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Behavioral response and adaptive cost in resistant and susceptible *Plutella xylostella* to Chlorantraniliprole

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Abstract

Diamides have been used worldwide to manage the diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), however some strains showed resistance to these molecules. Also, pheromone traps could be used to manage this pest, hence reducing the use of insecticides in the field. Resistant DBM strains may have biological disadvantages in comparison to susceptible strains in areas without sprays, including reduction in fitness or behavioral changes. Therefore, the aim of this study was to investigate whether DBM strains resistant to chlorantraniliprole showed adaptive costs that could alter male attraction to the sex pheromone, in comparison to susceptible strains in the laboratory and semi-field conditions. First, the LC_1, LC_{10}, LC_{25}, and LC_{50} of DBM to chlorantraniliprole were established, which were 0.003, 0.005, 0.007, and 0.011 mg a.i. liter^{-1}, and 5.88, 24.80, 57.22, and 144.87 mg a.i. liter^{-1} for the susceptible and resistant strains, respectively. Development and reproduction of DBM strains subjected to those concentrations were compared. Later, male response to the sex pheromone was investigated in a Y-tube in the laboratory and in a greenhouse to pheromone traps. Resistant DBM strain showed an adaptive cost in comparison to the susceptible strain that can result in a delay in population growth in the field when selection pressure is absent. Conversely, resistant males have no olfactory response alteration in comparison to susceptible males, consistently at 3 ($P = 0.6848$) and 7 days ($P = 0.9140$) after release, suggesting that pheromone traps continue to be a viable alternative to manage DBM in an IPM system.

Keywords: trade-off, reproduction, survival, semiochemicals, male attraction

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Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a major cruciferous pest worldwide (Cardoso *et al*., 2010; Ribeiro *et al*., 2014) due to its biological and behavioral traits, such as short life cycle, high fecundity, genetic plasticity, large dispersion ability, and others (Barros *et al*., 1993; Talekar & Shelton, 1993; Castelo-Branco *et al*., 1996). In the last decade, the average annual cost to manage DBM has varied from US$4–5 billion, and most of this cost was related to insecticide sprays (Furlong *et al*., 2013).

The use of diamides (chlorantraniliprole, cyantraniliprole, and flubendiamide), a relatively new chemical group, has increased worldwide since 2006 for the control of *P. xylostella* (Hirooka *et al*., 2007). Diamides act selectively to activate the insect ryanodine receptor in the endoplasmic reticulum of insects, leading to irreversible muscle contraction, paralysis, and feeding cessations (Nauen, 2006). However, within a short period of time, DBM populations showed resistance, especially to chlorantraniliprole, in many countries including Brazil, China, Korea, USA, Philippines, India, Japan, Vietnam, and

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Thailand (Troczka *et al*., 2012; Wang & Wu, 2012; Ribeiro *et al*., 2014; Steinbach *et al*., 2015; Nauen & Steinbach, 2016; Qin *et al*., 2018). Additionally, in 2012 there were 79 insecticide molecules to which DBM populations had shown resistance (Sun *et al*., 2012), whereas this number reached 95 molecules in 2018 (IRAC, 2018) and for this reason DBM is considered the most difficult cruciferous pest to control. In this context, alternative control tactics are necessary to efficiently manage *P. xylostella* in the field (Shakeel *et al*., 2017).

Besides the use of insecticides to manage DBM populations, the use of synthetic sexual pheromone to capture males and monitor DBM populations was shown to be a feasible alternative, hence reducing the use of chemical control in the field (Koshihara, 1986; Tanaka *et al*., 1990; Miluch *et al*., 2013, 2014). The use of pheromones in brassica crops provides data regarding population fluctuation (e.g. monitoring) and aids in the decision of when spraying is necessary (e.g. control) in an IPM system (Baker *et al*., 1982; Jutsum & Gordon, 1989; Hallett *et al*., 1995; Michereff *et al*., 2000). This population monitoring in IPM could lead to fewer sprayings in comparison to conventional systems. This therefore results in less selection pressure and delays the evolution of insecticide resistance within the pest population, as well as less exposure of natural enemies to insecticides and maintaining DBM biological control throughout the season.

Insecticide resistance is related to different mechanisms and genotypes, which results in a variety of insect phenotypes and confers a pleiotropic effect (Paris *et al*., 2011; Martins *et al*., 2012; Sanil & Shetty, 2012). Previous works have shown that resistance to diamides in some DBM strains is related to specific target-site mutations, while metabolic processes involving glutathione *S*-transferase and higher cytochrome P450-dependent monooxygenase activity in the resistant strains have also been suggested (Troczka *et al*., 2012; Steinbach *et al*., 2015; Wang *et al*., 2016; Kang *et al*., 2017). Resistant DBM strains may have biological disadvantages in comparison to susceptible strains in areas without the selective pressure of the insecticide, including reduction in insect fitness, known as a trade-off between survival and reproduction (Coustau *et al*., 2000; Arnaud & Haubruge, 2002; Jia *et al*., 2009; Castañeda *et al*., 2011; Ferreira *et al*., 2013; Ribeiro *et al*., 2014). This adaptive cost in the absence of the insecticide can contribute to resistance management, since products may be rotated (e.g. mode of action) or even stopped in order to explore lower fitness of resistant individuals and promote the increase of susceptibility within the populations, which in turn are more easily chemically controlled when necessary (Roush & McKenzie, 1987; French-Constant & Bass, 2017). In addition to fitness costs in resistant insect populations, behavioral traits might also be affected, since insect behavior is a result of the interaction of genes and the environment (Matthews & Matthews, 2010). For example, some variations in the DBM male response were observed in populations in Taiwan, Japan, Canada, and Indonesia (Maa, 1986; Zilahi-Balogh *et al*., 1995). These behavioral changes in DBM males may have been a result of the interaction between seasonal environmental factors and the genetic difference among insect populations, as well as the combination of chemical compounds and dose used for control (Talekar & Shelton, 1993).

