

# Vitality and Stability of Insecticide Resistance in Adult *Propylaea japonica* (Coleoptera: Coccinellidae)

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## Abstract

*Propylaea japonica* (Thunberg) was a dominant species among the predacious ladybirds in the fields and active from March to November during a year in Fuzhou, China. Stability of insecticide resistance and vitality in adult *P. japonica* were investigated. The field ladybird *P. japonica* in Fuzhou, China, showed 9- to 16-fold resistance ratios to chlorpyrifos, 13- to 2,083-fold to methamidophos, 32- to 230-fold to fenvalerate, and 4- to 49-fold to avermectins, respectively, based on the field monitoring during 2004, 2009, and 2012, as compared with insecticide-susceptible  $F_{39}$  progenies. The resistance levels in the field *P. japonica* were high during May and November but low during summer. The population growth tendency index in field *P. japonica* was 0.8-fold as high as that in insecticide-susceptible *P. japonica*. The field *P. japonica* also showed high tolerance to the insecticide as compared with pest *Lipaphis erysimi* Kaltendach and two parasitoids *Diaeretiella rapae* and *Pachyneuron aphidis*. Stable insecticide resistance levels and high vitality were found first in adult *P. japonica* with 1-, 30-, or 60-d-old adults, or among the adults developed from the eggs produced by newly emerged adults or by 60-d-old adults. Increased activity of glutathione *S*-transferases, carboxylesterases, and cytochrome P450 monooxygenases might be involved in the resistance of *P. japonica*. The results indicated that, in certain areas, inclusion of *P. japonica* for pest control in the integrated pest management would be highly recommended.

**Key words:** *Propylaea japonica*, vitality, stability, insecticide resistance

Due to the indiscriminate use of insecticides in vegetable fields, the pest problem becomes exacerbated. Therefore, reports on the efforts to search for alternative control measures are increasing. The rotational use of insecticides, biological control, integration of chemical and biological control systems, as well as biorational pesticides were been evaluated for pest management programs (Koss et al. 2005, Galvan et al. 2006). Some Coccinellidae are important natural enemies of pest species, especially whitefly, aphids, mealybugs, scales, and mites. For over a century, predacious coccinellids have been widely used in biological control mainly by three forms, i.e., importation, augmentation, and conservation (Obrycki and Kring 1998). The toxicity and selectivity of insecticides toward pest insects and their natural enemies (predators and parasitoids) are critical to integrated pest management (IPM). The susceptibility to insecticides, including organophosphates (OPs), pyrethroids, cabamates, and other different classes of commercial insecticides, was reported in predacious ladybirds (Coleoptera: Coccinellidae), for instance in *Adalia bipunctata* L. (Olszak 1999, Jalali et al. 2009, Jansen et al. 2010, Garzón et al. 2015), *Episyrrhus balteatus* (De Geer), *Coccinella septempunctata*

L., *Propylea quatuordecimpunctata* L., and *Chrysoperla carnea* (Stephens) (Yu et al. 2014, Jansen 2015); *Micraspis discolor* (Fabricius) (Hasan et al. 2001), *Coleomegilla maculata* De Geer (Duan et al. 2002), *Stethorus punctum picipes* (Casey), and *Harmonia axyridis* (Pallas) (James 2003, Youn et al. 2003, Galvan et al. 2006); *Adonia variegata* Goeze (Aldoghairi et al. 2004); and *Serangium japonicum* (Li et al. 2015). The studies on selective toxicity of insecticides toward aphids (or other pest insects) and ladybirds were also studied (Sechser et al. 2002, Xue and Li 2004, Lucas et al. 2004, Cole et al. 2010). In addition, the effects of insecticides on the biological characteristics in predacious ladybirds were also reported (Kalushkov 1999, Olszak 1999).

In general, the populations of the natural enemy are suppressed as a result of indiscriminate insecticide applications. Previous studies and our results showed that insecticide tolerance was less common in natural enemies than arthropod herbivores, and in parasitoid wasps than predacious insects and mites. Several species of predators (e.g., ladybirds, mites, and spiders) and parasitoid wasps could survive a certain insecticides (Croft 1977, Croft and Strickler 1983, Georgioui and Taylor 1986, Wu and Miyata 2005a, b).

Only a few reports referred to the study on the evolution and physiological mechanisms of insecticide resistance in Coleoptera pest insects. There were no significant insecticide resistance to three OPs and three pyrethroids in the field-collected populations of maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae), but moderate (10- to 100-fold) to very high levels (>1,000-fold) of resistance to pyrethroids in the DDT- and pyrethroid-resistant population of *S. zeamais* were found (Ribeiro et al. 2003). The resistance levels to OPs malathion and chlorpyrifos-methyl were low (resistant ratios = 1.04–4.35) in stored grain pests *Oryzaephilus mercator* (Coleoptera: Silvanidae) and *Oryzaephilus surinamensis*. Cholinesterases seem to be involved in this resistance (Silva and Lapenta 2011). Glutathione S-transferases (GSTs) (> 2-fold increase) might be involved in pyrethroid resistance of *S. zeamais* (Fragoso et al. 2003). Resistance ratios for the insecticides methyl parathion, telfuthrin, carbofuran, terbufos, and chlorpyrifos were 28.0, 9.3, 8.7, 2.6, and 1.3, respectively, in western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Esterases and GSTs might be involved in the resistance mechanisms to the insecticides in *D. virgifera virgifera* (Wright et al. 2000) and in *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Kostaropoulos et al. 2001), respectively. Although the insecticide resistance in Coleoptera pest insects was reported, few study on the resistance levels to insecticide, in particular on the resistance dynamic changes among years and seasons, could be found in ladybirds in the literatures.

