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Full Length Research Paper

Susceptibility of *Plutella xylostella* (Lepidoptera: Plutellidae) to emamectin benzoate and *lambda*-cyhalothrin in the greater Accra region of Ghana

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Plutella xylostella **is a serious pest of brassicae and a threat to cabbage production in Ghana. Following complaints of farmers about the failure of insecticides to control the diamondback moth, we investigated the susceptibility of the diamondback moth to emamectin benzoate and *lambda*-cyhalothrin insecticides which are commonly used in the diamondback moth control in the greater region of Accra, Ghana. We also investigated the carboxylesterases levels in these populations of the Diamondback moth. Five populations of *P. xylostella* were collected from greenhouses and outdoor crops at Haatso farm, University of Ghana farm, Korle-Bu farm, Dzorwulu farm, Opeibea farm and bred in the laboratory for two generations and the progeny used for laboratory bioassay using topical application and compared with a reference susceptible strain. Protein levels of the individual strains were determined and carboxylesterase activities were measured using a spectrophotometer at 600 nm. *P. xylostella* collected in Opeibea farm exhibited the highest level of resistance of 62.4-fold and 10.5-fold for *lambda*-cyhalothrin and Emamectin benzoate respectively while the carboxylesterase activity levels ranged from 7.8 to 79.2 nmol/ 10 min/ µg protein in the various populations with Dzorwulu population exhibiting the highest level of activity. The current findings have serious implications for brassica production in Ghana considering the fact that farmers rely mainly on insecticides for diamondback moth control. A coordinated resistance management program needs to be implemented with the involvement of pesticide industry, local pesticide regulatory authorities, scientists and farmers.**

Key words: *Plutella xylostella,* Emamectin benzoate, *lambda*-cyhalothrin, insecticide resistance, brassica, diamondback moth.

INTRODUCTION

The diamondback moth, *Plutella xylostella* L., is considered to be the most destructive and widespread insect pest of cruciferous crops worldwide and it causes considerable reduction in yield and quality of marketable heads in cabbage (Brempong-Yeboah, 1992, Talekar and Shelton, 1993). In Ghana, cabbage growers rely mainly on high dosages of synthetic insecticides to control the diamondback moth (Mawuenyegah, 1994). Farmers in their attempt to protect their crops and investment resort to the use of various insecticides in unnecessarily very high quantities and spray at very short intervals as well (Brempong-Yeboah, 1992). The indiscriminate use of these chemicals has created several problems such as the pollution of the environment, toxic residues in fresh vegetables, destruction of indigenous natural enemies resulting in resurgence of secondary pests and the development of resistant strains of pests.

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Conduction of baseline susceptibility studies is important in developing an effective resistance management scheme for different locations (Denholm et al., 1999). This study therefore reports the susceptibility of five field populations of the DBM to two insecticides. A pyrethroid (*lambda*-cyhalothrin) was chosen to ascertain the claim by farmers that this class of insecticides is ineffective in DBM control. The new product (Emamectin benzoate) an avermectin, was chosen because there has been no baseline data on its efficacy against the DBM in the Greater Accra Region. Carboxylesterase activity experiments were also conducted to determine the possible roles of detoxifying enzymes in resistance in the DBM in the region.

MATERIALS AND METHODS

Insects used for the assays

About 400 larvae and pupae of reference strain was obtained from Gomoa Feteh in the Central region of Ghana where there is a suspected minimal use of insecticides. This strain was bred in the laboratory for more than 17 generations and used as the reference strain. Also, about 200-300 larvae and pupae were collected from five cabbage farms located at Haatso farm, University of Ghana farm, Korle-Bu farm, Dzorwulu farm, Opeibea farm (all in parts of Greater Accra Region). These were bred in laboratories for two generations and the progeny used for the assays.

Insecticides and chemicals

Commercial formulations of *lambda*-cyhalothrin (Karate®) (2.5% EC) and Emamectin benzoate (Attack®) (1.9% EC) were purchased from AGRIMAT House and Aglow-Accra, all commercial agrochemical companies in Ghana. All other chemicals used for the enzyme assays are high standard technical grade products obtained from Sigma, Japan.