There is a great variation in DBM strains resistant to diamides (Qi & Casida, 2013; Steinbach *et al*., 2015, Troczka *et al*., 2017) and this may be a result of variations in selection pressure, mode of action of the insecticides used and quantity of products sprayed to control DBM, resistance mechanisms presented by different DBM strains, and other factors. In addition, we know that there is a trade-off between insect reproduction and survival and that resistant insects may show behavioral changes, for instance in sexual receptivity or attraction. Resistant DBM individuals could then be less attracted to the sex pheromone used to monitor this pest in the field. In this context, the aim of the present study was to investigate whether DBM strains resistant to chlorantraniliprole showed reproductive adaptive costs that could alter male attraction to the sex pheromone, in comparison to susceptible strains in the laboratory and semi-field conditions.

Material and methods

Experimental insects

The susceptible strain of *P. xylostella* used herein (REC-Suc) was first established at the Laboratory of Insect–Toxicant Interactions (LIIT) from the Universidade Federal Rural de Pernambuco (UFRPE) in 1998 and was maintained without insecticide selection pressure. Resistant strain individuals (CGD-Res) were collected in 2017 from a conventional kale (*Brassica oleracea* var. Acephala) field, in Chã Grande County, Pernambuco State, Brazil (08°14′18″ S, 35°27′42″ W), which regularly received insecticide sprays to manage this pest and reported that diamide-based insecticides were ineffective. Strains were maintained at the Laboratory of Insect Behavior of the UFRPE, wherein experiments were conducted under temperature of 25°C ± 1, 70 ± 10% relative humidity, and photoperiod of 12L:12D.

The susceptible strain was in the 472nd generation and the resistant strain in the 11th generation at the time of the experiments. Each strain was reared separately in the laboratory. Both susceptible and resistant DBM strains were maintained on organic kale leaves offered daily to adults for oviposition and larvae as food, according to Silva-Torres *et al*., (2010), but the resistant DBM strain was kept under selection pressure by exposure to kale leaves treated with the LC_{50} of chlorantraniliprole estimated from the concentration-response curve. After consumption, untreated kale leaves were used to feed the resistant larvae until the end of the cycle and for adult oviposition.

Susceptibility of P. xylostella *to chlorantraniliprole*

In order to establish the concentration-response curves of *P. xylostella* strains both susceptible and resistant to chlorantraniliprole (Prêmio® 20SC, DuPont Brasil Ltda), bioassays were conducted to estimate sub-lethal concentrations for use in subsequent trials. We tested the concentrations of 0.0019, 0.0039, 0.0078, 0.0156, 0.0312, 0.0625, 0.125, and 0.25 mg of a.i. liter^{-1} on the susceptible strain, whereas for the resistant strain we used 7, 14, 28.1, 56.25, 112.5, 225, 450, and 900 mg of a.i. $^{-1}$ of chlorantraniliprole according to Ribeiro *et al*. (2014).

Larvae were exposed by feeding on discs of kale leaves previously treated with the insecticide at each concentration. The control treatment and insecticide dilutions were prepared using 0.01% Triton X and water. Kale leaf discs (5 cm diameter) were initially washed in 5% sodium hypochlorite, rinsed in tap water, immersed for 30 s in the respective insecticide concentration, and left to dry at room temperature. Then, 27 small glass Petri dishes were prepared, consisting of three replicates per concentration (treatment) and the control. When dry, the leaf discs were transferred to Petri dishes lined with

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filter paper, which also received 12 24 h-old first instar larvae of *P. xylostella* each. Petri dishes were placed in a BOD at a temperature of 25 ± 5°C, relative humidity of 70 ± 10%, and photoperiod of 12:12 h (light:darkness) for a period of 96 h. Mortality was measured by counting the dead larvae, which were those that when touched with a paintbrush could not move (Ribeiro *et al*., 2017). The experimental design was completely randomized, in a scheme of nine treatments (eight concentrations and the control) for each strain, performed in triplicate. Mortality data were corrected using the mortality of the control treatment (Abbott, 1925) [$(M_c(\%) = ((\%M_o - \%M_t)/100 - \%M_t) \times 100]$, in which: M_c = corrected mortality, M_o = observed mortality, and M_t = control mortality). Thus mortality data were submitted to Probit analysis (Finney, 1971) using the POLO-Plus software (LeOra Software, 2005). Also, the resistance ratio and its 95% confidence interval were calculated according to Robertson *et al*. (2007). The values of LC_1, LC_{10}, LC_{25}, and LC_{50} were estimated, which were used in the subsequent tests, and the LC_{50} value was used to maintain the selection pressure on the resistant strain colony in the laboratory.

Developmental times of P. xylostella *treated with chlorantraniliprole*

This bioassay sought to compare developmental times of the resistant and susceptible strains of *P. xylostella*. Kale leaf discs treated with the corresponding LC_1, LC_{10}, and LC_{25} of chlorantraniliprole provided to each strain were used for this purpose. The control treatment consisted of distilled water and all dilutions were prepared using 0.01% Triton X and water. Thus, each leaf disc (5 cm diam.) was immersed in the respective dilution as previously described, left to dry at room temperature, and transferred to a Petri dish lined with filter paper moistened with distilled water. Next, each Petri dish received 12 24 h-old first instar larvae of *P. xylostella* and was then transferred to a BOD with a temperature of 25 ± 5°C, relative humidity of 70 ± 10%, and photophase of 12 h for a period of 96 h. After this period, leaf discs were replaced daily with non-treated discs until pupation of *P. xylostella* larvae. Pupa was weighted after 24 h of formation and individualized in glass tubes (2 × 6 cm) sealed with PVC film until adult emergence. Small holes were made in the PVC film with the aid of an entomological pin to allow gas exchange inside the tubes. After adult emergence, 12 couples (<48 h-old) were formed per treatment (insecticide concentrations and strains) and placed in small breeding cages. Adult cages were constructed of acrylic cups (vol. 350 ml), placed upside down, and sealed using a disc (5.5 cm diameter) of sponge soaked in water and lined with filter paper and a kale leaf disc of the same size as an oviposition substrate. On the top of the cage, there was a small hole where a piece of sponge soaked in a 10% honey solution was offered as food for the adults. Cages were placed on a plastic tray filled with water to keep the sponges damp and maintain the humidity within the cages. Leaf discs containing eggs were replaced daily and transferred to new dishes to count the number of eggs laid (fecundity) and larval eclosion (fertility) during seven consecutive days, which is considered the peak oviposition period of *P. xylostella* (Barros *et al*., 1993).

