

# Interaction between *Phaenotoma scabriventris* Nixon and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae): endoparasitoids of *Liriomyza* leafminer

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The exotic parasitoid, *Phaenotoma scabriventris* Nixon, was imported from Peru for the biological control of invasive *Liriomyza* species in vegetable and ornamental crops in Kenya where *Opius dissitus* Muesebeck is the most abundant indigenous *Liriomyza* parasitoid. Both species are solitary larva-pupal endoparasitoids attacking the same larval stage. In order to assess whether these two species compete or co-exist, an interaction study involving sole, sequential and simultaneous releases of the two species on polyphagous *Liriomyza huidobrensis* (Blanchard) was conducted in the laboratory at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Simultaneous releases of 50 individuals each of the two parasitoids resulted in significantly higher total parasitism rate ( $61.96 \pm 4.60$ ) than in the single release of *P. scabriventris* ( $34.94 \pm 8.50$ ). Simultaneous release of 25 individuals of each species resulted in a parasitism rate ( $44.52 \pm 2.75$ ) similar to that obtained for single releases of 50 individuals of *O. dissitus* ( $42.57 \pm 3.35$ ) and *P. scabriventris* ( $34.94 \pm 8.50$ ). No significant effect was observed in total parasitism between sequential and single releases of 50 individuals of each species. The specific parasitism rate of each parasitoid species in the simultaneous release of 50 individuals of each species was not significantly different from when each species was released alone. The first introduced parasitoid in sequential releases achieved the same parasitism rate as when released alone. However, the second released species gave a significantly lower parasitism rate than when released alone and compared to the first released species. The F1 progeny sex ratio was balanced for *P. scabriventris* but male-biased in *O. dissitus*. The sex ratios of both parasitoid species were not significantly affected, neither in simultaneous nor sequential releases, except in one of the sequential release where *P. scabriventris* was released second, with its sex ratio significantly female-biased. Non-reproductive host mortality was not important for both parasitoids when used alone and in combined releases compared to the natural mortality observed in the control. These findings suggest that *P. scabriventris* has no detrimental effect on *O. dissitus* and its release into Kenya's agricultural ecosystems will enhance the management of *Liriomyza* leafminer.

**Key words:** parasitism, competition, host discrimination, exotic parasitoid, indigenous parasitoid.

## INTRODUCTION

*Liriomyza* leafmining flies (Diptera: Agromyzidae) are among the most economically important pests of vegetable and ornamental plants worldwide (Spencer 1985; Murphy & LaSalle 1999; Burgio *et al.* 2007). Of particular importance are the three most invasive species, *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard and *Liriomyza trifolii* (Burgess) established in Africa, Asia and Latin America (Spencer 1990; Murphy & LaSalle 1999; Burgio *et al.* 2007). In Kenya, these species are frequently the most polyphagous species

of economic importance, causing extensive damage to a wide range of high-value vegetable and floriculture crops (Njuguna *et al.* 2001; KEPHIS 2007; Chabi-Olaye *et al.* 2008). These pests are the most important cause of Kenya's fresh vegetables and flowers interception in the European market due to their inclusion in the European Union list of quarantine pests (Kedera & Kuria 2003; Chabi-Olaye *et al.* 2008). Currently, the most devastating *Liriomyza* species in Kenya is *L. huidobrensis*, representing over 90 % of all *Liriomyza* species collected in vegetable-production systems (Chabi-Olaye *et al.* 2008; Foba *et al.* 2013). Depending on plant

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type, its developmental stage and altitude, infestation can range between 10 and 80 %, and is higher in cultivated than in wild habitats (Chabi-Olaye *et al.* 2008).

In Kenya, natural control by indigenous parasitoids has failed to provide adequate suppression of the invasive *Liriomyza* species. The diversity and abundance of indigenous parasitoids associated with *Liriomyza* species is low, with the solitary, larva-pupal endoparasitoid, *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), being the most abundant, representing 42 % of them (Chabi-Olaye *et al.* 2008). However, the total parasitism rate by all the indigenous parasitoid species is very low, not exceeding 5.2 % in both cultivated and wild habitats across all agro-ecological zones in Kenya (Chabi-Olaye *et al.* 2008). Classical biological control has therefore emerged as the most promising solution to the *Liriomyza* species menace in Kenya.

