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Preliminary Study on Resistance of the Rice Stem Borer (*Chilo Suppressalis*) to Fipronil

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Abstract: By means of topical application, fipronil resistance was surveyed in the rice stem borer, *Chilo suppressalis*, from 12 diferent areas in east China from 2001 to 2004. The rice stem borers in most regions of Jiangsu and Anhui were still susceptible to fipronil. But in Wuxi, Jiangsu Province and Cixi, Zhejiang Province, their sensitivity became decreased (resistance ratios were 3.1 and 3.6, respectively), and the medium level of resistance (resistance ratio was 21.2) was found in Cangnan, Zhejiang Province. So, it was still at the early stage for fipronil resistance development in this pest. Synergism experiments showed that piperonyl butoxide(PBO) had a little effect on both susceptible and resistant borers (synergism ratios were 1.1–1.2). Though triphenyl phosphate (TPP) and diethyl meleate (DEM) had no effect on the susceptible borers, they had significant synergism on fipronil in the resistant population to fipronil (synergism ratios were 1.8 and 1.6, respectively), indicating esterase and glutathion S-transferase may be involved in the resistance mechanism. Bioassay with currently used insecticides indicated that triazophos (because of high resistance), trichlorphon and acephate had very low toxicity to resistant borers. But diazinon, pyridaphenthion, decamethrin and avermectin showed high toxicity and had no cross resistance to fipronil, which could be considered as substitute insecticides in the resistance managment.

Key words: *Chilo suppressalis*; fipronil; resistance; relative toxicity

The rice stem borer (*Chilo suppressalis* Walker) is one of the most important rice pests. For control of this pest, nereistoxin and organic phosphate insecticides, such as monosultap (or bisultap), methamidophos and triazophos etc had been applied for a long period in China, which had led to serious resistance [1–4]. The novel phenylpyrazole insecticide, fipronil, exhbits excellent control effect against many pests, particular to those lepidopterons with high resistance to pyrethroid, organic phosphate and carbamate insecticides. Therefore, it had been introduced as a substitute when the rice stem borer developed high resistance against monosultap in Zhejiang and south Jiangsu in 1997–1998 [5, 6]. For delaying the development of resistance to fipronil, protective resistance management must be put into action. So, we made survey on the resistance with borers collected from different regions, selected effective insecticides, analyzed the possible resistance mechanism, and proposed some suggestion for rational use of insecticides in controlling the pest.

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MATERIALS AND METHODS

Insects

The rice stem borers were collected from Cangnan and Cixi County in Zhejiang Province, Changshu, Wuxi, Gaochun, Jiangpu, Jiangning, Lishui, Jiangdu, Dongtai and Funing in Jiangsu Province from April to May in 2001–2004. The susceptible strain (Th-S) of the rice stem borer was originally collected from the mountain area of Taihu County, Anhui Province, where few pesticides were traditionally used for rice pest control. Thereafter the stem borers were raised in laboratory with the seeding method reported by Shang et al [7]. The rearing conditions were 28±1℃, light cycle 16 h light / 8 h dark and relative humidity > 80%.

Insecticides and chemicals

Fipronil (98% a.i.) was obtained from Rhone-Poulenc Company in France. Triazophos (98% a.i.) was the product of Shenyang Research Institute of

Chemical Industry in China. Pyridaphenthion (90% a.i.) was supplied by Chizhou Xinsaide Chemical Reagent Limited Company in Anhui. Diazinon (95% a.i.) was supplied by Heben Pesticide Limited Company in Zhejiang. Trichlorphon (90% a.i.) was supplied by Shandong Pesticide Factory. Acephate (99.2% a.i.) was supplied by Suzhou Chemical Reagent Company in Jiangsu. Avermectin (96% a.i.) was supplied by Huabei Pharmacy Factory. Decamethrin (99.9% a.i.) was a gift from Professor Jiang Mugeng, Nanjing Agricultural University. Triphenyl phosphate (TPP, Reagent grade) was from Beijing Chemical Reagent, diethyl meleate (DEM, chemical grade) was from Shanghai Chemical Reagent in China and piperonyl butoxide (PBO, reagent grade) was from Sigma Chemical.

Bioassay and synergism

The bioassay followed the micro topical application technique reported by Chen et al [8]. Fourth-instar larvae of the rice stem borers were used as test insects in this study. Insecticides were diluted into a series of concentrations with acetone and a droplet of 0.04 μL insecticide solution was applied topically on the middle-abdomen notum of the larvae with a hand microapplicator (Burkard Manufacturing, Richmansworth, England). Thirty larvae were treated with each concentration. Control larvae were treated with acetone alone. In the synergism experiments, borers were treated with synergist (DEM 1.6 μg / insect, PBO and TPP 3.2 μg / insect) 1 h prior to the insecticide application. The treated larvae were then reared and the mortality was recorded in 72 h for fipronil and Avermectin, and 48 h for other insecticides. Insects were considered dead if no movement was observed after being touched gently. LD_{50} values were calculated according to mortality probit analysis method. The synergism ratio (SR) was calculated as follows:

SR=LD_{50} value of insecticide alone / LD_{50} value of insecticide after synergist.