In the immature stage, larval survival was measured after 96 h of exposure to chlorantraliniprole, in addition to developmental times (larval and pupal), larval viability, pupal viability, and weight. In adults, we measured the sex ratio, fecundity, and fertility during ten consecutive days. Data on the developmental times, larval survival and viability, pupal viability and weight, and adult sex ratio were submitted to analysis of variance (ANOVA) in a factorial design (4 × 2), consisting of the two strains (susceptible and resistant) and four concentrations (LC_1, LC_{10}, LC_{25}, and control), using the PROC GLM of SAS followed by the Tukey test ($P < 0.05$) for mean comparison (SAS Institute, 2002). Data of fecundity and fertility were submitted to ANOVA with repeated measures using the PROC GLM of SAS.

Olfactory response of P. xylostella *males in the laboratory*

In this assay, virgin males of both DBM strains were individualized in small glass vials (2 × 6 cm) immediately after emergence, fed with 10% honey solutions and used in olfactory tests after being sexually mature when they were 2–3 days old (Colares *et al*., 2013). We tested the male olfactory response in a two-choice Y-tube olfactometer (Magalhães *et al*., 2012), where the volatile treatments offered were: (i) resistant female × susceptible female, (ii) susceptible female × clean air, (iii) resistant female × clean air, (iv) susceptible female × synthetic sex pheromone, (v) resistant female × synthetic sex pheromone, and (vi) synthetic sex pheromone × clean air. The treatments with females were composed of a group of three 48–72 h-old females calling (releasing sex pheromone by the time of the assays). The sex pheromone treatment was composed of one-fourth of the synthetic pheromone septum [SKU PLUXYL001-G: (Z)-11-Hexadecenyl acetate, (Z)-11-Hexadecen-1-ol, (Z)-11-Hexadecenal and (Z)-11-Tetradecen-1-ol; Alpha Scents Inc.]. All tests were conducted in a dark room, with red light filter, from 20:00 to 24:00 h (midnight), due to the crepuscular mating behavior of *P. xylostella* (Yamanda & Koshihara, 1980). Before the assays, males and females remained in the test room for at least 1 h for acclimatization. Males were tested individually in the olfactometer, and for each treatment, there were 40 replicates.

Treatments were placed inside glass vials (150 ml), which were connected to the arms of the olfactometer by PTFE tubing. Charcoal-filtered and humidified air was pushed through the system by an aquarium air pump (Aleas® – AP9802, Chaozhou, Guangdong, China) regulated at a flow rate of 0.55 liter min^{-1} by flowmeters (KI, Hatfield, PA, USA) positioned on each arm of the olfactometer, and a suction pump (Marconi®, Recife, Brazil) was used to pull the air from the system at a rate of 1.0 liter min^{-1}, also regulated by a flowmeter.

One DBM adult male was introduced in the stem arm of the olfactometer and its response to odors offered in each arm of the olfactometer was observed for 10 min. A positive response was considered after the DBM male passed the bifurcation point of the Y-tube into one arm of the olfactometer and traveled at least 3 cm into its length and stayed there for at least 20 s. The first choice was measured along with the total time that the male spent in each Y-tube arm. Each DBM male was tested only once, and males which did not show a response within 5 min were replaced and not considered in the analysis. After every five replications, the position of the treatments offered was rotated to avoid any bias in the insect response. In addition, after every ten trials, the Y-tube apparatus was cleaned with soap and water, rinsed with 70% alcohol, and dried at room temperature.

Data on the first choice were submitted to the non-parametric Proc FREQ (SAS) followed by a χ^2 ($\alpha = 0.05$), with the null hypothesis that no difference exists between

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the two odor sources offered (1:1 ratio). Residence time of the males in each arm of the olfactometer was analyzed by the Wilcoxon rank-sum test ($P < 0.05$) using Proc NPAR1WAY of SAS (SAS Institute, 2002).

Response of DBM males to the synthetic sex pheromone in the greenhouse

This test sought to investigate whether there was any difference in the attraction of DBM males of both strains to the synthetic sex pheromone trap in semi-field conditions.

Experiments were conducted at the UFRPE in greenhouses measuring 90 m^2, closed laterally with anti-aphid screens, and covered with agricultural plastic. During the experiments, environmental conditions were monitored with the DataLogger Hobo® (Onset Computer, Bourne, MA, USA) at 30 min intervals, registering a mean temperature of 27.42°C (min = 20.5°C, max = 34°C), 70.60% RH, and natural photoperiod of 12:12 h (L:D).

