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Effects of endophytic *Beauveria bassiana* (Ascomycota: Hypocreales) on biological, reproductive parameters and food preference of the soybean pest *Helicoverpa gelotopoeon*

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ABSTRACT

Objectives: We studied *Beauveria bassiana* ability to endophytic colonize soybean and its effects on some biological and reproductive parameters and on the food preference of *Helicoverpa gelotopoeon*, a polyphagous plague of several agronomic crops.
Methods: A suspension of 1×10^8 conidia ml^{-1} of *B. bassiana* (LPSC 1098), was inoculated in soybean plants using the leaf spraying method. *H. gelotopoeon* survival, developement, fecundity and fertility were registered to evaluate fungal effect. Also, food preference was determined by the free-choice method.
Results: *B. bassiana* as an endophyte adversely affected the duration of the larval stages (L1, L2, L3, L4) and of the adult stages, as well as the total duration of the life cycle. Furthermore, the oviposition period, fecundity and fertility of *H. gelotopoeon* decreased. This is the first report of decreased consumption of soybean leaves by *H. gelotopoeon* due to endophytic *B. bassiana*.
Conclusions: This investigation shows that endophytic fungi could be considered as useful microorganisms for the integrated pest management.

1. Introduction

The soybean (*Glycine* max (L.) Merril.) (Fabaceae) is currently one of the most relevant extensive crops in Argentina and worldwide, due to the increase in area sown, productivity and yield in the last few years. During its cycle, this crop is affected by a diverse group of insect pests, which find in these plants the essential resources for their life. These pests produce different degrees of damage that may have a great impact on crop yield due to the losses caused (Caccia et al., 2014).

Noctuidae belongs to the Order Lepidoptera, represents a diverse family within the group that includes the highest number of insects of agricultural relevance. *Helicoverpa gelotopoeon* Dyar (Lepidoptera: Noctuidae) is a polyphagous plague of several crops including soybean, which causes damage to pods, leaves, and stems. Populations of *H. gelotopoeon* have increased lately in the Argentinean Pampean region causing problems in soybean crops, both during early vegetative stages and grain formation (Herrero et al., 2018).

The most commonly employed method to reduce the plague is the use of plaguicides, which changes the structure of the assemblages in the agroecosystem, kill beneficial microorganisms and natural enemies, promote the development of resistant pest populations and may have unwished impact on human health (Bellotti et al., 2005; Caccia et al., 2014; Holguín and Bellotti, 2004; Valerio, 2006).

The entomopathogenic *Beauveria bassiana* (Bals.)Vuill. was successfully introduced endophyticaly in many different plant species and is already used as an alternative control method against many insects (Batta, 2013), such as *Helicoverpa armigera* (Hubner) in broad bean (Jaber and Vidal, 2010) and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) in cotton and tomato (Castillo Lopez and Sword, 2015; Powell et al., 2009). In the case of leguminous plants, endophytic *B. bassiana* has been utilized against pests includes dipterans in broad bean (Akutse et al., 2013) and coleopterans in *Phaseous vulgaris* (Mutune et al., 2016).

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The objetive of the present study was to assess the effect of *B. bassiana* inoculated in soybean plants as an endophyte, on some biological and reproductive parameters and on the food preference of the pest lepidopteran *H. gelotopoen*.

2. Materials and methods

2.1. Inoculation of B. bassiana in soybean plant

B. bassiana LPSC 1098 (GenBank KT163259) obtained from "Instituto Spegazzini" collection (LPSC), Argentina was selected to perform bioassays. The selection of this entomopathogen was due to its proved endophytic efficiency in soybean plants under laboratory conditions (Russo et al., 2018 in press). Ten days cultures maintained on Petri dishes containing potato dextrose agar (PDA; Britania S.A., Buenos Aires) at 25 °C in the dark were utilized to obtain conidia.

