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Safety evaluation of the entomopathogenic bacterium *Brevibacillus laterosporus* for the green lacewing *Chrysoperla agilis* (Neuroptera: Chrysopidae)

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Abstract

The safety of the entomopathogenic bacterium *Brevibacillus laterosporus* for the natural insect predator *Chrysoperla agilis* was evaluated in this study. For this purpose, laboratory bioassays were conducted exposing different larval instars and adults of the chrysopid to bacterial spore preparations, in order to evaluate the possible effects on survival, longevity, immature development, and adult reproductive performance. The sub-lethal effects were investigated by feeding the bacterium directly to adults and larvae of *C. agilis* or to mealworm beetles (*Tenebrio molitor*) used as hosts for chrysopids (tritrophic interaction). Direct feeding of *B. laterosporus* spores to different lacewing larvae instars and to adults did not cause mean mortality levels significantly different from untreated control, and slight though not significant effects of treatments were generally observed on insect longevity, development, fecundity and egg hatching. In the case of lacewing larvae feeding on treated mealworm beetles, adult emergence percentage was reduced approximately 12 %, in comparison with untreated control. Based on these results, the use of *B. laterosporus* for pest management in the agroecosystem, appears to be compatible with chrysopids.

Key words: bioinsecticide; side-effects; sub-lethal effects; pest management; bacteria.

Introduction

The need to identify effective and eco-sustainable active substances for the management of crop pests is leading to the discovery of new species and strains of entomopathogenic bacteria, fungi, virus, microsporidia, and nematodes, with novel bioinsecticidal properties and target range (Ruiu, 2018). On the other hand, for a more complete evaluation of a new entomopathogen and its potential, effects in the agricultural ecosystem should be considered in the broadest sense. This involves the evaluation of the possible side-effects against non-target organisms, including natural predators and parasites (Lacey et al., 2001).

Brevibacillus laterosporus is a bacterial species represented by several strains exhibiting varying levels of pathogenicity against a variety of insect targets in different orders, including Coleoptera, Lepidoptera, and Diptera (Ruiu, 2013). The insecticidal action is mostly related to the production of diverse toxins, most of which act in the insect gut after ingestion (Marche et al., 2018; Glare et al., 2019).

While future applications of this microorganism and its products in agriculture appear promising, knowledge about the possible effects on non-target organisms is still limited. Previous studies have demonstrated a slight susceptibility of the muscoid fly parasitoid wasp *Muscidifurax raptor* Girault and Sanders (Hymenoptera: Pteromalidae) exposed to higher doses than those active against target pests (Ruiu et al., 2007), but no information on the possible effects on other beneficial insects is available.

Chrysopid species are primary predators against several plant pests including aphids, lepidopterans, mites, thrips, and whiteflies (Principi and Canard, 1984, Senior and McEwen, 2001). In addition to predation by natural populations, different species belonging to the *Chrysoperla carnea* group have been employed successfully in pest management programs that involve augmentation releases in the field (Pappas et al., 2011). Appropriate integrated pest management strategies should ensure protection and enhancement of their populations in the agricultural ecosystem (Cordeiro et al., 2010) and the safety of plant protection products, including entomopathogens, for chrysopids is therefore

59 necessary. While several studies considering the side-effects of several chemically active substances
60 against these beneficial insects have been conducted (Michaud and Grant, 2003), knowledge about
61 the possible non-target toxicity or pathogenicity associated with entomopathogenic microbials is still
62 limited. On the other hand, some studies have been dedicated to evaluating the safety of some insect
63 pathogens for chrysopids, including fungi, for example, *Beauveria bassiana* (Donegan and Lighthart,
64 1989) and *Metharizium anisopliae* (Ríos-Moreno et al., 2018), and bacteria, including *Bacillus*
65 *thuringiensis* (Rodrigo-Simón et al., 2006; Lövei et al., 2009). No information on susceptibility of
66 chrysopids to *B. laterosporus* is available.
67 *Chrysoperla agilis* Henry et al. (Neuroptera: Chrysopidae) is a natural biocontrol agent belonging to
68 the *Chrysoperla carnea* group. *C. agilis* opulations are distributed in Western Europe, overlapping
69 with other lacewing species within the same phylogenetic group, including *C. carnea sensu stricto*
70 (Stephens), *Chrysoperla lucasina* (Lacroix), *Chrysoperla mediterranea* (Hölzel) and *Chrysoperla*
71 *pallida* Henry et al. (Noh and Henry, 2010). Because of its wide distribution in agricultural
72 ecosystems and a broad range of hosts, the predatory role of *C. agilis* in the bio-containment of pests
73 in agriculture is considered significant (Pappas et al., 2013).
74 The purpose of this study was to evaluate the susceptibility of *C. agilis* to a *B. laterosporus* strain that
75 shows significant pathogenicity against different insect pests. In particular, our work investigated the
76 lethal and sublethal effects (development of immatures, adult emergence and reproductive
77 performance) in lacewings exposed to a diet containing bacterial spores. To evaluate possible indirect
78 effects on *C. agilis* larvae feeding on insect prey previously exposed to *B. laterosporus*, the tritrophic
79 interaction of host-predator-bacterium was also considered.
80
81 **2 Materials and methods**
82 **2.1 Bacterial preparations**
83 Entomopathogenic *B. laterosporus* strain UNISS 18 (= NCIMB 41419) was selected for this work
84 because of its well documented insect pathogenic properties (Marche et al., 2018). Spore

