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EFFECTS OF AN ORGANOPHOSPHOROUS INSECTICIDE ON SURVIVAL, FECUNDITY, AND DEVELOPMENT OF *HYLYPHANTES GRAMINICOLA* (SUNDEVALL) (ARANEAE: LINYPHIIDAE)

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Abstract—The effects of an organophosphorous insecticide, methamidophos, on fecundity and development of the spider *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae) were assessed under laboratory conditions. Susceptibility of adults of both sexes to the insecticide and its influence on fecundity of females and development of offspring were investigated. At 48 h after topical application in adults, the median lethal dose (LD50) and 10% lethal dose (LD10) were 0.35 and 0.12 μg/spider, respectively, for males and 0.52 and 0.16 μg/spider, respectively, for females. Methamidophos had detrimental effects on fecundity of females; number of eggs per clutch, total egg mass, and clutch size decreased significantly. The hatching rate of eggs from LD10-treated females was slightly higher than the rate in the controls, but the hatching rate of eggs from LD50-treated females was lower than the rate in the controls. However, no significant differences were observed in hatching time and development time across treatments. Development time of spiderlings from LD50-treated females was significantly longer than the time in the controls, and body sizes of the first spiderlings from insecticide-treated females were larger than those in the controls. However, matured offspring were smaller than those in the controls. It was concluded that methamidophos has long-term effects on *H. graminicola*, and that this may affect the development of spider populations in the field.

Keywords—Methamidophos   *Hylyphantes graminicola*   Fecundity   Development

INTRODUCTION

Spiders are one of the most abundant, beneficial arthropods in agricultural ecosystems [1–5]. Most are polyphagous predators and feed on various insects, including pest species. Therefore, spiders are a potentially important group of natural predators in agricultural ecosystems. However, natural biological control by spiders has been disrupted, both directly and indirectly, because of their high sensitivity to insecticides [6–10]. To emphasize the beneficial effect of spiders in fields and to reduce environmental pollution, it is important to select insecticides with high efficacy on the target pest but with minimal effects on nontarget species. Therefore, it is important to know the ecotoxicological effects of insecticide application on spiders. Many reports have shown that insecticides cause rapid decreases of spider populations in the field [11–18]. However, few studies have examined the sublethal and long-term effects of these chemicals on spiders [19–26], although similar studies have been carried out with other beneficial predators, such as ladybirds (Coccinellidae) and lacewings (Chrysopidae) [4].

Spiders in the sprayed fields may receive varying sublethal doses of the insecticides, which may interfere with their normal activity and reproduction and with the development of survivors. Many factors, such as fecundity of females and body size, influence the development of spider populations. It has been shown that body size is strongly correlated with fecundity in female spiders [27,28] and with mating success in males [29,30]. In addition, smaller spiders are more vulnerable to a wider range of predators and are restricted to the capture of a smaller range of prey items [21]. Wick and Freier [11] found a tendency toward decreased activity of spiders in the year after insecticide application in winter wheat. Nevertheless, little attention has been paid to the effects of insecticides on the fecundity and development of spiders.

In China, organophosphorous insecticides are the most widely used type of insecticide, and among these, methamidophos is one of the most popular. The production of methamidophos was estimated to have been nearly 70,000 tons in 1999 [31]. This insecticide is highly poisonous, and since 2004, its use is being phased out in China. However, use of methamidophos continues, mainly because it is inexpensive and efficient in pest control, and in 2006, its market demand so far has been 12,500 tons. Methamidophos is neurotoxic and can lead to behavioral, physiological, reproductive, and developmental changes in organisms [32–34]. The aims of the present study were to evaluate the effects of methanidophos on survival, fecundity, and development of spiders. The reproduction of female spiders and development of their offspring at sublethal doses (median lethal dose [LD50] and 10% lethal dose [LD10]) were compared to the control treatment.

MATERIALS AND METHODS

Materials

The methamidophos used in the present experiments was a formulated insecticide (151 g active ingredient/L; Sanonda, Jingzhou, Hubei, China). In all bioassays, the insecticide was diluted in acetone.

