FIELD RELEASE AND RECOVERY OF LEAFMINER ENDOPARASITOID *PHAEDROTOMA SCABRIVENTRIS* NIXON (HYMENOPTERA: BRACONIDAE), ITS INTERACTIONS WITH LOCAL PARASITOIDS AND BEHAVIORAL RESPONSES TO HOSTS IN VEGETABLE PRODUCTION SYSTEMS IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This work is dedicated to my husband E. Ngam, my daughters B. and A. Ngam, my parents Mr. and Mrs. G. Foba and the rest of my family– the wind beneath my wings

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LIST OF ABREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
HIVs	Herbivore Induced Volatiles
a.s.l	Above sea level
CIP	International Potato Center
df	Degree of freedom
EU	European Union
F1	First generation
GDP	Gross Domestic Product
GIS	Geographic Information System
HIVs	Herbivore Induced Volatiles
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenyan Agricultural Research Institute
KEPHIS	Kenyan Plant Health Inspectorate Service
KarU	Karatina University College
L: D	Light-Darkness photoperiod proportion
LMF	*Liriomyza* leafminers
MRLs	Maximum Residue Levels
PI	Preference index
RH	Relative humidity
SE	Standard error
SNK	Student-Newman-Keuls
VOCs	Volatile Organic Compounds

ABSTRACT

Liriomyza leafminers (LMF) (Diptera: Agromyzidae) are severe pests of vegetables and ornamentals worldwide. Parasitoids complex regulate LMF populations in their native and invaded areas while in Kenya, previous studies reveal low LMF parasitism (<6%) across all agro ecological zones. The endoparasitoid *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) was therefore imported from Peru to improve on LMF management at different agroecological zones of vegetable production in Kenya. This study evaluated the potential of *P. scabriventris* for biological control of LMF, its interactions with local parasitoids and behavioural responses to hosts in vegetable production systems in Kenya. Using single, sequential and simultaneous release laboratory assays of *P. scabriventis* and *O. dissitus* on *L. huidobrensis*-infested *Vicia faba* plants, individual parasitism rates of both parasitoid species were not affected. However, the sequence release resulted in the second released species achieving a significantly lower parasitism rate than when released alone, simultaneously or as first released species. The F1 progeny sex ratios of both parasitoid species were not significantly affected whether in single, simultaneous or sequential releases and was female biased for *P. scabriventris* and male biased for *O. dissitus*. Montly field surveys of infested incubated LMF host plants from November 2011 - November 2012 revealed three *Liriomyza* species (*L. huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess)), represented 99.8% of total LMF recorded. Of these, *L. huidobrensis* was the greatest challenge to vegetable production, being the most abundant and most polyphagous species at all elevations and seasons. *Liriomyza* species were collected from all infested incubated leaves of 20 crops surveyed with Fabaceae and Solanaceae crops being the most infested hosts. Nine parasitoid species were recorded with 31% total parasitism. *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), *P. scabriventris* and *O. dissitus*, were the most abundant at all elevations. *Phaedrotoma scabriventris* was recovered from all released sites.

Surveys from November – December 2013 revealed spread *P. scabriventris* away from the release sites to 40 km, 30 km and 50 km at high, mid and low elevations, respectively. *Diglyphus isaea* was more abundant at the high- and midlands at all seasons compared to the lowlands while the lowland favoured abundance of *P. scabriventris* and *O. dissitus* during the long rain compared to high- and midlands at other seasons. These parasitoids performed better on kidney bean and French bean at all elevations. Using dual choice olfactometer assays, *P. scabriventris* was significantly attracted to odours from *L. huidobrensis* damaged host plants; *Phaseolus vulgaris* L., *Pisum sativum* L. and *V. faba* L. and avoided mechanically damaged ones when compared against clean air controls. However, *P. scabriventris* preferred neither the clean air controls nor any of the healthy plants. These findings suggest that *P. scabriventris* has no detrimental effect on *O. dissitus* and its release into Kenya's agricultural ecosystems will enhance LMF management. The finding that *L. huidobrensis* dominates at all elevations challenges the long held consideration of *L. trifolii* as the dominant LMF in Kenya. Recovery of *P. scabriventris* from all release sites and its spread to different *Liriomyza*-infested crops two year post release, suggest its successful establishment and potential for widespread. Behavioural responses of *P. scabriventris* to the three *L. huidobrensis* host plant species provide evidence for differences in the parasitoid's response pattern to *L. huidobrensis* host plants and the volatile profiles induced by the different treated plant species. These results are discussed in relation to biological control and wider ecological implications. For improved LMF management, it is important to determine if there are genetic differences between *L. huidobrensis* identified in Kenya and that in countries where the species is still limited to higher elevations. Continuous field evaluation of the effectiveness of *P. scabriventris* performance and spatial distribution in Kenya agro-ecological zones over the years are necessary. Expansions of chemical, electrophysiological and behavioral studies

are also needed to unravel the chemical basis of host finding and location in *P. scabriventris*.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of the study

Production of vegetables and ornamentals significantly contribute to economic development of Africa because of the high economic returns, nutritive value of vegetables and its ability to generate significant employment (Weinberger & Lumpkin, 2007; Ekesi, 2010). In Kenya, the horticultural sector is one of the fastest growing and the largest agricultural sector generating annual revenue of $ 2 billion in 2013 with an annual contribution to Gross Domestic Product (GDP) of 25.3%, in 2013 (HCDA, 2013; KFC, 2014; KNBS, 2014). Flowers and vegetables constituted the biggest horticultural export representing 48.9% and 35.2% respectively of export value (HCDA, 2013). However, severe infestation of the invasive *Liriomyza* leafminers (LMF) (Diptera: Agromyzidae) are among the most important insect pests of economic importance on vegetables and flowers, limiting their production from achieving its full potential (Njuguna et al., 2001; KEPHIS, 2007; Chabi-Olaye et al., 2008). *Liriomyza* leafminers are the most important cause of interception of Kenya's fresh vegetables and flowers in European market due to their quarantine status (Kedera & Kuria, 2003; EPPO, 2013; EUROPHYT, 2014).

Liriomyza species are polyphagous invasive pest from South America and are among the most important pests of numerous field crops, ornamentals and vegetables worldwide (Spencer, 1985; Parrella, 1987; Murphy & LaSalle, 1999). In Kenya, three species; *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard and *Liriomyza trifolii* (Burgess) are important and account for over 90% of all *Liriomyza* species collected from a variety of infested incubated host plants in both cultivated and wild habitats across different elevations of vegetable production (Spencer, 1985; KEPHIS, 2007; Chabi-Olaye et al., 2008). Yield losses in Kenya's vegetable crops have been documented to be between 10 to 100% depending on the plant type, leafminers' developmental stage, elevation and the level of infestation (Chabi-Olaye et al., 2008).

Farmers’ major approach to control LMF in export vegetables and flowers is limited to routine insecticide applications in Kenya (Gitonga et al., 2010). However, none of the insecticides used at recommended doses in various pea production areas in Kenya is effective against the larval stage of *L. huidobrensis*, the most aggressive and abundant of all the invasive *Liriomyza* species (Guantai et al., 2015). Indiscriminate application of synthetic insecticides even at concentrations below recommended doses had led to low levels of parasitism (less than 6%) by the major indigenous parasitoid species associated with the pest across all elevations in Kenya (Chabi-Olaye et al., 2008; Gitonga et al., 2010; Guantai et al., 2015).

In both their native and invaded areas, LMF populations are regulated by a complex of parasitoid species (Murphy & LaSalle, 1999; Rauf et al., 2000; Mujica & Kroschel, 2011). However, the diversity and abundance of indigenous parasitoids associated with *Liriomyza* species in Kenya is low, with the solitary larva-pupa, endoparasitoid, *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), being the most abundant, representing 42% of them (Chabi-Olaye et al., 2008).

Integrated Pest Management (IPM) 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; Gitonga *et al*., 2010; Guantai *et al*., 2015). In order to enhance the biological control of LMF in Kenya, the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya in partnership with the International Potato Center (CIP), Peru, South America and Kenyan Agricultural Research Institute (KARI) imported the solitary endoparasitoid *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae).from Peru. After additional experiments, the Kenyan Plant Health Inspectorate Service (KEPHIS) granted to ICIPE an authorization of releasing the parasitoid at low, mid and high elevations of vegetable production system in 2010.

The objective of this study was therefore to evaluate the potential of *P. scabriventris* for classical biocontrol against LMF, its interactions with local parasitoids and behavioral responses to hosts in vegetable production systems in Kenya.

1.2 Statement of the problem

Production of vegetables and ornamentals in Kenya is severely constrained by infestation of the invasive *Liriomyza* species. Also, the quarantine status of these pests resulted to restrictions to exports resulting in loss of export markets and loss of revenue to many smallholder families. Efforts to control these pests through use of synthetic insecticides are plagued by development of resistance and elimination of natural enemies amongst others, which further worsened the pest status of *Liriomyza* species. Therefore, the management of these major agromyzid pests requires sustainable methods of which biological control with exotic parasitoids and semiochemicals are key aspects.

1.3 Justification of the study

Classical biological control programs involving importation of exotic parasitoids into a backdrop of indigenous parasitoid populations, introduces the risk of interspecific competition leading to ecological disruption. The chances of interspecific competition may be higher considering that *P. scabriventris* and *O. dissitus* are solitary endoparsitoids, preferring and attacking the same larval stage and emerging from the pupal stage of the host. Understanding the interspecific interactions between these two parasitoid species in their quest to parasitize similar host stage is therefore necessary since this might affect the outcome of the classical biological control of the pests.

It has long been known that *L. trifolii*, introduced from Florida, USA, through chrysanthemum cuttings is the main *Liriomyza* species infesting ornamentals and vegetables in Kenya. However, a countrywide survey in Kenya and numerous interceptions of fresh vegetables and flowers in the European markets revealed the existence of other invasive species namely *L. huidobrensis* and *L. sativae*. Although, these pests are known to infest hosts at different elevations as influenced by seasons, its continued to depict high adaptability with complex histories of invasion and establishing in many countries worldwide, exhibiting interspecific interactions, and causing damage to a wide range of plants at different agroecological zones. Since variations in climatic conditions are related to the elevation gradient according to the agroecological zones, and species richness and the structure of species assemblages change with respect to elevation, understanding how LMF and its associated parasitoids respond to gradient with regards to climate and vegetation within localized areas may be useful in the pest management

The success of a biological control agent depends in part on its ability to establish, co-exist with other existing parasitoids and its eventual spread to occupy the range of its host. However, many factors influence the establishment, spread and performance of released exotic parasitoids in a new habitat of which availability of host plants amongst others can also play a role. Plant volatile organic compounds (VOCs) are well known to play important roles in plant-insect herbivore-natural enemy tritrophic interactions. The utilisation of VOCs as host plant and oviposition substrate location cues has been demonstrated for the LMF parasitoids *O. dissitus* and *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and not for *P. scabriventris.*

1.4 Hypotheses

1. There are no interactions between *P. scabriventris* and *O. dissitus* in their quest to parasitize similar host which might affect the outcome of the classical biological control of *Liriomyza* species pests in Kenya.
2. Distribution of *Liromyza* leafminers is not affected by elevation and vegetable production systems in Kenya.
3. Establishment and spread of *P. scabriventris* is not dependent on elevation and vegetable production systems in Kenya.
4. There is no difference in the behavioural response of *P. scabriventris* to volatiles induced by *L. huidobrensis* host plants.

1.5 Objectives

1.5.1 General objective

To evaluate the potential of *P. scabriventris* for classical biocontrol against LMF, its interactions with local parasitoids and behavioral responses to hosts in vegetable production systems in Kenya.

1.5.2 Specific objectives

1. To determine the interaction between *P. scabriventris* and the indigenous parasitoid *O. dissitus*.
2. To determine the species composition, distribution and seasonal abundance of LMF under different vegetable production systems and agro-ecological zones in Kenya.
3. To determine the LMF parasitoid species composition and seasonality with focus on post release establishment and spread of *P. scabriventris* under different vegetable production systems and agro-ecological zones in Kenya.
4. To elucidate the behavioral response of *P. scabriventris* to *L. huidobrensis*-induced host plant volatiles

CHAPTER TWO

LITERATURE REVIEW

2.1 Species composition of *Liriomyza* leafminers

The genus *Liriomyza* (Diptera: Agromyzidae), commonly referred to as leafmining flies, is believed to be of neotropic origin and was restricted to the New World until the mid 1970s. Since then, several species of the genus have been spreading to Africa, Asia and Latin America (Murphy & LaSalle, 1999; EPPO, 2006). Leaf mining is generally the most widespread feeding behaviour shared by more than 75% of the species (Spencer, 1973). The genus contains about 330 species (Spencer, 1973; Kang et al., 2009; Liu et al., 2009). More than 20 species of *Liriomyza* (6% of the described 330 species) are economically important. Six species, namely *L. huidobrensis*, *L. sativae*, *L. trifolii*, *L. bryoniae* (Kaltenbach), *L. strigata* (Meigen) and *L. longei* Frick are polyphagous, an uncommon characteristic among the family Agromyzidae (Morgan, 2000; Van der Linden, 2004).

Over the past three decades, three highly polyphagous *Liriomyza* species; *L. huidobrenis*, *L. sativae* and *L. trifolii* have invaded many new areas worldwide (Shepard et al., 1998; Rauf et al., 2000; Bjorksten et al., 2005) and are the predominant invasive *Liriomyza* species, frequently reported in Kenya, including other African countries (Chabi-Olaye et al., 2008; Gitonga et al., 2010). In Kenya, the three species attack a variety of crops of commercial value, which include pea, bean, tomato, potato and okra and a variety of cut flowers (Chabi-Olaye et al., 2008).

Morphological identification of the three *Liriomyza* species is based on the distiphallic structure, a terminal part of the aedaegus (Chaput, 2000). The distiphallus is a very small, fragile structure enclosed by membranes and requires careful dissection and subsequent examination under a high-powered microscope. Separation of *Liriomyza* species using this structure can only be made for the differentiation of *L. huidobrensis* from *L. sativae* and *L. trifolii* and for male insects only (Spencer, 1990; OEPP/EPPO, 2005). *Liriomyza huidobrensis* are distinguished from *L. sativae* and *L. trifolii* by larger body size, overall dark colour; larger discal cell, relatively short distal section of vein M3+4, darkened femora (yellow in *L. sativae* and *L. trifolii*) and the male genitalia (OEPP/EPPO, 2005).

An easier identification of the three species based on morphological features is described in Spencer (1973) and it is based on the position of the two vertical setae on the head, colour of the frons and orbits, colour of the femur and the position of the middle furrow on the six visible abdominal tergites (EPPO/CABI, 2006). *Liriomyza huidobrensis* is distinguished from *L. sativae* and *L. trifolii* by having both vertical setae lying on the black ground of the frons, yellow frons and orbits, yellow femur with variably darkened brownish striations and the second visible abdominal tergite is divided by the yellow middle furrow. In *L. sativae* the outer vertical seta lies on the black ground which may just reach the inner vertical seta and which otherwise is on the yellow ground, the frons and orbits are yellow, the femur is bright yellow and the second visible abdominal tergite is divided by a yellow middle furrow, same as in *L. huidobrensis*. In *L. trifolii*, both vertical setae lie on the yellow ground, the frons and orbits are yellow with slight brownish striations, the femur is occasionally yellow and the second, third, fourth and fifth abdominal tergites are divided by the yellow middle furrow (EPPO/CABI, 2006).

2.2 Ecology of *Liriomyza* leafminers

Since the early 1970s, there has been a rapid movement of the three invasive *Liriomyza* species from the New World to the Afro-tropical regions. *Liriomyza sativae* and *L. trifolli* are the dominant agromyzid pests in lowland areas (Spencer, 1989; Rauf et al., 2000; Andersen et al., 2002) while *L. huidobrensis* is known to adapt and predominantly infest hosts at colder higher elevations, mostly above 1,000 m a.s.l. (Spencer, 1989; Rauf et al., 2000; Mujica & Kroschel, 2011). In Kenya, *L. huidobrensis* is the most important species (80%) in the highland, whereas *L. trifolii* and *L. sativae* are more frequently and constantly distributed in the mid- and lowlands (Chabi-Olaye et al., 2008).

2.3 Biology of *Liriomyza* leafminers

Most *Liriomyza* species are polyphagous holometabolous insects and for the three most invasive species frequently reported in Africa, mating occurs 24 hours after adult emergence and a single mating is sufficient to fertilise all the laid eggs (Murphy & La Salle, 1999). Eggs are laid singly but in close proximity and on lower leaf surface and mainly occur during morning hours (Parrella, 1987; Weintraub & Horowitz, 1995). Mean egg production per female ranges from less than 100 to more than 600, depending on environmental conditions, hosts and leafminer species (Parrella, 1987). Optimal temperatures for egg laying range between 21 and 32 °C (Chaput, 2000). Only females are able to create feeding punctures, causing the destruction of a larger number of cells, which are visible to the naked eye as white speckles measuring between 0.13 and 0.15 mm in diameter (EPPO/CABI, 2006). Males are unable to puncture leaves but feed on punctures made by females (Parrella, 1987). Oviposition punctures are smaller (0.05 mm) and are more uniformly round (EPPO/CABI, 2006). Eggs take between two to five days to hatch into larvae depending on the prevailing temperatures, relative humidity and availability of food (Bográn, 2005). Larvae feed on leaf tissue making a winding tunnel (serpentine leaf-mine) or blotch on the leaf (Bográn, 2005). The leaf-mines become increasingly larger in size as the larvae feed and grow through three immature stages (instars) (Bográn, 2005). Pupation mostly occurs externally, on the foliage or in the soil just beneath the surface (Bográn, 2005). Pupal development is completed in five to 12 days and the adult emerges from the puparium, mainly in the early morning hours. Both sexes emerge simultaneously.

The life cycle varies with host and temperature and many generations can occur each year as long as temperatures are above 10 °C (Bográn, 2005). Egg to adult development may take only two weeks at 35 °C or as long as eight weeks at 16 °C (Bográn, 2005). *Liriomyza huidobrensis* grows best under moderate temperatures, below 20 °C while *L. trifolii* prefers warmer summer temperatures, above 24 °C (Bográn, 2005).

2.4 Geographical distribution of *Liriomyza* leaminers in Africa

Liriomyza leafminers are invasive pests from South America and are among the most important pests of numerous field crops, ornamentals and vegetables worldwide (Spencer, 1985; Parrella, 1987; Murphy & LaSalle 1999). Three species, *L. huidobrensis*, *L. sativae* and *L. trifolii* are frequently reported from African countries including Kenya, Tanzania, Ethiopia, Zimbabwe, Sudan, Mauritius, Reunion, Tanzania, South Africa, Morocco, Egypt, Seychelles, Comoros, Senegal, Nigeria and Cameroon (Spencer, 1985; Chabi-Olaye et al., 2008) (Figure 2.1). *Liriomyza trifolii* was introduced into Kenya in the late 1970s through chrysanthemum, *Chrysanthemum* spp.; (Asteraceae) cuttings from Florida, USA (Spencer, 1985). In Kenya, *L. huidobrensis* has been reported as serious pest of horticulture especially on ornamentals and passion fruits (KEPHIS, 2005; Chabi-Olaye et al., 2008). *Liriomyza sativae* was also recently recorded in Kenya (Chabi-Olaye et al., 2008). Adult flies are capable of limited flight and dispersal over long distance is by way of planting material of host species.

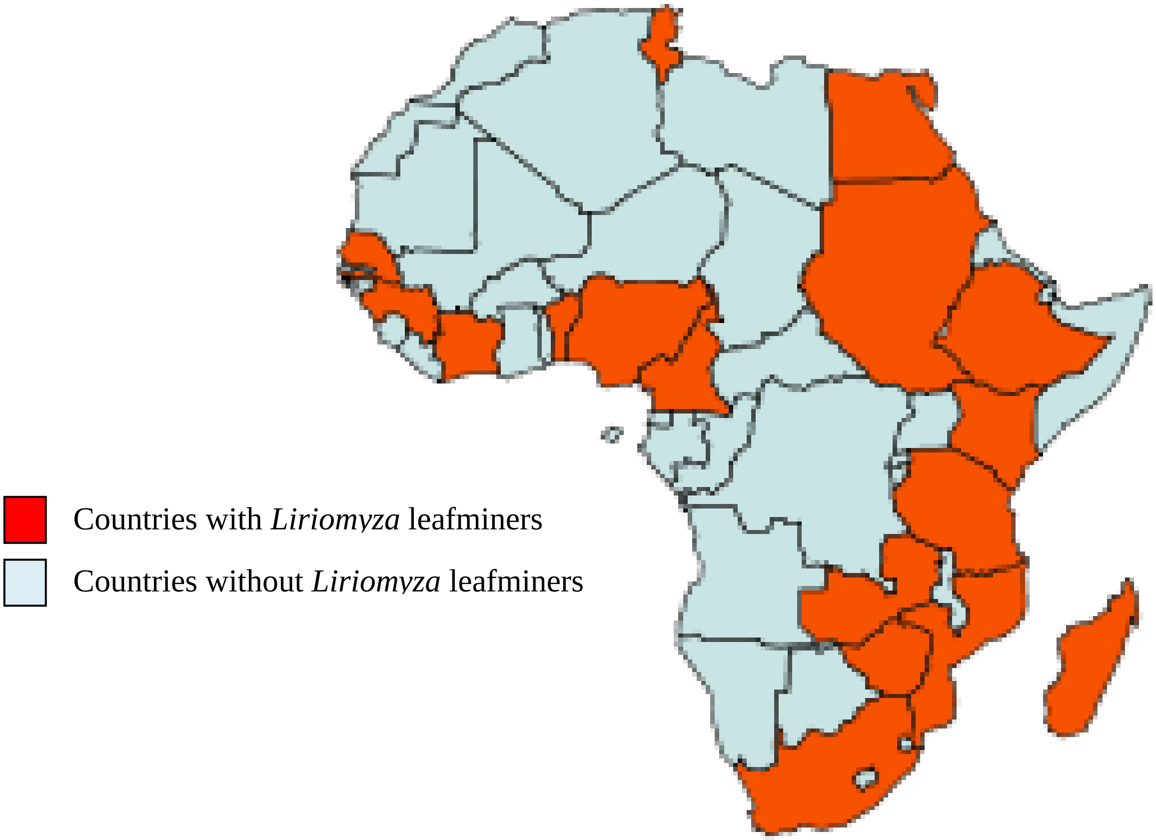

Figure 2.1: Geographical distribution of *Liriomyza* leafminers in Africa http://www.infonet-biovision.org/default/ct/83/pests.

2.5 Economic importance of *Liriomyza* leafminers

Liriomyza species are capable of causing both direct and indirect damage to plants. Larval mining in palisade parenchyma tissue reduces the photosynthetic capacity of plants by up to 62%, and severely infested leaves may fall (Johnson *et al.*, 1983). Further damage to leaves is caused by females, which use the ovipositor to make feeding punctures and oviposition punctures in the upper and/or lower surfaces of the leaves, promoting production of leaf exudates on which both females and males feed. Females make oviposition punctures to insert eggs below the leaf surface (Parrella & Bethke, 1984). Egg-laying females may also act as vectors for diseases (Deadman et al., 2002;

Bjorksten et al., 2005) such as *Alternaria alternata* (Fr.) Keissl. Mined leaves are unattractive and unmarketable. In young plants and seedlings, mining may cause considerable delay in plant development leading to yield loss (Bográn, 2005). Some damage may be tolerated in crops where foliage is not marketed, such as cut flower and seed production crops (Bográn, 2005).

In addition to the damage they cause, *Liriomyza* species, especially *L. huidobrensis* are listed as quarantine pests in the European Union (EU), thus, there are official controls to avoid their spread (Murphy & LaSalle, 1999). Furthermore, their quarantine status poses limits to new overseas market opportunities for East African farmers, especially Kenyan smallholder farmers.

2.6 Management strategies for *Liriomyza* leafminers

2.6.1 Monitoring of *Liriomyza* leafminers

A prerequisite for a successful IPM approach is to better understand the LMF species composition, abundance and the effect of seasons, host crop production systems and agroecology on the pest (Mwatawala et al., 2006). Several methods for LMF population assessment have been studied, and collecting puparia in trays placed beneath plants is one of them Johnson et al. (1980). Leafminers can also be monitored by foliage examination for the presence of mines and larvae (Chabi-Olaye et al., 2008; Mujica & Kroschel, 2011). Zehnder & Trumble (1984) used yellow sticky traps to monitor LMF adults. Sequential sampling of plants has been used by Zehnder & Trumble (1985). Regular monitoring seems necessary to maintain recurrence in addressing problems by the *Liriomyza* complex.

2.6.2 Chemical control of *Liriomyza* leafminers

Synthetic and natural insecticides for leafminer control have been extensively researched and are commonly used by farmers regardless of production scale and crop (Liu et al., 2009). Dimethoate, abamectin, imidacloprid, alphacypermethrin and beta-cyfluthrin are the most common insecticides used against LMF and other pests' complex in vegetable production systems in Kenya (Gitonga et al., 2010). Foliar application of insecticides is often frequent in susceptible crops. Insecticide susceptibility varies greatly, both spatially and temporally. In Kenya, none of the insecticides used in various pea production areas could kill the larval stage of *L. huidobrensis* when recommended doses were applied, while majority of them induced mortality of parasitoids at concentrations below recommended doses (Gitonga et al., 2010; Guantai et al., 2015). The most widely reported reason for the first leafminer outbreaks in their adventive ranges was the indiscriminate use of insecticides which adversely affect their natural enemies (Murphy & LaSalle, 1999). However, biopesticides especially botanicals like neem and pyrethrum are safer to use because they are highly biodegradable compared to synthetic pesticides.

2.6.3 Cultural practices for control of *Liriomyza* leafminers

Cultivars of tomato, cucumber: *Cucumis sativus* L. (Cucurbitaceae), cantaloupe: *Cucumis C. melo* Ser (Cucurbitaceae) and beans vary in their susceptibility to leafminer damage (Hanna et al., 1987). However, the differences tend to be moderate, and not adequate for reliable protection. Nitrogen level and reflective mulches are sometimes said to influence leafminer populations, but responses have not been consistent (Chalfant et al., 1977; Hanna et al., 1987). Placement of row covers over cantaloupe has been reported to prevent damage by leafminer (Orozco-Santos et al., 1995). Hand-picking, destruction of mined leaves and other plant material after harvest has been found to significantly manage leafminer flies damage (Varela et al., 2003). Varela et al. (2003) also reported that ploughing and flooding the soil followed by hoeing could kill or release much of the buried pupae, which are then killed by solarization or exposure to natural enemies.

2.6.4 Biological control of *Liriomyza* leafminers

In both their native and invaded areas, LMF population is regulated by a complex of parasitoid species (Waterhouse & Norris 1987; Murphy & LaSalle, 1999; Mujica & Kroschel, 2011). In a study to consolidate the available information and provide an analysis of the published work regarding investigations of natural enemies of *Liriomyza* in Texas, Liu et al. (2009) identified approximately 140 species of parasitoids, a few species of predators (including nematodes), and some entomopathogens. Noyes (2003) listed over 300 species of parasitoids associated with leafminers, and over 80 species that attack various *Liriomyza* species. In the Neotropics, Salvo & Valladares (1998) identified 69 parasitoid species of Agromyzidae leafminers in natural, urban and agricultural habitats of Central Argentina. Peruvian coast also hosts a very rich parasitoid community of about 63 species (Mujica & Kroschel, 2011). These parasitoid species complex have a wide adaptation to different host plants and *Liriomyza* species and regulate the pest populations to below economic threshold levels (Waterhouse & Norris, 1987; Murphy & LaSalle, 1999; Mujica & Kroschel, 2011).

In central Argentina, Colombia, Mexico and Peru, *Chrysocharis caribea* Boucek (Hymenoptera: Braconidae) are an extremely important source of mortality on agromyzid leafminers with an average of 30 to 55% parasitism (Valladares et al., 2001; Kroschel, 2008). *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *H. arduine* have also been found to cause 35.0 to 72.9% mortality to *Liriomyza* leafminers in Chile, Peru and Argentina, and are of global distribution (Murphy & LaSalle, 1999; Kroschel, 2008). *Diglyphus begini* Ashmead (Hymnoptera: Eulophidae) is also used in the United States for augmentative biological control of leafminers (Sher et al., 2000). Neuenschwander et al. (1987) reported that invading leafminer populations declined naturally after a few years in Senegal due to the action of local natural enemies. However, in Kenya the diversity of parasitoids associated with *Liriomyza* species is low in all horticultural production systems and includes mainly *O. dissitus*, *D. isaea*, *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) and *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) (Chabi-Olaye et al., 2008). Afrotropical populations of *Liriomyza* species suffer little mortality, less than 6% parasitism from these native parasitoids in both cultivated and wild habitats across all agro ecological zones (Chabi-Olaye et al., 2008), suggesting that introduction of specific Neotropical parasitoids may be required to achieve effective suppression of these pests.