For introducing *B. bassiana* as endophyte into soybean plants, the preparation of the conidia solution (1.10^8 conidia/ml) and the inoculation of soybean plants using the leaf spraying method were performed according to (Russo et al., 2018 in press). For the experiments, we used plants seven days after inoculation (Russo et al., 2018 in press). Before the beginning of the experiments, plants were assessed for the presence of endophytic *B. bassiana* (Russo et al., 2015).

2.2. Breeding of Lepidoptera

Eggs of *H. gelotopoeon* were provided by AgIdea, Buenos Aires, Argentina (www.agidea.com.ar) and maintained under controlled conditions (25 °C, 60% RH, 14:10 h LD photoperiod) until hatching. In order to prevent cannibalism, third instar caterpillars were individualized in Petri dishes with artificial diet as food source (Patana 1977, modified), which consisted in bean flour, beer yeast, methylparaben, ascorbic and sorbic acids, streptomycin, formaldehyde, vitamin complex, agar and distilled water. The diet was replaced every two days and in order to avoid desiccation moistened filter paper was placed into Petri dishes (Barrionuevo et al., 2012). Pupae were placed in polypropylene containers (6 cm diam × 12 cm height) until adult emergence and then placed into copulation cages (12 cm diam × 25 cm height), which inside contained folded paper to allow rest and oviposition. In the upper part of the cage, cotton embedded in a sucrose solution was provided as food source (Greene et al., 1976). Egg collection and diet replacement of the adults were performed daily. We utilized to performed bioassays eggs of *H. gelotopoeon* belonging to the first laboratory generation.

2.3. Effect of B. bassiana as endophyte

2.3.1. Biological parameters

Two groups of 100 eggs each were used to assess the sex ratio and the duration of the life cycle.

Eggs were daily observed to record incubation period. After hatching, newborn caterpillars were individualized and located in Petri dishes. Soybean leaves previously inoculated with *B. bassiana* were offered to one group and non-inoculated leaves were offered to the other group (controls).

Larvae were examined daily to check mortality and the presence of cephalic capsules were observed to determine if they had molted to the next stage. Pupae were sexed according to Angulo and Weigert (1975) and transferred to plastic containers to encourage adult development. Adults in couples (1 male and 1 female) were transferred to individual 2000 cc coupling cages to allow oviposition, as previously described. All insects were maintained under the most appropriate laboratory conditions of temperature, relative humidity, and photoperiod for the species (25 °C, 60% RH, 14:10 h LD photoperiod).

The following parameters were registered: a) length of the different developmental stages (egg, larvae, pupa, and adult); b) number of individuals at age × (nx); c) mortality (dx), as the number of dead individuals during age × of the original cohort; and d) sex ratio in the case of adults. Dead individuals were placed in humid chambers to confirm death due to *B. bassiana*.

2.3.2. Reproductive parameters

The following reproductive parameters were calculated: a) "duration of the oviposition period"; b) "age-specific survival rate (lx) from birth to death" for insects fed with inoculated and with non-inoculated plants (controls), as the "number of days lived at age x"; c) "age-specific fecundity (mx)", as the number of eggs produced daily by individuals at age × (Chi, 1988); and d) fertility, as the "number of eggs hatched/number of laid eggs × 100" (Schneider et al., 2009).

2.4. Food preference

Two soybean discs (3 cm diam), one corresponding to leaves from plants colonized with *B. bassiana* and the other to non-colonized leaves that were used as controls, were offered simultaneously to the larvae of *H. gelotopoeon*. As previously mentioned, the presence of the fungus as endophyte was confirmed in the inoculated discs prior to use. Thereafter, the discs were placed on a wet filter paper inside a Petri dish (90 mm) (Magrini et al., 2015) and a larva (L2) was then added in the center of each dish. Dishes were incubated for 24 h at 25 °C, 60% RH and a 14:10 h LD photoperiod. Three repetitions of 30 individuals each were performed. The existence of food preference was determined by the "free-choice method" (Ling et al., 2008; Napal et al., 2009). Discs were scanned initially to determine their originalarea and after 24 h to evaluate consumption. The total area consumed was calculated as the difference among the original surface of the leaf disc and the remaining surface after feeding (Milanovic et al., 2014), using ImageJ (Bailer, 2006).

