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High resistance levels in brazilian *Plutella xylostella* populations: needs for adjustments in field concentration

Níveis de alta resistência em populações de *Plutella xylostella* no Brasil: necessidade de ajuste da dose de campo

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ABSTRACT - *Plutella xylostella* cause severe damage on cruciferous plants all over the world. Farmers in several regions of Brazil report increasing inefficiency of chemical control, even when using high insecticide concentrations. We evaluate here the susceptibility of brazilian diamondback moth populations to the Premio® (Chlorantraniliprole), Dipel® (*Bacillus thuringiensis* var. *kurstaki*) and Lannate® BR (Oxime Methylcarbamate) insecticides, frequently used in Brazil. Susceptibility bioassays with five field-collected and two laboratory diamondback moth populations were conducted with increasing concentrations of insecticides up to ten times above the recommended concentration. Extremely high and region-dependent resistances were found in field populations against Chlorantraniliprole and *B. thuringiensis*, with Resistance Ratios up to 370.0 times for the Camocim de São Felix population. For Oxime Methylcarbamate, we were not able to do Probit analyses for the field populations because of very low mortality rates. Laboratory populations showed resistance to the three tested insecticides with all LC_{50} concentrations exceeding the recommended doses by at least 3 times. Our results show strong and variable resistance to the three tested insecticides according to the region of origin. To maintain efficient pest control in a large country like Brazil, local levels of resistance need therefore to be monitored by the authorities and reassessment and adjustments of regional doses of insecticides should be implemented as a public policy, to prevent massive spread of insecticides in the field, as well as increases in cases of resistance.

Keywords: Insecticide. Control failure. Resistance monitoring.

RESUMO - *Plutella xylostella* causa graves danos em plantas crucíferas em todo o mundo. Agricultores em várias regiões do Brasil relatam uma crescente ineficiência no controle químico, mesmo com o uso de altas concentrações de inseticidas. O objetivo deste trabalho foi avaliar a suscetibilidade de populações brasileiras da traça-das-crucíferas aos inseticidas Premio® (Chlorantraniliprole), Dipel® (*Bacillus thuringiensis* var. *kurstaki*) e Lannate® BR (Metilcarbamato de oxina), frequentemente usados no Brasil. Os bioensaios de suscetibilidade foram realizados com cinco populações de traça-das-crucíferas coletadas em campo e duas de laboratório e foram conduzidos com concentrações crescentes de inseticidas até dez vezes acima da concentração recomendada. Resistências extremamente altas e dependentes da região foram encontradas em populações de campo para o Chlorantraniliprole e *B. thuringiensis*, com Razões de Resistência de até 370,0 vezes para a população de Camocin de São Felix . Para Metilcarbamato de oxina, não foi possível realizar a análise Probit para as populações de campo devido baixa mortalidade. Populações de laboratório demonstraram resistência aos três inseticidas testados com concentrações de CL_{50} excedendo as doses recomendadas em pelo menos 3,0 vezes. Os resultados demonstraram resistência forte e variável aos três inseticidas testados de acordo com a região de origem. Para manter o controle de pragas eficiente em um país grande como o Brasil, os níveis locais de resistência precisam ser monitorados pelas autoridades e reavaliações e ajustes de doses regionais dos inseticidas deveriam ser implementadas como política pública para evitar a disseminação massiva de inseticidas em campo, assim como, aumentos dos casos de resistência.

Palavras chaves: Inseticida. Falha de controle. Monitoramento de resistência.

