

# Insecticide susceptibility of the green plant bug, *Apolygus lucorum* Meyer-Dür (Homoptera: Miridae) and two predatory arthropods

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**Abstract:** The green plant bug (*Apolygus lucorum* Meyer-Dür) is a key pest of Bt cotton in China. Along with biological control, chemical control is one of the most important strategies in *A. lucorum* Integrated Pest Management (IPM). The goal of this study was to evaluate the toxicity of eight conventional insecticides to *A. lucorum* and to assess the susceptibility of two generalist predators *Chrysopa sinica* (Jieder) and *Propylaea japonica* (Thunbery) to insecticides that are commonly used in *A. lucorum* management. *Via* glass-vial and leaf-dip bioassay, toxicity tests with selected insecticides at two different life-stages of *A. lucorum* indicated significant differences between the LD<sub>50</sub> or LC<sub>50</sub> values for these compounds within different insecticidal classes. Phenylpyrazole fipronil had the highest toxicity to 4th-instar nymphs and adults of *A. lucorum*, whereas neonicotinoid imidacloprid had the lowest toxicity among the insecticides. Females were more tolerant to insecticides than were males, as shown by the higher LD<sub>50</sub> values for females. Furthermore, laboratory tests showed that endosulfan had the highest selectivity to *C. sinica* and *P. japonica*: the selective toxicity ratios (STRs) were superior to other tested insecticides, particularly imidacloprid, and were 5.396 and 4.749-fold higher than baseline STRs, respectively. From this study, we conclude that fipronil can potentially be used to efficiently control *A. lucorum*. An alternative control agent worth consideration is endosulfan, owing to its relative safety to non-targeted natural enemies.

**Key words:** *Apolygus lucorum*, *Chrysopa sinica*, *Propylaea japonica*, safety evaluation, toxicity

## Introduction

Plant bugs (Homoptera: Miridae) occur worldwide and are one of the most destructive pests of cotton (*Gossypium hirsutum* L.) (Wheeler 2001). Since the widespread cultivation of Bt cotton in the Yellow River and Yangtze River valleys of eastern and southern China, cotton crops have suffered seriously from green plant bug (*Apolygus lucorum* Meyer-Dür) attacks while greatly reducing insecticide use for the control of *Helicoverpa armigera* (Hübner) (Wu 2007; Wu *et al.* 2008; Lu *et al.* 2012a; Lu *et al.* 2013). Feeding by *A. lucorum* adults and nymphs results in bud blast, flower abortion, and missing or shrunken squares and bolls. These abnormalities arise primarily from the activity of polygalacturonase in the salivary glands of *A. lucorum*, and cause extremely large cotton yield losses of up to 20–30% (Lu *et al.* 2007; Zhang *et al.* 2013). This pestiferous bug of Bt cotton also feeds on other perennial crops (e.g. beans, the Chinese date, the tea plant, cherry, apple, and peach), specifically at the flowering stage in the major Chinese cotton-growing regions (Lu *et al.* 2012b; Pan *et al.* 2013; Zhang *et al.* 2013). High-density outbreaks of *A. lucorum* frequently occur in cotton fields (Lu *et al.* 2010, 2011). Lu *et al.* (2008a, 2010) reported that *A. lucorum* infection levels were 10 per 100 plants at the seedling

stage, and its peak density ranged from 50 to 200 per 100 plants during the squaring and flowering stages. If the published economic thresholds for *A. lucorum* (seedling stage: 5 bugs · 100<sup>-1</sup> plants; mid-period of cotton growth: 10 bugs · 100<sup>-1</sup> plants) proposed by Zhang *et al.* (1986) had been followed, the need to control *A. lucorum* in Bt cotton fields would have been urgent based on the data of Lu *et al.* (2008a, 2010). Hence, suitable techniques for managing *A. lucorum* populations on Bt cotton are needed.

One of the important strategies used in the Integrated Pest Management (IPM) of *A. lucorum* is chemical control. The pest's numbers are often kept below the economic threshold with the use of chemicals. Currently, chemical insecticides are the most accessible control option to Chinese cotton growers, primarily because chemical insecticides are effective, easy to operate, cost-effective, and reliable against the target pests (Zhao 2000; Tan *et al.* 2012). Few alternative control measures are available (Hardee and Bryan 1997; Wu 2007). Wu *et al.* (2002) have confirmed that the population density of *A. lucorum* in chemically-controlled plots was far lower than that in non-chemically controlled plots. Traditionally, more than four broad-spectrum insecticide applications for *A. lucorum* are administered every growing season with

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organophosphates, carbamates, synthetic pyrethroids, and a few organochlorines (e.g. endosulfan) being the most common insecticides in various agricultural areas of China (e.g. Shandong which is a major province of cotton agriculture) (Zhang *et al.* 2009; Guo *et al.* 2010). In addition, neonicotinoids, the dominant insecticidal classes, are widely used as foliar sprays and seed treatments against piercing-sucking pests of Bt cotton (Kumar *et al.* 2012; Tan *et al.* 2012). So far, most cotton growers also frequently choose neonicotinoids to control *A. lucorum* in China (Liu *et al.* 2007). As a well-known insecticide with a novel mechanism of action, fipronil has been shown to be highly effective against a broad range of economically important insect pests in agriculture, including sucking pests in cotton (Zhao and Salgado 2010; Kumar *et al.* 2012; Shi *et al.* 2012; Ahmed *et al.* 2015). The aforementioned chemicals were used in the present research to assess their toxicity to *A. lucorum*.