Reports on potential of entomopathogenic fungi and nematodes as biocontrol agents of leafminers are few (Migiro et al., 2010; Wekesa et al., 2011). Entomopathogenic fungi may act as endophytes, antagonists of plant pathogens, associated with the rhizosphere, and possibly even as plant growth promoting agents (Vega et al., 2009). Some fungal endophytes protect host plants against pathogens and herbivores (Arnold & Lewis, 2005; Rudgers et al., 2007), and many fungi traditionally known as insect pathogens have been isolated as endophytes, including species of *Acremonium* (Hypocreales: Hypocreaceae), *Beauveria* (Hypocreales: Clavicipitaceae) *Cladosporium* (Capnodiales: Davidiellaceae), *Clonostachys* (Hypocreales: Bionectiaceae) and *Isaria* (Eurotiales: Trichocomaceae) (Vega, 2008; Vega et al., 2009). Toxic activities of some fungal endophytes on leafminers have been reported (Faeth & Hammon, 1996; Gaylord et al., 1996). Foliar applications of the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Chromadorea: Steinernematidae) significantly reduced adult development of *L. trifolii* (Harris et al., 1990). Fewer leaf mines by *L. trifolii* on gerbera: *Gerbera* L. (Asteraceae) and sunflower: *Helianthus* L. (Asteraceae) following application of *Isaria fumosorosea* Wise (Eurotiales: Trichocomaceae) than pesticide treatment has also been reported (Wekesa et al., 2011). In Kenya, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) isolate ICIPE 20 caused mortality of 90, 97 and 98% in *L. trifolii, L. huidobrensis* and *L. sativae*, respectively Migiro et al. (2010).

2.6.5 Importance of the endoparasitoid *Phaedrotoma scabriventris* for biological control of *Liriomyza* leafminers

Phaedrotoma scabriventris (Plate 2.1) is an important exotic natural enemy of *Liriomyza* species that originates from the Neotropics (Serantes de González, 1974, Salvo & Valladares 1995). The parasitoid has been described for the Neotropics in the countries Argentina, Brazil, Chile, and Peru, where it is often found as the dominant parasitoid of *Liriomyza* species, especially *L. huidobrensis*, representing up to 50% of the total parasitism (Serantes de González, 1974; Salvo & Valladares, 1995). Apart from its ability to parasitize *Liriomyza* species, it has been found to possess many other qualities that make it a potential candidate for use in classical biocontrol programs for *Liriomyza* species*;* this includes its wide range of geographical and ecological distribution, its low density in Agromyzidae occurring in natural and urban habitats and its significant importance as parasitoid of Agromyzidae flies that occur as pests in agricultural crops (Salvo, 1996; Salvo et al., 2005). It has been reported as the most important parasitoid of leafminers, causing 20 to 51.6% mortality in Argentina, Brazil and Peru (Valladares et al., 1999; Kroschel, 2008). Salvo (1996) recorded *P. scabriventris* as an important parasitoid of leafminers in Cordoba, Central Argentina at 800 m a.s.l., with an average annual temperature of 16 ºC (maximum 24 ºC, minimum 9 ºC). In natural habitats of Central Argentina, Valladares & Salvo (2001) examined the temporal community dynamics of Agromyzidae and their parasitoids, and found a frequent dominance of *P. scabriventris* during winter. *Phaedrotoma scabriventris* was recovered from faba bean: *Vicia faba* L.(Fabaceae) infested with *L. huidobrensis* in the Central Highlands of Peru, between 3,200 to 4,042 m of elevation (highlands of Peru) and with an annual average maximum temperature of 23 ºC and a minimum of 4 ºC. Also, this species occurs in potato and vegetable production systems in the central coast of Peru (500 m a.s.l.), which are highly infested by *L. huidobrensis* (Mujica & Cisneros, 1997). Previous laboratory studies on the preference and performance of *P. scabriventris* on *Liriomyza* species and host-plant interactions of this parasitoid showed it as a very promising candidate for *Liriomyza* suppression in vegetable production systems in Kenya (Chabi-Olaye, et al., 2013). The studies showed that, *P. scabriventris* oviposited and developed in three invasive *Liriomyza* species found in Kenya (Chabi-Olaye, et al., 2013). *Phaedrotoma scabriventris* is, therefore, an important natural enemy of leafminer species parasitiszing *Liriomyza* species across a wide range of agro-ecological zones at varying temperatures. This makes *P. scabriventris* a good candidate for use as a biocontrol agent of *Liriomyza* species, especially the three devastating invasive species attacking vegetable and ornamental crops at different elevations (low-, mid- and highlands) in Kenya.

Plate 2.1: Adult female of *Phaedrotoma scabriventris* searching for *Liriomyza huidobrensis* larvae to parasitize

2.7 Biology of *Phaedrotoma scabriventris*

Phaedrotoma scabriventris is a koinobiont parasitoid attacking leafmining larvae and emerging from puparia. Its developmental time (from eggs to adult) range from 12 days at 30 ºC to 31.9 days at 15 ºC with lowest number of progenies at 10 °C (36.2 progenies per female) and highest at 15 °C (151.2 progenies per female). At temperatures above 15 °C, progeny development decreased gradually, but does not differ significantly at 20 °C (122.9 progenies per female) while at 25 °C and 30 °C, 85.2 and 81.4 progeny per female are produced, respectively (Mujica et al., 2009). The sex ratio is highly affected by temperature; female progeny increased with increasing temperature with a female:

male sex ratio of 0.66:1 at 10 ºC and 1.31:1 at 30 ºC, respectively. A balanced sex ratio of 1:1 is registered at 20 ºC (Mujica et al., 2009).

2.8 Host insect, host plant and parasitoid interactions

The interactions between herbivores, host plants and their natural enemies are best understood when considered within a tritrophic context (Zhao & Kang, 2002a, 2002b; Kang et al., 2009). The volatiles released because of adult, larval feeding damage, leafminer-larvae size and host plant diversity are important factors in host finding and host selection by herbivorous insects as well as parasitoids (De Moraes et al., 2000; Takken & Dicke, 2006; Wei et al., 2007). The chemical compounds produced by the plants which act as cues in long and short distance range can be classified according to their effect on the host-location behaviour of insects into categories such as attractants, repellents, feeding and oviposition stimulants, and deterrents (Kang et al., 2009). Although both *Liriomyza* leafminers and their associated parasitoids are known to be generalists (Valladares & Salvo, 2001; Videla et al., 2006; Valencia, 2008), clear preferences of LMF parasitoids species such as *D. isaea* and *O. dissitus* on certain host crops have been observed (Zhao & Kang 2002a; Wei & Kang, 2006) with such information lacking for *P. scabriventris*. The differential attraction may be related to distinct plant specific induced volatile profiles. An understanding of the chemical basis of the differential attraction may offer potential for identifying attractants for parasitoids from host plants for use in sampling and monitoring populations of parasitoids.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Study sites

Laboratory studies were conducted at ICIPE, Nairobi, Kenya. Field sites were selected based on elevation namely; highland >1,800 m a.s.l (Nyeri and Kirinyaga Counties, Central Kenya), midland from 1,000 to 1,800 m a.s.l (Kajiado County, Rift Valley Region) and lowland <1,000 m a.s.l (Makueni County, Eastern Region) in Kenya (Figure 3.1) (Hassan, 1998). In each elevation, three locations with high productivity of vegetables, reliable irrigation schemes and highly infested fields of about 70% of leaves sampled were selected for the study. This was to allow for continued field monitoring of LMF and associated parasitoids throughout the year. The three elevations were located at least 65 km apart and locations within an elevation were approximately 5 km apart.

In the highland, surveys were conducted at three locations: Sagana (S0°21'9.972", E37°5'13.632") in Kirinyaga County, Kabaru (S0°17'48.408", E37°6'28.116") and Naromoru (S0°11'9.312", E37°6'36.972") in Nyeri County (Figure 3.1). The mean minimum to maximum temperature range from 8 to 24°C and mean rainfall is from 1,500 to 2,000 mm per annum.

In the midland, survey locations were Namelok (S2°43'6.096", E37°27'39.06"), Empiron (S2°50'57.948", E37°32'15.144") and Inkisanjani (S2°53'56.4", E37°34'51.564") in Kajiado County (Figure 3.1). The mean minimum to maximum temperature range from 16 to 26°C and mean rainfall is from 450 to 1,200 mm per annum.

In the lowland, survey locations were Kwakyai (S2°23'6.612", E38°0'9.504"), Kikoo (S2°23'50.928", E37°59'8.052") and Mangelete (S2°41'57.552", E38°7'32.268") in Makueni County (Figure 3.1). The mean minimum to maximum temperature range from 21 to 31°C and mean rainfall is from 800 to 1,200 mm per annum.

Generally, most agricultural crop production areas in Kenya are characterized by four cropping seasons namely the long rains, the short rains, the cold dry and the hot dry seasons. The seasons vary with respect to elevation and have been inconsistent especially at the mid and low elevations since 1980s (Hassan, 1998, Jaetzold et al., 2006).

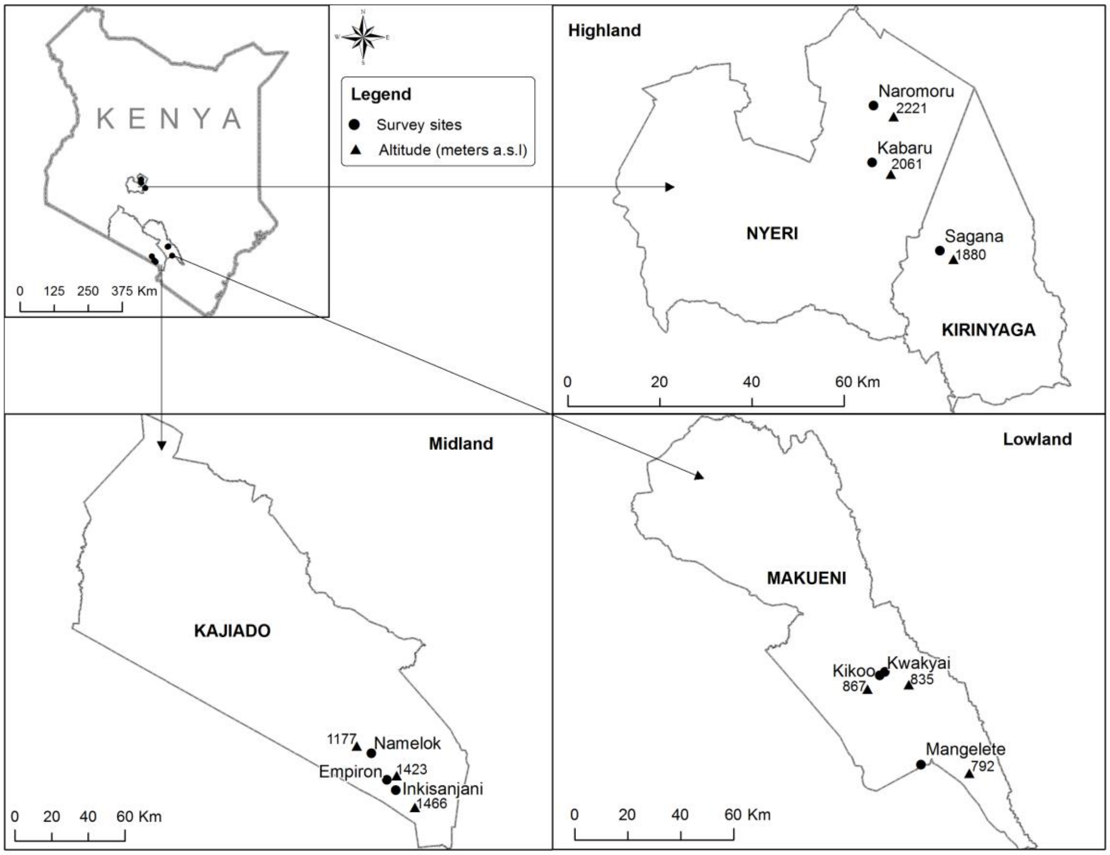

Figure 3.1: Study sites in high-, mid- and lowlands of Kenya

3.2 Host plants used in *Liriomyza huidobrensis*, *Phaedrotoma scabriventris* and *Opius dissitus* rearing and experiments

Three plant species in the family Fabaceae, namely *Phaseolus vulgaris* L.: common kidney bean, *Pisum sativum* L.: snow pea and *Vicia faba* L.: faba bean were used for laboratory studies. Four seeds of each plant species were planted individually in 7.5 cm diameter × 7.3 cm depth plastic pots containing 1: 5 manure: soil mixture in screen houses at ICIPE, Kenya. Experimental conditions were maintained at 25 ± 2 °C, 60 ± 9% RH and a photoperiod of 12L: 12D (Plate 3.1). The plants were watered daily and used at growth stages of days 14 to17 in all experiments and insect rearing. Faba bean was used as a positive control since it has been reported to be the best host for rearing *L. huidobrensis* and *P. scabriventris* (Videla et al., 2006; Chabi-Olaye et al., 2013).

Plate 3.1: *Vicia faba* growing in a screen house at ICIPE

3.3 Rearing of *Liriomyza huidobrensis*, *Phaedrotoma scabriventris* and *Opius dissitus*

The *L. huidobrensis* host used in this study was maintained and supplied by the insectary, ICIPE, Nairobi. It was cultured on 14-day-old faba bean at 25 ± 2 °C, 60 ± 9% RH and a photoperiod of L12:12D. Its colony was initiated from naturally occurring individuals collected from wild crucifers at Nyeri (0°21'S, 36°57’E, 2,200 m a.s.l), Central Kenya in 2007. *Liriomyza huidobrensis* was selected for this study because it represents the most abundant (>80%) *Liriomyza* species identified from both cultivated and wild host plants in Kenya (Chabi-Olaye et al., 2008).

The exotic parasitoid, *P. scabriventris* was imported into Kenya from a laboratory culture at the CIP in Peru, South America, in December 2008. *Phaedrotoma scabriventris* colony was maintained in the quarantine unit at ICIPE, Nairobi on *L. huidobrensis* late second and third instar larvae infesting faba bean plants under the same environmental conditions as described above.

A colony of the indigenous parasitoid, *O. dissitus* was initiated from *Liriomyza*-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, on *L. huidobrensis* late second and third instar larvae infesting faba bean plants. After emergence, adults of both parasitoid species were fed on 10% honey solution until maturity and mating before their introduction to *L. huidobrensis*-infested faba bean plants. Colonies of *O. dissitus* and *P. scabriventris* were placed in separate rearing rooms to avoid species mixture.

CHAPTER FOUR

INTERACTION BETWEEN *PHAEDROTOMA SCABRIVENTRIS* NIXON AND *OPIUS DISSITUS* MUESEBECK (HYMENOPTERA: BRACONIDAE): ENDOPARASITOIDS OF *LIRIOMYZA* LEAFMINERS

ABSTRACT

The exotic parasitoid, *Phaedrotoma 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. 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, Nairobi. Simultaneous releases of 50 individuals each of the two parasitoids resulted in significantly higher total parasitism rate (61.96±4.60) than in the single release of *P. scabriventris* (34.94±8.50). Simultaneous release of 25 individuals of each species resulted in a parasitism rate (44.52 ± 2.75) similar to that obtained for single releases of 50 individuals of *O. dissitus* (42.57 ± 3.35) and *P. scabriventris* (34.94 ± 8.50). 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 for *O. dissitus*. 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* leafminers.

4.1 Introduction

Liriomyza leafminers are among the most economically important pests of vegetable and ornamental plants worldwide (Spencer, 1985; Murphy & LaSalle, 1999). Of particular importance are the three most invasive species, *L. huidobrensis*, *L. sativae* and *L. trifolii* established in Africa, Asia and Latin America (Spencer, 1990; Murphy & LaSalle, 1999). 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 since they are included 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). Depending on the plant type, leafminers' developmental stage and elevation, infestation can range between 10 and 100%, and is higher in cultivated than 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-pupa, endoparasitoid, *O. dissitus*, 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 6% in both cultivated and wild habitats across all agro-ecological zones in Kenya (Chabi-Olaye et al., 2008). Consequently, there is need for classical biological control solution against the *Liriomyza* species menace in Kenya.

Phaedrotoma scabriventris, a solitary larva-pupa endoparasitoid is an important parasitoid parasitizing and suppressing populations of *Liriomyza* species in their original areas of Peru, Argentina, Brazil and Chile, South America. It is often the dominant parasitoid of *L. huidobrensis* in these areas, representing about 50% of total parasitism (Serantes de González, 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 *Liriomyza* species in Kenya.

However, classical biological control programs, that 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 endoparsitoids, preferring and attacking the same larval stage 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 (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 (Duyck et al., 2004; 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 parasitoid as well as the performance of the indigenous ones (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).

Understanding 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. scabriventirs* 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 Kenya including other East African countries.

4.2 Materials and methods

4.2.1 Rearing of *Liriomyza huidobrensis*, *Phaedrotoma scabriventris* and *Opius dissitus*

The *L. huidobrensis* host, the exotic parasitoid, *P. scabriventris* and the indigenous parasitoid, *O. dissitus* used in this study were maintained and supplied by the insectary, as described in Section 3.3.

4.2.2 Preparation of *Liriomyza huidobrensis, Phaedrotoma scabriventris* and *Opius dissitus* 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 faba bean 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 to faba bean 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 after adult emergence (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 faba bean (four plants per 7.5 cm diameter × 7.3 cm depth pot) plants were exposed to a colony of 200 adult *L. huidobrensis* with a male to female ratio of 1: 2 for 24 hours in transparent perspex cages (45 cm × 40 cm × 40 cm). Potted plants were removed from the exposure cages and held in similar empty cages for five days until second and third larval developmental stages were attained. 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 developmental stages (Plate 4.1).

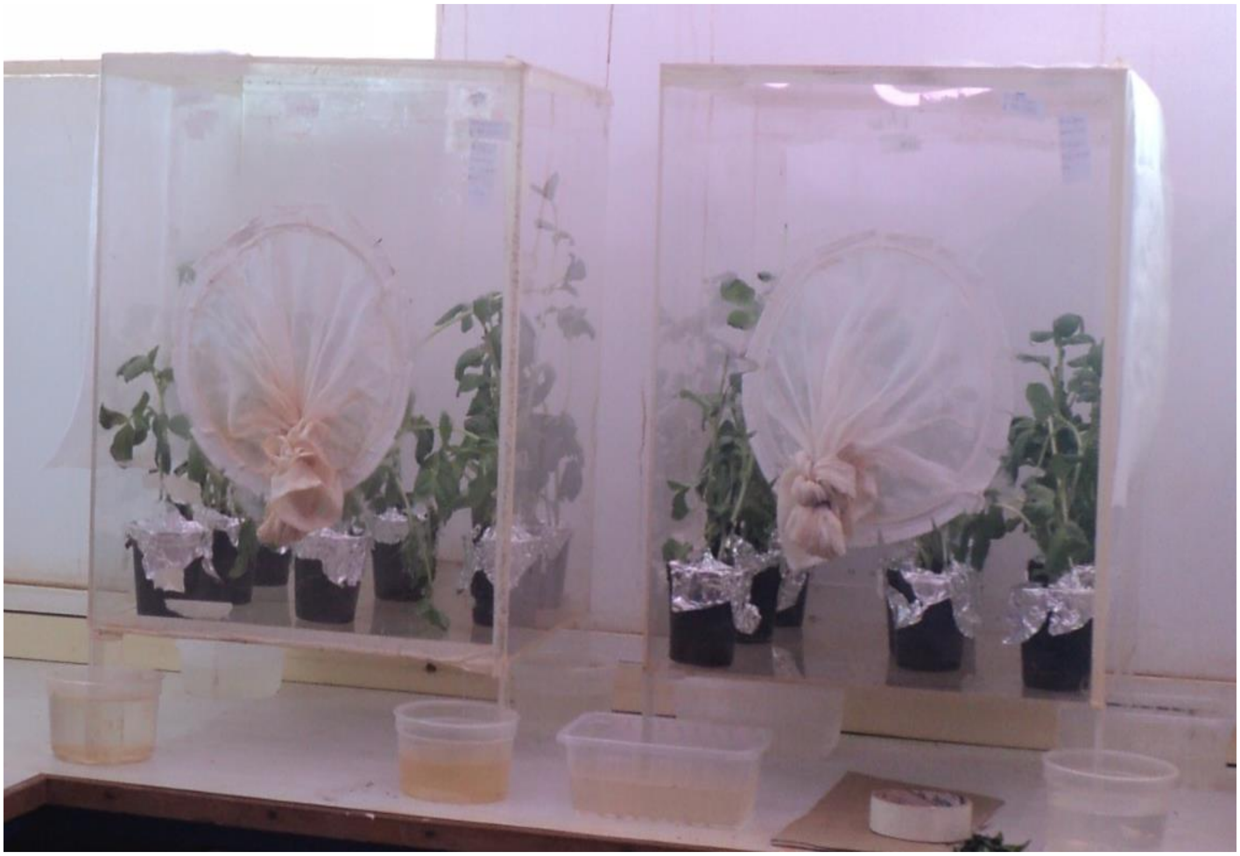

Plate 4.1: Exposure cages for *Phaedrotoma scabriventris*, *Opius dissitus* on *Liriomyza huidobrensis* larvae infesting faba bean plants

4.2.3 Assessment of *Phaedrotoma scabriventris* and *Opius dissitus* performance on *Liriomyza huidobrensis*

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* second and third instar larvae as well as a control where no parasitoid was released to measure the background effects of natural mortality. Each parasitoid species was alowed 24 hours 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 4.1. During the photophase, the releases were done under a 36W Sylvania Aquastar fluorescent white light and fluorescent cool purple light bulb supplied by Uganda Electricals (K) LTD, Kenya. Leafminer larvae were held in the experimental cages for seven to eight days and allowed to pupate. Prior to adult emergence, the pupae were collected and individually incubated in gelatin capsules (2.20 cm height, 0.7 cm diameter and 0.8 cm^3 volume) (Plate 4.2). 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 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.

Table 4.1: Summary of Phaedrotoma scabriventris and Opius dissitus release strategies on Liriomyza huidobrensis larvae

Treatment (T)	Release pattern
Single release	
O. dissitus only (T1)	50 adults *O. dissitus* at 1: 2 (15 M*: 35 F*) for 24 hour s
P. scabriventris only (T2)	50 adults *P. scabriventris* at 1: 2 (15 M: 35 F) for 24 hour s
Sequential release	
O. dissitus first, *P. scabriventris* second (T3)	50 adults *O. dissitus* at 1: 2 (15 M: 35 F) for 24 hours followed by 50 adults *P. scabriventris* at 1: 2 (15 M: 35 F) for another 24 hours
P. scabriventris first, *O. dissitus* second (T4)	50 adults *P. scabriventris* at 1: 2 (15 M: 35 F) for 24 hours followed by 50 adults *O. dissitus* at 1: 2 (15 M: 35 F) for another 24 hours
Simultaneous release	
O. dissitus and *P. scabriventris* (T5)	50 adults *O. dissitus* and 50 adults of *P. scabriventris*, both species at 1: 2 (15 M: 35 F) for 24 hours
O. dissitus and *P. scabriventris* (T6)	25 adults *O. dissitus* and 25 adults of *P. scabriventris* both species at 1: 2 (7 M: 18 F) for 24 hours
Control	
L. huidobrensis reared alone (T7)	No parasitoid species released

*M: male and F: female

Plate 4.2: Gelatine capsules containing individual pupa of *Liriomyza huidobrensis*, *Phaedrotoma scabriventris* and *Opius dissitus*

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 of each of the two parasitoid species (T5) with sequential releases of 50 individuals of each species (T3 and T4). Each specific parasitism rates 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 performance 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.

4.2.4 Non-reproductive host-killing

Non-reproductive host-killing behavior 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.

4.2.5 Data analyses

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

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

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

$$TP_{PsOd} = \left(\frac{C_{Ps} + C_{Od}}{C_{Ps} + C_{Od} + C_{Lh}}\right) \times 100$$

Where SP_{Ps} = the specific parasitism rate of *P. scabriventris*, C_{Ps} = 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 TP_{PsOd} = the total parasitism of *P. scabriventris* and *O. dissitus*.

The data on parasitism rates recorded for each treatment was first arcsine transformed and then subjected to one-way Analysis of variance (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 (χ^2) 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 statistical software (R Development Core Team, 2013).

4.3 Results

4.3.1 Interspecific competition between *Phaedrotoma scabriventris* and *Opius dissitus*

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 4.2). 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 4.2). In the sequential introductions (T3 and T4), no significant difference was observed in total parasitism between the release sequences (Table 4.2). Simultaneous release of 25 individuals of the two parasitoid 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 4.2).

The total specific parasitism rates of *P. scabriventris* and *O. dissitus* did not differ significantly when released alone (Table 4.2). 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 4.2). The first introduced species in sequential releases achieved similar specific parasitism rates as when used alone (T1 vs T3 and T2 vs T4)and significantly higher specific parasitism rates compared to the second released species (T3: $\chi^2 = 89.89$, df = 1, P <0.0001 and T4: $\chi^2 = 15.14$, df = 1, P <0.0001) (Table 4.2). When comparing the specific parasitism rates of *P. scabriventris* and *O. dissitus* in the simultaneous release treatment (T5) to the sequential release 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 4.2).

Table 4.2: Mean ± SE (Standard Error) for total and specific parasitism rate of *Phaedrotoma scabriventris* and *Opius dissitus* on *Liriomyza huidobrensis* per treatment

Treatment(T) regime	*O. dissitus* specific parasitism (%)	*P. scabriventris* specific parasitism (%)	χ^2	P-value	Total parasitism (%)
Single release					
O. dissitus only (T1)	42.6±3.4a	-	-	-	42.6±3.4ab
P. scabriventris only (T2)	-	34.9±8.5a	-	-	34.9±8.5a
Sequential release					
O. dissitus first, *P. scabriventris* second (T3)	38.3±2.3abA	11.2±0.7cB	89.89	<0.0001	49.5±2.9ab
P. scabriventris first, *O. dissitus* second (T4)	17.0±2.2cA	28.3±2.4aB	15.14	<0.0001	45.2±3.7ab
Simultaneous release					
O. dissitus and *P. scabriventris* (T5)	38.4±5.6abA	23.5±1.1abB	19.67	<0.0001	62.0±4.6b
O. dissitus and *P. scabriventris* (T6)	29.6±4.0bA	15.0±1.6bcB	23.48	<0.0001	44.5±2.8ab
Control					
L. huidobrensis 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).

The sex ratio of *P. scabriventris* in the F1 progeny was not significantly different when used alone (χ^2 = 1.14, df = 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 4.3). 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 (χ^2 = 3.88, df = 1, P = 0.049) compared to the male progeny (Table 4.3). Sex ratio of *O. dissitus* was significantly more male biased ($\chi^2 = 41.28$, df = 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 4.3). 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 to T7) (Table 4.3). From all the treatments, only one insect (parasitoid or pest) were obtained from each capsulated pupa either by natural adult emergence or by dissection.

Table 4.3: Effect of presence and sequence of introduction of *Phaedrotoma scabriventris* and *Opius dissitus* on the F1 progeny sex ratios of the host and parasitoids: Mean ± SE (Standard Error)

Insect species	Sex ratios	Treatment						
		T1*	T2	T3	T4	T5	T6	T7
L. huidobrensis	%* Male	44.3±2.8aA*	44.7±1.9aA	48.6±1.5aA	50.1±0.9aA	46.8±2.8aA	50.2±3.2aA	47.2±1.3aA
	% Female	55.7±2.8aA	55.3±1.9aA	51.4±1.5aA	49.9±0.9aA	53.2±2.8aA	49.8±3.2aA	52.8±1.3aA
	χ^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
O. dissitus	% Male	71.4±3.9aA		72.1±1.9aA	71.0±2.6aA	73.0±3.1aA	70.1±7.8aA	
	%Female	28.6±3.9aB		27.9±1.9aB	29.0±2.6aB	27.0±3.1aB	29.9±7.8aB	
	χ^2	41.2824		42.735	15.6957	40.9074	31.6712	
	P	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	
P. scabriventris	%Male		48.4±3.1aA	37.4±0.7aA	45.6±1.6aA	41.2±3.7aA	49.6±9.0aA	
	%Female		51.6±3.1aA	62.6±0.7aB	54.4±1.6aA	58.8±3.7aA	50.4±9.0aA	
	χ^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 ± SE.

