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EFFECTS OF METHAMIDOPHOS ON THE PREDATING BEHAVIOR OF *HYLYPHANTES GRAMINICOLA* (SUNDEVALL) (ARANEAE: LINYPHIIDAE)

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Abstract—The effects of an organophosphorous insecticide, methamidophos, on the pest control potential of the spider *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae) were investigated in the laboratory with the fruit flies (*Drosophila melanogaster* Meigen). The influence of methamidophos on predation by *H. graminicola* was very obvious in female spiders, which preyed on fewer prey in the 8 h after exposure to the insecticide but subsequently recovered. On the other hand, the predation rates in male spiders were not affected by the insecticide within 24 h of treatment. However, a 10% lethal dose (LD10) of methamidophos resulted in an enhanced predation rate per day for male spiders, whereas a 50% lethal dose reduced the predation rate. In addition, it was shown that the functional response of *H. graminicola* to the fruit fly was a type II response, and the type of functional response of insecticide-treated females changed from type II to type I, with no change in the response of male spiders. The attack rate of males treated with the LD10 dosage of insecticide was significantly higher than the controls, which suggests that the insecticide stimulates the performance of spiders. Prey utilization of males treated with low doses of insecticide was lower than the control, which indicates that the insecticide did not result in these spiders eating more prey, but killing more.

Keywords—Methamidophos *Hylyphantes graminicola* Predation rate Functional response Prey utilization

INTRODUCTION

Hylyphantes graminicola (Sundevall) (Araneae: Linyphiidae) is one of the most common species of spiders in agricultural fields in China. It is abundant in cereal, rice, corn, cotton, soybean, and vegetable crops and even in orchards [1,2]. This species builds sheetlike webs in the interstices of the soil or among branches of the vegetation and mainly hunts for prey actively on the ground and at the base of plants. Their prey include many pests such as aphids, leafhoppers, planthoppers, moth larvae, and corn borers. Consequently, they are known to be a potentially important group of natural predators of insect pests [1,2].

Insecticide application has great effects on spiders at the level of both population and individual. The effects of insecticides on spiders have been studied in the laboratory [3–5] and in the field [6–8], mostly concentrating on the lethal effects. Only a few studies actually investigated the effects of insecticide other than their direct toxicity to the spiders [9–18]. Several studies have shown that sublethal doses of insecticides might also affect spiders' behavior [15–18], which will directly and indirectly influence their pest control potential. Knowledge of the pest control potential of spiders is essential if they are to be used as bioagents in integrated pest management, so it is important to study whether the pest control potential of spiders is affected by insecticides.

One of the crucial elements in evaluating the control potential of a predator to the pest population is predation rate, which varies in response to prey density. The relationship between an individual predator's consumption rate and prey density is termed functional response, and this is a key factor regulating the population dynamics of predator–prey systems. Functional response of a predator reflects its searching ability, handling effect, and the maximum number of prey. Holling [19] classified functional responses as type I (predation rate increasing linearly), II (predation rate increasing hyperbolically), and III (predation rate increasing sigmoidally), and the functional response of most beneficial arthropods is either type II or type III [20]. The type II response is common in spiders, but the type III response also occurs [21].

In this study, we were concerned with the effects of an insecticide on the predation behavior of these spiders, and the predation rate, handling time, and attack coefficient in the functional response and the prey utilization of *H. graminicola* were determined under experimental conditions.

MATERIALS AND METHODS

Test materials and their maintenance

A number of female adults of *H. graminicola* were collected from Haidian Park (Beijing, China) during April 2005. They were kept individually in glass tubes (15 mm in diameter and 100 mm long) covered with a plug of cotton and with a 20-mm layer of moist sponges at the bottom to maintain high humidity. They were maintained in an illumination incubator at 25°C, 80% relative humidity (RH), and a 14:10 h light:dark photoperiod regime. The offspring of these female spiders were reared following methods outlined by Deng et al. [13] and were used as the test individuals. Wild-type fruit flies *Drosophila melanogaster* Meigen from stock cultures were provided as their prey.