85 suspensions used in bioassays were routinely produced by cultivation in LB broth, shaken at 180
86 rpm at 30 °C for 48-72 h to achieve culture sporulation.
87 For this purpose, a pre-culture (25 ml) was inoculated with a heat-activated spore suspension (1 ml),
88 and then used to inoculate a second culture in sporulation medium T3, as described elsewhere
89 (Marche et al., 2017). Spores were harvested by centrifugation at 15,000 x g at 4° C for 15 minutes
90 and resuspended in water to achieve the concentration needed in bioassays.
91
92 **2.2 Insect bioassays**
93 All insect bioassays were conducted in a climatic room at 25° C with a photoperiod of L16:D8.
94 Lacewings used in bioassays were provided by the insect rearing facility of the University of
95 Sassari where a colony of *C. agilis* established in 2016 from field-collected individuals is
96 maintained according to methods described by Pasqualini (1975) with some adaptations (Loru et al.,
97 2013). *Tenebrio molitor* L. (Coleoptera*:* Tenebrionidae) larvae (mealworm beetles) and *Musca*
98 *domestica* L. (Diptera: Muscidae) adults employed in bioassays were furnished by the same insect
99 facility.
100
101 **2.2.1 Toxicity bioassays**
102 Ingestion assays were conducted exposing lacewing adults and larvae to bacterial suspension drops
103 because it represents the way they may contact a bioinsecticide sprayed on plants against pests.
104 *C. agilis* larvae of each instar (1^{st}, 2^{nd}, and 3^{rd}), immediately after moult, were placed individually
105 into plastic jars (2 cm diameter x 3 cm high) where a 4-µl drop of a 20% fructose solution
106 incorporating *B. laterosporus* spores (10^9 spores/ml) was administered daily for 5 d. From the sixth
107 day on, larvae of *T. molitor* (1 larva per day) were provided to lacewing larvae as food. Mortality
108 was assessed daily for 10 d.
109 Newly emerged male and female *C. agilis* adults were maintained individually in plastic jars (2 cm
110 diameter and 3 cm high) and exposed daily to a 4-µl drop of a 20% fructose solution incorporating

111 *B. laterosporus* spores (10^9 spores/ml). Honeybee pollen was provided *ad libitum* to each adult
112 beginning on the sixth day and insect viability was verified daily for 10 d. These experiments
113 included 50-60 individual *C. agilis* adults and larvae.
114 Experiments also were conducted on *M. domestica* adults in order to verify the pathogenicity of the
115 spore suspensions used in experiments with lacewings. Newly emerged *M. domestica* adults were
116 grouped into four groups of 10 individuals per cage (10 x 10 x10 cm) and exposed to drops (10
117 µl/fly/day) of a 30% saccharose solution containing *B. laterosporus* spores (10^9 spores/ml) from the
118 same spore culture used in the lacewing experiments. Insect mortality was checked daily for 7 d,
119 comparing treated flies and untreated controls (Mura and Ruiu, 2017).
120
121 **2.2.2 Bioassays testing for sublethal effects**
122 Lacewing larvae and adults were challenged for 5 d with *B. laterosporus* spores as previously
123 described in the toxicity bioassays. Surviving individuals were maintained in the laboratory to
124 investigate the possible sub-lethal effects.
125 To test immature stages, second and third instar larvae surviving treatments (n = 20-30), were
126 maintained individually in jars, fed on *T. molitor* larvae (1 larva/individual/day), through pupation
127 and adult emergence. Treated and control insects were checked daily, and the dates of larval moult,
128 pupation, adult emergence, and death during the bioassay, were recorded.
129 Individual adult insects surviving *B. laterosporus* challenge (n = 20) were transferred into larger
130 plastic cages (10 cm diameter x 10 cm high) with two sides covered with gauze to allow ventilation.
131 Treated and control females were paired with treated and control males (1 pair per cage),
132 respectively, to allow mating and oviposition. The inner surface of the cage was covered with paper
133 on which females could lay their eggs, which were removed and counted daily. During this period,
134 lacewing adults in the cage were provided *ad libitum* with honeybee pollen and a piece of cotton
135 soaked in water. Death of each male or female individual in a cage was recorded during the
136 bioassay to assess insect longevity. A group of eggs (n=10) from each control and treated cages was

