BioAssay

BIOLOGICAL CONTROL

Effect of *Bacillus thuringiensis* on the biological characteristics of the predator *Orius insidiosus* Say (Hemiptera: Anthocoridae) feeding on eggs of *Plutella xylostella* L. (Lepidoptera: Plutellidae)

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Efeito de *Bacillus thuringiensis* nas características biológicas do predador *Orius insidiosus* Say (Hemiptera: Anthocoridae) alimentado com ovos de *Plutella xylostella* L. (Lepidopteta: Plutellidae)

RESUMO - A traça-das-crucíferas, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), é considerada uma das pragas mais importantes de brassicáceas (Brassicaceae) em todo o mundo. A praga ocorre todos os anos no Brasil, onde o seu manejo é feito principalmente com controle químico por causa de sua conveniência e rápida ação inicial. O uso indiscriminado de inseticidas afeta organismos não-alvo e tem sido associado com o aumento da resistência de populações de *P. xylostella*. Estas preocupações despertaram o interesse para o uso de outras estratégias de controle, que incluem o uso de microrganismos entomopatogênicos como *Bacillus thuringiensis* Berliner e do predador *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). O objetivo foi avaliar o efeito de *B. thuringiensis* (*B. thuringiensis aizawai* GC 91, Agree®) nas características biológicas de *O. insidiosus*. Os predadores foram alimentados com ovos de *P. xylostella* tratados com água destilada (controle) ou com suspensão de *B. thuringiensis* (0,7 g/0,5 L). Os seguintes aspectos biológicos foram avaliados: duração, sobrevivência e consumo durante o período ninfal; e consumo, número de ovos por fêmea e viabilidade de ovos durante a fase adulta. Os parâmetros de tabela de vida de fertilidade também foram avaliados para os tratamentos com ovos tratados e não tratados com *B. thuringiensis*. Os resultados mostraram que dentre os parâmetros de *O. insidiosus* a duração de ninfas de segundo ínstar, consumo ninfal e longevidade de fêmeas foram afetados pela presença de *B. thuringiensis* nos ovos tratados. Fêmeas alimentadas com ovos tratados mostraram menor número da progênie, que consequentemente levou a uma menor taxa de crescimento populacional.

PALAVRAS-CHAVE – controle biológico, biologia de insetos, controle microbiano

ABSTRACT - The diamondback moth (*Plutella xylostella*) is considered one of the most important pests of cruciferous crops (Brassicaceae) around the world. The moth occurs all year round in Brazil, where it is mainly managed by chemical control because of its convenience and quick onset of action. The indiscriminate use of pesticides affects non-target organisms and has also been associated with increased resistance ratio of *P. xylostella* populations. These concerns have thus sparked interest in the use of alternative control strategies, which include the use of entomopathogenic microorganisms such as *Bacillus thuringiensis* and predators such as *Orius insidiosus*. The aim was to evaluate the effect of *B. thuringiensis* (*B. thuringiensis aizawai* GC 91, Agree®) on the biological characteristics of *O. insidiosus*. The predators were fed eggs of *P. xylostella* treated with distilled water (control) or with a suspension of *B. thuringiensis* at a concentration of 0.7 g/0.5 L. The following biological aspects were evaluated: the duration, survival rate, and consumption of the nymphal period; and the consumption, number of eggs per female, and egg viability for adult insects. Fertility life table parameters were also

evaluated for eggs with and without *B. thuringiensis* treatment. The results showed that *O. insidiosus* parameters, such as duration of the second nymphal instar, nymph consumption, and female longevity were affected by the presence of *B. thuringiensis* in the treated eggs. Females fed with treated eggs showed a lower progeny number, which consequently led to a lower population growth rate.

KEYWORDS - Biological control, Insect biology, Microbial control.

The diamondback moth (*Plutella xylostella*) (L.) (Lepidoptera: Plutellidae) is considered one of the most important pests of cruciferous crops (Brassicaceae) around the world (Castelo Branco & Gatehouse 2001; Sarfraz *et al*. 2011). This pest occurs all year round in Brazil, where it is mainly managed by chemical control because of its convenience, quick onset of action, and effectiveness (Castelo Branco & Amaral 2002; Dias *et al*. 2004). However, the indiscriminate use of pesticides also affects non-target organisms such as beneficial insects, animals, and humans (Goulart *et al*. 2012). The increasing environmental concern, the high cost of pesticides, and an elevated resistance ratio of *P. xylostella* populations have spurred the interest in using entomopathogenic organisms and predators in the biological control of agricultural pests (Carvalho *et al*. 2012).