Bioassay

Third-instar DBM larvae weighing between 2.5 and 3.4 mg were used for all assays. Six serial dilutions with 3 replications of each of the test insecticides were prepared in 100% acetone. For Karate®, 200 mL/L, 100 mL/L, 50 mL/L, 25 mL/L, 12.5 mL/L were used whilst 10 mL/L, 1 mL/L, 0.1 mL/L, 0.01 mL/L, 0.001 mL/L were the dilutions used for Attack® . A 0.5 µL of each solution was topically applied to the nota region of each larva (Maa and Liao, 2000). Each test included a control where the larvae were treated with 100% acetone. The larvae were transferred into clean, plastic 90 mm Petri dishes, which were each lined with filter paper and containing fresh cabbage leaf discs. For each treatment there were three replicate Petri dishes each containing 10 larvae. All treatments were maintained at room temperature (27.0 ± 2.0°C) with a photoperiod of 12 h: 12 h (L:D). Mortality was recorded after 24 and 48 hours. Dead insects included moribound larvae which could not move in coordinated manner when prodded with a probe. Control mortality was at most 10% in all experiments.

Data analysis

The data on insect mortality was analyzed using an US EPA Probit analysis programme version 1.5 to determine the LD50, the slope and the fiducial limits.

Enzyme preparation

Samples of larvae of *P. xylostella* from the various locations were assayed for carboxylesterase activity by enzyme-naphthyl acetate-diazo blue coupling reaction (Owusu, 1992). Twenty third-instar larvae of each strain (population) weighing between 2.5 and 3.4 mg were singly homogenized on ice in 0.3 mL of potassium phosphate buffer pH 7.0 in a glass well. The resultant homogenate was used as enzyme source for carboxylesterase assay, which was based on method adopted for homopterans by Owusu et al. (1993).

Protein determination

The protein content of all the homogenates used for the carboxylesterase activity studies was determined by the method adopted with slight modifications by Owusu et al. (1994).
Reagent B (2 mL) (50 mL of 2% Na_2CO_3 in 0.1M NaOH added to 0.5 mL each of $CuSO_4$ and $NaC_4O_6.2H_2O$ to obtain a total ratio of 50:1) was added to 0.1 mL portion of the larval homogenate in a test tube, shaken for about 10 s and allowed to stand for 20 min. After 17 min, 0.25 mL of phenol: water mixture in a ratio of 1:1 was added to the test tube and left to stand for an extra 23 min to ensure a blue colour development. Absorbance was measured at 750 nm using a spectrophotometer (Camspec M106 spectrophotometer) against a control without enzyme homogenate.

Enzyme assay

This was done in accordance with the method of Owusu et al. (1995a). Larval homogenate (100 µL) was incubated in test tubes with 100 µL of 30 mM of either Þ-naphthyl or ß-naphthyl acetate in absolute ethanol for 10 min at 40ºC in a water bath with a stirrer. After which a mixture of 0.5 mL sodium dodecyl sulphate (SDS) and fast blue B salt (FBS) was added to stop the reaction and effect colour development. Absorbance was read at 600 nm using a spectrophotometer (Camspec M106 spectrophotometer) against a control (containing buffer only) (Owusu, 1992). For individuals with higher activity

Table 1. *Response of five Plutella xylostella populations to two insecticides by topical application* n ‡, total number of larvae tested. FL§, fiducial limits. RF♠, resistance factor: (LD_{50} field population) / (Field Recommended Rate of the insecticide). FRR, Field recommended rate; SSP, susceptible strain; OFP, Opeibea farm; HFP, Haatso farm; SFP, University of Ghana (Legon) farm; DFP, Dzorwulu farm; KFP, Korle-Bu farm.

Insecticide/population	n ‡	Slope ± SE	LD_{50} (µgai/larva)	95% FL§	FRR (ml/L)	RF♠
Emamectin benzoate					0.01	
OFP	180	0.75 ± 0.20	0.10	0.03 – 0.85		10.0
HFP	180	0.62 ± 0.16	0.02	0.01 – 0.16		0.001
SFP	180	0.77 ± 0.18	0.01	0.00 – 0.04		1.0
DFP	180	0.89 ± 0.22	0.06	0.02 – 0.26		6.0
KFP	180	1.04 ± 0.25	0.04	0.01 – 0.14		4.0
***Lambda*-cyhalothrin**					0.025	
OFP	180	3.32 ± 1.06	1.56	1.08 – 3.18		62.4
HFP	180	1.65 ± 0.51	1.05	0.61 – 3.17		42.0
SFP	180	2.88 ± 0.76	1.08	0.74 – 1.84		43.2
DFP	180	2.42 ± 0.67	1.20	0.79 – 2.46		48.0
KFP	180	2.14 ± 0.63	1.35	0.85 – 3.56		54.0

Table 2. *Mean carboxylesterase activity and projected resistance levels of P. xylostella populations.*

