CROP PROTECTION

A Survey of Insecticide Susceptibility in *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil

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Avaliação da Susceptibilidade a Inseticidas em *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) no Distrito Federal, Brasil

RESUMO – O nível de suscetibilidade a inseticidas em populações de *Plutella xylostella* (L.) coletadas no Distrito Federal foi avaliado em 1995 e 1996. Uma homogeneidade na suscetibilidade a acefato e fentoato foi observada nas populações amostradas. Uma delas, altamente suscetível a *Bacillus thuringiensis*, teve decréscimo no seu nível de suscetibilidade em duas gerações, após 10 aplicações do produto. Para esta população foi observado um aumento de 36 vezes na CL_{50}. Movimentos da praga entre as áreas de cultivo podem ter sido, em parte, responsáveis pelos resultados observados.

PALAVRAS-CHAVE: Insecta, traça-das-crucíferas, resistência a inseticidas

ABSTRACT - The level of insecticide susceptibility of strains of *Plutella xylostella* (L.) from the Federal District was evaluated in 1995 and 1996. A homogeneity in acephate and phentoate susceptibility was observed in the surveyed strains. One strain, highly susceptible to *Bacillus thuringiensis* lost its susceptibility after 10 insecticide sprays in two generations in the field with a 36-fold increase in its LC_{50}. Movements of the pest could have accounted for the observed results.

KEY WORDS: Insecta, diamondback moth, insecticide resistance.

Plutella xylostella (L.), the diamondback moth (DBM) has been reported as an important brassica pest since the beginning of this century and several insecticides have been employed for its control. The successive launch of insecticides occurred because, frequently, populations resistant to the new products were selected in a few months or years. The first report of resistance to insecticides in DBM came from Java. In that country, resistance to DDT appeared three years after the introduction of that insecticide in the field and resistance was suspected because increased doses of DDT were needed to control the pest (Arkersmit 1953). After this first detection of resistance, DBM populations resistant to insecticides were reported all over the world (Lee & Lee 1979, Liu *et al.* 1982, Hama 1987, Guan-Soon 1990, Ikin *et al.* 1993, Shelton *et al.* 1993a), including Brazil (Campos *et al.* 1997, Castelo Branco & Gatehouse 1997). The highest levels of resistance were generally associated with areas of intensive brassica cultivation (Cheng 1986, Tabashnik *et al.* 1987).

The objective of the work described here was to understand the spatial distribution of susceptibility to insecticides in DBM populations from the Federal District, Brazil. In order to obtain these results, the levels of susceptibility to *Bacillus thuringiensis,* and the ornanophosphorous (OPs) phenthoate and acephate were measured in strains collected in 1995 and 1996 from areas of brassica production.

Materials and Methods

Diamondback moth strains were collected from cabbage at four different geographical regions in the Federal District in 1995 and 1996 which were: Embrapa Hortaliças (CNPH), Vargem Bonita; Planaltina and Brazlândia. Field collections of 100 to 300 third - fourth instar larvae and pupae of DBM were made in grower areas located in the places described in Table 1. Strains were named according to the site of collection using the three-four initial letters. When more than one strain was collected in one site a number was added to differentiate one from another. Farmers were interviewed to determine the number of sprays and insecticides used up to the time of sampling. Pyrethroids and OPs insecticides were generally applied weekly in the early dry season (April to June) and two to three times per week in the late dry season (July to October). Other insecticides were sprayed weekly. Little or no insecticide was sprayed in the rainy season (November to March). Table 1 provides a summary of the information of the collected strain.

The field collected strains were bioassayed in the first or second laboratory generation. Three formulated insecticides were used in laboratory bioassays. They were: acephate, *B. thuringiensis* and phenthoate. Thrid instar larvae were used in all bioassays. A leaf-dip bioassay, by using cabbage leaf disk, was carried out to determine the susceptibility of the selected strains to the insecticides described above. Depending on the number of insects obtained in the rearing procedure, each population was exposed to one or more insecticides. Commercial formulations were serially diluted in water assuming a spray volume of 400 litres/ha. A surfactant, Agral® (ICI do Brasil - Zeneca), at a rate of 20 ml/100 litres of water was added to the solutions.

