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Imidacloprid Affects *Pardosa Pseudoannulata* Adults and Their Unexposed Offspring

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Abstract Imidacloprid is a nicotine-based, systemic, widely used insecticide. In order to investigate the effects of imidacloprid on the spider *Pardosa pseudoannulata* (Araneae: Lycosidae), specimens were exposed to different concentrations of imidacloprid (12.5, 25, 50, 100, 200 mg/L) by the dipping method. Surviving spiders were used to determine the fecundity, development time of unexposed offspring, predation, and the activities of detoxification enzymes. Significant reductions were observed in survival rate and fecundity of spiders exposed to imidacloprid. The development times of unexposed offspring (F_1) were prolonged significantly with increased concentrations of imidacloprid. Spiders exposed to concentrations of imidacloprid above 25 mg/L showed significantly weaker predation on *Drosophila melanogaster* than the control group, but a low dose of imidacloprid (12.5 mg/L) increased predation ability. The activities of carboxyl esterase, acetyl cholinesterase, and the mixed-function oxidase were significantly inhibited by imidacloprid. With increasing concentrations of imidacloprid, the activities of all three kinds of enzymes were decreased significantly. These results suggest that imidacloprid can stimulate the performance of spiders (in low concentration) and has chronic toxicity to the spiders.

Keywords *Pardosa pseudoannulata* · Imidacloprid · Fecundity · Development times · Predation · Activities of detoxification enzymes

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Pesticides are commonly used to control pest species for health and economic benefits, but worldwide use has led to increased contamination of aquatic habitats (Jones et al. 2009; Shi et al. 2011). It is generally recognized that agrochemicals can seriously affect local populations and the community structure of amphibians, reducing survival, altering feeding and swimming activity, causing a high incidence of deformities, and decreasing growth and development of larvae (Bridges 2000; Brunelli et al. 2009).

Insecticide application has strong effects on spiders at both the population and individual levels (Mansour et al. 1992; Dinter and Poehling 1995). Several studies have shown that sublethal doses of insecticides might also affect the behavior of spiders and can cause reductions in prey-capture ability, development time, and reproductive rate (Deng et al. 2007; Desneux et al. 2007), which will directly and indirectly influence their potential for use in pest control (Everts et al. 1991; Marc et al. 1999). However, a few studies have shown that a low dose of insecticides stimulated the predation behavior of spiders (Toft and Jensen 1998). This phenomenon is termed hormesis.

Hormesis is the name given to the stimulatory effects caused by low levels of potentially toxic agents (Stebbing 1982). Hormesis has often been associated with homeopathy, marginalized, and/or ignored; yet it is a very real phenomenon, and is still not fully understood by many (eco)toxicologists (Chapman 2001; Yu et al. 2010). Hormetic responses have been reported in hundreds of studies of a broad range of species (protozoa, bacteria, fungi, plants, invertebrates, and vertebrates including humans), biological endpoints (e.g., survival, growth, reproduction), and both inorganic and organic chemicals (Calabrese 2008; Calabrese 2010). There are also many instances of actual hormesis that researchers do not discover because they are not conducting tests with a

sufficiently low range of concentrations (Chapman 2001; Calabrese 2008).

In spiders, the observed effects of pesticides also involve changes at the cellular level, including the inhibition of carboxylesterase (CarE) and acetylcholinesterase (AChE) (Babczynska et al. 2006; Peng et al. 2010). The activities of CarE, AChE and MFO are widely used as biomarkers of pesticide exposure in a range of biota (Hyne and Maher 2003; Peng et al. 2010).

The relationship between an individual predator's consumption rate and prey density is termed the functional response, and is a key factor regulating the population dynamics of predator–prey systems (Lengwiler and Benz 1994). Holling (1959) 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 (Mandour et al. 2006). The type II response is common in spiders (Marc et al. 1999).

Imidacloprid is a nicotine-based, systemic, widely used insecticide. It was first introduced into China in the early 1990s, and rapidly became the primary insecticide for controlling *Nilaparvata lugens* (Zeng and Wang 2010). Studies on the safety of natural enemies of pests such as spiders, predatory beetles, and bugs when imidacloprid is used against sucking insects have yielded contradictory results (Mizell and Sconyers 1992; James and Price 2002; Zeng and Wang 2010). The objective of this study was to determine the effects of imidacloprid on spiders.

Pardosa pseudoannulata is one of the most common species of wandering spiders in agricultural fields in China (Wang et al. 2006). This study describes the effects of various doses of imidacloprid on life-history traits of this spider, including mortality, fecundity, development time of untreated F_1 individuals, and predation rate. The activity patterns of several detoxification enzymes were also determined.