*Phaenotoma scabriventris* Nixon (Hymenoptera: Braconidae), a solitary larva-pupal endoparasitoid is an important parasitoid parasitizing and suppressing populations of *Liriomyza* species in their original areas of Peru, Argentina, Brazil and Chile. It is often the dominant parasitoid of *L. huidobrensis* in these areas, representing about 50 % of total parasitism (Serantes de Gonzales 1974; Salvo & Valladares 1995) and having a wide geographical and ecological distribution (Salvo 1996; Salvo *et al.* 2005). Under laboratory conditions, *P. scabriventris* imported from Peru, accepted, developed and controlled effectively the three most important *Liriomyza* species found in Kenya (Chabi-Olaye *et al.* 2013). It is therefore proposed as a promising candidate in classical biological control against these species in Kenya.

However, classical biological control programmes, which require importation of exotic parasitoids into a backdrop of indigenous parasitoid populations, introduce the risk of interspecific competition leading to ecological disruption (Boettner *et al.* 2000; Louda *et al.* 2003). The chances of interspecific competition may be higher considering that *P. scabriventris* and *O. dissitus* are solitary endoparasitoids, preferring and attacking the same larval stage (second and third instars) and emerging from the pupal stage of the host (Bordat *et al.* 1995a; Chabi-Olaye *et al.* 2013).

Various studies have demonstrated that two species with highly similar fundamental niches (*i.e.* the niches potentially occupied in the absence

of competitors) will often compete strongly with each other when they first meet or when resources are limited (Mackauer 1990; Reitz & Trumble 2002; Duyck *et al.* 2004; Bajpai *et al.* 2006; Tian *et al.* 2008; Harvey *et al.* 2013). Such competition between introduced and indigenous parasitoids resulting from multiple sharing of a single host may affect the establishment and efficiency of the introduced parasitoids as well as the performance of the indigenous ones (Godfray 1994; Boettner *et al.* 2000; Reitz & Trumble 2002; Louda *et al.* 2003; Harvey *et al.* 2013).

In their native areas of South America, *Liriomyza* species are naturally controlled by a complex of more than 60 parasitoid species without any lethal interspecific competition occurring among them (Waterhouse & Norris 1987; Murphy & LaSalle 1999; Mujica & Kroschel 2011). Integrated pest management approaches based on conservation of existing natural enemies and introduction of additional species, offer viable alternatives to the application of insecticides which are ineffective in controlling *Liriomyza* species (Kang *et al.* 2009; James *et al.* 2010; Gitonga *et al.* 2010; Guantai 2011).

Understanding the interspecific interactions between the exotic *P. scabriventris* and the indigenous *O. dissitus* parasitoid species in their quest to parasitize similar host is therefore necessary since this might affect the outcome of the classical biological control of the pests. This study evaluated the effect of introductions and sequence of releases of *P. scabriventris* on the specific parasitism rates of *O. dissitus* and *vice versa*. Results from this study could help optimize the use of these natural enemies in the management of *Liriomyza* species in vegetable-production systems of East Africa.

## MATERIAL AND METHODS

### *Insect rearing*

The *L. huidobrensis* host used in this study was maintained and supplied by the International Centre of Insect Physiology and Ecology (ICIPE) insectary, Duduville campus, Nairobi, Kenya. It was cultured on 14-day-old faba bean (*Vicia faba* L.) at  $25 \pm 2$  °C,  $60 \pm 9$  % RH and a photoperiod of 12L:12D. Its colony was initiated from naturally occurring individuals collected from wild crucifers at Nyeri (0°21'S 36°57'E, 2200 m a.s.l.), Nyeri County, Kenya, in 2007. *Liriomyza huidobrensis* was selected for this study because it represents the most abundant (>80 %) *Liriomyza* species across a

wide range of agro-ecological zones for vegetable production in Kenya (Foba *et al.* 2013).

The exotic parasitoid, *P. scabriventris* was imported into Kenya from a laboratory culture at the International Potato Centre (CIP) in Peru, in December 2008. The *Phaedrotoma scabriventris* colony was maintained in the quarantine unit at ICIPE, Duduville, Nairobi, on *L. huidobrensis* late second and third-instar larvae infesting *V. faba* bean plants for about 60 generations between the time of importation and the commencement of the experiments.

The colony of the indigenous parasitoids, *O. dissitus* was initiated from *Liromyza*-infested French bean, tomato and water melon leaves collected from Masinga (0°55'S 37°32'E, 1069 m a.s.l.) and Kivaa (0°50'S 37°40'E, 1008 m a.s.l.), Machakos County, Kenya, between April and May 2011. *Opius dissitus* was also maintained in the quarantine unit at ICIPE, Duduville, Nairobi, on *L. huidobrensis* late second- and third-instar larvae infesting *V. faba* plants for about 17 generations between the time of collection and the commencement of the experiments. After emergence, adults of both parasitoid species were fed on 10 % honey solution until maturity and mating before their introduction to *L. huidobrensis*-infested *V. faba* plants. Colonies of *O. dissitus* and *P. scabriventris* were placed in separate rearing rooms to avoid species mixture.

