FUNGAL ECOLOGY 6 (2013) 293–301

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Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae)

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ARTICLE INFO

Article history:
Received 27 September 2012
Revision received 23 November 2012
Accepted 7 December 2012
Available online 9 April 2013
Corresponding editor: Fernando Vega

Keywords:
Beauveria
Colonization
Fungal endophytes
Fusarium
Gibberella
Host plant
Hypocrea
Leafminer flies
Negative effects
Trichoderma

ABSTRACT

Ten fungal isolates belonging to the genera *Beauveria* (3), *Hypocrea* (1), *Gibberella* (1), *Metarhizium* (2), *Trichoderma* (1) and *Fusarium* (2) were evaluated in the laboratory to determine whether they could become endophytic in two pea leafminer (*Liriomyza huidobrensis*) host plants (*Vicia faba* and *Phaseolus vulgaris*), and to assess their possible negative effects on leafminers. *Beauveria* (ICIPE279), *Hypocrea*, *Gibberella*, *Fusarium* and *Trichoderma* isolates colonized roots, stems and leaves of both host plant species. *Beauveria* isolates G1LU3 and S4SU1 colonized roots, stems, and leaves of *P. vulgaris* but only the root and stem of *V. faba*. Isolates of *Metarhizium* failed to colonize the two host plants. The effects of endophytically colonized fungal pathogens on mortality, oviposition, emergence and longevity of *L. huidobrensis* were investigated after endophytic colonization of *V. faba* plants. All the fungal isolates that succeeded in colonizing the host plant were pathogenic to *L. huidobrensis*, causing 100 % mortality within 13.2 ± 0.7–15.0 ± 0.6 d. However, *Hypocrea* outperformed the other isolates ($p < 0.0001$) in reducing longevity of the progeny (11.2 ± 1.0 vs. 17.8 ± 1.4 d in the control), the number of pupae (80.0 ± 6.7 vs. 387.0 ± 21.7 pupae in the control), and adult longevity (3.8 ± 1.0 vs. 9.9 ± 1.8 d in the control). Adult emergence was significantly reduced ($p < 0.0001$) in *Hypocrea* (21.4 %) and *Beauveria* (38.0 %) treatments compared to the control (82.9 %).

Introduction

Liriomyza spp. (Diptera: Agromyzidae) leafminers are exotic pests of horticultural crops in Africa and have invaded large parts of the continent from the New World (Murphy & LaSalle 1999). The most economically important species include *Liriomyza sativae*, *Liriomyza trifolii* and *Liriomyza huidobrensis* (Chabi-Olaye *et al.* 2008). *Liriomyza* species are listed as quarantine pests in overseas markets, especially the European Union (EPPO 2006), and therefore prevent access of Kenyan horticultural products to new market opportunities (KEPHIS 2006). Yield losses of 20–100 % have been reported, depending on crop and location (EPPO 2006). The currently adopted management strategy for their control is largely based on use

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http://dx.doi.org/10.1016/j.funeco.2013.01.003

of synthetic chemical insecticides. In many cases pesticides are not effective due to the development of resistance, elimination of natural enemies and pose health risks due to pesticide residues. Biological control through the use of parasitoids and fungal entomopathogens is being considered as part of an integrated strategy for leafminer management in Kenya (Migiro *et al.* 2010). Fungal entomopathogens are generally applied in an inundative approach in the crop (Lacey & Goettel 1995). The high cost of fungal production is a limiting factor for the adoption of the technology by growers, in addition to short survival of the inoculum in the environment. Another biocontrol strategy based on fungal entomopathogens consists of disseminating the pathogen among target pest populations by using devices that attract insects to baited stations where they are contaminated with the pathogen and then return to the environment where they can transmit the pathogen to healthy individuals (Vega *et al.* 2007). Such an approach was evaluated against *L. huidobrensis* by Migiro *et al.* (2010). It is now established that many fungal entomopathogens play additional roles such as endophytes in nature (Vega *et al.* 2009). Fungal endophytes are heterotrophic microorganisms that live inside plants primarily for nutrition, protection and reproduction (Carroll 1988; Azevedo *et al.* 2000; Backman & Sikora 2008). They have been isolated from many crops including wheat (Larran *et al.* 2002a), bananas (Pocasangre *et al.* 2000; Cao *et al.* 2002), soybeans (Larran *et al.* 2002b), coffee (Vega *et al.* 2008), and tomatoes (Larran *et al.* 2001). Among the fungal entomopathogens, *Beauveria bassiana* (Hypocreales: Clavicipitaceae) has been reported as an endophyte in maize (Bing & Lewis 1991, 1992a, b; Lomer *et al.* 1997; Cherry *et al.* 2004; Orole & Adejumo 2009), sugar beet (Fuller-Schaefer *et al.* 2005), banana (Akello *et al.* 2008a), coffee (Vega *et al.* 2008), tomato (Leckie 2002; Ownley *et al.* 2004) and cotton (Ownley *et al.* 2004). *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) has been recorded as an endophyte of sugar beet (Fuller-Schaefer *et al.* 2005) and maize (Akello unpublished data).

