Journal of Pest Science
https://doi.org/10.1007/s10340-020-01302-x

ORIGINAL PAPER

Effect of entomopathogenic fungi introduced as corn endophytes on the development, reproduction, and food preference of the invasive fall armyworm *Spodoptera frugiperda*

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Received: 27 June 2020 / Revised: 29 October 2020 / Accepted: 6 November 2020

Abstract

Fall armyworm (FAW), *Spodoptera frugiperda*, is a migratory polyphagous pest that causes major damage to economically important cultivated grasses, such as corn. Native to the neotropics in America but recently reported as an invasive pest in Africa and Asia, FAW imposes a serious threat to food security and sustainable crop productivity due to lack of effective management. In this study, the introduction of entomopathogenic fungi as endophytes was explored as an alternative more sustainable management strategy against FAW in corn. The study determined (1) the effect of isolates and inoculation methods on the ability of entomopathogenic fungi to colonize corn plants, and (2) the effect of colonized plants on *S. frugiperda* survival, development, reproduction, and food preference. Although all tested isolates (twelve of *Beauveria bassiana* and one each of *Metarhizium anisopliae* and *Metarhizium robertsii*) colonized inoculated plants, there was a highly significant interaction between isolates and inoculation methods. Highest plant colonization was obtained by *Beauveria bassiana* isolate (LPSc 1098) using foliar spray. Endophytic *B. bassiana* caused significant reductions in larval and pupal survival, length of different developmental stages, total *S. frugiperda* lifespan, and leaf area consumed by third instar larvae. Plant colonization also significantly reduced female longevity, fecundity, and fertility. This is the first report for the negative effects of endophytic *B. bassiana* on *S. frugiperda* growth, reproduction, and food preference. Our results highlight the promising potential of incorporating entomopathogenic fungi as endophytes in integrated pest management practices to protect corn against FAW if their efficacy is also confirmed under field conditions.

Keywords *Beauveria bassiana* · Fungal endophytes · Integrated pest management (IPM) · Invasive pest · *Metarhizium anisopliae* · *Metarhizium robertsii*

Key message

- All isolates were recovered as endophytes from inoculated plants, but colonization varied significantly among fungal isolates, inoculation methods, and plant tissues.
- Highest plant colonization was obtained by *B. bassiana* (LPSc 1098) using foliar spray.
- *B. bassiana* colonization significantly reduced larval and pupal survival, length of different developmental stages, female reproductive parameters, and leaf consumption by third-instar larvae.
- These results provide the first report for the adverse effects of endophytic *B. bassiana* on *S. frugiperda* growth, reproduction, and food preference.

Communicated by Nicolas Desneux.

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Published online: 16 November 2020

Introduction

Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae), commonly known as the fall armyworm (FAW), is a migratory polyphagous pestnative to the neotropics of the Americas. It has been reported to attack 353 host plant species from 76 plant families, but the greatest damage is commonly recorded on cultivated grasses, particularly corn and sorghum (Montezano et al. 2018). The ability to form large populations, the great voracity and displacement of larvae, and the high dispersion rate of adults make *S. frugiperda* one of the most important noctuid pests of corn in North and South America and result in huge economic losses (Castro et al. 2009). More recently, *S. frugiperda* has been reported as an invasive pest in many parts of Africa and Asia and is likely to become endemic due to the ideal climate conditions and abundance of suitable host plants which enable several pest generations in a single season (FAO 2019). It is estimated that FAW can cause crop losses of up to $US13 billion per annum across Africa in cereals such as corn, rice, and sorghum (Abrahams et al. 2017). The pest thus imposes a serious threat to food security and sustainable crop productivity, especially in light of the absence of effective management strategies. Current control measures largely focus on heavy and indiscriminate application of broad-spectrum insecticides, which not only negatively affects human health and the environment, but also often results in inconsistent FAW control (Stokstad 2017; Harrison et al. 2019). Subsequently, an alternative more sustainable management strategy against FAW is urgently needed.

Entomopathogenic fungi have been long recognized and developed as important biological control agents (de Faria and Wraight 2007). Found infecting and killing various groups of arthropods in a diverse array of geographic, climatic, and agro-ecological zones, these fungi offer a promising alternative to conventional chemical control (Lacey et al. 2015). Yet, their worldwide commercial adoption for effective pest biocontrol is still hindered by limited field efficacy due to high susceptibility to ultraviolet light, low moisture, and difficulties in reaching cryptic stages of the target pests. The ability of different genera of entomopathogenic fungi to colonize a variety of host plants as endophytes provides an exciting opportunity to improve their efficacy (Vega 2018). It also allows for a multifaceted application of these fungi for dual biocontrol of insect and pathogen pests as well as plant growth promotion (Jaber and Ownley 2018) in combination with other groups of biocontrol agents (e.g., parasitoids and predators, Akutse et al. 2014; Jaber and Araj 2018; González-Mas et al. 2019) and environmentally safe control measures (e.g., botanicals, Jaber et al. 2018) in Integrated Pest Management (IPM) programs.

Previous studies have demonstrated the endophytic ability of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) to control *Spodoptera littoralis* on colonized alfalfa, tomato, melon, and wheat (Resquín-Romero et al. 2016; Sánchez-Rodríguez et al. 2018) and of *B. bassiana* against *Spodoptera exigua* on colonized tomato (Shrivastava et al. 2015), but not against *S. frugiperda* feeding on colonized corn plants. A scientific note has previously reported the pathogenicity of *B. bassiana* against third instar larvae of *S. frugiperda* exposed to the fungus before and after its establishment as an endophyte in corn, but not when fed with endophytically colonized plants or plant parts (Ramirez-Rodriguez and Sánchez-Peña 2016). This study was therefore conducted to (1) examine the effect of fungal isolates and inoculation methods on the ability of entomopathogenic fungi (*B. bassiana*, *Metarhizium anisopliae*, and *Metarhizium robertsii*) to endophytically and systemically colonize different tissues (root, stem, and leaf) of corn plants and (2) determine the effect of endophytic plant colonization on the survival, development, reproduction, and food preference of *S. frugiperda*.

