Resistance of Brazilian diamondback moth populations to insecticides

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ABSTRACT: *Plutella xylostella* is a recurring pest on cruciferous crops around the world. In Brazil, it typically requires large number of insecticide sprays, which may lead to fast evolution of resistance. The aim of this study was to assess the susceptibility of Brazilian diamondback moth populations to the insecticides abamectin, deltamethrin, and spinosad. Leaf dip bioassays were used to determine mortality data obtained after 48 h of exposure to insecticides and subjected to Probit analysis. The population from Bonito, state of Pernambuco, Brazil, had the highest toxicity ratio (20.2 - fold) to abamectin compared to the reference population. The LC_{50} values for deltamethrin ranged from 85.2 to 360.1 mg L^{-1}, demonstrating a high survival of populations in relation to this insecticide field dose rate (7.5 mg L^{-1}). The toxicity ratios of the estimated LC_{50}s, however, were very low (varying from 2.2 - to 4.2 - fold). Most populations exhibited toxicity ratios for spinosad, ranging from 2.3 - to 5.1 - fold, while both the LC_{50} and LC_{95} values reflected a high susceptibility to the spinosad field dosage (120 mg L^{-1}). Only the Bonito - PE population resisted to abamectin, while all *P. xylostella* populations were resistant to deltamethrin, but particularly susceptible to spinosad because of the absence of selection pressure with it in these areas.

Key words: *Plutella xylostella*, Lepidoptera, chemical control, susceptibility

Resistência de populações brasileiras de traça das brássicas a inseticidas

RESUMO: *Plutella xylostella* é uma praga recorrente em brássicas de todo o mundo. No Brasil, normalmente exige grande número de pulverizações de inseticidas, que pode levar à rápida evolução da resistência. Avaliou-se a suscetibilidade de populações brasileiras da traça das crucíferas aos inseticidas abamectina, deltametrina e espinosade. Bioensaios de imersão de folhas foram utilizados para determinar a mortalidade, sendo os dados obtidos após 48 h de exposição aos inseticidas e submetidos à análise de Probit. A população de Bonito-PE apresentou a maior razão de toxicidade (20,2 vezes) para abamectina em relação à população de referência. Os valores de CL_{50}s para deltametrina variaram entre 85,2 - 360,1 mg L^{-1}, demonstrando alta sobrevivência das populações a este inseticida com relação à dose de campo (7,5 mg L^{-1}). Entretanto, as razões de toxicidade das CL_{50} estimadas foram muito baixas (variando de 2,2 a 4,2 vezes). A maioria das populações apresentou razões de toxicidade para espinosade, variando de 2,3 para 5,1 vezes, embora os valores de CL demonstram alta suscetibilidade delas à dose de campo para espinosade (120 mg L^{-1}). Apenas a população de Bonito – PE apresentou resistência a abamectina, enquanto todas as populações de *P. xylostella* estão resistentes a deltametrina, mas suscetíveis ao espinosade particularmente devido à ausência de pressão de seleção com este nestas áreas.

Palavras-chave: *Plutella xylostella*, Lepidoptera, controle químico, suscetibilidade

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), ranks in the top 20 most resistant insect species reported so far, which has developed resistance to virtually all insecticide classes (Sarfraz and Keddie, 2005). It is the most important Brassicaceae pest in many parts of the world (Talekar and Shelton, 1993). In Brazil, it occurs year round (Melo et al., 1994), particularly because cruciferous vegetables are usually staggered grown throughout the year. To reduce losses inflicted by the diamondback moth, most farmers have opted for the chemical method, the most used for control (França et al., 1985), which can apparently produce the best results through intensive sprays of insecticides (Castelo Branco et al., 2003). However, this practice has been inefficient over the time because up to four applications a week have apparently not reduced the losses caused by the diamondback moth (Castelo Branco et al., 2001). In many other parts of the world, up to 15 or 20 sprays per season are not uncommon, independently of the pest presence (Guan-Soon, 1990). The indiscriminate use of insecticides in Brazil has contributed to increasing environmental pollution, high levels of residues on products, and intoxication cases (Araújo et al., 2000). In general, these drawbacks are associated with another more aggravating problem, the evolution of insecticide resistance in insect populations (Georghiou, 1983), resulting from high selection pressure with pesticides. In the case of *P. xylostella,* several generations a year and its high migration potential have contributed to faster selection of resistant populations to several classes of insecticides (Sarfraz and Keddie, 2005).

