Monitoring Field Populations of Plutella xylostella (Lepidoptera: Plutellidae) for Resistance to Eight Insecticides in China

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Monitoring field populations of *Plutella xylostella* (Lepidoptera: Plutellidae) for resistance to eight insecticides in China

*Tiantian Jiang, Shunfan Wu, Tingting Yang, Cong Zhu and Congfen Gao**

Abstract

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the main destructive insect pest of brassicaceous vegetables around the world. It has developed resistance to various classes of insecticides. However, the current status of insecticide resistance in *P. xylostella* has not been examined in China. In this study, concentration-mortality responses of *P. xylostella* to 8 insecticides, including abamectin, chlorantraniliprole, spinosad, beta-cypermethrin, chlorfenapyr, diafenthiuron, chlorfluazuron and the bio-pesticide *Bacillus thuringiensis kurstaki* (Btk) were evaluated. The results showed that almost all of the tested populations had developed high to very high resistance to abamectin and beta-cypermethrin, with resistance ratios ranging from 62.9 to 1494.7-fold. Chlorantraniliprole was very effective against *P. xylostella* in most tested populations except those from Taihe and Wuxi. Approximately 61% of tested populations displayed moderate resistance to spinosad, while other field populations showed minor changes (3-fold) in their susceptibility to this insecticide. Obvious variation (93-fold) of susceptibility to chlorfenapyr existed in field populations of which 32% displayed low level resistance, and 36% exhibited moderate resistance. Only one field population (Wuxi) showed very high resistance to chlorfenapyr (RR = 260.1). Diafenthiuron and chlorfluazuron were highly effective against all of the tested populations with resistance ratios (RR) ranging from 0.4 to 8.7 – fold. Decreased susceptibility ranging to moderate resistance to Btk was observed (RR = 3.8 – 35.3). Significant correlations were detected between the values of logLC_{50} of chlorantraniliprole and 4 insecticides (abamectin, spinosad, beta-cypermethrin and chlorfenapyr). The results of this study provided valuable information for choosing alternative insecticides and for integrated resistance management of *P. xylostella*.

Key Words: resistance; abamectin; *Bacillus thuringiensis;* beta-cypermethrin; chlorantraniliprole; spinosad; integrated pest management

Resumen

La polilla de la col, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), es la plaga principal de insectos destructivos de hortalizas brassicaceous por todo el mundo. Esta ha desarrollado resistencia a diversas clases de insecticidas. Sin embargo, no se ha examinado el estado actual de resistencia a los insecticidas en *P. xylostella* en China. En este estudio, se evaluó la dosis-respuesta de *P. xylostella* a 8 insecticidas, incluyendo abamectina, clorantraniliprol, espinosad, beta-cipermetrina, clorfenapir, diafentiuron, clorfluazuron y Btk. Los resultados mostraron que casi todas las poblaciones analizadas habían desarrollado entre alta y muy alta resistencia a la abamectina y beta-cipermetrina, con relaciones de resistencia (RR) de 62.9-1,494.7 veces. Clorantraniliprol fue muy eficaz contra *P. xylostella* en la mayoria de las poblaciones probadas, excepto los de Taihe y Wuxi. Aproximadamente el 61% de las poblaciones analizadas mostraron resistencia moderada a spinosad, mientras que otras poblaciones de campo mostraron cambios menores (3 veces) en su susceptibilidad a este insecticida. Variación obvia (93 veces) en la susceptibilidad a chlorfenapyr existía en poblaciones de campo de las cuales el 32% está representada bajo nivel de resistencia, y el 36% exhibió una resistencia moderada. Sólo una población de campo (Wuxi) mostró resistencia mas alta a chlorfenapyr (RR = 260.1). Diafentiurón y chlorfluazuron fueron mas eficaces contra todas las poblaciones analizadas, con una tasa de resistencia (TR) de 0.4 hasta 8.7 veces. Se observó una disminución en la sensibilidad de resistencia moderada al biopesticida, *Bacillus thuringiensis kurstaki* (Bt), (RR = 3.8-35.3). Los resultados de este estudio provee información valiosa para la elección de los plaguicidas alternativos y para el manejo integrado de la resistencia de *P. xylostella*.

Palabras Clave: resistencia; abamectina; *Bacillus thuringiensis*; beta -cypermethrin; chlorantraniliprole; spinosad; manejo integrado de plagas

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most important pests of cruciferous crops worldwide; particularly cabbage, broccoli and cauliflower (Talekar & Shelton 1993). The overall management costs for DBM has been estimated at US$ 4~5 billion (Zalucki et al. 2012). Insecticidal control is the major measure for suppression of DBM damage (Furlong et al. 2013; Sarfraz & Keddie 2005). However, the pest's short generation time, genetic plasticity, high fecundity and particularly the intensive selection pressure, have resulted in DBM exhibiting resistance to various kinds of insecticides, including recently introduced compounds with new modes

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of action, such as spinosad, avermectins, indoxacarb, the bio-pesticide *Bacillus thuringiensis* Cry toxins, and the anthranilic diamide chlorantraniliprole (Hu et al. 2012; Li et al. 2006; Pu et al. 2010; Sayyed & Wright 2006; Sukonthabhirom & Siripontangmun 2013; Troczka et al. 2012; Wang et al. 2013; Wang & Wu 2012; Zhao et al. 2006). In order to provide successful resistance management strategies, it is necessary to monitor and understand the status of insecticide resistance. This study aimed to assess the current status of insecticide resistance to eight commonly used insecticides in DBM populations collected from various locations in China from 2012 to 2013, particularly in the Yangtze's river region.

