Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

PEST MANAGEMENT

Insecticide Resistance in Populations of the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), from the State of Pernambuco, Brazil

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Keywords
Insect, chemical control, survey, leaf-dipping bioassay, lethal ratio

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Edited by Raul N Guedes – UFV

Received 02 May 2010 and accepted 09 August 2010

Abstract

The diamondback moth *Plutella xylostella* (L.) has a great economic importance in Brassicaceae crops in many parts of the world. Recurrent infestations of this pest in growing areas of Pernambuco state, Brazil, have led farmers to frequently spray their crops with insecticides. However, control failures by several insecticides have been alleged by farmers. The objective of this study was to check whether resistance to insecticides could explain these control failures in *P. xylostella*. Populations of *P. xylostella* from Pernambuco were collected between January and April 2009. The resistance ratios of *P. xylostella* populations were compared among five different active ingredients: abamectin, methomyl, lufenuron, indoxacarb, and diafenthiuron by leaf dipping bioassays using foliar discs of kale leaves. Mortality data were submitted to probit analysis. The *P. xylostella* populations showed variable response and significant resistance to one or more insecticides. The population from Bezerros County exhibited the highest resistance ratios to indoxacarb (25.3 times), abamectin (61.7 times), and lufenuron (705.2 times), when compared to the reference population. The populations from Bonito and Jupi Counties were 33.0 and 12.0 times more resistant to lufenuron and abamectin, respectively, when compared with the reference population. Resistance to methomyl was the least common, but not less important, in at least four populations. These results indicated that control failures were associated with resistance by some of the evaluated insecticides, reinforcing the need for resistance management in areas of the state of Pernambuco.

Introduction

The diamondback moth, *Plutella xylostella* (L.), is considered the most destructive pest of Brassicaceae not only in Brazil, but also in several other regions of the world (Talekar & Shelton 1993, Castelo Branco & França 2001). To reduce yield losses caused by this insect pest, many growers mainly use chemical control because of the lack of reliable alternatives and the availability of relatively cheap insecticides (Talekar & Shelton 1993). However, over the years, this type of control has proved to be ineffective because even 15 to 20 insecticide applications during a crop cycle have not reduced the losses caused by *P. xylostella* (Pérez *et al* 2000). In Brazil, it is very common to adopt three insecticide sprays a week, as observed in areas close to Distrito Federal (Castelo Branco *et al* 2001) and usually up to four applications per week have been observed in Pernambuco (Oliveira *et al* 2011). Besides the consequences to the environment and human health, the inappropriate use of insecticides has selected populations of *P. xylostella* resistant to insecticides (Talekar & Shelton 1993, Castelo Branco *et al* 2001, Sayyed *et al* 2004, Attique *et al* 2006, Khaliq *et al* 2007).

In addition to the resistance problem, other characteristics of *P. xylostella*, such as the high migratory capacity and biotic potential, overlapping generations, and the availability of hosts due to staggered cropping (Talekar & Shelton 1993, Sarfraz *et al* 2006), have hampered the insect pest control, contributing in this way for selecting resistant populations as already reported for the classes of pyrethroids, organophosphates, carbamates (Yu & Nguyen 1992), and biological insecticides such as the *Bacillus thuringiensis* (Tabashnik *et al* 1990, Shelton *et al* 1993a).

In Brazil, resistant populations of *P. xylostella* have been restricted to Distrito Federal, particularly to pyrethroids, organophosphates, and *B. thuringiensis* (Castelo Branco & Gatehouse 1997). More recently, resistance to pyrethroids and avermectins has also been reported in populations from the States of Espírito Santo and Pernambuco (Oliveira *et al* 2011), which is a concern for both states, particularly due to insecticide overuse. Despite these reports, several other insecticides such as methomyl, diafenthiuron, indoxacarb, and lufenuron are frequently used in Pernambuco, but no cases of resistance are reported yet to these compounds. Nevertheless, claims of control failures are very common for these insecticides in many areas of the state. The objectives of the current work were to survey the susceptibility of *P. xylostella* populations originated from Pernambuco and to test the hypothesis of resistance evolution to abamectin, methomyl, diafenthiuron, indoxacarb, and lufenuron.