Test spiders and their maintenance

The spider *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae), a common species in agricultural fields of China, was selected as a test species. It is abundant in various crops, such as cereal, rice, corn, cotton, soybean, and vegetables, and occurs in orchards. Its biology and life history have been stud-

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ied intensively [35]. Female *H. graminicola* ($n = 35$) were collected from rice fields in Haidian Park (Beijing, China) during March 2005. They were kept individually in glass tubes (diameter, 15 mm; length, 100 mm), which were closed with a plug of cotton and included a 10-mm bottom of moist sponge to maintain high humidity, in an illumination incubator (25°C; relative humidity, 80%; photoperiod, 14:10-h light:dark). Wild-type fruit flies (*Drosophila melanogaster* Meigen) were provided twice a week as food. Female spiders readily produced eggs under these conditions. After oviposition, the female spiders were removed, and the eggs were left undisturbed until they hatched. Spiderlings passed through their first instars in the egg sacs and were transferred individually to glass tubes within 1 or 2 d. They were fed with fly larvae and an artificial dietary supplement (one egg, 60 ml of honey, 5 g of agar, 0.25 g of compound vitamin, 0.12 g of microelement, and 500 ml of water). After two molts, the juvenile spiders were offered live vestigial flies twice a week. After maturation, these spiders were used in the experiments during June 2005 to October 2005.

Toxicity tests

In all bioassays, the formulated insecticide (methamidophos) was topically applied [21,33]. In brief, two droplets (0.5 µl each) of insecticide solution were applied to the dorsal abdomen of spiders using a 5-µl microsyringe, and an acetone-only control was employed. To reduce possible variation in response to treatments caused by differences in gut fullness, spiders were starved for 3 d before insecticide exposure. Before treatment, the spiders were weighed, and all test spiders were in the range of 4.0 to 5.0 mg for females and of 2.0 to 3.0 mg for males. No food was offered during tests. All experiments were carried out at 25°C and 80% relative humidity.

Six dosages of methamidophos solution (together with pure acetone as a control) were applied after the pretest experiments: 2.00, 1.26, 0.80, 0.50, 0.32, and 0.20 µg/female spider, and 1.00, 0.63, 0.40, 0.25, 0.16, and 0.10 µg/male spider. For each sex, 20 individuals were tested for each dosage, plus another 20 individuals as controls. After insecticide application, spiders were kept in Petri dishes with one or two pieces of moist sponge to maintain humidity. Percentage spider mortality was recorded after 24 and 48 h. The control mortality after 48 h was zero. The LD50 and LD10 were estimated by probit regression analysis using SPSS software (Ver. 13.0 for Windows®; SPSS, Chicago, IL, USA).

Effect on fecundity of female spiders

For this experiment, the LD50 of 0.52 µg/spider and the LD10 of 0.16 µg/spider were chosen as the treatment dosages.

After the final molt, 44 female spiders with similar body weights (4.0–5.0 mg) were placed at random into three groups: The control, LD10-treated, and LD50-treated group. Taking account of insecticide-induced death, the numbers of spiders assigned to the three groups were uneven: 12 spiders at 0.16 µg/spider, 22 spiders at 0.52 µg/spider, and 10 spiders in the control group. Thereafter, female spiders were kept in Petri dishes with a male spider for 1 or 2 d and fed with sufficient live vestigial flies. During the next two months, egg-laying and egg-hatching events were recorded. Clutch size, number of eggs, and emergence of spiderlings also were recorded. Fecundity and percentage emergence of the three groups were compared.

Table 1. Probit regression analysis of 10% lethal dose (LD10), median lethal dose (LD50), and 95% confidence interval for *Hylyphantes graminicola* after 48 h of exposure to methamidophos

	Male	Female
Mean weight (mg ± standard error)	2.27 ± 0.03	4.50 ± 0.03
LD10 (µg/spider)	0.12 (0.07–0.16)	0.16 (0.08–0.24)
LD50 (µg/spider)	0.35 (0.28–0.44)	0.52 (0.40–0.65)
LD50 (µg/mg fresh weight of spider)	0.15	0.11

Effect on development of offspring

To test the effects of methamidophos on the development of offspring from spiders treated with insecticide, some spiderlings emerging from the eggs of the previous experiment were offered adequate food, and the others were preserved in ethanol for measurement of body size. The spiderlings were checked daily, and molting events were recorded. After maturity, they were preserved in ethanol as well. Abdomen length and cephalothorax width of juvenile and mature individuals were measured with an ocular micrometer under a binocular microscope.

Data analysis

For the toxicity test, a regression analysis was used to estimate the LD10 and LD50 with a 95% confidence limit and the slope ± standard error of the regression. A nonparametric Kruskal–Wallis test was used to test the significance of the differences in fecundity of females in the control, LD10-treated, and LD50-treated groups and the emergence of eggs. Comparison was made between the development period, together with the abdomen length and cephalothorax width, of offspring from LD10- and LD50-treated females and the control offspring using one-way analysis of variance (ANOVA). For cases in which the tests were significant, multiple comparisons between treatments were carried out using Duncan's multiple-range test. All analyses were performed using SPSS software.