analysed to evaluate hatching rate. For this purpose, eggs were kept individually in a jar for a week up to hatching. This analysis was repeated 5 times during the bioassay with different cohorts of eggs.

2.2.3 Tritrophic bioassays

In this experiment, mealworm beetles were used to evaluate the possible indirect effects on *C. agilis* larvae feeding on an insect prey previously exposed to *B. laterosporus*. Mealworm beetles were maintained for minimum 1 week on sterile dry wheat bran without a source of water to encourage their subsequent feeding on a 2-cm diameter ball of bran moistened with a *B. laterosporus* suspension (10^9 spores/ml) or water (untreated control), replaced daily. Treated mealworm beetles were maintained under these conditions for at least 3 d before being administered to lacewing larvae.

Second instar *C. agilis* larvae (n = 30) were maintained individually in plastic jars and fed treated or untreated (control) mealworm beetles, replaced daily (1 larva/individual/day) for 7 days. From the eighth day on, larvae were fed on untreated mealworm beetles and were inspected daily to assess mortality, date of moult, pupation, and adult emergence. Pupal weight was also recorded.

2.3 Statistical analysis

Data were analysed using R software (R Development Core Team, 2016).

We used *t*-tests to compare data means of treated and control groups of different experiments. General Linear Models (GLM) of ANOVA, followed by Least Significant Difference (LSD) tests for post-hoc comparison of means when needed, were used to analyse data on immature (larval and pupal) development time and percentage of adult emergence in experiments involving direct feeding of *B. laterosporus* to either second or third instar lacewing larvae.

3. Results

163 **3.1 Toxicity bioassays**

164 Direct feeding by different lacewing larvae instars and adults for 5 d on a fructose solution
165 containing *B. laterosporus* spores did not result in mean mortality levels that were significantly
166 different from untreated controls ($F_{7,33} = 0.53$; $P = 0.8091$). Larval mortality ranged on average
167 between 7 and 10% for treated insects and between 3 and 6% for the controls, while mean adult
168 mortality was 9–13%, for both treated and control insects (Fig. 1).
169 The insecticidal properties of the spore suspensions were confirmed in bioassays with *M.*
170 *domestica*, producing 100% adult mortality within 5 d in comparison with the control mortality of
171 approximately 5% ($t = 39.19$; $df = 4$; $P < 0.001$).
172
173 **3.2 Sublethal bioassays**
174 The sublethal effects on lacewings that survived exposure to *B. laterosporus* for 5 d during the second
175 or third larval instar are shown in Table 1. Larval development from egg hatch to pupation was not
176 affected by treatment in comparison with control ($F_{3,59} = 0.24$; $P = 0.8699$). Pupal development was
177 significantly influenced by exposure of second instar larvae to *B. laterosporus* ($F_{3,59} = 5.04$; $P =$
178 0.0037). An average reduction in pupal development of approximately 1 d was observed (Treatment:
179 $F_{1,59} = 6.00$; $P = 0.0176$; interaction Instar x Treatment: $F_{1,59} = 9.11$; $P = 0.0039$). Adult emergence
180 of treated insects did not differ from control ($F_{3,12} = 0.12$; $P = 0.9458$).
181 Viability and reproductive performance of lacewing *C. agilis* adults fed *B. laerosporus* spores
182 incorporated in a fructose solution are shown in Table 2. Exposure to the bacterium did not cause
183 significant changes in the longevity of either sex (males: $t = 1.088$; $df = 30$; $P = 0.1426$); females: $t =$
184 -0.56; df = 24; P = 0.2900). Treated lacewing females that survived exposure to the bacterium
185 exhibited fecundity levels comparable with control ($t = 0.939$; $df = 15$; $P = 0.1955$). Similarly, no
186 differences in egg hatch rate were observed in treated and control groups ($t = 0.138$; $df = 10$; $P =$
187 0.4466).
188

3.3 Tritrophic bioassays

The sub-lethal effects on immature lacewing development after second instar larvae were exposed to mealworm beetles treated with *B. laterosporus* are reported in Table 3. Treatment did not result in significant changes in development time of second ($t = 0.535$; $df = 23$; $P = 0.2988$) and third ($t = 0.713$; $df = 20$; $P = 0.2421$) instar larvae, nor were there differences in pupal weight ($t = -1.141$; $df = 17$; $P = 0.1349$). A slight though not significant slowing of pupal development time was observed in treated lacewings ($t = -1.637$; $df = 11$; $P = 0.0649$). Treatment of larvae reduced the average percentage of adult emergence by approximately 12% in comparison with the untreated control ($t = 1.964$; $df = 6$; $P = 0.0400$).

