Susceptibility of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to acetamiprid and selected insecticides by foliar treatment and strategy for resistance management

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ABSTRACT

The dependence on pyrethroids and organophosphates to control the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on cabbage and cauliflower in Ghana since the 1990's, and the absence of effective resistance management strategies has led to reported cases of widespread development of DBM resistance to these insecticides since 2002. The study was undertaken to identify alternative insecticides to the pyrethroids and organophosphates, and recommend a strategy that would effectively manage DBM resistance to insecticides in Ghana. The susceptibility of the DBM to acetamiprid (neonicotinoid), chlorfluazuron (benzoylphenyl urea) and *Bacillus thuringiensis* subsp. kurstaki Berliner (Btk) (microbial) was compared with susceptibility to esfenvalerate and phenthoate, which were selected as reference pyrethroid and organophosphate compounds, respectively. The Osaka susceptible strain of DBM and the leaf-dipping method were used. The results showed that esfenvalerate was most toxic after 72-h treatment. However, when the end-points for the insecticides were considered, chlorfluazuron was the most toxic after 168-h treatment and Btk the next most toxic after 96-h treatment. Acetamiprid was the least toxic to the DBM by foliar treatment. The low toxicity of acetamiprid would subject the DBM to a low selection pressure, and as a result delay the development of resistance in the pest. Acetamiprid should, therefore, be a preferred alternative for DBM control in Ghana. Chlorfluazuron and Btk by virtue of their toxicity against the DBM and favourable safety levels should also be a preferred alternatives for DBM control in Ghana. An effective resistance management strategy against the DBM in Ghana would be the rotational use of acetamiprid, chlorfluazuron and Btk for DBM control since these insecticides show no cross-resistance to each other.

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Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an economically important pest of crucifers with a high fecundity and rapid turnover of generations. It is the most destructive pest of cruciferous crops in Ghana and globally (Ninsin, 1997; Shelton *et al*., 1997). To manage the DBM effectively, growers rely solely on the use of insecticides in the ab-

sence of other effective methods. However, the ability of the DBM to develop resistance to insecticides has rendered many insecticides ineffective, and made it the second most resistant arthropod pest worldwide (Vasquez, 1995; Nauen, 2012).

Despite the earlier detection of insecticide resistance in DBM on cabbage in Ghana by Obeng-Ofori *et al*. (2002), widespread resistance persists in field populations of the pest (Odhiambo *et al*., 2010), which is causing DBM control failures (Botwe, Eziah & Owusu, 2012). Factors that are contributing to the continued presence of insecticide resistant in DBM populations in the field include the over-reliance on organophosphate and pyrethroid insecticides for DBM control since the 1990s (Ninsin, 1997), and the non-existence of effective insecticide resistance management (IRM) strategies in Ghana. It is, therefore, imperative to identify insecticides of different chemistries to be used to formulate IRM strategies that would effectively manage DBM resistance in Ghana.

Acetamiprid is a neonicotinoid insecticide registered in Japan for use against a wide range of insect pests, including the DBM (Matsuda & Takahashi, 1996). The neonicotinoid insecticides have established themselves worldwide as key components in control programmes, because of their novel mode of action as agonists of the nicotinic acetylcholine receptor (Yamamoto *et al*., 1995), unique chemical and biological properties, such as excellent uptake and translocation in plants and favourable safety profile (Maienfisch *et al*., 2001). As a result of a higher hydrophobicity (Yamamoto, 1996), acetamiprid has characteristic properties different from other neonicotinoid insecticides, notably a comparatively high activity against lepidopterous pests (Takahashi *et al*., 1998). Acetamiprid is formulated for both soil and foliar application (Matsuda & Takahashi, 1996), and is an integral component of DBM management programmes in Japan (Ninsin, Mo & Miyata, 2000).

This paper reports the results of experiments that compared the susceptibility of DBM to acetamiprid, chlorfluazuron (benzoylphenyl urea) and *Bacillus thuringiensis* subsp. kurstaki Berliner (Btk) (microbial) with susceptibility to phenthoate and esfenvalerate, which were selected as reference organophosphate and pyrethroid compounds, respectively, and recommends how acetamiprid, chlorfluazuron and Btk should be used in a strategy to manage DBM resistance in Ghana.