Within row, means followed by the same low case letter are not significantly different at P = 0.05 (Tukey's test).

Within column 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 tables 4.1 and 4.2 above.

4.3.2 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 4.4).

Table 4.4: Mean ± SE (Standard Error) for non-reproductive mortality of *Liriomyza huidobrensis* per treatment

Treatment(T) regime	Non-reproductive mortality (%)
Single release	
O. dissitus only (T1)	33.0±1.1a
P. scabriventris only (T2)	42.3±1.0a
Sequential release	
O. dissitus first, *P. scabriventris* second (T3)	30.1±1.1a
P. scabriventris first, *O. dissitus* second (T4)	37.5±1.1a
Simultaneous release	
O. dissitus and *P. scabriventris* (T5)	30.2±1.1a
O. dissitus and *P. scabriventris* (T6)	36.2±1.4a
Control	
L. huidobrensis reared alone (T7)	30.4±1.2a

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

4.4 Discussion

Introduction of exotic parasitoids is often the most desirable option in controlling invasive pests especially in areas where indigenous natural enemies have negligible effect in controlling the pest (Lenteren, 1997). This study represents the first laboratory evidence of parasitism rates of both *O. dissitus* and *P. scabriventris* on *L. huidobrensis*. In the current 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 (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. *Phaedrotoma scabriventris* seems to have an edge over *O. dissitus* in maintaining a balanced sex ratio of its offspring when host resources are abundant as in T2 and T4 and when given equal opportunity with *O. dissitus* to parasitize a given quantity of host as in T5 and T6. *Phaedrotoma scabriventris* seems to also have an edge over *O. dissitus* by producing more females when host resources are limited as in T3. The ability of parasitoids to determine the gender 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. *Phaedrotoma scabriventris*, therefore, demonstrated the ability to maximize its population fitness under limited resource condition (Chow & Heinz, 2005). These 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 Kenya could improve on the natural control of the pest.

From all the treatments, only one adult emerged from each capsulated pupa. In addition, only one individual was found in all dissected pupae that were harboring parasitoids. These findings confirm the solitary nature of both parasitoid 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 as observed in this study, 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; 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 (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 observed in this study 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; 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 result was reported by Collier et al. (2002) when using *Encarsia formosa* Gahan and *Encarsia pergandiella* (Howard) (Hymenoptera: Aphelinidae). 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 used in this study 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 Kenya.

Both parasitoid species did not cause any significant non-reproductive mortality of the host 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) behaviors 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 (Hemiptera: Aphididae) 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* (Zeller) (Lepidoptera: Gelechiidae) by egg-larval endoparasitoid, *Copidosoma koehleri* (Blanchard) (Hymenoptera: Encyrtidae) affected their development even when the probings did not lead to oviposition (Keinan et al., 2012). Non-reproductive host-killing behavior 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 behavior, killed about 44.7% of their host larvae, the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae).

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. dissutus* and *P. scabriventris* should be considered in the evaluation of their performance against *L. huidobrensis*.

4.5 Conclusion

In conclusion, both *P. scabriventris* and *O. dissitus* showed interspecific co-existence and host discrimination abilities during their reproductive process. However, further studies are warranted 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* leafminers 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 be evaluated based on their parasitism rate.

CHAPTER FIVE

SPECIES COMPOSITION, DISTRIBUTION AND SEASONAL ABUNDANCE OF *LIRIOMYZA* LEAFMINERS (DIPTERA: AGROMYZIDAE) UNDER DIFFERENT VEGETABLE PRODUCTION SYSTEMS AND AGRO-ECOLOGICAL ZONES IN KENYA

ABSTRACT

A longitudinal study to identify the species of *Liriomyza* leafminer, their distribution, relative abundance, and seasonal variation, including their host range, was conducted in vegetable fields at three elevations in Kenya from November 2011 to November 2012. Three main species were identified: *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *Liriomyza trifolii* (Burgess), of which *L. huidobrensis* was the most abundant across all elevations irrespective of the cropping season and accounting for over 90% of the total *Liriomyza* specimens collected. *Liriomyza* species were collected from all infested incubated leaves of 20 crops surveyed belonging to seven families: Fabaceae, Solanaceae, Cucurbitaceae, Malvaceae, Brassicaceae, Amaranthaceae, and Amaryllidaceae. However, more than 87.5% of the *Liriomyza* species were obtained from *Pisum sativum* L., *Phaseolus vulgaris* L., *Solanum lycopersicum* L., and *S. tuberosum*, thereby demonstrating that Fabaceae and Solonaceae crops are the most important hosts with regard to *Liriomyza* species richness and relative abundance. *Liriomyza huidobrensis* had the widest host range (20 crops), followed by *L. sativae* (18 crops) and *L. trifolii* (12 crops). Although *L. trifolii* has been considered the dominant *Liriomyza* leafminer in Kenya, this study suggests that this may not be the case anymore, as *L. huidobrensis* dominates at all elevations.

5.1 Introduction

Production of vegetables for both domestic and export markets in Kenya is a major source of income, employment and food for smallholder farmers, especially women (HCDA, 2010, 2013). The horticultural sector in Kenya is one of the fastest growing agricultural sectors and in 2012, horticultural exports generated above US$ 1.08 billion in foreign exchange (HCDA, 2013). Flowers and vegetables constituted the biggest horticultural export representing 48.9% and 35.2%, respectively of export value (HCDA, 2013). However, severe infestation of *Liriomyza* leafminers is among the most important production constrain on vegetables and flowers, limiting the horticultural sector from achieving its full potential (Njuguna et al., 2001; KEPHIS, 2007; Chabi-Olaye et al., 2008). LMF pests are the most important cause of interception of Kenya's fresh vegetables and flowers in European market due to their quarantine status (Kedera & Kuria, 2003; EPPO, 2013; EUROPHYT, 2014).

Farmers' approach to control LMF in export vegetables and flowers is limited to routine insecticide applications. According to Gitonga et al. (2010), dimethoate, abamectin, imidacloprid, alphacypermethrin and beta-cyfluthrin are the most common insecticides used against LMF and other pests' complex in vegetable production systems in Kenya. However, while various studies reported that abamectin, alphacypermethrin and beta-cyfluthrin can effectively control LMF (Murphy & La Salle, 1999; Weintraub, 2001; Kaspi & Parrella, 2005), a recent study in Kenya by Guantai et al. (2015) revealed that, at recommended doses, none of the aforementioned insecticides used in Kenya is effective against the larval stage of *L. huidobrensis*, the most aggressive and abundant of all the invasive LMF species identified in Kenya. Indiscriminate application of synthetic insecticides have led to low levels of parasitism, less than 6% by the major parasitoid species associated with the pest across all elevations in Kenya (Chabi-Olaye et al., 2008). Furthermore, high cost, human and animal health hazards, environmental risks and rejection of export products due to high pesticides residue levels are associated with such routine chemical pesticides application (Braun & Shepard, 1997; PIP, 2013; RASFF, 2013). There is therefore a need to develop sound, environmental friendly and efficient IPM techniques to control LMF pest in Kenya and East Africa. However, a prerequisite for a successful IPM approach is to better understand the LMF species composition, abundance and the effect of seasons, vegetable production systems and agroecology on the pest (Mwatawala et al., 2006).

It has long been known that *L. trifolii*, introduced from Florida, USA, through chrysanthemum cuttings at Masongaleni, Makueni County in 1976 is the main *Liriomyza* species infesting ornamentals and vegetables in Kenya (Spencer, 1985). Heavy infestation by *L. trifolii* over the years and its subsequent spread throughout the country and to other host plants and abroad resulted in closure of many flower farms, loss of jobs and loss of overseas markets due to quarantine requirements (Spencer, 1985; IPPC, 2005). The perception that *L. trifolii* was the most important invasive *Liriomyza* species in Kenya persisted over the years (IPPC, 2005). A countrywide survey in Kenya by Chabi-Olaye et al. (2008) revealed the existence of other invasive species; *L. huidobrensis* and *L. sativae*, with *L. trifolii* dominating at the low and mid elevations and *L. huidobrensis* dominating at the high elevation. *Liriomyza huidobrensis* is known to adapt and predominantly colonize hosts at colder higher elevations, mostly above 1,000 m a.s.l. (Spencer, 1989; Rauf et al., 2000; Mujica & Kroschel, 2011) while *L. sativae* and *L. trifolii* are the dominant pest in low and mid elevations (Spencer, 1989; Rauf et al., 2000; Andersen et al., 2002). However, neo tropical populations of these LMF have continued to depict high adaptability with complex histories of invasion and establishing in many countries worldwide, exhibiting interspecific interactions causing damage to many crops and growing ever more difficult to control (Costa-Lima et al., 2010; Yildirim & Unay, 2011; Gao et al., 2011). In Kenya, most agricultural crop production systems have four cropping seasons, including the long rains, the short rains, the cold dry and the hot dry seasons, which vary with respect to elevation and have been inconsistent especially at the mid and low elevations since 1980s (Hassan, 1998; Jaetzold et al., 2006). Thus, production of vegetable crops as host plants of *Liriomyza* species is not equally important across elevations. In addition occurrence and relative abundance of leafminers in relation to seasons and host plants may reflect the impacts of climate and their distinct preference for host plants (Johansen et al., 2003; Tran et al., 2005; Tran et al., 2007). The varying ecosystems may affect the distribution of *Liriomyza* species and functional diversity, thus, also affecting activities of associated natural enemies. This suggests that the inventories of LMF pest on host crops may have changed significantly over time. However, none of the previous studies carried out in Kenya covered the seasonal variation of LMF species across the elevations, vegetable production systems and host plants.

The aim of the present study was to identify and determine the LMF species composition, their seasonal variation, relative abundance and their host range in various agro-ecological zones and vegetable production systems in Kenya.

5.2 Materials and methods

5.2.1 Study sites

Three field sites were selected based on elevations and other factors as described in Section 3.1.

5.2.2 Field surveys and sampling to determine leafmier incidence, species composition, distribution and seasonal abundance at highland, midland and lowland in vegetable production systems in Kenya

Monthly field surveys were carried out from November 2011 to November 2012 to determine the incidence, species composition, abundance and distribution of *Liriomyza* species on crops in three locations across each of the three different elevations of vegetable production in Kenya as described by Chabi-Olaye et al. (2008) with some modifications.

During each observation date at each elevation, LMF infestation, LMF species composition and abundance was evaluated. In order to determine the LMF infestation at the field level, the sampling area per field was subdivided into four equal quadrants and from each quadrant, 50 leaves from the middle stratum of different plants were picked at random and examined for leafminer infestation (leaves with punctures and/or mines) and the total number infested was recorded. Leaves from the middle stratum of plants were preferred for sampling to leaves from the upper or lower parts of plants because upper leaves are most often clean or have mostly punctures and leaves from the lower part have old mines with larvae already dropped into soil for pupation, while the leaves from the middle stratum have pupae, “live mines” (mines containing larva) and punctures.

As for LMF abundance and species composition, a maximum of 25 infested leaves per quadrant, with developing or developed mines containing leafminer larvae (“live mines”) were picked, giving a total of 100 leaves per field for laboratory incubation and observations. The infested leaves per field were immediately stored in perforated plastic paper bags and afterwards transferred onto damp paper towels contained in plastic rearing containers (19 ×13 × 8 cm) to prevent drying of leaves (Plate 5.1). The rearing containers were closed with lids containing muslin windows (16 × 9.5 cm) for ventilation and were parked in large cooler boxes to prevent overheating before transportation to the laboratory. After five to 10 days, pupae were collected from the rearing containers per field using soft camel hair brushes as they formed, counted and incubated in bulk in plastic petri dishes with labels until adults of leafminer flies emerged. Laboratory conditions were maintained at 25 ± 2 °C and 80 ± 5% R.H. Adult *Liriomyza* species were preserved in 80% ethanol and identification done using conventional taxonomic keys and identification keys from the LMF project at ICIPE, Nairobi, Kenya. The *Liriomyza* species were further sent to the Royal Museum of Central Africa, Belgium for confirmation. Voucher specimens of identified adult *Liriomyza* species are stored in the entomological museum at the ICIPE, Nairobi.

Plate 5.1: Plastic rearing containers used in rearing *Liriomyza* leafminers and associated parasitoids

5.2.3 Data analysis

The proportion of leafminer infested leaves (leafminer incidence) was calculated as the number of infested leaves multiplied by 100 and divided by 200 (total number of leaves picked at random from the middle stratum of the plants from the four quadrants per field). Since the leaves were sampled over time, to avoid pseudo-replication, the proportion of leafminer infested leaves were averaged and the averages were used as the data for analysis (Hurlbert, 1984). Proportions were arcsine transformed and then subjected to two-way ANOVA to assess the effect of cropping season and elevation. Means were separated using Student-Newman-Keuls (SNK) test. The data were analyzed in R version 3.0.2 statistical software (R Development Core Team, 2013).

5.3 Results

5.3.1 Leafminers incidence in farmer fields at highland, midland and lowland in vegetable production systems in Kenya

Liriomyza leafminer infested crops were found in all three surveyed locations at each elevation throughout the study period. There was significant interaction between elevation and season in terms of incidence of leafminer puncture and mines on leaves, $F_{6, 24} = 10.8$, $P < 0.0001$, thus the variation in infestation levels between elevations depended upon the season. The leafminer incidence ranged from 35.7 to 71.7% across the different elevations and seasons (Table 5.1). Infestation levels varied significantly between seasons at highland ($F_{3, 8} = 43.1$, $P < 0.0001$) and midland ($F_{3, 8} = 7.0$, $P = 0.$) and no significant differences at lowland ($F_{3, 8} = 1.3$, $P = 0.331$). At highland, infestation was highest in dry hot season and lowest in the long rainy season while at midland, infestation was highest in the short rainy season and lowest in dry cold season (Table 5.1). The dry hot season recorded relatively higher LMF infestation than the other seasons (Table 5.1). During the dry hot season, of all the 20 crops sampled, the highest leafminer infestation was recorded on French bean (79.5% of leaves), kidney bean (79.1%), snow pea (70.0%) and potato (69.5%) at the high elevation, with the lowest observed on potato (0.5%), okra (10.0%) and onion (14.0%) at the mid elevation (Table 5.2).

Table 5.1: Incidence of leafminer infested leaves during different cropping seasons at low, mid and high elevations in vegetable production systems in Kenya

Elevation	LMF infested leaves (Mean ± SE %) per season			
	Short rains	Dry hot	Long rains	Dry cold
Highland	53.0±1.5b	71.7±0.9a	35.7±4.2c	56.6±2.0b
Midland	56.6±3.8a	53.6±2.1a	55.1±5.5a	36.0±4.1b
Lowland	49.6±1.5a	55.7±5.0a	48.0±2.3a	49.2±2.2a

Within row, means followed by the same lower case letter are not significantly different at P = 0.05 (Student-Newman-Keuls Test).

Table 5.2: Leafminers percentage infestation (Mean ± SE %) in farmer's fields on different host plants across low, mid and high elevations in vegetable production systems in Kenya

Highland

Crop	n[*]	Short rains	n	Dry hot	n	Long rains	n	Dry cold
Courgette	3	52.7±3.5	1	43.5	4	58.0±12.2	1	68.0
French bean	20	44.6±3.4	13	79.5±4.9	12	32.0±4.0	13	54.8±3.0
Garden pea	1	51.5	5	64.9±11.1	4	28.5±2.6	5	53.7±4.3
Kidney bean	3	60.2±18.5	13	79.1±7.2	8	30.7±6.7	3	65.5±9.4
Potato	16	53.7±3.6	21	69.7±5.0	23	31.1±2.7	6	59.4±4.9
Snow pea	30	57.9±3.0	26	70.0±4.1	39	36.7±2.9	23	56.2±2.2
Spinach	-	-	-	-	-	-	1	80.0
Sugar snap	5	63.8±6.7	8	72.4±6.7	5	53.3±4.9	4	53.5±5.8
Sweet pepper	1	45.0	1	54.5	1	29.5	1	35.5
Tomato	1	15.0	1	35.0	3	40.8±2.3	2	58.3±21.3

Midland

Crop	N	Short rains	n	Dry hot	n	Long rains	N	Dry cold
Courgette	-	-	1	25.0	-	-	-	-
Cowpea	-	-	1	80.5	-	-	-	-
French bean	19	57.6±5.2	39	55.6±4.2	15	46.4±4.0	14	28.7±4.2
Garden pea	-	-	1	-	-	-	-	-
Kidney bean	9	86.9±2.5	30	60.6±4.2	17	61.7±3.8	10	32.5±5.6
Okra	-	-	1	10.0	-	-	-	-
Onion	-	-	1	14.0	-	-	-	-
Potato	1	36.5	1	0.5	1	56.5	1	39.5
Tomato	15	39.2±2.3	53	51.7±3.6	29	54.8±3.3	32	39.6±3.7
Watermelon	-	-	-	-	1	35.0	2	45.8±7.3

Lowland

Crop	N	Short rains	n	Dry hot	n	Long rains	N	Dry cold
Bitter gourd	1	60.5	-	-	-	-	-	-
Brinjale eggplant	-	-	1	82.0	-	-	-	-
Butternut squash	-	-	-	-	-	-	1	39.0
Cowpea	1	23.5	7	39.6±10.8	3	46.5±11.0	-	-
Dolichos bean	-	-	1	79	-	-	-	-
French bean	3	69.5±7.2	-	-	-	-	-	-
Kale	-	-	3	12.8±4.7	1	71.5	-	-
Kidney bean	16	48.6±3.0	38	63.7±3.4	13	53.9±3.8	10	41.3±3.1
Okra	1	35.5	9	49.9±4.0	12	37.9±4.1	2	45.8±2.8
Pumpkin	1	28.0	2	49.0±17.0	1	57.5	-	-
Sweet pepper	-	-	1	18.5	1	41.0	1	18.5
Tomato	17	50.2±3.8	66	55.9±2.6	36	49.3±2.3	42	52.0±1.8
Watermelon	1	40	4	53.0±13.7	6	45.2±8.4	2	50.8±6.8

n[*]: Number of sampled fields

5.3.2 *Liriomyza*s species composition, abundance and distribution from infested leaves at highland, midland and lowland in vegetable production systems in Kenya

A total of 46,879 Agromyzidae *Liriomyza* leafminer adult flies composed of *L. huidobrensis, L. sativae L. trifolii*, *L. bryoniae* and unidentified *Liriomyza* species, were recorded on the 20 crops sampled belonging to seven families namely; Fabaceae, Solanaceae, Cucurbitaceae, Malvaceae, Brassicaceae, Amaranthaceae and Amaryllidaceae. The most abundant species was *L. huidobrensis*, representing overall 90.5% of all LMF species collected, and with 94.4%, 92.4% and 84.4% at high, low and mid elevations, respectively (Table 5.3). The relative abundance of *L. sativae* was much lower than that of *L. huidobrensis*, representing overall only 6.2% of LMF species collected, but relatively more common in the lowland (5.8%) and midland (9.5%) compared to highland (3.7%) (Table 5.3). *Liriomyza trifolii* was rarely found during this study, representing overall only 2.7% of LMF, with 1.0%, 5.7% and 1.5% of LMF at the highland, midland and lowland, respectively (Table 5.3).

Overall, *L. huidobrensis*, *L. sativae* and *L. trifolii* represented 99.4% of all *Liriomyza* species in the study sites. Seasonal comparisons of these species at each elevation revealed that *L. huidobrensis* was more abundant at all elevations, accounting for more than 70% of the three *Liriomyza* species throughout the study period (Table 5.4). *Liriomyza sativae* was generally more abundant during the hot dry season at all elevations and less abundant during the dry cold season at the high and mid elevations and during the short rains at the low elevation (Table 5.4). *Liriomyza trifolii* was most abundant in the dry hot season at the mid elevation and remained relatively low at both high and the low elevations in all cropping seasons (Table 5.4).

Table 5.3: Species composition, abundance and distribution of *Liriomyza* leafminer flies identified from infested leaves at low, mid and high elevations in vegetable production systems in Kenya

Liriomyza spp.	Number of LMF specimen per elevation			
	Highland (n*=327)	Midland (n=294)	Lowland (n=304)	Total
L. huidobrensis	26,788	7,257	10,134	44,179
L. sativae	583	850	516	1,949
L. trifolli	66	479	89	634
Liriomyza spp. (unidentified)	77	19	6	102
L. bryoniae	10	3	2	15
Total	27,524	8,608	10,747	46,879

n^*: number of sampled fields

Table 5.4: Species composition and abundance of the most frequent and invasive *Liriomyza* species at low, mid and high elevations during different cropping seasons in vegetable production systems in Kenya

	Number of LMF specimen per elevation and season (% of LMF species)		
	Highland		
Season	*L. huidobrensis*	*L. sativae*	*L. trifolii*
Short rains	6,604 (97.4%)	139 (2.1%)	36 (0.5%)
Dry hot	5,435 (94.0%)	334 (5.8%)	15 (0.3%)
Long rains	4,569 (98.6%)	60 (1.3%)	6 (0.1%)
Dry cold	10,180 (99.4%)	50 (0.5%)	9 (0.1%)
	Midland		
Season	*L. huidobrensis*	*L. sativae*	*L. trifolii*
Short rains	814 (94.1%)	36 (4.2%)	15 (1.7%)
Dry hot	2,355 (71.1%)	580 (17.5%)	379 (11.4%)
Long rains	2,002 (87.7%)	210 (9.2%)	71 (3.1%)
Dry cold	2,086 (98.2)	24 (1.1%)	14 (0.7%)
	Lowland		
Season	*L. huidobrensis*	*L. sativae*	*L. trifolii*
Short rains	1,249 (99.8%)	3 (0.2%)	0 (0.0%)
Dry hot	4,056 (92.7%)	270 (6.2%)	51 (1.2%)
Long rains	3,103 (93.0%)	204 (6.1%)	28 (0.8%)
Dry cold	1,726 (97.2%)	39 (2.2%)	10 (0.6%)

5.3.3 *Liriomyza* species host plants diversity at highland, midland and lowland in vegetable production systems in Kenya

A total of 20 different vegetable crops were sampled from the different elevations of which all were identified as host plants of leafminers (Tables 5.5 to 5.7). More than 87.5% of the total *Liriomyza* species were reared from six plant species belonging to two families: Fabaceae (snow pea, sugar snap, common bean and French bean) and Solonaceae (tomato and potato) from all the elevations (Tables 5.5 to 5.7). Crops from the family Fabaceae were the most commonly grown at high elevation and consequently were the most common *Liriomyza* host plants at the high elevation, with snow pea being the most affected while kidney bean was the least affected (Table 5.5). At the mid elevation, the Solonaceae and the Fabaceae were the most affected crop families, with tomato being the most affected and potato the least affected of the Solonaceae while French bean and cowpea were the most and least affected, respectively of the Fabaceae (Table 5.6). The Solonaceae and the Fabaceae were also the most affected crop families at the low elevation, with tomato and sweet pepper being the most and the least affected of the Solonaceae while kidney bean and dolichos bean were the most and the least affected of the Fabaceae, respectively (Table 5.7). The least affected crops were spinach, at high elevation (Table 5.5), onion and okra, at the mid elevation (Table 5.6) and kale, at the low elevation (Table 5.7). *Liriomyza huidobrensis* had the widest host range (20 crops) and had the highest abundance on these crops (Tables 5.5 to 5.7), followed by *L. sativae* (18 crops) and *L. trifolii* (12 crops) (Tables 5.5 to 5.7). These three *Liriomyza* species were consistently reared in high abundance on snow pea from the high elevation while tomato was the most affected host at the low and mid elevations (Tables 5.5 to 5.7). *Liriomyza bryonae* and the unidentified *Liriomyza* species were less abundant and were reared from four and nine crops respectively (Tables 5.5 to 5.7). The number of host plants varied across the elevations. The lowland had the highest number of crop species surveyed: 13 host plants while 10 crop species each were surveyed in the highland and midland (Tables 5.5 to 5.7).

Table 5.5: *Liriomyza* species abundance on different host plants at high elevation

Plant family/scientific name	Common name	n^*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	**Liriomyza* species	Total
Fabaceae								
Pisum sativum L.	Snow pea	118	12443 (45.2%)	274 (1.0%)	28 (0.1%)	3 (0.0%)	30 (0.1%)	12778 (46.4%)
Pisum sativum L.	Sugar snap	22	3076 (11.2%)	43 (0.2%)	9 (0.0%)	-	-	3128 (11.4%)
Phaseolus vulgaris L.	French bean	58	2148 (7.8%)	93 (0.3%)	20 (0.1%)	4 (0.0%)	27 (0.1%)	2292 (8.3%)
Pisum sativum L.	Garden pea	15	2039 (7.4%)	7 (0.0%)	2 (0.0%)	-	5 (0.0%)	2053 (7.5%)
Phaseolus vulgaris L.	Kidney bean	27	1311 (4.8%)	36 (0.1%)	1 (0.0%)	1 (0.0%)	5 (0.0%)	1354 (4.9%)
Fabaceae total		240	21017 (76.4%)	453 (1.7%)	60 (0.2%)	8 (0.0%)	67 (0.2%)	21605 (78.5%)
Solanaceae								
Solanum tuberosum L.	Potato	66	3383 (12.3%)	98 (0.4%)	3 (0.0%)	-	10 (0.0%)	3494 (12.7%)
Solanum lycopersicum L.	Tomato	7	508 (1.9%)	14 (0.1%)	3 (0.0%)	2 (0.0%)	-	527 (1.2%)
Capsicum L.	Sweet pepper	4	188 (0.7%)	6 (0.0%)	-	-	-	194 (0.7%)
Solanaceae total		77	4079 (14.8%)	118 (0.4%)	6 (0.0%)	2 (0.0%)	10 (0.0%)	4215 (15.3%)
Cucurbitaceae								
Cucurbita pepo L	Courgette	9	1659 (6.0%)	10 (0.0%)	-	-	-	1669 (6.1%)
Cucurbitaceae total		9	1659 (6.0%)	10 (0.0%)	-	-	-	1669 (6.1%)
Amaranthaceae								
Spinacia oleracea L.	Spinach	1	33 (0.1%)	2 (0.0%)	-	-	-	35 (0.1%)
Amaranthaceae total		1	33 (0.1%)	2 (0.0%)	-	-	-	35 (0.1%)
Total for all crops		327	26788 (97.3%)	583 (2.1%)	66 (0.2%)	10 (0.0%)	77 (0.3%)	27524 (100.0%)

n^*; number of sampled fields

**Liriomyza* species: unidentified specimen

Table 5.6: *Liriomyza* species abundance on different host plants at mid elevation

Plant family/scientific name	Common name	n*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	**Liriomyza* species	Total per crop
Solanaceae								
Solanum lycopersicum L.	Tomato	129	3592 (41.7%)	595 (6.9%)	383 (4.5%)	3 (0.0%)	18 (0.2%)	4591 (53.3%)
Solanum tuberosum L.	Potato	4	153 (1.8%)	5 (0.1%)	1 (0.0%)	-	-	159 (1.9%)
Solanaceae total		133	3745 (43.5%)	600 (7.0%)	384 (4.5%)	3 (0.0%)	18 (0.2%)	4750 (55.2%)
Fabaceae								
Phaseolus vulgaris L.	Kidney bean	66	1456 (16.9%)	132 (1.5%)	58 (0.7%)	-	-	1646 (19.1%)
Phaseolus vulgaris L.	French bean	87	1706 (19.8%)	84 (1.0%)	29 (0.3%)	-	1 (0.0%)	1820 (21.1%)
Pisum sativum L.	Garden pea	1	1 (0.0%)	-	-	-	-	1 (0.0%)
Vigna unguiculata (L.) Walp	Cowpea	1	-	-	1 (0.0%)	-	-	1 (0.0%)
Fabaceae total		155	3163(36.7%)	216 (2.5%)	88 (1.0%)	0 (0.0%)	1 (0.0%)	3468 (40.3%)
Cucurbitaceae								
Cucurbita pepo L.	Courgette	1	176 (2.0%)	33 (0.4%)	3 (0.0%)	-	-	212 (2.5%)
Citrullus lanatus (Thunb) Matsun and Nakai	Watermelon	3	153 (1.8%)	-	-	-	-	153 (1.8%)
Cucurbitaceae total		4	329 (3.8%)	33 (0.4%)	3 (0.0%)	-	-	365 (4.2%)
Malvaceae								
Abelmoschus esculentus (L.) Moench	Okra	1	12 (0.1%)	1 (0.0%)	4 (0.1%)	-	-	17 (0.2%)
Malvaceae total		1	12 (0.1%)	1 (0.0%)	4 (0.1%)	-	-	17 (0.2%)
Amarilidaceae								
Allium cepa L.	Onion	1	8 (0.1%)	-	-	-	-	8 (0.1%)
Amarilidaceae total		1	8 (0.1%)	-	-	-	-	8 (0.1%)
Total for all crops		294	7257 (84.3%)	850 (9.9%)	479 (5.6%)	3 (0.0%)	19 (0.2%)	8608 (100.0%)

n*; number of sampled fields, **Liriomyza* species: unidentified specimen

Table 5.7: *Liriomyza* species abundance on different host plants at low elevation

