Seasonal changes of methamidophos susceptibility in *Plutella xylostella* and its parasitoid *Cotesia plutellae*  

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**ABSTRACT**

Methamidophos resistance was monitored in field populations of *Plutella xylostella* and *Cotesia plutellae* collected on corresponding hosts from Fujian Province, China. Methamidophos resistance levels in two insect species were high during autumn and spring and low in summer. Resistance to methamidophos was 15.3- and 12.6-fold higher in *F₀* parents of *P. xylostella* and *C. plutellae* than in their susceptible *F₁₁* progeny, respectively. The bimolecular rate constant (kᵢ) values of acetylcholinesterase (AChE) to methamidophos, dichlorvos, and carbofuran were 4.6-, 6.3-, and 7.7-fold higher in *F₁₁* progeny of *P. xylostella*, and 3.7-, 4.5-, and 3.7-fold higher in *F₁₁* progeny of *C. plutellae* than those in their *F₀* parents, respectively. Compared with susceptible *F₁₁* progeny, the resistance ratios for methamidophos were 4.2-29.8 and 3.8-13.1 in 21 field populations of *P. xylostella* and *C. plutellae*, respectively. The molecular rate constant (kᵢ) values of AChE to methamidohos, dichlorvos, and carbofuran were 2.0-21.6, 3.6-9.5-fold higher in *F₁₁* progeny of *P. xylostella*, and 1.8-7.6-, 1.9-4.6-, and 2.2-7.6-fold higher in *F₁₁* progeny of *C. plutellae* than those in 21 field populations, respectively. We found significant correlative variations of resistance as well as significant correlative variations of kᵢ values of AChE to organophosphorus insecticides between two species of insects in space and time. There were no obvious differences in *Kₐ* and *Vₘₐₙ* of AChE between *F₀* parents and *F₁₁* progeny of *P. xylostella* and *C. plutellae*, respectively. But carboxylesterase activity was 1.6-fold higher in *F₀* parents of *C. plutellae* than in *F₁₁* progeny, and glutathione S-transferase activity was 1.5-fold higher in *F₀* parents of *P. xylostella* than in *F₁₁* progeny. From these results, insensitive AChE to methamidophos was thought to be the most important resistance mechanism in *P. xylostella* and *C. plutellae*.

**INTRODUCTION**

Indiscriminate use of insecticides for a pest can eliminate its parasitoids and predators and exacerbate the pest problem. Efforts are now being made to search for alternative control measures. Biological control, along with integration of chemical and biological control systems for arthropods by the use of integrated pest management-compatible pesticides, has received more and more attention in pest management programs (Hill and Foster 2000, Villameueva-Jimenez et al. 2000). Satisfactory controls of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), were achieved using *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), a larval parasitoid of *P. xylostella*, in combination with compatible agents such as *Bacillus*
thuringiensis (Bt) toxin formulation (Chilcutt and Tabashnik 1999). It was identified that C. plutellae had the greatest control potential among the parasitoids recorded on P. xylostella (Talekar and Shelton 1993). Also, there have been studies regarding the toxicity of organophosphates, carbamates, pyrethroids, fipronil, avermectin, benzoylphenyl urea compounds and Bts against adults of C. plutellae (Mani and Krishnamoorthy 1984, Kao and Tseng 1992, Miyata et al. 2004).

Studies on insecticide resistance and its biochemical basis in natural enemies, particularly in parasitoids, remain spare. A high level of malathion resistance in Anisopteromalus calandrae (Howard) was associated with increased activity of a malathion-specific carboxylesterase (Baker et al. 1998). Malathion resistance in Habrobracon hebetor Say was related to both an increased activity of the esterase E3 and the null alleles of the esterase E1 and E2 (Perez-Mendoza et al. 2000). Information about insecticide resistance in C. plutellae is limited. Activities of microsomal monoxygenase and glutathione S-transferase (GST) were far higher in P. xylostella than those in its parasitoids C. plutellae and Diadegma semiclausum Hellen, but the activities of carboxylesterase (CarE) were similar among the parasitoids and their hosts (Chiang and Sun 1991).