Materials and methods

Diamondback moth (DBM) strain

The Osaka susceptible strain (OSS) of the DMM was used for the laboratory study at Nagoya University, Nagoya, Japan. The OSS was originally collected from Katano City, Osaka Prefecture, Japan and has been reared in the laboratory free from any insecticidal pressure after field collection in 1969 (Noppun, Miyata & Saito, 1983). With complete susceptibility to various insecticides, the OSS is used as the standard reference susceptible strain by the Japan Plant Protection Association (Noppun, Miyata & Saito, 1983). The DBM were reared in the laboratory (Ninsin *et al*., 2000) at 25 ± 1 °C and 50 per cent relative humidity, under 16h : 8h (light : dark) photoperiod. Moths were reared in adult cages and fed on 5 per cent 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 till pupation, after which the pupae were returned to the adult cages for adult emergence.

Insecticides

The following commercially available insecticides were used: acetamiprid 200 g kg^{-1} SP (Mospilan®, neonicotinoid, Nippon Soda Co. Ltd., Japan); phenthoate 500 g l^{-1} EC (Elsan®, organophosphate, Nissan Chemical Co. Ltd., Japan); esfenvalerate 50 g l^{-1} EC (Sumialpha®, pyrethroid, Sumitomo Chemical Co. Ltd., Osaka, Japan); chlorfluazuron 50 g l^{-1} EC (Atabron®, benzoylphenyl urea, Ishihara Sangyo Kaisha Co. Ltd., Japan); *Bacillus thuringiensis* subsp. kurstaki Berliner 70 g kg^{-1} WP (Toarow CT®, microbial, Toagosei Co. Ltd., Japan).

Bioassay for susceptibility test

Leaf-dipping method Ninsin, Mo & Miyata. (2000) was used. Cabbage (*Brassica oleracea* capitata L. cv Chuseikanran) leaves measuring 5 cm × 5 cm were dipped for 10 s in various concentrations of insecticide solutions, which had been prepared with distilled water containing 200 µl l^{-1} spreading agent (Linoh®, Nihon Noyaku Co. Ltd., Osaka, Japan). The 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- 24-h-old 3rd-instar larvae were introduced into each cup. Seven concentrations were prepared for each insecticide. A minimum of five replicates per concentration and control were made for the insecticide concentrations. The end-points for larval exposure to the insecticides after which data on mortality were taken were 72 h for acetamiprid, phenthoate and esfenvalerate, 96 h for Btk and 168 h for chlorfluazuron (Fahmy, Sinchaisri & Miyata, 1991). Larvae that did not respond when prodded with a pencil were considered dead. There was usually no mortality in the control, but when control mortality was observed, this was less than 10 per cent. The mortality data were subjected to probit analysis (Finney, 1971) to determine the median lethal concentration (LC_{50}) values and 95 per cent confidence intervals (CI). When LC_{50} values were compared, they were judged as significantly different if the respective 95 per cent CI did not overlap.

Results and discussion

The results showed that after 72 h of exposure to the insecticides, esfenvalerate was most toxic to the DBM (Table 1). However, when the end-points for the tests were considered, chlorfluazuron was the most toxic insecticide, followed by Btk, when LD_{50} for the other insecticides were considered in dilution times (Table 1). Acetamiprid was the least toxic insecticide (Table 1).

Acetamiprid was the least toxic insecticide by foliar treatment because the neonicotinoids are more effective as stomach poisons due to their excellent systemic activity and low hydrophobicity (Yamamoto, 1996). The low toxicity of acetamiprid by foliar treatment is, however, favourable to resistance management since the DBM is subjected to a low acetamiprid selection pressure (Ninsin, 2011), which contributes to a delayed acetamiprid-resistance development in the DBM (Ninsin, 2004a). On the

Table 1

Response of the Osaka Susceptible Strain of Plutella Xylostella *to Acetamiprid and Selected Insecticides as Insecticide*

Insecticide	*72 hours after treatment*		*96 hours after treatment*		*168 hours after treatment*	
	LC50 (mg l^{-1}) (95% CI)	*Slope (± Se)*	*LC50 (mg l^{-1}) (95% CI)*	*Slope (± Se)*	*LC50 (mg l^{-1}) (95% CI)*	*Slope (± Se)*
Acetamiprid	58.9 (53.0 – 66.0)	2.57 (± 0.24)	-	-	-	-
Phenthoate	4.17 (3.07 – 6.56)	1.54 (± 0.18)	-	-	-	-
Esfenvalerate	0.524 (0.410 – 0.736)	1.84 (± 0.15)	-	-	-	-
Chlorfluazuron	2.29 (1.68 – 3.24)	0.99 (± 0.11)	-	-	0.094 (0.055 – 0.507)	1.08 (± 0.20)
Btk [b]	119,000	2.29 (± 0.24)	233,000	1.92 (± 0.22)		

