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CROP PROTECTION

Locomotor response of diamondback moth (*Plutella xylostella*) populations to a neurotoxic insecticide under laboratory conditions

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ABSTRACT: *The present study aimed to evaluate changes in the locomotor activity of diamondback moth (Plutella xylostella) populations exposed to surfaces treated with the insecticide chlorantraniliprole under laboratory conditions. Diamondback moth populations from municipalities incorrectly using the insecticide [Camocim de São Félix, PE (CSF); Sairé, PE (SR); and Lajedo, PE (LJ)] and two laboratory populations from Recife, PE (RCF), and Viçosa, MG (VÇS) were exposed to dry insecticide residues in increasing concentrations. The following behavioral parameters of the populations were analyzed: walking time (WT), mean walking speed (MWS), and rest time (RT). Regarding RT, the SR and CSF populations demonstrated a behavior of irritability and increased WT with increased exposure concentration. The RCF population presented a contrasting response. The MWS values in the SR and CSF populations showed a decreasing trend with increased exposure concentration. The LJ and RCF populations showed no changes in terms of MWS. The RT values in the SR population decreased with increased insecticide concentration. In general, the SR and CSF populations presented a behavioral pattern different from that of laboratory populations. The changes in locomotor activity observed may result in lower control efficacy of the insecticide due to repellency or escape of insects. Regarding the effect of insecticide concentrations used in the populations, a defined pattern was not observed, and the effect alternated between an increase, a decrease, and an undefined pattern for the variables studied.*
Key words: *control failure, sublethal dose, behavioral response, synthetic insecticide, chlorantraniliprole.*

Resposta locomotora de populações de traça-das-crucíferas (*Plutella xylostella*) a inseticida neurotóxico em condição de laboratório

RESUMO: *O presente estudo teve por objetivo averiguar, em condições de laboratório, alterações na atividade locomotora de populações de traça-das-crucíferas, expostas a superfícies tratadas com o inseticida clorantraniliprole. Populações de Plutella xylostella provenientes dos municípios com uso inadequado de inseticida: Camocim de São Félix–PE (CSF), Sairé–PE (SR), Lajedo–PE (LJ) e duas populações de laboratório: Recife–PE (RCF) e Viçosa–MG (VÇS) foram expostas a resíduos secos do inseticida, em concentrações crescentes. Estas populações tiveram os seguintes parâmetros comportamentais analisados: Tempo de caminhamento (TC), Velocidade Média de caminhamento (VMC) e Tempo de Repouso (TR). Com relação ao TC, as populações de SR e CSF demonstraram comportamento de irritabilidade, aumentando o tempo de caminhamento com o aumento da concentração de exposição. A população de RCF apresentou resposta contrária. Em relação ao parâmetro VMC, as populações de SR e CSF apresentaram tendência de redução nos valores com o aumento da concentração de exposição. As populações de LJ e RCF não apresentaram mudanças deste comportamento. Para a variável TR, a população de SR apresentou redução nesses valores, com o aumento da concentração do inseticida. De forma geral, as populações de SR e CSF apresentaram um padrão de comportamento diferente das populações de laboratório. Estas alterações na atividade locomotora podem resultar numa menor eficácia de controle do inseticida devido à irritabilidade ou fuga. Quanto ao efeito das concentrações usadas do inseticida entre populações, de forma geral, não foi possível observar um padrão definido, tendo este efeito alternado entre aumento, redução e padrão não definido para as variáveis estudadas.*
Palavras-chave: *falha de controle, dose sub-letal, resposta comportamental, inseticida sintético, clorantraniliprole.*

INTRODUCTION

Diamondback moth (*Plutella xylostella*) is a major pest in brassica crops, causing the largest economic losses to those crops worldwide and requiring approximately 5.0 billion dollars annually for its control (ZALUCKI et al., 2012; FURLONG et al., 2013). Several factors such as succession planting, simultaneous planting of other brassicas, biological aspects (e.g., short development cycles,

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high genetic and phenotypic plasticity, and high migration capacity) (ZAGO et al., 2014; LI et al., 2019), and partially, generalized chemical control recommendations for large geographical areas, contributed to population outbreaks of diamondback moths in crop areas.