2.5. Statistical analyses

The analysis of survival and fertility of each cohort were estimated according to Chi (1988) using the TWOSEX-MS Chart software (2008). The duration of each stage of *H. gelotopoeon*, as well as the comparison of the reproductive aspects between the different treatments and the analysis of the preference test were contrasted by a T test ($P < 0.05$). InfoStat, (2004) was utilized to perform data analysis.

3. Results

3.1. Biological and reproductive parameters of H. gelotopoeon

The development, survival, and mortality at all stages of two *H. gelotopoeon* cohorts, one fed with soybean leaves that were colonized by *B. bassiana* and the other, with non-inoculated leaves (control), are shown in Table 1. In both cohorts, 100% of the eggs were hatched and five stages were recognized during the larval period. The highest number of deaths was recorded during the second larval stage. From that stage, mortality decreased until the pupal stage, when it increased again in both cohorts. The adult stage was reached by 62% of the individuals fed with inoculated leaves and by 87% of the individuals from control populations. After incubation in a humid chamber, the presence of *B. bassiana*

Table 1
Average duration on each stage (days), number of individuals (nx), survival (lx), mortality (dx) of *Helicoverpa gelotopoeon* fed with soybean inoculated with *Beauveria bassiana* and not inoculated (control).

Life cycle stages	Control				Inoculated			
	Duration	nx	lx	dx	Duration	nx	lx	dx
Egg	5.42 ± 0.49 a	100	1	0	5 ± 0.50 a	100	1	0
L1	3.83 ± 0.40 a	100	1	3	3.38 ± 0.48 b	100	1	4
L2	4.83 ± 1.07 a	97	0.97	6	4.25 ± 1.25 b	96	0.96	18
L3	4.59 ± 1.57 a	91	0.91	1	3.38 ± 1.63 b	80	0.78	2
L4	4.14 ± 1.47 a	90	0.9	0	3.44 ± 1.67 b	78	0.76	0
L5	3.59 ± 1.35 a	90	0.9	0	3.51 ± 1.75 a	78	0.76	0
Overall larval stage	20.98 ± 5.15 a	90	0.9	0	17.96 ± 6.09 b	78	0.76	0
Pupa	6.18 ± 2.47 a	90	0.9	3	8 ± 2.88 a	78	0.78	15
Adult	4.47 ± 1.82 a	87	0.87	0	3.44 ± 2.56 b	63	0.63	0
Duration life cycle	37.05 ± 8.93 a				32.85 ± 10.59 b			
Sex ratio F:M	1:1.05				1:1.10			

Values followed by different letters within a same row are significantly different according to Student's T-test ($P < 0.05$).

was confirmed in 50% of the dead individuals that were fed with inoculated plants.

The mean duration of the larval stages L1, L2, L3, L4 and of the adult stages, as well as the total duration of the life cycle, were significantly lower in individuals fed with inoculated soybean than in non-inoculated (controls) ($p_{L1} < 0.0001$, $p_{L2} = 0.0201$, $p_{L3} = 0.0005$, $p_{L4} = 0.0162$, $pA = 0.0006$ and $p_{LC} = 0.0068$ respectively). In adults, the sex ratio was 1 female: 1.10 males in individuals fed with inoculated soybean leaves and 1 female: 1.05 males in the controls, thus showing a similar ratio between males and females in both treatments. The oviposition period was 1.95 ± 1.23 days and 2.7 ± 0.73 days for insects fed with inoculated and non-inoculated leaves, respectively (Fig. 1). During this period, females fed with inoculated leaves deposited an average of 460.7 eggs, with a range from 231 to 898 eggs (Fig. 1), while females fed with non-inoculated leaves deposited an average 743.45 eggs, with a range from 500 to 1071 eggs (Fig. 1). This parameter showed significant differences among both treatments ($p = 0.0033$). Fertility showed significant differences between both treatments ($p < 0.0001$), being 45.25 ± 0.40 for females fed with inoculated leaves and 74.62 ± 0.32 for control females (Fig. 2). The highest values were found on the fourth day of treatment in the control females.