Application of insecticide

The organophosphorous insecticide methamidophos used in the experiments was a formulated insecticide (151 g activity ingredient/L, Sanonda, Jingzhou, China). In these experiments, 50% and 10% lethal dose (LD50 and LD10, respectively) over 48 h of methamidophos to spiders were chosen as the treatment

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dosages. Preliminary experiments with spiders of the same weight range (4.0–5.0 mg for females and 1.0–2.0 mg for males) as used in the experiments had established the LD50 and LD10 values as 0.52 and 0.16 μg methamidophos per female spider, and 0.35 and 0.12 μg methamidophos per male spider, respectively [13]. In all bioassays, insecticides were diluted in acetone.

Spiders were conditioned by starving 3 d before the tests to standardize hunger level. The test spiders were immobilized by CO_2 treatment before application. Two droplets (each 0.5 μl) of insecticide solution or acetone as control were topically applied on the dorsal abdomen of the spiders with a 5-μl microsyringe.

Predation rate experiment and analysis

In the tests, about 60 female and male adults of *H. graminicola* were grouped respectively into three groups: control (treated with acetone, $n = 14$), LD10-treated group (treated with LD10 of methamidophos, $n = 16$), and LD50-treated group (treated with LD50 of methamidophos, $n = 30$). Taking account of insecticide-induced death, the numbers of spiders assigned to the three groups were uneven. After application, these spiders were put into separate containers with 10 fruit flies in each. The containers were 50 mm in diameter and 60 mm high, covered with two layers of gauzes and a 5-mm moist sponge in the bottom. The numbers of prey killed and consumed by spiders were recorded at 4, 8, 12, 18, and 24 h, when 10 new prey were added. The data relating to spiders that died during the tests were ignored. All tests were carried out in an illuminated incubator (25°C, 80% RH).

The mean predation rate of spiders in the intervals 0 to 4 h, 0 to 8 h, 0 to 12 h, 0 to 18 h, and 0 to 24 h after application was calculated by dividing the number of prey killed by the time interval. Data on predation rates of spiders at different intervals and with different treatments were analyzed with the Kruskal–Wallis test of nonparametric analysis and SPSS software (Ver 13.0 for Windows®, SPSS, Chicago, IL, USA).

Functional response experiment and analysis

The same containers used in predation rate tests were used as experimental arenas. Initial prey densities of 1, 2, 3, 4, 5, and 6 fruit flies per container, with at least five replicates per density, were established for male and female adults of *H. graminicola*, and females additionally had 8 and 10 fruit flies per container densities. Spiders were put into containers with prey after receiving a topical application of methamidophos. The number of prey killed and consumed was recorded every 24 h for 5 d, and prey were refreshed every day. Fruit flies for the tests had just emerged from pupae for 1 d, and a separate test with the lowest and highest fly densities demonstrated that the death rate of these flies was zero in 24 h. Therefore no adjustments were made to correct for the mortality observed in the experiments.

The data of predator functional response of spiders in each group were analyzed in two stages. First, the shape of the functional response curve was determined by logistic regressions of proportion of prey killed in 24 h against the number of initial prey [22,23]. Second, the random predator equation was fitted if a type II response was determined.

A polynomial function from Juliano [24] was first fitted,

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{[1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)]}$$

where N_e is the number of prey killed in 24 h, N_0 is the initial prey density, and N_e/N_0 is the probability of prey eaten. Parameters P_0 to P_3 were obtained by the method of maximum likelihood of logistic regression. The sign of the linear coefficient (P_1) determines the shape of the functional response type (I, II, or III), and log likelihood ratio tests were applied to determine the significance of these parameters. If the linear terms are significantly negative, it is sufficient to indicate a type II functional response; if not significantly negative, it is a type I response. On the other hand, it is sufficient to indicate a type III functional response if the linear terms were significantly positive. The general shapes of the functional response curves of *H. graminicola* in each group were determined in this way.