Bioassays were conducted at 20°C and a photoperiod of 13L:11D as described in Castelo Branco & Gatehouse (1997). Larval mortality to phenthoate was assessed after 24h, to acephate after 48h and to *B. thuringiensis* after 72h. Larvae that did not show coordinated movement or did not move when touched with a paint brush were considered dead. Data from the insecticides acephate, *B. thuringiensis* and phentoate were corrected for mortality using Abbott's formula (Abbott 1925). If mortality in the control treatment was higher than 10%, the replication was discarded. Concentration-mortality data were analysed by the probit method (Finney 1971), using the computer programme POLO (Russel *et al.* 1977) to estimate $LC_{50's}$ and their 95% confidential limits (referred to as CL hereafter), slopes and chi-squares. Strains were considered significantly different if their 95% CL of the LC_{50} did not overlap.

Results and Discussion

B. thuringiensis was not frequently used in the Federal District. In this work we found that only two growers used the product and in both cases the insecticide was not used alone (Table 1). Pyrethroids and OP compounds were the

Table 1. Place and date of collection of diamondback moth strains and insecticides used for pest control in the Federal District, Brazil, 1995 and 1996.

Place of collection	Strain	Date of collection	Insecticides
Brazlândia	BZD1[1]	June, October 1996	Metamidophos from April 1995 to June 1996. Deltamethrin from June to October 1996.
	BZD2[1]	June 1996	Deltamethrin from June 1995 to June 1996.
	BZD3[1]	October 1996	*B. thuringiensis* plus abamectin from March 1995 to October 1996.
	BZD4[2]	June, October 1996	Deltamethrin from January 1994 to June 1996. Chlorfluazuron plus metamidophos from June to October 1996.
	BZD5[1]	August 1996	Deltamethrin from June 1995 to June 1996.
CNPH	CNPH[3]	June, October 1995 June,	Acephate from September to October 1995. August and October 1996. Cartap from August to October 1995. Chlorfluazuron from January 1996.
Planaltina	PLA1[1]	August, October 1996	Phenthoate from 1995 to August 1996. Phenthoate plus *B. thuringiensis* from August to October 1996.
	PLA2[1]	August 1996	Phenthoate from 1995 to August 1996.
Vargem Bonita	VARG[1]	June, October 1996	Deltamethrin from 1995 to October 1996.

[1]Insecticides were sprayed once or twice a week in the dry season (April to October). Insecticides were rarely sprayed in the rainy season (November to March).
[2]Deltamethrin was sprayed weekly in the rainy season and once or twice a week in the dry season. Chlorfluazuron plus metamidophos were sprayed weekly.
[3]Acephate or Cartap were sprayed weekly in a plot measuring 240 m^2 between August and November 1995. Chlorfluazuron was sprayed weekly in a cabbage plot measuring 600 m^2 between January and June 1996 and six times between June and August 1996 in a broccoli plot measuring 1200 m^2.

most common insecticides (Table 1).

DBM strains collected in CNPH and Brazlândia (BZD1 and BZD2) fields in June differed significantly in their level of susceptibility to *B. thuringiensis* from strain PLA1 collected in Planaltina in August. The lowest LC_{50} was found in strain PLA1 which was therefore the most susceptible one (Table 2).

The LC_{50} from strains collected in CNPH, Brazlândia (BZD3) and Planaltina (PLA1) in Ocotober 1996 ranged from 0.7 to 1.8 g a.i./ha (Table 2). At that time, strain PLA1 was the least susceptible one (Table 2).

Strain CNPH collected in June and October 1996 showed an LC_{50} of 0.4 and 0.7 g a.i./ha respectively and no significant difference in the level of susceptibility was observed between the two sample dates (Table 2). On the other hand, strain PLA1 collected in August and October 1996 showed a significant difference in its level of susceptibility to *B. thuringiensis* between the two sample dates. (Table 2). After 10 insecticide sprays of *B. thuringiensis*, strain PLA1 collected in October showed a 36-fold increase in its LC_{50}. This means that this change in the level of susceptibility occurred after only approximately two generations of DBM in the field, assuming a mean of 25 days/generation.

Two hypotheses may explain the apparent quick loss of susceptibility to *B. thuringiensis* in population PLA1 after 10 sprays of the insecticide. The first hypothesis proposes that the less susceptible genotypes present in strain PLA1 before the first spray could have been sufficient for a rapid increase in their frequency as a result of the frequent sprays and the level of susceptibility to the insecticide was associated with insecticide sprays (Cheng 1986, Tabashnik *et al.* 1987, Castelo Branco & Gatehouse 1997). As the environmental conditions were favourable (no rain and temperatures in the region of 28°C), a rapid increase in population size in the area was observed and confirmed by the grower who, one week before sampling, had used doses of *B. thuringiensis* that were three times the recommended field rate. This spray at such a high dose rate could have contributed to the observed increase in the LC_{50}.