The survival curve (lx) showed a larger decrease in the number of insects fed with leaves with *B. bassiana* as endophyte, resulting in the death of the last individual three days before than in the control treatment (Fig. 3).

In addition to showing lower fecundity rates, the reproduction in insects fed with inoculated leaves began on day 36 extending over a period of 5 days and reaching a population growth peak during day 39 (Fig. 3a). In the case insects fed with non-inoculated leaves, reproduction began on day 37, extended for a period of one week, reaching the maximum growth rate on day 41 (Fig. 3b).

3.2. Food preference

Significant differences ($T = 6.24$, $df = 51$, $p < 0.0001$) were observed in the consumption of inoculated an non-inoculated leaves, showing that endophytic colonization of *B. bassiana* decreased the consumption of soybean leaves by *H. gelotopoeon* (Fig. 4).

4. Discussion

In this investigation, we studied the food preference of *H. gelotopoeon*, one of the main pests of soybean plants, as well as the different lethal and sub-lethal effects produced by endophytic *B. bassiana* LPSc 1098. *B. bassiana* adversely affected the survival, fertility, adult longevity, and the life cycle period and of the oviposition period of *H. gelotopoeon*. These results are in agreement with Castillo Lopez and Sword (2015) and Powell et al. (2009), who observed that *H. zea* that fed on cotton and tomato plants colonized by *B. bassiana* suffered a reduction in the survival rate at early larval stages. In agreement with our results, Jaber and Vidal (2010) also observed lower survival rates for *H. armigera* fed with plants of *V. faba* inoculated with *B. bassiana*.

In the present study, as in those performed by Powell et al. (2009) with the larvae of *H. zea*, the presence of *B. bassiana* in dead individuals was confirmed after being placed in a humid chamber. On the contrary other authors (Castillo Lopez and Sword, 2015; Jaber and Vidal 2010) did not detect the presence of the fungus.

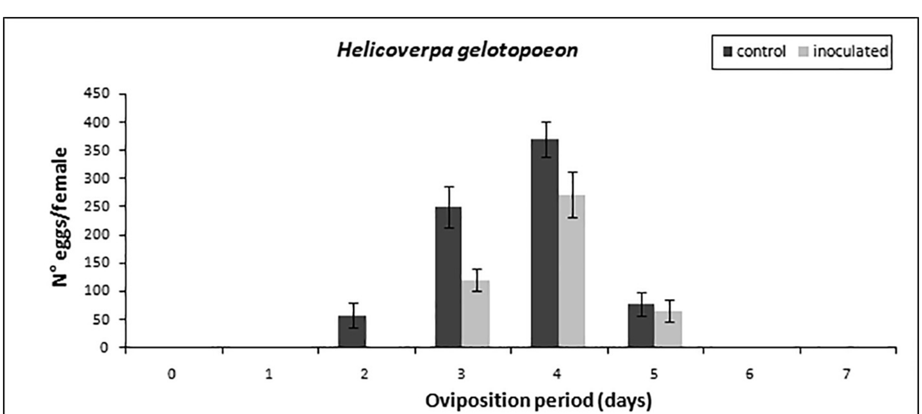

Fig. 1. *Helicoverpa gelotopoeon* fecundity of females fed with soybean inoculated with *Beauveria bassiana* and not inoculated (control). Bars indicate ± SEM.