Diamondback moth is highly destructive, and chemical control is the main strategy for controlling this pest owing to its practicality, ease of use, and immediate result (VILAS BOAS et al., 2004). However, an improper insecticide use has recently been observed, with up to 4 applications/week (OLIVEIRA et al., 2011; XIA et al., 2014) and >20 during the planting cycle (PÉREZ et al., 2000). Constant exposure to insecticides has contributed to the selection of resistant individuals (ZHANG et al, 2016; QUIN et al., 2018; MÜLLER, 2018). According to YIN et al. (2019) the diamondback moth presents resistance to several types of insecticides, and ZHANG et al. (2016) have reported different levels of resistance to 95 active ingredients in the species.

Insecticide resistance is divided as physiological, biochemical, and behavioral resistance (GEORGHIOU & TAYLOR, 1977). The former two are associated with changes in the target sites of insecticides, reduction of the penetration capacity of insecticides into the body, and increase in the metabolization capacity of those products by insects (PANINI et al., 2016). Behavioral resistance can develop in insect populations during exposure to insecticides; the insects develop the ability to detect and/or recognize surfaces treated with a certain compound that would be lethal for them (NANSEN et al., 2016; ZALUCKI & FURLONG, 2017). There is a high behavioral plasticity in insects resulting from exposure to stimuli, which can modulate odor sensitivity (ANDERSON & ANTON, 2014; GADENNE et al., 2016). Factors that can lead to behavioral changes in insects include adopted management practices, local environmental variables, inter and intraspecific competition of species spatial and temporal distribution, insects life history-related characteristics, and insecticides used (DESNEUX et al., 2007; NANSEN et al., 2016). Effects produced by lethal compounds are perceived by the sensory organs and central nervous system of the insects, causing irritability and/or repellency on physiologically sensitive individuals (HAYNES, 1988), or stimulating biological processes capable of increasing insect survival (RIX et al., 2016; ZHAO et al., 2018).

Among the neurotoxic insecticides commercially available and used to control Lepidopterans, insecticides from the diamide class act by binding to the ryanodine receptors, promoting the permanent opening of calcium channels and contributing to the uncontrolled release of calcium in muscle cells, thereby killing the insect (TEXEIRA & ANDALORO, 2013). Since its introduction in the Brazilian market in 2009, the insecticide chlorantraniliprole has widely been used to control diamondback moth. However, the improper use of this insecticide has affected its efficiency due to the high selection pressure exerted by the pesticide. The improper use has; therefore, allowed the evolution of resistant populations to diamondback moth, particularly in the northeast region of Brazil (SILVA et al., 2012).

With the emergence of chlorantraniliprole-resistant populations, several studies have shown changes in the locomotor activity of insects when exposed to surfaces treated with this insecticide—for example, the study by PLATA-RUEDA et al. (2019) on coffee borer beetle (*Hypothenemus hampei*), and studies by NANSEN et al. (2016), CHANDI & SINGH (2017), and PASSOS et al. (2019) on diamondback moth. Verifying changes in the locomotor activity of agricultural pests in response to exposure to the insecticide in each location is an issue, considering that this behavior can reduce the contact of the pest with the insecticide, thereby reducing the absorption of insecticide and causing a lower control efficacy (GUEDES et al., 2011). These changes caused by xenobiotics may be caused by proteomic and biochemical changes in insects (ROAT et al., 2017), with both biochemical and behavioral consequences (BANTZ et al., 2018). Therefore, the present study was conducted to evaluate possible changes in the locomotor activity of larvae of different diamondback moth populations exposed to surfaces treated with increasing concentrations of the insecticide chlorantraniliprole under laboratory conditions.

MATERIALS AND METHODS

Insects

Five diamondback moth populations were used in the behavioral response bioassays—three were from areas of kale and cabbage planting in the municipalities of Sairé, PE (SR), Camocim de São Félix, PE (CSF), Lajedo, PE (LJ) and the remaining two were laboratory populations, both provided by the Entomology Departments of the Federal Rural University of Pernambuco-UFRPE (RCF) and the Federal University of Viçosa-UFV (VÇS). Larvae and pupae were collected, and they were raised in the laboratory according to the methodology described by BARROS & VENDRAMIM (1999).

Insecticides

The bioassays were conducted using chlorantraniliprole in a concentrated suspension containing 200 g a.i./L, purchased from an agricultural store.

