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Interactions between *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae), parasitoids of *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae)

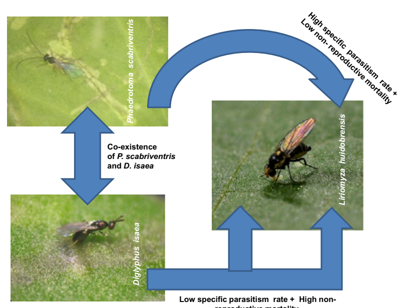
K.S. Akutse [a,b], J. Van den Berg [b], N.K. Maniania [a], K.K.M. Fiaboe [a,*], S. Ekesi [a]

[a] International Centre of Insect Physiology and Ecology (icipe), P. O. Box 30772-00100, Nairobi, Kenya
[b] Unit of Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

HIGHLIGHTS

- *Phaedrotoma scabriventris* used alone: high specific parasitism + low non-reproductive mortality.
- *Diglyphus isaea* used alone: low specific parasitism + high feeding-stinging mortality.
- *Phaedrotoma scabriventris* and *D. isaea* are predicted to be able to co-exist.
- Combination of *P. scabriventris* and *D. isaea*: high total parasitism + high total non-reproductive mortality.

GRAPHICAL ABSTRACT



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ABSTRACT

Liriomyza leafminer flies represent a serious threat to horticultural production in East Africa. Total field parasitism rates recorded in Kenya are below 5%, with the indigenous ectoparasitoid *Diglyphus isaea* Walker being one of the key parasitoid species. The International Centre of Insect Physiology and Ecology (*icipe*), in collaboration with the International Potato Centre (CIP), imported into Kenya the endoparasitoid *Phaedrotoma scabriventris* Nixon to improve natural control of leafminers. The objective of this study was to investigate the interactions between *D. isaea* and *P. scabriventris* when used together for the biological control of *Liriomyza* species. These interactions were studied under laboratory conditions, using treatments that involved single, simultaneous and sequential releases of the different parasitoid species onto plants infested by *L. huidobrensis* larvae. While used separately, parasitism rates of *D. isaea* and *P. scabriventris* were 30.4 ± 10.9% and 63.6 ± 7.7% respectively. However, when used simultaneously, the total parasitism rate increased to 77.0 ± 5.3%. Although *P. scabriventris* had no effect on *D. isaea*, the presence of *D. isaea* reduced the specific parasitism rate of *P. scabriventris*. In addition, both parasitoids induced leafminer mortality through larval-feeding and stinging. However, feeding and stinging mortality induced by *D. isaea* (41.9 ± 9.1%) was significantly higher compared to *P. scabriventris* (11.9 ± 8.7). Similarly, pupal mortality due to feeding and stinging activity was 49.1 ± 6.5% and 21.6 ± 1.9% when exposed to *D. isaea* and *P. scabriventris* respectively. The implication for simultaneous use of both parasitoids in East Africa is discussed.

* Corresponding author. Fax: +254 20 8632001.
E-mail address: kfiaboe@icipe.org (K.K.M. Fiaboe).

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1. Introduction

The growth of the horticultural industry in Kenya is limited by a number of arthropod pests. Among these are the invasive leafminer species *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard and *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). These pests pose the greatest challenge as they damage major vegetable and ornamental crops, serve as vectors for plant pathogens and constitute quarantine pests in European markets (Johnson et al., 1983; Parrella et al., 1984; Matteoni and Broadbent, 1988; Chandler and Thomas, 1991; Kapadia, 1995; Deadman et al., 2002; Chabi-Olaye et al., 2008; EPPO, 2009).

The management of leafminers worldwide, and particularly in East Africa, has commonly relied on the routine use of synthetic chemical insecticides (Chandler, 1981, 1984; Gitonga et al., 2010). However, the indiscriminate and frequent use of these chemicals resulted in insecticide resistance of flies (Parrella et al., 1984; Murphy and LaSalle, 1999), pollution of the environment as well as elimination of their natural enemies (Johnson et al., 1980). Chemical control is also not very effective since flies usually escape insecticide applications due to their high mobility. Furthermore, *Liriomyza* larvae are inaccessible to many pesticides because they develop inside leaves and pupate in soil (Mujica and Kroschel, 2011, 2013). The horticultural industry is also under additional pressure since the introduction of a maximum residue level (MRL) of pesticides on export produce set up by the European Union.

Biological control using parasitoids and entomopathogenic fungi is being considered as alternative to leafminer management in East Africa (Chabi-Olaye et al., 2008, 2013; Migiro et al., 2010). Surveys carried out in Kenya between 2007 and 2008 indicated the presence of various indigenous leafminer parasitoid species. The most important were *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), *Diglyphus isaea* Walker, *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) (Chabi-Olaye et al., 2008). However, according to Chabi-Olaye et al. (2008), the total parasitism rate of these species in the field was below 5%. It is within this context that the koinobiont larval endoparasitoid, *Phaedrotoma scabriventris* Nixon (Braconidae: Opiinae) was introduced into *icipe*'s quarantine facilities in Kenya during 2008 to improve the total parasitism rates achieved by the indigenous natural enemy complex in Kenya (Chabi-Olaye et al., 2013).