In our previous study, significant seasonal resistant changes to OPs, pyrethroids, and cabamates, high in May and November, but low in September, were found in crucifer vegetable pest *Plutella xylostella* (Lepidoptera: Noctuidae) (Wu and Jiang 2004, Wu et al. 2004) and *Phyllotreta striolata* (Coleoptera: Chrysomelidae) (Zhou and Wu 2004). The occurrence of major pests and insecticides used may influence the resistance levels of natural enemies to insecticides. Among predatory species, it has been shown that the natural enemies could also develop resistance to insecticides corresponding to that in the pest insects (Georghiou 1986, Pathan et al. 2010, Sayyed et al. 2010, Alperkumral et al. 2011, Rodrigues et al. 2013, Torres et al. 2015). The resistance to parathion-methyl and bifenthrin in *Stethorus gilvifrons* (Coleoptera: Coccinellidae) (Alperkumral et al. 2011) and to pyrethroid in *Eriopis connexa* (Torres et al. 2015) was reported. Elevated detoxification and insensitivity target were thought to be involved in resistance to insecticides in spider mites (van Leeuwen and Tirry 2007). The comparative studies in terms of detoxification enzymes and insensitivity were very limited for predaceous coccinellids (Alperkumral et al. 2011). Resistance-related genes were studied by gene expression profile analysis of responses to insecticide in *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) (Zhang et al. 2012) and *Propylaea japonica* (Thunberg) (Tang et al. 2014) based on transcriptome and digital gene expression analysis. Multiple biochemical resistance mechanisms, including elevated carboxylesterases (CarE) and GST activity and target-site insensitivity (insensitive acetylcholinesterase [AChE]) were involved in the resistance mechanisms in *S. gilvifrons* (Alperkumral et al. 2011). As compared with susceptible strain, insecticide-resistant *E. connexa* showed significantly higher survival rate (Torres et al. 2015). To study the role of detoxification enzyme in insecticide resistance, the synergistic effects of enzyme inhibitor, including piperonyl butoxide (PBO), diethyl maleate (DEM), or triphenyl phosphate (TPP), on chemical insecticides were studied in *P. japonica* or *A. bipunctata* (Jansen et al. 2010). In our previous study, by using enzyme inhibitors PBO, DEM, TPP, S,S,S-tributyl phosphorothioate (DEF), three detoxification enzymes, CarE, GST, and cytochrome P450 monooxygenase (P450), were confirmed to be involved in the toxicological

defense mechanisms in some predaceous coccinellids, *P. japonica*, *Verania discolor*, *Coccinella repanda*, *Chilomenes quadriplagiata*, *C. septempunctata*, and *Listronotus maculicollis*. However, only the field-collected resistant populations of the ladybirds were used in the researches. The insecticide-susceptible strain was lack (Wu and Miyata 2005a, b, Wu et al. 2007, Kang et al. 2008). Because the insecticide-susceptible strain was lack, the studies forward on insecticide resistance in predaceous ladybirds were very limited. Although resistance-related gene expression were studied (Tang et al. 2014), the study on the developments and dynamics of insecticide resistance could not be found in *P. japonica*.

Chemical insecticides were main control measure in the field of Shangjie, Minhou County (Sj), Fujian, China. No any natural enemy insect as a biological control agent was used in the field by the farmers in Sj. During spring and autumn when the climate is suitable for the development of insects, heavy insecticide spray happened in the fields. Integration of predators and insecticides for pest control in crop ecosystems has been focused on IPM, but due to incompatibilities, it has rarely been achieved (Tabashnik and Johnson 1999, Wright and Verkerk 2010). Potential utility as an aphid predator should be those exhibiting resistance to insecticides in the fields (Torres 2012). *P. japonica* was one of the most important predatory enemies against pest insects, such as aphids, whitefly species, scale insects, and small caterpillars in China (Tang et al. 2014), and a prevalent mobile predator of aphids in wheat, cotton, and maize, and moves among these crops in agricultural systems (Ouyang et al. 2015). *P. japonica* was active from March to November during a year in the commercial fields of Fuzhou, China, as a dominant species among the predaceous ladybirds in the fields according to our field observation. Five predator coccinellid species (*P. japonica*, *V. discolor*, *C. repanda*, *C. quadriplagiata*, and *C. septempunctata*) showed high AChE insensitivity (similar to herbivorous pest insects, but far lower than parasitoids). In addition, *P. japonica* and *V. discolor* displayed similar or higher tolerance to methamidophos, fenvalerate, and avermectin, as compared with herbivorous pest insects, and far higher tolerance to insecticides as compared with the other three ladybirds in Sj (Wu and Miyata 2005a, b, Wu et al. 2007). The AChE sensitivities to paraoxon, malaoxon, mathamidophos, and chlorpyrifos in insecticide-susceptible *P. japonica* were far higher than those in insecticide-resistant *P. japonica*, and the mutations of amino acid residuals in the *ace1* of resistant *P. japonica* might be involved in the resistance to the four OPs (Wang et al. 2018). It was speculated that high tolerance to insecticides and dominant species under insecticide pressure in *P. japonica* might be related to both insecticide resistance and high vitality. However, feeding, conservation, and application were focused on mainly in predaceous ladybird. It was needed to study the vitality of the ladybirds in the potential utility as an aphid predator to evaluate the *P. japonica*'s applications in IPM. In present study, the field monitoring and stability of insecticide resistance and vitality of *P. japonica* were studied in this study.

## Materials and Methods

### Sources of Insects

A field population of *P. japonica* was collected from the commercial crucifer fields of Shangjie, Fujian, China (Sj) (34°48' N, 113°18' E), 20 km away from Fujian Agriculture and Forestry University, Fuzhou, Fujian, China (FAFU), in October 2009. OPs, pyrethroids, and avermectins were heavily sprayed in controlling the pest insects in the field in Sj. No specific permissions were required for our collection of *P. japonica*, because the scientists were welcome to collect

the insect sample from the farmer's crucifer fields in order to control the pest insects. The field studies did not involve endangered or protected species. The field-collected *P. japonica* were subsequently reared on aphids, *L. erysimi* at  $25 \pm 1^\circ\text{C}$  in an PRX-205B environmental chamber (Ninbo Saifu Experimental Instrument Co., Ltd., China). The aphids were reared on seedlings of *Raphanus sativus* L. in a separate  $25^\circ\text{C}$  environmental chamber at  $25^\circ\text{C}$  and were used as food of *P. japonica*. The development time of a *P. japonica* from egg, larva, pupa to newly emerged adult was approximately 20–25 d. For this study, the field *P. japonica* collected in November 2009 was designated as  $F_0$  parents and reared starting in November 2009.  $F_{39}$  progenies of the field *P. japonica* were used as a related-susceptible (S) population. In addition to  $F_0$  parents and  $F_{20}$  progenies, other field *P. japonica* populations, collected from the same fields in 2004 and 2012, were also used as the R population. The density of ladybirds on commercial crucifer was low due to prior insecticide application. But, many ladybirds could be collected from the weeds outside of the fields.

To compare the susceptibilities to insecticide field *P. japonica* and aphid, other predatory ladybirds and parasitoids, *L. erysimi*, predatory ladybirds *C. quadriplagiata* and *M. discolor*, *D. rapae* (parasitoid of *L. erysimi*), and hyperparasitoid wasps *Pa. aphidis* (parasitoid of *D. rapae* pupae) were collected from crucifer fields of Sj in October 2004 together with the field *P. japonica*. The field-collected apterous adults of *L. erysimi* and parasitized *L. erysimi* were reared on *Brassica oleracea* in an environment chamber at  $25 \pm 1^\circ\text{C}$  for a photoperiod of 16:8 (L:D) h before they were used in experiments.  $F_1$  progenies of apterous adult of aphid and  $F_0$  progenies of newly emerged adults of parasitoids were used for bioassays. The field-collected *C. quadriplagiata* and *M. discolor* were fed on aphids for 48 h in an environment chamber at  $25^\circ\text{C}$  at a photoperiod of 16:8 (L:D) h before the beetles were used for bioassay.