Behavioral bioassay

The behavioral bioassay was conducted at the Laboratory of Applied Entomology of the Academic Unit of Garanhuns (UAG-UFRPE). Third-instar diamondback moth larvae were collected into Petri dishes (diameter, 9.0 cm), lined with filter paper treated with dry residues of chlorantraniliprole at a concentration of 0, 1, 2, 4, 6, 8, and 10 times the recommended field dose (7.5 mL/100 L). The filter paper discs used in the tests were treated with 1 mL of the insecticide solution prepared with a dispersant (Tween 80 at 0.001%). Control discs were loaded with distilled water along with the dispersant. After the filter paper discs were completely dry, the discs loaded with the treatments were glued to the bottom of the Petri dishes to prevent insects from moving underneath the filter paper, which would render it difficult to locate them using the ViewPoint® camera tracking system (methodology adapted from CORDEIRO et al. (2010)). The inner walls of the Petri dishes were coated with water-soluble gel (K-Y Jelly, Johnson & Johnson®) to prevent the larvae from escaping. The Petr dish thus prepared was kept under the video camera of the ViewPoint® tracking system for 5 minutes. Before starting recording, the larvae were placed at the center of the dish, and after 1 min of acclimatization, the test was commenced (CHANDI & SINGH, 2017). The design was completely randomized, with 18 repetitions for each population and for each concentration. Each repetition comprised a single insect, and at each repetition the larvae were replaced. In these trials, the behavioral variables analyzed were walking time (WT), mean walking speed (MWS), and rest time (RT). Data about the behavioral parameters were subjected to analysis of variance, and the means were grouped by the SCOTT & KNOTT test at 5% probability after verifying the assumptions of normality of the data and of homogeneity of variance (FERREIRA, 2011).

RESULTS

The experimental analyses were performed to verify whether both the high concentrations used in the field and high number of applications can cause irritability in insects and thereby result in control failure.

Regarding WT, for the behavior of each population, three patterns of responses were observed: 1) Increased WT associated with increased doses of insecticide (SR and CSF); 2) Decreasing trend in WT with increased doses (RCF), and 3) Undefined pattern (LJ and VÇS).

Results demonstrated that only in surfaces treated with a concentration 10 times higher than the recommended one, were observed no significant difference in diamondback moth populations behavior. (Table 1).

Regarding the MWS variable, three patterns were observed within the populations: 1) No influence of insecticide (LJ and RCF); 2) Decreasing trend influenced by contact with the insecticide (CSF and SR), and 3) Undefined pattern (VÇS) (Table 2). When in contact with the insecticide, the highest value of MWS was observed in the SR population (1.82 cm/s), and the lowest value in the VÇS laboratory population (Table 2).

Regarding the RT variable, the populations showed a significant difference in the other treatments, with the exception of that observed when the surface was treated with 10 times the recommended dose (Table 3). When studying the effects of doses on populations, two trends were reported: 1) decrease in RT (SR), and 2) undefined pattern for RT in the other populations. The highest

Table 1 - Walking time (WT) (s) of third-instar larvae of diamondback moth after contact with filter paper discs (diameter 9 cm) containing dry residues of the insecticide chlorantraniliprole in different doses.

P**	Control ± SD*	7.5 mL 100 L^{-1} ± SD	15 mL 100 L^{-1} ± SD	30 mL 100 L^{-1} ± SD	45 mL 100 L^{-1} ± SD	60 mL 100 L^{-1} ± SD	75 mL 100 L^{-1} ± SD
SR	121.04±84.03^{bD}	174.76±58.67^{bC}	264.11±67.41$^{a\,A}$	204.93±70.58$^{b\,B}$	224.47±74.99$^{b\,B}$	261.78±61.93$^{a\,A}$	241.29±85.22$^{a\,A}$
LJ	258.86±74.15$^{a\,A}$	199.60±81.75$^{b\,B}$	290.35±10.69$^{a\,A}$	284.30±18.66$^{a\,A}$	282.55±21.95$^{a\,A}$	264.23±53.28$^{a\,A}$	266.94±34.13$^{a\,A}$
CSF	125.76±69.71$^{b\,C}$	291.09±10.43$^{a\,A}$	278.62±35.80$^{a\,A}$	217.22±89.70$^{a\,B}$	206.62±69.54$^{b\,B}$	245.68±47.90$^{a\,B}$	232.21±54.71$^{a\,B}$
RCF	284.77±17.53$^{a\,A}$	264.05±32.12$^{a\,A}$	209.97±94.03$^{b\,B}$	251.43±34.68$^{a\,A}$	231.43±69.04$^{b\,B}$	214.62±67.15$^{a\,B}$	238.55±70.26$^{a\,B}$
VÇS	137.16±73.77$^{b\,B}$	180.75±77.99$^{b\,B}$	280.29±39.54$^{a\,A}$	285.99±15.56$^{a\,A}$	139.09±81.34$^{c\,B}$	149.53±68.90$^{b\,B}$	283.22±20.43$^{a\,A}$