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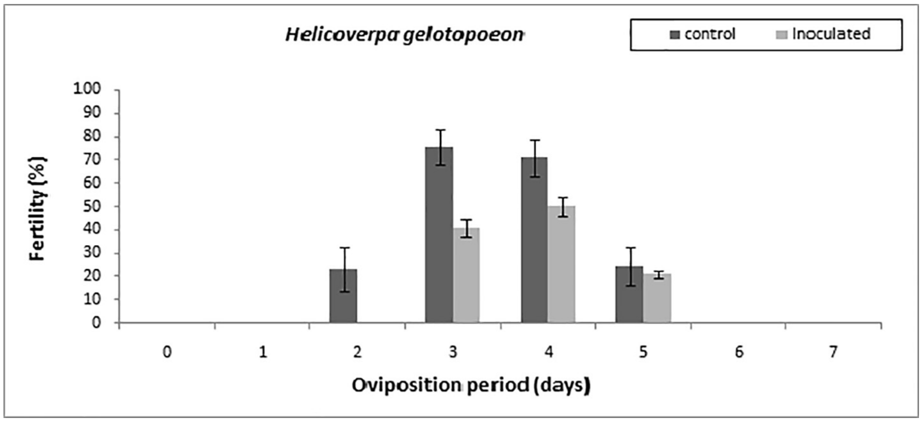

Fig. 2. *Helicoverpa gelotopoeon* fertility of females fed with soybean inoculated with *Beauveria bassiana* and not inoculated (control). Bars indicate ± SEM.

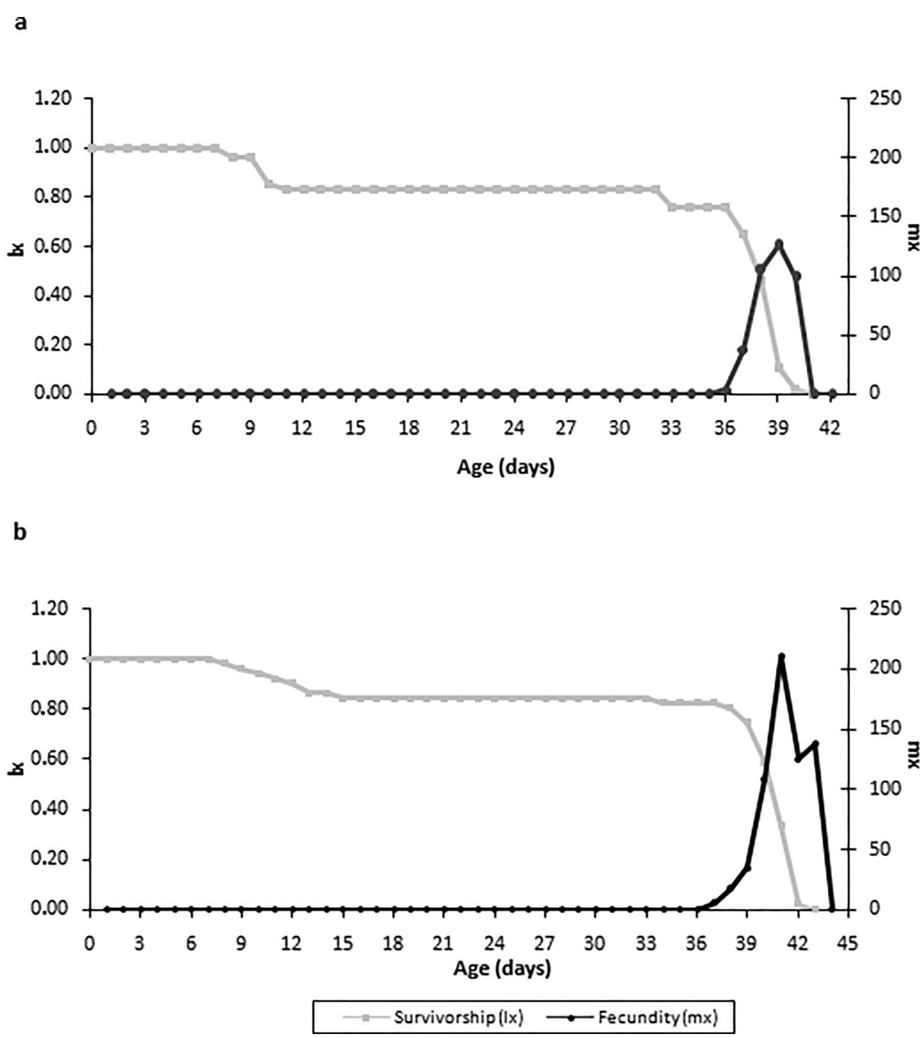

Fig. 3. Survival (*l*x) and fecundity *m*x curves of *Helicoverpa gelotopoeon* females reared with inoculated soybean plants (a) and not inoculated (control) (b).