= MATERIALS AND METHODS =

Sources of Insects

Field populations of C. plutellae and P. xylostella were collected from commercial cauliflower, Brassica oleracea variety italica L., in vegetable growing districts in Jianxin (Jx), Hongtan (Ht), and Xingdian (Xd) in Fuzhou, and in Shangjie (Sj) and Ganzhe (Gz) in Minhou, Fujian, China. Insecticides were heavily used in these districts, and the field populations were used as insecticide-resistant populations. Organophosphate (OP), carbamate, and pyrethroid insecticides were used six times per month in the crucifer vegetable fields in January, July, and August. High control doses were used because of high insecticide resistance in P. xylostella. OPs have been used in the fields for the past 20 years. In recent years, OPs were sometimes used in combination with other insecticides such as avermectin or fipronil to control P. xylostella. Newly collected field populations (F₀ parents) of P. xylostella and C. plutellae collected from Jx were introduced into the insectarium under field conditions in April 2001. C. plutellae was reared on P. xylostella, and P. xylostella was fed on cauliflower. The insects in the insectarium were free from any insecticides after April 2001. The insectarium was constructed with a stainless steel net and a glass roof at the Fujian Agriculture and Forestry University, Fuzhou, Fujian, which excluded external P. xylostella and C. plutellae populations. Two insectarium populations of the two species of insects, F₁ and F₁₁ progeny, were collected from the insectarium in November 2001 and April 2002, respectively. F₁₁ progeny were tentatively used as susceptible populations in this experiment.

Chemicals

Bovine serum albumin, reduced glutathione (GSH), 1-chloro-2,4-nitrobenzene (CDNB), glutathione, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), eserine, and acetylthiocholine (ACh) iodide were obtained from Sigma (St. Louis, MO). Analytical grade 1-naphthyl acetate (α-NA) was obtained from Shanghai Reagent Co., Ltd. (Shanghai, China). The other chemicals used in the experiments were of analytical grade. Methamidophos (technical grade, 90% pure) and carbofuran (technical grade, 98% pure) were gifts from Shangming Insecticide Co., Ltd. (Fujian, China). Dichlorvos (technical grade, 92.5% pure) was from Sanonda Co., Ltd. (Hubei, China).
Bioassays

Field-collected pupae of *P. xylostella* were reared in an environmental chamber at 25°C with a photoperiod of 16:8 (L:D) h, and the third instar of F1 progeny were used for the bioassay. Parasitized larvae of *P. xylostella* and cocoons of *C. plutellae* collected from the same group as the host in the field, were reared in an environmental chamber at 25°C with a photoperiod of 16:8 (L:D) hours for several days before the bioassay. Adults (F0 parents) of newly emerged *C. plutellae* were used for the bioassay.

The bioassay for *C. plutellae* was conducted by a dry film method reported by Chiang and Sun (1991). An aliquot of 0.2 ml of acetone solution of insecticide was used to coat the inner surface of a glass vial (1.5cm in diameter, 10cm in length). The glass vials were rolled to evaporate the acetone evenly. After the solvent was fully evaporated, treated vials were used for the bioassay. Control vials were treated with acetone only. Adults of *C. plutellae* were introduced into the vial and left in contact with the insecticide for 1 hour, and mortality was recorded. All treated parasitoids were then transferred to clean vials, provided with 15% honey solution for food, and mortality was recorded again after 8 hours. LC50 values at 1 and 9 hours were calculated based on the mortality at 1 hour after contact with insecticide, and 8 hours after transferring to clean vials, respectively. The definition of death was that the adults could not respond to a pencil tip prodding. No control mortality was observed during the bioassay.

A leaf-dipping technique was adopted for our bioassay of *P. xylostella* from the method of Fahmy et al. (1991). A stock solution of 5% methamidophos was prepared by dissolving it in a mixture of methyl alcohol and acetone 1:1 (vol:vol) containing 5% Tween 80. The test solution was diluted with water containing 0.02% Triton X-100 to make desired concentrations of insecticide. Cabbage leaves (5 by 5cm) were dipped for 10 s in an insecticide solution and left to air to dry at 25°C. Treated leaves were then introduced into a plastic cup (200 ml), and 15 third instars were placed on each leaf. For the control, cabbage leaves were dipped in distilled water containing the spreading agent only. At each insecticide concentration, at least three replications were made. Mortality was recorded at 48 hours after treatment. Larvae which did not respond to pencil tip prodding were judged to be dead.

