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Sublethal effects of imidacloprid on the fecundity, longevity, and enzyme activity of *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus)

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Abstract

The aphid species *Sitobion avenae* and *Rhopalosiphum padi* are the most important pests in wheat growing regions of many countries. In this study, we investigated the sublethal effects of imidacloprid on fecundity, longevity, and enzyme activity in both aphid species by comparing 3-h exposure for one or three generations. Our results indicated that 3-h exposure to sublethal doses of imidacloprid for one generation had no discernible effect on the survival, fecundity, longevity, or enzyme activity levels of aphids. However, when pulse exposures to imidacloprid were sustained over three generations, both fecundity and longevity were significantly decreased in both *S. avenae* and *R. padi*. Interestingly, the fecundity of *R. padi* had almost recovered by the F5 generation, but its longevity was still deleteriously affected. These results indicated that *R. padi* laid eggs in shorter time lags and has a more fast resilience. The change in reproduction behavior may be a phenomenon of *R. padi* to compensate its early death. If this is stable for the next generation, it means that the next generation is more competitive than unexposed populations, which could be the reason underlying population outbreaks that occur after longer-term exposure to an insecticide. This laboratory-based study highlights the sublethal effects of imidacloprid on the longevity and fecundity of descendants and provides an empirical basis from which to consider management decisions for chemical control in the field.

Keywords: imidacloprid, aphid, survival, fecundity, sublethal effects, enzyme activity

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Introduction

Wheat is one of the most important cereal crops and staple food for humans (Joshi & Sharma, 2009). In China, where wheat is the third most important food crop, wheat production is heavily affected by several insect pests. Aphids are one of the most important economic pests of wheat; aphipds have been reported as pests in all major wheat growing regions worldwide (Gao *et al.*, 2006). *Sitobion avenae* and *Rhopalosiphum padi* are the problematic pest aphid species and impact on a variety of wheat cultivars in various regions. These aphids damage wheat by directly feeding on the plants, and damage wheat indirectly by acting as vectors for the transmission of multiple plant pathogenic viruses (Lu & Gao, 2009, Lu *et al.*, 2013). Under favorable conditions, wheat aphids reproduce rapidly, and they have been estimated to cause damage to 1–3% of the global wheat production (Metcalf *et al.*, 1962).

Control of *S. avenae* and *R. padi* in China relies primarily on the application of insecticides. Imidacloprid belongs to a new class of chemical insecticides: the neonicotinoids. These act on the nervous system, as do 80% of other insecticides. Their

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molecular target is the nicotinic acetylcholine receptor (Nauen *et al.*, 2003, Sohrabi *et al.*, 2012). Imidacloprid is known to be effective against several sucking pests, including aphids (Joshi & Sharma, 2009, Robson *et al.*, 2010), and is commonly used as a foliar spray against aphids on a variety of crops such as cotton, wheat, and cucumber.

In addition to the lethal effects of insecticides, exposure to low doses of insecticide residues is highly likely as a consequence of the widespread use of pesticides (Brown, 1989). Such low concentrations of insecticides can occur at the edges of treated plots, as well as outside of treated areas as a result of unexpected weather changes or if the chemical is applied improperly. When insects are exposed to sublethal pesticide concentrations, some survivors may subsequently develop resistance to the chemical. In addition, the neurotoxic effects of sublethal doses of insecticides can also impact on the behavior and fecundity of individuals, and alter the activity of detoxifying enzymes (Haynes, 1988).

Numerous studies have investigated the effects of sublethal doses of various insecticides on the life history traits and enzyme activity of a variety of insects (Xing *et al.*, 2011, Guo *et al.*, 2013, He *et al.*, 2013). To date, however, there is no published information on the sublethal effects of imidacloprid on fecundity and enzyme activity in *S. avenae* and *R. padi*. In this study, we compared the sublethal effects of both instantaneous (3-h for one generation) and continuous (3-h pulses for three generations) exposure to imidacloprid (LC_{10} and LC_{25}) on survival, fecundity, and enzyme activity in *S. avenae* and *R. padi*, in order to evaluate the use of this insecticide in the management of these pest species.

Materials and methods

Insect rearing

Laboratory populations of both aphid species were from field-caught populations (wheat field of Agricultural Experiment Station belongs to China Agricultural University) and maintained in the laboratory since 2005 without exposure to insecticides. More than 20 generations were reared before experimentation. No specific experimental permissions were required prior to conducting this research because wheat aphids are not a protected species. The two colonies were reared on wheat seedlings at 18–25°C, a photoperiod of 17:7 h (L:D) and a relative humidity of 50–70% (Lu & Gao, 2007, Lu & Gao, 2009).

