Egyptian Journal of Biological Pest Control

SCIENTIFIC (SHORT) NOTE

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# Laboratory test of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin s.l. (Hypocreales: Clavicipitaceae) baits for the biocontrol of the Toad grasshopper pest, *Bufonacris claraziana* (Saussure) (Orthoptera: Tristiridae)

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## Abstract

**Background:** The Toad grasshopper pest, *Bufonacris claraziana* (Saussure) (Orthoptera: Tristiridae) is an endemic species of the Argentine Patagonia region. Among the Tristiridae family, it is the only species recognized as harmful to agricultural and livestock activities in the country. Outbreaks of *B. claraziana* have become a recurring phenomenon in the recent years, affecting different areas of the Patagonian provinces. The aim of this study was to evaluate, as laboratory bioassays, the mortality in young nymphs of this species treated with bait formulations of the entomopathogenic fungus *Beauveria bassiana* (Hypocreales: Clavicipitaceae).

**Results:** The two treatments performed, one of them (I) with conidia of *B. bassiana* only and the other (II) with conidia of *B. bassiana* plus canola oil as phagostimulant, produced significantly higher mortality than the control ($p < 0.05$). At 10 days from the start of the bioassay, the mortality registered in the treatment II (53.33%) was higher than that in the treatment I (23.33%) ($p < 0.05$). This difference was similar at 15 days post-treatment, mortality reached in treatment II (93.33%) higher than in treatment I (73.33%).

**Conclusions:** Results demonstrated that the combination of canola oil with wheat bran makes the bait with conidia of *B. bassiana* more attractive for nymphs of *B. claraziana*, enhancing mortality over a shorter interval of time.

**Keywords:** Toad grasshopper, *Bufonacris claraziana*, Baits, Canola oil, Entomopathogenic fungus

## Background

The Toad grasshopper *Bufonacris claraziana* (Saussure) (Orthoptera: Tristiridae) is an endemic species of the Argentine Patagonia region. It is distributed from Neuquén and Río Negro provinces in the North of the region to Santa Cruz province in the South (Cigliano and Lange 2019). Together with *Elasmoderus wagenknechti* (Liebermann) (Orthoptera: Tristiridae) in Coquimbo, Chile (Cepeda-Pizarro et al. 2007) they are the only species among the South America-endemic Tristiridae family that are known to be harmful to agricultural and livestock production when outbreaks occur. *B. claraziana* is a wingless polyphagous species and both nymphs and adults usually make considerable marching displacements through the Patagonian steppe. It is normally an early-season species (hatchings may begin as early as late July or early August after an obligatory embryonic diapause, still well into winter in Patagonia). Although not new in historical

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terms (COPR 1982), outbreaks of *B. claraziana* appear to have become a more frequent phenomenon in the recent past, the last two of which recorded in the 2016–17 and 2019–20 seasons, affecting several localities in western Chubut and Santa Cruz provinces (Mariottini et al. 2019).

Since the early days of agricultural and livestock activities in Argentina different grasshopper species have been considered as problematic (Cigliano et al. 2014). Despite such a long expanse of time and the development and use of biocontrol agents elsewhere in the world (Zhang and Hunter 2017), chemical insecticides are still the only available option for grasshopper control in the country. The only biological alternative that seems to be working against other grasshopper species in the country, the microsporidium, *Paranosema locustae* (Canning) (Microsporidia), is not an option for *B. claraziana* due to a lack of susceptibility (Lange et al. 2020). One of the most relevant entomopathogens for practical pest control purposes consists of the fungi genera *Metarhizium* and *Beauveria*. Fungal species such as *B. bassiana* and *M. anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) are two of the most important microbials currently used against a wide range of arthropod, mainly insect pests, and the most common species developed as mycopesticides (Mishra et al. 2013). Some of the advantages associated with the use of fungi in biocontrol strategies against insect pests are host specificity, negligible effect on non-target organisms, easy mass production and a relatively low cost, low environmental impact, without residual activities or mammalian toxicity, and less vulnerability to resistance development (Goettel et al. 2021). Despite the general mode of infection through the integument, there is evidence that *B. bassiana* may infect insects *per os*, particularly insects with chewing mouthparts (Feng et al. 1994), like grasshoppers. Baits are used in North America to control rangeland grasshoppers and Mormon crickets (*Anabrus simplex* Haldeman; Orthoptera: Tettigoniidae) (Latchininsky et al. 2007). A typical bait of these formulations consists of wheat bran or other solid carrier impregnated with a chemical or biological insecticides. An advantage of using baits might be protection of the fungal pathogen from degradation due to UV light and/or other environmental factors. Adding attractants and phagostimulants could result in improving bait formulations, making bait treatment even more efficacious (Latchininsky and VanDyke 2006). Vegetable oils have kairomones attractant properties for grasshoppers primarily due to the presence of linoleic and linolenic fatty acids. These fatty acids are dietary essentials for grasshoppers and, once volatilized, can be detected by the insects' olfactory chemical-receptors (Bomar and Lockwood 1994).

