Efficient elimination of insecticide-susceptible diamondback moth (DBM), *Plutella xylostella* (L.) by esfenvalerate from a population generates high esfenvalerate-resistance in the DBM

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ABSTRACT

The study was undertaken at Nagoya University, Nagoya, Japan, to determine in what way the low selection concentration of esfenvalerate influenced the speed and magnitude of resistance development in the DBM. KOBII-esfenvalerate selected strain and KOBII-nonselected strain, which were developed from the KOBII population, esfenvalerate 50 g/l EC and the leaf-dipping method were used for the study. The esfenvalerate concentration that caused hundred per cent mortality in the KOBII-nonselected strain, which is homogenous in homozygous susceptible (*ss*) individuals, was used to estimate the proportion of *ss* individuals in the esfenvalerate selected strain. Concentration of 6.25 mg/l esfenvalerate eliminated all *ss* individuals from the KOBII-nonselected strain. The 6.25 mg/l esfenvalerate showed that there were about 2.5 per cent *ss* individuals in the KOBII-esfenvalerate selected strain. Two generations after selection with the 10 mg/l esfenvalerate, which yielded 273-fold resistance, up to 97.5 per cent of the individuals in the esfenvalerate-selected strain were heterozygous resistant (*rs*) and homozygous resistant (*rr*). A field population of DBM, exposed to the recommended insecticide dilution for field application against DBM in Japan, which is 1,000 – 2,000 times dilution and translated into 25 mg/l – 50 mg/l esfenvalerate, a high frequency of *rr* individuals accumulated and generated a DBM population resistant to esfenvalerate in the field. Proactive management of the development of insecticide resistance is important.

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Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive pest of cabbage worldwide (Shelton *et al*., 1997; Srinivasan *et al*., 2011). The absence of alternative effective control methods against the DBM has compelled farmers to rely on the use of insecticides (Ninsin *et al*., 2000). A sustainable strategy for managing the DBM still remains elusive because of the development of insecticide resistance (Ninsin *et al*., 2000). Insecticide resistance in the DBM makes potent insecticides ineffective and deprive farmers the use of such active ingredients for DBM control. Studies to understand the development of resistance to insecticide active ingredients are, therefore, needed to help farmers better manage resistance in the DBM.

During selection in the laboratory for resistance to esfenvalerate (pyrethroid), phen-

thoate (organophosphate), cartap (nereistoxin analogue) and acetamiprid (neonicotinoid) in a field-collected DBM population, resistance to esfenvalerate progressed fastest and attained the highest magnitude of 222-fold resistance compared to 140-fold phenthoate-resistance, 15-fold cartap-resistance and 9.5-fold acetamiprid-resistance (Ninsin, 2004). The maximum selection concentration for each of the insecticide was 10 mg/l esfenvalerate, 300 mg/l phenthoate, 250 mg/l cartap and 550 mg/l acetamiprid (Ninsin, 2004). Since the intensity is central in determining rates of resistance evolution (Groeters & Tabashnik, 2000), it was expected that esfenvalerate-resistance would be the slow to develop due to lower selection concentration used for the laboratory selection (Ninsin, 2004).

Understanding the underlying cause of the accelerated resistance development in the DBM to esfenvalerate would be useful in helping to develop effective resistance management strategies. The study was, therefore, undertaken to determine how the low esfenvalerate concentration used by Ninsin (2004) influenced the speed and magnitude of resistance development in the DBM.

Materials and methods

Insects and insecticide

The DBM strains used for this laboratory study at Nagoya University, Nagoya, Japan, were KOBII-nonselected and KOBII-esfenvalerate selected. The strains were developed from a field population of DBM (KOBII) collected on 12 June 2000, from cabbage fields in Iwaoka-cho, Kobe City, Japan (Ninsin & Miyata, 2003). About 300 larvae and pupae of KOBII were collected and reared in the laboratory as reported by Ninsin *et al*. (2000) at 25 ± 1 °C and 50 per cent relative humidity, under 16.00 : 8.00 (light : dark) photoperiod . The moths were fed on 5 per cent honey solution and the larvae on 2-3-day-old radish, *Raphanus sativus* L var. Osaka 40 nichi, seedlings.

The procedures for establishing the two DBM strains from KOBII population are described by Ninsin (2004). The KOBII-nonselected strain was established by maintaining a part of KOBII population in the laboratory without exposure to any insecticide for over 19 generations. For this study, the strain had an LC_{50} (95 per cent CI) of 0.698(0.56-0.886) mg/l esfenvalerate (Ninsin, 2004), which was not significantly different from the LC_{50} (95% CI) of 0.524 (0.410-0.736) mg/l esfenvalerate for the Osaka susceptible strain (OSS) (Ninsin, 2015).

The OSS has full susceptibility (i.e. homogenous in homozygous susceptible [*ss*] individuals) to a wide range of insecticides, so it is used as the standard reference susceptible strain by the Japan Plant Protection Association (Noppun, Miyata & Saito, 1983). Since the OSS is homogenous in *ss* individuals, the KOBII-nonselected strain was, therefore. considered as being homogenous in *ss* individuals to esfenvalerate. The esfenvalerate selected strain was developed by exposing a part of KOBII population once to 2 mg/l esfenvalerate at F_{12} and three times to 10 mg/l esfenvalerate at F_{14}, F_{17} and F_{22}. The KOBII-esfenvalerate selected strain was used at F_{24} when it had attained 273-fold esfenvalerate-resistance (Ninsin, 2004). The insecticide used for this study was esfenvalerate - 50 g/l emulsifiable concentrate (EC) (Sumialpha®, pyrethroid, Sumitomo Chemical Co. Ltd., Osaka, Japan).