## Chemicals

Two different techniques were used to evaluate susceptibilities of insects to the insecticides assayed. The chemicals used in dry film method were as follows: chlorpyrifos (96% pure) and methamidophos (90% pure) from Shangming Insecticide Co., Ltd., Fujian, China; fenvalerate (technical grade, 96% pure) from Sumitomo Chemical Co., Ltd., Osaka, Japan; and avermectin (technical grade, 95.7% pure) from North China Pharmaceutical Group Corporation Aino Co., Ltd., Hebei, China. The following chemicals were used in leaf-dipping method: methamidophos (50% EC) from Shangming Insecticide Co., Ltd.; fenvalerate (20% EC) from Saronda Insecticide Co., Ltd., Hubei, China; and avermectin (1.8% EC) from Shijiazhuang Insecticide Co., Ltd., Hebei, China. The other chemicals used in enzyme assays were of analytical grade, and obtained from Sigma Chemical Co., Ltd. Methamidophos has been banned in China in vegetable field. However, this insecticide had been used in vegetable fields in past. We used it as one of the standard insecticides in this experiment.

## Experimental Population Life Tables in S and R *P. japonica*

$F_{39}$  and a field-collected *P. japonica* were used as S and R *P. japonica*, respectively. The experiments were conducted in an environmental chamber at  $25 \pm 1^\circ\text{C}$  with a photoperiod of 16:8 (L:D) h. Life table tests were conducted according to Liu et al. (2008). Fifty eggs ( $F_0$  parents) were collected from S and R populations of *P. japonica*, respectively. After the first instar larvae (neonate) were hatched, the neonate were put in a big glass vial (20 ml) and reared

on *L. erysimi* in an environmental chamber at  $25 \pm 1^\circ\text{C}$  with a photoperiod of 16:8 (L:D) h, one neonate per vial. As soon as the adults emerged, one pair of male and female adults *P. japonica* (that emerged on the same day) was selected randomly and introduced into a big glass vial (20 ml), which was confined by fine mesh nylon net. The pair of adults was fed on *L. erysimi* in the big glass vial for egg laying in an environmental chamber. The eggs produced by the pair of R or S adults in a big vial were calculated every day until the adults died. The pair of male and female *P. japonica* that did not produce the eggs was considered copulation failure. Total six pairs of adults for R or S were reared, respectively. In total, 54–70 neonates (first instar larvae,  $F_1$  progenies) were randomly picked with three replicates and reared continually on *L. erysimi* at  $25^\circ\text{C}$ . The life table parameters, as shown in Table 3, were recorded until the neonates of the next generation ( $F_2$  progenies) were hatched. Population growth tendency index (*I*) value = neonate number in present generation/neonate number in last generation. Relative fitness of R *P. japonica* was calculated by *I* value in R *P. japonica* divided by *I* value in S *P. japonica*, whereas relative fitness of S *P. japonica* was defined as 1.

## Bioassays of *P. japonica*

### Bioassay

Dry film method was used (Wu et al. 2007). An aliquot of 2.0 ml acetone solution of insecticide was poured into a glass vial (1.2 cm in diameter, 10-cm long) and capped with a rubber plug. The solution in the vial was swirled for 10 s, and then, the excess solution was poured off and the vial placed upside down on a wire rack. The vial was air-dried for 4–5 h to leave a dry coating on the inner surface of the vial to be used for the bioassay. Control vials were treated with only acetone in the same manner. Adult insects that were reared in environmental chamber were introduced into the vial and left in contact with the insecticide for 48 h at  $25^\circ\text{C}$ . Insect mortality was recorded 48 h after treatment. Three concentration replicates were performed with 10 ladybird individuals per replication. Each  $\text{LC}_{50}$  was calculated based on five concentration levels, and their corresponding mortalities with 150 adult ladybirds. Insects that did not respond to pencil tip prodding were judged to be dead.

### Field resistance monitoring

The field-collected adult *P. japonica* were fed on aphids for 48 h under insecticide-free condition in an environment chamber at  $25^\circ\text{C}$  at a photoperiod of 16:8 (L:D) h before the beetles were used for bioassay. It was not known whether or not the beetles had previous contacts with insecticide before the ladybirds were collected. Any of such occurrences might affect the response to insecticides in bioassays. However, there was no mortality in the control at 48 h during bioassay. The  $\text{LC}_{50}$  values were calculated based on the mortality rate at 48 h after the insects were treated with the insecticides. The *P. japonica* developed well during Spring and Autumn under field condition but were suppressed by heat stress during Summer because of high temperature in the field. The susceptibility to insecticide of field *P. japonica* during summer (i.e., June, July, and August) should be collected to study the effects of high temperature on the insecticide resistance. However, because the density of field *P. japonica* was very low, we collected the field *P. japonica* in 3–10 September from the fields, which were developed from the eggs produced in August in the fields, because the developmental time of *P. japonica* from egg to adult stages was about 15 d during August in the field condition, based on our observation.

### Susceptibility to insecticide among the adults with different ages

According to our observation, when the adult *P. japonica* was reared on *L. erysimi* at 25°C at a photoperiod of 16:8 (L:D) h in environmental chamber, the longevity of adult *P. japonica* could be as long as about 6 mo. The averaged adult mortality of *P. japonica* was about 20 and 70% after the adults were reared at 25°C for 2 and 5 mo, respectively. It was speculated that the susceptibility to insecticides might be varied with the adult's age because physiological statuses of the adults might be varied with their age during 1–60 d. It needed to study if the susceptibility of adults to insecticides would be affected adult's age. In addition, we found that the duration of egg laying in some of adults could be as long as 5 mo at 25°C. It needed to study the differences between the two type adults that were developed from two types of eggs, i.e., from the eggs produced by newly emerged adults or from the eggs produced by 60-d-old adults. Therefore, two kinds of experiments (experiments A and B) were studied. A field population of adult *P. japonica* (F<sub>0</sub> adults) collected from the commercial field in Sj in October 2012 was reared on *L. erysimi* at 25°C in environmental chamber, and F<sub>1</sub> adults were used for bioassays.

In experiment A, the newly emerged (within 24 h) F<sub>1</sub> adults of *P. japonica* were reared at 25°C in environmental chamber for producing F<sub>2</sub> eggs, and F<sub>2</sub> adults were obtained. After F<sub>2</sub> adults reared at 25°C for 1, 30, or 60 d, respectively, the 1-, 30-, or 60-d-old adults were used for bioassays, respectively. In experiment A, the adults (with 1-, 30-, or 60-d-old) were developed from the eggs, which were produced by newly emerged adults.

