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Bt_i-based insecticide enhances the predatory abilities of the backswimmer *Buenoa tarsalis* (Hemiptera: Notonectidae)

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Abstract The backswimmer *Buenoa tarsalis* (Hemiptera: Notonectidae) is a naturally occurring predator of immature stages of mosquitoes. These aquatic predators can suffer from non-targeted exposure to insecticides that are commonly used in aquatic environments to control mosquitoes. Here, we evaluated whether insecticide formulations containing the bacterium *Bacillus thuringiensis* var. *israelensis* (Bt_i) or the organophosphate pirimiphos-methyl would affect the survival and the predatory abilities of *B. tarsalis*. First, we conducted survival bioassays to estimate the median survival time (LT_{50}) of *B. tarsalis* when exposed to Bt_i-based insecticide (at 0.25 and 25 mg a.i./L) and pirimiphos-methyl (at 1, 10 and 1000 mg a.i./L). The highest concentrations of the insecticides were equivalent to the label-recommended field rates. Second, the predatory abilities of *B. tarsalis* exposed to insecticides were evaluated at three prey densities (3, 6 and 9 mosquito larvae/100 mL water) just after insecticide exposure or after a 24 h recovery time. While the survival of *B. tarsalis* was significantly reduced with pirimiphos-methyl concentrations ≥10 mg a.i./L, the Bt_i-exposed predators exhibited similar survival as unexposed predators. Interestingly, after a recovery time of 24 h, *B. tarsalis* sublethally exposed to pirimiphos-methyl or Bt_i-based insecticide consistently killed more *A. aegypti* larvae (at the intermediate density) than unexposed predators. However, for the without-recovery bioassays, the pirimiphos-methyl-exposed predators exhibited reduced predatory abilities at the lowest prey density. Because they do not reduce the survival or the predatory abilities of *B. tarsalis*, Bt_i-based insecticides can be considered a safe insecticide to use in the presence of backswimmers.

Keywords *Bacilus thuringiesnsis* · aquatic predators · Natural enemies · Pesticides

Introduction

Vector insects that convey human pathogens are recognized as a major health problem in tropical countries (WHO 2009). Among these insects, species belonging to the genera *Culex* and *Aedes* are widely distributed in Asia, Africa and Latin America, but the mosquito *Aedes aegypti* is especially notable due to its ability to transmit different types of arboviruses that include dengue fever, yellow fever, chikungunya and Zika (Moreira et al. 2009; Ndiaye et al. 2016; Barreto et al. 2016). The recent increase in human birth defects and deaths caused by mosquito-borne viruses has been reported as consequence of human movement (Adams and Kapan 2009), worldwide distribution of the vectors (Staples et al. 2009), and neglect, in the last decades, of prevention plans that include public awareness and the

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adoption of controversial methods to control mosquito populations (Rodríguez et al. 2007; Lima et al. 2011; Petersen et al. 2016).

In the absence of a vaccine against many diseases, the most common strategy currently used to reduce populations of mosquitoes is chemical control (Pridgeon et al. 2008; Liu 2015). Although insecticides have extinguished many diseases transmitted by mosquitoes around the world, the extensive use of neurotoxic insecticides such as pyrethroids and organophosphates has been controversial due to increased insecticide resistance in vector insects in Asia and Latin America (Rodríguez et al. 2007; Martins et al. 2009; Lima et al. 2011; Bellinato et al. 2016, Haddi et al. 2017). Consequently, there is a need for compounds with different action mechanisms or biological control agents as alternative approaches to manage insecticide resistance in mosquito populations (Quiroz-Martínez and Rodríguez-Castro 2007; Coelho et al. 2009; Shaalan and Canyon 2009; Kroeger et al. 2013; Swale et al. 2016). Furthermore, the beneficial interactions between pesticides and naturally occurring predators have been proposed as potential tools to enhance the control of aquatic insects (Relyea and Hoverman 2008; Holmstrup et al. 2010; Janssens and Stoks 2013).