Bacillus thuringiensis Berliner (*Bt*)-based biopesticides are the most important agents used in the biological control of *P. xylostella* (Zago *et al*. 2014), and predatory bugs of the genus *Orius* (Hemiptera: Anthocoridae) were detected in different crop areas, suggesting that these might be suitable for use in the biological control of *P. xylostella* (Guedes 2006; Brito *et al*. 2009).

Since its discovery in 1911, the entomopathogenic bacteria *B. thuringiensis* has been the most extensively studied microorganism in studies of pathology and microbial control. These bacteria are currently considered as one of the most important insect pathogens used in the control of agricultural pests (Bravo *et al*. 2013). *B. thuringiensis* is pathogenic to Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Isoptera, and Orthoptera. *B. thuringiensis* is already a useful alternative or supplement to synthetic chemical pesticide application in commercial agriculture, for pest management (Schnepf *et al*. 1998).

It is estimated that the genus *Orius* is composed of 75 species that are distributed worldwide, and it includes predators of small arthropods such as thrips, mites, whiteflies, aphids, Lepidoptera eggs, and small caterpillars (Lattin 2000; Studebaker & Kring 2003). Some of the characteristics that make these predators ideal for use in biological control include high efficiency of searching, rapid reproduction, ability to quickly aggregate in the presence of prey, and the capacity to survive with a low density of prey (Bush *et al*. 1993).

Although the harmful effects of bioinsectides on its natural enemies are lower than those of chemical pesticides (Glare & O'Callagham 2000; De Bortoli *et al*. 2012), and in view of the lack of studies on the selectivity of *B. thuringiensis*-based products specifically for the use of *O. insidiosus* (Say) (Hemiptera: Anthocoridae), studies demonstrating the interactions between these biological control agents are necessary to assist in the development of strategies to control *P. xylostella* in the field. Thus, the aim was to evaluate the effect of the commercial product based on *B. thuringiensis* (Agree®) on the biological characteristics of the predator *O. insidiosus*, in laboratory conditions.

Materials and Methods

The study was performed at the Laboratory of Biology and Insect Rearing (LBIR) of the Department of Plant Protection, College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, Sao Paulo, Brazil. The environmental conditions were controlled at a temperature of 25 ± 1ºC, 70 ± 10% relative humidity, and a photoperiod of 12L:12D.

Rearing of *P. xyslostella.* Pupae were obtained from the breeding stock of the LBIR, and following emergence, the adults were released into cages containing an 8-cm disc of kale placed over a moist filter paper disc of the same size. This paper was placed over an overturned transparent plastic cup in such a way that the kale leaf was elevated inside the transparent cage, where egg-laying took place. A 2.3-cm opening made on the receptacle's lid was used to hold a sponge soaked in an aqueous solution containing 10% honey, which was attached to the opening in a small voile sachet. Each cage had a lateral square-shaped opening (10 × 10 cm), which was covered with voile fabric. The discs made of kale leaf, where eggs were laid, were removed from the cages and transferred to Petri dishes until hatching. The caterpillars were then moved to plastic boxes (30 × 15 cm) containing kale leaves that were replenished as required until the larva reached the pupal stage. Pupae were collected using a paintbrush and stored in test tubes (8 × 2 cm), sealed with plastic film with small holes for aeration.

Rearing of *O. insidiosus*. Field collection of *O. insidiosus* adult insects was performed on corn stalks (*Zea mays* L.), beggarticks (*Bidens pilosa* L.), and amaranths (*Amaranthus* sp.) using the tapping method.

The predators were maintained in glass containers (1.7 L) sealed with organza fabric. Every second day, they were fed non-viable eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae). Inflorescences of beggarticks (*Bidens pilosa* L.) treated with 0.12% sodium hypochlorite for 4 min as proposed by Bueno *et al*. (2006) were used as egg-laying substrate. Paper towels were added to the containers to minimize cannibalism and to serve as housing.

