Cross-resistance assessment in cartap- and esfenvalerate-selected strains of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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Abstract

Effective control of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has become critical due to the genetic ability of the insect pest to develop resistance to insecticides. Alternating or rotating the use of insecticides that do not show cross-resistance is an important component of an effective resistance management strategy, as it helps prevent resistance development or regain susceptibility in an already resistant arthropod pest population. In this study, cross-resistance to selected insecticides in cartap- and esfenvalerate-selected strains of DBM was assessed in the laboratory, using the leaf-dipping method. The esfenvalerate-selected strain exhibited moderate cross-resistance to abamectin and a very low cross-resistance to cartap. The cartap-selected strain also displayed a very low cross-resistance to esfenvalerate but showed no cross-resistance to abamectin. Alternating cartap and abamectin would therefore help to effectively manage insecticide-resistance development in the DBM.

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive pest of cabbage and other cruciferous crops in Ghana (Ninsin, 1997; Odhiambo, 2005) and other parts of the world (Shelton *et al*., 1997; Srinivasan *et al*., 2011). In order to effectively control the DBM and satisfy consumers who continue to attach high cosmetic value to cruciferous vegetables, insecticides are mainly used against the pest. However, the need to rely on insecticides for DBM control has resulted in the pest developing resistance to all classes of insecticides and made the DBM the second most resistant arthropod pest worldwide (Vasquez, 1995; Nauen, 2012). As a consequence, useful insecticides have been rendered ineffective against the DBM. It is therefore necessary to manage insecticide-resistance development in the DBM so that existing insecticides could be relied upon to effectively control the pest.

The rotational use of insecticides that do not show cross-resistance is an important component of an effective resistance management strategy (Saito *et al*., 1995). In the course of rotating insecticides that do not show cross-resistance, the susceptibility of resistant insects will be restored if resistance is not stable thereby allowing the insecticide to which the insects had developed resistance be reintroduced for insect pest control (Ninsin and Tanaka, 2005). As a result, the rotational

use of insecticides that do not show cross-resistance helps prevent resistance development or regain susceptibility when resistance has already developed in an arthropod pest population.

It is necessary to identify, through laboratory studies, insecticides that show no cross-resistance to other insecticides so that they are incorporated into resistance management strategies to effectively manage insecticide-resistance development in the DBM. Cartap and esfenvalerate are two of the insecticides used for DBM control around the world. Due to insecticide-resistance development in the DBM, decreased susceptibilities of field populations of DBM to cartap and esfenvalerate have been observed (Ninsin and Miyata, 2003; Eziah *et al*., 2008). Thus, strategies for managing resistance development to cartap and esfenvalerate in the DBM are needed.

This laboratory study was undertaken to evaluate cross-resistance in cartap- and esfenvalerate-selected strains of DBM for resistance management. The cartap-selected strain was evaluated for cross-resistance to abamectin and esfenvalerate, while the esfenvalerate-selected strain was evaluated for cross-resistance to abamectin and cartap.

Materials and methods

DBM strains and their maintenance

Two insecticide-selected strains of DBM, KOBII-cartap-selected and KOBII-esfenvalerate-selected, and the reference Osaka Susceptible Strain (OSS) were used for this study. The two insecticide-selected strains were developed from a field population of DBM (KOBII) originally collected on 12 June 2000, from cabbage fields in Iwaoka-cho, Kobe City, Japan (Ninsin and Miyata, 2003). About 300 larvae and pupae of KOBII were collected and reared in the laboratory at Nagoya University, Nagoya, Japan, as previously reported (Ninsin *et al*., 2000) at 25 ± 1°C and 50% relative humidity, under 16.00 : 8.00 hours (light : dark) photoperiod. The moths were reared in adult cages and fed on 5% honey solution. Eggs were collected on 2-3-day-old radish, *Raphanus sativus* L var. Osaka 40 nichi, seedlings. After hatching, the larvae were also fed on 2-3-day-old radish seedlings in larval boxes until pupation, after which pupae were returned to adult cages for emergence.

The procedure for establishing the two insecticide-selected strains of KOBII are fully described by Ninsin (2004). Briefly, the insecticide selected strains were developed by exposing sub-populations of KOBII to various concentrations of the insecticides. Before selection started, the susceptibility of KOBII to cartap and esfenvalerate were comparable to the susceptibility of the reference OSS to the two insecticides (Ninsin, 2004). For the cartap-selected strain, a sub-population of KOBII was exposed to 50, 62.5 and 250 mg/l cartap at F_{12}, F_{19} and F_{21}, respectively. In the case of the esfenvalerate-selected strain, a sub-population of KOBII was exposed to 2 mg/l esfenvalerate at F_{12} and 10 mg/l esfenvalerate at F_{14} and F_{17}.