Previous work by Mariottini et al. (2019) showed that adults of *B. claraziana* were not only susceptible to three strains of *B. bassiana* but also caused a significant increase in mortality in treated individuals respect to controls. With the aim of advancing in the eventual use of entomopathogenic fungi as potential biological controllers of this grasshopper pest, the main objective of the present study was to evaluate the mortality rate in young nymphs (3rd instar) of *B. claraziana* from different bait formulation with the most promising strain of *B. bassiana* previously tested and canola oil as phagostimulant.

## Methods

Individuals of *B. claraziana* used in this experience were collected with an entomological net as 3rd instar nymphs in early September 2019 at Cushamen (42° 10′2 3″ S 70° 39′ 39″ W), western Chubut province (Fig. 1a–c). Once in the laboratory, they were kept under controlled conditions of temperature and photoperiod as described in Mariottini et al. (2019).

The fungal strain used was *B. bassiana* LPSc 1227 (Gen Bank accession number: MG012792). This isolate of *B. bassiana* was obtained from cadavers of the South American locust *Schistocerca cancellata* (Serville) (Orthoptera: Acrididae) found in nature near La Banda (27° 44′ 07″ S; 64°14′ 36″ W), Santiago del Estero province, northwestern Argentina (Pelizza et al. 2020), and was deposited in the culture collection of the Spegazzini Institute (LPSc 1227), La Plata National University, Argentina.

Conidia from *B. bassiana* strain were obtained from cultures on potato-dextrose-agar medium (Britania S.A., Buenos Aires, Argentina) after incubation for 10 days at 25 °C in the dark. Afterward, the conidia were harvested with disposable cell scrapers (Fisherbrand™) according to Pelizza et al. (2020). They were harvested and placed in test tubes containing 0.01% (v/v) polyoxyethylene sorbitan monolaurate (Tween 80™; Merck). Suspensions were adjusted to $1 \times 10^8$ conidia $ml^{-1}$ using a Neubauer haemocytometer. Viability of conidia used in the tests was determined after 24 h as described by Goettel and Inglis (1997). The conidia were considered to have germinated once the germ tube had reached half a conidium's length. Each assessment was made in triplicate per treatment and 300 conidia counted under each experimental condition.

Two treatments and one control were performed (Table 1). In all cases, three replicates (on different dates) of 10 individuals of 3rd-instar nymphs of *B. claraziana* were used and they were kept in wire-screened, aluminum cages (30 × 20 × 20 cm) (Fig. 1d). Both control and treated grasshoppers were placed under controlled conditions of temperature, relative humidity, and photoperiod (26 °C, 60%, 14:10-h L: D).