Material and Methods

P. xylostella *populations*

The susceptible population of *P. xylostella* was obtained from the rearing stock kept at the Laboratório de Biologia de Insetos, from the Universidade Federal Rural de Pernambuco (UFRPE) for about ten years without any insecticide exposure after collection in the Chã Grande County, PE (herein, named Chã Grande I). Seven populations of *P. xylostella* were collected from January to April 2009 in cultivated areas from the agreste of Pernambuco (Fig 1). These counties were chosen because of their importance in brassica production and the history of insecticide intensive use to control *P. xylostella*. Approximately 50-200 larvae and pupae of each *P. xylostella* population were collected and transported to the Laboratório de Interações Insetos-Tóxicos of the Departamento de Agronomia from UFRPE, where they were reared according to Barros & Vendramim (1999).

Insecticides

Bioassays were performed with the insecticides abamectin (36g/l, Milênia Agrociências S/A, Paraná), methomyl (215g/l, DuPont do Brasil, São Paulo), lufenuron (50g/l, Syngenta, São Paulo) diafenthiuron (500g/kg, Syngenta, São Paulo), and indoxacarb (300g/kg, DuPont do Brasil, São Paulo), obtained from manufacturers and pesticide stores in Recife, PE.

Bioassays

Leaf dip bioassays based on previous work (Shelton *et al* 1993a, Zhao *et al* 2002) were performed. For each population, preliminary tests were performed with the insecticides to establish the range of concentration where the concentration-response relationship occurs. At least four different concentrations of insecticides were tested in the preliminary tests and seven concentrations were used in the definitive bioassays. The concentrations were achieved by diluting the given insecticide in distilled water + Triton X-100 (0.01%). The control treatment consisted just of distilled water + Triton X-100 (0.01%). After preparing the treatments, leaf discs (19.64 cm^2) of kale, *Brassica oleracea* var. *acephala*, were immersed in the solutions. The leaves were then placed on paper towels until completely dried and transferred to Petri dishes (60 mm) covered with moistened filter paper (60 mm). Ten 2^{nd} instar larvae per Petri dish were transferred

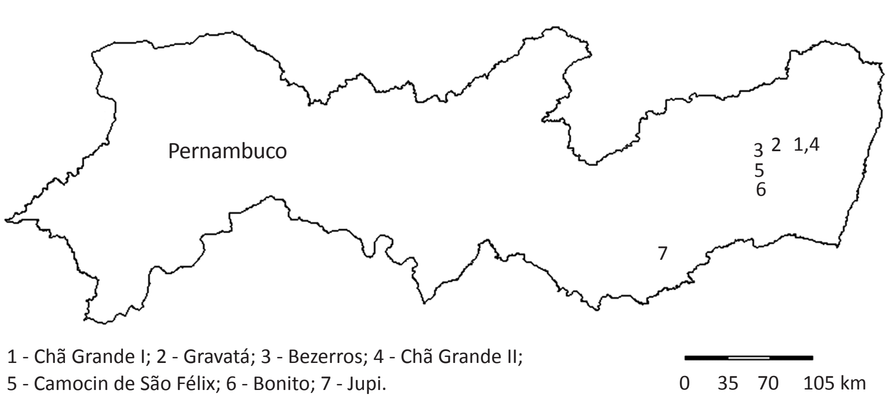

Fig 1 Locations in the agreste of Pernambuco state where *Plutella xylostella* populations were collected.

with the aid of a soft brush. Each treatment was replicated three times and designed as a complete random design. Each bioassay was repeated at least twice, performed inside a growth chamber (BOD) (27 ± 0.2ºC; 65 ± 5% RH, and 12h photophase). Mortality data were assessed 48h after placing the larvae on the treated leaves, except for lufenuron, which was evaluated after 96h. Those larvae whose whole body failed to respond when touched were considered dead (Tabashnik *et al* 1990).

Data analysis

Bioassays that showed mortality rates above 10% in the control plots were discarded. The mortality data were corrected according to Abbott's formula (Abbott 1925) and subjected to probit analysis (Finney 1971) at P > 0.05 using the program POLO-Plus 2.0 (LeOra-Software 2005). The resistance ratios were calculated by the "lethal ratio test" and considered significant when the confidence interval (CI) at 95% did not include the value one as proposed by Robertson & Preisler (1992). For each insecticide, the population with the lowest LC_{50} was used as the reference population for making comparisons with the others. Insecticide resistance level was classified by using RRs in terms widely accepted as follows: susceptibility (RR = 1), tolerance to low-resistance (RR = 2-10), moderate resistance (RR = 11-30), high resistance (RR = 31-100), and very high resistance (RR > 100). The Pearson correlation coefficient (r) was used to test for the existence of a relationship between Log LC_{50}s and LC_{95}s among the tested insecticides (Sokal & Rohlf 1994) using PROC CORR (SAS Institute Inc. 1999) at P < 0.05.

