall symmetry of behavior (i.e., switching between left and right limbs). No differences in the timing or symmetry of the palps or first leg movements were observed between the control and dosed males ($P > 0.05$, Mann-Whitney Test for each analysis).

Following the exposure of males to a female's silk, the responses of the males to normal females was examined by immediately transferring them to a second arena that was prepared as described for the chemoexploratory tests. They were allowed 10 min to acclimate before a female was introduced as far as possible from the test male. Trials were run for an hour or until the spiders stopped copulating.

Males of both treatments courted in response to females, but when females indicated readiness to mate with a first-leg wave, dosed males did not approach the female. Instead, all the dosed males either retreated from the female only to begin courtship again, or would simply continue courting. On the other hand, 38 of the 40 control males mated within 20 min and the remaining males successfully mated within 45 min. Thirty eight of the dosed males that ignored the female's mating signal were attacked and killed. Only two dosed males eventually mated at 42 and 48 min after the female's introduction into the arena. It is interesting to note that the effect of malathion on the spiders' nervous system was very specific and only affected the transition from courtship to mating behavior and not the timing or symmetry of the courtship sequence. Females were not tested to determine if ingestion of malathion-exposed males affected their behavior.

In a second experimental series the responses of dosed and undosed females to a standard male stimulus were explored. The test arena for females was a rectangular glass container with a 20 × 15 cm floor area. This arena replaced the circular arena used for testing male responses because the flat walls would not distort the video playback of a courting male that was used as a stimulus for the females.

The standard male stimulus was generated by videotaping a single male's courtship dance in response to a female's silk. Males were videotaped in the same arena used for testing females. The camera angle was adjusted so it would be similar to a female's view of a courting male on a flat surface. A speaker in contact with and under the floor of the arena served as a microphone to record the courtship vibrations.

The resulting 48 sec sequence was digitized at 30 frames per second and prepared so it would play in a continuous loop. The video was presented to the female along one of the 15 cm wide arena walls and was adjusted to approximate the size of a typical male. Courtship vibrations were played back using the speaker positioned under the floor of the females' test arena so that females were exposed to both visual and vibrational components of a standard male courtship display (silk from males was not introduced). These precautions eliminated variations in male courtship response as a confounding factor in the analysis. The false bottom of the test arena had a one-cm grid to facilitate distance measurements.

Females ($n = 20$) were individually introduced into the test arena and allowed 10 min to acclimate before the video was played. Tests ran for 10 min, The arena was washed and the substratum was replaced between runs. As before, the runs were videotaped and digitized at 10 frames per second for later analysis.

The time for the female to turn and face the screen and her distance from the screen were recorded as well as when she first touched the screen, attacked the screen, or had no response. Fifteen of the 20 control females and 17 of the dosed females responded to the video. For animals that responded, there were no differences between the control and dosed animals for times to face the screen, first touch, or attack ($P > 0.05$, Mann-Whitney test for each test). The apparent lack of an effect on female behavior can be explained since this test only assayed the female's participation in courtship. Thus, if the female's response to the pesticide is similar to that the male's, we would expect her courtship behavior to be unaffected (males showed normal courtship but couldn't switch to mating).

In the third experimental series, fresh males and females were dosed with malathion and their responses were compared to normal pairs. Twelve pairs for each treatment were observed for 60 min trials. The circular arenas used for testing the responses of dosed males were used in these trials and were cleaned and prepared as before.

Males were introduced first into the arena and allowed 10 min for acclimation. Females were then introduced and the recordings began immediately. All 12 of the control males began courting the female in 8.3 min ± 8.2 SD while only 9 of the dosed males courted the females (14.5 min ± 17.4 SD). The time until courtship started did not differ between the two treatments ($P > 0.05$, Mann-Whitney Test). Mating success differed between the two groups. Only one of the control pairs failed to mate compared to seven of the dosed pairs ($P < 0.05$, Chi Square Test). The mean time to copulation for control spiders was 12.4 min ± 9.7 SD while dosed spiders took 27.7 min ± 20.8 SD to accomplish the task.

This study indicates that a single dose of a neurotoxic pesticide interferes with the mating behavior of both male and female *R. rabida* and it suggests several areas for future research. The effects of chronic exposure and the possible persistence of behavioral anomalies deserve further attention, along with studies comparing potential fitness consequences for spiders from different families or with dissimilar feeding and mating strategies. A variety of other pesticides including other neurotoxins, hormone disruptors, biocontrol agents, herbicides and fungicides should also be examined to determine if sublethal exposure affects the behavior or reproductive physiology of spiders. These data suggest that the behavioral effects of pesticide exposure should be considered by future researchers.

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