Zentrum für Entwicklungsforschung (ZEF)

Host acceptability, suitability, interaction and establishment of two introduced *Liriomyza* leafminer endoparasitoids: *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) and *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae) in Kenya

Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

von

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Nairobi, Kenia

Bonn 2018

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Tag der mündlichen Prüfung: 20.08.2018

Abstract

Liriomyza (Diptera: Agromyzidae) leafminers are pests of economic importance to the production of horticultural crops in East Africa. In the scope of a classical biological control program in East Africa, two parasitoids *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) and *Chrysocharis flacilla* Walker (Hymenoptera: Eulophidae) were introduced from Peru. This dissertation aims to assess the performance and impact of the parasitoids in biological control of *Liriomyza* leafminer species. Specifically, the research work aims to assess acceptability and suitability of the two parasitoids in three *Liriomyza* species, the potential interactions with a dominant local parasitoid, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), their establishment and spread after field release. In chapter two and three, performance data of the two exotic parasitoids in three *Liriomyza* leafminer flies are presented. Results show that the two parasitoids successfully accepted, parasitized, and completed their development in the three *Liriomyza* species in 15.97 ± 1.48 and 23.96 ± 0.88 d under controlled environments. Both parasitoids preferred to oviposit in *L. huidobrensis* (Blanchard) causing parasitism rates of between 27.96 ± 3.86 and 77.73 ± 4.39% across the hosts. *Chrysocharis flacilla* further induced significant non-reproductive pupal host mortality. Parasitoid F1 progenies were female-biased across the three hosts for *C. flacilla* except for a balanced sex ratio for *H. arduine* when reared on *L. sativae* Blanchard. *Liriomyza huidobrensis* yielded larger parasitoids and thus a good candidate for parasitoid mass rearing.

In chapters four and five, the potential interactions between the two exotic parasitoids and the local parasitoid *D. isaea* were evaluated. Interaction results between *H. arduine, C. flacilla,* and *D. isaea* show perfect co-existence with no negative effect on performance of either of the parasitoids except on *D. isaea's* when *C. flacilla* came after *D. isaea*. *Halticoptera arduine* and *C. flacilla* had superior parasitism rates over *D. isaea*. Host parasitism rate more than doubled (53.27 ± 4.99 and 72.96 ± 4.12%) when each of the exotic parasitoids occurred together with *D. isaea* as opposed to when *D. isaea* acted alone. Both *D. isaea* and *C. flacilla* caused significant non-reproductive host mortality. Presence of *H. arduine* and *C. flacilla* positively affected *D. isaea* reproduction as only female parasitoids cause host suppression.

In chapter six, results of parasitoid establishments and spread in three agro ecological systems are presented. *Liriomyza huidobrensis* is the dominant species across the three agro ecological systems. Further, a complex of 14 parasitoid species is associated with *Liriomyza* leafminers. *Halticoptera arduine* and *C. flacilla* are confirmed to have established during the survey period of between January 2013 and June 2014. There is an increased in pest suppression of up to 40%, with *C. flacilla* causing substantive specific field parasitism of 3.5%. Seasons, cropping systems and host plants affected parasitoid establishment. *Halticoptera arduine* moved 10 km away from the release point.

Chapter seven discusses the implications of the findings in the management of *Liriomyza* leafminers. This work shows promising candidates as biological control agents against *Liriomyza* leafminers. This research will hopefully influence the private and public sector in the East African region to adopt sustainable pest management strategies that will conserve the parasitoids which might lead to a reduction in the (over) reliance on synthetic pesticide by small holder producers of horticultural crops.

Zusammenfassung

Liriomyza-Blattminierer (Diptera: Agromyzidae) sind wirtschaftlich bedeutende Schädlinge für die Produktion von gartenbaulichen Kulturen in Ostafrika. Im Rahmen eines klassischen biologischen Bekämpfungsprogramms in Ostafrika wurden zwei Parasitoide, *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) und *Chrysocharis flacilla* Walker (Hymenoptera: Eulophidae), aus Peru eingeführt. Diese Dissertation zielt darauf ab, die Leistung und Wirkung der Parasitoide bei der biologischen Bekämpfung von *Liriomyza*-Minierfliegenarten zu untersuchen. Die Forschungsarbeiten zielen insbesondere darauf ab, die Akzeptanz und Eignung der beiden Parasitoide für drei *Liriomyza*-Minierfliegenarten, die möglichen Wechselwirkungen mit dem dominanten lokalen Parasitoiden *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) und deren Etablierung und Verbreitung nach Freisetzung im Feld zu bewerten.

In Kapitel zwei und drei dokumentieren die Leistung der beiden exotischen Parasitoid bei drei *Liriomyza*-Minierfliegenarten. Die Ergebnisse zeigen, dass die beiden Parasitoide die drei *Liriomyza*-Arten erfolgreich akzeptierten, parasitierten und ihre Entwicklung unter kontrollierten Bedingungen jeweils in 15,97 ± 1,48 und 23,96 ± 0,88 Tagen vervollständigten. Beide Parasitoide bevorzugten die Eiablage in *L. huidobrensis* (Blanchard), was zu Parasitierungsraten von 27,96 ± 3,86 und 77,73 ± 4,39% führte. *Chrysocharis flacilla* induzierte weiterhin eine signifikante nicht-reproduktive Puppenwirtsmortalität. Die Parasitoid-F1-Nachkommenschaft war bei den drei Wirten für *C. flacilla* weiblich, mit Ausnahme eines ausgeglichenen Geschlechterverhältnisses für *H. arduine*, wenn sie auf *L. sativae* Blanchard aufgezogen wurde. *Liriomyza huidobrensis* brachte größer Parasitoide hervor und ist somit ein guter Kandidat für die Massenproduktion von Parasitoiden.

In Kapitel vier und fünf wurden mögliche Interaktionen zwischen den beiden exotischen Parasitoiden und dem lokalen Parasitoiden *D. isaea* untersucht. Wechselwirkungsergebnisse zwischen *H. arduine*, *C. flacilla* und *D. isaea* zeigen eine perfekte Koexistenz ohne negative Auswirkungen auf die Leistung beider Parasitoide, außer bei *D. isaea* wenn *C. flacilla* nach *D. isaea* eingeführt wurde. *Halticoptera arduine* und *C. flacilla* erzielten höhere Parasitierungsraten als *D. isaea*. Die Parasitierungsraten mehr als verdoppelten sich (53,27 ± 4,99 und 72,96 ± 4,12%) wenn jeder der exotischen Parasitoide zusammen mit *D. isaea* auftraten, als wenn *D. isaea* alleine auftrat. Sowohl *D. isaea* als auch *C. flacilla* verursachten eine signifikante nicht-reproduktive Wirtsmortalität. Die Anwesenheit von *H. arduine* und *C. flacilla* beeinflusste die Reproduktion von *D. isaea* positiv, da nur weibliche Parasitoide eine Bekämpfung des Wirts verursachen.

In Kapitel 6 werden die Ergebnisse Etablierung und Verbreitung der Parasitoide in drei agroökologische Systemen präsentiert. *Liriomyza huidobrensis* ist die dominierende Spezies in den drei agroökologischen Systemen. Ferner ist ein Komplex von 14 Parasitoidenarten mit *Liriomyza*-Blattminierern assoziiert. *Halticoptera arduine* und *C. flacilla* haben sich während des Erhebungszeitraums zwischen Januar 2013 und Juni 2014 in ihrer Leistung bestätigt. Es kam zu einer erhöhten Schädlingsunterdrückung von bis zu 40% mit *C. flacilla*, die eine wesentliche und spezifische Parasitierung von 3,5% erzielt. Jahreszeiten, Anbausysteme und Wirtspflanzen beeinflussten die

Etablierung von Parasitoiden. *Halticoptera arduine* bewegte sich bis zu 10 km vom Freigabepunkt weg.

Kapitel sieben diskutiert die Implikationen der Ergebnisse in der Handhabung von *Liriomyza*-Minierfliegen. Diese Arbeit zeigt vielversprechende biologische Antagonisten gegen *Liriomyza*-Minierfliegen. Diese Forschung kann möglicherweise Einfluss auf den privaten und den öffentlichen Sektor in der ostafrikanischen Region haben was die Entwicklung einer nachhaltigeren Bekämpfungsstrategie von Minierfliegen unter Einbeziehung der Parasitoide betrifft. Dies kann u.U. die übermäßige Abhängigkeit der Kleinproduzenten gartenbaulicher Kulturen von synthetischen Pestiziden in der Schädlingsbekämpfung reduzieren.

Dedication

This work is dedicated to my beloved late mother, Grace Wangui, who went to be with the Lord as I penned down this thesis. You were a great inspiration to my life and your memories will forever live with us. May your soul rejoice with angels as we purpose to meet again.

List of Acronyms and abbreviations

CABI	Commonwealth Agricultural Bureau International
CIP	International Potato Centre
EC	European Commission
EPPO	European Plant Protection Organization
EU	European Union
EUROPHYT	European Union Notification System for Plant Health Interceptions
FVO	Food and Veterinary Office
GDP	Gross Domestic Product
HCDA	Horticultural Crops Development Authority
Icipe	International Center of Insect Physiology and Ecology
IPM `	Integrated Pest Management
IPPC	International Plant Protection Convention
KEPHIS	Kenya Plant Health Inspectorate Service
KFC	Kenya Flower Council
KHC	Kenya Horticultural Council
KIPPRA	Kenya Institute for Public Policy Research and Analysis
KNBS	Kenya National Bureau of Statistics
LMF	*Liriomyza* leafminer flies
NARS	National Agricultural Research Station
NHP	National Horticulture Policy
PaDIL	Pests and Diseases Image Library
RASFF	Rapid Alert System for Food and Feed
UN	United Nations
USA	United States of America
USD	United States Dollars
ZEF	Center for Development Research

1.0. Introduction

1.1. Background to the study

Agriculture accounts for a third of Gross Domestic Product (GDP) for the African continent employing 65-75% of the population (UN 2013). The sector is key to driving sustainable, equitable growth across the continent and significantly contributes to poverty eradication and food security (Blein 2013). Horticulture is an important sub-sector of agriculture, generating high economic returns, nutritive value and significant employment opportunities (Weinberger & Lumpkin 2007, Ekesi et al. 2010, UN 2013). The sub-sector is ranked high at the national development plans in most sub-Sahara African countries. In Kenya, agriculture accounts for about 25% of the country's GDP and was valued at USD 4.12 billion in 2016, with an estimated 75% of the population directly or indirectly depending on it (KNBS 2017). The majority of this population lives in the rural areas, with 46% of the population living below the poverty line (Kristjanson 2010, Kabubo et al. 2012). In Kenya, the horticulture sub-sector is one of the major employer and source of revenue valued at slightly over USD 1 billion in 2016 (KNBS 2017) after tea and coffee. Kenya is one of the major producers and exporters of horticultural products in the world (Jaffee et al. 2005, HCDA 2009, Ekesi 2010). The European Union (EU) is the largest destination for horticultural produce while the USA and Asia are emerging markets. Eighty percent of the horticultural production is done by small scale farmers and growth in the sub-sector is therefore expected to have a greater impact on a large section of the population than any other sector in Kenya (Minot & Ngigi 2004, HCDA 2009).

Production and access of horticultural produce to market is considerably hindered by several constraints. Ranking high among these constraints is infestation by the invasive leafminer species of the *Liriomyza* genus notably; *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) (Diptera: Agromyzidae) (Chabi-Olaye et al. 2008). These pests are listed as quarantine pests in the EU Plant Health Directive 2000/29 and categorized as regulated pests by the European Plant Protection Organization (EPPO) requiring official controls (EU 2000, EPPO 2011). The said three species have been identified as the most invasive leafminers with high economic importance in Kenya (Spencer 1985, Chabi-Olaye et al. 2008, Musundire et al. 2011, Foba et al. 2015b).

1.2. Literature review

1.2.1. Species composition and identification of *Liriomyza* leafminers

The genus *Liriomyza*, commonly referred to as Leafminer flies, contains more than 300 species distributed worldwide (Spencer 1973, Liu et al. 2009, Mujica & Kroschel 2011). Of the 300 *Liriomyza* species, 23 are of economic importance causing damage to a wide range of agricultural, horticultural, and ornamental crops. Of these, *L. huidobrensis*, *L. trifolii*, *L. sativae*, *L. bryoniae* (Kaltenbach), *L. strigata* (Meigen) and *L. longei* Frick are among the most important species of the genus *Liriomyza* in many parts of the world (Morgan 2000, Van der Linden 2004, Kang 2009). The genus is believed to be of neotropic origin and described as a new World pest until the 1970s

(Waterhouse & Norris 1987, Murphy & LaSalle 1999). In the past three decades, three highly polyphagous leafminer fly species, *L. huidobrensis, L. sativae* and *L. trifolii* have invaded new areas worldwide through trade in plant host commodities and are presently reported from several countries of Africa, Asia and Latin America (Shepard et al. 1998, Rauf et al. 2000, Bjorksten et al. 2005, CABI 2007, Chabi-Olaye et al. 2008, Gitonga et al. 2010). The first report of *Liriomyza* leafminer flies (LMF) in Kenya was in the 1970's with the accidental introduction of the serpentine leafminer, *L. trifolii* through the importation of *Chrysanthemum* spp. (Asteraceae) cuttings from Florida, USA (Spencer 1985). Subequently, other *Liriomyza* species, including *L. huidobrensis* and *L. sativae*, became common pests with the growth of the horticultural sectory.

Identification of the three *Liriomyza* species is based on morphological features. The body size, color, discal cell and distal section of vein M3+4, femora and the male genitalia (Figs.1 (a), (b) and (c)) are the main morphological features used for *Liriomyza* identification (Chaput 2000, Miura et al. 2004, Sappanukhro et al*.* 2011). Spencer (1973) further described identification of *Liriomyza* species using the position of the two vertical setae on the head, color of the frons and orbits, color of the femur and the position of the middle furrow on the six-visible abdominal tergites. Based on these features, *L. huidobrensis* (Fig 2 (a)) is distinguished from *L. sativae* (Fig. 2 (b)) and *L. trifolii* (Fig. 2 (c)) by having both vertical setae lying on the back 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. The outer vertical setae in *L. sativae* (Fig. 2 (b)) lies on the black ground which may just reach the inner vertical setae and which otherwise is on the yellow ground, the frons and orbits is yellow, the femur is bright yellow and the second visible abdominal tergite is divided by the yellow middle furrow same as in *L. huidobrensis*. Both vertical setae in *L. trifolii* (Fig. 2 (c)) lie on the yellow ground, the frons and orbits is yellow with slight brownish striations, the femur is yellow occasionally and the second, third, fourth and fifth abdominal tergites are divided by the yellow middle furrow (PaDIL 2005).

Source: Alice Ames
Department of Primary
Industries, Victoria

Fig 1.(a)

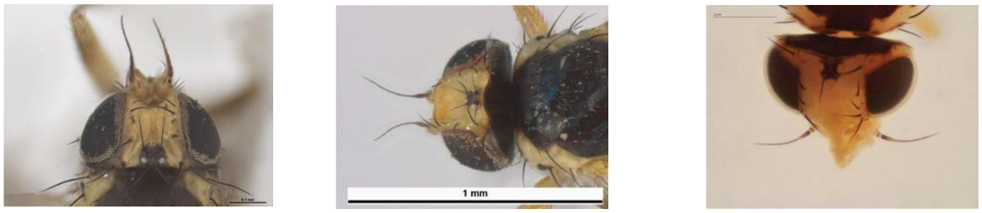
Source: Amy Carmichael
Queensland University of
Technology

Fig. 1 (b)

Source: Alice Ames
Department of Primary
Industries, Victoria

Fig. 1 (c)

(L to R Dorsal view of *L. huidobrensis*, *L. sativae* and *L. trifolii*)



Source: Sarah McCaffrey Museum Victoria

Source: Amy Carmichael Queensland University of Technology

Source: Alice Ames Department of Primary Industries, Victoria

Fig. 2 (a) Fig. 2 (b) Fig. 2 (c)

(L to R; head top view of *L. huidobrensis*, head dorsal view of *L. sativae* and dorsal view of *L. trifolii*)

1.2.2. Ecology and distribution of *Liriomyza* leafminers

Adult leafminers are capable of limited flight and dispersal over long distances but easily disperse on planting material of host species (CABI 2007). Fresh plant materials in trade such as cut flowers and vegetables present dispersal means to other geographical areas. Since the early 1970s, there has been rapid movement of New World invasive *Liriomyza* leafminers from their native origins to the Afro-tropical regions. *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* are the three most important *Liriomyza* species occuring in Afro tropical countries including Tanzania, Ethiopia, Zimbabwe, Sudan, Mauritius, Reunion, Tanzania, South Africa, Morocco, Egypt, Seychelles, Comoros, Senegal, Nigeria and Cameroon (Chabi-Olaye et al. 2008, Foba et al. 2015b). Distribution of *Liriomyza* species in Kenya is wide spread since the first report of *L. trifolii*. Whereas *L. trifolii* was described as the most dominant species in Kenya in 1970s (Spencer 1983), a field survey by Chabi-Olaye et al. (2008) in 2007, described *L. huidobrensis*, *L. sativae* and *L. trifolii* as predominant in the high, mid and low land areas, respectively. Similar distribution patterns of *L. huidobrensis* in the highlands of Indonesia had been described by Rauf et al. (2000), while *L. sativae* and *L. trifolii* have been described as predominant in the mid and low land areas (Spencer 1989, Rauf et al. 2000, Andersen et al. 2002). Recent field surveys in Kenya have shown that *L. huidobrensis* is the most dominant (90%) of all *Liriomyza* species across the low, mid and high elevation areas (Foba et al. 2015b).

1.2.3. Biology of *Liriomyza* leafminers

LMF exhibit a holometabolous life cycle with off-white and slightly translucent eggs, white to creamy colored larvae, pupae and adults of varying colors. Most of the species in this genus are polyphagous with overlapping host range. In all of the three most invasive species frequently reported in Africa, mating occurs 24 h after adult emergence and a single mating is sufficient to fertilize all the eggs (Murphy & LaSalle 1999). Multiple mating is, however, required by females for maximum egg production (Kotze & Dennill 1996). Eggs are laid singly close to each other on the lower leaf surface in small uniformly round oviposition punctures (0.05 mm) (Bethke & Parrella 1985) mainly during the morning hours (Parrella 1987, Weintraub & Horowitz 1995). Females can lay upto 600 eggs, depending on environmental conditions, hosts and leafminer species (Parrella 1987). Optimal temperatures for egg laying range between 21 and 32°C with reduced egg laying at temperatures below 10°C (Chaput 2000). In younger females, eggs are laid at a rate of 30 to 40 per day, with the numbers decreasing as flies age (Murphy & LaSalle 1999). Eggs take between 2 to 5 days to hatch into larvae depending on the prevailing temperatures, relative humidity, and availability of food (Parrella 1987). Larvae feed on leaf tissues making a winding tunnel or blotch on the leaf. The leaf-mines become increasingly larger as the larvae feed and grow through three immature stages (instars) before pupation occurs externally, either on the foliage, in the soil just beneath the surface the plant or in the leaves (Parrella 1987, EPPO/CABI 2006). Pupal development is completed in 5 to 12 days depending on the prevailing temperatures and the adult emerges from the puparium, principally in the early morning hours (Parrella 1987). Both sexes emerge simultaneously and in equal sex ratios (Parrella 1987, Mau & Kessing 2002). Adults are active during the day and rarely after the 1800 h (Parrella et al*.* 1983, Fagoonee & Toory 1984, Parrella 1984).

The life cycle of LMF varies with host and temperature and many generations can occur per year if temperatures are above 10°C. Egg to adult development may take as little as two weeks at 35°C facilitating rapid populations build up, or upto eight weeks at 16°C (Varela et al*.* 2003, CABI 2007). Developmental time of different *Liriomyza* leafminers varies. Under greenhouse conditions of 27^{o}C, *L. huidobrensis* egg stage last three days, larval stages three to five days while the pupal stage lasts for nine days (Parrella & Bethke 1984). Development time required by *L. sativae* egg and larval stages is about seven to nine days at 25-30^{o}C while pupal development takes about nine days at the same temperatures under laboratory conditions (Capinera 2007). At 25^{o}C, *L. trifolii* egg stage requires three days for development, while the larval stages require five days and pupal stage nine days under laboratory conditions (Minkenberg 1989).

1.2.4. **Economic importance of *Liriomyza* leafminers**

Liriomyza leafminers are pests of both cultivated and wild host plants. The latter playing not only an important role in the carry-over of the pest infestations but also as refuges for leafminers' natural enemies. The economic importance of *Liriomyza* leafminers on vegetable crops is considerable (Spencer 1985, Murphy & LaSalle 1999, EPPO 2006, Burgio et al. 2007, Hernandez et al. 2010, Mujica & Kroschel 2011). Severe crop loss and damage of both commercial and indigenous vegetable and ornamental hosts in the families of Amaranthaceae, Amaryllidaceae, Asteraceae, Brassicaceae, Fabaceae, Compositae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Graminaceae, Liliaceae, Malvaceae, Rosaceae and Solanaceae have been associated with LMF (Rauf et al. 2000, CABI 2007, Musundire et al. 2011, Chabi-Olaye et al. 2008, López et al. 2010, KEPHIS 2014, EUROPHYT 2017). Yield losses ranging between 20 and 100% are associated with *Liriomyza* leafminers depending on crop, level of infestation and location (EPPO 2006). In Kenya, *L. huidobrensis* cause damage between 10 and 80% and is higher in cultivated than wild habitats (Chabi-Olaye et al. 2008) and this damage results from the feeding and oviposition punctures by adult leafminers and tunneling by larvae (Parrella 1987, Wei et al. 2000). Only females create feeding punctures as white speckles measuring between 0.13 and 0.15 mm in diameter, causing destruction of a larger number of cells (Parrella 1987). Leaf puncturing may occur with equal frequency on the upper and lower leaf surfaces, but this may depend on the species of *Liriomyza* (Parrella & Bethke 1984). Larval mining is generally the most widespread feeding behavior shared by more than 75% of the species where males are unable to puncture leaves but feed at punctures made by females (Parrella 1987). Larval mining in palisade parenchyma tissue reduces the photosynthetic capacity of plants up to 62% (Johnson 1993) and can severely reduce plant yield, transport viral and fungal diseases (Civelek & Önder 1999) and even kill the plants at high fly density (Spencer & Steyskal 1986). Adult leafminers act as vectors for diseases such as *Alternaria alternata* (Fr.) Keissl and in young plants and seedlings, may lead to yield loss and reduction in value (Musgrave et al. 1975, Zitter & Tsai 1977, Matteoni & Broadbent 1988, Deadman et al. 2002, Bjorksten et al. 2005).

In East Africa, severe crop damage by LMF is reported in commercial and and indigenous vegetables crops including; *Pisum* sp. L., *Phaseolus vulgaris L.* (Fabaceae), *Solanum melongena* L., *S. lycopersicum* L., *S. tuberosum* L., *S. nigrum* L. (all Solanaceae), *Abelmoschus esculentus* (L.) Moench (Malvaceae), *Ocimum basilicum* L. (Lamiaceae), *Amaranthus* sp, (Amaranthaceae), and *Cleome gynandra* L. (Cleomaceae); fruits such as *Passiflora edulis* Sims (Passifloraceae) and ornamental plants such as *Eryngium* spp. (Apiaceae), *Gypsophila* spp. (Caryophyllaceae), *Chrysanthemum* sp. (Asteraceae) (Chabi-Olaye et al. 2008, Foba et al. 2015b, Farmerstrend 2016, EUROPHYT 2018). Of the three most important *Liriomyza* leafminers in Kenya, *L. huidobrensis* is rated more invasive and aggressive than *L. trifolii* and *L. sativae* (Weintraub and Horowitz 1995). More than 80% of all the species in the high, mid and lowlands of Kenya comprise of *L. huidobrensis* (Foba et al. 2015b). In addition to direct damage, *Liriomyza* spp. are notifiable pests in the European Union and official controls are applied to avoid their introduction (Murphy & LaSalle 1999, EU 2000). LMF have been a major cause of non-compliances of Kenya's fresh produce in the European markets (IPPC 2005, Chabi-Olaye et al. 2008, FVO 2014). Therefore, they are a cause of barrier to trade that calls for sustainable solutions. Furthermore, their quarantine status limits overseas market opportunities for vegetables and ornamentals fresh produce from smallholder farmers in East Africa.

1.2.5. Management strategies of *Liriomyza* leafminers

Integrated Pest Management (IPM) potentially can provide economical, sustainable, and environmentally friendly strategies for the management of *Liriomyza* leafminers due to complexity of their biology. Application of synthetic insecticides has largely been practiced by many farmers (Chandler 1984, Parrella & Robb 1985). Control using insecticides is usually complicated by the pests' biology, development time; smallness and high mobility of the adults; a relatively long pupal stage occurring in the concealed places; high reproductive capability; and egg and larval stages within and protected by leaf tissue (Shiming & Hongwei 1999). A mine created by the larva remains in the leaf if the leaf survives; thus, insecticide application may have little use in preserving the aesthetic value of ornamentals or for preventing yield reduction in vegetables. Insecticide applications have commonly been responsible for outbreaks of *Liriomyza.* Moreover, they are often more toxic to the pests' natural enemy complex rather than to the leafminers themselves (Parella et al. 1984, Murphy & LaSalle 1999, Chabi-Olaye et al. 2008, Guantai et al. 2015). Outbreaks may also result as a consequence of leafminers receiving sub-lethal insecticide doses that may only physiologically stimulate but not kill them (Oatman & Kennedy 1976, Guantai et al. 2015), often resulting in the rapid development of insecticide resistance (Tran & Takagi 2005b, Liu et al. 2009, Musundire et al. 2011). Commonly used insecticide compounds by vegetable farmers to control leafminers in Kenya, include; abamectin, cyromazine, carbamates, organophosphate and pyrethroids but often with little success (Kabira 1985, Gitonga et al. 2010). Resistance of *Liriomyza* leafminers to different insecticides like cyromazine, carbamates, organophosphates, pyrethroids, and triazine in some parts of the world have been reported (MacDonald 1991, Kotzee & Dennill 1996, Guantai et al. 2015). Not only has the use of insecticides compounded the management of *Liriomyza* leafminers but also present food safety concerns to vegetable consumers. The EU for example regulates the allowable pesticide residues present in fresh farm produce for human consumption (EC 2005, RASFF 2015).

Biopesticides and botanicals such as neem and pyrethrym form sustainable alternatives to the use of synthetic insecticieds due to their safety and ease of degradation. Neem-based insecticides applied as drench or as foliar spray reduce fecundity and longevity of leafminer flies and disrupt the development of the maggots (Van Randen 1996). Entomopathogenic nematodes and fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) have shown potential against *Liriomyza* leafminers (Head et al. 2000, Migiro et al. 2010, Akutse et al. 2013). In addition, farmers continuously engage the use of cultural control strategies to manage *Liriomyza* leafminers. In tomato, cucumber, cantaloupe, and beans crop varieties vary in their susceptibility to leaf mining (Hanna et al. 1987, Mujica & Kroschel 2011) while the placement of row covers over cantaloupe prevents damage by leafminer (Orozco-Santos et al. 1995). However, the impact of such differences in susceptibility as well as varying nitrogen levels, and the use of reflective mulches tend to be moderate, inconsistent, and not adequate for reliable protection from infestation (Chalfant et al. 1977, Hanna et al. 1987). Hand-picking, destruction of mined leaves and other plant materials after harvest, ploughing, and flooding may significantly reduce leafminer flies damage in vegetable and ornamental crops (Leuprecht 1993, Varela et al. 2003). Finally, trapping of adult *Liriomyza* leafminers using yellow sticky traps can also achieve some level of control (Weintraub 2001).

1.2.6. Biological control of *Liriomyza* leafminer flies

Agromyzid leafminers have a rich natural enemy fauna, particularly in their native areas of origin (Waterhouse & Norris 1987, Murphy & LaSalle 1999). Natural enemies are an important factor in regulating *Liriomyza* populations in pesticide-free areas (Johnson 1993, Shepard et al. 1998, Murphy & LaSalle 1999, Rauf et al. 2000, Mujica & Kroshel 2011). More than 300 species of parasitoids are associated with leafminers worldwide (Noyes 2004) and of these over 80 species are known to attack various LMF species (Liu et al. 2009). *Diglyphus begini* Ashmead (Hymenoptera: Eulophidae) is one of the widely used parasitoid species for augmentative biological control against LMF (Sher et al. 2000). In commercial greenhouses in Europe, *Dacnusa sibirica* Telenga, *Opius pallipes* Wesmael (both Hymenoptera: Braconidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) have been used as natural enemies of the pest (Van der

Linden 2004). *Diglyphus isaea* is an effective parasitoid at higher temperatures and has been as well considered for controlling leafminers in tropical environments (Minkenberg 1989). In Kenya and South Africa, large-scale mass-production programmes of *D. isaea* have been developed to support biological control efforts of the LMF through augmentative biological control approaches (Musundire et al. 2011). In Senegal, a decline of the invading leafminer populations was observed after a few years of action following the introduction of exotic parasitoids (Neuenschwander et al. 1987). Similarlry, in Mozambique, sufficient control of *Ophiomyia spencerella* (Greathead), *O. phaseoli* Tyron and *O. centrocematis* de Meijere (all Diptera: Agromyzidae) was achieved by the locally occurring *Eucoilidea nitida* (Benoit) and *Opius melanagromyzae* (Fischer) (both Hymenoptera: Braconidae) (Davies 1998). In Kenya, however, the diversity of parasitoids associated with *Liriomyza* leafminers in field crops, is low and mainly comprises of four parasitoids species namely; *D. isaea*, *Neochrysocharis formosa* (Westwood), *Hemiptarsenus varicornis* (Girault) (both Hymenoptera: Eulophidae) and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) accounting for a total parasitism rate of less than 6% in both cultivated and wild habitats across all agro-ecological zones (Chabi-Olaye et al. 2008).

In the native origin of *Liriomyza* leafminers, the use of parasitoids as biological agents has achieved considerable success in managing Agromyzid leafminers (Murphy & Lassalle 1999, Mujica & Kroschel 2011). In Peru, 63 parasitoids are associated with leafminers causing up to 85% parasitism rates (Mujica & Kroschel 2011). Of theses, the most important parasitoids in LMF control are *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae), *Chrysocharis flacilla* Walker (Hymenoptera: Eulophidae) and *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) (De Santis 1979, Sanchez & Redolfi 1985, Salvo & Valladares 1997, Cisneros & Mujica 1997, Neder de Român 2000, Salvo et al. 2005, Mujica & Kroschel 2011) with *Liriomyza* field mortality of upto 66.7% (Salvo & Valladares 1999, Mujica & Kroschel 2011). Between 2010 and 2012, the three parasitoid species were introduced from Peru to Kenya in an effort to boost suppression of invasive *Liriomyza* leafminers in a biological control program for East Africa at the International Center of Insect Physiology and Ecology (*icipe*). This dissertation focus on two of the three parasitoids namely; *H. arduine* and *C. flacilla*.

1.2.7. Exotic parasitoids

Halticoptera arduine and *C. flacilla* are larval endoparasitoids attacking the late 2nd and 3rd instar stages of a range of Agromyzid leafminer species completing their development within the host (Redolfi & Ascencios 1988). After host parasitization, the host continues to develop into the pupal stage until the adult parasitoid emerges from the host pupa. Shortly after emergence, adult female parasitoids begin to search for leafminer hosts larvae from infested plant leaf surfaces. Upon larval encounter, *H. arduine* female parasitoids deposit up to three eggs in a host but only one offspring develops per host (Arellano & Redolfi 1989). *Halticoptera arduine* sex ratio is affected by host density and fertilization of females. Unfertilized *H. arduine* females produce only males (Kroschel et al. 2016). Temperatures of $<10^{o}$ C and $>30^{o}$ C affect the reproduction and development of *H. arduine* (Prudencio 2010). In its native origin, *H. arduine* occurs from the coast to the highlands at up to of 4,045 meters above sea level (m.a.s.l.) with mean temperatures of 8 and 24^{o} C (maximum 30^{o} C and minimum 4^{o} C)

(De Santis 1979, Arellano & Redolfi 1989, Neder de Roman 2000, Mujica & Kroschel 2011). It is also found occurring in hot dry and semi-arid and desert regions and in winter and summer seasons (Mujica & Kroschel 2011). *Halticoptera arduine* attacks more than seven Agromyzid leafminer host species of *Amuauromyza* sp. Hendel, *Cerodontha dorsalis* Loew, *Japanagromyza* sp. Sasakawa, *L. huidobrensis*, *L. sativae*, *L. graminivora* Hering, unidentified *Liriomyza* sp, *Melanagromyza* sp. Hendel and an unidentified Agromyzidae occurring in 25 host plants (Mujica & Kroschel 2011). In the Peruvian coast, *H. arduine* is the dominant species accounting for 48.2% of all parasitoid species of the Agromyzids leafminers on vegetable crops, causing upto 66.7% parasitism rates of *L. huidobrensis* under field conditions (Mujica 2007). *Halticoptera arduine* was therefore regarded as a potential candindate for classical biological control of LMF in varying elevations (>900 asl to 1,800 m.a.s.l.) in Kenya.