The most important natural enemies of mosquitoes are fishes and insect predators such as odonates, water bugs and backswimmers that play an important role in reducing numbers of mosquitoes (Quiroz-Martínez and Rodríguez-Castro 2007; Relyea and Hoverman 2008; Shaalan and Canyon 2009). However, insecticides with broad-spectrum action can affect these non-target organisms (Schulz 2004; Relyea and Hoverman 2008; Kuivila et al. 2012; Gutiérrez et al. 2016a, b). In addition, little information is available on the sublethal effects of insecticides in these animals because most studies focus on lethal effects (Marina et al. 2014; Halstead et al. 2015). Although lethality is important in risk assessment for any animal species, the sublethal effects on behaviors such as predatory ability in non-target species is also important in assessing pesticide risks (Desneux et al. 2007; Rasmussen et al. 2013). Therefore, effective insecticides that control mosquitoes and have low impacts on the mosquitoes' natural enemies are required for more rational control of mosquitoes in aquatic environments.

Commercial larvicides based on the bacterium *Bacillus thuringiensis* var. *israelensis* (Bt_i) are very commonly used worldwide to control vector insects (Crickmore 2005; Lacey 2007; Jakob and Poulin 2016) due to their high toxicity to mosquitoes, and low or lack of toxicity to most aquatic organisms (Boisvert and Boisvert 2000; Lacey and Merritt 2004; Lagadic et al. 2014, 2016). However, despite being effective against mosquitoes, there is a lack of information regarding the lethal and mainly sublethal effects of Bt_i-based commercial insecticides in non-target aquatic insects. Thus, this study was conducted to evaluate whether insecticide formulations (one containing the *Bacillus thuringiensis* var. *israelensis* (Bti) and another with organophosphate pirimiphos-methyl) would affect the survival and predatory ability of the backswimmer *Buenoa tarsalis* (Hemiptera: Notonectidae), an important natural predator of immature stages of mosquitoes.

Material and methods

Test organisms

Adults of *B. tarsalis* were collected from artificial ponds at the fish-farm station of the Department of Animal Biology, Federal University of Viçosa (UFV, Viçosa, MG, Brazil, 20°45′S, 42°52′W) using a D-net. Second instar larvae (L2) of *A. aegypti* (strain PP-Campos, Campos dos Goytacazes, RJ, Brazil) were obtained from a colony maintained in the Department of General Biology of the Federal University of Viçosa. The insects were maintained under controlled conditions (25 ± 2 °C, 12 h of scotophase) for 48 h before the experiments. All bioassays were conducted using mineral water (Hélios, Dona Eusébia—MG, Brazil) as a medium to dilute the insecticides. The properties of the water were as follows: HCO_3^- 8.32 mg/L, Na^+ 2.028 mg/L, Ca^{2+} 1.381 mg/L, K^+ 1.381 mg/L, NO_3^- 1.82 mg/L, Mg^{2+} 0.631 mg/L, Cl^- 1.69 mg/L, SO_4^{2-} 0.55 mg/L, F^- 0.02 mg/L, pH 5.61, and conductivity 25.5 µS/cm.

Insecticide efficiency in controlling A. aegypti larvae and impact in the survivability of B. tarsalis

First, we exposed (for 24 h) groups of *A. aegypti* larvae to a Bt_i-based commercial insecticide (Bt-Horus SC® [12 g a.i./L], BTHEK Biotecnologia Ltda, Brasília, Brazil) or to a pirimiphos-methyl commercial formulation (Actellic 500 EC® [500 g a.i./L], Syngenta Ltda, São Paulo, Brazil) in order to evaluate the efficacy of these products against mosquitoes. Groups of 25 *A. aegypti* second instar larvae (L2) were exposed for 24 h to the insecticides at their respective label rates for application in water bodies to control mosquitoes (Bt_i: 25 mg of a.i./L; pirimiphos-methyl: 1000 mg of a.i./L). The larvae were exposed in a 500-mL glass beaker containing 300 mL of insecticide solution or mineral water as a control. Four replicates were used for every treatment.

For the survival bioassays with *B. tarsalis*, adult males and females of the predator were exposed to concentrations 1, 10 and 1000 mg of a.i./L of pirimiphos-methyl and 0.25 and 25 mg of a.i./L of Bt_i-based insecticide. In the control treatment, the predators were exposed to mineral water as described above. These bioassays were conducted in

mineral water using unaged adult insects that faced starvation condition during all the experimental time. Groups of 10 *B. tarsalis* were placed in a 500-mL glass beaker containing 300 mL of insecticide solution; the beaker was covered with fine mesh netting (i.e., organza) to prevent insect escape. Four replicates of ten insects each were used for every treatment, and mortality was assessed every 6 h for 16 days. In each evaluation, the individuals who remained motionless after repeated mechanical stimuli with a pipette were considered dead and removed from the containers to avoid cannibalism.