Although insecticide treatments may be substantial at present, natural predators in Bt cotton fields are an additional and necessary source of biological control for *A. lucorum* populations. The integration of chemical and biological control is key for the success of the IPM program (Planes *et al.* 2013; Saber and Abedi 2013). Insecticides provide effective control of key pests, but at the same time these insecticides often have adverse impacts on other beneficial organisms (Kumar *et al.* 2012; Moens *et al.* 2012). In Chinese Bt cotton-planting regions, the most common generalist predators are *Chrysoperla sinica* (Jieder) and *Propylaea japonica* (Thunberg). These predators are the most consistently abundant. They attack *A. lucorum* and several other pests, such as aphids, thrips, mites and whitefly, at all developmental stages (Deng *et al.* 2003). Currently, little is known about the toxicity of chemical insecticides used in Bt cotton on these two predatory species. Therefore, knowledge of the selective toxicity of the chemicals on predators and *A. lucorum* is crucial. Environmentally sound, less hazardous and sustainable chemicals for the control of *A. lucorum* are urgently needed.

The objectives of our study were to: assess the efficacy of eight common insecticides to *A. lucorum*, establish baselines, and further determine whether the susceptibility of females was different from that of males with regard to selected insecticides. Specifically, the latter laboratory assay was focused on the selective toxicity of these insecticides to *C. sinica* and *P. japonica* to assess the potential compatibility of biological and chemical control for improved IPM programs of *A. lucorum*. Overall, our aims are to supply accurate information to cotton growers for the rational application of insecticides in the chemical control of *A. lucorum*, and to reduce pesticide use in Bt cotton-planting regions.

## Materials and Methods

### Test insects

*Apolygus lucorum* adults were obtained from established laboratory colonies collected from a Xiajin County cotton field (36°55'30"N, 115°56'59"E) in Shandong Province, China. Sweep nets were used to catch the *A. lucorum* adults from the

fields, from July to October, 2009. The population colony was maintained on fresh asparagus bean pods (*Phaseolus vulgaris* L.) under constant laboratory conditions of 25°C, 70% relative humidity (RH) and a photoperiod of 14 : 10 (L : D) h, as described in Lu *et al.* (2008b) and Feng *et al.* (2012). Eggs laid in asparagus bean pods were placed in plastic insect boxes (23 × 15 × 9 cm) along with moist paper, until the eggs hatched. The nymphs and subsequent adults were reared with the asparagus bean pods in the controlled environment of the laboratory. The bean pods were washed in detergent and soaked in a 0.5% sodium hypochlorite solution as described in Snodgrass (1996). This procedure was done to remove or oxidize any insecticide residue on them in advance. Pods were replaced every 2 days and those containing *A. lucorum* eggs were transferred to a new rearing box to initiate a new generation. Supplementary nutrition consisted of a 10% honey solution on water-soaked piece of cotton which was changed daily.

*Chrysoperla sinica* and *P. japonica* adults were obtained from laboratory colonies that were established with the eggs of insects collected from the experimental cotton field and nearby corn field in the 2000s in Xiajin (Shandong), China. Newly hatched larvae were maintained in plastic containers (3 × 15 × 9 cm) with laboratory reared cotton aphids (*Aphis gossypii* Glover) until eclosion and were then transferred to wooden cages (50 × 50 × 50 cm) fitted with four fine nylon mesh screens for maintenance at 25°C with 70% RH and a 14 : 10 (L : D) h photoperiod. All predators were reared under insecticide-free conditions until the desired developmental testing stages.

### Insecticides

The technical grade insecticides with a purity higher than 95% used in the bioassays were: 95% endosulfan (organochlorine) (Makhteshim-Agan Industries Ltd., Shanghai, China), 98% bifenthrin (synthetic pyrethroid) (Yangnong Chemical Co., Ltd., Jiangsu, China), 97% chlorpyrifos (organophosphate) (Dow AgroSciences LLC., Indianapolis, IN, USA), 95% malathion (organophosphate) (Sinochem Ningbo Chemical Co., Ltd., Zhejiang, China), 98% methomyl (carbamate) (Changlong Chemicals Co., Ltd., Jiangsu, China), 95% carbosulfan (carbamate) (Luba Chemicals Co., Ltd., Shandong, China), 96% fipronil (phenylpyrazole) (Bayer CropScience Ltd., Zhejiang, China), and 96.8% imidacloprid (neonicotinoid) (Hisun Chemical Co., Ltd., Zhejiang, China). For contact bioassays, the insecticides were used directly after being dissolved in acetone. In leaf-dip bioassays, an emulsible concentrate was prepared by dissolving each insecticide in a 1 : 9 mixture of Tween-80 (Jiangsu Hai'an Petrochemical Plant, Nantong, China)/acetone used as the solvent. The emulsifier Tween-80 in the solvent was used to avoid the precipitation of solutions after a period of time.