#### *Preparation of leafminer host and parasitoids for experiments*

Prior to the experiments, newly emerged adults of the two parasitoid species (*P. scabriventris* and *O. dissitus*) were fed on 10 % honey solution for two to three consecutive days for maturity and mating before introducing to *L. huidobrensis*-infested *V. faba* plants.

Adult *L. huidobrensis* flies were fed on 10 % sugar solution soaked in cotton wool in a Petri dish for three consecutive days for maturity and mating before introducing *V. faba* plants. The pre-experimental periods adopted for both host and parasitoid species were based on previous studies which indicated that the highest oviposition by females occurred between two to three days (EPPO/CABI 2006; Chabi-Olaye *et al.* 2013).

The following procedures were used in preparing *L. huidobrensis* hosts for exposure to parasitoids. Ten uninfested 14-day-old potted *V. faba* (4 plants per 7.5 cm diameter × 7.3 cm depth pot) plants

were exposed to a colony of 200 adult *L. huidobrensis* of mixed sexes in male to female ratio of 1:2 for 24 h in transparent Perspex cages (45 cm × 40 cm × 40 cm). Potted plants were isolated from the exposure cages and held in similar empty cages for 5 days until second and third larval developmental stages. This exposure regime was used to provide parasitoids with plants containing uniform and appropriate host developmental stages. Prior to exposure of the leafminer larvae to the parasitoids, the base of the potted plants were covered with aluminium foil to prevent the developing pupae from dropping into the soil during their later development stages.

#### *Assessment of parasitoid performance*

Interactions between *P. scabriventris* and *O. dissitus* in parasitizing *L. huidobrensis* larvae were studied following the procedures described by Wang & Messing (2002) and Bader *et al.* (2006) with some modifications. Treatment comparisons included single (sole), combined (simultaneous) and sequential releases of parasitoid species on *L. huidobrensis* larvae as well as a control where no parasitoid was released to measure the background effects of natural mortality. Each parasitoid species spent 24 h in the experimental cages before being removed. A total of 50 adult parasitoids of each species in male to female ratio of 1:2 were released in each treatment. However, in one of the simultaneous release treatments, 25 adults of each parasitoid species were released to determine the performance of 50 combined individual parasitoids when used under single and mixed species release regimes. A summary of the treatment combinations is shown in Table 1. The releases were done under a 36 W Sylvania Aquastar fluorescent white light and a fluorescent cool purple light bulb supplied by Uganda Electricals Ltd, Kenya, during the photophase. Leafminer larvae were held in the experimental cages for 7 to 8 days and allowed to pupate. Prior to adult emergence, the pupae were collected and individual pupae were incubated in gelatin capsules (2.20 cm height, 0.7 cm diameter and 0.8 cm<sup>3</sup> volume). After emergence, unhatched pupae were dissected under a dissecting microscope and the content inspected for the presence or absence of any developmental stages of *L. huidobrensis* or parasitoid species. The results of the dissection were used to correct the actual parasitism rates. The number of adult parasitoids collected was pooled over the experimental period

**Table 1.** Summary of parasitoids release strategies.

Treatment (T)	Release pattern
<b>Single release</b>	
<i>O. dissitus</i> only (T1)	50 adults <i>O. dissitus</i> at 1:2 (15♂:35♀) for 24 h
<i>P. scabriventris</i> only (T2)	50 adults <i>P. scabriventris</i> at 1:2 (15♂:35♀) for 24 h
<b>Sequential release</b>	
<i>O. dissitus</i> first, <i>P. scabriventris</i> second (T3)	50 adults <i>O. dissitus</i> at 1:2 (15♂:35♀) for 24 h followed by 50 adults <i>P. scabriventris</i> at 1:2 (15♂:35♀) for another 24 h
<i>P. scabriventris</i> first, <i>O. dissitus</i> second (T4)	50 adults <i>P. scabriventris</i> at 1:2 (15♂:35♀) for 24 h followed by 50 adults <i>O. dissitus</i> at 1:2 (15♂:35♀) for another 24 h
<b>Simultaneous release</b>	
<i>O. dissitus</i> and <i>P. scabriventris</i> (T5)	50 adults <i>O. dissitus</i> and 50 adults of <i>P. scabriventris</i> , both species at 1:2 (15♂:35♀) for 24 h
<i>O. dissitus</i> and <i>P. scabriventris</i> (T6)	25 adults <i>O. dissitus</i> and 25 adults of <i>P. scabriventris</i> both species at 1:2 (7♂:18♀) for 24 h
<b>Control</b>	
<i>L. huidobrensis</i> reared alone (T7)	No parasitoid species released

and a mean specific and total parasitism rates were generated for each treatment. All the treatments were arranged in a randomized complete block design and replicated five times.