Chrysocharis flacilla has a wide ecological distribution in its native origin occurring from 0 - 4,045 m.a.s.l. It thrives in warm and dry climates and areas with mean temperatures of 18 - 20^{o}C (maximum 30^{o}C and minimum 4^{o}C) (Aguilera 1972, Arce De de Hamity & Neder de Roman 1984, Arellano & Redolfi 1989, Neder de Roman 2000, Mujica & Kroschel 2011). It is associated with eight species of the Agromyzidae LMF on 21 host plants, causing parasitism rates of between 30-55% (Mujica & Kroschel 2011), and is a dominant parasitoid of *L. huidobrensis* in the Peruvian potato fields (Arce de Hamity & Neder de Roman 1984, Neder de Roman 2000).

However, little is know on *H. arduine* and *C. flacilla* biology including their searching behaviors or potential use in classical biological control programs. Hence in addition to *H. arduine,* also *C. flacilla* was therefore considered as a potential candindate for classical biological control of LMF in varying elevations (>900 to 1,800m m.a.s.l.) in Kenya.

1.3. Parasitoids interactions

A natural enemy in a classical biological control suppresses the abundance of an arthropod pest to a level that is economically tolerable in the target environment. If multiple parasitoid introductions occur in the background of existing natural enemies, it becomes necessary to understand the potential consequences of interactions between the different species. The outcome of such interactions is dependent upon the degree of aggregation of encounters between the interacting parasitoids and their host(s), and to a lesser extent on the form of limitation of the indigenous or primary parasitoid species (Mills 2003). When both the indigenous and the introduced parasitoids show highly aggregated search behavior, then most forms of the interactions will lead to greater suppression of the pest(s). However, parasitoids effectiveness in controlling the target pest can be reduced by competitive interactions among conspecifics or between individuals of different species where a single insect host can be attacked by a range of parasitoids species to support their populations (Briggs 1993, Godfray 1994, Bogran et al. 2002). Competition may be indirect which may be witnessed when competing for host or direct interference competition, which may be resolved through contests (Hardy et al. 2013). These contests are mostly ritualized non-injurious agonistic behaviors (Briffa & Sneddon 2010, Kokko 2013).

Competition is one of the factors which may influence parasitoid foraging behavior and may occur when an individual fitness is decreased by the presence of another individual which increases its own fitness (Grover 1997). Host size also influences competition ability of interacting species (Luck & Podoler 1985, Murdoch et al. 1996, Yu et al. 1990). To avoid competition, parasitoid species over time become discriminative to hosts parasitized by conspecifics or by themselves (Salt 1961, Lenteren 1981, Van Alphen et al. 1987). Host utilization mediating competitive interactions among parasitoids suggest that interactions among parasitoids may cause local displacement of competitively inferior species (Yu et al. 1990, Vargas et al. 1993, Murdoch et al. 1996, Itioka et al. 1997). Negative interactions amongst biological control agents and competitors may play a significant role both for the success of biological control programs and for non-target effects. In fact, some well-documented examples of displacement have occurred among introduced biological control agents, and some of them showed complex ecological processes as responsible for displacement (Bigler et al. 2006). Obviously, a successful biological control agent by itself may have dramatic consequences on the composition of a given natural enemy complex. It may be questioned whether displacement of an exotic or population changes of indigenous natural enemies associated with the control of the pest, can be considered as relevant non-target effects (Bigler et al. 2006). For parasitoids to coexist, niche differentiation between the species or host specifity for competing species is needed (Bokonon-Ganta et al. 1996, Snyder et al. 2005, Hackett et al. 2009).

1.4. Statement of the problem

The growth of the horticultural sector in East Africa is severely constrained by infestation of invasive *Liriomyza* LMF, with reported yield losses of upto 80-100%. Their quarantine status in the export markets posses a threat to the livelihood of smallholder farming families engaged in horticultural activities and for generating foreign currency to the respective national Government(s). Management of the pest using synthetic insecticides is plagued by their high costs, resistance development in the pests, elimination of natural enemy fauna and consumer food safety concerns. Management of Agromyzid *Liriomyza* LMF requires the adoption of Integrated Pest Management (IPM) strategies as a sustainable solution which has not been fully exploited in Africa.

1.5. Justification

IPM approaches based on the conservation of existing natural enemies and in the case of invasive pests, the introduction of exotic natural enemy species offer viable alternatives to the application of insecticides (Kang et al. 2009). In Kenya and the East Africa region as a whole, management of *Liriomyza* LMF in horticultural production systems is characterized by the routine application of synthetic insecticides, causing food safety, human and environmental concerns. The diversity of *Liriomyza* LMF parasitoids in East Africa is is low and the existing indigenous parasitoids only cause low pest suppression (<6%) of. Increasing pest suppression by boosting parasitization through exploration and introduction of exotic parasitoids is a sustainable solution to pest management. However, this should take into consideration the competitive risks of any potential candidate to the indigenous species, as well as potential non-target impacts to other endemic pests (Bokonon-Ganta et al. 2005). To avoid ecological disruption of the indigenous parasitoid populations arising from the introduction of exotic biological control agent(s) to a new ecosystem, an assessment of its/ their performance and impact to the indigenous biological control agents is required (Boettner et al. 2000, Louda et al. 2003).

Studies on *Liriomyza* LMF parasitoids in classical biological control programs in sub-Saharan Africa are scarce. In the same breadth, few data derived from field experiments address the question central to interspecific competition among parasitoids and its impact on host dynamics. Little evidence is available on the potential negative effect to the outcomes of a biological control program by interspecific interactions. Understanding the the impact of multiple parasitoid species on the population dynamics of a shared host as well as coexistence of introduced and native parasitoid species is crucial in the selection of appropriate exotic biological control agents for release. The biology of *H. arduine* and *C. flacilla* is yet to be fully understood and we expect to contribute to this.

In view of this, *icipe*, with permission form the relevant authorties of the Government of Kenya, imported three *Liriomyza* LMF parasitoid species, namely *P. scabriventris*, *H. arduine,* and *C. flacilla* from Peru into its quarantine facilities in Nairobi, Kenya in the context of an East African LMF IPM program. Previous parasitoid introduction studies, guides on the number of parasitoid species for release . For instance, an introduction of the equivalent of 3% of *Opius pallipes* Wesmael (Hymenoptera: Braconidae) to the total larvae in the first leafminer generation was sufficient to achieve control (Woets & van den Linden,1982). Notable case studies of classical biological control against insect pests from we can also learn from includes the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in traopical Africa and mango mealybug, *Rastrococcus invadens* Williams () in West Africa (Cock et al. 2015)

1.6. Hypotheses

Halticoptera arduine and *C. flacilla* will not be limited to accept, oviposit and complete their development within three dominant *Liriomyza* LMF species occurring in Kenya

Halticoptera arduine and *C. flacilla* will not negatively interact with the existing indigenous parasitoid *D. isaea*.

Halticoptera arduine and *C. flacilla* will not be limited to successfully establish themselves in three different agro ecological vegetable production zones of Kenya.

1.7. Objectives

1.7.1 General objective

The study was designed to assess the acceptability and suitability of two exotic parasitoids; *H. arduine* and *C. flacilla* on three important *Liriomyza* LMF species; *L. huidobrensis*, *L. sativae* and *L. trifolii*, their interactions with the indigenous parasitoid; *D. isaea* under laboratory conditions and after field establishment following releases in a classical biological control program in Kenya.

1.7.2. Specific objectives

1. To evaluate acceptability and suitability of *H. arduine* and *C. flacilla* using *L. huidobrensis*, *L*. *sativae,* and *L*. *trifolii* as hosts.

2. To evaluate interactions between *H. arduine* and *C. flacilla* and the indigenous parasitoid *D. isaea*
3. To evaluate potential field establishment of *H. arduine* and *C. flacilla* in three vegetable production systems at high, mid and low elevations in Kenya.

2.0. Acceptability and suitability of three *Liriomyza* species *as host f*or the endoparasitoid *Halticoptera arduine* (Hymenoptera: Pteromalidae)

This chapter has been published as Muchemi et al, Journal of Environmental Entoomology, 47 (3), 2018, 684-691, doi: 10.1093/ee/nvy050.

2.1. Abstract

In the scope of using *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) in a classical biological control program in East Africa, laboratory bioassays were conducted to evaluate the acceptability and suitability of the three economically important LMF species to the exotic parasitoid. Searching time, number of oviposition attempts, F1 parasitoid developmental period, parasitism rates, sex ratio, host mortality and body size indices were assessed. *Halticoptera arduine* parasitized and developed successfully in the three *Liriomyza* species reported in East Africa. Female parasitoids took on average between 10.45 ± 0.83 to 15.80 ± 0.91 (means ± SE) seconds to encounter their first host and made significantly more oviposition attempts on *L. huidobrensis* (Blanchard) than *L. sativae* Blanchard and *L. trifolii* (Burgess) (Diptera: Agromyzidae) (P = 0.0006). Parasitoid development period from egg to adult ranged between 19.32 ± 0.96 and 22.86 ± 0.27 days. Parasitism rate ranged from 27.96 ± 3.86 to 44.10 ± 4.56 in the three host species, and was significantly higher in *L. huidobrensis* than in *L. sativae* (P = 0.0397). *Halticoptera arduine* did not induce significant non-reproductive host mortality in any of the three *Liriomyza* hosts. A female-biased parasitoid sex ratio was observed in *L. huidobrensis*, a balanced sex ratio in *L. sativae and* a male-biased in *L. trifolii*. Parasitoids progeny were significantly larger on *L. huidobrensis* for both tibia and wing length than *L. sativae* and *L. trifolii* (P = 0.0109 and P = 0.0192 respectively). The implication for the environmentally friendly management of *Liriomyza* leafminers in East Africa is discussed.

2.2. Introduction

Liriomyza (Diptera: Agromyzidae), commonly referred to as Leafminer flies contains more than 300 species distributed worldwide (Spencer 1973, Liu et al. 2009, Mujica & Kroschel 2011). The genus is believed to be of Neotropic origin which had restricted distribution to the New World until the mid 1970's (Waterhouse & Norris 1987, Murphy & LaSalle 1999). Twenty-three species are of economic importance causing damage to a wide range of horticultural and ornamental crops (Morgan et al. 2000, Van der Linden 2004). Several species of the *Liriomyza* have since invaded new areas worldwide (Shepard et al. 1998; Rauf et al. 2000; Bjorksten et al. 2005). *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) was first reported in Kenya in 1976 through *Chrysanthemum* spp. (Asterales: Asteraceae) cuttings from Florida USA, and was subsequently recorded in other localities from the coastal areas to the highlands (Spencer 1985). Three highly polyphagous leafminer fly species, *Liriomyza huidobrensis* (Blanchard)*, Liriomyza sativae* Blanchard (Diptera: Agromyzidae) and *L. trifolii* (Burgess) (Diptera: Agromyzidae) are currently the predominant invasive species frequently reported from Kenya (Chabi-Olaye et al. 2008, Gitonga et al. 2010, Foba et al. 2015b).

Adult female leafminers cause damage on leaves by puncturing using their ovipositor on which they feed from leaf exudates and insert eggs. Males are unable to puncture leaves but feed from punctures produced by females. The punctures may act as pathway for diseases vectors such as *Alternaria alternata* (Fr.) Keissl (Pleosporales: Pleosporaceae) (Zitter & Tsai 1977, Parrella et al. 1984, Matteoni & Broadbent 1988, Deadman et al. 2002, Bjorksten et al. 2005). Larval mining of leaves is the most destructive feeding behavior and may lead to leaf fall in severe infestation, delay in plant development and yield loss (Johnson et al. 1983, EPPO 2013). The larval stage is also difficult to control due to its concealed nature in plant tissues. *Liriomyza* species are categorization as quarantine pest (EC 2000, IPPC 2005, Anderson & Hofsvang 2010, EPPO 2013, EUROPHYT 2015) posing a trade barrier in fresh horticultural products.

In Kenya, *Liriomyza* species attack a variety of horticultural commercial value crops including snow pea (*Pisum sativum* L.), French bean (*Phaseolus vulgaris* L.), runner bean (*Phaseolus coccineus*), tomato (*Lycospersicon esculentum* Miller), potato (*Solanum tuberosum*), *Eryngium* sp., *Gypsophila* sp. and *Carthamus* sp. cut flowers (Chabi-Olaye et al. 2008, KEPHIS 2014, Foba et al. 2015b, Guantai et al. 2015). Horticulture is an important sector of agriculture providing employment to millions of people and generating foreign currency (McCulloch & Ota 2002, NHP 2012, KHC 2015). It contributes on average 25.3% of Kenya's Gross Domestic Product (GDP and earned the country $ 2 billion in 2013 (KNBS 2015, KFC 2014).

Several pesticide including organophosphates, carbamates, pyrethroids and triazines (cryomazine) have been identified and adversely used for LMF control which if used exclusively could quickly generate resistance (Weintraub & Horowitz 1997, Price & Nagle 2002, Guantai et al. 2015). The indiscriminate use of insecticides is likely to be one of the reasons for the leafminer outbreaks in their invaded ranges with negative effects to their natural enemies (Murphy & LaSalle 1999, Gitonga et al. 2010). In recent years, pesticide residues above acceptable levels have become a technical trade to barrier in horticultural products in the European market (RASFF 2014).

In their native ranges, natural enemies are important in regulating *Liriomyza* species populations (Shepard et al. 1998, Murphy & LaSalle 1999, Rauf et al. 2000, Mujica & Kroshel 2011). More than 300 species of parasitoids are associated with leafminers worldwide (Noyes 2003). In Peru, a complex of 63 parasitoid species is associated with *Liriomyza* leafminers causing high leafminer mortality of between 20 and 55% (Mujica & Kroschel 2011). In contrast, the diversity of existing *Liriomyza* parasitoids in East Africa field crops is low with parasitism rates below 6% (Chabi-Olaye et al. 2008). Parasitoids associated with *Liriomyza* leafminers mainly comprises of *Diglyphus isaea* (Walker), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae) and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae), (Chabi-Olaye et al. 2008, Guantai et al. 2015, Foba et al. 2015c).

In view of improving biological control of *Liriomyza* leafminers and further boosting the parasitism rates in East Africa, *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) was imported from Peru into Kenya by the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, in collaboration with the International Potato Centre (CIP), under Leafminer IPM program.

Halticoptera arduine is a larval endoparasitoid of Agromyzid leafminers which completes its development within the host. After parasitization, the host larva continues to develop into pupal stage until the adult parasitoid emerges from the host pupal case. Shortly after emergence, adult female parasitoids begin searching for leafminer larvae on leaf surfaces and on encounter, deposits up to three eggs in one host but only one offspring develops per host (Arellano & Redolfi 1989). Sex ratio of *H. arduine* is affected by host density and fertilization of females where unfertilized females produce only males (Kroschel et al. 2016). Low (< 10^{o}C) and high (> 30^{o}C) temperatures also affect the reproduction and development of *H. arduine* (Prudencio 2010). However, not much on *H. arduine* biology including its searching behavior, host specificity studies or its use in classical biological control programs has been reported. In its native origins, *H. arduine* is adapted to a wide range of ecological areas efficiently parasitizing up to seven species of Agromyzid leafminer fly species in a range of twenty five host plants causing up to 67% parasitism rates (San- chez & Redolfi 1989, Neder de Roman 2000, Mujica & Kroschel 2011, Kroschel et al. 2016).

The potential performance of the exotic parasitoid in controlling local *Liriomyza* species needed an evaluation before its consideration for a classical biological control program. This acceptability research will address the questions as to whether *H. arduine* will search and accept to oviposit in three local *Liriomyza* host species. The suitability studies will provide answers to the question on whether eggs will complete development within the hosts, parasitoid performance and their fitness in these three host species. The objective of this study was therefore to evaluate the acceptability and suitability of *H. arduine* to three *Liriomyza* species found in Kenya before its consideration for introduction as a biological control agent.

2.3. Materials and methods

2.3.1.Plant materials

Fourteen days-old potted plants of faba bean, *Vicia faba* L. and rose coco beans, *Phaseolus vulgaris* L. (Fabales: Fabaceae) were raised and supplied from screen house at the International Center of Insect Physiology and Ecology (*icipe*) Duduville campus in Kenya in conical plastic pots (5.5-cm diameter and 7.3-cm height) with five plants per pot.

2.3.2. Insect colonies

2.3.2.1. Leafminer colonies

Colonies of *L. huidobrensis* were initiated from field collections in Nyeri (0^{0}21'S, 36^{0}57'E, 2200 m.a.s.l) in Central Kenya highlands of Nyeri County and reared on *V. faba. Vicia faba* was chosen because it had been found as the best host plant for laboratory rearing and maintenance of *L. huidobrensis* (Videla & Valladares et al. 2006, Chabi-Olaye, Mwikya et al. 2013).

Liriomyza sativae and *L. trifolii* colonies were initiated from field collections in Kibwezi (02°15′S 37°49′E, 965 m.a.s.l), Makindu (02°16′S 37°48′E, 991 m.a.s.l) and Masongaleni (02°22′S 38°08′E, 714 m.a.s.l) in the Eastern low-lying counties of Kenya

(Chabi-Olaye et al., 2013) and reared on rose coco beans. Musundire et al. (2012a) and Okoth (2011) reported that *L. sativae and L. trifolii* preferred *P. vulgaris* compared to other tested host plants for oviposition and development and this led to the use of *P. vulgaris* as experimental host plant. The colonies were reared in Perspex cages (60 cm length x 60 width x 60 cm) (made at *icipe*, Nairobi, Kenya) under species specific controlled temperatures and humidity that were optimum for their development (Okoth 2011, Musundire et al. 2012a, Foba et al 2015a) (25 ± 2°C and 55 ± 5% RH for *L. huidobrensis* and 27± 2°C and 55 ± 5% RH for *L. sativae* and *L. trifolii*). Adult leafminers were fed on 10% sugar solution for two days after emergence before exposure to host plants for experimental use.

2.3.2.2. *Halticoptera arduine* colony

Initial culture of *H. arduine*, a solitary endoparasitoid of *Liriomyza* leafminers, was obtained from the International Potato Center (CIP) in Peru, where they were maintained on *L. huidobrensis*. The parasitoid was maintained in the quarantine facility at *icipe* on *L. huidobrensis* at 25^0C ±1 and 55-60% RH for twelve generations after establishment before its experimental use. Newly emerged parasitoids were maintained on honey solution as source of food for two days before their exposure to the host insect larvae.

2.3.3. Experimental procedure

The first set of acceptability experiments were conducted separately from the second set of suitability experiments which were sequentially conducted after the acceptability experiments. The acceptability experiment study was conducted using methodology described by Chabi-Olaye et al. (2013) in the assessment of *P. scabriventris* acceptability to three *Liriomyza* species. Procedures described by Chabi-Olaye et al. (2013) were used for suitability studies with slight modifications. Chabi-Olaye et al. (2013) used excised plants with only two infested leaves and immersed in water in 10-ml glass vial and 10 female parasitoids per replicate. In the present study however, 50 infested whole potted plants (10 pots x 5 plants/pot) were held per Perspex cage and 50-two day-old *H. arduine* adults (1 male: 2 females) were released per replicate.

In host acceptance experiments, faba bean plants were exposed to two day-old adults of *L. huidobrensis* for 24-h for egg laying and maintained in a cage for five to six days to get a cohort of same age larvae. A two-leaved faba bean stem infested by between 10-15 2nd-3rd larval stages of *L. huidobrensis* was excised above the soil base, and inserted into a glass vial (30 ml) in upright position supported by moist cotton wool. The set up was placed in clear Perspex cage (15 x 15 x 20 cm) with the top and sides covered by fine insect netting (150 x 150 µm) for aeration in controlled environment (25°C ±1 and 55-60% RH) using a thermostatic electric heater (Xpelair, UK) and humidifier (Fig. 2.1).

Fig. 2. 1. (a) b) c)

a-c. Right to left: a) experimental set up of a two-leaved Rose coco *Liriomyza* infested host plant held in a 10- ml glass vial of water in a perspex cage in readiness for introduction of a parasitoid in acceptability observations, b) A closeup of the introduced parasitoid searching for oviposition sites on a *L. huidobrensis* infested faba bean plant, and c) a close up of the introduced parasitoid making oviposition attempts in a host larvae beneath a faba infested leaf.

A two day-old naive mated female adult of *H. arduine* was introduced into the cage. The behavioral activities of the parasitoid on the infested plant (time spent on host searching and encounter and number of oviposition attempts) were directly recorded by visual observationfor a 2-h period per replicate.

After the 2-h period, the female parasitoid was removed and the larvae incubated (25°C ±1 and 55-60% RH) in plastic Petri dish for three days to allow for pupae development. After six days, each individual pupa was incubated (25°C ±1 and 55-60% RH) in gelatin capsule (2.20 cm height and 0.7 cm diameter) for eight to sixteen days until adult leafminer or parasitoid emergence. Under these experimental conditions, average developmental times were 14 and 25 days for leafminer and parasitoid respectively. The number of female parasitoids with successful oviposition in each host was confirmed by emergence or recovery of a parasitoid. The experiment was replicated 40 times and the same experimental set up was repeated using *L. sativae* and *L. trifolii* on *P. vulgaris*.

In our second experiment, 200 two-days-old *L. huidobrensis* (at the ratio of 1 male: 2 females) were exposed to ten pots of two weeks-old faba bean plants (5 plants/pot) for 24-h in Perspex cages (30 cm x 30 cm x 45 cm) with two sides covered by fine insect netting (150 x 150 µm) for aeration. The exposure of *L. huidobrensis* was done in ten cages, five of which received the parasitoid treatment and five represented control with no parasitoids. Infested plants were held for five to six days to allow the development of same age cohort of 2nd-3rd larval stages of leafminer. A batch of 50 two-day-old adult *H. arduine* parasitoids (at the ratio of 1 male: 2 females) were released in the five Perspex cages containing the infested plants for 24-h before removal. The host larvae were held on the plants for five to six days for pupae development. Each pupa was capsulated and incubated (25°C ±1 and 55-60% RH) in transparent gelatin capsules (2.20 cm height and 0.7 cm diameter) for adult leafminers and parasitoids emergence. The control allowed for assessment of leafminer natural mortality (Fig. 2.2).

a) b) c)

Fig. 2.2. Process of suitability experiment. a-c: Right to left: a) host plants (Rose coco) infested by *Liriomyza* in a Perspex cage in readiness for parasitoids release, b) A close up of *Liriomyza* infested rose coco leaves with active mines ready for parasitoid release, d) Host pupae being capsulated in gelatin capsules for incubation.

The same methodology was repeated for *L. sativae* and *L. trifolii* hosts reared on *P. vulgaris* at different times. This experiment thus, consisted of three treatments and each of the treatment represented a *Liriomyza* host species replicated five times alongside with a control in a completely randomized design (CRD).

In both experiments, the number of pupae, emerged leafminer adults and first generation parasitoids (F1 offspring), F1 parasitoids developmental period and sex ratio were recorded. Pupae without exit holes and with unemerged insects were dissected under Leica EZ4D binocular microscope (Leica Microsystems Switzerland Ltd 2007; Glattbrugg, Switzerland; LAS EZ V 1.5.0 software (LEITZ, Glattbrugg, Switzerland)) following the methodology described by Heinz & Parrella (1990) to correct parasitism rate and non-reproductive host mortality. Non-reproductive host mortality was expressed as a percentage of unviable pupae over the total pupae in each treatment as described by Foba et al. (2015a).

The right forewing and right hind tibia of ten randomly selected male and female F1 parasitoids were detached from the point of contact with thorax and images taken using Leica EZ4D microscope camera (Leica Microsystems Switzerland Ltd 2007; Glattbrugg, Switzerland; LAS EZ V 1.5.0 software (LEITZ, Glattbrugg, Switzerland). Wings and hind tibia were spread in 70% ethanol and the lengths measured at X35 magnification (Heinz & Parrella 1990, Honek 1993, Videla et al. 2006, Okoth et al. 2014).

2.3.4. Data analyses

For each *Liriomyza* species, absolute numbers of F1 progeny males and females parasitoids and dead pupae were analyzed using Chi-square test in R version 3.0.2 statistical software (R Development Core Team 2013) to determine differences in sex ratio and significance level of non-reproductive mortalities. Count data on searching time, oviposition attempts, developmental time and number of parasitoids in a progeny and percentage data on parasitism rates, sex ratios and mortalities were log and arcsine transformed respectively before being subjected to one-way Analysis of variance (ANOVA). Where there was significant difference between *Liriomyza* species in regards to time taken for a female parasitoid to first encounter host, oviposition attempts made on larval hosts in a 2-h observation period, proportion of female parasitoids that successfully oviposited in the hosts, number of parasitoids in F1 progeny from each host, developmental time of F1 parasitoids, parasitism rates, pupal mortality and body size indices, means were separated using Tukey-Kramer HSD (honest significant difference) test ($P<0.05$) (SAS 2013, JMP V11, 2013).

2.4. Results

2.4.1. Host acceptance

Results of *H. arduine* acceptability to *Liriomyza* host species after 2-h are presented in Table 2.1. The parasitoid accepted and successfully deposited eggs in the three *Liriomyza* species, with up to 97.50 ± 2.50 (means ± SE) % of females laying eggs in *L. sativae* and *L. trifolii*. The number of oviposition attempts per female within 2-h observation period were also high, ranging from 57.30 ± 2.06 to 66.20 ± 3.48, with a significantly higher number of oviposition attempts on *L. huidobrensis* compared to *L. sativae* and *L. trifolii* ($F_{2,117} = 4.07$, $P = 0.0196$). Females parasitoids took a short period of time (as low as 10.45 ± 0.83 to 13.30 ± 1.37 seconds) to search and encounter their first host for oviposition with significantly shorter time ($F_{2,117} = 7.98$, $P = 0.0006$) spent on *L. trifolii* than on *L. sativae.* However, searching and encountering time on *L. huidobrensis* was not significantly different from that observed on *L. sativae* and *L. trifolii.* Significantly more females parasitoids successfully oviposited in *L. sativae* and *L. trifolii* than in *L. huidobrensis* ($F_{2,117} = 14.91$, $P < 0.0001$) (Table 2.1).

2.4.2. Host suitability

The three *Liriomyza* hosts tested were found suitable for *H. arduine*. The parasitoid took between 19.32 ± 0.96 and 22.86 ± 0.27 days to complete development from egg to adult in the three hosts. *Halticoptera arduine* parasitized significantly more *L. huidobrensis* than *L. sativae* ($F_{2,12} = 4.05$, $P = 0.0452$), however the parasitism in *L. trifolii* was similar to the two other hosts (Table 2.2). *Liriomyza* hosts affected sex ratio of *H. arduine* F1 progeny with a significant female biased sex ratio when reared on *L. huidobrensis* ($\chi^2 = 18.84$, $P < 0.0001$) compared to a balanced sex ratio when reared on *L. sativae* ($\chi^2 = 0.00$, $P = 1.00$) and a male biased when reared on *L. trifolii* ($\chi^2 = 18.84$, $P < 0.0001$). Across the treatments, the proportion of female parasitoids' in F1 were not significantly different ($F_{2,79} = 4.67$, $P = 0.4798$) (Table 2.2).

Table 2.1. Acceptability parameters of three *Liriomyza* species to *Halticoptera arduine* (Mean ± SE) under laboratory conditions (25°C ±1 and 55-60% RH)

Variable indicator	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
Time taken (s) to search and encounter first host	13.30 ± 1.37ab	15.80 ± 0.91a	10.45 ± 0.83b
Mean number of oviposition attempts per female parasitoid	66.20 ± 3.48a	57.38 ± 1.73b	57.30 ± 2.06b
Proportion of female parasitoids with successful oviposition (%)	65.00 ± 7.64b	97.50 ± 2.50a	97.50 ± 2.50a

(s)- second, Within row, means followed by the same letter are not significantly different at P ≤ 0.05 (Tukey-Kramer test).

Table 2.2. Host suitability - effect of *Liriomyza* host species on *Halticoptera arduine* developmental time, parasitism rate and sex ratio (Mean ± SE) under laboratory conditions (25°C ±1 and 55-60% RH)

Variable indicator	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
F1 developmental time (d)	19.32 ± 0.96c	21.23 ± 0.16b	22.86 ± 0.27a
Parasitism rate (%)	44.10 ± 4.56a	27.96 ± 3.86b	32.28 ± 3.65ab
Proportion of female parasitoids in F1 progeny (%)	57.94 ± 9.32aA	49.32 ± 5.59aA	45.31 ± 6.43aB
Proportion of male parasitoids in F1 progeny (%)	42.03 ± 9.32aB	50.68 ± 5.59aA	54.69 ± 6.43aA
Mean number of parasitoids in F1 progeny	33.0 ± 5.11b	61.80 ± 2.15a	61.00 ± 4.83a

(d)-days, within row and for the same variable, means followed by the same lower case letter are not significantly different at P ≤ 0.05 (Tukey-Kramer test). For each *Liriomyza* species, means followed by same upper-case letter for male and female are not significantly different at P ≤ 0.05 (Chi-square test).

In the three *Liriomyza* hosts studied, *H. arduine* did not induce any significantly different non-reproductive host mortality in three hosts from the control (P_{Lh} = 0.0639, P_{Ls} = 0.2345, and P_{Lt} = 0.6155) (Table 2.3).

Table 2.3. Host suitability - non-reproductive host mortality by *Halticoptera arduine* in three *Liriomyza* host species (Means ± SE) under laboratory conditions (25°C ±1 and 55-60% RH)

	Liriomyza huidobrensis	*Liriomyza sativae*	*Liriomyza trifolii*
Host mortality in presence of parasitoids (%)	50.55 ± 4.58Aa	37.32 ± 2.97Aa	40.81 ± 2.36Aa
Natural host mortality in control (%)	33.32 ± 2.99Aa	41.77 ± 2.17Aa	39.40 ± 1.98Aa
χ^2	3.43	1.41	0.25
P value	0.0639	0.2345	0.6155

Within rows (columns), means followed by the same lower (upper) case letter are not significantly different at P≤0.05 (Tukey-Kramer) and (Chi-square) test in that respect.

Parasitoid fitness on the various *Liriomyza* hosts in F1 offspring are presented in Table 2.4. The forewing of *H. arduine* measured between 1.27 ± 0.04 and 1.43 ± 0.10 mm for the females and 1.19 ± 0.03 to 1.34 ± 0.02 mm for the males. The hind tibia length of the female parasitoids measured between 0.35 ± 0.01 and 0.40 ± 0.01 while that in the males measured between 0.34 ± 0.01 and 0.41 ± 0.02. Female parasitoids reared on *L. huidobrensis* had significantly longer forewing than those reared on *L. trifolii* while those of *L. sativae* did not significantly differ from either of the two hosts ($F_{2,27}$ = 4.59, P = 0.0192). Similarly, female parasitoids reared on *L. huidobrensis* had significantly longer hind tibia than those reared on *L. sativae* and *L. trifolii* ($F_{2,27}$ = 5.37, P = 0.0109) (Table 2.4). On the other hand, male parasitoids reared on *L. trifolii* and *L. huidobrensis*, had significantly longer forewing than those reared on *L. sativae* ($F_{2,27}$ = 7.94, P = 0.0019) while their hind tibia were significantly longer for those reared on *L. trifolii* than from *L. sativae.* Hind tibia from *L. huidobrensis* were not significantly different from the two former hosts ($F_{2,27}$ = 7.14, P = 0.0032). There was no significance difference in parasitoid wing and tibia lengths between male and females within host (*L. huidobrensis*: χ^2 = 0.01, P = 0.9137 and χ^2 = 0, P = 1; *L. sativae*: χ^2 = 0.05, P = 0.8316 and χ^2 = 0, P = 1; *L. trifolii*: χ^2 = 0.0.01, P = 0.9294 and χ^2 = 0, P = 1 respectively) (Table 2.4).