Materials and methods

Study organisms

Corn seeds of the hybrid DK747 (DEKALB-Monsanto) were surface-sterilized by soaking in a solution of 70% ethanol for 2 min, followed by sodium hypochlorite (commercial bleach 55 g CIL^{-1}) for 2 min, and finally rinsed twice in sterile distilled water. Prior to sowing, seeds were soaked in sterile distilled water for 24 h at 4 °C. Seeds were planted in 330 cm^3 plastic containers with a mixture of soil, perlite, and vermiculite at a ratio of (1:1:1). The planting substrate was autoclaved thrice for 45 min at 121 °C with a 24h interval between each autoclaving process and allowed to cool before use. All plants were maintained under controlled conditions in a greenhouse at 25 °C, 75% Relative Humidity (RH), and 12:12h light/dark photoperiod. Plants were watered as needed but not fertilized during the course of experiments.

Twelve isolates of *B. bassiana* sensu stricto: LPSc 1060, LPSc 1061, LPSc 1062, LPSc 1063, LPSc 1066, LPSc 1067, LPSc 1080, LPSc 1082, LPSc 1083, LPSc 1086, LPSc 1098, LPSc 1156 (GenBank accession numbers MG712618, MG712619, MG712620, MG712624, MG712621, KF500409, MG712623, KJ7722495, MG712625, MG712626, KT163259, MG712627, respectively), an isolate of *M. anisopliae* LPSc 907 (GenBank accession number KT163258), and an isolate of *M. robertsii* LPSc 963 (GenBank accession number KJ772494) were used in this study. All fungal isolates were obtained from the Culture Collection of "InstitutoSpegazzini" (LPSc), La Plata,

Buenos Aires, Argentina. These isolates were selected due to their pathogenicity against several insect pests (Pelizza et al. 2012a, b). Fungal isolates were maintained on Potato Dextrose Agar (PDA) plates at 25 °C in darkness.

Eggs of *S. frugiperda* were provided by AgIdea (www.agidea.com.ar), Pergamino city, Buenos Aires, Argentina. The insect pest was reared in a bioterium under controlled conditions (25 ± 2 °C, 70–75% RH and 14:10 h light/dark photoperiod) using a bean-based artificial diet prepared as described in Murúa et al. (2003) and replaced every two days. Pupae were sexed and maintained in containers lined with moistened filter paper until adult emergence. Emerged adults were used to start the next generation for insect bioassays as described below.

Plant inoculation with entomopathogenic fungi

Conidia were harvested by scraping the surface of 10-day-old cultures with a sterile scalpel after flooding plates with sterile distilled water containing Tween 80 (0.01% v/v, Merck®). The conidia were then filtered through several layers of sterile cheese cloth into sterile test tubes containing sterile distilled water with 0.01% Tween 80. The conidial suspension for each fungal isolate was adjusted to 1×10^8 conidia mL^{-1} using a Neubauer hemocytometer according to Gurulingappa et al. (2010). Conidial viability was assessed for each fungal isolate prior to plant inoculation by carrying out a germination test, as described by Lane et al. (1988), and only suspensions with ≥ 95% germination were used.

Three inoculation methods (foliar spray, root dipping, and seed immersion) were tested as described in Russo et al. (2015). For foliar spray and root dipping, surfaced-sterilized corn seeds were sown in plastic pots filled with sterile mixture of the planting substrate as mentioned above. A glass hand sprayer (30-ml capacity) was used to spray each three-week-old seedling with an average of 3 ml conidial suspension of each fungal isolate (leaf surfaces received a deposition rate equivalent to 5×10^5 conidia cm^{-2}). Control plants were sprayed with 3 ml of sterile 0.01% Tween 80 solution. The spray was mainly directed to the leaves, but may have incidentally coated the stems as well. The top of each pot was covered with aluminum foil while spraying to avoid conidial runoff to the soil. For root dipping, three-week-old seedlings were removed from pots and rinsed three times with sterile distilled water. Prior to plant inoculation, the root ends were cut and individually placed in test tubes with 2 ml conidial suspension of each fungal isolate for 24 h. Roots of control plants were dipped in sterile 0.01% Tween 80 solution for 24 h. Treatment and control plants were then replanted in respective pots.

For seed immersion, surface-sterilized seeds were immersed in 10 ml conidial suspension of each fungal isolate for 24 h. Seeds were then dried on sterile paper towels in a sterile laminar flow cabinet for 30 min before being sown in 330 cm^3 plastic containers filled with sterile mixture of the planting substrate as described above. Control seeds were immersed in sterile 0.01% Tween 80 solution for 24 h before sowing. One plant per plastic container was used.

The experiment was run as a complete randomized design with a factorial arrangement. Two main factors, fungal isolate and inoculation method, were included. The first factor had 15 levels (twelve *B. bassiana* isolates, *M. anisopliae, M. robertsii* and the control). The second factor had three levels: foliar spray, root dipping, and seed immersion. A total of 45 treatment combinations (fungal isolate × inoculation method) were used. Each treatment combination had a total of 40 plant replicates, ten of which were destructively sampled for assessment of endophytic colonization per sampling day (i.e., 7, 14, 21, and 28 days after inoculation).