Once the type of functional response was determined, nonlinear least squares regression was used for parameter estimation of the random predator equation [25],

$$N_e = N_0\{1 - \exp[-a(t - t_hN_e)]\}$$

where N_e represents the number of prey killed in 24 h, N_0 represents initial prey density, and t represents the total time for the predator to attack (24 h). Parameter a represents the attack coefficient, and t_h represents handling time. The random predator equation was used here because it allows for consumed prey not being replaced during the assays. The parameters of functional response (a and t_h) were then compared within the treated and control groups, and separation of statistically different parameter estimates was made by 95% confidence intervals. If comparisons produced 95% confidence intervals that included zero, parameter estimates were not significantly different [24]. A two-way analysis of variance (ANOVA) was performed to determine the effects of insecticide and prey density on the number of prey killed by adults. In all statistical analyses, the significance level was set at $p = 0.05$, and all the analyses were carried out by SPSS software.

Prey utilization experiment and analysis

Spiders were weighed before and after the functional response tests to examine the effects of insecticide on prey utilization. The average dry weight to live weight ratio of fruit fly was established by drying five groups of 10 flies at 60°C for 72 h. The ratio of spider growth to the dry weight of food obtained from prey killed (prey utilization) could be calculated according to these data. A nonparametric Kruskal–Wallis test was performed to test the significance of differences between the treated and control groups.

RESULTS

Effect of methamidophos on predation rate of H. graminicola

Predation rates of male and female adults of *H. graminicola* in five continuous intervals of 24 h are presented in Figure 1. Compared with females, males had a lower predation rate. In females, predation rates of insecticide-treated groups were significantly lower than the controls in the intervals 0 to 4 h and 4 to 8 h (Kruskal–Wallis test, $p < 0.05$). Subsequently, the insecticide-treated females showed no significant difference from the controls. However, insecticide did not affect male spider predation rate within 24 h of treatment; any differences were nonsignificant (Kruskal–Wallis test, $p > 0.05$). As a whole, insecticide had an inchoate negative effect on predation rate of *H. graminicola* for a short time, but the spiders soon recovered and reached the control level.

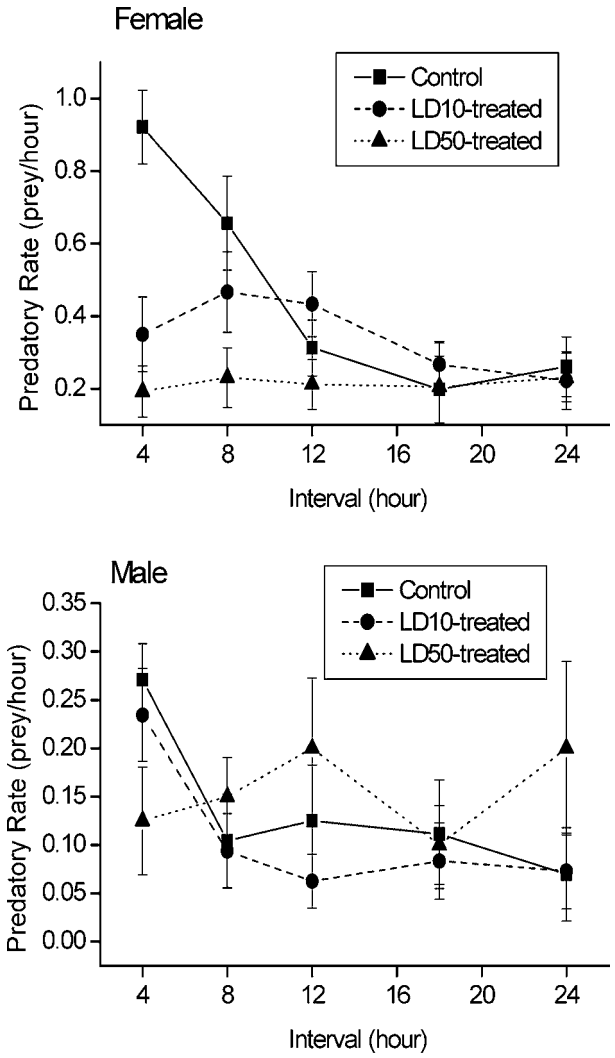

Fig. 1. Predation rates of female and male adult *Hylyphantes graminicola* in five continuous intervals of 24 h after insecticide application with 50% and 10% lethal doses (LD) of methamidophos doses.