Population/ site	Mean CaE activity (± S.E) (nmol/ 10 min/ µg protein)	RR	Projected resistance level
SSP (susceptible)	7.79 ± 0.67^{a}		Susceptible
OFP	23.46 ± 6.65^{a}	3.012	Moderately resistant
HFP	50.80 ± 20.50^{b}	6.521	Resistant
SFP	17.88 ± 4.29^{a}	2.295	Moderately resistant
DFP	79.22 ± 30.33^{b}	10.169	Highly resistant
KFP	10.94 ± 2.80^{a}	1.404	Susceptible

Source: Projected resistance level, Key to resistance classification by Owusu *et al*. (1996). CaE, carboxylesterase; RR, activity ratio: (Mean CaE activity of field population) / (Mean CaE activity of SSP population); LSD= 0.04296; Means followed by the same letters are not significantly different (P = 0.05) from one another; SSP, susceptible strain; OFP, Opeibea farm; HFP, Haatso farm; SFP, University of Ghana, (Legon) farm; DFP, Dzorwulu farm; KFP, Korle-Bu farm.

above the measurable range, the homogenate was appropriately diluted with phosphate buffer before measurement (Owusu et al., 1993). The assays were run in duplicate. The carboxylesterase activity was then expressed as nmoles α-naphthol produced/ 10 min/ µg protein.

RESULTS

Susceptibility of the DBM to insecticides

Diamondback moth populations were found to differ in their susceptibility to the two insecticides assayed. There were also wide variations in the response of the insect populations from the different sites (Table 1). The slope of regression values obtained from the Probit analysis for Attack® and Karate® were low. This suggests that the populations were quite heterogeneous in their responses to the insecticides tested.

The DBM populations were generally susceptible to Attack® insecticide. The Opeibea DBM populations showed a 10.0-fold resistance to Attack® while University of Ghana (Legon) DBM population was completely susceptible (1.0-fold resistance). The insect populations were however, resistant to Karate® insecticide with the Opeibea population exhibiting the highest level of resistance (62.4-fold) to Karate® insecticide while the least resistant population was from Haatso recording a 42.0-fold resistance (Table 1). These levels of resistance were however not significantly different from one another from the probit analysis.

Carboxylesterase activity in field populations of diamondback moth

Varying levels of carboxylesterase activity were observed in the different populations of *P. xylostella* studied (Table 2). The results showed significant differences ($P < 0.05$)

in enzyme activity among the various field populations (susceptible strain, Haatso farm, University of Ghana farm, Korle-Bu farm, Dzorwulu farm, Opeibea farm) (N= 120; df= 5; F pr.= 0.008). The carboxylesterase activity of the field populations ranged from 1.4 - to 10.2- fold higher compared to the susceptible strain (Table 2). The OFP population showed approximately a 3-fold increase (23.5 nmol/ 10 min/ µg protein) in activity over the susceptible strain (7.8 nmol/ 10 min/ µg protein). Though the highest carboxylesterase activity was observed in the DFP population (79.2 nmol/ 10 min/ µg protein), there was no significant difference between the DFP population and HFP population (50.8 nmol/ 10 min/ µg protein) but was significantly ($P<0.05$) different from the other populations (Table 2). The activity from the DFP was approximately 10-fold higher compared with the susceptible population. The HFP also showed a high Carboxylesterase activity, which was significantly different from the remaining populations. All projected resistance levels were detected among the *P. xylostella* populations sampled (Table 2). One of the populations (KFP) was susceptible and two (OFP and SFP) were moderately resistant. The resistant population was HFP whilst the highly resistant population was DFP (Table 2).

DISCUSSION

The high levels of resistance observed in the DBM populations from the fields studied are not surprising considering the rate at which these insecticides were used in insect pest control. The current study reveals that the DBM populations were more resistant (≤ 62.4-fold) to Karate® insecticide compared to Attack® insecticide (≤ 10.0-fold) probably due to the fact that the former insecticide has been used much longer for DBM control in these areas than the latter. Jacinter (2005) also reported of high resistance ratios for pyrethroids insecticides in DBM populations in Southern Ghana where the current populations were sampled. Similar high resistance ratios for pyrethroids for DBM populations in commercial farms were reported in Sydney region, Australia (Eziah et al., 2008) which supports the current findings. The results of the current findings may be due to the prolonged usage of pyrethroids in the control of the DBM. The high resistance ratios observed for the pyrethroid (karate) for the various populations in the present study is significant considering the fact that even in areas where this insecticide had never been used high levels of resistance were observed. In Haatso farm where insecticides were relatively minimally used, a 42-fold resistance to karate® insecticide was observed in the population. This observation was not surprising considering the fact that the DBM can migrate long distances (Talekar and Shelton, 1993) and may therefore transfer the resistant genes into such populations. These findings showed that resistance to synthetic pyrethroids is widespread and may be stable in the areas studied. Therefore, the use of synthetic pyrethroids in DBM control in the region should be discontinued but may be rotated with other newer insecticides such as Bt products, spinosad and indoxacarb which have been reported to be effective in DBM control in Australia (Eziah et al., 2008) so as not to exacerbate the resistance problem. During the current study, it was revealed that Attack®, an avermectin, has been introduced for DBM control just two years prior to the current study hence, the much relatively lower resistance ratios recorded in the various DBM populations.