Assessment of endophytic colonization of corn by entomopathogenic fungi

Endophytic colonization with the tested fungal entomopathogens was evaluated at 7, 14, 21, and 28 days after inoculation by destructively sampling root, stem, and leaf tissues of plants. For each treatment combination, ten plant replicates were sampled per each sampling day. Plants (after thorough washing with running tap water) were surface-sterilized by successive immersion in 70% ethanol for 2 min, followed by sodium hypochlorite (commercial bleach 55 g CIL^{-1}) for 2 min, and finally rinsed twice in sterile distilled water. Imprints of surface-sterilized plant material were made and the final rinse water was plated onto PDA media and incubated at 25 °C for 10 days to determine the efficiency of the surface-sterilization procedure in eliminating epiphytic microorganisms (Schulz et al. 1998). Plant material was dried on sterile paper towels in a laminar flow cabinet. Each surface-sterilized plant tissue was cut with a sterile scalpel into 1 cm^2 pieces. An average of six pieces were sampled from each tissue and then evenly plated onto Petri dishes containing 20 ml of PDA with 0.1% stock antibiotics. The antibiotic stock consisted of 0.02 g of each of three antibiotics (tetracycline, streptomycin, and penicillin; Vega et al. 2008). All Petri dishes were incubated at 25 °C in the dark and examined every ten days to record fungal outgrowth. Fungal outgrowth from plated plant samples was identified as *B. bassiana*, *M. anisopliae*, or *M. robertsii* based on differential growth on semi-selective media, colony morphology, and microscopic examination of conidia (Humber 1997). Data were expressed as percent colonization frequency = (number of plant pieces showing fungal outgrowth/total number of plated plant pieces) × 100 (Petrini and Fisher 1987). A total of 120 plant replicates and 2160 plant pieces were examined for each inoculated fungal isolate, with a

total of 1680 plant replicates and 30,240 plated plant pieces for all tested isolates.

Effect of endophytic colonization of corn on *S. frugiperda* development and reproduction

Two groups of 50 eggs, obtained from a laboratory colony of *S. frugiperda*, were used for this bioassay. Eggs were examined daily until hatching. First-instar neonate larvae (hatching within 24 h) were randomly and individually placed in Petri dishes lined with moistened filter paper to favor feeding and avoid cannibalism. Corn plants that showed the highest rate of systemic endophytic colonization by the most successful (effective) inoculation method were used for this bioassay. Half of the neonate larvae were offered excised leaves (5 × 2 cm) from inoculated plants in which endophytic colonization by the fungal isolate was confirmed seven days after inoculation, whereas the other half received leaves from non-inoculated (control) plants. Leaves were replenished daily and filter papers were replaced with new ones as necessary.

The following parameters were recorded: (1) length of different developmental stages (egg, larval, pupal, and adult), (2) number of individuals at age x (n_x), (3) mortality (proportion of individuals of the original cohort that died at age x, d_x), and (4) adult sex ratio. The larvae were checked daily for mortality and moulting until pupation. The presence of cephalic capsules was observed to verify if larvae had moulted to the next developmental stage. Pupae were sexed according to Angulo and Weigert (1975) and transferred as couples (1 male:1 female) to 500 cm^3 containers to allow adult emergence. Paired adults were transferred to copulation cages with folded paper to allow egg laying and handling of egg masses. A small piece of cotton soaked in a 10% sugar solution was placed on the upper part of cages as a food source. Egg collection and diet replacement for adults were carried out daily until female adult death.

During the reproductive stage, the following parameters were recorded: (1) duration of the oviposition period, (2) age-specific survival rate from birth to death (the number of days lived at age x, l_x), (3) age-specific fecundity (the number of eggs produced daily by individuals at age x as m_x, Chi 1988) but modified to include only viable (hatched) eggs instead of all (hatched and unhatched) eggs according to Muo et al. (2015), and (4) fertility (the number of eggs hatched/the number of eggs laid × 100, Schneider et al. 2009). Other population parameters, such as the net reproductive rate (R_0), the intrinsic rate of increase (r), and the finite rate of increase (λ), were also calculated using the TWOSEX-MSChart computer program (Chi 2008). This program includes a routine for the estimation of standard error of population parameters using the Jackknife technique. Cadavers were surface sterilized as mentioned before then incubated in moist sterile chambers at 25 °C in the dark and inspected daily by microscopic examination to observe mycosis which confirms death due to the fungal entomopathogenic isolate inoculated into plants as endophyte. The entire bioassay was repeated once over time.

Effect of endophytic colonization of corn on *S. frugiperda* food preference

Corn plants previously inoculated with the fungal isolate showing the highest plant colonization rate through the most effective inoculation method were used for this bioassay as described above. Fragments of corn leaves (6 × 3 cm) obtained from inoculated or non-inoculated (control) plants were simultaneously offered to larvae. The presence or absence of the fungus as endophyte within inoculated and non-inoculated plants was confirmed prior to use.

Food preference was determined by the "free-choice method" (Ling et al. 2008; Napal et al. 2009). Leaf fragments from inoculated and non-inoculated (control) plants were scanned to determine initial leaf area. Two equally spaced leaf fragments (inoculated and control) were placed on a wet filter paper in each Petri dish (90 mm diameter) and a third instar larva (L3) was introduced to the center of the dish (Magrini et al. 2015). The larva was left for 24 h, and leaf fragments were scanned again to evaluate consumption. Leaf area consumed was calculated as the difference between the initial leaf area and the remaining leaf area after larval feeding (Milanovic et al. 2014) using ImageJ (Bailer 2006). Three repetitions of 30 individuals (replicates) each were made on different dates.

Statistical analyses

Data were tested for normality and homogeneity of the variance prior to statistical analyses. Percentage values of plant colonization frequency were angular transformed to stabilize the variance. Differences in percent colonization frequency were analyzed using three-way Analysis of Covariance (ANCOVA), with fungal isolate, inoculation method and plant tissue as main factors, and time as a covariate. Significant differences among treatment means ($P < 0.05$) were compared with Tukey´s test. The mortality and reproductive parameters of each *S. frugiperda* cohort were analyzed according to Chi (1988) using TWOSEX-MSChart (Chi 2008). Student´s t test ($P < 0.05$) was used to compare the length of each developmental stage (egg, larval, pupal, and adult), the food preference, and the population parameters of *S. frugiperda* reared on colonized and non-colonized (control) leaves. A two-way ANOVA (with treatment and sex as main factors) was used to analyze adult longevity (the total number of days lived as adults from emergence to death), followed by Tukey´s test for separation of treatment means.

All analyses were performed using InfoStat version 2004 (InfoStat 2004).