Cabbage plants were grown in 5 liters plastic pots containing a mixture of soil and humus (2:1), to which 5 g of N:P:K fertilizer (04-14-08) were added per pot, and these were used in the experiments after 90 days. Pots containing the cabbage plants were placed 30 cm apart from each other, and a total of 63 plants were used. Next, a Delta trap (15 × 10 × 28 cm) (Imenes *et al*., 2002) baited with a septum of the synthetic pheromone (SKU PLUXYL001-G) was positioned in the center of the greenhouse, 20 cm above the plant canopy. Each week 200 adult virgin males (48–72 h-old) of the respective strain (susceptible and resistant) were released at sunset. There were eight consecutive releases for each DBM strain. The sticky floor of the Delta trap was replaced between releases, and the pheromone septum was replaced every 15 days. The number of males captured by the trap was counted after 3 and 7 days of release. Data were submitted to the Student's *t*-test ($\alpha = 0.05$) (SAS Institute, 2002), with the null hypothesis that there was no difference between the attraction of DBM males of the two strains, those resistant and susceptible.

Results

Susceptibility of P. xylostella *to chlorantraniliprole*

Following exposure to chlorantraniliprole in the laboratory, the corrected mortality data of both DBM strains assumed the Probit model ($P > 0.05$). After 472 and 11 generations in the laboratory, the initial LC_{50} values obtained were 0.0035 and 144.871 mg a.i. liter^{-1} for the susceptible and resistant strains, respectively. In addition, the LC_1, LC_{10}, and LC_{25} values estimated were 0.00014, 0.0006, and 0.00139 mg a.i. liter^{-1} for the susceptible strain, and 5.883, 24.802, and 57.223 mg a.i. liter^{-1} for the resistant strain (table 1). Thus, the resistant ratio ($LC_{50\ Res}/LC_{50\ Sus}$) for the resistant strain was about 41,391 times more resistant to chlorantraniliprole than the susceptible strain.

Developmental times of P. xylostella *treated with chlorantraniliprole*

There was a significant effect of insecticide concentrations on larval survival after 96 h of exposure ($F_{3,\ 88} = 17.12$, $P < 0.0001$), but there was no difference between DBM strains ($F_{1,\ 88} = 0.30$, $P = 0.5865$) and there was also no interaction of these factors ($F_{3,\ 88} = 0.59$, $P = 0.6261$) (table 2). Larval survival

Table 1. Susceptibility of *P. xylostella* strains to chlorantraniliprole after 96 h exposure.

Strain	N[1]	Slope ± SE[2]	LC_1 (CI95%)[3] (mg a.i. liter^{-1})	LC_{10} (CI95%)	LC_{25} (CI95%)	LC_{50} (CI95%)	χ^2 (DF)[4,5],
REC – Suc	598	1.68 ± 0.17	1.4×10^{-4} ($5.0 \times 10^{-5} - 3.0 \times 10^{-4}$)	6.0×10^{-4} ($2.9 \times 10^{-4} - 9.9 \times 10^{-4}$)	1.39×10^{-3} ($0.8 \times 10^{-3} - 2.0 \times 10^{-3}$)	3.5×10^{-3} ($2.5 \times 10^{-3} - 4.6 \times 10^{-3}$)	6.5 (7)
CGD – Res	257	1.67 ± 0.43	5.88 (0.0–34.06)	24.8 (0.07–82.92)	57.22 (0.9–141.88)	144.87 (14.26–274.51)	8.5 (7)
Camocim[6]	458	3.06 ± 0.28	35	78	123	204.32 (176.91–236.64)	2.23 (7)
CGD[7]	296	3.27 ± 0.39	–	–	–	77.21 (63.59–93.60)	1.15 (6)

[1]Number of insects tested.
[2]Standard error.
[3]95% confidence interval.
[4]χ^2 ($P > 0.05$).
[5]Degrees of freedom.
[6]Camocim de São Félix-PE, Ribeiro *et al*. (2014).
[7]CGD (Chã Grande-PE), Ribeiro *et al*. (2017).

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Table 2. Biological and reproductive parameters (mean ± SE) of *Plutella xylostella* resistant and susceptible subjected to chlorantraniliprole.

Strain	Conc.[1]	Larval survival (%)	Larval period (days)	Larval viability (%)	Pupal period (days)[1]	Pupal weight (mg)	Sex ratio	Fecundity	Egg viability (%)
REC-Suc	Control	93.07 ± 1.73 A[2]	5.65 ± 0.12 A	86.81 ± 2.17 A	3.97 ± 0.05 AB	5.53 ± 0.72 A[3]	0.49 ± 0.03 A	237.0 ± 7.45 A	78.42 ± 2.15 A
	LC1	93.06 ± 1.73 A	6.07 ± 0.17 AB	84.03 ± 2.40 AB	4.09 ± 0.07 A	5.39 ± 0.14 A	0.52 ± 0.03 A	223.0 ± 5.67 A	74.08 ± 2.04 AB
	LC10	86.11 ± 2.96 A	6.55 ± 0.14 B	73.61 ± 3.38 B	3.80 ± 0.06 B	5.51 ± 0.09 A[3]	0.51 ± 0.02 A	177.7 ± 13.41 B	69.17 ± 2.22 B
	LC25	74.31 ± 4.16 B	7.24 ± 0.24 C	59.72 ± 4.08 C	3.83 ± 0.04 A	5.34 ± 0.10 A[3]	0.48 ± 0.02 A	168.6 ± 12.88 B	68.54 ± 1.99 B
	Stat.	$F_{3, 44} = 9.77$; $P < 0.0001$	$F_{3, 44} = 15.73$; $P < 0.0001$	$F_{3, 44} = 15.65$; $P < 0.0001$	$F_{3, 44} = 5.32$; $P = 0.0033$	$F_{3, 44} = 0.76$; $P = 0.5239$	$F_{3, 88} = 0.56$; $P = 0.64$	$F_{3, 44} = 10.35$; $P < 0.0001$	$F_{3, 44} = 4.68$; $P = 0.0052$
CGD-Res	Control	90.97 ± 1.91 A	5.91 ± 0.16 A	85.42 ± 2.74 AB	4.07 ± 0.04 A	5.35 ± 0.04 A	0.53 ± 0.02 A	220.4 ± 8.22 A	76.73 ± 2.30 A
	LC1	92.36 ± 1.91 A	6.10 ± 0.16 A	89.58 ± 1.50 A	4.00 ± 0.04 A	5.18 ± 0.09 A	0.53 ± 0.04 A	219.1 ± 5.38 A	71.93 ± 1.12 A
	LC10	90.28 ± 2.26 A	6.83 ± 0.14 B	79.17 ± 3.48 B	3.99 ± 0.03 A[3]	5.20 ± 0.08 A	0.52 ± 0.04 A	186.9 ± 17.74 A	71.87 ± 2.24 A
	LC25	77.08 ± 3.72 B	8.00 ± 0.22 C[3]	63.19 ± 2.80 C	4.31 ± 0.07 B[3]	4.82 ± 0.07 B	0.51 ± 0.03 A	175.6 ± 16.37 A	71.63 ± 3.35 A
	Stat.	$F_{3, 44} = 7.73$; $P = 0.0003$	$F_{3, 44} = 29.51$; $P < 0.0001$	$F_{3, 44} = 18.08$; $P < 0.0001$	$F_{3, 44} = 8.76$; $P = 0.0001$	$F_{3, 44} = 9.06$; $P < 0.001$	$F_{3, 88} = 0.56$; $P = 0.64$	$F_{3, 44} = 2.08$; $P = 0.1169$	$F_{3, 44} = 1.06$; $P = 0.3769$