Inflorescences of beggarticks containing eggs of the predator were transferred to Petri dishes (15-cm diameter) containing corrugated paper (housing). A cotton ball pre-soaked in distilled water was also added to avoid desiccation and death of eggs and nymphs. Every second day, nymphs were fed eggs of *A. kuehniella*. The Petri dish plates were

sealed with polyethylene film with small holes equipped with a stylus for aeration.

Experimental design. The experiments included two treatments; the first treatment consisted of predators fed with *P. xylostella* eggs soaked in water (control) and the second involved eggs soaked in a *B. thuringiensis* suspension. The concentration of the suspension was as recommended by the manufacturer of the commercial product based *B. thuringiensis* (*B. thuringiensis aizawai* GC 91; Agree®; Biocontrole, Indaiatuba, Sao Paulo, Brazil) for the control of the cabbage moth (0.7 g/0.5 L). As described by the manufacturer, this bioinsecticide contains the toxins Cry1Ac, Cry1C, Cry1D, and Cry2.

Eighty first-instar nymphs obtained from the laboratory stock were placed in Petri dishes (6 × 2 cm) containing a cotton ball (approximately 1 cm^2) pre-soaked in distilled water, a piece of white sulfite paper (0.5 cm^2) to serve as shelter, and *P. xylostella* eggs as food source. Each treatment group was presented daily with 30 up-to-24-h eggs, which were glued to sky blue Bristol boards (0.4 × 2.0 cm). After 24 h, the boards were replaced with new ones and daily consumption was noted. Parameters evaluated included duration, survival rate, and consumption of the nymphal period. When the adult stage was reached, couples were isolated in Petri dishes (6 × 2 cm), with each couple representing a replicate. Each couple was presented daily with 40 eggs placed on sky blue Bristol boards (0.4 × 2.0 cm); after 24 h, the boards were replaced with new ones. Parameters evaluated included consumption, number of eggs per female, and egg viability. The experiments followed a completely randomized design, with 20 replicates per treatment.

Statistical analysis. The results were subjected to tests of normality (Kolmogorov), homogeneity of variance (Bartlett's test), and when required, the data was transformed to meet ANOVA requirements. Nymph viability was analyzed using the chi-square test. All analyses, including the t-test to evaluate significance between treatments, were performed using SAS software (SAS Institute 2002).

The parameters used to generate fertility life tables were based on the data obtained on the biological characteristics of *O. insidiosus* (Birch 1948).

Analysis of fertility life tables and comparison of means were performed using PROC GLM of the SAS Institute (2002), according to Maia *et al.* (2000). The adult survival was compared between treatments using the Kaplan–Meier method (PROC LIFETEST, SAS Institute [2002]).

Results and Discussion

There was no statistical difference in the duration of nymphal stages between treatments, except for the second stage (t_{89} = -2.33; P = 0.0222), which was longer for predators fed with *P. xylostella* eggs soaked in water. However, the nymphal period was similar (t_{89} = 1.48; P = 0.1431), lasting 12.7 and 13.2 days for water- and *Bacillus*-treated eggs, respectively (Table 1). Nymph viability was similar between the treatments (χ^2 = 0.13; P = 0.1175), with values at 66.7% for the control and 63.8% for *B. thuringiensis*-treated eggs. Hafez et al. (1995) found that the nymphal period of *O. albidepennis* (Reuter) (Hemiptera: Anthocoridae) was significantly longer for insects that were fed eggs or larvae treated with β-exotoxin. However, these findings were not considered relevant because β-exotoxin cannot be present in *B. thuringiensis*-based products because of its teratogenic and carcinogenic potential (Glare & O'Callagham 2000).

There were significant differences in consumption in the first (t_{89} = 5.30; P < 0.0001), second (t_{89} = 2.84; P = 0.0055),

Table 1. Duration and consumption *Orius insidiosus* nymphs fed with eggs treated with a suspension of *B. thuringiensis*

Instar		Eggs + Water	Eggs + Suspension
Duration (days)[1]	1st instar	2.0 ± 0.03	2.0 ± 0.08
	2nd	2.1 ± 0.05*	1.9 ± 0.08
	3rd	2.0 ± 0.10	2.0 ± 0.08
	4th	2.2 ± 0.08	2.2 ± 0.09
	5th	4.2 ± 0.10	4.4 ± 0.08
	Nymph period	12.7 ± 0.18	13.2 ± 0.27
Consumption	1st instar	4.0 ± 0.34	8.0 ± 0.61*
	2nd	5.3 ± 0.49	7.5 ± 0.57*
	3rd	8.2 ± 0.70	10.1 ± 0.75
	4th	12.8 ± 0.83	13.7 ± 0.86
	5th	25.7 ± 1.21	30.0 ± 1.19*
	Nymph period	55.6 ± 1.67	69.3 ± 1.72*