Materials and Methods

Subadult spiders were collected from cotton fields in Jiufeng Forest Park, Wuhan (114°31′N, 30°52′E), China, from May 2010 to May 2011. The spiders were kept individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) with a layer of sponge (1.5 cm thick) moistened with distilled water on the bottom. The tubes were plugged with cotton. The spiders were kept in the chambers at 24°C and relative humidity of 60%–80% under a light: dark cycle of 14:10 h (lights turned on at 08:00 h). We fed the spiders with adults of *Drosophila melanogaster* and *Tendipes* sp. every 2 days.

We created a series of imidacloprid solutions corresponding to 12.5, 25, 50, 100, and 200 mg/L by dissolving 20 mg of 93.8% imidacloprid in 100 ml acetone, making five two-fold serial dilutions. In all bioassays, the insecticides were diluted in acetone. The subadults of *P. pseudoannulata* (n = 590) were placed in one of six groups: control group (treated with acetone, n = 60), and 12.5 mg/L (n = 80), 25 mg/L (n = 100), 50 mg/L (n = 100), 100 mg/L (n = 100), and 200 mg/L (n = 150) of imidacloprid. Equal numbers of females and males were used in each group. Spiders were immersed in different concentrations of imidacloprid, each time for 20 s (Watanabe 1993). After 24 h, we recorded the number of dead spiders. The surviving spiders were used to assay the fecundity and development time of unexposed offspring in the subsequent experiment.

After being dipped in imidacloprid, the surviving spiders were fed with adults of *D. melanogaster* and *Tendipes* sp. every 2 days. Two days post-maturation (determined by completion of their final molt), females and males were placed together for mating. Males were removed after the female deposited the first egg sac. We collected all egg sacs laid by the females, and counted the total number of eggs (Chen et al. 2011).

Once the 2nd-instar spiderlings (F_1) dispersed from the abdomen of females, they were kept individually and fed with adults of *D. melanogaster* and *Tendipes* sp., and the sponges were replaced every 2 days. Molts were recorded, and the time between molts was used as the development time of untreated F_1.

In the predation test, 325 adult female *P. pseudoannulata* were allocated to one of six groups: control group (treated with acetone, n = 20), and 12.5 mg/L (n = 25), 25 mg/L (n = 30), 50 mg/L (n = 35), 100 mg/L (n = 55), and 200 mg/L (n = 150) of imidacloprid. Spiders were starved for 3 days before the tests to standardize the hunger level, and were dipped in different concentrations of imidacloprid for 20 s (Watanabe 1993). Taking account of insecticide-induced deaths, the numbers of spiders assigned to the five groups were uneven. Initial prey densities of 10, 20, 30, 40, 50, and 60 fruit flies per container, with at least three replicates per density level, were established for adults of *P. pseudoannulata*. Spiders were placed in containers with prey, and after 2 days received an application of imidacloprid. The number of prey killed was recorded every 24 h for 5 days. The functional responses of spiders in each group were analyzed according to Stewart et al. (2002).

For the measurement of detoxification enzyme activities, adults of *P. pseudoannulata* (n = 225) were placed into six groups: control group (treated with acetone, n = 30), 12.5 mg/L (n = 30), 25 mg/L (n = 30), 50 mg/L (n = 30), 100 mg/L (n = 30), and 200 mg/L (n = 75) of imidacloprid. The spiders were also dipped in different

concentrations of imidacloprid, according to Watanabe (1993). In this experiment, the spiders surviving after 5 days immersed in imidacloprid were used to make the preparations for assaying the activity of enzymes. CarE activity was measured using the method described by Peng et al. (2010). AChE activity was measured essentially according to the method of Ellman et al. (1961), and MFO activity was measured according to Chang and Hodgson (1975). CarE, AChE and MFO concentrations are expressed per mass of protein. Protein concentrations in the preparations were estimated using the colorimetric method of Bradford (1976).

Data are expressed as mean ± SE. The relationships between imidacloprid concentrations and mortality rates were tested by Finney's probit analysis, using the EPA software. The differences in development time, total number of eggs, attack coefficient, handling time and enzyme activities were compared using Duncan's multiple range tests (SAS Institute 1997). The data were tested for homogeneity of variance, using Levene's test of equality of error variances. A nonparametric Kruskal–Wallis test was performed to test the significance of differences between the treated and control groups for predation.