RESULTS

Toxicity test

Because no individuals died in the control test within 48 h of acetone application, no adjustment for control mortality was necessary. The methamidophos LD10 and LD50 for *H. graminicola* after 48 h of exposure were calculated using probit regression analysis and are shown in Table 1. The LD50 is the most commonly used index for the susceptibility of an organism to toxicants. It may be expressed in terms of dose per individual, which gives an indication of susceptibility in the field, or in terms of dose per unit body weight, which gives a measure of the intrinsic susceptibility of the species to the toxicant [36]. In terms of µg/spider, females were more tolerant than males, whereas this relationship was reversed in terms of µg/mg fresh weight. This difference was the result of differences in fresh weight, because females usually are heavier than males.

Effect on fecundity of female spiders

The fecundity of *H. graminicola* females exposed to the LD10 ($n = 11$) or to the LD50 ($n = 10$) was compared with that of control females ($n = 10$) (Table 2). The effects of methamidophos on total number of eggs and on clutch sizes

Table 2. Comparisons of fecundity of females and emergence of eggs in *Hylyphantes graminicola* between different doses of methamidophos and controls[a]

Treatment	Control	LD10 treated	LD50 treated
Spiders (*n*)	10	11	10
Total eggs (*n*)	293.60 ± 52.90	250.82 ± 38.41	227.10 ± 40.97
Clutch size (*n*)	8.60 ± 1.47	8.18 ± 1.11	8.10 ± 1.30
Eggs per clutch**(*n*)	33.88 ± 2.00	29.77 ± 1.29	28.35 ± 1.08
Hatching rate** (%)	72.08 ± 3.87	77.17 ± 2.94	50.07 ± 4.08
Hatching time (d)	5.25 ± 0.07	5.34 ± 0.05	5.43 ± 0.11

[a] LD10 = 10% lethal dose; LD50 = the median lethal dose. Asterisks indicate significant differences in fecundity of females and emergence of eggs was detected using the Kruskal–Wallis test (**$p < 0.01$).

were not significant (Kruskal–Wallis test, $p > 0.05$), although it appeared that LD50- and LD10-treated females laid fewer eggs than the control females. However, the mean numbers of eggs per sac from females exposed to the LD10 and LD50 were significantly smaller than in the case of control females (Kruskal–Wallis test, $p < 0.01$), indicating that methamidophos had negative effects on the fecundity of females.

The effects of methamidophos on the hatching rates of eggs from LD10- and LD50-treated and control females differed significantly (Kruskal–Wallis test, $p < 0.01$) (Table 2). Surprisingly, eggs from LD10-treated females had slightly higher hatching rates than those from the controls, whereas the hatching rates of eggs from LD50-treated females were much lower than those of the controls. Hatching time of eggs was not significantly affected by insecticide treatment (Kruskal–Wallis test, $p > 0.05$).

Effect on development and size of spiders

Male spiders mature faster than female spiders, because male spiders need only four molts to reach maturity and females need five. No significant difference was observed between development times of spiderlings from the first to fifth instar (Kruskal–Wallis test, $p > 0.05$) (Fig. 1). Considering the whole development time from egg to adult, female spiders from the control treatment took more days to mature compared with those from the LD10 treatment, but fewer days compared with those from the LD50 treatment (Kruskal–Wallis test, $p < 0.05$). No significant difference was found in the development time of male spiders (Kruskal–Wallis test, $p > 0.05$).

The effects of the LD10 and LD50 on abdomen length and cephalothorax width of first-instar spiderlings were significantly different (Fig. 2). Abdomen lengths of spiderlings from LD10- and LD50-treated females were larger than those of the controls (Kruskal–Wallis test, $p < 0.01$), whereas differences of cephalothorax width were not significant when compared to the controls (Kruskal–Wallis test, $p > 0.05$). On the other hand, abdomens of adult offspring from LD10- and LD50-treated females were much shorter compared with abdomens of the controls (ANOVA, $p < 0.05$), except that males from LD10-treated females showed no significant differences (ANOVA, $p > 0.05$). Cephalothorax width of adult offspring from control, LD10-treated, and LD50-treated females also showed significant differences. The LD50 exposure produced significant decreases in cephalothorax width of female adult offspring (ANOVA, $p < 0.05$) but no effects in males (ANOVA, $p > 0.05$), whereas the effects of the LD10 exposure were not significant (ANOVA, $p > 0.05$).