Predation bioassay

Recently collected adult females of *B. tarsalis* were acclimated under the conditions described above. Then, the insects were exposed to insecticide solutions (1 mg a.i./L of pirimiphos-methyl or 25 mg a.i./L of Bt_i-based insecticide) or to mineral water (control treatment) for 24 h. The insecticide concentrations chosen were considered sublethal because they did not reduce the survival of the insects when compared with the control in the survival bioassay. Subsequent to insecticide exposure, females were submitted to predation experiments either immediately (i.e., without recovery) or were maintained separately in glass containers with 150 mL of mineral water without insecticides for 24 h until the onset of the experiment (i.e., with recovery time). To assess predatory ability in both treatments, second instar larvae of *A. aegypti* were offered in three densities (3, 6 or 9 larvae) in mineral water. At least five insects were used in each combination among prey availability, insecticide type and insecticide recovery time. The number of preyed larvae was evaluated every 20 min for 2 h and the densities were re-established after each evaluation. The total of *A. aegypti* larvae preyed by *B. tarsalis* during these experiments were also compared among insecticide-exposed and unexposed predators.

Statistical analysis

The results of the survival bioassays were subjected to survival analysis performed using the Kaplan–Meier estimator (Log-rank method) with SigmaPlot 12.0 software (Systat Software, San Jose, California, USA). The median value for the LT_{50} estimations were pairwise compared using the posthoc Holm-Sidak's test Tukey's HSD test ($P < 0.05$). The data obtained in the predation bioassays was subjected to repeated measure analyses of variance to determine the effects of insecticides, prey densities and recovery time. The number of preyed larvae during each 20 min interval was used as the replicate (within-sample variation) to avoid problems of pseudoreplication in time (Paine 1996 and von Ende 1993). The GLM procedure with the PROFILE statement was used for this analysis (SAS Institute 2008). The total of *A. aegypti* larvae preyed by *B. tarsalis* was subjected to analyses of covariance with the availability (i.e., density level) of *A. aegypti* larvae as the independent variable and the in each insecticide recovery situation as a covariate (PROC GLM procedure).

Results

Survival bioassay

The label rates of Bt_i and pirimiphos-methyl killed 100% of *A. aegypti* larvae after 24 h of exposure, confirming the high efficiency of both compounds. The analysis of survival showed significant differences between the survival of *B. tarsalis* males exposed to the two higher concentrations of both pirimiphos-methyl and those exposed to all other treatments (Log-Rank: $\chi^2 = 184.36$, df = 7, $P < 0.001$). The mean of male survival time for the control (84.0 ± 9.4 h) was approximately 2.9 and 13.7 times higher than the survival time of males exposed to pirimiphos-methyl at 10 mg/L (29.0 ± 4.5 h) and 1000 mg/L (6.1 ± 1.1 h), respectively (Fig. 1a, b). Similar reductions in longevity were found in females exposed to the two higher concentrations of pirimiphos-methyl (Log-Rank: $\chi^2 = 166.20$, df = 7, $P < 0.001$), where the mean of survival time in the control (133.60 ± 11.7 h) was approximately 4.6 and 26.2 times higher than the survival time of females exposed to 10 mg/L (29.0 ± 4.5 h) and 1000 mg/L (5.1 ± 1.0 h) (Fig. 1c, d). Furthermore, it is worth to note that *B. tarsalis* females survived longer than *B. tarsalis* males in all bioassays. There were no significant differences between the control and the lowest Bt_i and pirimiphos-methyl concentrations ($P > 0.05$) for either males or females. For this reason, the lowest Bt_i and pirimiphos-methyl concentrations were chosen to test the effects on the predation abilities of *B. tarsalis*.

