Host plants, herbivores and natural enemies in Kenyan horticulture: tritrophic interactions involving *Liriomyza* leafminers (Diptera: Agromyzidae)

BY

ROBERT MUSUNDIRE

Submitted in partial fulfilment of the requirements for the degree
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Declaration

I, Robert Musundire, declare that the thesis which I hereby submit for the degree *Philosophiae Doctor* (Entomology) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature...

Date....10/12/2010..

Dedication

This work is dedicated to my parents Mr. A.L. Musundire and the late Ms C. Kufandikamwe. The way you raised me made it possible for these heights to be reached. To the late Cicilia, the little messages you left behind inspired me throughout and I am proud to have achieved what you always wished for your only son. To my father, thank you for all the necessary support you gave me throughout my early academic life. To my surviving grandmothers, thank you for taking care of me and though in your advanced ages, your wisdom was always there to show me the way during difficult times. Finally, to my family and friends, thank you for being supportive during the entire period of my study. Courtney all those years of absence, surely, you must have been wondering.

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Host plants, herbivores and natural enemies in Kenyan horticulture: tritrophic interactions involving *Liriomyza* leafminers (Diptera: Agromyzidae)

Student:	Robert Musundire
Supervisor:	Dr. Kerstin Krüger
Co-supervisor:	Dr. Adenirin Chabi-Olaye (*icipe*)
Department:	Zoology and Entomology, University of Pretoria
Degree:	Philosophiae Doctor

Abstract

In the Afrotropical region, the accidental introduction of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii*, like in other parts of the world, has caused considerable economic losses, especially in the production of vegetables and ornamental plants. The management of these *Liriomyza* species and other agromyzid pests requires various sustainable methods of which biological control by parasitoids is a key aspect.

To determine the potential of Afrotropical parasitoids for biological control, data on distribution of Agromyzidae, host plant records and associated parasitoid species was collated from museum collections, available literature and own observations. Most of the 599 agromyzid records comprising 301 species are from East (36 %) and Southern Africa (34 %). Host plant records include 48 plant families. The paucity of parasitoids (105 records) associated with only 7 % of agromyzid species suggests a lack of sampling effort.

Although the exotic biological control agent *Diglyphus isaea* (Hymenoptera: Eulophidae) successfully established after introduction to control *Liriomyza* species in Kenya, no studies have been undertaken on host plant-leafminer-parasitoid interactions. In view of the limited information available on local parasitoids, the tritrophic interactions between the three *Liriomyza* species, four crops and *D. isaea* were assessed in the current study.

Body size, which has previously been positively linked with leafminer fitness, was determined for the three *Liriomyza* species reared on *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba* using wing morphometric and hind tibia measurements. Two distinct leafminer morphospecies (*L. huidobrensis* cluster and *L.*

sativae, *L. trifolii* cluster) were recognized across all plant species based on canonical discriminant analyses. Unlike *L. sativae* and *L. trifolii*, rearing *L. huidobrensis*, the largest of the three species, on different host plant species did not result in differences in the size of adult progeny. Hind tibia length was strongly correlated with wing length and could reliably be used as indicators of body size.

To assess plant-leafminer-parasitoid interactions at the third trophic level, rate of parasitism, host feeding and sex ratios of *D. isaea* were determined in no-choice and choice experiments. Results suggest that larval size of the host is not necessarily positively linked with parasitism but that plant related factors influenced parasitism.

To determine why abundance and efficacy of *D. isaea* differ in different habitats in Kenya, the role of olfactory cues of host plants in host finding by the parasitoid has been examined in a Y-tube olfactometer experiment. Results showed that interactions between host plant and leafminer species influence the degree of attraction to leafminer-infested plants. To explain the behavioural pattern by *D. isaea* in the olfaction experiment, an analysis of volatile components emitted by leafminer-damaged plants, showed considerable qualitative variations within the same host plant species infested with different *Liriomyza* species for all plant species except for *S. lycopersicum*.

In conclusion, the suitability of *D. isaea* for controlling *Liriomyza* species is variable and depends mostly on host plant species and leafminer size. A need exists for more intensive collaborative research in the afrotropic zone to identify other suitable biological control candidates.

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GENERAL INTRODUCTION

Field and greenhouse vegetable production worldwide has been continuously under threat since the early 1950s from New World *Liriomyza* species (Diptera: Agromyzidae), collectively termed leafminers (Spencer, 1973, 1989; Parrella, 1982; Minkenberg & van Lenteren, 1986; Murphy & LaSalle, 1999; Kang *et al.*, 2009; Liu *et al.*, 2009). Of these, three highly polyphagous species, *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess), have been found to attack vegetable and ornamental plants in many parts of the world (Chaput, 2000). These species are known to have originated in the warmer parts of the New World but have subsequently been spreading to Africa, Asia, and various oceanic islands (Murphy & LaSalle, 1999; EPPO, 2006).

The serpentine leafminer, *L. trifolii* was reported to have been accidentally introduced into Kenya in 1976 and has subsequently been recorded in a number of localities from the coastal areas to the highlands (Spencer, 1985). Reports from interception of produce in Europe indicate that the pea leafminer, *L. huidobrensis,* and the vegetable leafminer, *L. sativae,* are also present in Kenya (B. Löhr, pers. comm.). A recent report by Chabi-Olaye *et al.* (2008) suggests that the three leafminer species are widely distributed in Kenya.

Damage caused by *Liriomyza* leafminers

Adult female leafminers make small punctures in the upper epidermis with their pointed ovipositors. These punctures cause a stippled and yellowish appearance of the leaves and are easily visible during heavy infestations (Chaput, 2000). The larvae of all three species produce mines on leaves, young tender stems and pods. Individual mines are of little significance as it is the entire larval mine network that results in considerable destruction of the leaf area (Fig. 1). Feeding damage of large/dense populations can thus severely weaken or even destroy both young and mature plants. Heavily damaged plants appear as if scorched by fire, especially broad beans (*Vicia faba* L. (Fabaceae)). Infested leaves are more susceptible to wind damage and infection by plant pathogens (Chaput, 2000). Furthermore, in heavily mined crops, accumulations of mines may necessitate more trimming, cleaning and culling before the produce can be marketed (Capinera, 2001).

Larvae of *L. huidobrensis* usually begin feeding on the upper leaf surface and move to the lower surface (spongy mesophyll) after a few millimetres of surface feeding (Parrella & Bethke, 1984). The mines are usually found along the midrib and lateral veins (Fig. 1) (Spencer, 1990). The larvae deposit frass in thin, broken to continuous lines down the middle of the mine. The placement of mines on the leaf underside, the location of mines along leaf veins and frass deposition pattern of *L. huidobrensis* larvae are distinctive for this species (Spencer, 1973). However, this pattern may be obscured when many larvae feed together on the same leaf (Spencer, 1973). Typically, mines of *L. sativae* follow a more loosely, irregular serpentine pattern (Fig. 2), while larvae of *L. trifolii* construct tightly coiled almost blotch-like mines (Fig. 3) (Collins, 2009).

The mine configuration of the three *Liriomyza* species is affected by both the physical and physiological condition of each leaf and the number of larvae mining the same leaf. Due to the high variation, mine patterns cannot be relied upon for identification of *Liriomyza* leafminer species (OEPP/EPPO, 2005).

Fig. 1. Damage by *Liriomyza huidobrensis* adults and larvae on Swiss chard (*Beta vulgaris*). Small spots are punctures made by adult females during feeding and oviposition. Whitish lines between leaf veins are mines formed during larval feeding.

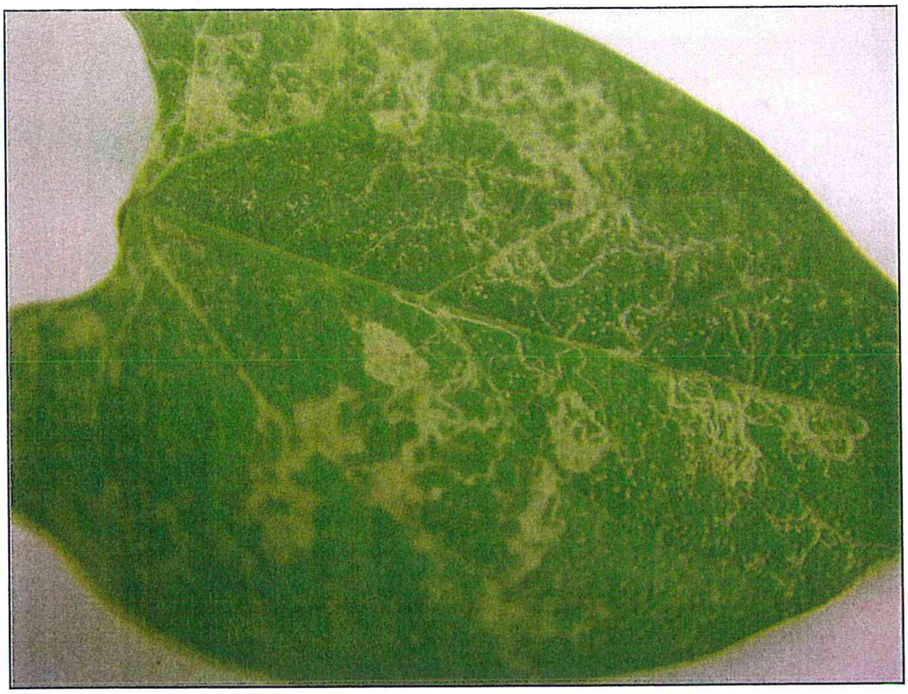

Fig. 2. Mines of *Liriomyza sativae* on *Phaseolus vulgaris.* Thin lines are mines formed by early-instar larvae.

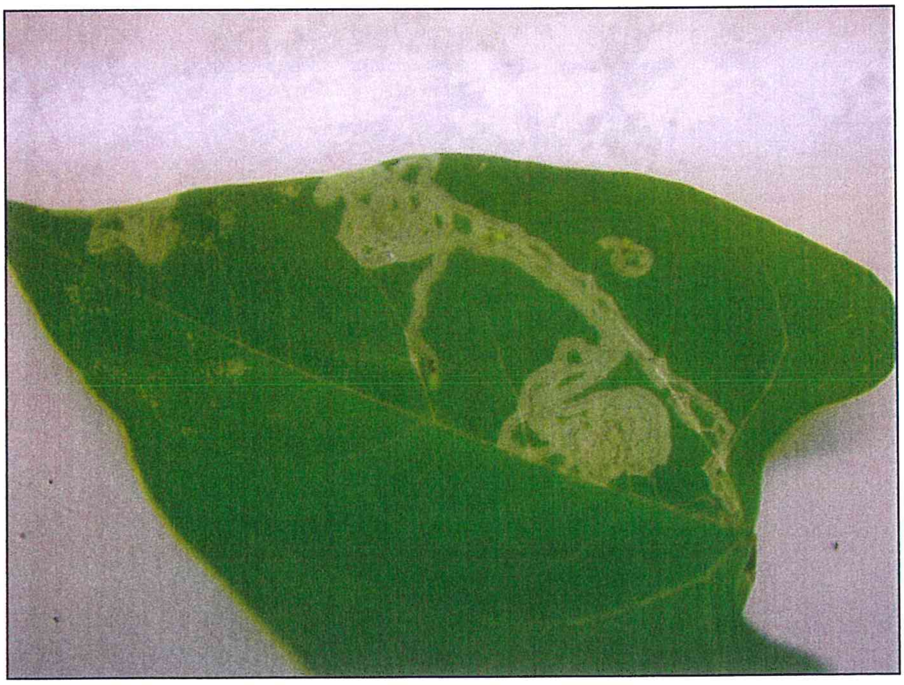

Fig. 3. Mines of *Liriomyza trifolii* on *Phaseolus vulgaris.* The tightly-coiled mines are typical of the larval feeding damage of this leafminer species.

Economic losses caused by *Liriomyza* leafminers

In Kenya, considerable *Liriomyza* leafminer damage has been reported from *Pisum sativum* L. (snowpeas), *Phaseolus coccineus* L. (runner beans), *Phaseolus vulgaris* L. (French beans) (Fabaceae), *Abelmoschus esculentus* (L.) Moench (okra) (Malvaceae), and cut flowers, with yield losses ranging from 50 to 100 % (B. Löhr pers. comm.). *Liriomyza huidobrensis* is a notifiable pest in the European Union, thus there are official controls to avoid its spread (Murphy & LaSalle, 1999). This leafminer species has therefore posed limits to new market opportunities for Kenyan horticultural producers due to strict quarantine requirements by the overseas markets. *Liriomyza sativae* and *L. trifolii* are major international pests of ornamental plants and many crops belonging to Solanaceae, particularly tomatoes, Cucurbitaceae and Fabaceae (Spencer, 1989).

The three leafminer species have also been reported to occur on wild host plants in both native and adventives ranges (Spencer, 1973; Spencer 1990). This is believed to increase the pest status of the leafminer species because they can survive in the absence of cultivated host plants (Spencer, 1990).

Description of the leafminer life cycle stages

Adult description

Adults of *Liriomyza huidobrensis* are distinguished from other pest species of *Liriomyza* particularly *L. sativae* and *L. trifolii* by larger body size, overall dark colour; larger discal cell, relatively short distal section of vein M_{3+4}, darkened femora (yellow in *sativae* and *trifolii*), and the male genitalia (Spencer, 1973). The adults of *L. trifolii* are smaller and have a more grayish upper thorax because of more bristles compared to *L. huidobrensis* and *L. trifolii*; the area behind the eyes is mostly yellow (Chaput, 2000). Adults of *L. sativae* are shiny black on the upper surface and the area between the eyes is yellow whereas the area just behind the eyes is black (Chaput, 2000). However, the identification of *L. sativae* and *L. trifolii* based on the colour patterns is very difficult and unreliable.

Morphological identification of the three-leafminer species is based on the distiphallic structure, a terminal part of the aedaegus (OEPP /EPPO, 2005). The distiphallus is a very small, fragile structure enclosed by membranes and requires careful dissection and subsequent examination under a high-powered microscope. However, separation of the three species using this structure can only be made for the differentiation of *L. huidobrensis* from *L. sativae* and *L. trifolii* and for male insects only (Spencer, 1973; Spencer, 1990; OEPP/EPPO, 2005; Collins, 2009).

Adult biology

The time between adult emergence and mating (premating interval) is inversely related to temperature and may differ for the sexes (Parrella, 1987). Mating in the majority of adult leafminers occurs soon after emergence with almost all females mating within 24 hours of emergence (Parrella, 1987). For maximum egg production, females undergo multiple matings (Parrella, 1987). Mating takes place especially during morning hours (Parrella, 1987). However, Chaput (2000) reports that mating may occur at any time of day but is most frequent during daylight hours and is dependent on cloud cover. Temperature, relative humidity and availability of food determine the pre-oviposition period, which may extend to 5 days (Parrella, 1987).

The behaviour of females is of most significance in perpertution of leafminer populations after mating. Females feed from all the punctures they make before laying eggs on a proportion of them (Parrella, 1987). Eggs are laid in oviposition punctures termed 'stipples' (Chaput, 2000). Feeding and oviposition by adults appear to occur most commonly during the morning hours and the frequency of activities is positively correlated to temperature (Fagoonee & Toory, 1984; Parella, 1984). Mean egg production per female ranges from less than 100 to more than 600, depending on environmental conditions and leafminer species (Parrella, 1987). Fecundity as reported by Parrella (1987) is strongly related to food source and temperature, with maximum oviposition occurring between 20-27 °C. However, according to Chaput (2000), in all three leafminer species optimal temperatures for egg laying range between 21 and 32 °C and egg laying is reduced at temperatures below 10 °C. In younger females, eggs are layed at a rate of 30 to 40 per day, with numbers decreasing as flies age (Chaput, 2000).

Eggs

Eggs of the three *Liriomyza* species are laid singly in punctures in the leaf epidermis (Parrella, 1987). Eggs of *L. sativae* and *L. trifolii* are similar but differ from those of *L. huidobrensis*. Freshly laid eggs of *L. trifolii* and *L. sativae* are creamy white and shaped like an elongated oval, small (0.2 mm in length) and hatch in 2 to 4 days, whereas eggs of *L. huidobrensis* are white, elliptical and measure about 0.23 mm in length and 0.13 mm in width and hatch in 3 to 5 days (Chaput, 2000; pers. obs.). Neither genus nor species identification is possible by examining the eggs (OEPP/EPPO, 2005).

Description of larvae

There are three larval stages, each of which is completed in 2 to 3 days. The full length of third-instar larvae is species- and environment-dependent and has important implications on leafminer-parasitoid relationships. In all three leafminer species, larvae are initially nearly colourless, becoming greenish and then yellowish as they mature. These characters are also of valuable importance for the natural enemies of leafminers as the colour patterns can be perceived by parasitoids especially in determining the suitable instar for parasitism. Black mouthparts are apparent in all instars, and can be used to differentiate between instars (Petitt, 1990; Head *et al.*, 2002). Larvae of the three leafminer species can be separated by the posterior spiracles. The larvae of *L. huidobrensis* have six to nine spiracles that appear as bulbs, while those of *L. sativae* and *L. trifolii* have only three spiracles (Parrella, 1987).