[1]Insecticide concentration tested (Table 1).
[2]Means followed by the same capital letter in the column (within each strain) do not differ statistically by Tukey's test ($P < 0.05$).
[3]Means followed by asterisks are statistically different between strains by ANOVA ($P < 0.05$).

was significantly lower when larvae fed on leaves treated with the LC_{25} in both the susceptible ($F_{3, 44} = 9.77$, $P < 0.0001$) and resistant ($F_{3, 44} = 7.73$, $P = 0.0003$) strains.

Duration of the larval period was significantly different between DBM strains ($F_{1,88} = 7.38$, $P = 0.008$) and insecticide concentrations ($F_{3,88} = 43.81$, $P < 0.0001$), but there was no interaction between these factors ($F_{3,88} = 1.58$, $P = 0.1988$) (table 2). In both DBM strains, there was a positive correlation with insecticide concentration and larval duration. Moreover, in the susceptible DBM strain, there was a significant difference among insecticide concentrations ($F_{3,44} = 15.73$, $P < 0.0001$); and the larval period (mean ± SE) varied from 5.65 ± 0.12 to 7.24 ± 0.24 days, with the shortest period obtained in the control and LC_1 treatments, and the longest period on the LC_{25} (table 2). Similarly, the duration of the larval period was affected by the insecticide concentration in the resistant DBM strain ($F_{3, 44} = 29.51$, $P < 0.0001$), with mean duration varying from 5.91 ± 0.16 to 8.00 ± 0.22 days, and the larval period did not differ between the LC_1 and the control treatments (table 2). When comparing the DBM strains, the LC_{25} was the only treatment which showed a significant difference in larval duration ($F_{1,22} = 5.37$, $P = 0.0302$), while the others were similar ($P > 0.05$) (table 2).

Larval viability showed a significant difference only among insecticide concentrations ($F_{3,88} = 32.79$, $P < 0.0001$), but was similar between DBM strains ($F_{1,88} = 2.55$, $P = 0.1136$), and there was no interaction of these factors ($F_{3,88} = 0.63$, $P = 0.5979$) (table 2). In the susceptible strain, larval viability varied from 59.72 ± 4.08 to 86.81 ± 2.17%, whereas in the resistant strain, it varied from 63.19 ± 2.80 to 89.58 ± 1.50% (table 2). In both DBM strains, there was a significant negative correlation between the insecticide concentration and larval viability. In addition, the highest larval viability was similar in the control and LC_1 treatments for the susceptible ($F_{3, 44} = 15.65$, $P < 0.0001$) and resistant strains ($F_{3, 44} = 18.08$, $P < 0.0001$) (table 2).

The pupal period was affected by the insecticide concentrations ($F_{3, 88} = 3.83$, $P = 0.0125$), DBM strains ($F_{1, 88} = 19.36$, $P < 0.0001$), and the interaction of these factors ($F_{3, 88} = 9.72$, $P < 0.0001$) (table 2). In the susceptible strain, the pupal period varied from 3.80 ± 0.06 to 4.09 ± 0.07 days, with the shortest pupal period obtained for LC_{10} and the other concentrations were statistically similar (table 2). In the resistant strain, the pupal period varied from 3.99 ± 0.03 to 4.31 ± 0.07 days, with the only significant difference in the LC_{25}, which had the longest pupal period (table 2). In addition, significant differences in the pupal period between strains were detected at LC_{10} ($F_{1,22} = 7.23$, $P = 0.0113$) and LC_{25} ($F_{1, 22} = 33.35$, $P < 0.0001$), being superior in the resistant strain for both concentrations.

Weight of the pupae was significantly different between DBM strains ($F_{1, 88} = 23.43$, $P < 0.001$), with pupae of the susceptible strain being slightly heavier than pupae of the resistant strain (table 2). There was no effect of concentrations on the pupal weight within the susceptible DBM strain ($F_{3, 44} = 0.76$, $P = 0.5239$), which varied from 5.34 ± 0.10 to 5.53 ± 0.72 mg. In contrast, there was a significant difference in pupal weight in the resistant strain ($F_{3, 44} = 9.06$, $P < 0.001$), which varied from 4.82 ± 0.07 to 5.35 ± 0.04 mg, with the lowest weight of pupae found for LC_{25} while the other treatments were similar (table 2). When comparing the DBM strains at each concentration, there was a significant reduction in pupal weight for the resistant strain in the control ($F_{1, 22} = 4.31$, $P = 0.0497$), LC_{10} ($F_{1,22} = 7.20$, $P = 0.0136$), and LC_{25} ($F_{1, 22} = 17.97$, $P = 0.0003$) treatments (table 2).