In experiment B, the 60-d-old F<sub>1</sub> adults were obtained to be allowed to produce F<sub>2</sub> eggs. The F<sub>2</sub> eggs were reared at 25°C in environmental chamber until adults were obtained, and the 1-d-old F<sub>2</sub> adults were used for bioassays. In experiment B, the adults (with 1-d-old) were developed from the eggs, which were produced by 60-d-old adults. In the experiments, the effects of different age on the *P. japonica*'s susceptibility to insecticides were studied.

### Bioassays of Other Ladybirds, Parasitoids, and *L. erysimi*

To compare the susceptibility with the insecticides among ladybirds, parasitoids, and aphid, the bioassays of other ladybirds, parasitoids, and *L. erysimi* were carried out. In the bioassays for the adults of *D. rapae*, *Pa. aphidis*, *C. quadriplagiata*, and *M. discolor*, the dry film method was used, which was same as that used for *P. japonica* (Wu et al. 2007). Mortality was recorded at 24 h for *D. rapae* and *Pa. aphidis* or 48h for *C. quadriplagiata* and *M. discolor* after treatment. In the bioassays for apterous adults of *L. erysimi*, the leaf-dipping method was used (Wu and Miyata 2005a, b). The insecticide solutions were prepared by using a 0.02 % Triton X-100 water solution. *B. oleracea* leaves (about 5 × 5 cm) were dipped for 10 s in an insecticide solution and left to air dry at 25°C. The treated leaves were then introduced into a plastic cup (200 ml), and 20 apterous adults placed on each leaf. For the control, cabbage leaves were dipped in distilled water containing the spreading agent only. Mortality was recorded at 24 h after treatment. Three concentration replicates were performed with 10 (for ladybirds), 15 (for parasitoids), or 20 (for aphids) individuals per replication. Each LC<sub>50</sub> was calculated based on five concentration levels, and their corresponding mortalities with 150 (for ladybirds), 225 (for parasitoids), or 300 (for aphids) adult insects. Insects that did not respond to pencil tip prodding were judged to be dead.

### Assays of Detoxification Enzymes

Whole body of adult *P. japonica* was homogenized at 4°C in a 66 mM, pH 7.5 sodium phosphate buffer with 0.2% (V/V) of Triton X-100. The mixture was centrifuged at 10,000 g at 4°C for 15 min.

Supernatants were used to assay activity of AChE, CarE, and GSTs. The assays of CarE, GST, and AChE were conducted by the method of Wu and Miyata (2005a, b). AChE activity was determined with acetylthiocholine iodide (ATCh) iodide as a substrate in the presence of (5,5'-dithiobis[2-nitrobenzoic acid] [DTNB]) in a 0.066 M phosphate buffer, pH 7.8, at 25°C. Optical density was measured at 412 nm. The reaction mixture (2.0 ml) consisted of 0.6 mM ATCh, 0.4 mM DTNB, and 0.05 ml aliquot of the enzyme solution. CarE activity toward  $\alpha$ -NA was measured in a 0.04 M phosphate buffer, pH 7.0 at 37°C, by measuring the optical density at 600 nm. The amounts of hydrolysate were calculated by using standard curve prepared with  $\alpha$ -naphthol. The reaction mixture (2.5 ml) contained  $3 \times 10^{-4}$  M  $\alpha$ -NA,  $4 \times 10^{-4}$  M serine, and a 0.025 ml aliquot of the enzyme solution. GST assay determined with 2,4-dinitrochlorobenzene (CDNB) as a substrate in a 0.066 M phosphate buffer, pH 7.4 at 25°C. Absorbance changes were recorded at 340 nm for 1 min. The reaction mixture (2.0 ml) consisted of 5.0 mM GSH, 0.5 mM CDNB, and 0.05 ml aliquot of the enzyme solution. Absorbance was converted subsequently to nanomoles by using the extinction coefficient of  $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$  for calculating the specific activity. In P450 production assay, the buffer used for the preparation of P450 was 0.1 M sodium phosphate buffer, pH 7.5, and contained 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM DL-dithiothreitol (DTT), 1 mM phenylmethanesulfonyl fluoride, 1 mM phenylthiourea (PTU), and 10% glycerol (v/v). Entire adults of *P. japonica* were homogenized at 4°C, and centrifuged at 10,000 g at 4°C for 15 min. Supernatants were filtered through glass wool. Filtrates were then centrifuged at 20,000 g at 4°C for 30 min. The precipitation (contained microsomal pellets) obtained from the 20,000 g were resuspended in a resuspension medium [0.1 M sodium phosphate buffer (pH 7.8)] contained 1 mM EDTA, 0.1 mM DTT, 1 mM PMSF, 1 mM PTU, and 10% glycerol and diluted to a final protein concentration of 3–5 mg/liter. The above procedures were done at 0–4°C. About 10-mg sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) was added into 2-ml aliquots of the resuspension for the reduction of both sample and reference. The content of the sample cell was then saturated with CO (CO was carefully bubbled through the sample). The difference spectrum was measured from 400 to 500 nm. P450 production was quantified with an extinction coefficient of  $91 \text{ mM}^{-1} \text{ cm}^{-1}$  based on the difference in absorbance at 450 and 490 nm (Omura and Sato 1964). The enzymatic activity was determined on 756MC Ultraviolet visible spectrophotometer (Shanghai Optical Instrument Co., Ltd., China). The enzyme assay was replicated at least three biological replicates with 10 insect individuals in each replication.

### Statistical Analysis

The insecticide bioassay, enzyme profile, and life table data were analyzed by probit analysis (Finney 1971) using a DPS data processing system (Tang and Feng 1997).

## Results

### Resistance Levels to Four Insecticides

Field populations of *P. japonica* developed significant resistance to chlorpyrifos, methamidophos, fenvalerate, and avermectin according to field monitoring during 2004, 2009, and 2012. As compared with LC<sub>50</sub> values of F<sub>39</sub> progenies, the resistance ratios were 8.9- to 37.2-fold for chlorpyrifos, 13.0- to 517-fold for methamidophos, 31.9- to 230-fold for fenvalerate, and 4.32- to 47.8-fold for avermectin. Significantly, seasonal changes of resistance levels to the four insecticides were found in the field populations of *P. japonica*, based on the field monitoring of insecticide resistance during 2004 and 2012. Resistance levels to the four insecticides were low during September

in field *P. japonica* populations but high during May and November. The resistance levels in September were about half of those in May and November for the four insecticides, even more, about 10% of those in May and November for methamidophos or avermectin in 2004 (Table 1). After the field *P. japonica* was reared in laboratory under insecticide-free condition for three generations, the resistance to chlorpyrifos, fenvalerate, and avermectin in  $F_4$  progenies declined significantly (half of those in  $F_0$  parents; Table 1). The resistance to the four insecticides declined greatly in  $F_{20}$  and  $F_{39}$ . The  $LC_{50}$  values to chlorpyrifos, methamidophos, fenvalerate, or avermectin were 6.7, 24, 4.9, or 2.7% in  $F_{20}$ , and 2.7, 2.2, 0.5, or 2.1% in  $F_{39}$  as high as those in  $F_0$  (Table 1). The resistance levels to methamidophos, fenvalerate, and avermectin in field *L. erysimi* collected from Sj during 2004 also displayed a significantly seasonal variation just like the situations found in field *P. japonica*.