[a] LC_{50} values judged as significantly different if the respective 95 per cent CI did not overlap
[b] LC_{50} values for Btk, a preparation of *Bacillus thuringiensis* subsp. *kurstaki* Berliner are given in dilution times

other hand, the high toxicity of esfenvalerate and phenthoate by foliar treatment is unfavourable to resistance management since the DBM is subjected to a high selection pressure (Ninsin, 2003; Ninsin, 2011), which accelerates the development of DBM resistance to both esfenvalerate and phenthoate (Ninsin, 2004a). Even if the DBM develops resistance to acetamiprid (Ninsin, 2004b), the resistance is unstable (Ninsin, Mo & Miyata, 2000; Ninsin & Tanaka, 2005). An unstable resistance means that when acetamiprid is not used to control DBM for a period, susceptibility to the compound will be restored in DBM populations, allowing the insecticide to be reused for DBM control. Given that resistance to organophosphates and pyrethroids is prevalent in field populations of DBM in Ghana (Obeng-Ofori *et al*., 2002; Odhiambo *et al*., 2010; Botwe, Eziah & Owusu, 2012) but acetamiprid elicits retarded resistance in the DBM (Ninsin, 2004a), it is recommended that acetamiprid should be registered in Ghana and its field efficacy validated for incorporation into DBM management programmes in Ghana.

Insecticide-resistance management requires prudent choice, and application of insecticides to increase the availability of susceptible individuals in field populations of insect pests so that insecticides are continuously effective. To accomplish this, insecticides that do not show cross-resistance to each other needs to be identified and used in a rotation. It has been shown that acetamiprid-resistant DBM show cross-resistance to phenthoate and esfenvalerate, but not to chlorfluazuron and Btk (Ninsin, 2003; Ninsin, 2004b). Chlorfluazuron is an insect growth regulator that inhibits chitin biosynthesis (Insecticide Resistance Action Committee [IRAC], 2012) and was the most toxic insecticide by foliar treatment (Table 1). Although the level of efficacy of chlorfluazuron is desirable, such a high efficacy suggests that it would exert a high selection pressure on the DBM and quickly result in the development of resistance.

Studies have shown that when the DBM develops resistance to chlorfluazuron (Fahmy, Sinchaisri & Miyara, 1991), the resistance is not stable (Fahmy & Miyata, 1992). It is, therefore, recommended that chlorfluazuron should also be registered in Ghana and

its field efficacy validated for DBM management in Ghana. Btk is a microbial disruptor of insect mid-gut membranes (IRAC, 2012). Since Btk is toxic against the DBM (Table 1), the insecticide was widely used for DBM control in the Greater Accra Region of Ghana (Ninsin, 1997). However, Btk use in recent times has decreased considerably because farmers found it to be ineffective against the DBM. Although the DBM has been shown to develop high resistance to Btk (Tabashnik et al., 1990), the resistance is unstable (Hama, Suzuki & Tanaka, 1992), so Btk could be reused for DBM control in Ghana.

Fahmy, Suzuki & Tanaka (1991) showed that chlorfluazuron-resistant DBM lack cross-resistance to Btk, whilst Sarnthoy *et al*. (1997) also showed that Btk-resistant DBM lack cross-resistance to chlorfluazuron. The lack of cross-resistance among acetamiprid, chlorfluazuron and Btk indicates that the mechanism by which the DBM develops resistance to each of the insecticides is different. Since DBM resistance to acetamiprid, chlorfluazuron and Btk is unstable, and there is no cross-resistance among the insecticides, it is recommended that acetamiprid, chlorfluazuron and Btk should be used in a rotation for DBM control in Ghana. The rotational use of acetamiprid, chlorfluazuron and Btk against the DBM would ensure effective control of DBM, allow insecticide susceptible individuals to be constantly present in field populations of DBM and prevent the development of resistance to any of the three insecticides.

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