Larval behaviour

The mature larvae in all three leafminer species cut a semicircular slit in the mined leaf just prior to formation of the puparium. The slit in *L. sativae* and *L. trifolii* is made in the upper surface of the leaf while in *L. huidobrensis* it is on the lower surface. In some cases depending on host plant species, larvae of all the three species usually emerge from the mine, drop from the leaf, and burrow into the soil to a depth of only a few centimetres to form pupae (Steck, 1999). However, in some instances for example on *Phaseolus vulgaris*, the larvae of *L. sativae* and *L. trifolii* emerge from the mine and pupate on the leaf surface (pers. obs.)

Description of pupae

The pupae of *L. sativae* and *L. trifolii* are oval, narrowing at the ends, yellow-brown in colour, distinctly segmented and measure about 1.2 mm in length and 0.55 mm in width depending on the host plant the larvae were feeding on. In *L. huidobrensis* the reddish brown puparium when reared on *P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*, measures about 1.5 mm in length and 0.75 mm in width (pers. obs.). Pupal development is completed in 5 to 12 days, whereupon the adult emerges from the puparium, principally in the early morning hours, both sexes emerging simultaneously (Chaput, 2000).

Life cycle

Under greenhouse conditions at 27 °C, for *L. huidobrensis* the egg stage last 3 days, larval stages 3 to 5 days while the pupal stage lasts for 9 days (Parrella & Bethke, 1984). Development time required by *L. sativae* egg and larval stages is about 7 to 9 days at 25-30 °C while pupal development takes about 9 days at the same temperatures under laboratory conditions (Capinera, 2007). In the laboratory and at 25 °C, *L. trifolii* egg stage requires about 3 days for development, while the larval stages require about 5 days and pupal stage about 9 days (Minkenberg, 1988).

Historical profile of pest status of leafminer species in Kenya

The most widely reported reason for the first leafminer outbreaks in their adventive ranges was the indiscriminate use of insecticides and non-target effects on their natural enemies (Murphy & LaSalle, 1999). Many growers of horticultural crops in Kenya have used avermectins (abamectin), triazines (cyromazine), carbamates, organophosphates and pyrethroids to control leafminers (Kabira, 1985). Due to zero tolerance for leafminer larvae on fresh produce, many farmers responded to leafminer attacks with heavy applications of these groups of pesticides. There have been reports of cases where chemicals were no longer effective in controlling leafminers (B. Löhr, pers. comm.) as a result of insecticide resistance (B. Löhr, pers. comm.).

Robin (1983) reported a similar case in Hawaii (USA) during 1978 and 1979 when watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai (Cucurbitaceae) growers in Oahu suffered crop losses due to *Liriomyza* damage. Attempts made by farmers to control leafminers with chemical insecticides failed because leafminers had become resistant to them (Robin, 1983). Similarly, Kotzee & Dennill (1996) reported that *L. trifolii* developed resistance to the chemical cyromazine, a triazine, in South Africa. Macdonald (1991) reported that *Liriomyza* leafminer had shown higher levels of resistance to most carbamate, organophosphate, and pyrethroid insecticides commonly used to kill larvae and adults in the United Kingdom.

Current biological control methods in leafminer pest management

The management of agromyzid leafminers became a topic of extensive research and more scientific debate in the early 1990s (Murphy & LaSalle, 1999; Ode & Heinz, 2002; Liu *et al.*, 2009). Some researchers believe that integrated pest management approaches based on conservation of existing natural enemies and introductions of additional species, offer viable alternatives to the application of insecticides. Biological control by native natural enemies is among the most important methods in agricultural pest management (Kang *et al.*, 2009). Research in various parts of the world revealed that natural enemies are important in regulating *Liriomyza* species in their native and adventive ranges (Neuenschwander *et al.*, 1987; LaSalle & Parella, 1991; Johnson, 1993; Murphy & LaSalle, 1999; Rauf *et al.*, 2000; Tran *et al.*, 2005).

Parasitoids recorded from *L. huidobrensis*, *L. sativae* and *L. trifolii* from around the world are diverse. For example, Waterhouse & Norris (1987) listed more than 40 species of parasitoids from northern America. The parasitoid genera *Opius* (Hymenoptera: Braconidae), *Chrysocharis* and *Diglyphus* (Hymnoptera: Eulophidae), and *Halticoptera* (Hymenoptera: Pteromalidae) are of global distribution (Murphy & LaSalle, 1999). There is evidence that species of these genera and many other *Liriomyza* parasitoids display a high degree of polyphagy, explaining why they can readily control alien invasive species (Murphy & LaSalle, 1999). In regions where concerted efforts of biodiversity conservation are undertaken, pools of potential biological control agents of leafminers can be found (LaSalle, 1993). Regional biodiversity can thus serve as a source of indigenous parasitoids that can contribute to the control of invading agromyzid pests (Murphy & LaSalle, 1999). However, in the Afrotropical region considerable research gaps on the diversity of natural enemies of agromyzids exist which limit our knowledge of conservation biological control techniques of leafminers pests.

Amongst the common parasitoids of leafminers, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) has been shown to be effective at higher temperatures (Minkenberg, 1989) and thus could be effectively used in controlling leafminers in tropical environments. This species occurs widely as a larval ectoparasitoid of leafmining diptera on herbaceous plants in Europe, Northern Africa and Japan (Minkenberg, 1989). It is usually a solitary larval ectoparasitoid of agromyzid leafminers including *L. huidobrensis*, *L. sativae* and *L. trifolii* (Musundire, 2002; Ode & Heinz, 2002; Liu *et al.*, 2009). The parasitoid has been reported to be an effective augmentative biological control agent where some damage to the leaves can be tolerated (Kang *et al.*, 2009). In some cases reduction in pesticide use combined with integrated pest and crop management often allows naturally occurring *D. isaea* to invade crops, and these can contribute significantly to leafminer control (Liu *et al.*, 2009).

In Africa, large-scale mass-production programmes of *D. isaea* have been developed to support biological control of leafminer efforts in Kenya and South Africa through augmentative biological control approaches (A. L. Owuor, Dudutech Pvt Ltd-Kenya, pers. comm.).

Description of *Diglyphus isaea*

Adult *D. isaea* are very variable in size and are a dark metallic green in colour (Fig. 4) (Bouček, 1988). One of the distinguishing characteristics of this parasitoid is the submarginal veins (SMV) of the fore wings, which have three or more dorsal satae. Fisher *et al.* (2005) give the full adult description.

The colour of the hind tibia varies between males and females. In males, the hind tibia has alternated yellow and black patches; sometimes the yellow is more pronounced (Bouček, 1988). In females, the yellow patches do not alternate with the black patches along the hind tibia. Yellow patches are only located near the tarsus end or the tronchanter (Fig. 4). The colour patterns of the hind tibia are often used to distinguish the sexes of this species (Bouček, 1988).

Female *D. isaea* search for suit
them through the leaf cuticle. The stin
female wasps then lay one or more eg
larvae of *D. isaea* feed externally or
continue feeding on the paralysed hos
2002). Microscopic examination and d
to be turgid with a parasitoid larval sta
mine stops growing and the leafmi
develops.

Fig. 4. Adults of *Diglyphus isaea*. T
are used to distinguish between mal

Behaviour of Diglyphus isaea

The amount of food available to each
variable size of adult parasitoids. A
control the sex of the eggs they lay. G
larvae and female eggs on large host
of available host larvae can affect the
is important for mass rearing *D. is*
parasitoids into crops as it determin
regenerating population (Ode & Heinz

Females of *D. isaea* and its congener *Diglyphus begini* Ashmed have been shown to feed on host larvae (host feeding) (Minkenberg & van Lenteren, 1986; Heinz & Parrella, 1989; Ode & Heinz, 2002). This allows them to produce optimal number of eggs and is important in boosting fecundity and longevity and maximising offspring production. Small host larvae are normally rejected or host-fed by the adult females (Minkenberg & van Lenteren, 1986).

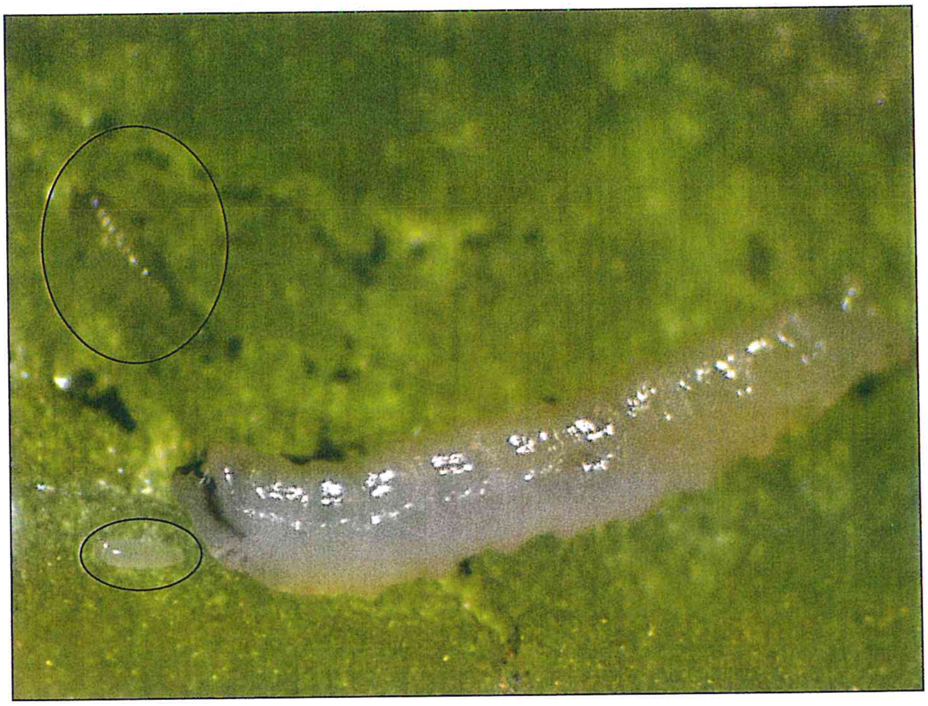

Fig. 5. Paralyzed *Liriomyza huidobrensis* larva with *Diglyphus isaea* larvae next to it. *Diglyphus isaea* larvae are translucent and elliptical shaped (black circled objects).

Life cycle

At 20 °C female *D. isaea* larvae develop from egg to pupa in 9 days (Minkenberg, 1989). The pupal stage at this temperature lasts 8 days. At 15 °C development time from egg to adult is 26-27 days, whilst it is shortened to 10-11 days at 25 °C. The development time of the parasitoid is shorter than that of host leafminer species at all temperatures (Bazzocchi *et al.*, 2003).

Plant, leafminer species and *Diglyphus isaea* interactions

Although *D. isaea* parasitizes and host feeds on several *Liriomyza* species on different crops as hosts, the distribution of parasitoids among crops is not uniform in the field (Zehnder & Trumble, 1984; Johnson & Hara, 1987; Chabi-Olaye *et al.*, 2008). Host plant associations of *D. isaea* are poorly understood in vegetable production systems in Kenya. The interactions between herbivores and their host plants, and between herbivores and their natural enemies, are best understood when considered within a tritrophic context (Kang *et al.*, 2009) because when locating their hosts parasitoids must search for potential hosts occurring on different plants growing in diverse habitats (Zhao & Kang, 2002a).

A number of host plant and leafminer-larvae related factors are important in host finding by *D. isaea*. These include visual cues from the plants (colour of leaves, mine shape, mine size), size of leafminer larvae and the volatiles that are released because of adult and larval feeding damage. Amongst these factors, chemical stimuli (volatiles released from plants damaged by adults and larvae) act as cues that direct many parasitoids to plant habitats and their hosts in the long to short distance range (Dicke & Minkenberg, 1991).

A number of studies have demonstrated that chemical information from plants plays key roles in host selection by herbivorous insects including leafminers and host location by the associated parasitoids (Vet & Dicke, 1992; De Moraes *et al.*, 2001; Smid *et al.*, 2002; Zhao & Kang, 2002a,b; Turlings & Wäckers, 2004; Bruce *et al.*, 2005; Takken & Dicke, 2006; Wei *et al.*, 2006; Wei *et al.*, 2007). The chemical compounds produced by the plants can be classified according to their effect on the host-location behaviour of insects into categories such as attractants, repellents, feeding and oviposition stimulants, and deterrents (Bernays & Chapman, 1994). These chemicals can be either constitutive or inducible. They play important roles in host selection by the leafminers and at the same time in host plant defence against them (Kang *et al.*, 2009).

Thesis organisation

This study aimed at (i) reviewing the agromyzid diversity, host plants and associated natural enemies within the Afrotropical region to evaluate future needs for classical, augmentative and conservation biological control efforts against invasive agromyzids especially *Liriomyza* species, in this region, and (ii) examining tritrophic interactions between *Liriomyza* species, their host plants and the parasitoid *D. isaea* to improve biological control of *Liriomyza* species in the Afrotropical region.

The thesis chapters are written in the form of research papers. Therefore, there is some overlap between chapters with regard to parts of the text. After the general introduction, the first chapter provides a review of the existing records on species diversity, host plants and distribution patterns of agromyzids and their associated hymenopteran parasitoids within the Afrotropical region. Chapter 2 deals with the effects of host plant on adult size of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* reared on four host plant species (*Phaseolus vulgaris* L., *Pisum sativum* L., *Vicia faba* L. (Fabaceae), *Solanum lycopersicum* L. (Solanaceae)). This is followed by Chapter 3, in which host feeding, parasitism and sex ratio of *D. isaea* on *Liriomyza* species reared on the four host plant species were determined. In Chapter 4, olfactory responses of *D. isaea* to the four host plant species infested with third-instar larvae of *Liriomyza* species was evaluated. In Chapter 5, herbivore-induced compounds emitted by *Liriomyza*-infested plants species were identified. At the end of the five chapters, a general discussion of all chapters is given, interpreting the results obtained during the study in the context of current knowledge, implications for *Liriomyza*-management practices and needs for future research, followed by general conclusions.

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CHAPTER ONE

Diversity of Agromyzidae and associated hymenopteran parasitoid species in the Afrotropical region: implications for biological control

Abstract

Agromyzidae (Diptera) is a family with many species of economic importance on agricultural plants. However, many species are attacked by hymenopteran parasitoids which are known to be habitat rather than species specific. In the Afrotropical region, information about agromyzid and parasitoid diversity in different habitats is scattered in literature. The aim was to provide a snapshot of this dispersed information and discuss future needs of biological control efforts against invasive agromyzids in the Afrotropics. From published information, 599 agromyzid records comprising 301 species from 20 genera were recorded from 48 plant families. Parasitoids (105 records) were associated only with 20 agromyzid species belonging to 10 different genera. The comparatively low parasitoid diversity in the Aftrotropical region is however, most likely a result of poor sampling effort and lack of taxonomic expertise for parasitoid species in this area. More research on native parasitoids and associated Agromyzidae is therefore required before embarking on extensive biological control programmes.

Introduction

The Agromyzidae is a dipteran family consisting of approximately 2,750 species (Tschirnhaus, 2000). Of these, about 110 species are known to be major pests of cultivated crops (Dempewolf, 2007). All known agromyzid larvae are internal feeders on living plant tissue and exhibit an array of different feeding habits such as leaf-mining, stem-mining, stem-tunnelling and cambium-mining (Dempewolf, 2007). Leaf mining is generally the most widespread feeding behaviour shared by more than 75 % of the species (Spencer, 1973).

The Afrotropical region, which includes Sub-Saharan Africa, the southern and eastern fringes of the Arabian Peninsula, the islands of Madagascar and the western Indian Ocean, represents a unique ecozone characterized by a diverse flora, fauna and climate. The subject of agromyzid diversity in this region was documented by Spencer (1973) and Cogan (1980), who recorded 19 genera. Since then, the distribution and pest status of several species have changed considerably.

The accidental introduction of the invasive *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* Blanchard and *Liriomyza trifolii* (Burgess) to the Afrotropical region has caused considerable economic losses to the production of vegetable and ornamental plants, (e.g. Kotzee & Dennill, 1996; Murphy & LaSalle, 1999; Musundire, 2002; Neuenschwander *et al.*, 1987; Spencer, 1985). For example, the polyphagous Neotropical species *L. trifolii* caused serious damage to *Chrysanthemum* (Asteraceae) species in the lowlands of Kenya, resulting in the cessation of production and consequent loss of substantial exports (Spencer, 1985). In Senegal, Neuenschwander *et al.* (1987) reported *L. trifolii* to be a major pest of indigenous vegetables. In South Africa, *L. trifolii* caused serious losses in tomatoes, *Solanum lycopersicum* L. (Solanaceae) (Kotzee & Dennill, 1996). *Liriomyza huidobrensis* has been shown to cause up to 40 % damage in faba bean (*Vicia faba* L., Fabaceae) in Zimbabwe (Musundire, 2002). *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae), has been shown to cause widespread damage in haricot bean (*Phaseolus vulgaris* L.) and was rated the most serious problem in East Africa (Abate, 1990). In Mozambique, the same pest has been recorded to cause a yield reduction of between 12 to 71 % on field beans depending on time of planting of the crop and level of infestation (Davies, 1998).