If the susceptibility to the three insecticides were compared among three ladybirds, *L. erysimi* and two parasitoids that were collected from Sj during same time (October 2004), as compared

with the insecticide's toxicity to *L. erysimi* (Table 2), methamidophos, fenvalerate, and avermectin were found to be less toxic to *P. japonica* (Table 1); methamidophos and fenvalerate to *M. discolor* (Table 2), and avermectin to *C. quadriplagiata* (Table 2). In particular, *P. japonica* and *M. discolor* showed very low susceptibilities to methamidophos and fenvalerate than *L. erysimi*. The parasitoids *D. rapae* and hyperparasitoid *Pa. aphidis* displayed very high susceptibilities to the three insecticides (Table 2).

### Comparisons on the Life Table Parameters Between R and S *P. japonica*

Among the life table parameters in R and S *P. japonica* at 25°C, although the survival rate during the developmental stages from neonate to pupae, female rate, and fecundity number per female in  $F_{39}$  *P. japonica* were higher than those in the field-collected population of *P. japonica*, there were no significant difference in the life table parameters between  $F_{39}$  and field *P. japonica* (*t*-test,  $P \leq 0.05$ ). However,  $F_{39}$  *P. japonica* displayed higher relative biological fitness (1.25-fold) than

**Table 1.** The susceptibility to insecticides in insecticide-resistant and -susceptible *P. japonica*

Insecticides	Collection date <sup>a</sup>	$LC_{50}$ (95% CL) (mg/liter) (48 h) <sup>b</sup>	Slope $\pm$ SD <sup>b</sup>	Ratios <sup>c</sup>	
Chlorpyrifos	$F_0$	124 (103–147)	2.57 $\pm$ 0.25	37.2	
	$F_4$	60.8 (45.7–80.9)	2.45 $\pm$ 0.37	18.3	
	$F_{20}$	8.25 (2.79–24.54)	3.28 $\pm$ 1.23	2.5	
	$F_{39}$	3.33 (1.49–7.42)	0.76 $\pm$ 0.27	1	
	May 12	54.3 (46.2–63.8)	2.79 $\pm$ 0.23	16.3	
	Sep. 12	29.6 (20.5–42.6)	7.15 $\pm$ 1.36	8.9	
	Oct. 12	50.1 (46.5–62.9)	1.53 $\pm$ 0.12	15.1	
Methamidophos	$F_0$	105 (67.0–166)	4.82 $\pm$ 1.00	45.6	
	$F_4$	89.9 (58.6–138)	5.12 $\pm$ 1.07	39.1	
	$F_{20}$	25.3 (22.9–28.1)	2.33 $\pm$ 0.12	11	
	$F_{39}$	2.30 (1.91–2.27)	2.30 $\pm$ 0.22	1	
	May 04	1170 (873–1570)	1.76 $\pm$ 0.21	509	
	Sep. 04	104 (78.5–137)	1.91 $\pm$ 0.25	45.2	
	Oct. 04	1189 (857–1648)	1.56 $\pm$ 0.20	517	
	Nov. 04	4790 (3620–6350)	1.89 $\pm$ 0.24	2083	
	May 12	93.8 (51.9–170)	4.60 $\pm$ 1.33	313	
	Sep. 12	30.0 (23.4–38.5)	7.49 $\pm$ 0.97	13.0	
Fenvalerate	$F_0$	65.6 (35.8–120)	4.64 $\pm$ 1.04	28.8	
	$F_4$	421 (274–646)	4.84 $\pm$ 0.95	188	
	$F_4$	231 (201–266)	3.00 $\pm$ 0.22	103	
	$F_{20}$	20.4 (12.3–33.9)	5.32 $\pm$ 1.45	9.1	
	$F_{39}$	2.24 (1.45–3.45)	1.25 $\pm$ 0.28	1	
	May 04	514 (390–677)	1.94 $\pm$ 0.26	230	
	Sep. 04	286 (227–359)	2.43 $\pm$ 0.31	128	
	Oct. 04	380 (249–580)	1.35 $\pm$ 0.18	158	
	May 12	264 (214–325)	2.53 $\pm$ 0.27	118	
	Sep. 12	71.5(42.2–121)	4.58 $\pm$ 1.09	31.9	
	Oct. 12	131 (109–158)	2.02 $\pm$ 0.23	58.5	
	Avermectin	$F_0$	87.5 (55.0–139)	5.07 $\pm$ 1.16	46.8
		$F_4$	32.6 (24.5–43.3)	7.37 $\pm$ 1.09	17.4
$F_{20}$		2.38 (1.63–3.48)	6.62 $\pm$ 1.20	1.3	
$F_{39}$		1.87 (1.16–3.00)	1.05 $\pm$ 0.18	1	
May 04		91.2 (70.0–119)	2.03 $\pm$ 0.34	48.8	
Sep. 04		8.08 (6.16–10.6)	2.33 $\pm$ 0.31	4.32	
Oct. 04		88.6 (66.7–118)	1.86 $\pm$ 0.25	47.4	
May 12		89.3 (58.2–137)	5.17 $\pm$ 1.09	47.8	
Sep. 12		33.2 (19.4–56.9)	4.46 $\pm$ 0.58	17.8	
Oct. 12		70.0 (58.9–83.2)	2.27 $\pm$ 0.33	37.4	

<sup>a</sup> $F_0$  were collected from the commercial fields in 2009. In field monitoring, the field adult *P. japonica* were collected from the commercial fields in 2004 and 2012, respectively.

<sup>b</sup>The values of  $\chi^2$  (df = 3) were ~1.81 to 7.50, and <7.82, which indicated that a significant fit between the observed and expected regression lines ( $P \leq 0.05$ ).

<sup>c</sup>Resistance ratio =  $LC_{50}$  in other populations /  $LC_{50}$  in  $F_{39}$ .

field *P. japonica*, because population growth tendency index (*I*) in  $F_{39}$  *P. japonica* were 1.26-folds as high as those in field *P. japonica* (Table 3).