Discussion

Brevibacillus laterosporus showed only slight effects on *C. agilis* larvae and adults directly exposed to bacterial spores at concentrations that cause high mortality in Diptera (Lethal Concentration$_{100}$) (Ruiu et al., 2007a; Marche et al., 2017). The bioassay methods used in this study involved the administration of spores incorporated in a fructose solution to simulate the way in which chrysopids would contact *B. laterosporus* as an insecticide sprayed in the field. Additionally, the continuous exposure to bacterial drops in the laboratory can be considered an extreme condition, given that natural feeding behavior of *C. agilis* larvae and adults may significantly reduce possible contacts with a bioinsecticide applied in the field. Chrysopid adults typically feed on plant resources such as pollen, nectar, and honeydew, so could be attracted by sugary baits, but the insecticidal formulations might be less attractive for these non-target insects (Wang et al., 2011). In addition to food preferences, contact between non-target insects and the active ingredient in the field may be affected by specific behaviors related to repellency or, more generally, to avoidance (Cordeiro et al., 2010).

Lacewing larvae feed on insect prey, which may pose an additional barrier to direct contact between the beneficial insect and the entomopathogen. When *C. agilis* larvae were offered mealworm beetles treated with *B. laterosporus* as food, no significant effects on development until pupation were

observed. However, slight changes in adult emergence rate were detected, suggesting a possible indirect effect of treated mealworms, due to the bacterium or, possibly due to deteriorated quality of the treated prey as food (Eubanks and Denno, 2000). Previous laboratory studies showed that development and survival of beneficial arthropods feeding on hosts that were pre-fed with a diet containing entomopathogenic bacteria like *Bacillus thuringiensis* could be affected under specific conditions (Blumberg et al., 1997; Ruiu, et al., 2007b; Salama et al., 1991; Sterk et al., 1999).

The general safety of *B. laterosporus* for *C. agilis* we recorded for this study is in line with results of several investigations conducted with *B. thuringiensis* and its insecticidal toxins against different chrysopid species. Given its highly specific mode of action, crystal toxin Cry1Ab from *B. thuringiensis* was found to have no direct effects on *C. carnea* after ingestion (Romeis et al., 2004), which is corroborated with no detected interaction of Cry toxins with midgut receptors of this non-target species (Rodrigo-Simón et al., 2006). On the other hand, significant differences between treated and control insects were observed in different laboratory conditions (Hilbeck et al., 1998). Similarly, several laboratory experiments assessing the safety of genetically modified (GM) plants, mostly expressing Cry proteins from *Bt*, for beneficial arthropods, have led to variable and sometimes conflicting results, probably in relation to the use of different non-target species and experimental conditions (Lövei et al., 2009). However, such effects observed in the laboratory are expected to be attenuated in natural field conditions (Bourguet et al., 2002).

The entomopathogenic action of bacteria on their targets is often complex and involves the combined effect of several toxins and virulence factors (Glare et al., 2019). Accordingly, the pathogenicity of *B. laterosporus* against invertebrate pests may rely on a wide range of molecules including enzymes (i.e., chitinases, proteases), insecticidal toxins homologous to Cry proteins, polyketides, and nonribosomal peptides (Glare et al., 2019). The insecticidal potential of the *B. laterosporus* strain employed in this study and the range of toxins and virulence factors it can express are well documented (Marche et al., 2018). The lack of toxicity and only slight sublethal effects we observed on *C. agilis* fed extreme spore concentrations support the compatibility of *B. laterosporus* with this

non-target species. According to our findings, the use of this bacterium in the pest management context appears promising.

It is not fully understood how *B. laterosporus* specifically evolved as a pathogen for certain insect species, while it is weakly active or inactive in others (Ruiu et al., 2007b). Among beneficial insects, microbiome studies revealed that *B. laterosporus* is a common resident of the honeybee body (Marche et al., 2017) and a beneficial role in favour of bee health was proposed, as a result of its antagonism against honeybee pathogens (Bartel et al., 2018; Marche et al., 2019 a and b). Based on the present knowledge, *B. laterosporus* can be considered a selective microbial species with potential for integrated pest management programs. Further research in the laboratory and in the field is needed to screen a wider range of non-target species in order to evaluate the safety of this microorganism in different agroecosystem contexts.