Results

The probit model fitted to mortality data from the different insecticides tested towards the populations of *P. xylostella* from Pernambuco (χ^2 is not significant, P > 0.05). The population of Chã Grande I (Lab) was the most susceptible to all insecticides, with a variation among the LC_{50}s of 0.01 mg/L for abamectin up to 48 mg/L for diafenthiuron (Table 1).

The LC_{50}s of diafenthiuron, methomyl, indoxacarb, abamectin, and lufenuron were 48-122.2, 0.4-7.3, 0.2-7.6, 0.01-0.74 and 0.02-18.31 mg/l, respectively (Table 1). The resistance ratios (RR) for diafenthiuron, methomyl, indoxacarb, abamectin, and lufenuron ranged from 1.3 to 2.5, 1.1 to 6.4, 0.8 to 25.3, 2.2 to 61.7, and 1.6 to 705.2 times, respectively, when compared with the reference population (Chã Grande I) (Table 1). Following the non-inclusion criterion of the value 1.0 in the confidence interval at 95% probability for the resistance ratio, only the populations from Gravatá, Jupi, and Bonito Counties exhibited significant resistance ratios for diafenthiuron (1.6, 2.3, and 2.5 times, respectively) (Table 1), and thus, they showed low resistance (RR ≤ 10 times) to this compound. Four populations exhibited significant resistance ratios to methomyl: Chã Grande II (RR = 2.7 times), Bezerros (RR = 4.0 times), Camocin (RR = 5.6 times), and Gravatá (RR = 6.4 times) (Table 1). The remaining populations, i.e., Bonito and Jupi, showed tolerance to low resistance ratio (RR ≤ 10 times).

For indoxacarb, only the populations from Bonito and Bezerros Counties showed significant resistance to this insecticide, with corresponding resistance ratios of 4.0 and 25.3 times when compared with the reference population. Bezerros County population exhibited a moderate resistance level (10 < RR ≤ 30 times) while the population from Bonito showed low resistance. All populations of *P. xylostella* tested with the insecticide abamectin showed significant resistance ratios. Among the populations, Bonito, Chã Grande II, and Camocin exhibited low resistance (RR ≤ 10 times), while Jupi and Bezerros showed respectively moderate resistance (10 < RR ≤ 30 times) and high resistance (30 < RR ≤ 100 times) (Table 1).

Among the tested insecticides, lufenuron was the one causing the highest resistance level (RR > 100 times), represented by Bezerros County population of *P. xylostella* (RR = 705.2 times) (Table 1). High resistance ratio was observed for the Bonito County population (RR = 33.1 times). The other populations were either susceptible of exhibited only low levels of lufenuron resistance.

Pairwise correlation analysis was performed among LC_{50}s and LC_{95}s across insecticides tested towards *P. xylostella* (Table 2). Significant correlation was only observed between the LC_{50}s of lufenuron and indoxacarb (r = 0.99, P = 0.002; n = 5; Table 2). Significant LC_{95}s correlation was observed between diafenthiuron and lufenuron (r = 0.90, P = 0.04; n = 5; Table 2) and diafenthiuron and indoxacarb (r = 0.81, P = 0.05; n = 6; Table 2), while no correlation was detected between lufenuron and indoxacarb (r = 0.85, P = 0.07; n = 5; Table 2).

Discussion

Growers have frequently sprayed Brassicaceae crops from Pernambuco with pesticides. Because they usually do not have access to technical assistance, label rate recommendations are often misused, leading to the selection of diamondback moth resistant individuals. To date, the resistance in *P. xylostella* populations from these areas is known for deltamethrin and abamectin (Oliveira *et al* 2011), although abamectin resistance levels were considered moderate in that study. The current work shows that resistance to abamectin and other insecticides, even to relatively new molecules, is already spreading in the region. Resistance levels ranged from low to high, depending on the insecticide tested. For instance, the first

Table 1 Insecticide resistance in populations of *Plutella xylostella* from state of Pernambuco, Brazil.