Results

Effect of fungal isolate and inoculation method on the endophytic colonization of corn by entomopathogenic fungi

Endophytic colonization of corn plants by the tested entomopathogenic fungi was determined using re-isolation of respective fungal isolates following surface-sterilization of plant tissues. No fungal growth was observed on final rinse water or plant imprint plates. This indicates the efficacy of surface sterilization in eliminating epiphytic microorganisms and confirms that the fungi growing out of surface-sterilized plant material were endophytic microorganisms originating from within plant tissues. None of the inoculated fungal isolates were recovered from control plants, whereas all tested isolates were successfully recovered as endophytes from inoculated plants (Fig. 1). However, there was a highly significant interaction between fungal isolates and inoculation methods ($P < 0.0001$; Table 1), as not all of the three tested inoculation methods were effective in establishing all tested fungal isolates as endophytes. For example, seed immersion was not successful in introducing LPSc 1067, LPSc 907, or LPSc 963 into plants, while foliar spray resulted in the highest rate of plant colonization for all tested isolates except LPSc 907 and LPSc 963 (Fig. 1). Highest rate of plant colonization was also consistently observed 7 days after inoculation, irrespective of fungal isolate and inoculation method. Percentage colonization of plants varied significantly among isolates and plant tissues (root, stem, and leaf) within each sampling date (7, 14, 21, and 28 days after inoculation) and decreased significantly

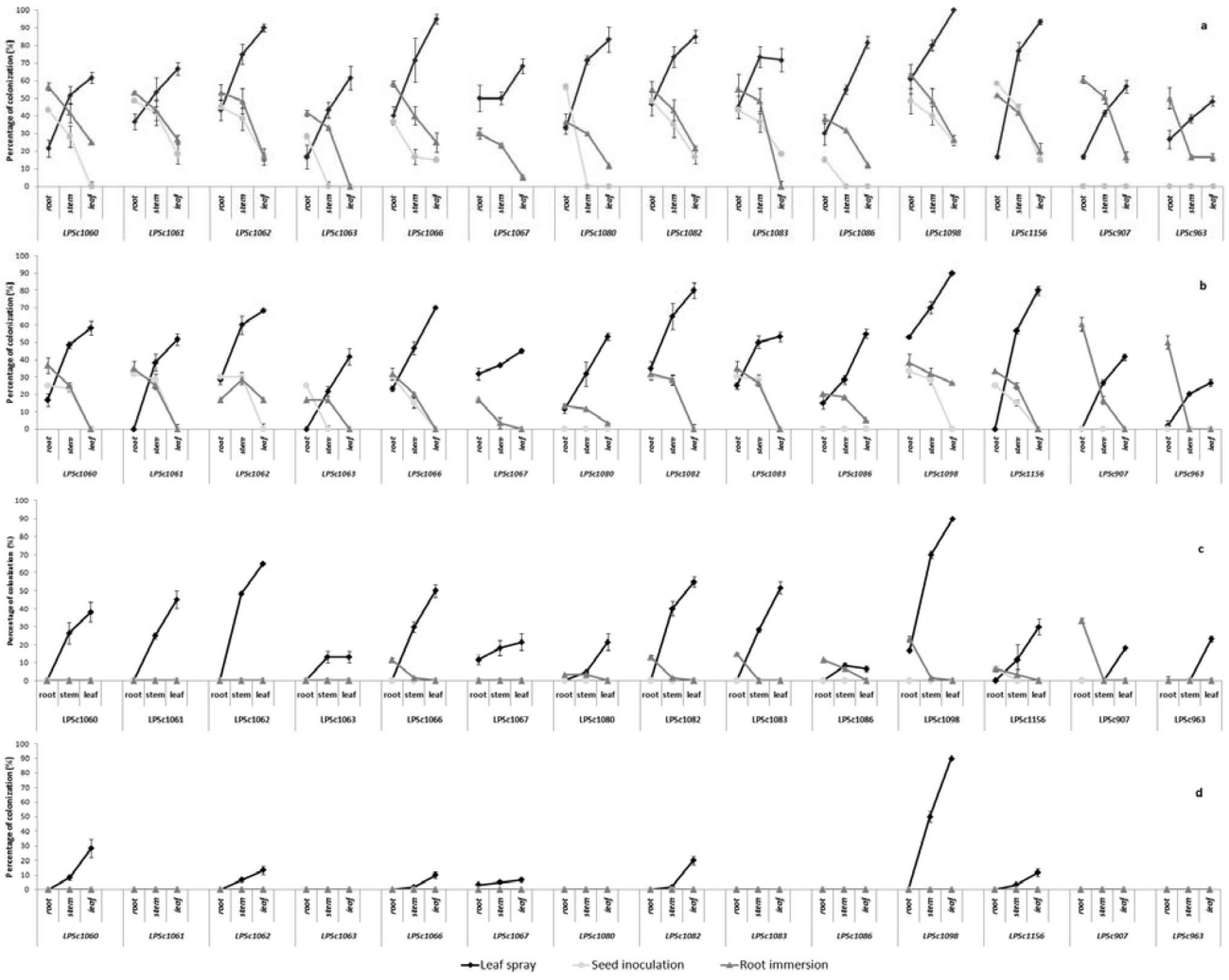

Fig. 1 Effect of fungal isolates and inoculation methods on mean (±SE) colonization (%) of different plant tissues (root, stem, and leaf) of corn by entomopathogenic fungi as endophytes at **a** 7, **b** 14, **c** 21, and **d** 28 days after inoculation (Tukey's test after three-way ANCOVA)

Table 1 Three-way ANCOVA for the effects of fungal isolate, inoculation method, and/or plant organ on colonization (%) of corn by entomopathogenic fungi as endophytes at 7, 14, 21, and 28 days after inoculation

	F	d.f	*P*	Coef
Isolate	53.54	13	< 0.0001	
Inoculation	811.65	2	< 0.0001	
Organ	22.49	2	< 0.0001	
Isolate × Inoculation	65.89	26	< 0.0001	
Isolate × Organ	3.91	26	< 0.0001	
Inoculation × Organ	375.95	4	< 0.0001	
Inoculation × Isolate × Organ	5.20	52	< 0.0001	
Time	3588	1	< 0.0001	− 0.03

over time (Fig. 1, Table 1). Yet at all sampling dates, LPSc 1098 was the best systemic plant colonizer, particularly when inoculated into plants through foliar spray (Tukey's test: $P < 0.05$, Fig. 1). This *B. bassiana* isolates colonized 100% of leaves, 80% of stems, and 60% of roots seven days after foliar spray (Fig. 1a). This isolate was therefore introduced into corn plants in further bioassays using the foliar inoculation method in order to examine the effect of plant colonization with entomopathogenic fungi as endophytes against *S. frugiperda*.