Determination of sublethal concentrations of imidacloprid

To ascertain what constituted sublethal doses of imidacloprid (95.3% a.i., Jiangsu Changlong Chemical Co., Ltd., China) to both aphid species, we followed the bioassay method of residual film in glass tubes as described by Shotkoski *et al.* (1990) and Shufran *et al.* (1997), with some modifications. Briefly, imidacloprid was diluted to five concentrations in analytical grade acetone. An aliquot of 200 µl of the imidacloprid-acetone solution was then applied to every glass tube (inner surface: 36.0 cm^2) and then was immediately rotated using a micro-rotator (American Wheaton Company). Twenty apterous adults (<24 h old) were introduced into each tube treated with imidacloprid with three replicates conducted for each treatment. A control group of aphids was introduced into tubes treated with acetone. All the treated aphids were allowed to walk in the tubes and mortality was checked after 3-h according to the methods of Lu and Gao (2009). Adults that did not move their legs when touched with a fine brush were assumed to have died (Moores *et al.*, 1996). The LC_{10}, LC_{25}, and LC_{50} values of imidacloprid to *R. padi* and *S. avenae* were then estimated using PoloPlus (LeOra Software, Berkeley, CA; LeOra 2003) with a Probit regression analysis method.

Sublethal effects of imidacloprid on the longevity and fecundity of S. avenae *and* R. padi

In demographic studies, concentrations of less than LC_{30} are usually considered to be 'sublethal' doses (Biddinger & Hull, 1999, Dastjerdi *et al.*, 2009, Bayram *et al.*, 2010, Sohrabi *et al.*, 2011). Hence, using the experimental protocol described above, about 60 young adult aphids (<24 h old, 20 aphids for each of three replicates) were treated with imidacloprid at concentrations of either LC_{10} or LC_{25} for 3-h. These were undertaken as single exposure events in either one or three consecutive generations. Subsequently, the aphids were confined individually in wheat leaf cages and kept under standard conditions. Survival and fecundity were assessed visually. We collected apterous adults of *S. avenae* and *R. padi* from each generation to determine enzyme activities. The collected samples were immediately frozen in liquid nitrogen and stored at −80°C until use the next day. Our experimental processes were consistent for all the treatments.

Assessment of families of enzymes activity

We determined the activity of three enzymes, achetylcholinesterase (AChE), carboxylesterase (CarE), and glutathione S-transferase (GSTs).

AChE: The enzymatic activity of AChE was determined with a previously described method (Ellman *et al.*, 1961), with some modifications (Lu *et al.*, 2013). Batches of approximately 30 frozen apterous adults of each species were manually homogenized in 100 µl ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5% (vol./vol.) Triton X-100. Homogenates were centrifuged at 4°C, 12,000 *g* (Eppendorf centrifuge 5417R, Germany) for 15 min. The supernatant was used as an enzyme source to measure the activity of AChE and protein contents. Briefly, for each reaction, 25 µl substrate ATCh (5 mM, Sigma Chemical Company, St Louis, USA) and 25 µl enzyme were incubated at 30°C for 15 min. The reaction was stopped by the addition of 900 µl DTNB (0.125 mM, Sigma) with 40% ethanol, and the optical density (OD) was measured at 412 nm by a Thermomax microplate reader (Tecan Spectra). The control samples contained no enzyme during the incubation period. After the addition of the color reagent, 25 µl enzyme solutions were added to the controls. Specific activity was expressed as nmol of ATCh hydrolyzed min^{-1} mg^{-1} protein using the extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}.

CarE: We performed three replicates for each assay. To determine the activity of CarE, the enzyme preparation method was similar to AChE, except 0.04 M phosphate buffer (pH7.0). CarE activity was measured via the method of van Asperen (Van Asperen, 1962) using α-naphthol (Sigma) as the substrate, with some modifications (Lu & Gao, 2009). For a separate sample, 900 µl homogenization buffer containing substrate (3×10^{-4} M) and eserine (3×10^{-4} M, Sigma), and 25 µl enzyme were added to each reaction. The mixture was incubated at 30°C for 15 min and the enzyme reaction was stopped by the addition of 450 µl fast blue B-SDS solution. Fast blue B

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Table 1. Toxicity of imidacloprid to *S. avenae* and *R. padi*.

Insecticides	Insects	N[1]	Slope ± SE[2]	LC_{10}[3]	LC_{25}[3]	LC_{50}[3]	χ^2 (df)[4]
Imidacloprid	*S. avenae*	360	1.60 ± 0.20	23.80 (13.66–34.27)	57.18 (41.10–73.08)	151.09 (120.79–196.17)	0.25 (3)
	R. padi	360	1.72 ± 0.20	4.09 (2.18–6.16)	9.24 (6.16–12.21)	22.74 (18.03–28.00)	1.71 (3)

[1]Number of tested aphids.
[2]SE = standard error.
[3]Expressed in ng cm^{-2}; 95% fiducial limits (FL) of LC_{10}, LC_{25}, LC_{50} are given in parenthesis, respectively.
[4]χ^2 testing linearity of dose-mortality responses; df = degree of freedom.