[1]Mean ± standard error; * indicates difference, $p < 0.05$

and fifth (t_{89} = 2.53; P = 0.0131) nymphal instars. In terms of the total nymphal period (t_{89} = 5.64; P < 0.0001), nymphs in the control group consumed 55.6 eggs and those in the bacteria-treated group consumed 69.3 eggs (Table 1). Brito *et al*. (2009) reported that nymphs of *O. insidiosus* consumed 86.99 *P. xylostella* eggs. However, no significant difference in consumption was observed for the adult stage (t_{35} = 0.73; P = 0.4705), with consumption values of 184.7 and 198.7 eggs for control and treated groups, respectively (Table 2).

On the basis of the results presented in Table 1, the mortality rates were 36.2% for the *B. thuringiensis*-group and 33.3% for the control group, which indicates that nymph survival was not affected by *B. thuringiensis*. Brito *et al*. (2009) obtained similar mortality values (32%), suggesting that this parameter was intrinsic to the insect and not related to the effect of the study reagent.

However, *B. thuringiensis* affected nymph consumption during the first, second, and fifth instars (Table 1), which was not observed in the adult phase (Table 2), indicating that the *Bt*-based bioinsecticide was not lethal to nymphs and adults of *O. insidiosus*. However, the presence of the microorganism affected the behavior of the predator during its young phase, causing it to increase egg consumption to guarantee survival. The predator recognized that the prey had its quality compromised due to the presence of the pathogen; yet the predator did not reject these eggs because these were the only food source available. As insect development continued and the adult stage was reached, these effects were no longer observed. The recovery of *Bt*-infected insects has been described in the literature (van Frankenhuyzen et al. 2009). However, in some cases, its effects on insect biology persist throughout the remainder of the cycle, as well as in subsequent generation (Polanczyk & Alves 2005).

The complexity of the mechanism of action of *B. thuringiensis* has not been completely elucidated and there have been reports suggesting that this bacterium is not pathogenic to its natural enemies (predators and parasitoids) (Carvalho *et al*. 2012; De Bortoli *et al*. 2012). However, some studies have reported on alteration in the patterns of predation and parasitism due to behavioral changes in its natural enemies (Glare & O´Callagham 2000).

So, Cunha *et al*. (2013) showed that the toxin Cry1Ac induced changes in the apical surface of columnar epithelial cells of the middle intestine of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), which suggests that *B. thuringiensis* can affect both pests and natural enemies. However, the intensity of the effect also depends on the affinity between the toxin and the apical membrane of the columnar cells of the insect's middle intestine. The lethal effect of *B. thuringiensis* on arthropods is complex, depending on several consecutive phases, beginning with dissolution of the crystal following ingestion (Fast and Milne 1979) at adequate pH (Gringorten *et al*. 1992), activation of protoxins (Mohan & Gujar 2003), binding to specific receptors located at the apical villi of the middle intestine (Benfarhat-Touzri *et al*. 2013; Valaitis & Podgwaite 2013), forming pores on these cells, and rupturing the middle intestine structures (Grochulski *et al*. 1995). Finally, germination of the spores ingested along with the crystal(s) initiates a septic process leading to the death of the target organism.

The tolerance to *B. thuringiensis* is associated with changes in the proteolytic process of the toxin(s) (Oppert *et al*. 1997), loss or modification of receptors (Gahan *et al*. 2001; Darboux *et al*. 2002), reversible binding to receptors (Aranda *et al*. 1996), and recovery of damaged epithelial intestinal cells (Forcada *et al*. 1999). Furthermore, Rahman *et al*. (2004) emphasized that melanization of the hemolymph as an immune response mechanism is related to tolerance to *B. thuringiensis*.

Studies on the trophic transfer of toxins showed that *O. insidiosus* acquired 17% of the toxin from its prey *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) (Torres & Ruberson 2008). In this study, the nymphs showed a higher consumption of bacteria-treated eggs and the acquired toxins from its prey were not sufficient to affect the reproductive characteristics of the females, which were similar between the treatments, as shown by the following parameters (Table 2): number of eggs/female (t_{34} = -1.55; P = 0.1312) and egg viability (t_{33} = -0.63; P = 0.5304), corresponding to 128.2 eggs and 92.3% for the control, and 101.8 eggs and 90.7% for bacteria-treated eggs.