RESULTS

Fipronil resistance monitoring in the rice stem borers from different regions

Fipronil resistance had been monitored with the rice stem borers collected from 12 counties in Jiangsu, Zhejiang and Anhui Provinces from 2001 to 2004. As listed in Table 1, the results revealed that fipronil was highly toxic to the rice stem borer. The LD_{50} to the Th-S population was only 2.5 ng per larva. The borers in most areas from Jiangsu were still susceptible to fipronil, except those from Wuxi, Jiangsu and Cixi, Zhejiang exhibited stronger tolerance [Resistance ratio (RR) were 3.1 and 3.6, respectively]. However, it was

Table 1. Fipronil resistance in *Chilo suppressalis* collected from different regions.

Population / Tested year	LD-*P* line	LD_{50} (95% confidence interval, μg/insect)	Resistance ratio (RR)
Taihu, Anhui / 2001	y =13.3718+3.1804x	0.0025 (0.0020–0.0029)	1.0
Taihu, Anhui / 2002	y =12.2020+2.8220x	0.0028 (0.0203–0.0039)	1.1
Funing, Jiangsu / 2002	y =13.4210+3.1453x	0.0025 (0.0019–0.0031)	1.0
Dongtai, Jiangsu / 2001	y =10.9986+2.3205x	0.0026 (0.0020–0.0035)	1.0
Jiangning, Jiangsu / 2001	y =14.9513+3.9591x	0.0031 (0.0026–0.0037)	1.2
Gaochun, Jiangsu / 2001	y =8.7597+1.5405x	0.0036 (0.0026–0.0051)	1.4
Jiangdu, Jiangsu / 2001	y =10.5909+2.3320x	0.0040 (0.0032–0.0050)	1.6
Lishui, Jiangsu / 2001	y =16.0942+4.7141x	0.0044 (0.0039–0.0051)	1.8
Changshu, Jiangsu / 2001	y =12.7483+3.3407x	0.0048 (0.0039–0.0059)	1.9
Jiangpu, Jiangsu / 2001	y =10.6779+2.4912x	0.0053 (0.0044–0.0063)	2.1
Wuxi, Jiangsu / 2001	y =11.5069+3.0861x	0.0078 (0.0064–0.0095)	3.1
Cixi, Zhejiang / 2001	y =9.5444+2.2273x	0.0091 (0.0077–0.0108)	3.6
Cangnan, Zhejiang / 2002	y =8.4451+2.7020x	0.0531 (0.0439–0.0668)	21.2
Cangnan, Zhejiang / 2003	y =8.8476+2.7516x	0.0400 (0.0326–0.0491)	16.0
Cangnan, Zhejiang / 2004	y =9.7432+2.8118x	0.0206 (0.0182–0.0233)	8.2

The resistance ratio was calculated based on the LD_{50} (0.0025 μg/insect) of the susceptible strain(Th-S) from Taihu in Anhui Province.

found that the field population in Cangnan County had developed resistance against fipronil (RR were 8.2–21.2). But the resistance had dropped in recent years (RR were from 21.2 in 2002 to 8.2 in 2004).

Synergism of TPP, PBO, and DEM on fipronil

The synergism of TPP, PBO, and DEM on fipronil in both susceptible and resistant populations was tested. The results (Table 2) demonstrated that PBO had a little effect on fipronil toxicity in both susceptible and resistant populations. TPP and DEM exhibited little synergism in susceptible population. But they showed good synergism in resistant population (SR were 1.8 and 1.6, respectively). The resistance ratio was depressed obviously.

Toxicity of several common insecticides to the rice stem borer from resistant region (Cangnan, Zhejiang)

The toxicity of several common organic phosphate insecticides, decamethrin and avermectin was evaluated and compared against the resistant and susceptible rice stem borers (Table 3).

The results in Table 3 showed that Cangnan-resistant population had developed medium and high level resistance to trichlorphon, fipronil and triazophos, but still kept susceptibility to other insecticides. Based on the relative toxicity index, it was found that acephate exhibited very low toxicity against this pest. Triazophos and trichlorphon had also very low toxicity to Cangnan population because of the resistance. Fipronil was still high toxic, even to Cangnan population, which had developed medium level resistance. Pyridaphenthion, diazinon, decamethrin and avermectin all showed high relative toxicity and no cross resistance existed to fipronil or triazophos.