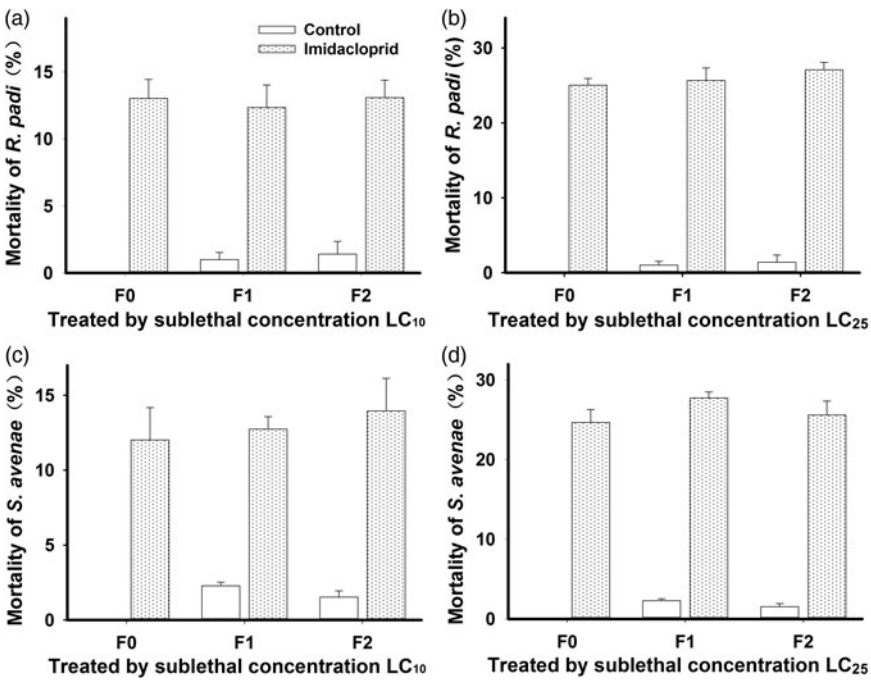

Fig. 1. Adult mortality of *S. avenae* and *R. padi* exposed to sublethal concentrations of imidacloprid for one generation or for three consecutive generations (F0 = treated adults from the first generation, F1 = treated adults from the offspring of the F0 generation, F2 = treated adults from the offspring of the F1. (a) Mortality of *R. padi* adults following exposure to the LC_{10} concentration of imidacloprid; (b) Mortality of *R. padi* adults following exposure to the LC_{25} concentration of imidacloprid; (c) Mortality of *S. avenae* adults following exposure to the LC_{10} concentration of imidacloprid; (d) Mortality of *S. avenae* adults following exposure to the LC_{25} concentration of imidacloprid.

was purchased from Fluka Chemical Company. The absorbance was determined at 600 nm on a Thermomax microplate reader. The OD values were converted to the production of naphthol nmol min^{-1} mg^{-1} protein through α-naphthol standard curves and protein values.

GSTs: The activity of GSTs was determined in apterous adults using CDNB (1-chloro-2,4-dinitrobenzene, Sigma) as a substrate according to a previously described method (Habig *et al.*, 1974). Batches of approximately 30 frozen apterous adults of each species were homogenized in 100 µl of ice-cold 0.1 M phosphate buffer (pH 6.5) containing 1 mM EDTA (ethylene diamine tetraacetie acid). Homogenates were centrifuged at 12,000 × *g* for 15 min at 4°C, and the supernatants were collected and used as enzyme sources (Lu & Gao, 2009). The total volume of assay mixture was 900 µl and contained 1 mM CDNB and 1 mM GSH (reduced glutathione, Sigma). The assay was initiated by the addition of 50 µl enzyme and the absorbance at 340 nm was monitored for 2 min with 10 s intervals using a spectrophotometer (Lambda Bio 40). Controls lacking any enzyme always accompanied each assay. Enzyme activity was calculated with an extinction coefficient of 9.6 mM^{-1} cm^{-1} and expressed as nmol min^{-1} at 25°C, while the specific activity was expressed as nmol min^{-1} mg^{-1} protein.

Protein: Protein content of the enzyme preparations were assessed with Braford Assays (Bradford, 1976) using bovine serum albumin (Sigma) as a standard.

Data analysis

The bioassay results were estimated using PoloPlus (LeOra Software, Berkeley, CA; LeOra 2003) with a Probit regression analysis method. Analysis of variance followed by Fisher's Least Significant Difference (LSD) multiple comparisons tests were performed to assess the significance of imidacloprid effects on fecundity, longevity, and enzyme activity ($P < 0.05$).

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Table 2. Longevity and fecundity of *S. avenae* offspring following one generation of 3-h exposure to sublethal concentrations of imidacloprid.[1]

Biological characteristics	Generations	Control	Imidacloprid	
			LC_{10} treatment	LC_{25} treatment
Progeny number (N)	F1	26.35 ± 3.73 A a	29.12 ± 4.75 A a	25.98 ± 4.56 A a
	F2	28.51 ± 3.47 A a	27.79 ± 2.54 A a	29.63 ± 4.08 A a
	F3	29.14 ± 3.44 A a	28.70 ± 3.05 A a	29.03 ± 3.23 A a
Adults longevity (d)	F1	17.71 ± 1.84 A a	17.88 ± 1.36 A a	17.13 ± 0.97 A a
	F2	18.59 ± 2.00 A a	19.10 ± 1.53 A a	18.33 ± 1.74 A a
	F3	19.31 ± 2.04 A a	18.57 ± 1.63 A a	18.63 ± 1.59 A a

[1]Means within a row followed by the same capital letters are not significantly different among treatments (LSD, $P < 0.05$); Means with in a column followed by the same lowercase are not significantly different among progeny number or among adults longevity (LSD, $P < 0.05$).