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INTRODUCTION

Brassicaceae are a family of vegetables with a large variety of cultivated species (ARIAS et al., 2014; FOURIE et al., 2016). In the Brazilian Northeast, reductions in production areas are frequent due to drought and abundant pest attacks, especially by the diamondback moth *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) causing yield losses (RIBEIRO et al., 2014; MELO et al., 2017). Considered an important factor in limiting Brassicaceae production, the diamondback moth can cause close to 100% damage in crop systems (TALEKAR; SHELTON, 1993; SHELTON et al., 1993). Thus, significant losses have been recorded in various parts of the world, mainly caused by the larval stage of the pest (ZALUCKI et al., 2012). In addition, pupae contamination can also reduce the commercial value of the cultivated material (TROCZKA et al., 2016). In tropical regions, damage caused by diamondback moths is potentiated due to favorable environmental conditions for their development, associated with their high genetic and biochemical plasticity and migratory capacity (TALEKAR; SHELTON, 1993; SARFRAZ; KEDDIE, 2005; LI et al., 2016). These factors together with the practice of staggered planting and simultaneous cultivation of different species of *Brassica* contribute directly to pest population outbreaks (BARROS; VENDRAMIN, 1999).

To reduce the losses caused by *P. xylostella*, farmers use almost exclusively chemical control due to its practicality, efficiency, and immediate results (FEYEREISEN, 1995; CASTELO BRANCO; MELO, 2002; MOTA-SANCHEZ; BILLS; WHALON, 2002; VILAS BÔAS et al., 2004; SANTOS et al., 2011; ZAGO et al., 2014; SINIARD; WADE; DRURY, 2016). However, intensive applications and non-rotation of active ingredients have selected high resistance levels of the diamondback moth populations for all insecticide groups used (NANSEN et al., 2016). Therefore, high cost of control and pest management has been estimated globally between 4 and 5 billion dollars per year (ZALUCKI et al., 2012; FURLONG; WRIGHT; DOSDALL, 2013).

As observed by Xia et al. (2014) in China, with the report of three or even four insecticide applications per week against *P. xylostella*, similar practices have been described in some Brazilian *Brassica* plantations of the Federal District and in Pernambuco State (CASTELO BRANCO; AMARAL, 2002). Several areas of production have been also abandoned due to the control failure and inefficiency of insecticides leading farmers to use (without success) up to 4 applications per week (OLIVEIRA et al., 2011; LIMA NETO et al., 2016).

Bio-ecological, genetic, and operational factors are responsible for fast evolutionary adaptation and the emergence of resistant herbivore insects, presenting the main obstacle to the safe control of agricultural pests (GEORGHIOU; TAYLOR, 1997). This research aimed to evaluate the susceptibility or resistance of diamondback moth populations collected in different municipalities in the north-east and the south-east of Brazil to the insecticides Brilhante® (Oxime Methylcarbamate), Premio® (Chlorantraniliprole) and Dipel® (*Bacillus thuringiensis* var. *kurstaki*) and to test the efficiency of field insecticide concentrations.

MATERIALS AND METHODS

Insects

Seven populations of *P. xylostella* were used for the susceptibility bioassays; five field and two laboratories populations. Four field populations within an area of 200 km were collected in *Brassica oleracea* var. *acephala* and *Brassica oleracea* var. *capitata* cultivation fields in the municipalities of Bezerros (PE), Sairé (PE), Camocim de São Félix (PE), Lajedo (PE) in the north-east (semi-arid region) and one, Venda Nova do Imigrante (ES), was collected in the south east of Brazil (tropical region) (Figure 1, Table 1). Two populations (one for each region), separated by more than 1600 km, provided by the Entomology Departments of Rural Federal University of Pernambuco (UFRPE) (Recife – Pernambuco) and Federal University of Viçosa (UFV) (Viçosa – Minas Gerais), maintained in the laboratory without any exposure to insecticides were also used in the bioassays as reference populations (Figure1).

All populations of diamondback moths were fed with insecticide-free leaves of *B. oleracea* var. *acephala* following the methodology described by Barros and Vendramim (1999). The collected populations were reproduced separately in cages and the tests were carried out with the second and third instar larvae.