The OSS after field collection in Katano City, Osaka Prefecture, Japan in 1969, has been reared in the laboratory without exposure to any insecticide. The strain has become fully susceptible to insecticides and is therefore used as the standard reference susceptible strain by the Japan Plant Protection Association (Noppun *et al*., 1983).

All DBM strains used for this study were reared as described above.

Insecticides

Since there is a greater risk of cross-resistance between insecticides of same chemistry because of target-site resistance mechanism, insecticides of different chemistries were used for this study. The following commercially available insecticides were used: cartap - 500 g/kg wettable powder (WP) (Padan® – nereistoxin analogue); esfenvalerate – 50 g/l emulsifiable concentrate (EC) (Sumialpha® - pyrethroid); abamectin - 18 g/l EC (Abamectin® – avermectin).

Technique for susceptibility test

The leaf-dipping method previously reported by Ninsin *et al.* (2000) was used. Cabbage, *Brassica oleracea capitata* L. cv *Chuseikanran,* leaves measuring 5 cm × 5 cm were dipped for 10 seconds in various concentrations of insecticide solutions. All insecticide solutions were prepared with distilled water containing 200 µl/l spreading agent (Linoh®, Nihon Noyaku Co. Ltd., Osaka, Japan). Control test cabbage leaves were dipped in distilled water containing only the spreading agent. The treated leaves were allowed to air-dry at 25 °C. Each leaf was put into a 200-cm^3 plastic cup padded with a slightly moistened 70-mm filter paper (Advantec®, Toyo Roshi Kaisha Ltd., Tokyo, Japan). Ten 12- to 24-hour-old third-instar larvae were introduced into each cup. A minimum of five insecticide concentrations were prepared for each insecticide, and four replicates for every concentration and control. Larval mortalities were recorded 72 h. after treatment for all insecticides. The larvae that did not respond when prodded with a pencil tip were considered dead. There was usually no mortality in the control, but when control mortality was observed, this was less than 10%. Data obtained were subjected to probit analysis (Finney, 1971) to determine the median lethal concentration (LC_{50}) and the 95% confidence interval (CI). The resistance level (resistance ratio [RR]) of the selected strains was calculated by dividing the LC_{50} of the insecticide-selected strain by the LC_{50} of the reference susceptible OSS.

Results

The KOBII-cartap-selected strain exhibited moderate resistance (RR=14.2) to cartap and very low cross-resistance (RR=2.6) to esfenvalerate (Table 1). The KOBII-esfenvalerate-selected strain which showed high resistance (RR=131) to esfenvalerate also displayed very low cross-resistance (RR=3.8) to cartap (Table 2). While the esfenvalerate-selected strain showed moderate cross-resistance (RR=16.6) to abamectin (Table 2), the cartap-resistance strain showed no cross-resistance (RR=1) to abamectin (Table 1).

Discussion

The observation by Ninsin (2004) that both cartap-resistance and esfenvalerate-resistance in the DBM were unstable indicated that the rotational use with insecticides that show no cross-resistance would effectively manage DBM resistance development to cartap and esfenvalerate. However, the cross-resistance observed between esfenvalerate and cartap in both the cartap- and esfenvalerate-selected strains suggests that these insecticides should not be alternated or rotated for DBM control. Studies by Cheng (1986) indicated that cartap-resistant DBM also showed cross-resistance to fenvalerate, a compound

TABLE 1
Responses of cartap-selected *Plutella xylostella* strain (KOBII-cartap-selected strain) and the reference Osaka susceptible *P. xylostella* strain (Osaka susceptible strain) to cartap, esfenvalerate and abamectin

Insecticide	KOBII-cartap-selected strain		Osaka susceptible strain		Resistance ratio[b]
	LC_{50}(mg/l) (95% CI)	Slope (±SE)	LC_{50}(mg/l) (95% CI)	Slope (±SE)	
Cartap	277 (222–353)	1.79 (±0.17)	19.5 (15.8–24.3)	1.76 (±0.20)	14.2
Esfenvalerate	1.38 (0.797–7.53)	1.20 (±0.27)	0.524 (0.410–0.736)[a]	1.84 (±0.15)	2.6
Abamectin	0.014 (0.008–0.084)	1.29 (±0.18)	0.014 (0.011–0.223)	1.43 (±0.48)	1