### Susceptibility to Insecticides in R *Propylaea japonica* With Different Ages

Although the  $LC_{50}$  values to chlorpyrifos, fenvalerate, or avermectin in the 60-d-old adults were slightly higher than those in the 1- or 30-d-old adults, no significant differences in the  $LC_{50}$  values to the

three insecticides were found among the adults that were developed from the eggs produced by  $F_0$  parents at the same time, but of different day old (1-, 30- or 60-d-old, respectively), based on the overlap of the 95%CL values of  $LC_{50}$  (Table 2). On the other hand, based on the overlap of the 95%CL values of  $LC_{50}$ , there were no significant differences in susceptibility to chlorpyrifos, fenvalerate, or avermectin between the 1-d-old adults that were developed from the eggs which were produced by the  $F_0$  parents 60 d after they were collected

**Table 2.** The susceptibility to insecticides in the field populations of *L. erysimi* collected from Sj in 2004

Insecticides	Collection date	$LC_{50}$ (95% CL) mg/liter) (24 or 48 h) <sup>a</sup>	Slope ± SD
<i>Lipaphis erysimi</i>			
Methamidophos	May 04	551 (426–714)	2.18 ± 0.23
	Sep. 04	196 (142–263)	2.37 ± 0.33
	Oct. 04	527 (409–678)	1.94 ± 0.27
	Nov. 04	820 (616–1091)	1.93 ± 0.26
Fenvalerate	May 04	532 (336–845)	1.43 ± 0.13
	Sep. 04	172 (132–219)	3.12 ± 0.28
	Oct. 04	345 (246–486)	1.54 ± 0.23
	Nov. 04	546 (319–762)	1.60 ± 0.20
Avermectin	May 04	4.91 (3.76–6.57)	2.39 ± 0.26
	Sep. 04	0.80 (0.61–1.04)	3.80 ± 0.50
	Oct. 04	1.03 (0.73–1.45)	2.05 ± 0.32
	Nov. 04	5.29 (3.72–7.54)	1.41 ± 0.15
<i>Chilomenes Quadriplagiata</i>			
Methamidophos	Oct. 04	9.94 (7.15–13.8)	1.52 ± 0.21
Fenvalerate	Oct. 04	57.3 (41.33–79.4)	1.91 ± 0.30
Avermectin	Oct. 04	19.7 (14.9–26.1)	1.24 ± 0.17
<i>Micraspis discolor</i>			
Methamidophos	Oct. 04	9,617 (7,556–12,240)	2.27 ± 0.32
Fenvalerate	Oct. 04	837 (635–1103)	1.97 ± 0.30
Avermectin	Oct. 04	2.76 (1.86–4.10)	1.43 ± 0.18
<i>Diaeretiella rapae</i>			
Methamidophos	Oct. 04	1.83 (1.51–2.18)	9.27 ± 1.30
Fenvalerate	Oct. 04	53.3 (41.5–68.8)	3.84 ± 0.61
Avermectin	Oct. 04	6.10 (5.06–7.43)	5.39 ± 0.80
<i>Pachyneuron Aphidis</i>			
Methamidophos	Oct. 04	0.67 (0.48–0.89)	6.18 ± 0.82
Fenvalerate	Oct. 04	38.1 (31.0–45.7)	5.28 ± 0.68
Avermectin	Oct. 04	5.34 (4.57–6.17)	5.06 ± 0.48

<sup>a</sup>The *L. erysimi* were bioassay by leaf-dipping method. The ladybirds and parasitoids were bioassay by dry film method. Mortality was recorded 24 h (for *L. erysimi*, *D. rapae*, and *Pa. aphidis*) or 48 h (for *C. quadriplagiata* and *M. discolor*), and  $LC_{50}$  values were calculated. The values of  $\chi^2$  (df = 3) were <7.82 (=0.62–5.86), which indicated a significant fit between the observed and expected regression lines ( $P \leq 0.05$ ).

**Table 3.** Experimental population life tables on resistant and susceptible strains of *P. japonica*

Biological characteristics	Field population <sup>a</sup>	$F_{39}$
Neonate number	54 (3) <sup>c</sup>	70(3)
Survival rate from neonate to second larvae (%)	93.8 ± 10.8a	97.9 ± 3.61a
Survival rate from second to third larvae (%)	97.9 ± 3.61a	97.9 ± 3.61a
Survival rate from third to fourth larvae (%)	97.8 ± 3.85a	100 ± 0.00a
Survival rate from fourth larvae to pupae (%)	97.9 ± 3.61a	100 ± 0.00a
Pupation rate (%)	97.8 ± 3.85a	95.8 ± 7.22a
Emergence rate (%)	100 ± 0.00a	100 ± 0.00a
Female rate (%)	48.9 ± 5.57a	52.3 ± 1.92a
Fecundity number/female	483 ± 81.6a	531 ± 77.8a
Next generation larvae	10,956	17,845
Population growth tendency index ( <i>I</i> )	203	255
Relative fitness	0.80	1.00

<sup>a</sup>The field *P. japonica* were collected from the commercial field in Oct. 2012, and the insect's susceptibility was showed in Table 1.

<sup>b</sup>Different letters indicate significant differences between field population collected in Oct. 2012 and  $F_{39}$  progenies (*t*-test,  $P \leq 0.05$ ).

<sup>c</sup>Number in parentheses indicate the number of replicates.

from the fields and the 1-d-old adults that were developed from the eggs which produced by the  $F_0$  parents immediately after they were collected from the fields (Table 4).

### Comparisons on the Resistance-Related Enzymes Activity

Corresponding to the declines of resistance levels to the three insecticides in  $F_{20}$  or  $F_{39}$  progenies of *P. japonica* after  $F_0$  parents (a field-collected population) were reared in laboratory under insecticide-free condition for 20 or 39 generation (Table 1), the GSTs and CarE activities and CYP450 content in  $F_{20}$  or  $F_{39}$  were significantly lower than those in two field populations of *P. japonica*. The enzyme activity in  $F_0$  and field *P. japonica* were about 3.8- to 5.7-fold for CarE activity, 1.6- to 2.4-fold for GSTs activity and 2.5- to 3.1-fold for P450 as high as those in insecticide-susceptible  $F_{39}$  generations (Table 5).

### Discussion

The field *P. japonica* in Sj, Fuzhou, China, had developed multiple-resistance to the four insecticides. In our previous resistance monitoring on *L. erysimi* and *D. rapae* which were collected from same vegetable production districts of Sj in October 2004, resistance ratios (RRs) of *L. erysimi* was far higher than those of *D. rapae*. The field population of *L. erysimi*'s displayed 57-, 42-, 93-, and 36-fold RRs to methamidophos, chlorpyrifos, fenvalerate, and avermectin, respectively (Chen and Wu 2005), whereas parasitoid *D. rapae* displayed 32-, 18-, and 39-fold RRs to methamidophos, fenvalerate, and avermectin, respectively (Wu and Miyata 2005a, b). However, the RRs to methamidophos, fenvalerate, and avermectin in the field

*P. japonica* collected from same vegetable production districts in October 2004 were 517-, 158-, and 47-folds, respectively (Table 1). The RRs values in *P. japonica* were significantly higher than those in herbivores *L. erysimi* and the parasitoid wasps. The results in this study provided a evidence that ladybirds (*P. japonica* in Table 1 and *M. discolor* in Table 2) as well as herbivores *L. erysimi* (Table 1) could develop their high resistance levels to insecticides just like herbivores pest (*L. erysimi* in Table 2) and displayed far high tolerance to methamidophos, fenvalerate, and avermectin than parasitoid wasps (*D. rapae* and *Pa. aphidis* in Table 2). The results indicated that, in certain areas, predatory ladybirds could sometimes be more tolerant to some insecticides than pest *L. erysimi*. The results were consistent with the results reported previously (Croft 1977, Croft and Strickler 1983, Georghiou and Taylor 1986).