Table 2.4. Host suitability - effect of *Liriomyza* host species on adult *Halticoptera arduine* fitness (Means ± SE) under laboratory conditions (25°C ±1 and 55-60% RH)

Parasitoid body size indices	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
Forewing length (mm)			
Females	1.43 ± 0.03aA	1.37 ± 0.04abA	1.27 ± 0.04bA
Males	1.29 ± 0.03aA	1.19 ± 0.03bA	1.34 ± 0.02aA
χ^2	0.0118	0.0452	0.0079
P value	0.9137	0.8316	0.9294
Hind tibia length (mm)			
Females	0.40 ± 0.01aA	0.36 ± 0.01bA	0.35 ± 0.01bA
Males	0.37 ± 0.01abA	0.34 ± 0.01bA	0.41 ± 0.02aA
χ^2	0	0	0
P value	1	1	1

Within rows (columns and for each parameter), means followed by the same lower (upper) case letter are not significantly different at P≤0.05 according to Tukey-Kramer (Chi-square) test.

2.5. Discussion

Parasitoids spend a significant amount of time searching for hosts, while a significant amount of time is spent probing the host to assess their suitability before making the decision to oviposit. The success of parasitoid-host relationship assumes a hierarchy of discrete steps that include habitat location, host location, host acceptance, host suitability (Doutt 1959, Vinson 1976) and host regulation (Vinson & Iwantsch 1980). Vinson (1975) considered host acceptance as the most important step for parasitoid success. *Halticoptera arduine*, the exotic endoparasitoid of LMF accepted to oviposit and developed successfully in the three common *Liriomyza* hosts found in Kenya. Previous studies on the searching behavior of *H. arduine* showed that females encountered *L. huidobrensis* larvae immediately on their introduction to *V. faba* infested plants (Prudencio 2010). This concurs with our observations on the three *Liriomyza* hosts. Plant physical and chemical characteristics have an effect on searching time, movement and foraging success of parasitoids (Andow & Prokrym 1990, Lukianchuk & Smith 1997, Lovinger et al. 2000, Suverkropp et al. 2001, Wang & Keller 2002). However, *H. arduine* searching behavior seemed not to be affected by host plant. Searching time on *L. huidobrensis* reared on *V. faba* was not different from the one on *L. sativae* and *L. trifolii*, both reared on *P. vulgaris*. The significant difference was rather between *L. sativae* and *L. trifolii* despite both being reared on the same plant. We therefore, hypothesize that pest-host interaction could have influenced the searching behavior of *H. arduine* and thus recommend further studies to elucidate potential difference in chemical profile resulting from infestation of *P. vulgaris* by *L. trifolii*.

Oviposition attempt by female parasitoids is considered as an important behavioral measurement for parasitoid host acceptance (Agboka et al. 2002). Considering the number of oviposition attempts made by *H. arduine* female on each of the *Liriomyza* hosts as selection decision for oviposition, it is evident that *L. huidobrensis* was a better choice for oviposition than *L. sativae* and *L. trifolii,* which also translated to a high parasitism rate on this host.

Among the factors which influence the success of parasitoid development is the stage or instar at parasitization (Hegazi & Khafagi 2005). *Halticoptera arduine* prefers late 2nd and early 3rd larval instars of *Liriomyza* hosts for oviposition and parasitization (Arellano & Redofi 1989) during which development to adult takes place. Results from this study showed that *H. arduine* completed its development within the three *Liriomyza* host species and our results were similar to that reported by Prudencio (2010) when reared on *L. huidobrensis.* This implies that *H. arduine* will successfully develop in the existing *Liriomyza* species occurring in Kenya’s agro-ecological systems and contribute to their natural control. However further studies are required to assess its potential competition, complementarities or coexistence with indigenous parasitoids already present in the system. Various indigenous and exotic leafminer parasitoids have been reported in Kenya and this include mainly *D. isaea*, *O. dissitus* and *P. scabriventris* which represent 95.18% of total parasitoids recorded at low, mid and high altitudes of Kenya (Foba et al. 2015c).

Male parasitoids only mate and do not contribute to pest mortality (Hassel et al. 1983; Comins & Welling 1985). Male biased populations of *H. arduine* have been reported by Arellano & Redolfi (1989), Neder de Român (2000) and Prudencio (2010) when *H. arduine* was reared on low host population of *L. huidobrensis* and from lower and higher temperature developmental thresholds. The reverse was true when *H. arduine* was reared in high population of *L. huidobrensis*. (Neder de Român, 2000). In contrast to these findings, our study revealed a female-biased and balanced sex ratio in *L. huidobrensis* and *L. sativae* hosts respectively. 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, Pascua & Pascua 2004, Chow & Heinz 2005, Abe & Kamimura 2012, Foba et al. 2015a). Our results therefore suggest better reproduction potential for *H. arduine* in regards to the local populations of *L. huidobrensis*, *L. sativae* and *L. trifolii* in Kenya.

With insects, there is generally a positive relationship between body size and performance (Clutton-Brock 1988, Honek 1993) and fitness (Stoepler et al. 2011). The success of parasitoid to develop and complete its life cycle in a suitable host is influenced by among other factors, host size (Lawrence 1990) which positively correlates with adult parasitoid size (Spencer 1990, Visser 1994, Lampson et al. 1996, Fidgen et al. 2000, Roitberg et al. 2001, Teder & Tammaru 2002). Additionally, performance of Agromyzid leafminer parasitoids depends on the body size of their hosts (Heinz & Parrella 1990, Spencer 1990, Salvo & Valladares, 1995, Ode & Heinz, 2002).

However, host size is not necessarily the only indicator of parasitoid performance and other factors such as host plants can influence parasitoid performance (Salvo & Valladares 2002, Tran et al. 2007, Musundire et al. 2010). In our present studies, *H. arduine* female progeny reared on *L. huidobrensis* were larger than those reared on *L.sativae* and *L. trifolii*. In similar studies, *Neochrysocahris okazakii* Kamijo (Hymenoptera: Eulophidae) reared on *Liriomyza chinensis* (Kato) were larger than those reared on *L. trifolii* (Tran & Takagi 2005a, Tran et al. 2007). Similarly *P. scabriventris* reared on *L. huidobrensis* were found to be larger than those reared on *L. sativae* and *L. trifolii* (Chabi-Olaye et al. 2013). It is assumed that larger hosts represent greater resource quantity for parasitoid development (Charnov 1982, Charnov & Skinner 1985) and performance (fecundity) (Musundire et al. 2012a) with greater longevity, better searching capacity and higher dispersal ability (Godfray 1994, Visser 1994). Studies by Spencer (1973) and Musundire et al. (2012a) have confirmed that *L. huidobrensis* is larger in body size than *L. sativae* and *L. trifolii*. This suggests that the body size of the host affected the fitness of the parasitoid in the present study.

Non-reproductive host mortality caused by parasitoids has been reported to constitute an important component in pest suppression in many studies (Sandlan 1979, Walter 1988, Tran & Takagi 2006, Bernardo et al. 2006, Akutse et al. 2015). However, this is more commonly reported amongst ectoparasitoids than in endoparasitoids. For example, Mafi and Ohbayashi (2010) observed that *Sympiesis striatipes* Ashmead (Hymenoptera: Eulophidae) an ectoparasitoid, caused non-reproductive mortality of 44.7 ± 4.2% on citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Similarly, female wasps of *D. isaea* were reported to cause significant non-reproductive mortality of *Liriomyza* larvae by host feeding and stinging without oviposition (Minkenberg 1989, Liu et al. 2013, Akutse et al. 2015). *Copidosoma koehleri* (Hymenoptera: Encyrtidae), an egg-larval endoparasitoid of potato tuber moth *Phthorimaea operculella* is one of the few exceptions of endoparasitoids reported to cause non-reproductive mortality (Keinan et al. 2012). In the present study, no significant non-reproductive host mortalities were recorded in *H. arduine* in any of the three host's studies. These results concur with the findings of Prudencio (2010) who reported non-reproductive mortalities as low as 7% in *H. arduine* when reared on *L. huidobrensis*. Similar findings of such poor endoparasitoid induced non-reproductive host mortality in LMF were reported by Chabi-Olaye et al. (2013) and Foba et al. (2015a) while studying the performance of *P. scabriventris* against *L. huidobrensis*.

2.6. Conclusion

This study has established that *H. arduine* can accept to oviposit and develop successfully in the three most economically important *Liriomyza* leafminers in East Africa and was not affected by host plant. The performance of the parasitoid should also be mainly based on its parasitism potential since it caused no significant nonreproductive mortality in the hosts. Parasitism rates,fitness, and sex-ratio parameters of the parasitoid were all promising. Considering that the three LMF species studied are the most important across Africa, we hypothesize that *H. arduine* can successfully establish and contribute to the control of *Liriomyza* leafminers in Africa in general and particularly in Kenya. Pilot sites and open-field release activities are warranted to test and confirm this hypothesis. However, prior to this, to ensure that local parasitoid populations are not displaced by the exotic species, interaction studies between *H. arduine* and the parasitoids already existing in the system are warranted.

3.0. Acceptability and suitability of three *Liriomyza* Leafminer species as host for the endoparasitoid *Chrysocharis flacilla* (Hymenoptera: Eulophidae)

This chapter has been published as Muchemi et al. *Journal of Economic Entomology*, 111 (3), 2018, 1137-1143, doi: 10.1093/jee/toy088.

3.1. Abstract

Liriomyza leafminers represent important threats to the horticulture sector in East Africa. Parasitism rates of local parasitoids are reported to be low and the endoparasitoid, *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae), was introduced in Kenya for a classical biological control program. Acceptability and suitability bioassays were conducted on the three economically important *Liriomyza* species in Africa (*Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae), and *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). Foraging behavior, developmental time, sex ratio, parasitism rates, host pupal mortality, and body indices of *C. flacilla* were assessed. Results showed that the three LMF species were accepted and suitable to *C. flacilla*. Foraging time was significantly shorter on *L. trifolii* than on *L. sativae* and *L. huidobrensis*. Ninety-eight per cent of females successfully oviposited in the three-host species. Female parasitoids were significantly aggressive in attempting to oviposit on *L. huidobrensis* than on *L. sativae* and *L. trifolii*. High parasitism rates ranging between 73 and 78% were observed from the three *Liriomyza* hosts, but no significant difference among hosts. *C. flacilla*-induced significant nonreproductive pupal mortality ranging from 23 to 35%, an attribute rare among endoparasitoids. In all three *Liriomyza* hosts, the parasitoid progeny was female biased. Parasitoid development period ranged between 16 and 24 d. Female parasitoids reared on *L. huidobrensis* were significantly bigger than those reared on *L. sativae* and *L. trifolii*. The acceptance to local *Liriomyza* leafminers and high host suppression ability is potential for considerations of *C. flacilla* in the management of *Liriomyza* spp. in Africa.

3.2. Introduction

Horticulture is an important sector of Kenya’s economy and the East African Region in general. In Kenya, the sector contributed 27.3% to the annual Gross Domestic Product in 2014 (KNBS 2016). Pest infestation including that of the introduced invasive *Liriomyza* leafminers is however one of the major constraints to production, and farmers rely on routine application of insecticides to manage these pests (Price & Nagle 2002, Gitonga et al. 2010). In addition, pesticide effectiveness has been reduced due to development of resistance to several groups of insecticide molecules by the pest rendering the management of *Liriomyza* leafminers ineffective. Natural enemies associated with *Liriomyza* leafminers have not been spared and their ability to regulate them has been reduced (Tran & Takagi 2005b, Liu et al. 2009, Gitonga et al. 2010, Guantai et al. 2015). Reports from various parts of the world have indicated that commonly used pesticides for the management of *Liriomyza* leafminers including the active ingredients of, Abamectin, Alpha-cypermethrin, Betacyfluthrin, Chlorpyrifos, Cymoxanil, Cypermethrin, Dimethoate, Imidacloprid, Tebuconazole, Thiosultap disodium, and Propineb are known to affect parasitoids of leafminers including *Opius* sp., *Phaedrotoma scabriventris* (Nixon) (Hym: Braconidae), *Gronotoma micromorpha* (Perkins) (Hym: Figitidae), *Hemiptarsenus varicornis* (Girault) (Hym: Eulophidae), and *Diglyphus isaea* (Walker) (Hym: Eulophidae) (Prijono et al*.* 2004, Bjorksten & Robinson 2005, Guantai et al. 2015). The fresh horticultural export market and smallholder farmers engaged in vegetable production, have immensely been impacted by the presence of pesticide residues. For instance, Kenyan beans and peas are hosts of *Liriomyza* leafminers and receive heavy pesticide applications (Okello & Swinton 2007, Gitonga et al*.* 2010, Foba et al*.* 2015a, Guantai et al. 2015). Consequently, between 2012 and 2015, these two horticultural products were intercepted more than 20 times in the European Union markets due to pesticide residues above the allowed limits (RASFF 2015, KEPHIS 2016). The need for alternative and sustainable pest management options for pests including leafminers is therefore of high importance for continued trade in vegetable production.

In Kenya, a field survey conducted in 2007 and 2012 showed that diversity of locally existing parasitoids associated with *Liriomyza* leafminers is low comprising mainly of *D. isaea* (Walker), *Neochrysocharis formosa* (Westwood), *H. varicornis* (Girault) (Hymenoptera: Eulophidae), and *Opius dissitus* (Muesebeck) (Hymenoptera: Braconidae) (Chabi-Olaye et al*.* 2008, Foba et al*.* 2015c). These parasitoids caused <6% parasitism rates of leafminers in 2007. However, in the native origin of *Liriomyza* species, natural enemies are important in regulating *Liriomyza* species populations (Shepard et al*.* 1998, Murphy & LaSalle 1999, Rauf et al*.* 2000, Mujica & Kroschel 2011). For instance, in the Neotropics of South America in Peru, a complex of 63 parasitoid species is associated with *Liriomyza* leafminers (Mujica & Kroschel 2011). These parasitoids cause high *Liriomyza* leafminers mortality of between 20 and 55% (Mujica & Kroschel 2011). Worldwide, more than 300 species of parasitoids are associated with leafminers (Noyes 2004).

In view of a leafminer Integrated Pest Management (IPM) program in East Africa, the International Center of Insect Physiology and Ecology (*icipe*) imported *Chrysocharis flacilla* from Peru into *icipe's* quarantine facilities. *C. flacilla* was identified as one of the most important parasitoid in its native origin of Peru with great potential in suppressing *Liriomyza* leafminers (Salvo et al*.* 2005, Mujica & Kroschel 2011). *C. flacilla* is reported to have a high degree of polyphagy infesting more than 33 host species in the family Agromyzid (Salvo & Valladares 1997). It is a larval endoparasitoid attacking the late second and early third larval stage of its host causing field parasitism rates of up to 40% (Mujica & Kroschel 2011). Adult *C. flacilla* has been recovered from host pupae 9 months after host parasitization indicating characteristics of diapause from a wide range of altitudinal gradients in coastland to the highlands (Redolfi de Huiza et al*.* 1985, Salvo 1996, Mujica & Kroschel 2011). Host finding factors employed by parasitoids include visual cues from plants, structure and habitat, and size of leafminer larvae (Godfray 1994, Finidori-Logli et al*.* 1996).Volatiles released by feeding leafminer larvae or damaged plants by adult leafminers may lead foraging parasitoids to host plant habits and their host pests (Dicke & Minkenberg 1991). However, the biology of *C. flacilla* has not yet been fully studied. The introduction of an exotic biological control agent, *C. flacilla* into the agricultural systems, required an evaluation to assess its acceptability and suitability to the local *Liriomyza* species. The objective of our study was therefore to evaluate the acceptability and suitability of *C. flacilla* to three economically important

Liriomyza species in Kenya; *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard, and *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) under laboratory conditions for its consideration as a potential agent in classical biological control program.

3.3. Materials and Methods

3.3.1. Plant Materials

Fourteen-day-old potted faba bean, *Vicia faba* (L.) (Fabales: Fabaceae) and Rose coco bean, *Phaseolus vulgaris* (L.) (Fabales: Fabaceae) were raised in conical plastic pots (5.5 cm in diameter and 7.3 cm in height) in the screen house of *icipe* Duduville campus, Nairobi, Kenya. Five plants per pot were supplied for leafminer exposure from which infested plants were derived for experimental use.

3.3.2. Insect Colonies

3.3.2.1. Leafminer colonies

Species of *Liriomyza* were morphologically confirmed using taxonomic manuals (IPPC 2016) and reference samples from the National Museums of Kenya. Voucher specimens of adult *Liriomyza* leafminers are preserved in 70% ethanol and 10% glycerin at the Biosystematic Unit of *icipe*. Colonies of *L. huidobrensis* were initiated from field collections in Nyeri in Central Kenya highlands of Nyeri County (0°21′S 36°57′E, 2,200 m.a.s.l) and reared on faba bean. *L. sativae* and *L. trifolii* colonies were initiated from field collections in the low lands of Kibwezi (02°15′S 37°49′E, 965 m.a.s.l), Makindu (02°16′S 37°48′E, 991 m.a.s.l), and Masongaleni (02°22′S 38°08′E, 714 m.a.s.l) in the Eastern low altitude Counties of Kenya (Chabi-Olaye et al. 2013) and reared on Rose coco bean. Faba bean has been reported as the most suitable host for *L. huidobrensis* rearing and maintenance (Videla et al. 2006, Chabi-Olaye et al. 2013, Okoth et al. 2014) while Rose coco bean was found as the most preferred host for *L. sativae* and *L. trifolii* oviposition and development (Okoth 2011, Musundire et al. 2012a). Insect colonies were reared in Perspex cages (60 cm length × 60 cm width × 60 cm height) under controlled temperatures and humidity (25 ± 2°C and 55 ± 5% RH for *L. huidobrensis* and 27 ± 2°C and 55 ± 5% RH for *L. sativae* and *L. trifolii*) using a humidifier and thermostatic heater (Xpelair, United Kingdom). Adult leafminers were fed on 10% sugar solution as source of food for two days for egg maturation and mating after emergence before exposure to host plants for experimental use.

3.3.2.2. *Chrysocharis flacilla* colony

The initial culture of *C. flacilla*, a solitary larval endoparasitoid of *Liriomyza* leafminers was obtained from the International Potato Center (CIP), Peru in 2012, where they were maintained on *L. huidobrensis*. At *icipe*, the parasitoid was maintained in the quarantine facility on *L. huidobrensis* at 21 ± 1°C and 55 ± 5% RH using a humidifier and thermostatic heater (Xpelair) for six generations before its experimental use. Newly emerged parasitoids were maintained on honey solution as source of food for 2 d for egg maturation and mating before their exposure to the host insect larvae. Voucher specimens are stored at *icipe*.

3.3.3. Experimental procedure

This study was conducted in two sets of experiments with the first set of acceptability experiments conducted separately from the second set of suitability experiments in controlled laboratory conditions of 21 ± 1°C, 55 ± 5% RH and 12L:12D photoperiod. The acceptability experiment study was conducted using methodology described by Chabi-Olaye et al. (2013) in the assessment of *P. scabriventris* acceptability to three *Liriomyza* species. Procedures described by Chabi-Olaye et al. (2013) were also used for suitability studies with slight modifications. Chabi-Olaye et al. (2013) used excised plants with only two infested leaves and immersed in water in 10-ml glass vial and 10 female parasitoids per replicate. In the present study, however, 50 infested whole potted plants (10 pots x 5 plants/pot) were held per Perspex cage and 50 2-d-old *C. flacilla* adults (1 male: 2 females) were released per replicate.

In host acceptance experiment, 200 2-d-old adults of *L. huidobrensis* at a ratio of 1:2 (male: female) were exposed to faba bean plants for a 24-h for egg laying and maintained in a cage for 5 to 6 d to get cohort of the same age larvae. A two-leaved faba bean stem infested by 10 to 15 third larval stages of *L. huidobrensis* was excised above the soil base, inserted into a glass vial (30 ml) and with the support of a moist cotton wool, held in upright position. The set up was placed in a clear Perspex cage (15 cm x 15 cm x 20 cm) with the top and sides covered by fine insect netting (150 x 150 µm) for aeration. A 2-d-old naive mated female adult of *C. flacilla* was introduced into the cage. The behavioral activities of the parasitoid on the infested plant (searching time and oviposition attempts) were recorded over a 2-h period. Female parasitoid was removed after the 2-h period and leaves with the host larvae were incubated in a plastic Petri dish for 6 d to allow pupae development. Individual pupae were incubated in a transparent gelatin capsule (2.2 cm height and 0.7 cm diameter) for adult emergence and to investigate developmental time (21 ± 1°C, 55 ± 5% RH and 12L:12D photoperiod). The number of emerged adult leafminers and parasitoids was recorded. The proportion of female parasitoids with successful oviposition was computed as a percentage of females with confirmed emergence or recovery after dissection of a parasitoid from host pupae and divided by total females presented to each *Liriomyza* species. The same experimental set up was repeated using *L. sativae* and *L. trifolii* on Rose coco plant and replicated 40 times for each host species.

In the second experiment assessing host suitability, 200 2-d-old *L. huidobrensis* (at a ratio of 1 male: 2 females) were exposed to 10 pots of 2-wk-old faba bean plants (5 plants/pot) for 24 h. Infested plants were held in Perspex cages (30 cm x 30 cm x 45 cm) with two sides covered by fine insect netting for aeration for 5 to 6 d for the development of same age cohort of second–third larval stages of leafminer. A batch of 50 2-d-old adult *C. flacilla* parasitoids (at the ratio of 1 male: 2 females) were released in the Perspex cages containing the infested plants with second–third larvae for 24 h under controlled laboratory conditions (21 ± 1°C, 55 ± 5% RH, 12L:12D photoperiod). The parasitized larvae were held on the plants for 5 to 6 d for pupae development. Pupae were then collected using fine camel hair brush and individual pupa incubated in transparent gelatin capsules (2.2 cm height and 0.7 cm diameter) for adult leafminers and parasitoids emergence. A control treatment with no parasitoid release was set up to allow for assessment of leafminer natural mortality and induced nonreproductive host pupal mortality by *C. flacilla*. The same experimental set up was repeated for *L. sativae* and *L. trifolii* reared on Rose coco plants. This experiment was set up in a Randomized Complete Design and replicated five times.

Host pupae without exit holes or with nonemerged insect were dissected under a binocular microscope (Leica Microsystems Switzerland Ltd 2007; Glattbrugg, Switzerland; LAS EZ V 1.5.0 software (LEITZ, Glattbrugg, Switzerland)) following the methodology described by Heinz & Parrella (1990) to correct parasitism rate and nonreproductive host pupal mortality. Pupae that were found to contain any dead stage of parasitoid were used to correct the final tally of parasitized pupae. Similarly, those which contained immature stages of the host were considered in the finally tally of viable pupae. It is only those which had no content that were considered as nonviable and used accordingly in our computations. The number of pupae and the number of emerged leafminer adults and first generation (F1) parasitoids offspring were recorded. F1 offspring developmental period and sex ratio were also recorded. Pupal mortality was expressed as a percentage of unviable pupae over the total pupae in each treatment as described by Akutse et al. (2015) and Foba et al. (2015a). Parasitoid-induced nonreproductive host mortality was corrected using Abbott formula (Abbott 1925).

Differences in body size of parasitoids that emerged from the three species of leafminer were determined by measuring the right forewing and right hind tibia of 10 randomly selected male and female F1 parasitoids. The right forewing and hind tibia were used as proxy to fitness (Roitberg et al. 2001, Musundire et al. 2012a, Akutse et al. 2015, Foba et al. 2015a) with better accuracy in measurement than body length (Salvo & Valladares 2005). Legs and wings were detached from the point of contact with thorax, spread in 70% ethanol and images taken and measured at 35× magnification using Leica EZ4D microscope (Leica Microsystems Switzerland Ltd 2007; LAS EZ V 1.5.0 software (LEITZ)) following the method described by Heinz & Parrella (1990), Honek (1993), Videla et al. (2006), and Okoth et al. (2014).

3.3.4. Data Analyses

For each *Liriomyza* species, absolute numbers of F1 progeny male and female parasitoids and dead pupae were analyzed using Chi-square test in R version 3.0.2 statistical software (R Development Core Team 2013) to determine differences in sex ratio and significance level of nonreproductive mortalities. Count data on searching time, oviposition attempts, developmental time and number of parasitoids in a progeny and percentage data on parasitism rates, sex ratios, and host pupae mortalities were log and arcsine transformed, respectively, before being subjected to one-way analysis of variance. Where there was significant difference between *Liriomyza* species in regards to time taken for a female parasitoid to first encounter host, oviposition attempts made on larval hosts in a 2-h observation period, proportion of female parasitoids that successfully oviposited in the hosts, F1 progeny from each host, developmental time of

F1 parasitoids, parasitism rates, pupal mortality, and body size indices, means were separated using Tukey–Kramer HSD test ($P < 0.05$) (SAS 2013, JMP V11, 2013).

3.4. Results

3.4.1. Host acceptance

All the three *Liriomyza* species were accepted by *C. flacilla*. Time taken by *C. flacilla* females to search and encounter its host for oviposition ranged from 9.13 ±1.19 to 39.15 ± 4.01 s on average and varied on hosts. Significantly shorter time was taken on *L. trifolii* followed by *L. huidobrensis* then *L. sativae* ($F_{2,117} = 35.67$, $P < 0.0001$). The parasitoids' number of oviposition attempts within the 2-h observation ranged between 53.13 ± 2.24 and 85.13 ± 4.71, with significantly more oviposition attempts on *L. huidobrensis* than on *L. sativae* and *L. trifolii* host larvae ($F_{2,117} = 20.89$, $P < 0.0001$) (Table 3.1). Successful oviposition by parasitoid females in the three *Liriomyza* species host larvae ranged between 82.50 ± 6.08% and 97.50 ± 2.50% with no significant differences across leafminer hosts ($F_{2,117} = 2.54$, $P = 0.0829$) (Table 3.1).

Table 3.1. Acceptability behavior of *Chrysocharis flacilla* to *Liriomyza* host species (Mean ± SE) under laboratory conditions (21 ± 1°C and 55 ± 5% RH)

Variable indicator	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
Time taken (s) to search and encounter first host	24.93 ± 2.98b	39.15 ± 4.01a	9.13 ± 1.19c
Mean number of oviposition attempts per female parasitoid	85.13 ± 4.71a	64.58 ± 3.20b	53.13 ± 2.24b
Proportion of female parasitoids with successful oviposition (%)	97.50 ± 2.50a	82.50 ± 6.08a	90.00 ± 4.80a

(s)- seconds; within rows, means followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey-Kramer HSD test)

3.4.2. Host suitability

All the three *Liriomyza* species were suitable for *C. flacilla* egg to adult development. The developmental time of *C. flacilla* ranged between 15.97 ± 1.48 to 23.96 ± 0.88 d depending significantly on host species. *Chrysocharis flacilla* significantly took fewer days to complete its life cycle development in *L. sativae* than in *L. huidobrensis* and *L. trifolii* ($F_{2,106} = 9.75$, $P < 0.0001$). The number of adult parasitoids emerging in F1 progeny was significantly higher in *L. huidobrensis* than *L. trifolii* and *L. sativae* ($F_{2,14} = 4.85$, $P < 0.0286$). Parasitism rates caused by *C. flacilla* were high (73.00 ± 6.81%, 73.56 ± 4.62% and 77.73 ± 4.39% in *L. sativae*, *L. huidobrensis*, and *L. trifolii*, respectively ($F_{2,14} = 0.22$, $P = 0.8048$) but did not significantly differ across the three *Liriomyza* hosts (Table 3.2). The sex ratios of the parasitoid's F1 progeny reared on all three *Liriomyza* hosts was female biased (Chi-square values for *L. huidobrensis* =

135.42, $P < 0.0001$; *L. sativae* = 55.07, $P < 0.0001$; *L. trifolii* = 5.75, $P = 0.01648$). The proportions of females were similar across the three LMF hosts ($F_{2,12} = 0.75$, $P = 0.5133$) (Table 3.2).

Table 3.2. Effect of *Liriomyza* host species on *Chrysocharis flacilla* developmental time, parasitism rate and sex ratio (Mean ± SE) under laboratory conditions (21 ± 1°C and 55 ± 5% RH)

Variable indicators	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
F1 developmental period (d) (*n* = 40)	20.13 ± 0.76a	15.97 ± 1.48b	23.96 ± 0.88a
Number of parasitoids in F1progeny	125.00 ± 13.26a	95.80 ± 11.38ab	70.00 ± 11.50b
Parasitism rate (%)	73.56 ± 4.62a	73.00 ± 6.81a	77.73 ± 4.39a
Proportion of female parasitoids in F1 progeny (%)	73. 17 ± 1.34a	69.69 ± 2.41a	72.39 ± 2.40a

RH - Relative humididty, F1 - first generation, *n*- number of parasitoids investigated per *Liriomyza* species, d - days; Within rows, means followed by the same lower case letter are not significantly different at $P \leq 0.05$ (Tukey-Kramer HSD test).

3.4.3. Non-reproductive host pupal mortality

Presence of *C. flacilla*-induced significant nonreproductive host pupal mortality in the three *Liriomyza* hosts compared to the control mortality (*L. huidobrensis*: 34.98 ± 3.42; *L. sativae*: 22.70 ± 1.20; *L. trifolii*: 28.53 ± 4.86; *P* values < 0.0001). This induced non-reproductive host pupal mortality was similar across the tested LMF host species ($F_{2,14} = 0.21$, $P = 0.809$; Table 3.3).

3.4.4. Parasitoid body indices

Results on F1 parasitoids body size showed that females reared on *L. huidobrensis* had significantly longer forewing ($F_{2,29} = 168.36$, $P < 0.0001$) and hind tibia ($F_{2,29} = 514.76$, $P < 0.0001$) than those reared on *L. sativae* and *L. trifolii* (Table 3.4). There was however no significant difference in the forewing and hind tibia lengths of F1 male parasitoids reared on the three *Liriomyza* hosts ($F_{2,29} = 1.83$, $P = 0.1791$ and $F_{2,29} = 0.43$, $P = 0.6555$; Table 3.4). There were no significant differences in forewing and hind tibia of male and female parasitoids reared from one host (*L. huidobrensis*: Chi-square = 0.003, $P = 0.9562$ for forewing and Chi-square = 0, $P = 1$ for hind tibia; *L. sativae*: Chisquare = 0, $P = 1$ for both forewing and hind tibia; *L. trifolii*: Chisquare = 0, $P = 1$ for both forewing and hind tibia; Table 3.4).

Table 3.3. Non-reproductive host pupal mortality by *Chrysocharis flacilla* on three *Liriomyza* host species (Mean ± SE) under laboratory conditions (21 ± 1^{o}C and 55 ± 5% RH)

	Liriomyza huidobrensis	*Liriomyza sativae*	*Liriomyza trifolii*
Mortality in presence of parasitoid (%)	34.98 ± 3.42aA	22.70 ± 1.20aA	28.53 ± 4.86aA
Mortality in the absence of parasitoid (%)	16.60 ± 4.05aB	8.82 ± 1.52aB	17.41 ±10.05aB
Chi - square	65.02	92.01	26.30
P value	0.0001	0.0001	0.0001

Within rows (columns), means followed by the same lower (upper) case letter are not significantly different at P ≤ 0.05 according to Tukey-Kramer test (Chi -square) test

Table 3.4. Effect of *Liriomyza* hosts species on body size of F1 adult *Chrysocharis flacilla* (Mean ± SE) under laboratory conditions (21 ± 1^{o}C and 55 ± 5% RH)

Body size indices	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
Fore wing length (mm)			
Females	1.52 ± 0.02aA	1.33 ± 0.03bA	1.30 ± 0.06bA
Males	1.40 ± 0.05aA	1.36 ± 0.03aA	1.30 ± 0.03aA
Chi -square	0.003	0.0	0.0
P value	0.9562	1.0	1.0
Hind tibia length (mm)			
Females	0.50 ± 0.01aA	0.43 ± 0.01bA	0.41 ± 0.02bA
Males	0.45 ± 0.02aA	0.44 ± 0.01aA	0.43 ± 0.01aA
Chi -square	0.0	0.0	0.0
P value	1.0	1.0	1.0

Within rows, means followed by the same lower case letter are not significantly different at $P \leq 0.05$ (Tukey-Kramer HSD test), within columns, and for each parameter, means followed by the same upper case letter are not significantly different (Chi -square test, $P \leq 0.05$).