### Toxicity determination for *Apolygus lucorum* via glass-vial bioassay

A standard glass-vial bioassay (Snodgrass and Scott 2000; Zhang *et al.* 2009) was used to determine the toxicity of insecticides to *A. lucorum* nymphs and adults. Five to

eight concentrations within a mortality range of 0–100% based on preliminary assays, were prepared from serial dilutions as  $\text{mg} \cdot \text{l}^{-1}$  of the active ingredient of the test compounds with acetone before treatment. Each insecticide was applied by pipetting 0.5 ml of the insecticide dissolved in 99.7% (v/v) acetone, into 20-ml glass scintillation vials (2.8 cm diameter, 6.1 cm high, 59.786  $\text{cm}^2$  inner area). Then, the treated vial was manually rotated until no more droplets were seen on the glass wall. At this point, the vial was kept at room temperature for 1 h to allow for the complete evaporation of the acetone before introducing *A. lucorum*. Vials in the control treatments received only 0.5 ml of acetone. A small piece of washed and sterilised asparagus bean pod (cut transversely, 3 mm thick) was added to each vial as food for *A. lucorum*. Next, five 4th-instar nymphs or active adults which were 2–4 days old were transferred into one vial, and the vial was then sealed by one layer of gauze to allow for air circulation. Three replications of 10 *A. lucorum* per concentration were conducted in each insecticide treatment. After the treatment, the vials were held during the test in an upright position under environmental conditions necessary for rearing hemipterans. Mortality was checked after 24 h by counting the number of surviving bugs. The tested insects were considered dead if they were unable to stand normally or walk, or there was no movement when disturbed with a fine needle.

#### Toxicity determination for *Apolygus lucorum* via leaf-dip bioassay

The leaf-dip method was adopted for the ingestion bioassay to evaluate the stomach toxicity in *A. lucorum*. Five to eight concentrations within a mortality range of 0–100% based on preliminary assays were prepared from serial dilutions using 0.1% (v/v) Tween-80 in distilled water. Fresh and uncontaminated top leaves of cotton (cut to a square area of  $2 \times 2$  cm) with approximately 2 cm of petiole were individually dipped into one of the solutions for 5 sec, removed, and dried for 2 h in the open air on filter paper. One treated leaf was inserted into 1% agar medium (ca. 1 ml) at the bottom of a tube (2.5 cm diameter, 8 cm high) to maintain moisture. Five 4th-instar nymphs or active adults which were 2–4 days old were introduced into each tube, and the tube was then sealed by one layer of gauze. Three replications of 10 individuals per concentration were conducted in each insecticide treatment. After the treatment, the tubes were kept in an upright position under the environmental conditions outlined for rearing hemipterans. Mortality was recorded after 48 h. Cotton leaves were dipped into 0.1% (v/v) Tween-80 in distilled water as an untreated control.

#### Toxicity determination for *Chrysopa sinica* and *Propylaea japonica*

The bioassay that was developed to determine the toxicity for two predators was modified from that of Xue and Li (2002), in which the test procedures were similar to those described above as the glass-vial bioassay applied in the toxicity determination for *A. lucorum*. Each vial contained

two active adult *C. sinica* or *P. japonica* which were 1–2 days old. Each vial was kept at a constant temperature of 25°C with 70% RH and an 14 : 10 (L : D) h photoperiod. Three replications of 10 *C. sinica* or *P. japonica* adults per concentration were conducted in each insecticide treatment. Mortality was assessed at 24 h after treatment.

#### Data analysis

Data were corrected for the control mortality using Abbott's (1925) formula before analysis. For each insecticide, regression lines,  $\text{LD}_{50}$  or  $\text{LC}_{50}$  values,  $\chi^2$  values, and 95% confidence limits were calculated using the Probit procedure in SAS/STAT version 9.2. The  $\text{LD}_{50}$  or  $\text{LC}_{50}$  values without overlapping 95% confidence limits are considered to be significantly different ( $p < 0.05$ ).

## Results

#### Toxicity of insecticides determined by the glass-vial bioassay

The toxicity of eight insecticides to 4th-instar nymphs and adults were investigated by the glass-vial bioassay to elucidate the contact toxicities of the tested compounds to the different life stages of *A. lucorum*. The slopes of the probit lines varied but were relatively steep in all cases, indicating a homogenous population response within each chemical. The three most toxic compounds for the 4th-instar nymphs were fipronil, bifenthrin, and chlorpyrifos, with  $\text{LD}_{50}$  values of 0.107, 0.125, and 0.251  $\text{mg} \cdot \text{m}^{-2}$ , respectively. Among eight tested insecticides, fipronil with an  $\text{LD}_{50}$  of 0.013  $\text{mg} \cdot \text{m}^{-2}$  was the compound most toxic to adults. The next most toxic insecticides were bifenthrin and methomyl with  $\text{LD}_{50}$  values of 0.132 and 0.196  $\text{mg} \cdot \text{m}^{-2}$ , respectively. The  $\text{LD}_{50}$  value for imidacloprid was the lowest and was significantly different from all the other seven  $\text{LD}_{50}$  values. Based on the non-overlapping confidence limits of  $\text{LD}_{50}$  values, slight but significant differences in susceptibility were observed among the eight tested insecticides. Fipronil was most toxic to the different life stages of *A. lucorum*. The relative toxicity indices for fipronil were 0.002 and 0.001, respectively, when we used the  $\text{LD}_{50}$  values for imidacloprid, the least toxic insecticide to *A. lucorum* (Table 1).