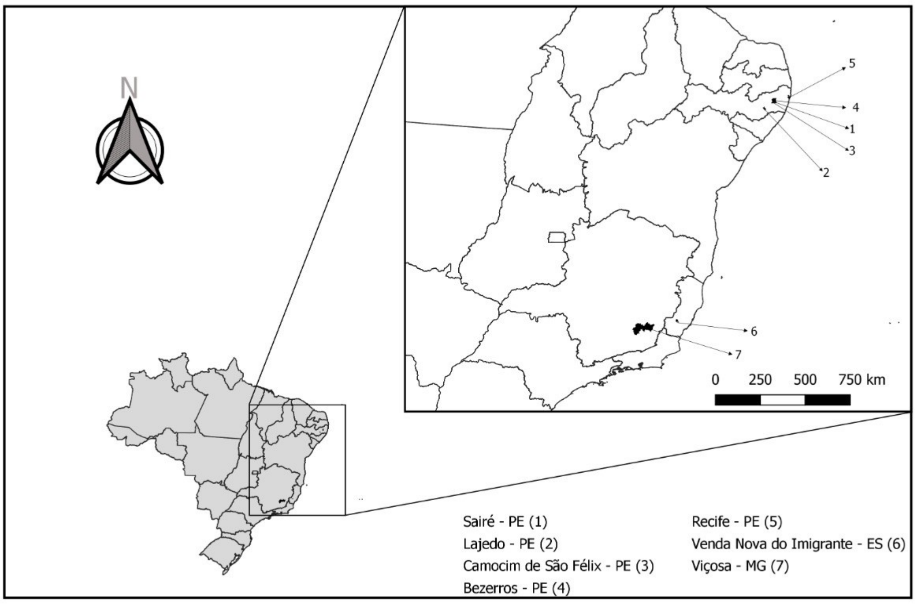

Figure 1. Geographical origin of Brazilian *Plutella xylostella* populations used in tests.

Table 1. Description of the population origin, year of collection, geographic coordinates, host, insect numbers and development stage of *Plutella xylostella*.

P	Y.C	G. L.	H.	E. I	S
SR - PE	2018	8° 19’24’’ S 35°44’02,615’’ W	Cabbage/Field	~300	Larvae
CSF-PE	2018	8°20’39’’ S 35°44’47,864’’ W	Cabbage/Field	~300	Larvae
BZR-PE	2018	8°14’13,665’’ S 35°46’15,762’’ W	Cabbage/Field	~300	Larvae
VNE-ES	2018	Espírito Santo	Cabbage/Field	~300	Larvae
RCF-PE	2018	Laboratory/ UFRPE	Cabbage	~100	Larvae
VÇS-MG	2018	Laboratory/ UFV	Cabbage	~100	Larvae
LJD-PE	2018	8°42’03’’ S 36°20’46’’ W	Cabbage/Field	~300	Larvae

(P) Population:SR) Sairé – PE, (CSF) Camocim de São Félix – PE, (BZR) Bezerros – PE, (RCF) Recife – PE, (LJD) Lajedo – PE, (VNE) Venda Nova do Imigrante – ES, (VÇS) Viçosa – MG)) (Y. C) Year of collection, (G. L) Geographic Coordinates, (H.) Host, (E. I) Estimated number of Individuals (S) Stage.

Insecticides

The bioassays were performed with insecticides purchased from farm suppliers: Chlorantraniliprole (Premio® 200 g a.i. / L Concentrated Suspension, DuPont do Brazil Ltda.); Oxime Methylcarbamate (Brilhante® BR, 215 g a.i. / L, Soluble Concentrate, Ouro Fino Química Ltda.) and *Bacillus thuringiensis* (Dipel WG®, *Bacillus thuringiensis*, var. *kurstaki*, HD-1 strain, Sumitomo Chemical do Brazil Representation).

Bioassay of susceptibility

To evaluate the resistance to commercial insecticides and as a consequence control failure likelihood, second and third instar larvae from the F2 field generation and from laboratory populations were exposed to a range of concentrations, using as base the concentration recommended by the manufacturers for control of *P. xylostella*. Solutions with two, four, six, eight, and ten times the recommended field concentration were prepared. The solutions used in the tests were obtained by diluting each formulation in distilled water containing 0.01% Tween 80 as spreader.

