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Monitoring and biochemical characterization of beta-cypermethrin resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae) in Sichuan Province, China

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ABSTRACT

The beet armyworm *Spodoptera exigua*, a major pest affecting numerous cultivated crops in China, has developed a serious resistance to many traditional chemical insecticides. The resistance levels of the field-collected populations from different districts in Sichuan Province, China, to nine insecticides were detected with a diet-incorporation bioassay. Compared to the Lab-ZN strain, five (in 2014) and three (in 2016) field populations displayed either high or extremely high levels of resistance to beta-cypermethrin. All the field populations collected in 2014 were susceptible to emamectin benzoate, hexaflumuron, methoxyfenozide, chlorantraniliprole, cyantraniliprole and indoxacarb but exhibited low or moderate levels of resistance to abamectin. The resistances of field populations collected in 2016 were significantly higher than two years earlier, especial for chlorantraniliprole and cyantraniliprole with RRs rising from 173.4- to 582.6-fold and 175.3- to 287.6-fold, respectively, even though the field populations had retained moderate or low levels of resistance to chlorpyrifos and hexaflumuron. The synergism experiment revealed that the resistance of the LS16 population to beta-cypermethrin may be mainly related to cytochrome P450 monooxygenases (P450s), which was responsible for the highest increase ratio of 37.97-fold, for piperonyl butoxide, rather than either carboxylesterase (CarE) or glutathione S-transferase (GST). The cytochrome P450 ethoxycoumarin *O*-deethylase activity of the LS16 population was also the strongest among the treatments ($P < 0.05$). Non-denaturing polyacrylamide gel electrophoresis (native PAGE) indicated that enhanced E11, E13 and E15-E16 bands in the LS16 population likely contribute to the development of resistance to beta-cypermethrin.

1. Introduction

The beet armyworm, *Spodoptera exigua* (Hübner), which originated in south-east Asia, has now become a serious pest of worldwide importance. This polyphagous pest attacks over 130 species of host plants representing over 30 different families, including major food crops such as Chinese cabbage, soybean, onion, cotton, scallion, ginger and pepper [1,2]. *S. exigua* is one of the three most important pest species in the genus *Spodoptera*; its larvae feed on the leaves, seedlings, and stems of host crops [3]. Chemical insecticides have been the most effective means of controlling it for the last two decades, with traditional organophosphorus (chlorpyrifos, profenofos, phoxim), carbamate (methomyl), pyrethroid (beta-cypermethrin, cyfluthrin, fenvalerate, fenpropathrin, deltamethrin), and benzoylurea (chlorfluazuron, chlorbenzuron, diflubenzuron, flufenoxuron) insecticides [4,5] being supplemented by newer insecticides (indoxacarb, methoxyfenozide, chlorantraniliprole and cyantraniliprole) in recent years [6–8].

However, this exclusive reliance on chemical control has resulted in many failure attempts to control this pest insect [9,10]. Field-evolved resistance to a number of insecticides has been widely reported [11–13], including the development of resistance to carbamates and pyrethroids in the United States [14,15], to spinosad in Thailand and Mexico [16,17], and to both conventional insecticides (endosulfan, organophosphates, and pyrethroids) and newer chemistries (spinosad,

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abamectin, emamectin benzoate, indoxacarb, methoxyfenozide, lufenuron, chlorantraniliprole and tebufenozide) [5,10,18,19].

Due to the different history and frequency of chemical control for pest insects in different countries and regions, resistance monitoring is critical to provide accurate information on resistance levels for the management of resistance, which has been become an essential component of any pest control program [20]. Monitoring the resistance status and diagnosing the potential resistance mechanisms of field populations of *S. exigua* has thus become an important focus of research in China, especially in Sichuan Province, where as yet little is known. The development of a better understanding of the biochemical mechanisms conferring insecticide resistance is indispensable for effective resistance management tactics. One of the most important factors governing resistance to insecticides is an increase in the metabolic processes that lead to higher detoxification of insecticides by enzymes such as mixed-function oxidase (MFO), carboxylesterase (CarE) and glutathione S-transferase (GST) [21]. Beet armyworm resistance to pyrethroid and organophosphorus insecticides is known to be related to increases in both the MFO [10,22] and CarE [23] activities; the MFO activity has also been linked to resistance to spinosad [24]. Meanwhile, a comparison of the different isozyme bands in susceptible and highly resistant field-collected populations using non-denaturing polyacrylamide gel electrophoresis (native PAGE) may provide useful clues for understanding the potential resistance mechanism [25].