#### Toxicity of insecticides determined by the leaf-dip method

For *A. lucorum* nymphs and adults, the dosage mortality equations for the eight insecticides tested by the leaf-dip method for 48 h responses, are summarised in table 2. From all the compounds for the 4th-instar nymphs, fipronil, with an  $\text{LC}_{50}$  of 0.153  $\text{mg} \cdot \text{l}^{-1}$ , was the most toxic insecticide to *A. lucorum*, and was significantly different in its toxicity from all other compounds tested. Bifenthrin was the second most toxic insecticide to nymphs, with an  $\text{LC}_{50}$  of 6.304  $\text{mg} \cdot \text{l}^{-1}$ . Methomyl and imidacloprid had  $\text{LC}_{50}$  values of 695.854 and 895.416  $\text{mg} \cdot \text{l}^{-1}$ , respectively, and were the least toxic and they were not significantly different from each other. For *A. lucorum* adults, the ranking of the

**Table 1.** Lethal dosage (LD) ( $\text{mg} \cdot \text{m}^{-2}$ ) data for the contact toxicity of technical insecticides to *Apolygus lucorum* 4th-instar nymphs or adults as determined by the glass-vial bioassay

Insecticides	4th-instar nymphs				Adults (Sexes combined)					
	$N^a$	$\chi^2$ (df)	Slope $\pm$ SE	LD <sub>50</sub> <sup>b</sup> (95% CL)	RT <sup>c</sup>	$N$	$\chi^2$ (df)	Slope $\pm$ SE	LD <sub>50</sub> (95% CL)	RT
Endosulfan	180	2.93 (5)	3.128 $\pm$ 0.305	0.607 (0.541–0.679) c	0.010	245	2.45 (7)	2.811 $\pm$ 0.635	0.462 (0.287–1.174) cd	0.035
Bifenthrin	242	5.08 (7)	3.787 $\pm$ 0.283	0.125 (0.112–0.136) a	0.002	210	4.16 (6)	2.734 $\pm$ 0.532	0.132 (0.102–0.174) b	0.010
Chlorpyrifos	238	4.94 (7)	2.731 $\pm$ 0.105	0.251 (0.219–0.312) b*	0.004	210	1.27 (6)	2.581 $\pm$ 0.382	0.817 (0.586–1.249) d	0.061
Malathion	210	4.37 (6)	3.538 $\pm$ 0.068	0.725 (0.704–0.736) d	0.012	185	2.41 (5)	2.882 $\pm$ 0.527	0.652 (0.431–1.246) d	0.049
Carbosulfan	210	3.97 (6)	1.929 $\pm$ 0.107	1.372 (1.119–1.695) e	0.024	210	4.24 (6)	2.035 $\pm$ 0.413	1.365 (1.132–1.591) de	0.103
Methomyl	155	2.71 (4)	2.551 $\pm$ 0.117	0.622 (0.585–0.653) c*	0.011	182	3.74 (5)	2.668 $\pm$ 1.475	0.196 (0.071–0.313) bc	0.014
Fipronil	210	3.12 (6)	2.735 $\pm$ 0.104	0.107 (0.091–0.133) a*	0.002	150	3.43 (4)	2.834 $\pm$ 0.355	0.013 (0.009–0.021) a	0.001
Imidacloprid	215	3.17 (6)	2.207 $\pm$ 0.172	58.011 (45.193–70.835) f*	1.000	240	3.38 (7)	2.295 $\pm$ 0.024	13.211 (12.981–13.455) f	1.000

<sup>a</sup>total number of tested individuals at 5 to 9 concentrations<sup>b</sup>within columns, data followed by a common letter indicate no significant difference based upon the presence of overlap in the 95% confidence limits (CL)<sup>c</sup>relative toxicity (RT) was determined by comparing the LD<sub>50</sub> value for each insecticide with the LD<sub>50</sub> value for imidacloprid, which was the least toxic agent to *A. lucorum*\*significant difference ( $p < 0.05$ ) between two different stages (adults and nymphs) for the same insecticide**Table 2.** Lethal concentration (LC) ( $\text{mg} \cdot \text{l}^{-1}$ ) data for the oral and residual toxicity of technical insecticides to *Apolygus lucorum* 4th-instar nymphs or adults as determined by the leaf-dip method