[a] Data cited from Ninsin (2015)
[b] Resistance ratio = LC_{50} of KOBII-cartap-selected strain divided by LC_{50} of Osaka susceptible strain

TABLE 2
Responses of esfenvalerate-selected *Plutella xylostella* strain (KOBII-esfenvalerate-selected strain) and the reference Osaka susceptible *P. xylostella* strain (Osaka susceptible strain) to esfenvalerate, cartap and abamectin

Insecticide	KOBII-esfenvalerate-selected strain		Osaka susceptible strain		Resistance ratio[b]
	LC_{50}(mg/l) (95% CI)	Slope (±SE)	LC_{50}(mg/l) (95% CI)	Slope (±SE)	
Esfenvalerate	68.6 (44.4–116)	0.75 (±0.18)	0.524 (0.410-0.736)[a]	1.84 (±0.15)	131
Cartap	74.8 (57.9–103)	1.66 (±0.28)	19.5 (15.8-24.3)	1.76 (±0.20)	3.8
Abamectin	0.233 (0.153–0.829)	1.29 (±0.50)	0.014 (0.011-0.223)	1.43 (±0.48)	16.6

[a] Data cited from Ninsin (2015)
[b] Resistance ratio = LC_{50} of KOBII-esfenvalerate-selected strain divided by LC_{50} of Osaka susceptible strain

which is similar to esfenvalerate but is a racemic mixture of four isomers in approximately equal concentrations (Kelly, 2003). On the other hand, Noppun *et al*. (1989) observed that fenvalerate-resistant DBM did not show cross-resistance to cartap. The absence of cross-resistance to cartap in fenvalerate-resistant DBM (Noppun *et al*., 1989) but its presence in the esfenvalerate-resistant DBM in this study may be due to the difference in the selecting compounds. Esfenvalerate is made up of 84% of the most insecticidally active *S,S*-isomer compared to 23% of the *S,S*-isomer in fenvalerate (Kelly, 2003) and so may have activated resistance mechanisms with a much broader activity than fenvalerate. The cross-resistance observed between cartap and esfenvalerate indicates that aspects of the mechanisms underlying DBM resistance to cartap and esfenvalerate are common. Cartap is a nereistoxin analogue, a nicotinic acetylcholine receptor channel blocker (Insecticide Resistance Action Committee [IRAC], 2012) while esfenvalerate is a pyrethroid which modulates the sodium channel by keeping sodium channels open (IRAC, 2012). Since cartap and esfenvalerate are of different chemistries it is unlikely that target-site resistance mechanism which is group specific is involved in the cross-resistance between the insecticides, but

instead resistance mechanisms which are not chemical group specific are involved. Although it appears that due to the very low cross-resistance between cartap and esfenvalerate, the insecticides could be alternated or rotated to manage the DBM, it is not prudent to do so, since utilizing them together will not restore the susceptibility of DBM to either insecticide. Alternating or rotating cartap and esfenvalerate would rather worsen resistance in the DBM population as the practice would select and increase the frequency of the genes responsible for the common resistance-mechanism.

The cross-resistance to abamectin in the esfenvalerate-resistant DBM also suggests that aspects of the mechanism of esfenvalerate-resistance in the DBM may be common to abamectin resistance. Thus, when esfenvalerate is being used for DBM control, abamectin should not be applied right after esfenvalerate use, as its application would also worsen resistance in the DBM population by selecting and increasing the frequency of the genes responsible for the common resistance-mechanism. The cross-resistance to abamectin in the esfenvalerate-resistant strain is also likely due to other mechanisms other than target-site resistance, since abamectin is a chloride channel activator that allosterically activates glutamate-gated chloride channels (IRAC, 2012) while esfenvalerate is a sodium channel modulator that keeps sodium channels open (IRAC, 2012).

The lack of cross-resistance to abamectin in the cartap-resistant strain and the observation by Feng *et al*. (2004) that an abamectin-selected strain of DBM did not show cross-resistance to cartap suggests a lack of common mechanisms underlying resistance in the DBM to abamectin and cartap. Thus, during DBM management, abamectin and cartap could be alternated or rotated. The unstable resistance observed in the DBM to cartap (Ninsin, 2004) and abamectin (Pu *et al*., 2010) suggests that the susceptibility of DBM to either insecticide would be restored during the rotational use of the insecticides, thereby allowing either insecticide to be reintroduced for DBM control.

Given that control of the DBM has become critical because of the numerous insecticides that the pest has developed resistance to (Vasquez, 1995; Nauen, 2012), there is an urgent need to continue searching for insecticides that do not show cross-resistance in the laboratory for field deployment to help effectively manage DBM resistance development.

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