Enzyme Assays

Whole bodies of fourth instar of *P. xylostella* and whole bodies of adults of *C. plutellae* were used for the biochemical assay of enzymes, except that heads of fourth instar of *P. xylostella* were used for the assay of acetylcholinesterase (AChE).

Enzyme Preparation

Insects were homogenized at 0°C in a 0.066 M sodium phosphate buffer, pH 7.8 (for AChE), pH 7.4 (for GST), and pH 7.0 (for CarE), and the homogenates were filtered through glass wool. Filtrates were centrifuged at 1 500 × g (for AChE and CarE) or 10 000 × g (for GST) at 4°C for 15 min. The buffer used in preparation of AChE contained 0.5% (vol:vol) Triton X-100. The buffer used for the preparation of GST contained 4 mM GSH. Supernatants were used as enzyme sources for measuring the activities and kinetics parameters of enzymes. Three insect samplings were made for each assay. The protein concentrations in the enzyme preparations were ≈5 mg/ml for both pests and parasitoid samples. Protein concentrations were determined by the method of Bradford (1976) by using bovine serum albumin as a standard.

AChE Assay

AChE activity was determined according to the method of Ellman et al. (1961) with ATCh iodide as a
substrate in the presence of 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 AChE, 0.4 mM DTNB, and 0.05 ml aliquot of the enzyme solution.

**CarE Assay**

CarE activity toward α-NA was measured in a 0.04 M phosphate buffer, pH 7.0, at 37°C, by measuring the optical density at 600 nm according to the method of van Asperen (1962). The reaction mixture (2.5 ml) contained $3 \times 10^{-4}$ M α-NA, $4 \times 10^{-4}$ M eserine, and a 0.025 ml aliquot of the enzyme solution.

**GST Assay**

For GST determination, the procedure of Habig (1981) was adopted with CDNB as a substrate in a 0.066 M phosphate buffer, pH 7.4, at 25°C. Absorbance changes were recorded at 30 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 mM$^{-1}$ cm$^{-1}$ for calculating the specific activity.

The enzyme assays were determined at the same concentration of substrate by using the same reaction system for *P. xylostella* and *C. plutellae*.

**Determination of Kinetic Parameters**

The apparent Michaelis-Menten constant ($K_a$) and maximal velocity ($V_{max}$) for AChE were determined by enzymatic activity measured at different substrate concentrations (from $5 \times 10^{-7}$ M to $1.0 \times 10^{-3}$ M), with values obtained using Lineweaver-Burk plots. The biomolecular rate constant ($k_i$) was estimated followed the method of Aldridge (1950). AChE solutions were incubated with the inhibitor for various times before tipping the inhibition mixture into a solution for AChE to measure residual activity. Experiments were all carried out in triplicate in the presence or absence of the inhibitor.

**Statistical Analysis**

The insecticide bioassay data were analyzed by probit analysis (Finney 1971) by using DPS data processing system (Tang and Feng 1997). The correlative coefficients between methamidophos resistance and $k_i$ values of AChE to three insecticides in both insect species also were calculated using the DPS data processing system. The significance of difference for the enzyme activities between resistant and susceptible insects was calculated by $t$-test (Tang and Feng 1997).