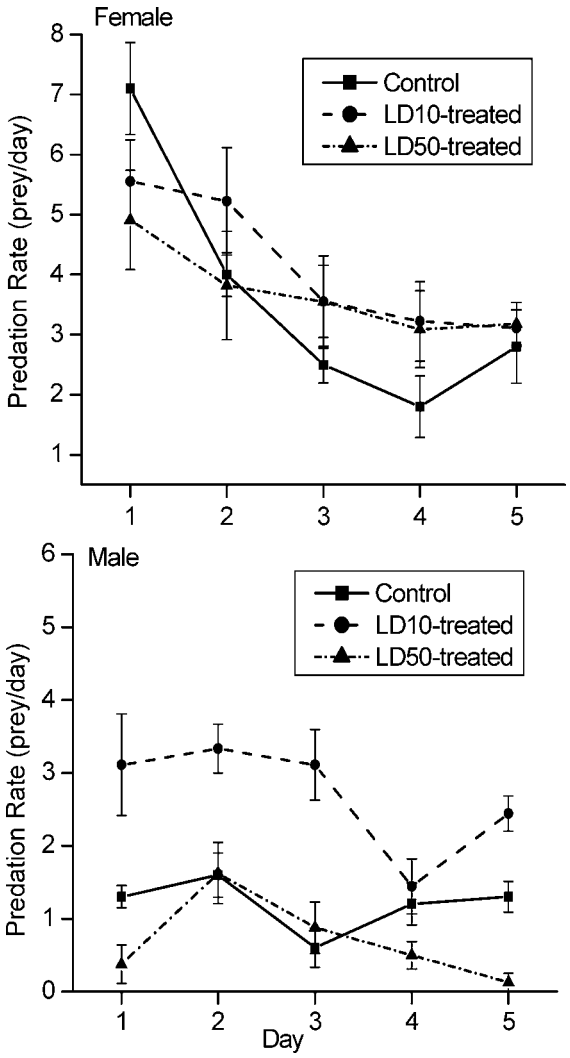

Fig. 2. Predation rates of female and male adults of *Hylyphantes graminicola* for 5 d after insecticide application of 50% and 10% lethal doses (LD) of methamidophos.

The mean numbers (±standard error [SE]) of prey killed in 24 h by three groups of female spiders were 10.31 ± 1.18 (control group), 7.93 ± 1.18 (LD10-treated group), and 5.15 ± 0.77 prey/h (LD50-treated group). The LD50-treated female spiders killed significantly fewer prey than the control group on the basis of one-way ANOVA ($p < 0.05$), whereas the LD10-treated group showed no significant difference (ANOVA, $p > 0.05$). In males, the mean predation rates in 24 h for the control, LD10-treated, and LD50-treated groups were 3.08 ± 0.29, 2.50 ± 0.38, and 3.70 ± 0.67 prey/h, respectively, with no significant difference between treatments (ANOVA, $p > 0.05$).

The long-term effects of methamidophos on predation rate were estimated on the basis of the number of prey killed at the highest prey density over 5 d, in the functional response experiments (Fig. 2). No significant negative effects were found in females compared with the control group (Kruskal–Wallis test, $p > 0.05$). However, the predation rate of LD10-treated group of males was significantly higher than the control and LD50-treated groups (Kruskal–Wallis test, $p < 0.05$), except on the fourth day. Simultaneously, the predation rate of the LD50-treated group showed a decreasing trend and was close to zero on the last day. As a result, it was seen that a low dose of methamidophos enhanced the predation rate of male spiders, whereas a high dose had an inhibiting effect.

Effect of methamidophos on functional response of H. graminicola

Coefficients for linear regression of proportion of prey killed to the number of prey offered (N_e/N_0) for female and male adults of *H. graminicola* under three treatments are presented in Table 1. Methamidophos proved to have no effect on the type of functional response of male spiders because all the linear coefficients were negatively significant, which indicates a type II response. However, the linear coefficients for LD10- and LD50-treated females were negative but not significant, which indicates a type I response. However, the type of functional response of control females was still type II, because of the significant negative linear coefficient.