Effect of endophytic colonization of corn on *S. frugiperda* development and reproduction

Mortality and length of different developmental stages of *S. frugiperda* reared on *B. bassiana* (LPSc 1098) colonized and non-colonized corn leaves are shown in Table 2. All eggs of the original cohorts hatched and six larval stages were recognized. Highest mortality (d_x) was observed during the second larval stage followed by pupal stage of insects feeding on endophytically colonized leaves compared to control. Overall, a significantly higher number ($n_x = 85$) of individuals fed on leaves from non-colonized (control) plants reached the adult stage compared to 65 of those fed on leaves from colonized plants (Table 2). Dead individuals were surface-sterilized and incubated in a dark moist chamber to induce mycosis. Mycosis was recorded in 65% of dead insects fed on colonized leaves, and the fungus growing on the surface of incubated cadavers was identified as the inoculated fungal isolate by microscopic examination.

Feeding on leaves of colonized plants significantly decreased mean length of second (L2, $P < 0.0001$), third (L3, $P = 0.0038$), fourth (L4, $P = 0.0073$), and sixth (L6, $P = 0.0075$) larval instars, in addition to pupal ($P < 0.0001$) and adult ($P < 0.0001$) stages, but not first (L1, $P = 0.57$) and fifth (L5, $P = 0.20$) larval instars. *Spodoptera frugiperda* lifespan was significantly shorter on average for insects reared on *B. bassiana* (LPSc 1098) colonized corn leaves (35.18 ± 15.05 days) compared to those reared non-colonized (40.85 ± 9.28 days) leaves ($P = 0.0013$; Table 2). Moreover, there was a significant interaction between treatment and sex for adult longevity ($P = 0.0002$), which was significantly reduced for females but not males reared on endophytically colonized leaves compared to control (Fig. 2).

Plant colonization with *B. bassiana* (LPSc 1098) significantly reduced *S. frugiperda* fecundity and oviposition period ($P < 0.0001$). Mean duration of oviposition period

Table 2 Mean (± SE) of mortality and length of different developmental stages of *Spodoptera frugiperda* fed on leaves of *Beauveria bassiana* (LPSc 1098) colonized (treated) and non-colonized (control) corn plants

Stage	Treated				Control			
	Length	n_x [a]	l_x [b]	d_x [c]	Length	n_x	l_x	d_x
Eggs	2.7 ± 0.46a[d]	100[e]	1	0	2.79 ± 0.40[a]	100	1	0
1st larval (L1)	3.43 ± 0.51a	100	1	6	3.41 ± 0.81[a]	100	1	3
2nd larval (L2)	3.21 ± 1.06b	94	0.94	15	3.96 ± 0.65[a]	97	0.96	4
3rd larval (L3)	3.35 ± 1.69b	79	0.79	2	3.81 ± 0.87[a]	93	0.93	3
4th larval (L4)	3.65 ± 1.91b	77	0.77	2	4.32 ± 1.37[a]	90	0.9	1
5th larval (L5)	3.69 ± 1.96a	75	0.75	0	3.7 ± 1.27[a]	89	0.89	0
6th larval (L6)	3.0 ± 1.16b	75	0.75	0	3.62 ± 1.97[a]	89	0.89	0
Total larval	20.95 ± 8.2b	75	0.75	0	22.2 ± 4.5[a]	89	0.89	0
Pupal	6.45 ± 1.34b	75	0.75	10	8.66 ± 2.80[a]	89	0.89	3
Adult	5.08 ± 3.56b	65	0.65	0	7.2 ± 2.74[a]	85	0.85	0
Total lifespan	35.18 ± 15.0b				40.85 ± 9.28[a]			
Sex ratio F:M	1:1.3				1:1.2			

[a] n_x = number of individuals at age x

[b] l_x = age-specific survival rate from birth to death (the number of days lived at age x)

[c] d_x = mortality (proportion of individuals of the original cohort that dies at age x)

[d] Means with different letters across treatments differ significantly at $P < 0.05$ (Student´s t test)

[e] Values obtained from the pooled data sets of two experimental repetitions

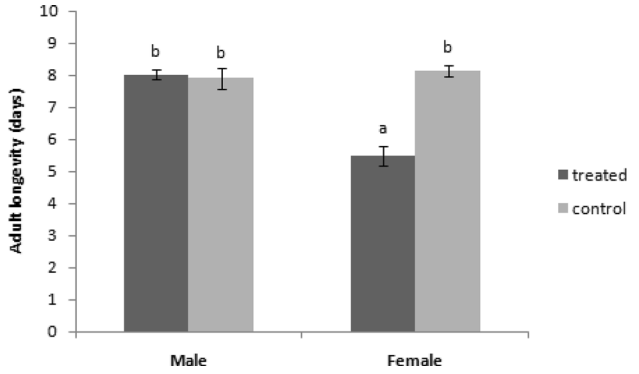

Fig. 2 Adult longevity of *Spodoptera frugiperda* males and females fed on leaves of *Beauveria bassiana* (LPSc 1098) colonized (treated) and non-colonized (control) corn plants. Bars indicate mean (±SE). Bars with different letters differ significantly at $P<0.05$ (Tukey's test after two-way ANOVA)

