Research Article

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Responses of striped stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), from Taiwan to a range of insecticides

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Abstract

BACKGROUND: Information on the insecticide susceptibility of striped stem borer, *Chilo suppressalis* (Walker), is essential for an effective pest management programme. An early detection of resistance development can prompt the modification of current control methods and increase the lifespan of insecticides through the rotation of chemicals with different modes of action. In this study, the susceptibility of this pest in Taiwan to four classes of insecticides has been examined.

RESULTS: Over 1000-fold resistance to carbofuran was detected in *C. suppressalis* collected from Chiayi and Changhua prefectures, with estimated LC_{50} values of >3 mg cm^{-2}. In addition, 61-fold resistance to cartap was found in the Chiayi population. On the other hand, all tested populations of rice stem borer were still relatively susceptible to chlorpyrifos, fipronil and permethrin, with LC_{50} values ranging from 30 to 553 ng cm^{-2}. *Chilo suppressalis* populations collected from the central parts of Taiwan have a higher degree of resistance to the tested insecticides than those from northern areas.

CONCLUSION: The occurrence of high resistance to carbofuran in the Chiayi and Changhua areas suggests that this compound should be replaced with chemicals having a different mode of action, such as chlorpyrifos, fipronil and permethrin, to which low cross-resistance has been detected.

Keywords: susceptibility; resistance; striped stem borer; *Chilo suppressalis*; pest management; cross-resistance

1 INTRODUCTION

The striped stem borer, *Chilo suppressalis* (Walker), is an important insect pest of rice in India, south-east Asia, China, Iran and southern Europe. It infests rice plants from the seedling stage to maturity and may cause deadhearts and whiteheads during the vegetative and reproductive stages respectively.[1,2] Before 1960, *C. suppressalis* was only a minor insect pest in northern Taiwan, while yellow stem borer, *Scirpophaga incertulas* (Walker), was the most pervasive and injurious rice stem borer throughout the island. However, the population of *C. suppressalis* started to spread out and caused serious damage in southern areas of Taiwan between 1958 and 1964, when the density of *S. incertulas* decreased dramatically owing to shifts in cultural practices, such as earlier transplanting and the synchronising of cultivation periods among farmers.[3] A demand for rice straw to export to Japan and an intercropping between the two annual rice crops in the 1980s have seen *C. suppressalis* become a major pest again, causing up to 10–20% deadhearts and >5% whiteheads in certain areas.[4]

Various tactics, including varietal resistance, *Bt* rice, cultural practices, insecticides and biological control, have been used to control rice stem borers, while insecticides are still preferred by farmers. Before 1990, carbofuran, EPN, monocrotophos and cartap were the most commonly used control agents in Taiwan for *C. suppressalis*.[2] Currently, fipronil is used to treat rice seedlings in nursery boxes,[5] while cartap, chlorpyrifos, fenthion and other insecticides have been recommended for controlling *C. suppressalis* in the field.[6] Under intensive use, EPN, carbofuran and cartap were found to lose their efficacy against *C. suppressalis* in the field during the early 1990s.[7] However, no further study has been performed to confirm the development of resistance in these striped stem borers to these chemicals.

In this paper, the susceptibility and resistance development potential of *C. suppressalis* to five insecticides with four different modes of action were evaluated. These results provide valuable information for effective resistance management and at the same time can prolong the lifespan of effective compounds for *C. suppressalis* control in Taiwan.

2 MATERIALS AND METHODS

2.1 Insects

Chilo suppressalis larvae were gathered from rice stalks showing damage symptoms, i.e. deadhearts or whiteheads, which were collected from different prefectures of western Taiwan (Fig. 1) in