Insecticides	4th-instar nymphs				Adults (Sexes combined)					
	$N^a$	$\chi^2$ (df)	Slope $\pm$ SE	LC <sub>50</sub> <sup>b</sup> (95% CL)	RT <sup>c</sup>	$N$	$\chi^2$ (df)	Slope $\pm$ SE	LC <sub>50</sub> (95% CL)	RT
Endosulfan	183	2.84 (5)	2.582 $\pm$ 0.316	83.378 (69.871–99.505) d*	0.093	180	2.97 (5)	2.245 $\pm$ 0.197	15.698 (13.335–18.472) b	0.044
Bifenthrin	211	2.15 (6)	1.402 $\pm$ 0.135	6.304 (4.457–8.915) b*	0.007	240	2.01 (7)	2.045 $\pm$ 0.087	34.041 (27.835–41.649) c	0.096
Chlorpyrifos	240	4.94 (7)	2.578 $\pm$ 0.034	52.852 (52.081–53.623) c*	0.059	210	2.13 (6)	2.081 $\pm$ 0.195	23.597 (13.915–40.043) bc	0.066
Malathion	210	3.57 (6)	2.273 $\pm$ 0.036	118.175 (116.147–120.242) e*	0.132	215	4.18 (6)	3.823 $\pm$ 0.191	198.933 (188.216–210.277) d	0.560
Carbosulfan	238	3.74 (7)	1.363 $\pm$ 0.127	140.764 (111.562–177.606) e	0.157	240	4.19 (7)	1.021 $\pm$ 0.116	131.714 (96.741–179.307) e	0.371
Methomyl	240	3.17 (6)	2.735 $\pm$ 0.224	695.854 (627.767–771.341) f*	0.777	240	3.74 (7)	2.955 $\pm$ 0.194	300.038 (231.076–389.572) f	0.845
Fipronil	210	3.19 (6)	2.655 $\pm$ 0.282	0.153 (0.121–0.195) a	0.0002	210	2.15 (6)	2.832 $\pm$ 0.623	0.195 (0.134–0.281) a	0.001
Imidacloprid	210	2.24 (6)	2.869 $\pm$ 0.447	895.416 (736.261–1143.069) f*	1.000	209	3.63 (6)	2.341 $\pm$ 0.269	355.295 (299.374–421.638) f	1.000

<sup>a</sup>total number of tested individuals at 5 to 9 concentrations<sup>b</sup>within columns, data followed by a common letter indicate no significant difference based upon the presence of overlap in the 95% confidence limits (CL)<sup>c</sup>relative toxicity (RT) was determined by comparing the LC<sub>50</sub> value for each insecticide with the LC<sub>50</sub> value for imidacloprid, which was the least toxic agent to *A. lucorum*\*significant difference ( $p < 0.05$ ) between two different stages of *A. lucorum* (adults and nymphs) for the same insecticide

insecticides from most toxic to least toxic was: fipronil, endosulfan, chlorpyrifos, bifenthrin, carbosulfan, malathion, methomyl, and imidacloprid. For fipronil, the  $LC_{50}$  value of  $0.195 \text{ mg} \cdot \text{l}^{-1}$  indicated that it was the most toxic insecticide to the adult tested insects. Conversely, methomyl and imidacloprid with  $LC_{50}$  values of 300.038 and 355.295  $\text{mg} \cdot \text{l}^{-1}$ , respectively, were the least toxic to the adults of *A. lucorum*. The results of bioassays also showed that the oral and residual toxicities of fipronil were the highest amongst the insecticides tested and that fipronil was the most toxic compound for nymphs as well as adults.

### Comparison of insecticide toxicity in males and females

Our results showed that the  $LD_{50}$  values for endosulfan, bifenthrin, chlorpyrifos, carbosulfan, methomyl, fipronil, and imidacloprid were almost always numerically higher for female adults compared to male adults. There were no significant differences, however, between insecticides based on the overlapping confidence limits of  $LD_{50}$  values. The relative toxicity ratio (*RTR*) values ranged from a low of 1.094- to 1.587-fold, which can be explained by the size difference between males and females. However, the organophosphate insecticide malathion was 1.706-fold more toxic to the male *A. lucorum* than to the female *A. lucorum*. The  $LD_{50}$  value for this organophosphate was sig-

nificantly higher for females ( $LD_{50} = 0.716 \text{ mg} \cdot \text{m}^{-2}$ ) compared to males ( $LD_{50} = 0.419 \text{ mg} \cdot \text{m}^{-2}$ ) (Table 3).

### Selective toxicity between predatory arthropods and *Apolygus lucorum*

The toxicity of endosulfan was lowest for *C. sinica* and *P. japonica* adults, with  $LD_{50}$  values of 2.482 and 2.181  $\text{mg} \cdot \text{m}^{-2}$ , respectively. Endosulfan, however, was relatively toxic to the target pests and was safe to two predators according to the selective toxicity ratio (*STR*) values, which were 5.396- and 4.749-fold, respectively. Thus, endosulfan had the highest selectivity to both *C. sinica* and *P. japonica*. Two predators were sensitive to fipronil. This insecticide exhibited high levels of insecticidal activity against *A. lucorum*, with  $LD_{50}$  values of only 0.002 and 0.055  $\text{mg} \cdot \text{m}^{-2}$ . Fipronil had a higher selectivity for *P. japonica* (*STR* = 5.018-fold), while fipronil had a low selectivity for *C. sinica* (*STR* = 0.167-fold). For another insecticide, bifenthrin, the *STR* values for *P. japonica* and *C. sinica* were 4.220-fold and 0.540-fold, respectively, indicating that bifenthrin was also selective for *P. japonica*. Among the insecticides tested, the *STR* values for imidacloprid were the lowest, specifically, 0.018-fold for *C. sinica* and 0.021-fold for *P. japonica*. To summarise, based on the *STR* values for the eight chemicals, endosulfan was relatively safe for *C. sinica* and *P. japonica*, which are common predators in Bt cotton fields (Table 4).