In order to determine whether each parasitoid release strategy influenced the performance of either or both parasitoid species, the total and specific parasitism rates were compared among treatments as well as comparing specific parasitism rates within treatments. Specific comparisons included comparing total parasitism rates in the simultaneous release of 50 individuals each of the two parasitoid species (T5) with sequential releases of 50 individuals of each species (T3 and T4). Each specific parasitism rate in the simultaneous release treatment (T5) was compared with their respective single releases (T5 *vs* T1 and T5 *vs* T2) as well as comparing specific parasitism rate of each species with one another in T5. Total parasitism rates in sequential releases (T3 and T4) were compared among themselves. Similarly, each specific parasitism rate in the sequential releases was compared with the specific parasitism rates in the single (T1 and T2) and simultaneous (T5) releases of 50 individuals of each species to determine the effect of release sequence. Comparisons were also made between total parasitism rates in simultaneous release of 25 individuals of each species (T6) with the two single releases of 50 individuals of each species (T1 and T2) to determine the perfor-

mance of the combined parasitoid species with each parasitoid species' single release at the same density. The effects of the parasitoid release strategies on the sex ratios of the F1 progeny of parasitoids and the host were also compared among and between treatments.

#### Non-reproductive host killing

Non-reproductive host killing behaviour due to physical attack such as host-stinging by parasitoid species is regarded as an additional crucial cause of host mortality (Sandlan 1979; Walter 1988; Tran & Takagi 2006). Thus, in this study, the pupal mortality rate was used and expressed as the numbers of unemerged pupae divided by total pupae multiplied by 100 in each treatment.

#### Data analyses

Specific parasitism rate for each parasitoid species and the total parasitism rate for both species were calculated using the following equations:

$$SP_{p_s} = \left( \frac{C_{p_s}}{C_{p_s} + C_{Lh}} \right) \times 100$$

$$SP_{O_d} = \left( \frac{C_{O_d}}{C_{O_d} + C_{Lh}} \right) \times 100$$

$$TP_{p_s O_d} = \left( \frac{C_{p_s} + C_{O_d}}{C_{p_s} + C_{O_d} + C_{Lh}} \right) \times 100$$

**Table 2.** Mean  $\pm$  S.E. for total and specific parasitism rate of *Phaenrotoma scabriventris* and *Opius dissitus* on *Liriomyza huidobrensis* for each treatment.

Treatment (T) regime	<i>O. dissitus</i> specific parasitism (%)	<i>P. scabriventris</i> specific parasitism (%)	$\chi^2$	P-value	Total parasitism (%)
<b>Single release</b>					
<i>O. dissitus</i> only (T1)	42.57 $\pm$ 3.35 a	—	—	—	42.57 $\pm$ 3.35 ab
<i>P. scabriventris</i> only (T2)	—	34.94 $\pm$ 8.5 a	—	—	34.94 $\pm$ 8.50 a
<b>Sequential release</b>					
<i>O. dissitus</i> first, <i>P. scabriventris</i> second (T3)	38.3 $\pm$ 2.3 abA	11.2 $\pm$ 0.7 cB	89.89	<0.0001	49.46 $\pm$ 2.90 ab
<i>P. scabriventris</i> first, <i>O. dissitus</i> second (T4)	17.0 $\pm$ 2.2 cA	28.3 $\pm$ 2.4 aB	15.14	<0.0001	45.20 $\pm$ 3.71 ab
<b>Simultaneous release</b>					
<i>O. dissitus</i> and <i>P. scabriventris</i> (T5)	38.4 $\pm$ 5.6 abA	23.6 $\pm$ 1.1 abB	19.67	<0.0001	61.96 $\pm$ 4.60 b
<i>O. dissitus</i> and <i>P. scabriventris</i> (T6)	29.6 $\pm$ 4.0 bA	14.9 $\pm$ 1.6 bcB	23.48	<0.0001	44.52 $\pm$ 2.75 ab
<b>Control</b>					
<i>L. huidobrensis</i> reared alone (T7)	—	—	—	—	—