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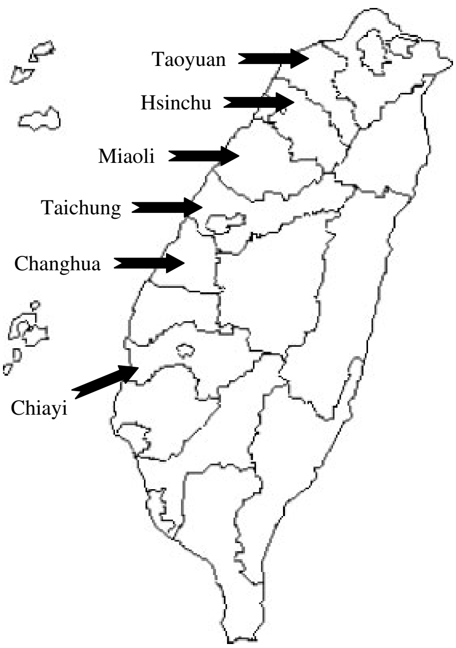

Figure 1. Collection sites of *Chilo suppressalis* in western prefectures of Taiwan.

Table 1. Collection dates and numbers of *Chilo suppressalis* collected from different prefectures during 2006 and 2007

Prefecture	2006		2007	
	Date	Number	Date	Number
Taoyuan	13 July	424	13 May	6
	31 October	221	6 November	14
Hsinchu	13 July	145	28 June	208
	14 October	36	12 September	114
Miaoli	23 June–10 July	338	3 October	48
Taichung	23 May–2 July	571	2 June	2
	29 October	50	15 October	2
Changhua	26 May	373	28 April	89
	6 October	508	1 October	355
Chiayi	20 October	66	18 October	252

2006 and 2007. The dates of collection and the numbers of larvae collected from each site at each time are listed in Table 1. This *C. suppressalis* population was first enlarged for one generation before bioassay by rearing the collected larvae separately in plastic containers (21 cm diameter × 7 cm high) with artificial diet modified from Cheng[8] with improved solubility of cholesterol and lecithin. The pupation of *C. suppressalis* was facilitated by putting corrugated papers in the container. Twenty pupae were put into zip-lock bags (34 × 24 cm^2) filled with air and sprayed with a blended solution of rice leaves, and egg masses laid on the bag surface were collected and treated with 0.5 g L^{-1} benomyl for 10 min. They were put on wet filter papers in a petri dish until their colour turned black, and were then moved into diet-filled containers for hatching. Both larvae and adults were reared in a growth chamber at 25 ± 1 °C and 16 : 8 h light : dark photoperiod.

2.2 Insecticides

Technical carbofuran (984 g kg^{-1}), chlorpyrifos (953 g kg^{-1}), cartap (950 g kg^{-1}) and permethrin (920 g kg^{-1}) were provided by Sinon Corporation (Taichung, Taiwan). Analytical-grade standard fipronil (989 g kg^{-1}) was provided by Bayer CropScience.

2.3 Bioassay

The susceptibility test of *C. suppressalis* was performed by a modified filter paper method[9,10] that could establish effectively the dose response and was much more convenient than the classic topical application suggested by FAO.[11] All insecticides were serially diluted with analytical-grade acetone, except cartap, which was diluted with distilled water. Whatman® No. 1 filter papers with a diameter of 7 cm were put in glass petri dishes with a diameter of 9 cm, and 0.5 mL of various insecticide concentrations was added to each petri dish. After 30 min air drying, 0.5 mL distilled water was added to keep the filter paper moist before ten fourth-instar larvae of *C. suppressalis* were placed in the dish. Five to six concentrations for each insecticide and at least four replicates for each concentration of all insecticides were performed. The control tests used water-impregnated filter papers for cartap and acetone-impregnated filter papers for the other insecticides. Mortality of rice stem borers in each petri dish was determined 24 h after treatment.

2.4 Data analysis

Mortality data in dose–response bioassays were analysed by probit analysis,[12] using a computer program developed in the Department of Entomology, National Chung-Hsing University.[13] The software provides the linearity of dose–mortality response and determines the slope, the lethal concentrations (LC), the 95% fiducial limits (FL) of LC_{50}, and chi-square of each line tested. The resistance ratio (RR) of field populations was calculated by dividing the LC_{50} of each field population by the corresponding LC_{50} of the Hsinchu population, which had not received insecticides for more than 20 years.