Agromyzid pests are controlled mainly chemically or using biological control methods. There is limited documentation available on pesticides used for the control of agromyzids in the Afrotropical region, but it appears that mostly broad spectrum insecticides have been applied (Abate, 1990; Davies, 1998; Musundire, 2002). In general, problems associated with chemical control include the development of resistance to pesticides by agromyzids such as the *Liriomyza* species (Parella *et al.*, 1984; Keil *et al.*, 1985; Murphy & LaSalle, 1999). In addition, the use of broad-spectrum pesticides to control primary pests has been reported to eliminate natural enemies of leafminers (Johnson *et al.*, 1980), which in turn has been reported to lead to pest outbreaks (Parrella, 1987).

The management of major agromyzid pests, therefore, requires sustainable methods of which biological control with parasitoids is a key aspect. Agromyzid leafminers are known to have rich natural enemy fauna, particularly in their areas of origin, and there is evidence that they can regulate leafminers in pesticide free areas (Waterhouse & Norris, 1987; Murphy & LaSalle, 1999). In central Argentina 46 hymenopteran parasitoid species from 29 agromyzid species in a natural habitat have been recorded (Valladares & Salvo, 2001). The same authors also noted that the parasitoids recorded were highly polyphagous.

Indigenous parasitoid species of agromyzid leafminers have also been found in the adventive areas of the invasive agromyzids. Burgio *et al.* (2007) reported 53 hymenopteran parasitoid species from Agromyzidae colonizing weeds in northern Italian agro ecosystems. In his work on the Agromyzidae of economic importance, Spencer (1973) lists parasitoids from different families, including Eulophidae, Pteromalidae, Tetracampidae, Braconidae and Eucoilidae that regulate *Liriomyza* species in areas other than those of *Liriomyza* species origin. Previous biological control programmes against *L. trifolii* and *L. sativae* have been documented by Greathead & Greathead (1992) and Johnson (1993). Evidence from a number of sources suggests that natural enemies can limit the distribution and incidence of leafminers. Neuenschwander *et al.* (1987) reported that invading leafminer populations declined naturally after a few years in Senegal due to the action of local natural enemies. Davies (1998) reported a significant control effect of locally occurring *Eucoilidea nitida* (Benoit) (Hymenoptera: Braconidae) and *Opius melanagromyzae* (Fischer) (Hymenoptera: Braconidae) on

Ophiomyia spencerella (Greathead), *O. phaseoli* Tryon and *O. centrocematis* de Meijere in Mozambique.

Efforts to contain the losses by invasive agromyzid pests in the Afrotropical region through biological control strategies should therefore include the use of existing native parasitoids of agromyzids in this region.

Diversity of natural enemies has been documented in detail in the Neotropical parts of the southernmost USA (Waterhouse & Norris, 1987; Schuster *et al.*, 1991; Schuster & Wharton, 1993; Noyes, 1998), central Argentina (Valladares & Salvo, 2001), the eastern Palaearctic region (Chen *et al.*, 2003; Fisher & LaSalle, 2005), Australia (Spencer, 1989), the western Palaearctic and Nearctic region (i.e. the greatest part) of North America (LaSalle & Parella, 1991; Belokobylskij *et al.*, 2004; Edwards & LaSalle, 2004; Gençer, 2004; Çikman *et al.*, 2006) and Hawaii (Johnson & Hara, 1987). Although information on the diversity of the agromyzid fauna and associated host plants and hymenopteran parasitoid species in the Afrotropical region exists, it is widely dispersed in literature, thus rendering it of less practical use in designing improved biological control programmes of specific agromyzid species in this region.

The present study was carried out to collate and assemble the dispersed information. In addition, examination of the diversity of Agromyzidae and their associated natural enemies in the Afrotropical region is made in relation to the future needs of classical, augmentative and conservation biological control efforts against agromyzids in the Afrotropical region.

Materials and Methods

This review is based on previously published information on the economically important agromyzids of the world. Some of the key references include Spencer (1959; 1960a,b; 1961a,b,c,d,e; 1963; 1964; 1965; 1973; 1977; 1985; 1989; 1990), Cogan (1980) and Dempewolf (2007). Literature data from Kenya and South Africa were verified with reliable records of Agromyzidae in the collections of the National Museums of Kenya and the National Collection of Insects of the ARC-Plant Protection Research Institute of South Africa. The data for Agromyzidae were also crossreferenced with the Species 2000 Biodiversity of World Diptera (BDWD), Catalogue of Life: 2008 Annual Checklist (Bisby *et al.*, 2008).

The diversity and distribution of the agromyzid species and associated natural enemies within the Afrotropical sub-regions are illustrated based on the records. Host plant families associated with the agromyzid species are listed. A discussion is given on the diversity of Afrotropical agromyzids and the occurrence of parasitoid species. Synonyms for agromyzid and parasitoid species are not given, but can be found in the Catalogue of Life: 2008 Annual Checklist (Bisby *et al.*, 2008) for agromyzid species and in the catalogue of the Universal Chalcidoidea Database (Noyes, 2003) for parasitoid species.

Results

Diversity and distribution of Agromyzidae within the Afrotropical region

In total, 301 agromyzid species belonging to 20 genera have been recorded from the Afrotropical region (Table 1). Of the 599 agromyzid species recorded from the Afrotropical region, 36 % are from East Africa, 34 % from Southern Africa, 7 % from West Africa, 11 % from East Islands (Comoros, Madagascar, Mauritius and Seychelles), 11 % from the Central African region, and 4 % from the Cape Verde Islands (Fig. 1.1).

The highest number of agromyzid species recorded from the Afrotropical region is from *Melanagromyza* Hendel with 97 species and 178 records, followed by *Ophiomyia* Braschnikov with 35 species and 72 records. The majority of records for these two genera are from countries in the Southern and East African regions (Table 1). The lowest number of records is from the genera *Hexomyza* Enderlein (2), *Penetagromyza* Spencer (2), *Amauromyza* Hendel (1), *Haplomyza* Hendel (1) and *Pseudoliriomyza* Spencer (1). *Melanagromyza* and *Liriomyza* Mik are the only two genera with records in all Afrotropical sub-regions.

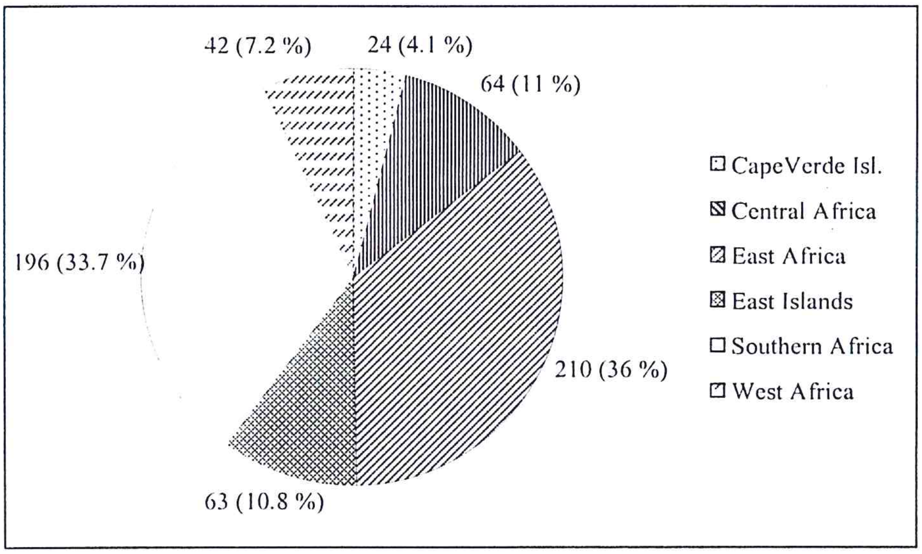

Fig. 1.1. Total number of agromyzid records and percent of total records (in parenthesis) within the Afrotropical sub-regions

Diversity and distribution of agromyzid hymenopteran parasitoids within the Afrotropical region

There are 105 parasitoid records with species belonging to 10 families and 28 genera. These are associated with 7 %, that is, 20 out of 301, agromyzid species, recorded from the Afrotropical region. Of the parasitoids records from the Afrotropical region, 58 % are from East Africa, 27 % from Southern Africa, 22 % from West Africa, 8 % from East Islands and 2 % from the Central African region (Fig. 1.2). No records exist from the Cape Verde Islands. Only 21 of the parasitoids recorded have been identified to species level (Table 1).

The majority of parasitoid records are from *Ophiomyia phaseoli* with 32 records and *Liriomyza trifolii* with 18 records. There are only 10 parasitoid records for *Melanagromyza*, the genus with highest number of agromyzid species records. Data on parasitoids attacking agromyzids of no economic importance are scarce.

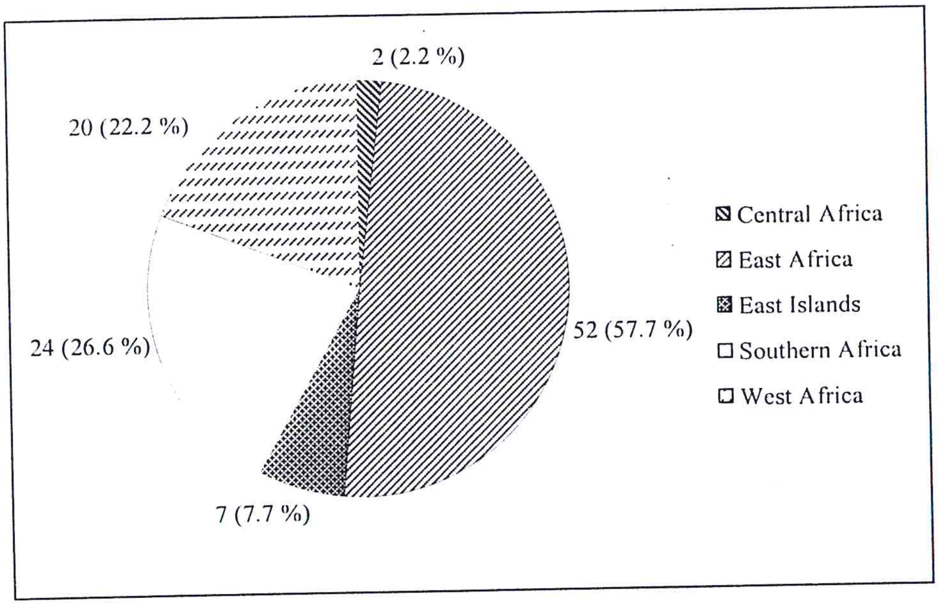

Fig. 1.2. Total number of parasitoid species records and percent of total records (in parenthesis) within the Afrotropical sub-regions

Host plants associated with agromyzids and their natural enemies

The agromyzid species of the Afrotropical region have been recorded from 48 plant families according to this review. The highest number of agromyzid records is from Fabaceae (86) and Asteraceae (Compositae) (80). There are 376 records of agromyzids with records of host plants. The majority of the host plant records are associated with the agromyzid genera *Liriomyza* (82), *Tropicomyia* (65) and *Melanagromyza* (58) (Table 1.1). However, records of host plants and agromyzid and parasitoid species associations are not available in most cases.

Discussion

Although the data used in this review may not be exhaustive, the number of records suggests that the family Agromyzidae has been studied comparatively more extensively in East and Southern Africa compared to Central and West Africa. However, this trend is likely to be due to inadequate sampling of agromyzids in these regions.

Results suggest that parasitoid sampling efforts concentrated on economically important agromyzid species. For example, *Melanagromyza* has the highest number of species but a low number of parasitoid records. In contrast, the highest number of parasitoid records is from the genus *Ophiomyia*, which comprises fewer species than *Melanagromyza* but includes many agriculturally important pest species on legumes. Similarly, *L. trifolii*, another important pest species, has a relatively high number of parasitoid records.

Parasitoids attacking agromyzids in natural habitats could nevertheless be important in controlling agromyzid pests. A study by Valladares & Salvo (2001) in Central Argentina on community dynamics of leafminers and their parasitoids revealed a diverse parasitoid fauna in a natural habitat. Furthermore, in northern Italy, Burgio *et al.* (2007) reported 53 parasitoid species from agromyzids colonizing weeds. Schuster *et al.* (1991) reported 17 parasitoid species on agromyzids infesting weed species, while Schuster & Wharton (1993) reported 14 species of parasitoids attacking *Liriomyza* species on non-sprayed tomatoes in Florida. These studies form a basis for undertaking intensive sampling in both agricultural and non-agricultural ecosystems in the Afrotropical region. A pressing challenge, though, is the identification of the collected parasitoid specimens to species level.

The scarcity of records of associations between agromyzids, their host plants and parasitoids makes it difficult to assess the importance of natural habitats in biological control in the Afrotropical region. Similarities in assemblages of many parasitoids attacking leafminers have been observed in managed and natural habitats (Gratton & Welter, 2001) and the frequent proximity of the two habitats potentially enhances control of agromyzids in agricultural areas through the possible exchange of natural enemies between the two habitats.

The agromyzid records show that the economically important genera *Ophiomyia* and *Liriomyza* are present in most countries of the Afrotropical region. It is most likely that these species occur in agro-ecosystems where pesticide use interferes with parasitoid abundance. On the other hand, natural ecosystems are an important reservoir of agromyzid and associated parasitoid species (Schuster *et al.*, 1991; Masetti *et al.*, 1997; Gratton & Welter, 2001; Valladares & Salvo, 2001; Burgio *et al.*, 2007). Conservation biological control may have high potential for agromyzid pest management in the Afrotropical region. In Uganda, Greathead (1971) recorded a number of hymenopteran parasitoids *Habrocytus* sp. (Pteromalidae), *Eurytoma* sp. (Eurytomidae) and *Eucoilidea* sp. (Eucoilidae) on *Melanagromyza bonavistae* Greathead, *M. chalcosoma* Spencer and *M. vignalis* Spencer (Diptera: Agromyzidae) from the pods of the bonavist bean (*Lablab niger* L.). Some parasitoids in the Eucoilidae (*Eucoilidea* spp.) family were also reported from the same country on *O. phaseoli*, an economically important pest of cultivated legumes. Given the polyphagous nature of some hymenopteran parasitoids of agromyzids (Murphy & LaSalle, 1999), parasitoids of agromyzids that are not major pests of agricultural crops, such as in the genera *Agromyza*, *Melanagromyza* and *Tropicomyia*, could form an important part of conserved natural enemies in non-cropped areas and cropping boundaries where pesticides are not applied.

The genus *Ophiomyia*, which has been suggested to have evolved in the Afrotropical region (Spencer, 1973), has been widely recorded on Fabaceae (legumes). The highest number of parasitoid records (30 %) is from *Ophiomyia*. Although agromyzid parasitoids, especially Eulophidae, are thought to be polyphagous, *Ophiomyia* and *Liriomyza* (a new invasive agromyzid species in the Afrotropical region) do not share all of their parasitoid species. The present records suggest that parasitoids associated with *Ophiomyia* are not associated with any of the modern genera of leafminers such as the *Liriomyza* species. Although the data available are insufficient to make any conclusion about *O. phaseoli*-parasitoid association patterns, it can be hypothesised that there might have been an early diversity in parasitoid species associated with this group especially in East Africa (where most records have been made), but that parasitoids associated with this mostly stem-tunnelling species seem unable to adapt to the more modern leaf-mining taxa. Hence, these parasitoid species are not expected to control any other invading agromyzid species such as the invasive *Liriomyza* species. This could be an interesting and important research area to pursue in future.

Scheffer *et al.* (2007) report the genus *Ophiomyia* to be polyphyletic with five species (*Ophiomyia* group A) related to *Tropicomyia* and *Hexomyza* (Enderlein) and two species (*Ophiomyia* group B) forming a sister group to *Melanagromyza*. In-depth studies of parasitoid diversity in such polyphyletic groups within the family

Agromyzidae could be vital in establishing relationships between parasitoid guilds of phylogenetically related agromyzid species. This knowledge could be effectively used in conservation biological control, which is a vital component of an integrated pest management programme.

When multiple species of natural enemies are released into a crop, or if a mass-reared species is introduced into a system containing existing natural enemy populations, competitive interactions are likely to occur (Kang *et al.*, 2009). For example, the parasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) has been recorded in Senegal, South Africa, Kenya and Zimbabwe (Neuenschwander *et al.*, 1987; Musundire, 2002; Chabi-Olaye *et al.*, 2008). Although this parasitoid is of Palaearctic origin (Minkenberg, 1989), it has been reported to have successfully established in these countries. However, without comprehensive studies on the diversity of the native agromyzid parasitoid fauna in the Afrotropical region, the presence of this introduced parasitoid could cause unwanted non-target effects, such as outcompeting native parasitoid species (van Lenteren *et al.*, 2006). Non-target effects could include larval ecto-parasitoids parasitizing leafminers that already contain a living endo-parasitoid (Mitsunaga & Yano, 2004). Although the parasitoids *Opius phaseoli* Fischer (Hymenoptera: Braconidae), *D. isaea*, *Hemiptarsenus albens* (Delucchi), *Meruana elegans* (Delucchi) and *Meruana liriomyzae* Bouček (Hymenoptera: Eulophidae) are potentially suitable candidates for mass-production for augmentative releases based on their association with agriculturally important agromyzids, these parasitoid-parasitoid interactions need to be investigated further.

