Crop Protection 63 (2014) 131–137

Contents lists available at ScienceDirect

Crop Protection

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

Resistance monitoring for eight insecticides in *Plutella xylostella* in central China

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form
12 March 2014
Accepted 14 March 2014
Available online 6 June 2014

Keywords:
Diamondback moth
Cabbage
Resistance ratio
Bioassay

ABSTRACT

The diamondback moth, *Plutella xylostella* L., is one of the most important pests of cruciferous vegetables in the world. Assessment of changing insecticide resistance is essential for maintaining control efficiency and resistance management. In this study, four field populations of *P. xylostella* were collected from cabbage (*Brassica oleracea*, var. *capitata* L.) fields in central China from 2010 to 2012 to monitor their resistance to abamectin, *Bacillus thuringiensis* (BT) subsp. *kurstaki* (WG-001), spinosad, chlorfluazuron, chlorfenapyr, diafenthiuron, indoxacarb and beta-cypermethrin by using a leaf-dipping bioassay method. The results indicated that chlorfenapyr and diafenthiuron showed high toxicity to *P. xylostella* in central China, with no obvious toxicity change during the three years. The resistance of *P. xylostella* to spinosad was at low to moderate levels of resistance in all three years. Resistance of *P. xylostella* to abamectin, chlorfluazuron and indoxacarb varied greatly among the four regions. *P. xylostella* exhibited moderate and high levels of resistance to abamectin, chlorfluazuron and indoxacarb. The resistance of this pest to BT (WG-001) and beta-cypermethrin was severe in the four regions. Our study was conducive for developing a more effective resistance management program for *P. xylostella*.

1. Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is one of the most notorious pests of cruciferous vegetables in the world (Talekar and Shelton, 1993; Wang and Wu, 2012). The annual cost of its management has been estimated to reach US $1 billion worldwide (Talekar and Shelton, 1993; Capinera, 2001; Gong et al., 2010a). The production area of cabbage (*Brassica oleracea*, var. *capitata* L.) and cauliflower (*Brassica oleracea* var. *botrytis* L.) grown in 1999 in China was 1.2 million ha (Shelton, 2001). The pest has been problematic in many parts of China, including central China, since the 1970s (Wu et al., 2012). At present, the control of *P. xylostella* in China is primarily dependent on the application of insecticides. Overall, several factors may have contributed to *P. xylostella* outbreaks in central China, including continuous planting of host crops, the use of susceptible plant varieties, and exclusive reliance on chemical control, which usually requires at least 3–4 sprays per month. Because of its genetic plasticity, high fecundity, rapid generation time in the subtropics and temperate zone, and the strong insecticide selection pressure it has been subjected to, it has acquired insecticide resistance rapidly in many areas of central China (Cao and Han, 2006). However, abamectin, spinosad, chlorfenapyr, indoxacarb, chlorantraniliprole and certain *Bacillus thuringiensis*-based products are still effective among the few insecticide options available in some areas, but the risk of resistance evolution to these insecticides is also high. Some field populations of *P. xylostella* have developed resistance to these newer chemicals, including spinosad, avermectins (abamectin and emamectin benzoate), indoxacarb and chlorantraniliprole (Wang and Wu, 2012; Zhao et al., 2002; Zhao et al., 2006; Heckel et al., 2004; Sayyed and Wright, 2006; Pu et al., 2010). In addition, *P. xylostella* was the first lepidopteran pest to evolve resistance to *Bacillus thuringiensis* (BT) subsp. *kurstaki* in the field (Tabashnik et al., 1990; Yu and Zhan, 1999; Sarfraz and Keddie, 2005; Han et al., 2012). Thus, there are only a few insecticides that remain effective in controlling this pest in China. Insecticide resistance monitoring should be an essential component of any pest control program. In order to provide a basis for developing resistance management strategies, it is necessary to evaluate the resistance of *P. xylostella* to commonly used insecticides in the field. Here, we report the results of a three-year study documenting the resistance to eight commonly used insecticides, including abamectin, BT (WG-

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http://dx.doi.org/10.1016/j.cropro.2014.03.011
0261-2194/

001), spinosad, chlorfluazuron, chlorfenapyr, diafenthiuron, indoxacarb and beta-cypermethrin in *P. xylostella* populations from four regions in three provinces of central China.

2. Materials and methods

2.1. Populations of P. xylostella

The field populations of *P. xylostella* used for resistance monitoring in this study were collected from cabbage fields in four different geographical regions of central China from 2010 to 2012. The sites included Luoyang (LY: E 112.4°, N 34.6°) in He'nan Province, Yueyang (YY: E 113.12°, N 29.36°) in Hu'nan Province, and Wuxue (WX: E115.56°, E 29.85°) and Yichang (YC: E 111.30°, N 30.7°) in Hubei Province (Fig. 1). More than 500 fourth instar larvae or pupae on 25 cabbage plants in every field were counted. In each geographical region more than five cabbage fields were surveyed. Insects were mass-mated in cages (50 cm × 40 cm × 30 cm) and their F1 larvae were used for bioassays. Adults were fed on 10% honey solution and allowed to lay eggs on radish seedling