Table 2 - Mean walking speed (MWS; cm/s) of third-instar larvae of diamondback moth after contact with filter paper discs (diameter 9 cm) containing dry residues completely treated with a dose of the insecticide chlorantraniliprole in different doses.

P**	Control ± SD*	7.5 mL 100 L^{-1} ± SD	15 mL 100 L^{-1} ± SD	30 mL 100 L^{-1} ± SD	45 mL 100 L^{-1} ± SD	60 mL 100 L^{-1} ± SD	75 mL 100 L^{-1} ± SD
SR	$1.82\pm0.71^{a\ A}$	$1.45\pm0.35^{a\ B}$	$0.74\pm0,33^{a\ C}$	$1.13\pm0.48^{a\ C}$	$1.05\pm0.37^{b\ C}$	$0.87\pm0.30^{b\ C}$	$0.96\pm0.41^{a\ C}$
LJ	$0.83\pm0.39^{c\ A}$	$0.89\pm0.48^{b\ A}$	$0.79\pm0.27^{a\ A}$	$0.76\pm0.21^{a\ A}$	$0.80\pm0.19^{b\ A}$	$1.01\pm0.32^{b\ A}$	$0.99\pm9.29^{a\ A}$
CSF	$1.34\pm0.72^{b\ A}$	$0.91\pm0.28^{b\ B}$	$0.92\pm0,32^{a\ B}$	$1.10\pm0.63^{a\ B}$	$1.33\pm0.59^{a\ A}$	$1.11\pm0.41^{b\ B}$	$1.07\pm0.35^{a\ B}$
RCF	$0.87\pm0.26^{c\ A}$	$0.94\pm0.28^{b\ A}$	$0.91\pm0.47^{a\ A}$	$1.01\pm0.34^{a\ A}$	$0.81\pm0.34^{b\ A}$	$1.05\pm0.44^{b\ A}$	$0.98\pm0.35^{a\ A}$
VÇS	$1.66\pm0.66^{a\ A}$	$1.30\pm0.37^{a\ B}$	$0.76\pm0.20^{a\ C}$	$1.06\pm0.42^{a\ C}$	$1.42\pm0.64^{a\ B}$	$1.58\pm0.41^{a\ A}$	$0.84\pm0.29^{a\ C}$

mean values of RT were observed in the SR (178.88 s), CSF (174.14 s), and VÇS (162.80 s) populations in the control treatment (Table 3).

DISCUSSION

Diamondback moth populations showed changes in the locomotor activity variables evaluated for the insecticide chlorantraniliprole. The same response pattern was not observed for all variables and for all populations studied. These responses can be defined as repellency (behavioral response of insects with little or no contact with the insecticide) or irritability (behavioral response after extensive contact with a pesticide) induced by xenobiotics when in contact with insects (SILVA et al., 2013; GUEDES et al., 2011; 2015; LOCKWOOD et al., 1989). In the present study, where the entire surface of the test dish was treated, a great variation of irritability behavior was observed. Although, the high plasticity of behavioral responses of the studied populations was not analyzed in this study, it may be attributed to post-transcriptional and epigenetic regulations that can modify the individual's gene expression without changing the genetic sequence rather than to mutations, which require a long time (BANTZ et al., 2018). A study of the behavior of the offspring of each population, depending on their history of contact with a particular insecticide should be performed to ascertain whether there would be a different response to xenobiotics because BANTZ et al. (2018) have explained that memory and stress can be transmitted to subsequent generations and influence them.

When exposed to surfaces treated with the insecticide, diamondback moth larvae collected from the municipalities of SR and CSF increased the WT, suggesting an evasive behavior from the treated site considering that WT in the control treatment was shorter. PLATA-RUEDA et al. (2019) verified similar observations in coffee borer beetle populations when in contact with surfaces treated with chlorantraniliprole. The changes reported were associated with the activity of toxic compounds found in the insecticide formulation on the nervous system of the insect, thereby possibly stimulating its mobility.