In conclusion, the family Agromyzidae has probably been more extensively studied than its associated natural enemies in the Afrotropical region. The recorded parasitoids belong to 10 families. Only 21 species out of the 105 recorded cases (20 %) have been identified to species level. Parasitoids (105 records) were associated only with 20 agromyzid species belonging to 10 different genera hence indicating a possible lack of diversity in parasitoids associated with agromyzid species in the Afrotropical region. The comparatively low parasitoid diversity in the Aftrotropical region is however, most likely a result of poor sampling effort and lack of taxonomic expertise for parasitoid species in this area. In addition, records on the distribution and abundance of parasitoids of agromyzids presented here with respect to insect hosts and crop habitats are largely lacking. More research on parasitoid fauna and associated Agromyzidae is therefore required before embarking on extensive biological control programmes.

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Table 1.1 Agromyzid species, origin, host plant species and their hymenopteran parasitoids in the Afrotropical region.

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
***Agromyza* Fallén 1810**					
abutilonis Spencer 1959	Kenya (3, 7, 31, 37), South Africa (3, 7, 31, 33), Uganda (3, 7, 23, 34, 37)	Malvaceae	*Abutilon mauritianum* (Jacq.) Medik (31)	-	-
		Malvaceae	*Abutilon* sp. (21, 23, 37), *Hibiscus* sp. (34, 37)		
abyssinica Spencer 1964	Ethiopia (3, 7, 32)	Convolvulaceae	*Ipomea* sp. (32)	-	-
albipila Becker 1908	South Africa (3, 7, 33)	-	-	-	-
catherinae Spencer 1959	South Africa (7, 23, 31, 32, 33, 37) Zimbabwe (3, 7, 33, 37)	Poaceae	*?Setaria megaphylla* (Steud.) T. Durand & Schinz (32, 33, 37)	-	-
confusa Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
cyeni Spencer 1959	DRC (3, 7, 23)	-	-	-	-
graminacea Spencer 1985	Kenya (3, 36)	-	-	-	-
infusca Spencer 1959	Ethiopia (3), Kenya (3), Tanzania (3, 7, 23)	-	-	-	-
malvaceivora Séguy 1951	Madagascar (3, 7, 21, 23, 30, 37)	-	-	-	-
munduleae (Séguy 1951)	Angola (3), Benin (3), Cameroon (3) Madagascar (3, 7, 21, 23, 30), Zambia (3)	Fabaceae	*Mundulea sericea* (Willd.) A. Chev. (21, 23, 30, 37)	-	-
ocularis Spencer 1961	Botswana (3, 23), Lesotho (3, 7), South Africa (3, 7, 28, 31)	-	-		-
oliviae Spencer 1959	Ethiopia (3, 7, 28, 31, 32), Tanzania (3, 7, 23, 28, 31)	-	-	-	-
pallidifrons Spencer 1959	DRC (3, 7, 23, 37), South Africa (3, 7, 31, 37), Zimbabwe (3, 7, 23, 37)	Poaceae	*Urochloa panicoides* P. Beauv. (30, 31, 37)	-	-
penniseti Spencer 1959	Cameroon (3, 7, 23, 34 , 36, 37), Nigeria (3, 7, 34, 36, 37), Senegal (3, 7, 23, 24, 36), South Africa (3, 7, 34), Uganda (3, 7, 34, 36, 37)	Poaceae	*Digitaria ciliaris* (Retz.) Koeler (23, 36, 37)	**Braconidae** *Opius* sp.	Nigeria (34)
		Poaceae	*Eleusine coracana* (L.)_Gaertn. (37)		
		Poaceae	*Pennisetum glaucum* (L.) R. Br. (37)		
		Poaceae	*Pennisetum pedicellatum* Trin. (34, 35, 36)		
		Poaceae	*Pennisetum purpureum* Schumach. (23,		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
			34, 36)		
somereni Spencer 1959	Kenya (3, 7, 23, 36)	-	-	-	-
sulfuriceps Strobl 1898	Niger (3, 7)	-	-		
susannae Spencer 1959	Malawi (7, 23), Mozambique (7, 23), South Africa (7, 23, 37), Zambia (37)	Poaceae	*Urochloa panicoides* P. Beauv. (37)	-	-
ugandae Spencer 1985	Uganda (3, 36)	-		-	-
uniseta Spencer 1959	Madagascar (3, 7, 23, 30)		-	-	-
verdensis Spencer 1959	Cape Verde Is (3, 7, 23, 33), Namibia (3)	-			
Amauromyza **Hendel 1931**				-	-
triseta (Spencer 1959)	South Africa (3, 7, 23)	-	-	-	-
Calycomyza **Hendel 1931**					
gigantissima (Spencer 1959)	DRC (3, 7, 23, 24, 31), South Africa (3, 7, 24, 31)	-	-	-	-
humeralis (von Roser 1840)	Cameroon (7, 23), Ethiopia (7, 32), South Africa (7)	Asteraceae Asteraceae Asteraceae Asteraceae	*Conyza bonariensis* (L.) Cronquist (15) *Dicrocephala* sp. (23) *Dichrocephala chrysanthemifolia* (Blume) DC (32) *Erigeron* sp. (23)	-	-
lantanae (Frick 1956)	South Africa (2, 17)	Verbenaceae	*Lantana camara* L. (2)	**Eulophidae** ?*Chrysonotomyia* sp. *Diglyphus ambiguus* Hansson & LaSalle 1996	South Africa (2) South Africa (14)
Cerodontha **Rondani 1861**					
aberdarensis Spencer 1985	Kenya (36)	-	-	-	-
abyssinica Spencer 1961	Ethiopia (3, 7, 31), South Africa (3, 7, 31)	-	-	-	-
africana Spencer 1985	Kenya (3, 36), South Africa (3, 36)	-	-	-	-
aristella (Spencer 1961)	South Africa (3, 7, 31, 33, 37), Zimbabwe (3, 7, 33, 37)	Poaceae	*Setaria megaphylla* (Steud.) T. Durand & Schinz (33, 37)	**Eulophidae** *Chrysocharis* sp.	Zimbabwe (33)
cariciphaga (Spencer 1963)	Cameroon (3, 7, 31, 36, 37)	Cyperaceae	*Carex* sp. (31, 36, 37)	-	-
caricivora (Groschke 1954)	Cameroon (3, 7, 23, 36), Ethiopia (3)	Cyperaceae	*Carex* sp. (23)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
denticornis (Panzer 1806)	DRC (7, 23, 24, 32, 36, 37), Lesotho (24), South Africa (7, 23, 24, 32, 36, 37), Tanzania (7, 37), Uganda (7, 23, 24, 36, 37)		-	-	-
elevata Spencer 1985	Ethiopia (3, 36), Kenya (3, 36), South Africa (3, 36)	Poaceae	-	-	-
geniculata (Fallen 1823)	South Africa (7, 23, 32, 37)	Cyperaceae	*Eriophorum* sp. (37)		
guineana Zlobin 1993	Guinea (3)		-	-	-
heringiella Spencer 1961	Ethiopia (3, 7, 31, 34, 36), Kenya (3, 34, 36), South Africa (3, 7, 31, 34, 36), Tanzania (3, 34, 36)	-	-	-	-
kakamegae Spencer 1985	Kenya (3, 36)	-	-	-	-
kenyana Zlobin 2001	Kenya (3, 39, 40)	-	-	-	-
kivuensis (Spencer 1959)	DRC (7, 23, 40)	-	-	-	-
magnificans (Spencer 1959)	DRC (3, 7, 23, 33), South Africa (3, 7, 33)	-	-	-	-
orbitona (Spencer 1960)	Gambia (9), Ghana (3, 7, 34, 36, 37), Kenya (3, 9, 36, 37), Nigeria (9) Réunion (3, 36), South Africa (3, 7, 24, 34, 36, 37), Uganda (36)	Poaceae Poaceae Poaceae	*Hyparrhenia cymbaria* (L.) Stapf (36, 37) *Oryza sativa* L. (34, 36, 37) *Zea mays* L. (36, 37)	**Ceraphronidae** *Ceraphron* cf. *fijiensis* (Ferrierre 1933) **Eucoilidae** *Eucoilidea* sp. **Eulophidae** *Achrysocharoides* sp. *Hemiptarsenus varicornis* (Girault 1913) *Sympiesis* sp. **Pteromalidae** *Callitula* sp.	Ghana (34) Ghana (34) Ghana (34) Ghana (34) Ghana (34) Ghana (34)
piliseta (Becker 1903)	Cape Verde Is. (7, 23, 36), Kenya (36, 37), Seychelles (7, 23), Tanzania (36), Zimbabwe (7, 23, 36, 37)	Cyperaceae	*Fimbristylis* sp. (23, 37)	-	-
pubicata (Spencer 1959)	DRC (7, 23, 36, 37), Kenya (3, 23,		-	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
	37)				
setariae (Spencer 1959)	Cameroon (3, 23, 37), Sierra Leone (3, 7, 23, 37)	Poaceae	*Setaria* sp. (23, 37)	-	-
stuckenbergiella Spencer 1977	South Africa (3, 7, 37)		-	-	-
***Chromatomyia* Hardy 1849**					
anonera (Séguy 1951)	Ethiopia (36), Madagascar (36)	Asteraceae	*Vernonia appendiculata* Less. (36)	-	-
elgonensis Spencer 1985	Kenya (3, 36)		-	-	-
horticola (Goureau 1851)	Cameroon (34, 37), Ethiopia (1, 5, 19), Kenya (34, 36, 37), Madagascar (34, 37), South Africa (1, 34, 36, 37)	Asteraceae	*Bidens* sp. (36)	**Eulophidae** *Meruana liriomyzae* Bouček 1988	Ethiopia (1, 5), South Africa (5)
		Asteraceae	*Chrysanthemoides* sp. (37)		
		Asteraceae	*Dahlia* sp. (36)		
		Asteraceae	*Erigeron* sp. (36)		
		Asteraceae	*Galinsoga* sp. (36)		
		Asteraceae	*Sonchus* sp. (36)		
		Brassicaceae	*Brassica rapa* L. (37)		
		Fabaceae	*Pisum* sp. (36)		
		Fabaceae	*Trifolium alexandrinum* L. (37)		
		Fabaceae	*Vicia faba* L. (37)		
		Solanaceae	*Petunia* sp. (36)		
		Solanaceae	*Solanum melongena* L. (37)		
		Solanaceae	*Solanum* sp. (36)		
nigrissima Spencer 1985	Kenya (3, 36, 37)	-	-	-	-
seneciophila Spencer 1985	Kenya (3, 36, 37), Tanzania (3, 36)	Asteraceae	*Senecio johnstonii* Oliv. (36, 37)	-	-
		Asteraceae	*Senecio* sp. (36, 37)		
seneciovora (Spencer 1959)	Cameroon (23, 33, 36, 37), Uganda (36)	Asteraceae	*Sonecio mannii* (Hook.f.) C. Jeffrey (36, 37)	-	-
		Asteraceae	*Senecio moorei* R.E.Fr. (23)		
subnigra Spencer 1985	Kenya (3, 36, 37)	-	-	-	-
***Haplomyza* Hendel**					
diminuella Spencer 1961	South Africa (7)	-	-		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
Hexomyza **Enderlein 1936**					
gymnosporivora (Spencer 1963)	Kenya (3, 36, 37), South Africa (7, 31, 36, 37)	Celastraceae	*Gymnosporia buxifolia* (L.) Szyszyl. (31, 36, 37)	-	-
Japanagromyza **Sasakawa 1958**					
meridiana Spencer 1961	South Africa (3, 7, 29)	-	-	-	-
nigrihalterata (Spencer 1959)	Kenya (3, 7, 23, 24), Rwanda (7)	-	-	-	-
parvula Spencer 1961	Kenya (37), Tanzania (7, 27, 37)	Fabaceae	*Crotalaria agatiflora* Schwief. (37)	-	-
***Liriomyza* Mik 1894**					
atrescens 1961b	Ethiopia (3, 7)	-	-	-	-
brassicae (Riley 1885)	Cape Verde Is. (7, 23, 33), Ethiopia (7, 33), Kenya (7, 34, 36, 37), Mauritius (1, 5, 23, 37), Mozambique (7, 34), Senegal (7, 23, 34), South Africa (7, 23, 33, 37), Zimbabwe (7, 32, 34, 36)	Brassicaceae Brassicaceae Brassicaceae Brassicaceae Brassicaceae Fabaceae Tropaeolaceae	*Brassica oleracea* L. (36, 37, 38) Brassica sp. (23, 38) *Erucastrum arabicum* Fisch. & C. A. Mey. (36, 37) *Nasturtium officinale* W. T. Aiton (38) *Nasturtium* sp. (23) *Pisum* sp. (36, 37, 38) *Tropeaolum majus* L. (15, 23)	**Eulophidae** *Meruana liriomyzae* Bouček 1988	Mauritius (1, 5)
diminuella Spencer 1961	South Africa (3)	-	-	-	-
emiliae Séguy 1951	Madagascar (3, 7, 21, 23, 30, 37)	Asteraceae Asteraceae Tropaeolaceae	*Emilia citrina* DC. (21, 23, 30, 37) *Emilia* sp. (37) *Tropaeolum* sp. (37)	-	-
enormis Spencer 1961	Madagascar (3, 7)	-	-	-	-
flavalis Spencer 1959	South Africa (3, 7, 23, 24, 33)		-	-	-
helichrysivora Spencer 1965	Kenya (3, 37), South Africa (3, 7, 33, 37), Uganda (3, 37)	Asteraceae Asteraceae	? *Helichrysum cooperi* Harv. (33) *Helichrysum foetidum* (L.) Moench (37)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Asteraceae	*Helichrysum nudifolium* (L.) Less. (33, 36)		
		Asteraceae	*Helichrysum odoratissimum* (L.) Sweet. (37)		
huidobrensis (Blanchard 1926)	Comoros (10), Kenya (6), Mauritius (10, 37), Réunion (10), Seychelles (10), South Africa (10, 17), Zimbabwe (16)	Polyphagous	Highly polyphagous	**Braconidae**	
		Aliaceae	*Allium cepa* L. (37)	*Dacnusa sibirica* Telenga 1934	Zimbabwe (16)
		Apiaceae	*Apium graveolens* L. (37)		
		Asteraceae	*Lactuca sativa* L. (37)	**Eulophidae**	
		Asteraceae	*Sonchus* spp. (37)	*Diglyphus isaea* (Walker 1838)	Kenya (6), Zimbabwe (16)
		Asteraceae	*Tagetes* spp. (37)		
		Brassicaceae	*Brassica oleracea* L. (37)	*Meruana* spp.	Zimbabwe (16)
		Caryophyllaceae	*Dianthus caryphyllus* L. (37)		
		Chenopodiaceae	*Spinacia oleraceae* L. (6, 37)		
		Fabaceae	*Pisum sativum* L. (6, 37)		
		Fabaceae	*Vicia faba* L. (16)		
		Solanaceae	*Solanum lycopersicum* L. (6, 37)		
		Solanaceae	*Solanum tuberosum* L. (6, 37)		
manni Spencer 1985	Kenya (3, 36)	Asteraceae	*Tithonia diversifolia* (Helmsl.) A. Gray (36)	-	-
melantherae Spencer 1959	Cameroon (3, 7, 23, 37)	Asteraceae	*Melanthera ?brownei* Sch. Bip. (23, 37)	-	-
mikaniopsidis Spencer 1961	Ethiopia (3, 7, 33)	-	-	-	-
mirifica Spencer 1963	Uganda (7, 31, 33)	-	-	-	-
mosselensis Spencer 1965	Lesotho (3, 7, 33), South Africa (3, 7, 33)	-	-	-	-
nana Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
novissima Spencer 1960	South Africa (7, 24, 31, 32, 33)	-	-	-	-
sativae Blanchard 1938	Cameroon (10), Kenya (6, 37), Mauritius (5, 37), Nigeria (9, 10) Réunion (5, 36, 37), Sudan (10), Zimbabwe (10)	Polyphagous	Polyphagous, Pest of vegetables and ornamental plants, e.g.	**Eulophidae** *Hemiptarsenus variconis* (Girault 1913)	Ethiopia (5), Ghana (5), Kenya (5), Senegal (5), Sudan (5), Tanzania (5)
		Asteraceae	*Chrysanthemum* spp. (37)		
		Chenopodiaceae	*Beta vulgaris* L. (37)		
		Cucurbitaceae	*Cucumis melo* L. (37)	*Meruana liriomyzae* Bouček 1988	Mauritius (5), Réunion (1, 5)
		Cucurbitaceae	*Cucumis sativus* L. (37)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Euphorbiaceae	*Ricinus communis* L. (37)		
		Fabaceae	*Phaseolus vulgaris* L. (6, 37)		
		Fabaceae	*Pisum sativum* L. (6, 37)		
		Malvaceae	*Abelmoschus esculentus* (L.) Moench (6, 18)		
		Solanaceae	*Capsicum frutescens* L. (37)		
		Solanaceae	*Solanum aethiopicum* L. (37)		
		Solanaceae	*Solanum lycopersicum* L. (6)		
		Solanaceae	*Solanum tuberosum* L. (6, 37)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
trifolii (Burgess 1880)	Benin (10, 37), Cote dIvoire (3, 7, 10, 37), Ethiopia (10, 37), Guinea (10), Kenya (5, 7, 10, 36, 37), Madagascar (10), Mauritius (10, 36), Mayotte (10), Nigeria (10, 37), Réunion (10, 36, 37), Senegal (10, 18, 36), South Africa (10, 18, 37), Sudan (10, 37), Tanzania (10, 36), Zambia (10, 37), Zimbabwe (5, 10, 37)	Polyphagous	Polyphagous, e.g.	**Braconidae**	
		Acanthaceae	*Peristrophe bicalyculata* (Retz) Nees (18)	*Opius dissitus* Muesebeck 1963	Senegal (18)
		Aliaceae	*Allium cepa* L. (18)	**Eucoilidae**	
		Amaranthaceae	*Amaranthus viridis* L. (18)	*Eucoilidea futura* Quinlan 1986	Senegal (18)
		Apiaceae	*Apium graveolens* L. (18)	**Eulophidae**	
		Apiaceae	*Daucus carrota* L. (18)	*Cirrospilus* sp.	South Africa (17)
		Asteraceae	*Bidens pilosa* L. (36)	*Cirrospilus* sp. nr *cinctiventris* Ferrière 1936	Senegal (18)
		Asteraceae	*Chrysanthemum* spp. (36)	*Closterocerus formosus* Westwood 1833	South Africa (17, 19, 20)
		Asteraceae	*Gerbera* sp. (36)	*Chrysonotomyia* sp. 1	Senegal (18)
		Asteraceae	*Lactuca sativa* L. (18)	*Chrysonotomyia* sp. 2	Senegal (18)
		Asteraceae	*Launaea cornuta* (Hochst. Ex Oliv. & Hiern) C. Jeffrey (36)	*Diglyphus isaea* (Walker 1838)	Senegal (18), South Africa (18)
		Asteraceae	*Tagetes minuta* L. (36)	*Hemiptarsenus albens* Delucchi 1962	Senegal (18)
		Asteraceae	*Tagetes patula* L. (18)	*Hemiptarsenus varicornis* (Girault 1913)	Senegal (18), Tanzania (34)
		Asteraceae	*Tithonia diversifolia* (Hemsl.) A. Gray (36)	*Meruana camerounensis* (Risbec 1955)	Cameroon (34)
		Asteraceae	*Tridax procumbens* L. (36)	*Meruana elegans* Delucchi 1962	Tanzania (34)
		Brassicaceae	*Brassica napus* L. (18)	*Meruana liriomyzae* Bouček 1988	Kenya (5), Zimbabwe (5)
		Chenopodiaceae	*Beta vulgaris* L. (18)	**Figitidae**	
		Chenopodiaceae	*Spinacia olaraceae* L. (37)	*Nordlanderia plowa* Quinlan 1986	Senegal (18)
		Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum.& Nakai (18)	**Ichneumonidae**	
		Cucurbitaceae	*Cucumis melo* L. (18)	*Allophrys* sp.	Senegal (18)
		Cucurbitaceae	*Cucumis sativus* L. (18)		
		Euphorbiaceae	*Ricinus communis* L. (18, 36)		
		Fabaceae	*Canavalia ensiformis* (L.) DC. (18)		
		Fabaceae	*Phaseolus* spp. (36)		
		Fabaceae	*Phaseolus vulgaris* L. (18)		
		Fabaceae	*Pisum sativum* L. (18)		
		Fabaceae	*Senna occidentalis* (L.) Link (18)		
		Fabaceae	*Trifolium repens* L. (37)		
		Fabaceae	*Vicia faba* L. (6)		
		Fabaceae	*Vigna unguiculata* (L.) Walp. (18)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Lamiaceae	*Ajuga remota* Benth. (36)		
		Lamiaceae	*Ocimum basilicum* L. (18)		
		Malvaceae	*Abelmoschus esculentus* (L.) Moench (6, 18)		
		Malvaceae	*Gossypium herbaceum* L. (18)		
		Passifloraceae	*Passiflora foetida* L. (18)		
		Poaceae	*Zea mays* L. (18)		
		Solanaceae	*Capsicum annuum* L. (18)		
		Solanaceae	*Capsicum frutescens* L. (18)		
		Solanaceae	*Datura metel* L. (18)		
		Solanaceae	*Solanum lycopersicum* L. (18, 36)		
		Solanaceae	*Solanum melongena* L. (18)		
		Solanaceae	*Solanum tuberosum* L. (18)		
		Solanaceae	*Sonchus bruneri* L. (18)		
volatilis Spencer 1965	Ethiopia (7, 33)	-	-	-	-
***Melanagromyza* Hendel 1920**					
acaciae Spencer 1963	Tanzania (3, 7, 31, 37)	Fabaceae	*Acacia drepanolobium* Harms ex Y.Sjöstedt (31, 37)	-	-
albisquama (Malloch 1927)	Cape Verde Is. (7, 23, 33, 36, 37), Mauritius (7, 24, 33, 37), Principe (7, 36), Uganda (36)	Fabaceae	*Desmodium incanum* DC. (24) *Desmodium* sp. (33, 37)	-	-
annae Spencer 1964	South Africa (3, 7, 32, 33)	-	-	-	-
aprilis Spencer 1959	DRC (3, 7, 23)	-	-	-	-
argentea Spencer 1964	DRC (3, 7), South Africa (3, 7, 32)	-	-	-	-
aurea Spencer 1959	DRC (3, 7, 23), South Africa (7)	-	-	-	-
ballardi Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
barbata Spencer 1960	South Africa (3, 7, 24)	-	-	-	-
blepharidis Spencer 1960	Kenya (3, 37), South Africa (3, 7, 24, 37)	Acanthaceae	*Blepharis* sp. (24, 33, 37)	-	-
bonavistae Greathead 1971	Kenya (36), Sudan (37), Tanzania (3, 7, 34), Uganda (7, 13, 34, 36)	Fabaceae Fabaceae	*Dolichos* sp. (37) *Lablab purpureus* (L.) Sweet (13, 34,	**Eucoilidae** *Eucoilidea* sp.	Uganda (13, 34)