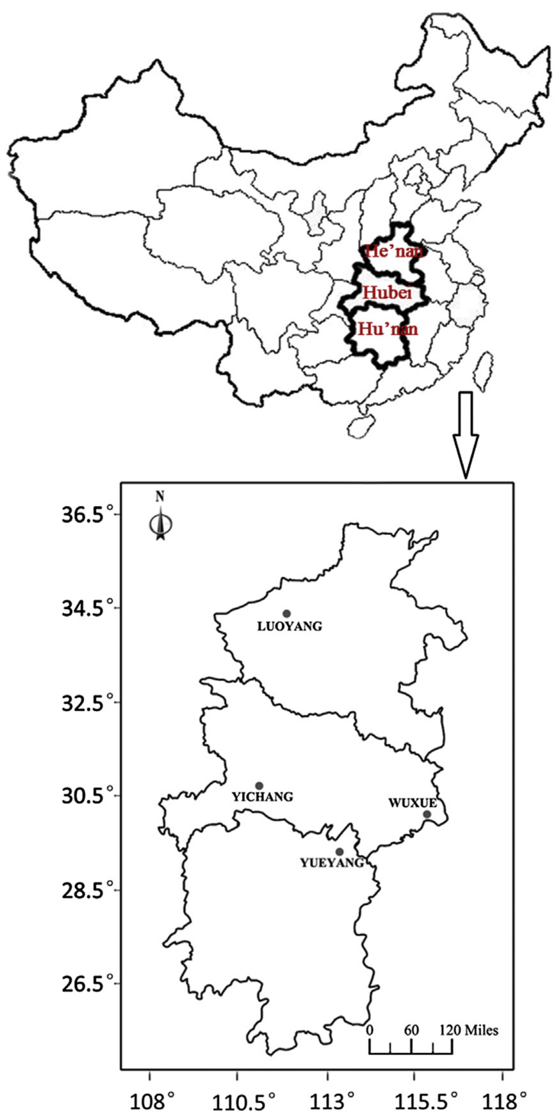

Fig. 1. Sampling sites of *P. xylostella* field populations from different provinces in central China. He'nan Province: Luoyang, Hu'nan Province: Yueyang, Hubei Province: Wuxue and Yichang.

(*Raphanus sativus* L.) (Wang and Zhu, 2012). All populations were maintained separately at 25 ± 1 °C and 60–70% RH (relative humidity) with a photoperiod of 16:8 h (L:D). Baseline susceptibility for the selected insecticides was presented in Technological Rules for Monitoring Insecticide Resistance in Crucifer Vegetables Diamondback Moth, *Plutella Xylostella* (Shao et al., 2013), with a strain obtained from Rothamsted Research (Harpenden, Hertfordshire, United Kingdom) which has been maintained free from insecticide exposure since 1995.

2.2. Insecticides

Eight commonly used insecticides, including 1.68% abamectin (Trade name, avermectin; Manufacturer, Guangdong Plant Protection Technology Co. Ltd.; Recommended application rate, 8.1–10.8 g ai/ha), 16000 IU/mg BT WG-001 (BTV; Hubei Biopesticide Engineering Research Center; 750.0–1125.0 g ai/ha), 2.50% spinosad (Tracer; DowAgrosciences; 12.4–24.8 g ai/ha.), 5.02% chlorfluazuron (Atabron 5E; Jiangsu Yangnong Chemical Group Co. Ltd.; 30.0–45.0 g ai/ha), 9.17% chlorfenapyr (Pirate; BASF Europe; 50.0–70.0 g ai/ha), 20.18% diafenthiuron (Polo; Guangdong Plant Protection Technology Co. Ltd.; 300.0–450.0 g ai/ha), 4.86% indoxacarb (AVAUNTTM; Guangdong Plant Protection Technology Co. Ltd.; 67.5–121.5 g ai/ha) and 4.5% beta-cypermethrin (Fastac; Shenzhen Noposion Agrochemicals Co. Ltd.; 10.1–27.0 g ai/ha), were provided by the Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, China.

2.3. Bioassays

The leaf-dipping bioassay method was used as described by Shelton et al. (1993), Liang et al. (2003) and Lin et al. (2008). Briefly, each insecticide was serially diluted to five required concentrations with distilled water containing 0.1% Triton X-100 (Beijing Solarbio Science and Technology Co. Ltd., China). Young cabbage (*B. oleracea*) leaf discs (6.5 cm in diameter) were cut from cabbage plants grown in greenhouse without any insecticides and dipped in different concentration of insecticide solutions for 10 s. The control discs were treated with 0.1% Triton X-100 solution only and the mortality of control was <10%. All dipped leaf discs were air-dried at room temperature for approximately 2 h. The discs were then placed individually in plastic Petri dishes (7.0 cm in diameter). A total of 10 third instar larvae were used in each dish, and four replicates were prepared (in total 240 third-larvae for each bioassay) and kept under standard conditions as previously described. The mortality was recorded 48 h later for abamectin, spinosad, chlorfenapyr, diafenthiuron, indoxacarb and beta-cypermethrin, and 72 h later for BT (WG-001) and chlorfluazuron. Larvae were considered dead if they could not be induced to move when stimulated with a probe.

2.4. Statistical analysis

Bioassay data were analyzed using PROC PROBIT (SAS Institute Inc., 1997). The resistance ratio (RR) was calculated by dividing the LC_{50} (median lethal concentration) value of each population by the LC_{50} value for baseline susceptibility provided by Technological Rules for Monitoring Insecticide Resistance in Crucifer Vegetables Diamondback Moth and was considered to be significant when the 95% fiducial limits (FL) did not include a value of 1.0 (Robertson and Preisler, 1992). The insecticide resistance levels were classified according to the standard established by Shen et al. (1991): susceptible ($RR < 3.0$), decreased susceptibility ($3.0 \le RR < 5.0$), low level of resistance ($5.0 \le RR < 10.0$), moderate level of resistance ($10.0 \le RR < 40.0$), high level of resistance ($40.0 \le RR < 160.0$), and extremely high level of resistance ($RR \ge 160.0$).