**RESULTS**

**Correlative Variations of Resistance to Methamidophos between *P. xylostella* and *C. plutellae***

The spatial and temporal variations of resistance to methamidophos in both *P. xylostella* and *C. plutellae* are shown in Figure 1. Significant resistance to methamidophos was found in field populations of *P. xylostella* and *C. plutellae* collected from Jx, Ht, and Xd in Fuzhou, and Sj and Gz in Minhou, Fujian, China. Resistance levels to methamidophos decreased markedly after *P. xylostella* and *C. plutellae* were reared under insecticide-free condition for 11 generations. Compared with their F$_{11}$ progeny, F$_0$ parents of *P. xylostella* displayed 15.3-fold resistance based on LC$_{50}$ value at 48 hours, and F$_0$ parents of *C. plutellae* displayed 10.2-
and 12.6-fold resistance based on LC₅₀ values at 1 and 9 hours, respectively. Compared with F₁₁ progeny of both species which originated from Jx, the field populations that were collected from Jx, Ht, Xd, Sj, and Gz, respectively, during October 1998 to December 2003, showed 4.5–29.8-fold resistance in *P. xylostella* based on LC₅₀ values at 48 hours, and 3.8–11.4- and 3.8–13.1-fold resistance in *C. plutellae* based on LC₅₀ values at 1 and 9 h, respectively. The field populations of *P. xylostella* displayed great variation in resistance to methamidophos from October 1998 to December 2003, high from autumn to spring and low in summer. *C. plutellae*, which originated from the same group of *P. xylostella*, showed the same tendency in methamidophos resistance as *P. xylostella*. There existed significant spatial and temporal correlative fluctuations of the resistance to methamidophos in both species by correlative test (*P* ≤ 0.01). Correlative coefficients were 0.921 between LC₅₀ (48 hours) of *P. xylostella* and LC₅₀ (1 hour) of *C. plutellae*, and 0.924 between LC₅₀ (48 hours) of *P. xylostella* and LC₅₀ (9 hours) of *C. plutellae*, respectively (Figure 1).

![Graph](image_url)

**Figure 1** Monitoring of resistance levels to methamidophos in *Plutella xylostella* and *Cotesia plutellae* collected from commercial fields of Fuzhou and Minhou, Fujian, China. Resistance ratio values represent LC₅₀ in F₈ parents, F₁ progeny and other field populations / LC₅₀ in F₁ progeny of *P. xylostella* and *C. plutellae*, respectively

**Correlative Variations of AChE Insensitivity in *P. xylostella* and *C. plutellae***

The *k* values of AChE increased dramatically after *P. xylostella* and *C. plutellae* were free from insecticides for 11 generations. AChE sensitivity in *P. xylostella* increased more rapidly than in *C. plutellae*. The *k* values of AChE in the parasitoid were far higher than those in its host *P. xylostella*. High AChE insensitivity from autumn to spring and low AChE insensitivity in summer were found in both field populations of *P. xylostella* and *C. plutellae*. Significant correlations (*P* ≤ 0.01) were found between
methamidophos resistance and AChE insensitivity to methamidophos, dichlorvos and carbofuran in *P. xylostella* and *C. plutellae*. The correlation coefficients were 0.929, 0.941, and 0.758 between LC₅₀ values (48 hours) to methamidophos, and *kₜ* values to dichlorvos, carbofuran, and methamidophos, respectively, in *P. xylostella*, and 0.836, 0.942, and 0.733 between LC₅₀ values (1 hour) to methamidophos, and *kₜ* values to dichlorvos, carbofuran and methamidophos, respectively, in *C. plutellae*. In addition, the correlation coefficients in *kₜ* of AChE between *P. xylostella* and *C. plutellae* were 0.918 for dichlorvos, 0.926 for carbofuran, and 0.966 for methamidophos. Significant correlations for AChE insensitivity to methamidophos, dichlorvos and carbofuran were found between *P. xylostella* and *C. plutellae* (*P* ≤ 0.01).

**Assays of AChE, CarE, and GST**

Comparisons of the kinetic parameters of AChE with the activities of CarE and GST between *F₀* parents and *F₁₁* progeny of *P. xylostella* and *C. plutellae* are summarized. There were no obvious differences in the *Kₗ* and *Vₘₘₚ* of AChE and the activity of GST between *F₀* parents and *F₁₁* progeny of *C. plutellae*, but CarE activity was 1.6-fold higher in *F₀* parents of *C. plutellae* than that in the *F₁₁* progeny. The *Kₗ* and *Vₘₘₚ* of AChE and CarE activity in *F₀* *P. xylostella* were similar to those in *F₁₁* progeny, but GST activity in *F₀* *P. xylostella* was 1.5-fold higher than that in its *F₁₁* progeny. *P. xylostella*, both in *F₀* parents and *F₁₁* progeny, showed lower *Vₘₘₚ* of AChE and CarE activity and higher *Kₗ* of AChE and GST activity compared with its parasitoid *C. plutellae*.