Cabbage leaves were washed under tap water and neutral detergent, then rinsed with distilled water and cut in discs (six cm of diameter). The discs were dried at room temperature. The tests consisted of four cabbage discs placed individually in a Petri dish. For each insecticide concentration, four repetitions were done. The discs were submerged in insecticide solutions for 30 seconds. Control treatment was distilled water plus 0.01% adhesive spreader. After submersion in the test solution, the discs were placed on paper towels to dry for approximately 2h at room temperature, afterwards, the cabbage discs were transferred individually to petri dishes with filter paper moistened with distilled water. On the cabbage discs, ten 2nd instar larvae were added. Petri dishes were sealed with PVC plastic film to prevent larvae from escaping and the film was pierced with an entomology pin to allow gas exchange. The bioassays were maintained in a Biological Oxygen Demand Chamber (BOD) at 25 ± 1.0 ° C, 12h photoperiod and 65% ± 10% relative humidity (susceptibility test method nº 18) (IRAC, 2010).

Mortality was assessed for four days for the insecticides chlorantraniliprole and *B. thuringiensis* and three days for oxime methylcarbamate insecticide after exposure to insecticide solutions following the IRAC protocol. The larvae were considered dead when not responding with movement, when touched with a fine brush.

Mortality results obtained in concentration-response tests were subjected to Probit analysis, according to Finney (1971), through the PROC PROBIT procedure of SAS 9.0 (2002), generating thus the concentration - response curves. Resistance ratios (RR) were obtained by dividing the lethal concentration (LC) value necessary to kill half of the tested populations (LC_{50}) by the lowest LC_{50} value found.

RESULTS AND DISCUSSION

Despite the low mortality rate for concentrations close to those recommended by the manufacturer, the Probit analysis done to determine the concentration response curve with regard of insect mortality, suited to concentration-mortality bioassays from insecticides Dipel (*B. thuringiensis*) and Premio (chlorantraniliprole) ($P < 0.05$) (Tables 2 and 3). The probit model, however, allowed only for the laboratory populations of Recife (PE) and Viçosa (MG) to construct a valid concentration-mortality relationship for the oxime methylcarbamate insecticide ($P < 0.05$) (Table 4). For this last insecticide, extremely low mortalities were observed in field population at all concentrations tested.

Table 2. Susceptibility of diamondback moth populations (*Plutella xylostella*) to the insecticide Premio® (chlorantraniliprole). Recommended concentration = 0.075 mL L^{-1}.

[a] P	[b] N	[c] Angular coefficient ± SD	[d] LC_{50} (IC 95%) mL L^{-1}	[e] LC_{95} (IC 95%) mL L^{-1}	[f] RR_{50} (IC 95%)	[g] χ^2	[h] p
SR	2240	2.92 ± 0.38	0.72 (0.63 – 0.85)	1.73 (1.45 -2.19)	144.0	58.18	0.001
LJD	1120	1.64 ± 0.50	0.65 (0.48 – 1.18)	2.44 (1.64 – 5.70)	130.0	10.59	0.0011
CSF	1120	1.33± 0.47	1.85 (1.23 – 5.40)	4.05(2.53–12.95)	370.0	7.82	0.0052
BZR	960	4.23 ± 0.76	0.69 (0.59 – 0.89)	1.39(1.11 – 1.96)	138.0	30.49	0.001
RCF	1120	2.57 ± 0.54	0.39(0.28 – 0.52)	1.53 (1.17 – 2.41)	78.0	22.59	0.001
VNE	1120	2.35 ± 0.45	0.60 (0.49 – 0.79)	1.85 (1.43 – 2.80)	120.0	26.23	0.001
VÇS	1120	6.01 ± 1.11	0.005(-0.10–0.07)	0.49 (0.38 – 0.78)	--	29.25	0.001

Table 3. Susceptibility of diamondback moth populations (*Plutella xylostella*) to the insecticide Dipel WG® (*Bacillus thuringiensis* var. *kurstakii*). Recommended concentration = 0.6 g L^{-1}.