Table 3. Longevity and fecundity of *R. padi* offspring following one generation of 3-h exposure to sublethal concentrations of imidacloprid.[1]

Biological characteristics	Generations	Control	Imidacloprid	
			LC_{10} treatment	LC_{25} treatment
Progeny number (N)	F1	56.18 ± 8.28 A a	51.41 ± 5.53 A a	49.26 ± 5.61 A a
	F2	53.56 ± 6.73 A a	36.84 ± 4.38 B b	29.86 ± 4.61 B b
	F3	52.85 ± 8.01 A a	48.78 ± 6.84 A ab	47.26 ± 6.53 A a
Adults longevity (d)	F1	12.18 ± 0.96 A a	12.57 ± 1.61 A a	12.73 ± 1.46 A a
	F2	13.16 ± 1.97 A a	12.53 ± 1.58 A a	12.96 ± 1.38 A a
	F3	13.31 ± 1.04 A a	13.57 ± 1.13 A a	13.23 ± 1.09 A a

[1]Means within a row followed by the different capital letters are significantly different among treatments (LSD, $P < 0.05$); Means with in a column followed by the different lowercase are significantly different among progeny number or among adults longevity (LSD, $P < 0.05$).

Results

Bioassays and determination of sublethal concentrations

The mortality of adult apterous aphids of both species increased with an increase in the dose of imidacloprid. The LC_{50} values of imidacloprid for *S. avenae* and *R. padi* were 151.09 and 22.74 ng cm^{-2}, respectively, indicating that imidacloprid was 6.64-fold more toxic to *R. padi* than to *S. avenae*. After 3-h of exposure, the LC_{10} and LC_{25} doses for *S. avenae* were 23.80 and 57.18 ng cm^{-2}, and for *R. padi* were 4.09 and 9.24 ng cm^{-2} (table 1). These LC_{10} and LC_{25} values were used during the subsequent experiments.

Adult mortality

Mortality of *S. avenae* adults treated with imidacloprid showed no obvious variation over pulse exposures for one or three generations. The mortalities of *S. avenae* adults treated by the LC_{10} and LC_{25} dosages were F0 = 12%, F1 = 13%, F2 = 14% for LC_{10} treatment and F0 = 25%, F1 = 28%, F2 = 26% for LC_{25} treatment, respectively (fig. 1A, B), and very similar results were obtained for *R. padi* adults (LC_{10}: F0 = 13%, F1 = 12%, and F2 = 13%; LC_{25}: F0 = 25%, F1 = 26%, and F2 = 27%; fig. 1C, D). Mortalities in the control groups were always below 5%, which is normal and acceptable for any bioassays of insect species, and indicated that acetone did not induce the mortality variations in the offspring of aphids.

The effect of imidacloprid on the longevity and fecundity of S. avenae *and* R. padi

Exposure to sublethal doses of imidacloprid did not affect the offspring longevity of the F1, F2, and F3 generations of both aphid species that were 3-h exposed to this chemical for only one generation. Their longevity varied from 17.13 to 19.31 days for *S. avenae* (table 2) and from 12.18 to 13.57 days for *R. padi* (table 3) among all the treatments. However, when pulse exposures to imidacloprid were sustained over three generations, the longevity was significantly decreased for both *S. avenae and R. padi* adults. For the F3, F4, and F5 generations, the longevity of both aphid species ranged by Control > LC_{10} treatment > LC_{25} treatment (tables 4 and 5).

Our results showed that the fecundity of *S. avenae* offspring was not obviously reduced by 3-h exposure to sublethal doses of imidacloprid for one generation (table 2). In contrast, the fecundity of *R. padi* F2 generation, but not the F1 and F3 generation, was significantly reduced after just one generation of 3-h exposure to sublethal doses of imidacloprid (table 3). When pulse-exposed to the insecticide for three subsequent generations, the fecundity of *S. avenae* was reduced for all generations, including the F3, F4, and F5 generations. In addition, for the F4 and F5 generations of *S. avenae*, the reduction in fecundity was greater for the LC_{25} treatment than the LC_{10} treatment (table 4). When pulse-exposed to imidacloprid for three subsequent generations, the fecundity of the F3 and F4 generations of *R. padi* were significantly reduced, with a stronger effect for the higher dosage (LC_{25}). Interestingly, imidacloprid did not reduce the fecundity of the F5 generation of *R. padi* adults, but reduced its longevity (table 5).