Predation bioassay

As shown in Table 1, the repeated measure analyses of variance revealed significant effects of insecticide ($F_{(1,2)} = 3.2$, $P = 0.0445$), density of prey ($F_{(1,2)} = 288.7$, $P < 0.0001$), recovery time ($F_{(1,1)} = 4.17$, $P = 0.0423$) and time ($F_{(5,111)} = 10.0$, $P < 0.0001$). The interactions of prey density with insecticide ($F_{(1,4)} = 3.0$, $P = 0.0204$) and with time ($F_{(10,222)} = 4.3$, $P < 0.0001$) were also significant (Table 1).

For the predation bioassays without recovery time (Fig. 2), exposure to pirimiphos-methyl significantly decreased the number of larvae preyed by *B. tarsalis* when these insects had the lowest prey availability (i.e., 3 larvae/

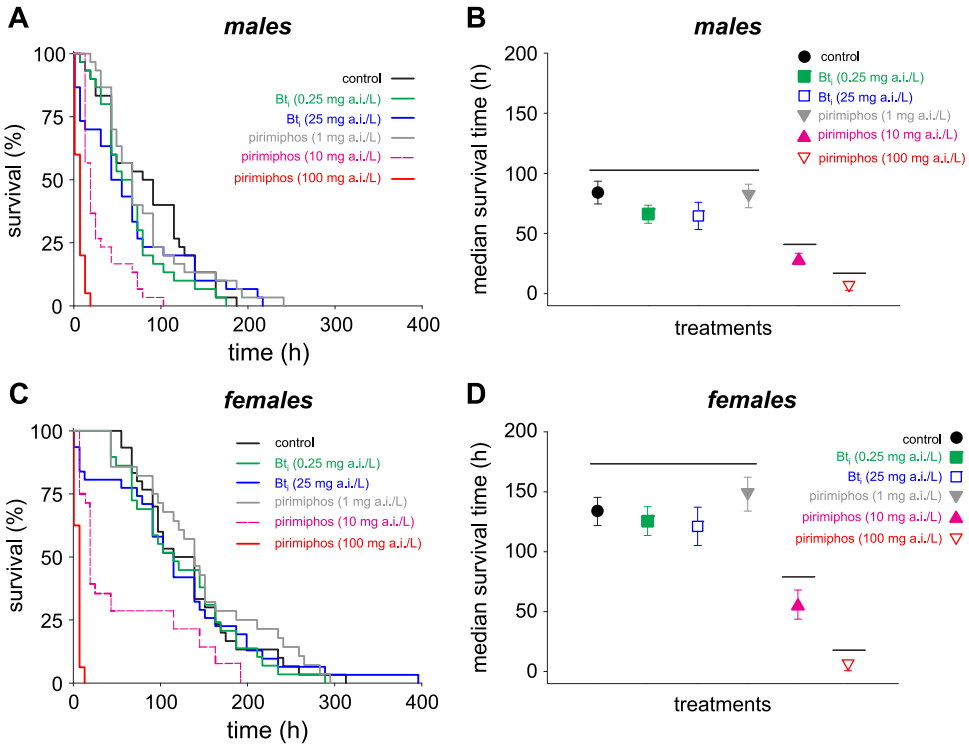

Fig. 1 Survival analysis of adult *Buenoa tarsalis* exposed to pirimiphos-methyl or to a Bt_i–based insecticide. **a**, **c** Show the survival curves for males and females, respectively. The estimated mean survival time (i.e., LT_{50}) for males (**b**) and females (**d**) is also presented. In **b**, **d**, *symbols* grouped by the same *horizontal line* do not differ according to a Tukey's HSD test ($P < 0.05$) and represent the average of four replicates of ten insects

Table 1 Repeated measures analysis of variance for predation of *Buenoa tarsalis* exposed to Bti-based insecticide (25 mg a.i./L) or pirimiphos-methyl (1 mg a.i./L)