3.5. Discussion

Our results indicated that the three invasive *Liriomyza* species in Kenya were accepted and suitable for the development of the exotic endoparasitoid, *C. flacilla*. *C. flacilla* foraging behavior, measured by its host searching and oviposition attempts, varied. However, females successfully oviposited equally in the three *Liriomyza* hosts. The number of oviposition attempts by female parasitoid in the three *Liriomyza* hosts, exceeded the number of larvae presented suggesting that the parasitoid could superparasitise same host. Future studies involving similar studies on *C. flacilla* could therefore reduce the time of host exposure to avoid superparasitism. Less time was spent by *C. flacilla* to search and encounter *L. trifolii* host than *L. sativae* yet both were reared on the same host plant, *P. vulgaris*. Similarly there were differences between searching time on *Liriomyza* hosts reared on different host plants, *V. faba* and *P. vulgaris*. We hypothesize therefore that, host plant frequently reported to play a major role in parasitoids' host finding (Andow & Prokrym 1990, Lukianchuk & Smith 1997, Lovinger et al. 2000, Suverkropp et al. 2001, Wang & Keller 2002, Gingras et al. 2008), may have affected *C. flacilla* host searching abilities despite both rearing host plants being in the family Fabaceae. The success of searching in foraging parasitoids may be as a result of learned, genetic, and sensory sources of information available to an insect (Gingras et al. 2008). However, we cannot rule out that the experience *C. flacilla* had acquired during its rearing host and host plant influenced our results even though searching for *L. trifolii* on *P. vulgaris* was fast than for *L. huidobrensis* on *V. faba*. Further studies are therefore warranted to assess potential learning effect on performance of *C. flacilla* on the three *Liriomyza* leafminers. It is also possible that *C. flacilla* may have been affected by the *Liriomyza* host species or the interaction between host plant and insect host, and further studies are warranted to elucidate this. *C. flacilla* is a successful colonizer of a wide host range, with more than 33 Agromyzid leafminers on 21 different host plants in which Fabaceae host plants are preferred (Salvo & Valladares 1997, Mujica & Kroschel 2011).

A high number of *C. flacilla* females (97.5%) successfully caused *Liriomyza* parasitism during the 2-h observation, and these results are similar to rates observed in *P. scabriventris* (92.2%) when reared on *L. huidobrensis* using *V. faba* (Chabi-Olaye et al. 2013) and *H. arduine* (97%) when reared on *L. sativae* and *L. trifolii* using *P. vulgaris* (Muchemi et al. 2018a).

Successful completion of *C. flacilla* development within the three dominant *Liriomyza* hosts under controlled laboratory conditions is promising for its use as potential biological control candidate in East Africa. Lucero (2008) observed a similar *C. flacilla* developmental period of 22–26 d when reared on *L. huidobrensis* at 20–30°C. Existing parasitoids in the target agro-ecosystems have shorter developmental periods than *C. flacilla*. For instance, developmental periods reported under similar tropical temperatures (20–25°C) for the parasitoids are 14 d for *O. dissitus* and *D. isaea*, 12 d for *N. formosa*, 11.1 d for *H. varicornis*, and 14.8–15.2 d for *P. scabriventris* (Bordat et al. 1995, Bazzocchi et al. 2003, Kafle et al. 2005, Hondo et al. 2006, Tran et al. 2006, Chabi-Olaye et al. 2013, Foba et al. 2015a). The longer developmental time in *C. flacilla* could be disadvantageous to it, should there be superparasitism or kleptoparasitism from the local parasitoids, and further studies are warranted to assess the interaction between this exotic parasitoid and the local ones.

The demonstrated ability of *C. flacilla* to cause high parasitism rates (up to 77.73%) in the three investigated *Liriomyza* host species suggests its potential as a biological control candidate for introduction in East Africa agro-ecosystems. Under the same laboratory conditions, our present findings revealed far higher parasitism rates for *C. flacilla* than for any other reported LMF parasitoids. For instance, the exotic and indigenous parasitoids, *P. scabriventris* and *O. dissitus* caused 64% and 43% parasitism rates on *L. huidobrensis*, respectively (Akutse et al. 2015, Foba et al. 2015a), while *D. isaea* caused 30% parasitism rates of *L. huidobrensis* (Akutse et al. 2015) and *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae*)* caused 44%, 32.3%, and 28% parasitism rates of *L. huidobrensis*, *L. trifolii*, and *L. sativae* in that respect (Muchemi et al. 2018b). The present parasitoid seems therefore to be the most promising leafminer parasitoid and further studies are warranted to also evaluate *C. flacilla* performance under field conditions in East Africa.

Further to host suppression through parasitization, *C. flacilla*induced significant nonreproductive host pupal mortality in the three *Liriomyza* hosts. This is an additional performance factor which needs to be accounted for when evaluating its performance. In fact, this is a unique attribute for *C. flacilla* compared to other *Liriomyza* endoparasitoids with negligible nonreproductive mortalities. For instance, the endoparasitoids *P. scabriventris* and *O. dissitus* were reported incapable of inducing significant nonreproductive mortalities (Chabi-Olaye et al. 2013, Akutse et al. 2015, Foba et al. 2015a). Host suppression is not only dependent on parasitism but also induced nonreproductive host pupal mortality (Bordat et al. 1995, Bernardo et al. 2006, Tran & Takagi 2006, Chabi-Olaye et al. 2013, Akutse et al. 2015, Foba et al. 2015a). With its substantive levels of nonreproductive host killing, coupled with high parasitism rates, *C. flacilla* demonstrated a high potential in *Liriomyza* leafminers suppression through biological control programs. *D. isaea*, a key local leafminer ectoparasitoid was also found to induce significant nonreproductive mortality in *L. huidobrensis* larvae of up to 49%, however, its parasitism rates of 30% were far below those of *C. flacilla* (Minkenberg 1989, Liu et al. 2013, Akutse et al. 2015). Nonreproductive mortality caused by parasitoids constitutes an important component in pest suppression, however; this is more frequently reported in ectoparasitoids (Sandlan 1979, Walter 1988, Bernardo et al. 2006, Tran & Takagi 2006, Mafi & Ohbayashi 2010, Akutse et al. 2015). Only a few endoparasitoids have this ability. For instance, *Copidosoma koehleri* (Blanchard) (Hym: Encyrtidae) is reported to have this potential on potato tuber moth, *Phthorimaea operculella* (Zeller) (Lep: Gelechiidae) (Keinan et al. 2012). Further studies are however recommended to assess how *C. flacilla* induces the nonreproductive host pupal mortality.

The female-biased sex ratio of *C. flacilla* F1 progeny is also a positive attribute to the successful buildup of its population. Our results confirm that of Lucero (2008) who reported a female-biased sex ratio for *C. flacilla* when reared on *L. huidobrensis* at temperatures between 15°C and 25°C. According to Lucero (2008), however, an increase in temperature to 30°C resulted to male-biased populations of *C. flacilla* and similar studies at higher temperatures are warranted for the colony introduced in Kenya to assess effect of temperature on the parasitoid’s sex ratio. A female-biased sex ratio in parasitoids confers high efficiency and performance compared to a male-biased one (Beddington et al. 1978, Mills & Getz 1996, Ode & Heinz 2002, Pascua & Pascua 2004, Chow & Heinz 2005, Abe & Kamimura 2012, Foba et al. 2015a). This therefore suggests a high-parasitoid performance, increased overall stability, and a good biological control candidate for leafminer suppression on the introduction of *C. flacilla* in the Afro tropical regions of East Africa as only females contribute to the mortality of pests.

Bigger female parasitoids were obtained when reared on *L. huidobrensis* than when reared on the smaller hosts, *L. sativae* and *L. trifolii*. Similar results were reported by Chabi-Olaye et al. (2013) and Musundire et al. (2012a) where bigger female parasitoids of *P. scabriventris* and *D. isaea* were obtained when reared on *L. huidobrensis* than when reared on *L. sativae* and *L. trifolii*. The bigger size of *L. huidobrensis* compared to *L. sativae* and *L. trifolii* could underline this finding (Musundire et al. 2012a). The fitness of Agromyzid leafminer parasitoids is reported to depend on the body size of their hosts (Heinz & Parrella 1990, Visser 1994, Ode & Heinz 2002, Stoepler et al. 2011, Musundire et al. 2012a) and the bigger the parasitoids, the better is their performance (Clutton-Brock 1988, Honek 1993, Musundire et al. 2012a). We therefore recommend that mass rearing of *C. flacilla* for biological control programs be done preferentially on *L. huidobrensis*. Furthermore, according to Foba et al. (2015b) and Fiaboe personal observation, *L. huidobrensis* represents between 50% and 90% of *Liriomyza* leafminers in Tanzania and Kenya, while in Uganda the pests remains the dominant species at high altitudes, suggesting an expected high fitness and high performance of the parasitoid in East Africa.

3.6. Conclusion

Chrysocharis flacilla was found to be more promising leafminer parasitoid compared to other important leafminer parasitoids reported in Africa on the three economically most important *Liriomyza* leafminers. The parasitoid exhibited the highest parasitism rate obtained for any single *Liriomyza* leafminers' parasitoid species studied so far, producing constantly a female-biased sex ratio. *C. flacilla* is so far the only endoparasitoid inducing a significant nonreproductive pupal mortality in addition to the high parasitism rates and the parasitoid performed equally well on host plants tested. The future of this parasitoid in a classical biological control program on various host plants and pest hosts is very promising under the prevailing farmers' management approach of indiscriminate application of insecticide. Over reliance on insecticides has far reaching consequences not only to the target pest but also to the nontarget organisms, the environment, and health of the consumers (Murphy & LaSalle 1999, Guantai et al. 2015, RASFF 2015). However, to ensure that local parasitoid populations are not displaced by the exotic species and explore potential complementarities or additive effect between the exotic and the indigenous parasitoids, interaction studies between *C. flacilla* and the parasitoids already existing in the system are warranted. Ultimately, since the current results are based on laboratory conditions, further field release studies are warranted to confirm the parasitoids performance under field conditions in East Africa.

4.0. Interaction between two leafminer parasitoids, *Halticoptera arduine* (Hymenoptera: Pteromalidae) and *Diglyphus isaea* (Hymenoptera: Eulophidae), in the management of *Liriomyza huidobrensis* (Diptera: Agromyzidae)

This chapter has been published as Muchemi et al. *Environmental Entomology*, 47 (3), 2018, 692-699. doi: 10.1093/ee/nvy051

4.1. Abstract

Liriomyza spp., leafminer flies (Mik; Diptera: Agromyzidae), are economically important quarantine pests that puncture and mine leaves and fruits of various horticultural crops worldwide, affecting yield and trade. *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae), a key parasitoid from the pests' areas of origin in South America, was introduced as a potential alternative management strategy. Prior to *H. arduine* release, its potential interactions with the dominant local ectoparasitoid, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), were assessed. *Halticoptera arduine* and *D. isaea* were released in single, sequential and simultaneous combinations on *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) to evaluate possible effect on the parasitism rate, reproduction and host mortality. The combination of both parasitoids did not significantly affect the specific parasitism rates of either of them, an indication that *H. arduine* and *D. isaea* can co-exist. Parasitism rates of the exotic *H. arduine* were significantly superior to the indigenous *D. isaea* in all release combinations except when both species were released simultaneously. While 50 individuals of *D. isaea* resulted only in 21.23±2.1% parasitism, 50 parasitoids composed of 25 *H. arduine* and 25 *D. isaea* caused 53.27±4.99%. Both parasitoids further induced significant non-reproductive host mortalities. Both parasitoids' F1 progenies sex ratios were female-biased in all parasitoid release combinations except in single release of *D. isaea* with a balanced sex ratio. The improvement in *D. isaea*'s sex ratio induced by the presence of *H. arduine* suggests a synergetic effect on *D. isaea*'s reproductive performance. The introduction of *H. arduine* in horticulture production systems may therefore improve natural control of *Liriomyza* leafminers in East Africa.

4.2. Introduction

Horticulture is a leading sector in Kenya's economy, contributing annual revenue of USD2 billion (KNBS 2016). In 2015, horticulture contributed 27.3% to Kenya's gross domestic product (GDP) (KIPPRA 2016, KNBS 2016), with export of horticultural products generating $1 billion in foreign exchange for the country (KNBS 2016). More than six million people are employed in the production, processing and marketing of horticultural products in Kenya (NHP 2012, KHC 2015). A major constraint to the growth of this sector is the occurrence of pests and diseases. Among the important pests is LMF (Diptera: Agromyzidae), originating from the neotropics. They have become a hindrance to the production and trade in ornamentals, fruits and vegetables in the East African region. LMF were first reported in Kenya in the early 1970s (Spencer 1973), with *L. huidobrensis* (Blanchard), *L. sativae* Blanchard (Diptera: Agromyzidae) and *L. trifolii* (Burgess) (Diptera: Agromyzidae) now widely distributed in the country. The three species represent above 95% of total *Liriomyza* species across East Africa, and known to infest a variety of food crops of commercial value including snow pea (*Pisum sativum*

L. (Fabales: Fabaceae)), Faba bean (*Vicia faba* L. (Fabales: Fabaceae)), French bean (*Phaseolus vulgaris* L. (Fabales: Fabaceae)), runner bean (*Phaseolus coccineus* L. (Fabales: Fabaceae)), tomato (*Solanum lycopersicum* L. (Solanales: Solanaceae)), Irish potatoes (*Solanum tuberosum* L. (Solanales: Solanaceae)) and a variety of cut flowers including *Chrysanthemum* spp. L. (Asterales: Asteraceae), *Eryngium* spp. L. (Apiales: Apiaceae), *Gypsophila* spp. L. (Caryophyllales: Caryophyllaceae) and *Carthamus* spp. L. (Asterales: Asteraceae) (Chabi-Olaye et al. 2008, Mujica & Kroschel 2013, KEPHIS 2014). Adult female leafminers make punctures on leaves using the ovipositor on which both females and males feed from leaf exudates. The punctures are also used to insert eggs below the leaf surface and may also act as vectors for diseases such as *Alternaria alternata* (Fr.) Keissl (Pleosporales: Pleosporaceae) (Parrella et al. 1984, Deadman et al. 2002, Bjorksten et al. 2005). Larval mining is generally the most destructive feeding behavior that reduces the photosynthetic capacity of plants and may lead to leaf fall in severe infestation (EPPO 2013). *Liriomyza* species have become main contributors to yield losses of greenhouse and field crops (Chabi-Olaye et al. 2008, Mujica & Kroschel 2013, KEPHIS 2014, Foba et al. 2015b). LMF are categorized as of quarantine significance to the main trading partners in the European Union (EU) (EU 2000, IPPC 2005, EPPO 2013). For example, in 2016, 23% of interceptions of Kenyan horticultural products destined for the EU were as a result of the presence of *Liriomyza* species (EUROPHYT 2016).

In an effort to manage LMFs, farmers in Kenya have mostly relied on the use of synthetic insecticides (Gitonga et al. 2010). This overreliance has resulted in the development of resistance by LMF to several groups of insecticides (Tran & Takagi 2005b, Liu et al. 2009, Guantai et al. 2015). Moreover, the accumulation of pesticide residues in horticultural products has become a food safety issue while the non-selective use of insecticides adversely affects natural enemies associated with LMF (Mujica & Kroschel 2005, Guantai et al. 2015).

Parasitoids and other natural enemies are important in regulating LMF populations in their native and invaded areas (Shepard et al. 1998, Murphy & LaSalle 1999, Rauf et al. 2000, Mujica & Kroshel 2011). Worldwide, more than 300 species of parasitoids are associated with agromyzids, and of these, more than 80 species are known to attack *Liriomyza* spp. (Noyes 2004), although the majority, have been reported from South America (Liu et al. 2009). For instance, along the Peruvian coast, LMFs' native region, a complex of 63 parasitoid species is associated with *Liriomyza* spp. (Mujica & Kroschel 2011). A rich complex of the endoparasitoids *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae), *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae), *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae), *Ganaspidium* spp. (Weld) (Hymenoptera: Eucoilidae) and the two ectoparasitoids *Diglyphus websteri* (Crawford) (Hymenoptera: Eulophidae) and *D. begini* (Ashmead) (Hymenoptera: Eulophidae) are the most important parasitoid species, representing more than 94.1% of total parasitoid species and causing LMF mortality of up to 55% in Peru, Argentina and Chile (Serantes de Gonzales 1974, Salvo & Valladares 1995, Cisneros & Mujica 1998, Neder de Roman 2000, Mujica & Kroschel 2011). Under the field conditions of the Peruvian coast, *H. arduine* was the most abundant and efficient parasitoid species, occurring on 25 host plants infested by a wide range of agromyzid LMF species, including the three key *Liriomyza* species identified in Kenya (Mujica &

Kroschel 2011). The parasitoid is adapted to a wide range of ecologies from the coastal region of Peru and Chile (less than 500 meters above sea level [m.a.s.l.]) to high altitudes of up to 4,050 m.a.s.l. in Argentina, Chile and Peru (Sanchez & Redolfi 1985, Neder de Roman 2000, Mujica & Kroschel 2011).

The diversity of indigenous parasitoids associated with *Liriomyza* spp. in East Africa in horticultural field crops is, low. In a field survey conducted in 2008 in Kenya, the two parasitoids *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) and *D. isaea* (Walker) were identified as the most important local LMF parasitoids, yet only causing parasitism rates of approximately 6%. *Diglyphus isaea* represents approximately 35% of all LMF parasitoids found in Kenya, closely followed by *O. dissitus* (Chabi-Olaye et al. 2008, Foba et al. 2015c). In the effort to improve biological control of *Liriomyza* leafminers in East Africa by boosting the parasitism rates, *H. arduine* was imported from Peru into Kenya by the International Centre of Insect Physiology and Ecology (*icipe*), in collaboration with the International Potato Centre (CIP), Peru. To avoid potential ecological disruptions to the local parasitoid populations as a consequence of the introduction of the exotic biological control agent, an assessment of its impact is necessary (Boettner et al. 2000, Louda et al. 2003). Interacting parasitoids may compete for resources, thereby affecting their performance (Godfray 1994, Hardy et al. 2013). The objective of this study was to evaluate the interactions between the exotic *H. arduine* and the indigenous *D. isaea* using *L. huidobrensis*. The results obtained in these studies will be a key criterion for consideration of *H. arduine* potential release as a biological control agent against LMF in East Africa. *Liriomyza huidobrensis* is the most abundant and widely distributed LMF species found in Kenya (Chabi-Olaye et al. 2013, Foba et al. 2015b).

4.3. Materials and methods

4.3.1.Plant materials

An open pollinated variety of Kenyan faba bean, *Vicia faba* L. (Fabales: Fabaceae) was used for the rearing of *L. huidobrensis* and its two parasitoid species. Five seeds were planted in plastic pots (5.5 cm diameter x 7.3 cm high) and filled with planting substrate (mixture of soil and manure 5:1 in a ratio). Potted plants were maintained in a screenhouse (2.8 m length x 1.8m width x 2.2 m height) at *icipe*’s Duduville campus in Nairobi, Kenya at 25 ± 2° C for two weeks. Two week-old plants were used for adult *L. huidobrensis* exposure, on which the parasitoid interaction experiments were conducted using procedures adapted from Akutse et al. (2015) and Foba et al. (2015a).

4.3.2. Insect colonies

4.3.2.1. Leafminer colony

The initial colony was started from field collections in Nyeri County (0°° 21’S, 36°57’E, 2,200 m.a.s.l.) of Central Kenya in 2007 and maintained on faba bean plants at *icipe*’s rearing unit. Two day-old adult LMF were released for 24h in Perspex cages (60 cm length × 60 width × 60 cm height) for egg laying on potted faba plants. The colony was cultured at 25 ± 2°C, 60 ± 5% relative humidity (RH) and a photoperiod of 12L: 12D. Infested plants were removed and held in wooden cages (45 cm wide × 45 cm long × 60 cm high) for five to six days for the development of 2nd and 3rd similar age cohort instar larvae of *L. huidobrensis*. Plants were cropped at the base of the stem and incubated on mesh trays to capture dropping pupae. Pupae were collected and incubated in Petri dishes for adult emergence. Adult LMF were fed on 10% sugar solution for two days after emergence for pre-oviposition period before experimental use as described in Chabi-Olaye et al. (2013), Akutse et al. (2015) and Foba et al. (2015a).

4.3.2.2. Parasitoid colonies

The initial culture of *H. arduine* was imported from CIP in 2012, where they were maintained on *L. huidobrensis*. In the *icipe* quarantine facility the parasitoid was also maintained on *L. huidobrensis* reared on faba beans at 21°C ±1 and 55% ± 5 RH for ten generations before experimental use. The initial culture of *D. isaea* was recovered from LMF-infested *P. sativum* L. (Fabales: Fabaceae) plants collected from farmer fields in Naromoru (0°18’ S, 036°84’ E, 1975 m.a.s.l) of Nyeri County, Kenya, in 2013. Cultures of *D. isaea* were then reared on *L. huidobrensis* at *icipe*’s insect rearing facilities (25°C ± 2 and 60% ± 5 RH) for five generations before experimental use as described by Akutse et al. (2015).

Adult parasitoids of both species were fed on 10% honey solution after emergence for two days to allow mating and egg maturation before experimental use and in mass production. Each insect species colony was reared in separate rooms to avoid contamination of colonies.

4.3.3. Experimental procedure

Bioassay experiments on parasitoid interactions were conducted in laboratories at *icipe*’s Duduville campus. Ten pots of two-week-old faba bean plants from the screenhouse were placed in aerated Perspex cages (30 cm × 30 cm × 45 cm). Two hundred adults of two-day old *L. huidobrensis* in the ratio of 1:2 (males: females) were exposed for 24h to the plants for egg-laying before being removed. Infested plants were held for five to six days (25 ± 2°C, 60 ± 5% RH and photoperiod (12L: 12D)) for the development of 2nd to 3rd LMF larval instars, thereby generating a similar age cohort. Pots were then covered with aluminum foil to minimize the loss of host larvae in potted soil. Two-day-old adult parasitoids in the ratio of 1:2 (male: females) were subsequently introduced in each of the respective treatments described in Table 1 for 24h before their removal by aspiration. LMF larvae were held on the plants for two to three days for pupa development under the same laboratory conditions as described above. To confirm the solitary nature of *H. arduine*, pupae were collected using a fine camel-hair brush, transferred singly into transparent gelatin capsules (2.2 cm height and 0.7 cm diameter) and then incubated for 30 days to allow emergence of adult LMF and parasitoids. Because *D. isaea* is an ectoparasitoid, which parasitizes its host by injecting venom into the larvae before depositing the eggs on or close to the host larvae, parasitized larvae were not able to drop for pupation. Thus, plant foliage was cropped and maintained in separate aerated lunch boxes (19 cm long × 13 cm wide × 8 cm high) for 20 days to allow adult emergence as described by Akutse *et al*. (2015). The leaves were later examined under the microscope to correct for *D. isaea* parasitism and host mortality. Pupae without exit holes and where insects failed to exit were dissected under the microscope following the methodology described by Heinz and Parrella (1990) to correct for parasitism rates and host mortality. The experiment was arranged in a randomized complete block design (RCBD) with six blocks and one replicate per block.

4.3.4. Assessment of parasitoid interactions

Interactions between *H. arduine* and *D. isaea* in parasitizing *L. huidobrensis* larvae were studied following the procedures described by Wang & Messing (2002), Bader et al. (2006), Akutse et al. (2015) and Foba et al. (2015a). Six parasitoid combinations comprising sole release of 50 *H. arduine* (T1), sole release of 50 *D. isaea* (T2), simultaneous release of 50 *H. arduine* and 50 *D. isaea* (T3), simultaneous release of 25 *H. arduine* and 25 *D. isaea* (T4), sequential release of 50 *H. arduine* before 50 *D. isaea* (T5), sequential release of 50 *D. isaea* before 50 *H. arduine* (T6) and a control without parasitoids (T7) were established (Table 4.1). The average number of larvae per treatment and per replicate was n =190. The number of adult parasitoids collected for each treatment was pooled per replicate and a specific mean and total parasitism rates generated. To assess the effect of parasitoid combinations on both parasitoids' performance, specific and total parasitism rates were computed and comparisons made among treatments as well as within treatments for specific parasitism rates.

Total parasitism rates in simultaneous release of 50 individuals of each of the two parasitoid species (T5) were compared 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 (T1 and T2) for both parasitoid species. Total parasitism rates in sequential release strategies (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 evaluate the performance of the combined parasitoid species with each parasitoid species' single release at the same parasitoid density. To assess effects of parasitoid release combinations on sex ratio, F1 progenies from each treatment were compared among and within treatments. Assessment of parasitoids' non-reproductive host mortality was done using procedures described by Wang & Messing (2002) and Foba et al. (2015a). The pupal mortality rate was expressed as the numbers of un-emerged host pupae divided by total pupae multiplied by 100 in each treatment.

Table 4.1. Release strategies, sequences and densities of *Halticoptera arduine* and *Diglyphus isaea* on *Liriomyza huidobrensis* under laboratory conditions (25 ± 2°C, 60 ± 5% RH and 12L: 12D photoperiod)

Treatments (T)	Parasitoid species release combinations
Sole releases	
T1-*H. arduine* alone	50 adults of *H. arduine* (1:2 for ♂and ♀)
T2-*D. isaea* alone	50 adults of *D. isaea* (1:2 for ♂and ♀)
Sequential releases	
T3-*H. arduine* first, *D. isaea* second	50 adults of *H. arduine*, followed by 50 adults of *D. isaea* (1:2 for ♂and ♀ of each species)
T4-*D. isaea* first, *H. arduine* second	50 adults of *D. isaea*, followed by 50 adults of *H. arduine* (1:2 for ♂and ♀ of each species)
Simultaneous releases	
T5-*H. arduine* and *D. isaea*	50 adults of *H. arduine* + 50 adults of *D. isaea* (1:2 for ♂and ♀ of each species)
T6-*H. arduine* and *D. isaea*	25 adults of *H. arduine* + 25 adults of *D. isaea* (1:2 for ♂and ♀ of each species)
Control	
T7-*L. huidobrensis* reared alone	No parasitoids released

♂: males, ♀: females

4.3.5. Data analyses

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

$$SpHa = \left(\frac{C_{Ha}}{C_{Ha} + c_{Lh}}\right) x\ 100$$

$$SpDi = \left(\frac{C_{Di}}{C_{Di} + c_{Lh}}\right) x\ 100$$

$$TPHaDi = \left(\frac{C_{Ha} + C_{Di}}{C_{Ha} + C_{Di} + c_{Lh}}\right) x\ 100$$

Where Sp_{Ha} = the specific parasitism of *H. arduine*; C_{Ha} =corrected number of *H. arduine*; C_{Lh} = the corrected number of *L. huidobrensis*; Sp_{Di} = the specific parasitism of *D. isaea*; C_{Di} = the corrected number of *D. isaea*; TP_{HaDi} = the total parasitism of *H. arduine* and *D. isaea*.

Percentage data on specific, total parasitism rates and sex ratios were arcsine transformed and subjected to one way Analysis of Variance (ANOVA). The non-reproductive (parasitoid induced) mortality was evaluated using Abbott's formula (Abbott 1925), while the level of observed mortalities was assessed by comparing each treatment with the control using the chi-square test ($P< 0.05$). Mean differences in parasitism rates across treatments were separated using Tukey-Kramer Honest significant difference (HSD) test at $P \le 0.05$ while differences in parasitoid species sex ratio within treatments were separated using Chi-square test ($P< 0.05$). The statistical programs used for these analyses were JPM (SAS, 2013) and R version 3.1 (R Core Development Team 2013).

4.4. Results

4.4.1. Effect of *Halticoptera arduine* and *Diglyphus isaea* combinations on parasitism rates

A total of 7,293 LMF pupae were kept individually in gelatin capsules each of which yielded a single parasitoid or LMF adult. The solitary nature was observed for both *H. arduine* and *D. isaea,* even when both parasitoid species were jointly released (T3, T4, T5 and T6).

In the single 50-parasitoid species releases (T1 and T2), the specific parasitism rate for *H. arduine* (42.40 ± 3.27%) was significantly two times higher than that for *D. isaea* (21.23 ± 2.10%) (χ^2 = 197.71, df = 1, P<0.0001). The presence of *H. arduine* did not affect the specific parasitism rate of *D. isaea*, and neither did *D. isaea* affect the specific parasitism rate of *H. arduine* (T3 and T4), (Table 4.2). From simultaneously introducing both parasitoid species at a density of 50 individuals/species (T5), their specific parasitism rates did not differ significantly from the same density in their sequential introduction (T3 and T4) for both parasitoid species. Similarly, simultaneous release of both parasitoid species at 50 individuals/species (T5) did not result in significantly different specific parasitism from T1 and T2.

The sequence of introducing the parasitoids (T3 and T4) had no significant effect on total parasitism when compared with the single release of 50 *H. arduine* (T1) and the simultaneous release of 100 total individuals of both parasitoid species (T5). However, the total parasitism in T5 (55.46%) was significantly higher compared with that resulting from the 50 *D. isaea* releases (T2) (21.23%). There was no significant effect in host parasitization of the simultaneous release of 25 individuals of each parasitoid species (T6) (53.27%) compared with 50 individuals of each species (T5). However, the specific parasitism by 25 *H. arduine* in a simultaneous release strategy (T6) was 1.9 times greater than that of 50 *D. isaea* (T2) (21.23%) under the same host conditions, and 2.3 and 1.6 times higher than the specific parasitism by 50 *D. isaea* when used in T5 and T3, respectively. Furthermore, total parasitism rate by simultaneous use of 25 each of *H. arduine* and *D. isaea* (T6) was significantly 2.5 times higher than use of 50 *D. isaea*

(T2) ($F_{5,35}$ = 4.88, P<0.01) (Table 4. 2). Except for T3, where specific parasitism rates of both parasitoid species were similar (χ^2 =1.87, df =1, P=0.1714), those of *H. arduine* were always significantly superior to those of *D. isaea* in all treatments where both parasitoids were jointly released, by 1.7 to 2.6 times (T4, T5 and T6) (Table 4.2).

4.4.2. Effect of *Halticoptera arduine* and *Diglyphus isaea* combinations on *Liriomyza huidobrensis* non-reproductive mortality

Liriomyza huidobrensis exhibited mortality in the presence of both *H. arduine* and *D. isaea* in single and combined release strategies that were significantly higher compared with the control where no parasitoids were released. However, the non-reproductive moralities of *L. huidobrensis* resulting from both parasitoid species did not differ significantly ($F_{5,33}$ = 1.33, P = 0.2823) among the release combinations (Table 4.3).

4.4.3. Effect of *Halticoptera arduine* and *Diglyphus isaea* combinations on parasitoid F1 sex ratios

Halticoptera arduine F1 progenies in all parasitoid release strategies were significantly female-biased (Table 4.4), but the proportion of males and females did not differ significantly among the treatments ($F_{4,29}$ = 0.73, P=0.5806). Sole releases of *D. isaea* resulted in a balanced sex ratio in the F1, but was significantly female-biased in combinations with *H. arduine* either sequentially (T3 and T4) or simultaneously (T5 and T6). As with *H. arduine*, the proportion of males and females of *D. isaea* did not differ among the treatments ($F_{4,29}$ = 0.48, P=0.75) (Table 4.4).

4.5. Discussion

For both parasitoid species, irrespective of the treatment, only one parasitoid specimen was recovered from each host pupa. This finding confirms the solitary nature of both *H. arduine* and *D. isaea*, corroborating earlier reports on *H. arduine* by Arrellano and Redolfi (1989) and preliminary studies(Muchemi et al. 2018c) while studying its performance on three LMF hosts (Akutse et al. 2015) reported on the solitary nature of *D. isaea* while carrying out interaction studies between *D. isaea* and *P. scabriventris*. Thus, our results indicate the possibility for host resource sharing of the two parasitoid species, which could result in higher levels of LMF control (Foba et al. 2015a). Interactions between parasitoids in the exploitation of a common resource can influence the performance and ability to control the target pest (Briggs 1993, Grover 1997, Bogran et al. 2002). The introduction of a new parasitoid species that shares the same resources as indigenous parasitoids can pose a risk of interspecific competition, possibly leading to ecological disruption and reduced performance of one or both species (Briggs et al. 1993, De Moraes et al. 1999, Pianka 2000, Louda et al. 2003, Shi et al. 2004, Tian et al. 2008, Jones et al. 2009, Harvey et al. 2013).

However, co-existence is common between different parasitoid species if the parasitoids attack different host life stages. 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.

Table 4.2. Mean (± S.E.) of total and specific parasitism rates of *Halticoptera arduine* (*Ha*) and *Diglyphus isaea* (*Di*) on *Liriomyza huidobrensis* following various release combinations under laboratory conditions (25 ± 2^{o}C, 60 ± 5% RH and 12L: 12D photoperiod).