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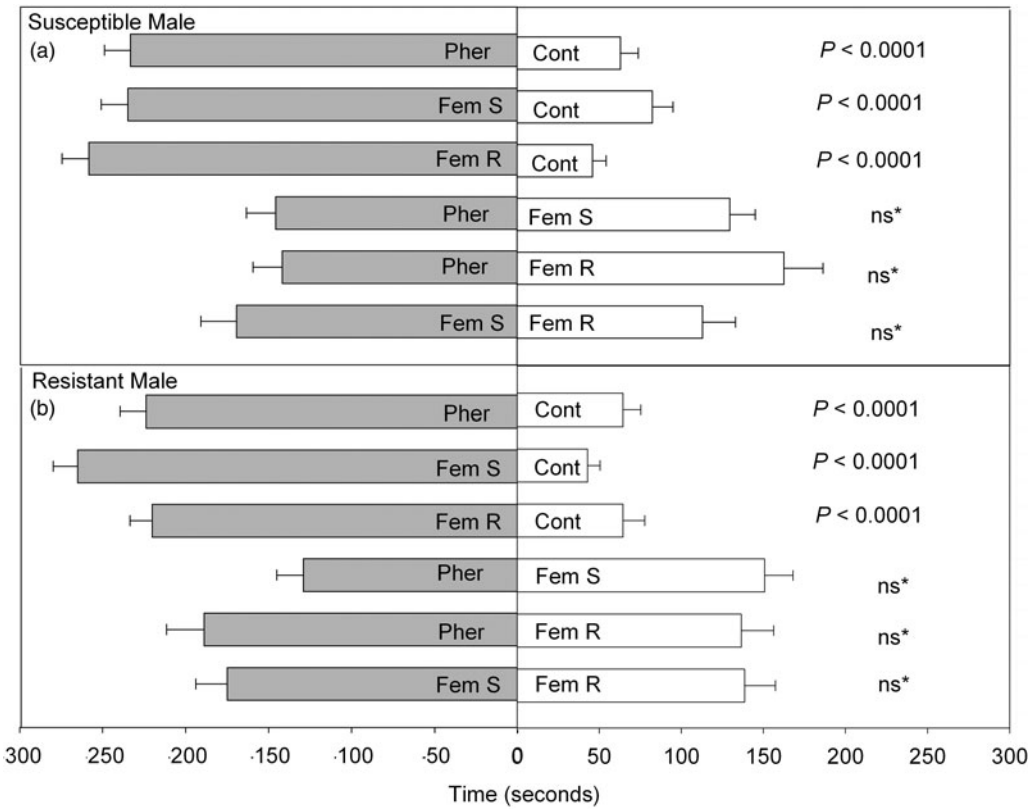

Fig. 1. Mean time (+SE) spent by (a) susceptible and (b) resistant *Plutella xylostella* males in the Y-tube olfactometer subjected to odors the following paired treatments: susceptible female (FemS), resistant female (FemR), synthetic sex pheromone (SKU PLUXYL001-G/Pher), and clean air (Cont.). Bars followed by *ns are not statistically significant (Wilcoxon rank-sum test; $P < 0.05$).

There was no effect of chlorantraniliprole concentrations ($F_{3,\ 88} = 0.56$, $P = 0.64$), DBM strains ($F_{1,\ 88} = 1.68$, $P = 0.1985$), or their interaction on the adult sex ratio, which varied from 0.48 ± 0.02 to 0.54 ± 0.02 females (table 2).

Female fecundity varied within the concentrations ($F_{3,\ 88} = 11.27$, $P < 0.0001$), but not between DBM strains ($F_{1,\ 88} = 0.02$, $P = 0.8989$), nor was there interaction of these ($F_{3,\ 88} = 0.50$, $P = 0.6825$) (table 2). Females of the susceptible strain laid significantly fewer eggs ($F_{3,\ 44} = 10.35$, $P < 0.0001$) when treated with the highest concentrations of chlorantraniliprole, LC_{10}, and LC_{25}, with means (±SE) of 177.75 ± 13.41 and 168.67 ± 12.88 eggs laid, respectively. Females treated with the control and LC_1 laid a mean of 237.0 ± 7.45 and 223.0 ± 5.67 eggs, respectively (table 2). In the resistant DBM strain, female fecundity was similar across all treatments ($F_{3,44} = 2.08$, $P = 0.1169$) (table 2).

Egg viability was affected by the insecticide concentrations ($F_{3,\ 88} = 4.67$, $P = 0.0045$), but not by the strains ($F_{1,88} = 0.10$, $P = 0.7528$) or their interaction ($F_{3,\ 88} = 0.76$, $P = 0.5169$) (fig. 2). Within the susceptible strain, egg viability was significantly higher for the control and LC_1 than the LC_{10} and LC_{25} treatments ($F_{3,\ 44} = 4.68$, $P = 0.0052$) (table 2). Moreover, the mean (±SE) egg viabilities in the susceptible strain were 78.42 ± 2.15% for the control, 74.08 ± 2.04% at LC_1, 69.17 ± 2.22% at LC_{10}, and 68.54 ± 1.99% at LC_{25}. Regarding the resistant DBM strain, there was no effect of insecticide concentration on egg viability ($F_{3,44} = 1.06$, $P = 0.3769$) (table 2).