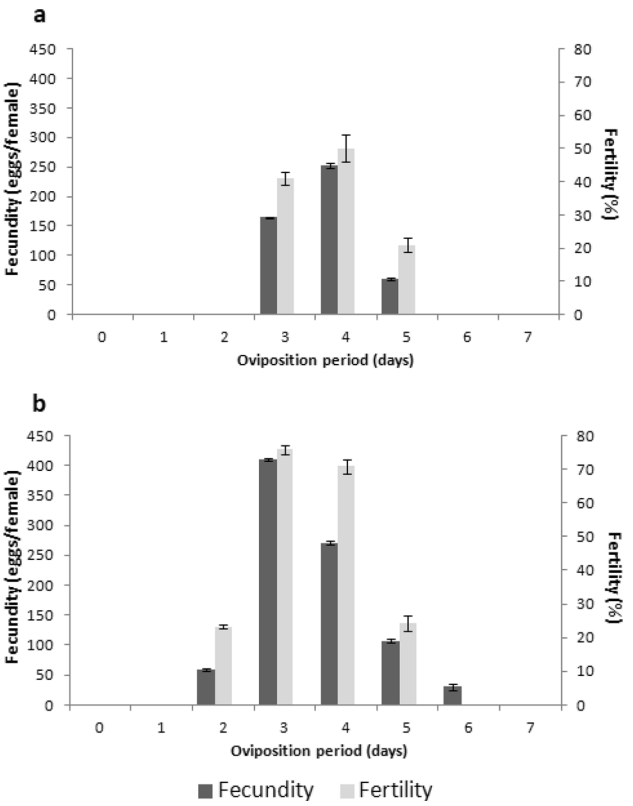

Fig. 3 Fecundity and fertility of *Spodoptera frugiperda* females fed on **a** leaves of *Beauveria bassiana* (LPSc 1098) colonized (treated) and **b** non-colonized (control) corn plants. Bars indicate mean (±SE)

was 2.2 ± 0.89 and 2.95 ± 0.51 days for females fed on *B. bassiana* (LPSc 1098) colonized and non-colonized leaves, respectively. Females deposited an average of 495.7 eggs with a range of 343–865 eggs when fed on colonized leaves (Fig. 3b), whereas females fed on non-colonized (control) leaves deposited an average 848 eggs with a range of 500–1100 eggs (Fig. 3a). Endophytic *B. bassiana* (LPSc 1098) also caused a highly significant decrease in mean fertility ($P<0.0001$) of *S. frugiperda* reared on colonized (43% ± 0.41; Fig. 3b) compared to non-colonized leaves (93% ± 0.04; Fig. 3a).

Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *S. frugiperda* are shown in Fig. 4a and b. Age-specific survival curve of *S. frugiperda* reared on leaves of colonized plants, but not on those of non-colonized controls, decreased noticeably at days 7 and 27 (Fig. 4a). The curve of age-specific fecundity showed that reproduction began after day 37, peaked to reach a maximum population growth rate on day 39, and ended on day 47 for insects reared on non-colonized leaves (Fig. 4b). On the other hand, the age-specific fecundity curve indicated a delay and a reduction in reproduction of insects reared on colonized leaves. For those insects, reproduction began after day 39, peaked on day 43, and ended on day 45 as shown in Fig. 4a.

Finally, significant differences (Student's test: $P<0.05$, Table 3) were also found between *S. frugiperda* population parameters calculated using the TWOSEX-MSChart. Endophytic colonization of plants with *B. bassiana* (LPSc 1098) significantly reduced *S. frugiperda* net reproductive rate (R_0) which was 107.5 offspring per individual in insects reared on colonized plants compared to 311.6 offspring per individual in those reared on non-colonized controls. Similarly, significantly lower values of the intrinsic rate of increase ($r=0.11$/day) and the finite rate of increase ($\lambda=1.12$/day) were obtained for insects fed on colonized plants (Table 3) than for those fed on control plants ($r=0.14$/day and $\lambda=1.15$/day).

Effect of endophytic colonization of corn on *S. frugiperda* food preference

Highly significant differences ($P<0.0001$, Fig. 5) were found in leaf area (mm^2) consumed by third instar larvae offered *B. bassiana* (LPSc 1098) colonized and non-colonized (control) leaves in a free-choice feeding experiment, indicating that the presence of *B. bassiana* (LPSc 1098) as an endophyte markedly reduced food preference and consequently consumption of corn plants by *S. frugiperda* (Fig. 5).

Discussion

All tested isolates of the fungal entomopathogens *B. bassiana*, *M. anisopliae*, and *M. robertsii* were able to colonize corn plants when inoculated by foliar spray, root dipping, or seed immersion, but percent plant colonization varied significantly among fungal isolates, inoculation methods, and plant tissues. Highest colonization rate of different plant tissues was obtained by *B. bassiana* isolate (LPSc 1098) using