P. scabriventris is the most important leafminer parasitoid in Argentina, Brazil and Peru where it has been reported to cause mortality of between 20% and 52% (Valladares et al., 1999, 2001; Kroschel, 2008). In Kenya, all the three important *Liriomyza* species (*L. huidobrensis*, *L. sativae* and *L. trifolii*) were found acceptable and suitable for the development of *P. scabriventris* although the parasitoid preferred *L. huidobrensis* (Chabi-Olaye et al., 2013). *P. scabriventris* was therefore considered for release against leafminer flies in the horticulture production systems in East Africa. Since *D. isaea* is one of the most important larval parasitoids found in Kenya, Uganda and Tanzania (Chabi-Olaye et al., 2008; our unpublished data), understanding the interactions between this parasitoid and the exotic *P. scabriventris* is important. A range of interactions have been described between parasitoids. These include exploitative competition, interference competition with priority effects, cleptoparasitism, facultative hyperparasitism, obligate hyperparasitism, complementarity, additive and synergetic effects (Mills, 2003). Many synovigenic parasitoids use their host insects not only as oviposition sites but also as food sources, a behavior called "host-feeding"; to improve their longevity, fecundity, and searching efficiency (DeBach, 1943; Syme, 1977; Jervis and Kidd, 1986). Liu et al. (2013) reported that *D. isaea* caused the death of host larvae not only by reproductive parasitizing host-killing, but also by non-reproductive host-killing through feeding and/or stinging without feeding and oviposition. Females prefer to parasitize larger larvae but also feed on smaller host larvae. Mafi and Ohbayashi (2010) also reported that feeding and stinging without oviposition by *Sympiesis striatipes* Ashmead (Hymenoptera: Eulophidae), an ectoparasitoid of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), killed 45 host larvae per female parasitoid, of which approximately 60% was caused by host feeding or stinging activity of adult females.

In addition to understanding the dynamics of interactions for the selection of effective biocontrol agents, there is need to evaluate risks posed by potential direct and indirect non-target impacts from the introduced natural enemies within an agricultural environment (Mackauer, 1990; Follett and Duan, 1999; Cusumano et al., 2011). The objective of this study was to investigate interactions between the ectoparasitoid *D. isaea* and the introduced endoparasitoid *P. scabriventris* with the aim of combined application for the biological control of *Liriomyza* species in horticultural production systems in East Africa.

2. Materials and methods

2.1. Host plants

A Kenyan open pollinated variety of Faba bean, *Vicia faba* L. (Fabales: Fabaceae) was used for the rearing of *L. huidobrensis*. Seeds were planted in plastic pots (8 cm diameter × 7.5 cm high) filled with the planting substrate (mixture of soil and manure 5:1 in a ratio). Pots were maintained in a screen-house (2.8 m length × 1.8 m width × 2.2 m height) at 25 ± 3 °C for 2 weeks. Two week-old plants were used for exposure to adult *L. huidobrensis*.

2.2. Insects

2.2.1. L. huidobrensis

L. huidobrensis was obtained from the Animal Rearing and Quarantine Unit (ARQU) of *icipe*. The colony originated from adult leafminers collected from wild crucifers on the *icipe* campus (01°13.3′S 36°53.8′E, 1600 m a.s.l.), reared on *V. faba* for 8–10 generations prior to experiments. Rearing colonies were maintained at 27 ± 2 °C with a photoperiod of 12L:12D and relative humidity of approximately 40%. In addition to the *V. faba* plant on which *L. huidobrensis* adults fed, a 10% sucrose solution was also provided. In order to obtain leafminer-infested plants with larvae of the appropriate size (2nd and 3rd instars) for parasitoids release, 10 potted plants were exposed to 200 two day-old *L. huidobrensis* adults (sex ratio 1:2 males:females) in Plexiglas cages (50 cm × 50 cm × 45 cm) for 24 h. Plants were removed and transferred to similar cages free of adult leafminers to allow development of larvae until the 2nd to 3rd instar (5–8 days post-exposure) before introducing the parasitoids. The exposed-infested potted plants with 2nd and 3rd instar larvae were used for experiments. Each Plexiglas cage contained 10 potted plants and represented a replicate and was maintained at 25–27 °C, at 50–60% RH and 12L:12D photoperiod. The trial was replicated five times.

2.2.2. D. isaea

The ectoparasitoid *D. isaea* used in the experiments was also obtained from the ARQU at *icipe*. The colony originated from adult *D. isaea* collected from leafminer-infested French bean, tomatoes and crucifers at Naivasha (S: 00.66731°; E: 036.38603°; Elevation: 1906 m a.s.l.), Kenya. *D. isaea* were reared on *L. huidobrensis*-infested *V. faba* in Plexiglas cages (50 cm × 50 cm × 45 cm) for 5–10