The effect of imidacloprid on enzyme activity in S. avenae *and* R. padi

After one generation of 3-h exposure there were no significant differences in the activity of the enzyme AChE in all the determined generations of either species (fig. 2A, D), however, the specific activity of CarE in the F1 generation for both

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Table 4. Longevity and fecundity of *S. avenae* offspring following three consecutive generations of 3-h exposure to sublethal concentrations of imidacloprid.[1]

Biological characteristics	Generations	Control	Imidacloprid	
			LC_{10} treatment	LC_{25} treatment
Progeny number (N)	F3	25.83 ± 4.28 A a	16.45 ± 3.82 B a	14.76 ± 3.74 B a
	F4	23.98 ± 3.21 A a	12.83 ± 1.61 B a	7.63 ± 1.06 C b
	F5	26.41 ± 4.03 A a	17.06 ± 3.28 B a	9.03 ± 1.95 C a
Adults longevity (d)	F3	14.93 ± 1.15 A a	9.52 ± 0.93 B a	5.03 ± 0.79 C a
	F4	15.62 ± 2.12 A a	10.10 ± 1.04 B a	6.37 ± 0.74 C a
	F5	15.31 ± 1.85 A a	9.86 ± 1.13 B a	6.48 ± 1.05 C a

[1]Means within a row followed by the different capital letters are significantly different among treatments (LSD, $P < 0.05$); Means with in a column followed by the different lowercase are significantly different among progeny number or among adults longevity (LSD, $P < 0.05$).

Table 5. Longevity and fecundity of *R. padi* offspring following three consecutive generations of 3-h exposure to sublethal concentrations of imidacloprid.[1]

Biological characteristics	Generations	Control	Imidacloprid	
			LC_{10} treatment	LC_{25} treatment
Progeny number (N)	F3	53.62 ± 7.52 A a	29.57 ± 5.21 B b	17.59 ± 3.95 C b
	F4	54.94 ± 8.26 A a	39.51 ± 5.75 B a	29.86 ± 4.61 C ab
	F5	52.49 ± 7.21 A a	45.13 ± 4.96 A a	44.25 ± 5.87 A a
Adults longevity (d)	F3	12.75 ± 1.03 A a	8.36 ± 1.39 B a	7.95 ± 1.04 B a
	F4	12.99 ± 1.38 A a	8.98 ± 1.15 B a	8.37 ± 1.03 B a
	F5	13.15 ± 1.12 A a	9.04 ± 1.26 B a	8.82 ± 1.23 B a

[1]Means within a row followed by the different capital letters are significantly different among treatments (LSD, $P < 0.05$); Means within a column followed by the different lowercase are significantly different among progeny number or among adults longevity (LSD, $P < 0.05$).

species increased following exposure to the LC_{25} dose of insecticide (*S. avenae* = 21.71%; *R. padi* = 32.66%; fig. 2B, E). In contrast, one generation of 3-h exposure to the sublethal dose of insecticide significantly inhibited the specific activity of GSTs in the F3 generation of *S. avenae* (fig. 2C), and the F0, and F3 generations of *R. padi* (fig. 2F).

Pulse exposure to imidacloprid for three consecutive generations did not alter the activity of AChE in *S. avenae* and *R. padi* (fig. 3A, D), but did increase the activity of CarE in the F1 and F3 generations for *S. avenae* (fig. 3B) and in F1 generation of *R. padi* (fig. 3E). After three consecutive generations of pulse exposure to imidacloprid, the specific activity of GSTs of *R. padi* was significantly inhibited by the LC_{10} concentration in F0, F1, F2, and F4 generations (fig. 3F). There was no parallel reduction in the activity of GSTs in any generation of *S. avenae* (fig. 3C).

Discussion

Sublethal effects in insects exposed to low dosage insecticides have been documented for most of insects and pesticide compounds. Most of these studies, however, have examined effects on survival, growth, and fecundity of single cohort insects, and suggested that insects exposure to sublethal insecticide concentrations over time is probably one of the causes of the resurgence of insect pests (Christopher Cutler *et al.*, 2009). In this study, an attempt was made to assess sublethal effects on two species of wheat aphid over subsequent generations during pulse-exposure to imidacloprid. Such multigenerational evaluations of sublethal effects are important in the case of imidacloprid, because it can be assumed that with current control practices sublethal concentrations of this chemical occur often. For example, the period of imidacloprid residues being retained on the wheat leaves can last for several weeks after an application. Hence, *S.avenae* and *R. padi* are likely often exposed to sublethal concentrations of imidacloprid in the field. More importantly, our study produced some interesting results on the effects of imidacloprid on both aphid species, some of which were not found in previous studies.

Our results revealed moderate mortality rates in the F0, F1, and F2 generations of *S. avenae* and *R. padi* when exposed to the sublethal doses of imidacloprid, and we observed that this mortality was higher than that in the untreated control aphids. After pulse exposures of three consecutive generations, the mortality was still reasonable in all generations (below 15% for the LC_{10} treatment and below 30% for the LC_{25} treatment). These results indicated that our established sublethal doses were adequate as they did not result in either high or low mortality of treated aphids.