Materials and Methods

INSECT STRAINS

Thirty one populations of DBM were collected from 18 different geographical regions in 7 provinces including Jiangxi, Shandong, Guangxi, Guangdong, Jiangsu, Anhui and Shanghai of China at 2012 and 2013 (Fig. 1; Table 1). Third or fourth instars, or pupae were collected from each sampling site. Adults that emerged from the collections were fed on 10% diluted sugar solution (10% weight-to-volume solution) in cotton wool and mated at random in cages (about 100 males and 100 females). Potted radish seedlings were placed in the cages for oviposition. Third instars of the next generation, which represent the progeny of field collected insects, were used for the resistance studies. The insectaries were maintained at 25 ± 1 °C and RH of 60%-70% with a photoperiod of 16:8 h L:D.

INSECTICIDES

All insecticides, except *Bacillus thuringiensis* used in this study were technical grade (Table 2).The *B.t. kurstaki* (Btk) strain (16,000 IU/mg wettable powder) was a commercially formulated product. These insecticides were dissolved in acetone or DMF (N, N-dimethyl formamide) as stock solutions and Bt wettable powder was dissolved in water.

BIOASSAYS

The concentration-mortality response of DBM to different insecticides were measured using the leaf-dip method (Mohan & Gujar 2003). The insecticides were diluted to generate serial dilutions with distilled water containing 0.1% Triton X-100 (to facilitate uniform treatment with active ingredient). Cabbage, Brassica oleracea L., leaves were washed with distilled water containing 10% acetone and then air dried for about 1 h. Cabbage leaf discs (diameter, 6.5 cm) were cut and dipped in an insecticide solution for 10s. Control discs were treated with 0.1% Triton X-100 solution only. The leaf discs were dried at room temperature for 1-2 h. One treated leaf disc with 10 third-instar larvae was placed in a plastic petri dish and then kept at 25 ± 1 °C, 60%-70% RH, and a photoperiod of 16:8 h L:D. For each concentration, 40 third-instar larvae with body length range from 5mm to 6 mm were exposed to the insecticides and the same amount larvae in the control group. Mortality was assessed after 48h except for the 96h mortality assessed for chlorfluazuron and Btk treatment. Larvae were considered dead if they could not make coordinated movements after gentle stimulation with a small paintbrush.

DATA ANALYSIS

Lethal concentration values (LC_{50}) and their 95% fiducial limits (FL) were estimated using POLO Plus program (Leora Software 2002). Mortality was corrected by Abbott's formula (Abbott 1925) for each probit analysis. A significant difference between LC_{50} values was indicated by non-overlapping 95% FLs. Correlation between variables was calculated using the Pearson method via the IBM SPSS Statistics software package (Corp. 2011), a *P*-value of less than 0.05 was thought to be statistically significant. The resistance ratio (RR) was calculated by dividing the LC_{50} value of a field population by the corresponding LC_{50} value of the susceptible strains. Guideline for Insecticide Resistance Monitoring of DBM on Cruciferous Vegetables (Shao et al. 2013) was the source of susceptibility baselines values to all tested insecticides in this study. Insecticide resistance levels were described using RRs as follows: susceptibility (RR < 3.0), decreased susceptibility (RR = 3.1 – 5.0), low resistance (RR = 5.1 – 10.0), moderate resistance (RR = 10.1 – 40.0), high resistance (RR = 40.1 – 160.0), and very high resistance (RR > 160.0) (Ban et al. 2012). Correlation between variables was calculated using the Pearson method via the IBM SPSS Statistics software package, a *P*-value of less than 0.05 was thought to be statistically significant.