[a] P	[b] N	[c] Angular coefficient ± SD	[d] LC_{50} (IC 95%) g L^{-1}	[e] LC_{95} (IC 95%) g L^{-1}	[f] RR_{50} (IC 95%)	[g] χ^2	[h] p
SR	2240	0.52 ± 0.06	1.21 (0.67 – 1.65)	6.80 (5.77 – 8.46)	2.63	63.97	0.001
LJD	840	0.53 ± 0.10	0.99 (-0.10 –1.71)	6.61 (5.10-10.06)	2.15	23.15	0.001
CSF	1120	1.33 ± 0.07	2.16 (0.97 – 3.14)	11.84(8.76-20.21)	4.69	17.78	0.001
BZR	960	0.69 ± 0.11	0.86 (0.29 – 1.30)	5.10 (4.11 - 6.88)	1.86	39.04	0.001
RCF	1120	0.76 ± 0.13	-0.46 (-1.82 –0.18)	3.19 (2.55 – 5,31)	----	20.29	0.001
VNE	840	0.31 ± 0.10	0.75 (- 3.28 –2.09)	10.02(6.69-27.34)	1.63	8.35	0.0038
VÇS	1120	0.63 ± 0.17	0.56 (-0,41 – 0.18)	5.19 (4.04 – 7.67)	1.21	25.33	0.001

(a) Population (b) Number of insects tested (c) Standard deviation (d) Lethal concentration causing death of 50% of the exposed individuals and confidence interval at 95% probability (e) Lethal concentration causing death of 95% of the exposed individuals and confidence interval at 95% probability (f) RR (IC 95 %) = Resistance Ratio calculated by dividing the LC_{50} of the study population by the LC_{50} of the most susceptible population and confidence intervals at 95% probability (g) Chi-square test (h) Significance of the test.

Table 4. Susceptibility of diamondback moth populations (*Plutella xylostella*) to the insecticide Brilhante®BR (Oxime methylcarbamate). Recommended concentration = 0.075 mL L^{-1}.

[b] N	[c] Angular coefficient ± SD	[d] LC_{50} (IC 95%) mL L^{-1}	[e] LC_{95} (IC 95%) mL L^{-1}	[f] RR_{50} (IC 95%)	[g] χ^2	[h] p
1120	0.28 ± 0.03	8.34 (7.42–9.62)	18.50(15.83-22.90)	2.61	62.27	0.001
1120	0.33 ± 0.03	3.19 (2.46–3.87)	11.88(10.24-14.40)	--	77.11	0.001

(a) Population (b) Number of insects tested (c) Standard deviation (d) Lethal concentration causing death of 50% of the exposed individuals and confidence interval at 95% probability (e) Lethal concentration causing death of 95% of the exposed individuals and confidence interval at 95% probability(f) RR (IC 95 %) = Resistance Ratio calculated by dividing the LC_{50} of the study population by the LC_{50} of the most susceptible population and confidence intervals at 95% probability (g) Chi-square test (h) Significance of the test.

Our results show clearly that concentrations recommended by manufacturers for the three tested insecticides do not demonstrate the expected control efficiency of cruciferous moth populations in the localities evaluated. According to our analysis, we needed to use more than 50 times the recommended concentration for chlorantraniliprole and almost 20 times the recommended concentration for the *B. thuringiensis* insecticide, to kill 95% of the Camocim de São Félix population. For the oxime methylcarbamate insecticide, the results showed the inefficiency of this product in the tested populations, even at high concentration. According to Davidson and Zahar (1973); Chediak et al. (2016) and Guedes (2017), mortality levels below 80% when using the recommended field concentration, classify the plant protection product as providing a high likelihood of control failure.