Although there have been a number of reports from China on insecticide resistance monitoring of *S. exigua* during the last twenty years, most have focused on a few populations sampled from very limited areas, and the methods used to monitor the resistance and the reference strains used were not consistent. Therefore, the resistance levels in many districts in China remain unclear. Our objective was thus to measure the resistance levels of *S. exigua* field populations in Sichuan Province, a large province in southwestern China, and establish the biochemical mechanisms involved to enable farmers to make the best use of insecticides and synergists and implement effective resistance management strategies. Our results are expected to contribute to the design of more effective resistance management measures to support efforts to control this important insect pest.

2. Materials and methods

2.1. Insects

The susceptible laboratory (Lab-ZN) strain of *S. exigua* established in our laboratory was obtained from the Department of Entomology of China Agricultural University (Beijing, China) in 2011, where the strain had been reared in the laboratory without exposure to any insecticide for approximately fifteen years. The field populations were collected from five administrative divisions across Sichuan Province in 2014 (Pengzhou, Shuangliu, Leshan, Ziyang and Suining) and three in 2016 (Pengzhou, Leshan, Ziyang). Approximately 200–400 individual 2nd to 5th-instar *S. exigua* were collected from each sampling site (Table 1). All stages were maintained with the same standard conditions of 27 ± 1 °C, 70–80% RH and a 16:8 h (L: D) photoperiod. The larvae and adults were reared with an artificial diet and 10% sugar solution, respectively. Pupa and newly laid eggs were sterilized with a 0.2–0.3% sodium hypochlorite disinfection solution [7]. The F1-F3 larvae were used for the bioassays.

2.2. Insecticides and chemicals

Nine industrial insecticides were used in this experiment: 98% chlorpyrifos (Hubei Sharonda Co., Ltd., Jingzhou, China), 95% abamectin (Hebei Weiyuan Bio-Chemical Co., Ltd., Weiyuan, China), 90% emamectin benzoate (Nanjing Red Sun Co., Ltd., Gaochun, China), 97% hexaflumuron and 97.8% methoxyfenozide (Dow AgroSciences Company Indianapolis, USA), 95% indoxacarb (Jiangsu Flag Chemical

Table 1
Locations, sampling dates, host plants and developmental stages of *S. exigua*.

Populations	Locations	Collection date	Sites	Host plants	Insect stage	Larvae number
PZ14	Junle Village, Hei Long Town	Aug. 2014	N31°03′6.75″, E103°58′2.46″	*Allium fistulosum* L. var. *giganteum* Makino	3rd–5th instar larvae	300
SL14	Yongan Village, Baiguo Town	Sept. 2014	N30°24′14.15″, E103°58′18.68″	*Allium fistulosum* L. var. *giganteum* Makino	2nd–5th instar larvae	400
LS14	Shizhongqu Village, Mouzi Town	Sept. 2014	N29°38′13.78″, E103°46′3.84″	*Allium fistulosum* L.	3rd–5th instar larvae	300
ZY14	Yanjiaba Village, Yanjiang District	Sept. 2014	N30°09′32.59″, E104°42′23.14″	*Allium fistulosum* L. var. *giganteum* Makino	3rd–5th instar larvae	250
SN14	Laochi Village, Chuanshan District,	Oct. 2014	N30°24′6.63″, E105°41′39.99″	*Allium fistulosum* L. var. *giganteum* Makino	3rd–5th instar larvae	200
PZ16	Junle Village, Hei Long Town	Jul. 2016	N31°03′6.75″, E103°58′2.46″	*Allium fistulosum* L. var. *giganteum* Makino	2nd–5th instar larvae	300
LS16	Shizhongqu Village, Mouzi Town	Jul. 2016	N29°38′13.78″, E103°46′3.84″	*Allium fistulosum* L.	3rd–5th instar larvae	400
ZY16	Yanjiaba Village, Yanjiang District	Sept. 2016	N30°09′32.59″, E104°42′23.14″	*Allium fistulosum* L. var. *giganteum* Makino	3rd–5th instar larvae	350