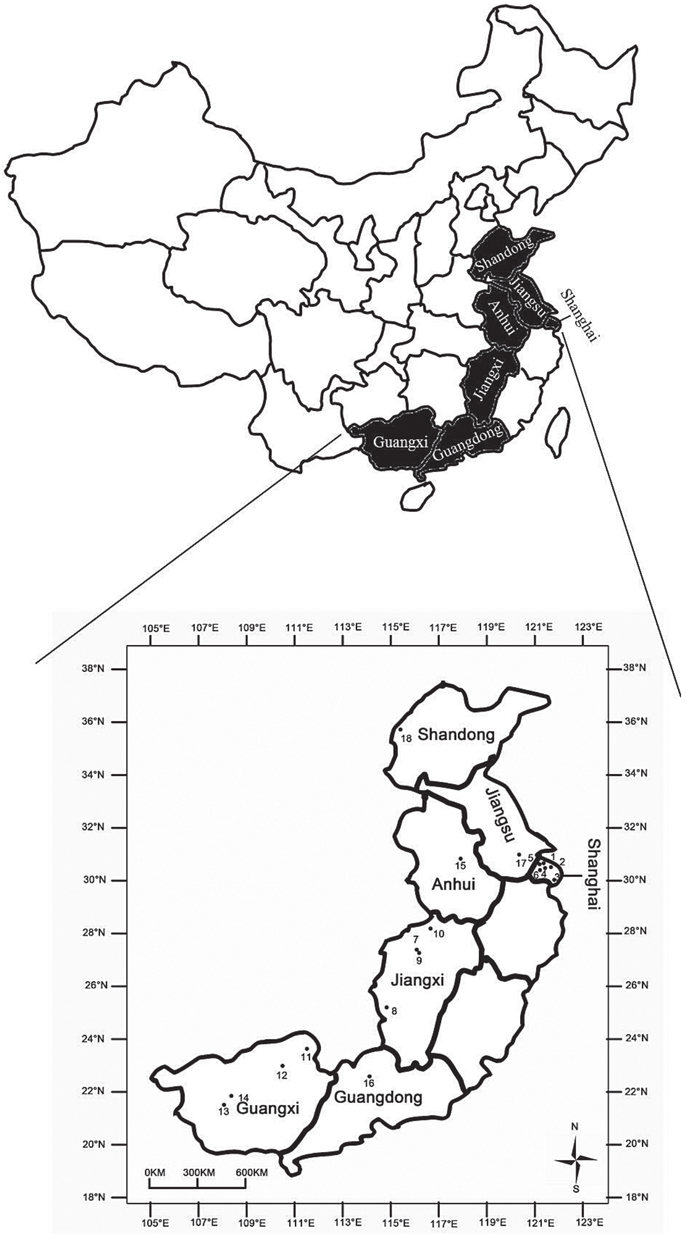

Fig. 1. Sampling sites of *Plutella xylostella* field populations in seven provinces of China.

Table 1. Sampling dates, host crops, and developmental stages of *Plutella xylostella* of 31 field populations of DBM collected from 18 sites in 7 provinces (Jiangxi, Shandong, Guangxi, Guangdong, Jiangsu, Anhui and Shanghai).

Populations	Locations	Site	Map ref. no.	Time of insect collection	Host plant	Developmental stage
BS12	Baoshan, Shanghai	31.24°N, 121.20°E	1	May 2012	*Brassica oleracea* L. var. botrytis L.	third and fourth instar larvae
BS13	Baoshan, Shanghai	31.24°N, 121.20°E	1	Apr 2013	*Brassica chinensis* L.	third, fourth instar larvae and pupae
CM12	Chongming, Shanghai	31.37°N, 121.23°E	2	Apr 2012	*Brassica oleracea* L. var. botrytis L.	third, fourth instar larvae and pupae
CM13	Chongming, Shanghai	31.37°N ,121.23°E	2	April 2013	*Brassica oleracea* L. var. botrytis L.	fourth instar larvae and pupae
JS12	Jinshan, Shanghai	30.47°N, 121.11°E	3	Jun 2012	*Brassica oleracea* L. var. botrytis L.	fourth instar larvae and pupae
JS13	Jinshan, Shanghai	30.47°N, 121.11°E	3	Jun 2013	*Brassica oleracea* L.var.capitata L.	fourth instar larvae and pupae
MH12	Minhang, Shanghai	31.11°N, 121.25°E	4	May , Jun 2012	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
MH13	Minhang, Shanghai	31.11°N, 121.25°E	4	May 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
PD12	Pudong, Shanghai	31.13°N, 121.32°E	5	May, Jun, Jul 2012	*Brassica capitata*L. *Brassica oleracea* L. var. botrytis L. *Brassica chinensis* L.	third ,fourth instar larvae and pupae
PD13	Pudong, Shanghai	31.13°N, 121.32°E	5	May 2013	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
SJ12	Songjiang, Shanghai	31.08°N, 121.07°E	6	Jun 2012	*Brassica oleracea* L. var. botrytis L.	fourth instar larvae and pupae
NC12	Nanchang, Jiangxi	28.32°N, 116.00°E	7	May 2012	*Raphanus sativus* L	fourth instar larvae and pupae
NC13	Nanchang, Jiangxi	28.32°N, 116.00°E	7	May 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
TH12	Taihe, Jiangxi	26.47°N, 114.54°E	8	Oct 2012	*Brassica oleracea* L. var. capitata, *Brassica chinensis* L.	fourth instar larvae and pupae
TH13A	Taihe, Jiangxi	26.47°N, 114.54°E	8	Jun 2013	*Brassica oleracea* L. var. capitata, *Brassica chinensis* L.	third, fourth instar larvae and pupae
TH13B	Taihe, Jiangxi	26.47°N, 114.54°E	8	Oct 2013	*Brassica oleracea* L. var. capitata, *Brassica chinensis* L.	third, fourth instar larvae and pupae
WA13	Wanan, Jiangxi	26.27°N, 141.46°E	9	Mar 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
DC13	Duchang, Jiangxi	29.19°N, 116.16°E	10	May 2013	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
GY12A	Guanyang, Guangxi	25.29°N, 111.09°E	11	Apr 2012	*Brassica oleracea* L. var. capitata, *Brassica oleracea* L. var. botrytis L.	fourth instar larvae and pupae
GY12B	Guanyang, Guangxi	25.29°N, 111.09°E	11	Oct, Nov 2012	*Brassica juncea*, *Brassica chinensis* L.	fourth instar larvae and pupae
GY13	Guanyang, Guangxi	25.29°N, 111.09°E	11	Apr 2013	*Brassica oleracea* L. var. capitata, *Brassica oleracea* L. var. botrytis L.	third, fourth instar larvae and pupae
LC12	Lingchuan, Guangxi	25.23°N, 110.19°E	12	May 2012	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
LC12	Lingchuan , Guangxi	25.23°N, 110.19°E	12	May 2012	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
NN12	Nanning, Guangxi	22.45°N, 108.28°E	13	Dec 2012	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
NN13	Nanning, Guangxi	22.45°N, 108.28°E	13	Apr 2013	*Brassica oleracea* L. var. botrytis L.	third, fourth instar larvae and pupae
SL13	Shanglin, Guangxi	23.25°N, 108.36°E	14	Jun, Jul 2013	*Brassica campestris* L. ssp.chinensis var.utilis Tsen et Lee	third, fourth instar larvae and pupae
HX12	Hexian, Anhui	31.44°N, 118.21°E	15	Oct 2012	*Brassica chinensis* L.	fourth instar larvae
HZ12	Huizhou, Guangdong	23.02°N, 114.34°E	16	Feb 2012	*Brassica oleracea* L. var. capitata	third, fourth instar larvae and pupae
HZ13	Huizhou, Guangdong	23.02°N, 114.34°E	16	Mar 2013	*Brassica oleracea* L. var. botrytis L.	third, fourth instar larvae and pupae
WX12	Wuxi, Jiangsu	31.27°N, 120.07°E	17	Oct 2012	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
WX13A	Wuxi, Jiangsu	31.27°N, 120.07°E	17	Mar 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
WX13B	Wuxi, Jiangsu	31.27°N, 120.07°E	17	Sep 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae
LC13	Liaocheng, Shandong	36.26°N, 115.59°E	18	May 2013	*Brassica oleracea* L. var. capitata	fourth instar larvae and pupae

