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Effect of infection by *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) on the feeding and oviposition of the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) on different host plants

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ABSTRACT

The effect of fungal infection by *Metarhizium anisopliae* on feeding and oviposition of adult *Liriomyza huidobrensis* was examined on three host plants, faba bean (*Vicia faba*), French bean (*Phaseolus vuklgaris*) and snow pea (*Pisum sativum*) in the laboratory. Flies were contaminated with dry conidia and allowed to feed and oviposit on the different host plants. Mortality in *L. huidobrensis* varied between 14% and 20% in the controls and between 77% and 100% in fungal treatments 120 h post-infection for the three host plants. *L. huidobrensis* made more punctures (47.3–52.6 cm^{-2}) in the control than in the fungal treatments (23.1–26.9 cm^{-2}) for the three host plants. The cumulative average number of punctures cm^{-2}/female by *L. huidobrensis* was higher in the controls than in fungal treatments from 72 h post-treatment in faba bean (12.2 vs. 8.2) and French bean (14.8 vs. 8.9), and from 48 h post-inoculation in snow pea (8.5 vs. 5.7). Female *L. huidobrensis* laid more eggs in the control (0.6–6.1) than in fungal treatments (0.2–1.5) across the host plants tested. The cumulative mean number of eggs cm^{-2}/female was significantly higher in the controls than in fungal treatments from 48 h post-treatment in faba bean (0.4 vs. 0.2) and French bean (0.1 vs. 0), and 96 h post-inoculation in snow pea (0.2 vs. 0.1). The host plant did not affect the average total number of punctures but had a significant effect on egg laying, with faba bean harboring greater number of eggs in both control and fungal treatments. A proper timeline application of the fungus before onset of feeding and oviposition peaks will be crucial in field suppression of the pest using *M. anisopliae*. In addition, a great consideration must be given to the target host plants prior to application of the fungus.

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1. Introduction

The pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), is an economically important pest of a variety of flower and vegetable crops worldwide (Spencer, 1973; Weintraub and Horowitz, 1995; Wei et al., 2000; Martin et al., 2005; Mckee et al., 2009). It is an invasive species in Africa and believed to be native to the western United States, attacking horticultural crops from over 14 plant families (Spencer, 1990; Wei et al., 2000; Martin et al., 2005; Mckee et al., 2009). The invasive nature and widespread occurrence of *L. huidobrensis* are attributed to its polyphagous nature, multivoltinism, and the extreme capacity to develop resistance to different pesticides (MacDonald, 1991; Shepard et al., 1998). Adults damage crops by puncturing the leaf surfaces to feed and lay eggs into the leaf tissue (Parrella, 1987). When the eggs hatch, the larvae tunnel within the leaf tissue and form damaging and disfiguring mines (Parrella, 1987). Due to the widespread and significant damage that *Liriomyza* leafminers cause on various crops around the world, there is a need to develop environmentally sound and sustainable methods of control for this pest.

Biological control of *Liriomyza* leafminers has mainly focused on the use of parasitoids (Waterhouse and Norris, 1987; Johnson, 1993; Murphy and La Salle, 1999). However, the potential of entomopathogenic fungi as biological control agents for dipteran *Liriomyza* has received increasing attention (Bordat et al., 1988; Borisov and Ushchekov, 1997; Migiro et al., 2010). Recently, Migiro et al. (2010) reported on the virulence of some of isolates of the entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin against *L. huidobrensis*. Although virulence of a pathogen is generally measured in terms of host mortality, factors such as feeding and reproduction potential that can be affected by fungal infection are equally important and need to be considered when evaluating a fungal pathogen (Fargues et al., 1991, 1994; Moore et al., 1992; Seyoum et al., 1994; Thomas et al., 1997; Ekesi and Maniania,

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2000; Ondiaka et al., 2008). The virulence of an entomopathogen may also be affected by the host plants or cultivars (Cory and Hoover, 2006). The objective of our study was to evaluate the effects of the fungal pathogen, *M. anisopliae* isolate ICIPE 20, on oviposition and feeding of *L. huidobrensis* on three different host plants: faba *bean,* French bean, and snow pea.

2. Materials and methods

2.1. Host plants

Three host plants namely faba bean, *Vicia faba* L. (var. *minor*), snow pea, *Pisum sativum* L. (var. *Oregon II*) and French bean, *Phaseolus vulgaris* L. (var. *Samantha)* (Fabales: Fabaceae) were used in the experiments. They were grown in a screen house (2.8 m length × 1.8 width × 2.2 m height) in 15 cm pots (5–8 plants per pot) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, using a mixture of compost manure/cow dung and clay soil in a ratio of 1:5, respectively. Plants used in the experiment were 2 week old.

2.2. Insects

Adult *L. huidobrensis* flies were obtained from the *icipe* Animal Rearing and Quarantine Unit, Nairobi, Kenya. The colony had originally been reared on faba bean for over 40 generations. Before use in the experiments, the leafminers were further reared separately for three generations on the three target host plants prior to the setting of this experiment following a modified method by Zhao and Kang (2000). Five hundred 2- to 3-day-old mated adult leafminers were released onto the different host plants and allowed to lay eggs for 24 h. The adults were then aspirated out of the rearing cages and plants containing eggs were held until pupae formation. The pupae falling off the leaves were collected and transferred into Perspex cages (150 × 150 × 200 mm) for adult emergence. Emerged adults were released onto fresh plants and the process repeated for at least three successive generations. The colonies were maintained in a rearing room at 25–27°C, 60–80% R.H. and 12 L:12 D photoperiod. Adults were fed a 10% sugar solution soaked on balls of cotton wool and placed at the bottom corner of the rearing cages. In all bioassays, 1- to 2-day-old naive adult flies were used.