Treatments (T) regime	T1	T2	T3	T4	T5	T6
Ha specific parasitism (%)	42.40 ± 3.27aA		29.72 ± 3.14aA	30.84 ± 3.70aA	40.14 ± 2.50aA	34.60 ± 5.27a A
Di specific parasitism (%)		21.23 ± 2.10aB	21.78 ± 2.67aA	18.68 ± 3.316aB	15.32 ± 1.99aB	18.67 ± 2.36aB
χ^2 values		197.71*	1.87	90.18	347.63	137.98
P-values		0.0001	0.1714	0.0001	0.0001	0.0001
Total parasitism (%)	42.40 ± 3.27a	21.23 ± 2.10b	51.50 ± 3.82a	49.52 ± 5.17a	55.46 ± 2.60a	53.27 ± 4.99a

Ha- *H. arduine*, Di- *D. isaea*.
* A comparison between T1 and T2. T1-50 *H. arduine* only, T2- 50 *D. isaea* only, T3-50 *H. arduine* first followed by 50 *D. isaea*, T4-50 *D. isaea* first followed by 50 *H. arduine*, T5 - 50 *H. arduine* plus 50 *D. isaea* simultaneously, T6- 25 *H. arduine* plus 25 *D. isaea* simultaneously. Within rows (columns), means followed by same lower (upper) case letter are not significantly different at P≤0.05 according to Tukey-Kramer (Chi-square) test.

Table 4.3. Effect of combinations of *Halticoptera arduine* and *Diglyphus isaea* on *Liriomyza huidobrensis* mortality rates (mean ± S.E.) under laboratory conditions (25 ± 2^{o}C, 60 ± 5% RH and 12L: 12D photoperiod)

Treatment (T) regime	*L. huidobrensis* non-reproductive mortality*	Significance level of treatment mortality versus control**	
		χ^2 values	P values
Single releases			
T1-*H. arduine*	15.79 ± 0.58a	10.72	0.001
T2-*D. isaea*	14.78 ± 1.76a	9.23	0.0024
Sequential releases			
T3-*H. arduine* first, *D. isaea* second	12.12 ± 3.81a	29.30	0.0001
T4-*D. isaea* first, *H. arduine* second	14.98 ± 3.74a	23.00	0.0001
Simultaneous releases			
T5-*H. arduine* and *D. isaea*	13.71 ± 2.03a	5.61	0.0178
T6-*H. arduine* and *D. isaea*	22.59 ± 4.85a	92.35	0.0001

*Host mortality induced by parasitoid through host stinging and/or feeding, besides direct parasitization. Means followed by the same letters within columns are not significantly different at P≤0.05 (Tukey-Kramer test). ** Comparison of observed mortality in each treatment (presence of parasitoid species) versus the control (absence of parasitoids)

Table 4.4. Effect of various combinations of *Halticoptera arduine* (*Ha*) and *Diglyphus isaea* (*Di*) on parasitoid F1 progeny sex ratios (mean ± S.E.) under laboratory conditions (25 ± 2^{o}C, 60 ± 5% RH and 12L: 12D photoperiod)

Parasitoid species	Sex ratio	T1	T2	T3	T4	T5	T6
H. arduine	%♀	65.02 ± 1.03aA		67.32 ± 1.41aA	66.21 ± 1.00aA	64.65 ± 1.37aA	61.94 ± 2.00aA
	%♂	34.98 ± 1.03aB		32.68 ± 1.41aB	33.79 ± 1.00aB	35.34 ± 1.37aB	38.08 ± 2.00aB
	χ^2	20.50		27.76	24.00	19.60	13.01
	P	<0.0001		<0.0001	<0.0001	<0.0001	0.0003
D. isaea	%♀		56.45 ± 3.71aA	58.27 ± 2.16aA	66.43 ± 1.98aA	58.07 ± 4.45aA	59.83 ± 3.55aA
	%♂		43.55 ± 3.71aA	41.73 ± 2.16aB	33.57 ± 1.98aB	41.93 ± 4.45aB	40.16 ± 3.55aB
	χ^2		3.75	6.22	25.21	6.88	9.21
	P		0.0529	0.0127	<0.0001	0.0087	0.0024

Key; ♀= females, ♂= males, T1= 50 *Ha*, T2= 50 *Di*, T3= 50 *Ha* first, 50 *Di* second, T4= 50 *Di* first, 50 *Ha* second, T5= 50 Ha +50 *Di*, T6= 25 *Ha* + 25 *Di*. Within rows, means followed by the same lower case letters are not significantly different at *P* < 0.05 (Tukey's test). Within columns, and for each parasitoid species, means followed by the same upper case letters are not significantly different at *P*< 0.05 (Chi-square test).

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 with single releases. In our study, the exotic parasitoid *H. arduine* had no detrimental effect on the specific parasitism performance of the local parasitoid *D. isaea,* whether released first, simultaneously or second. Similarly, the local parasitoid's presence did not affect the specific parasitism of the exotic species independently of combination sequence, indicating co-existence of both species. Possibly, the fact that *H. arduine* is an endo- and *D. isaea* an ecto-parasitoid might have reduced the risk of direct competition of the immature stages of both species. The co-existence could also be related to host discrimination abilities in one or both parasitoids (Bakker et al. 1985). Further studies are warranted to assess the potential host discrimination abilities in both parasitoids as well as the potential mechanisms involved. Beyond the co-existence observed, the introduction of *H. arduine* considerably boosted the total parasitism. Releases of 50 individual parasitoids composed of 25 *H. arduine* and 25 *D. isaea* resulted in 2.5 times more parasitism than when 50 individuals of the indigenous parasitoid species were used. At the same host density, releases of 25 or 50 *H. arduine* resulted in the same level of parasitism, warranting further studies on parasitoid-host density functional and numerical responses. This could guide optimizing the number of parasitoids required for releases in the case of inundative release strategies, such as in greenhouse environments. Moreover, *H. arduine* proved to be superior in LMF control than *D. isaea,* with between 1.4 and 2.6 times higher specific parasitism rates.

Such relatively low reproductive performance of *D. isaea* compared with an endoparasitoid have also been reported by Akutse et al. (2015) while studying the interactions between *P. scabriventris* and *D. isaea* using *L. huidobrensis* as host. Yildirim et al. (2011) and Boot et al. (1992) also reported and modeled low *D. isaea* parasitism rates on *L. bryoniae* (Kaltenbach) and *L. sativae*, respectively, in field studies. Similarly, Mujica & Kroschel (2011) found that *H. arduine* co-existed with a complex of 60 parasitoid species in the field and was more important in controlling the majority of LMF including several *Diglyphus* species. Along the Peruvian coast*, H. arduine* was found parasitizing different leafminer species with up to 66.7% parasitism rates under field conditions (Mujica & Kroschel, 2011).

In parasitoids, non-reproductive host mortality is considered an additional and important mortality factor in pest suppression (Honda et al. 2006). Female parasitoids induce this additional mortality through paralyses of the host by stinging, often followed by host feeding (Jervis & Kidd 1986, Liu et al. 2013, Akutse et al. 2015). In our study, both *H. arduine* and *D. isaea* caused insignificant non-reproductive mortality of *L. huidobrensis* in single and combined release strategies. This insignificant non-reproductive mortality by *H. arduine* is consistent with earlier results observed during our host performance studies on three LMF species (Muchemi et al. 2018c). However, the significant mortalities of *L. huidobrensis* in the presence of *D. isaea* are in line with previous reports by Minkenberg (1989), Liu et al. (2013) and Akutse et al. (2015). In addition, the observed mortalities of *L. huidobrensis* in the presence of *H. arduine* were also notable, though not significant compared with the control. Further studies on the effect of parasitoid density on host non-reproductive mortalities are warranted.

In our study, reproduction of the exotic *H. arduine* always resulted in a female-biased sex ratio, irrespective of the presence of the other parasitoid species. However, for the indigenous *D. isaea*, the sex ratio of its F1 became female-biased only in the presence of *H. arduine*, indicating a synergistic potential when combining both parasitoid species. Since only female parasitoids cause host mortality (Pascua & Pascua 2004, Chow & Heinz 2005, Abe & Kamimura 2012, Foba et al. 2015a), the female-biased parasitoid populations suggest better reproductive and parasitism performances.

4.6. Conclusion

Halticoptera arduine host parasitization is superior to the indigenous *D. isaea* with synergistic improvement on *D. isaea* reproduction potential when both parasitoid species were released simultaneously. Thus, if successfully introduced into the East African horticultural production systems, *H. arduine* might not only co-exist with *D. isaea,* but also raise the total parasitism rates to achieve economically important pest control levels. Low pest pressure may cause small holder farmers to shift away from heavy broad-spectrum pesticides use. Upon receiving the official release permit from the Kenyan authorities, we will therefore assess the performance of the parasitoids under East African field conditions.

5.0. Interaction between *Chrysocharis flacilla* and *Diglyphus isaea* (Hymenoptera: Eulophidae), two Parasitoids of *Liriomyza* Leafminers

This chapter has been published as Muchemi et al (2018). *Journal of Economic Entomology*, 111(2): 556–563.

5.1. Abstract

Agromyzid LMF are a major threat to horticultural production in East Africa with low natural control reported. The endoparasitoid *Chrysocharis flacilla* (Walker; Hymenoptera: Eulophidae) was introduced from Peru into quarantine facilities at *icipe* in Kenya for a leafminer classical biological control program. Interaction assays with one of the dominant local parasitoids, *Diglyphus isaea* (Walker; Hymenoptera: Eulophidae), using *Liriomyza huidobrensis* (Blanchard; Diptera: Agromyzidae) was assessed through sole, simultaneous and sequential releases. *C. flacilla* resulted to superior host parasitism rates over *D. isaea*. When used separately, specific parasitism rates of *D. isaea* and *C. flacilla* were 26.33 ± 2.07% and 60.27 ± 2.53% respectively but, when simultaneously used, the total parasitism rose to 72.96 ± 4.12%. Presence of *C. flacilla* after *D. isaea* reduced significantly parasitism rate of *D. isaea*. Both parasitoids caused separately and simultaneously additionally significant nonreproductive host mortalities of between 48.33 ± 3.75% and 69.33 ± 3.92 for *D. isaea* and *C. flacilla* respectively. Sex ratios of *C. flacilla* and *D. isaea* F1 progenies were female biased and were not affected by interspecific interactions. Implications of these results for subsequent combined use of *C. flacilla* and *D. isaea* against *Liriomyza* leafminers in East Africa are discussed.

5.2. Introduction

Liriomyza huidobrensis (Blanchard; Diptera: Agromyzidae), *Liriomyza sativae* (Blanchard; Diptera: Agromyzidae), and *Liriomyza trifolii* (Burgess; Diptera: Agromyzidae) are the most important leafminer species which contribute to yield and aesthetic losses of up to 100% in vegetables and ornamentals (IPPC 2005, Foba et al. 2015b). *Liriomyza* leafminers are categorized as quarantine pest in the European Union, the main export market of vegetables and ornamentals from East Africa (EU 2000, IPPC 2005, EPPO 2013). Farmers have largely relied on the use of insecticides in the management of these pests. However, over time, they have developed resistance to insecticides which not only are inefficient against the leafminer flies but also adversely affect their associated natural enemies (Tran & Takagi 2005b, Liu et al. 2009, Gitonga et al. 2010, Guantai et al. 2015). In addition, the increased pesticide usage on vegetables has resulted in food safety concerns due to pesticides residues, maximum residue limits being above required levels in vegetable products (RASSF 2015).

Parasitoids make a strong contribution to the service of pest control and have become widely used in pest management (Heinz 2004, Mills & Wajnberg 2008, Pennisi 2010). In the native area of the pests, *Chrysocharis flacilla* (Walker; Hymenoptera: Eulophidae) is one of their dominant parasitoid accounting for 19.5% of total parasitoids with parasitism rates of up to 33.3% under field habitats (Mujica & Kroschel 2011). In addition, *C. flacilla* is a polyphagous parasitoid attacking up to 33 species but all of which belong to the same Agromyzidae family (Salvo & Valladares 1997, Mujica & Kroschel 2011). In East Africa, the diversity of local parasitoids associated with *Liriomyza* leafminers in horticultural field crops is low, with total parasitism rates under field condition below 6% (Chabi-Olaye et al. 2013). In natural habitats, it is possible for more than one parasitoid to share a common host resource (Godfray et al. 1994, Brodeur & Rosenheim 2000, Harvey et al. 2013). In such situations, interactions between parasitoids are inevitable and could either enhance pest suppression where synergetic and additive effects occur or have dire consequences on inferior parasitoid where competition occurs (Talekar & Yang 1993, Pianka 2000, Bogran et al. 2002, Reitz & Trumble 2002, Tian et al. 2008).

Biological control projects have successfully contributed to the protection of the flora and fauna of many natural ecosystems, and are presently a component in many recovery and restoration efforts worldwide (Hoddle et al. 2013). According to van Lenteren et al. (2006) and van Driesche et al. (2010), many classical biological control programs involving the introduction of exotic parasitoids into new areas against insect pests have rarely resulted in negative effects to the environment or beneficial nontargets. The authors reported 81% success rate in the protection of biodiversity by classical biological programs targeting insect pests, 62% achieved complete control of target pests and 19% achieved partial control. 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).

The introduction of exotic parasitoids into a backdrop of existing parasitoid populations has the risk of causing interspecific competition that may lead to ecological disruption (Boettner et al. 2000, Mills & Wajnberg 2008, Pennisi 2010, Peralta et al. 2014). Considering that *C. flacilla* and *Diglyphus isaea* (Walker; Hymenoptera: Eulophidae) are solitary parasitoids preferring and attacking the same larval stage of the host (Salvo & Valladares 2002, Liu et al. 2009, Peralta et al. 2011, Akutse et al. 2015), it is possible that interspecific competition may occur. Such interspecific competition can influence the size, structure, and stability of parasitoid communities (Jalali et al. 1988, Louda et al. 2003, Hardy et al. 2013), and may also affect the establishment of the introduced parasitoid as well as reduce the performance of existing parasitoids in pest population regulation thereby affecting the overall success of biological control programmes (Dent 2000, Follett et al. 2000, Bogran et al. 2002, De Moraes & Mescher 2005, Peri et al. 2011). Critical parasitoid performance parameters such as specific and total parasitism rates, nonreproductive mortality and progeny sex ratio are commonly used to assess parasitoids interspecific interactions (Akutse et al. 2015, Foba et al. 2015a). Parasitism rate is the most important parameter used to evaluate performance of a parasitoid and its potential in controlling a particular pest. Specific parasitism rate displayed by a particular parasitoid species in absence or presence a second parasitoid provides a direct measurement to assess whether the presence of the latter affected or not its performance (Chabi-Olaye et al. 2001, Akutse et al. 2015, Foba et al. 2015a). Total parasitism has also been reported to be higher when various species of parasitoids are used compared to each one used separately (Hunter et al. 2002, Akutse et al. 2015, Foba et al. 2015a, Wang et al. 2015). However, various studies have shown that two species with similar niches will often compete strongly with each other, resulting in ecological disruptions (Duyck et al. 2004, Bajpai et al. 2006, Tian et al. 2008, Harvey et al. 2013, Xu et al 2013, Foba et al. 2015a). In various parasitoids, and particularly ecto-parasitoids like *D. isaea*, induced host nonreproductive mortality, through host feeding and stinging, have been reported to be very critical in parasitoids' performance measurement (Jervis & Kidd 1986, Mafi & Ohbayashi 2010, Liu et al. 2013, Akutse et al. 2015). In parasitoids, progeny sex ratio is critical for population stability and parasitoid efficiency; with a female-biased sex ratio preferred over a male-biased one, as only females directly contribute to the mortality of pests both through parasitism and nonreproductive mortality (Mills & Getz 1996, Ode & Heinz 2002, Chow & Heinz 2005, Guo et al. 2014, Foba et al. 2015a). Furthermore, depending on suitability of environment, including host availability, weather conditions and competition, parasitoids have the potential of modulating their progeny's sex ratio towards a male or female bias (Chow & Henz 2005).

In an effort to improve leafminers parasitism rates in East Africa through biological control, *C. flacilla* was imported from Peru into Kenya by the International Centre of Insect Physiology and Ecology (*icipe*), in collaboration with the International Potato Center (CIP), under the Leafminer IPM program. Under laboratory conditions, *C. flacilla*, accepted, developed and caused high host parasitization of the three most important *Liriomyza* species found in Kenya (Muchemi et al. 2018a). *D. isaea* is one of the dominant and important local LMF ectoparasitoid within the Kenyan agro-ecosystems, accounting for more than 60% of total parasitoids (Guantai et al. 2015, Foba et al. 2015c). To explore the potential benefit of introducing the new parasitoid to the existing complex and avoid potential negative impacts on existing parasitoid populations arising from it, studies on its interaction with the existing biological control agent are required (Boettner et al. 2000, Louda et al. 2003). This study was therefore designed to evaluate interspecific interaction between *C. flacilla* and the locally existing *Liriomyza* parasitoid, *D. isaea* for its consideration as a potential biological control agent in Kenya to boost parasitism rates of *Liriomyza* leafminers in East Africa.

5.3. Materials and Methods

5.3.1. Plant Materials

An open pollinated variety of Kenyan Faba bean, *Vicia faba* L. (Fabales: Fabaceae) was used for the rearing of *L. huidobrensis* and parasitoid species. Five seeds were planted in plastic pots (5.5 cm diameter × 7.3 cm high) filled with the planting substrate (mixture of soil and manure in a ratio of 5:1). Potted plants were maintained in a screen house (2.8 m length × 1.8 m width × 2.2 m height), ICIPE Duduville campus, Nairobi, Kenya at 25 • ± 2°C for 2 wk. Two-week-old plants were used for exposure to adult *L. huidobrensis* on which the parasitoid interaction experiments were conducted using procedures adapted from Akutse et al. (2015) and Foba et al. (2015a).

5.3.2. Insect Colonies

5.3.2.1. Leafminer Colony

L. huidobrensis was selected for this study because it is the most abundant LMF in Kenya representing more than 90% of *Liriomyza* species occurring across a wide range of vegetable production agro ecological zones (Chabi-Olaye et al. 2013, Foba et al. 2015a, Foba et al. 2015b). A colony of *L. huidobrensis* was initiated from field collections from, Nyeri County (0°21′S, 36°57′E, 2200 m.a.s.l) in Central Kenya highlands since 2007 and reared on faba bean plants at *icipe*. Two-day-old adult leafminer flies were released in Perspex cages (60 cm length × 60 width × 60 cm height) with potted faba plants for 24 h for egg laying. The colony was cultured at 25 ± 2°C, 60 ± 5% relative humidity (RH) and a photoperiod of 12:12 (L:D) h. Infested plants were removed and held in wooden cages (45 cm wide × 45 cm long × 60 cm high) for 5 to 6 d for the development of similar age second to third cohort of instar larvae. Plants were cropped at the base of the stem and incubated on mesh trays for pupae dropping. Pupae were incubated in Petri dishes for adult emergence. Adult leafminers were fed on 10% sugar solution for 2 d after emergence to allow for pre-oviposition period before experimental use (Chabi-Olaye et al. 2013, Foba et al. 2015a, Akutse et al. 2015).

5.3.2.2. Parasitoid Colonies

The initial culture of *C. flacilla* was imported from the CIP, Peru in 2012, where they were maintained on *L. huidobrensis*. At *icipe* quarantine facility, the parasitoid was maintained on *L. huidobrensis* 2nd to 3rd instar larvae reared on faba bean at 21 ± 1°C and 55 ± 5% RH for six generations before experimental use.

Initial culture of the indigenous parasitoid, *D. isaea* was recovered from *Pisum sativum* L. (Fabales: Fabaceae) collected from farmer fields in Naromoru (0°18′ S, 036°84′ E, 1975 m.a.s.l) of Nyeri County in 2013. Cultures of *D. isaea* were then reared on *L. huidobrensis* at *icipe* insect rearing facilities (25 ± 1°C and 60 ± 5% RH) for five generations before experimental use as described by Akutse et al. (2015).

Adult parasitoids of both species were fed on 10% honey solution after emergence for 2 d. Parasitoid species and host pest were reared in isolated separate rooms in laboratory to avoid contamination.

5.3.3. Experimental Procedure

Ten pots containing each five un-infested 2-wk-old *V. faba* plants from the screen house were placed per aerated Perspex cage (30 cm x 30 cm x 45 cm). Each cage contained a treatment and each treatment was replicated six times. Two-hundred-two-day old *L. huidobrensis* adults in the ratio of 1:2 (males: females) were exposed in each cage for 24 h to the plants for egg laying before their removal. Infested plants were held for 5 to 6 d at 25 ± 2°C, 60 ± 5% RH and photoperiod 12:12 (L:D) h for the development of similar second to third age cohort instar larvae. Pots were covered at the basis of the plants with aluminum foil to minimize the loss of host larvae in potted soil. From each cage, an average of 176 leafminer pupae that dropped from the infested leaves were collected for incubation.

Two-day-old adult parasitoids were then introduced in each of the six respective treatments comprising of sole release of 50 *C. flacilla* (T1), sole release of 50 *D. isaea* (T2), simultaneous release of 50 *C. flacilla* and 50 *D. isaea* (T3), simultaneous release of 25 *C. flacilla* and 25 *D. isaea* (T4), sequential release of 50 *C. flacilla* before 50 *D. isaea* (T5), sequential release of 50 *D. isaea* before 50 *C. flacilla* (T6) and a control without parasitoids (T7) as described in Table 5.1 for 24 h before their removal by aspiration. Leafminer larvae were held on the plants for 2–3 d for pupae development at 21 ± 2°C, 55 ± 5% RH and photoperiod 12:12 (L:D) h. To confirm the solitary nature of parasitoids, pupae were collected using fine camel hair brush, transferred singly into transparent gelatin capsules (2.2 cm height and 0.7 cm diameter) and then incubated for 30 d to allow for the emergence of adult leafminers or *C. flacilla* as described in Akutse et al. (2015) and Foba et al. (2015a). Pupae without exit holes and where insects failed to completely exit were dissected under binocular microscope following the methodology described by Heinz and Parrella (1990) to correct for *C. flacilla* parasitism and host mortality. *D. isaea* always parasitizes its host by injecting the venon into the larvae before depositing the eggs on or close to the parasitized larvae. Parasitized larvae were therefore not able to drop for pupation and instead, plant foliage was cropped and maintained in separate aerated lunch boxes (19 cm long x 13 cm wide x 8 cm high) for 20 d to allow for adult emergence (Akutse et al. 2015). The leaves were later examined under microscope to correct for *D. isaea* parasitism and host mortality. Host parasitized by *D. isaea* did not pupate while those parasitized by *C. flacilla* pupated before yielding either adult *C. flacilla* or adult leafminer. Results from dissection of un-hatched pupae were used to correct number of host parasitized by *C. flacilla* while number of dead *D. isaea* found on leaves were used to correct *D. isaea*’s parasitism. The experiment was set in a Randomized Complete Block Design (RCBD).

5.3.4. Assessment of Parasitoid Interactions

Interactions between *C. flacilla* and *D. isaea* in parasitizing *L. huidobrensis* larvae were evaluated using the number of emerged insects for both the host pest and parasitoids in each treatment following procedures described by Wang & Messing (2002), Bader et al. (2006), Akutse et al. (2015) and Foba et al. (2015a). Six parasitoid combinations were set as described in Table 1. Parasitoids were introduced at the ratio of 1:2 (male:female). To assess the effect of parasitoid combinations on leafminer suppression, specific and total parasitism rates were determined. Specific parasitism rates were calculated as the corrected number of each parasitoid species divided by sum total of parasitoids and leafminer multiplied by 100 from each treatment. Total parasitism rates were calculated as the corrected number of total sum of both parasitoids divided by sum total of parasitoids and leafminer multiplied by 100 from each treatment where both parasitoids occurred. To assess effect of parasitoid release combinations on sex ratio, F1 parasitoid progeny from each treatment were sexed into males and females and their proportions determined as a percentage of the number of total parasitoids. To assess parasitoid’s actual nonreproductive host mortality on *L. huidobrensis*, total number of pupae in control was used to correct for natural pupal mortality in treatments using Abbott’s formula (Abbott 1925, Akutse et al. 2015).

Table 5.1. Summary of parasitoid release strategies

Treatments (T)	Parasitoid release pattern
Single release	
C. flacilla only (T1)	50 *C. flacilla* alone for 24 h at 1:2 (15♂:35♀)
D. isaea only (T2)	50 *D. isaea* alone for 24 h at 1:2 (15♂:35♀)
Sequential release	
C. flacilla first, *D. isaea* second (T3)	50 *C. flacilla* for 24 h, followed by 50 *D. isaea* for 24 h at 1:2 (15♂:35♀ of each species)
D. isaea first, *C. flacilla* second (T4)	50 *D. isaea* for 24 h, followed by 50 *C. flacilla* for 24 h at 1:2 (15♂:35♀ of each species)
Simultaneous release	
C. flacilla and *D. isaea* (T5)	50 *C. flacilla* + 50 *D. isaea* for 24 h at 1:2 (15♂:35♀ of each species)
C. flacilla and *D. isaea* (T6)	25 *C. flacilla* + 25 *D. isaea* for 24 h at 1:2 (15♂:35♀ of each species)
Control	
L. huidobrensis reared alone (T7)	No parasitoids release

♂: males, ♀: females
Adapted from Foba et al. (2015)a and Akutse *et al*. (2015)

5.3.5. Data Analyses

Data on specific and total parasitism rates, nonreproductive pupae host mortality and sex ratio were normalized by Arcsine transformation before being subjected to data analysis. Within treatments specific parasitism rates between both species were compared using Chi square test. For the same parasitoid species however, specific parasitism rate, total parasitism rate, actual nonreproductive host mortality and sex ratio in the different treatments were compared using one-way Analysis of Variance (ANOVA). Where differences were observed in ANOVA, means were separated using Tukey-Kramer HSD test. The significance of nonreproductive mortality for each treatment was assessed by comparing natural mortalities in the control with mortalities in presence of parasitoid using Chi-square test. Count numbers of male and female parasitoids from each treatment were log-transformed (x + 0.5) before analysis. Differences in progeny sex ratio of parasitoids within treatments were assessed using Chi-square test at $P \leq 0.05$. The statistical programs used for these analyses were SAS JPM-version 11 statistical package (SAS 2013) and R version 3.1 statistical software package (R Development Core Team 2013).

5.4. Results

From a total of 7,401 *L. huidobrensis* capsulated pupae and *D. isaea* puparia observed from leaves, the parasitized pupae resulted each to a single *C. flacilla* or *D. isaea* parasitoid per pupa, even in simultaneous (T3 and T4) and sequential (T5 and T6) releases.

Specific parasitism rates on *L. huidobrensis* varied in treatments where both *C. flacilla* and *D. isaea* parasitoids occurred (T1, T3, T4, T5, T6 and T2, T3, T4, T5, T6) ($F4,29$ = 5.39, $P \leq 0.05$ and $F_{4,29} = 4.39$, $P \leq 0.05$, respectively) (Table 5.2). Overall, specific parasitism rates caused by *C. flacilla* on *L. huidobrensis* in T1 versus T2, and within T3, T4, T5 and T6 were significantly superior to those of *D. isaea* ($P < 0.0001$) (Table 5.2). *C. flacilla* specific parasitism rate was 2.3 times higher than *D. isaea* parasitism rate in single releases. The exotic parasitoid's specific parasitism rates remained constantly above the indigenous parasitoid's parasitism rate in all interspecific combinations, with up to 3.2 times the latter's specific parasitism rate in simultaneous release scenario. The presence of *C. flacilla* in simultaneous parasitoids releases (T5 and T6) and in sequential release of *D. isaea* after *C. flacilla* (T3) had no significant effect on *D. isaea* specific parasitism except in sequential introduction of *C. flacilla* after *D. isaea* (T4) where its parasitism rate became significantly lower than when *D. isaea* was used alone (T1). Similarly, interspecific interaction with *D. isaea* did not reduce significantly *C. flacilla* specific parasitism rate in any of the parasitoid combinations. Under the same host conditions, 25 individuals of *C. flacilla* in simultaneous release caused significantly less parasitism rate than 50 *C. flacilla.* In the contrary, 25 *D. isaea* under the same host conditions caused similar parasitism rates as 50 *D. isaea* in simultaneous release strategy. Total parasitism rate was significantly higher when both parasitoid species were used simultaneously at high densities ($F_{5,35} = 11.75$, $P < 0.0001$). There was improved total parasitism rates in parasitoid combinations compared to sole release of *D. isaea* (T1) (Table 5.2).

Across all release combinations, *C. flacilla* and *D. isaea* caused significant nonreproductive mortalities of host pupae compared to the control (*C. flacilla*: χ^2 = 136.67, $P = 0.01$; *D. isaea*: χ^2 = 67.96, $P = 0.0001$). The highest nonreproductive mortality was recorded during simultaneous release of 50 individuals of each parasitoid species, with 86.32 ± 3.72% of host mortality.

Nonreproductive host pupal mortality was significantly higher in simultaneous use of 50 parasitoids of each species and sole release of *C. flacilla* than in simultaneous introduction of low density parasitoids of 25 *D. isaea* and 25 *C. flacilla* (T6) ($F_{5,34} = 4.40$, $P = 0.0042$). There was however no significant difference between nonreproductive mortalities in simultaneous release of 25 individuals of each species and the sole release of 50 individuals of *D. isaea*. Nonreproductive host pupa mortality in 50 *D. isaea* (T2) and sequential introduction of 50 *C. flacilla* before or after 50 *D. isaea* (T3 and T4) were statically similar and not different from pupa mortality observed in 50 *C. flacilla* (T1) and in combined use of 50 *C. flacilla* and 50 *D. isaea* In both parasitoid species, sex ratios in F1 progenies were significantly female-biased (*C. flacilla*: χ^2 = 184.51, $P <$ 0.0001; *D. isaea*: χ^2 = 64.03, $P < 0.0001$) (Table 5.3). Sex ratio was not affected by the different parasitoid release combinations tested (*C. flacilla*: $F4,29 = 1.38$, $P = 0.2682$; *D. isaea*: $F4,29 = 0.58$, $P = 0.7161$) (Table 5.3).

(Fig. 5.1).

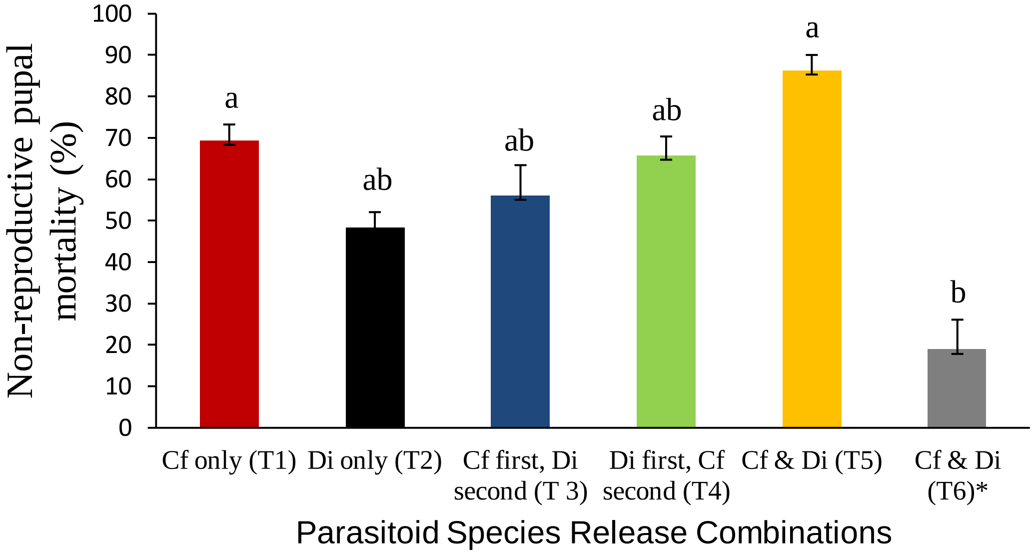


Fig. 5.1. Nonreproductive pupal mortality of *Liriomyza huidobrensis* caused by *Diglyphus isaea* (*Di*) and *Chrysocharis flacilla* (*Cf*) in various release combinations.