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Declaration of Competing Interest

Authors do not have any competing interests to declare.

References

Bartel, L.C., Abrahamovich, E., Mori, C., López, A.C., Alippi, A.M., 2018. *Bacillus* and *Brevibacillus* strains as potential antagonists of *Paenibacillus larvae* and *Ascosphaera apis*. J. Apicult. Res. 58, 117-132.

265 Blumberg, D., Navon, A., Keren, S., Goldenberg, S., Ferkovich, S.M., 1997. Interactions among
266 *Helicoverpa armigera* (Lepidoptera: Noctuidae), its larval endoparasitoid *Microplitis croceipes*
267 (Hymenoptera: Braconidae), and *Bacillus thuringiensis*. J. Econ. Entomol. 90, 1181–1186.
268 Bourguet, D., Chaufaux, J., Micoud, A., et al., 2002. *Ostrinia nubilalis* parasitism and the field
269 abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (Zea mays). Environ.
270 Biosaf. Res. 1, 49-60.
271 Cordeiro, E.M.G., Corrêa, A.S., Venzon, M., Guedes, R.N.C., 2010. Insecticide survival and
272 behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*.
273 Chemosphere 81, 1352-1357.
274 Donegan, K., Lighthart, B., 2003. Effect of several stress factors on the susceptibility of the
275 predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen
276 *Beauveria bassiana*. J. Invertebr. Pathol. 54, 79-84.
277 Eubanks, M.D., Denno, R.F., 2000. Health food versus fast food: The effects of prey quality and
278 mobility on prey selection by a generalist predator and indirect interactions among prey
279 species. Ecol. Entomol. 25, 140-146.
280 Glare, T.R., Durrant, A., Berry, C., Palma, L., Ormskirk, M.M., Cox, M.P., 2019. Phylogenetic
281 determinants of toxin gene distribution in genomes of *Brevibacillus laterosporus*. Genomics,
282 DOI: 10.1016/j.ygeno.2019.06.020
283 Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A., Bigler, F., 1998. Toxicity of *Bacillus*
284 *thuringiensis* Cry1Ab Toxin to the Predator *Chrysoperla carnea* (Neuroptera: Chrysopidae).
285 Environ. Entomol. 27, 1255-1263.
286 Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents:
287 Do they have a future? Biol. Control 21, 230-248.
288 Loru, L., Fois, X., Sassu, A., Pantaleoni, R.A., 2013. An individual marking technique for green
289 lacewings (Neuroptera: Chrysopidae). Fla. Entomol. 96, 628-630.

290 Lövei, G.L., Andow, D.A., Arpaia, S., 2009.Transgenic insecticidal crops and natural enemies: A
291 detailed review of laboratory studies. Environ. Entomol. 38, 293-306.
292 Marche, M.G., Camiolo, S., Porceddu, A., Ruiu, L., 2018. Survey of *Brevibacillus laterosporus*
293 insecticidal protein genes and virulence factors. J. Invertebr. Pathol. 155, 38-43.
294 Marche, M.G., Mura, M.E., Falchi, G., Ruiu, L., 2017. Spore surface proteins of *Brevibacillus*
295 *laterosporus* are involved in insect pathogenesis. Sci. Rep. 7, 43805.
296 Marche, M.G., Mura, M.E., Ruiu, L., 2016. *Brevibacillus laterosporus* inside the insect body:
297 Beneficial resident or pathogenic outsider? J. Invertebr. Pathol. 137, 58-61.
298 Marche, M.G., Satta, A., Floris, I., Lazzeri, A.M., Ruiu, L., 2019a. Inhibition of *Paenibacillus*
299 *larvae* by an extracellular protein fraction from a honeybee-borne *Brevibacillus laterosporus*
300 strain. Microbiol. Res. 227, 126303.
301 Marche, M.G., Satta, A., Floris, I., Pusceddu, M., Buffa, F., Ruiu, L., 2019b. Quantitative variation
302 in the core bacterial community associated with honey bees from *Varroa*-infested colonies. J.
303 Apicult. Res. 58, 444-454.
304 Michaud, J.P., Grant, A.K., 2003. IPM-compatibility of foliar insecticides for citrus: Indices derived
305 from toxicity to beneficial insects from four orders. J. Insect Sci. 3, 18.
306 Mura, M.E., Ruiu, L., 2018. *Brevibacillus laterosporus* pathogenesis and local immune response
307 regulation in the house fly midgut. J. Invertebr. Pathol. 145, 55-61.
308 Noh, S., Henry, C.S., 2010. Sexually monomorphic mating preferences contribute to premating
309 isolation based on song in European green lacewings. Evolution 64, 261–270.
310 Pappas, M.L., Broufas, G.D., Koveos, D.S., 2011. Chrysopid predators and their role in biological
311 control. J. Entomol. 8, 301-326.
312 Pappas, M.L., Karagiorgou, E., Papaioannou, G., Koveos, D.S., Broufas, G.D., 2013.
313 Developmental temperature responses of *Chrysoperla agilis* (Neuroptera: Chrysopidae), a
314 member of the European carnea cryptic species group. Biol. Control 64, 291-298.