Populations of *P. xylostella* have a long history of resistance evolution to virtually all classes of insecticides (Khaliq et al., 2007; Shelton et al., 2000; Talekar and Shelton, 1993), including the biological insecticides such as the *Bacillus thuringiensis* (Berl.) (Shelton et al., 1993). In the recent years, groups of insecticides have been developed and showed excellent activities towards *P. xylostella* populations resistant to traditional products such as organophosphates, carbamates, and pyrethroids. Among those, the avermectins (França and Medeiros, 1998), insect growth regulators (Oouchi, 2005), emamectin benzoate, spinosad, and indoxacarb (Wilson et al., 2006) are included. But, cases of resistance to these insecticides have already been reported in the literature such as to spinosad (Zhao et al., 2002; Zhao et al., 2006).

The first evidence of resistant populations of diamondback moth to insecticides in Brazil was reported in 1997 for pyrethroids, organophosphates, and *B. thuringiensis* in the Federal District area (Castelo Branco et al., 1997). Despite that, surveys from other regions do not exist, particularly in the Northeast region where indiscriminate use of pesticides is high and control failures are very common among growers. Therefore, the present work aimed to evaluate the current susceptibility of *P. xylostella* populations to deltamethrin, spinosad, and abamectin. Deltamethrin use has been long in Northeast Brazil to control the diamondback moth and sprays with this insecticide have been nowadays very reduced in the region due to lack of efficacy. The abamectin is not registered to control *P. xylostella* in Brazil, but farmers use it sporadically in some areas while spinosad is used in a lesser extent because it is costly.

Material and Methods

Eight populations of diamondback moth from three crucifer-growing regions in Brazil were bioassayed to compare their susceptibility to deltamethrin, abamectin, and spinosad (Figure 1, Table 1). These populations were reared individually in the laboratory in the absence of insecticides treatments, but assayed not beyond the 7th generation. The Chã-Grande I - PE population has been under laboratory rearing with no insecticide selection pressure since 1998. The *P. xylostella* rearing procedures were based on those described in Barros and Vendramin (1999). Adults were kept in cylinder cages and fed 10% honey-water. Eggs were collected on collard discs every day and transferred to plastic cages containing organic *Brassica oleracea* var. *acephala* fresh leaves. The number of eggs was adjusted to ensure that overcrowding did not occur to reduce the fitness of the larvae. The insecticides used in this study were abamectin, deltamethrin, and spinosad.

Leaf dip bioassays as previously reported (Shelton et al., 1993; Shelton et al., 2000) were used for each population of *P. xylostella*, but insecticide-free collard (*Brassica oleracea* var. *acephala*) leaves were used instead. Concentration-response lines were established with the insecticides abamectin, deltamethrin, and spinosad for the different populations of *P. xylostella* through bioassays to assess mortality. Preliminary assays were done with every insecticide for each population to determine an "all or none" response, i.e., the concentration range where a concentration-response relationship exists.

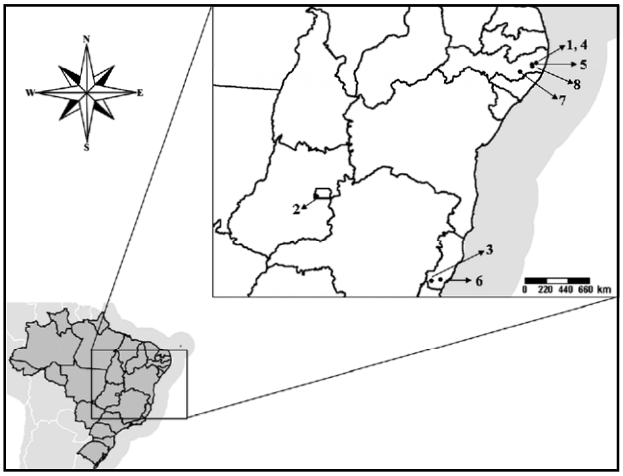

Figure 1 – Site collection of diamondback moth, *Plutella xylostella,* populations.