The interaction term (insecticide × density) from the two-way ANOVA was significant ($p < 0.05$), which indicated the significant effects of insecticide on the predation of spiders. Results of nonlinear least square regression analysis revealed that there was a significant fit (χ^2 goodness of fit test, $p < 0.05$) between the observed and expected numbers of prey killed by spiders applied with acetone plus LD10 or LD50 of

Table 1. Coefficients of linear regression analysis of proportion of prey killed to the number of prey offered for female and male spiders exposed topically to acetone and 50% and 10% lethal doses (LD50 and LD10, respectively) of methamidophos. Parameters P_1 were obtained by the method of maximum likelihood of logistic regression, p is the significance value

Sex	Treatment	P_1(±SE)[a]	p
Female	Control	−1.548 ± 0.527	0.003
	LD10	−1.831 ± 1.494	0.291
	LD50	−0.449 ± 0.832	0.590
Male	Control	−0.969 ± 0.307	0.002
	LD10	−0.284 ± 0.068	<0.001
	LD50	−0.170 ± 0.074	0.022

[a] Standard error.

insecticide (Fig. 3). In males, significant differences in the attack coefficient (a) estimates of the three groups were observed (Table 2). The attack coefficient of the LD10-treated group was significantly higher than of the control, whereas that of the LD50-treated group was lower. However, no significant differences in handling time (t_h) estimates were observed. In females, although the two parameters are not directly comparable, insecticide application affected predation because the number of prey killed at the highest prey density in the control group was higher than in the insecticide-treated groups.

Effect of methamidophos on prey utilization of H. graminicola

The mean numbers (±SE) of prey utilization by the control and LD10- and LD50-treated groups of females were 0.811 ± 0.054, 1.074 ± 0.080, and 0.984 ± 0.093, respectively, with no significant difference between insecticide-treated and control groups (Kruskal–Wallis test, $p > 0.05$). At the same time, prey utilization by male spiders was significantly different from the control and LD10- and LD50-treated groups (Kruskal–Wallis test, $p < 0.05$), and the mean numbers of prey utilization by the three groups were 0.259 ± 0.029 (control), 0.187 ± 0.042 (LD10-treated), and 0.306 ± 0.065 (LD50-treated). The prey utilization of LD10-treated males was lower than the control.

DISCUSSION

Methamidophos had significant effects on the predating behavior of *H. graminicola* through predation rate, functional response, and prey utilization. Interestingly, the responses of male and female spiders to the insecticide were quite different. First, the predation rate of females in the first 24 h was affected only initially by the insecticide, whereas no significant response of males was found. As to the long-term effect of the insecticide on predation rate, the response of males was significant whereas that of females was not. Second, the types of functional responses of male and female spiders were also affected differently by the insecticide. The functional responses of insecticide-treated females changed from type II to I, whereas the types of functional responses of male spiders were not affected. Finally, prey utilization of female spiders was not significantly influenced, whereas that of males was affected significantly. The different responses by sex to the insecticide have been reported before [26,27], and the discrepancy of body mass between sexes was presented to explain this diversity. However, because the equal dose of the insecticide was applied to all individuals in terms of their body mass at this point, another intrinsic mechanism should influence the responses of female and male spiders to the insecticide, and it requires more detailed research to test and develop these responses.

Another finding of this study was that the low dose of

Table 2. Mean (±standard error)[a] estimates of the attack coefficient (a) and handling time (t_h) for *Hylyphantes graminicola* with 10% lethal dose (LD10) and 50% lethal dose (LD50) of methamidophos and acetone treatments

Sex	Treatment	a (h^{-1})	t_h (h)
Female	Control	0.114 ± 0.039	1.231 ± 0.737
	LD10	—	—
	LD50	—	—
Male	Control	0.044 ± 0.004 B	7.381 ± 0.467 A
	LD10	0.092 ± 0.030 C	6.089 ± 0.771 A
	LD50	0.018 ± 0.004 A	3.551 ± 2.105 A

[a] Means in each column followed by a different letter are significantly different for the 95% confidence interval at $p < 0.05$.