Results and Discussion

The mortality rates of *P. pseudoannulata* differed significantly among different treatments ($p < 0.05$), and rose with increasing concentrations of imidacloprid (Table 1). The nominal LC_{50} of imidacloprid for *P. pseudoannulata* was calculated as 40.44 mg/L, according to Finney's probit analysis, using the EPA software.

Females exposed to imidacloprid produced fewer eggs than the control group. This effect was strongest in females exposed to 200 mg/L (Fig. 1).

The total development time of unexposed offspring (F_1) from the 3rd to the 7th instar was significantly longer in spiders exposed to imidacloprid compared to the control (Table 2). Development times also differed among

Table 1 Mortality rates of subadult *Pardosa pseudoannulata* exposed to different concentrations of imidacloprid

Concentrations of imidacloprid (mg/L)	Number exposed	Number of deaths	Mortality rate (%)
0 (Control)	60	0	0
12.5	80	10	12.5
25	100	28	28.0
50	100	48	48.0
100	100	66	66.0
200	150	130	86.67

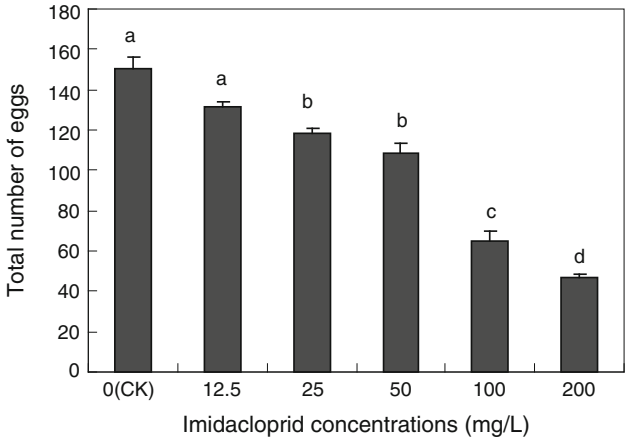

Fig. 1 Total number of eggs produced by females of *Pardosa pseudoannulata* exposed to different concentrations of imidacloprid (control group, n = 8; 12.5 mg/L, n = 7; 25 mg/L, n = 9; 50 mg/L, n = 7; 100 mg/L, n = 6; 200 mg/L, n = 5). *Different letters* above columns indicate significant differences among treatments ($p < 0.05$)

different concentrations of imidacloprid, but not significantly. With higher doses of imidacloprid, the development time of the spiderlings lengthened.

In the spiders, significant differences in the estimated attack coefficient (a) of the six groups were observed (Table 3). The attack coefficients of most of the treated groups were significantly lower than that of the control, except that of the group treated with the concentration of 12.5 mg/L, which was higher. Significant differences in estimated handling time (t_h) were also observed. The handling times of the treated groups were longer than that of the control, but the difference was not significant between the group treated with 12.5 mg/L and the control (Table 3).

Imidacloprid can inhibit the activities of detoxification enzymes in *P. pseudoannulata*. The activities of CarE, AChE and MFO in the imidacloprid-treated spiders were mostly significantly lower than those of the control group. In an exception, the activity of CarE was highest for the concentration of 12.5 mg/L. With increasing concentrations of imidacloprid above this level, the activities of all three kinds of enzymes decreased significantly (Table 4).

Low doses of insecticides may induce an increased predation behavior of spiders, which is called physiological resurgence or hormesis (Luckey 1968, Stebbing 1982). This phenomenon was also reported by Toft and Jensen (1998), although the effect was not statistically significant. Yu et al. (2010) demonstrated that low-dose imidacloprid induced the occurrence of hormesis in *Myzus persicae*, and decreased the control power of *Pirata subpiraticus* on *M. persicae*. In the present study, we obtained a result similar to that of Toft and Jensen (1998). A low dose of imidacloprid stimulated the predation behavior of the spiders,

Table 2 Development times of untreated offspring in adults of *Pardosa pseudoannulata* exposed to different concentrations of imidacloprid (d)