Within column, means followed by the same lower case letter are not significantly different at  $P = 0.05$  (Tukey's test). Within row for each treatment, means followed by the same upper case letter are not significantly different at  $P = 0.05$  (chi-square goodness of fit test).

where  $SP_{p_s}$  = the specific parasitism rate of *P. scabriventris*,  $C_{p_s}$  = the corrected number of *P. scabriventris*,  $C_{Lh}$  = the corrected number of *L. huidobrensis*,  $SP_{Od}$  = the specific parasitism rate of *O. dissitus*,  $C_{Od}$  = the corrected number of *O. dissitus* and  $T_{p_sOd}$  = the total parasitism of *P. scabriventris* and *O. dissitus*.

The data on parasitism rates recorded for each treatment were first arcsine transformed and then subjected to one-way ANOVA. Data on non-reproductive host killing rates were normal, thus, one-way ANOVA was applied directly to them. The number of adult parasitoids and host in each treatment were log transformed and then subjected to one-way ANOVA. Means among treatments were separated using Tukey's test. Chi-square goodness of fit test was used to determine the within treatment effect for the specific parasitism rates and sex ratios. All data were analysed in R version 3.0.2<sup>®</sup> statistical software Development Core Team 2013).

**RESULTS**

**Interspecific competition on parasitoid species**

There were significant differences between single, sequential and simultaneous release treatments with regard to total parasitism rates ( $F_{5,24} = 3.60$ ,  $P = 0.014$ ), with the simultaneous release of 50 individuals of each species (T5) significantly recording the highest total parasitism of over 61% (Table 2). A significant difference was observed between total parasitism in T5 and T2 where *P. scabriventris* was used alone but similar to the other parasitoid release treatments (Table 2). In the sequential introductions (T3 and T4), no significant difference was observed in total parasitism between the release sequences (Table 2). Simultaneous release of 25 individuals of the two parasitoids species (T6) led to a total parasitism rate similar to the specific parasitism of 50 *P. scabriventris* (T2) and 50 *O. dissitus* (T1) when used alone (Table 2).

The total specific parasitism rates of *P. scabriventris* and *O. dissitus* did not differ significantly when released alone (Table 2). The specific parasitism rates of *P. scabriventris* and *O. dissitus* in the simultaneous release treatment of 50 individuals of each species (T5) were not significantly different compared to each parasitoid's respective specific parasitism when released alone (T2 and T1) (Table 2). The first introduced species in sequential

releases achieved similar specific parasitism rates (T1 *vs* T3 and T2 *vs* T4) as when used alone and significantly higher specific parasitism rates compared to the second released species (T3:  $\chi^2 = 89.89$ , d.f. = 1,  $P < 0.0001$  and T4:  $\chi^2 = 15.14$ , d.f. = 1,  $P < 0.0001$ ) (Table 2). When comparing the specific parasitism rates of *P. scabriventris* and *O. dissitus* in the simultaneous release treatment (T5) to the sequential treatment where each species was released first (T4 and T3), no significant difference was observed. However, when released second, their specific parasitisms were significantly lower ( $F_{5,24} = 3.60$ ,  $P = 0.014$ ) than in treatments where they were used alone (Table 2).

The sex ratio of *P. scabriventris* in the F1 progeny was not significantly different when used alone ( $\chi^2 = 1.14$ , d.f. = 1,  $P = 0.286$ ) (T2). This equal proportion of males and females were not affected by the different parasitoid release combinations ( $F_{4,20} = 1.20$ ,  $P = 0.342$ ) (Table 3). However, in combination where *P. scabriventris* was released second (T3) and where lower *P. scabriventris* parasitism rates were recorded, it produced a significantly female-biased F1 progeny ( $\chi^2 = 3.88$ , d.f. = 1,  $P = 0.049$ ) compared to the male progeny (Table 3). Sex ratio of *O. dissitus* was significantly more male-biased ( $\chi^2 = 41.28$ , d.f. = 1,  $P < 0.0001$ ) when used alone and even with different treatment combinations, with no significant effects due to presence of *P. scabriventris* or sequence of parasitoid introduction ( $F_{4,20} = 0.042$ ,  $P = 0.996$ ) (Table 3). The proportions of males were approximately similar to that of females for the host (*L. huidobrensis*) and did not significantly differ ( $F_{6,28} = 1.143$ ,  $P = 0.364$ ) among the various treatments (T1–T7) (Table 3).