3 RESULTS AND DISCUSSION

This study revealed the variations in insecticide susceptibility in different populations of *C. suppressalis* and the potency of these compounds with different modes of action. Table 2 shows the susceptibility of six field populations of *C. suppressalis* from western prefectures of Taiwan to five insecticides representing four modes of action, i.e. carbofuran, chlorpyrifos, cartap, permethrin and fipronil.

3.1 Carbofuran

There were significant differences in the susceptibility of six field populations of *C. suppressalis* to carbofuran (Table 2). Among them, the Hsinchu population was the most susceptible to this acetylcholinesterase inhibitor (AChEI), with an LC_{50} of 2.73 µg cm^{-2}; according to the owner of the farm where the stem borer was collected, no insecticides have been applied to his paddy field for the past 20 years. In general, the susceptibility to carbofuran of this stem borer decreased from the north (Miaoli and Taoyuan) to the south (Taichung, Changhua and Chiayi), probably as a result of a higher frequency of insecticide applications in the south than in the north of Taiwan (Fig. 2). This insecticide dramatically lost its effect against Changhua and

Table 2. Susceptibility of six field populations of *Chilo suppressalis* to five insecticides

Insecticide/population	LC_{50}	95% FL	Slope (SE)	χ^2 (df)	RR[a]
Carbofuran	(μg cm^{-2})				
Taoyuan	111.9	93.28–138.5	1.03 (0.24)	0.15 (3)	41.1
Hsinchu	2.73	0.91–4.68	1.48 (0.30)	2.78 (3)	1.0
Miaoli	61.71	51.06–82.50	1.23 (0.45)	0.19 (3)	22.6
Taichung	1120	568.4–356 200	1.03 (0.29)	5.03 (4)	410.3
Changhua	3269	1547–395 700	0.91 (0.33)	0.47 (2)	1198.2
Chiayi	UD[b]	–	–	–	UD[b]
Chlorpyrifos	(ng cm^{-2})				
Taoyuan	9.87	7.80–12.21	3.87 (0.65)	0.71 (2)	0.5
Hsinchu	21.05	18.19–24.17	4.36 (0.54)	1.20 (3)	1.0
Miaoli	11.04	8.70–13.90	3.76 (0.63)	0.74 (2)	0.5
Taichung	31.70	22.74–42.49	2.71 (0.35)	0.92 (2)	1.5
Changhua	75.87	52.10–133.6	1.86 (0.44)	0.53 (2)	3.6
Chiayi	97.05	77.56–126.2	3.01 (0.40)	3.57 (4)	4.6
Cartap	(ng cm^{-2})				
Taoyuan	83.15	69.25–99.26	2.07 (0.37)	0.17 (2)	1.7
Hsinchu	47.94	0.53–105.8	1.80 (0.36)	2.13 (2)	1.0
Miaoli	185.1	122.5–277.5	1.82 (0.36)	0.58 (2)	3.9
Taichung	1076	864.0–1374	2.19 (0.34)	0.38 (2)	22.5
Changhua	477.5	393.7–548.8	2.10 (0.36)	0.20 (2)	10.0
Chiayi	2922	2226–3695	3.51 (0.46)	4.74 (4)	61.0
Permethrin	(ng cm^{-2})				
Hsinchu	91.21	77.69–106.8	2.70 (0.43)	0.19 (2)	1.0
Miaoli	76.78	31.44–140.4	1.56 (0.24)	3.95 (3)	0.8
Taichung	465.2	378.1–580.9	1.39 (0.19)	0.88 (4)	5.1
Changhua	474.0	400.2–565.2	2.36 (0.38)	0.19 (2)	5.2
Chiayi	554.0	426.3–690.0	2.74 (0.44)	0.38 (2)	6.1
Fipronil	(ng cm^{-2})				
Hsinchu	30.14	28.19–32.35	3.22 (0.41)	0.16 (3)	1.0
Taichung	62.75	46.12–81.07	1.94 (0.32)	0.42 (2)	2.1
Changhua	68.60	59.37–78.22	3.63 (0.49)	0.75 (3)	2.3
Chiayi	44.69	36.25–54.44	2.78 (0.37	1.09 (3)	1.5

[a] RR: resistance ratio = LC_{50} of field population/LC_{50} of the Hsinchu susceptible population for each insecticide.
[b] UD: undetectable. Under the highest attainable concentration (100 mg mL^{-1}), the mortality only reached 8.3% (5/60) and an LC_{50} value could not be obtained for the Chiayi population.