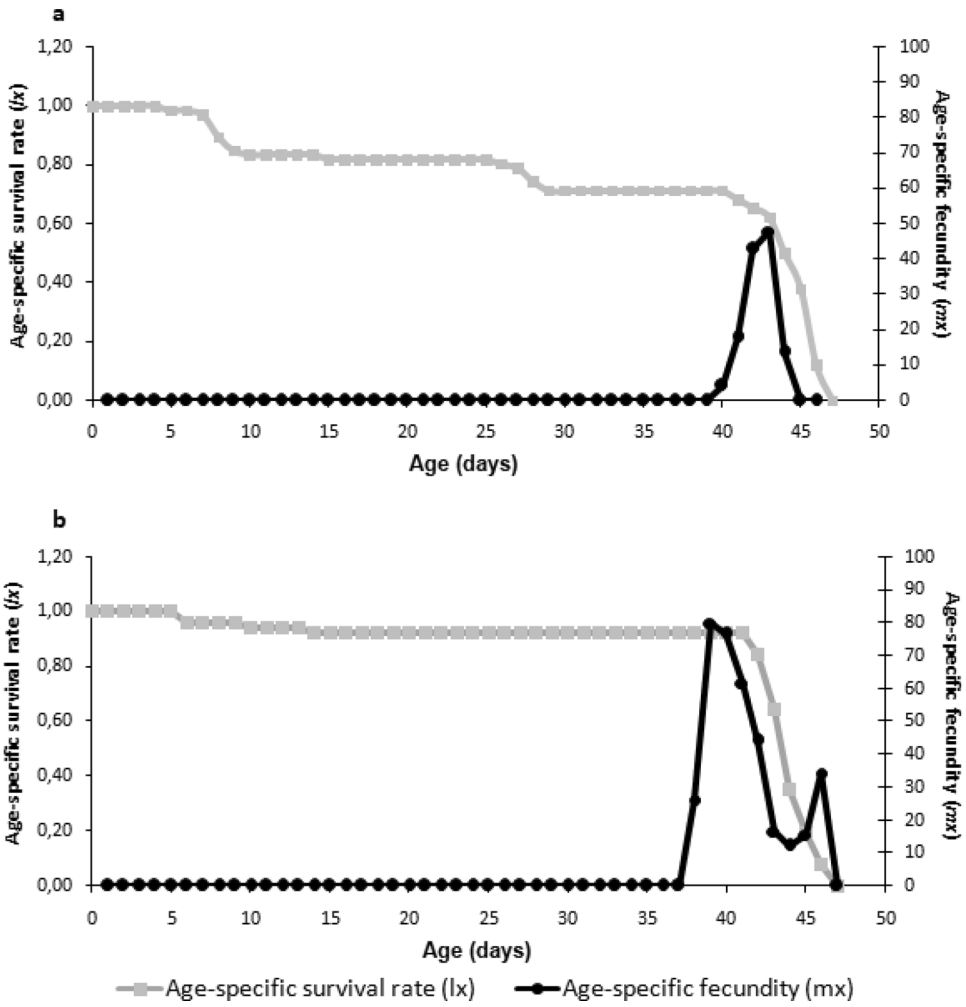

Fig. 4 Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *Spodoptera frugiperda* fed on **a** leaves of *Beauveria bassiana* (LPSc 1098) colonized (treated) and **b** non-colonized (control) corn plants

Table 3 Mean (±SE) of population parameters of *Spodoptera frugiperda* fed on leaves of *Beauveria bassiana* (LPSc 1098) colonized (treated) and non-colonized (control) corn plants

Parameter	Treated	Control	*T*	*P*
Net reproductive rate (R_0)	107.50 ± 30.5b	311.60 ± 57.5a	3.14	0.0024
Intrinsic rate of increase (r)	0.11 ± 0.01b	0.14 ± 0.01a	3.62	0.005
Finite rate of increase (λ)	1.12 ± 0.01b	1.15 ± 0.01a	3.46	0.008

Means (±SE) obtained by the Jackknife method embedded in the TWOSEX-MSChart (Chi 2008). Means followed by different letters within the same row differ significantly at $P < 0.05$ (Student´s t test)

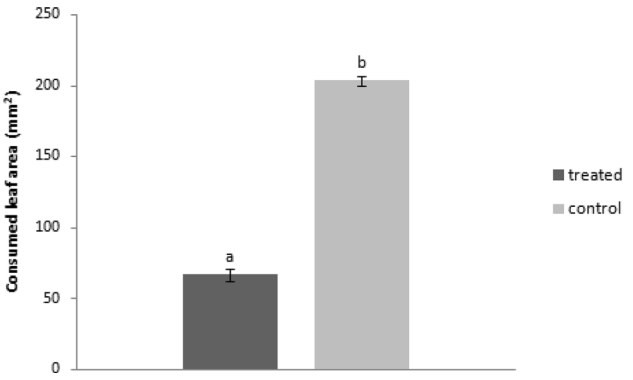

Fig. 5 Total area consumed (mm^2) by *Spodoptera frugiperda* third instar larvae freely offered *Beauveria bassiana* (LPSc 1098) colonized (treated) and non-colonized (control) leaves. Bars indicate mean (±SE). Mean values were obtained from the pooled data sets of three experimental repetitions. Bars with different letters differ significantly at $P < 0.05$ (Student´s t test)

foliar spray. When sprayed into plant foliage, this isolate systemically colonized 100, 80, and 60% of leaves, stems, and roots of plants, respectively, seven days after inoculation. Although there was a significant decline in plant colonization over time, regardless of fungal isolate or inoculation method, more than 90 and 50% of leaf and stem tissues,

respectively, remained colonized by *B. bassiana* (LPSc 1098) across all sampling dates at 7, 14, 21, and 28 days after foliar inoculation. Using light and electron microscopy, it has been previously shown how *B. bassiana* is able to penetrate the leaf surface and move within the xylem vascular elements throughout the corn plant from foliar inoculation sites (Wagner and Lewis 2000). Multipartite interactions with other bacterial and fungal endophytes naturally colonizing plant hosts might explain the inability of some of the tested fungal isolates to extensively colonize plant tissues or persist for long periods of time (Schulz et al. 2015). Our study further demonstrates that foliar spray was the best inoculation method for all tested *B. bassiana* isolates, but not the two *Metarhizium* isolates (LPSc 907 and LPSc 963), to enter and systemically colonize different plant tissues. This is not surprising given that *Metarhizium* species have been often reported to be almost exclusively restricted to plant roots, whereas *Beauveria* species are found throughout the plant with a higher prevalence in aboveground plant tissues (Behie et al. 2015). Indeed, our findings are in agreement with several previous studies (reviewed in Jaber and Ownley 2018 and Vega 2018) showing that the extent and persistence of plant colonization with fungal entomopathogens may well be influenced by fungal species and strain, host plant species and tissues, and inoculation method, among other factors. When applied as endophytes though, an extensive and persistent plant colonization by these entomopathogens would certainly constitute the basis for the degree of plant protection they confer against insect pests.