Insecticide Population	N (DF)	Slope ± SE	LC_{50} (CI 95%) mg/l	LC_{95} (CI 95%) mg/l	$\chi^{2\,(1)}$	RR_{50} [(2)] (CI 95%)
Diafenthiuron						
Chã Grande I	381 (6)	1.7 ± 0.19	48.0 (38.55 – 57.71)	418.3 (290.76 – 718.40)	3.3	---
Camocin	240 (6)	1.6 ± 0.42	61.9 (38.44 – 249.76)	703.7 (196.99 – 39686)	1.2	1.3 (0.6 – 2.7)
Chã Grande II	444 (6)	2.8 ± 0.34	65.5 (52.09 – 76.50)	258.8 (213.68 – 347.60)	5.8	1.4 (1.0 – 1.8)
Bezerros	202 (5)	1.4 ± 0.29	76.0 (25.45 – 129.67)	1117.8 (677.66 – 3027.25)	2.8	1.6 (0.8 – 3.3)
Gravatá	205 (5)	3.0 ± 0.45	77.4 (55.51 – 96.20)	278.9 (220.16 – 406.46)	3.2	1.6 (1.2 – 2.2)*
Jupi	268 (5)	2.6 ± 0.28	109.7 (91.37 – 128.80)	471.2 (365.89 – 689.89)	2.7	2.3 (1.7 – 3.0)*
Bonito	348 (5)	2.3 ± 0.23	122.2 (99.25 – 144.84)	635.9 (498.42 – 892.70)	2.4	2.5 (1.9 – 3.4)*
Methomyl						
Chã Grande I	317 (7)	1.7 ± 0.15	0.4 (0.30 – 0.57)	3.7 (2.26 – 7.51)	7.6	----
Bonito	236 (6)	2.4 ± 0.54	0.6 (0.24 – 0.64)	2.3 (1.55 – 5.44)	5.9	1.1 (0.4 – 2.5)
Jupi	280 (6)	1.8 ± 0.18	0.6 (0.42 – 0.86)	4.9 (2.69 – 13.92)	7.8	1.4 (0.6 – 3.1)
Chã Grande II	333 (5)	3.4 ± 0.51	1.1 (0.74 – 1.38)	3.4 (2.58 – 5.74)	5.0	2.7 (1.2 – 5.6) *
Bezerros	579 (5)	3.0 ± 0.34	1.1 (0.78 – 1.30)	3.8 (2.82 – 6.12)	5.8	4.0 (3.1 – 5.2) *
Camocin	185 (6)	2.5 ± 0.40	2.3 (1.48 – 3.23)	10.9 (7.48 – 20.34)	3.2	5.6 (2.4 – 13.0) *
Gravatá	261 (7)	5.8 ± 1.26	7.3 (5.79 – 8.37)	14.1 (11.98 – 19.91)	6.8	6.4 (4.9 – 8.3) *
Indoxacarb						
Chã Grande I	405 (5)	2.1 ± 0.17	0.2 (0.24 – 0.36)	1.8 (1.34 – 2.54)	3.9	----
Chã Grande II	319 (5)	2.5 ± 0.22	0.2 (0.20 – 0.29)	1.1 (0.85 – 1.62)	4.6	0.8 (0.6 – 1.1)
Camocin	224 (7)	1.8 ± 0.26	0.3 (0.17 – 0.39)	2.3 (1.32 – 5.87)	4.7	0.9 (0.6 – 1.4)
Jupi	356 (7)	1.5 ± 0.13	0.3 (0.17 – 0.42)	3.1 (1.51 – 11.17)	13.8	0.9 (0.6 – 1.2)
Bonito	283 (5)	1.4 ± 0.15	1.2 (0.88 – 1.59)	18.6 (11.21 – 39.73)	2.8	4.0 (3.3 – 4.9)*
Bezerros	366 (7)	3.2 ± 0.44	7.6 (5.69 – 8.96)	24.8 (19.78 – 38.08)	7.5	25.3 (19.4 – 32.9)*
Abamectin						
Chã Grande I	379 (6)	1.9 ± 0.17	0.01 (0.01 – 0.02)	0.08 (0.06 – 0.13)	4.4	---
Bonito	216 (6)	2.7 ± 0.45	0.02 (0.01 – 0.03)	0.10 (0.07 – 0.26)	7.1	2.2 (1.5 – 3.0) *
Chã Grande II	338 (5)	1.5 ± 0.16	0.04 (0.03 – 0.06)	0.58 (0.37 – 1.15)	1.4	4.0 (2.8 – 5.6) *
Camocin	345 (5)	1.8 ± 0.19	0.05 (0.03 – 0.07)	0.46 (0.33 – 0.75)	3.6	4.5 (3.2 – 6.4) *
Jupi	513 (9)	1.0 ± 0.07	0.14 (0.10 – 0.19)	6.66 (4.00 – 12.65)	4.7	12.0 (8.0 – 17.7) *
Bezerros	330 (5)	1.7 ± 0.17	0.74 (0.56 – 0.93)	7.26 (5.03 – 12.19)	2.6	61.7 (44.3 – 85.7) *
Lufenuron						
Chã Grande I	366 (11)	0.5 ± 0.05	0.02 (0.01 – 0.05)	66.55 (16.66 – 570.61)	0.6	---
Chã Grande II	137 (5)	1.4 ± 0.19	0.04 (0.02 – 0.09)	0.69 (0.24 – 5.88)	5.8	1.6 (1.0 – 2.7)
Camocin	262 (7)	0.6 ± 0.09	0.10 (0.01 – 0.36)	44.23 (7.48 – 3480)	13.4	4.0 (1.6 – 10.1)*
Bonito	230 (6)	0.7 ± 0.10	0.86 (0.05 – 2.98)	283.53 (58.78 – 20131)	11.1	33.1 (12.7 – 86.1)*
Bezerros	228 (6)	0.9 ± 0.13	18.31 (11.79 – 29.70)	1104.55 (384.72 – 7156.26)	2.7	705.2 (429.4 – 1158.3)*