DISCUSSION

As a predator of insect pests, *H. graminicola* is an important nontarget, beneficial species affected by pesticides. Suscepti-

bility of this species to methamidophos was assessed in the present study. Female spiders had a lower LD50 in terms of µg/mg fresh weight compared with male spiders, which showed that female spiders were intrinsically more susceptible. However, LD50s in terms of µg/spider were the opposite, indicating that individual females were more tolerant to methamidophos in the field. This gender-specific difference has been observed in other spiders. Dinter and Poehling [20] found that females of *Erigone atra* and *Oedotharax apicatus* were intrinsically more susceptible to fenvalerate and lambda-cyhalothrin compared with male spiders but were more tolerant in terms of µg/mg fresh weight.

A sublethal application of insecticides may induce an increased fecundity of pests, which is called physiological resurgence or hormoligosis [37]. This phenomenon has been proved in some pests, such as spider mites [38], but in natural enemies, the effects were negative [39,40]. The present study also demonstrated these effects. The number of eggs per clutch from LD10- and LD50-treated female spiders was statistically lower than that in the controls. The adverse effects became greater as the dose increased. The effects of the insecticide on hatching of eggs paralleled the effects on fecundity. Hatching rate of eggs from LD50-treated females was distinctly affected, but eggs from LD10-treated females had a slightly higher hatching rate compared with the controls. To our knowledge, this promoting effect has not been reported previously in natural enemies. It is unknown whether it is hormoligosis (i.e., low sublethal doses of insecticide having stimulatory effects on hatching of eggs). Much more detailed information is required to test this hypothesis.

The insecticide also had long-term effects on the spider offspring. The present results show that applications of an insecticide did not significantly affect the development time of offspring but did affect their size. In general, body sizes of spiderlings from LD10- and LD50-treated females were larger than those of the control. This result may be caused by the decreased egg mass per clutch and the reproductive trade-off in spiders, whereby relative egg number is inversely related to the size of the eggs produced [28]. However, no advantage of body size in spiderlings was found when these offspring matured, because the body sizes of adults from insecticide-treated females were no larger than those from the controls. The abdomen of female offspring from LD10- and LD50-treated females was much shorter than those from the controls. This is particularly important for female spiders, because fecundity is positively correlated with body size [29], suggesting that the fecundity of these offspring also might decrease. This may have implications for the development of populations in fields where pesticides are applied. On the other hand, large body size could enhance the ability of males to succeed in
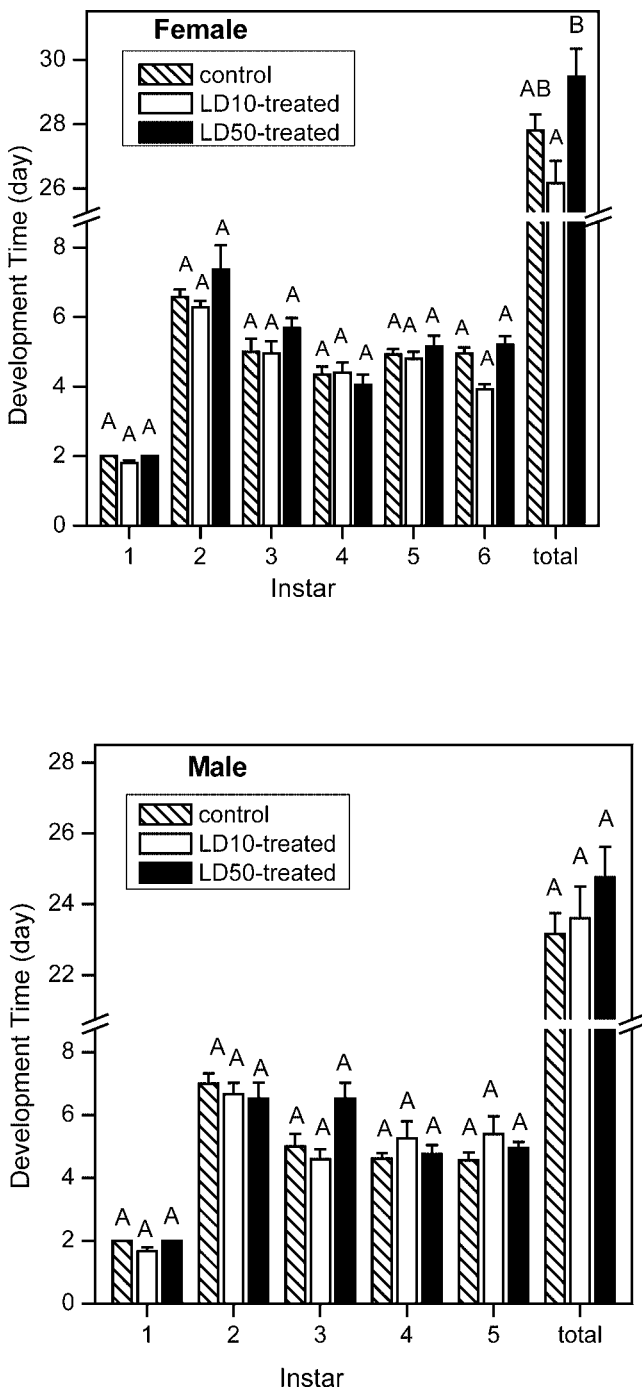
Fig. 1. Development time of female and male offspring from female spiders treated with the 10% lethal dose (LD10) and with the median lethal dose (LD50) compared with those of the controls. Error bars represent one standard error of the mean. Statistical differences were detected by analysis of variance, followed by Duncan's multiple-range test; comparisons significant at $p < 0.05$ are indicated by different capital letters.