Our results, on the other hand, provide the first report for the negative effects of the presence of an entomopathogenic fungus, *B. bassiana*, as an endophyte on the survival, development, and reproduction of *S. frugiperda* feeding on leaves of colonized corn plants. Endophytic *B. bassiana* (LPSc 1098) caused significant reductions in 2nd instar larval and pupal survival, length of different developmental stages, and total *S. frugiperda* lifespan as exhibited by individuals reared on colonized compared to non-colonized leaves. Plant colonization also significantly reduced female longevity, fecundity and oviposition period duration, in addition to fertility. Due to the negative effects of endophytic *B. bassiana* (LPSc 1098), there was a noticeable decrease in age-specific survival rate as well as a delay and a decrease in age-specific fecundity rate of *S. frugiperda* when fed with leaves obtained from colonized plants. *S. frugiperda* individuals fed on colonized plants also had significantly lower net reproductive rate, intrinsic rate of increase, and finite rate of increase compared to individuals fed on non-colonized control plants. Mechanisms underlying endophytic entomopathogenic fungi-mediated adverse effects against herbivores are often attributed to production of secondary metabolites, induction of plant defenses, and mycosis (Vidal and Jaber 2015; Jaber and Ownley 2018; Vega 2018). In the present study, mycosis was evidenced by fungal growth emerging from surface-sterilized cadavers of insects fed with colonized leaves after being placed in dark moist chambers. A number of previous studies have similarly reported insect mycosis following feeding on *B. bassiana*-endophytically colonized plants by other chewing lepidopteran pests such as *Helicoverpa zea*, *Helicoverpa armigera*, and *Tuta absoluta* (Powell et al. 2007, 2009; Vidal and Jaber 2015; Klieber and Reineke 2016). Alternatively, Shrivastava et al. (2015) reported that the antiherbivore properties of endophytic *B. bassiana* against the beet armyworm *S. exigua* might be partly due to higher levels of terpenoids which were induced in colonized tomato plants. Although not tested in our study, the possibility that plant colonization with *B. bassiana* may have also led to enhanced levels of corn terpenoid defense compounds against *S. frugiperda* cannot be excluded.

The negative effects of fungal entomopathogens when introduced as endophytes against insect pests could also be a result of antibiosis and feeding deterrence by fungal secondary metabolites produced *in planta* as has been widely proposed by several studies (reviewed in Jaber and Ownley 2018). However, only a very few of these studies have actually detected such metabolites in plants colonized by entomopathogenic fungi (Jaber and Ownley 2018; Vega 2018). For example, Resquín-Romero et al. (2016) detected traces of destruxin A in *M. brunneum*-colonized tomato leaves and also within *S. littoralis* larvae fed discs obtained from those leaves. In our study, the significant detrimental effect of corn colonization with *B. bassiana* on *S. frugiperda* food preference, as indicated by the marked reduction in leaf area consumed by third instar larvae when offered a choice to feed on colonized or control leaves, could possibly be caused by antifeedant or deterrent properties of *in planta*-produced *B. bassiana* metabolites (reviewed in Ownley et al. 2010). Cherry et al. (2004) proposed a similar explanation for the reduced tunneling and feeding damage observed by the corn stem-borer, *Sesamia calamistis*, on *B. bassiana*-colonized corn plants, whereas Bing and Lewis (1991) attributed reduced tunneling by another corn stem-borer, *Ostrinia nubilalis*, to the systemic and persistent *B. bassiana* colonization of plants. In the latter study, the fungal isolate (ARSEF 3113) was recovered from most internal plant tissues and provided the greatest level of *O. nubilalis* suppression until harvest only when inoculated into corn via foliar application (Bing and Lewis 1991). Such a possibility of applying small amounts of fungal inoculum and obtaining a systemic season-long protection against insect pests makes endophytism a cost-effective delivery route for entomopathogenic fungal biocontrol agents. However, in order to ensure prolific and persistent levels of plant colonization, particular attention should be paid to selecting fungal isolates and inoculation methods most adapted to introducing these entomopathogens as endophytes into specific host

plants (Jaber and Ownley 2018). Notably, when the same fifteen fungal isolates and three inoculation methods tested here were investigated in soybean by Russo et al. (2018a), *B. bassiana* (LPSc 1098) was the most successful plant colonizer using foliar spray as well. The study further demonstrated that foliar inoculation with *B. bassiana* (LPSc 1098) promoted the growth and increased the yield of soybean plants under filed conditions (Russo et al. 2018a). The same *B. bassiana* isolate was also shown to negatively affect the survival, development, and reproductive parameters of the soybean pest *Helicoverpa gelotopoeon* following endophytic establishment by foliar spray (Russo et al. 2018b). Similar to the results obtained in the present study, endophytic colonization of soybean plants by *B. bassiana* (LPSc 1098) caused significant reduction in leaf area consumed by this pest. This fungal isolate was originally isolated from *Triatoma infestans* Klug (Hemiptera: Reduviidae) in Chaco Province, Argentina and is particularly characterized by high sporulation rate and biocidal capacity, which are major determinants of fungal virulence against insect pests (Pelizza et al. 2012a, b, 2018). Such superior endophytic fungal isolates with high virulence against one or more pests in addition to growth promotion potential could possibly be developed as biocontrol agents against multiple pests as well as biofertilzers for wider application in IPM programs and sustainable agriculture (Jaber and Ownley 2018).

Previous studies reported on the pathogenicity of *B. bassiana* isolates against *S. frugiperda* eggs and early larval instars when topically applied to immature stages of the insect (Ramirez-Rodriguez and Sánchez-Peña 2016; Akutse et al. 2019). The present study demonstrates, for the first time, the adverse effects of *B. bassiana* (LPSc 1098) introduced into corn plants as an endophyte on several growth and reproductive parameters in addition to the survival and food preference of *S. frugiperda* when fed on colonized plant tissues. Although these effects were only investigated in greenhouse trials, our results highlight the promising potential of incorporating entomopathogenic fungi as endophytes in IPM practices to protect corn plants against this invasive pest if their efficacy is confirmed under field conditions as well.

Acknowledgements This study was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP 0018; PIP 0205), Agencia Nacional de Promoción Científica y Tecnológica (PICT 2018-2100), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), and Universidad Nacional de La Plata (UNLP, 11/N 903). Comments from the Editor-in-Chief (Nicolas Desneux) and three anonymous reviewers are appreciated.

Authors contribution MLR, SAP, and LRJ conceived and designed research. MLR, FV, ACS, and MNC conducted experiments and analyzed data. MLR and LRJ wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed while conducting this research.

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