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Toxicity of chlorantraniliprole to Cry1Ac-susceptible and resistant strains of *Helicoverpa armigera*

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ABSTRACT

Transgenic Bt cotton expressing Cry1Ac is important in controlling various agricultural pests, including *Helicoverpa armigera*. Especially for transgenic crops that are cultivated in large expanses, avoiding resistance development is a key for ensuring sustainability of Bt technologies. Integrated pest management, in which transgenic crops are strategically combined with rational pesticide use, may help to prevent *H. armigera* resistance acquisition in Bt cotton. In this study, we evaluated the toxicity of a novel insecticide (chlorantraniliprole) on Cry1Ac-susceptible and resistant individuals of *H. armigera*. More specifically, we assessed the effect of chlorantraniliprole on the activity of two enzymes and conducted laboratory bioassays to determine its toxicity on *H. armigera* larvae. Chlorantraniliprole increased esterase and glutathione-*S*-transferase activities in Cry1Ac susceptible and resistant populations of *H. armigera*. Cry1Ac resistant populations XJ-F (Cry1Ac resistance ratio 21.8-fold), XJ-10.0 (95.8-fold) and BTR (3536.5-fold) did not show cross-resistance to chlorantraniliprole, with LC_{50} values of 0.0733 (μg/mL) in XJ-F, 0.0545 (μg/ml) in XJ-10.0 and 0.0731 (μg/mL) in BTR, which were close to that in the susceptible strain 96S (0.0954 μg/mL). Our work shows that chlorantraniliprole could be considered to be integrated in Bt cotton management schemes to delay the *H. armigera* resistance development.

1. Introduction

The cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is an important pest of cotton in China. Several *H. armigera* strains prove resistant to a variety of insecticides [1,2]. At present, transgenic cotton expressing Cry1Ac provides an efficient method to control this pest, and has turned to be a favored *H. armigera* management strategy among Chinese cotton growers since 1997 [3,4]. However, given the large-scale adoption of *Bacillus thuringiensis* Berliner transgenic cotton (Bt cotton) throughout China, the risk of *H. armigera* resistance development to Bt toxins is ever more critical [5–11]. Currently, 'high-dose refuge' strategies are accepted as a resistance management tactic [12]. Nevertheless, when refuges are absent or prove inadequate, new insecticides with distinct a mode of action than Bt toxins should prove useful to either delay *H. armigera* resistance development to Cry1Ac or control resistant *H. armigera* strains.

Chlorantraniliprole is a new anthranilic diamide insecticide, which effectively controls pest insects belonging to Lepidoptera, Coleoptera, Diptera and Hemiptera [13–18], and has been shown to be effective against insecticides that have developed resistance to older classes of chemistry [19]. Chlorantraniliprole causes feeding cessation, lethargy, muscle paralysis and ultimately death by activating the ryanodine receptor. It can also affect calcium homeostasis by triggering release of internal Ca^{2+} within the cell [20]. This was difference from the older classes of chemistry. On the other hand, Cry1Ac kills insects by perforating their midgut, thereby targeting a cadherin-like protein, the latter which requires Ca^{2+} to stabilize itself [21,22]. So, two insecticides might cause the change of Ca^{2+}. To explore the potential of chlorantraniliprole for use in *H. armigera* resistance management programs, one needs to understand its toxicity on both susceptible and Bt resistant individuals and its compatibility with the mode of action of Cry1Ac.

In order to avoid cross-resistance with other toxins, an exploration of the mode of action of a novel product is recommended. However, nothing is known about the biochemical basis of toxicity of chlorantraniliprole, and more specifically of its effects on esterase and glutathione-*S*-transferase. Both enzymes play key roles in the metabolism and detoxification of insecticides, with insecticides commonly interfering in their activities [23–25]. If both insecticides have same effect on the activities of both enzymes, they will display a certain degree of cross resistance [26–29], a close examination of the effect of chlorantraniliprole on both detoxification

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enzymes in susceptible and resistant *H. armigera* strains is crucial to determine the potential of this insecticide for inclusion in resistance management strategies against this pest.

In this paper, the effects of chlorantraniliprole on esterase and glutathione-*S*-transferase in Cry1Ac susceptible and resistant strains of *H. armigera* were evaluated to clarify the change of two enzymes induced by chlorantraniliprole and Cry1Ac resistance. This change was confirmed by toxicity of chlorantraniliprole and can advise in the evaluation of the cross resistance between it and other insecticides.

2. Materials and methods

2.1. Study insects

A total of four different *H. armigera* populations were maintained in the laboratory. As susceptible strain, we selected the 96S *H. armigera* strain, which was initially collected from Xinxiang County (Henan Province, China) in 1996 and cultured on artificial diet in the laboratory [30]. Additionally, three resistant strains (BtR, XJ-F and XJ-10.0) were kept under laboratory conditions. The BtR strain was obtained by subjecting 96S individuals to solubilized Cry1Ac pro-toxin [31], and selected by 0.6 g/L 25 generations. A XJ-F strain was collected from Xiajin County (Shandong Province, China) in 2004 and subsequently cultured on artificial diet. Lastly, XJ-10.0 was obtained by subjecting XJ-F individuals to Cry1Ac pro-toxin at concentration of 10.0 mg/L, and selected 16 generations. All insect colonies were feeding at 27 ± 2 °C, 75 ± 10% RH and a photoperiod of 14:10 (L:D) h.