When both aphid species exposed to residue film treated with sublethal concentrations of imidacloprid for 3-h of one generation, a no significant differences were found for fecundity (progeny number) or adult longevity, with the exception that the progeny number of the F2 generation of *R. padi* was reduced. However, the effect on reduced progeny disappeared in the following (F3) generation. These results indicated an inhibited effect on the F2 generation reproduction of *R. padi*, which was contradictory to most previous studies. Christopher Cutler *et al.* (2009) reported that sublethal concentrations of imidacloprid have a stimulatory effect on *Myzus persicae* second generation reproduction following several days' exposure. In addition, the LC_{25} concentration of imidacloprid increased reproduction of *M. persicae*, although it was not clear if this effect was statistically significant (Wang *et al.*, 2008). Nevertheless,

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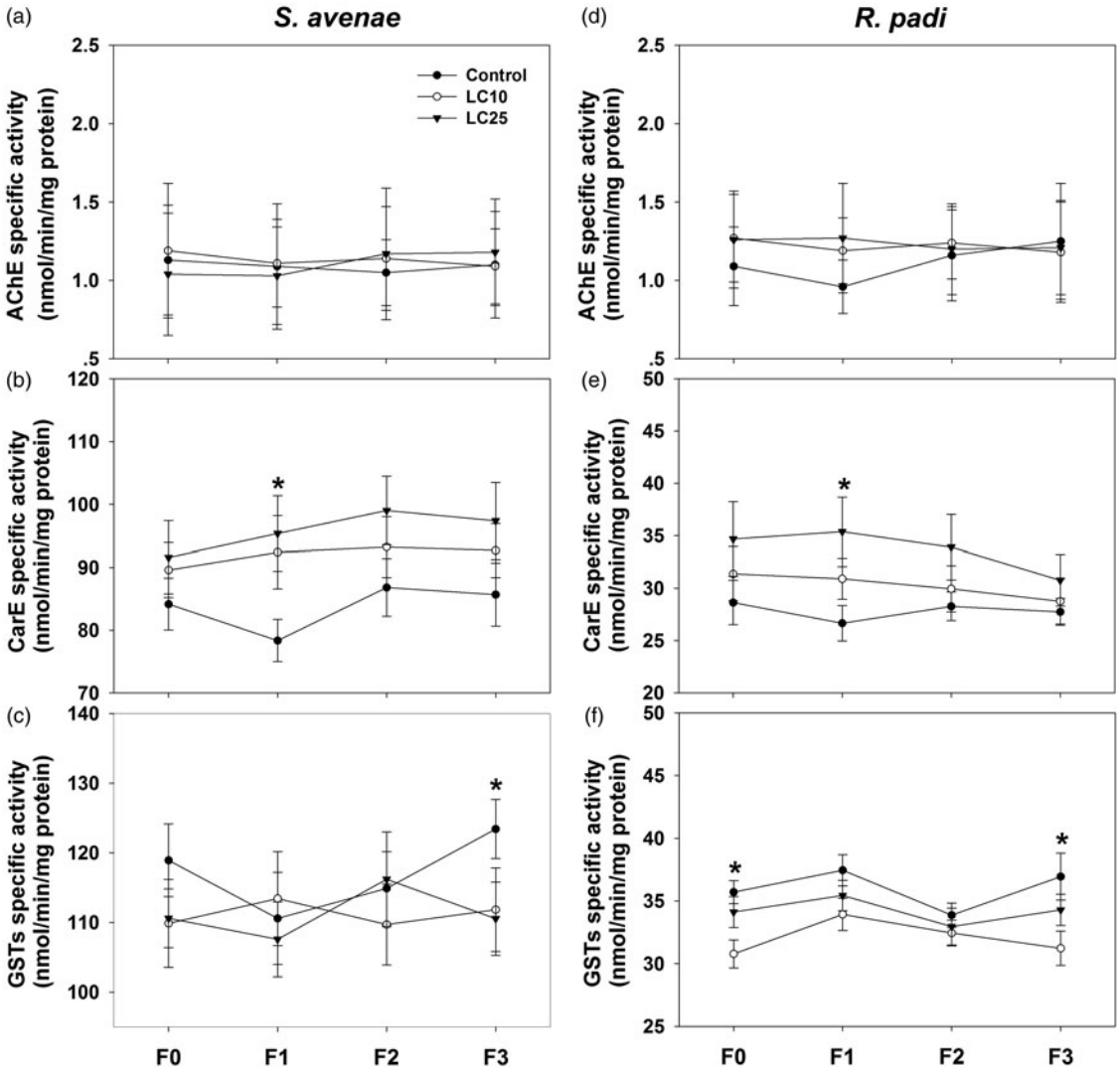

Fig. 2. Effect of one generation of exposure to sublethal concentrations of imidacloprid on the specific activity of (a) AChE in *S. avenae*; (b) CarE in *S. avenae*; (c) GSTs in *S. avenae*; (d) AChE in *R. padi*; (e) CarE in *R. padi*; and (f) GSTs in *R. padi*.

several studies showed that sublethal concentrations of imidacloprid reduced fecundity of *Brevicoryne brassicae* (Moharamipour *et al*., 2003, Lashkari *et al*., 2007).