Sources of variation	df	F	*P*	
Between samples				
Insecticide (I)	2	3.2	0.0445*	
Density (D)	2	288.7	<0.001*	
Recovery time (RT)	1	4.1	0.0423*	
I x D	4	3.0	0.0204*	
I x RT	2	1.5	0.23	
D x RT	2	1.3	0.28	
I x D x RT	4	0.62	0.64	
Error	115	–	–	
	df_{den}/df_{num}	Wilks' lambda	F_{approx}	*P*
Within samples				
Time (T)	5/111	0.68	10.0	<0.0001*
T x I	10/222	0.90	1.2	0.32
T x D	10/222	0.70	4.3	<0.0001*
T x RT	5/111	0.96	1.0	0.44
T x I x D	20/369	0.85	0.9	0.53
T x I x RT	10/222	0.89	1.4	0.20
T x D x RT	10/222	0.96	0.5	0.88
T x I x D x RT	20/369	0.87	0.7	0.79

*Significant at $P < 0.05$

100 mL of water) (Fig. 2), but pirimiphos-methyl exposure did not affect the predatory abilities of *B. tarsalis* at the intermediate (i.e., 6 larvae/100 mL of water) or highest (i.e., 9 larvae/100 mL of water) prey densities (Fig. 2b, c). When the predators were exposed to the Bti-based insecticide, the predator's capacities for preying on mosquito larvae at the lowest and intermediate densities were not significantly different from those recorded for unexposed predators (Fig. 2a, b). Surprisingly, at the highest density of prey, the predatory abilities of *B. tarsalis* exposed to Bt_i-based insecticide were not affected over time (Fig. 2), which could suggest significant differences from the abilities of the unexposed and pirimiphos-methyl-exposed predators that killed significantly fewer mosquito larvae (Fig. 2). However, the total of *A. aegypti* larvae preyed by these Bt_i-exposed predators were significantly different from the total of larvae preyed by predators exposed to pirimiphos-methyl or by unexposed predators (Table 2; Fig. 3).

When *B. tarsalis* were exposed to insecticides and had a recovery time of 24 h (Fig. 4), the exposure to pirimiphos-methyl did not impact their predatory abilities at the lowest or highest densities of prey (Fig. 4a, c). However, at intermediate prey availability (i.e., six *A. aegypti* larvae/100 mL of water), the pirimiphos-methyl-exposed predators ate significantly more *A. aegypti* larvae than the unexposed predators (Fig. 4b). In this experimental scenario, although the exposure to the Bt_i-based insecticide did not impact the predators' abilities at the lowest prey availability (Fig. 4a),

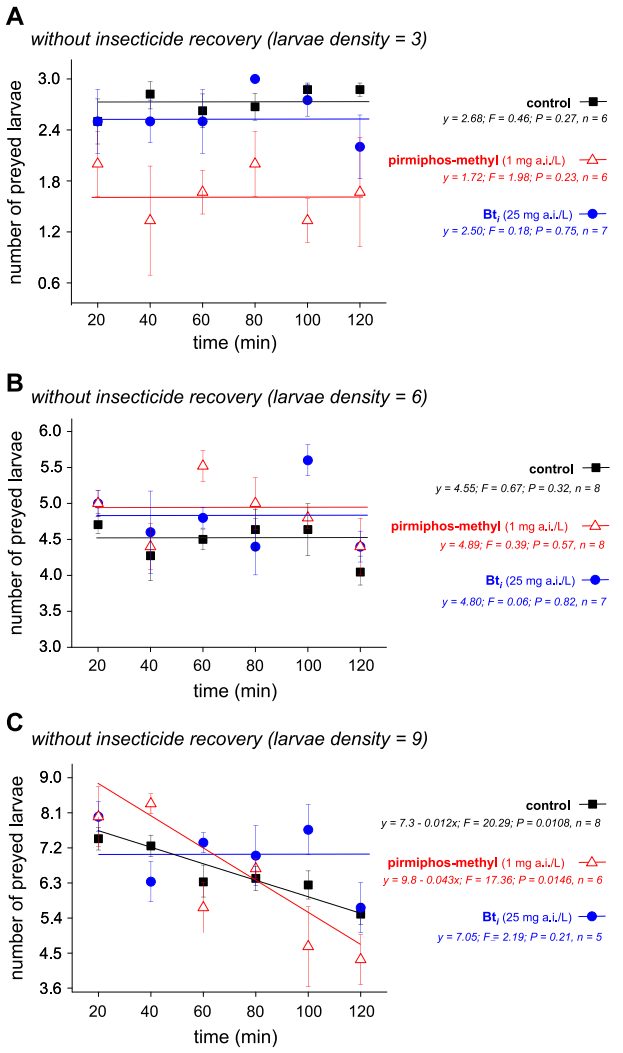

Fig. 2 Number of *Aedes aegypti* larvae preyed upon by *Buenoa tarsalis* females just after 24 h of exposure to pirimiphos-methyl and a Bt_i-based insecticide. The predators' abilities were assessed at larval densities of 3 (**a**), 6 (**b**) and 9 (**c**) larvae/100 mL of water. Larval densities were reestablished after every evaluation. Data are the mean ± SE