Table 2. List of the eight insecticides in the assay of the resistance levels of 31 field populations of *Plutella xylostella*.

Insecticide	Group	Content of active ingredient	Supplier	IRAC mode of action
Abamectin	Abamectin	92%	Jiangsu Fengyuan Bio-engineering Co.,Ltd	Chloride channel activators
Chlorantraniliprole	Diamides	95.30%	DuPont Agricultural Chemicals Limited	Ryanodine receptor modulators
Spinosad	Spinosyns	90%	Liyang Zhongnan Chemical industry Co.,Ltd	Nicotinic acetylcholine receptor allosteric activators
Beta-cypermethrin	Pyrethroids Pyrethrins	95%	Nanjing Red Sun Co., Ltd.	Sodium channel modulators
Diafenthiuron	Diafenthiuron	97.1%	Xinxing Chemical industry Co.,Ltd	Inhibitors of mitochondrial ATP synthase
Chlorfluazuron	Benzylpthenylureas	97.2%	Nanjing Red Sun Co., Ltd.	chitin synthesis inhibitors
B.t.kurstaki	B.t.	16000 IU/mg	Hubei Kangxin Agro-industry Co., Ltd.	Microbial disruptors of insect midgut membranes
Chlorfenapyr	Pyrrole Insecticides	98.7%	American Cyanamid Co.	Uncouplers of oxidative phosphorylation via disruption of the proton gradient

Results

RESISTANCE TO ABAMECTIN

The toxicities (LC_{50} values) of abamectin against DBM ranged from 0.63 mg/L in GY12A to 29.89 mg/L in WX13B (Table 3). The resistance ratio of abamectin in comparison to the baseline exceeded 100-fold in most of populations except GY12A (31.4-fold) and JS12 (62.9-fold). The highest resistance was 1494.7-fold in WX13B. Based on LC_{50} values, the resistance in populations from the six locations (Guanyang, Huizhou, Pudong, Jinshan, Taihe and Wuxi) to abamectin showed significant increase from 2012 to 2013.

RESISTANCE TO CHLORANTRANILIPROLE

LC_{50} values of chlorantraniliprole against DBM were in the range of 0.15 mg/L in WA13 and 37.27 mg/L in WX13B (Table 3). Most of the field populations were susceptible (RR < 3.0) to chlorantraniliprole during the 2 years, except for the BS13 (4.6-fold), HZ13 (5.3-fold) and PD13 (3.4-fold) with decreased susceptibility to low resistance. However, there was a progressive decreased susceptibility to chlorantraniliprole from 2012 to 2013. TH12 (24.8-fold) and TH13A (22.7-fold) had developed moderate level of resistance to chlorantraniliprole. TH13B (63.4-fold), WX12 (61.4-fold), WX13A (85.4-fold) and WX13B (162.0-fold) populations had developed high to very high level of resistance to chlorantraniliprole.

RESISTANCE TO SPINOSAD

LC_{50} values of spinosad against DBM ranged from 0.54 mg/L in SJ12 to 4.13 mg/L in WX12 (Table 3). The LC_{50} value of spinosad against DBM tended to increase with year from the data collected from the same locations, such as Huizhou, Guanyang, Taihe, Jinshan and Wuxi (Table 3). Most of populations (94%) exhibited low or moderate resistance to spinosad except 2 populations from GY12A (4.7-fold) and SJ12 (4.5-fold).

RESISTANCE TO BETA-CYPERMETHRIN

The LC_{50} values of field populations to beta-cypermethrin ranged from 89.02 mg/L in HZ12 to 4338.21 mg/L in TH12 (Table 3).The collected DBM, except the population of HZ12 which showed moderate resistance level (RR = 25.1), had developed high to very high resistance to beta-cypermethrin with resistance ratio varied from 76.2 (GY12A) to 1222.0-fold (TH12). The resistance increased significantly between 2 consecutive years in some locations, such as Huizhou, Jinshan, Pudong and Wuxi, while it tended to decrease from one year to the next in some other locations, such as Chongming, Nanchang and Taihe.

RESISTANCE TO CHLORFENAPYR

LC_{50} values of chlorfenapyr against DBM were in the range of 1.14 mg/L in GY12A and 106.64 mg/L in WX13B (Table 3).The resistance ratios to chlorfenapyr ranged from 2.9 to 31.0-fold in most field populations. However, 3 populations (WX12, WX13A and WX13B) collected from the same place (Wuxi, Jiangsu province) showed high to very high level resistance to chlorfenapyr. Generally, there was a progressive increase in resistance to chlorfenapyr over the 2-year period.