Bioassay technique

The leaf-dipping method described by Ninsin, Mo & Miyata (2000) was used. Eleven esfenvalerate concentrations, ranging from 0.1953 mg/l to 200 mg/l, were prepared with distilled water containing 200 µl/l spreading agent (Linoh®, Nihon Noyaku Co. Ltd., Osaka, Japan). Leaves of cabbage, *Brassica oleracea capitata* L. cv *Chuseikanran,* measuring 5 cm × 5 cm were dipped for 10 sec in the esfenvalerate concentrations. Control 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 of DBM were introduced into each cup. Four replicates were prepared for each esfenvalerate concentration and control. Larval mortalities were recorded 72 after treatment. The larvae that did not respond when prodded with a pencil were considered dead. There was no larval mortality in the control setups for either, the KOBII-nonselected or KOBII-esfenvalerate selected strains. Mortalities recorded were tabulated against the respective esfenvalerate concentrations. The concentration that caused 100 per cent mortality in the KOBII-nonselected strain was used to estimate the proportion of *ss* individuals in the KOBII-esfenvalerate selected strain.

Results

A concentration of 6.25 mg/l esfenvalerate caused a hundred per cent mortality in the KOBII-nonselected strain (Table 1). Thus, 6.25 mg/l is the concentration of esfenvalerate required to eliminate all *ss* individuals from a field population of DBM. The 6.25 mg/l esfenvalerate caused 2.5 per cent mortality in the KOBII-esfenvalerate selected strain (Table 1). This shows that two generations after the esfenvalerate selected strain had been exposed to the third 10 mg/l esfenvalerate selection pressure (Ninsin, 2004), there were about 2.5 per cent *ss* individuals within the KOBII-esfenvalerate selected strain. Thus, the 7.5 per cent to 55 per cent mortality caused by the 12.5 mg/l to 200 mg/l esfenvalerate concentrations in the KOBII-esfenvalerate selected strain (Table 1) are expected to be resistant individuals, i.e. heterozygous resistant (*rs*) and homozygous resistant (*rr*).

TABLE 1

Mortalities in a field population (KOBII) of Plutella xylostella not selected with any insecticide (KOBII-nonselected strain) and KOBII selected with esfenvalerate (KOBII-esfenvalerate selected strain) after exposure to concentrations of esfenvalerate for 72 hours

Concentration of esfenvalerate (mg/l)	0.1953	0.3906	0.7813	1.563	3.125	6.25	12.5	25	50	100	200
KOBII-nonselected mortality (%)	7.5	30	55	75	82.5	100	-	-	-	-	-
KOBII-esfenvalerate selected mortality (%)	-	-	-	-	0	2.5	7.5	7.5	10	3 5	55

Discussion

The development of insecticide-resistance in an insect population is hastened by the accelerated elimination of a high percentage of *ss* individuals leaving behind a high frequency of *rr* individuals in the population (Matsumuura, 1985). The exposure of a pest population to an insecticide concentration that eliminates all *ss* individuals is, therefore, crucial to the speed and magnitude of resistance development. According to Ninsin (2011), concentration of 31.25 mg/l phenthoate, 250 mg/l cartap and 350 mg/l acetamiprid is needed to eliminate all *ss* individuals from the KOBII field population. These concentrations of phenthoate, cartap and acetamiprid are much higher than the 6.25 mg/l esfenvalerate needed to eliminate all the *ss* individuals from the KOBII population. Thus, the exposure of KOBII population to the maximum selection concentration of 10 mg/l esfenvalerate provided adequate selection pressure to accelerate the development of resistance to esfenvalerate compared to the development of resistance to phenthoate, cartap and acetamiprid (Ninsin, 2004) and showed how efficient esfenvalerate can accumulate resistant genes in a DBM population. The efficiency of esfenvalerate in eliminating the *ss* individuals with the concomitant accumulation of resistant individuals in the population is due to the chemical characteristics of the insecticide,

which is evidenced in its superior contact toxicity.

The 273-fold esfenvalerate-resistance recorded for the KOBII-esfenvalerate resistant strain could have been higher had it not been for the presence of the 2.5 per cent *ss* individuals in the selected strain. The *ss* individuals increased the sensitivity of the strain and reduced the resistance level. The presence of the *ss* individuals within the esfenvalerate selected strain when it was expected that only *rs* and *rr* individuals would be available, since selection was done with 10 mg/l esfenvalerate that eliminates all *ss* individuals, is due to reversion of esfenvalerate-resistance (Ninsin, 2004). There was reversion of esfenvalerate-resistance because the study was conducted two generations after the last selection with the 10 mg/l esfenvalerate. In the absence of further selection with esfenvalerate, the *rs* individuals within the strain mated to produce *ss*, *rs* and *rr* progenies.

The 10 mg/l esfenvalerate used for the selection by Ninsin (2004) represents 5,000 times dilution of esfenvalerate 50 g/l EC. It is, therefore, expected that a higher esfenvalerate-resistance than the 273-fold resistance generated by the 10 mg/l esfenvalerate would be obtained if the insecticide recommendation for DBM control in Japan, which is 1,000 - 2,000 times insecticides dilution, is used for resistance selection in the laboratory or to control field pest. The recommended insecticide dilution translates into 25 - 50 mg/l esfenvalerate, which would retain in the KOBII-esfenvalerate selected strain individuals that are only *rr* or the most would be *rr*. Thus, recommended insecticide concentrations could generate high levels of resistance in field populations of DBM. There is, therefore, the need for a proactive approach to insecticide resistance management by implementing strategies that would maintain a high percentage of *ss* individuals in a pest population and, thereby, prevent the development of insecticide resistance.

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