Crop Protection 42 (2012) 217–222

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes

Jianya Su [a,b,*], Tiancai Lai [a], Jia Li [a]

[a] College of Plant Protection, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu 210095, China
[b] Jiangsu Key Laboratory of Pesticide Science, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 3 June 2011
Received in revised form
14 June 2012
Accepted 24 June 2012

Keywords:
Spodoptera litura
Chlorantraniliprole
Baseline susceptibility
Mixed function oxidase
Esterase
Glutathione-S-transferase

ABSTRACT

The cutworm *Spodoptera litura* is a major insect pest of vegetables and cotton in China, and has been reported to develop resistance to various classes of insecticides. Chlorantraniliprole, which has been registered recently in China, provides a novel option for control of this pest. The susceptibilities of *S. litura* collected from Southeast China to chlorantraniliprole were determined by diet incorporation assay with neonates. The susceptibility variation among 12 field populations was low (<4-fold), with median lethal concentration (LC_{50}) values varying from 28.4 to 102.5 μg/l. However, all the 12 field populations were less susceptible to chlorantraniliprole than a laboratory susceptible population. The most tolerant populations were sampled from Guangdong and Anhui Provinces where *S. litura* had been frequently challenged by insecticides. However, no correlation was found between LC_{50} values and the number of applications of this chemical. Biochemical assays were performed to determine the potential mechanisms involved in the tolerance observed in field populations. Most field populations showed significantly enhanced activities of mixed function oxidase enzymes compared with the susceptible strain. Only a few populations had higher activities of esterase and glutathione-S-transferase than the susceptible strain. No correlations were observed between activities of metabolic enzymes and chlorantraniliprole toxicity, suggesting that these detoxification enzymes were not the main cause of the field tolerance observed in this study, and there might be other mechanisms conferring the tolerance variation to chlorantraniliprole in *S. litura*.

1. Introduction

The cutworm *Spodoptera litura* (Fabricius) is a polyphagous insect pest of many crops in Asia. In recent years frequent outbreaks have been more common in the middle and lower reaches of the Yangtze River and the southern region of China (Zhou, 2009). Frequent insecticide applications are made to suppress populations due to the serious damage it potentially causes to economically important crops such as cotton, vegetables, soybean and others. The extensive application of insecticide has led to the rapid development of insecticide resistance in the field. The first case of insecticide resistance to benzene hexachloride (BHC) in *S. litura* was reported in 1965 (Srivastava and Joshi, 1965). Currently, high levels of resistance to conventional insecticides, including organochlorines, organophosphates, carbamates and pyrethroids, has been reported in China (Zhou, 1984; Wu et al., 1995; Huang et al., 2006), Korea (Kim et al., 1998), India (Ranakrishnan and Saxena, 1984; Murugesan and Dhingra, 1995; Rao and Dhingra, 1996; Armes et al., 1997; Kranthi et al., 2001, 2002) and Pakistan (Ahmad et al., 2007a; Saleem et al., 2008). Recently, resistance to some newer insecticides such as abamectin, spinosad and indoxacarb has also been documented (Chen et al., 2008; Ahmad et al., 2008; Shad et al., 2010). The development of insecticide resistances was implicated as the major reason for field control failures. The introduction of chlorantraniliprole which has a novel insecticidal mechanism added an alternative insecticide for managing *S. litura* (Bentley et al., 2010).

Chlorantraniliprole is a novel insecticide discovered by DuPont, also known as rynaxypyr (Bentley et al., 2010). Studies have shown that chlorantraniliprole has exceptional insecticidal activity on a range of lepidopteran pests and many other orders, such as Coleoptera, Diptera, Isoptera and Hemiptera (Sattelle et al., 2008; Lahm et al., 2009). Chlorantraniliprole activates the unregulated release of internal calcium stores leading to Ca^{2+} depletion, feeding cessation, lethargy, muscle paralysis and finally insect death (Lahm

* Corresponding author. College of Plant Protection, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu 210095, China. Tel.: +86 25 84395269.
E-mail addresses: sjy@njau.edu.cn, jianyasu@gmail.com (J. Su).

0261-2194/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cropro.2012.06.012

et al., 2005). It blocks the feeding of lepidopteran larvae rapidly, the feeding cessation time being equivalent to that of nerve agents. Accordingly, it was ranked as the fastest-acting insecticide for lepidopteran control (Hannig et al., 2009). In addition, the low ecotoxicology to non-target organisms such as birds, fish, mammals, earthworms and many other arthropods (Lahm et al., 2007; Larson et al., 2012) and no cross-resistance with other older classes of chemistry (Cao et al., 2010; Sial et al., 2010; Wang et al., 2010) make it an excellent pest management tool.

It is critically important to establish the susceptibility of field populations to newly developed insecticides before their widespread use. The objective of this study was to determine the susceptibility of field populations of *S. litura* to chlorantraniliprole in China, as well as to evaluate the detoxification activities of mixed function oxidases (MFO), glutathione S-transferases (GST) and esterases (EST) to determine the potential mechanisms involved in the susceptibility variation to chlorantraniliprole.

2. Materials and methods

2.1. Insects

The susceptible population (XW-Sus) of *S. litura* was used as the reference population. It was provided by the Institute of Plant Protection, Jiangsu Academy of Agricultural Science, China. XW-Sus had been reared on artificial diet in the laboratory without exposure to any insecticide for more than 8 years. Twelve field populations of *S. litura* were collected from Southeast China. Approximately 100–200 individuals of 2nd to 5th-instars of *S. litura* were collected from each sampling site (Fig. 1, Table 1). The collected insects were bred in the laboratory and the larvae of the first generation were used for susceptibility bioassay and detoxification enzyme assay.