**Table 3.** Lethal concentration (*LD*) ( $\text{mg} \cdot \text{m}^{-2}$ ) data for the contact toxicity of technical insecticides to female and male *Apolygus lucorum* as determined by the glass-vial bioassay

Insecticides	Gender	N <sup>a</sup>	$\chi^2$ (df)	Slope $\pm$ SE	$LD_{50}$ <sup>b</sup> (95% CL)	<i>RTR</i> <sup>c</sup>
Endosulfan	female	240	1.92 (7)	2.802 $\pm$ 0.236	0.110 (0.096–0.126) a	1.103
	male	245	2.45 (7)	2.459 $\pm$ 0.112	0.099 (0.088–0.113) a	
Bifenthrin	female	210	2.69 (6)	1.991 $\pm$ 0.095	0.041 (0.037–0.044) a	1.196
	male	210	3.11 (6)	2.168 $\pm$ 0.253	0.034 (0.027–0.041) a	
Chlorpyrifos	female	210	2.68 (6)	2.011 $\pm$ 0.255	0.369 (0.303–0.452) a	1.186
	male	211	3.17 (6)	2.207 $\pm$ 0.052	0.312 (0.294–0.332) a	
Malathion	female	180	2.91 (5)	2.932 $\pm$ 0.098	0.716 (0.663–0.775) a	1.706
	male	185	2.76 (5)	3.524 $\pm$ 0.141	0.419 (0.401–0.438) b	
Carbosulfan	female	210	4.17 (6)	2.451 $\pm$ 0.586	0.753 (0.650–0.874) a	1.094
	male	215	3.03 (6)	2.135 $\pm$ 0.288	0.687 (0.634–0.745) a	
Methomyl	female	210	2.24 (6)	2.158 $\pm$ 0.045	0.061 (0.058–0.065) a	1.263
	male	180	2.79 (5)	1.944 $\pm$ 0.092	0.049 (0.046–0.053) a	
Fipronil	female	215	3.97 (6)	2.454 $\pm$ 0.822	0.019 (0.016–0.024) a	1.199
	male	240	3.72 (7)	2.367 $\pm$ 0.142	0.016 (0.014–0.017) a	
Imidacloprid	female	210	2.31 (6)	2.165 $\pm$ 0.248	45.581 (28.545–65.897) a	1.587
	male	215	2.14 (6)	2.624 $\pm$ 0.075	28.738 (19.570–42.202) a	

<sup>a</sup>total number of tested individuals at 5 to 9 concentrations

<sup>b</sup>within columns, data followed by a common letter indicate no significant difference based upon the presence of overlap in the 95% confidence limits (CL)

<sup>c</sup>relative toxicity ratio (*RTR*) determined by comparing the  $LD_{50}$  value for each insecticide in females with the  $LD_{50}$  value in males

**Table 4.** Lethal concentration (LD) ( $\text{mg} \cdot \text{m}^{-2}$ ) data for the contact toxicity of technical insecticides to *Chrysopa sinica*/*Propylaea japonica* as determined by the glass-vial bioassay

Predators	Insecticides	N <sup>a</sup>	$\chi^2$ (df)	Slope $\pm$ SE	LD <sub>50</sub> <sup>b</sup> (95% CL)	LD <sub>50</sub> of <i>A. lucorum</i>	STR <sup>c</sup>
<i>C. sinica</i>	endosulfan	180	1.87 (5)	2.762 $\pm$ 0.294	2.482 (1.903–3.395) f*	0.462 (0.287–1.174)	5.396
	bifenthrin	180	1.62 (5)	2.774 $\pm$ 0.631	0.071 (0.049–0.099) b*	0.132 (0.102–0.174)	0.540
	chlorpyrifos	180	1.95 (5)	2.858 $\pm$ 0.514	0.055 (0.042–0.075) b*	0.817 (0.586–1.249)	0.068
	malathion	182	1.69 (5)	2.746 $\pm$ 0.496	0.107 (0.087–0.135) c*	0.652 (0.431–1.246)	0.165
	carbosulfan	178	2.73 (5)	3.614 $\pm$ 0.667	0.667 (0.443–1.219) e	1.365 (1.132–1.591)	0.489
	methomyl	180	1.87 (5)	2.937 $\pm$ 0.523	0.065 (0.054–0.079) b	0.196 (0.071–0.313)	0.343
	fipronil	180	1.71 (5)	2.895 $\pm$ 0.623	0.002 (0.002–0.005) a*	0.013 (0.009–0.021)	0.167
	imidacloprid	180	1.57 (5)	2.982 $\pm$ 0.553	0.243 (0.182–0.368) d*	13.211(12.981–13.455)	0.018
<i>P. japonica</i>	endosulfan	184	2.83 (5)	2.257 $\pm$ 0.626	2.181 (0.662–7.225) e	0.462 (0.287–1.174)	4.749
	bifenthrin	180	2.64 (5)	2.449 $\pm$ 0.375	0.553 (0.406–0.842) d*	0.132 (0.102–0.174)	4.220
	chlorpyrifos	182	2.35 (5)	2.551 $\pm$ 0.387	0.467 (0.352–0.665) d	0.817 (0.586–1.249)	0.570
	malathion	180	1.83 (5)	2.677 $\pm$ 0.429	0.585 (0.429–0.912) d	0.652 (0.431–1.246)	0.898
	carbosulfan	180	1.72 (5)	2.618 $\pm$ 0.562	0.228 (0.174–0.311) bc*	1.365 (1.132–1.591)	0.162
	methomyl	180	3.46 (5)	1.771 $\pm$ 0.366	0.169 (0.128–0.215) b	0.196 (0.071–0.313)	0.819
	fipronil	180	2.41 (5)	2.585 $\pm$ 0.550	0.055 (0.035–0.077) a*	0.013 (0.009–0.021)	5.018
	imidacloprid	178	2.98 (5)	2.021 $\pm$ 0.345	0.273 (0.217–0.382) c*	13.211(12.981–13.455)	0.021