such exposure resulted in higher numbers of preyed-upon larvae at intermediate (Fig. 4b) and the highest density of larvae (Fig. 4c). However, when the total of *A. aegypti* larvae preyed by these Bt_i-exposed predators were submitted to analyses of covariance, only the predators that faced intermediate prey densities exhibited significant predatory abilities (Table 2; Fig. 5).

Discussion

Our findings suggest that the *Bt_i*-based insecticide formulations at the recommended rate (25 mg a.i./L) are efficient in controlling *A. aegypti* and can be used in natural aquatic environments due to their safety with the natural predator *B. tarsalis*. For the first time, this study demonstrates that the recommended concentrations of a Bt_i-based insecticide neither reduced the survival of backswimmers nor affected their predatory abilities. However, predators exposed to the organophosphate pirimiphos-methyl above 10 mg/L exhibited reduced survival abilities. Furthermore, when these predators had no time to recover after sublethal exposure (1 mg/L) to pirimiphos-methyl and faced low prey availability, they were less able to prey upon mosquito larvae when compared to unexposed predators.

Although most studies have demonstrated high selectivity of Bt_i against many beneficial aquatic organisms (Boisvert and Boisvert 2000; Lacey and Merritt 2004; Lagadic et al. 2016), field studies have shown that Bt_i can significantly affect non-target insects either by direct toxicity (Davis and Peterson 2008; Painter et al. 1996) or indirectly through starvation (Jakob and Poulin 2016). In our study, the Bt_i-based insecticide did not cause reductions in the survival of males or females of the backswimmer *B. tarsalis* exposed to a concentration recommended to control mosquitoes. However, the exposure of such predators to pirimiphos-methyl at the recommended rates reduced significantly their survival abilities, when compared with the survival abilities of the unexposed predators. These results might be a consequence of different mechanisms between organophosphate- and Bt_i-based insecticides. While organophosphate insecticides such as pirimiphos-methyl are neurotoxic inhibitors of cholinesterases with high toxicity to various aquatic organisms, including mosquitoes' predators (Fukuto 1990; Relyea and Hoverman 2008), the Bt_i toxins act on the midgut cell receptors of insects (Melo et al. 2014) and are considered non-toxic to most aquatic organisms

Table 2 Analyses of covariance for the total number of *Aedes aegypti* larvae preyed by *Buenoa tarsalis* exposed to Bti-based insecticide (25 mg a.i./L) or pirimiphos-methyl (1 mg a.i./L)

Sources of variation	df	Total of preyed larvae	
		F	*P*
Model	11	62.3	<0.0001*
Error	124	–	–
Insecticide (I)	2	3.2	0.0434*
Density (D)	1	622.8	<0.0001*
Recovery time (RT)	1	3.5	0.0461*
I x D	2	1.7	0.19
I x RT	2	1.3	0.28
D x RT	1	2.4	0.13
I x D x RT	2	1.0	0.40