[(1)]Chi-square ($P > 0.05$); [(2)]Resistance ratio: ratio of LC50 estimates between resistant and susceptible populations according to Robertson & Preisler (1992) and 95% RR confidence interval. *Resistance ratio is significant if confidence interval do not comprise the value 1.0.

reports of *P. xylostella* resistant to lufenuron in Brazil are herein identified, showing high resistance ratios for Bonito and Bezerros populations, 33.0 and 705 times, respectively. High resistance to lufenuron particularly observed for the Bezerros County population may be the result of frequent sprays of insecticides in the vegetable

Table 2 Correlation coefficients of pairwise comparisons between log LC_{50} and LC_{95} values of the evaluated insecticides towards *Plutella xylostella* populations.

	Abamectin	Diafenthiuron	Indoxacarb	Lufenuron
		LC50		
Diafenthiuron	0.26^{ns}			
Indoxacarb	0.67^{ns}	0.33^{ns}		
Lufenuron	0.82^{ns}	0.58^{ns}	$0.99^{0.002}$	
Methomyl	0.34^{ns}	-0.09^{ns}	0.08^{ns}	0.13^{ns}
		LC95		
Diafenthiuron	0.34^{ns}			
Indoxacarb	0.24^{ns}	$0.81^{0.05}$		
Lufenuron	0.22^{ns}	$0.90^{0.04}$	0.85^{ns}	
Methomyl	0.23^{ns}	-0.22^{ns}	-0.39^{ns}	-0.10^{ns}

^{ns}Not significant

crops. But also, there has been a history of intense use of insect growth regulators (IGRs) in the region, which may explain the very high resistance to lufenuron found in the Bezerros County population. The insecticide is still under use in the area and these results may suggest the need to implement strategies to overcome resistance. The responses to lufenuron were not similar across the populations, with some of them exhibiting high susceptibility such as the Chã Grande II population. This insecticide has been effective in the control of other *P. xylostella* populations elsewhere as showed by Lima & Barros (2000) and Thuler *et al* (2007).

The Bezerros County population still exhibited moderate and high resistance to indoxacarb and abamectin, respectively. The moderate level of resistance (RR = 25.3 times) to indoxacarb was lower than that observed by Sayyed & Wright (2006) in the Cameron Highlands, Malaysia. The resistance to indoxacarb in Bezerros population is possibly associated with its frequent use, despite the rotation usually adopted with methomyl- and *B. thuringiensis*-based products. Moreover, eventual cross-resistance with insect growth regulators is still possible because strong correlation was observed in this study between lufenuron and indoxacarb LC's, which might be due to either simultaneous selection for resistance to each insecticide or cross-resistance in which resistance to one insecticide confers resistance to the other (Pérez *et al* 2000). Sayyed & Wright (2006) showed that esterases were involved in the resistance of *P. xylostella* to indoxacarb, which was unexpected since indoxacarb is a pro-insecticide that is known to be activated through hydrolysis by esterases (Wing *et al* 2000). Therefore, it is possible that resistance to indoxacarb in this population may be associated with a metabolic detoxification mechanism of lufenuron or other IGRs other than that usually used to activate the indoxacarb. Nevertheless, further studies will determine whether or not a mechanism is present conferring cross-resistance among these insecticides or if more than one mechanism is conferring multiple resistances, and thus clarifying the factors that possibly have led to this resistance scenario in the Bezerros County population.