Table 3 - Rest time (RT) (s) of third-instar larvae of diamondback moth after contact with filter paper discs (diameter 9 cm) containing dry residues completely treated with a dose of the insecticide chlorantraniliprole in different doses.

P**	Control ± SD*	7.5 mL 100 L^{-1} ± SD	15 mL 100 L^{-1} ± SD	30 mL 100 L^{-1} ± SD	45 mL 100 L^{-1} ± SD	60 mL 100 L^{-1} ± SD	75 mL 100 L^{-1} ± SD
SR	$178.88\pm84.02^{a\ A}$	$125.18\pm58.65^{a\ B}$	$35.83\pm67.39^{b\ D}$	$95.02\pm70.56^{a\ C}$	$75.48\pm75.03^{b\ C}$	$38.13\pm61.93^{b\ D}$	$58.65\pm85.21^{a\ D}$
LJ	$41.18\pm74.16^{b\ B}$	$100.32\pm81.74^{a\ A}$	$9.55\pm10.69^{b\ B}$	$15.66\pm18.62^{b\ B}$	$17.38\pm21.95^{c\ B}$	$35.70\pm53.27^{b\ B}$	$32.98\pm34.13^{a\ B}$
CSF	$174\pm69.71^{a\ A}$	$8.85\pm10.43^{b\ C}$	$21.31\pm35.78^{b\ C}$	$82.70\pm89.68^{a\ B}$	$93.31\pm69.58^{b\ B}$	$54.20\pm47.88^{b\ B}$	$67.72\pm54.71^{a\ B}$
RCF	$15.18\pm17.54^{b\ B}$	$35.89\pm32.12^{b\ B}$	$89.93\pm94.05^{a\ A}$	$48.51\pm34.65^{b\ B}$	$68.48\pm69.04^{b\ A}$	$85.31\pm67.15^{b\ A}$	$61.39\pm85.21^{a\ A}$
VÇS	$162.80\pm73.79^{a\ A}$	$119.18\pm78.01^{a\ A}$	$19.63\pm39.56^{b\ B}$	$13.93\pm15.58^{b\ B}$	$160.86\pm81.33^{a\ A}$	$150.40\pm68.91^{a\ A}$	$16.70\pm20.42^{a\ B}$

*SD = Standard Deviation, **P = Population. [a]Different superscript values in the same column indicate statistical difference, as determined by the Scott & Knott test ($P<0.05$); [A]Different superscript values in the same line indicate statistical difference, as determined by the Scott & Knott test ($P<0.05$). #Concentrations used in the study refer to increasing concentrations of insecticide in relation to the concentration recommended by the insecticide manufacturer (7.5 mL/100 L water).

According to GUEDES et al. (2015) and SILVA et al. (2013), changes in the behavior of insects exposed to surfaces treated with insecticides are motivated by one or several compounds reported in the formulations, which induce the behavior of escaping areas with lethal compounds, thereby increasing their chances of survival.

The modification of the walking pattern has already been reported in other insects as a strategy used to increase chances of survival. Results for walking distance observed in the predators *Orius tristicolor*, *Amphiareus constrictus*, and *Blaptostethus pallescens* showed a reduction in distance when the insects were exposed to surfaces treated with chlorantraniliprole (PEREIRA et al., 2014). The authors hypothesized that reducing the walking distance would be an adaptive response learned by insects to reduce their exposure to toxic residues of insecticides.

BANTZ et al. (2018) suggested that insects can learn and modify their behavior at each generation, which may lead to the evolution of behavioral resistance. Therefore, the changes may be owing to the selection pressure exerted by insecticides. Consequently, insects can avoid or evade areas with lethal effects, reducing the efficacy of the chemical control method. According to DESNEUX et al. (2007), the evasion behavior in areas treated with insecticides is responsible for minimizing their exposure, leading them to a higher survival rate in the field.