315 Pasqualini, E. 1975. Prove di allevamento in ambiente condizionato di *Chrysopa carnea* Steph.
316 (Neuroptera, Chrysopidae). Bollettino dell'Istituto di Entomologia della Università degli Studi
317 di Bologna 32, 291-304.
318 Principi, M.M., Canard, M., 1984. Feeding Habits, in: Canard, M., Semeria, Y., New, T.R. (Eds),
319 Biology of Chrysopidae. Dr. W Junk Publishers, The Hague, The Netherlands, pp. 76-92.
320 R Development Core Team, 2016. R: a language and environment for statistical computing. Vienna:
321 R Foundation for Statistical Computing. Available at http://www.R-project.org.
322 Ríos-Moreno, A., Quesada-Moraga, E., Garrido-Jurado, I., 2018. Treatments with *Metarhizium*
323 *brunneum* BIPESCO5 and EAMa 01/58-Su strains (Ascomycota: Hypocreales) are low risk for
324 the generalist predator *Chrysoperla carnea*. J. Pest Sci. 91, 385-394.
325 Rodrigo-Simón, A., De Maagd, R.A., Avilla, C., Bakker, P.L., Molthoff, J., González-Zamora, J.E.,
326 Ferré, J., 2006. Lack of detrimental effects of *Bacillus thuringiensis* cry toxins on the insect
327 predator *Chrysoperla carnea*: A toxicological, histopathological, and biochemical analysis.
328 Appl. Environ. Microbiol. 72, 1595-1603.
329 Romeis, J., Dutton, A., Bigler, F., 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect
330 on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). J.
331 Insect Physiol. 50, 175-183.
332 Ruiu, L., 2013. *Brevibacillus laterosporus*, a Pathogen of Invertebrates and a Broad-Spectrum
333 Antimicrobial Species. Insects 4, 476–492.
334 Ruiu, L., 2018. Microbial biopesticides in agroecosystems. Agronomy 8(11), 235.
335 Ruiu, L., Floris, I., Satta, A., Ellar, D.J., 2007a. Toxicity of a *Brevibacillus laterosporus* strain
336 lacking parasporal crystals against *Musca domestica* and *Aedes aegypti*. Biol. Control 43, 136-
337 143.
338 Ruiu, L., Satta, A., Floris, I., 2007b. Susceptibility of the house fly pupal parasitoid *Muscidifurax*
339 *raptor* (Hymenoptera: Pteromalidae) to the entomopathogenic bacteria *Bacillus thuringiensis*
340 and *Brevibacillus laterosporus*. Biol. Control 43, 188-194.

Salama, H.S., El-Moursy, A., Zaki, F.N., Aboul-Ela, R., Abdel-Razek, A., 1991. Parasites and predators of the meal moth *Plodia interpunctella* Hbn. as affected by *Bacillus thuringiensis* Berl. J. Appl. Entomol. 112, 244–253.

Senior, L.J., McEwen, P.K., 2001. The use of Lacewings in Biological Control, in: McEwen, P.K., New T.R. and Whittington A.E. (Eds.), Lacewings in the Crop Environment. Cambridge University Press, Cambridge, pp. 296-302.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., et al., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. BioControl 44, 99-117.

Wang, X.-G., Johnson, M.W., Opp, S.B., Krugner, R., Daane, K.M., 2011. Honeydew and insecticide bait as competing food resources for a fruit fly and common natural enemies in the olive agroecosystem. Entomol. Exp. Appl. 139, 128-137.

Table 1 – Means (± SE) of larval development time, pupal development time, and percentage of adult emergence of *Chrysoperla agilis* exposed to *Brevibacillus laterosporus* at different developmental stages.