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		 Fabaceae Fabaceae	36, 37) *Pachyrhizus* sp. (37) *Vigna unguiculata* (L.) Walp. (13, 34, 36)	**Eulophidae** *Pediobius* sp. **Eurytomidae** *Eurytoma* sp. **Pteromalidae** *Pteromalus* subgenus *Habrocytus* sp.	Uganda (13) Uganda (13, 34) Uganda (13, 34)
brassica Spencer 1990	Zimbabwe (34, 37)	Brassicaceae	*Brassica* sp.(34, 37), *Brassica* spp. (37)	-	-
bulbifrons Spencer 1959	Malawi (3, 7, 23, 34)	-	-	-	-
caeruleana Spencer 1959	DRC (23), Rwanda (3, 7, 23), Tanzania (3, 7, 23)	-	-	-	-
candidipennis (Lamb 1912)	Kenya (3, 7, 23, 24, 34, 36, 37), Malawi (3, 24, 37), Nigeria (3, 7, 34, 36, 37), Seychelles (3, 7, 23, 24, 34, 36, 37), South Africa (3, 7, 24, 34, 36, 37), Tanzania (3, 7, 23, 24, 34, 36)	Fabaceae	*Vigna unguiculata* (L.) Walp. (34, 36, 37)	-	-
chalcosoma Spencer 1959	Kenya (7, 23, 34, 36), Madagascar (24, 30, 37), Malawi (7, 23, 24, 34, 36, 37), Nigeria (23, 24, 30, 37) South Africa (7, 24, 37), Uganda (7, 13, 23, 24, 30, 34, 36), Zanzibar (23, 24, 30, 37)	Fabaceae Fabaceae Fabaceae Fabaceae Fabaceae Fabaceae	*Cajanus cajan* (L.) Millsp. (13, 23, 30, 34, 36, 37) *Cajanus* sp. (24) *Flemingia grahamiana* Wight & Arn. (23, 30, 34) *Flemingia* sp. (24, 30) *Lablab purpureus* (L.) Sweet (13, 23, 37) *Vigna unguiculata* (L.) Walp. (E. Mey.) (13, 34, 36, 37)	**Eucoilidae** *Eucoilidea* sp. **Eurytomidae** *Eurytoma* sp. **Pteromalidae** *Pteromalus* sp.	Uganda (12, 13) Uganda (13) Uganda (13)
compositana Spencer 1959	Cape Verde Is. (7, 23), DRC (3, 7, 23, 27), Kenya (7, 23, 27, 36, 37), Malawi (7, 23, 27), Mauritius (23, 27), Mozambique (7, 29), South Africa (7, 27, 29, 36), Tanzania (7, 27), Zambia (24)	Asteraceae Asteraceae Asteraceae	*Bidens pilosa* L. (23) *Senecio ruderalis* Harv. (23, 37) *Senecio* sp. (23, 27)	-	-
cotyledonus Spencer 1960	South Africa (3, 7, 24, 37)	Crassulaceae	*Cotyledon orbiculata* L. (26, 30, 37)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
crassocephali Spencer 1985	Kenya (3, 36)	Asteraceae	*Crassocephalum* sp. (36)	-	-
crotalariae Hering 1957	South Africa (7, 15, 23, 31), Uganda (7, 23)		*Crotalaria capensis* Jacq (15)		
crotalariana Spencer 1961	Ethiopia (7, 32), Uganda (7, 37)	Euphobiaceae	*Croton macrostachyus* Hochst ex Delile (32, 34, 36, 37)	-	-
		Fabaceae	*Crotalaria laburnifolia* (32)		
		Fabaceae	*Crotalaria* sp. (37)		
cunctans (Meigen 1830)	DRC (3, 7, 23), Madagascar (3, 7, 30), South Africa (3, 7, 23), Uganda (3, 7, 23)	-	-	-	-
curiosa Spencer 1959	DRC (3, 7, 23)	-	-	-	-
curvibucca Spencer 1959	DRC (7, 23), South Africa (3, 37)	-	-	-	-
cussoniae Spencer 1964	South Africa (3, 7, 32, 37)	Araliaceae	*Cussonia* sp. (32, 37)	-	-
cyrtanthi Spencer 1960	South Africa (3, 7, 24, 37)	Amaryllidaceae	*Cytanthus sauguinens* (Lindl.) (24, 37)	-	-
cyrtorchidis Spencer 1985	Kenya (3, 36, 37)	Orchidaceae	*Cyrtorchis arcuata* (Lindl.) Schltr. (36, 37)	-	-
		Orchidaceae	*Rangaeris amaniensis* (Kraenzl.) Schltr. (37)		
dakarensis Spencer 1959	Senegal (3, 7, 23)	-	-	-	-
damnata Spencer 1961	Mozambique (3, 7, 29)	-	-	-	-
devia Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
difficilis Spencer 1959	Mozambique (3, 7, 23)	-	-	-	-
drakensbergi Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
elgonensis Spencer 1965	Kenya (3, 7, 33)	-	-	-	-
elongata Spencer 1959	Zimbabwe (3, 7, 23)	-	-	-	-
frigida Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
frontata Spencer 1960	South Africa (3, 7, 24)	-	-	-	-
funebris (Lamb 1912)	Seychelles (3, 7, 23)	-	-	-	-
fuscalis Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
galactoptera Bezzi & Lamb 1926	Rodriguez (3, 7, 23)	-	-	-	-
genata Spencer 1959	South Africa (3, 7, 23)	-	-	-	-
generosa Spencer 1961	Ethiopia (3, 7, 36), Kenya (3, 36)	-	-	-	-
gerberae Spencer 1960	South Africa (3, 7, 24, 29, 37)	Asteraceae	*Gerbera jamesonii*	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
			Bolus ex Hook. f. (23, 24, 25, 28, 37)		
gerberivora Spencer 1960	South Africa (3, 7, 23, 25, 37)	Asteraceae Asteraceae	*Gerbera jamesonii* Bolus ex Hook. f. (23, 37) *Gymnosporia buxifolia* (L.) Szyszyl. (37)	-	-
ghanensis Spencer 1965	Ghana (3, 7, 33)	-	-	-	-
gracilis Spencer 1959	DRC (7, 23)	-	-	-	-
gynurae Spencer 1959	Madagascar (3, 7, 23, 30, 37)	Asteraceae	*Crassocephalum rubens* (Juss. ex Jacq.) S. Moore (23, 30)	-	-
heatoni Spencer 1990	Kenya (3, 37)	Asteraceae	*Senecio moorei* R.E.Fr. (37)	-	-
indubita Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
insolita Spencer 1959	Cape Verde Is. (3, 7, 23)	-	-	-	-
inulivora Spencer 1961	South Africa (3, 7, 29, 37)	Asteraceae Asteraceae	*Helichrysum nudifolium* (L.) Less. (29, 37) *Inula glomerata* Oliv. & Hiern. (37)	-	-
kenyensis Spencer 1959	Kenya (3, 7, 23, 33, 36, 37)	Euphorbiaceae	*Croton* sp. (23, 28, 36, 37)	-	-
laburuiboriae Spencer 1985	Kenya (36)	-	-	-	-
leguminosarum Spencer 1985	Kenya (36)	-	-	-	-
lindneri Spencer 1961	Ethiopia (7, 27, 33), South Africa (7, 33), Tanzania (7, 27, 33)	-	-	-	-
livida Spencer 1985	Kenya (3, 36)	-	-	-	-
longibucca Spencer 1959	Zimbabwe (3, 7, 23)	-	-	-	-
lustralis Spencer 1959	Cape Verde Is. (3, 7, 23)	-	-	-	-
luthulii Spencer 1964	South Africa (3, 7, 32)	-	-	-	-
metallica (Thomson 1869)	Cape Verde Is. (3, 7, 27, 32), DRC (3, 7, 23, 37), Ethiopia (3, 7, 37), Kenya (3, 36, 37), Madagascar (3, 7, 30, 37), Mauritius (3, 7, 37), Mozambique (3, 7, 28, 37), Rodriguez (3, 7, 23, 37), Seychelles (3, 7, 37), South Africa (3, 7, 23, 24,	Asteraceae Asteraceae Asteraceae Rubiaceae	*Ageratum conyzoides* L. (36, 37) *Bidens pilosa* L. (36, 37) *Inula glomerata* Oliv.& Hiern (24, 37) *Pavetta* sp. (32)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
	28, 29, 32, 37), Tanzania (3, 7, 27, 37), Uganda (3, 36, 37)				
montana Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
mugungae Spencer 1959	DRC (7, 23)	-	-	-	-
nairobensis Spencer 1959	Kenya (3, 7, 23, 33, 36)	-	-	-	-
natalensis Spencer 1959	South Africa (3, 7, 23)	-	-	-	-
neutralis Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
nicolaudis Spencer 1959	Cape Verde Is. (3, 7, 23)	-	-	-	-
nigrimaculata Spencer 1959	DRC (23, 27), Nigeria (27), Tanzania (27), Uganda (23, 27)	-	-	-	-
nudifolii Spencer 1965	South Africa (3, 7, 33, 37)	Asteraceae	*Helichrysum nudifolium* (L.) Less. (33, 37)	-	-
obscura Spencer 1977	Principe (7)	-	-	-	-
ocellata Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
ochrasquamata Spencer 1961	Mozambique (3, 7, 29)	-	-	-	-
ornatissima Spencer 1961	South Africa (3, 7, 29)	-	-	-	-
parvisetula Spencer 1959	Madagascar (3, 7, 23, 30)	-	-	-	-
perinetensis Spencer 1959	Madagascar (3, 7, 23, 30)	-	-	-	-
principensis Spencer 1977	Principe (7, 35)	-	-	-	-
provecta (Meijere 1910)	DRC (3, 7, 23, 24), Ethiopia (3, 7, 31, 36), Kenya (36), Mozambique (3, 7, 31), South Africa (3, 7, 24, 36), Tanzania (3, 7, 23, 24), Uganda (36), Zimbabwe (3, 7, 32)	Asteraceae	*Bidens pilosa* L. (23)	-	-
pubescentis Spencer 1959	Burundi (3, 7, 23), DRC (3, 7, 23), Kenya (3, 7, 23), Zimbabwe (3, 7, 23, 32)	-	-	-	-
purpurea Spencer 1959	South Africa (3, 7, 23)	-	-	-	-
purpureana Spencer 1959	Madagascar (7, 23, 30), South Africa (30)		-	-	-
ruandae Spencer 1959	Burundi (3, 7, 23, 37), Ethiopia (3, 7, 37), Ghana (3, 7, 37), Kenya (37), Rwanda (3, 7, 23, 37)	Asteraceae	*Senecio moorei* R. E. Fr. (37)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
rutshurensis Spencer 1959	DRC (3, 7, 23), Rwanda (3, 7, 23)	-	-	-	-
scottburghensis Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
seneciocaulis Spencer 1960	Ethiopia (3, 7, 34, 37), South Africa (3, 7, 24, 31, 34, 37)	Asteraceae Asteraceae Asteraceae Asteraceae Solanaceae	*Senecio juniperinus* L. f. (24, 37) *Senecio pterophorus* DC. (31) *Senecio ruderalis* Harv. (24, 37) *Senecio* sp. (28) *Solanum* sp. (24)	-	-
setulana Spencer 1959	DRC (3, 7, 23)	-	-	-	-
setulifera Spencer 1959	Madagascar (23, 30)	-	-	-	-
sojae (Zehntner 1900)	South Africa (3, 7, 33, 34)	Fabaceae Fabaceae	*Glycine max* (L.) Merr. (34) *Medicago sativa* L. (34)	-	-
solanidis Spencer 1959	South Africa (7), Tanzania (7), Uganda (3, 7, 23, 37)	Solanaceae Solanaceae	*Solanum melongena* L. (23, 37)	-	-
spenceriana Zlobin 2001	Kenya (3)	-	-	-	-
spungaberensis Spencer 1959	Mozambique (3, 7, 23), Zimbabwe (23)		-	-	-
stuckenbergi Spencer 1959	Madagascar (3, 7, 23, 30, 33)	-	-	-	-
suborbitalis Spencer 1959	DRC (7, 23), Zimbabwe (7, 23)	-	-	-	-
tamsi Spencer 1977	Principe (3, 7)	-	-		
thunbergiae Spencer 1960	South Africa (3, 7, 24, 37)	Acanthaceae Acanthaceae	*Thunbergia natalensis* Hook. (37) *Thunbergia* sp. (24, 37)	-	-
verdata Spencer 1961	Cape Verde Is. (3, 7, 29)	-	-	-	-
vignalis Spencer 1959	Kenya (3, 7, 24, 27, 31, 32, 34, 37), Mali (3, 7, 37), Nigeria (3, 34, 37), Sierra Leone (3, 7, 23, 24, 27, 32, 34, 37), South Africa (3, 7, 27, 31, 32, 34, 37), Sudan (23, 24, 27, 34, 37), Tanzania (7, 27)	Fabaceae Fabaceae Fabaceae	*Glycine sp.* (31, 34, 37) *Vigna* sp. (23, 27, 37) *Vigna unguiculata* (L.) Walp. (23, 24, 34, 37)	**Eucoilidae** *Eucoilidea* sp. **Eurytomidae** *Eurytoma* sp. **Pteromalidae** *Pteromalus* sp.	 Uganda (13) Uganda (13) Uganda (13)
viridissima Spencer 1959	Ghana (31), South Africa (3, 7, 24, 29, 33), Zimbabwe (3, 7, 23, 24, 29, 31)	Acanthaceae	*Thunbergia* sp. (24, 33)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
volubilis Spencer 1965	Cape Verde Is. (3, 7, 33)	-	-	-	-
wulfi Spencer 1959	DRC (3, 7, 23)	-	-	-	-
zomandoae Spencer 1961	Madagascar (3, 7, 30)	-	-	-	-
***Napomyza* Haliday 1840**					
drakensbergensis Spencer 1963	South Africa (3, 7, 31, 33)	-	-	-	-
munroi Spencer 1960	Lesotho (7), South Africa (7, 24)	-	-	-	-
renovata Spencer 1960	Kenya (37), South Africa (37)	Ranunculaceae	*Ranunculus multifidus* Forssk. (37)	-	-
strana Spencer 1960	Tanzania (3, 7, 33)	-	-	-	-
subeximia Spencer 1985	Kenya (3, 36, 37)	Ranunculaceae	?*Ranunculus multifidus* Forssk. (36, 37)	-	-
vivida Spencer 1965	Lesotho (3, 7, 33)	-	-	-	-
***Ophiomyia* Brazchnikov 1897**					
aberrans (Spencer 1959)	DRC (3, 7, 23, 37), South Africa (3, 7, 24, 37)	-	-	-	-
acutalis Spencer 1959	DRC (3, 7, 23, 33)	-	-	-	-
albivenis Spencer 1959	South Africa (3, 7, 23)	-	-	-	-
anomala Spencer 1961	South Africa (3, 7, 29)	-	-	-	-
atralis (Spencer 1961)	Kenya (3, 28, 29, 31, 32) , South Africa (7, 37)	Asteraceae Asteraceae Scrophulariaceae	*Cyanthillium cinereum* (L.) H. Rob. (37) *Vernonia appendiculata* Less. (37) *Striga hermonthica* (Delile) Benth. (31, 32, 37)	-	-
beckeri (Hendel 1923)	South Africa (7, 15, 23, 27, 33, 37), Tanzania (7, 8), Zimbabwe (7, 8, 23, 27)	Asteraceae	*Sonchus oleraceus* L. (15, 23, 33, 37)	-	-
camarae Spencer 1963	South Africa (13)	Verbenaceae	*Lantana camara* L. (13)	-	-
centrosematis (de Meijere 1940)	Kenya (7, 12, 34, 36, 37), Mozambique (8), Tanzania (7, 12, 27, 34, 36, 37), Uganda (7, 12, 34, 36, 37)	Fabaceae Fabaceae Fabaceae Fabaceae	*Centrosema pubescens* Benth. (12) *Crotalaria pallida* Aiton (12, 36, 37) *Glycine max* Siebold & Zucc (12, 34) *Phaseolus lunatus* L. (12, 34, 36)	**Braconidae** *Opius melanagromyzae* Fischer 1963 **Eucoilidae**	 Uganda (12, 34)