Plant family/scientific name	Common name	n*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	**Liriomyza* species	Total
Solanaceae								
Solanum lycopersicum L.	Tomato	161	7416 (69.0%)	356 (3.3%)	53 (0.5%)	2 (0.0%)	1 (0.0%)	7828 (72.8%)
Solanum melongena L.	Brinjale eggplant	1	122 (1.1%)	25 (0.2%)	-	-	1 (0.0%)	148 (1.4%)
Capsicum L.	Sweet pepper	3	15 (0.1%)	3 (0.0%)	-	-	-	18 (0.2%)
Solanaceae Total		165	7553 (70.3%)	384 (3.6%)	53 (0.5%)	2 (0.0%)	2 (0.0%)	7994 (74.4%)
Fabaceae								
Phaseolus vulgaris L.	Kidney bean	77	1319 (12.3%)	43 (0.4%)	2 (0.0%)	-	1 (0.0%)	1365 (12.7%)
Vigna unguiculata (L.) Walp	Cowpea	11	159 (1.5%)	18 (0.2%)	12 (0.1%)	-	1 (0.0%)	190 (1.8%)
Phaseolus vulgaris L.	French bean	3	33 (0.3%)	1 (0.0%)	-	-	-	34 (0.3%)
Lablab purpureus (L.) Sweet	Dolichos bean	1	4 (0.0%)	-	-	-	-	4 (0.0%)
Fabaceae Total		92	1515 (14.1%)	62 (0.6%)	14 (0.1%)	-	2 (0.0%)	1593 (14.8%)
Cucurbitaceae								
Citrullus lanatus (Thunb) Matsun and Nakai	Watermelon	13	358 (3.3%)	20 (0.2%)	4 (0.0%)	-	-	382 (3.6%)
Momordica charantia L	Bitter gourd	1	145 (1.4%)	-	-	-	-	145 (1.4%)
Cucurbita maxima Duchesne	Pumpkin	4	42 (0.4%)	5 (0.1%)	-	-	-	47 (0.4%)
Cucurbita moschata Duchesne ex Poir.	Butternut squash	1	30 (0.3%)	-	-	-	-	30 (0.3%)
Cucurbitaceae Total		19	575 (5.4%)	25 (0.2%)	4 (0.0%)	-	-	604 (5.6%)
Malvaceae								
Abelmoschus esculentus (L.) Moench	Okra	24	439 (4.1%)	40 (0.4%)	12 (0.1%)	-	2 (0.0%)	493 (4.6%)
Malvaceae Total		24	439 (4.1%)	40 (0.4%)	12 (0.1%)	-	2 (0.0%)	493 (4.6%)
Brassicaceae								
Brassica oleracea acephala L.	Kale	4	52 (0.5%)	5 (0.1%)	6 (0.1%)	-	-	63 (0.6%)
Brassicaceae Total		4	52 (0.5%)	5 (0.1%)	6 (0.1%)	-	-	63 (0.6%)
Total for all crops		304	10134 (94.3%)	516 (4.8%)	89 (0.8%)	2 (0.0%)	6 (0.1%)	10747 (100.0%)

n*; number of sampled fields and **Liriomyza* species: unidentified specimen

5.4 Discussion

Leafminer incidence and the resulting counts of emerging LMF were recorded in all the survey sites across the three elevations of vegetable production in Kenya. This suggests a widespread distribution of LMF in Kenya. With regard to elevations, LMF was more abundant in the highland relative to mid- and lowlands. This may be attributed to the fact that the LMF collections were dominated by *L. huidobrensis* which is known to adapt and predominantly infest hosts at colder higher elevations, mostly above 1,000 m a.s.l. (Spencer, 1989; Rauf et al., 2000; Mujica & Kroschel, 2011). However, this species was not only predominant at the high elevation but also at the warmer mid and the low elevations, a finding which contrasts previous studies in Kenya indicating that *L. sativae* and *L. trifolli* predominate in the two later elevations (Chabi-Olaye et al., 2008). This finding suggests that *L. huidobrensis* is more aggressive and is adapting to warmer areas and may be displacing *L. trifolli* which has long history of establishing in Kenya as well as *L. sativae* at the low and the mid elevations. Species displacement is a potentially widespread phenomenon, receiving much attention from ecologists because it affects the structure of communities (Reitz & Trumble, 2002; Reitz, 2007; Abe & Tokumaru, 2008). This phenomenon is common in *L. sativae* and *L. trifolli* under field conditions. However, Chen & Kang (2004, 2005) found that *L. huidobrensis* replaced *L. sativae* as the predominant pest in all areas of varying elevations in China. Subsequent studies by Gao et al. (2011) in the Chinese province of Hainan, revealed that *L. trifolii* displaced *L. sativae* which was the predominant pest on cowpea. Similarly, *L. sativae* was also displaced by *L. trifolii* in the western United States (Trumble & Nakakihara, 1983; Palumbo et al., 1994). However, in Japan, the opposite occurred, where *L. trifolii* was displaced by *L. sativae* (Abe & Kawahara, 2001; Abe & Tokumaru, 2008). Thus, given that *L. huidobrensis* was the most abundant, in addition to its wider distribution as determined in this study, the species constitutes the greatest threat to vegetables and ornamentals production in Kenya. Additionally, the spread of *L. huidobrensis* from higher elevation to lower elevations and its high adaptation could serve as an indicator that the same may be obtained in neighboring Uganda where, according to an unpublished data, LMF are currently not considered to be of high importance, with *L. sativae* dominating countrywide while *L. huidobrensis* is limited to higher elevations (K.K.M. Fiaboe, personal communication). The three most important LMF species in Kenya are *L. huidobrensis*, *L. sativae* and *L. trifolii*, representing 99.4% of total LMF species. This implies that *L. bryonae* and the unidentified *Liriomyza* species are currently negligible in vegetable production systems of Kenya. This, in addition to the low population of *L. sativae* and *L. trofolii* during this survey suggests that special attention should be given to *L. huidobrensis* control. Further field surveys will be required in Kenya as well as neighboring countries to assess the status of LMF species composition and abundance over several years in vegetable productions systems.

Although different host plants present an array of chemical, nutritional, and morphological challenges for larval development, the three most abundant *Liriomyza* species identified in this study are highly polyphagous, attacking plants in several families (Murphy & LaSalle, 1999; Andersen et al., 2002; Tran et al., 2006). In the current survey, *L. huidobrensis, L. sativae* and *L. trifolii* were identified from 20, 18 and 12 different infested crops, respectively. This may suggest that, the current host range for *L. huidobrensis* and *L. sativae* is relatively high compared to that of the long established *L. trifolii* in Kenya. Unlike *L. trifolii* and *L. sativae* larvae which are relatively small and feed on the upper mesophyll of the leaves, that of *L. huidobrensis* is larger and more aggressive by feeding in the lower mesophyll, mine into the petioles and pods, and causing more damage to plant photosynthetic area thus, severe yield reduction is inevitable (Weintraub & Horowitz, 1995). Being the most polyphagous species, in addition to its aggressiveness and high abundance at all elevations, *L. huidobrensis* constitutes the greatest LMF challenge to vegetables and ornamentals production in Kenya.

Although most *Liriomyza* species are polyphagous with broad and diverse host range (Murphy & LaSalle, 1999; Andersen et al., 2002; Tran et al., 2006), they also exhibit host plant preference (Zhao & Kang, 2003; Tokumaru & Abe, 2005). In the present study, snow pea (Fabaceae) was the most highly attacked *Liriomyza* host plant in the high elevation while tomato (Solonaceae) was the most highly attacked *Liriomyza* host plant at both mid and low elevations. This finding is consistent with previous studies, indicating that the fabaceaes and solanaceaes are the most suitable host for

Liriomyza species development (Tran et al., 2007; Chabi-Olaye et al., 2008; Mujica & Kroschel, 2011). Kale, onion and sweet pepper had lower LMF abundance. Chabi-Olaye et al. (2008) also found that field infested onion and kale leaves in Kenya resulted to very few numbers of *Liriomyza* species identified while Martin et al. (2005) found that Asian broccoli: *Brassica alboglabra* L. (Brassicaceae) was the least preferred host for the chrysanthemum leafminer. *Liriomyza huidobrensis* was the only *Liriomyza* species identified from onion and butter squash infested leaves. *Liriomyza huidobrensis* was also found infesting onion in California (Reitz &, Trumble 2002) and previous study in Kenya also revealed *Liriomyza* species infesting butter squash (Chabi-Olaye et al., 2008). Although host availability varied across elevations in this study, *Liriomyza* species can potentially exploit new host plants encountered in newly colonized habitats especially under conditions where their common hosts are rare or absent (Via, 1984). While the factors accounting for the differential LMF attraction and infestation are unclear, it is known that the plant hosts volatiles may play a significant role in pest attraction (Arimura et al., 2005; Takken & Dicke, 2006; Wei et al., 2007). Furthermore, differential host plant preference between LMF and their natural enemies, where some of the preferred host plants of the pest might be less suitable to natural enemies' colony development and/or performance against LMF, may also contribute to the observed differences (Knodel-Montz et al., 1985; Minkenberg & Ottenheim, 1990; Wei et al., 2000). Therefore, an understanding of the semiochemical basis for attraction between most important host plants (e.g., Fabaceae and Solanaceae) and less important plants (e.g., Brassicaceae and Amarilidaceae) as well as the semiochemical and morphological effects of these LMF host plants on the most important and efficient natural enemies could provide a solid foundation for management of LMF. Further studies to elucidate the parameters above will also help in minimizing the over reliance on synthetic insecticides currently used in LMF control in Kenya.

The occurrence and relative abundance of leafminers in relation to seasons and host plants may reflect the impacts of climate and their distinct preference for host plants (Murphy & LaSalle, 1999; Johansen *et al*., 2003). *Liriomyza* species can be present in the fields throughout the year as showed in this study. Apart from *L. huidobrensis* whose abundance remained relatively high throughout all the cropping seasons at all elevations, *L. trifolii* and *L. sativae* were more abundant during the hot dry season as compared to the other seasons across all elevations. This finding is consistent with previous studies indicating that *L. sativae* was very abundant in the dry season compared to the rainy season in South Vietnam and in Ho Chi Minh City, Vietnam (Tran et al., 2005, 2007). While Rauf & Shepard (1999) found that infestations by *L. huidobrensis* occurred heavily during the dry season compared to the rainy season, the present study however, revealed that the species remained relatively abundant throughout the cropping seasons at all elevations. This may suggests, that *L. huidobrensis* is not only adapting at all elevations, more aggressive and polyphagous, but is also consistently high at all elevations throughout the different cropping seasons despite the varied climatic conditions and crops available.

5.5 Conclusion

In conclusion, *Liriomyza* species pose a threat to vegetables and ornamentals at different elevations in Kenya. Of all LMF identified in this study, *L. huidobrensis* constitutes the greatest challenge to vegetable production by being consistently the most abundant and most polyphagous species at all elevation areas and all seasons. Additionally, the species is adapting to warmer climates at the mid and low elevations, thus, displacing *L. trifolii*, which is long established at these elevations in Kenya. While a quick action concerning the management of these pests should be put in place, the results of this study should serve as a signal to other countries like Uganda where it is still considered a high elevation pest. It is also important to determine if there are genetic differences between *L. huidobrensis* identified in Kenya to that in countries where the species is still limited to higher elevations for effective future management. Further studies to assess the species composition, abundance and possible displacement over time as well as to elucidate the semiochemical and morphological effects of host plants on LMF species infestation and their associated natural enemies are warranted.

CHAPTER SIX

LIRIOMYZA LEAFMINER PARASITOIDS COMPLEX IN DIFFERENT AGRO-ECOLOGICAL ZONES IN KENYA WITH SPECIAL FOCUS ON RELEASE, ESTABLISHMENT AND SPREAD OF *PHAEDROTOMA SCABRIVENTRIS* NIXON (HYMENOPTERA: BRACONIDAE)

ABSTRACT

Liriomyza leafminers (Diptera: Agromyzidae) are severe pests of vegetables and ornamentals worldwide. Previous studies revealed low leafminer parasitism across all agro ecological zones. The present paper reports on the complex of parasitoids at different elevations of vegetable production in Kenya after release of the exotic endoparasitoid *Phaedrotoma scabriventris* Nixon. Monthly surveys were carried out from 1st to 11th month post release. Nine parasitoid species were recorded with 31.23 ± 1.03% total parasitism rate around the release sites, from a total of 20 different vegetable *Liriomyza* infested crops belonging to seven families. *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), *P. scabriventris* and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), were the most abundant at all elevations, with 67.32%, 18.63% and 9.23% of total parasitoids recorded around release sites, respectively. *Phaedrotoma scabriventris* was recovered from all release sites and spread to up to 40, 30 and 50 km at high, mid and low elevations, respectively away from the release sites. Specific parasitism rates of *P. scabriventris* around release sites were significantly different across elevations, with more than five times higher in lowland (12.33%) and midland (6.36%) than in highland (1.51%). The 31.23 ± 1.03% total parasitism rate observed in this study compared to previous surveys in Kenya indicates a considerable improvement in leafminer parasitism. Implications of these findings on leafminer flies' management in vegetables and ornamentals production in Kenya are discussed.

6.1 Introduction

Liriomyza leafminers are invasive pest from South America and are among the most important pests of numerous field crops, ornamentals and vegetables worldwide (Spencer, 1985; Parrella, 1987; Murphy & LaSalle, 1999). In Kenya, three species; *L. huidobrensis*, *L. sativae* and *L. trifolii* are economically important and account for over 90% of all *Liriomyza* species collected from a variety of infested incubated host plants in both cultivated and wild habitats across different elevations of vegetable production (KEPHIS, 2007; Chabi-Olaye et al., 2008; Foba et al., 2015a) (Chapter 5). Depending on the plant type, leafminers' developmental stage, cropping season and elevation, LMF infestation ranges between 35.7 to 71.7% and yield losses between 10 to 100% (Chabi-Olaye et al., 2008; Foba et al., 2015a). Yield loss is higher in cultivated crops than wild hosts (Chabi-Olaye et al., 2008).

Control of LMF in Kenya has relied on indiscriminate application of synthetic insecticides (Gitonga et al., 2010). However, these insecticides do not kill the most destructive phase of the pest embedded within the leaf epidermis. In Kenya, none of the insecticides used in various pea production areas could kill the larval stage of *L. huidobrensis* when recommended doses were applied, while majority of them induced mortality of parasitoids at concentrations below recommended doses (Gitonga et al., 2010; Guantai et al., 2015). The quarantine status of LMF and the introduction of Maximum Residue Levels (MRLs) by EU put Kenyan horticulture farmers under pressure, to seek for alternative management strategies to produce clean products for export into EU markets.

In both their native and invaded areas, LMF populations are regulated by a complex of parasitoid species (Waterhouse & Norris, 1987; Murphy & LaSalle, 1999; Mujica & Kroschel, 2011). Noyes (2003) listed over 300 species of leafminer parasitoids, and over 80 species that attack various *Liriomyza* species. In the Neotropics, Salvo & Valladares (1998) identified 69 parasitoid species of Agromyzidae leafminers in natural, urban and agricultural habitats of Central Argentina. The Peruvian coast also hosts a very rich parasitoid community of about 63 species (Mujica & Kroschel, 2011). These parasitoid species complex have a wide adaptation to different host plants and *Liriomyza* species and regulate the pest populations to below economic threshold levels (Waterhouse & Norris, 1987; Murphy & LaSalle, 1999; Mujica & Kroschel, 2011). However, in Kenya the diversity of parasitoids associated with *Liriomyza* species is low in all horticultural production systems and includes mainly *O. dissitus*, *D. isaea*, *N. formosa* and *H. varicornis* (Chabi-Olaye et al., 2008).

Afrotropical populations of *Liriomyza* species suffer little mortality, less than 6% parasitism from these native parasitoids in both cultivated and wild habitats across all agro ecological zones (Chabi-Olaye et al., 2008; Guantai et al., 2015), suggesting that introduction of specific Neotropical parasitoids may be necessary to achieve effective suppression of these pests.

In order to enhance biological control of the invasive *Liriomyza* species in Kenya, ICIPE in partnership with CIP and KARI, imported three endoparasitoids; *H. arduine*, *C. flacilla* and *P. scabriventris* from Peru in December 2008. Of these parasitoids, *P. scabriventris* was the first to successfully establish in the quarantine facilities of ICIPE. *Phaedrotoma scabriventris* accounts for >50% of total parasitism of *Liriomyza* species especially *L. huidobrensis*, infesting different host plants across a wide range of agro-ecological zones; <500 to >4,000 m a.s.l. and temperature range of 4 to 24 °C in its native areas Peru, Chili, Brazil and Argentina (Valladares & Salvo, 2001; Videla *et al*., 2006; Valencia, 2008). Laboratory studies at ICIPE, indicated that the three major leafminer species present in East Africa were accepted and suitable for *P. scabriventris* colony establishment and the exotic parasitoid can co-exist with indigenous parasitoids recorded in East Africa (Chabi-Olaye et al., 2013; Akutse et al., 2015; Foba et al., 2015b) (Chapter 4). The present study was conducted to determine the leafminer parasitoid species composition in the different elevations post the release of *P. scabriventris* in vegetable production systems in Kenya. In addition, the establishment and spread of the released *P. scabriventris* in vegetable production systems in Kenya was studied.

6.2 Materials and methods

6.2.1 Release, establishment and spread of *Phaedrotoma scabriventris*

6.2.1.1 Rearing of *Phaedrotoma scabriventris*

The solitary endoparasitoid, *P. scabriventris* used in this study was imported into Kenya from a laboratory culture of CIP in Peru, South America, in December 2008. The parasitoid was maintained and supplied by the insectary, ICIPE, Kenya until its field release in November and December 2011 as described in Section 3.3.

6.2.1.2 Study sites

Three sites were selected based on elevation and other factors as described in Section 3.1.

6.2.2 Release of *Phaedrotoma scabriventris* at highland, midland and lowland in vegetable production systems in Kenya

Prior to field release in November and December 2011, newly emerged males and females of *P. scabriventris* were fed on 10% honey solution and kept together for two days to ensure mating. A total of 15,000 adult *P. scabriventris* of two-day old in the ratio of 1: 1 (male: female) were released in 15 farmers' vegetable fields per elevation: 1,000 adults released per farmer field. The distance between farmers' fields per location was approximately 300 m apart. In order to minimize the risk of failure of establishment, field owners were advised on subsequent insecticide use and conservation of *P. scabriventris* alongside other indigenous parasitoids. *Phaedrotoma scabriventris* was released in farmers' fields where no insecticides were sprayed at least two weeks before and after release. In addition, farmers training and awareness campaigns were conducted before, during and after release. In addition, extension officers were trained to disseminate the information to other farmers in the county.

6.2.3 Establishment of *Phaedrotoma scabriventris* at highland, midland and lowland in vegetable production systems in Kenya

Monthly field surveys to determine the establishment of *P. scabriventris*, abundance, distribution and seasonal variation of indigenous parasitoids associated with *Liriomyza* species were carried out from January 2012 to November 2012 on several vegetable crops in the three localities across each elevation of vegetable production in Kenya. Field surveys on establishment of *P. scabriventris* were conducted at a radius of about 4.5 km around release sites at each elevation. A total of 10 famers' crop fields were surveyed from each location during each field evaluation date.

6.2.4 Spread of *Phaedrotoma scabriventris* away from its release sites

Field surveys to determine the spread of *P. scabriventris* were conducted between November and December 2013 (two years after its release). This period also corresponded with the short rainy season, and hence, the surveys included sampling from none irrigated crops. Sampling points were systematically determined along transects in four cardinal directions including north, south, east and west in order to determine the dispersal of *P. scabriventris* from the initial released points (Gichini et al., 2008). Using Geographic Information System (GIS) tools, concentric circles were drawn on the map at a distance of 10, 20, 30, 40 and 50 km around each release point. On each circle, coordinates of four sampling points were established, one on each of the four cardinal directions. With a GPS and map of each elevation area, these sampling points were then located on the ground. A maximum of four fields including both cultivated crops and/or weeds depending on availability were surveyed within 2.5 km radius of each selected point. All pre-identified sampling points were visited except when inaccessible.

6.2.5 Sample collection and processing

In each field visited, the sampling area was subdivided into four equal quadrants and from each quadrant, a maximum of 25 infested leaves per quadrant, with developing or developed mines containing leafminer larvae (“live mines”) were actively picked, giving a total of 100 leaves per field for laboratory incubation and observations. Sampled leaves were processed as described in Section 5.2.2. Emerged adult parasitoids were preserved in 80% ethanol amended with 10% glycerin. Identification was done using keys as decribed in Section 5.2.2. The adult parasitoids were further sent to the Royal Museum of Central Africa, Belgium for confirmation. Voucher specimens of identified adult parasitoid species are stored in the entomological museum at ICIPE, Kenya.

6.2.6 Data analysis

Parasitoid abundance was expressed as percentage of total number of parasitoids identified per elevation and cropping season. The parasitism rates were expressed as proportion of emerged adult parasitoids out of all insects (emerged adult parasitoids and LMF) per elevation and cropping season. The monthly parasitism rates were analysed using a One-Way ANOVA to examine the effect of elevation for each recorded parasitoid species. Estimated parasitism rates at each elevation were further compared using SNK test. The spread of *P. scabriventris* at different elevations were assessed at different distances and in all four cardinal directions. In order to determine the spread of *P. scabriventris* at different distances outward from the release points, data collected from the fields were pooled according to field distance from the release site. The data were analyzed in R version 3.0.2 statistical software (R Development Core Team, 2013).

6.3 Results

6.3.1 Leafminer parasitoids species diversity, abundance and establishment of *Phaedrotoma scabriventris* in various agro-ecological zones in Kenya

A total of 9,282 hymenopteran individuals composed of nine LMF parasitoid species was recorded from *Liriomyza* leafminer infested leaves collected from release sites. The nine species belonged to four families namely Eulophidae (*D. isaea*, *N. formosa*, *Meruacesa* sp. Walker, and *H. varicornis*), Braconidae (*O. dissitus*, *P. scabriventris* and an unidentified Opiinae species), Pteromalidae (*Halticoptera* sp.) and an unidentified Figitidae species. Out of this total, 3,942 (42.47%) were recorded in the highland, 2,383 (25.67%) in the midland and 2,957 (31.86%) in the lowland. *Diglyphus isaea* was the most abundant parasitoid species at all elevation: 3,460 (87.77%), 1,601 (67.18%) and 1,188 (40.18%) at high, mid and low elevations, respectively. *Phaedrotoma scabriventris* was recovered in all release sites. At high and mid elevations, it reached 101 (5.50%) and 210 (19.22%) respectively, being two folds higher than *O. dissitus*, a closely related indigenous parasitoid known to be dominant in the study areas. At low elevation, *P. scabriventris* reached 1,054 (35.64%), not only doubling *O. dissitus* population, but also approaching *D. isaea* population. Overall, *D. isaea, P. scabriventris* and *O. dissitus* represented 95.18% of all parasitoid species recorded around the release sites.

Seasonal comparisons of all parasitoid species associated with *Liriomyza* species at each elevation revealed that *D. isaea* was the most abundant parasitoid species during all cropping seasons, followed by *P. scabriventris*, then *O. dissitus* (Table 6.1). *Diglyphus isaea* remained relatively high, exceeding 80% at high elevation and exceeding 66% at mid elevation throughout all the seasons (Table 6.1). However, at low elevation, *D. isaea* was most abundant during the dry cold season (50.97%) and least abundant during the short rainy season (11.61%) (Table 6.1). *Phaedrotoma scabriventris* was more abundant during the dry hot, long rainy and short rainy seasons compared to the dry cold season at high elevation (Table 6.1). At mid elevation, *P. scabriventris* was less abundant during the short rainy season compared to the other seasons and at low elevation, it was generally more abundant during all the cropping seasons (Table 6.1). *Opius dissitus* was more abundant during the dry hot and long rainy seasons compared to the dry cold and short rainy seasons at both high and mid elevations (Table 6.1). However, at low elevation, it was most abundant during the short rainy seasons compared to the other three seasons (Table 6.1).

Table 6.1: Species composition, abundance and distribution of parasitoids associated with *Liriomyza* species at low, mid and high elevations during different cropping seasons in Kenya

Season	n*	Counts of parasitoid species per elevation								
		D. isaea	*P. scabriventris*	*O. dissitus*	*N. formosa*	*Meruacesa* sp.	Opiinae species	*H. variconis*	Figitidae species	*Halticoptera* sp.
					Highland					
Dry hot	89	643 (80.98%)	62 (7.81%)	37 (4.66%)	22 (2.77%)	25 (3.15%)	-	2 (0.25%)	3 (0.38%)	-
Long rain	99	385 (82.09%)	31 (6.61%)	26 (5.54%)	13 (2.77%)	8 (1.71%)	-	6 (1.28%)	-	-
Dry cold	59	1,578 (92.17%)	60 (3.50%)	24 (1.40%)	15 (0.88%)	30 (1.75%)	-	5 (0.29%)	-	-
Short rain	55	854 (88.31%)	64 (6.62%)	14 (1.45%)	19 (1.95%)	15 (1.55%)	-	1 (0.10%)	-	-
Total for highland	302	3,460 (87.77%)	217 (5.50%)	101 (2.56%)	69 (1.75%)	78 (1.98%)	-	14 (0.36%)	3 (0.08%)	-
					Midland					
Dry hot	128	633 (66.56%)	156 (16.40%)	114 (11.99%)	27 (2.84%)	10 (1.05%)	-	9 (0.95%)	-	2 (0.21%)
Long rain	63	451 (66.62%)	130 (19.20%)	67 (9.90%)	6 (0.89%)	21 (3.10%)	-	2 (0.30%)	-	-
Dry cold	59	346 (66.54%)	156 (30.00%)	16 (3.08%)	0 (0.00%)	1 (0.19%)	-	1 (0.19%)	-	-
Short rain	25	171 (72.77%)	16 (6.81%)	13 (5.53%)	35 (14.89%)	-	-	-	-	-
Total for midlalnd	275	1,601 (67.18%)	458 (19.22%)	210 (8.81%)	68 (2.85%)	32 (1.34%)	-	12 (0.50%)	-	2 (0.08%)
					Lowland					
Dry hot	132	633 (41.37%)	537 (35.10%)	302 (19.74%)	32 (2.09%)	17 (1.11%)	4 (0.26%)	5 (0.33%)	-	-
Long rain	73	209 (38.56%)	193 (35.61%)	100 (18.45%)	14 (2.58%)	14 (2.58%)	12 (2.21%)	-	-	-
Dry cold	58	315 (50.97%)	194 (31.39%)	65 (10.52%)	3 (0.49%)	17 (2.75%)	21 (3.40%)	3 (0.49%)	-	-
Short rain	22	31 (11.61%)	130 (48.69%)	79 (29.59%)	16 (5.99%)	-	11 (4.12%)	-	-	-
Total for lowland	285	1,188 (40.18%)	1,054 (35.64%)	546 (18.46%)	65 (2.20%)	48 (1.62%)	48 (1.62%)	8 (0.27%)	-	-
Total for all elevations	862	6,249	1,729	857	202	158	48	34	3	2

n*; number of sampled field

On average, an overall parasitism rate of 31.23 ± 1.03% was recorded on *Liriomyza* species as an aggregate of all survey sites, with 26.06 ± 1.72%, 31.85 ± 1.77% and 36.11 ± 1.82 at high, mid and low elevations, respectively (Table 6.2). Total parasitism around the released sites was significantly different across elevations ($F_{2, 859} = 9.39$, $P <0.001$). Species specific parasitism rates across elevations was significantly different only for *D. isaea, P. scabriventris* and *O. dissitus*, $F_{2, 859} = 8.51$, $P < 0.001$ for *D. isaea*, $F_{2, 859} = 66.96$, $P < 0.001$ for *P. scabriventris* and $F_{2, 859} = 37.89$, $P < 0.001$ for *O. dissitus* parasitism. The specific parasitism rate of *D. isaea* was higher at high and mid elevations, with 21.25 ± 1.61% and 20.36 ± 1.53% respectively, compared to low elevation (13.69 ± 1.24%) (Table 6.2). The specific parasitism rate of *P. scabriventris* was about 10 times higher in lowland (12.33 ± 1.08%) compared to highland (1.51 ± 0.33%) and two times higher compared to midland (6.36 ± 0.81%) (Table 6.2). The specific parasitism rate of *O. dissitus* was about five times higher in lowland (7.67 ± 0.89%) compared to highland (1.57 ± 0.48%) and two times higher compared to midland (3.11 ± 0.49%) (Table 6.2).