### Non-reproductive host killing

The non-reproductive host mortalities recorded in the different combinations of parasitoids were not significantly different from the natural mortality obtained in the control ( $F_{6,28} = 2.48$ ,  $P = 0.048$ ) (Table 4).

## DISCUSSION

Introduction of exotic parasitoids is often the most desirable option for controlling invasive pests especially in areas where indigenous natural enemies have negligible effect in controlling the pest (van Lenteren 1997). This study represents the first laboratory evidence of parasitism rates of both *O. dissitus* and *P. scabriventris* on *L. huidobrensis*.

In the present study, no significant differences in specific parasitism were found between both parasitoid species when used separately, indicating similar parasitism potential of *P. scabriventris* and *O. dissitus* under laboratory conditions. Both parasitoid species are closely related such that *P. scabriventris* was initially named *Opius scabriventris* (Fischer 1977; PEET 2003). This could possibly account for the observed similarities in their specific parasitism rates.

Although the same level of specific parasitism was observed for both species, the sex ratios of their F1 progeny were different, with *P. scabriventris* having a balanced sex ratio while *O. dissitus* produced a male-biased progeny. This result confirms that of Bordat *et al.* (1995b) who reported 33.3 % female progeny at 25 °C for *O. dissitus*. However, Chabi-Olaye *et al.* (2013) reported a more female-biased sex ratio in *P. scabriventris*. In parasitoids, a balanced or female-biased sex ratio infers stability and higher efficiency compared to a male-biased one, as only females directly contribute to the mortality of pests (Beddington *et al.* 1978; Mills & Getz 1996; Ode & Heinz 2002; Chow & Heinz 2005). In addition to this advantage of *P. scabriventris* over *O. dissitus* regarding their sex ratios, *P. scabriventris* displayed a dynamic reaction by producing a significantly more female-biased progeny under the experimental conditions where it was disadvantaged by being introduced as the second parasitoid with limited resources. In most of the treatments, sex ratios of *P. scabriventris* and *O. dissitus* were not affected by the presence of one another except in the case where *P. scabriventris* was released second after *O. dissitus* (T4) which produced a more female-biased progeny compared to its sex ratio in the other treatments. *Phaenodrotoma scabriventris* seems to have an edge over *O. dissitus* in maintaining a balanced sex ratio of its offspring: 1) when host resources are abundant as in T2 and T4, 2) when given equal opportunity with *O. dissitus* to parasitize a given quantity of host as in T5 and T6, and 3) producing more females when host resources are limited as in T3. The ability of parasitoids to determine the sex of its offspring in variable conditions allows a female to maximize her returns in terms of fitness (Chow & Heinz 2005). Kaitala & Getz (1992) and Wogin (2011) showed that parasitoids could co-exist with one another by biasing their sex ratio towards females when host resources are limited. *Phaenodrotoma scabriventris* therefore demonstrated

**Table 3.** Effect of presence and sequence of introduction of *Phaedrotoma scabriventris* and *Opius dissitus* on the F1 progeny sex ratios of the host and parasitoids.

Insect species	Sex ratios	Treatment						
		T1*	T2	T3	T4	T5	T6	T7
<i>L. huidobrensis</i>	% ♂	44.3 ± 2.8 aA*	44.7 ± 1.9 aA	48.6 ± 1.5 aA	50.1 ± 0.9 aA	46.8 ± 2.8 aA	50.2 ± 3.2 aA	47.2 ± 1.3 aA
	% ♀	55.7 ± 2.8aA	55.3 ± 1.9 aA	51.4 ± 1.5 aA	49.9 ± 0.9 aA	53.2 ± 2.8 aA	49.8 ± 3.2 aA	52.8 ± 1.3 aA
	$\chi^2$	3.3088	1.9516	0.0748	0	1.4886	0	1.8618
	P	0.06891	0.1624	0.7845	1	0.2224	1	0.1724
<i>O. dissitus</i>	% ♂	71.4 ± 3.9 aA		72.1 ± 1.9 aA	71.0 ± 2.6 aA	73.0 ± 3.1 aA	70.1 ± 7.8 aA	
	% ♀	28.6 ± 3.9 aB		27.9 ± 1.9 aB	29.0 ± 2.6 aB	27.0 ± 3.1 aB	29.9 ± 7.8 aB	
	$\chi^2$	41.2824		42.735	15.6957	40.9074	31.6712	
	P	< 0.0001		<0.0001	<0.0001	<0.0001	<0.0001	
<i>P. scabriventris</i>	% ♂		48.4 ± 3.1 aA	37.4 ± 0.7 aA	45.6 ± 1.6 aA	41.2 ± 3.7 aA	49.6 ± 9.0 aA	
	% ♀		51.6 ± 3.1 aA	62.6 ± 0.7 aB	54.4 ± 1.6 aA	58.8 ± 3.7 aA	50.4 ± 9.0 aA	
	$\chi^2$		1.1395	3.8788	0.8675	3.508	1.3158	
	P		0.2858	0.0489	0.3517	0.06107	0.2513	

\*Percentages indicated in the Table are means ± S.E.