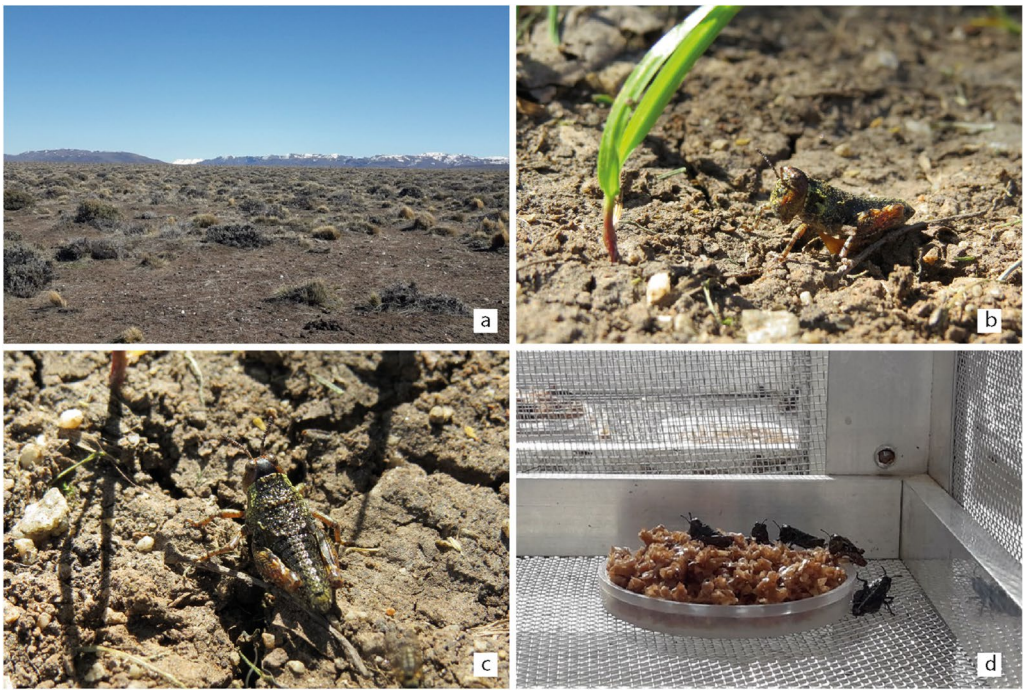


**Fig. 1** **a** Sample site in Chubut province, **b**, **c** young nymphs of *Bufonacris claraziana*, **d** nymphs of *Bufonacris claraziana* feeding on wheat bran baits with conidia of *Beuaveria bassiana*

Mortality rate of nymphs was recorded each 24 h for 15 days. Dead grasshoppers with no external mycelium were surface-sterilized by dipping them successively in 70% ethanol (10–15 s), 0.5% sodium hypochlorite solution (1 min), and sterile distilled water (1 min, two consecutive baths) according to Lacey and Brooks (1997). Next, they were placed in a sterile culture chamber consisting of a Petri dish (60 mm diameter) with a filter-paper disk that was periodically moistened with sterile distilled water and incubated at 25 °C in the absence of light. Mycosis was confirmed by microscopic examination of dead grasshoppers.

Cumulative mortality of treatments and control at the end of the experience were compared using the Kruskal Wallis test (Kruskal and Wallis 1952). Also, percent of mortality observed in each one of the treatments at 10, 15, and 20 days since the start of the experience were compared using one-way analysis of variance (ANOVA). For later comparisons the LSD Fisher test ($p < 0.05$) was used. Median survival time (MST) and survival curves were calculated through the Kaplan–Meier survival analysis. Pairwise comparisons between survival curves were made by Log-rank test. The Infostat software was used (Di Rienzo et al. 2011).

## Results

High responsiveness was observed in both treatments, producing a significantly higher mortality rates than the control (Kruskal Wallis Test, $H = 7.20$; $p = 0.0036$) (Fig. 2). In the expanse of the experience (15 days), the mortality achieved in individuals treated with baits of *B. bassiana* with canola oil (Treatment II) was of (93.33%) (Only two individuals remained alive after 15 days of treatment). In treatment I the percentage of mortality reached (73.33%). No mortality was recorded in the control.

Significant differences were registered between treatments in percentage of mortality reached at 5, 10, and 15 days from the start of the bioassay (ANOVA, $F$ 30.05, $df$ 17, $p < 0.0001$). At 5 days, mortality recorded in both treatments was similar (ANOVA, $F = 0.00$; $df = 5$; $p = > 0.9$) (Fig. 3). At 10 days, mortality registered in treatment II (53.33%) was higher than that registered in treatment I (23.33%) (LSD Fisher $p < 0.05$). This difference was similar at 15 days, mortality reached in treatment II (93.33%) was higher than in treatment I (73.33%). Percentages of mortality achieved in individuals treated with baits of *B. bassiana* without canola oil were similar at 5 and 10 days from the start of the bioassay (LSD Fisher $p > 0.05$), while in individuals treated with *B. bassiana*

**Table 1** Treatments with *Beauveria bassiana* against nymphs of *Bufonacris claraziana* carried out under laboratory conditions

| Treatment I | Treatment II | Control |
|---|---|---|
| Two hundred grams of wheat bran were sprinkled with a suspension of $1 \times 10^8$ conidia/ml of *Beauveria bassiana*. This bait was mixed and then introduced into wire-screened aluminum cages housing 10 third instar *Bufonacris claraziana* nymphs | Two hundred grams of wheat bran were sprinkled with a suspension of $1 \times 10^8$ conidia/ml of *B. bassiana* plus canola oil. This bait was mixed and then introduced into wire-screened aluminum cages housing 10 of 3rd-instar *B. claraziana* nymphs | *B. claraziana* nymphs were fed on wheat bran mixed with canola oil, but without the addition of fungal inoculum |