RESISTANCE TO DIAFENTHIURON

LC_{50} values of diafenthiuron against DBM were in the range of 10.78 mg/L in WA13 and 31.01 mg/L in TH13B (Table 3). The results showed that all of the field populations were susceptible to diafenthiuron.

Table 3. The resistance levels of 31 *Plutella xylostella* field populations to eight insecticides.

Population	Abamectin		Chlorantraniliprole		Spinosad		Beta-cypermethrin	
	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]
baseline	0.02 (0.01-0.03)		0.23 (0.18-0.28)		0.12 (0.09-0.14)		3.55 (3.05-5.21)	
BS13	3.89 (2.57-5.39)	194.7	1.06 (0.61-1.51)	4.6	1.35 (0.84-1.99)	11.3	1185.00 (809.29-1639.15)	333.8
CM12	3.37 (2.01-4.91)	168.3	0.22 (0.14-0.33)	0.9	0.72 (0.53-0.98)	6.0	727.33 (501.17-1033.25)	204.9
CM13	3.25 (1.86-4.8)	162.6	0.36 (0.24-0.47)	1.5	1.48 (1.04-1.92)	12.3	608.03 (410.75-835.66)	171.3
DC13	3.15 (2.33-4.02)	157.6	0.35 (0.25-0.46)	1.5	1.67 (1.17-2.15)	13.9	1285.12 (938.60-1695.22)	362.0
GY12A	0.63 (0.31-0.95)	31.4	0.26 (0.18-0.37)	1.1	0.57 (0.41-0.74)	4.7	270.41 (187.76-363.79)	76.2
GY12B	7.29 (4.17-10.14)	364.4	0.34 (0.25-0.45)	1.5	1.33 (0.87-1.82)	11.1	1448.27 (1021.01-1815.14)	408.0
GY13	5.33 (3.86-7.04)	266.5	0.32 (0.23-0.41)	1.4	1.79 (1.29-2.34)	14.9	956.88 (704.18-1247.39)	269.5
HX12	4.23 (2.89-5.74)	211.5	0.44 (0.31-0.60)	1.9	1.14 (0.76-1.52)	9.5	1124.96 (795.11-1560.76)	316.9
HZ12	11.00 (6.81-16.80)	549.9	0.29 (0.20-0.40)	1.3	1.48 (1.05-2.00)	12.3	89.02 (55.27-129.11)	25.1
HZ13	27.28 (17.75-39.21)	1364.0	1.22 (0.83-1.69)	5.3	2.14 (1.50-2.73)	17.8	542.16 (364.29-822.51)	152.7
JS12	1.26 (0.80-1.71)	62.9	0.20 (0.13-0.27)	0.9	0.75 (0.50-1.01)	6.2	472.80 (326.79-635.21)	133.2
JS13	3.58 (2.55-4.69)	178.8	0.50 (0.35-0.65)	2.2	1.25 (0.88-1.63)	10.4	941.34 (707.65-1196.65)	265.2
LC12	2.09 (1.45-2.82)	104.7	0.40 (0.23-0.69)	1.7	0.91 (0.65-1.19)	7.6	458.70 (261.44-734.29)	129.2
LC13	3.34 (2.42-4.32)	167.2	0.40 (0.28-0.52)	1.7	1.37 (0.97-1.82)	11.4	NA[b]	
MH12	2.58 (1.73-3.63)	128.9	0.31 (0.23-0.40)	1.4	1.03 (0.74-1.35)	8.6	613.61 (467.81-778.17)	172.9
MH13	3.34 (2.35-4.37)	167.0	0.39 (0.29-0.51)	1.7	1.60 (1.08-2.16)	13.3	961.68 (663.05-1276.76)	270.9
NC12	3.38 (0.64-5.92)	169.1	0.32 (0.22-0.44)	1.4	0.72 (0.48-1.00)	6.0	1588.09 (1110.04-2135.28)	447.4
NC13	4.88 (3.37-6.74)	243.9	0.41 (0.28-0.54)	1.8	2.06 (1.45-2.89)	17.1	1206.85 (870.52-1597.62)	340.0
NN12	2.65 (1.83-3.47)	132.4	0.42 (0.29-0.60)	1.8	1.17 (0.80-1.54)	9.7	598.50 (209.84-1025.16)	168.6
NN13	2.85 (1.91-3.83)	142.5	0.55 (0.36-0.76)	2.4	0.91 (0.66-1.19)	7.6	1238.53 (853.75-1953.38)	348.9
PD12	2.00 (1.33-2.76)	100.1	0.22 (0.15-0.29)	1.0	0.68 (0.49-0.89)	5.7	600.57 (419.82-804.87)	169.2
PD13	4.04(2.92-5.31)	202.2	0.78 (0.54-1.03)	3.4	1.85 (1.31-2.48)	15.4	1105.28 (832.44-1515.19)	311.4
SJ12	2.59 (1.68-3.74)	129.4	0.23 (0.17-0.29)	1.0	0.54 (0.37-0.71)	4.5	783.59 (525.66-1093.72)	220.7
SL13	6.42 (4.70-8.51)	321.2	0.54 (0.40-0.72)	2.4	1.58 (1.14-2.07)	13.1	986.78 (726.06-1268.93)	278.0
TH12	13.48 (9.49-18.98)	674.1	5.70 (3.43-8.11)	24.8	1.33 (0.95-1.72)	11.1	4338.21 (3008.95-5437.88)	1222.0
TH13A	11.36 (7.76-15.38)	568.1	5.21 (3.61-6.92)	22.7	1.55 (1.11-2.02)	12.9	2995.59 (1976.14-4009.93)	843.8
TH13B	26.34 (20.39-37.41)	1317.1	14.70 (10.59-18.42)	63.4	1.92 (1.53-2.34)	16.0	2412.66 (1101.31-3465.94)	679.6
WA13	2.46 (1.50-3.44)	122.8	0.15 (0.11-0.20)	0.7	0.75 (0.51-0.95)	6.2	483.48 (314.47-745.51)	136.2
WX12	15.10 (10.83-18.87)	754.8	14.12 (9.30-22.35)	61.4	4.13 (3.06-5.41)	34.4	1479.23 (842.36-2138.63)	416.7
WX13A	13.13 (8.94-22.11)	656.3	19.63 (13.48-26.18)	85.4	3.76 (2.61-6.50)	31.3	1782.50 (1277.23-2380.36)	502.1
WX13B	29.89 (23.82-38.68)	1494.7	37.27 (18.53-107.77)	162.0	4.01 (3.14-4.99)	33.4	3426.57 (2753.74-4433.93)	965.2