Abamectin was the only insecticide tested in this study that is not registered for Brassicaceae crops in Brazil, and in spite of that has been largely used by growers in cropping areas of Pernambuco. In China (Zhang *et al* 2001, Pu *et al* 2010) and Malaysia (Iqbal *et al* 1996), where abamectin is registered for *P. xylostella* control, cases of field resistance have already been reported. In Brazil, resistance to this insecticide has been identified in populations of *P. xylostella* from both the Distrito Federal and Pernambuco State (Castelo Branco & Melo 2002, Oliveira *et al* 2011). In the current study, all populations exhibited significant resistance to the insecticide abamectin at different extents and such differences can be explained by the varying field rate dosis and application frequencies to which these populations have been subjected. The particularly high resistance ratio estimated for the Bezerros population to abamectin may be due to previous strong selection pressure exerted with insect growth regulator and pyrethroid insecticides in these areas. Nevertheless, cross-resistance is still possible between these two classes of insecticides as reported by Cao & Han (2006). Selection of *P. xylostella* for resistance to tebufenozide, an insect growth regulator, led to abamectin resistance associated with enhanced mixed-function oxidases (MFOs) (Qian *et al* 2008).

Resistance of *P. xylostella* populations to diafenthiuron was a minor problem in this study. This insecticide has been fairly used in Pernambuco, which may explain the observed outcomes. No resistance to this insecticide was reported in Taiwan populations during a three-year assessment particularly because of its little use (Kao & Cheng 2001). To date, no case of diafenthiuron resistance is reported in the literature for *P. xylostella* to the best of our knowledge. Nevertheless, diafenthiuron monitoring and management should be established in these areas because the apparent onset of resistance observed in the current study, i.e., LC_{95} values close to field rate dose. Despite the reported cases of high resistance in populations of *P. xylostella* to methomyl (Sun *et al* 1978, Yu & Nguyen 1992, Shelton *et al* 1993b), populations of *P. xylostella* from Pernambuco showed relatively low resistance levels. This is likely a consequence of insecticide rotation by growers and the cessation of insecticide applications in off-season, which can significantly reduce the resistance level of a population (Murai *et al* 1992). However, the use of methomyl should be closely monitored because its frequency is high during the cropping season in Pernambuco.

The current study shows that insecticide resistance is present in populations of *P. xylostella* from Pernambuco, but other circumstances such as incorrect use of insecticides should be investigated to further clarify the control failures in several fields. Misleading crop management practices in this area is an important factor that has caused these insecticide resistance problems. Most growers from the region are characterized as small-scale farmers that cultivate no more than 10 ha, and usually do not use other control methods to reduce pest densities in their fields. Reliance only in the chemical control has led them to spray more and more often their crops. Also, constant food availability for *P. xylostella,* either by staggered or abandoned crops, has maintained resistance genes in the area allowing the local selection of resistant individuals.

From the geographical standpoint, it appears that migration or dispersal in the region may be of secondary importance and the current results show that this hypothesis may be plausible, because resistance variation among local populations were very high. If long distance migration has occurred in the region, one might expect more homogeneous response for resistance among the populations. Recent work (Roux *et al* 2007) suggested that very limited migration probably occurs in areas of favorable growing conditions, what appears to be the case in Pernambuco. The association of the high number of generations of *P. xylostella* in the region and high frequency of insecticide use with the potential low migration can lead to local selection of different resistance mechanisms, and this would explain the great variability among populations. However, these hypotheses need further investigation to elucidate the current resistance outcomes.

These results can be used as a basis for further studies on toxicology, particularly on management of resistance in *P. xylostella* populations from Pernambuco, leading to the selection of more effective insecticides to be used in rotation within a resistance management program. Also, adopting an integrated pest management program is an urgent need, but very challenging. At first, simple measures such as plowing cultural remains as soon as possible by every grower, following the manufacturers' labels, monitoring pest densities using economic injury thresholds, and making use of pesticides, when indeed they are needed, have to be established for the area leading to resistance reduction or regression. Additionally, extra work is required to further characterize these resistance cases in insect populations from Pernambuco, particularly the potential co-occurrence of insecticide resistance mechanisms to two or more insecticides, which will fine-tune a resistance management program in the area.

Acknowledgments

To Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco - FACEPE for providing the assistantship to the first author and to the Conselho Nacional de Devenvolvimento Científico e Tecnológico - CNPq for the financial support to the project (Universal 470825/2008-1, H.A.A.S.).

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