When pulse-exposed to sublethal concentrations of imidacloprid for three consecutive generations, a significant decrease was found on the fecundity and the adult longevity of both aphid species. However, in previous studies, Sohrabi *et al*. (2011) reported that imidacloprid increased the fecundity of *Bemisia tabaci*, while Beers and Himmel (2002) similarly reported that imidacloprid caused an increase in mite populations when applied to control psyllids on pears. Similar observations have been found in green peach aphids exposed to residues of azinphosmethyl (Lowery *et al*., 1986) and in citrus thrips on leaves that were contaminated with dicofol or malathion residues (Morse & Zareh, 1991). Our observations seem to be in opposition to the phenomena reported in most studies, which support the hormesis whereby reproductive stimulation of pests or beneficials occurs after exposure to sublethal doses of certain synthetic insecticides (Luckey, 1968). It is interesting that, as in the present imidacloprid experiment, the fecundity of *R. padi* almost recovered to normal levels by the F5 generation, but its longevity remained strongly affected by the pulse-exposure for three generations. These results indicated that *R. padi* laid eggs in shorter time lags and has a more fast resilience. Recovery of fecundity after exposure to sublethal concentration of insecticides has previously been reported in insect species exposed to pesticides (Desneux *et al*., 2004*a*, Desneux *et al*., 2007), although it is not always found (Desneux *et al*., 2004*b*, Moser & Obrycki, 2009). However, the phenomenon of insects that has no fertility reduction but its longevity is largely affected by sublethal concentrations of insecticides was not found in previous reports. This change in reproduction behavior may be a phenomenon of aphids to compensate its early death.

Exposure to sublethal concentrations of insecticides affects the longevity and fecundity of insects via biochemical, physiological, and behavioral mechanisms (Desneux *et al*., 2007, Miao *et al*., 2014). Daniels *et al*. (2009) reported that a

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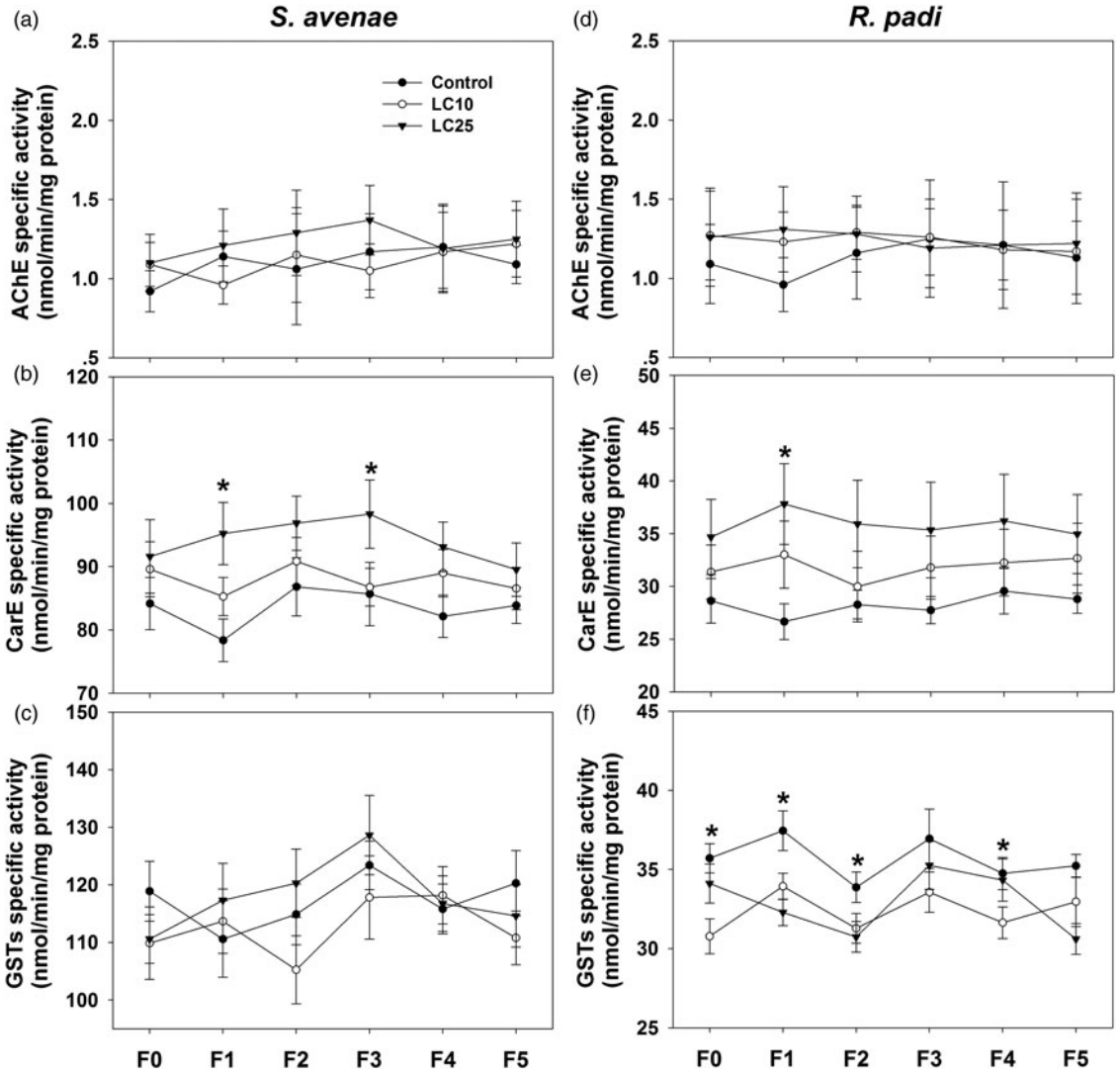