The contribution of *P. scabriventris* specific parasitism to total parasitism was highest at lowland (34.14%) and similar to *D. isaea* contribution to total parasitism at lowland (37.91%) compared to *O. dissitus* specific parasitism contribution to total parasitism at the same elevation (21.24%) (Table 6.2). *Phaedrotoma scabriventris* specific parasitism contribution to total parasitism was lowest at highland (5.79%) (Table 6.2). The specific parasitism rate of each of the remaining six parasitoid species was < 1% across all the elevations (Table 6.2). Specific parasitism rate of *P. scabriventris* increased from <10% during the long rainy and dry cold seasons to 24.15% during the short rainy season of monitoring in lowland (Table 6.3).

Table 6.2: Estimated parasitism rates (Mean ± SE %) of *Liriomyza* leafminer flies parasitoid species at low, mid and high elevations in vegetable production systems in Kenya

Parasitoid species	Parasitism rate (%Mean±SE)		
	Highland (n*=302)	Midland (n=275)	Lowland (n=285)
D. isaea	21.25±1.61a	20.36±1.53a	13.69±1.24b
P. scabriventris	1.51±0.33c	6.36±0.81b	12.33±1.08a
O. dissitus	1.57±0.48c	3.11±0.49b	7.67±0.89a
N. formosa	0.80±0.24a	1.13±0.41a	0.88±0.23a
Meruacesa sp.	0.71±0.23a	0.46±0.17a	0.89±0.38a
Opiinae species	-	-	0.38±0.15
H. variconis	0.19±0.09a	0.43±0.37a	0.26±0.18a
Figitidae species	0.02±0.02	-	-
Halticopera sp.	-	0.01±0.01	-
Total	26.06±1.72b	31.85±1.77a	36.11±1.82a

n^*; number of sampled fieldsWithin row, means followed by the same lower case letter are not significantly different at P = 0.05 (Student-Newman-Keuls Test).

Table 6.3: Estimated parasitism rates (Mean ± SE %) of *Liriomyza* leafminer flies parasitoid species at low, mid and high elevations during different cropping seasons in vegetable production systems in Kenya

Season	n*	Parasitoid species per elevation (Mean ± SE)									
		D. isaea	*P. scabriventris*	*O. dissitus*	*N. formosa*	*Meruacesa* sp.	Opiinae species	*H. variconis*	Figitidae species	*Halticoptera* sp.	Total
						Highland					
Dry hot	89	20.76 ±2.98	1.53 ±0.64	1.93 ±0.90	0.83 ±0.35	1.49 ±0.72	-	0.06 ±0.06	0.08 ±0.06	-	26.68 ±3.20
Long rain	99	20.92 ±3.14	1.70 ±0.67	2.48 ±1.19	1.13 ±0.62	0.16 ±0.09	-	0.44 ±0.27	-	-	26.82 ±3.37
Dry cold	59	24.16 ±3.22	1.11 ±0.47	0.4 ±0.16	0.22 ±0.08	0.57 ±0.19	-	0.13 ±0.08	-	-	26.59 ±3.52
Short rain	55	19.54 ±3.35	1.54 ±0.66	0.61 ±0.35	0.80 ±0.33	0.62 ±0.38	-	0.02 ±0.02	-	-	23.13 ±3.45
Total	302	21.25 ±1.16	1.51 ±0.33	1.57 ±0.48	0.80 ±0.24	0.71 ±0.23	-	0.19 ± 0.09	0.02 ±0.02	-	26.06 ±1.72
						Midland					
Dry hot	128	18.13 ±2.07	4.84 ±1.10	3.00 ±0.63	1.11 ±0.79	0.41 ±0.20	-	0.91 ±0.79	-	0.02 ±0.02	28.42 ±2.49
Long rain	63	18.03 ±3.11	7.46 ±1.49	5.35 ±1.46	0.63 ±0.29	1.14 ±0.60	-	0.01 ±0.01	-	-	32.61 ±3.52
Dry cold	59	25.68 ±3.89	8.79 ±2.14	1.30 ±0.78	-	0.02 ±0.02	-	0.01 ±0.01	-	-	35.80 ±4.25
Short rain	25	25.05 ±4.81	5.64 ±2.70	2.34 ±1.13	5.12 ±1.59	-	-	-	-	-	38.15 ±6.12
Total	275	20.36 ±1.53	6.36 ±0.81	3.11 ±0.49	1.13 ±0.41	0.46 ±0.17	-	0.43 ±0.37	-	0.01 ±0.01	31.85 ± 1.77
						Lowland					
Dry hot	132	15.04 ±1.94	13.39 ±1.75	7.72 ±1.21	0.83 ±0.28	0.31 ±0.12	0.16 ±0.10	0.47 ±0.38	-	-	37.92 ±2.70
Long rain	73	12.1 ±2.61	9.26 ±1.57	7.09 ±1.74	1.24 ±0.69	2.44 ±1.43	0.35 ±0.28	-	-	-	32.49 ±3.77
Dry cold	58	16.48 ±2.47	9.26 ±1.76	5.00 ±1.73	0.11 ±0.07	0.62 ±0.21	0.85 ±0.59	0.24 ±0.18	-	-	32.57 ±3.50
Short rain	22	3.45 ±0.90	24.15 ±5.37	16.33 ±4.84	1.99 ±0.98	-	0.60 ±0.60	-	-	-	46.53 ±6.86
Total	285	13.69 ±1.24	12.33 ±1.08	7.67 ±0.89	0.88 ±0.23	0.89 ±0.38	0.38 ±0.15	0.26 ±0.18	-	-	36.11 ±1.82
Total for all elevations	862	18.46 ±0.86	6.63 ±0.48	4.08 ±0.38	0.93 ±0.17	0.69 ±0.16	0.13 ±0.05	0.29 ±0.14	0.01 ±0.01	-	31.23 ±1.03

n*; number of sampled fields

All parasitoids identified were collected on *Liriomyza* species infesting a total of 20 different vegetable host crops belonging to seven families namely; Fabaceae, Solanaceae, Cucurbitaceae, Malvaceae, Brassicaceae, Amaranthaceae and Amaryllidaceae from the three elevations (Tables 6.4 to 6.6). Total parasitoid abundance was highest on Fabaceae at high and mid elevations and on Solanaceae at low elevation (Tables 6.4 to 6.6).

The host plant range for *D. isaea* included all infested *Liriomyza* host plants surveyed with the exception of onion at the mid elevation (Tables 6.4 to 6.6). This parasitoid was also the most abundant on all its host crop surveyed (Tables 6.4 to 6.6). The host plant range for the exotic *P. scabriventris* and its closest allied *O. dissitus* were almost similar and also high: 18 crops each (Tables 6.4 to 6.6). While no *P. scabriventris* was collected on butternut squash, *O. dissitus* was not collected on leafminer infesting bitter gourd leaves at low elevation, and like *D. isaea*, both parasitoid species were not collected on leafminer infesting onion leaves surveyed at mid elevation (Tables 6.5 and 6.6). Amongst all the crops surveyed, the bean (French bean and kidney bean) yielded the highest abundance of *P. scabriventris* and *O. dissitus,* while snow pea and tomato yielded the highest *D. isaea* abundance (Tables 6.4 to 6.6).

Table 6.4: Parasitoid species abundance associated with *Liriomyza* infested crops at high elevation

Plant family/scientific name	Common name	n^*	*D. isaea*	*P. scabriventris*	*O. dissitus*	*N. formosa*	*Meruacesa* sp.	*H. variconis*	Figitidae species	Total
Amaranthaceae										
Spinacia oleracea L.	Spinach	1	19 (0.48%)	1 (0.03%)	3 (0.08%)	-	-	-	-	23 (0.58%)
Amaranthaceae total		1	19 (0.48%)	1 (0.03%)	3 (0.08%)	-	-	-	-	23 (0.58%)
Cucurbitaceae										
Cucurbita pepo L.	Courgette	9	128 (3.25%)	5 (0.13%)	1 (0.03%)	2 (0.05%)	3 (0.08%)	-	-	139 (3.53%)
Cucurbitaceae total		9	128 (3.25%)	5 (0.13%)	1 (0.03%)	2 (0.05%)	3 (0.08%)	-	-	139 (3.53%)
Fabaceae										
Phaseolus vulgaris L.	French bean	46	768 (19.48%)	78 (1.98%)	28 (0.71%)	17 (0.43%)	12 (0.30%)	5 (0.13%)	-	908 (23.03%)
Pisum sativum L.	Garden pea	15	110 (2.79%)	7 (0.18%)	2 (0.05%)	2 (0.05%)	8 (0.20%)	1 (0.03%)	-	130 (3.30%)
Phaseolus vulgaris L.	Kidney bean	26	164 (4.16%)	60 (1.52%)	12 (0.30%)	9 (0.23%)	12 (0.30%)	-	-	257 (6.52%)
Pisum sativum L.	Snow pea	107	1427 (36.20%)	25 (0.63%)	8 (0.20%)	22 (0.56%)	27 (0.68%)	7 (0.18%)	-	1516 (38.46%)
Pisum sativum L.	Sugar snap	22	496 (12.58%)	7 (0.18%)	5 (0.13%)	9 (0.23%)	11 (0.28%)	-	-	528 (13.39%)
Fabaceae total		216	2965 (75.22%)	177 (4.49%)	55 (1.40%)	59 (1.50%)	70 (1.78%)	13 (0.33%)	-	3339 (84.70%)
Solanaceae										
Solanum tuberosum L.	Potato	65	204 (5.18%)	29 (0.74%)	40 (1.01%)	8 (0.20%)	4 (0.10%)	1 (0.03%)	3 (0.08%)	289 (7.33%)
Capsicum L.	Sweet pepper	4	39 (0.99%)	-	-	-	-	-	-	39 (0.99%)
Solanum lycopersicum L.	Tomato	7	105 (2.66%)	5 (0.13%)	2 (0.05%)	-	1 (0.03%)	-	-	113 (2.87%)
Solanaceae total		76	348 (8.83%)	34 (0.86%)	42 (1.07%)	8 (0.20%)	5 (0.13%)	1 (0.03%)	3 (0.08%)	441 (11.19%)
Total for all crops		302	3460 (87.77%)	217 (5.50%)	101(2.56%)	69 (1.75%)	78 (1.98%)	14 (0.36%)	3 (0.08%)	3942 (100.00%)

n^*; number of sampled fields

Table 6.5: Parasitoid species abundance associated with *Liriomyza* infested crops at mid elevation

Plant family/scientific name	Common name	n^*	*D. isaea*	*P. scabriventris*	*O. dissitus*	*N. formosa*	*Meruacesa* sp.	*H. variconis*	*Halticoptera* sp.	Total
Amarilidaceae										
Allium cepa L.	Onion	1	-	-	-	-	1 (0.04%)	-	-	1 (0.04%)
Amarilidaceae total		1	-	-	-	-	1 (0.04%)	-	-	1 (0.04%)
Cucurbitaceae										
Cucurbita pepo L.	Courgette	1	3 (0.13%	-	3 (0.13%)	-	-	-	-	6 (0.25%)
Citrullus lanatus (Thunb.) Matsun and Nakai	Watermelon	3	22 (0.92%)	15 (0.63%)	-	-	-	-	-	37 (1.55%)
Cucurbitaceae total		4	25 (1.05%)	15 (0.63%)	3 (0.13%)	-	-	-	-	43 (1.80%)
Fabaceae										
Vigna unguiculata (L.) Walp	Cowpea	1	3 (0.13%)	2 (0.08%)	1 (0.04%)	-	-	-	-	6 (0.25%)
Phaseolus vulgaris L.	French bean	78	540 (22.66%)	90 (3.78%)	34 (1.43%)	35 (1.47%)	5 (0.21%)	1 (0.04%)	-	705 (29.58%)
Pisum sativum L.	Garden pea	1	3 (0.13%)	-	-	-	-	-	-	3 (0.13%)
Phaseolus vulgaris L.	Kidney bean	57	261 (10.95%)	132 (5.54%)	64 (2.69%)	7 (0.29%)	8 (0.34%)	1 (0.04%)	-	473 (19.85%)
Fabaceae total		137	807 (33.86%)	224 (9.40%)	99 (4.15%)	42 (1.76%)	13 (0.55%)	2 (0.08%)	-	1187 (49.81%)
Malvaceae										
Abelmoschus esculentus (L.) Moench	Okra	1	17 (0.71%)	1 (0.04%)	-	-	-	-	-	18 (0.76%)
Malvaceae total		1	17 (0.71%)	1 (0.04%)	-	-	-	-	-	18 (0.76%)
Solanaceae										
Solanum tuberosum L.	Potato	4	8 (0.34%)	3 (0.13%)	-	-	-	1 (0.04%)	-	12 (0.50%)
Solanum lycopersicum L.	Tomato	128	744 (31.22%)	215 (9.02%)	108 (4.53%)	26 (1.09%)	18 (0.76%)	9 (0.38%)	2 (0.08%)	1122 (47.08%)
Solanaceae total		132	752 (31.56%)	218 (9.15%)	108 (4.53%)	26 (1.09%)	18 (0.76%)	10 (0.42%)	2 (0.08%)	1134 (47.59%)
Total for all crops		275	1601 (67.18%)	458 (19.22%)	210 (8.81%)	68 (2.85%)	32 (1.34%)	12 (0.50%)	2 (0.08%)	2383 (100.00%)

n^*; number of sampled fields

Table 6.6: Parasitoid species abundance associated with *Liriomyza* infested crops at low elevation

Plant family/scientific name	Common name	n*	*D. isaea*	*P. scabriventris*	*O. dissitus*	*N. formosa*	*Meruacesa* sp.	Opiinae species	*H. variconis*	Total
Brassicaceae										
Brassica oleracea acephala L.	Kale	4	8 (0.27%)	2 (0.07%)	2 (0.07%)	-	-	-	-	12 (0.41%)
Brassicaceae total		4	8 (0.27%)	2 (0.07%)	2 (0.07%)	-	-	-	-	12 (0.41%)
Cucurbitaceae										
Momordica charantia L	Bitter gourd	1	11 (0.37%)	6 (0.20%)	-	-	-	-	-	17 (0.57%)
Cucurbita moschata Duchesne ex Poir.	Butternut squash	1	14 (0.47%)	-	1 (0.03%)	-	-	-	-	15 (0.51%)
Cucurbita maxima Duchesne	Pumpkin	4	5 (0.17%)	2 (0.07%)	5 (0.17%)	1 (0.03%)	2 (0.07%)	-	-	15 (0.51%)
Citrullus lanatus (Thunb.) Matsun and Nakai	Watermelon	13	36 (1.22%)	55 (1.86%)	31 (1.05%)	-	1 (0.03%)	-	-	123 (4.16%)
Cucurbitaceae total		19	66 (2.23%)	63 (2.13%)	37 (1.25%)	1 (0.03%)	3 (0.10%)	-	-	170 (5.75%)
Fabaceae										
Vigna unguiculata (L.) Walp	Cowpea	11	18 (0.61%)	34 (1.15%)	27 (0.91%)	2 (0.07%)	-	-	1 (0.03%)	82 (2.77%)
Lablab purpureus (L.) Sweet	Dolichos bean	1	3 (0.10%)	9 (0.30%)	13 (0.44%)	-	1 (0.03%)	-	-	26 (0.88%)
Phaseolus vulgaris L.	Kidney bean	65	172 (5.82%)	347 (11.73%)	178 (6.02%)	8 (0.27%)	6 (0.20%)	2 (0.07%)	1 (0.03%)	714 (24.15%)
Fabaceae total		77	193 (6.53%)	390 (13.19%)	218 (7.37%)	10 (0.34%)	7 (0.24%)	2 (0.07%)	2 (0.07%)	822 (27.80%)
Malvaceae										
Abelmoschus esculentus (L.) Moench	Okra	24	77 (2.60%)	62 (2.10%)	34 (1.15%)	3 (0.10%)	5 (0.17%)	1 (0.03%)	-	182 (6.15%)
Malvaceae total		24	77 (2.60%)	62 (2.10%)	34 (1.15%)	3 (0.10%)	5 (0.17%)	1 (0.03%)	-	182 (6.15%)
Solanaceae										
Solanum melongena L.	Brinjale eggplant	1	6 (0.20%)	2 (0.07%)	1 (0.03%)	-	3 (0.10%)	-	1 (0.03%)	13 (0.44%)
Capsicum L.	Sweet pepper	3	8 (0.27%)	2 (0.07%)	1 (0.03%)	-	-	-	-	11 (0.37%)
Solanum lycopersicum L.	Tomato	157	830 (28.07%)	533 (18.03%)	253 (8.56%)	51 (1.72%)	30 (1.01%)	45 (1.52%)	5 (0.17%)	1747 (59.08%)
Solanaceae total		161	844 (28.54%)	537 (18.16%)	255 (8.62%)	51 (1.72%)	33 (1.12%)	45 (1.52%)	6 (0.20%)	1771 (59.89%)
Total for all crops		285	1188 (40.18%)	1054 (35.64%)	546 (18.46%)	65 (2.20%)	48 (1.62%)	48 (1.62%)	8 (0.27%)	2957 (100.00%)

n*; number of sampled fields

Although the total parasitoids abundance on snow pea, crop frequently surveyed at high elevation, was highest, total parasitism on it was not the highest: 21.76 ± 2.48% compared to total parasitism on French bean: 55.25 ± 4.99% and kidney bean: 39.79 ± 6.17%. At the mid elevation, tomato, kidney bean and French bean infested leaves yielded the highest total parasitism: 25.98 ± 2.26% on tomato versus 37.44 ± 4.31 on kidney bean and 37.33 ± 3.59% on French bean amongst the Fabaceae and Solanaceae crops, respectively. Although, tomato, crop frequently surveyed at low elevation yielded the highest parasitoid abundance, total parasitism rate on it was not highest: 28.80 ± 2.11% compared to 53.93 ± 4.15% on kidney bean which was about half of the number of surveyed fields for tomato.

6.3.2 Spread of *Phaedrotoma scabriventris* from release sites

At high elevation, *P. scabriventris* was recovered from 10 to 40 km away from the release sites (Table 6.7). At mid elevation, it was recovered at 10 to 30 km while at low elevation, it was recovered at 10 to 50 km away from the release sites (Table 6.7). At the three elevations, as expected, results were different between sampling radii pooled from the cardinal directions away from the release sites (Table 6.7). Specific parasitism rates of *P. scabriventris* at the longest distance recovered away from the initial release sites were 3.60 ± 2.50, 0.05 ± 0.03 and 4.35% at high, mid and low elevations, respectively.

Of all the seven host crops surveyed, *P. scabriventris* was recovered on four crops namely kidney bean, potato, tomato and okra. Of all these surveyed crops, *Liriomyza* infested Fabaceae crops were the most common crop surveyed and yielded the highest *P. scabriventris* abundance.

Table 6.7: Spread of *Phaedrotoma scabriventris* at high, mid and low elevations in vegetable production systems in Kenya

Distance from the release site (km)	n*	*P. scabriventris* recovered
	Highland	
10 km	3	Yes
20 km	10	Yes
30 km	5	Yes
40 km	10	Yes
50 km	16	No
	Midland	
10 km	12	Yes
20 km	5	No
30 km	5	Yes
40 km	3	No
50 km	-	-
	Lowland	
10 km	17	Yes
20 km	13	No
30 km	4	No
40 km	2	No
50 km	1	Yes

n*; number of sampled fields

-; site was inaccessible

6.4 Discussion

Nine LMF parasitoid species including the exotic *P. scabriventris* were recorded in this study with >30% average total parasitism. This results indicates considerable progress in LMF biological control in Kenya since previous parasitism was <6% (Chabi-Olaye et al., 2008). The diversity of LMF parasitoids in Kenya from this study is higher (nine species) compared to Chabi-Olaye et al. (2008) who reported only four parasitoid species namely *O. dissitus*, *D. isaea*, *N. formosa* and *H. varicornis* in Kenya. In addition to this, the *Meruacesa* sp. found during the survey is new to science (Dr. Alex Gumovski, personal communication) and further description is being carried out by the Royal Museum of Central Africa in Belgium for its publication as new species. This increase in LMF parasitoid species diversity may be due to the extensive nature of the current study compared to study by Chabi-Olaye et al. (2008). The high leafminer parasitism observed in this study may be due to considerable farmer awareness campaigns on conservation of parasitoids rolled out prior to *P. scabriventris* release. While invading leafminer populations have been observed to decline naturally after a few years due to the action of local natural enemies, classical biological control agents especially parasitoids are often very effective against these exotic pests (Caltagirone, 1981; Neuenschwander et al., 1987; Murphy & Lasalle, 1999). For example, parasitism of invasive LMF in Senegal reached 90% due to the combined action of five indigenous and five exotic parasitoid species (Neuenschwander et al. 1987). Thus, IPM approaches based on conservation of existing parasitoids and introduction of additional species could potentially result to effective management of the invasive LMF infesting vegetables and ornamentals in Kenya.

The indigenous ectoparasitoid *D. isaea* was the most abundant species and accounted for the highest LMF parasitism at all elevations. It was highly abundant at high elevation, followed by mid elevation, then low elevation and its parasitism was significantly higher in high and mid elevations compared to low elevation. This dominance of *D. isaea* at all elevations is unusual in Kenya as compared to previous studies which indicated that *O. dissitus* was the most dominant parasitoid species on LMF (Chabi-Olaye et al., 2008). In addition, *D. isaea* being relatively more abundant in higher elevation than in low elevation contradicts the normal expectation based on its biological information in literature. For instance Cheah (1987), Minkenberg (1989), Van der Linden (2004) and Haghani et al. (2007) reported that cooler temperatures as observed at high elevation in Kenya hamper its reproduction and development. Thus, the findings of this study suggest that *D. isaea* is adapting to colder areas and can perform at all the studied elevations.

The exotic endoparasitoid *P. scabriventris* was recovered from all release sites and was the second highest in terms of abundance of all the parasitoids identified on LMF with highest parasitism rate recorded at low elevation compared to mid and high elevations. The fact that *P. scabriventris* has quickly reached the top two within the first year post release in terms of parasitoid abundance confirms reports from its original zone. This parasitoid is often the dominant parasitoid of *L. huidobrensis* in Argentina, Brazil, Chile, and Peru, South America where it represents up to 50% of total parasitism (Serantes de González, 1974; Salvo & Valladares, 1995). This is promising for the improvement of biological control of LMF in Kenya and East Africa over the years. However, follow up surveys will be required to assess the progress in performance over the years since its specific parasitism over the first year post release is still low. On the other hand, *P. scabriventris*, being more abundant and performing better at lower elevations in Kenya is in contrast with reports on its distribution from its original zone. In South America, the parasitoid is known to have a wide geographical and ecological distribution and was recorded between 500 m and 4,042 m a.s.l, with varying temperatures but being more effective at higher colder elevations (Mujica & Cisneros, 1997; Valladares & Salvo, 2001; Salvo et al., 2005). For instance, in Argentina, Salvo (1996) recorded *P. scabriventris* as an important parasitoid of LMF in Cordoba, Central Argentina at 800 m a.s.l., with an average annual temperature of 16 ºC (maximum 24 ºC, minimum 9 ºC). Mujica & Cisneros (1997) recorded the same parasitoid as an important parasitoid of *L. huidobrensis* in the Central Highlands of Peru between 3,200 and 4,042 m a.s.l., with an annual average maximum temperature of 23 ºC and a minimum of 4 ºC. Furthermore, under laboratory conditions, Mujica et al. (2009) reported best performance of *P. scabriventris* between 15 and 20 °C, with significant decrease in progeny at temperatures above 20 °C. Valladares & Salvo (2001) reported higher performance of the *P. scabriventris* under cooler winter and fall conditions at temperatures below 15 °C in Argentina. It was, therefore, expected that the parasitoid will perform better in higher elevations than at lower elevations in Kenya. It is hypothesized that this unexpected result could be due to the short term observation and in the long run, *P. scabriventris* performance can improve at high elevation and at least reach same levels as currently observed at low elevations. According to various authors, at higher temperatures, the sex ratio of *P. scabriventris* is more female biased and its life cycle is shorter than at low temperatures (Mujica *et al*., 2009; Chabi-Olaye *et al*., 2013; Foba et al., 2015b; Akutse *et al*., 2015) (Chapter four). The female biased sex ratio and shorter life cycle repored in other studies may have caused the faster establishment of *P. scrabriventris* in the low elevation.

The indigenous endoparasitoid *O. dissitus*, a taxonomically related species to *P. scabriventris* was the third most abundant parasitoid species across all elevations of vegetable production in Kenya. A similar trend in abundance and parasitism of this parasitoid species as for *P. scabriventris* was recorded with a higher parasitism rate in low elevation compared to mid and high elevations. This observation fits *O. dissitus* biological knowledge as the species is expected to perform better between 20 and 25 °C (Bordat et al., 1995b). However, Li et al. (2012) reported higher performance of *O. dissitus* in South Florida during cooler conditions of temperature between 15 and 20 °C like that of *P. scabriventris*.

Phaedrotoma scabriventris was recovered from all release sites and up to 40, 30 and 50 km at high, mid and low elevations, respectively away from the release sites one to two years after its initial release. However, during the same period, its specific parasitism rates were low at all elevations. *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) released against *Plutella xylostella* L. (Lepidoptera: Plutellidae) in East Africa, established around release sites and spread up to 30 km away from the initial release sites of the parasitoid within two years of its release with a specific parasitism rate of 5.9% at that radii (Gichini et al., 2008). Release of *Apoanagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) from Central America against the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in West Africa, spread 170 km in five months after its release (Neuenschwander & Hammond, 1988), a far faster spread than obtained in the present study for *P. scabriventris*. However, cases where despite slower establishment and spread coupled with low initial specific parasitism rates, released biological control agents eventually build up their populations and became important natural control agents of pests are known (Masoodi et al., 1989; Murakami & Gyoutoku, 1995; Nyambo et al., 2008). For instance, following repeated releases of *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) in 2005 in Kenya against *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), the parasitoid was not recovered in the first two years post release in any of the release sites. However, from 2008, the parasitoid became common in the field crops and became a major parasitoid of the pest under field conditions across the country (Nyambo et al., 2008). Murakami & Gyoutoku (1995) also reported initial low populations levels of the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), with high levels of parasitism achieved only seven to 11 years post release. First recovery of *Bathyplectes anurus* (Hymenoptera: Ichneumonidae), a larval parasitoid of the alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae) was made only two years after its release in Japan (Shoubu et al. 2005). The recovery of *P. scabriventris* from all release sites and 30 to 50 km away from the release sites within the first and second years post release only indicates considerable success in its establishment and spread. Taking into account the factors mentioned above, further field studies should be continued to evaluate the effectiveness of *P. scabriventris* performance and spatial distribution in Kenya agro-ecological zones over the years.

All 20 crops surveyed were at least host to one of the nine parasitoid species recovered; with *D. isaea* being the most abundant on all its hosts (19 crops) while *P. scabriventris* and *O. dissitus* were reared on 18 crops each. Chabi-Olaye et al. (2008) also reported a high diversity in host range for the indigenous LMF parasitoids in Kenya. Although LMF parasitoids are known to be generalists attacking several LMF species on different host crops (Valladares & Salvo, 2001; Videla et al., 2006; Valencia, 2008), clear preferences of these parasitoids based on host crops and location have been observed (Salvo & Valladares, 1997; Murphy & Lasalle, 1999; Tran et al., 2006). In this study, the Fabaceae, especially bean and the Solanaceae, especially tomato were the most important hosts on which the highest total parasitoids were recovered. Other field based studies have also demonstrated that the Fabaceae crops especially bean, are the most suitable hosts for *Liriomyza* species development and their associated natural enemies (Mujica & Kroschel, 2011; Tran et al., 2007; Tokumaru & Abe, 2005) (Chapter five).