Within rows, means followed by the same low case letter are not significantly different at  $P = 0.05$  (Tukey's test).Within columns for each species, means followed by the same upper case letter are not significantly different at  $P = 0.05$  (chi-square goodness of fit test).

\*T1, T2, T3, T4, T5, T6 and T7 see description on Table 1 and 2.

**Table 4.** Mean  $\pm$  S.E. for non-reproductive mortality of *Liriomyza huidobrensis* per treatment

Treatment (T) regime	Non-reproductive mortality (%)
<b>Single release</b>	
<i>O. dissitus</i> only (T1)	33.01 $\pm$ 1.05 a
<i>P. scabriventris</i> only (T2)	42.33 $\pm$ 1.00 a
<b>Sequential release</b>	
<i>O. dissitus</i> first, <i>P. scabriventris</i> second (T3)	30.13 $\pm$ 1.14 a
<i>P. scabriventris</i> first, <i>O. dissitus</i> second (T4)	37.47 $\pm$ 1.08 a
<b>Simultaneous release</b>	
<i>O. dissitus</i> and <i>P. scabriventris</i> (T5)	30.15 $\pm$ 1.08 a
<i>O. dissitus</i> and <i>P. scabriventris</i> (T6)	36.17 $\pm$ 1.40 a
<b>Control</b>	
<i>L. huidobrensis</i> reared alone (T7)	30.39 $\pm$ 1.16 a

Means followed by the same letter are not significantly different at  $P = 0.05$  (Tukey's test).

the ability to maximize its population fitness under limiting resource condition (Chow & Heinz 2005). Thus, our findings suggest differences in the reproductive strategy between the two species, with *P. scabriventris* having an edge over *O. dissitus*, indicating that the introduction of *P. scabriventris* for the management of *Liriomyza* species in vegetable-production areas in East Africa could improve on the natural control of the pest.

From all the treatments, only one adult emerged from each capsulated pupa. Even in the simultaneous or sequential release treatments, only a single parasitoid of both species hatched from each capsulated pupa. In addition, only one individual was found in all dissected pupae that were harbouring parasitoids. These findings confirm the solitary nature of both parasitoids species (Bordat *et al.* 1995a; Chabi-Olaye *et al.* 2013). As such, both solitary parasitoids will then be expected to co-exist in the same ecosystem in host resource sharing, which would result in high level of *Liriomyza* species suppression. In nature, *Liriomyza* species have many natural enemies co-existing and regulating their population in both their invaded and native areas, with no case of lethal interspecific interaction (Waterhouse & Norris 1987; Johnson 1993; Murphy & LaSalle 1999; Mujica & Kroschel 2011).

Given that irrespective of the number of parasitoid released and species involved (T1 *vs* T6 and T2 *vs* T6), total parasitism did not vary significantly. This suggests that the presence of *P. scabriventris* did not reduce the parasitism potential of *O. dissitus* and *vice versa* when used alone, and can co-exist in parasitizing the same host stage. Various authors

have reported that co-existence is common between different parasitoid species if the parasitoids attack different host life stages or if they exhibit differences in their developmental time, with the one having a shorter developmental time always outcompeting the one with a longer one (Briggs *et al.* 1993; De Moraes *et al.* 1999; Shi *et al.* 2004; Tian *et al.* 2008). According to Harvey *et al.* (2013), co-existence between two or more species sharing the same host and stage may be due to the degree of specificity, searching efficiency, egg load and the ability to discriminate between hosts parasitized by each other in ways that dilute competition. In addition, Stiling & Cornelissen (2005) showed that the introduction of two or more biocontrol agents increased pest mortality by 12.97 % and decreased pest abundance by 27.17 % compared to single releases. The co-existence between both species suggests that the introduction of *P. scabriventris* would lead to an improved total parasitism of *Liriomyza* species.

