Notas Científicas

Induced-feeding bioassays for detection of *Bacillus thuringiensis* insecticidal activity against *Epilachna paenulata* (Coleoptera)

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Abstract – The objective of this work was to establish and test the induced-feeding bioassay in order to detect *Bacillus thuringiensis* insecticidal activity against *Epilachna paenulata* (Coleoptera: Coccinellidae). Larvae were induced to swallow high concentrations of spore-crystal suspensions of seven exotic and 30 Argentine *B. thuringiensis* strains. The great majority of strains showed no toxicity *to E. paenulata larvae*, and observed mortality was lower than 30%. Induced-feeding bioassay is feasible, and should be used for prospecting strains that produce right combinations of Cry proteins needed to an efficient pest control.

Index terms: biological control, Coccinellidae, Cucurbitaceae, ladybird beetle, spore-crystal toxicity.

Bioensaios de alimentação induzida para determinar a atividade inseticida de *Bacillus thuringiensis* contra *Epilachna paenulata* (Coleoptera)

Resumo – O objetivo deste trabalho foi estabelecer e testar o bioensaio de alimentação induzida para a determinação da atividade inseticida de *Bacillus thuringiensis* contra larvas de *Epilachna paenulata* (Coleoptera: Coccinellidae). As larvas foram induzidas a ingerir concentrações elevadas de suspensões esporo-cristal de *7* cepas exóticas e 30 argentinas de *B. thuringiensis*. A maioria das cepas não apresentou toxicidade a larvas de *E. paenulata*, com mortalidade inferior a 30%. O bioensaio de alimentação induzida se mostrou efetivo, e pode ser usado para identificar cepas que produzam combinações adequadas de proteínas Cry, necessárias para o controle de pragas.

Termos para indexação: controle biológico, Coccinellidae, Cucurbitaceae, joaninha, toxicidade esporo-cristal.

Ladybird beetle [*Epilachna paenulata* Germar, (Coleoptera: Coccinellidae)] is a pest of cucurbits, such as pumpkin. Larvae and adults of *E. paenulata* eat cucurbit leaf tissue, causing plants to become completely stripped of leaves and to show shallow holes on the fruit surface. Currently, the most common method for controlling this pest relies on the use of synthetic insecticides, in spite of the damage they may cause to non-target organisms and to the environment.

Bioinsecticides are viable alternatives for insect control in agriculture and, among them, *Bacillus thuringiensis* Berliner is the most widely used. This bacterium is characterized by the production, during sporulation, of parasporal crystals composed of insecticidal proteins (Cry), which are biodegradable, highly specific to target insects, and safe for humans, other vertebrates, and for plants (Crickmore, 2006; Sauka & Benintende, 2008). Additionally, the proteinaceous toxins produced by this bacterium are widely used in genetically modification of plants in order to obtain insect-resistant varieties (Sauka & Benintende, 2008).

Information regarding the susceptibility of *Epilachna* spp. to *B. thuringiensis* is very limited. A single work (Peña et al., 2006) reported the toxicity of a Mexican strain to *E. varivestis*, determined by the leaf dip technique. Since the toxicity levels of *B. thuringiensis* to coccinellids are low or null (McManus et al., 2005; Bozsik, 2006), the present work was focused on establishing and testing a novel type of bioassay, where larvae are induced to swallow high concentrations of spore-crystal suspensions, for screening the toxicity of *B. thuringiensis* to *E. paenulata*. These bioassays were called induced-feeding bioassays.

Thirty monosporic *B. thuringiensis* isolates collected from leaves, stored product dust, soils, dead insect larvae and spider webs from different ecological regions of Argentina were selected from the Bacterial Collection of Instituto de Microbiología y Zoología Agrícola of Instituto Nacional de Tecnología Agropecuaria. Twin strains were previously discarded to avoid overestimated distribution of frequencies, using sodium dodecyl sulphate–polyacrylamide gels and PCR (Sauka et al., 2006).

Reference strains *B. thuringiensis* svar *tenebrionis* DSM2803 and svar *israelensis* IPS-82 were supplied by the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Mexico; *B. thuringiensis* svar *kurstaki* HD-1 and HD-73, by the Agricultural Research Service, Peoria, USA; and *B. thuringiensis* svar *kumamotoensis* HD-867, *B. sphaericus* 1593 and 2362, by the Bacillus Genetic Stock Center, Columbus, USA. All the strains were cultured in 100 mL of BM medium (Benintende & Cozzi, 1996), until complete autolysis was observed at 340 rpm and 30°C. Spore-crystal complexes were obtained by centrifugation for 15 min at 12,000 *g* and 4°C, and pellets were freeze-dried. Powders of spore-crystal complexes were kept at -20°C until further use.