High resistance levels were found in the field *P. japonica* in 2004 and 2012. However, the resistance to the four insecticides was not stable after the insects were reared under insecticide-free conditions for long time. After *P. japonica* were reared under insecticide-free condition for 20 or 39 generations, the resistance levels to the four insecticides declined greatly (Table 1). If the field *P. japonica* were reared under insecticide-free conditions for four generations, the resistance levels did not decline so heavily in  $F_4$  progenies, i.e., the resistance levels were stable from  $F_0$  to  $F_4$ . The  $LC_{50}$  values to chlorpyrifos, fenvalerate, or avermectin in  $F_4$  were about half of that in  $F_0$ , and there was no significant changes in  $LC_{50}$  values to methamidophos between  $F_0$  and  $F_4$ . No great declines of resistance levels to the four insecticides in  $F_4$  *P. japonica* would be important for the field *P. japonica* to keep their resistance level to survival from insecticide pressure under field condition.

**Table 4.** The susceptibility to insecticides in adult of field *P. japonica* with different age

Insecticides	Age of adults (d)	$LC_{50}$ (95% CL) (mg/liter) (48 h)	Slope $\pm$ SD	Ratios <sup>c</sup>
Chlorpyrifos	1 <sup>a</sup>	50.1 (46.5–62.9)	1.53 $\pm$ 0.12	15.1
	30 <sup>a</sup>	59.4 (52.6–67.0)	2.84 $\pm$ 0.18	17.8
	60 <sup>a</sup>	39.0 (23.7–64.2)	4.77 $\pm$ 1.11	11.7
	1 <sup>b</sup>	43.0 (26.1–70.6)	4.93 $\pm$ 1.19	12.9
Fenvalerate	1 <sup>a</sup>	131 (109–158)	2.02 $\pm$ 0.23	58.5
	30 <sup>a</sup>	126 (107–148)	2.52 $\pm$ 0.21	56.3
	60 <sup>a</sup>	79.8 (43.0–148)	4.54 $\pm$ 1.33	35.6
	1 <sup>b</sup>	152 (129–178)	2.52 $\pm$ 0.21	67.9
Avermectin	1 <sup>a</sup>	70.0 (58.9–83.2)	2.27 $\pm$ 0.33	37.4
	30 <sup>a</sup>	66.4 (38.7–114)	4.46 $\pm$ 1.05	35.5
	60 <sup>a</sup>	46.0 (35.8–59.0)	2.63 $\pm$ 0.33	24.6
	1 <sup>b</sup>	47.6 (29.1–77.9)	5.12 $\pm$ 1.27	25.5

<sup>a</sup>The adults of *P. japonica* were developed from the eggs produced by newly emerged adults, and the 1-, 30-, or 60-d-old adults were used for bioassays, respectively.

<sup>b</sup>The adults of *P. japonica* were developed from the eggs produced by 60-d-old adults, and the 1-d-old adults were used for bioassays.

<sup>c</sup>Ratio =  $LC_{50}$  in different populations tested in Table 2/ $LC_{50}$  in  $F_{39}$  in Table 1.

**Table 5.** Comparisons on the resistance-related enzymes between resistant strains and susceptible strains of *P. japonica*

Enzyme activity <sup>a</sup>	$F_0$	Ratios	Field population <sup>b</sup>	Ratios	$F_{20}$	Ratios	$F_{39}$
AChE	—	—	1.71 $\pm$ 0.13a	1.0	—	—	1.67 $\pm$ 0.22a
CarE	276 $\pm$ 9.11a	5.7	182 $\pm$ 7.47c	3.8	208 $\pm$ 6.39b	4.3	48.2 $\pm$ 6.42d
GSTs	66.7 $\pm$ 2.09a	2.4	43.1 $\pm$ 2.37b	1.6	36.9 $\pm$ 11.6b	1.4	27.4 $\pm$ 3.60c
P450	0.22 $\pm$ 0.049a	3.1	0.184 $\pm$ 0.0080a	2.5	0.072 $\pm$ 0.045b	1.0	0.074 $\pm$ 0.0027b

<sup>a</sup>The units of enzyme activity were nmol/min/mg pro for AChE, CarE, and GSTs. The unit of P450 content was nmol/mg pro. Different letters after the mean  $\pm$  SD values indicate significant difference among  $F_0$ ,  $F_{20}$ , field population, and  $F_{39}$  (Dacunt test,  $P < 0.05$ ).

<sup>b</sup>The field population were collected from the commercial field in Oct. 2012.

Because the susceptibility to the three insecticides in field *L. erysimi* was determined by leaf-dipping method (Table 2), the control doses in the fields could be implicated by the  $LC_{50}$  values. During October 2004, the  $LC_{50}$  values to methamidophos, fenvalerate, and avermectin in field *P. japonica* (Table 1) were far higher than those in field *L. erysimi* (Table 2). The  $LC_{50}$  values in field *P. japonica* were obtained based on 48 h mortality by using dry film method, whereas the  $LC_{50}$  values in field *L. erysimi* were obtained based on 24 h mortality by using leaf-dipping method. Although different bioassays were used in *P. japonica* and *L. erysimi*, our results implicated that the field *P. japonica* had low susceptibility to the three insecticides as compared with the field *L. erysimi*. In addition, *P. japonica* displayed far lower susceptibility to the three insecticide as compared with two parasitoids *D. rapae* and *Pa. aphidis* and one ladybird *C. quadriplagiata* (Tables 1 and 2). On the other hand, the susceptibility to the insecticides of  $F_{39}$  *P. japonica* was far higher than those of the field populations of *L. erysimi* and *C. quadriplagiata*, and similar to those in two parasitoids *D. rapae* and *Pa. aphidis*. The facts implicated that the field *P. japonica* had developed high resistance levels to the insecticides because of insecticide application.