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Fabaceae	*Phaseolus vulgaris* L. (34, 36)	*Eucoilidaea* sp.	Uganda (12, 34)
		Fabaceae	*Vigna unguiculata* (L.) Walp. (12, 34, 36)		
colei Spencer 1965	South Africa (3, 7, 33, 37)	Lamiaceae	*Ocimum gratissimum* L. (36, 37)	-	-
		Lamiaceae	*Solenostemon scutellarioides* (L.) Codd (33, 37)		
crotalariella Spencer 1990	Kenya (3, 37)	Fabaceae	*Crotalaria agatiflora* Schwief. (37)	-	-
decembris (Spencer 1959)	Cape Verde Is. (7, 23, 24), South Africa (24)	-	-	-	-
helichrysi Spencer 1960	South Africa (3, 7, 24, 37)	Asteraceae	*Helichrysum argyrosphaerum* DC. (32, 37)	-	-
kenyae Spencer 1985	Kenya (3, 36)	-	-	-	-
kilembensis Spencer 1985	Uganda (3, 36)	-	-	-	-
kilimanii Spencer 1985	Kenya (3, 36)	-	-	-	-
labiatalis Spencer 1959	Cameroon (3, 7, 23)	Labiatae	*Labiatae* sp. (23)	-	-
lantanae (Froggatt 1919)	Kenya (3, 7, 23, 31), South Africa (2, 3, 34), Tanzania (34), Uganda (34), Zambia (3)	Verbenaceae	*Lantana camara* (L.) (23)	**Braconidae**	
		Verbenaceae	*Lantana* sp. (23, 37)	*Bracon* sp.	South Africa (2)
				Opius sp.	South Africa (2)
				Eucoilidae	
				indet. sp. (2223)	South Africa (2)
				Eulophidae	
				Euderus sp. (2220)	South Africa (2)
				Eupelmidae	
				Eupelmus sp. 2219	South Africa (2)
				Eupelmus sp. 2221	South Africa (2)
lucidata Spencer 1961	South Africa (3, 7, 29)	-	-	-	-
lunatica Spencer 1961	South Africa (3, 7, 29)	-	-	-	-
mesonotata Spencer 1961	Ethiopia (3, 7)	-	-	-	-
nigerrima Spencer 1959	Kenya (3, 7, 23)	-	-	-	-
ocimi Spencer 1965	Guinea (3, 7, 33, 37)	Lamiaceae	*Ocimum gratissimum* L. (33, 37)	-	-
ocimivora Spencer 1985	Kenya (12, 36, 37)	Lamiaceae	*Ocimum lamiifolium* Hoscht. ex Benth (36, 37)	-	-
perversa Spencer 1965	South Africa (3, 7, 33)	-	-	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
phaseoli (Tryon 1888)	Botswana (12), DRC (3, 7, 23), Ethiopia (1, 3, 12), Kenya (3, 12, 22, 34, 36, 37), Madagascar (12), Malawi (3, 7, 8, 23), Mauritius (3, 12, 23, 34), Mozambique (3, 8), Nigeria (4) Rwanda (3, 7), Senegal (3, 7, 23, 37), South Africa (3, 7, 23), Tanzania (12), Uganda (3, 7, 12, 23, 34, 36, 37), Zimbabwe (3, 7, 8, 12, 23, 37)	Fabaceae	*Cajanus cajan* L. (12)	**Braconidae**	
		Fabaceae	*Crotalaria juncea* L. (12)	*Opius liogaster* Szépligeti 1932	Ethiopia (1), Mauritius (34) Zimbabwe (12, 34)
		Fabaceae	*Crotalaria laburnifolia* L. (1)		
		Fabaceae	*Crotalaria pallida* Aiton (12)		
		Fabaceae	*Glycine max* (L.) Merr. (1, 12)	*Opius melanagromyzae* Fischer 1963	Madagascar (12), Mauritius (12), Mozambique (8), Uganda (12)
		Fabaceae	*Lablab purpureus* (L.) Sweet (12)		
		Fabaceae	*Phaseolus lunatus* L. (12, 23)		
		Fabaceae	*Phaseolus* spp. (12)		
		Fabaceae	*Phaseolus vulgaris* L. (1, 8, 22, 23)	*Opius phaseoli* Fischer 1963	Kenya (22), Ethiopia (1)
		Fabaceae	*Vigna mungo* (L.) Hepper (12)		
		Fabaceae	*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi (12)	**Eucoilidae**	
		Fabaceae	*Vigna unguiculata* (L.) Walp. (1, 4, 12, 23)	*Eucoilidea nitida* Benoit 1956	Mozambique (8)
				Eulophidae	
				Aprostocetus sp.	Ethiopia (1)
				Cirrospilus sp.	Ethiopia (1)
				Chrysonotomyia sp. nr *erythraea* (Silvestri 1914)	Mozambique (34) Ethiopia (1)
				Closterocerus formosus Westwood 1833	Ethiopia (1, 34), Kenya (34), Tanzania (34), Uganda (34)
				Meruana liriomyzae (Boucek 1988)	Ethiopia (1), Mauritius (1)
				Pediobius metallicus (Nees, 1834)	Ethiopia (1)
				Eupelmidae	
				Eupelmus sp.	Ethiopia (1)
				Eupelmus sp. nr. *urozonus* Dalman 1820	Ethiopia (1)
				Eurytomidae	
				Eurytoma sp.	Ethiopia (1), Kenya (12)
				Pteromalidae	

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
				Callitula filicornis Delucchi 1962	Ethiopia (1)
				Halticoptera ?circulus (Walker 1833)	Ethiopia (1)
				Herbertina sp.	Ethiopia (1)
				Sphegigaster brunneicornis (Ferrière 1930)	Ethiopia (1), Gambia (1)
				Sphegigaster stepicola Bouček 1965	Ethiopia (1)
				Tetracampidae	
				Epiclerus sp. nr *nomocerus* (Masi 1934)	Ethiopia (1)
pretoriensis Spencer 1960	South Africa (3, 7, 24)	-	-	-	-
productella Spencer 1960	South Africa (3, 7, 24, 29)	-	-	-	-
pulicaria (Meigen 1830)	Kenya (36, 37)	Asteraceae	*Sonchus oleraceaus* L. (36)	-	-
		Asteraceae	*Sonchus* sp. (37)		
rhodesiensis Spencer 1959	Zimbabwe (3, 7, 23, 31)	-	-	-	-
solanivora Spencer 1961	Ethiopia (3, 7, 32, 37), Kenya (3, 37), Madagascar (3, 7, 30, 31, 32, 37), South Africa (3, 7, 31, 32, 33, 37)	Solanaceae	*Solanum incanum* L. (30, 32, 37)	-	-
		Solanaceae	*Solanum lasiocarpum* Dunal (30, 31, 32, 37)		
		Solanaceae	*Solanum panduriforme* E. Mey (33)		
spencerella Greathead 1969	Kenya (3, 7, 12, 22, 34, 36, 37), Mozambique (8), Nigeria (3, 7, 34, 37), Tanzania (3, 7, 12, 34, 37), Uganda (3, 7, 12, 34, 37)	Fabaceae	*Lablab purpureus* (L.) Sweet (12, 34)	**Braconidae**	
		Fabaceae	*Phaseolus lunatus* L. (12, 34)	*Opius phaseoli* Fischer 1963	Kenya (25)
		Fabaceae	*Phaseolus* sp. (22, 34, 36, 37)	**Eucoilidae**	
		Fabaceae	*Phaseolus vulgaris* L. (8, 12, 22, 34)	*Eucoilidea nitida* Benoit 1956	Mozambique (8)
		Fabaceae	*Vigna mungo* (L.) Hepper (12)		
		Fabaceae	*Vigna* sp. (34, 37)	*Eucoilidea* sp.	Uganda (12, 34)
		Fabaceae	*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi (12)		
		Fabaceae	*Vigna unguiculata* (L.) Walp. (12, 34)		
spuriosa Spencer 1960	South Africa (3, 7, 32)	-	-	-	-
strigalis Spencer 1963	Kenya (3, 7, 11, 31, 32, 34, 37), Tanzania (7, 11, 32, 34, 37), Uganda (7, 11, 34, 37)	Scrophulariaceae	*Alectra asperrima* Benth (11)	**Eucoilidae**	
		Scrophulariaceae	*Cycnium tubulosum* Engl. N.E. Br. (11)	*Eucoilidea* sp.	Kenya (12), Uganda (11, 12, 34)
		Scrophulariaceae	*Striga asiatica* (L.) Kuntze (11)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Scrophulariaceae	*Striga hermonthica* (Delile) Benth. (12, 31, 34, 37)	*Opius* sp.	Tanzania (12), Uganda (11)
		Lamiaceae	*Coleus* sp. (32)	-	-
verdalis Spencer 1959	Cape Verde Is. (3, 7, 23, 33), Ethiopia (3, 7), South Africa (24)	-	-	-	-
visenda Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
***Penetagromyza* Spencer 1959**					
aloes Spencer 1959	South Africa (3, 7, 23, 37)	Asphodelaceae	*Aloe petricola* Pole-Evans (23, 37)	-	-
similans Spencer 1961	South Africa (3, 7)	-	-	-	-
***Phytobia* Lioy 1864**					
brincki Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
flavosquamata (Spencer 1959)	Nigeria (3, 7, 23, 33), South Africa (3, 7)	-	-	-	-
humeralis Spencer (von Roser, 1840)	Ethiopia (32), South Africa (15, 24)	Asteraceae Asteraceae	*Conyza bonariensis* (L.) Cronquist (15) *Dichrocephala chrysanthemifolia* (Blume) DC. (32)	- -	- -
nigeriensis Spencer 1977	Nigeria (3, 7)	-	-	-	-
nigrita (Malloch 1914)	Tanzania (3, 7, 27)	-	-	-	-
ruandensis (Spencer 1959)	Rwanda (3, 7, 23, 33)	-	-	-	-
setariae Spencer & Sasakawa 1961	South Africa (23)	Poaceae	*Setaria* sp. (23)	-	-
shizukoae Spencer 1965	South Africa (3, 7, 33)	-	-	-	-
***Phytoliriomyza* Hendel 1931**					
asiatica Spencer 1985	Kenya (37)	-	-	-	-
gilleti Spencer 1985	Kenya (37)	-	-	-	-
immoderata Spencer 1963	South Africa (3, 7, 31)	-	-	-	-
intermedia Spencer 1985	Kenya (3, 36, 37)	-	-	-	-
jacarandae Steyskal & Spencer 1978	South Africa (17)	-	-	**Eulophidae** *Cirrospilus ?ambiguus* *Diglyphus* sp.	 South Africa (17) South Africa (17)