Finidori-Logli et al. (1996) and Wei & Kang (2006) have respectively, showed that female *D. isaea* and *O. dissitus* were strongly attracted to the odours arising from *Liriomyza*-infested bean plants. Plant volatiles are employed by both herbivores and their natural enemies in searching for food and oviposition site (De Moraes et al., 2000). Exploiting such volatile substances to attract parasitoids can improve biological control in the field (Vet & Dicke, 1992; Cortesero et al., 2000; Aldrich et al., 2003). Thus, understanding the basis for this preference on bean and tomato may be exploited in improving biological control of LMF.

6.5 Conclusion

In conclusion, *P. scabriventris* established at all release sites and became in less than a year, the second most abundant leafminer parasitoid. Two year post its release, its spread up to 50 km away from the release sites on different *Liriomyza* infested crops. The 31% total average parasitism rate observed in this study compared to the <6% parasitism rate in previous surveys in Kenya indicates a considerable improvement in leafminer parasitism. Thus, integrated pest management (IPM) approaches based on conservation of existing parasitoids and introduction of additional species could potentially result to effective management of the invasive leafminer flies in vegetables and ornamentals in Kenya. Further studies are warranted to assess the progress in the parasitism rates and contribution of the various parasitoids recorded, as well as the spread of the newly introduced species. These parasitoids may offer viable alternatives to the application of insecticides or at least reduction of their application frequency, which is currently the most widely used control approach against leafminer flies in Kenya.

CHAPTER SEVEN

BEHAVIORAL RESPONSE OF *PHAEDROTOMA SCABRIVENTRIS* TO *LIRIOMYZA HUIDOBRENSIS*-INDUCED HOST PLANT VOLATILES

ABSTRACT

Plant volatile organic compounds (VOCs) are well known to play important roles in plant-insect herbivore-natural enemy tritrophic interactions. The utilisation of VOCs as host plant and oviposition substrate location cues has been demonstrated for the *Liriomyza* (Diptera: Agromyzidae) leafminer parasitoids *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) and *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae). However, similar information for another dominant *Liriomyza* leafminer parasitoid *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) is largely unknown. Using dual choice olfactometer assays, it was observed that whereas *P. scabriventris* was significantly attracted to odours from *L. huidobrensis* damaged host plants; *Phaseolus vulgaris* L., *Pisum sativum* L. and *Vicia faba* L., the parasitoid avoided mechanically damaged ones when compared against clean air controls. However, *P. scabriventris* preferred neither the clean air controls nor any of the healthy plants. These results suggest that like in similar tritrophic systems, herbivore induced plant volatiles mediate the plant- *Liriomyza*-*P. scabriventris* tritrophic interaction and should be exploited to lure the parasitoid to infested plants.

7.1 Introduction

Phaedrotoma scabriventris is a solitary larval-pupae endoparasitoid found in Peru, South America and newly introduced into Kenya for biological control of LMF. Recent data presented in Chapter six has shown that the parasitoid has established and spread up to 30 to 50 km from the initial release sites across varying elevations of vegetable production in Kenya. Although both LMF and their associated parasitoids are known to be generalists (Valladares & Salvo, 2001; Videla et al., 2006; Valencia, 2008), clear preferences of *P. scabrivenris* on certain host crops have been observed in the field (Chapter six). Thus, understanding the basis for this preference may be exploited in improving biological control of LMF.

Plant volatiles are employed by both herbivores and their natural enemies in search of food and oviposition sites (De Moraes et al., 2000). Several studies have shown that insect herbivores are attracted by odours released from the host plants (Guerin & Visser, 1980; Light et al., 1988; Cossé et al., 1995). In addition, plants release Herbivore Induced Volatiles (HIVs) as a result of feeding by herbivores. The same are used by natural enemies of the herbivores to locate their hosts (Turlings et al., 1995; De Moraes et al., 1998; Pare´ & Tumlinson, 1999). Exploiting such volatile substances to attract parasitoids can improve biological control programs in the field (Vet & Dicke, 1992; Cortesero et al., 2000; Aldrich et al., 2003). Studies on the foraging behavior of LMF parasitoids have been carried out but they are limited to few species such as *D. isaea* and *O. dissitus* (Zhao & Kang, 2002a; Wei & Kang, 2006). Such information is lacking for *P. scabriventris*.

Liriomyza huidobrensis constitutes the greatest threat to the production of vegetables and ornamentals because it is the most polyphagous and abundant *Liriomyza* species occurring in all cropping seasons at varying elevations in Kenya (Foba et al., 2015a) (Chapter five). While snow pea is the most susceptible host plant to *L. huidobrensis* (Foba et al., 2015a) (Chapter five), other studies have demonstrated that the fabaceaes are the most suitable hosts for *Liriomyza* species development and their associated natural enemies (Tokumaru & Abe, 2005; Tran et al., 2007; Mujica & Kroschel, 2011). Moreover, strong attraction of female *D. isaea* and *O. dissitus* to the volatiles from *Liriomyza*-infested bean (*P. vulgaris*) plants have been reported (Finidori-Logli et al., 1996; Wei & Kang, 2006).

Recent field surveys have demonstrated that *P. scabriventris* was mostly recovered on *Liriomyza*-infested kidney bean leaves compared to *Liriomyza*-infested snow pea leaves; the most susceptible *L. huidobrensis* host plant, across varying elevations of vegetable production in Kenya (Foba et al., 2015a) (Chapters five and six). This may imply that kidney bean plants infested with LMF appear to attract more of the endoparasitoid *P. scabriventris* than its counterpart snow pea, suggesting differential response tactics to this pest and the parasitoid. While plants have been shown to display a wide range of defensive tactics to protect themselves from attack by pathogens and herbivores, including exploitation of infochemicals to advertise pest presence on them and thereby recruit the natural enemies of these pests (Kang et al., 2009), reasons for the observed field infestation levels remain unclear. Differential attraction may as well be related to distinct plant-specific induced volatile profiles. Therefore, an understanding of the chemical basis of the differential attraction may offer potential for identifying attractants for parasitoids from host plants such as kidney bean and snow pea for use in sampling and monitoring populations of parasitoids. As an initial step towards this goal, this study investigated the behavioral response of *P. scabriventris* to odours from *L. huidobrensis*-infested kidney bean and snow pea plants for an improved biological control system of LMF.

7.2 Material and methods

7.2.1 *Liriomyza huidobrensis* host plants, rearing of *Liriomyza huidobrensis* and *Phaedrotoma scabriventris*

Four seeds of the test host plants namely the common kidney bean, snow pea and faba bean were sown individually in 7.5 cm diameter × 7.3 cm depth plastic pots containing a 1: 5 manure: soil mixture at 25 ± 2 °C, 60 ± 9% RH and a photoperiod of 12L: 12D in screen houses at ICIPE, Kenya. The plants were watered daily and used at growth stages of days 14 to 17 (four fully developed true leaves for kidney bean and faba bean, and six fully developed true leaves forsnow pea) in all experiments and pest/parasitoid rearing. Faba bean was used as the positive control since it is the best known host crop for rearing *L. huidobrensis* and *P. scabriventris* (Videla et al., 2006; Chabi-Olaye et al., 2013).

The host pest *L. huidobrensis* and the solitary endoparasitoid *P. scabriventris* were reared on faba bean plants and *L. huidobrensis* infesting faba bean plants, respectively, as described by Chabi-Olaye et al. (2013) and Foba et al. (2015b) (Chapters four and five). Parasitoids and leafminer colonies were maintained in separate environmental chambers maintained at 25 ± 2 °C, 60 ± 9% RH and a photoperiod of 12L: 12D under 36W Sylvania Aquastar fluorescent white light and fluorescent cool purple light bulb supplied by Uganda Electricals (K) LTD, Kenya in the quarantine unit at ICIPE. Female parasitoids from pupae that had been isolated in petri dishes were mated within 24 hours of emergence and then kept in perspex cages (45 cm × 40 cm × 40 cm) with access to honey solution (10%) under the same environmental conditions described above. The parasitoids were held in the cages in the bioassay room for 24 hours prior to each experiment in order to allow them get acclimatized with the experimental room. All parasitoids used in the experiments were two to four days old naïve female adults, which had no previous exposure to either leafminers or plants. Each individual was used only once in the experiments.

7.2.2 Odour sources used for behavioural assays of *Phaedrotoma scabriventris*

The plant treatments acting as odour sources used for behavioral experiments were prepared in the same way as described by Wei et al. (2006). Briefly, these includes 1) clean oven bag (blank or negative control); 2) healthy, undamaged plants; 3) mechanically damaged plants induced by making scratch lines and punctures with a needle along the entire length of the developed true leaves per plant to simulate *L. huidobrensis* damage; 4) plants on which *L. huidobrensis* second to third instar larvae had fed. To achieve treatment 4, 150 adult *L. huidobrensis* with sex ratio of 1 male: 2 females were exposed to 10 potted intact plants contained in a 45 cm × 40 cm × 40 cm transparent perspex cage maintained in an environmental chamber of the same conditions as described above for 24 hours. The pest-plant system was held for five to seven days for the pest to reach second to third instar larvae.

7.2.3 Behavioural response of *Phaedrotoma scabriventris* to *Liriomyza huidobrensis*-induced host plant volatiles in an olfactometer

A four-arm olfactometer was used to investigate the responses of *P. scabriventris* females to the different plant odour sources in the absence of any visual cues as described by Suazo et al. (2003) with some modifications (Figure 7.1)**.** Each odour source was connected to two arms of the olfactometer and the blank connected to the other two arms. Charcoal filtered humidified air was delivered into the olfactometer at 683 mL/min/quadrant (flow rate). The air was drawn from the centre of the olfactometer at 1167 mL/min. Female parasitoids were released one at a time at the centre of the olfactometer and the behaviour (total time spent in each odour zone) of each parasitoid was recorded for 10 min for all plant species treated as described above against a blank (air). The olfactometer was rotated 90° after five parasitoids had been tested to avoid positional bias. The tubes and plant treatments were replaced with clean ones after 10 replicate trails. Thirty parasitoids were tested individually for each odour source against a blank set up. A 36W Sylvania Aquastar fluorescent white light bulb was placed 1 m above the centre of the olfactometer for uniform illumination. After use, the olfactometer was washed sequentially with tap water, distilled water and methanol and dried in a hood for later use. The same environmental conditions as described above were maintained in the bioassay room. The study was conducted between 8:30 to 11:30 and 14:30 to 16:00 hours (this time was arrived at following preliminary experiments which showed optimal activity of the parasitoid).

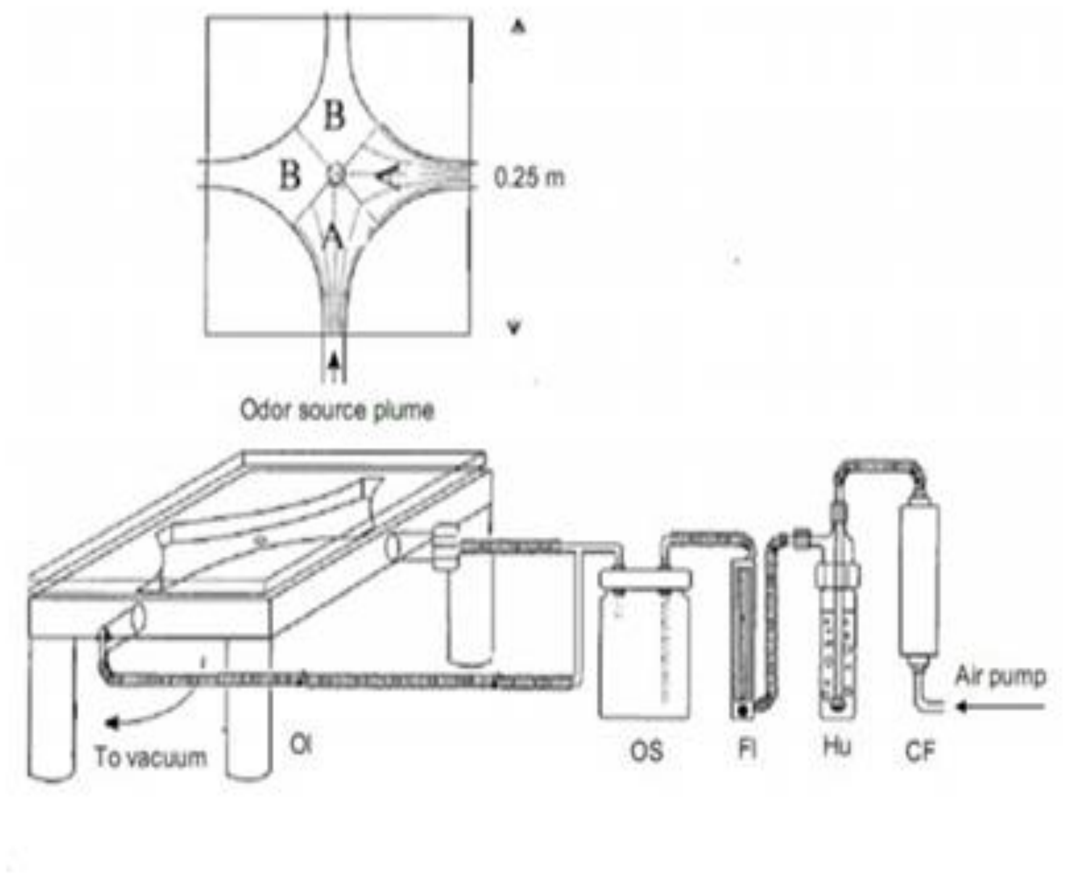

Figure 7.1: Four-arm olfactometer used in the bioassay system

The main arena of the olfactometer consisted of two odour zones; “A”s = odour zone, “B”s = control zone, OI = Olfactometer, OS = odour source, Fl = flowmeter, Hu = humidifier and CF = Charcoal filter; modified from Suazo et al. (2003).

7.2.6 Data analysis

A preference index (*PI*) for all the dual choice assay data was calculated according to the formula:

$$PI = [(SS - NSS) / (SS + NSS)] \times 100$$

where *SS* is the time spent by *P. scabriventris* parasitoid in the test odour zone and *NSS* the time spent in the control odour zone (Carlsson et al., 1999; Nyasembe et al., 2012). The *PI* would be zero if equal time was spent by the parasitoid in each side of the olfactometer chamber and ± 100 if all parasitoids preferred one odour zone of the olfactometer. A positive value indicates more time spent in the test odour zone while a negative value indicates the converse. One-Sample t- Test was used to show differences in the parasitoids response time to both test and control odours. A One-Way ANOVA followed by Tukey’s test was used to compare the parasitoids’ *PI* for the different categories of host plant odours within each host plant and similar test odours across host plants. All data were analysed in R version 3.0.2 statistical software (R Development Core Team, 2013).

7.3 Results

Phaedrotoma scabriventris significantly preferred all the three *L. huidobrensis*-infested plants compared to the clean air controls (*L. huidobrensis*-infested *P. vulgaris*: t = 9.02, df = 29, $P < 0.001$, *L. huidobrensis*-infested *V. faba*: t = 7.80, df = 29, $P < 0.001$, and *L. huidobrensis*-infested *P. sativum*: t = 3.49, df = 29, $P < 0.01$) (Figure 7.2). Contrary to the leafminer damaged plants, mechanically damaged ones were significantly less preferred over the air controls (mechanically damaged *P. vulgaris*: t = -3.10, df = 29, $P < 0.01$, mechanically damaged *V. faba*: t = -4.82, df = 29, $P < 0.001$ and mechanically damaged *P. sativum*: t = -3.96, df = 29, $P < 0.001$) (Figure 7.2). The parasitoid preferred neither the clean air controls nor any of the undamaged plants (undamaged *P. vulgaris*: t = 0.28, df = 29, P = 0.7843, undamaged *V. faba*: t = 0.82, df = 29, P = 0.4181 and undamaged *P. sativum*: t = -0.36, df = 29, P

$= 0.7248$) (Figure 7.2). Significant differences were found in the response of *P. scabriventris* to volatiles from the different categories of host plant odours within each host plant relative to the blank (*V. faba*: $F_{2, 87} = 30.00$, $P < 0.001$, *P. sativum*: $F_{2, 87} = 12.06$, $p < 0.001$) and *P. vulgaris*: $F_{2, 87} = 22.38$, $p < 0.001$ (Figure 7.3). For each plant species, *L.* huidobrensis-infested plants were significantly preferred by the parasitoids over the blank ($F_{2, 87} = 3.547$, $P = 0.0331$) compared to healthy undamaged plants over the blank ($F_{2, 87} = 0.324$, $P = 0.724$) and mechanically damaged plants over the blank ($F_{2, 87} = 0.194$, $P = 0.824$) (Figure 7.4). A significantly higher preference was displayed by *P. scabriventris* for *L. huidobrensis*-infested *P. vulgaris* over the blank compared to infested *P. sativum* over the blank (Figure 7.4).

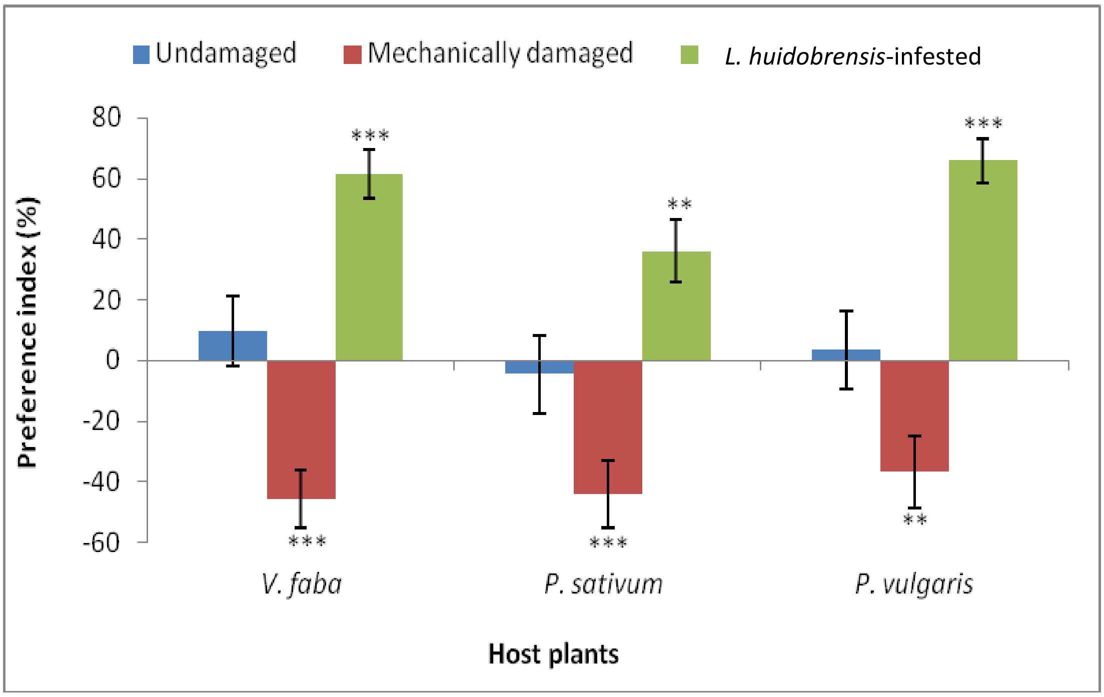

Figure 7.2: Olfactometer responses of *Phaedrotoma scabriventris* to odours from all the different tested host plant treatments expressed as the mean preference index (PI ± SE) using One-Sample t-Test

The asterisks indicate the significance levels with ** = significant at 0.01 and *** = significant at <0.001.*Phaedrotoma scabriventrisPhaedrotoma scabriventris*

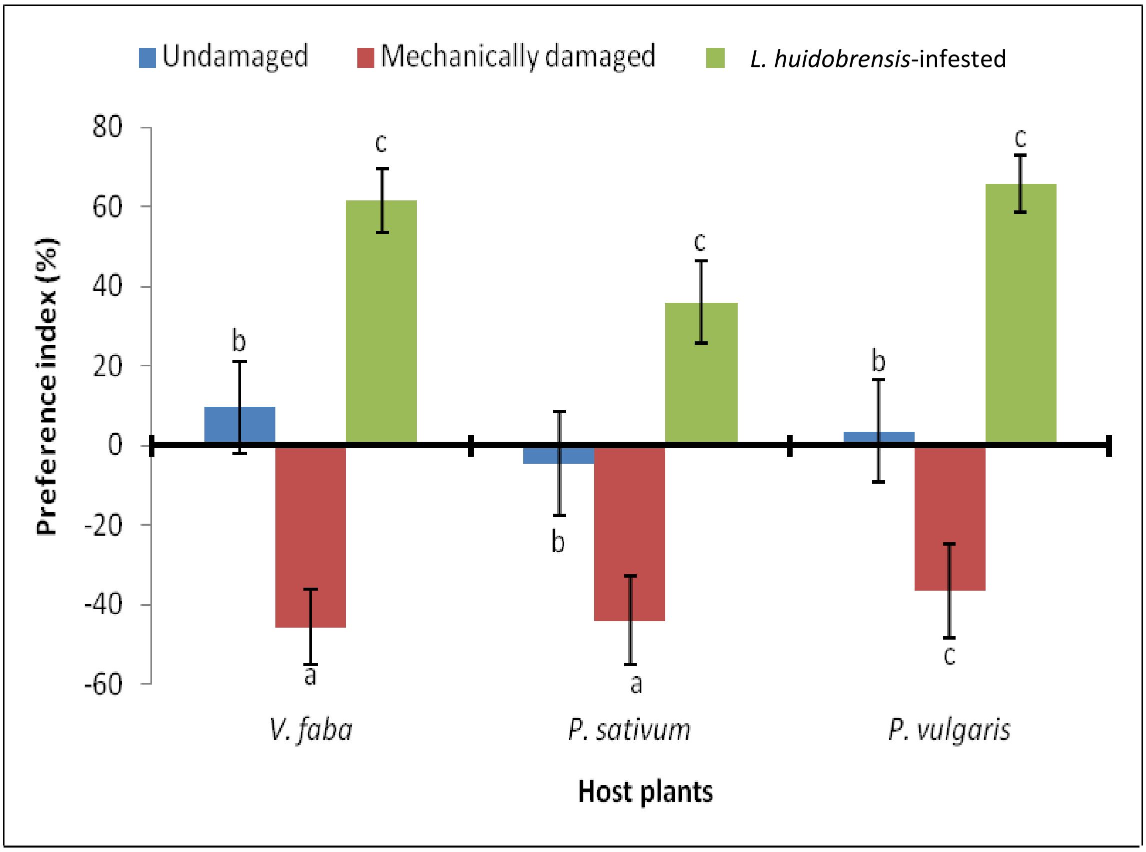

Figure 7.3: Olfactometer responses of *Phaedrotoma scabriventris* to odours from all the different tested host plant treatments expressed as the mean preference index (*PI* ± SE) using One-Way ANOVA within each plant species

Means followed by the same letter per plant treatment are not significantly different.*Phaedrotoma scabriventrisPhaedrotoma scabriventris*

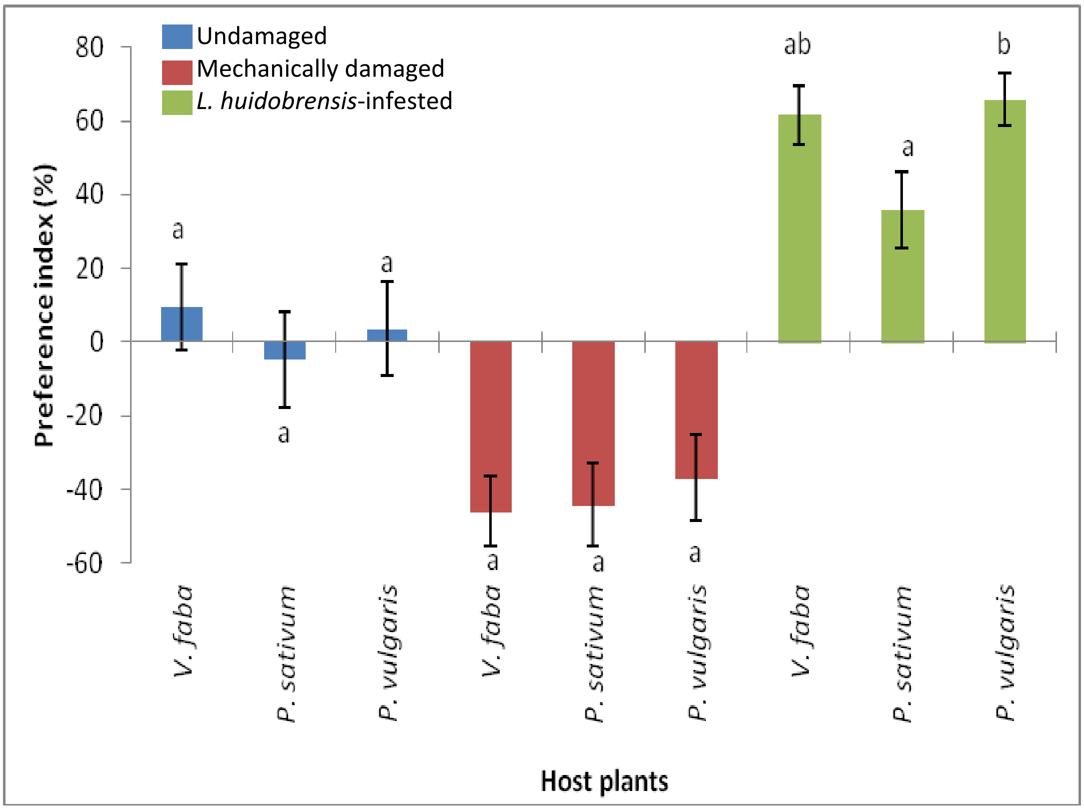

Figure 7.4: Olfactometer responses of *Phaedrotoma scabriventris* to odours from all the different tested host plant treatments expressed as the mean preference index (PI ± SE) using One-Way ANOVA based on the damage status of the plant

Means followed by the same letter per plant treatment are not significantly different.

7.4 Discussion

Parasitoids will spend longer time in the arm of the device containing the preferred odour (Du et al., 1996; Wei & Kang, 2006). *Liriomyza huidobrensis*-infested host plants were generally more attractive to *P. scabriventris* compared to mechanically damaged and healthy undamaged plants against the blank (control). These findings are in line with previous studies by Wei & Kang (2006) reporting that *L. huidobrensis*-infested bean plants (*P. vulgaris* L. cv Naibai) elicited the strongest response to the endoparasitoid *O. dissitus*, compared to the blank. Petitt et al. (1992) also demonstrated through olfactometric assays that *O. dissitus* preferentially landed on leafminer-infested, rather than non-infested, potted lima bean plants. Herbivore-inflicted injuries induce plants to release volatiles, and their associated parasitoids and predators use these chemicals as major cues to locate their hosts (Turlings et al., 1990; De Moraes et al., 1998; Ngi-Song et al., 2000). Thus, attractiveness of *P. scabriventris* to *L. huidobrensis*-infested plants suggests possible plant induced cues to advertise the presence of the pests on the plant for attack by the parasitoid

Attractiveness of *P. scabriventris* to *L. huidobrensis*-infested plants was highest on common kidney bean and faba bean compared to snow pea. Differences in attractiveness of the parasitoid to the infested plants may suggest differences in chemical profiles probably reflecting differences in metabolic pathways involved in signal transduction among the plants. Thus, analyses and testing of the chemical profiles of these plants will be required to shed light on this pattern. The strong preference of *P. scabriventris* to common kidney bean compared to snow pea is not surprising since field observations reveal the parasitoid to be abundant on the former as compared to the later (Section 6.3). Preference of *P. scabriventris* to faba bean may be due to the effect of prolonged exposure of the parasitoid to this plant since insects are known to genetically adapt to hosts on which they have been cultured over time through inherent learning. For instance, Petitt et al. (1992) demonstrated that female *O. dissitus* that had been exposed to lima bean plants preferred the odour of infested lima bean plants over that of infested aubergine or cotton plants but, an oviposition experience on these non-natal host plant species caused a dramatic change in preference that was due, at least in part, to associative learning. Although *P. scabriventris* used in the current study was not pre-reared on the non-natal host plant species, common kidney bean and snow pea, attractiveness of this parasitoid to these leafminer-infested host plants during its first exposure suggests that it has some level of behavioural plasticity in host selection involving association of chemical cues mostly likely involved in its host location as previously found for other parasitic wasps (Vet & Dicke, 1992; Meiners et al., 2003; Olson et al., 2003). Thus, it is possible that *P. scabriventris* is able to distinguish between the odours of plants with different types of damage by inherent and associative learning, thereby enabling them to distinguish those that have been damaged by potential host pests, *L. huidobrensis*, from those damaged by another factor, mechanically damaged plants and healthy undamaged plants as observed in this study.