<sup>a</sup>total number of tested individuals at 5 to 9 concentrations

<sup>b</sup>within columns, data followed by a common letter indicate no significant difference based upon the presence of overlap in the 95% confidence limits (CL)

<sup>c</sup>selective toxicity ratio (STR) estimated by comparing the LD<sub>50</sub> value for each insecticide in the predator (*C. sinica* or *P. japonica*) with the LD<sub>50</sub> value in *A. lucorum*

\*significant difference ( $p < 0.05$ ) between the LD<sub>50</sub> value in the predator and the LD<sub>50</sub> value in *A. lucorum* for the same insecticide

## Discussion

Chemical control relying on insecticides with various modes of action is thought to be a potentially effective measure against *A. lucorum* (Liu *et al.* 2008; Tan *et al.* 2012). The key data of our study showed that there were significant differences between the LD<sub>50</sub> or LC<sub>50</sub> values for the insecticides tested against *A. lucorum* within each insecticidal class (Tables 1, 2). Many reports have confirmed that fipronil exhibits high toxicity against highly damaging agricultural pests, including cotton plant bugs (Zhang *et al.* 2009; Zhao and Salgado 2010; Ma *et al.* 2012). This conclusion, in relation to *A. lucorum*, is exemplified by the results demonstrating that fipronil had the highest acute oral and contact toxicity to 4th-instar nymphs and adults. It is noteworthy that since the mid-1990s, fipronil has been recommended for the management of another harmful mirid species of cotton in the U.S., *Lygus lineolaris* (Palisot de Beauvois), and has significantly enhanced the effectiveness of the management of this harmful mirid species (Snodgrass *et al.* 2008). Neonicotinoids have become an important chemical group for the control of plant-sucking insects (e.g. aphids, thrips, and whiteflies), and act as competitive inhibitors of nicotinic acetylcholine

receptors in the central nervous system (Wang *et al.* 2007; Shi *et al.* 2011). Chinese farmers have almost exclusively relied on applying imidacloprid or acetamiprid rotated with other conventional insecticides to control *A. lucorum* in cotton fields, orchards, and tea-planted fields in North China. However, there is no clear reason for doing so, because our results show that imidacloprid is the least toxic agent to *A. lucorum* out of all of the insecticides tested in this study. We speculate that the most likely reason for the low toxicity of imidacloprid is that its toxicity is temperature dependent (Ma *et al.* 2012). At a low tested temperature (the tested temperature in this study was 25°C), the toxicity of imidacloprid to *A. lucorum* was surprisingly much lower than it was at a high temperature (e.g. 35°C), almost resulting in a loss of efficacy. Hence, during the application of imidacloprid for controlling *A. lucorum*, the influence of temperature on the toxicity of this chemical should be taken into consideration.

The conventional insecticides, namely, organochlorines, pyrethroids, organophosphates, and carbamates, which are most commonly used for the low-cost control of cotton pests, had a certain degree of toxicity to *A. lucorum*. These conventional insecticides, including endo-

sulfan, bifenthrin, and chlorpyrifos etc. had been recommended previously and have effectively controlled the other mirid species *Latrodectus hesperus* Knight and *L. linearis* for years (Snodgrass and Scott 2000; Udayagiri *et al.* 2000). There are few data available on the differences in the susceptibility of *A. lucorum* to conventional insecticides, which have also been observed in earlier reports by Zhang *et al.* (2009) and Guo *et al.* (2010). Unfortunately, conventional insecticides are likely to be more damaging to natural enemies, particularly in fields where survival from the sprays is low (Easterbrook 1997). Additionally, extensive and intensive use of insecticides will likely have adverse effects on human health and the environment and may result in the development of insecticide resistance in abundant target pest populations (Węgorok *et al.* 2011). Therefore, in these cases, the use of insecticides should be restricted as much as possible by developing new formulation technologies, new pesticide types, and new application methods for pesticides, together with improved pest monitoring, established treatment thresholds, and improved non-chemical control measures.