**Fig. 2** Nymphs of *Bufonacris claraziana* infected with *Beauveria bassiana*

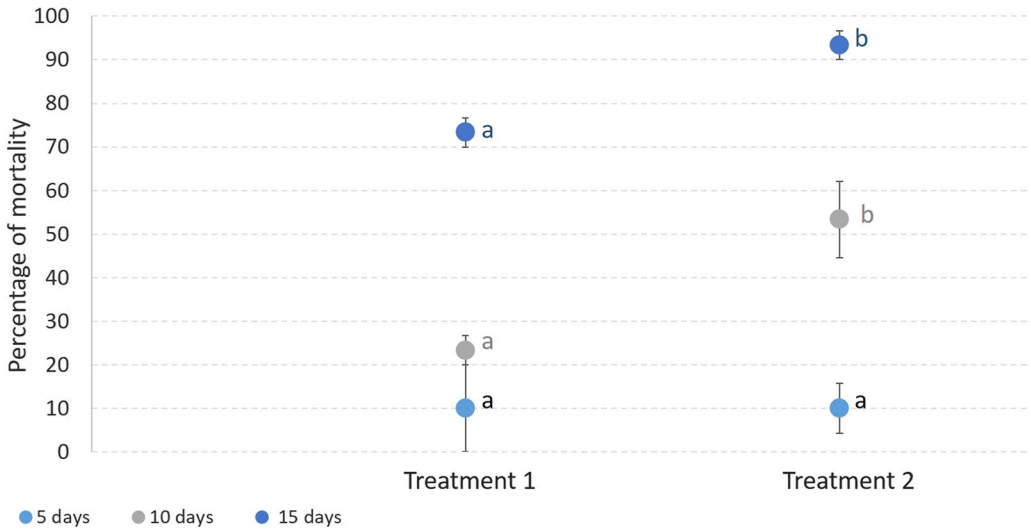


**Fig. 3** Percentage of mortality of nymphs of *Bufonacris claraziana* treated with different baits containing *Beauveria bassiana* conidia at 5, 10, and 15 days after treatment. Different letters showed significant differences (LSD Fisher $p < 0.05$)

baits plus canola oil, the mortality increased significantly between 5 and 10 days after the bioassay began (LSD Fisher $p < 0.05$).

Complementing these results, the survival curves of Kaplan–Meier, show a significant difference between the survival of the treated individuals compared to the control individuals (log-rank test $p < 0.05$) (Table 2). The lowest survival was registered in the individuals of the II treatment (log-rank test $p < 0.05$) (Fig. 4).

**Table 2** Results of long rank test ($X^2$) for the comparison of the Kaplan–Meier curves of each treatment

| | Treatment I | Treatment II | Control |
|---|---|---|---|
| Treatment I | | | |
| Treatment II | $X^2$: 4.88<br>$p$: 0.027 | | |
| Control | $X^2$: 16.8<br>$p$: 0.000 | $X^2$: 24.1<br>$p < 0.000$ | |

## Discussion

Results of this study showed that the two treatments administered produced significant mortality rates in the nymphs of *B. claraziana* compared to the control. They additionally showed that baits with *B. bassiana* plus canola oil were more effective, producing not only high mortality rate but also a short interval of time. The treatment with the combination of conidia of *B. bassiana* plus wheat bran and canola oil enhanced mortality. Canola oil possibly made the bait more attractive to *B. claraziana* nymphs. If so, they presumably consumed more bait and consequently more conidia, which resulted in a high percentage of mortality after 10 days. Similar results were obtained by Latchininsky et al. (2007), who reported that there are certain vegetable oils such as olive, canola, corn, and flax that have phagostimulant properties on nymphs and adults of the North American grasshopper pest, *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae). Also, Pelizza et al. (2019) evaluated under laboratory bioassay and field cage conditions the most effective treatment option using different baits with three different strains of *B. bassiana* against the Melanoplinae grasshopper pest *Dichroplus maculipennis* (Blanchard) (Orthoptera: Acrididae). The authors showed that under both laboratory and field conditions, the combination of *B. bassiana* plus wheat bran and canola oil improved the performance of conidia with a high percentage of mortality and a shorter survival time. Both canola oil and wheat bran likely provided protection to conidia against UV rays from solar radiation, allowing propagules to remain viable long and thus causing high percentage of infection (Pelizza et al. 2019). Latchininsky and VanDyke (2006) remarked that one of the advantages of using baits might be protection of the pathogen from degradation due to UV light and/or other environmental factors. Adding attractants and phagostimulants could result in improving bait formulations, making bait treatment more efficacious (Latchininsky et al. 2007).