2.3. Fungal isolate

M. anisopliae isolate ICIPE 20 used in this study has been reported earlier to be virulent to *L. huidobrensis* (Migiro et al., 2010). The isolate was cultured on Sabouraud Dextrose Agar (SDA) in 9-cm Petri dishes and incubated at 25 ± 2°C in complete darkness. Conidia were harvested from 3-week-old cultures by scraping using a clean sterile spatula. The viability of conidia was determined before any bioassay by spread-plating 0.1 ml of suspension titrated 3×10^6 conidia ml^{-1} onto 9-cm Petri dishes containing SDA medium. A sterile microscope cover slip was placed on each plate, and the plates were incubated in complete darkness at 25 ± 2°C and examined after 20 h. Percentage germination of conidia was determined by assessing whether a germ tube had formed in 100 random conidia on the surface area covered by each coverslip under the light microscope (400 X). Four replicate plates of the isolate were maintained.

2.4. Inoculation of insects

Two-week-old plants of the three hosts were cut at the stem with each shoot left with only four leaves. The foliage was cleaned with a solution of sodium hypochlorite (2%) and rinsed thrice in sterile distilled water. For each host plant, two shoots were placed in a 20-ml universal bottle containing tap water to prevent them from drying. The bottles were then plugged with cotton wool to prevent flies from drowning and to maintain humidity near saturation in the bottles. Each bottle was transferred into a clean ventilated Perspex cage (150 × 150 × 200 mm). Adult flies were contaminated using the technique described by Migiro et al. (2010). Briefly, flies were exposed to 0.1 g of dry conidia evenly spread on a cotton velvet cloth covering the inner side of a cylindrical plastic tube (70 mm length × 48 mm diameter). For each host plant, 20 flies (10 males and 10 females) were transferred into the cylindrical tube and allowed to walk on the velvet for 1 min, after which five of the male and female insects were transferred from the velvet into the cages containing the different host plants. Insects in the control treatments were exposed to fungus-free velvet cloth before being transferred into similar cages. The treatments were maintained at 25 ± 2°C, 60–70% RH and 12 L:12 D photoperiod. The experiment was replicated five times with 10 flies (5 males + 5 females)/host plant/replicate. Test insects were provided with fresh shoots after 24, 48, 72, 96, 120 h post-inoculation. The experiment was repeated twice. Flies surviving fungal infection for the different post-infection times were recorded. Dead flies were removed from the container, surface-sterilized and placed into 9-cm diameter Petri dish lined with moistened filter paper to favor development of mycosis on the surface of the cadaver. Only mortality by mycosis was considered for analysis. Leaves containing eggs and punctures were cut off the shoots daily, transferred by treatments into Petri dish which was sealed with Parafilm, placed in a plastic bag and stored at 4 °C until samples were processed. The number of feeding punctures and eggs was recorded using a modified egg staining technique described by Simonet and Pienkowski (1977) for potato leafhopper in alfalfa. Five hundred ml of lactophenol and acid fuchsin solution was made by mixing 100 ml distilled water, 100 ml lactic acid, 200 ml glycerin, 100 ml melted phenol crystals and 0.5 g of acid fuchsin stain. The solution was heated to 95 °C, after which leaves were dipped into the boiling solution for 5 min. The stained leaves were then transferred into 9-cm glass Petri dishes and left overnight. To remove excess stain, leaves were rinsed four times with warm water. Eggs and punctures on the entire top and bottom leaf surfaces for different time intervals were counted using a dissecting microscope at 85 X magnification. Since leaf size varied with host plant, leaf area was converted into cm^2 unit area for comparison purpose. Forty leaves per host plant were randomly selected and scanned (Canoscan LiDE 50) and the pixels corresponding to the leaf image were recorded using Adobe Photoshop. The leaf area in pixels was then converted to square centimeters (cm^2).

2.5. Statistical analyses

Percent mortality data were corrected for control mortality (Abbott, 1925) and normalized by arcsine transformation (Sokal and Rohlf, 1981) before being subjected to analysis of variance (ANOVA) using PROC GLM. Student–Newman–Keul's (SNK) was used to separate the means as a post-ANOVA procedure. *t*-test was used to compare percent mortality between the infected and uninfected *L. huidobrensis*. Data on the overall number of punctures and eggs cm^{-2} among host plants were analyzed using PROC GLIMMIX with a normal error distribution and an identity link function. Following a significant *F* test, means were separated by the macro PDmix 800 for SAS (Saxton, 1998) using the Tukey–Kramer comparison procedure. The cumulative mean number of eggs and punctures per cm^{-2}/female between the infected and uninfected-*L. huidobrensis* at the different times post- infection for the different host plants was analyzed using the Student *t*-test. All analyses were performed using the SAS version 9.2 (SAS Institute, 2003) and

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