Fig. 3. Effect of three consecutive generations of exposure to sublethal concentrations of imidacloprid on the specific activity of (a) AChE in *S. avenae*; (b) CarE in *S. avenae*; (c) GSTs in *S. avenae*; (d) AChE in *R. padi*; (e) CarE in *R. padi*; and (f) GSTs in *R. padi*.

sublethal dose of thiamethoxam had detrimental biochemical effects on the performance of the bird cherry-oat aphid (*R. padi*). In this study, we report that a sublethal dose of 3-h exposure to imidacloprid for one generation, to a certain extent, induced the activity of the CarE enzyme but inhibited the activity of the GST enzymes in *S. avenae* and *R. padi*. And this effect became more strongly pronounced with pulse-exposures to the sublethal concentrations of imidacloprid (both LC_{10} and LC_{25}) for three consecutive generations. An external stimulus (imidacloprid) induced the CarE activities, which probably cause enhanced sequestration of insecticidal esters. This phenomenon may be a stress response of aphids to the external stimuli. Devonshire & Moores (1982) found that CarE is relatively inefficient in degrading insecticidal esters in resistant *Myzus persicae* populations. Its effect is mediated by sequestering a substantial proportion of a toxic dose of insecticide. In addition, external stimuli induced CarE activities are often found in other insect species, such as *Bemisia tabaci* (Byrne *et al*., 2003) and *Plutella xylostella* (Xing *et al*., 2011). As to the GST activities being inhibited after long exposure, similar results were found in *Porcellio scaber* (Drobne *et al*., 2008). They found that GST activity increased at a low exposure concentration of imidacloprid but decreased at higher concentration levels. *Plutella xylostella* also exhibited significantly decreased GST activity after exposure to sublethal concentrations (LC_{10} and LC_{25}) of chlorantraniliprole (Xing *et al*., 2011). So, we hold the opinion that both increased and decreased GST activities are possible in chemically-stressed animals, depending on the type of the chemical and the time and dose of the exposure. Moreover, AChE is an important biochemical marker in ecotoxicology. Hence, AChE activity was determined as a marker of insect health although it is not the molecular target of imidacloprid and has no role in the detoxification of insecticides. Our results showed that the lack of variation of AChE activities indicated a lack of alteration in the nerve communication of both aphid species, in spite of either a single or a prolonged exposure to imidacloprid.

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In conclusion, this study produced a novel empirical perspective on the sublethal effects of imidacloprid on *S. avenae* and *R. padi*. Short-term exposure (3-h exposure for one generation) to sublethal concentrations of imidacloprid had no obvious effect on the longevity, fecundity, or enzyme activities of *S. avenae* or *R. padi*, whereas pulse-exposure over three consecutive generations did adversely affect all of these traits: decreasing longevity and fecundity and altering the activity of several key enzymes.

Based on these results, it is worth nothing that imidacloprid is likely to have no obvious resistance risk and population outbreak of *S. avenae* exposure to low dose in the short-term (several generations) in laboratory. Of note, the fecundity of *R. padi* recovered in the F5 generation, but its longevity was still strongly deleteriously affected. The change in reproduction behavior may be a phenomenon of aphids to compensate its early death. If this is stable for the next generation, it means that the next generation would potentially be more competitive than unexposed populations, which may be a key factor leading to population outbreaks after longer-term exposure to an insecticide. However, sublethal effects on insects are very complex factors to assess the total effect on population and resistance, and varied with different insecticides and different insect species. This laboratory-based study highlights that, sublethal doses of imidacloprid can reduce the longevity and fecundity of descendants. Nevertheless, given that the genetic variation in field populations is naturally greater than that of laboratory strains, the situation in the field may be more complex (Jackson & Wilkins, 1985). Therefore, further investigations on the spatial and temporal effects of sublethal doses of this insecticide on wheat aphids in the field are needed.

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