Insect eggs are not directly susceptible to *B. thuringiensis*, despite the high mortality compared to that observed in the controls (Ali & Watson, 1982). Larvae of *Boarmia* (*Ascotis*) *selenaria* Denis & Schiffermuller (Lepidoptera: Geometridae) hatched from eggs treated with *Bt kurstaki*, although only 2.2% survived. This mortality can be explained by the fact that the neonate larvae of some species feed on chorion immediately after hatching.

Some fertility life table parameters were affected by

Table 2. Consumption, number of eggs/female, and egg viability of *O. insidiosus* fed on eggs treated with a suspension of *B. thuringiensis*.

	Treatments	
Biological characteristics	**Eggs + water**	**Eggs + suspension**
Consumption/adult	184.7 ± 10.43	198.7 ± 15.20
Eggs/female	128.2 ± 8.63	101.8 ± 13.62
Egg viability (%)	92.3 ± 2.25	90.7 ± 1.22

[1]Mean ± standard error; * indicates difference, p < 0.05

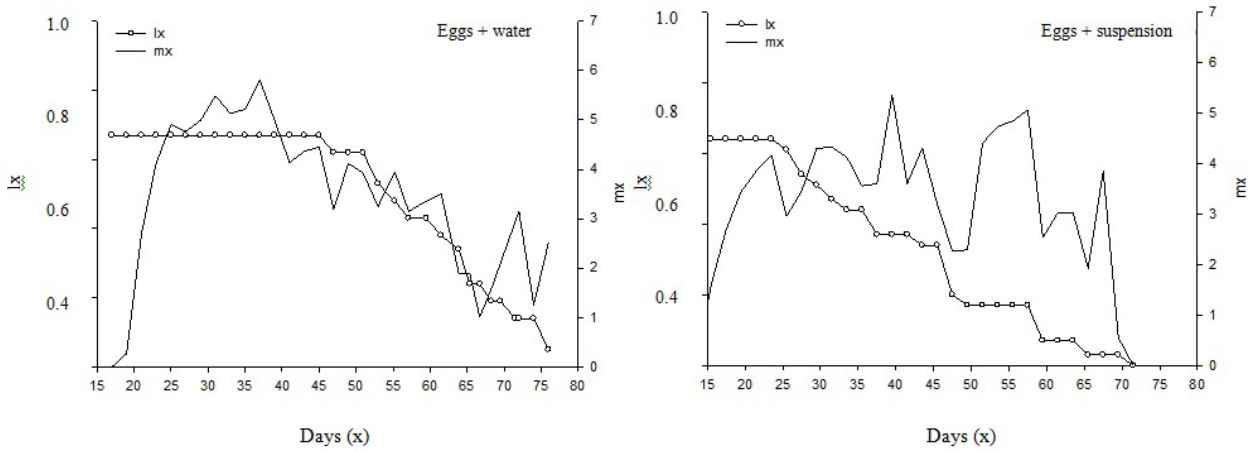

Figure 1. Number of nymphs per female (mx) and survival rate (lx) of *O. insidiosus* fed on eggs of *P. xylostella* immersed in water and immersed in suspension of *B. thuringiensis*.

the presence of bacteria (*Bt*) in the predator's food. The difference in net reproduction rate (R_0) was significant between the treatments, which corresponded to 60.0 for the treatment eggs + water and 35.2 for the treatment eggs + suspension. These findings indicate that the bacteria affect the development and mortality rates of *O. insidiosus* (Table 3). There were also significant differences in mean generation time between the treatments; values were equivalent to 33.7 days for the treatment eggs + water and 27.7 days for the treatment eggs + suspension. The reproduction period of predatory bugs is generally longer in the presence of more adequate prey (Evans, 1982). The contaminated food (egg) or some other inert component of the product may have induced the early development of the natural enemy.