Olfactory response of P. xylostella *males in the laboratory*

The first choice of DBM males in the Y-tube was affected by the different treatments offered. Males of the susceptible strain preferred volatiles of the synthetic pheromone compared to clean air ($\chi^2 = 7.9121$, $P = 0.0049$). In addition, susceptible males also preferred volatiles of females in comparison with clean air, regardless of the female strain (susceptible: $\chi^2 = 5.333$, $P = 0.0209$; and resistant: $\chi^2 = 5.333$, $P = 0.0209$). Similarly, resistant males preferred the pheromone ($\chi^2 = 6.545$, $P = 0.0105$) and the females (susceptible: $\chi^2 = 4.2660$, $P = 0.0389$; and resistant: $\chi^2 = 9.448$, $P = 0.0021$) compared to clean air. In contrast, regardless of male strain, there was no significant difference in the first choice of males when they were offered the synthetic pheromone and the females (resistant and susceptible strains), in the different paired combinations ($P > 0.05$).

When considering the residence time, males of both strains spent more time in the spaces with volatiles of the synthetic pheromone or females compared to the clean air. Susceptible males stayed longer in the arms of the Y-tube with pheromone volatiles ($\chi^2 = 40.1653$, $P < 0.001$) and female volatiles (susceptible: $\chi^2 = 32.7583$, $P < 0.001$; and resistant: $\chi^2 = 52.9959$, $P < 0.001$) when compared with clean air (fig. 1a). Similarly, resistant males also stayed longer in arms with pheromone volatiles ($\chi^2 = 40.9678$, $P < 0.001$) and female volatiles (susceptible: $\chi^2 = 52.8536$, $P < 0.001$; and resistant: $\chi^2 = 40.2032$, $P < 0.001$) (fig. 1b). On the other hand, when volatiles of the pheromone were paired either with susceptible or resistant females, males

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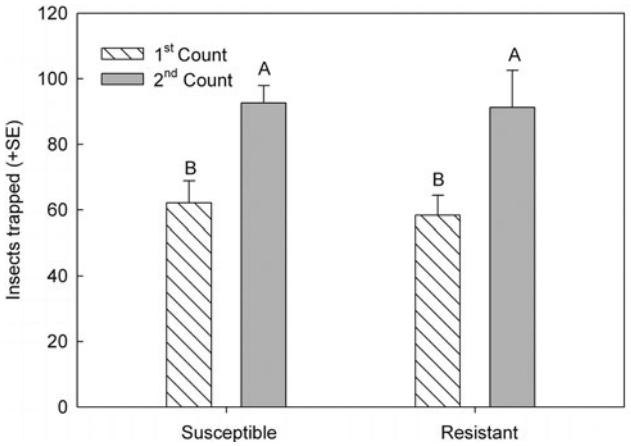

Fig. 2. Mean number (+SE) of resistant and susceptible *Plutella xylostella* males captured in a Delta trap baited with a septum of the synthetic pheromone (SKU PLUXYL001-G), 3 (1st count) and 7 (2nd count) days after release in a greenhouse.

of both strains showed no preference between these treatments and spent about the same amount of time on either arm of the Y-tube (fig. 1a, b).

Response of DBM males to the synthetic sex pheromone in the greenhouse

There was no effect of DBM strains on the mean number of males captured by the pheromone trap in the greenhouse (fig. 2). This result was consistent at 3 days ($t_{14} = 0.41$, $P = 0.6848$) and 7 days ($t_{14} = 0.11$, $P = 0.9140$) after release. In contrast, there was a significant increase in the number of males trapped over time, with more males captured 7 days after release in the susceptible ($t_{14} = 3.57$, $P = 0.0031$) and resistant ($t_{14} = 2.56$, $P = 0.0227$) strains. The mean numbers (±SE) of males trapped varied from 62.12 ± 6.71 to 92.62 ± 5.31 in the susceptible strain, and from 58.37 ± 6.07 to 91.25 ± 11.32 in the resistant population at 3 and 7 days after release, respectively.

Discussion

Since diamides debuted on the market to manage lepidopteran pests in 2006 (Nauen, 2006), studies have shown that some species have developed resistance to these insecticides as *P. xylostella* in Brazil (Ribeiro *et al.*, 2017), *Adoxophyes honmai* (Lepidoptera: Tortricidae) in Japan (Uchiyama & Ozawa, 2014), and *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy and Brazil (Roditakis *et al.*, 2015; Silva *et al.*, 2016). This resistance to diamides has increased rapidly over the last decade (Wang & Wu, 2012; Jiang *et al.*, 2015; Ribeiro *et al.*, 2017), and could be noticed even within a short period of time in our tests. Herein, a resistant field-collected population of *P. xylostella* (CGD-Res) was assessed for fitness costs and behavioral response. Although the grower cultivates both conventional and organic Brassicaceae in this site, resistance to diamides is still high as observed here ($LC_{50} = 144.871$ mg a. i. liter^{-1}), suggesting that the eventual flow of individuals from the organic plot (and *vice versa*) may not be impacting the local resistance to diamides. This shows that diamide resistance has not reduced since Ribeiro *et al.*'s (2017) reports, which included another CGD-Res population. In view of that, fitness associated with diamide resistance (Ribeiro *et al.*, 2014) might not be contributing to reduce resistance, and thus other tactics than chemical use, such as pheromone approaches, might aid for this purpose. For this, behavioral responses of both colonies to sex pheromone was assessed after evaluation of other biological parameters. To our knowledge, this is the first report regarding possible fitness constraints and behavioral olfactory response of resistant strains of *P. xylostella*, including laboratory and semi-field tests.