The failure of chemical control using recommended doses of various insecticides and resistance is often reported in the literature for *P. xylostella* populations and can be caused by insect resistance. This resistance is an important factor that directly limits management strategies (ZHANG et al., 2016), compromising the fundamental and most widely used tool to control this insect (JIANG et al., 2015).

The results found in the concentration-mortality bioassays for the chlorantraniliprole insecticide (Table 2) showed differences between populations in the concentration that caused 50% of mortality, with the lowest observed for the Viçosa population (0.005 mL L^{-1}). The highest value was found for the Camocim de São Felix population (PE) (1.85 mL L^{-1}). These values indicate that more than 320 times the amount of insecticide is necessary to eliminate 50% of the Camocim de São Felix (most resistant), than to eliminate 50% of the Viçosa (MG) population (most susceptible). When the comparison is made with the manufacturer's recommended concentration (0.075 mL L^{-1}), it was found that the concentration needed to cause 95% of mortality (4.0 mL L^{-1}) in the Camocim de São Felix population represents 54 times the recommended field concentration, but even the Viçosa population (laboratory) requires 6.6 times the recommended field concentration. Also, for all other tested populations, concentrations highly exceeding the recommended field dose of chlorantraniliprole were necessary to obtain 95% of mortality.

For chlorantraniliprole insecticide, failure of control and resistance in various parts of the world has been reported, such as the Philippines, Thailand, Brazil, India, the United States, Japan, Korea, and Vietnam (TROCZKA et al., 2012; RIBEIRO et al., 2014; TROCZKA et al., 2016, STEINBACH et al., 2015). According to Hu et al. (2014), only 10 generations of this pest insect treated with the manufacturer's recommended dose of chlorantraniliprole would be sufficient for the emergence of individuals with resistance ratios of up to 88 times under laboratory conditions. Thus, control failure can be explained by frequent use of the same active ingredient even for a short period of time. Together with the short developmental cycle of the diamondback moth this would lead to high levels of resistance under field conditions, so that the selection pressure exerted by insecticide use is very high (ZHAO et al., 2006; SPARKS et al., 2012).

In northeastern Brazil, Ribeiro et al. (2014) and Silva et al. (2012) observed some *P. xylostella* populations with high levels of resistance to the chlorantraniliprole insecticide. Their attention was drawn to the fact that these populations from the Midwestern region of Pernambuco State had high levels of resistance to the insecticide shortly after its release for use in Brazil. In our study we confirmed that the field populations of this Brazilian northeastern region showed indeed high and alarming levels of resistance to the insecticide chlorantraniliprole, especially the populations from the Agreste de Pernambuco region as observed for the Camocim de São Félix population (RR_{50} = 370.0 times), Sairé population (RR_{50} = 144.0 times) and Bezerros population (RR_{50} = 138.0 times) when compared with the Viçosa population. We observed that geographical origin of the insect population could explain resistance results. The southern field population of Venda Nova do Imigrante, tested in our study, even with a high level of resistance detected, was more susceptible to the insecticide (RR_{50} = 120.0 times).

For the results of the concentration-mortality tests with the insecticide Dipel (*B. thuringiensis*) (Table 3), it was found that the highest LC_{50} was observed again for the Camocim de São Felix population (2.16 g L^{-1}) and the Recife population was the most susceptible, still with an LC_{95} concentration 5.16 times higher than the recommended field concentration. However, for this biological insecticide, the resistance ratio between these two populations is 4.62, indicating a less variable resistance between the examined populations compared to chlorantraniliprole. Regarding the values of LC_{95}, 19.73 times the recommended field concentration was necessary to kill 95% of the individuals of the population of Camocim de São Felix (11.84 g L^{-1}). A similarly high value was also observed for the Venda Nova do Imigrante population (10.02 g L^{-1}), needing 16.76 times more insecticide than the recommended concentration to kill 95% of the tested insects. For all other populations tested, the values needed for 95% insect mortality were also largely above the recommended field concentration for this insecticide (Table 3).