Development stages	Concentration of imidacloprid (mg/L)					
	0 (Control)	12.5	25	50	100	200
3rd instar	7.15 ± 0.11a	8.72 ± 0.15a	8.87 ± 0.39a	9.03 ± 0.76a	10.26 ± 0.31a	10.54 ± 0.25a
4th instar	8.00 ± 0.16a	9.12 ± 0.23a	9.37 ± 0.54a	9.97 ± 0.37b	11.02 ± 0.11b	11.28 ± 0.21b
5th instar	8.78 ± 0.18a	10.27 ± 0.19a	10.31 ± 0.26b	10.54 ± 0.15b	11.89 ± 0.29b	12.32 ± 0.41b
6th instar	8.56 ± 0.17a	10.12 ± 0.29a	10.21 ± 0.63a	10.87 ± 0.27a	12.07 ± 0.31b	13.12 ± 0.38b
7th instar	11.18 ± 0.35a	13.07 ± 0.38b	14.21 ± 0.35b	15.01 ± 0.47b	16.83 ± 0.16b	17.03 ± 0.71b
Total duration	43.67 ± 1.21a	51.3 ± 2.37b	52.97 ± 3.14b	55.42 ± 5.17b	62.07 ± 2.39b	64.29 ± 3.17b

Duncan's multiple range tests. Data indicated with different letters denote a significant difference ($p < 0.05$)

Table 3 Estimates of the attack coefficient (a) and handling time (t_h) for adult female *Pardosa pseudoannulata* exposed to different concentrations of imidacloprid

Concentrations of imidacloprid (mg/L)	a (h^{-1})	t_h (h)
0 (Control)	1.122 ± 0.012b	0.038 ± 0.004e
12.5	1.301 ± 0.017a	0.039 ± 0.006e
25	1.008 ± 0.013c	0.041 ± 0.005d
50	0.987 ± 0.009d	0.047 ± 0.008c
100	0.875 ± 0.013e	0.052 ± 0.011b
200	0.677 ± 0.018f	0.078 ± 0.015a

Duncan's multiple range tests. Data indicated with different letters denote significant differences among different concentrations of imidacloprid

and their attack efficiencies were enhanced (Table 3). One potential explanation for our observation is that the activities of some enzymes (such as CarE) in the spiders increased when treated by a low dose of insecticide (with higher activity of CarE than the control, see Table 4), which resulted in an increase in the attack rates of these predators.

Bioassays of the effect of different concentrations of imidacloprid in in vivo pretreatment experiments showed that most concentrations effectively inhibited enzymes. The metabolic abilities of detoxifying enzymes induced by the insecticides varied with the type of insecticide, resulting in variations in insect susceptibility to different insecticides (Yang et al. 2005).

Detoxification enzymes, including CarE and MFO, are important determinants for growth and survival in herbivores (Prasad et al. 1995). Wang et al. (2006) found that *P. pseudoannulata* is generally more susceptible to insecticides when levels of CarE are lower. Our results showed that a low dose of imidacloprid might cause an increase of CarE activity and lead to an increase in the prey-capture ability of *P. pseudoannulata*. AChE is a target enzyme of organophosphate and carbamate insecticides (Peng et al. 2010). Here, we found that *P. pseudoannulata* had lower AChE activity when treated with imidacloprid, and these results were negatively related to their higher susceptibility to the insecticides that were tested. The observed significant inhibition of MFO activity in *P. pseudoannulata* at the level of spectral and catalytic activity due to in vitro treatment of imidacloprid, and its further increase by the addition of an NADPH-generating system, indicate that imidacloprid and its metabolites have an inhibitory effect on CYP 450 (Rastogi et al. 1997).

In general, the present study demonstrated that imidacloprid can lead to the improvement of prey-capture ability (with a low dose), but shows chronic toxicity to the spiders, with effects including reduction of survival and fecundity, inhibition of enzyme activities, and prolongation of the development time of unexposed offspring. Our findings will be useful in evaluating the influence of the insecticides

Table 4 The activities of CarE, AChE and MFO in adults of *Pardosa pseudoannulata* exposed to different concentrations of imidacloprid

Concentrations of imidacloprid (mg/L)	Activities of enzymes		
	CarE (μmol/mg/30 min)	AChE (μmol/mg/15 min)	MFO (μmol/mg/30 min)
0 (Control)	3.56 ± 0.012a	6.176 ± 0.008a	5.271 ± 0.008a
12.5	3.63 ± 0.013a	5.897 ± 0.004a	4.137 ± 0.025a
25	2.23 ± 0.008b	4.091 ± 0.007b	4.027 ± 0.011b
50	1.69 ± 0.007c	3.8 ± 0.011c	3.278 ± 0.010c
100	1.37 ± 0.021de	3.514 ± 0.018d	2.071 ± 0.049d
200	1.06 ± 0.006e	2.917 ± 0.005e	0.979 ± 0.023e

Duncan's multiple range tests. Data indicated with different letters denote significant differences among different concentrations of imidacloprid

on the spiders and in developing better management strategies.

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