The highest peaks of egg-laying (mx) occurred between 20 and 40 days after birth, when insects fed with eggs + water laid 48% of their eggs. In the case of the treatment egg + suspension, up to 40 days, females laid approximately 52% of their eggs. This can be explained by the fact that to guarantee survival of the progeny, females laid a higher number of eggs during this period once they realized that the feed was not adequate. It is possible that the shorter egg-laying periods observed for eggs treated with water as compared to eggs + *Bt* could favor perpetuation of the species since female longevity was shorter for these treatments. This female behavior of ensuring survival of its descendants was evident when results showed a second increase in the number of eggs between 50 and 60 days for the treatment eggs + suspension (Fig. 1).

Adult survival was affected by different treatments. The egg + suspension treatment showed a drop in survival at day 12, followed by an increase in mortality as the insects aged, maintaining the same behavior until the end of the phase (Fig. 2). Females fed on eggs contaminated with *B. thuringiensis* died earlier. The mean survival of *O. insidiosus* females fed on eggs + water was 52.3 days, whereas those fed on eggs + suspension survived for 34.3 days. In a study with another species of predator, adults of *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) showed a mean survival rate

Table 3. Life table parameters (mean ± CI)[1] *O. insidiosus* fed on eggs of *P. xylostella* dipped in water or suspension of *B. thuringiensis*.

Parameters	Feed	
	eggs + water	eggs + suspension
R_0	60.0 ± 9.26*	35.2 ± 10.55
T	33.7 ± 2.90*	27.7 ± 3.25
r_m	0.121 ± 0.0091	0.129 ± 0.0108
λ	1.129 ± 0.0122	1.137 ± 0.0103
Dt	5.7 ± 0.45	5.4 ± 0.43

* indicates difference ($p < 0.05$).
$R_0=\sum(l_x m_x)$; number of eggs per female per generation, when l_x = mated proportion of females alive at age x ; e m_x = age-specific fecundity multiplied by the sex ratio (0.63 and 0.55 sex ratio for larva+water and larva+suspension). $T=\sum(xl_x m_x)/\sum(l_x m_x)$; $r_m=\text{Ln } R_0/T$; $\lambda=e^{rm}$.

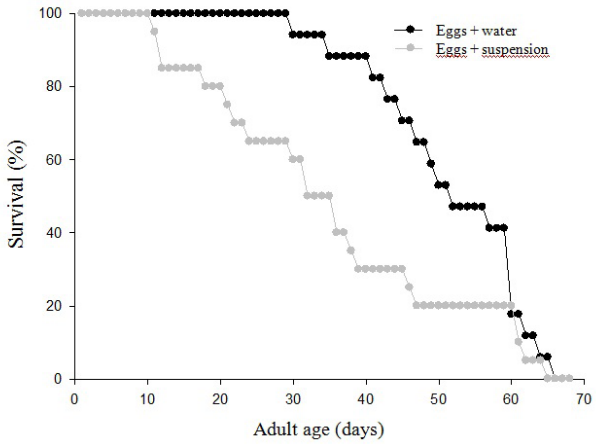

Figura 2. Survival of female *O. insidiosus* reared with *P. xylostella* eggs dipped in suspension of *B. thuringiensis* or water. Significant difference between the survival curves for females by Wilcoxon test ($df = 1$; $\chi^2 = 4.4126$; $P = 0.0357$).

of 89.4% after exposure to *Bt*, which was similar to that observed in the controls at 94.8% (Simões *et al*. 1998). Showing that the survival may or may not be influenced by the *Bt* and that depends of the predator species and *Bt* protein.

The parameters of duration of the second instar, nymph consumption, and female longevity in *O. insidiosus* were affected by the presence of *B. thuringiensis* in the consumed eggs. Females of *O. insidiosus* fed on eggs of *P. xylostella* soaked in *B. thuringiensis* generated a lower number of progeny, which consequently resulted in a decreased population growth rate. So, *B. thuringiensis* affect the biological characteristics of the predator *O. insidiosus* feeding on eggs of *P. xylostella*.