Similarly to the reduction in susceptibility of the pest populations for the insecticide chlorantraniliprole, it was also found that the field populations showed reduced susceptibility to the insecticide Dipel WG®, and again the field populations from the region Agreste of Pernambuco proved to be most resistant. Similar results were found by Zago et al. (2014) for Brazilian populations of diamondback moths, which showed differentially reduced susceptibility levels of various populations for both insecticides Dipel® WP and Xentari®WDG. Reports of variations in resistance levels for *B. thuringiensis* insecticides were found by Mohan and Gujar (2002) (India), Zhang et al. (2016) and Jiang et al. (2015) (China), and can be associated to the geographical differences of the regions that limit the pest migration and the genetic constitution of the populations.

In the case of the oxime methylcarbamate insecticide, the Probit model fit only for laboratory populations ($P < 0.005$). Little mortality was registered in field populations even when high concentrations, until 10 times the recommended field concentration, were used. For the Viçosa population, the LC_{50} value was 3.19 mL L^{-1} and for the Recife population, the LC_{50} value was 8.34 mL L^{-1} (Table 4). Regarding the resistance ratios, the Recife population was 2.61 times more resistant to the oxime methylcarbamate insecticide when compared to the Viçosa population. We observed that the Recife population presented the highest value of LC_{95}, (18.50 mL L^{-1}), which exceeds 17.50 times the recommended field concentration by the manufacturer to control the pest. Liu, Tzeng and Sun (1982), and Yu and Nguyen (1982) observed that Thailand and American populations of diamondback moths exhibited high levels of resistance to the insecticide Metomil with the same active ingredient. Similarly, Castelo Branco and Melo (2002) found that pest populations in the Brasilia Federal District had high levels of resistance to insecticides from the Carbamate group. In another study done in the Pernambuco State, Santos et al. (2011) found low resistance levels for Metomil, but also warned for the risk of an increase in resistance levels: since the insecticide was efficient in controlling the pest, it had been used alone by producers more frequently and without active principle rotation. Less than 10 years after, the results found here, show that their concern was justified. It is therefore necessary to improve regulatory policy of plant protection product homologations and do a revision of the recommended concentrations to be sure that efficiency of control and food security can be maintained.

CONCLUSIONS

In summary we show here that cruciferous moth populations showed varying levels of susceptibility, but all field populations showed resistance to the three tested insecticides chlorantraniliprole, *B. thuringiensis* and most strikingly to oxime methylcarbamate. For the populations tested, we confirm thus decreased efficiency of the insecticides.

The consequences of this inefficiency are severe for consumers and the local environment. Even though the importance of promoting food security in order to feed the ever-growing world population is undeniable, the modern dilemma now is that food security, food safety and healthy nutrition need to be rendered compatible (WALLS et al., 2019). Brazilian legislation (Law nº 7.802, of July 11, 1989), as well as European Union legislation (regulation (EC) nº 1107/2009 of the European Parliament and of the Council, of October 21, 2009; (stricter because it divides its territory into three areas)), are basically very well written to promote the protection of the environment and populations. Although toxicological assessment is not the only parameter considered by the authorities when approving plant protection products, when using larger doses than recommended, safety for the environment and human populations can be compromised. Our results can serve as a basis for a discussion on the adoption of regionalized toxicological parameters such as pre-harvest interval, maximum residue level and acceptable daily intake.

That is the reason why it is important to monitor insect population responses to pesticides in order to identify areas where changes in control strategies must be done to avoid that the frequent use of the same molecule induces the development of insect resistance ensuring their survival. Outbreaks due to surviving insects may encourage producers to increase the amounts of insecticide, leading to a vicious circle with higher health risks and even more resistance developing.

It is therefore necessary to develop new control strategies that will help slow down the emergence of resistant individuals, and thus contribute to efficient insecticide use and reduce health risks for farmers and agro-ecosystem poisoning.

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