[a] Resistance Ratio
[b] Not Assayed

Table 3. (Continued) The resistance levels of 31 *Plutella xylostella* field populations to eight insecticides.

	Chlorfenapyr		Diafenthiuron		Chlorfuazuron		Btk	
Population	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]	LC_{50} (mg/L) (95%FL)	RR[a]
baseline	0.41 (0.20-0.79)		22.11 (18.52-46.11)		0.33 (0.11-0.58)		0.26 (0.03-0.50)	
BS13	3.91 (2.54-6.01)	9.5	19.74 (14.31-25.16)	0.9	0.28 (0.17-0.31)	0.8	2.62 (1.69-3.72)	10.0
CM12	1.32 (0.95-1.71)	3.3	NA		NA		NA	
CM13	2.10 (1.26-2.92)	5.1	20.42 (6.53-32.32)	0.9	0.96 (0.69-1.24)	2.9	NA	
DC13	4.54 (3.25-5.97)	11.1	20.53 (15.23-26.28)	0.9	0.69 (0.49-0.93)	2.1	NA	
GY12A	1.14 (0.64-1.63)	2.9	NA		NA		NA	
GY12B	2.29 (1.71-2.88)	5.6	20.68 (12.12-28.81)	0.9	0.35 (0.23-0.47)	1.1	NA	
GY13	3.82 (2.68-5.07)	9.3	29.93 (22.22-38.21)	1.4	0.41 (0.28-0.57)	1.2	1.82 (1.27-2.42)	6.9
HX12	1.66 (1.17-2.19)	4.0	27.3 (18.75-36.3)	1.2	0.24 (0.16-0.32)	0.7	NA	
HZ12	NA		NA		NA		0.99 (0.62-1.53)	3.8
HZ13	5.41 (3.38-9.19)	13.2	17.79 (11.86-26.62)	0.8	0.14 (0.09-0.21)	0.4	1.40 (0.78-2.07)	5.3
JS12	1.68 (1.24-2.13)	4.2	29.61 (16.81-43.53)	1.3	NA		NA	
JS13	4.16 (2.86-5.59)	10.2	22.38 (15.16-29.66)	1.0	0.48 (0.32-0.66)	1.4	2.45 (1.68-3.19)	9.4
MH12	2.06 (1.42-2.82)	5.1	12.11 (7.30-17.06)	0.6	NA		NA	
MH13	6.11 (4.18-7.98)	14.9	27.44 (18.80-36.21)	1.3	1.84 (1.15-2.49)	5.6	3.90 (2.67-5.49)	14.9
NC12	2.86 (2.01-4.85)	7.2	11.12 (7.14-15.27)	0.5	0.15 (0.06-0.24)	0.5	NA	
NC13	5.96 (4.45-7.80)	14.5	23.06 (16.19-31.48)	1.1	1.01 (0.68-1.51)	3.1	4.33 (2.74-5.82)	16.5
NN12	1.86 (1.29-2.46)	4.5	12.87 (9.20-16.88)	0.6	0.25 (0.17-0.35)	0.8	NA	
NN13	2.95 (1.90-4.06)	7.2	11.47 (7.49-15.44)	0.5	0.22 (0.15-0.31)	0.7	4.02 (2.65-6.04)	15.4
PD12	2.17 (1.35-3.16)	5.4	NA		NA		NA	
PD13	5.22 (3.71-7.08)	12.7	23.91 (17.77-30.06)	1.1	1.20 (0.87-1.57)	3.7	7.90 (5.21-17.41)	30.2
SJ12	1.42 (1.05-1.82)	3.6	18.29 (12.50-24.5)	0.8	NA		NA	
SL13	4.32 (3.22-5.56)	10.5	21.57 (15.42-28.52)	1.0	0.89 (0.60-1.17)	2.7	3.80 (2.67-5.16)	14.5
TH12	4.24 (2.62-5.79)	10.3	17.14 (10.41-23.26)	0.8	0.22 (0.13-0.30)	0.7	NA	
TH13A	5.12 (3.48-6.89)	12.5	21.05 (14.72-27.34)	1.0	0.25 (0.17-0.35)	0.8	1.56 (0.98-2.17)	5.9
TH13B	12.70 (6.72-17.35)	31.0	31.01 (23.30-46.73)	1.4	0.41 (0.30-0.57)	1.2	1.73 (1.27-2.24)	6.7
WA13	2.26 (1.40-3.14)	5.5	10.78 (6.96-14.56)	0.5	0.21 (0.14-0.29)	0.6	NA	
WX12	20.95 (13.81-29.15)	51.1	25.99 (16.75-35.57)	1.2	2.76 (1.81-5.05)	8.4	NA	
WX13A	32.12 (22.13-42.88)	78.3	22.09 (16.55-28.11)	1.0	2.86 (1.74-4.60)	8.7	2.21 (1.41-3.13)	8.4
WX13B	106.64 (59.82-141.57)	260.1	27.12 (19.70-34.90)	1.2	1.61 (1.15-2.09)	4.9	9.18 (6.46-17.87)	35.3