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References

Ali, A.A. & T.F. Watson. 1982. Efficacy of Dipel and *Geocoris punctipes* (Hemiptera: Lygaeidae) against the tobacco budworm (Lepidoptera: Noctuidae) on cotton. J. Econ. Entomol. 75:1002–1004

Aranda, E., J. Sanchez, M. Peferoen, L. Guereca & A. Bravo. 1996. Interactions of *Bacillus thuringiensis* crystal proteins with the midgut epithelial cells of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 68:203–212

Benfarhat-Touzri, D., M. Saadaoui, L. Abdelkefi-Mesrati, I. Saadaoui, H. Azzouz & S. Tounsi. 2013. Histopathological effects and determination of the putative receptor of *Bacillus thuringiensis* Cry1Da toxin in *Spodoptera littoralis* midgut. J. Invertebr. Pathol. 112: 142–145

Birch, L.C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17:15–26

Bravo, A., I. Gomez, H. Porta, B.I. Garcia-Gomez, C. Rodriguez-Almazan, L. Pardo & M. Soberon. 2013. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microb. Biotechnol. 6:17–26

Brito, J.P., A.M. Vacari, R.T. Thuler & S.A. De Bortoli. 2009. Aspectos biológicos de *Orius insidiosus* (Say, 1832) predando ovos de *Plutella xylostella* (L., 1758) e *Anagasta kuehniella* (Zeller, 1879). Arq. Inst. Biol. 76:627–633

Bueno, V.H.P., S.M. Mendes & L.M. Carvalho. 2006. Evaluation of a rearing-method for the predator *Orius insidiosus*. Bull. Insectol. 59:1–6

Bush, L., T.J. Kring & J.R. Rubserson. 1993. Suitability of greenbugs, cotton aphids, and *Heliothis virescens* eggs for the development and reproduction of *Orius insidiosus*. Entomol. Exp. Appl. 67:217–222

Carvalho, V.F.P., A.M. Vacari, A.F. Pomari, C.P. De Bortoli, D.G. Ramalho & S.A. De Bortoli. 2012. Interaction between the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae) and the entomopathogenic bacteria *Bacillus thuringiensis*. Environ. Entomol. 41:1454–1461

Castelo Branco, M. & P.S.T. Amaral. 2002. Inseticidas para o controle da traça-das crucíferas Hortic. Bras. 20:410–415

Castelo Branco, M. & A.G. Gatehouse. 2001. A survey of insecticide susceptibility in *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) in the Federal District, Brazil. Neotrop. Entomol. 30:327–332

Cunha, F.M., L. Wandeley-Teixeira, A.A.C. Teixeira, F.A.B. Santos, L.C. Alves & F.H. Caetano. 2013. Insect/Bt-cotton interactions: are immunological variables and hemocyte ultrastructure in *Podisus nigrispinus* (Hemiptera: Pentatomidae) affected? Int. J. Pest Manag. 59:157–164

Darboux, I., Y. Pauchet, C. Castella, M.H. Silva-Filha, C. Nielsen-Leroux, J.F. Charles & D. Pauron. 2002. Loss of the membrane anchor of the target receptor is a mechanism of bioinsecticide resistance. PNAS 99:5830–5835

De Bortoli, S.A., A.M. Vacari, G.O. Magalhães, W. Dibelli, C.P. De Bortoli & M.P. Alves. 2012. Subdosages of *Bacillus thuringiensis* on *Plutella xylostella* (Lepidoptera: Plutellidae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Rev. Caatinga 25:50–57

Dias, D.G.S., C.M.S. Soares & R.G. Monnerat. 2004. Avaliação de larvicidas de origem microbiana no controle da traça-das-crucíferas em couve-flor. Hort. Bras. 22:553–556

Evans, E.W. 1982. Timing of reproduction by predatory stinkbugs (Hemiptera: Pentatomidae): patterns and consequences for a generalist and a specialist. Ecology 63:147–158

Fast, P.G. & R. Milne. 1979. *Bacillus thuringiensis* parasporal toxin – dissolution of crystals with retention of toxicity. J. Invertebr. Pathol. 34:319–319

Forcada, C.; Alcacer, E.; Garcera, M.D.; Tato, A. & Martinez, R. 1999. Resistance to *Bacillus thuringiensis* Cry1Ac toxin in three strains of *Heliothis virescens*: proteolytic and SEM study of the larval midgut. Arch. Insect Biochem. Physiol. 42:51–63

Gahan, L.J., F. Gould & D.G. Heckel. 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens*. Science 293:857–860

Glare, T.R. & M. O_Callaghan. 2000. *Bacillus thuringiensis*: biology, ecology and safety. Jonh Wiley, Chichester, 350 p

Goulart, R.M., H.X.L. Volpe, A.M. Vacari, R.T. Thuler & S.A. De Bortoli. 2012. Insecticide selectivity to two species of *Trichogramma* in three different hosts, as determined by IOBC/WPRS methodology. Pest Manag. Sci. 68:240–244