2.2. Insecticides and chemicals

Cry1Ac pro-toxin (extracted and purified solubilized pro-toxin from *B. thuringiensis* HD73) was provided by the Biotechnology Group in Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS) (Beijing, China). Chlorantraniliprole 96% powder was purchased from DuPont Company (Wilmington, Delaware, USA).

1-Naphthyl acetate, fast blue RR salt and reduced glutathione (GSH) were from Sigma Chemical Co. (St. Louis, MO), 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) were from Shanghai Chemical Reagent Co., Ltd., in China. The bradford dye reagent and bovine serum albumin (fraction V) were purchased from Pierce (Rockford, IL). Triton X-100 was purchased from Fluka (Sydney, Australia). All other chemicals were of analytical quality and purchased from commercial suppliers.

2.3. Determination of esterase and glutathione-S-transferase activities

2.3.1. Enzyme preparation

Five mid-guts of 5th instar *H. armigera* larvae were homogenized in 1.5 mL homogenization buffer (PBS, 0.02 M, pH7.0 with 0.05% Triton X-100, for EST; 0.02 M, pH 7.0, for GST experiments). The homogenate was centrifuged at 10,000*g* and 4 °C for 10 min and the resultant supernatant was collected as enzyme solution and stored at 4 °C until use. The storage time is lesser than 12 h.

2.3.2. Detoxification enzyme assay

For determination of the esterase activity, we adopted existing protocols [32]. Enzyme activitiy was determined through kinetic analysis using a microplate reader (SPECTRA max 340-PC), and 100 μL of 1-naphthyl acetate solution (10 mM), 100 μL fast blue RR Salt (1 mM) and 50 μL PBS was added into each microplate well. The reaction was initiated by addition of 50 μL enzyme solution. Optical density (OD) at 450 nm was recorded at 25 s intervals for 10 min.

Glutathione-*S*-transferase activity was measured using established protocols [33]. One hundred microliters of CDNB (20 mM) or DCNB (40 mM) and 100 μL GSH (40 mM) were pipetted into the microplate wells, and then 100 μL enzyme solution (for DCNB) or 10 μL enzymes solution and 90 μL PBS (for CDNB) was added. The OD value at 340 nm was recorded at 25 s interval for 10 min. All reactions were done at 27 °C. For both enzymes, 30 larvae were tested and enzyme activity was valued by the absorbance change rate per minute ($mOD \cdot min^{-1}$).

2.3.3. Protein assay

Total protein content of the enzyme solution was determined by the Bradford method using bovine serum albumin as the standard [34]. Absorbance was measured at 595 nm.

2.4. Toxicity trials

We determined toxicity of Cry1Ac and chlorantraniliprole in all *H. armigera* strains, by subjecting larvae to artificial diet that had been mixed with various concentrations of Cry1Ac and chlorantraniliprole. Artificial diets without Cry1Ac or chlorantraniliprole were used as control. About 1–1.5 g artificial diet was put in each well of a 24-well plate. Next, one 4-day old *H. armigera* larva from either strain was placed in the well, which was subsequently covered with a transparent plastic cover. Ninety-six larvae were tested for each concentration. Larval mortality was measured after 7 days, both dead larvae and those with a body mass of less than 5 mg were recorded as dead [35].

2.5. Statistical analyses

All the enzyme assays above were with six replications. Enzyme activity was showed as means ± SD ($mOD\ min^{-1}\ mg^{-1}$ protein). Statistical differences in enzyme activity between treatments or strains were determined using a one way ANOVA, followed by a Tukey's honestly significance difference (HSD) test for mean separation. All statistical analyses were executed using the software package SAS [36]. For bioassays, LC_{50} values and the slope of the concentration- mortality for each assay were estimated by probit analysis [37], using POLO-PC software [38]. Significant differences of LC_{50} were determined by non-overlapping 95% confidence limits.

3. Results

3.1. Effect on detoxification enzyme activity

The change of the activity of esterase and glutathione-*S*-transferase (CDNB and DCNB) induced by chlorantraniliprole and Cry1Ac resistance was shown in Table 1.

The activity of EST was significantly lower for resistant *H. armigera* strains than susceptible strain ($F = 89.67$; $df = 3,8$; $P < 0.05$) when chlorantraniliprole is absent. In all strains, the effect of chlorantraniliprole was greater at higher concentrations. Low concentration (0.001 μg/mL) caused significant change in 96S (1.30-fold) and XJ-10.0 (1.18-fold), but did not cause significant increase in BTR and XJ-F. The increase in 96S (1.84-fold) and XJ-10.0 (2.08-fold) caused by 0.1 μg/mL chlorantraniliprole was higher than the increasing in BTR (1.36-fold) and XJ-F (1.75-fold). The increasing induced by chlorantraniliprole did not change the lower of the activity of EST for resistant *H. armigera* strains than susceptible strain ($F = 355.6$; $df = 3,8$; $P < 0.05$).