Some fungal endophytes protect host plants against plant pathogens (Ownley *et al.* 2010) and herbivores, including insects (Arnold *et al.* 2003; Arnold & Lewis 2005; Schulz & Boyle 2005; Rudgers *et al.* 2007; Vega 2008; Vega *et al.* 2008). For example, exposure of two aphid species, *Rhopalosiphum padi* and *Metopopophium dirhodum* (Hemiptera: Aphididae), and wheat stem sawfly *Mayetiola destructor* (Diptera: Chloropidae) to wild barley infected with *Neotyphodium coenophialum* (Hypocreales: Clavicipitaceae) reduced their survival (Clement *et al.* 1994, 2005). Wheat leaves colonized by either *B. bassiana* or *Aspergillus parasiticus* (Eurotiales: Trichocomaceae) reduced the growth rate of *Chortoicetes terminifera* (Orthoptera: Acrididae) nymphs (Gurulingappa *et al.* 2010). Endophytic *B. bassiana* in banana significantly reduced larval survivorship of banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae), resulting in 42–87 % reduction in plant damage (Akello *et al.* 2008b). Reduction in feeding and reproduction by *Aphis gossypii* (Hemiptera: Aphididae) has also been reported on cotton endophytically colonized by either *B. bassiana* or *Lecanicillium lecanii* (Hypocreales: Clavicipitaceae) (Gurulingappa *et al.* 2010). Another possible role for fungal endophytes could include plant growth promotion as well as impact on tritrophic interaction (Harish *et al.* 2008; Vega *et al.* 2008; Paparu *et al.* 2009). Fungal entomopathogens that become established as endophytes can, therefore, play an important role in the regulation of insect populations. The objectives of this study were to determine whether selected fungal isolates are able to endophytically colonize *Vicia faba* and *Phaseolus vulgaris* plants, and to assess their negative effects on the life history of *L. huidobrensis* following endophytic colonization of the host plant.

Materials and methods

Fungal cultures

Ten fungal isolates in six genera, obtained from the International Centre of Insect Physiology and Ecology (*icipe*)'s Arthropod Germplasm Centre, were used in this study: *Beauveria* (3), *Hypocrea* (1), *Gibberella* (1), *Metarhizium* (2), *Trichoderma* (1) and *Fusarium* (2). Eight of the fungal isolates were isolated from the aboveground parts of maize, sorghum and Napier grass and were able to endophytically colonize maize and bean seedlings (Akello 2012). *B. bassiana* isolate ICIPE279 and *Metarhizium anisopliae* isolate ICIPE30 were isolated from a Coleopteran larva and *Busseola fusca* pupa, respectively. The isolates were cultured on potato dextrose agar (PDA), except *Metarhizium* which was cultured on Sabouraud dextrose agar (SDA), and were maintained at 25 ± 2 °C in complete darkness. Conidia were harvested by scraping the surface of 2–3-week-old sporulating cultures with a sterile spatula. The harvested conidia were then mixed in 10 ml sterile distilled water containing 0.05 % Triton X-100 and vortexed for 5 min to produce homogenous conidial suspensions. Conidial counts were made using a Neubauer Hemacytometer (Goettel & Inglis 1997). The conidial suspension was adjusted to 1×10^8 conidia ml^{-1} through dilution prior to inoculation of seeds.

Spore viability was determined before any bioassay by plating 0.1 ml of 3×10^6 conidia ml^{-1} onto 9-cm Petri dishes containing SDA or PDA. A sterile microscope cover slip (2 × 2 cm) was placed on the top of the agar in each plate. Plates were incubated in complete darkness at 25 ± 2 °C and were examined after 16–20 hr. The percentage germination of conidia was determined from 100 randomly selected conidia on the surface area covered by each cover slip under the light microscope (400×) using the method described by Goettel & Inglis (1997). Conidia were deemed to have germinated when the length of the germ tube was at least twice the diameter of the conidium. Four replicates were used for each isolate.

Plant inoculation and colonization of endophyte isolates

Inoculation was done by soaking seeds of *V. faba* (a local Kenyan Open Pollinated variety) and *P. vulgaris* (Brown Rose Coco) in conidial suspensions titrated at 10^8 ml^{-1} for 2 hr. Prior to inoculation, seeds were surface-sterilized in 70 % ethanol for 2 min followed by 1.5 % sodium hypochlorite for 3 min and rinsed with sterile distilled water three times. For the controls, sterilized seeds were soaked in sterile distilled water for 2 hr. The last rinse water was plated out to assess the effectiveness of the surface sterilization procedure (Schultz *et al.* 1998). Seeds