Table 1 – Origin and collection year of diamondback moth, *Plutella xylostella,* populations in Brazil.

Map Reference	State	County	Region	Host	Place	Month and year of collection
1	Pernambuco	Chã Grande I	Northeast	Cabbage	Laboratory	May 1998
2	Distrito Federal	Brasília	Middle west	Cabbage	Laboratory	September 2004
3	Espírito Santo	Alegre	Southeast	Collard	Laboratory	November 2006
4	Pernambuco	Chã Grande II	Northeast	Cabbage	Field	August 2008
5	Pernambuco	Camocin	Northeast	Cabbage	Field	May 2008
6	Espírito Santo	Vargem Alta	Southeast	Cabbage	Field	September 2007
7	Pernambuco	Garanhuns	Northeast	Cabbage	Field	February 2008
8	Pernambuco	Bonito	Northeast	Cabbage	Field	May 2008

Leaves of organic collard were used in the bioassays after sanitization in sodium hypochlorite (5%) and thorough rinsing in clean water. Leaf discs of 5 cm diameter were cut out with a sharp metal cylinder, and dipped for one minute into each concentration, and left to dry at room temperature. Later, discs were transferred into Petri dishes (60 × 15 mm), containing filter papers (5 cm) moistened with H_2O. At least seven insecticide concentrations (+Triton X-100 at 0.01%, as spreader) with three replicates were used. The control comprised leaf discs treated with Triton X-100 solution at 0.01%. Following, ten late 2nd-instar larvae of *P. xylostella* were transferred to each dish with the aid of a soft brush. The Petri dishes were maintained inside a growth chamber at the temperature of 27 ± 0.2°C and R.H. of 65 ± 5%. The mortality was evaluated 48 h after exposure and the bioassays were repeated at least one more time. The mortality criterion was based on the movement of larvae for at least the body length after being prodded by a fine brush (Kabir et al., 1993). When control mortality was greater than 10%, bioassays were discard and repeated. Mortality data were subjected to probit analysis (Finney, 1971) after correction (Abbott, 1925) using the POLO-PC program (Leora Software, 1987). Toxicity ratios and their 95% confidence intervals were calculated following the method described by Robertson and Preisler (1992), and considered significant ($p < 0.05$) when the confidence interval did not include the value 1.0. The Pearson correlation coefficient (r) was used to test the pairwise relationship between the Log LC$_{50}$s as well as the LC$_{95}$s among insecticides (Sokal and Rohlf, 1995) using the PROC CORR (SAS Institute, 2001).

Results and Discussion

There was a variation in susceptibility among the populations for the insecticides evaluated, based on the inclusion criterion of the value 1.0 in the 95% CI of the toxicity ratio (Table 2, 3 and 4). Because of the lack of a general standard susceptible population, toxicity ratios were compared with the most susceptible population for each insecticide. Based on the LC$_{50}$ estimates for the *P. xylostella* populations, the Chã Grande I-PE population was the most susceptible to the abamectin insecticide (Table 2), while Vargem Alta-ES was the most susceptible to deltamethrin (Table 3), and Garanhuns-PE to spinosad (Table 4).

The LC$_{50}$ values of abamectin for *P. xylostella* populations varied from 0.007 to 0.136 mg L^{-1} (Table 2). Among the populations, six showed toxicity ratios to abamectin (2.1 - to 20.2 - fold). Only the Bonito - PE population, however, presented a relatively high toxicity ratio (20.2 - fold)

Table 2 – Susceptibility of *Plutella xylostella* populations to abamectin.