Some of the key aspects for the success of a mycoinsecticide lie in three main areas: formulation, application, and understanding the host–pathogen relationship in the field (Dakhel et al. 2020). Results demonstrated that the combination of canola oil with wheat bran makes the bait with conidia of *B. bassiana* more attractive for nymphs of *B. claraziana*, attractiveness that can conceivably be enhanced by the aridity, the low environmental humidity, and the thermal amplitude of the Patagonian steppe (Oliva et al. 2017), where *B. claraziana* inhabits.

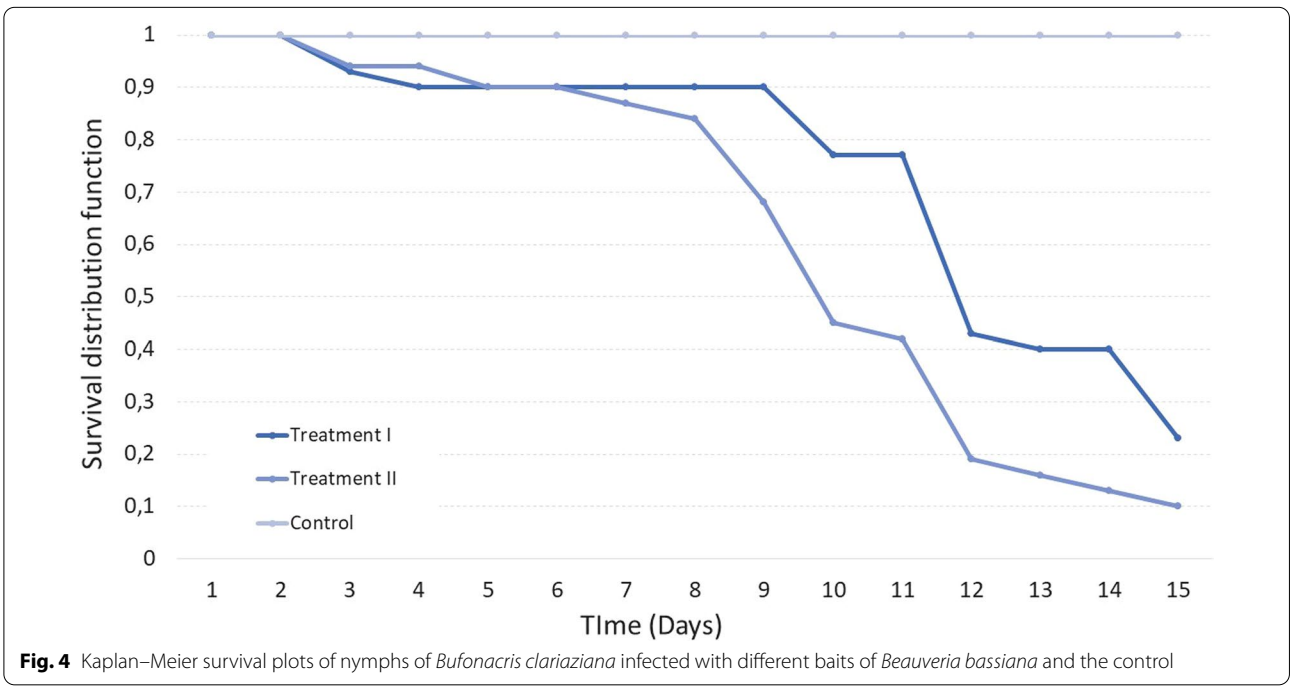


**Fig. 4** Kaplan–Meier survival plots of nymphs of *Bufonacris clariaziana* infected with different baits of *Beauveria bassiana* and the control

## Conclusion

It can be concluded from the results of the present study and the work by Mariottini et al. (2019) that the use of *B. bassiana* in both baits and spray increased the mortality of this species of grasshopper pest in Patagonia. The use of wheat bran and canola oil baits would be a good option for the control of *B. claraziana*. Field essays should be conducted to fully explore the actual potential of *B. bassiana* baits for the management of *B. claraziana*.

**Acknowledgements**
We appreciate the assistance in the field provided by Agrarian Engineers Marcela Schiappacassi and Franco Armento of the National Service of Agrifood Health and Quality (SENASA). This study was partially funded by the National Agency for Scientific and Technological Promotion (PICT Start Up 2020-0008).

**Author contributions**
The three authors contributed equally in the made of the bioassay, in the data analysis and in the writing the manuscript. All authors read and approved the final manuscript.

**Funding**
Not applicable.

**Availability of data and materials**
All data generated or analyzed during this study are included in this published article.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no conflict of interest.

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Received: 8 July 2022 Accepted: 10 September 2022
Published online: 19 September 2022

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