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. (Lepidoptera: Plutellidae)

RESUMO - A traça-das-crucíferas, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), é considerada uma das pragas mais importantes de brasiílias (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 ninfa de segundo instar, 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 they 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 bioinsecticides on its natural enemies are lower than those of chemical pesticides (Glaire & O’Callaghan 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 Biological and Insect Rearing (LBIR) of the Department of Plant Protection, College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, São Paulo, Brazil. The environmental conditions were controlled at a temperature of \( 25 ± 1^\circ \)C, \( 70 ± 10\% \) relative humidity, and a photoperiod of 12L:12D.

Rearing of \( P. \) xylostella. 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 cm²) pre-soaked in distilled water, a piece of white sulfite paper (0.5 cm²) 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 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 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 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*₂ = 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 (χ² = 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’Callaghan 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*

<table>
<thead>
<tr>
<th>Instar</th>
<th>Eggs + Water</th>
<th>Eggs + Suspension</th>
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<tbody>
<tr>
<td>1⁰ instar</td>
<td>2.0 ± 0.03</td>
<td>2.0 ± 0.08</td>
</tr>
<tr>
<td>2⁰</td>
<td>2.1 ± 0.05*</td>
<td>1.9 ± 0.08</td>
</tr>
<tr>
<td>3⁰</td>
<td>2.0 ± 0.10</td>
<td>2.0 ± 0.08</td>
</tr>
<tr>
<td>4⁰</td>
<td>2.2 ± 0.08</td>
<td>2.2 ± 0.09</td>
</tr>
<tr>
<td>5⁰</td>
<td>4.2 ± 0.10</td>
<td>4.4 ± 0.08</td>
</tr>
<tr>
<td>Nymph period</td>
<td>12.7 ± 0.18</td>
<td>13.2 ± 0.27</td>
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<thead>
<tr>
<th>Consumption</th>
<th>Eggs + Water</th>
<th>Eggs + Suspension</th>
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<tbody>
<tr>
<td>1⁰ instar</td>
<td>4.0 ± 0.34</td>
<td>8.0 ± 0.61*</td>
</tr>
<tr>
<td>2⁰</td>
<td>5.3 ± 0.49</td>
<td>7.5 ± 0.57*</td>
</tr>
<tr>
<td>3⁰</td>
<td>8.2 ± 0.70</td>
<td>10.1 ± 0.75</td>
</tr>
<tr>
<td>4⁰</td>
<td>12.8 ± 0.83</td>
<td>13.7 ± 0.86</td>
</tr>
<tr>
<td>5⁰</td>
<td>25.7 ± 1.21</td>
<td>30.0 ± 1.19*</td>
</tr>
<tr>
<td>Nymph period</td>
<td>55.6 ± 1.67</td>
<td>69.3 ± 1.72*</td>
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¹Mean ± standard error, * indicates difference, *p* < 0.05
and fifth ($t_{n5} = 2.53; P = 0.0131$) nymphal instars. In terms of the total nymphal period ($t_{n} = 5.64; P < 0.0001$), 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 ($t_{a} = 0.73; P = 0.4705$), 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 Frankenhuysen 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’Callaghan 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 Frankinliella 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 ($t_{n} = -1.55; P = 0.1312$) and egg viability ($t_{a} = -0.63; P = 0.5304$), 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 (Ascoitis) seleanaria 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>
<thead>
<tr>
<th>Table 2. Consumption, number of eggs/female, and egg viability of O. insidiosus fed on eggs treated with a suspension of B. thuringiensis.</th>
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<tr>
<td><strong>Treatments</strong></td>
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<tr>
<td>Biological characteristics</td>
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<tr>
<td>Consumption/adult</td>
</tr>
<tr>
<td>Eggs/female</td>
</tr>
<tr>
<td>Egg viability (%)</td>
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</table>

1 Mean ± standard error; * indicates difference, $p < 0.05$
the presence of bacteria (Bt) in the predator’s food. The difference in net reproduction rate (R0) 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) of O. insidiosus fed on eggs of P. xylostella dipped in water or suspension of B. thuringiensis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Feed</th>
<th>eggs + water</th>
<th>eggs + suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0</td>
<td>60.0 ± 9.26*</td>
<td>35.2 ± 10.55</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>33.7 ± 2.90*</td>
<td>27.7 ± 3.25</td>
<td></td>
</tr>
<tr>
<td>rm</td>
<td>0.121 ± 0.0091</td>
<td>0.129 ± 0.0108</td>
<td></td>
</tr>
<tr>
<td>λ</td>
<td>1.129 ± 0.0122</td>
<td>1.137 ± 0.0103</td>
<td></td>
</tr>
<tr>
<td>Dt</td>
<td>5.7 ± 0.45</td>
<td>5.4 ± 0.43</td>
<td></td>
</tr>
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</table>

* indicates difference (p < 0.05).

R0 = Σ(lmx); number of eggs per female per generation, when l = mated proportion of females alive at age x; e = m = age-specific fecundity multiplied by the sex ratio (0.63 and 0.55 sex ratio for larva+water and larva+suspension), T = Σ(xlmx) / Σ(lmx); rm = Ln R0 / T; λ = e^rm.

**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.

**Figure 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; χ^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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