Larvae of *S. litura* were reared on an artificial diet at 27 ± 1 °C and 60–70% RH with 14L:10D photoperiod (Lai and Su, 2011). Adults were fed 10% sugar solution under the same temperature and light conditions with a relative humidity of 90%. Newly laid eggs were sterilized with 5% formaldehyde to prevent viral pathogens.

2.2. Insecticide and chemicals

Chlorantraniliprole (Rynaxypyx™ 200 g/l SC, Coragen; DuPont Agricultural Chemicals Ltd., Shanghai, China) was commercially available. NADPH, *p*-nitrophenol (PNP), Glutathione (GSH) and α-naphthol were purchased from Bio Basic Inc. (Buffalo, USA). Coomassie Brilliant Blue G-250 and α-naphthyl acetate (α-NA) were obtained from Shanghai Chemical Factory, China. 2, 4-Dinitrochlorobenzene (CDNB) and EDTA were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). DTT was from Beijing Solarbio Science and Technology Co., Ltd, China. Bovine serum albumin (BSA) and PMSF were obtained from Sino-American Biotechnology Co. Ltd. (Shanghai, China).

2.3. Diet incorporation bioassay

The chlorantraniliprole susceptibilities of field populations of *S. litura* were assayed with neonates (<10 h old) using a diet incorporation method. Seven concentrations of chlorantraniliprole using two fold dilutions were prepared with distilled water. After preparing the diet, a quantity of 5 ml of diluted chlorantraniliprole was mixed thoroughly with 45 ml of artificial diet in a glass cup (below 54 °C), then approximately 2.5 ml of the diet was dispensed into cylindrical tissue culture tubes (2 × 8 cm). Five neonates were placed in each culture tube, and 20 tubes were prepared for each concentration. A total of 100 larvae were used for each concentration. After larval inoculation all bioassay tubes were closed with cotton pads and placed in an incubator maintained at 27 ± 1 °C, 60–70% RH, and a 14L:10D photoperiod. Larval mortality was evaluated after 3 d. Larvae were recorded as dead if they did not respond with head movements or peristaltic contractions when touched with camel's-hair brush. To ensure that offspring of many females were assayed, larvae hatching from any given egg mass were systematically distributed among various concentrations (i.e., a maximum of about 5 larvae per egg mass were exposed to every concentration).

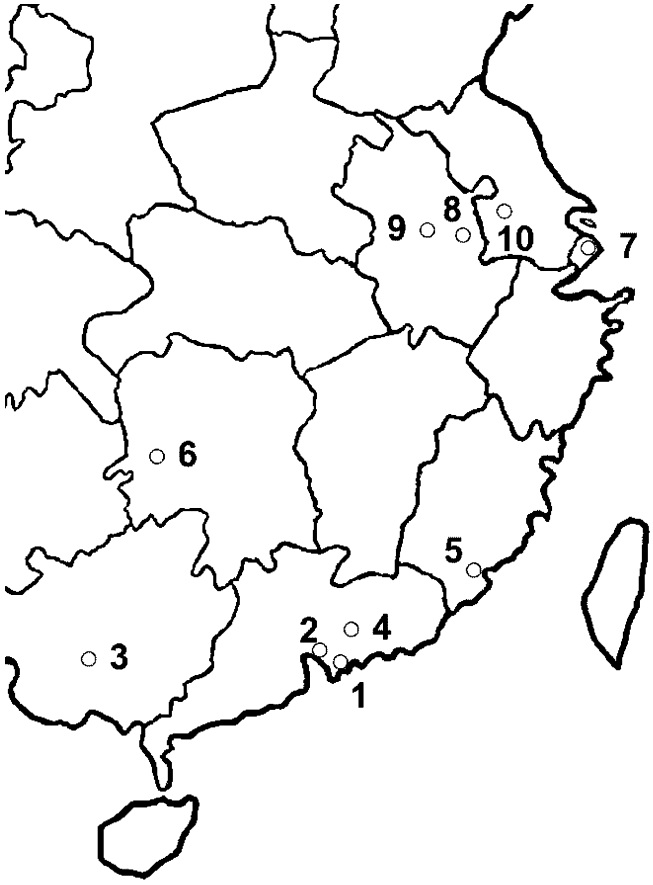

Fig. 1. Sampling sites of *S. litura* field populations in South-east China.

2.4. Enzyme preparation

The 3rd-instar larvae (13–15 mg) were separated and starved for 3 h to remove digested food particles. The whole larvae were homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 1 mM PTU, 1 mM PMSF and 20% glycerol). Insects were chilled on ice before homogenization. Sixteen larvae were homogenized in 2 ml of buffer. The homogenate was then centrifuged at 4 °C, 10, 000 *g* for 15 min, and the solid debris and cellular material were discarded. The supernatant was decanted into a clean eppendorf tube, placed on ice and used immediately for assaying mixed function oxidase (MFO), glutathione S-transferase (GST), esterase (EST) and total protein content. Four enzyme samples were prepared for each populations assayed.

2.5. Enzyme activity assays

The activity of mixed function oxidase (MFO) was assayed using the protocols of Rose et al. (1995). One hundred μl of 2 mM