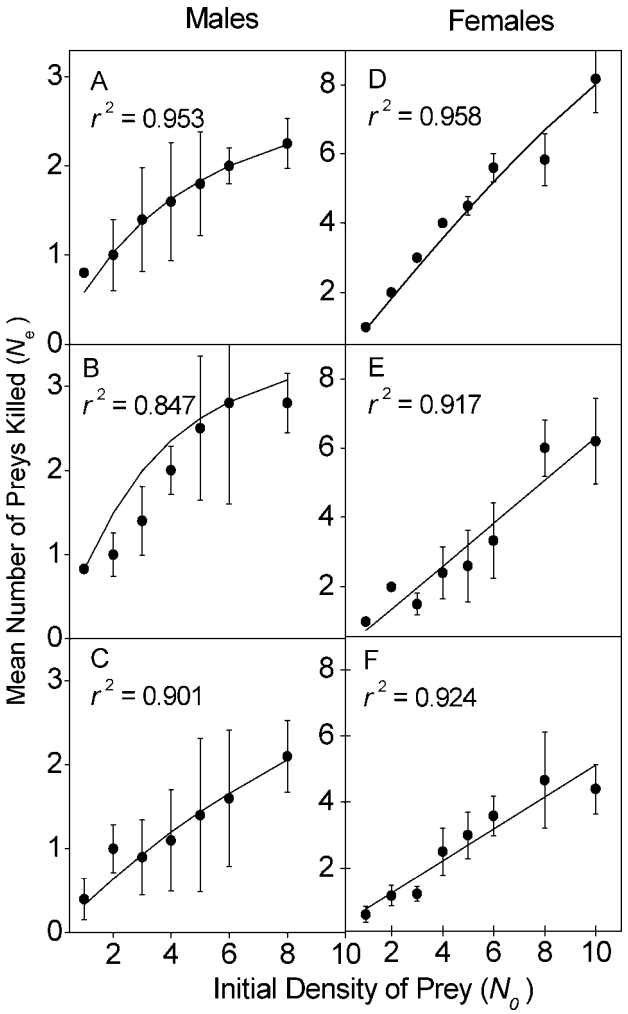

Fig. 3. Functional response curves of female and male spiders, treated with acetone (control) and 50% and 10% lethal doses (LD50 and LD10, respectively) of methamidophos, to densities of their prey at 25°C. (**A–C**) Functional responses of male spiders with acetone and LD10 and LD50 of insecticide. (**D–F**) Females treated with acetone and LD10 and LD50 of insecticide. The symbols were the observed number of prey killed by spiders, and the bars indicate 95% confidence intervals. The lines in the figures were the fitted model of type II functional response by nonlinear regression, except that in panels E and F, it was the fitted model of type I functional response by linear regression.

methamidophos stimulated the predation behavior of male spiders, and their predation rate per day was significantly enhanced. Their attack efficiency was enhanced, also. To our knowledge, this promoting effect has only been reported once [14], but no statistical significance was shown. This study tested this effect, and showed statistical significance. However, this enhanced potential did not result from more prey being eaten by the spiders, but from more prey being killed, because prey utilization decreased.

Methamidophos is neurotoxic and acute [28], and it was expected that this insecticide could affect the behavior of spiders quickly. It should not be surprising that the predation rate of female spiders decreased quickly after application of methamidophos, although this decline was not observed in the males, mainly because the predation rate of the control spiders was very low as a baseline (Fig. 1). It is shown that the females play a more important role than the males in pest control because their predation efficiency is greater. The results of this study showed that methamidophos had positive effects on the predation behavior of male spiders and short-term negative effects on that of female spiders. Consequently, the overall effects of methamidophos on the predation behavior of spiders were not as great as expected. These results are referenced to evaluate the influence of insecticide in the field; however, this result should be applied cautiously in the field because many other factors (biotic and abiotic) influence the pest potential of spiders, such as temperature, crop structure, and the presence of other nonpest prey.

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