Table 5.2. Mean ± S.E for total and specific parasitism rate of *Chrysocharis flacilla* (*Cf*) and *Diglyphus isaea* (*Di*) on *Liriomyza huidobrensis* for each treatment

Treatment (T) regime	*C. flacilla* specific parasitism (%)	*D. isaea* specific parasitism (%)	χ^2	P value	Total parasitism (%)
Single release					
50 *Cf* only (T1)	60.27 ± 2.53a	-		-	60.27 ± 2.96ab
50 *Di* only (T2)	-	26.33 ± 2.07a	-	-	26.33 ± 3.68c
Sequential release					
50 *Cf* first, 50 *Di* second (T3)	47.82 ± 3.96abA	17.80 ± 3.62abB	155.84	<0.0001	65.62 ± 2.97ab
50 *Di* first, 50 *Cf* second (T4)	40.13 ± 4.49abA	11.29 ± 2.07bB	257.06	<0.0001	51.42 ± 5.19b
Simultaneous release					
50 *Cf* & 50 *Di* (T5)	55.39 ± 3.52aA	17.57 ± 1.03abB	246.33	<0.0001	72.96 ± 4.12a
25 *Cf* & 25 *Di* (T6)	32.20 ± 1.80bA	17.74 ± 2.35abB	71.78	<0.0001	49.94 ± 3.47b

Within columns, means followed by the same lower case letters are not significantly different at P≤0.05 (Tukey-Kramer test). Within rows and between parasitoid species, means followed by same upper case letters are not significantly different at P≤0.05 (Chi-square test).

Table 5.3. Effect of presence and sequence of introduction of *Chrysocharis flacilla* and *Diglyphus isaea* on the F1 progeny sex ratios of the parasitoids

Parasitoid species	Sex ratio	Parasitoid release combinations / Treatments					
		T1	T2	T3	T4	T5	T6
C. flacilla	% ♀	76.50 ± 2.20aA		68.43 ± 1.96aA	76.76 ± 1.78aA	78.27 ± 4.55aA	72.74 ± 7.06aA
	% ♂	23.50 ± 2.20aB		31.56 ± 1.96aB	23.24 ± 1.78aB	21.73 ± 4.55aB	27.26 ± 2.89aB
	χ^2	184.51		46.04	156.10	174.33	115.56
	P value	0.0001		0.0001	0.0001	0.0001	0.0001
D. isaea	% ♀		67.10 ± 3.90aA	62.37 ± 4.73aA	61.43 ± 1.84aA	63.09 ± 2.28aA	66.63 ± 2.89aA
	% ♂		32.90 ± 3.90aB	37.63 ± 4.73aB	38.57 ± 1.84aB	36.91 ± 2.28aB	33.37 ± 2.89aB
	χ^2		64.03	9.80	7.28	10.37	28.12
	P value		0.0001	0.02	0.01	0.001	0.0001

♀ females, ♂ males. T1, T2, T3, T4, T5 and T6 see description on Table 5.1.Within rows (columns), means followed by the same lower (upper) case letter are not significantly different at $P \leq 0.05$ according to Tukey-Kramer (Chi-square) test.

5.5. Discussions

The present study has shown the superior performance of *C. flacilla* in parasitizing *L. huidobrensis* than that of the indigenous *D. isaea*. While the single release of *C. flacilla* resulted in more than 60% parasitism, *D. isaea* under the same conditions yielded two times less parasitism. The current results for *D. isaea* are similar to parasitism rates reported by various authors. Boot et al. (1992), Yildirim et al. (2011) and Akutse et al. (2015) consistently reported 30% parasitism rate of *D. isaea* on *L. sativae*, *Liriomyza bryoniae* (Kaltenbach; Diptera: Agromyzidae) and *L. huidobrensis*.

The study has also confirmed the solitary nature of *C. flacilla* and *D. isaea* parasitoids as previously reported, whether used alone or in combination (Ode & Heinz 2002, Salvo & Valladares 2002, Liu et al. 2009, Peralta et al. 2011, Akutse et al. 2015). Furthermore, while both parasitoids belong to the same taxonomic family and targeting the same host stage, they have a differential spatial requirement. Because *C. flacilla* is an endoparasitoid (Salvo & Valladares 2002, Peralta et al. 2011) while *D. isaea* is an ectoparasitoid (Liu et al. 2009, Akutse et al. 2015), only *C. flacilla* emerged from the host pupae while *D. isaea* emerged directly from the leaves, reducing risk of cannibalism between larvae of the two species. Further studies are however required to assess the possibility and frequency of same host larvae being parasitized by both species through establishment of exact positioning of egg laid.

The presence of *D. isaea* had no detrimental effect on *C. flacilla* specific parasitism rate in any of the parasitoid combinations compared to the sole release of the exotic parasitoid whether introduced before, after or simultaneously. This suggests that when released in the field, the exotic parasitoid, *C. flacilla*, may not suffer any reduced performance resulting from interaction with the local parasitoid, *D. isaea*. We hypothesize that, beyond the cryptic positioning of *C. flacilla* eggs that protects them from any potential direct attack (stinging or feeding) by adult female *D. isaea*, the high fecundity of *C. flacilla* (can oviposit 13.8 eggs/female/d at optimum temperature of 20°C) (Mujica et al. 2009), as compared to the low fecundity of *D. isaea* (can only oviposit 3.5 eggs/ female/d) (Zhang et al. 2011) could justify the higher performance of *C. flacilla* compared to *D. isaea*. We therefore hypothesize that the relatively low number of *D. isaea* eggs and subsequent larvae could result into low pressure on *C. flacilla* in the system. Finally, it is also possible that female *D. isaea* successfully discriminates against larvae already parasitized by *C. flacilla*. Various studies demonstrated that female parasitoids use their antennae and/or ovipositor to probe host and assess whether it is already parasitized or not prior to their own oviposition, and, after their oviposition, often mark their own parasitized hosts in an effort to avoid superparasitism and multiparasitisms (Van Lenteren 1981, Agboka et al. 2002). However, other parasitoids are reported to either ignore or prefer previously parasitized hosts (Chabi-Olaye et al. 2001, 2004). However, further studies are warranted to elucidate how *C. flacilla* escapes potential detrimental effects in the presence of *D. isaea*. These studies should purposely localize the positions of both parasitoids eggs in regard to already parasitized hosts and observe behavior of female parasitoids during oviposition in or on previously parasitized hosts. The effect that *C. flacilla* had on the performance of *D. isaea* depended, however, on sequence of release. For instance, while the introduction of *C. flacilla* as second parasitoid in the system (T4) reduced significantly *D. isaea*'s specific parasitism rates compared to its sole use, the use of *C. flacilla* in simultaneous release (T5) and as first parasitoid in the system (T3) did not affect *D. isaea's* specific parasitism rate. This finding contrasts with the common situations where the first parasitoid to oviposit into a host becomes the dominant one due to resource depletion (Mills 2003, Couchoux & Van Nouhuys 2013, Mustu & Kilincer 2015). Foba et al. (2015a) for instance, showed that the first parasitoid introduced in interaction studies between *Phaedrotoma scabriventris* (Nixon) (Hymenoptera: Braconidae) and *Opius dissitus* (Muesbeck) (Hymenoptera: Braconidae) using *L. huidobrensis* always had a higher parasitism rate than the second parasitoid. We hypothesize that, considering the low oviposition rate of *D. isaea*, when introduced as second parasitoid in the system or when used simultaneously, *D. isaea* female discriminate and avoid already parasitized hosts by *C. flacilla* and this could be the reason why no difference was found in its parasitism rates when compared to its sole use. However, when *D. isaea* was first used and *C. flacilla* had the choice 24 h later, *C. flacilla* females might not have avoided hosts already occupied by *D. isaea*. Therefore, it is also possible that *C. flacilla* adult females could have laid eggs in hosts already parasitized by *D. isaea*. However, further studies are required to understand the host searching behavior of *C. flacilla* in presence of already parasitized host larvae by a different parasitoid species such as *D. isaea* monitored. We also hypothesize that this difference in the effect of *C. flacilla* on *D. isaea* parasitization may be due to density of host larvae available during the present experiment, implying that in systems with abundance of hosts, no detrimental effect on specific parasitism might be observed (Montoya et al. 2006, Finke & Snyder 2008, Tylianakis et al. 2008, Wilby & Orwin 2013). Further studies to assess the mechanism of *C. flacilla* host discrimination as well as effect of host densities on its parasitism and host selection are recommended.

In addition to the fact that significantly lower specific parasitism rates of *D. isaea* were obtained only when *C. flacilla* was exploiting host resource already used by *D. isaea*, our results further demonstrated a boost to the overall host suppression. In parasitoid combinations where 50 individual *C. flacilla* were added to 50 *D. isaea*, the total parasitism rates obtained ranged between 2.0 and 2.8 times the total parasitism rates of sole use of 50 *D. isaea*. In the native and invaded areas of *Liriomyza* species, parasitoids play an important role in regulating their populations (Shepard et al. 1998, Murphy & LaSalle 1999, Rauf et al. 2000, Mujica & Kroshel 2011). Over 300 species of parasitoids are associated with leafminers worldwide (Noyes 2004). In Peru, a complex of 63 parasitoid species is associated with *Liriomyza* leafminers causing parasitism rates of between 20 and 55% (Mujica & Kroschel 2011). The introduction of more than one parasitoid into ecosystems has also shown increased suppression of various target pests. For instance, the introduction of more than one *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) parasitoids including *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae), *Tetrastichus sokolowaskii* (= *Oomyzus sokolowaskii*) Kurdjumov (Hymenoptera: Eulophidae), *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), and *Cotesia plutellae* Kurdjumov (Hym., Braconidae) in many parts of the world has led to its suppression and increased production of crucifers (Talekar & Shelton 1993, Shi et al. 2004, Momanyi et al. 2006). The introduction of *C. flacilla* into horticultural agro ecosystems is likely to further boost *Liriomyza* leafminers host suppression in East Africa. However, more studies are warranted to evaluate interaction between *C. flacilla* and *O. dissitus,* the most important local *Liriomyza* parasitoids in the lower agro-ecological elevations of Kenya (Foba et al. 2015c). The present study was conducted under laboratory conditions where parameters were controlled and it is therefore likely that performance under field conditions could be different from current results. Various parameters like temperature, altitude, host plant, host diversity and abundance, timely host detection, among others, under field conditions have been reported to affect parasitoid performance (Malhi et al. 2010, Rehman & Powel 2010, Kessler et al. 2011). For instance, while laboratory studies demonstrated superiority of *P. scabriventris* and *O. dissitus* over *D. isaea* in terms of parasitism rate under same experimental conditions as in the present study (Akutse et al. 2015, Foba et al. 2015a), under field conditions however, Foba et al (2015c) confirmed the superiority of *P. scabriventris* and *O. dissitus* only at low altitude levels while both were dominated by *D. isaea* at high altitude levels. Further field studies are therefore warranted to assess the performance of *C. flacilla* under various agro-ecological conditions.

The phenomenon of nonreproductive host mortality amongst endoparasitoids of *Liriomyza* is not as common as it is in ectoparasitoids (Minkenberg 1989, Akutse et al. 2015). In addition to host parasitization, our results confirm *C. flacilla'*s ability to cause significant nonreproductive host mortality, a rare attribute in endoparasitoids. Nonreproductive mortality from *C. flacilla* was significant and in equal measures to the one caused by *D. isaea*. In addition, the combined nonreproductive mortality is high than that of each parasitoid used alone, and higher than the combined mortality reported by Akutse et al. (2015) for *D. isaea* and *P. scabriventris*. We hypothesize therefore that the introduction of *C. flacilla* in areas where *D. isaea* is reported could result in an increase in *Liriomyza* pest suppression not only through parasitism but also a synergy in nonreproductive mortality caused by both parasitoids.

Although interacting species have the potential to negatively alter individual species reproduction, in our present study, the F1 progenies derived from all the treatment combinations for both parasitoid species were female biased, suggesting that the interaction between both parasitoids did not have an effect on progeny sex ratio. Our results are consistent with observations made by Akutse et al. (2015) and Foba et al. (2015a) who also found no effect of leafminer parasitoids combination on their progeny sex ratio for *P. scabriventris* versus *D. isaea* and *P. scabriventris* versus *O. dissitus*, respectively.

5.6. Conclusions

Interaction between *C. flacilla* and the locally existing *D. isaea* on *L. huidobrensis* under laboratory conditions showed no detrimental effect on *C. flacilla* performance no matter the combination approach while at the same time *C. flacilla* co-exists with *D. isaea* when introduced simultaneously or as first parasitoid. However, *C. flacilla* significantly reduces the specific parasitism rate of *D. isaea* when introduced as last parasitoid in the system. The total parasitism from combination of both parasitoids was higher than in sole use of *D. isaea*. Both parasitoids had female biased sexratios and produced significant nonreproductive mortalities when used alone and these were not altered but rather improved by combining both parasitoids. The introduction of *C. flacilla* could therefore contribute considerably to LMF suppression in East Africa agro ecosystems and further studies are required to assess its potential under field conditions.

6.0. Field establishment of *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) and *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae); in Kenya

6.1 Abstract

LMF are invasive pests of economic importance to horticultural crops in East Africa. Parasitoids are important in regulating *Liriomyza* pest populations. *Halticoptera arduine* (Hymenoptera: Pteromalidae) and *Chrysocharis flacilla* (Hymenoptera: Eulophidae) were imported from Peru into Kenya for a leafminer biological control program. Parasitoids were mass reared in the laboratory and released in farmer fields across three agro ecological elevations. Field survey around release sites were conducted every month in 2013 after *H. arduine* release and twice on quarterly basis in 2014 following *C. flacilla* release. Three species of *Liriomyza* leafminers, reared from 20 plant species were most important leafminers. Of the three, *Liriomyza huidobrensis* (Diptera: Agromyzidae) was the dominant species across the elevation zones. 14 parasitoid species including the introduced *H. arduine* and *C. flacilla* were reared from *Liriomyza* hosts. Significant numbers of *H. arduine* were recovered in 2013 in the first three months of wet hot and dry hot seasons across the high and mid agro ecological elevations. *Chrysocharis flacilla* was recovered during the dry hot and dry cold seasons in 2014 across the three agro ecological elevations. Average field parasitism rates across the three agro ecological elevations ranged between 21.54 ± 2.02 and 40.00 ± 2.21% during 2013 and 2014. Overall, *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) caused the highest parasitism rates compared to other parasitoids. Parasitism by *C. flacilla* reached 3.5% and compared to that of *D. isaea* in the low elevation. Seasons significantly affected establishment of both *H. arduine* and *C. flacilla* ($P<0.0001$) but *C. flacilla* was further impacted by cropping systems ($P<0.0001$) and host plants ($P<0.0001$). By end of the first year, *H arduine* had spread 10 Km from the release point in the low elevation zone. The potential for *H. arduine* and *C. flacilla* in biological control program against *Liriomyza* leafminers in East Africa is discussed.

6.2. Introduction

Agriculture in Kenya generates an average of 25% of the Gross Domestic Product (GDP) where tea, coffee, and horticulture are the top foreign exchange earners (KNBS 2017). In 2016, the horticulture sector contributed slightly over USD 1 billion to the national GDP from the export of fruits, vegetables, and ornamentals (KNBS 2017, KFC 2016). This sector directly employs more than six million people in production, processing, and marketing (NHP 2012, KHC 2015). Therefore, it is a source of food security, income, and means to poverty alleviation in Kenya. The continued growth of the horticultural sector in many developing economies of the sub-Saharan Africa is challenged by production, access to markets and pest infestations. Infestation by *Liriomyza* leafminers (Diptera: Agromyzidae) is of economic importance worldwide because of their polyphagous nature and invasive potential in natural and cultivated ecosystems (Spencer 1985, Parrella 1987, Murphy & LaSalle 1999, Rauf et al. 2000). The three most important species in Kenya are *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) (Lachaal 2006, Liu et al. 2009, Gitonga et al.

2010, Foba et al. 2015b). *Liriomyza huidobrensis* is the most dominant species in vegetable production systems in Kenya contrary to earlier reports of *L. sativae* and *L. trifolii* dominance in the mid and low elevations respectively (Neuenschwander et al. 1987, Chabi-Olaye et al. 2008). Crop losses and damage associated with LMF infestation is dependent on the crop, season, and region and can be as high as 100% (CABI 2007, Chabi-Olaye et al. 2008, Foba et al. 2015b). LMF are important in trade and are categorized as quarantine in the European Union (EU) markets (EU 2000, IPPC 2005, EPPO 2013). The EU is the main trading partner for East African horticultural products and presence of LMF in the export produce has been a cause of interceptions in the EU. For instance, between 2013 and 2017, an average of 10% of annual interceptions is due *Liriomyza* species (EUROPHYT 2018). Due to this importance and severity of infestation, farmers, have the tendency to extensively use synthetic insecticides against these pests (Gitonga et al. 2010). This further impairs trade in fresh produce due to exceedances in pesticide residues (Maximum Residue Limits (MRLs)) (RASFF 2015). The most destructive larval stages are usually concealed within the leaf tissues and few of the active ingredients registered in Kenya against *Liriomyza* leafminers cause mortality of the larval stage (Gitonga et al. 2010, Guantai et al. 2015). In most cases, insecticides have proved ineffective due to the ability of *Liriomyza* leafminers to build resistance to several groups of insecticide molecules (Tran & Takagi 2005b, Liu et al. 2009, Guantai et al. 2015).

Efficient and environmental friendly management approaches are therefore necessary to overcome the increasing food safety concerns, environmental degradation, loss of revenue, high cost of production and food insecurity in the East African region. In the native origins of Agromyzid leafminers, parasitoids play an important role in regulating pest populations. Circumstantial evidence, suggests that parasitoids can have significant impact after their introduction (Murphy & LaSalle 1999). Biological control, as part of integrated pest management (IPM) is a reliable and economical management strategy for LMF in their native and invaded areas (Johnson 1993, Shepard et al. 1998, Murphy & LaSalle 1999, Rauf et al. 2000, Mujica & Kroshel 2011). More than 300 species of parasitoids are associated with leafminer worldwide (Murphy & LaSalle 1999, Noyes 2004). In the Neotropics of South America, the native origin of *Liriomyza* leafminers, a complex of more than 63 parasitoid species is associated with LMF in the natural habitats (Mujica & Kroschel 2011). In the Central coast of Peru, three most important parasitoids associated with *Liriomyza* leafminers include *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae), *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) and *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae) (Salvo & Valladares 2007, Mujica & Kroschel 2011). In Peru, these parasitoids have a wide ecological range from the coastal lowlands to the highlands of 4,045 m a.s.l (Aguilera 1972, De Santis 1983, Neder de Roman & Arce de Hamity 1984, Arellano & Redolfi 1989, Mujica & Kroschel 2007). Parasitoids in this region cause LMF parasitism rates of between 20% and 55% (Mujica & Kroschel 2011). Worldwide, notable parasitoid introductions in biological control programs against *Liriomyza* leafminers in protected commercial and field cultivated ecosystems have been successful (Neuenschwander 1987, Petcharat et al. 2002, Liu et al. 2009). Most of the introductions however, have occurred in the native origins of *Liriomyza* species in

America, Europe, and Asia in the 70's and 80's (Liu et al. 2009) with few recent reports in the invaded areas of Africa.

In East Africa, records of LMF and their parasitoids are scanty. In the early 1970, the first report of *Liriomyza* introduction in Kenya through *Chrysanthemum* cuttings (Spencer 1985) was reported. Since then, few records on *Liriomyza* are reported until when authors including Chabi-Olaye et al. (2008), Musundire et al. (2012a), Akutse et al. (2015) and Foba et al. (2015ab) reported work on *Liriomyza* leafminers. Diversity of parasitoids associated with LMF on horticultural field crops across all agro ecological zones in Kenya is low. Main species include of *Diglyphus isaea* (Walker), *Neochrysocharis formosa* (Westwood), *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae), and *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) and are associated with parasitism rates less than 6% (Chabi-Olaye et al. 2008, Akutse et al. 2015, Foba et al. 2015b, Guantai et al. 2015). The low parasitoid diversity and parasitism rates warranted the introduction of parasitoids against LMF as part of an IPM program in Kenya. To enhance biological control of *Liriomyza* leafminers in Kenya, the International Centre of Insect Physiology and Ecology (*icipe*), imported *P. scabriventris, H. arduine* Walker and *C. flacilla* from Peru.

Introduction of *P. scabriventris* resulted to its successful establishment in three agro ecological elevations of Kenya (Foba et al 2015b). Laboratory assays of *H. arduine* and *C. flacilla* to the existing *Liriomyza* leafminers and their interaction with existing parasitoids showed promising results (Muchemi et al, 2018abc&d). Field studies will evaluate establishment and impact of *H. arduine* and *C. flacilla* against *Liriomyza* leafminers in three agro ecological elevations. In view of this, we hypothesize that the performance and establishment of *H. arduine* and *C. flacilla* will not be affected by factors across agro ecological elevations and that suppression of LMF in the three vegetable production systems will be realized further. Field results are expected to contribute to a change in farmer attitude on application of insecticides and make it possible for successful LMF classical biological program.

6.3. Materials and Methods

6.3.1. Study sites

Three agro ecological zones were selected based on elevation for the release of parasitoids namely; the lowlands, <1000 m. a.s.l (Eastern region of Kenya in Makueni County with mean temperature of 21 - 31^{o}C and mean annual precipitation of 800 - 1200 mm per annum); the midlands, 1000 - 1800 m. a.s.l (Rift valley in Kajiado County with mean temperature of 16 - 26^{o}C and precipitation of 450 - 1200 mm per annum); the highlands >1800 m. a.s.l (Central Kenya in Nyeri County with mean temperature of 8 - 24^{o}C and annual precipitation of 1500 - 2000 mm) (Fig. 6.1) (Hassan et al. 1998, Foba et al. 2015b). Agro ecological zones were geographically separated by 65 and 250 KM apart. In each of the agro ecological zones, three locations based on water supply reliability, intensity of vegetable production and LMF infestation were selected for the release and monitoring of parasitoids In the lowlands: Kikoo (2^{o} 23'50.928'' S, 37^{o} 59'8.052'' E), Kwakyai (2^{o} 23'6.612'' S, 38^{o} 0'9.504'' E) and Mangelete (2^{o} 41'57.552'' S, 38^{o} 7'32.268'' E); midlands: Namelok (2^{o} 43'6.096'' S, 37^{o} 27'39.06'' E), Empiron (2^{o} 50'57.948'' S, 37^{o}32'15.144'' E), Inkisanjani (2^{o} 53'56.4'' S, 37^{o} 34'51.564'' E); and highlands: Sagana (0^{o} 21’9.972’’ S, 37^{o} 5’13.632’’ E), Kabaru (0^{o} 17’48.408’’ S, 37^{o} 6’28.116’’E), Naromoru (0^{o} 11’9.312’’ S, 37^{o} 6’36.972’’ E). The zones were characterized by four cropping seasons of wet cold (March to May), wet hot (October to December), dry cold (June to September) dry hot (January to February) which varied according to elevation.

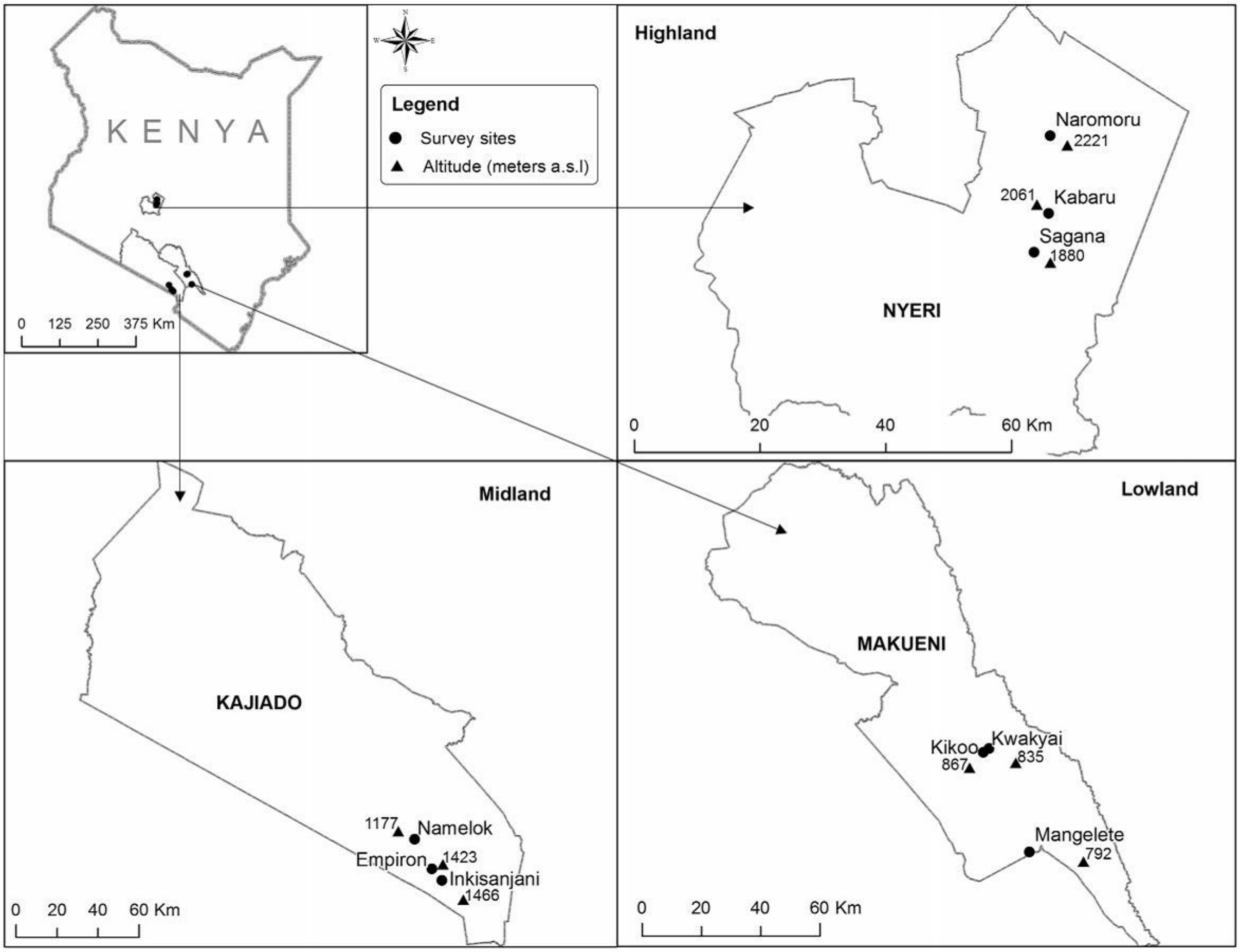


Fig 6.1. Study sites in the high, mid, and low elevation agro ecological zones of Kenya (source: Foba et al. 2015b)

6.3.2. Mass rearing of insects

6.3.2.1. Host inset colonies

The initial colony of *L. huidobrensis* was started from field collections in Nyeri County (0^{o} 21’S, 36^{o}57’E, 2, 200 m a.s.l.) of Central Kenya in 2007 and reared on faba bean plants at *icipe*’s rearing unit. Two-day-old adult *L. huidobrensis* were released for 24h in Perspex cages (60 cm length × 60 width × 60 cm height) for egg laying on potted faba plants. The colony was cultured at 25 ± 2^{o}C, 60 ± 5%, relative humidity (RH), and a photoperiod of 12L: 12D. Infested plants were removed and held in wooden cages (45 cm wide × 45 cm long × 60 cm high) for five to six days for the development of 2nd and

3rd similar age cohort instar larvae of *L. huidobrensis*. For continued rearing of the host pest, some of the infested faba plants were cropped at the base of the stem and incubated on mesh trays to capture dropping pupae. Pupae were collected and incubated in Petri dishes for adult emergence. Adult flies were provided with 10% sugar solution for two days after emergence to allow for pre-oviposition period before exposure to faba bean for further exposure to faba bean.

6.3.2.2. Parasitoid colonies

Mass rearing of *H. arduine* and *C. flacilla* parasitoids was started in 2012 and 2013 respectively following the approval for release by the Ministry of Agriculture in Kenya.

An open pollinated variety of Kenyan faba bean, *Vicia faba* L. (Fabales: Fabaceae) was used for the rearing *L. huidobrensis* and the parasitoids. Five seeds par plastic pots (5.5 cm diameter x 7.3 cm high) were planted and filled with planting substrate (mixture of soil and manure 5:1 in a ratio). Potted plants were kept in screen house (2.8 m length x 1.8m width x 2.2 m height) at *icipe*'s Duduville campus in Nairobi, Kenya at 25 ± 2° C for two weeks.

The initial cultures of *H. arduine* and *C. flacilla* were imported from Peru in 2012, where they were maintained on *L. huidobrensis*. At *icipe* the parasitoids were also maintained on *L. huidobrensis* reared on faba beans at 25 ± 2° C, 55 ± 5% RH and 21°C ±1 and 55% ± 5 RH respectively for one year before release. Two-day old *L. huidobrensis* were exposed to two-week-old potted faba bean for 24 h in aerated Perspex cages and held in screen house for 5-6 days to allow development of *L. huidobrensis* 2-3rd larval stage of same age cohort. Infested faba bean plants were cropped at the base of the stem and the soil media, placed in clean 30 ml glass vial in upright position into clear aerated Perspex cage (60 cm length × 60 width × 60 cm height). Two-day old *H. arduine* and *C. flacilla* parasitoids were introduced in separate the cages for 24 h (25 ± 2° C, 55 ± 5% RH and 21 ± 2° C, 55 ± 5% RH for *H. arduine* and *C. flacilla* respectively). The plants were removed from the cage and from vials and spread on a mesh wire tray on which sterilized sand was spread to allow development and dropping of host pest pupae. After two days, host pupae were collected in clear plastic Petri dishes and incubated at room temperatures for adult leafminer and parasitoid emergence four and up to 30 days respectively). Adult parasitoids of both species were fed on 10% honey solution after emergence for two days to allow mating and egg maturation before use in mass production. Each insect species colony was reared in separate rooms to avoid colonies contamination.

Similar cycles of pest and parasitoid exposures were repeatedly synchronized to allow parasitoids emergence from several cages to get enough number of parasitoids for release.

6.3.3. Parasitoids field release

Prior to field release, newly emerged parasitoids were denied oviposition and maintained in Perspex cages on 10% honey solution for a few days. Sex ratio of newly emerged parasitoids was established by assessing sex of ten randomly picked individuals from a cage. 540 individuals of *H. arduine* and 1,000 of *C. flacilla* adults were each put overnight in separate aerated clear plastic jars and honey provided as droplets on a white paper stuck inside the jar as source of food during transportation. Each jar held parasitoid individuals for one famer filed.

From each of the three locations in each of the three agro ecological elevations, five farmer fields at least 200 meters apart were randomly selected based on availability of active mines (having leafminer larvae) and less application of insecticides. A week before the actual transfer of the parasitoids from *icipe* rearing facility to specific fields, farmers and stakeholders from each location and local administration, the regulatory agency in the Ministry of Agriculture, KEPHIS, and extension services were sensitized on proper use of insecticides and conservation of parasitoids after field introduction. The awareness was meant to minimize the risk of failure of parasitoid establishment after field release. A total of 16, 740 and 45,000 adult parasitoids of *H. arduine* and *C. flacilla* were released in five of the selected farmer fields in each of three locations in every agro ecological of low, mid, and high elevations during December 2012 and 2013 for *H. arduine* and *C. flacilla* respectively.

6.3.4. Monitoring for parasitoid establishment post release

Field surveys for *H. arduine* establishment were conducted within a radius of 5 Km from the release points of each location and one month after release on monthly basis between January and December 2013 for *H. arduine* and between March and June of 2014 after *C. flacilla* release. Based on availability of *Liriomyza* infested host plants, ten randomly farmer field plots, not less than 20 m^2 and 200 m between each other were selected in each location for LMF and parasitoids sampling. Thirty farmers' plots from each agro ecological zone were sampled during each sampling period. Four equal quadrants measuring $1m^2$ were randomly selected from each farmer field. From each quadrant, a maximum of 25 leaves with active mines from three plant strata (upper, middle, and lower) were randomly picked, giving 100 per field for laboratory incubation and observations. Field collected infested leaves were held in sample plastic bags and preserved in cooler box. Later in the same day, leave samples were transferred into aerated lunch boxes (19 cm ×13 cm × 8 cm) lined with paper towels to absorb excess leaf moisture. In this form, they were transferred from the field to the laboratory for incubation (25 ± 2^oC and 55 ± 5% RH) at *icipe* Duduville campus, Nairobi, Kenya for four weeks. During this period, LMF pupae from each field were incubated in separate Petri dishes for insect emergence. Similarly, insects emerging directly from leaves were collected from the lunch boxes daily and preserved. Emerged insects were allowed to harden before preservation in 70% ethanol amended with 10% glycerin, and identification was done using conventional taxonomic keys and identification keys from the *Liriomyza* leafminers project at *icipe*. The adult parasitoids were sent to the Invertebrate taxonomy department of the National Museum of Kenya and further samples were sent to the Royal Museum of Central Africa, Belgium, for confirmation. Voucher specimens of adult LMF and parasitoids are found at *icipe* Biosystematics Unit. In addition, information on the Geographical Position System (GPS) coordinates; host plants (both cultivated and wild), weather parameters, name, and date when pesticide if any was applied in field before sampling and cropping system were collected.