Gringorten, J.L., R.E. Milne, P.G. Fast, S.S. Sohi & K. van Frankenhuyzen. 1992. Suppression of *Bacillus thuringiensis* delta-endotoxin activity by low alkaline PH. J. Invertebr. Pathol. 60:47–52

Grochulski, P., L. Masson, S. Borisova, M. Pusztai-Carey, J.L. Schwartz, R. Brousseau & M. Cygler. 1995. *Bacillus thuringiensis* CrylA(a) insecticidal toxin: crystal structure and channel formation. J. Mol. Biol. 254:447–464

Guedes, I.V. 2006. Resposta funcional e numérica do predador *Orius insidiosus* (Say., 1832) (Hemiptera: Anthocoridae) com diferentes presas. 2006. 69 f. Master Degree (Agricultural Entomology), Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, Sao Paulo, Brazil

Hafez, M., S.H. Salama, R. Aboul-Ela, F.N. Zaki & M. Ragaei. 1995. The potential of the predator *Orius albidepennis* on *Agrotis ypsilon* as affected by *Bacillus thuringiensis*. J. Islam. Acad. Sci. 8:79–84

Lattin, J.D. 2000. Economic importance of minute pirate bugs (Anthocoridae). In C.W.S. Schoefer & A.R. Panizzi (eds.), Heteroptera of economic importance. CRC Press, Florida, pp. 607–637

Maia, A.H.N., A.J.B. Luiz & C. Campanhola. 2000. Statistical inference on associated fertility life table parameters using Jackknife technique: computational aspects. J. Econ. Entomol. 93:511–518

Mohan, M. & G.T. Gujar. 2003. Local variation in susceptibility of the diamondback moth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. Crop Prot. 22:3495–504

Oppert. B., K.J. Kramer, R.W. Beeman, D. Johnson & W.H. Mcgaughey. 1997. Proteinase-mediated insect resistance to *Bacillus thuringiensis*. J. Biol. Chem. 272:23473–23476

Polanczyk, R.A. & S.B. Alves. 2005. Interação entre *Bacillus thuringiensis* e outros entomopatógenos no controle de *Spodoptera frugiperda*. Manejo Integr. Plagas Agroecol. 74:24–33

Rahman, M.M., H.L.S. Roberts, M. Sarjan, S. Asgari & O. Schmidt. 2004. Induction and transmission od *Bacillus thuringiensis* tolerance in the flour moth *Ephestia kuehniella*. PNAS 101: 2696–2699.

Sarfraz, R.M., L.M. Dosdall, A.B. Keddie & J.H. Myers. 2011. Larval survival, host plant preferences and developmental responses of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) on wild brassicaceous species. Entomol. Sci. 14:20–30

SAS Institute. 2002. SAS/STAT User`s Guide, version 9.00 TS level 2MO. SAS Institute Inc., Cary, NC

Schnepf, E., N. Crickmore, J. van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler & D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol. Biol. Ver. 62:775–806

Simões, J.C., I. Cruz, & L.O. Salgado. 1998. Seletividade de inseticidas às diferentes fases de desenvolvimento do predador *Doru luteipes* (Scudder) (Dermaptera: Forficulidade). An. Soc. Entomol. Bras. 27:289–294

Studebaker, G.E. & T.J. Kring. 2003. Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae), measured by field, greenhouse and petri dish bioassays. Fla. Entomol. 86:178–185

Torres, J.B. & J.R. Ruberson. 2008. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heretopterans. Transgenic Res. 17:345–354

Valaitis, A.P. & J.D. Podgwaite. 2013. *Bacillus thuringiensis* Cry1A toxin-bilding glicoconjugates present on the brush border membrane and in the peritrophic membrane of the Douglas-fir tussock moth are peritrophins. J. Invertebr. Pathol. 112:1–8

van Frankenhuyzen, K. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J. Invertebr. Pathol. 101:1–16

Zago, H.B., H.A.A. Siqueira, E.J.G. Pereira, M.C. Picanço & R. Barros. 2014. Resistance and behavioural response of *Plutella xylostella* (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* formulations. Pest Manag. Sci. 70:488–495

Available online: www.bioassay.org.br/ojs/index.php/bioassay/article/view/142