A seasonal change of resistance level to insecticides, high in May and November, but low in September, was found in the field *P. japonica* population (Table 1) and *L. erysimi* (Table 2) based on the field monitoring in 2004 and 2012. Similar phenomenon was found in *Pl. xylostella* (Wu and Jiang 2002, Wu et al. 2004), *Ph. striolata* (Zhou and Wu 2004), and *D. rapae* (parasitoid of aphids *L. erysimi*; Wu and Miyata 2005a, b). The fitness costs caused by insecticide resistance had been verified in many insects (Bourguet et al. 2004, Berticat et al. 2008, Zhang et al. 2015a, b). The majority studies in this field were conducted at the temperature, which is conducive to the survival and reproduction of insects. The results in this study indicated that the biological fitness of insecticide-susceptible *P. japonica* at 25°C (a suitable temperature for the survival and reproduction of *P. japonica*) were higher than that of the resistant field *P. japonica* (Table 3). The comparisons on the biological fitness of insecticide-resistant and -susceptible *P. japonica* under heat stress condition were not conducted in this study. In our previous report, significant biological and physiological fitness cost existed in insecticide-resistant *Pl. xylostella* under heat stress (Zhang et al. 2015a, b). It suggested that the increase of insecticide resistance might induce biological fitness cost in the *R. Pl. xylostella*. The sharp declines of insecticide resistance during summer might be associated with the significantly lower fitness under heat stress (Zhang et al. 2015a, b). It was speculated that sharp declines of resistance level to insecticides might be related to the lower biological fitness in insecticide-resistant *P. japonica*. The related research under heat stress should be carried out in our future study. It had some guiding for natural enemies utilization and pest control. It might be important to adopt the control strategy in IPM according to the seasonal change of resistance insecticide of the field *P. japonica*.

On the other hand, although lower fitness cost was found in insecticide-resistant *P. japonica* (0.8 as high as that in susceptible *P. japonica*), high vitality in the tolerance to insecticides and stability of insecticide resistance were found among the *R. P. japonica* with different ages (Table 4). According to our observation in laboratory, the longevity of adult *P. japonica* could be as long as 6 mo at 25°C, and the duration of egg laying of adults could last for 5 mo when the adults were fed on aphids. Long durations of the adult's longevity and egg laying would be benefit for *P. japonica* to control pea aphids in the field. The results in this study indicated that the resistance levels in the field *P. japonica* did not vary whatever the adult's age were 1-, 30-, or 60-d old, or whatever the adults were developed from

the eggs, which were produced by 1- or 60-d-old adults (Table 4). The facts, i.e., high and stable resistance levels upon the adult's age or physiological statue, indicated that the adult *P. japonica* showed high vitality and would be benefit for *P. japonica* to survival from the control doses of the insecticides in the field.

The P450, GSTs, and esterase were best known detoxification enzymes involved in the metabolic resistance mainly occurs due to an increase in the expression or activity of the three major enzyme families. P450 (Scott and Wen 2001, Ramoutar et al. 2009, Stevenson et al. 2012) can metabolize numerous substrates and carry out multiple oxidative reactions, and are more commonly linked to the resistance to OPs, pyrethrins and avermectins in insects than GSTs and CarE. GSTs (Enayati et al. 2005, Ramoutar et al. 2009) and CarE (Montella et al. 2012) have already been confirmed to be correlated with resistance to OPs and pyrethroids. Besides metabolic resistance, insensitive AChE played an important role in the resistance to OPs and carbamates in insects (Fournier and Muterio 1994). In our previous report (Wu and Miyata 2005a, b), seven parasitoids showed significantly higher affinity (Km values) and activity (Vmax values) of AChE and lower sensitivity ( $k_i$  values) as compared with four predatory ladybirds and six herbivorous pests. The  $k_i$  values of AChE to methamidophos, dichlorvos, and carbofuran in four predatory ladybirds were similar to those in six herbivorous pests, and about 10- to 60-fold lower than those in seven parasitoids. There existed significant correlations between the susceptibility to methamidophos and the  $k_i$  values of AChE among 18 species of insects (Wu and Miyata 2005a, b). MFO might play the most important role in the 18 species of insects, and CarE or GST might be important in the tolerance in some insect species (Wu and Miyata 2005a, b, Wu et al. 2007). In the results reported by Ramoutar et al. (2009), the synergism ratios to bifenthrin by PB, EDF, and DEM were 4.5–5.9, 0–3.2, and 2.5–3.2, respectively, in field populations of ladybird *L. maculicollis*, whereas in the field population of *P. japonica*, the synergism ratios were 10.9, 1.7, and 2.0, respectively, to methamidophos, 19.8, 8.7, and 4.2, respectively, to fenvalerate, and 13.4, 2.2, and 1.6, respectively, to avermectin. MFO played a critical role in the metabolic detoxification in *P. japonica* (Wu et al. 2007).

Usually, GSTs and CarE were thought to be associated with the resistance to OPs and pyrethroids, and P450 were linked to the resistance to OPs, pyrethroids, and avermectins as described above. It was suggested that increased activity of P450 monooxygenases, GSTs, and CarE might be involved in the resistance of the field *P. japonica* with multiple-resistance to the four insecticides, and P450 might play most important role in metabolic resistance (Table 5). In addition,  $F_{39}$  *P. japonica* displayed 75, 64, 78, and 41 times higher AChE sensitivity to paraoxon, malaoxon, chlorpyrifos, or methamidophos, respectively, than  $F_0$  *P. japonica* (Wang et al. 2018). Insensitive AChE was also involved in the resistance of *P. japonica* to the two OPs.

Because of chemical sprays, the density of ladybird population in the treated crop was low. The ladybirds for this study were collected from the weeds grown on the periphery of the crucifer fields. However, the field *P. japonica* had developed high resistance and tolerance to three different classes of insecticides, i.e., OPs, pyrethrins, and avermectins. Furthermore, adult *P. japonica* showed stable resistance levels from  $F_0$  to  $F_4$  generation. The adult *P. japonica* showed stable resistance levels whatever in 1- or in 60-d-old adults, long adult's longevity and long duration of egg laying (>5 mo upon our observation). In addition, Ladybirds were found in the weeds in S<sub>j</sub> year-round, except winter. In particular, *P. japonica* could be collected from the weeds in August, when *L. erysimi* could hardly be found in the vegetable field due to high temperature. Based on our years of observation, besides *L. erysimi*, *P. japonica* can also feed on

*Bemisia tabaci*, which develop well in August. In addition, perhaps, *P. japonica* could live on other organisms (e.g., microorganisms, animals, or plant) in addition to aphid and *B. tabaci*, as many ladybirds were found in the weeds grown on the periphery of the crucifer fields. High vitality and polyphagous will be benefit for the adaptability of *P. japonica* to survival from the insecticides in the fields. Further study on the biology and ecology of *P. japonica* appears necessary in order to find the cause(s) of *P. japonica*'s high resistance and tolerance to certain insecticides. The present study demonstrated that, in certain areas, predatory ladybirds could sometimes be high resistance to insecticides and more tolerant to particular insecticides than pest *L. erysimi*. Therefore, inclusion of ladybirds for pest control in the IPM studies would be highly recommended.

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