Fig. 2. Abdomen length (**a**) and cephalothorax width (**b**) of offspring treated with the 10% lethal dose (LD10) and with the median lethal dose (LD50) compared with those of the controls. Error bars represent one standard error of the mean. The numbers 1, 2, and 3 on the *x*-axis represent spiderlings, females, and males, respectively. Statistical differences were detected by analysis of variance, followed by Duncan's multiple-range test; comparisons significant at $p < 0.05$ are indicated by different capital letters.

mating competition. However, male offspring from LD50-treated females had shorter cephalothorax width and, thus, smaller size, making them less competitive in mating. The decreasing abilities of spiders in terms of both fecundity and reproductive competition likely are detrimental to the development of spider populations and may be one of explanations for the outbreaks of the pest (their prey) after spraying of insecticide.
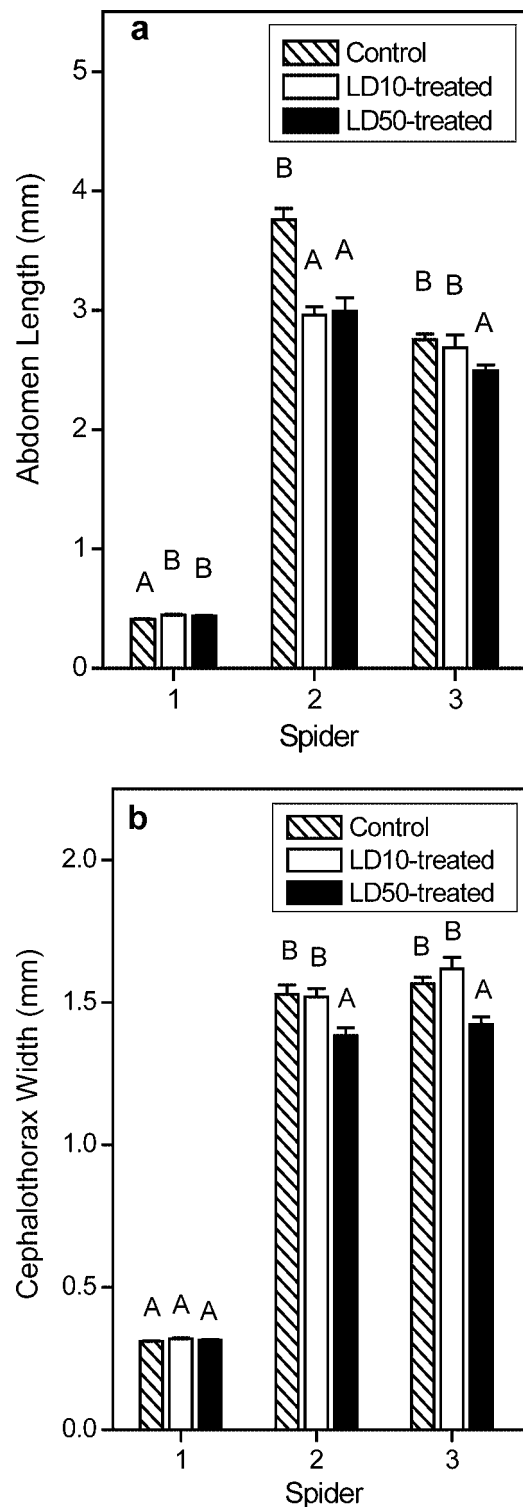
From the present results, it can be concluded that application of insecticide adversely influences the fitness of the spider *H. graminicola* and its offspring, that methamidophos at high dose can cause long-lasting side effects on spiders, and that the possible stimulatory effects of low sublethal doses of insecticide on the pests have not been proved.

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