DISCUSSION

The novel phenylpyrazole insecticide fipronil has the characteristic of unique action mechanism and prominent control effect against the rice stem borers. For rational use of this insecticide, it is necessary to carry out resistance monitoring and management. In our work, we found that the rice stem borer from Cangnan in Zhejiang had developed resistance to fipronil (RR 21.2), and then went on successive monitoring. It was found that resistance level of Cangnan population dropped continuously in recent years. Investigation on insecticides use status in the area revealed that the import fipronil was used in a

Table 2. Synergism of PBO, TPP and DEM on fipronil in the resistant and susceptible stem borers (2003).

Treatment	Susceptible		Resistant		Resistance ratio
	LD_{50} (95% confidence interval, μg/insect)	SR	LD_{50} (95% confidence interval, μg/insect)	SR	
Fipronil	0.0023 (0.0021–0.0032)	-	0.0400 (0.0326–0.0491)	-	17.4
Fipronil+PBO	0.0021 (0.0018–0.0029)	1.1	0.0324 (0.0253–0.0415)	1.2	15.4
Fipronil+DEM	0.0026 (0.0022–0.0033)	0.9	0.0247 (0.0194–0.0331)	1.6	9.5
Fipronil+TPP	0.0025 (0.0020–0.0035)	0.9	0.0223 (0.0166–0.0328)	1.8	8.9

SR (synergism ratio) was calculated as LD_{50} of insecticide alone / LD_{50} of insecticide plus synergist.

Table 3. Toxicity of several common insecticides against *Chilo suppressalis* from resistant and susceptible regions.

Insecticide	LD_{50} of susceptible borer (95% confidence interval, μg/insect)	LD_{50} of resistant borer (95% confidence interval, μg/insect)	Resistance ratio	Toxicity index
Triazophos	0.0059 (0.0039–0.0071)	1.7577 (1.3268–2.3286)	297.0	1.0
Acephate	0.5615 (0.3771–0.9267)	1.5233 (1.1646–1.9921)	2.7	1.2
Trichlorphon	0.1093 (0.0589–0.1826)	1.4002 (1.1119–1.7837)	12.8	1.3
Diazinon	0.0492 (0.0383–0.0631)	0.2732 (0.2177–0.3428)	5.6	6.5
Pyridaphenthion	0.0351 (0.0220–0.0461)	0.2073 (0.1637–0.2625)	5.9	8.4
Fipronil	0.0025 (0.0020–0.0029)	0.0400 (0.0326–0.0491)	16.0	44.0
Decamethrin	0.0022 (0.0017–0.0028)	0.0050 (0.0032–0.0077)	2.3	352.0
Avermectin	0.0003 (0.0002–0.0004)	0.0007 (0.0005–0.0009)	2.3	2514.0

great quantity when the rice stem borer of Cangnan developed resistance against monosultap in 1997. Thereafter, the cheap insecticide mixtures without fipronil had been developed and used extensively. So, the use of fipronil decreased in succession. This is corresponding to the resistance development trend we had monitored. This situation revealed that not only the rice stem borer has risk to develop high resistance to fipronil, but also the resistance development could be delayed by using more than one insecticide alternately.

Synergistic test revealed that TPP and DEM had distinct synergism in resistant population. This means that esterase and glutathione S-transferase may be involved in resistance mechanism. However PBO had a little synergism in resistant population (SR was 1.2), which was only 1.09-fold higher than that in susceptible strain. The effect of PBO on fipronil seems varied with insects. Scott et al [9] reported that PBO didn't exhibit synergism on fipronil in cross-resistant strain of German cockroach, but this was contrary in cross-resistant strain of housefly. Valles et al and Liu et al also came to a similar conclusion [10,11]. Thus, it can be deduced that fipronil is metabolized differently by microsomal oxidases in different insects. The present study showed that the resistance ratio decreased in small extent with PBO treatment, implying that microsomal oxidases might be involved in resistance mechanism but not as a major factor.

Toxicity test of several common insecticides showed that the borers from resistant region (Cangnan) had developed high- and medium-level resistance to triazophos, trichlorphon and fipronil. But this population didn't exhibit significant resistance to other insecticides. Triazophos, trichlorphon and acephate had much low toxicity to be used in controlling the resistant population. Fipronil remained high toxic though medium-level resistance had developed. Therefore, it might be kept on using to control the borers. But it would be necessary to put resistance management into action and reduce its use. Pyridaphenthion, diazinon, decamethrin and avermectin showed high toxicity and had no cross resistance to fipronil, which could be considerated as substitute insecticides in the resistance management.

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