Population	n(1)	DF(2)	Slope ± SE(3)	LC$_{50}$ (CI 95%) mg L^{-1}	LC$_{95}$ (CI 95%) mg L^{-1}	χ^{2}(4)	TR(5) (CI 95 %)(6)
Chã Grande I-PE	253	5	2.12 ± 0.26 a	0.007 (0.005 - 0.009)	0.040 (0.028 - 0.068)	2.16	—
Brasília-DF	293	5	2.55 ± 0.27 b	0.008 (0.007 - 0.010)	0.036 (0.027 - 0.054)	1.86	1.2 (0.9 - 1.7)
Alegre-ES	350	5	1.89 ± 0.22 a	0.014 (0.011 - 0.017)	0.105 (0.075 - 0.174)	1.84	2.1 (1.5 - 3.0)*
Chã Grande II-PE	268	5	1.83 ± 0.23 a	0.016 (0.012 - 0.022)	0.126 (0.072 - 0.309)	3.87	2.4 (1.6 - 3.5)*
Camocin-PE	319	5	2.04 ± 0.27 a	0.019 (0.014 - 0.024)	0.124 (0.090 - 0.206)	3.16	2.9 (2.0 - 4.1)*
Vargem Alta-ES	297	5	2.25 ± 0.26 a	0.019 (0.015 - 0.023)	0.103 (0.077 - 0.158)	1.96	2.9 (2.0 - 4.0)*
Garanhuns-PE	327	5	1.83 ± 0.23 a	0.022 (0.018 - 0.027)	0.174 (0.115 - 0.331)	4.16	3.3 (2.4 - 4.6)*
Bonito-PE	264	5	2.61 ± 0.31 b	0.136 (0.110 - 0.161)	0.579 (0.443 - 0.866)	3.74	20.2 (14.7 - 27.8)*

[1] Number of insects bioassayed. [2] Degree of Freedom. [3] Standard Error. [4] Chi Square ($p > 0.05$). [5] Toxicity ratio – calculated between the estimated LC$_{50}$ of susceptible and tolerant populations through Robertson and Preisler (1992) method. [6] Confidence interval at 95% of the toxicity ratio. *Toxicity ratio significant (confidence interval does not include the value 1.0).

Table 3 – Susceptibility of *Plutella xylostella* populations to deltamethrin.

Population	n(1)	DF(2)	Slope ± SE(3)	LC$_{50}$ (CI 95%) mg L^{-1}	LC$_{95}$ (CI 95%) mg L^{-1}	χ^{2}(4)	TR(5) (CI 95 %)(6)
Vargem Alta-ES	320	6	3.50 ± 0.35b	85.19 (75.16 - 97.03)	251.75 (201.70 - 345.17)	4.12	—
Brasília-DF	353	5	2.97 ± 0.43a	94.95 (82.55 - 107.46)	338.71(256.13 - 546.85)	3.52	1.1 (0.7 - 1.8)
Camocin-PE	301	5	2.81 ± 0.29a	185.82 (154.80 - 218.26)	715.42 (565.85 - 991.30)	3.93	2.2 (1.3 - 3.6)*
Alegre-ES	305	5	2.72 ± 0.29a	222.90 (188.44 - 263.83)	896.81 (673.35 - 1361)	1.43	2.6 (0. 8 - 8.8)
Chã Grande II-PE	302	5	2.46 ± 0.28a	258.75 (217.70 - 306.46)	1204 (878.58 - 1938)	2.35	3.0 (1.8 - 5.1)*
Bonito-PE	258	5	2.35 ± 0.31a	261.87 (215.98 - 314.86)	1311 (917.48 - 2339)	2.47	3.1 (1.8 - 5.2)*
Chã Grande I-PE	313	5	2.43 ± 0.28a	276.76 (234.29 - 327.51)	1312 (947.42 - 2150)	3.39	3.3 (1.0 - 10.2)*
Garanhuns-PE	376	5	3.01 ± 0.36a	360.10 (317.22 - 411.38)	1266 (962.90 - 1938)	2.27	4.2 (2.6 - 7.0)*

[1] Number of insects bioassayed. [2] Degree of Freedom. [3] Standard Error. [4] Chi Square ($p > 0.05$). [5] Toxicity ratio – calculated between the estimated LC$_{50}$ of susceptible and tolerant populations through Robertson and Preisler (1992) method. [6] Confidence interval at 95% of the toxicity ratio. *Toxicity ratio significant (confidence interval does not include the value 1.0).

Table 4 – Susceptibility of *Plutella xylostella* populations to spinosad.