Odours from healthy undamaged plants and clean bags (blank control) were found unattractive to *P. scabriventris*. Wei & Kang (2006) also reported similar results when studying preference of the parasitoid *O. dissitus* to bean as leafminer host. Healthy plant species in the Fabaceae family release few volatile compounds in low concentrations (Wei et al., 2007). This may explain why the parasitoid responses to healthy host plants were not strong in this study.

Given that mechanically damaged plants were not preferred by *P. scabriventris*, suggests release of stress-related volatiles, mainly from tissue damage not associated with host finding in the parasitoids. However, an opposing effect was observed by Wei & Kang (2006) whereby, the parasitoid *O. dissitus* significantly preferred volatile extracts collected from injured plants to the solvent control. However, this opposing pattern may reflect physiological differences between the parasitoid species and chemical profiles release due to the damage intensity of host plant species.

Plants are known to release a specific cocktail of HIVs in response to attack by leafminers and they also produce a quantitatively different cocktail of volatiles in response to mechanical damage (Wei et al., 2006). Hands (2013) provided evidence that volatile compounds from leafminer-infested plant foliage strongly affected the behavior of leafminer parasitoids (*D. isaea* and *O. dissitus*) in the absence of visual cues. Thus, *P. scabriventris* was likely responding to one or more of the several additional volatile compounds emanating from the leafminer-infested and mechanically damaged plants as opposed to uninfested plants. Analyses of the chemical profiles from these plant species treated differently will lend support for the observed behavioural results obtained in this study. Identification of attractive compounds can be used to increase the parasitoid's population in field crops. Being an eco-friendly approach, this could reduce over reliance on usage of insecticides which currently is the most widely used control method against LMF in Kenya.

7.5 Conclusion

In conclusion, the behavioural responses of *P. scabriventris* to the three *L. huidobrensis* host plant species observed in this study provide evidence for differences in the parasitoid's response pattern to *L. huidobrensis* host plant species and the volatile profiles induced by the different treated plant species. However, further investigation involving headspace volatile collection from the different plant treatments, chemical identification via electrophysiological and mass spectrometry and behavioural studies will provide insight into the role of individual and blends of compounds in the volatiles mediating host finding and location in *P. scabriventris*, which can be used to improve on LMF management.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

8.1 General discussion

Assessment of interaction between *O. dissitus* and *P. scabriventris* showed that the individual parasitism rates of both parasitoid species were not affected when used alone. This indicates similar parasitism potential of these parasitoids under laboratory conditions. However, in sequence release, the second released species achieved a significantly lower parasitism rate than when released alone, simultaneously or as first released 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 F1 progeny sex ratio was balanced for *P. scabriventris* but male biased for *O. dissitus* whether in single, simultaneous or sequential release. 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. *Phaedrotoma 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. 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.

Leafminer incidence and LMF adults were recorded in all the survey sites across the three elevations of vegetable production in Kenya. This suggests a widespread distribution of LMF in Kenya. The finding that *L. huidobrensis* dominates at all elevations challenges the long held consideration of *L. trifolii* as the dominant *Liriomyza* species in Kenya. This finding suggests that *L. huidobrensis* is more aggressive and is adapting to warmer areas and may be displacing *L. trifolli* and *L. sativae* at the low and mid elevations. *Liriomyza* species were collected from all infested incubated leaves of 20 crops surveyed. Of all the crops, 87.5% of the *Liriomyza* species were obtained from pea, bean, tomato and potato, thereby highlighting these crops as the most important hosts with regard to *Liriomyza* species richness and relative abundance. While the factors accounting for the differential LMF attraction and infestation are unclear, it is known that the plant hosts volatiles may play a significant role in pest attraction.

Nine LMF parasitoid species including the exotic *P. scabriventris* were recorded in this study with 31.2% mean total parasitism rate. This indicates considerable progress in LMF biological control in Kenya since previous parasitism was 5.2%. The diversity of LMF parasitoids in Kenya from this study is higher (nine species) compared to only four parasitoid species in an earlier countrywide survey in Kenya. This increase in LMF parasitoid diversity may be attributed to the extensive nature of the current research work. The indigenous ectoparasitoid *D. isaea* was the most abundant species and accounted for the highest LMF parasitism at all elevations in Kenya. This high dominance of *D. isaea* at all elevations is unusual in Kenya as compared to previous studies which indicated *O. dissitus* as being the most dominant parasitoid species on LMF. In addition, *D. isaea* being relatively more abundant in higher elevation than in low elevation contradicts the normal expectation based on its biological information in literature, suggesting that *D. isaea* is adapting to colder areas and can perform at all the elevation.

Phaedrotoma scabriventris was recovered from all released sites as the second most abundant species and up to 40 km, 30 km and 50 km at high, mid and low elevations, respectively away from the release sites. The recovery of *P. scabriventris* from all release sites and its spread away from the release sites on different *Liriomyza* infested crops, are evidence of successful establishment and spread of *P. scabriventris.* The fact that *P. scabriventris* has quickly reached the top two within the first year post its release in terms of parasitoid abundance confirms reports from its original zone as being the dominant parasitoid over *L. huidobrensis* representing up to 50% of total parasitism. This is promising for the improvement of biological control of LMF in Kenya and East Africa over the years. In this study, beans and tomato were the most important hosts on which the highest total parasitoids were recovered. Of the crops surveyed, *Liriomyza* infested kidney bean yielded the highest proportion of *P. scabriventris* at all elevations. Other field based studies have also reported bean to be the most suitable host for *Liriomyza* species development and their associated natural enemies. Thus, understanding the basis for this preference on bean and tomato may be exploited in improving biological control of LMF.

Phaedrotoma scabriventris was more attracted to *L. huidobrensis*-infested plants compared to undamaged and mechanically damaged plants against the blank. Attractiveness of *P. scabriventris* to infested plants suggests that the presence or action of the pests in the plants may be facilitating easy detection by the parasitoid. Differences in attractiveness of the parasitoid to the infested plants may suggest differences in chemical profiles probably resulting from differences in metabolic pathways.

8.2 Conclusions

1. Introduction of *P. scabriventris* into vegetable production systems in Kenya could potentially provide increased parasitism and mortality of *Liriomyza* species since it co-exists with *O. dissitus* without affecting each others performance.
2. *Liriomyza* leafminers especially *L. huidobrensis* pose a threat to vegetables and ornamentals at different elevations in Kenya. The finding that *L. huidobrensis* dominates at all elevations challenges the long held consideration of *L. trifolii* as the dominant *Liriomyza* species in Kenya.
3. Intergrated Pest Management approaches based on conservation of existing parasitoids and introduction of additional species could potentially result to effective management of the invasive LMF infesting vegetables and ornamentals in Kenya. These approaches may offer viable alternatives to the application of insecticides or at least reduction of their application frequency, which is currently the most widely used control approach against LMF in Kenya.
4. The behavioural responses of *P. scabriventris* to the three *L. huidobrensis* host plant species observed in this study provide evidence for differences in the parasitoid’s response pattern to *L. huidobrensis* host plant species and the volatile profiles induced by the different treated plant species.

8.3 Recommendations

1. Further studies are warranted to assess host discrimination abilities in *P. scabriventris* and *O. dissitus* during their entire reproduction process in parasitizing *L. huidobrensis*.
2. *Liriomyza huidobrensis* being the most economically important *Liriomyza* pest across all elevations of vegetable production in Kenya should serve as a signal to other countries like Uganda where it is still considered a high elevation pest.
3. It is also important to determine if there are genetic differences between *L. huidobrensis* identified in Kenya and that in countries where the species is still limited to higher elevations for effective future management.
4. Continuous monitoring of *Liriomyza* species composition, abundance and possible displacement over time are warranted.
5. Further studies for continuous field evaluation of the effectiveness of *P. scabriventris* performance and spatial distribution in Kenya agro-ecological zones over the years are necessary.
6. Investigations to elucidate the morphological effects of the most important and less important host plants on LMF species infestation and their associated parasitoids are essential.
7. Expansions of chemical, electrophysiological and behavioral studies are needed to unravel the chemical basis of host finding and location in *P. scabriventris* for improved management of LMF.
8. Development of a test kit for molecular identification and characterisation of LMF larvae and associated parasitoids at the field level is also recommened for effective and quick management of LMF.

8.4 Limitations

1. Release permit for *P. scabriventris* from KEPHIS took up to one year for its approval; this delayed the commencement of the planned objectives for this study.

2. Experts’ knowledge was needed in identification and confirmation of LMF and its associated parasitoids reared from infested incubated host plants leaves.
3. Spread of *P. scabriventris* was conducted two years after its release.
4. Unfavourable weather conditions affected laboratory bioassays.

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APPENDICES

Appendix 1: List of publications

Appendix 2: Permit granted by KEPHIS to release *Phaedrotoma scabriventris* in the study sites in Kenya.

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POPULATION ECOLOGY

Species Composition, Distribution, and Seasonal Abundance of *Liriomyza* Leafminers (Diptera: Agromyzidae) Under Different Vegetable Production Systems and Agroecological Zones in Kenya

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ABSTRACT A longitudinal study to identify the species of *Liriomyza* leafminer, their distribution, relative abundance, and seasonal variation, including their host range, was conducted in vegetable fields at three altitudes in Kenya from November 2011 to November 2012. Three main species were identified: *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *Liriomyza trifolii* (Burgess), of which *L. huidobrensis* was the most abundant across all altitudes irrespective of the cropping season and accounting for over 90% of the total *Liriomyza* specimens collected. *Liriomyza* species were collected from all infested incubated leaves of 20 crops surveyed belonging to seven families: Fabaceae, Solanaceae, Cucurbitaceae, Malvaceae, Brassicaceae, Amaranthaceae, and Amaryllidaceae. However, more than 87.5% of the *Liriomyza* species were obtained from only four of these crops: *Pisum sativum* L., *Phaseolus vulgaris* L., *Solanum lycopersicum* L., and *Solanum tuberosum*, thereby demonstrating that Fabaceae and Solonaceae crops are the most important hosts with regard to *Liriomyza* species richness and relative abundance. *L. huidobrensis* had the widest host range (20 crops), followed by *L. sativae* (18 crops) and *L. trifolii* (12 crops). Although *L. trifolii* has been considered the dominant *Liriomyza* leafminer in Kenya, this study suggests that this may not be the case anymore, as *L. huidobrensis* dominates at all altitudes.

KEY WORDS *Liriomyza huidobrensis; Liriomyza sativae*, *Liriomyza trifolii*, Altitude, Host plant

Introduction

Production of vegetables for both domestic and export markets in Kenya is a major source of income, employment, and food for smallholder farmers, especially women (HCDA 2010, 2013). The horticultural sector in Kenya is one of the fastest-growing agricultural sectors, and in 2012, horticultural exports generated above US$1.08 billion in foreign exchange (HCDA 2013). Flowers and vegetables constituted the biggest horticultural export, representing 48.9 and 35.2%, respectively, of export value (HCDA 2013). However, *Liriomyza* leafmining flies (LMF; Diptera: Agromyzidae) are among the most important insect pests of economic importance on vegetables and flowers, limiting the horticultural sector from achieving its full potential (Njuguna et al. 2001, KEPHIS 2007, Chabi-Olaye et al. 2008). LMF pests are the most important cause of interception of Kenya's fresh vegetables and flowers in European market due to their quarantine status (Kedera and Kuria 2003, Chabi-Olaye et al. 2008, EPPO 2013, FVO 2013, EUROPHYT 2014).

Farmers' approach to control LMF in export vegetables and flowers is limited to routine insecticide applications. According to Gitonga et al. (2010), dimethoate, abamectin, imidacloprid, alphacypermethrin, and beta-cyfluthrin are the most common insecticides used against LMF and other pests' complex in vegetable production systems in Kenya. However, while various studies reported that abamectin, alphacypermethrin, and beta-cyfluthrin can effectively control LMF (Kabira 1985, Murphy and La Salle 1999, Weintraub 2001, Kaspi and Parrella 2005), a recent study in Kenya by Guantai (2011) revealed that, at recommended doses, none of the aforementioned insecticides used in Kenya is effective against the larval stage of *Liriomyza huidobrensis*, the most aggressive and abundant of all the invasive LMF species identified in Kenya. Indiscriminate application of synthetic insecticides had led to low levels of parasitism (<5.2%) by the major parasitoid species associated with the pest across all altitudes in Kenya (Chabi-Olaye et al. 2008). Furthermore, high cost, human and animal health hazards, environmental risks, and rejection of export products due to high pesticides residue levels are associated

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with such routine chemical pesticides application (Braun and Shepard 1997, Gitonga et al. 2010, Okoth et al. 2014, PIP 2013, RASFF 2013). There is therefore a need to develop sound, environmental-friendly, and efficient integrated pest management (IPM) techniques to control LMF pests in Kenya and East Africa. However, a prerequisite for a successful IPM approach is to better understand the LMF species composition; abundance; and the effect of seasons, vegetable production systems, and agroecology on the pest (Mwatawala et al. 2006).

It has long been known that *Liriomyza trifolii* (Burgess), introduced from Florida, USA, through chrysanthemum cuttings at Masongaleni, Makueni County, in 1976 is the main *Liriomyza* species infesting ornamentals and vegetables in Kenya (Spencer 1985). Heavy infestation by *L. trifolii* over the years and its subsequent spread throughout the country and to other host plants and abroad resulted in closure of many flower farms, loss of jobs, and loss of overseas markets due to quarantine requirements (Spencer 1985, IPPC 2005). The perception that *L. trifolii* was the most important invasive *Liriomyza* species in Kenya persisted over the years (IPPC 2005). A countrywide survey in Kenya by Chabi-Olaye et al. (2008) revealed the existence of other invasive species: *Liriomyza huidobrensis* (Blanchard) and *Liriomyza sativae* Blanchard, with *L. trifolii* dominating at the low and mid altitudes and *L. huidobrensis* dominating at the high altitude. *L. huidobrensis* is known to adapt and predominantly colonize hosts at colder higher elevations, mostly above 1000 m. a.s.l. (Spencer 1989, Shepard et al. 1998, Sivapragasam and Syed 1999, Rauf et al. 2000, Weintraub 2001, Andersen et al. 2002, Chabi-Olaye et al. 2008, Tantowijoyo and Hoffmann 2010, Mujica and Kroschel 2011), whereas *L. sativae* is the dominant pest in lowland areas (Andersen et al. 2002, Rauf et al. 2000, Spencer 1989). However, neotropical populations of *L. huidobrensis*, *L. sativae*, and *L. trifolii* have continued to depict high adaptability with complex histories of invasion and establishing in many countries worldwide, exhibiting interspecific interactions causing damage to many crops and growing ever more difficult to control (Chaney 1995, Kang 1996, Costa-Lima et al. 2010, Yıldırım and Ünay 2011, Geo et al. 2011). In Kenya, most agricultural crop production systems have four cropping seasons, including the long rains, the short rains, the cold dry, and the hot dry seasons, which vary with respect to altitude and have been inconsistent especially at the mid and low altitudes since 1980s (Hassan 1998, Jaetzold et al. 2006). Thus, production of vegetable crops as host plants of *Liriomyza* species is not equally important across altitudes. In addition, occurrence and relative abundance of leafminers in relation to seasons and host plants may reflect the impacts of climate and their distinct preference for host plants (Murphy and LaSalle 1999, Johansen et al. 2003, Tran et al. 2005, Tran et al. 2007, Rauf and Shepard 1999). The varying ecosystems may affect the distribution of *Liriomyza* species and functional diversity, thus also affecting activities of associated natural enemies. This suggests that the inventories of LMF pest on host crops may have changed significantly over time. However, none of the previous studies carried out in Kenya covered the seasonal variation of LMF species across the altitudes, vegetable production systems, and host plants.

The aim of the present study was to identify and determine the LMF species composition, their seasonal variation, relative abundance, and their host range in various agroecological zones and vegetable production systems in Kenya.

Materials and Methods

Study Sites. Three sites were selected based on altitudes, namely, highland >1,800 m. a.s.l (Nyeri County, Central Kenya), midland from 1,000 to 1,800 m. a.s.l (Kajiado County, Rift Valley Region), and lowland <1000 m. a.s.l (Makueni County, Eastern Region), in Kenya (Hassan 1998). In each altitude, three locations with high productivity of vegetables and reliable irrigation schemes were selected for the study. This was to allow for continued field monitoring throughout the year. The three altitudes were located at least 65 km apart, and locations within altitudes were approximately 5 km apart. Details on locations' coordinates, altitudes, mean annual temperature and rainfall, LMF host crops grown, and seasons are provided in Table 1. Generally, most agricultural crop production areas in Kenya are characterized by four cropping seasons, namely, the long rains, the short rains, the cold dry, and the hot dry seasons, which vary with respect to altitude and have been inconsistent especially at the mid and low altitudes since 1980s (Hassan 1998, Jaetzold et al. 2006; Table 1). In the different altitude levels, the production of vegetable crops as host plants for *Liriomyza* species was not equally important, hence the sampled crops varied in each altitude level.

Field Surveys and Sampling Methods. Monthly field surveys were carried out from November 2011 to November 2012 to determine the species composition, abundance, and distribution of *Liriomyza* species on crops in three locations across each of the three different altitudes of vegetable production in Kenya.

During each observation date at each altitude, LMF infestation and LMF species composition and abundance were evaluated. To determine the LMF infestation at the field level, the sampling area per field was subdivided into four equal quadrants and from each quadrant, 50 leaves from the middle stratum of different plants were picked at random and examined for leafminer infestation (leaves with punctures and/or mines) and the total number infested was recorded. Leaves from the middle stratum of plants were preferred for sampling to leaves from the upper or lower parts of plants because upper leaves are most often clean or have only punctures and leaves from the lower part have old mines with larvae already dropped into soil for pupation, while the leaves from the middle stratum have pupae, "live mines" (mines containing larva) and punctures.

As for LMF abundance and species composition, a maximum of 25 infested leaves per quadrant, with developing or developed mines containing leafminer

Table 1. Summary of comparable attributes per altitude area

Attributes	Highland	Midland	Lowland
Location and GPS coordinates[a]	Sagana (S0° 21'9.972", E37° 5'13.632", 1880 m a.s.l) Kabaru (S0° 17'48.408", E37° 6'28.116", 2061 m a.s.l) Naromoru (S0° 11'9.312", E37° 6'36.972", 2221 m a.s.l)	Namelok (S2° 43'6.096", E37°27'39.06", 1177 m a.s.l) Empiron (S2°50'57.948", E37°32'15.144", 1423 m a.s.l) Inkisanjani (S2°53'56.4", E37°34'51.564", 1466 m a.s.l)	Kwakyai (S2° 23'6.612", E38°0'9.504", 835 m a.s.l) Kikoo (S2°23'50.928", E37°59'8.052", 867 m.a.s.l) Mangelete (S2° 41'57.552", E38°7'32.268", 792 m a.s.l)
Minimum to maximum temperature[b]	8 to 24°C	16 to 28°C	21 to 31°C
Mean rainfall range[b]	~1500 to 2000 mm	~450 to 1200 mm	~800 to 1200 mm
Common *Liriomyza* host crops grown[a]	Potato (*Solanum tuberosum* L.); kales (*Brassica oleracea acephala* L.); cabbage (*Brassica oleracea* L.); pea varieties (*Pisum sativum* L.); French bean (*Phaseolus vulgaris* L.); kidney bean (*Phaseolus vulgaris* L.)	Kidney bean varieties; tomato (*Solanum lycopersicum* L.); French bean	Tomato; common bean; cowpea (*Vigna unguiculata* (L.) Walp); brinjal (*Solanum melongena* L.); sweet pepper (*Capsicum spp*. L.); green gram (*Vigna radiate* (L.) R. Wilczek); okra (*Abelmoschus esculentus* (L.) Moench); courgette (*Cucurbita pepo* L.); butternut squash (*Cucurbita moschata* Duchesne ex Poir.); bitter gourd (*Momordica charantia* L.); pumpkin (*Cucurbita maxima* Duchesne); watermelon (*Citrullus lanatus* (Thunb) Matsun and Nakai)
Short rainy season[a]	Nov 2011 Oct- Nov 2012	Nov 2011 and 2012	Nov 2011 and 2012
Dry and hot season[a]	Jan-Feb 2012 Sept 2012	Jan-Mar 2012 Sept–Oct 2012	Jan-Mar 2012 Sept-Oct 2012
Long rainy season[a]	Mar-June 2012	April–June 2012	April-June 2012
Cold and dry season[a]	July-Aug 2012	July-Aug 2012	July-Aug 2012

[a] Our field observation.
[b] Hassan 1998, Jaetzold et al. 2006.

larvae ("live mines"), were picked, giving a total of 100 leaves per field for laboratory incubation and observations. The infested leaves per field were immediately stored in perforated plastic paper bags and afterwards transferred onto damp paper towels and then placed in plastic rearing containers (19 by 13 by 8 cm) to prevent drying of leaves. The rearing containers were closed with lids containing muslin windows (16 by 9.5 cm) for ventilation and were parked in large cooler boxes to prevent overheating before transportation to the laboratory. After 5 to 10 d, pupae were collected from the rearing containers per field using soft camel hair brushes as they formed, counted, and incubated in bulk in plastic petri dishes with labels until adults of leafminer flies emerged. Laboratory conditions were maintained at 25 ± 2°C and 80 ± 5% R.H. Adult *Liriomyza* species were preserved in 80% ethanol and identification done using conventional taxonomic keys and identification keys from the *Liriomyza* leafminer flies project at the International Center of Insect Physiology and Ecology (ICIPE), Duduville campus, Nairobi, Kenya. The *Liriomyza* species were further sent to the Royal Museum of Central Africa, Belgium, for confirmation. Voucher specimens of identified adult *Liriomyza* species are stored in the entomological museum at the ICIPE, Duduville campus, Nairobi, Kenya.

Data Analysis. The proportion of leafminer-infested leaves (leafminer incidence) was calculated as the number of infested leaves multiplied by 100 and divided by 200 (i.e., total number of leaves picked at random from the middle stratum of the plants from the four quadrants per field). As the leaves were sampled over time, to avoid pseudo-replication, the proportion of leafminer-infested leaves was averaged and the averages were used as the data for analysis (Hurlbert 1984). Proportions were arcsine-transformed and then subjected to two-way ANOVA to assess the effect of cropping season and altitude. Means were separated using Student Newman–Keuls test. The data were analyzed in R version 3.0.2 statistical software (R Development Core Team 2013).

Results

Leafminer Fly Incidence in Farmer Fields. *Liriomyza* leafminer-infested crops were found in all three surveyed locations at each altitude throughout the study period. There was significant interaction between altitude and season in terms of incidence of leafminer puncture and mines on leaves, $F_{6,\ 24} = 10.8$, $P < 0.0001$, thus the variation in infestation levels between altitudes depended on the season. The leafminer incidence ranged from 35.7 to 71.7% across the different altitudes and seasons (Table 2). Infestation levels varied significantly between seasons at highland ($F_{3,\ 8} = 43.1$, $P < 0.0001$) and midland ($F_{3,\ 8} = 7.0$, $P = 0$), and no significant differences at lowland ($F_{3,\ 8} = 1.3$, $P = 0.331$). At highland, infestation was highest in the dry hot season and lowest in the long rainy season, whereas at midland, infestation was highest in the short rainy season and lowest in the dry cold season (Table 2). The dry hot season recorded relatively higher LMF infestation than the other seasons (Table 2). During the dry hot season, of all the 20 crops sampled, the highest leafminer infestation was recorded on French bean (79.5% of leaves), kidney bean (79.1%), snow pea (70.0%), and potato (69.5%) at the high altitude, with the lowest observed on potato (0.5%), okra (10.0%), and onion (14.0%) at the mid altitude (Table 3).

Table 2. Incidence of leafminer-infested leaves during different cropping seasons at low, mid, and high altitudes of vegetable production systems in Kenya

Altitude	LMF-infested leaves (mean ± se %) per season			
	Short rains	Dry hot	Long rains	Dry cold
Highland	53.0 ± 1.5b	71.7 ± 0.9a	35.7 ± 4.2c	56.6 ± 2.0b
Midland	56.6 ± 3.8a	53.6 ± 2.1a	55.1 ± 5.5a	36.0 ± 4.1b
Lowland	49.6 ± 1.5a	55.7 ± 5.0a	48.0 ± 2.3a	49.2 ± 2.2a

Within row, means followed by the same lowercase letter are not significantly different at $P = 0.05$ (Student–Newman–Keuls test).

Leafminer Flies Species Composition, Abundance, and Distribution From Infested Leaves. A total of 46,879 Agromyzidae *Liriomyza* leafminer adult flies composed of *L. huidobrensis*, *L. sativae* *L. trifolii*, *Liriomyza bryoniae* (Kaltenbach), and unidentified *Liriomyza* species were recorded on the 20 crops sampled belonging to seven families, namely, Fabaceae, Solanaceae, Cucurbitaceae, Malvaceae, Brassicaceae, Amaranthaceae, and Amaryllidaceae. The most abundant species was *L. huidobrensis*, representing overall 90.5% of all LMF species collected, and with 94.4, 92.4, and 84.4% at high, low, and mid altitudes, respectively (Table 4). The relative abundance of *L. sativae* was much lower than that of *L. huidobrensis*, representing overall only 6.2% of LMF species collected, but relatively more common in the lowland (5.8%) and midland (9.5%) compared to highland (3.7%; Table 4). *L. trifolii* was rarely found during this study, representing overall only 2.7% of LMF, with 1.0, 5.7, and 1.5% of LMF at the highland, midland, and lowland, respectively (Table 4). Overall, *L. huidobrensis*, *L. sativae*, and *L. trifolii* represented 99.4% of all *Liriomyza* species in the study sites. Seasonal comparisons of these species at each altitude revealed that *L. huidobrensis* was more abundant at all altitudes, accounting for more than 70% of the three *Liriomyza* species throughout the study period (Table 5). *L. sativae* was generally more abundant during the hot dry season at all altitudes and less abundant during the dry cold season at the high and

Table 3. Leafminer fly percentage infestation (mean ± se %) in farmers' fields on different host plants across low, mid, and high altitudes of vegetable production systems in Kenya

Crop	*n*	Short rains	*n*	Dry hot	*n*	Long rains	*n*	Dry cold
Highland								
Courgette	3	52.7 ± 3.5	1	43.5	4	58.0 ± 12.2	1	68.0
French bean	20	44.6 ± 3.4	13	79.5 ± 4.9	12	32.0 ± 4.0	13	54.8 ± 3.0
Garden pea	1	51.5	5	64.9 ± 11.1	4	28.5 ± 2.6	5	53.7 ± 4.3
Kidney bean	3	60.2 ± 18.5	13	79.1 ± 7.2	8	30.7 ± 6.7	3	65.5 ± 9.4
Potato	16	53.7 ± 3.6	21	69.7 ± 5.0	23	31.1 ± 2.7	6	59.4 ± 4.9
Snow pea	30	57.9 ± 3.0	26	70.0 ± 4.1	39	36.7 ± 2.9	23	56.2 ± 2.2
Spinach	–	–	–	–	–	–	1	80.0
Sugar snap	5	63.8 ± 6.7	8	72.4 ± 6.7	5	53.3 ± 4.9	4	53.5 ± 5.8
Sweet pepper	1	45.0	1	54.5	1	29.5	1	35.5
Tomato	1	15.0	1	35.0	3	40.8 ± 2.3	2	58.3 ± 21.3
Midland								
Courgette	–	–	1	25.0	–	–	–	–
Cowpea	–	–	1	80.5	–	–	–	–
French bean	19	57.6 ± 5.2	39	55.6 ± 4.2	15	46.4 ± 4.0	14	28.7 ± 4.2
Garden pea	–	–	1	–	–	–	–	–
Kidney bean	9	86.9 ± 2.5	30	60.6 ± 4.2	17	61.7 ± 3.8	10	32.5 ± 5.6
Okra	–	–	1	10.0	–	–	–	–
Onion	–	–	1	14.0	–	–	–	–
Potato	1	36.5	1	0.5	1	56.5	1	39.5
Tomato	15	39.2 ± 2.3	53	51.7 ± 3.6	29	54.8 ± 3.3	32	39.6 ± 3.7
Watermelon	–	–	–	–	1	35.0	2	45.8 ± 7.3
Lowland								
Bitter gourd	1	60.5	–	–	–	–	–	–
Brinjal eggplant	–	–	1	82.0	–	–	–	–
Butternut squash	–	–	–	–	–	–	1	39.0
Cowpea	1	23.5	7	39.6 ± 10.8	3	46.5 ± 11.0	–	–
Dolichos bean	–	–	1	79	–	–	–	–
French bean	3	69.5 ± 7.2	–	–	–	–	–	–
Kale	–	–	3	12.8 ± 4.7	1	71.5	–	–
Kidney bean	16	48.6 ± 3.0	38	63.7 ± 3.4	13	53.9 ± 3.8	10	41.3 ± 3.1
Okra	1	35.5	9	49.9 ± 4.0	12	37.9 ± 4.1	2	45.8 ± 2.8
Pumpkin	1	28.0	2	49.0 ± 17.0	1	57.5	–	–
Sweet pepper	–	–	1	18.5	1	41.0	1	18.5
Tomato	17	50.2 ± 3.8	66	55.9 ± 2.6	36	49.3 ± 2.3	42	52.0 ± 1.8
Watermelon	1	40	4	53.0 ± 13.7	6	45.2 ± 8.4	2	50.8 ± 6.8

n = number of sampled fields.