[a] Resistance Ratio
[b] Not Assayed

RESISTANCE TO CHLORFLUAZURON

LC_{50} values of chlorfluazuron against DBM were in the range of 0.14 mg/L in HZ13 and 2.86 mg/L in WX13A (Table 3). Three populations (MH13, WX12 and WX13A) from 2 locations showed low level resistance to chlorfluazuron. Three populations (NC13, PD13 and WX13B) exhibited decreased susceptible to chlorfluazuron. Most of populations remained susceptible to this insecticide.

RESISTANCE TO BTK

LC_{50} values of Btk against DBM ranged from 0.99 mg/L in HZ12 to 9.18 mg/L in WX13B thereby showing a narrow range (9.2-fold) of susceptibility among the 14 sampled field populations (Table 3). Compared with the LC_{50} of baseline (0.26 mg/L), 6 of 14 field populations exhibited moderate level resistance to Btk. Other populations showed decreased susceptibility and low level resistance to this insecticide.

PAIR-WISE CORRELATIONS BETWEEN TOXICITIES OF CHLORANTRANILIPROLE AND OTHER INSECTICIDES

The pair-wise correlations between the values of $\log LC_{50}$ of chlorantraniliprole and $\log LC_{50}$ of the seven insecticides were analyzed. The results showed significant correlation between DBM susceptibility to chlorantraniliprole and the 4 insecticides (abamectin, $R^2 = 0.788$, $P = 0.000$; spinosad, $R^2 = 0.758$, $P = 0.000$; chlorfenapyr, $R^2 = 0.869$, $P = 0.000$; and beta-cypermethrin, $R^2 = 0.668$, $P = 0.000$) (Table 4). In addition, significant correlation were presented at spinosad and chlorfenapyr with the other insecticides except for Btk. Btk only showed significant correlation with chlorfluazuron ($R^2 = 0.574$, $P = 0.040$) (Table 4).

DISCUSSION

In this study, field populations of DBM were surveyed for their susceptibilities to eight insecticides. The results suggested that the patterns of pesticides usage for controlling DBM were very similar across most places. Conventional synthetic pyrethroids beta-cypermethrin and abamectin may no longer be effective against DBM due to developed resistance. Because of extensive indiscriminate use of conventional insecticides and some newer insecticides (chlorantraniliprole, spinosad, chlorfenapyr), DBM has developed resistance to almost all kind of insecticides (Gong et al. 2010; Nehare et al. 2010; Santos et al. 2011; Sayyed et al. 2004; Wang et al. 2013; Zhao et al. 2002; Zhou et al. 2011). The study presented here suggests an overall increase in resistance for all the tested DBM populations to abamectin, chlorantraniliprole, spinosad, chlorfenapyr, chlorfluazuron and Bt from 2012 to 2013.

Abamectin had been widely used to control DBM until loss of efficacy in DBM was reported. In 1994, it had been observed that the DBM populations collected from Malaysia had developed high to very high levels of resistance (65-195 fold) to abamectin (Iqbal et al. 1996). In China, high to very high levels of resistance to abamectin in DBM populations was found in Shantou (RR = 75.9) and Guangzhou (RR = 122.4) (Zhou et al. 2011). A DBM field population collected from Tonghai in Yunnan province showed an extreme 5000-fold resistance to abamectin (Pu et al. 2010). Our results corroborate earlier findings that DBM populations in China have developed high levels of resistance to abamectin due to prolonged widespread use. Furthermore, according to the monitoring results, resistance to abamectin increased significantly in some locations from 2012 to 2013, such as Huizhou, Guanyang, Taihe, Jinshan and Wuxi. The similar resistance tendency to beta-cypermethrin was also detected and the level of resistance reported in this study was higher compared to previous report (Zhou et al. 2011), the reason might be beta-cypermethrin has been still extensively used as an admixture with other insecticides in China. Based on the above results, we suggest that abamectin and beta-cypermethrin should be suspended for controlling DBM in China due to high resistance.

Chlorantraniliprole was the first commercialized ryanodine receptor insecticide from a novel class of chemistry, the anthranilic diamides(Cordova et al. 2006; Nauen 2006). Although most the tested populations of DBM exhibited susceptibility to low resistance levels to chlorantraniliprole (RR = 0.7-5.3), resistance to chlorantraniliprole of DBM increased noticeably in individual locations (Taihe and Wuxi). Similarly, a very high level of resistance was reported in 2011 for a population from Zengcheng (RR > 2000-fold) in the Guangdong province of South China (Wang et al. 2013; Wang et al. 2010; Wang & Wu 2012). In our study, obvious variations of susceptibility (up to 242-fold between WA13 and WX13B populations) existed among the populations, which was similar to previous studies (Wang & Wu 2012). However, the resistance ratios of the Zengcheng population declined to 25-fold after 6 generations without selection (Wang et al. 2013). It indicated that a very high-level resistance to chlorantraniliprole in a field population was unstable. Hence, chlorantraniliprole should be rotated with other insecticides with different modes of action in the locations where very high level of resistance was found.