Population	n(1)	DF(2)	Slope ± SE(3)	LC_{50} (CI 95%) mg L^{-1}	LC_{95} (CI 95%) mg L^{-1}	$\chi^{2(4)}$	TR(5) (CI 95 %)(6)
Garanhuns-PE	240	5	2.96 ± 0.33b	0.014 (0.012 - 0.017)	0.052 (0.040 - 0.075)	1.96	—
Chã Grande I-PE	312	5	2.07 ± 0.22a	0.023 (0.019 - 0.028)	0.144 (0.100 - 0.246)	3.61	1.6 (0.8 - 3.1)
Brasília-DF	295	5	1.90 ± 0.22a	0.025 (0.020 - 0.031)	0.183 (0.119 - 0.351)	3.29	1.7 (0.8 - 3.3)
Vargem Alta-ES	273	5	3.75 ± 0.41b	0.033 (0.028 - 0.037)	0.090 (0.074 - 0.118)	3.48	2.3 (1.2 - 4.3)*
Bonito-PE	300	5	2.05 ± 0.25a	0.038 (0.031 - 0.046)	0.240 (0.162 - 0.438)	3.17	2.6 (1.4 - 5.0)*
Camocin-PE	366	6	2.45 ± 0.31a	0.047 (0.040 - 0.056)	0.220 (0.151 - 0.404)	1.34	3.3 (1.7 - 6.2)*
Alegre-ES	385	5	2.65 ± 0.36a	0.062 (0.046 - 0.077)	0.258 (0.200 - 0.381)	3.51	4.3 (2.2 - 8.4)*
Chã Grande II-PE	318	5	1.99 ± 0.24a	0.074 (0.060 - 0.090)	0.496 (0.346 - 0.863)	2.53	5.1 (2.7 - 9.9)*

[1]Number of insects bioassayed. [2]Degree of Freedom. [3]Standard Error. [4]Chi Square ($p > 0.05$). [5]Toxicity ratio – calculated between the estimated LC_{50} of susceptible and tolerant populations through Robertson and Preisler (1992) method. [6]Confidence interval at 95% of the toxicity ratio. *Toxicity ratio significant (confidence interval does not include the value 1.0).

when compared with the most susceptible population (Chã Grande I - PE), which may indicate an onset of resistance. High resistance levels of diamondback moth to abamectin have been reported in areas of Malaysia (Sayyed et al., 2004), which suggests that Brazilian populations may also evolve quickly to resistance. Abamectin is not registered to control pests of Brassicaceae in Brazil. In spite of this, growers have used it in instances where other insecticides have showed control failures as in the Agreste region of Pernambuco State. Indeed, the area where the Bonito-PE population was collected, the farmer was spraying abamectin-based products to control the diamondback moth until few days before the colony collection. The indiscriminate use of this product may actually worsen the resistance problems in these regions, even before its eventual registration.

The observed toxicity ratio suggests that proactive monitoring and management programs need to be implemented to sustain the potential use of insecticides not yet registered to control *P. xylostella* in Brazil. Also, the resistance ratio of abamectin for Bonito-PE population may be an indicative that resistance frequencies are close to a critical value, requiring a particular attention to the use of this product in the area. The broad use of insect growth regulators (IGRs) in some regions, such as the Agreste of Pernambuco, may have contributed to the fast abamectin resistance evolution. Populations selected with tebufenozide in laboratory resulted in cross-resistance to abamectin (Cao and Han, 2006). But, the results of the present work suggest that this hypothesis needs further investigation, because resistance to abamectin was restricted to a unique site. In fact, the other populations were particularly susceptible to it and toxicity ratio may reflect only the natural variability of those populations to abamectin.

The LC_{50} values for deltamethrin were very high (85.2 to 360.1 mg L^{-1}) among the populations when compared with the field rate dosage, although the toxicity ratios to deltamethrin were low (2.2 - to 4.2 - fold), particularly because the lack of a general standard susceptible population of *P. xylostella* to this insecticide in this study (Table 3). For instance, the Vargem Alta - ES population, the most susceptible population to deltamethrin, showed an LC_{50} of 85.2 mg L^{-1}, which is about 11-fold higher than the field rate dosage (7.5 mg L^{-1} of abamectin). Field rate dosage must be able to kill a high percentage, generally 95% of a pest population regardless of its population density (Knipling, 1979). This efficacy loss has been observed at several other places in Brazil (Castelo Branco et al., 2003) without a clear explanation, but it is associated with resistance evolution in the evaluated populations. The LC_{50} values showed that these *P. xylostella* populations have been subjected to high selection pressure with deltamethrin or other pyrethroids over many years. Indeed, control failures with pyrethroids have been a constant in many of those areas, particularly in the Pernambuco State, which has a long history of pyrethroids use, mainly deltamethrin. Possibly other pyrethroids have likely lost their efficacies towards local *P. xylostella* populations.