6.3.5. Spread of parasitoids at high, mid, and low elevations post release

Field surveys to determine the spread of *H. arduine* were conducted one year after its release in 2013 which coincided with the short rainy season. During this time of the year, there was abundant filed vegetation for *Liriomyza* infestation and their parasitoids. Sampling was systematically decided along transects in four cardinal directions of north, south, east, and west using Geographic Information System (GIS) tools. Using Google earth map and as described by Gichini et al. (2008) and Foba et al (2015c), release points were marked by a circle on release locations at a radius of 10, 20, 30, 40, and 50 km (Fig. 6.2). From each circle, four sampling points on each of the four cardinal directions were established using coordinates and with the use of a GPS unit, the points were located on the ground. Within a radius of not < 2.5 Km of each pre-identified point, four fields with suitable LMF host plants were sampled for host pest and parasitoid except when the terrain proofed inaccessible. Sample collection, processing, and identification of LMF and associated parasitoids were done as described in section 6.3.4 above.

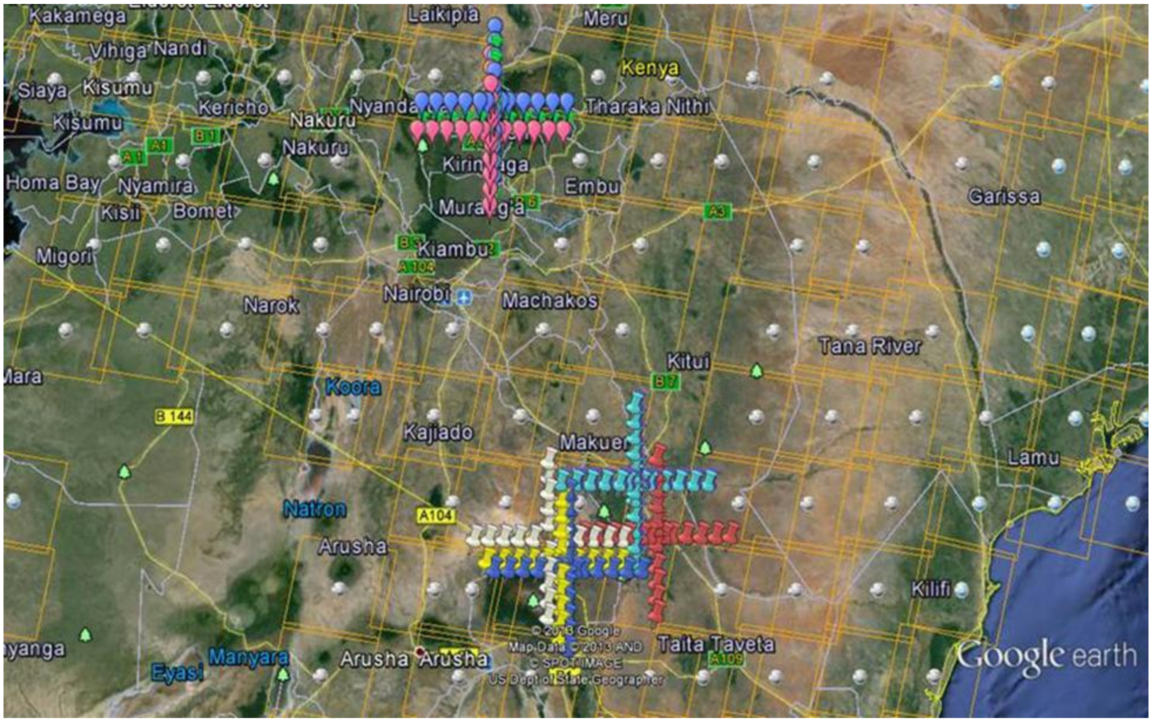


Fig. 6.2. Marked cardinal points on Google map of Kenya in the high, mid, and low elevation areas from which monitoring for parasitoid establishment and spread were conducted.
(Source: Google earth)

6.4. Data analyses

The parasitism rates in each of the three agro ecological elevation for each year were expressed as a proportion of emerged adult parasitoids out total insects (i.e. emerged adult parasitoids and leafminer flies multiplied by 100. Specific parasitism rates for each parasitoid species were expressed as a percentage of total number of adult parasitoid species dividend by the sum of adult parasitoids and LMF multiplied by 100 per year, season, host plant, and elevation. Count data on LMF and parasitoid species were square root (x + 0.5) transformed and percentages arcsine transformed before analysis. Data on host plants and farmer practices from different locations in each agro ecological

elevation during the four cropping seasons were pooled together (n = 1073 for the whole survey) and subjected to correspondence analysis (Pearson χ^2 test at $P < 0.05$).
Simple descriptive statistics were used to build trends of LMF hosts and parasitoids over the sampling period. Count data on LMF and parasitoid species were square root (x + 0.5) and percentages arcsine transformed before analysis using Analysis of Variance (ANOVA). Where significance was observed, mean differences in *Liriomyza* leafminers and parasitoids abundance, diversity and parasitism rates were separated using Tukey Krammer HSD test ($P \leq 0.05$). Distribution of host plants within the agro ecological zones was estimated by Pearson χ^2 method in Generalized Linear Model (GLM). GLM with poison distribution was used to test for the effect of explanatory factors (seasons, cropping system (mono, intercrop, or host plants adjacent to sampled field), pesticides, and host plants) on the abundance and establishment of LMF and parasitoids. Where significant models occurred, means were separated using Wilcoxon/ Kruskal-Wallis test ($P<0.05$) in JMP-version 11 data analysis software (SAS, 2013). In most cases, actual, rather than transformed data are presented.

6.5. Results

6.5.1. *Liriomyza* leafminer host plants diversity and farmer practices across agro ecological zones

Leaves from 1,073 *Liriomyza* infested host plants were collected for incubation during the field survey. LMF were recovered from 20 plants species (both wild and cultivated) belonging to six plant families. The distribution of the host plants in the three agro ecological elevations was significantly different ($\chi^2 = 10.52$, $P < 0.005$). Overall, the Solanaceae (46.37%) and Fabaceae (42.55%) plant families were the most often sampled and cultivated host plants from farmer fields. Less than 1% of host plants sampled were wild plants. Tomato (*Solanum lycopersicum* L. (Solanales: Solanaceae)) and beans (French bean and local bean cultivars) (*Phaseolus vulgaris* L. (Fabales: Fabaceae)) were the most sampled host plants in the low and mid elevation zones while potatoes (*Solanum tuberosum* L. (Solanales: Solanaceae)) and French beans were often sampled LMF host plants in the high elevation zone.
The diversity of host plants during the survey period varied across the elevation. Twelve host plant species were sampled in the highlands compared to nine in the mid and low elevations (Table 6.1).

Table 6.1. Diversity of LMF host plants in three agro ecological zones of high, mid, and low elevations of Kenya.

			High elevation		Mid elevation		Low elevation		Total	%
Binomial name	Common	Family	Frequ	%	Frequ	%	Frequ	%		
Bidens pilosa.	Blackjack	Asteraceae	1	0.29	0	0	0	0.00	1	0.28
Galinsoga parviflora Cav.	Macdonald		2	0.57	0	0.00	0	0.00	2	
Cucurbita moschata	Butternut	Cucurbitaceae	0	0.00	0	0.00	7	1.84	7	5.21
Cucurbita pepo.	Courgettes		26	7.47	1	0.29	0	0.00	27	
Cucurbita pepo.	Pumpkin		0	0.00	0	0.00	1	0.26	1	
Citrullus lanatus	Water melon		0	0.00	5	1.45	15	3.95	20	
Unidentified wild cucurbit	Cucurbit		0	0.00	1	0.29	0	0.00	1	
Ricinus communis.	Castor weed	Euphorbiaceae	1	0.29	0	0.00	2	0.53	3	0.28
Vigna unguiculata Walp.	Cowpeas	Fabaceae	0	0.00	0	0.00	9	2.37	9	42.55
Phaseolus vulgaris	French beans		88	25.29	83	23.99	0	0.00	171	
Pisum sativum	Garden peas		25	7.18	1	0.29	0	0.00	26	
Phaseolus vulgaris	Local beans		51	14.66	88	25.43	46	12.11	185	
Pisum sativum	Snow peas		52	14.94	0	0.00	0	0.00	52	
Pisum sativum	Sugar snaps		14	4.02	0	0.00	0	0.00	14	
Abelmoschus esculentus	Okra	Malvaceae	0	0.00	0	0.00	57	15.00	57	5.31
Solanum melongena L.	Aubergines	Solanaceae	0	0.00	0	0.00	6	1.58	6	46.37
Solanum nigrum	Night shade		0	0.00	1	0.29	0	0.00	1	
Capsicum annuum	Green Pepper		2	0.57	0	0.00	0	0.00	2	
Solanum tuberosum L.	Potatoes		73	20.98	3	0.87	0	0.00	76	
Solanum lycopersicum L.	Tomatoes		13	3.74	163	47.11	237	62.37	413	
Total count	**20**		**348**		**346**		**380**		**1074**	**100**

Distribution of host plants during the survey period was significantly affected by seasons (Pearson χ^2 test = 181.77, P < 0.0001). During the dry cold season, high number of host plants (380) were sampled and this number compared to the wet hot season (293). Fewer host plants were sampled during the wet cold (203) and dry hot (193) seasons (Fig.6.3).

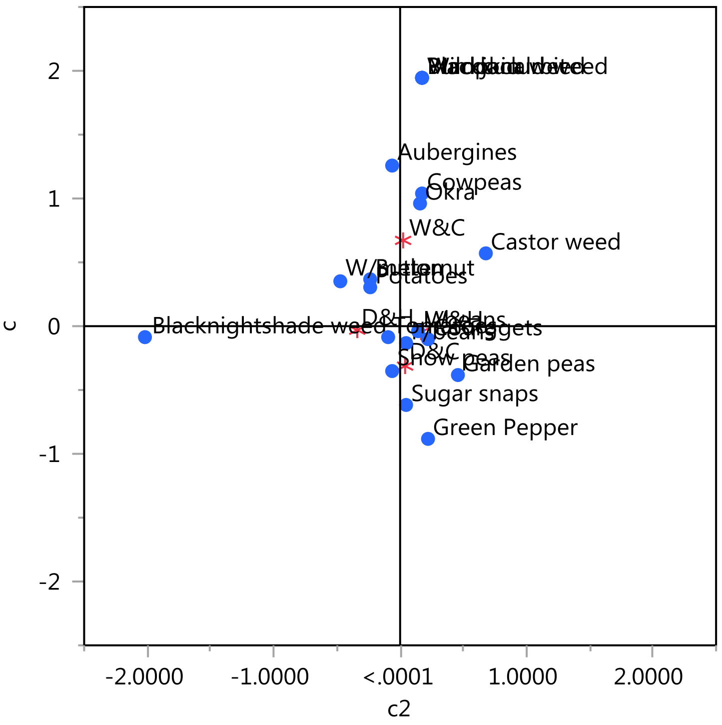


Fig. 6.3. Distribution of host plants over the sampling period in different seasons of Dry and Cold (D&C), Dry and Hot (D&H), Wet and Cold (W&C) and Wet and Hot (W&H)

1,136 pesticide molecules (both insecticides and fungicides) were reported by farmers as having been applied during the sampling period to manage various pests across the three agro ecological elevations. A significant number of pesticide molecules were applied in the mid elevation zone than the low and high elevation agro ecological elevations ($F_{2,\ 1070}$ = 48.06, P < 0.0001). Despite the variation in the usage of pesticides across the farming regions, these pesticides did not significantly affect the abundance of both LMF and parasitoids.

6.5.2. Abundance of *Liriomyza* leafminers

During the survey period of between January 2013 and June of 2014, 49,184 LMF fly specimens were reared from the incubated leaves. Of the total *Liriomyza* samples, three dominant species were identified as *L. huidobrensis*, *L. sativae,* and *L. trifolii* with varying incidences of 86.16, 10.69, and 3.15% in that respect. *Liriomyza huidobrensis* was the most dominant species across the elevations and its distribution across the elevations was significant in the high than in low and mid elevation zones ($F_{2,\ 1043}$ = 60.72, P < 0.0001) (Table 6. 2). *Liriomyza sativae* was significantly high in the mid and low elevation zones than in the high elevation ($F_{2,1043}$ = 33.54, P < 0.0001) while *L.*

trifolii was significantly high and different in the mid elevation zone than in the low and high elevation zones ($F_{2,1043}$ = 77.27, P < 0.0001) (Table 6.2). A significantly high number of *Liriomyza* leafminers were reared from filed samples collected in the high elevation than from the mid elevation zone while the number of LMF recovered in the low elevation zone compared to the former two zones ($F_{2,1073}$ = 6.77 P < 0.001).

Between January 2013 and June 2014, four distinctive seasons (dry hot, dry cold, wet hot and wet cold) were experienced during the sampling period within the agro ecological zones. Across the seasons, the population of LMF was significantly different. During the dry cold season, *L. huidobrensis* was significantly high during the dry cold than during the dry hot, wet cold and wet hot seasons ($F_{3,1072}$ = 19.35, P< 0.0001). The dry hot season significantly favored *L. sativae* population than the dry and wet cold seasons. In the dry cold and wet hot seasons, the population of *L. sativae* compared to that during dry hot and wet cold seasons ($F_{3,1072}$ = 3.22, P< 0.05). The population of *L. trifolii* was significantly favored by the dry hot season than wet hot season while during the dry cold and wet cold seasons, its populations were comparable to the dry hot and wet hot seasons ($F_{3,1072}$ = 2.68, P< 0.05) (Table 6. 2).

Two more species of *Liriomyza* leafminer; *Liriomyza bryoniae* (Kaltenbach) and *L. chinensis* (Kato) were recovered from the high elevation zone but the numbers were very low and insignificant for statistical comparisons.

6.5.3. Diversity and abundance of *Liriomyza* parasitoids post *Halticoptera arduine* and *Chrysocharis flacilla* field release

15 parasitoids species were recovered from *Liriomyza* infested host plant materials collected during the survey period. Of the 15 species, one was a hyperparasitoid, *Cheiloneurus carinatus* Compere (Hymenoptera: Encyrtidae) (Table 6.3). The hyperparasitoid was recovered from the three elevation zones from LMF pupae. A handful field sample of *Liriomyza* pupae from the high elevation zone resulted in 96 individuals of *C. carinatus* unlike those recovered from mid and low elevation zones with a single recovery (Table 6.3). The parasitoids included indigenous species; *Diglyphus isaea*, *Hemiptarsenus varicornis*, *Neochrysocharis formosa*, *Meruacesa* sp (Hymenoptera: Eulophidae) and *Opius dissitus* (Hymenoptera: Braconidae), introduced species; *Phaedrotoma scabriventris*, *H. arduine* and *C. flacilla,* new associations; *Pachyneuron? muscarum* (Hymenoptera: Pteromalidae), *Pteromalus* sp. (Hymenoptera: Pteromalidae), *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) and three unidentified species of braconid, scelionid and platygastroid (Table 6.3). Of the 14 recovered parasitoids, *Diglyphus isaea* was the most dominant parasitoid species followed by *O. dissitus, N. formosa, P. scabriventris, Meruacesa* sp. and *H. arduine* in that order with more than 1% of the total parasitoids. The remaining eight species were few and constituted less than 1% of the total parasitoids. Among them was the latest introduction of an exotic parasitoid, *C. flacilla* (Table 6.3).

Table 6.2. Incidences of *Liriomyza* leafminer species across seasons and agro ecological elevations of Kenya (Mean ± SE)

Parameter	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*	Total composition of *Liriomyza* leafminer
Liriomyza species across elevations				
High elevation (*n*=339)	92.07 ± 0.74a	7.21± 0.72b	0.73 ± 0.18c	53.95 ± 3.85a
Mid elevation (*n*=333)	75.15 ± 1.35c	17.27 ± 0.98a	7.58 ± 0.72a	36.95 ± 2.76b
Low elevation (*n*=372)	82.33 ± 1.4b	14.36 ± 0.92a	3.31 ± 0.37b	46.38 ± 2.98ab
Liriomyza composition by species (%)	86.16	10.69	3.15	
Liriomyza species across seasons				
Dry cold	55.78 ± 3.40A	4.51 ± 0.62AB	1.22 ± 0.21AB	
Dry hot	31.29 ± 2.78B	6.98 ± 0.78A	2.07 ± .42A	
Wet cold	22.80 ± 2.51B	3.67 ± 0.95B	1.79 ± 0.36AB	
Wet hot	35.43 ± 3.43B	4.86 ± 0.48AB	1.07 ± 0.18B	

Means followed by the same lower (upper) case letters and for each parameter across rows (columns) are not significantly different (P<0.05) (Tukey-Kramer HSD test) for each parameter, *n*- sample size.

Abundance of parasitoid species across ecological zones was significantly different during the study period. *Diglyphus isaea* recovered in the high elevation zone was significantly different being 1.4 and 2.9 times more than those recovered from the mid and low elevation zones respectively ($F_{2,1073}$ = 26.47, P<0.0001) (Table 6.3). *Opius dissitus* was significantly high in the low agro ecological zone compared to the mid and high elevation zones ($F_{2,1073}$ = 26.43, P<0.0001). *Neochrysocharis formosa*, recovered from the low elevation zone was significantly high than those from the high elevation zone while in the mid elevation zone, *N. formosa* population compared to that in the high and low elevation zones. *Meruacesa* sp. population was significantly high from the high elevation zone than in mid elevation areas but in the low elevation zone, the population levels compared to those in the high and mid elevation zones ($F_{2,1073}$=4.74, P<0.01). Population levels of *P. scabriventris, H. arduine* and *C. flacilla* were not different across the agro ecological zones during the survey period ($F_{2,1072}$ = 0.2799, P = 0.7559, $F_{2,1073}$ = 0.32, P = 0.7287 and $F_{2,1073}$ = 0.03, P = 0.9682 respectively) (Table 6.3).

6.5.4. Establishment of *Halticoptera arduine* and *Chrysocharis flacilla* post release

Much of the recovery of *H. arduine* done during the first three months between January and March overlapping the dry hot and wet cold seasons in the three ecological zones of 2013 compared to 2014 (Fig. 6.4). In the high elevation agro ecological zone, *H. arduine* was recovered the first month after its release reappeared in the system during the cold dry season of July and September in 2013 and only to appear in the hot dry and cold dry seasons in March and during the cold dry season in June of 2014. In the mid elevation agro ecological zone, *H. arduine* was recovered during the dry hot season of January to March and dry cold season in May to July of 2013 and no recovery of it was made in the system until June 2014. In the low elevation agro ecological zone, *H. arduine* was recovered during the dry hot season of January and March, cold dry season of June to September and wet hot season of November and December in 2013. No recoveries of *H. arduine* were done in 2014 (Fig. 6.4).

Establishment of *C. flacilla* after its release in December 2014 was characterized by its consecutively recovery during the cold wet and dry cold seasons in March and June of 2014 from all the three agro ecological zones (Fig. 6.4).

Table 6.3. Diversity and abundance of *Liriomyza* leafminer parasitoids recovered between January 2013 and June 2014 post parasitoid release (Mean ± SE)

Recovered parasitoid	High elevation	Mid elevation	Low elevation	Species composition (%)	Status
Diglyphus isaea	49.06 ± 0.63a	33.89 ± 0.47b	17.05 ± 0.25c	46.15	Local (Chabi-Olaye et al. 2008)
Opius dissitus	18.31 ± 0.28c	31.21 ± 0.31b	50.48 ± 0.36a	38.13	Local (Chabi-Olaye et al. 2008)
Neochrysocharis formosa	24.05 ± 0.09b	30.75 ± 0.08ab	45.20 ± 0.10a	8.09	Local (Chabi-Olaye et al. 2008)
Phaedrotoma scabriventris	34.27 ± 0.05a	28.32 ± 0.95a	37.41 ± 0.28a	2.67	Local (Foba et al. 2015b)
Meruacesa sp	48.82 ± 0.05a	17.06 ± 0.02b	34.12 ± 0.03ab	1.59	Local (Foba et al. 2015b)
Halticoptera arduine	37.76 ± 0.08a	36.36 ± 1.10a	25.87 ± 0.62a	1.34	Introduced in present studies
Chrysocharis flacilla	33.98 ± 0.02a**	31.07 ± 0.03a**	34.95 ± 0.02a**	0.96	Introduced in present studies
*Cheiloneurus carinatus**	96.97 ^{ns}	1.01 ^{ns}	2.02 ^{ns}	0.93	A hyperparasitoid
Pachyneuron? *Muscarum*	0.00 ^{ns}	60.00 ^{ns}	40.00 ^{ns}	0.05	1st record
Braconid	0.00 ^{ns}	0.00 ^{ns}	100.00 ^{ns}	0.03	Unidentified
Pteromalus sp.	50.00 ^{ns}	0.00 ^{ns}	50.00 ^{ns}	0.02	1st record
H. varicornis	0.00 ± 0.00a	50.00 ± 0.00a	50.00 ± 0.00a	0.02	Local (Chabi-Olaye et al. 2008)
Scelionidae	0.00 ^{ns}	100.00 ^{ns}	0.00 ^{ns}	0.02	Unidentified
Pediobius furvus	100.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.01	1st record
Platygastroidae	0.00 ^{ns}	0.00 ^{ns}	100.00 ^{ns}	0.01	Unidentified

Means followed by the same lower case letters within rows are not significantly different ($P<0.05$, Tukey Kramer HSD Test), ^{ns} indicates the number was negligible only recovered in isolated cases and insufficient for means comparison, * reported as hyperparasitoid (Ackonor and Mordjifa 1999) and first report of LMF parasitoid in Kenya, ** data collected during 2014 survey period

6.5.5. *Liriomyza* leafminer parasitism rates post *Halticoptera arduine* and *Chrysocharis flacilla* release

The overall field parasitism rates from the three agro ecological zones ranged between 25.6% and 40% during the survey period (Table 6.4). During the 2013 monthly sampling period after the release of *H. arduine*, *Liriomyza* parasitism rates across elevations were not significantly different ($F_{2,\ 901} = 2.46$, $P = 0.0864$). In 2014, and following the release of *C. flacilla*, *Liriomyza* field parasitism rates in the same study sites were significantly different across the ecological zones ($F_{2,169} = 9.82$, $P < 0.0001$). Significantly, high LMF parasitism rate was recorded in the mid and low agro ecological elevations than in the high agro ecological elevation. Overall, the total LMF parasitism rates during the entire survey period, were significantly different across the three agro ecological elevations and high in the mid agro ecological elevation than in the high agro ecological elevation ($F_{2,1071} = 6.47$, $P < 0.001$). Total LMF field parasitism rates in the low agro ecological elevation compared to those observed in high and mid agro ecological elevations (Table 6.4).

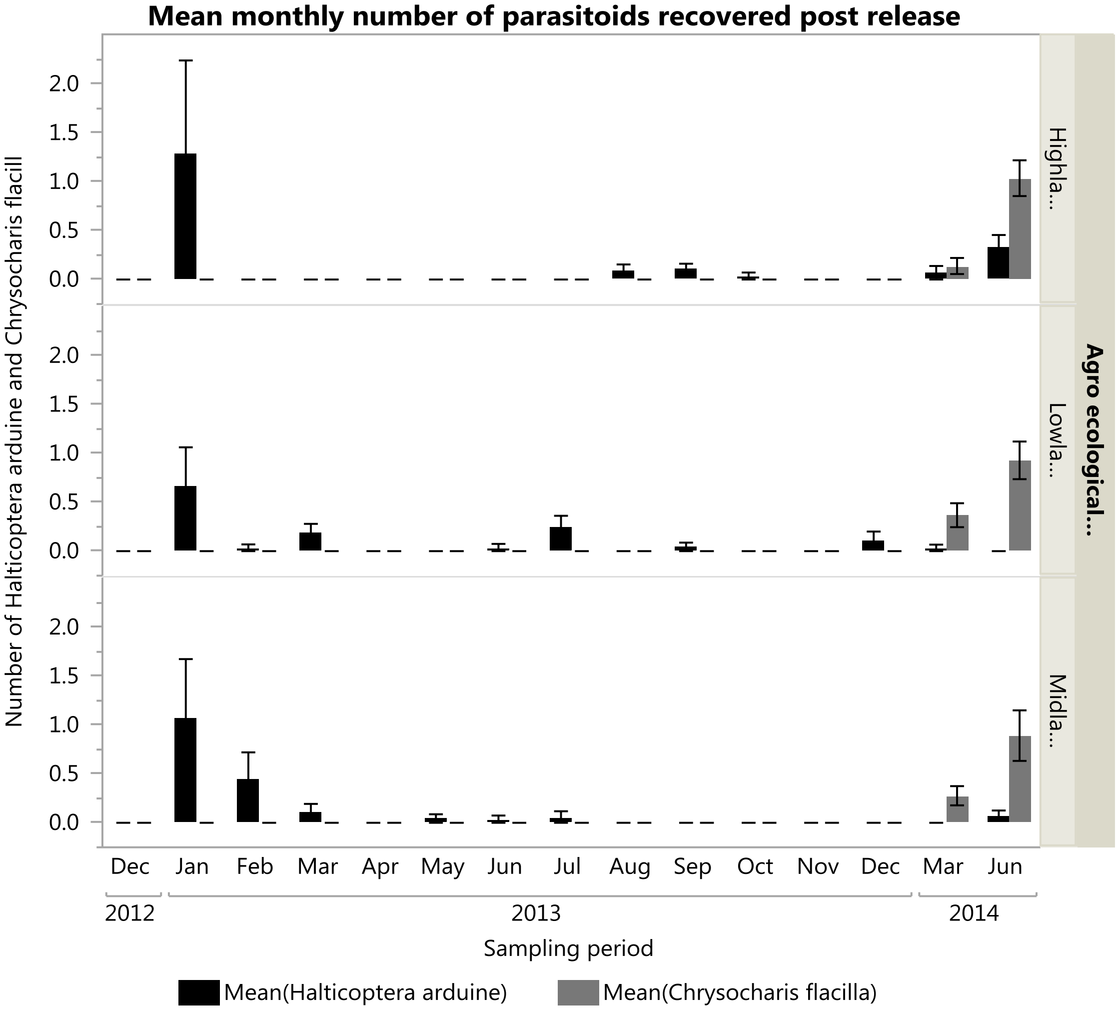


Fig. 6.4. Monthly recovery of introduced parasitoids between January 2013 and June 2013 post release

Table 6. 4. *Liriomyza* leafminer parasitism rates post release of *Halticoptera arduine* and *Chrysocharis flacilla* in between January 2013 and June 2014.

Agro ecological zones	*Liriomyza* leafminer parasitism rates post *H. arduine* release in 2013	*Liriomyza* leafminer parasitism rates post *C. flacilla* release in 2014	Total *Liriomyza* leafminer parasitism rates
High elevation zone	25.60 ± 1.20a	21.54 ± 2.02b	24.93 ± 1.06b
Mid elevation zone	29.24 ± 1.20a	40.00 ± 2.21a	31.00 ± 1.08a
Low elevation zone	26.42 ± 1.00a	32.59 ± 2.52a	27.93 ± 0.94ab

Within columns, means followed by the same lower case letter are not significantly different ($P<0.05$) (Tukeys Krammer HSDD test).

During the first year of survey post *H. arduine* release in 2013, 13 parasitoids species were associated with significantly different LMF specific field parasitism rates. *Diglyphus isaea*, *O. dissitus*, *N. formosa* and *P. scabriventris* were the main cause of *Liriomyza* parasitism in all the three agro ecological zones each contributing more than 1% specific parasitism rates in 2013 (Table 6.5a). Specific field parasitism rates caused by *D. isaea*, *O. dissitus, N. formosa* and *Meruacesa* sp. were significantly different across the three agro ecological zones. *Diglyphus isaea* specific parasitism rates in the high and mid elevation zones were significantly high than in the low elevation zone ($F_{2,901} = 33.74$, $P<0.0001$).

Field parasitism rates by *O. dissitus* were significantly high in the low elevation zone than in mid and high elevation zones ($F_{2,901} = 34.62$, $P < 0.0001$). *Neochrysocharis formosa* caused significantly high specific parasitism rate in the low than in the high elevation zone while its specific field parasitism rate in the mid elevation zone, compared with that in the high and low elevation zones ($F_{2,901} = 8.90$, $P < 0.0001$). *Meruacesa* sp. caused significantly high field parasitism rates in the high elevation zone than in the mid elevation zone ($F_{2,901} = 7.48$, $P < 0.001$). Specific field parasitism rate of *P. scabriventris* and *H. arduine* were less than 1% and did not different significantly across the three agro ecological elevations (*P. scabriventris*: $F_{2,901} = 0.23$, $P = 0.7981$; *H. arduine*: $F_{2,901} = 0.15$, $P = 0.8641$ respectively). Specific field parasitism rates for the other parasitoids (*P*? *muscarum*, *Pteromalus* sp., *H. varicornis*, *P. furvus*, unidentified braconid, scelionid, and platygastroid were low and lacking in some of the agro ecological zones for statistical comparisons (Table 6.5a).

Table 6.5a. Specific *Liriomyza* leafminer field parasitism rates across agro ecological zones in 2013 (X±SE)

Parasitoids species	Specific parasitism rates (%)		
	High elevation zone	Mid elevation zone	Low elevation zone
D. isaea	15.38 ± 1.08a	14.82 ± 1.17a	5.14 ± 0.63b
O. dissitus	6.11 ± 0.82c	10.48 ± 0.90b	15.09 ± 0.93a
N. formosa	1.82 ± 0.49b	2.68 ± 0.53ab	3.89 ± .57a
P. scabriventris	0.81 ± 0.30a	0.78 ± 0.28a	1.14 ± 0.36a
Meruacesa sp	0.56 ± 0.24a	0.13 ± 0.20b	0.46 ± 0.19ab
H. arduine	0.39 ± 0.23a	0.30 ± 0.18a	0.32 ± 0.18a
Pachyneuron? muscarum	-	0.01 ± 0.4^{ns}	0.01 ± 0.04^{ns}
Unidentified Braconid	-	-	0.81 ± 0.09^{ns}
Pteromalus sp.	0.01 ± 0.04^{ns}	-	0.00 ± 0.02^{ns}
H. varicornis	-	0.01 ±0.04^{ns}	1.39 ± 0.09^{ns}
Unidentified Scelionidae	-	0.03 ± 0.05^{ns}	-
P. furvus	0.01 ± 0.04^{ns}	-	-
Unidentified Platygastroidae	-	-	0.06 ± .08^{ns}

Within rows, means followed by the same lower case letter are not significantly different (P<0.05) (Tukeys Krammer HSDD test), -indicates no record of the parasitoid, ^{ns}- insignificant.

In 2014 survey post *C. flacilla* release, eight parasitoid species were associated with *Liriomyza* parasitism (Table 6.5b). Although *D. isaea* specific field parasitism rate was slightly lower compared to its parasitism in 2013, it was significantly high in the high elevation zone than in low elevation zone. However, in mid elevation zone, its parasitism rates compared with the high and low elevation zones ($F_{2,169}$ = 7.36, P < 0.001). Parasitism rates by *O. dissitus* were high for mid and low elevation zones in 2014 compared to 2013 and significantly high in low and mid elevation zones than in the high elevation zone ($F_{2,169}$ = 18.32, P < 0.0001). As compared to 2013, *P. scabriventris* field parasitism rate in 2014 were more than 1% but did not differ significantly across agro ecological elevations ($F_{2,169}$ = 0.38, P = 0.6829). *Neochrysocharis formosa* specific field parasitism rates were significantly high in the mid elevation zone than it was in both the high and low elevation zones ($F_{2,169}$ = 6.64, P < 0.01). Unlike in 2013, *Meruacesa* sp. field parasitism rate in 2014 was more than 1% and significantly high in mid elevation zone than in both the high and low elevation zones ($F_{2,169}$ = 5.21, P < 0.01). *Halticoptera arduine,* having been in the system the entire period of 2013, had its specific field parasitism rates equally low and less than 1% in 2014Parasitism rates by

H. arduine in 2014 did not differ significantly across the three elevations ($F_{2,169}$ = 2.70, P = 0.0701). *Chrysocharis flacilla,* having been in the system six months into 2014, recorded field parasitism rates above 2% in all the three agro ecological elevations. However, these parasitism rates by *C. flacilla* did not differ across the elevation zones ($F_{2,169}$ = 0.72, P= 0.486) (Table 6.5b).

Table 6.5b. Specific *Liriomyza* leafminer field parasitism rates across agro ecological elevations in 2014 (X±SE).