Table 4. Species composition, abundance, and distribution of *Liriomyza* leafminer flies identified from infested leaves at low, mid, and high altitudes of vegetable production systems in Kenya

Liriomyza spp.	Number of LMF specimen per altitude			Total
	Highland ($n = 327$)	Midland ($n = 294$)	Lowland ($n = 304$)	
L. huidobrensis	26,788	7,257	10,134	44,179
L. sativae	583	850	516	1,949
L. trifolli	66	479	89	634
Liriomyza spp. (unidentified)	77	19	6	102
L. bryoniae	10	3	2	15
Total	27,524	8,608	10,747	46,879

n = number of sampled fields.

Table 5. Species composition and abundance of the most frequent and invasive *Liriomyza* species at low, mid, and high altitudes during different cropping seasons of vegetable production systems in Kenya

Number of LMF specimen per altitude and season (% of LMF species)			
Season	*L. huidobrensis*	*L. sativae*	*L. trifolii*
Highland			
Short rains	6,604 (97.4%)	139 (2.1%)	36 (0.5%)
Dry hot	5,435 (94.0%)	334 (5.8%)	15 (0.3%)
Long rains	4,569 (98.6%)	60 (1.3%)	6 (0.1%)
Dry cold	10,180 (99.4%)	50 (0.5%)	9 (0.1%)
Midland			
Short rains	814 (94.1%)	36 (4.2%)	15 (1.7%)
Dry hot	2,355 (71.1%)	580 (17.5%)	379 (11.4%)
Long rains	2,002 (87.7%)	210 (9.2%)	71 (3.1%)
Dry cold	2,086 (98.2)	24 (1.1%)	14 (0.7%)
Lowland			
Short rains	1,249 (99.8%)	3 (0.2%)	0 (0.0%)
Dry hot	4,056 (92.7%)	270 (6.2%)	51 (1.2%)
Long rains	3,103 (93.0%)	204 (6.1%)	28 (0.8%)
Dry cold	1,726 (97.2%)	39 (2.2%)	10 (0.6%)

mid altitudes and during the short rains at the low altitude (Table 5). *L. trifolii* was most abundant in the dry hot season at the mid altitude and remained relatively low at both high and the low altitudes in all cropping seasons (Table 5).

***Liriomyza* Species Host Plants Diversity.** A total of 20 different vegetable crops were sampled from the different altitudes, of which all were identified as host plants of leafminer flies (Tables 6–8). More than 87.5% of the total *Liriomyza* species were reared from six plant species belonging to two families: Fabaceae (snow pea, sugarsnap, common bean, and French bean) and Solonaceae (tomato and potato; Tables 6–8) from all the altitudes. Crops from the family Fabaceae were the most commonly grown at high altitude and consequently were the most common *Liriomyza* host plants at the high altitude, with snow pea being the most affected, whereas kidney bean was the least affected (Table 6). At the mid altitude, the Solonaceae and the Fabaceae were the most affected crop families, with tomato being the most affected and potato the least affected of the Solonaceae, whereas French bean and cowpea were the most and least affected, respectively, of the Fabaceae (Table 7). The Solonaceae and the Fabaceae were also the most affected crop families at the low altitude, with tomato and sweet pepper being the most and the least affected of the Solonaceae, whereas kidney bean and dolichos bean were the most and the least affected of the Fabaceae, respectively (Table 8). The least affected crops were spinach (Amaranthaceae) at high altitude (Table 6), onion (Amariliidaceae) and okra (Malvaceae) at the mid altitude (Table 7), and kale (Brassicaceae), at the low altitude (Table 8). *L. huidobrensis* had the widest host range (20 crops) and had the highest abundance on these crops (Tables 6–8), followed by *L. sativae* (18 crops) and *L. trifolii* (12 crops; Tables 6–8). These three *Liriomyza* species were consistently reared in high abundance on snow pea from the high altitude, whereas tomato was the most affected host at the low and mid altitudes (Tables 6–8). *L. bryonae* and the unidentified *Liriomyza* species were less abundant and were reared from four and nine crops, respectively (Tables 6–8). The number of host plants varied across the altitudes. The lowland had the highest number of crops surveyed, i.e., 13 host plants, whereas 10 host plants each were surveyed in the highland and midland (Tables 6–8).

Discussion

Leafminer incidence and the resulting counts of emerging LMF were recorded in all the survey sites across the three altitudes of vegetable production in Kenya. This suggests a widespread distribution of LMF in Kenya. With regard to altitudes, LMF was more abundant in the highlands relative to mid and lowlands. This may be attributed to the fact that the LMF

Table 6. *Liriomyza* species abundance on different host plants at high altitude

Plant family/scientific name	Common name	*n*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	*Liriomyza* species[a]	Total
Fabaceae								
Pisum sativum L.	Snow pea	118	12,443 (45.2%)	274 (1.0%)	28 (0.1%)	3 (0.0%)	30 (0.1%)	12,778 (46.4%)
Pisum sativum L.	Sugar snap	22	3,076 (11.2%)	43 (0.2%)	9 (0.0%)	–	–	3,128 (11.4%)
Phaseolus vulgaris L.	French bean	58	2,148 (7.8%)	93 (0.3%)	20 (0.1%)	4 (0.0%)	27 (0.1%)	2,292 (8.3%)
Pisum sativum L.	Garden pea	15	2,039 (7.4%)	7 (0.0%)	2 (0.0%)	–	5 (0.0%)	2,053 (7.5%)
Phaseolus vulgaris L.	Kidney bean	27	1,311 (4.8%)	36 (0.1%)	1 (0.0%)	1 (0.0%)	5 (0.0%)	1,354 (4.9%)
Fabaceae total		240	21,017 (76.4%)	453 (1.7%)	60 (0.2%)	8 (0.0%)	67 (0.2%)	21,605 (78.5%)
Solanaceae								
Solanum tuberosum L.	Potato	66	3,383 (12.3%)	98 (0.4%)	3 (0.0%)	–	10 (0.0%)	3,494 (12.7%)
Solanum lycopersicum L.	Tomato	7	508 (1.9%)	14 (0.1%)	3 (0.0%)	2 (0.0%)	–	527 (1.2%)
Capsicum L.	Sweet pepper	4	188 (0.7%)	6 (0.0%)	–	–	–	194 (0.7%)
Solanaceae total		77	4,079 (14.8%)	118 (0.4%)	6 (0.0%)	2 (0.0%)	10 (0.0%)	4,215 (15.3%)
Cucurbitaceae								
Cucurbita pepo L	Courgette	9	1,659 (6.0%)	10 (0.0%)	–	–	–	1,669 (6.1%)
Cucurbitaceae total		9	1,659 (6.0%)	10 (0.0%)	–	–	–	1,669 (6.1%)
Amaranthaceae								
Spinacia oleracea L.	Spinach	1	33 (0.1%)	2 (0.0%)	–	–	–	35 (0.1%)
Amaranthaceae total		1	33 (0.1%)	2 (0.0%)	–	–	–	35 (0.1%)
Total for all crops		327	26,788 (97.3%)	583 (2.1%)	66 (0.2%)	10 (0.0%)	77 (0.3%)	27,524 (100.0%)

[a] *Liriomyza* species: unidentified specimen.
n = number of sampled fields.

Table 7. *Liriomyza* species abundance on different host plants at mid altitude

Plant family/scientific name	Common name	*n*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	*Liriomyza* species[a]	Total per crop
Solanaceae								
Solanum lycopersicum L.	Tomato	129	3,592 (41.7%)	595 (6.9%)	383 (4.5%)	3 (0.0%)	18 (0.2%)	4,591 (53.3%)
Solanum tuberosum L.	Potato	4	153 (1.8%)	5 (0.1%)	1 (0.0%)	–	–	159 (1.9%)
Solanaceae total		133	3,745 (43.5%)	600 (7.0%)	384 (4.5%)	3 (0.0%)	18 (0.2%)	4,750 (55.2%)
Fabaceae								
Phaseolus vulgaris L.	Kidney bean	66	1,456 (16.9%)	132 (1.5%)	58 (0.7%)	–	–	1,646 (19.1%)
Phaseolus vulgaris L.	French bean	87	1,706 (19.8%)	84 (1.0%)	29 (0.3%)	–	1 (0.0%)	1,820 (21.1%)
Pisum sativum L.	Garden pea	1	1 (0.0%)	–	–	–	–	1 (0.0%)
Vigna unguiculata (L.) Walp	Cowpea	1	–	–	1 (0.0%)	–	–	1 (0.0%)
Fabaceae total		155	3,163(36.7%)	216 (2.5%)	88 (1.0%)	0 (0.0%)	1 (0.0%)	3,468 (40.3%)
Cucurbitaceae								
Cucurbita pepo L.	Courgette	1	176 (2.0%)	33 (0.4%)	3 (0.0%)	–	–	212 (2.5%)
Citrullus lanatus (Thunb) Matsun and Nakai	Watermelon	3	153 (1.8%)	–	–	–	–	153 (1.8%)
Cucurbitaceae total		4	329 (3.8%)	33 (0.4%)	3 (0.0%)	–	–	365 (4.2%)
Malvaceae								
Abelmoschus esculentus (L.) Moench	Okra	1	12 (0.1%)	1 (0.0%)	4 (0.1%)	–	–	17 (0.2%)
Malvaceae total		1	12 (0.1%)	1 (0.0%)	4 (0.1%)	–	–	17 (0.2%)
Amarilidaceae								
Allium cepa L.	Onion	1	8 (0.1%)	–	–	–	–	8 (0.1%)
Amarilidaceae total		1	8 (0.1%)	–	–	–	–	8 (0.1%)
Total for all crops		294	7,257 (84.3%)	850 (9.9%)	479 (5.6%)	3 (0.0%)	19 (0.2%)	8,608 (100.0%)

[a] *Liriomyza* species: unidentified specimen.
n = number of sampled fields.

collections were dominated by *L. huidobrensis*, which is known to adapt and predominantly colonize hosts at colder higher elevations, mostly above 1000 m. a.s.l. (Spencer 1989, Shepard et al. 1998, Sivapragasam and Syed 1999, Rauf et al. 2000, Weintraub 2001, Andersen et al. 2002, Chabi-Olaye et al. 2008, Tantowijoyo and Hoffmann 2010, Mujica and Kroschel 2011). However, this species was not only predominant at the high altitude but also at the warmer mid and the low altitudes, a finding that contrasts previous studies in Kenya indicating that *L. sativae* and *L. trifolli* predominate in the two later altitudes (Chabi-Olaye et al. 2008). This finding suggests that *L. huidobrensis* is more aggressive and is adapting to warmer areas and may be displacing *L. trifolli*, which has long history of establishing in Kenya, as well as *L. sativae* at the low and the mid altitudes. Species displacement is a potentially widespread phenomenon, receiving much attention from ecologists

Table 8. *Liriomyza* species abundance on different host plants at low altitude

Plant family/scientific name	Common name	*n*	Number of LMF (% of LMF per crop)					
			L. huidobrensis	*L. sativae*	*L. trifolii*	*L. bryoniae*	*Liriomyza* species[a]	Total
Solanaceae								
Solanum lycopersicum L.	Tomato	161	7,416 (69.0%)	356 (3.3%)	53 (0.5%)	2 (0.0%)	1 (0.0%)	7,828 (72.8%)
Solanum melongena L.	Brinjal eggplant	1	122 (1.1%)	25 (0.2%)	–	–	1 (0.0%)	148 (1.4%)
Capsicum L.	Sweet pepper	3	15 (0.1%)	3 (0.0%)	–	–	–	18 (0.2%)
Solanaceae total		165	7,553 (70.3%)	384 (3.6%)	53 (0.5%)	2 (0.0%)	2 (0.0%)	7,994 (74.4%)
Fabaceae								
Phaseolus vulgaris L.	Kidney bean	77	1,319 (12.3%)	43 (0.4%)	2 (0.0%)	–	1 (0.0%)	1,365 (12.7%)
Vigna unguiculata (L.) Walp	Cowpea	11	159 (1.5%)	18 (0.2%)	12 (0.1%)	–	1 (0.0%)	190 (1.8%)
Phaseolus vulgaris L.	French bean	3	33 (0.3%)	1 (0.0%)	–	–	–	34 (0.3%)
Lablab purpureus (L.) Sweet	Dolichos bean	1	4 (0.0%)	–	–	–	–	4 (0.0%)
Fabaceae total		92	1,515 (14.1%)	62 (0.6%)	14 (0.1%)	–	2 (0.0%)	1,593 (14.8%)
Cucurbitaceae								
Citrullus lanatus (Thunb) Matsun and Nakai	Watermelon	13	358 (3.3%)	20 (0.2%)	4 (0.0%)	–	–	382 (3.6%)
Momordica charantia L	Bitter gourd	1	145 (1.4%)	–	–	–	–	145 (1.4%)
Cucurbita maxima Duchesne	Pumpkin	4	42 (0.4%)	5 (0.1%)	–	–	–	47 (0.4%)
Cucurbita moschata Duchesne ex Poir.	Butternut squash	1	30 (0.3%)	–	–	–	–	30 (0.3%)
Cucurbitaceae total		19	575 (5.4%)	25 (0.2%)	4 (0.0%)	–	–	604 (5.6%)
Malvaceae								
Abelmoschus esculentus (L.) Moench	Okra	24	439 (4.1%)	40 (0.4%)	12 (0.1%)	–	2 (0.0%)	493 (4.6%)
Malvaceae total		24	439 (4.1%)	40 (0.4%)	12 (0.1%)	–	2 (0.0%)	493 (4.6%)
Brassicaceae								
Brassica oleracea acephala L.	Kale	4	52 (0.5%)	5 (0.1%)	6 (0.1%)	–	–	63 (0.6%)
Brassicaceae total		4	52 (0.5%)	5 (0.1%)	6 (0.1%)	–	–	63 (0.6%)
Total for all crops		304	10,134 (94.3%)	516 (4.8%)	89 (0.8%)	2 (0.0%)	6 (0.1%)	10,747 (100.0%)

[a] *Liriomyza* species: unidentified specimen.
n = number of sampled fields.

because it affects the structure of communities (Reitz and Trumble 2002, Reitz 2007, DeBach 1966, Abe and Tokumaru 2008). This phenomenon is common in *L. sativae* and *L. trifolli* under field conditions. However, Chen and Kang (2004, 2005) found that *L. huidobrensis* replaced *L. sativae* as the predominant pest in all areas of varying altitudes in China. Subsequent studies by Gao et al. (2011) in the Chinese province of Hainan revealed that *L. trifolii* displaced *L. sativae*, which was the predominant pest on *Vigna unguiculata* L. Similarly, *L. sativae* was also displaced by *L. trifolii* in the western United States (Trumble and Nakakihara 1983, Palumbo et al. 1994). However, in Japan, the opposite occurred, where *L. trifolii* was displaced by *L. sativae* (Abe and Kawahara 2001, Abe and Tokumaru 2008). Thus, given that *L. huidobrensis* was the most abundant, in addition to its wider distribution as determined in this study, the species constitutes the greatest threat to vegetables and ornamentals production in Kenya. Additionally, the spread of *L. huidobrensis* from higher altitudes to lower altitudes and its high adaptation could serve as an indicator that the same may be obtained in neighboring Uganda, where, according to our unpublished data, LMF are currently not considered to be of high importance, with *L. sativae* dominating countrywide, whereas *L. huidobrensis* is limited to higher altitudes (K.K.M. Fiaboe, personal communication). The three most important LMF species in Kenya are *L. huidobrensis*, *L. sativae*, and *L. trifolii*, representing 99.4% of total LMF species. This implies that *L. bryonae* and the unidentified *Liriomyza* species are currently negligible in vegetable production systems of Kenya. This, in addition to the low population of *L. sativae* and *L. trofolii* during this survey, suggests that special attention should be given to *L. huidobrensis* control. Further field surveys will be required in Kenya as well as neighboring countries to assess the status of LMF species composition and abundance over several years in vegetable productions systems.

Although different host plants present an array of chemical, nutritional, and morphological challenges for larval development, the three most abundant *Liriomyza* species identified in this study are highly polyphagous, attacking plants in several families (Spencer 1990, Murphy and LaSalle 1999, Andersen et al. 2002, Tran et al. 2006). In our survey, we identified *L. huidobrensis*, *L. sativae*, and *L. trifolii* from 20, 18, and 12 different infested crops, respectively. This may suggest that the current host range for *L. huidobrensis* and *L. sativae* is relatively high compared to that of the long-established *L. trifolii* in Kenya. Unlike *L. trifolii* and *L. sativae* larvae, which are relatively small and feed on the upper mesophyll of the leaves, that of *L. huidobrensis* is larger and more aggressive by feeding in the lower mesophyll, mine into the petioles and pods, and causing more damage to the plant photosynthetic area; thus, severe yield reduction is inevitable (Weintraub and Horowitz 1995). Being the most polyphagous species, in addition to its aggressiveness and high abundance at all altitudes, *L. huidobrensis* constitutes the greatest LMF challenge to vegetables and ornamentals production in Kenya.

Although most *Liriomyza* species are polyphagous with a broad and diverse host range (Spencer 1990, Murphy and LaSalle 1999, Andersen et al. 2002, Tran et al. 2006), they also exhibit host plant preference (Zhao and Kang 2003, Tokumaru and Abe 2005). In the present study, snow pea (Fabaceae) was the most highly attacked *Liriomyza* host plant in the high altitude, whereas tomato (Solonaceae) was the most highly attacked *Liriomyza* host plant at both mid and low altitudes. This finding is consistent with previous studies, indicating that the fabaceaes and solanaceaes are the most suitable host for *Liriomyza* species development (Mujica and Kroschel 2011, Tran et al. 2007, Tokumaru and Abe 2005, Chabi-Olaye et al. 2008). Kale (Brassicaceae), onion (Amarilidaceae), and sweet pepper (Solonaceae) had lower LMF abundance. Chabi-Olaye et al. (2008) also found that field infested onion and kale leaves in Kenya resulted to very few numbers of *Liriomyza* species identified, whereas Martin et al. (2005) found that Asian broccoli (*Brassica alboglabra* L.; Brassicaceae) was the least preferred host for the chrysanthemum leafminer. *L. huidobrensis* was the only *Liriomyza* species identified from onion and butter squash infested leaves. *L. huidobrensis* was also found infesting onion in California (Reitz and Trumble 2002), and a previous study in Kenya also revealed *Liriomyza* species infesting butter squash (Chabi-Olaye et al. 2008). Although host availability varied across altitudes in this study, *Liriomyza* species can potentially exploit new host plants encountered in newly colonized habitats, especially under conditions where their common hosts are rare or absent (Via 1984). While the factors accounting for the differential LMF attraction and infestation are unclear, it is known that the plant hosts volatiles may play a significant role in pest attraction (Mattiacci et al. 2001, Hartmann 2004, Arimura et al. 2005, Takken and Dicke 2006, Wei et al. 2007). Furthermore, differential host plant preference between LMF and their natural enemies, where some of the preferred host plants of the pest might be less suitable to natural enemies' colony development and/or performance against LMF, may also contribute to the observed differences (Fagoonee and Toory 1983, Knodel-Montz et al. 1985, Minkenberg and Ottenheim 1990, Wei et al. 2000). Therefore, an understanding of the semiochemical basis for attraction between most important host plants (e.g., Fabaceae and Solanaceae) and less important plants (e.g., Brassicaceae and Amarilidaceae) as well as the semiochemical and morphological effects of these LMF host plants on the most important and efficient natural enemies could provide a solid foundation for management of LMF. Further studies to elucidate the parameters above will also help in minimizing the over reliance on synthetic insecticides currently used in their control in Kenya.

The occurrence and relative abundance of leafminers in relation to seasons and host plants may reflect the impacts of climate and their distinct preference for host plants (Murphy and LaSalle 1999, Johansen et al. 2003). *Liriomyza* species can be present in the fields throughout the year, as shown in this study. Apart from *L. huidobrensis*, whose abundance remained relatively high throughout all the cropping seasons at all altitudes, *L. trifolii* and *L. sativae* were more abundant during the hot dry season as compared to the other seasons across all altitudes. This finding is consistent with previous studies, indicating that *L. sativae* was very abundant in the dry season compared to the rainy season in Southern Vietnam and in Ho Chi Minh City (Tran et al. 2005, Tran et al. 2007). While Rauf and Shepard (1999) found that infestations by *L. huidobrensis* occurred heavily during the dry season compared to the rainy season, the present study, however, revealed that the species remained relatively abundant throughout the cropping seasons at all altitudes. This may suggest that *L. huidobrensis* is not only adapting at all altitudes, more aggressive, and polyphagous, but is also consistently high at all altitudes throughout the different cropping seasons despite the varied climatic conditions and crops available.

In conclusion, *Liriomyza* species pose a threat to vegetables and ornamentals at different altitudes in Kenya. Of all LMF identified in this study, *L. huidobrensis* constitutes the greatest challenge to vegetable production by being consistently the most abundant and most polyphagous species at all altitude areas and all seasons. Additionally, the species is adapting to warmer climates at the mid and low altitudes, thus displacing *L. trifolii*, which is long-established at these altitudes in Kenya. Whereas a quick action concerning the management of these pests should be put in place, the results of this study should serve as a signal to other countries like Uganda where it is still considered a high-altitude pest. It is also important to determine the molecular background of *L. huidobrensis* identified in Kenya compared to that in countries where the species is still limited to higher altitudes to test for genetic differences between populations for effective future management. Further studies to assess the species composition, abundance, and possible displacement over time as well as to elucidate the semiochemical and morphological effects of host plants on LMF species infestation and their associated natural enemies are warranted.

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

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The exotic parasitoid, *Phaedrotoma 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 endoparasioids 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 ± 4.60) than in the single release of *P. scabriventris* (34.94 ± 8.50). Simultaneous release of 25 individuals of each species resulted in a parasitism rate (44.52 ± 2.75) similar to that obtained for single releases of 50 individuals of *O. dissitus* (42.57 ± 3.35) and *P. scabriventris* (34.94 ± 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.

Phaedrotoma 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 endoparsitoids, 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. scabriventirs* 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 ± 2 °C, 60 ± 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. huidobrenis*-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^3 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
Single release	
O. dissitus only (T1)	50 adults *O. dissitus* at 1:2 (15♂:35♀) for 24 h
P. scabriventris only (T2)	50 adults *P. scabriventris at* 1:2 (15♂:35♀) for 24 h
Sequential release	
O. dissitus first, *P. scabriventris* second (T3)	50 adults *O. dissitus* at 1:2 (15♂:35♀) for 24 h followed by 50 adults *P. scabriventris* at 1:2 (15♂:35♀) for another 24 h
P. scabriventris first, *O. dissitus* second (T4)	50 adults *P. scabriventris at* 1:2 (15♂:35♀) for 24 h followed by 50 adults *O. dissitus* at 1:2 (15♂:35♀) for another 24 h
Simultaneous release	
O. dissitus and *P. scabriventris* (T5)	50 adults *O. dissitus* and 50 adults of *P. scabriventris*, both species at 1:2 (15♂:35♀) for 24 h
O. dissitus and *P. scabriventris* (T6)	25 adults *O. dissitus* and 25 adults of *P. scabriventris* both species at 1:2 (7♂:18♀) for 24 h
Control	
L. huidobrensis 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 performance 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:

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

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

$$TP_{P_sOd} = \left(\frac{C_{P_s} + C_{Od}}{C_{P_s} + C_{Od} + C_{Lh}} \right) \times 100$$

Table 2. Mean ± S.E. for total and specific parasitism rate of *Phaedrotoma scabriventris* and *Opius dissitus* on *Liriomyza huidobrensis* for each treatment.

Treatment (T) regime	*O. dissitus* specific parasitism (%)	*P. scabriventris* specific parasitism (%)	χ^2	*P*-value	Total parasitism (%)
Single release					
O. dissitus only (T1)	42.57 ± 3.35 a	–	–	–	42.57 ± 3.35 ab
P. scabriventris only (T2)	–	34.94 ± 8.5 a	–	–	34.94 ± 8.50 a
Sequential release					
O. dissitus first, *P. scabriventris* second (T3)	38.3 ± 2.3 abA	11.2 ± 0.7 cB	89.89	<0.0001	49.46 ± 2.90 ab
P. scabriventris first, *O. dissitus* second (T4)	17.0 ± 2.2 cA	28.3 ± 2.4 aB	15.14	<0.0001	45.20 ± 3.71 ab
Simultaneous release					
O. dissitus and *P. scabriventris* (T5)	38.4 ± 5.6 abA	23.6 ± 1.1 abB	19.67	<0.0001	61.96 ± 4.60 b
O. dissitus and *P. scabriventris* (T6)	29.6 ± 4.0 bA	14.9 ± 1.6 bcB	23.48	<0.0001	44.52 ± 2.75 ab
Control					
L. huidobrensis 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_{Ps} = the specific parasitism rate of *P. scabriventris*, C_{Ps} = 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_{PsOd} = 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® 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. *Phaedrotoma 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. *Phaedrotoma 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
L. huidobrensis	%* ♂	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
	χ^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
O. dissitus	% ♂	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	
	χ^2	41.2824		42.735	15.6957	40.9074	31.6712	
	P	< 0.0001		<0.0001	<0.0001	<0.0001	<0.0001	
P. scabriventris	% ♂		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	
	χ^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 ± S.E. for non-reproductive mortality of *Liriomyza huidobrensis* per treatment

Treatment (T) regime	Non-reproductive mortality (%)
Single release	
O. dissitus only (T1)	33.01 ± 1.05 a
P. scabriventris only (T2)	42.33 ± 1.00 a
Sequential release	
O. dissitus first, *P. scabriventris* second (T3)	30.13 ± 1.14 a
P. scabriventris first, *O. dissitus* second (T4)	37.47 ± 1.08 a
Simultaneous release	
O. dissitus and *P. scabriventris* (T5)	30.15 ± 1.08 a
O. dissitus and *P. scabriventris* (T6)	36.17 ± 1.40 a
Control	
L. huidobrensis reared alone (T7)	30.39 ± 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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Our Ref: PH/3/59 Vol. 13/ 312 **Date:** 10th October 2011

The Director
African Insect Science for Food and Health (ICIPE)
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RE : Permission to conduct trials on application for permission to expand release of the endoparasitoid *Phaedrotoma scabriventris* in different agro-ecosystems of horticultural production in Kenya by *Icipe*.

Reference is made to your application for permission to expand release of the endoparasitoid *Phaedrotoma scabriventris* in different agro-ecosystems of horticultural production in Kenya. We note that The Kenya Standing Technical Committee on Imports and Exports considered the application at a meeting held on 15th April 2011 and granted ICIPE approval to the requests.

Following submission of the trial protocol and the identification and approval of the inspection sites; you may proceed with the trial under the following conditions;

1. Icipe will be required to report directly to KSTCIE on the progress of the trial.
2. All the trials will be monitored by KEPHIS and KSTCIE sub-committee and ICIPE will bear the costs for approval, monitoring of the exercise.
3. The trials will be conducted in Loitoktok, Kibwezi, Makindu, Makueni areas as approved during inspection.
4. Any changes to the methodologies submitted should be officially communicated to KEPHIS.

Abed Kagundu
HEAD, PHYTOSANITARY AND BIOSAFETY SERVICES