The asterisk indicates significant difference at $P \leq 0.05$

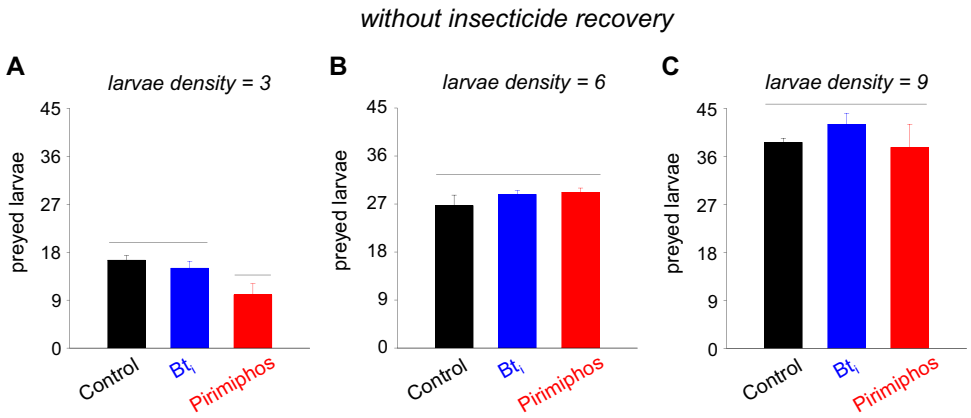

Fig. 3 Total number of *Aedes aegypti* larvae preyed upon by *Buenoa tarsalis* females in a 2 h interval just after 24 h of exposure to pirimiphos-methyl and a Bt_i-based insecticide. The predators' abilities were assessed at larval densities of 3 (**a**), 6 (**b**) and 9 (**c**) larvae/100 mL of water. Larval densities were reestablished after every evaluation. Data are the mean ± SE

(Boisvert and Boisvert 2000; Lacey and Merritt 2004; Lagadic et al. 2014, 2016).

Sublethal effects of insecticides on the behaviors of aquatic insects that prey upon mosquito larvae have been rarely investigated and most studies have focused only on the acute toxicity of the compounds (Relyea and Hoverman 2008; Marina et al. 2014; Halstead et al. 2015; Gutiérrez et al. 2016a, b). In the same way, most studies with backswimmers exposed to Bt_i have mainly evaluated the lethal effects (Purcell 1981; Olejnicek 1986; Aly and Mulla 1987; Quiroz Martinez et al. 1996), and no information is available about possible effects of Bt_i on the behavior of such insects. Recently, food web alterations mediated by Bt_i-based insecticides indirectly affected the abundance of non-target insect predators (Jakob and Poulin 2016). However, as recorded with other insecticides, it is reasonable to think that sublethal exposure to Bt_i might also directly affect the predatory abilities of aquatic predators such as dragonflies (Painter et al. 1996).

Comparatively to the predation of unexposed predators, the sublethal concentrations of both Bt_i and pirimiphos-methyl induced *B. tarsalis* to prey on more mosquitoes when the predators had 24 h of recovery after insecticide exposure, which might be a compensatory response to stress or physiological costs associated with the insecticide detoxification process (Kliot and Ghanim 2012). In addition, Bt_i did not affect the predatory ability of *B. tarsalis* even without any recovery time at all prey densities. As expected, when the predators had not any time to recover from sublethal concentrations of pirimiphos-methyl, their predatory abilities were reduced, demonstrating that neurotoxic compounds can be harmful for non-target insects. However, it is worth noting that in field conditions, the sublethally exposed predators may have the chance of recovery from insecticide exposure, which in certain situations can cause positive responses, as we recorded here for predators that faced sublethal exposure to pirimiphos-methyl or Bt_i preyed, after a recovery time of 24