Overall, most evaluated biological parameters in both strains were affected in their respective sublethal concentrations. However, individuals of the resistant strain were slightly favored in comparison to those of the susceptible strain treated with chlorantraniliprole, and the response of both strains was concentration dependent for most parameters as expected. Both larval and pupal periods were elongated in the resistant strain at the highest sublethal concentrations as also observed by Ribeiro *et al.* (2014) when compared with the susceptible strain. Conversely, in the absence of chlorantraniliprole, susceptible pupae were heavier than resistant ones, suggesting an energetic cost for resistant larvae that ended up in lighter pupae. Heavier pupae produce larger adults, which in turn tend to be more fecund and live longer than smaller ones (Honek, 1993; Beukeboom, 2018). Fecundity of susceptible individuals was higher than that of resistant individuals, though not statistically significant. Thus, our findings corroborate with the hypothesis of a trade-off between survival and reproduction, when resistant individuals are not under selection pressure. This trade-off, also called the adaptive cost, explains the rapid reversion of resistance conditions in the absence of selection pressure (Crow, 1957; Han *et al.*, 2012; Sun *et al.*, 2012; Ribeiro *et al.*, 2017; Steinbach *et al.*, 2017). Nevertheless, this study observed less alterations in the resistant strain regarding the biological parameters compared with that of Ribeiro *et al.* (2014). This suggests that CGD-Res strain used here may present a lower adaptive cost than the population evaluated by those authors. This might explain the high resistance to diamides still found at the Chã Grande site. Environmental factors affecting both local populations of DBM may contribute to differential fitness observed between both studies as discussed next.

Insecticide resistance that has been related to adaptive costs are usually dependent on environmental conditions where

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insect strains are found, hence having an effect on population growth parameters. In this regard, Steinbach *et al*. (2017) showed that higher temperatures (≈30°C) had a profound effect on overall fitness and population growth parameters of either susceptible or diamide-resistant DBM strains. In addition, Saeed *et al*. (2018) have found that life-table parameters such as fecundity and net reproductive rate were affected by the temperature, suggesting that effective management tactics should be applied to prevent significant yield loss to cruciferous crops when the temperature is around 20°C, which was found to be the optimal condition for DBM population build-up. Results of such laboratory studies are very useful; however, they do not reflect precisely how DBM populations fluctuate in the field, and only help to predict what could happen under conditions that are more natural. Therefore, further studies should address how natural environmental conditions as well as insecticide selective pressure may interact and influence the fitness of DBM strains and management practices.

One of the strategies recommended to mitigate resistant populations is the alternation of insecticide modes of action (MoA) (Onstad, 2014). However, for the DBM, this is quite difficult because this pest has shown resistance to more than 90 molecules recommended for its control (Steinbach *et al*., 2017; IRAC, 2018). Additionally, the lower adaptive cost of the CGD-Res strain may not favor the alternation of MoA; and thus, alternative control methods are needed to manage this pest in such situations. In this context, the use of sex pheromones has been suggested not only to monitor DBM populations with baited traps, but also to control it via mating disruption since the early 1980s (Baker *et al*., 1982; Chow *et al*., 1984) and has been partially adopted in Asian countries ever since.

Even though previous studies have shown that males may show differences in their response to sex pheromones (Maa, 1986; Zilahi-Balogh *et al*., 1995; Trimble *et al*., 2004), probably due to differences in composition, as well as genetic and environmental factors, this is the first report of behavioral response of resistant *P. xylostella* to sex pheromone compared to susceptible strain that we know. No effect of synthetic pheromone or female strain was observed on the attraction of *P. xylostella* males. A very consistent attraction of *P. xylostella* males was found, regardless of the strain, to volatiles released by females (resistant and susceptible) and to the synthetic pheromone compared to clean air. Males showed no preference between females and the sex pheromone, suggesting equivalence in the volatile ratio (female/pheromone) tested in the olfactometer, and that the synthetic sex pheromone used in this study is as attractive to males as calling females of either strain. This suggests that resistant males are attracted to and could mate with susceptible females, and this may help to obtain heterozygous decedents. Thus, it would reduce the frequency of resistance alleles in the population and delay the evolution of resistance in the field (Onstad, 2014). However, this would depend on the male's competition toward females, whether resistance males take advantage over the susceptible ones, yet to be unveiled.

Moreover, the greenhouse assessment showed that resistant DBM males respond similarly to those of the susceptible strain, and the number of insects trapped was similar over time in both DBM strains. This suggests that pheromone traps can be effective in monitoring DBM populations regardless of their susceptibility to chlorantraniliprole. Therefore, resistance to chlorantraniliprole has not impaired males of detecting volatiles that indicate the presence of a mate, which is probably not related to diamides mode of action (ryanodine receptors) (Nauen, 2006) or insect resistance mechanisms (target mutation and metabolism) found in *P. xylostella* strains (Wang *et al*., 2016; Kang *et al*., 2017; Ribeiro *et al*., 2017; Troczka *et al*., 2017). Insect volatile chemoreception is regulated by different genes, sensilla, olfaction receptor neurons, and odorant binding proteins (Steinbrecht, 1996; Li *et al*., 2013; Yi *et al*., 2016; Yipeng *et al*., 2018) that are not targets of diamides. Therefore, it is unexpected that mutations leading to resistance to diamides may cause alteration in male attraction to calling females or the synthetic sex pheromone.

In conclusion, DBM strains resistant to chlorantraniliprole show an adaptive cost in comparison to susceptible strains that can result in a delay in population growth in the field when selection pressure is absent. In contrast, resistant adult males showed no olfactory response alteration. They are equally attracted to virgin females or the synthetic sex pheromone regardless of their strain. Therefore, resistant males can copulate with susceptible females or can be captured in sex pheromone traps in infested areas. The use of pheromone traps continues to be a viable alternative to manage this pest population in an IPM system, especially when DBM has developed resistance to pesticides such as chlorantraniliprole. Consequently, there is a reduction in the number of sprayings, which contributes to the increase in insect susceptibility in the area, since without selection pressure the frequency of resistant alleles tends to drop rather quickly (Tabashnik *et al*., 1994; Hollingsworth *et al*., 1997; Onstad, 2014; Sparks & Nauen, 2015).

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