Spinosad was an effective insecticide for control DBM in China. In this study, DBM showed decreased susceptibility to moderate level of resistance to spinosad. This finding is consistent with previous research (Attique et al. 2006; Eziah et al. 2008). However, because of the extensive application of this chemical in DBM control, where most farmers had used spinosad for DBM control since late 1990s, very high resistance has been reported in Californian and Cameron High-lands (Sayyed et al. 2004; Sayyed & Wright 2006; Zhao et al. 2006). Although spinosad has a unique mode of action and has no cross-resistance to conventional insecticides, subsequent resistance of DBM to spinosad needs to be investigated.

There was a progressive increase in resistance to chlorfenapyr from 2012 to 2013. Chlorfenapyr has not been used as frequently as abamectin and beta-cypermethrin in China. Consequently, the development

Table 4. Pair-wise correlation coefficient analysis between $\log LC_{50}$ values of the insecticides.

	Abamectin	Chlorantraniliprole	Spinosad	Beta-cypermethrin	Chlorfenapyr	Diafenthiuron	Chlorfluazuron
Chlorantraniliprole	0.788**						
Spinosad	0.777**	0.758**					
Beta-cypermethrin	0.496**	0.668**	0.439*				
Chlorfenapyr	0.768**	0.869**	0.878**	0.647**			
Diafenthiuron	0.338	0.349	0.522**	0.244	0.410*		
Chlorfluazuron	0.173	0.389	0.752**	0.132	0.645**	0.576**	
Btk	-0.263	0.122	0.227	0.469	0.330	0.027	0.574*

*Means positive correlation between LC_{50} values of insecticides at 95% significant level.
**Means positive correlation between LC_{50} values of insecticides at 99% significant level.

of resistance to this insecticide was not so rapid. But Wuxi population in Jiangsu province showed a very special information where very high level of resistance to chlorfenapyr was observed (RR = 260.1, WX13B). It suggested that resistance monitoring must be strengthened for this population.

Resistance to diafenthiuron and chlorfluazuron was maintained at comparatively susceptible levels. As diafenthiuron and chlorfluazuron have not being extensively used in China, resistance to them was not as high. They might be used as alternatives in regions where others insecticides are no longer effective owing to resistance. Although Btk had been used to control DBM for a long time, all of the tested field populations were not found to have a high level of resistance to this insecticide. It might be due to the overall effectiveness of Btk being as a result of multiple genes, each of them making a small contribution to overall resistance (Bourguet 2004). But high and extremely high level resistance to Bt had been reported by Xia et al. (2014) in central China (Xia et al. 2014), this may be the result of this microbial insecticide was developed by Hubei Academy of agricultural Science, which lead to its widely application over the past 30 years.

Based on the results, the resistance level of DBM populations to chlorantraniliprole, abamectin, spinosad, chlorfenapyr and beta-cypermethrin showed a tendency that the DBM populations collected in autumn exhibited higher resistance level than the populations collected in the same location in spring or early summer in the same year, such as, the populations collected in Wuxi or Taihe in 2013 and Guanyang in 2012 (Table 3). Due to suffering from continuous and high selection pressure, the populations collected in autumn would be more tolerant to these insecticides. In this study, the Wuxi population, collected from Jiangsu province in 2013 September, had developed a very high level of resistance to most of the tested insecticides, including some newer insecticides, such as chlorantraniliprole and chlorfenapyr. Wang & Wu (2012) have reported DBM population in Zengcheng showing resistance to chlorantraniliprole as high as 2,000-fold (Wang & Wu 2012). They attributed rapid evolution of very high level of resistance to chlorantraniliprole by DBM in Zengcheng to intensive use and misuse of the insecticide. However, the rice stem borer in different geographic populations have been inconsistent with their responses to this insecticide, and the obvious variation of susceptibility to chlorantraniliprole also existed in *Chilo suppressalis* Walker (Su et al. 2014).

The existing variation in resistance to chlorantraniliprole may be brought about by the genetic diversity of the test populations, different insecticide exposure histories or other factors. To analyse the relationship between chlorantraniliprole resistance and insecticide exposure history, the pair-wise correlation between LC_{50} values of chlorantraniliprole and the other seven insecticides were analyzed. Significant correlation were found between chlorantraniliprole resistance and the resistance to the abamectin, spinosad, chlorfenapyr and beta-cypermethrin ($R^2 > 0.6$), indicating that the resistance to chlorantraniliprole in DBM populations was related to the resistance of these same populations to other insecticides. The significant correlation between the resistance to different insecticides implies that the mechanisms of the insecticide are likely to share some common biochemical pathways and genetic mutations resulting in resistance for one insecticide (abamectin, spinosad, chlorfenapyr or beta-cypermethrin) was highly likely to increase the resistance against chlorantraniliprole as well. The correlation structure among insecticides would be great value in agriculture applications by facilitating the design of effective insecticides combinations and strategies.

Although insecticides play a vital role in DBM management, their extensive use have resulted in the insect developing resistance to many of these chemical insecticides. In our study, DBM collected in Wuxi in 2013 September, have developed high resistance to most of the tested insecticides. However, diafenthiuron and chlorfluazuron remained effective against DBM in all tested populations. Therefore, it is crucial to integrated pest management that monitoring resistance of widely diverse field populations of DBM in China should be strengthened. Moreover, the sequence of insecticide application should be adjusted according to changes in insecticide susceptibilities of DBM. To prevent the resistance development, the author suggested that the combination of insecticide rotation and mixture of insecticides could be practiced in the field control of DBM infestation.

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