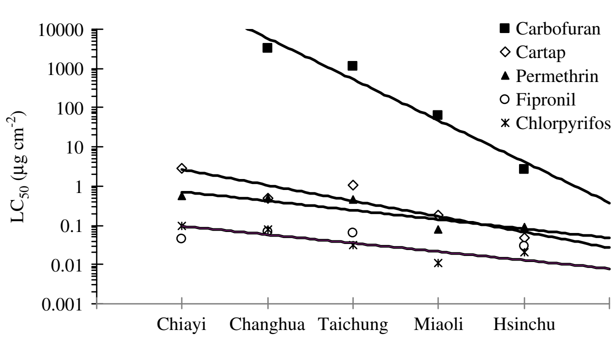

Figure 2. Correlation of geographic variation and insecticide susceptibility to carbofuran, cartap, permethrin, fipronil and chlorpyrifos in *Chilo suppressalis*.

Chiayi populations: the mortalities of these two populations were 38.3 and 8.3%, respectively, under the highest concentration (100 mg mL^{-1}) feasible for bioassay. Therefore, the LC_{50} value of the Changhua population (3269 μg cm^{-2}) was estimated by extrapolation, and the LC_{50} value of the Chiayi population was even higher than that of the Changhua population. Compared with the LC_{50} of the Hsinchu population, the Taichung, Changhua and Chiayi populations have developed respectively 410-fold, 1200-fold and >1200-fold resistance to carbofuran, which is consistent with the failure of this insecticide to control *C. suppressalis* in the 1990s.[7] To prevent the misuse of this chemical, replacement with insecticides having different modes of action should be carried out in these areas to prevent subsequent environmental impact caused by carbofuran resistance.

3.2 Chlorpyrifos

Chlorpyrifos has been a very effective control agent against all *C. suppressalis* populations collected from western Taiwan. The LC_{50} values of the six populations to chlorpyrifos range from 9.87 to 97.05 ng cm^{-2}, i.e. only about tenfold difference in susceptibility (Table 2). It is worth noting that the *C. suppressalis* populations with more than 1000-fold resistance to carbofuran had less than tenfold resistance to chlorpyrifos (Table 2). A similar situation has also been observed in the triazophos-selected Cn-R strain of *C. suppressalis*, which had developed 787-fold resistance to triazophos and yet only sixfold resistance to methomyl.[14] Although both carbamates and OPs share the same mode of action by competing with acetylcholine and inhibiting the activity of acetylcholinesterase (AChE) in target insects, no obvious cross-resistance between these two classes of insecticides was found in *C. suppressalis* populations from Taiwan and mainland China. Most cases of carbamate resistance have been mediated by target-site mutation, and, so far, OP resistance has been related to metabolic changes.[15] However, a recent report has demonstrated that the triazophos resistance of *C. suppressalis* was related to the point mutation of A314S on CsAChE.[16] Unpublished data of the present authors also indicate that part of the carbofuran resistance in *C. suppressalis* in Taiwan is associated with a point mutation near the C-terminal end of CsAChE. These results suggest that the absence of obvious cross-resistance between chlorpyrifos and carbofuran or between triazophos and methomyl in *C. suppressalis* could be due to the fact that they bind to different sites of AChE, and so suggest the possibility of an alternative use of these two subclasses of AChEI for *C. suppressalis* control.