They attributed insect mortality to indirect effects that occurred during the consumption of the inoculated plant, such as the production of secondary metabolites or the induction of a systemic response in the plant, which inhibits feeding behavior in insects.

A reduced fecundity and fertility was observed in those insects fed with inoculated leaves compared to the control plants. These results agree with those obtained by Mutune et al. (2016), who found a reduction in the oviposition rate of *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae) fed with *P. vulgaris* plants with endophytic *B. bassiana*. Akutse et al. (2013) also reported reduced fertility of dipterans fed with beans colonized with *B. bassiana*. These results are also in agreement with those obtained for *H. armigera* fed with *V. faba* with *Acremonium strictum* Gams (Hypocreales) as endophyte (Jaber and Vidal, 2010). In contrast,

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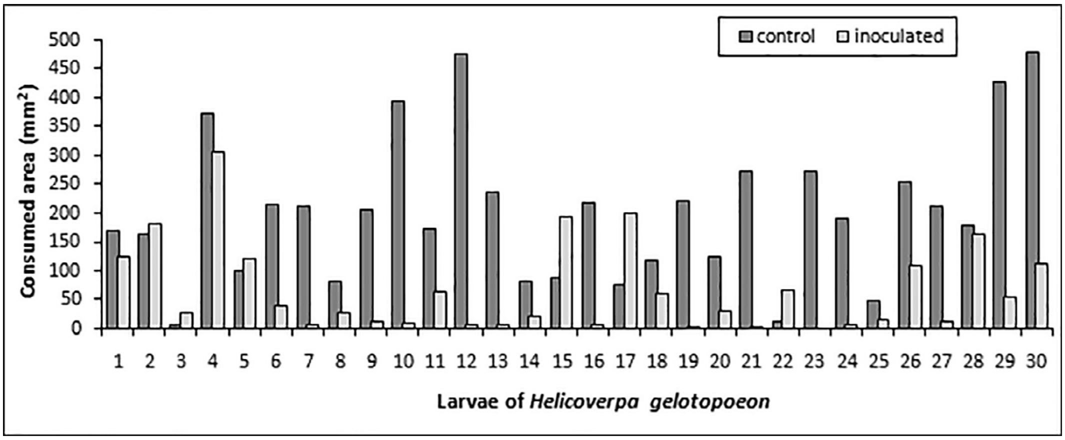

Fig. 4. Food preference of *Helicoverpa gelotopoeon* fed with soybean inoculated with *Beauveria bassiana* and not inoculated (control).

Raps and Vidal (1998) reported no significant differences in the life span and survival of adults of *Plutella xylostella* L. (Lepidoptera: Plutellidae) fed with inoculated and non-inoculated *Helianthus annus* plants. These authors observed that insects fed with inoculated plants, presented a shorter life cycle and a lower longevity of adults, as also reported in Dipterans by Akutse et al. (2013). There was a delay in the emergence of adults in insects fed with inoculated leaves, since the period in the pupae stage was longer than in the controls, as also registered by Jaber and Vidal (2010).

In this study, we observed that the larvae of *H. gelotopoeon* preferred to consume leaves non-inoculated with *B. bassiana*. Castillo Lopez and Sword (2015) also observed feeding preference in *H. zea* for non-inoculated leaves.

5. Conclusions

The entomopathogenic fungi *B. bassiana* has the potential to protect soybean plants against pest insects, by a twofold effect. On one hand, by decreasing the preference and consumption of leaves by these insects, and on the other, due to the ingestion itself of plants inoculated with *B. bassiana*. As a result, there can be a decrease in the number of lepidopteran individuals, and thus population increase is prevented. This study suggests the potential application of this endophyte could results in ameliorate soybean plants production and health. Although studies under field conditions are required to support the current finding, this appears to be an interesting tool that should be considered for pest biocontrol.

Conflicts of interest

None.

Acknowledgments

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