An important aspect of the pyrethroid resistance is its stability (Georghiou, 1983; Chen and Sun, 1986). Although this has not been assessed in this study, the Chã Grande I - PE population, with an LC_{50} of 276.8 mg L^{-1}, which correspond to 39 times the field dosage rate, is a strong case to support that hypothesis, despite of its maintenance in laboratory without any insecticide selection pressure for about ten years. Nevertheless, Sayyed and Wright (2006) stated that the toxicity ratio to deltamethrin in a diamondback moth population decreased from 498 to 47 μg mL^{-1}, when they compared the generation F_2 and F_8. Therefore, it is likely that the LC_{50} for deltamethrin in the Chã Grande I - PE population was manyfold higher at the collection time.

Ten years after introduction of spinosad in Asia to control *P. xylostella,* Attique et al. (2006) and Khaliq et al. (2007) observed that toxicity ratios varied from 1.0- to 12.0-fold, during survey for resistance in *P. xylostella* populations from Pakistan. Eziah et al. (2008) verified that Australian field populations of *P. xylostella* were susceptible to spinosad. In the present study, although five populations had toxicity ratios to spinosad (2.3 - to 5.1 - fold), both field and laboratory populations surveyed were susceptible to the insecticide (Table 4). Lethal concentration (LC_{50}) values varied from 0.014 mg L^{-1} (Garanhuns-PE) to 0.074 mg L^{-1} (Chã Grande II-PE), a 5.3-fold difference in susceptibility, which are very low values compared with the field dose rate (120 mg L^{-1}). Additionally, these values are still below the discriminating dose (10 mg L^{-1}), which separate the SS and RS genotypes from RR genotype in *P. xylostella* (Zhao et al., 2002). These values possibly reflect the natural variability of populations when responding to spinosad, and particularly because the laboratory populations were never exposed to this insecticide. Despite this observation, resistance to spinosad has already been reported in many populations of *P. xylostella* worldwide (Sayyed et al., 2004; Zhao et al., 2002; Zhao et al., 2006), which suggests that spinosad use in Brazil must be cautiously pursued. Castelo Branco et al. (2003) obtained 100% mortality 72 hours after spraying 96 mg L^{-1} of spinosad on some Brazilian populations of the diamondback moth. Sayyed et al. (2004) reported that the majority of growers from Malaysia used to spray spinosad to control diamondback moth since the end of 1990 and, because of the high spinosad pressure towards this pest, up to 171 - fold toxicity ratio to spinosad was reported in Malaysian field populations (Sayyed and Wright, 2006). Additionally, insects from other orders such as *Musca domestica* (Shono and Scott, 2003) and *Franklinella occidentalis* have already been reported to resist to spinosad (Zhang et al., 2008). These authors suggest that resistance to spinosad can rapidly appear in diamondback moth populations from Brazil, and thus spinosad use must be monitored after its introduction. For a while, the high cost of spinosad may prevent growers from using it indiscriminately, therefore delaying the onset of resistance.

No evidence of cross-resistance among the evaluated insecticides was observed. There was no correlation between Log LC_{50}s of abamectin and deltamethrin ($r = 0.142$; $p > 0.05$, $n = 8$), abamectin and spinosad ($r = -0.033$; $p > 0.05$, $n = 8$), and deltamethrin and spinosad ($r = -0.060$; $p > 0.05$, $n = 8$). Same trend was observed for pairwise correlation analysis of Log LC_{95}s. Sayyed et al. (2005) showed no evidence of cross-resistance, neither between deltamethrin and spinosad nor between deltamethrin and abamectin. Therefore, rotational use of spinosad and abamectin, together with the current use of Bt-based products, can be applied in Brazilian areas where pyrethroids have failed in the control of *P. xylostella*. However, a monitoring program designed to detect changes in the susceptibility, which may result from repeated use and prolonged exposure to these insecticides under field conditions, will be essential to these insecticides long-term sustainability.

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