Carboxylesterase enzymes in *P. xylostella* sampled from the different locations in Accra showed varying levels of activity. These variations may reflect the level of insecticide resistance in the moths and also the trend and intensity of insecticide use on vegetables, particularly cabbages cultivated in different locations (Brempong-Yeboah, 1992). The high carboxylesterase activity levels observed from DFP and HFP may be attributed to the exposure of the insects to regular and frequent use of insecticides. A major factor accounting for the pattern of insecticide use is the ever-increasing demand for unblemished vegetables (Brempong-Yeboah, 1992). Comparing the carboxylesterase values from the current study to the key to resistance classification (Owusu et al., 1996), DBM populations at Opeibea were projected as moderately resistant (3-fold resistance) and that of Dzorwulu was projected as highly resistant. Obeng-Ofori et al. (2002) reported a moderately resistant *P. xylostella* population on cabbages at Dzorwulu (the same site used in the current study) and a highly susceptible population at Haatso (the same site used in the current study). Avicor (2009) reported a moderately resistant *Bemisia tabaci* population on okra at Haatso and recorded 4-fold resistance. This might indicate that the carboxylesterase values and projected resistance levels recorded in the present study at Haatso and Dzorwulu are progressively increasing. Carboxylesterase activities were relatively lower compared to resistance factors from the toxicity test in all the populations. For instance, carboxylesterase activity of *P. xylostella* on cabbage at Dzorwulu was almost quadruple and 8-fold that at Opeibea and Korle-Bu, respectively. This might stem from the possibility that some carboxylesterase isozymes in *P. xylostella* at Opeibea and Korle-Bu did not effectively hydrolyse the 1-naphthyl acetate substrate used in the current study. Wheelock et al. (2005) has reported that some CarE isozymes do not hydrolyse p-nitrophenyl acetate or α-naphthylacetate at all and even if they do, hydrolysis may be ineffectively done. Also there is the likelihood that apart from carboxylesterases, other enzymes such as oxidases and glutathione-s-transferases may be involved in DBM resistance as reported elsewhere (Maa *et al*., 1997).

There was also a significant positive correlation between carboxylesterase activity of *P. xylostella* populations and the LD_{50} values for Karate® insecticide

and a relatively higher significant positive correlation between carboxylesterase activity of the DBM and the LD_{50} values for Attack® insecticide.

The results of this study have serious implications for brassica growers in Ghana since the development of resistance is a major constraint to effective control of most pests with pesticides. Farmers may be compelled to apply higher doses of these insecticides at higher frequencies in order to achieve some level of control which may further exacerbate the resistance problem. Early detection of resistance should provide pest control practitioners the option to develop a long term strategy to arrest further resistance development and institute a sound integrated approach to solving pest problems (Obeng-Ofori et al., 2002). It is suggested that national monitoring network should be established to monitor insecticide resistance in major insect pests in all national agricultural systems where application of pesticides constitute a major approach to pest control (Obeng-Ofori et al., 2002)

Conclusion

The significant increase in the resistance ratios compared with earlier studies confirmed that DBM populations have developed resistance to the pyrethroid (karate) assayed. This insecticide should therefore be removed from the Ghanaian market and replaced with other products such as *Bacillus thuringiensis* and the neonicotinoids which have been reported in other studies to be efficacious against the DBM but these should be used in a rotational manner.

The low carboxylesterase activity recorded suggests that apart from carboxylesterase, other enzymes such as oxidases and glutathione-S-tranferase which have been reported to be involved in DBM resistance may be involved and should be investigated.

For effective management of DBM, resistance development must be delayed. This could be achieved by strategically applying insecticides at the most vulnerable developmental stages of the DBM. The use of insecticides with short persistence is preferred to avoid prolonged exposure to insecticides, which is a prerequisite to resistance development. A more integrated approach using IPM principles such as cultural practices, host plant resistance should also be incorporated to delay resistance development. It will also be rewarding setting up a monitoring network to monitor resistance in major insect pests in all national agricultural systems where insecticide application constitutes a major approach to pest control.

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