In sequential releases, the first introduced parasitoid, whether *P. scabriventris* or *O. dissitus*, achieved similar specific parasitism rates as when released alone and significantly higher than the second introduced species. This suggests that the first introduced parasitoid species always has an advantage in utilizing the available host resource as compared to the second one. The result of this study contrasts with others where the second introduced female has an advantage in interspecific competition or a particular species dominated the other regardless of order of release (Collier & Hunter 2001; Collier *et al.* 2002; Pedata *et al.* 2002; Xu *et al.* 2013). For instance, Collier



& Hunter (2001) showed that both *Encarsia sophia* (Girault and Dodd) and *Eretmocerus hayati* (Zolnerowich and Rose) (Hymenoptera: Aphelinidae) prevailed in competition when ovipositing secondly by multiparasitism and host-feeding. On the other hand, Xu *et al.* (2013) showed that *E. hayati* prevailed over *E. sophia* in interspecific competition studies regardless of the order that the hosts were exposed to the female of these parasitoid species. Same results were reported by Collier *et al.* (2002) and Pedata *et al.* (2002) when using *Encarsia formosa* Gahan and *Encarsia pergandiella* (Howard). In the present study, the second introduced parasitoid species achieved significantly lower parasitism rates than the first introduced species and significantly lower than when used alone. This is in line with Agboka *et al.* (2002) and Karamaouna & Copland (2009) who reported that previous parasitism reduces the available host resources for subsequent female parasitoid. It can be concluded that none of the species was dominant over the other. This may be linked to host discrimination abilities of the two species and suggest a complementary effect of both parasitoids if used together in vegetable-production areas of East Africa. Since this avoidance behaviour was not particularly studied between *P. scabriventris* and *O. dissitus*, we recommend that further studies be carried out to assess the self, intra and interspecific host discrimination behaviour of both parasitoids.

Both parasitoid species did not cause any significant non-reproductive mortality of the hosts when released alone or in sequential and simultaneous releases compared to the natural mortality observed in the control. In many species of hymenopterous parasitoids, non-reproductive (host stinging, paralysis or feeding) behaviours of the female wasps cause important additional mortality (Sandlan 1979; Walter 1988; Tran & Takagi 2006). For instance, Byeon *et al.* (2009) showed that the solitary endoparasitoid *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) killed by host-feeding and stinging 3.3 *Aphis gossypii* Glover per day and up to 73.9 aphids during their life span (21.3 days). Also, repeated probings during host examination of the potato tuber moth *Phthorimaea operculella* by egg-larval endoparasitoid, *Copidosoma koehleri* (Hymenoptera: Encyrtidae) affected their development even when the probings did not lead to oviposition (Keinan *et al.* 2012). Non-reproductive host killing behaviour has also been

reported in some parasitoids of leafminers. For instance, Tran & Takagi (2006) showed that the solitary endoparasitoid, *Neochrysocharis okazakii* Kamijo (Hymenoptera: Eulophidae) caused 37.1 % mortality of *Liriomyza chinensis* (Kato) through non-reproductive host killing. Mafi & Ohbayashi (2010) found that one female of the ectoparasitoid, *Sympiesis striatipes* Ashmead (Hymenoptera: Eulophidae), through non-reproductive behaviour, killed around 44.7 % of their host larvae, the citrus leafminer *Phyllocnistis citrella* Stainton.

While the results of the present study showed no significant non-reproductive mortality for *O. dissitus* on *L. huidobrensis*, Bordat *et al.* (1995b) reported a significantly higher mortality of parasitized pupae while studying the performance of the same parasitoid on *L. trifolii*. On the other hand, the result of this study agreed with those of Chabi-Olaye *et al.* (2013) who reported no significant non-reproductive effect of *P. scabriventris* on *L. huidobrensis* mortality in the laboratory. This suggests that only parasitism rates of *O. dissitus* and *P. scabriventris* should be considered in the evaluation of their performance against *L. huidobrensis*.

In conclusion, both *P. scabriventris* and *O. dissitus* showed interspecific co-existence and host discrimination abilities during their reproductive process. However, further studies are needed to assess this host discrimination during their entire reproduction process. When both parasitoid species were used simultaneously, an improved total parasitism rate was obtained compared to each species specific parasitism used alone. The introduction of *P. scabriventris* into vegetable-production systems could potentially provide increased parasitism and mortality of *Liriomyza* leafminer flies since it co-exists with *O. dissitus* without affecting their performance. No significant non-reproductive mortality was induced by any of the parasitoids; hence their performance should only be evaluated based on their parasitism rate.

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