Our results (Tables 1, 2) suggest that various stages of *A. lucorum* were relatively susceptible to fipronil, bifenthrin, and endosulfan. This information will be useful for *A. lucorum* control. It seems plausible that the greater toxicity of these insecticides was due to their different sites of action in the nervous system of the targeted insect pest. Fipronil and endosulfan are well-known GABA-gated chloride channel-blocking insecticides, while the target site of bifenthrin is the voltage-sensitive sodium channel (Barčić *et al.* 2006; Zhao and Salgado 2010; Shi *et al.* 2012). In addition, aside from its direct (lethal) effect on *A. lucorum*, studies have demonstrated that exposure to lethal concentrations of pesticides have long-term, adverse effects not only on the individual behavior (e.g. survival, fecundity, and egg hatching) of *A. lucorum* but also on its population dynamics (Liu *et al.* 2008; Tan *et al.* 2012). All of the above information helps assess the value of these insecticides as a control tool for *A. lucorum*. The information also provides a basis for exercising rational chemical control in Bt cotton fields in China.

In comparison with female *A. lucorum*, males were sensitive to all of the insecticides tested, and there were differences in their sensitivity to different insecticides (Table 3). Previous studies with different insecticides also showed differential susceptibility between the sexes of tested insects (López *et al.* 2008; Arthur 2012). It was suggested by Zhang (1988) that females were less sensitive to organophosphates and carbamates than males owing to the lower quantity of acetylcholinesterase in females. We did not find any evidence to suggest that the mechanisms of toxicity of these selected insecticides involved the inhibition of acetylcholinesterase. Acetylcholinesterase is an enzyme that plays a major role in nerve conduction in insects. Thus, the differential sensitivity between the sexes may stem from differences in the physiological state of insects, as measured by body weight (Tan *et al.* 2012). In all the cases, the female *A. lucorum* weighed significantly more compared to the males (Men *et al.* 2008). A similar result was demonstrated by López *et al.* (2008). They indicated that the higher female  $LC_{50}$  values for insecticides in

the cotton fleahopper (*Pseudatomoscelis seriatus*) were likely a result of the higher body weights of females. Thus, possible physiological mechanisms responsible for the phenomenon remain unknown and require further work. Based on our findings, however, it is noteworthy that the tolerance of females to insecticides may also help magnify the risk of insecticide resistance evolving in *A. lucorum* as a result of the females' survival, following the spraying of insecticide in fields.

Our studies focused on the lethality of insecticides to *C. sinica* and *P. japonica* adults. We have provided important information for fully understanding the relative safety of the tested chemicals to two non-target arthropods in cotton. There were no previous data on this subject available in the literature. In reality, an insecticide's utility increases if it controls *A. lucorum* while having a minimal impact on beneficial organisms. In our experiment with two predators, the *STR* values were highest for endosulfan, indicating that endosulfan was safe to *C. sinica* and *P. japonica*. Zhu *et al.* (1998) have previously concluded that endosulfan is also a desirable insecticide for controlling resistant cotton aphids, based on the *STR* values for endosulfan in *P. japonica*. Interestingly, fipronil showed the highest toxicity to the two predators, but it was selective for *P. japonica* only. Moreover, a similar phenomenon was also observed in the bifenthrin treatment. Our results confirm the findings of earlier studies which indicated that fipronil had a mild effect on generalist predators (e.g. ladybird beetles, chrysopids, and spiders) when fipronil was used under field conditions (Kumar *et al.* 2012; Seagraves and Lundgren 2012). Conversely, imidacloprid had the lowest *STR* values and was found to be the least selective of the tested compounds with regard to the two generalist predators. The conclusion that the predators are highly susceptible to neonicotinoids, particularly imidacloprid, is also based on observations from other studies (Al-Kherb 2011; Kumar *et al.* 2012; Seagraves and Lundgren 2012). The above information is disappointing for cotton growers given that neonicotinoids are efficacious against some key piercing-sucking pests of Bt cotton (Tan *et al.* 2012). An appreciation of the lethal effects of insecticides on two important beneficial arthropods could help in the design of IPM programs with an emphasis on biological control for *A. lucorum* (Desneux *et al.* 2007). Meanwhile, to assess the total effect of toxicants on the natural enemies of *A. lucorum*, a long-term study is needed to evaluate the potential sublethal effects.

Laboratory bioassays are a convenient and useful way for screening a range of insecticides to determine which insecticides should be applied for the chemical control of *A. lucorum*. The accuracy and usefulness of the bioassays will depend on the correlation of bioassay results with methods that more closely approximate the effects of insecticides used under field conditions (Fitzgerald 2004; Zhang *et al.* 2009). To sum up, the development of insecticide toxicity curves in our study provides a useful basis for selecting appropriate insecticides to implement scientific chemical control of *A. lucorum* and minimise the adverse impacts on beneficial arthropods, human health, and the natural environment. Of the insecticides tested in the present study, endosulfan (in reasonable doses), with

its reduced impact on beneficial arthropods, showed the greatest promise for the control of *A. lucorum*, followed by fipronil and bifenthrin. Although our bioassays were conducted under relatively controlled conditions, we believe that our results provided valuable insights regarding likely trends. Further work addressing more precise impacts of insecticides on *A. lucorum* and non-targeted predators, as well as examining rational pest-control strategies, such as insecticide rotations and mixtures, would strengthen our understanding of the IPM of this pest.

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