Parasitoids species	Specific parasitism rates (%)		
	High elevation zone	Mid elevation zone	Low elevation zone
D. isaea	10.23 ± 1.96a	7.16 ± 1.51ab	3.30 ± 1.27b
O. dissitus	6.04 ± 1.44b	23.45 ± 2.32a	23.88 ± 2.58a
P. scabriventris	1.72 ± 0.95a	1.66 ± 1.03a	1.09± 0.78a
N. formosa	0.42 ± 0.46b	3.32 ± 0.59a	0.65 ± 1.33b
Meruacesa sp	0.22 ± 0.35b	1.47 ± 0.89a	0.05 ± 0.17b
H. arduine	0.84 ± 0.72a	0.07 ± 0.20a	0.01 ± 0.08a
C. flacilla	2.08 ± 1.02a	2.72 ± 1.10a	3.55 ± 1.292a
Pachyneuron? *muscarum*	-	0.15 ± 0.30	-

Within rows, means followed by the same lower case letter are not significantly different (P<0.05) (Tukeys Krammer HSDD test), - indicates no record of the parasitoid, ns-insignificant.

6.5.6. Effect of explanatory factors on *Liriomyza* abundance and parasitoids establishment

Seasons, elevation, host plant, and cropping systems had significant impact on the abundance of *Liriomyza* leafminers and parasitoid species including *H. arduine* and *C. flacilla* and parasitism rates. Elevation had a significant effect only in the abundance of LMF (P< 0.01) but not in the parasitoid abundance. Pesticides applied during the survey period were significantly high in the mid elevation zone than in the low and high elevation zones and did not significantly impact on the establishment of parasitoids (Table 6.6).

The introduced parasitoids were significantly affected by seasonal variability during the survey period. *Phaedrotoma scabriventris* (P<0.01), *H. arduine* (P<0.0001) and *C. flacilla* (P<0.0001) (Table 6.6). High and significantly different numbers of the total parasitoids ($F_{3,1073}$=10.09, P<0.0001) were recovered during the dry cold season than during the dry hot, wet cold and wet hot seasons ($F_{3,1073}$=15.52, P<0.0001) (Table 6.7). Of special interest to our study was the significant effect on introduced parasitoid species by seasons. *Phaedotroma scabriventris* being in the system two years before our survey, recorded significantly high abundance during the wet cold and wet hot seasons than in the dry and cold season but abundance in the dry hot season compared to those recovered from wet seasons and dry cold season across elevation ($F_{3,1073}$=3.1726, P<0.0235). Abundance of *H. arduine* parasitoids during the wet hot season was significantly high than those recovered during the hot cold and wet cold seasons. However, during the dry hot season, the numbers of *H. arduine* compared to the other three seasons ($F_{3,1073}$=4.1438, P< 0.0062). In 2014 survey period, *C. flacilla* establishment was assessed only during the dry cold and wet cold seasons in March and June. The populations of *C. flacilla* were significantly different and high during dry cold season than during wet cold season ($F_{1,63}$=4.45, P=0.0389) (Table 6.7).

The cropping systems significantly affected *Liriomyza* leafminers (P<0.05), total field parasitism rate (P<0.01) and abundance of *C. flacilla* (P<0.0001). Habitat was observed to have an impact on *Liriomyza* leafminers and *C. flacilla* parasitoids. *Liriomyza* leafminers were significantly abundant in pure stand fields as par the time of sampling where no other vegetation was available within or in the vicinity than in mono crop fields with other vegetation in the vicinity and in intercropped fields with more than one plant species. Significant high number of *C. flacilla* parasitoids were recovered from farmer fields that were intercropped with more than one plant species than in mono crop farmer fields with no other vegetation around the fields ($F_{2,1072}$ = 6.8239, P<0.0012). Absence of other plant species around the sampled fields resulted to *C. flacilla* populations that were comparable to the latter two scenarios. In reverse to the observation made on *C. flacilla*, *Liriomyza* leafminers populations were significantly concentrated in farmer fields which had no diverse habitats within the vicinity as compared to habitats with one plants species and with diverse habitats (intercrop) across elevations ($F_{2,1072}$ = 14.51, P<0.0001) (Table 6.7).

Table 6.6. Sources of variation in the establishment of introduced parasitoids (*GLM Pearsons Chis/Df* with poison distribution)

Source of variation	Number of *Liriomyza* leafminers		Total number of parasitoids*		Parasitism rates		*Phaedotroma scabriventris*		*Halticoptera arduine*		*Chrysocharis flacilla*	
	Pearson Chisq/Df	P values	Pearson Chisq/Df	P values	Pearson Chisq/Df	P values	Pearson Chisq/Df	P values	Pearson Chisq/Df	P values	Pearson Chisq/Df	P values
Seasons	25.78	0.0001	17.48	0.001	-	-	10.73	0.01	26.40	0.0001	134.92	0.0001
Elevation	8.87	0.01	2.72	0.26 ^{ns}	-	-	2.49	0.28^{ns}	1.16	0.563^{ns}	0.13	0.94 ^{ns}
Cropping system**	6.05	0.05	0.88	0.64^{ns}	11.10	0.01	4.74	0.09^{ns}	0.37	0.83^{ns}	22.90	0.0001
Host plant	67.82	0.0001	42.45	0.01	38.26	0.01	18.76	0.47^{ns}	9.54	0.96^{ns}	52.69	0.0001
Pesticides used	2.61	0.11^{ns}	0.34	0.56 ^{ns}			0.93	0.334^{ns}	0.06	0.80^{ns}	3.43	0.06^{ns}

^{ns} indicates non-significance, * total number of parasitoids over the entire sampling period, ** type of cropping system in the sampling field.

Table 6.7. Significance level of explanatory factors with influence on *Liriomyza* leafminer and parasitoid abundance

Season	Mean number of *Liriomyza* leafminers	Mean number of parasitoids	Mean number of *P. scabriventris*	Mean number of *H. arduine*	Mean number of *C. flacilla*
DC	61.50 ± 3.08a	12.25 ± 0.69a	0.15 ± 0.04b	0.07 ± 0.02b	0.71 ± 0.05a
DH	40.20 ± 3.07b	9.04 ± 0.68b	0.28 ± 0.05ab	0.09 ± 0.04ab	*
WC	28.56 ± 3.26b	7.68 ± 0.54b	0.33 ± 0.08a	0.01 ± 0.01b	0.61 ± 0.55b
WH	41.35 ± 3.75b	9.70 ± 0.57b	0.37 ± 0.07a	0.32 ± 0.11a	*
Cropping systems					
Intercropping	38.21 ± 3.05c	ns	ns	ns	0.23 ± 0.04a
Monocropping	48.26 ± 2.34 b	ns	ns	ns	0.09 ± 0.01ab
Pure stand fields	110.84 ± 13.90a	ns	ns	ns	0.00 ± 0.00b

DC-Dry Cold, DH- Dry Hot, WC- Wet cold, WH- Wet Hot, * no data collected during 2014 seasons, ns- no significant effect was noted, within columns, means followed by the same lower case letter are not significantly different, P≤ 0.05 (Tukeys Krammer HSDD test)

Host plants were found to be a source of variation that affected the establishment of parasitoids. From a total of twenty cultivated and wild host plants sampled during the survey period, local beans, *P. vulgaris* recorded significantly high and different number of parasitoids than any other host plant (P<0.0001). A variety of the *Pisum sativum* L. (Fabales: Fabaceae), sugar snaps, recorded a significantly high number of *C. flacilla* than any other LMF host (P<0.0001) (Table 6.8). However, host plants were not a source of variation for any other parasitoid.

6.5.7. Spread of *Halticoptera arduine*

Spread of *H. arduine* was conducted between November and December of 2013, a year post release in all the three agro ecological elevations. Within a radius of 50 km of the four cardinal directions in each agro ecological elevation release locations, *H. arduine* was recovered from only one point in the low elevation zone 10 Km North of Kikoo release point and only one individual adult emerged from plant materials of the local bean. Due to time limit and logistic constraints, spread of *C. flacilla* was not conducted following its establishment in the vegetable production systems.

Table 6.8. Significance levels of host plants on *Chrysocharis flacilla* establishment

	Deviation from the mean (Wilcoxon / Kruskal Wallis test (Rank sum))	
Host plant common name	Total parasitoids	*Chrysocharis flacilla*
Aubergine	1.208	1.059
Blackjack	-1.577	-0.248
Black nightshade	0.605	-0.248
Butternut	1.285	2.527
Castor weed	-1.401	-0.434
Courgettes	-2.372	-1.323
Cowpeas	2.287	1.986
French beans	2.739	-1.090
Garden peas	-0.144	0.374
Green Pepper	-0.451	-0.353
Local beans	4.416*	0.649
Macdonald eye	-0.488	-0.353
Okra	0.282	0.900
Potatoes	-3.191	-0.784
Pumpkin	-1.577	-0.248
Snow peas	-1.533	-1.277
Sugar snaps	-1.580	8.214*
Tomatoes	-2.340	-1.453
Water melon	-1.007	-0.209
Wild cucurbit	1.605	-0.248
Chi Square approximation values	77.28	85.15

*significant level of mean deviation (P<0.0001) using 1-way Test, Chi Square approximation

6.6. Discussions

Three *Liriomyza* leaf miners; *L. huidobrensis*, *L. sativae*, and *L. trifolii* were the major species reared from the host plants collected across the three elevations zones. In addition, two minor *Liriomyza* leafminers; *L. bryoniae* and *L. chinensis* were recovered in the high elevation zones. Their potential threat to vegetable production and their role needs further evaluation. In our current study, *L. huidobrensis* was the dominant species across the three elevation production areas. Our findings are consistent with the findings reported by Foba et al. (2015b). However, these findings are inconsistent with the findings by Chabi-Olaye et al. (2008) where *L. sativae* and *L. trifolii* were shown as the predominant *Liriomyza* species in the mid and low elevation areas in that respect. *Liriomyza huidobrensis* is a species known to be invasive, adapt, and colonize hosts at colder and higher elevations of above 100 m a.s.l in the native origins (Spencer 1989, Rauf et al. 2000, Weintraub 2001, Andersen et al. 2002, Chabi-Olaye et al. 2008, Tantowijoyo & Hoffmann 2010, Mujica & Kroschel 2011). Our results suggest *L. huidobrensis* ability to adapt to different climatic regimes and its likelihood to displace other *Liriomyza* species in the mid and low elevations. Species displacement within the *Liriomyza* genus leafminers complex has been reported in several parts of the world. In USA, for instance, *L. trifolii* is reported to have displaced *L. sativae* and in the mainland of China, *L. huidobrensis* was reported to displace *L. sativae* in many production areas as *L. trifolii* displaced *L. sativae* on *Vigna unguiculata* L. (cowpea) in Hainan province (Gao et al. 2011). Species displacement from their natural habitats is a worrying concern in the world of ecologists since this is the cause of ecosystems imbalances and impacts on species biodiversity (Reitz & Trumble 2002, Abe & Tokumaru 2008). Our observations further points towards a potential species displacement threat in the low and mid elevation areas of Kenya and other regions of East Africa with similar climatic conditions. This therefore calls for concerted efforts in the development of management strategies targeting the invasive *L. huidobrensis*. Further studies are called for to elucidate whether natural enemies associated with the previously dominant *L. sativae* and *L. trifolii* from the mid and low elevation regions will evolve adaptation mechanisms alongside the rapidly encroaching *L. huidobrensis*.

Our present study showed the successful establishment of the two exotic parasitoids following their release in Kenya. This success is attributed to the availability of a wide range of *Liriomyza* host species occurring on a wide range of host plants distributed across agro ecological vegetable production systems. Successful introduction of *Liriomyza* parasitoids have occurred elsewhere in other parts of the world. For instance, *Opius pallipes* Wesmael (Hymenoptera: Braconidae) was introduced in the Netherlands and achieved control of *L. trifolii* in green house tomato (Woets & van den Linden 1982). *Ganaspidium utilis* Baerdsley (Hymenoptera: Eucoilidae) introduced in 1977 had a major impact on *L. trifolii* and *L. sativae* on watermelons in Hawaii. In the same region, *Neochrysocharis diastatae* (Howard) (Hymenoptera: Eulophidae) significantly affected on *L. trifolii* and *L. sativae* on several vegetable crops (Johnson 1993). In Africa, classical examples of successful introduction of exotic LMF parasitoids in the background of existing parasitoids includes *P. scabriventris* in Kenya (Foba et al. 2015b) and *O. dissitus* in Senegal (Neuenschwander et al. 1987). The introduced parasitoid species in our current study is expected to boost *Liriomyza* suppression in East Africa alongside other existing parasitoid species (Johnson & Hara 1987). Equally, same note, tremendous increase in the suppression of the LMF by parasitization has been shown in our present study. Compared to earlier parasitism rate of less than 6% by the main local parasitoids; *D. isaea* and *O. dissitus* in 2007 (Chabi-Olaye et al. 2008) through 31% following the introduction of *P. scabriventris* in 2012 (Foba et al. 2015b), up to 40% LMF parasitism rate was reported in this study. However, when compared to field parasitism rates of the key local parasitoids, parasitism rates by *H. arduine* and *P. scabriventris* remained low except for *C. flacilla* which was introduced last in the system at 3.5%. Parasitism rates by *C. flacilla* in our current studies are by far more promising compared to any of the two earlier introduced parasitoids; *P. scabriventris* and *H. arduine* and even better than some of the local parasitoids; *N. formosa* and *Meruacesa* sp. It is expected that once these parasitoids establish and adapt to the local environments, they are likely to have a bigger impact on pest suppression by parasitism.

In addition to the above key finding, this study has also established a richer diversity of 14 *Liriomyza* parasitoids species in Kenya as compared to earlier reports by Chabi-Olaye et al. (2008) and Foba et al. (2015b) of nine parasitoid species associated with *Liriomyza* leafminers. Of these, three are new records not known to be associated with *Liriomyza* leafminer; *Pachyneuron*? *Muscarum, Pteromalus* sp., and *Pediobius furvus* while three were unidentified hymenopterans not previously associated with LMF in Kenya. At the same time, this study documented *C. carinatus,* a possible gregarious hyperparasitoid of LMF parasitoids as a new record in Kenya. This species is known to be a hyperparasitoid of *Coccodiplosis coffeae* Barnes (Diptera: Cecidomyiidae), *Xyplugaster pseudococci* Risbec and other undetermined parasitoids (Acknor and Mordjifa 1999). This new report suggests a potential threat to the *Liriomyza* parasitoid complex and their pest suppression ability in presence of the hyperparasitoid. Natural enemies of parasitoids are major ingredient of the fourth trophic level in insect communities and are likely to limit the performance of parasitoids in suppressing the pest host (Harvey 2008, Poelman et al. 2012). Further studies are however warranted to evaluate potential threat and impacts on the performance of introduced parasitoids posed by this hyperparasitoid.

In our current studies, both parasitoids have demonstrated potential performance in field suppression of targeted host pest within the survey period. In their native origins of South America, *Halticoptera arduine* and *C. flacilla* are known to be efficient and abundant LMF parasitoids (Mujica & Kroschel 2011). Because of this promising performance, farmers are likely to decrease the indiscriminate application of insecticides and consequently reduced pesticide residues on fresh produce hence market access which will contribute to food safety among the consumers. Continuous farmer awareness campaigns are integral to the sustenance of introduced parasitoids in pest control.

The establishment and performance of introduced natural enemies is however influenced by a couple of factors (Waage 1983, Courchamp et al. 1999) Amongst them are climate, cropping systems, host plant, host density and farming practices, poor economic returns, multiple pests affecting the host crop, thermal tolerance, and competitive interactions among natural enemies amongst others (Klapwijk et al. 2005, Liu et al. 2009) some of which we suspect might have affected effective spread of *H. arduine* in all directions after its release. In our current studies, host plants, cropping systems and climate impact on parasitoids establishment. Host plants have a strong influence on the vulnerability of herbivores to parasitoids (Barbosa et al. 2001), and where parasitoid respond to plant traits, it can in turn provide a selection pressure on herbivores (Stamp 2001, Mulatu et al. 2004, Singer et al. 2004). Host plants in six plant families, were earlier reported by Foba et al. (2015b) and were similarly recorded in our current studies. In addition, two wild plant species in the families of Asteraceae and Euphorbiaceous were an addition to the list. This extended host range confirms the polyphagous nature of *Liriomyza* leafminers (Shepard et al. 1998, Murphy & LaSalle 1999, Morgan et al. 2000, van der Linden 2004, Tran et al. 2006). In future and with expanded survey programs, it is likely that more host plant species will be included in the current list from Kenya. Since host nutrition on which the herbivore is reared is likely to be a factor in parasitoid establishment, where the slow-growth, high mortality hypothesis predicts that herbivores feeding on plants with low nutritional quality or digestibility are more susceptible to natural enemy attack (Clancy & Price 1987), we will assume that the assemblage of parasitoids followed the pattern of high quality host distribution. In our current studies, *C. flacilla* was strongly observed on *P. sativum* and cucurbits host plants over potatoes. Besides, the abundance of host plant was dependent on climatic regimes and it is likely to have influenced the abundance of *H. arduine* and *C. flacilla* parasitoids. *Pisum sativum* was the abundant host plant in the high elevation zone during the cold and wet seasons which impacted positively on *C. flacilla* while cucurbits grown during wet and hot seasons favored establishment of *H. arduine*.

Different authors have reported consistent observations regarding host nutrition both under laboratory and field conditions. For instance, female parasitoids of *O. dissitus* were attracted to *L. trifolii* host reared on cucurbits rather than on tomatoes or lettuce (Olivera & Bordat 1996). Similarly, *D. isaea* parasitism was reported to be affected by the host plant nutrition on which the host insect was reared (Musundire et al. 2012b, Videla et al. 2006) and in laboratory experiment, parasitism rate of *H. arduine* was suspected to be influenced by host-pest interactions (Muchemi et al. 2018a). Elsewhere, field observations showed significant high parasitoid population of *Chrysocharis parksi* Crawford (Hymenoptera: Eulophidae) on *L. sativae*-infested tomatoes over *L. trifolii*-infested celery, which was the most dominant pest under similar situation (Zehnder & Trumble 1984). *Phaedotroma scabriventris* was reported to cause higher mortality and was more suitable in host crops which had bigger *Liriomyza* pests (Videla et al. 2006). In Argentina, consistently higher densities of *L. huidobrensis* have been reported to occur on specific crops when several host species were simultaneously available in the field (Salvo & Valladares 1995, Valladares et al. 1996, 1999) suggesting host preferences in *Liriomyza* species illustrating the influence of host plants on parasitoids (Murphy & LaSalle 1999).

Our results further indicated that crop farming preferences within the sampled localities had an impact on the establishment of parasitoids. Tomatoes and beans (French and local beans) were the main crops under production in the low and mid elevation zones due to their commercial value while in the high elevation zone, potatoes, beans, and peas were often sampled in the fields. Infestation by LMF and associated parasitoids is expected to follow host plant distributions as dictated by crop production preference. Similar host plant distribution patterns were reported by Gitonga et al. (2010), Guantai et al. (2015) and Foba et al. (2015b) in Kenya. Related to host plant distribution is the relative application of pesticides. Farmer awareness before release of parasitoids is likely to have played a positive role in the reduced usage of pesticides. There is restricted use of pesticides on these host plants due to the residues restrictions in the export market especially amongst the producers of French beans and peas for export (Brancato et al. 2017). However, in the local market, there is no restriction on pesticide residues hence products such as tomatoes destined to this market, end up receiving high pesticide usage as noted in the mid elevation zones.

Cropping system structure is an important aspect in biological pest control due to its influence on natural enemy's population. In complex cropping system, parasitism of pest is higher with lower crop damage than in simple cropping system with a high percentage of agricultural use (Thies et al. 2003). Undisturbed and complex habitats contribute to the success of biological agents as observed in our current studies where intercropped fields supported high numbers of *C. flacilla* parasitoids. Because of the intense pressure from modern farming practices, many agricultural cropping systems are poor for natural enemies to thrive (Thies & Tscharntke 1999, Landis et al. 2000). Simple cropping systems are poor in the successful establishment of imported natural enemies and in controlling the target pest than in more stable cropping systems (Stiling 1990). High densities of host pest were observed in our current studies in monocropping systems. Although it is not always possible, habitat diversification and management can be important for enhancement of *Liriomyza* parasitoids. Through experimental and comparative methodologies, Salvo et al. (2005) analyzed the parasitoid assemblages of *L. huidobrensis* in relation to natural, urban, and cultivated habitats and showed that overall; parasitism and parasitoid species-richness were lower in simple than in complex habitats. Therefore, complex habitats with disturbed habitats are likely to influence the success of introduced parasitoids in the vegetable production systems of Kenya. The patterns of the seasons in Kenya have been inconsistent since the 1980's (Hassan 1998, Jaetzold et al. 2006, Foba et al 2015b) which is likely to be a consequence of climate change.

6.7. Conclusion

Halticoptera arduine and *C. flacilla* parasitoids have successfully established in Kenya confirming the prediction of their potential spread in tropical areas of Africa (Mujica et al. 2016). Their potential in suppressing the pest holds positive impacts for the small holder farmers. Further survey is warranted to establish the potential of *C. flacilla* to spread given that it has shown high establishment within six months' post release. In addition, it would be necessary to assess, the impact of the introduced parasitoids, *P. scabriventris*, *H. arduine* and *C. flacilla* over a given period in suppressing *Liriomyza* leafminers. We would recommend the up scaling of release efforts in other vegetable production areas not covered in our current studies to hasten their distribution and further boost LMF suppression.

7.0. Synthesis

Management of leafminers worldwide, and particularly in East Africa, has relied on the use of synthetic chemical insecticides (Chandler 1984, MacDonald 1991, Gitonga et al. 2010). However, the frequent use of these chemicals resulted in insecticide resistance of the flies (Parella et al. 1984; Murphy & LaSalle 1999), pollution of the environment as well as elimination of their natural enemies (Johnson et al. 1980, Murphy & LaSalle 1999). Chemical control is also not very effective since flies usually escape insecticide applications due to their high mobility. Furthermore, *Liriomyza* larvae, once inside the leaf tissues, are inaccessible to many pesticides and subsequently pupate in the soil (Mujica & Kroschel 2011). In recent years, market access of fresh produce from many developing countries into the EU has been constrained by exceedance of maximum residue levels of pesticides (RASF 2014). Many livelihoods of small-scale growers who engage in the production of fresh produce for the export markets are endangered. The growing concerns of high LMF resistance to insecticides, pesticide residues in fresh food products and loss of biodiversity have become a global issue. Considering the risks associated with synthetic chemical pesticides, there is need for a more sustainable pest management against LMF.

Biological control using parasitoids has been considered as an alternative to leafminer management in East Africa and beyond (Migiro et al. 2010), with most research focusing on augmentative releases and less on classical biological control of LMF in Africa.

This dissertation evaluates the process of introducing two exotic parasitoids with neotroprics origin, i.e. *H. arduine* and *C. flacilla*, against LMF in Kenya. The acceptance of the exotic parasitoids to utilize the most important invasive LMF species, namely *L. huidobrensis*, *sativae* and *L. trifolii*, and the suitability of these hosts for the development of the parasitoids is a significant move towards the process of parasitoid introduction. Given the high performance of the parasitoids in host parasitization and reproduction potential on *L. huidobrensis*, this study highly recommends the use of *L. huidobrensis* for parasitoid mass rearing. Because only female parasitoids in addition to host parasitization cause host killing, female biased progenies improve parasitoid fitness and guarantee a stable population (Pascua & Pascua 2004, Abe & Kamura 2012). However, only *C. flacilla* and unlike other endoparasitoids associated with *Liriomyza* leafminer, caused additional host mortality, a positive attribute for a biological control agent. Thus, performance of *C. flacilla* may not only be based on host parasitization but also on such non-reproductive host mortality. Findings from this research on acceptability and suitability of *H. arduine* and *C. flacilla* on three species of *Liriomyza* leafminer are good indicators for consideration for introduction into new geographical areas. Further to this, these results have improved the understanding of the biology and ecology of the two parasitoid species and their use in the management of LMF.

An assessment of the interaction between the exotic parasitoids to the existing indigenous parasitoid is a necessary precursor to the introduction of natural enemies to new geographical areas (Bokonon-Ganta et al. 2005). The assessment between *H. arduine* and *C. flacilla* and *D. isaea,* the latter one of the most important indigenous LMF parasitoids in Kenya (Chabi-Olaye et al. 2008, Musundire et al. 2011, Akutse et al. 2013, Foba et al. 2015a), proofed co-existence between these parasitoid species. Although performance through parasitization of the two exotic parasitoids under laboratory conditions was superior to *D. isaea* (Akutse et al. 2015, Foba et al. 2015a, Muchemi et al. 2018b&d), the presence of *D. isaea* significantly boosted overall host suppression. Synergetic effects to the indigenous parasitoid reproduction potential were recorded as well. Overall, no negative interspecific interactions that could have led to out-competition of the less competitive species irrespective of the shared host resources (Wang & Messing 2003, Tian et al. 2008) was observed. Rather, there was co-existence between the exotic and indigenous parasitoid species thus posing little risk of species displacement following releases of the two introduced species. Based on these findings, specific recommendations for the field release of the two exotic parasitoids in three agro ecological farming systems were made.

The subsequent field releases of the two exotic parasitoids against LMF in Kenya in a classical biological control program were a success. While *L .huidobrensis* has been identified as the preferred host for parasitoid rearing, it is an also a dominant species of LMF across agro ecological systems of Kenya (Foba et al. 2015b) and may therefore be a significant stabilizing host for the introduced parasitoids. However, this dominance is a potential threat to biodiversity loss when other LMF species are displaced by *L. huidobrensis*. This observation of *L. huidobrensis* dominance across vegetable production systems of Kenya contradicts earlier report by Chabi-Olaye et al. (2008) where *L. huidobrensis* was found to be dominant in the high elevation zones, while *L. sativae* and *L. trifolii* were dominant in mid and low elevation zones respectively. Above this, *H. arduine* and *C. flacilla* successfully utilized the local *Liriomyza* hosts in the system occurring on twenty plant host species to become established in Kenya as predicted by Mujica et al. (2016). Five new association of *Liriomyza* parasitoids species were made in Kenyan as compared to the previously reported nine parasitoid species (Chabi-Olaye et al. 2008, Foba et al. 2015c). In the assessment of factors impacting parasitoids establishment, seasons significantly influenced both introduced parasitoid species while host plant and cropping systems/ landscape only had an influence on *C. flacilla* establishment. Weather patterns in Kenya are characterized by cold and hot seasons with or without precipitation (Hassan et al. 1998). Thus, the previous laboratory rearing temperature regimes corresponded well to the high and low temperatures and favored development of *H. arduine* and *C. flacilla*. Hence, agro-ecological systems in Kenya have the suitable environmental conditions to support each of the two parasitoid species at different times of the year to facilitate pest suppression. Notwithstanding this, landscape is one of the factors known to greatly influence foraging behavior of parasitoids (Cronin & Reeve, 2005, Tscharnkte et al. 2008). While pure stand fields may support high densities of host pests and aggregation of specific natural enemies, intercropped fields on the other hand, can support a greater diversity of natural enemies (Thies et al. 2013). In line with this study, intercropped fields favor the establishment of *C. flacilla* more than mono or pure stand fields indicating the need for mixed cropping landscapes to provide foraging avenues and reservoirs for parasitoids and natural enemies. In the surveys a range of insecticide, compounds were found to be used by small scale growers across different agro ecological systems, though none of them seem to have had an impact on parasitoid establishment. However, in other studies, pesticide compounds had detrimental effects on *Liriomyza* parasitoids and other natural enemies (Gitonga et al. 2010, Guantai et al. 2015), though this could potentially be mitigated by sensitizing small-scale farmers, and other stakeholders in the fresh produce export value chain on the risks of pesticides. On a local level, this research has established that the two exotic parasitoids have the potential to successfully impact on populations of *Liriomyza* hosts in different agro-climatic zones of the Afro tropics. Their ability to complete development in local LMF hosts, cause pest mortality, and suppression and establish in three different agro ecological vegetable production areas in a classical biological control program is promising, though the potential threat posed by the indigenous encyrtid hyperparasitoid wasp *Cheiloneurus carinatus* Compere to the introduced parasitoids needs further evaluation.

On a global scale, the contributions from this dissertation are valuable in the discipline of agricultural sciences. However, they may not be enough to allow full understanding of *H. arduine* and *C. flacilla* biology and their interactions with other parasitoids occurring in the afro tropical areas unless further studies involving different *Liriomyza* hosts, other indigenous parasitoid species, and field impact assessments are done. Yet, results from this study indicate that the exotic parasitoids pose little risk on one dominating indigenous LMF parasitoid species; on the contrary, their introduction has lead to a boosting of target pest suppression. Thus, an IPM strategy to the overreliance on chemical pesticides against LMF by small-scale growers could be demonstrated in Kenya. This strategy has the potential of being replicated in other regions experiencing similar pest challenges and with similar climatic regimes. In addition, knowledge gained through the analysis of parasitoid interactions allows for future improvement of classical biological control against LMF in Africa. The dissertation as well allows for broader gains in environmental conservation, human health, food safety and improved economic returns to the small-scale producers of horticultural crops in developing countries.

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Acknowledgement

I acknowledge the financial support from the German Federal Ministry for Economic Cooperation and Development (BMZ) (Grant number: 09.7860.1-001.00; Contract number: 81121261) that enabled research to be done in Kenya. I am also grateful to the Dissertation and Research Internship Program (DRIP) of the capacity building of *icipe* for the award of my PhD scholarship and *icipe* for hosting my research work. I cannot fail to acknowledge the Kenyan Government for its support to facilitate the present work in Kenyan soil.

I am also truly grateful to the German Federal Ministry for Economic Cooperation and Development (BMZ) for funding the Leafminer Project in which this research was conducted. I am also indebted to the Faculty of Agriculture in the University of Hohenheim for accepting my PhD candidature at the institute of Phytomedicine. My acknowledgements as well go to the Faculty of Agriculture of the University of Bonn for accepting my transfer of the PhD program and awarding the doctorate degree.

Specifically, I am grateful to Prof. Christian Borgemeister, Director, Center for Development Research (ZEFc), University of Bonn, and former Director General of *icipe* for accepting to be my promoter and having initiated the collaborative capacity building for National Agricultural Research Station (NARS) in Kenya. You have played a pivotal role in the review of manuscripts from this research program and my dissertation. Am also grateful to Prof. Dr. Dr. Claus P. W. Zebitz, the chair Entomology Department of the Institute of Phytomedicine in Hoheinheim University for his acceptance as supervisor to my PhD candidature. Your valuable advice and guidance during data collection, publications, and thesis will undoubtedly be inscribed in my life. To Dr. Fiaboe K. K. M, the coordinator of LMF project at *icipe*, you are a great mentor in research; writing and you never relented on exerting pressure when it seemed not well with me. I appreciate and will forever cherish working with you.

I am equally grateful to Dr. Sunday Ekesi, Director Research and partnerships at *icipe* the role you played in the initiation stages of *Liriomyza* leafminer (LMF) research program and the direction you gave during publications from this PhD program. To the late Dr. Chabi-Olaye Adenirin, I lack the words to appreciate you for initiating me into the LMF program as a PhD scholar, may his soul rest in peace. I am also grateful to Prof. Krochel Jurgen and Dr. Norman Mujica of the International Potato center (CIP) for accepting to share with us the initial colonies of the exotic parasitoids from Peru which formed the basis for my research work.

To my predecessor scholars in the LFM project at *icipe*, Drs. Fauster Akutse and Foba Ngichop for their role in providing guidance in insect rearing, research techniques and their unending commitments in the review of manuscripts of this study. To all the staff of LMF project at *icipe* laboratories notably; Faith Nyamu, Nicholas Mwikya, Nancy Mwende, Raphael Mukiti, Raphael Kioko, SosPeter Wafula, Druscilla Obonyo, Joseph Mucheru and all the interns who worked in the project for short period, your support in the provision of research materials and technical assistance during laboratory and field activities made me accomplish a big mileage in my life.

I am truly grateful to staff members and scholars at the Institute of Phytomedicine notably Sandra Garken, Maggein, Helene, and Irina amongst others not mentioned for their support during my stay in Germany. They made the stay possible by organizing for

My travel logistics, accommodation and supported my recovery when I fell ill during one of visits to the University.

To my family and friends, your encouragements made my academic journey enjoyable than I expected. Finally, to my dear wife Leah, son Cedric and daughter Trisha, your kind and loving support has made my studies so much less burdensome and you are truly an inspiration to me.

God, bless you all.