Treated lacewing larval instar	Development time (days)		Adult emergence[c] %
	Larvae[a]	Pupae[b]	
2nd instar			
Treated	18.4 ± 0.47 a[d]	9.8 ± 0.16 a	82.5 ± 4.74 a
Control	18.8 ± 0.37 a	11.1 ± 0.37 b	80.0 ± 2.36 a
3rd instar			
Treated	18.9 ± 0.64 a	10.7 ± 0.33 ab	82.5 ± 3.03 a
Control	18.7 ± 0.58 a	10.2 ± 0.25 ab	85.0 ± 3.73 a

[a] calculated from egg hatching to pupation

[b] calculated from pupation to adult emergence

[c] calculated on the initial number of larvae

[d] Different letters in a column indicate significantly different means (GLM ANOVA, followed by LSD test, $P < 0.05$).

Table 2 - Means (± SE) of longevity, fecundity, and percentage of egg hatching of *Chrysoperla agilis* adults surviving exposure to *Brevibacillus laterosporus*

Treatment[a]	Longevity (days)[b]		n[c]	Eggs/female	Egg hatching[d]
	Male	Female			%
Treated	26.5 ± 2.02 a[e]	27.9 ± 1.98 a	19	352.9 ± 32.85 a	77.1 ± 5.65 a
Control	24.7 ± 2.46 a	31.1 ± 2.28 a	19	291.9 ± 56.14 a	78.3 ± 6.54 a

[a] Newly emerged adults were exposed for 5 days to a 20% fructose solution containing *B. laterosporus*.

[b] Days from adult emergence to death.

[c] Number of ovipositing females.

[d] Egg hatching was evaluated at different time intervals during the oviposition period. Mean values are reported.

[e] Means in each column followed by different letters, are significantly different (*t*-test, $p < 0.05$).

Table 3 - Means (± SE) of larval instar and pupal development time, pupal weight, and percentage of emergence of *Chrysoperla agilis* exposed to mealworm beetles treated with *Brevibacillus laterosporus*

Treatment	Larval development time[a] (days)		Pupal weight (mg)	Pupal development time[b] (days)	Adult emergence[c] %
	2nd instar	3rd instar			
Treated	4.8 ± 0.22 a[d]	4.2 ± 0.20 a	7.6 ± 0.20 a	11.2 ± 0.32 a	72.5 ± 1.58 a
Control	4.9 ± 0.24 a	4.4 ± 0.23 a	7.1 ± 0.36 a	10.6 ± 0.15 a	82.5 ± 1.44 b

[a] time between moults

[b] calculated from pupation to adult emergence

[c] calculated on the initial number of larvae

[e] Means in each column followed by different letters, are significantly different (*t*-test, $p < 0.05$).