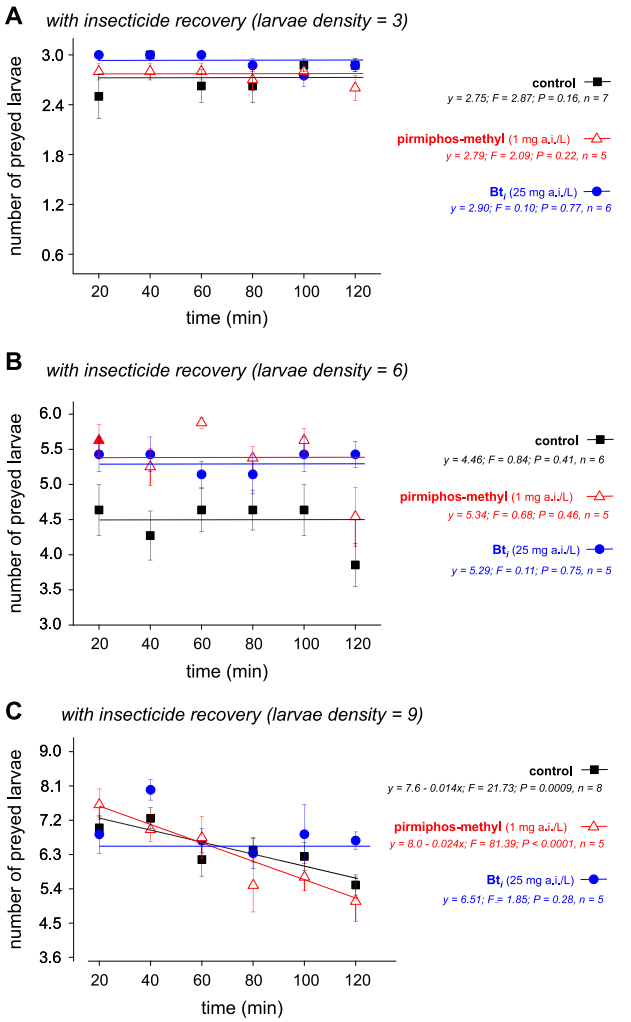

Fig. 4 Impact of a 24-h recovery time on *Buenoa tarsalis* females that were exposed to pirimiphos-methyl and a Bt_i–based insecticide. The predators' abilities were assessed at larval densities of 3 (**a**), 6 (**b**) and 9 (**c**) larvae/100 mL of water. Larval densities were reestablished after every evaluation. Data are the mean ± SE

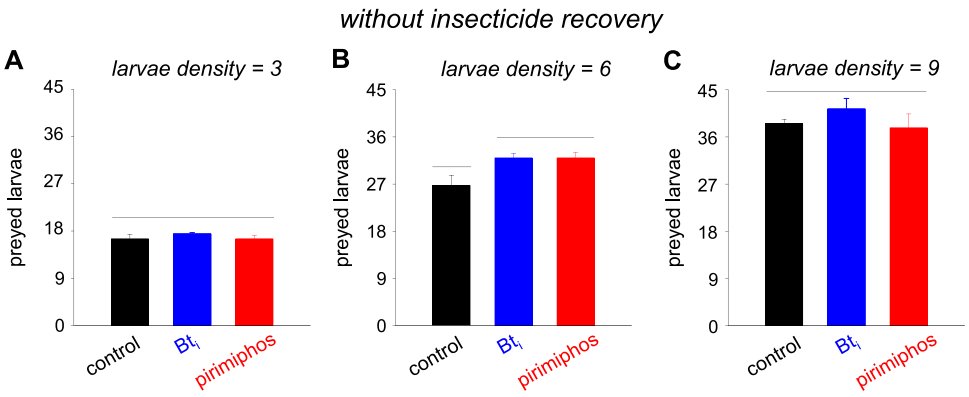

Fig. 5 Total number of *Aedes aegypti* larvae preyed upon by *Buenoa tarsalis* females in a 2 h interval 24 h after the exposure (for 24 h) to pirimiphos-methyl and a Bt_i-based insecticide. The predators' abilities were assessed at larval densities of 3 (**a**), 6 (**b**) and 9 (**c**) larvae/100 mL of water. Larval densities were re-established after every evaluation. Data are the mean ± SE

h, at intermediate prey availabilities. Our results reinforce the idea that the Bt_i effects on non-target insect predators found in field studies are not due to direct effects of Bt_i on the physiology and behavior of these organisms but instead the indirect effects such as changes in food availability as demonstrated elsewhere (Jakob and Poulin 2016).

Synergistic interactions between natural stressors and insecticides (including Bt_i) have been reported in aquatic insects, but the effects are investigated mainly in mosquitoes (Campero et al. 2007; Relyea and Hoverman 2008; Holmstrup et al. 2010; Qin et al. 2011; Janssens and Stoks 2013; Beeck et al. 2016). In this sense, our study is the first to show a direct effect of insecticides on the survival and behavior of backswimmers *B. tarsalis*. The Bt_i-based commercial insecticides seem to be safer for this natural predator of mosquitoes and might be a suitable option for chemical control of mosquitoes in combination with biological control agents. Compounds such as organophosphates should be acknowledged as a potential threat to backswimmers and possibly to other non-target insects in as much as they can directly impair the survival and important behaviors of these insects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation.

Informed consent All the authors of this manuscript accept that the paper is submitted for publication in the Ecotoxicology journal and report that this paper has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

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