Collections of *E. paenulata* from highly infested areas of Buenos Aires and Mendoza provinces, Argentina, were used to start a colony under insectary conditions. *Epilachna paenulata* were continuously laboratory-reared on a natural diet of pumpkin (*Cucurbita maxima* Duchesne) leaves, maintained in a growth chamber at 25±1°C, 70–75% relative humidity and a photoperiod of 16:8 light-dark cycle.

Susceptibility of second-instar *E. paenulata* larvae to *B. thuringiensis* isolates and reference strains was tested under laboratory conditions using induce-feeding bioassays. *Epilachna paenulata* larvae were individually placed without food during 24 h in a well from a 24-well plate (Nunc 143982). Bioassays were performed using highly concentrated spore-crystal suspensions (1 mg mL^{-1}), homogenized into a 20% sucrose solution, used as a feeding inductor, and dispersed in 2 µL droplets on a small piece of Parafilm (Menasha, WI, USA). Thirty larvae were tested for each spore-crystal suspension. Each starved larva was allowed to feed one complete droplet and then transferred to a plastic box containing pumpkin leaves. Only a 20% sucrose solution was fed to the control larvae. At least two replicates were performed on different days for each spore-crystal suspension. Mortality percentages were registered after 72 h at 25±1°C, 70–75% relative humidity, and a photoperiod of 16:8 (light:dark). Data were corrected for control mortality using Abbott (1925) correction factor.

After a reliable bioassay technique was established, the corrected means of mortality percentage and their standard errors indicated that the great majority of the microorganisms showed no toxicity against *E. paenulata* larvae (Table 1), which proved themselves to be only slightly susceptible to some *B. thuringiensis* toxins. None of the exotic or native strains caused mortality higher than 30%, despite the highly concentrated spore-crystal suspensions tested. This

Table 1. Toxic activity of *Bacillus thuringiensis* spore-crystal complexes against second-instar *Epilachna paenulata* larvae.

Microorganism	Target[1]	Mortality[2] (%)
Bacillus thuringiensis		
svar *kurstaki* HD-1	L and D	0
svar *kurstaki* HD-73	L	0
svar *israelensis* IPS-82	D	0
svar *tenebrionis* DSM2803	C	25.0±5.0
svar *kumamotoensis* HD-867	C	0
INTA 7-3	L and D	0
INTA Mo1-12	L and D	0
INTA Mo5-8	L and D	0
INTA Mo32-3	L and D	0
INTA TA24-6	L and D	0
INTA H39-19	D	0
INTA H41-1	D	20.0±0.0
INTA Mo4-4	Neither L nor D	25.0±5.0
INTA Mo14-4	Neither L nor D	0
INTA Mo21-1	Neither L nor D	0
INTA Pol49-1	Neither L nor D	30.0±0.0
INTA Fr7-4	Neither L nor D	15.0±5.0
INTA Fr8-1	Neither L nor D	10.0±0.0
INTA 33-5	Neither L nor D	10.0±0.0
INTA 50-6	Neither L nor D	10.0±0.0
INTA 51-3	Neither L nor D	10.0±0.0
INTA 77-10	Neither L nor D	0
INTA 103-23	Neither L nor D	20.0±0.0
INTA H2-1	Neither L nor D	20.0±0.0
INTA H6-2	Neither L nor D	20.0±0.0
INTA H7-2	Neither L nor D	0
INTA H11-2	Neither L nor D	0
INTA H13-2	Neither L nor D	15.0±5.0
INTA H22-2	Neither L nor D	0
INTA H25-2	Neither L nor D	0
INTA H27-3	Neither L nor D	10.0±0.0
INTA H46-3	Neither L nor D	5.0±5.0
INTA H47-3	Neither L nor D	10.0±0.0
INTA H48-12	Neither L nor D	0
INTA TA1-2	Neither L nor D	10.0±0.0
Bacillus sphaericus		
1593	D	0
2362	D	0

[1]C, Coleoptera; D, Diptera; L, Lepidoptera. [2]Mean±SD.

result agrees with previous studies that showed that the toxicity levels of *B. thuringiensis* to coccinellids are relatively low or null (McManus et al., 2005; Bozsik, 2006; Peña et al., 2006).

Bacillus thuringiensis INTA H41-1 strain, native to Argentina, showed 20.0% mortality. Characterization of this strain in a parallel work indicated that it is very similar to *B. thuringiensis* svar *israelensis* IPS-82 in terms of protein composition, morphology of the parasporal crystal, plasmid patterns and mosquitocidal activity. Strains belonging to svar *israelensis* have previously shown toxicity to other Coleoptera species (Méndez-López et al., 2003). This result may represent the first step along the way to the identification of the Cry proteins or other virulence factors involved in the insecticidal activity of this strain for *E. paenulata*.

This study is the first report of an induced-feeding bioassay where the toxic activity of *B. thuringiensis* strains to *E. paenulata* is screened. Although the susceptibility of *E. paenulata* larvae to *B. thuringiensis* did not prove to be high, this method is feasible, and may be used for prospecting strains with the right combination of Cry proteins needed to control this or other pests.

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