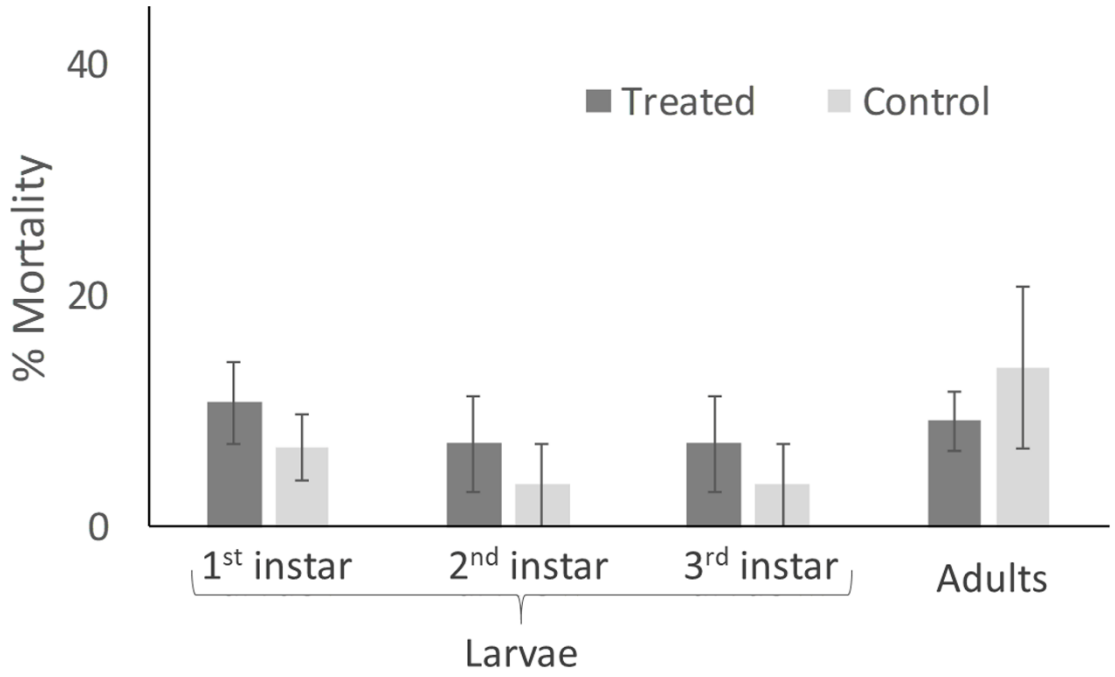

Fig. 1 – Mean (± SE) percentage mortality of *Chrysoperla agilis* larvae and adults after 5 days exposure to *Brevibacillus laterosporus* spores. No significant differences between means were found (GLM ANOVA, followed by LSD test: $P > 0.05$).

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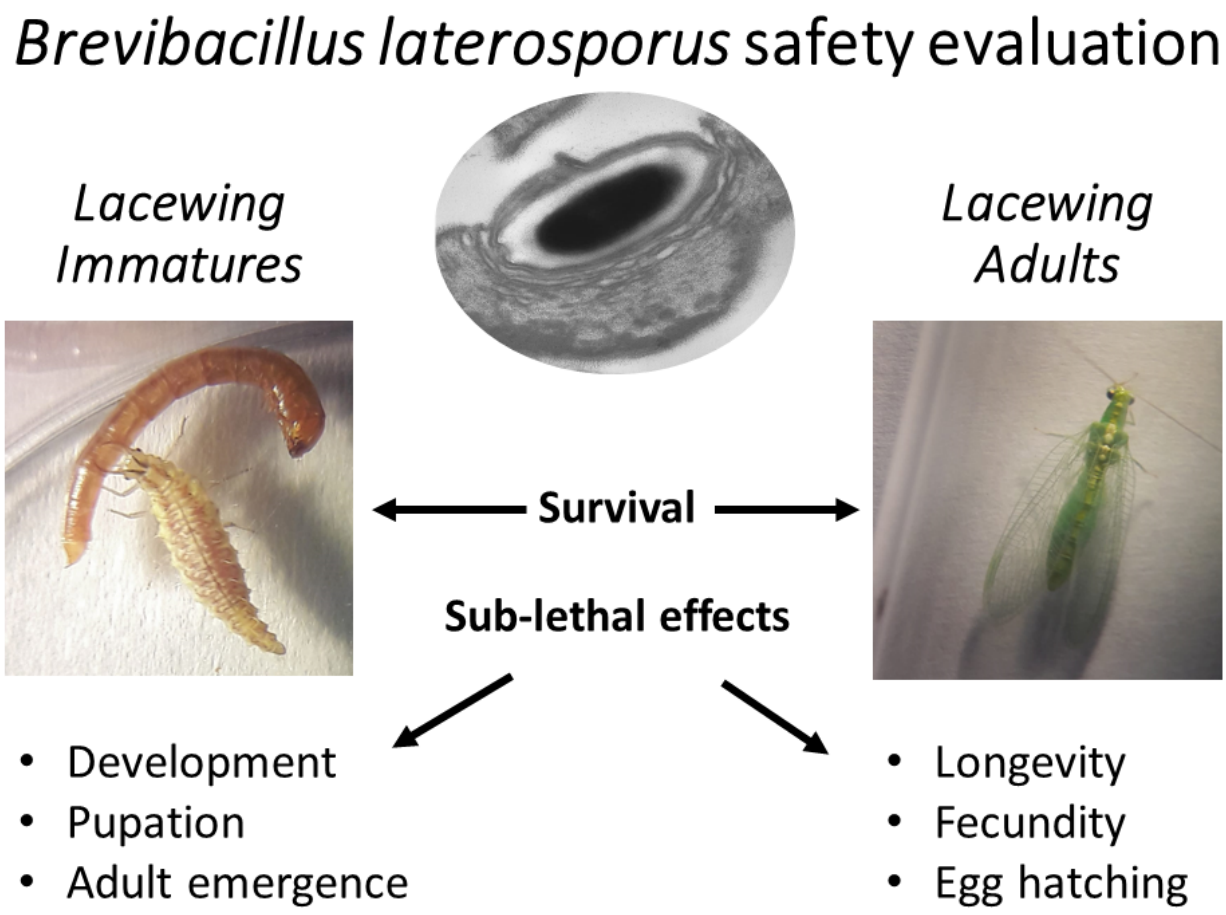

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