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
perpusilla (Meigen 1830)	Cape Verde Is. (7, 23, 24), Lesotho (7, 24), South Africa (7, 29)	-	-	-	-
***Phytomyza* Fallén 1810**		-		-	-
africana Spencer 1959	Tanzania (3, 7, 23)	-	-	-	-
anonera Séguy 1951	Ethiopia (7, 32), Madagascar (7, 21, 23, 30, 32)	Asteraceae	*Bidens pilosa* L. (21)		
		Asteraceae	*Vernonia appendiculata* Less. (21, 23, 30, 32)		
atricornis Meigen 1838	Cameroon (3, 23), Cape Verde Is. (3, 23), DRC (3, 23), Eritrea (3), Ethiopia (3, 32), Kenya (3, 23, 32), South Africa (3, 15, 23, 32)	Asteraceae	*Vernonia* sp. (32)	**Eulophidae** *Meruana liriomyzae* Bouček 1988	Eritrea (5)
		Asteraceae	*Bidens pilosa* L. (15)		
		Asteraceae	*Chrysanthemum tricolor* Daisy (23)		
		Asteraceae	*Cotula abyssinica* Sch. Bip. Ex A. Rich. (32)		
		Asteraceae	*Conyza bonariensis* (L.) Cronquist (15)		
		Asteraceae	*Dahlia* sp. (15)		
		Asteraceae	*Galinsoga parviflora* L. (15)		
		Asteraceae	*Glebionis carinata* (Schousb) Tzvelev (23)		
		Asterceae	*Mikaniopsis clematoides* (A. Rich.) Milne-Redh. (32)		
		Asteraceae	*Sonchus oleracerus* L. (15, 23)		
		Brassicaceae	*Brassica* sp. (23)		
		Brassicaceae	*Erysimum* sp. (23)		
		Brassicaceae	*Nasturtium* sp. (23)		
		Chenopodiaceae	*Chenopodium* sp. (15)		
		Fabaceae	*Lathyrus* sp. (23)		
		Fabaceae	*Medicago sativa* L.(23)		
		Plantaginaceae	*Linaria* sp. (23)		
		Solanaceae	*Petunia* sp. (15, 23)		
		Solanaceae	*Solanum lycopersicum* L. (23)		
		Solanaceae	*Solanum tuberosum* L. (23)		
		Solanaceae	*Solanum ?nigrum* L.(15)	-	-
basiflavus Yang & Yang 1989	Madagascar (30)			-	-
caffra Macquart 1846	South Africa (3, 7)	-	-		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
clematidella Spencer 1959	Kenya (3, 7, 23, 36, 37)	Ranunculaceae	*Clematis* sp. (23, 36, 37)	-	-
clematisi Spencer 1964	Kenya (37), Ethiopia (7, 32, 37)	Ranunculaceae	*Clematis simensis* Fresen. (32, 37)	-	-
eximia Spencer 1964	South Africa (7, 32)	-	-	-	-
horticola Goureau 1851	Cameroon (3, 7, 34, 37), Cape Verde Is. (3, 7, 37), Ethiopia (3, 7, 37), Kenya (3, 7, 34, 37), Madagascar (3, 7, 21, 23, 34, 37), South Africa (3, 7, 34, 37)	Alliaceae Asteraceae Brassicaceae Solanaceae	*Allium cepa* L. (37) *Bidens pilosa* L. (21) *Brassica oleracea* L. (37) *Solanum lycopersicum* L. (37)	-	-
knowltoniae Hering 1957	South Africa (3, 7, 15, 23, 31, 32, 37)	Loasaceae Ranunculaceae	*Kissenia capensis* Endl. (23, 31, 37) *Knowltonia capensis* (L.) Huth. (15, 31)	-	-
multifidi Spencer 1985	Kenya (3, 36)	Ranunculaceae	*Ranunculus multifidus* Forssk. (36)	-	-
natalensis Spencer 1985	Kenya (36, 37), South Africa (3, 7, 32, 36, 37)	Ranunculaceae	*Ranunculus multifidus* Forssk. (31, 36)	-	-
orobanchia Kaltenbach 1864	Ethiopia (3, 7, 37)	Orobancaceae	*Orobanche* sp. (37)	-	-
philoclematidis Hering 1957	Kenya (3, 37), South Africa (7, 15, 23, 31, 33, 36, 37)	Ranunchulaceae Ranunculaceae	*Clematis brachiata* Thunb. (15, 23, 31, 36) *Clematis simensis* Fresen. (37)	-	-
ranunculina Spencer 1963	Ethiopia (3, 7, 31, 32, 36, 37), South Africa (7, 32, 36, 37)	Ranunculaceae	*Ranunculus multifidus* Forssk. (31, 32, 37)	-	-
renovata Spencer 1960	Kenya (3, 36), South Africa (3, 7, 31, 32, 36)	-	-	-	-
seneciovora Spencer 1959	Cameroon (3, 7, 23), Ethiopia (3, 7), Tanzania (3, 7)	Asteraceae	*Senecio mannii* (Hook.f.) (23, 28)	-	-
varii Spencer 1964	South Africa (3, 7, 32, 37)	Ranunculaceae	*Clematis brachiata* Thunb. (32, 37)	-	-
vitalbae Kaltenbach 1872	South Africa (3, 7, 15, 23, 36)	Ranunculaceae	*Clematis brachiata* Thunb. (15, 23, 36)	-	-
vitalbella Hering 1957	Ethiopia (3, 7, 32, 36, 37), South Africa (3, 7, 15, 23, 32, 37)	Ranunculaceae Ranunculaceae	*Clematis brachiata* Thunb. (15, 23, 36) *Clematis simensis* Fresen. (36)	-	-
***Pseudoliriomyza* Spencer 1966**					
cordiae (Spencer 1959)	Tanzania (3, 7, 23, 37)	Boraginaceae	*Cordia ovalis* R.Br. ex DC. (23, 37)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
***Pseudonapomyza* Hendel 1920**					
acanthacearum (Spencer 1959)	Cameroon (3, 7, 23, 37), Kenya (3), South Africa (3, 7, 33), Sudan (3, 7), Tanzania (3, 7)	Acanthaceae Acanthaceae	*Acanthaceae* sp. (23) *Justicia diclipteroides* Lindau. (23, 37)	-	-
afrospicata Zlobin 2002	Gambia (3)	-	-	-	-
alternantherae (Séguy 1951)	Cameroon (3, 7, 28, 29, 30), Ethiopia (3), Madagascar (3, 7, 21, 23, 28, 29, 30, 37), South Africa (3, 7, 28, 29, 36)	Amaranthaceae Amaranthaceae	*Achyranthes aspera* L. (30, 37) *Alternanthera sessilis* (L.) R. Br. ex DC. (23, 30, 37)	-	-
asiatica Spencer 1961	Cape Verde Is. (3, 7, 34, 36, 37), Ethiopia (3, 7, 34, 36, 37), Kenya (3, 36, 37), South Africa (3, 7, 28, 29, 34, 36, 37), Tanzania (36)	Poaceae Poaceae Poaceae Poaceae	*Cynodon dactylon* L. (Pers.) (34, 37) *Eragrotis* sp. (34) *Oryza sativa* L. (37) *Zea mays* L. (29, 34, 37)	-	-
asystasiae Spencer 1965	Cape Verde Is. (3), Kenya (3, 37), South Africa (3, 7, 33, 37), Zimbabwe (3, 33, 37)	Acanthaceae	*Asystasia gangetica* L. (T. Anderson) (33, 37)	-	-
atra (Meigen 1830)	Cape Verde Is. (7), Ethiopia (23)	-	-	-	-
confusa Zlobin 1993	Cape Verde Is. (3, 39)	-	-	-	-
diminua (Spencer 1961)	Madagascar (7, 27, 30), Tanzania (7, 27)	-	-	-	-
embuensis Spencer 1985	Kenya (3, 36)	-	-	-	-
embui Spencer 1985	Kenya (3, 36)	-	-	-	-
gambia Zlobin 2002	Gambia (3)	-	-	-	-
gilletti Spencer 1985	Kenya (3, 36)	-	-	-	-
grandiosa (Spencer 1961)	Madagascar (3, 7, 30)	Poaceae	*Sorghum bicolor* (L.) Moench (34, 37)	-	-
hispanica Spencer 1973	Ethiopia (37), Kenya (36, 37), Tanzania (36, 37)		-	-	-
hohmanni Spencer 1965	Namibia (3, 7, 33), South Africa (3, 7, 33)			-	-
hypoestis Hering 1957	DRC (3, 7, 23), Ethiopia (3, 7), South Africa (3, 7, 15, 23, 28, 29, 33, 37)	Acanthaceae Acanthaceae	*Hypoestis aristata* Soland ex Roem. & Schult. (15, 23, 28, 37) *Hypoestis ?verticillaris* (Linn. F. Soland. (33)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
hypoestivora (Séguy 1951)	Madagascar (7, 21, 23, 28, 29, 30, 37), Zimbabwe (7, 33)	Acanthaceae Acanthaceae	*Hypoestes betsiliensis* S. Moore. (21, 23, 30, 37) *Hypoestis* sp. (33)	-	-
insularis Zlobin 1993	Cape Verde Is. (3, 39)	-	-	-	-
justiciae Spencer 1990	Kenya (3, 37)	Acanthaceae	*Hypoestes aristata* Soland ex Roem. & Schult. (37)	-	-
lucentis Spencer 1959	Cape Verde Is. (3, 7, 23)	-	-	-	-
matopi Spencer 1965	Zimbabwe (3, 7, 33, 34, 37)	Acanthaceae	*Dicliptera monroi* S. Moore (33, 34, 37)	-	-
media (Spencer 1961)	Madagascar (3, 7, 30)	-	-	-	-
nigralis Spencer 1961	Ethiopia (3, 7)	-	-	-	-
ovalis Zlobin 2002	Tanzania (3)	-	-	-	-
perspicua Spencer 1963	South Africa (3, 7, 31)	-	-	-	-
ruiruensis Spencer 1985	Kenya (3, 36)	-	-	-	-
similis Spencer 1985	Kenya (3, 36), Tanzania (3, 27, 36)	-	-	-	-
spinosa Spencer 1973	Cape Verde Is. (39), Kenya (36), Lesotho (7, 34), Nigeria (7, 34, 39), South Africa (3, 7, 34, 36, 37, 39), Tanzania (36)	Poaceae Poaceae Poaceae	*Hordeum vulgare* L. (34, 36, 37) *Triticum aestivum* L. (34, 36, 37) *Zea mays* L. (34, 37)	-	-
subspinosa Spencer 1985	Kenya (3, 36)	-	-	-	-
urundensis (Spencer 1959)	Burundi (3, 7, 23), DRC (3, 23, 37), Ethiopia (3, 7, 32, 33, 37), South Africa (3, 7, 37), Zimbabwe (3, 7, 33)	Acanthaceae Acanthaceae	*Hypoestes aristata* Soland ex Roem & Schult. (37) *Hypoestes triflora* Roem. Schult. (32, 33, 37)	-	-
vernoniae (Séguy 1951)	Madagascar (3, 7, 21, 23, 37)	Asteraceae	*Vernonia appendiculata* Less. (21, 23, 37)	-	-
zeae Spencer 1973	Ghana (3, 7, 34, 37)	Poaceae	*Zea mays* L. (34, 37)	-	-
***Ptochomyza* Hering 1942**					

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
asparagivora Spencer 1964	Ethiopia (3, 7, 32, 36, 37), Kenya (3, 36, 37), South Africa (3, 37)	Asparagaceae Asparagaceae Asparagaceae Asparagaceae Asparagaceae	*Asparagus acutifolius* L. (36, 37) *Asparagus africanus* Lam. (32, 36, 37) *Asparagus densiflorus* (Kunth) Jessop (37) *Asparagus falcatus* L. (36, 37) *Asparagus stipularis* Forssk. (36, 37)	-	-
***Tropicomyia* Spencer 1973**					
capeneri (Hering 1957)	Senegal (3, 7, 15, 34, 37), South Africa (3, 7, 15, 23, 34, 36, 37)	Fabaceae	*Vigna unguiculata* (L.) Walp. (34, 37)	-	-
cassinis (Hering 1957)	South Africa (3, 7, 15, 23, 34, 36, 37)	Celastraceae	*Mystroxylon aetiopicum* (Thunb.) Loes (15, 23, 34, 37)	-	-
ceratiosicyi (Hering 1957)	South Africa (3, 7, 15, 23, 34, 37)	Achariaceae	*Ceratiosicyos ecklonii* Nees (15, 23, 34, 37)	-	-
clutiae (Spencer 1963)	Ethiopia (3, 7, 31), South Africa (3, 7, 31, 34, 36)	Euphorbiaceae	*Clutia pulchella* L. var. *pulchella* (31, 34, 37)	-	-
crotalariae (Hering 1957)	Ethiopia (37), South Africa (3, 15, 23, 31, 36, 37), Uganda (37)	Fabaceae Fabaceae Fabaceae	*Crotalaria capensis* Jacq. (31) *Crotalaria ?juncea* L. (23) *Crotalaria capensis* Jacq. (31, 37)	-	-
crotonella (Spencer 1964)	Ethiopia (3, 7, 32, 34, 36, 37), Kenya (3, 36, 37)	Euphorbiaceae	*Croton* sp. (36, 37)	-	-
dicksoni (Hering 1957)	South Africa (3, 7, 15, 23, 34, 36)	-	-	-	-
eulophiae Spencer 1990	Kenya (3, 37)	Orchidaceae	*Eulophia porphyroglossa* (Rchb. f.) Bolus (37)	-	-

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
flacourtiae (Séguy 1951)	Cameroon (3, 7, 23, 34, 37), Ethiopia (3, 7, 34, 37), Kenya (3, 34, 36, 37), Madagascar (3, 7, 21, 23, 30, 34, 36, 37), Nigeria (3, 7, 23, 34, 37), Sierra Leone (3, 7, 23, 34, 37), South Africa (3, 7, 15, 23, 34, 37), Tanzania (3, 7, 34, 37)	Apocynaceae	*Cryptostegia madagascariensis* Bojer ex Decne. (21, 23, 34, 37)	**Eulophidae** *Chrysonotomyia purpurissata* Kerrich 1970	Kenya(34), Tanzania (34)
		Apocynaceae	*Mascarenhasia arborescens* A. DC. (21, 23, 37)		
		Apocynaceae	*Pachypodium rutenbergianum* Vatke (37)		
		Apocynaceae	*Thevetia peruviana* (Pers.) K. Schum. (36)		
		Crassulaceae	*Kalanchoe ? pubescens* Baker (23)		
		Crassulaceae	*Kalanchoe pubescens* Baker (37)		
		Dioscoreaceae	*Dioscorea* sp. (21, 23, 30, 34, 37)		
		Fabaceae	*Clitoria* sp. (23)		
		Fabaceae	*Crotalaria* sp. (23, 27)		
		Fabaceae	*Senna occidentalis* (L.) Link (23, 37)		
		Fabaceae	*Senna septemtrionalis* (Viv.) H. S. Irwin & Barneby (21, 23, 37)		
		Maesaceae	*Maesa emirnensis* A. DC. (21, 23, 37)		
		Malvaceae	*Gossypium* spp. (23, 34, 37)		
		Nyctaginaceae	*Bougainvillea* sp. (21, 23, 34, 36)		
		Orchidaceae	*Eulophia porphyroglossa* (Rchb. f.) Bolus (36)		
		Passifloraceae	*Passiflora caerulea* L. (15, 23, 37)		
		Passifloraceae	*Passiflora edulis* Sims. (21, 23, 36)		
		Passifloraceae	*Passiflora ? foetida* L. (23)		
		Passifloraceae	*Passiflora* sp. (23)		
		Pittosporaceae	*Pittosporum stenopetalum* Baker (21, 23, 37)		
		Rosaceae	*Crataegus laevigata* (Poir.) DC. (37)		
		Rubiaceae	*Coffea arabica* L. (23, 34, 36)		
		Rubiaceae	*Coffea* sp. (21, 23)		
		Rubiaceae	*Mussaenda arcuata* Lam. ex Poir. (21, 23, 37)		
		Rutaceae	*Citrus* sp. (23, 34, 37)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
		Salicaceae	*Flacourtia indica* (Burm. f.) Merr. (21, 23, 30, 37)		
		Solanaceae	*Solanum giganteum* Jacq. (21, 23, 37)		
		Solanaceae	*Solanum lasiocarpum* Dunal (23)		
		Thiaceae	*Camellia sinensis* (L.) Kuntze var. *sinensis* (21, 23, 34, 37)		
		Verbenaceae	*Duranta erecta* L. (23, 37)		
gloriosae Spencer 1990	Kenya (3, 37)	Colchicaceae	*Gloriosa superba* L. (37)	-	-
gymnosporiae Hering (1957)	Ethiopia (3, 7, 32), South Africa (3, 7, 15, 23, 24, 31, 34, 37)	Asteraceae	*Crassocephalum rubens* (Juss. Ex Jacq.) S. Moore (23)	-	-
		Celastraceae	*Celastrus* sp. (24, 37)		
		Celastraceae	*Gymnosporia buxifolia* (L.) Szyszyl. (15, 23, 31, 34, 37)		
		Celastraceae	*Gymnosporia* sp. (31)		
		Rhamnaceae	? *Rhamnus prinoides* L'Hér. (31)		
haemanthi (Spencer 1963)	Ethiopia (3), South Africa (3, 7, 31, 34, 37)	Amaryllidaceae	*Scadoxus puniceus* (L) Friis & Nordal (31, 34, 37)	-	-
kalanchoes Spencer 1985	Kenya (3, 36, 37)	Crassulaceae	*Kalanchoe densiflora* Rolfe (36, 37)	-	-
laburnifoliae (Spencer 1964)	Ethiopia (3, 7, 31, 32, 34, 37)	Fabaceae	*Crotalaria laburnifolia* L. (32, 34, 37)	-	-
nigriclava (Bezzi and Lamb 1926)	Mauritius (3, 7, 23), Rodriguez (3, 7, 23, 34)	-	-	-	-
philocroton (Hering 1957)	South Africa (3, 7, 15, 34, 36, 37)	Euphorbiaceae	*Croton gratissimus* Prain (15, 34)	-	-
		Euphorbiaceae	*Croton macrostachyus* Hochst. ex Delile (23)		
		Euphorbiaceae	*Croton* sp. (37)		
thunbergivora (Spencer 1963)	Ethiopia (3, 7, 32), South Africa (3, 7, 31, 34, 37)	Acanthaceae	*Thunbergia natalensis* Hook (31, 34, 37)	-	-
		Cucurbitaceae	*Cucurbita pepo* L. (32, 37)		
		Cucurbitaceae	*Melothria tomentosa* Cogn. (32)		
vigneae (Séguy 1951)	Cape Verde Is. (3, 7,34, 37), Ethiopia (3, 7, 32), Madagascar (3, 7, 21, 23, 30, 31, 34), Senegal (7, 23, 30, 31, 32), South Africa (7, 32, 37), Sudan	Asparagaceae	*Asparagus densiflorus* (Kunth) Jessop (37)	-	-
		Fabaceae	*Crotalaria incana* L. (23, 30)		
		Fabaceae	*Dipogon lignosus* (L.) Verdc. (37)		