**DISCUSSION**

The hymenopteran parasitoid *A. calandrae* showed stable resistance to malathion after 23 generations of rearing with no selection pressure (Baker 1995). In our studies the resistance levels to methamidophos in both *C. plutellae* and *P. xylostella* collected in Jx, Fuzhou, were not stable (Figure 1). A significant reversal of the susceptibilities and AChE sensitivities (*kₜ*) in the two species of insects occurred when they were released from exposure to insecticides for 11 generations. However, there were no apparent changes in *Kₗ* and *Vₘₘₚ* of AChE between *F₀* parents and *F₁₁* progeny in both species. Significantly higher *kₜ* values for AChE in *F₁₁* progeny might indicate that insensitive AChE was found in field populations of both species of insects. In our studies, correlation of resistance to methamidophos between *P. xylostella* and *C. plutellae* was found. Correlations between resistance to methamidophos and AChE insensitivity to dichlorvos, carbofuran, and methamidophos also were found in field populations of *P. xylostella* and *C. plutellae*. Variations of AChE insensitivity played the most important role in the development of insecticide resistance in *P. xylostella* and *C. plutellae*.

Because an insect’s physiology could be affected by weather conditions, insecticide toxicity might vary due to the season when the experiment was executed. Thus, simultaneous tests to determine toxicity differences between both species to methamidophos were conducted in the field and in the insecticide-free insectarium where the same weather conditions occurred. The LC₅₀ values in the field populations of *P. xylostella* (1 260 mg/L) and *C. plutellae* (96.7 mg/L) collected from Jx in November 2001 were far higher than those in the *F₁* progeny of *P. xylostella* (216 mg/L) and *C. plutellae* (16.7 mg/L) collected from the insectarium in November 2001. The LC₅₀ values in the field populations of *P. xylostella* (1 780 mg/L) and *C. plutellae* (83.6 mg/L) collected from Jx in April 2002 were also far higher than those in *F₁₁* progeny of *P. xylostella* (106 mg/L) and *C. plutellae* (9.07 mg/L) collected from the insectarium in April 2002. Significant differences in susceptibility to methamidophos between the field and insectarium populations were found. These differences in susceptibility to methamidophos in field populations of *P. xylostella* and *C.
plutellae were as high as 16.8- and 9.2-fold, respectively, compared with \( F_{11} \) progeny. But the differences in \( LC_{50} \) values among the 21 field populations at different seasons during October 1998 to December 2003 were only from two-to four-fold higher, depending on the populations and pesticides. Thus, it is concluded that although significant seasonal variations existed, the insects’ resistance to methamidophos played a more important role. Because we could not obtain susceptible strains of \( P. xylostella \) and \( C. plutellae \), \( F_{11} \) progeny of the two species of insects were used as susceptible strains. The resistance ratio (RR) values to methamidophos in the field populations of two species of insects were moderate in this study. Higher RR values to methamidophos might have been found if the susceptible \( P. xylostella \) and \( C. plutellae \) had been reared under insecticide-free conditions for a longer time.

The seasonal changes in methamidophos resistance and AChE insensitivity in the two species of insects (high resistance in autumn and spring and low resistance in summer) might be related to the seasonal changes in their exposure to insecticides. The difference in resistance levels we found in different geographic populations or populations having different collection times (from 4.2 to 29.7-fold in \( P. xylostella \) and from 3.8 to 12.6-fold in \( C. plutellae \)) might be related to selection pressure from insecticides as well as food and climate. The environmental temperature in Fuzhou was suitable for the development of both \( P. xylostella \) and \( C. plutellae \) during spring and autumn, but it was unsuitable during summer. Higher densities of \( P. xylostella \) resulted in increased insecticide use during spring and autumn, whereas lower density populations and lower insecticide use occurred during summer in the study. In this study, RR Values declined sharply from April to July. The sharp decline in methamidophos resistance was probably related to a sharp decline in insecticide use in fields after May. Wakisaka et al. (1992) reported that the net reproductive rate and intrinsic rate of natural increase of a population of \( P. xylostella \) from central Japan maintained almost identical values between 25°C and 30.5°C, and declined considerably at 33°C. In both tropical and temperate populations of \( P. xylostella \), larvae developed well between 15°C and 30°C, but their development was severely inhibited at 32.5°C (Shirai 2000). Parasitism of \( P. xylostella \) by \( C. plutellae \) reached its highest point at 30°C and then declined significantly when the temperature was > 30°C (Talekar and Yang 1991). According to statistical data during 1960-1990 provided by Fujian Meteorological Bureau, the average monthly temperature in July in Fuzhou and Minhou was as high as 28.9°C. The daytime temperature in July could be higher than 35°C and last for a considerable time. The highest recorded temperature was 41°C. Ecological fitness of the two species of insects could be low for the development of \( P. xylostella \) and \( C. plutellae \) under the high temperature found in the field during July and August. Low ecological fitness in methamidophos-resistant strains caused by high temperature could be involved in the rapid decline of methamidophos resistance in the field populations of \( P. xylostella \) and \( C. plutellae \) during July. This will be a future subject for study.