According to the second hypothesis, the increase in the LC_{50} of strain PLA1 which was observed from August to October could be attributed to a dilution of the high-susceptibility genotypes observed in June by interbreeding with less susceptible ones from nearby populations following local movements as proposed by Caprio & Tabashnik (1992), as well as to selection by intensive spraying between the two sampling dates. Immigration of less susceptible genotypes into cultivated brassica areas has often been reported to result in significant changes in insecticide susceptibility in DBM strains in Japan (Hama 1990, Mizukoshi 1994).

Table 2. Dose-response date for *B. thuringiensis* in diamondback moth populations from the Federal District, Brazil in 1996.

Strain	n	LC50 (CL95%)[1]	Slope ± SD	χ^2	df	RF[2]
			June or August 1996			
PLA1	391	0,05 (0,02 – 0,10)	1,26 ± 0,19	4,94	6	1,0
CNPH	155	0,4 (0,2 – 0,7)	1,36 ± 0,20	3,77	4	8,0
BZD1	258	0,6 (0,3 – 0,9)	1,55 ± 0,37	2,44	5	12,0
BZD2	308	1,4 (0,9 – 2,0)	1,49 ± 0,25	3,41	5	28,0
			October 1996			
CNPH	233	0,7 (0,5 – 1,1)	2,13 ± 0,31	0,55	5	1,0
BZD3	210	1,3 (0,7 – 1,9)	1,69 ± 0,30	0,35	4	1,8
PLA1	194	1,8 (1,3 – 2,3)	3,24 ± 0,59	4,47	5	2,5

[1]g.a.i/ha
[2]RF= Resistance factor. Calculated for each sample date.

To acephate, strain CNPH sampled in June 1995 and in August 1996 showed $LC_{50's}$ of 150 and 195 g a.i./ha respectively. No significant difference in the level of susceptibility was detected (Table 3). In this case the LC_{50} remained stable, despite no OP sprays being used to control DBM in the previous ten months in that area (Table 1). In strains sampled in June or August 1996, the LC_{50} to acephate showed values that ranged from 170 to 333 g.a.i./ha (Table 3). Strain BZD1, sprayed with metamidophos [the first compound formed when acephate degradation begins (Bouchardy & Lavy 1982)], showed an intermediate LC_{50}. No significant differences in the level of susceptibility to acephate were detected among the strains surveyed (Table 3).

Two hypotheses could explain the apparent stability of the level of susceptibility to acephate in strain CNPH. The first is that the level of susceptibility to these insecticides did not change or changed very slowly in absence of sprays, i.e. the time elapsed between the samples was too small to detect any change. The same hypothesis was proposed by Tabashnik *et al.* (1990) to explain the apparent stability of susceptibility to *B. thuringiesis* in Hawaii.

The second hypothesis is that continual immigration of individuals of less susceptible genotypes to acephate into the area did not allow an increase in the level of susceptibility to these insecticides in strain CNPH. Also movements of the pest could explain the fact that a strain that was sprayed several times with the OP metamidophos (BZD1) did not

Table 3. Dose-response date for acephate in diamondback moth populations from the Federal District, Brazil in 1995 and 1996.

Strain	n	LC50 (CL95%)[1]	Slope ± SD	χ^2	df	RF
			June 1995[2]			
CNPH	242	150 (85 - 1990)	2,14 ± 0,32	1,39	4	1,0
			June or August 1996			
CNPH	182	195 (120 - 263)	2,57 ± 0,47	5,18	6	1,3
BZD1	220	255 (173 - 338)	2,57 ± 0,43	2,48	4	1,7
BZD2	247	333 (124 - 540)	2,57 ± 0,47	4,00	4	2,2
VARG	313	170 (131 - 213)	2.37 ± 0.26	1,91	5	1,1

[1]g.a.i./ha

show any significant difference in its level of susceptibility in relation to no sprayed strains (BZD2, CNPH and VARG) (Table 3). Similar results was found by Shelton *et al.* (1993b) in Florida. The authors collected DBM strains in areas sprayed and not sprayed with *B. thuringiensis.* A strain sampled from a field that was not cultivated year round, had low DBM populations and was not frequently sprayed with the insecticide, had similar levels of susceptibility to *B. thuringiensis* when compared with a strain sampled from an area where brassicas were cultivated year round, had high DBM populations and where the insecticide was sprayed weekly or more frequently. As OPs compounds are not used in all fields at the same time (Table 1), populations resistant to this compound is selected in some areas and not in others. The dispersion of these resistant and susceptible insects among the cultivated fields could cause in some occasions a homogeniety in the level of susceptibility as described by Comins (1977).