3.3 Cartap

Cartap, a broad-spectrum insecticide with good activity against rice stem borers and other insect pests on rice,[17] has been used to control *C. suppressalis* in Taiwan for more than 20 years.[2] As with carbofuran, there were significant differences in the toxicity of cartap to the tested populations. The LC_{50} values varied from 47.94 ng cm^{-2} for the most susceptible Hsinchu population to 2922 ng cm^{-2} for the least susceptible Chiayi population (Table 2). The Chiayi population of *C. suppressalis* has only developed 61-fold resistance to cartap, and the resistance levels of the Taichung and Changhua populations were under 23-fold. Although cartap is suspected to have lost its efficacy in the field,[7] the rate of resistance development to cartap appears to be much slower than to carbofuran (Table 2). Therefore, a proper rotation design for insecticides with different modes of action and continuous monitoring for resistance development would provide a chance to manage cartap resistance, and extend its lifespan for controlling *C. suppressalis*.

3.4 Permethrin

Permethrin, a synthetic pyrethroid with low mammalian toxicity, might be an alternative choice for controlling early-instar larvae of *C. suppressalis* before they migrate from leaves into stems. Table 2 shows that the susceptibilities among different field populations of *C. suppressalis* could be divided into two groups. The populations of Hsinchu (LC_{50} = 91.21 ng cm^{-2}) and Miaoli (LC_{50} = 76.78 ng cm^{-2}) were more sensitive than the populations of Taichung (LC_{50} = 465.2 ng cm^{-2}), Changhua (LC_{50} = 474.0 ng cm^{-2}) and Chiayi (LC_{50} = 553.9 ng cm^{-2}). Although the LC_{50} value of the Miaoli population was smaller than that of Hsinchu, there was no significant difference between them. Compared with the Hsinchu population, only 5–6-fold resistance to permethrin was detected in the Changhua and Chiayi populations, which have extremely high resistance to carbofuran. The limited permethrin resistance in both carbofuran-resistant populations suggests that permethrin will be a good candidate for rotation by treating rice seedlings before transplanting into the rice paddy to avoid harmful impact on the environment. In addition, a few more pyrethroids, e.g. *S*-fenvalerate and fenpropathrin, which have been found to possess high efficacy against both susceptible and resistant *C. suppressalis*,[18] can also be considered in rice seedling treatment.

3.5 Fipronil

Fipronil, a novel phenylpyrazole systemic insecticide effective against piercing-sucking and chewing insects,[19] has been used for the control of many insects.[20–26] It was first recommended in 2003 for *C. suppressalis* control on rice seedlings[5] in Taiwan, and all populations tested in this study are highly susceptible, with LC_{50} values ranging from 30.14 to 68.60 ng cm^{-2} (Table 2), with only twofold difference observed among the three populations from the central part and the Hsinchu population of striped stem borers. The relatively short application history of fipronil (since 2003) in Taiwan may have contributed to its high susceptibility in all populations tested. In China, fipronil has been used for rice pest control since 1997. Ten years later, bioassay data still indicated a high susceptibility to fipronil in most field populations, except for the samples collected from Ruian and Cangnan, Zhejiang, in 2006.[27] In subsequent studies, LD_{50} values less than 1 ng larva^{-1} also showed that the field rice stem borer is still sensitive to this insecticide.[28] Although fipronil is highly toxic to aquatic animals and other non-target insects, such as bees, it can be applied on rice seedlings to prevent early infestation of *C. suppressalis* after transplanting into rice paddy.

4 CONCLUSIONS

After long periods of usage and intensive applications, human-made selective pressure has become the main driving force for target pests to develop resistance,[29] causing a serious threat to effective pest control as well as impacting on the environment. One approach to slow down the advance of resistance is to rotate insecticides with different modes of action, so avoiding persistent applications of insecticides sharing a common biochemical target. To date, the control of rice insect pests, including stem borers, in Taiwan still relies heavily on insecticides. Therefore, the development of resistance to each insecticide and the potency of cross-resistance to insecticides with different modes of action are valuable data to resistance management programmes for *C. suppressalis*. The present bioassay data indicated that *C. suppressalis* from the Chiayi and Changhua prefectures have developed enormous resistance to carbofuran, while they are still highly susceptible to chlorpyrifos, fipronil and permethrin. In view of their aquatic toxicity, fipronil and permethrin are suggested for application on rice seedling boxes to prevent the early infestation of *C. suppressalis*, while chlorpyrifos can be used to replace carbofuran in the rice paddy.

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