Lenormand et al. (1999) reported that there was a correlative variation between the frequency of resistance alleles (at the \( Ace0.1 \) coding for a modified acetylcholinesterase) and insecticide treatment, in the mosquito \( Culex pipiens \) L., which accompanied the change of seasons during a year. During periods of exposure to insecticides in the summer, resistance alleles were selected for the treated area that produced the steep cline and high frequency of resistance alleles. Owing to the cost of resistance and gene flow, this frequency decreased when the treatment was interrupted, leading to shallow clines and lower frequencies of resistance alleles in winter. But there was no significant correlation between the frequency of resistance alleles (at the Ester locus for overproduction of detoxifying esterase) and insecticide treatment during a year (Lenormand et al. 1999). Results from our field monitoring confirmed that there were correlative variations between resistance to methamidophos and resistant AChE in \( C. plutellae \) and its host \( P. xylostella \) that accompanied the seasonal variations of selection pressure from insecticides during a year.

CarE might be involved in the resistance to methamidophos in \( C. plutellae \) because of high CarE activity.
in F₀ parents compared with F₁₁ progeny. Previous research showed that malathion resistance in P. xylostella was only related to malathion CarE, and not to nonspecific esterases (Doiichuangngm and Thornhill 1989). Glutathion conjugation was confirmed as a major detoxifying reaction for parathion and methyl parathion in a parathion-selected strain and a methyl parathion-selected strain of P. xylostella (Kao and Sun 1991). Our results showed that GST might be involved in the biochemical mechanism of methamidophos resistance because of high GST activity in F₀ parent P. xylostella, whereas CarE activity was low. In a P. xylostella population with multiple resistance to OPs, carbamates, and pyrethroids from a cabbage field in northern Florida in 1999 (Yu and Nguyen 1992), the activities of microsomal oxidases, GST, and reductase were obviously higher in the field strain than in the susceptible strain, but the activities of CarE and non-specific esterases were similar to those in the susceptible strain. The kᵢ of AChE (3.6 × 10⁵ M⁻¹ min⁻¹) for dichlorvos in the field strain was 2.8 times as high as that (1.0 × 10⁵ M⁻¹ min⁻¹) of the susceptible strain. The kᵢ for dichlorvos of AChE (3.6 × 10⁴ M⁻¹ min⁻¹) in the field strain in northern Florida was 11.1 times as high as that (3.24 × 10⁴ M⁻¹ min⁻¹) in the field P. xylostella (F₀ parents) in Jianxin, Fuzhou. This indicated that a very high level of insensitive AChE was present in the field P. xylostella in Jianxin, Fuzhou.

In this study, because OPs, carbamates, pyrethroids, avermectin, fipronil, and imidacloprid were heavily sprayed in the field in Jianxin, Fuzhou, it could be speculated that P. xylostella and C. plutellae developed a multiple-resistance mechanism in the field. The high GST level in P. xylostella and high level of CarE in C. plutellae might be involved in the multiple resistance mechanism because higher enzyme activities were found in F₀ parents compared with their susceptible F₁₁ progeny. In addition, other resistance mechanisms, such as microsomal monoxygenase or penetration, also might be involved. Nevertheless, in the view of the significant correlation between resistance to methamidophos and AChE insensitivities to dichlorvos, carbofuran, and methamidophos, AChE insensitivity could play the most important role in the correlative evolution of methamidophos resistance in P. xylostella and C. plutellae. In addition, the increase of the activity of detoxification enzymes might be another factor contributing to increased resistance.

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