To phenthoate no significant differences in the level of susceptibility were found among strains collected in unsprayed areas (strains CNPH and BZD6) and strains collected in areas sprayed with phenthoate weekly (strains PLA1 and PLA2) (Table 4). In this case the level of susceptibility to this insecticide was not influenced by insecticide sprays. One cause of this result could be the movements of DBM among sprayed and no sprayed areas.

When the immigration of DBM into a cultivated field in CNPH was evaluated, it was observed that less than 16 males were captured until 14 May. After that time the number of trapped males increased and more than 30 adults were caught in six of the following eight weeks (Fig. 1). In spite of these consistent and latterly high catches of moths in the trap, the number of larvae on the cabbage plants remained low due to weekly chlorfluazuron sprays. In the samples taken between March and June a maximum of 0,47 larvae/plant was recorded and no larvae were found in the last seven weeks of the experiment (Fig. 1). This fact indicates that the trapped males were immigrants that came from outside areas and confirms that movements of DBM among cultivated brassica fields occurs.

In summary the results obtained here showed the occurrence of immigration of DBM into cabbage fields; it was suggested that movement of the pest can influence the level of susceptibility to insecticides. In some cases this movement could cause a homogeneity in the level of susceptibility to some insecticides as observed here to acephate and phenthoate and as observed to *Helicoverpa armigera* Hübner in Austrália (Daly 1993). In other cases movements of the pest, associated with high numbers of insecticide sprays could cause a rapid decrease in the level of susceptibility to some insecticides, as observed here with *B. thuringiensis.* Because DBM has developed resistance to several insecticides in several places, it is important to develop successful insecticide resistance management strategies. To do that we need extensive information about the extension of the movements of DBM and their impact in spreading

Table 4. Dose-response date for phenthoate in diamondback moth populations from the Federal District, Brazil in 1996.

Strain	n	LC50 (CL95%)[1]	Slope ± SD	χ^2	df	RF
			August 1996			
BZD5	273	48 (30 - 69)	2,84 ± 0,42	0,40	5	1,0
CNPH	282	48 (30 - 70)	2,33 ± 0,32	4,10	5	1,0
PLA1	211	162 (41 - 367)	1,55 ± 0,21	7,11	5	3,3
PLA2	273	147 (68 - 244)	1,55 ± 0,21	8,35	5	3,0

[1]g.a.i/ha

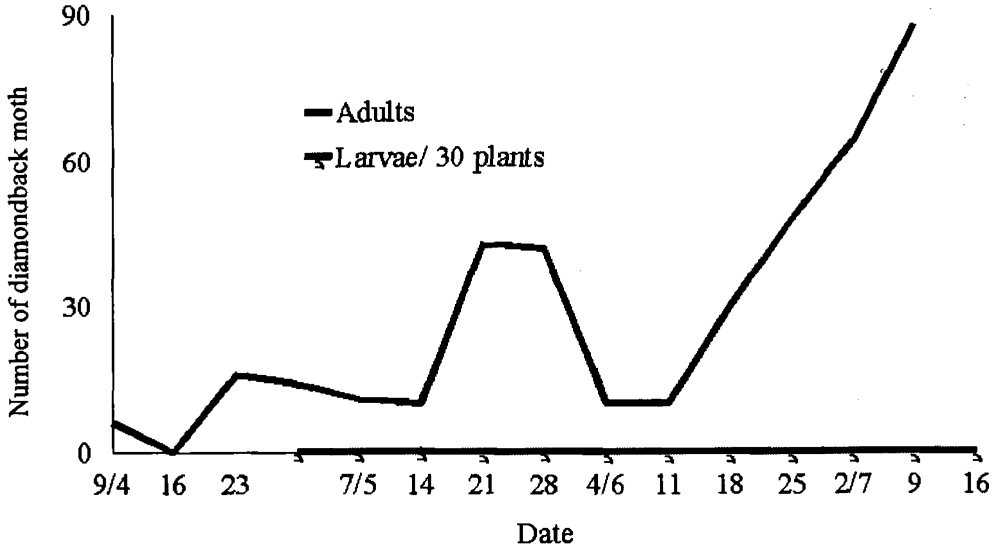

Figure 1. Number of diamondback moth males caught in a pheromone trap in a cabbage plot sprayed wekly with chlorfluazuron and number of larvae on 30 plants. Federal District, Embrapa Hortaliças, 1996.

insecticide resistance. Techniques of molecular biology may be useful in this work, as it can estimate gene flow among populations.

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