The RCF laboratory population showed reduced WT on surfaces treated with chlorantraniliprole. Reduction of locomotor activity on treated surfaces has been reported as a strategy used by insects to minimize exposure to insecticides. BRAGA et al. (2011) reported this behavior in some populations of *Sitophilus zeamais* and termed it as behavioral avoidance, which may be dependent on stimulus such as irritability. Due to selection pressure, the reduction of insect locomotor activity on treated surfaces is an alternative strategy to minimize the exposure of insects to insecticides, and this behavior has been verified in numerous insect species (GUEDES et al. 2011).

CASTRO et al. (2018) found that *Spodoptera exigua* populations avoided areas treated with chlorantraniliprole, and this behavior was a result of the repellency and irritability exerted by the insecticide. In addition to changes in locomotion, other responses have been reported when insects are exposed to surfaces treated with insecticides (xenobiotics), allowing them to avoid food and oviposition on plant leaves treated with insecticides (ZHANG et al., 2004). ZHANG et al. observed this behavior in *Helicoverpa armigera* on cotton leaves treated with *Bacillus thuringiensis* and reported that the larvae moved on to places with lower concentration of the insecticide.

Chlorantraniliprole significantly affected the MWS of CSF and SR populations, reducing it on surfaces treated with the insecticide. BECKEL et al. (2004) reported that individuals of *Rhyzopertha dominica* (Coleoptera: *Bostrichidae*) populations showed a reduction in the MWS on surfaces treated with the insecticide deltamethrin; this fact was correlated with the insects' goal to avoid or decrease their contact with the insecticide. However, NANSEN et al. (2016) observed different results from those observed in that study. NANSEN et al. verified an increase in the MWS of diamondback moth larvae that presented physiological resistance to gamma-cyhalothrin on surfaces treated with it and recommended the need for understanding whether the behavioral response of insects indeed occurred in relation to areas treated with insecticides or whether insects only respond to behavioral adaptation to avoid contact with treated surfaces.

The CSF and SR populations showed a reduction in the RT on surfaces treated with chlorantraniliprole when compared with the control treatment. Decreased RT is associated with the irritability and repellency induced by insecticides on the insects, compelling the insects to escape from treated surfaces (DESNEUX et al., 2007). The compounds present in insecticide formulations may cause evasion or shorter time when the insects halt on surfaces with high concentrations of insecticides (HOY et al., 1998; GUEDES et al., 2015).

According to ROYAUTÉ et al. (2014, 2015), insect populations can develop, maintain, or change behavioral patterns when subjected to surfaces treated with insecticides, thereby promoting the selection of individuals with possible behavioral adaptations to the conditions imposed. They mentioned that insect populations of the same species possibly share the same behavior; however, behavioral expressions often change based on the exposure to environments with insecticide applications and the duration of exposure, resulting in various behavioral and physiological changes. Our results are consistent with these abovementioned results, thereby contributing to the knowledge that behavioral changes occur as a result of the behavioral identity developed by each population according to their agricultural environment and duration of exposure to the insecticide.

Changes in insect behavioral patterns, along with other mechanisms that characterize insecticide resistance, contribute to a reduction in insecticide efficacy; and consequently, render it challenging to control pest populations and maintain them at the below-threshold levels to prevent economic losses (GUEDES et al., 2011). Results of this study showed that insecticides might influence the behavioral patterns of several populations of the same pest in different manner. A same pattern of irritability was not observed, both within laboratory populations and within field populations. Insecticide management practices in each crop possibly influence the behavioral response of insects in the corresponding regions. Therefore, establishing a relationship between the history and frequency of insecticide use by producers in the field and the response of insect offspring to insecticides is crucial for the development of more effective chemical control approaches that minimize instances of control failure.

CONCLUSION

Diamondback moth populations presented different behavioral patterns for the variables analyzed when exposed to surfaces treated with increasing concentrations of chlorantraniliprole. Behavioral responses alternated between decrease, increase, and an undefined pattern of locomotor activity.

Behavioral responses may reduce the exposure of certain pest populations to insecticides, thereby increasing the likelihood of pest survival. These responses differ based on the region, history of pest management, and contact of the insects with the insecticide.

In this study, behavioral differences were observed among the different populations of diamondback moths, reinforcing the need for adopting a regionalization of control recommendations, considering that each population reacts differently to insecticide exposure.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest of personal, academic, political or financial interests relating to the publication.

AUTHORS' CONTRIBUTIONS

All authors have made contributions to the conception and design of the manuscript. All authors have participated in revising the manuscript critically and have given final approval of the version to be submitted.

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