Agromyzid species	Agromyzid distribution	Host plant family	Host plant species	Parasitoids	Parasitoid distribution
	(7, 34)	Fabaceae	*Lablab purpureus* (L.) Sweet (34)		
		Fabaceae	*Pueraria montana* (Lour.) Merr. (32, 37)		
		Fabaceae	*Senna bicapsularis* (L.) Robx (23)		
		Fabaceae	*Senna occidentalis* (L.) Link (21, 23, 30)		
		Fabaceae	*Vigna angivensis* Baker (21, 23, 30)		
		Fabaceae	*Vigna mungo* (L.) Hepper (21, 23, 30)		
		Fabaceae	*Vigna unguiculata* (L.) Walp (30 31, 32, 34)		
		Xanthorrhoeaceae	*Kniphofia* nr *galpinii* (37)		

Abate (1991)[1], Baars & Heystek (2003)[2], Bisby et al. (2008)[3], Bottenberg et al. (1998)[4], Bouček (1988)[5], Chabi et al. (2008)[6], Cogan (1980)[7], Davies (1998)[8], Deeming & Mann (1999)[9], EPPO (2006)[10], Greathead & Milner (1971)[11], Greathead (1969)[12], Greathead (1971)[13], Hasson & LaSalle (1996)[14], Hering (1957)[15], Musundire (2002)[16], NCI (2009)[17], Neuenschwander et al. (1987)[18], Noyes (1998)[19], Noyes (2003)[20], Séguy (1951)[21], Songa & Ampofo (1999)[22], Spencer (1959)[23], Spencer (1960a)[24], Spencer (1960b)[25], Spencer (1961a)[26], Spencer (1961b)[27], Spencer (1961c)[28], Spencer (1961d)[29], Spencer (1960e)[30], Spencer (1963)[31], Spencer (1964)[32], Spencer (1965)[33], Spencer (1973)[34], Spencer (1977)[35], Spencer (1985)[36], Spencer (1990)[37], Stegmaier (1967)[38], Zlobin (1993)[39], Zlobin (2001)[40]

Numbers in parentheses represent the reference where the record has been made.

Reference for plant names: USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database].
National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl (01 February 2009)
Additional references for parasitoids were made from "The Chalcidoidea Specimen Database of the Biosystematics Division, ARC-Plant Protection Research Institute, Pretoria, South Africa" (NCI)

CHAPTER TWO

Host plant effects on morphometric characteristics of *Liriomyza huidobrensis, L. sativae* and *L. trifolii* (Diptera: Agromyzidae)[1]

Abstract

Body size of herbivorous insects may be directly related to host plant characteristics (e.g. nutrition, chemical composition) and positively linked to performance of the herbivore and its parasitoids. Differences in adult body size of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* reared on *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba* were assessed to determine the effect of host plants on adult size. Based on univariate, canonical and linear discriminant analyses of wing morphometric and hind tibia variables, *Liriomyza huidobrensis*, the largest species, showed no differences in the variables measured when reared on the four plant species. However, different plant species seem to influence wing and hind tibia measurements in *L. trifolii* to a greater extent than in *L. huidobrensis* and *L. sativae*. The first two canonical variates could not separate the populations of *L. huidobrensis* and *L. sativae* reared on different plant species, but showed separation of *L. trifolii* populations into two clusters: firstly, insects reared on *V. faba* and secondly, insects reared on *P. vulgaris* and *S. lycopersicum*. Hind tibia length strongly correlated with wing length. Both can be used to determine adult size of the three *Liriomyza* species. The performance of *Liriomyza* species from this study partially provides new evidence for existence of differential preference by *Liriomyza* species on the four studied host plant species. This therefore, warrants larger scale field studies to test the positive preference - performance of *Liriomyza* species on these four plant species and possible consequences at the third tritrophic level.

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[1] **In press as**: Musundire, R., Chabi-Olaye, A. & Krüger, K. (in press) Host plant effects on morphometric characteristics of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* (Diptera: Agromyzidae). *Journal of Applied Entomology*. The content of the article has been slightly modified for a more uniform presentation of chapters in the thesis.

Introduction

Host plant quality may directly affect performance, e.g. survivorship, fitness and fecundity, of herbivorous insects (Parrella *et al.*, 1983; Via, 1984; Smith & Hardman, 1986; Awmack & Leather, 2002), for example through differences in leaf nutrition (Minkenberg & Ottenheim, 1990). The size of herbivorous insects has been positively linked with performance and is influenced by genetic factors and environmental conditions, which include the host plants they are feeding on (Honék, 1993). In addition, herbivore host size directly affects traits at the third trophic level, for example, the performance (host feeding, parasitism, and sex allocation) of parasitoids of leafminers (Ode & Heinz, 2002).

Leafminer species belonging to the genus *Liriomyza* Mik (Diptera: Agromyzidae) are important pests of various horticultural crops worldwide (Spencer, 1985; Murphy & LaSalle, 1999; Burgio *et al.*, 2007; Chabi-Olaye *et al.*, 2008). The economically important species *L. huidobrensis* (Blanchard), *L. sativae* Blanchard, and *L. trifolii* (Burgess), originate from neotropic sub-regions of Central and South America. They are characterized by their high degree of polyphagy and have been spreading amongst others to several countries of Africa, Asia and Europe (Spencer, 1985, 1990; Murphy & LaSalle, 1999; EPPO, 2006; Burgio *et al.*, 2007).

In Kenyan horticultural production systems, the three species attack a variety of crops of commercial value, including snow peas (*Pisum sativum* L.), French beans (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), runner bean (*Phaseolus coccineus* L.) (Fabaceae), tomato (*Solanum lycopersicum* L.) and potato (*Solanum tuberosum* L.). (Solanaceae), and a variety of cut flowers (Chabi-Olaye *et al.*, 2008). Depending on the plant and location, the pest infestation ranged between 10 and 80 %, and was higher in cultivated than wild habitats (Chabi-Olaye *et al.*, 2008). The quarantine species *L. huidobrensis* was the most important species (80 %) in the highland vegetable production areas on snow pea and potato (Chabi-Olaye *et al.*, 2008).

Host plant preferences in the polyphagous leafminers have previously been reported. Valladares *et al.* (1996) observed that *L. huidobrensis* consistently occurred at higher densities on particular crops when several host species were simultaneously available in a field. Scheirs *et al.* (2004) reported *L. trifolii* preferentially selects *S.*

lycopersicum and *Solanum americanum* Mill. for feeding and ovipostion compared to *Physalis pubescens* L. (Solanaceae) and *Bidens pilosa* L. (Asteraceae). In addition, *L. trifolii* preferred *Sonchus oleraceus* L. and *Jacobaea vulgaris* Gaertn. to *Sonchus arvensis* L. (Asteraceae) (Mayhew, 1998). Laboratory and field data obtained by Videla *et al.* (2006) suggest that *L. huidobrensis* attained larger body size on crops were it was more abundant, thus supporting a positive host preference- performance linkage pattern.

For the biological control of *Liriomyza* species with parasitoids such as *Diglyphus isaea* (Walker) and *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae), the host larval size is important in determining subsequent activities of the parasitoids such as rejection, host-feeding and parasitism (Ode & Heinz, 2002). A better understanding of the relationship between host plant and leafminer size could provide useful insights in understanding the performance of parasitoids of *Liriomyza* species as leafminer size influences parasitoid size (Salvo & Valladares, 2002).

In the current study, we seek to test the positive host preference- performance linkage hypothesis pattern based on observations of the spatial variation of *Liriomyza* species in Kenyan horticultural agro ecosystems (Chabi- Olaye 2008). Further, we seek to discuss the implications of the differential host plant related *Liriomyza* size effect on parasitoid performance.

To determine the effect of host plants on adult size, which serves as an indirect indicator of performance (fecundity) (Honék, 1993), we compared wing morphometric variables and hind tibia length of different populations of *L. huidobrensis*, *L. sativae*, and *L. trifolii* on four economically important host plant species (*P. vulgaris, P. sativum, S. lycopersicum* and *V. faba*). In addition, we determined the most suitable parameters to use as proxy measures for adult *Liriomyza* body size to facilitate future studies in this field.

Materials and Methods

Plants

Four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III), *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open-pollinated variety) were used in the experiments. Plants were grown in a leafminer-free screen house at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya. Ten grams of fertilizer (di-ammonium phosphate 18: 46: 0 (N: P: K)) was mixed with red clay potting soil sufficient to fill 50 pots (11 cm diameter and 9 cm depth). One gramm of a top-dressing of calcium ammonium nitrate (27 % nitrogen) was applied per pot one week after germination of *P. vulgaris*, *P. sativum* and *V. faba* plants. These plant species were directly seeded in the pots, while *S. lycopersicum* was first established in a nursery tray measuring 60 cm × 60 cm before being transplanted two weeks after germination into the same sized pots used for the other plant species. Top-dressing to *S. lycopersicum* plants was applied one week after transplanting as in other plant treatments. All plants were grown in a temperature controlled greenhouse at 27 ± 2 °C and approximately 30 % relative humidity (R.H.). Two-week-old *P. vulgaris*, *P. sativum* and *V. faba* plants and four-week-old *S. lycopersicum* plants were used in experiments. Plants used in the experiments were standardized with regard to size and leaf area as far as possible.

Insect rearing

A culture of *L. huidobrensis* was initiated from adult leafminers occurring naturally on wild crucifers in the proximity of the *icipe* campus (01°13.3′S 36°53.8′E, 1600 m). Colonies of *L. sativae* and *L. trifolii* were established from insects collected from Kibwezi, (02°15′S 37°49′E, 965 m), Makindu (02°16′S 37°48′E, 991 m) and Masongaleni (02°22′S 38°08′E, 714 m) in the eastern low-lying districts of Kenya. *Liriomyza huidobrensis* was reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris* for approximately 8-10 generations prior to experiments. All leafminer species were maintained at 27 ± 2 °C with a photoperiod of 12L: 12D and relative humidity of approximately 30 %. Species were identified using PCR-restriction fragment length polymorphism (PCR-RFLP) adapted from Scheffer and Lewis (2001, 2005) and Kox et al. (2005) with assistance from B. Wagener (*icipe*).

To avoid bias involving the original plant species on which leafminer species were reared, the leafminer population used for experimentation was obtained by rearing leafminers on each of the four host plant species for three generations. *Liriomyza sativae* and *L. trifolii* did not produce enough progeny on *P. sativum*. Hence, the effect of this host plant species on *L. sativae* and *L. trifolii* was not evaluated.

Assessment of plant leaf areas and larval densities

Subsets of plants of each species were sampled to assess host plant effects and larval densities and on leafminer wing morphometric and hind tibia variables. To determine leaf area, all leaves/leaflets from 10 plants of each species were scanned using a Canon scanner LiDE 50. Images were analyzed using Adobe Photoshop 8.0

Larval densities per plant leaf area were evaluated to determine the range of plant leaf areas where larval densities did not vary within and between *Liriomyza* and plant species. Ten potted plants of each of the plant species were exposed individually (no choice trial) to 20 three-day-old adults (10 males and 10 females) of each of the three leafminer species in a wooden cage (50 cm × 50 cm × 45 cm) fitted with a wire screen mesh on top for ventilation at 27 ± 2 °C and approximately 30 % R.H. To avoid negative effects of intraspecific competition among *Liriomyza* larvae (Parella *et al.*, 1983), the exposure time was 24 hours based on the fly density of 10 males and 10 females (adapted from Mayhew, 1998).

Thereafter the leafminer adult flies were removed and plants transferred to a holding room for the development of mines at 27 ± 2 °C and approximately 30 % R.H. When larvae had developed to the first instar, each leaflet on the plant was detached, its leaf area determined by scanning and analysis in Adobe Photoshop 8.0. Subsequently, the number of larvae per leaflet was determined by dissecting the mines and direct counting of larvae under a Leica EZ4D dissecting microscope. For each host plant, the relationship between plant leaf area (cm^2) and *Liriomyza* larval density was determined and the range of crop leaf areas from which pest density did not significantly vary between and within plant species were used for the morphometric analyses.

Univariate and morphometric analyses

In order to determine the effect of host plant species on adult size of the three *Liriomyza* leafminer species, morpometric analyses were performed on *L. huidobrensis*, *L. sativae* and *L. trifolii* each reared on four different plant species, *P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*.

Plants with leaf areas ranging between 50 to 70 cm^2 (determined from the preliminary experiments) were used for experiments to determine leafminer size.

Leafminer adults for measurements were obtained by exposing 10 potted plants of each of the plant species individually to 20 three-day-old adults (10 males and 10 females) of each of the three leafminer species in a wooden cage (50 cm × 50 cm × 45 cm) fitted with a wire screen mesh on top for ventilation at 27 ± 2 °C and approximately 30 % R.H.

Each of the 10 potted plants was exposed for a period of 24 hours. This exposure method, as determined in the experiment on the relationship between plant leaf areas and larval densities, allowed for standardization of larval densities at low levels to avoid competition and the development of almost the same aged cohort of larvae. After the 24-hour period adult leafminers were removed and plants transferred to a holding room for the development of mines as described above.

Ten days after infestation, all leaves from an individual exposed plant were incubated in a ventilated perspex cage (20 cm × 20 cm × 20 cm) until adult emergence. Four days after first adult emergence a total of 30 adult males and 30 adult females of each plant species (3 males and 3 females from each exposed plant; 10 plants per species and leafminer combination) were randomly sampled from each population for wing morphometric analysis and measurement of the length of the hind tibia. Measurements for males and females were combined in the analyses (n = 57 – 61 per leafminer and plant species combination).

The right wing of each individual insect was detached at the point of contact of the wing and the thorax while the right hind leg was detached for measurement of the hind tibia (Fig. 1). The dissections were done under a Leica EZ4D microscope. Each of the dissected wings was mounted dorso-ventrally on a microscope slide and covered with a cover slip. The hind tibia was mounted in such a way that the outside length of the hind tibia could be measured. Images of the wing and the hind tibia were taken with a Leica EC3 camera (Leica Microsystems Switzerland Ltd 2007) mounted on the microscope at × 35 magnification. Measurements were analyzed using LAS EZ V1.5.0 software. Three measurements were taken for each distance and the mean length was used in the analysis to account for measurement errors.

Six quantitative characters, namely wing length (M1, landmarks 1-2), diagonal length of discal cell (M2, landmarks 3-4), length of the penultimate vein (M3, landmarks 5-6), length of M_{3+4} vein (M4, landmarks 6-7), wing width (M5, landmarks

7-8) and length of the hind tibia were measured for each insect (Fig. 1). Five landmarks on the wing were selected based on their capacity to define major elements of shape and their reliability of measurement (Shiao, 2004). The distances between the landmarks were computed to characterize the wing as estimates of size differentiation in the leafminers.

Morphometric analyses were performed using the Statistical Analysis System (SAS software version 9.1.3, 2002-2003 SAS Institute Inc). To determine whether the overall shape information of the insects varied significantly among leafminer and plant species, differences in the measurements of different body parts were first analyzed by Generalized linear models (GLM) (PROC GLM) and then by Canonical discriminant analysis (CANDISC). In the CANDISC analysis, the five wing measurements were used as continuous, numeric predictors or discriminating variables and host plant species as the grouping variables. The procedure was stratified over the three leafminer species. The objective of this procedure was to establish the relationship between the five predictors and the grouping variable. Specifically, we determined how many dimensions (canonical variates) were needed to express this relationship and assessed how well the predictors separated the groups in the classification.

Linear discriminant analysis with cross validation was performed to examine the degree of accuracy (goodness) of the resulting classification. Cross validation omits the first observation from the data set, develops a classification function using the remaining observations then classifies the omitted observation. Thereafter, it returns the first observation to the data set, omits the second observation and repeats the same procedure. This process continues with all observations in the data set (Fernandez, 2001). Cross validation is generally used to compensate for optimistic apparent error, that is, the number of misclassified observations in the data set divided by the total number of observations in the data set (Fernandez, 2001).

Differences in average hind tibia length among populations of leafminers were analyzed by PROC GLM (SAS software version 9.1.3, 2002-2003 SAS Institute Inc). *F* statistics were used for tests of significance and means were separated using the Tukey test. The significance level was set at $P = 0.05$. Within each plant and leafminer species, two-dimensional relationships were explored by plotting variables pairwise in scatter plots to determine the correlations that existed between any two of them.

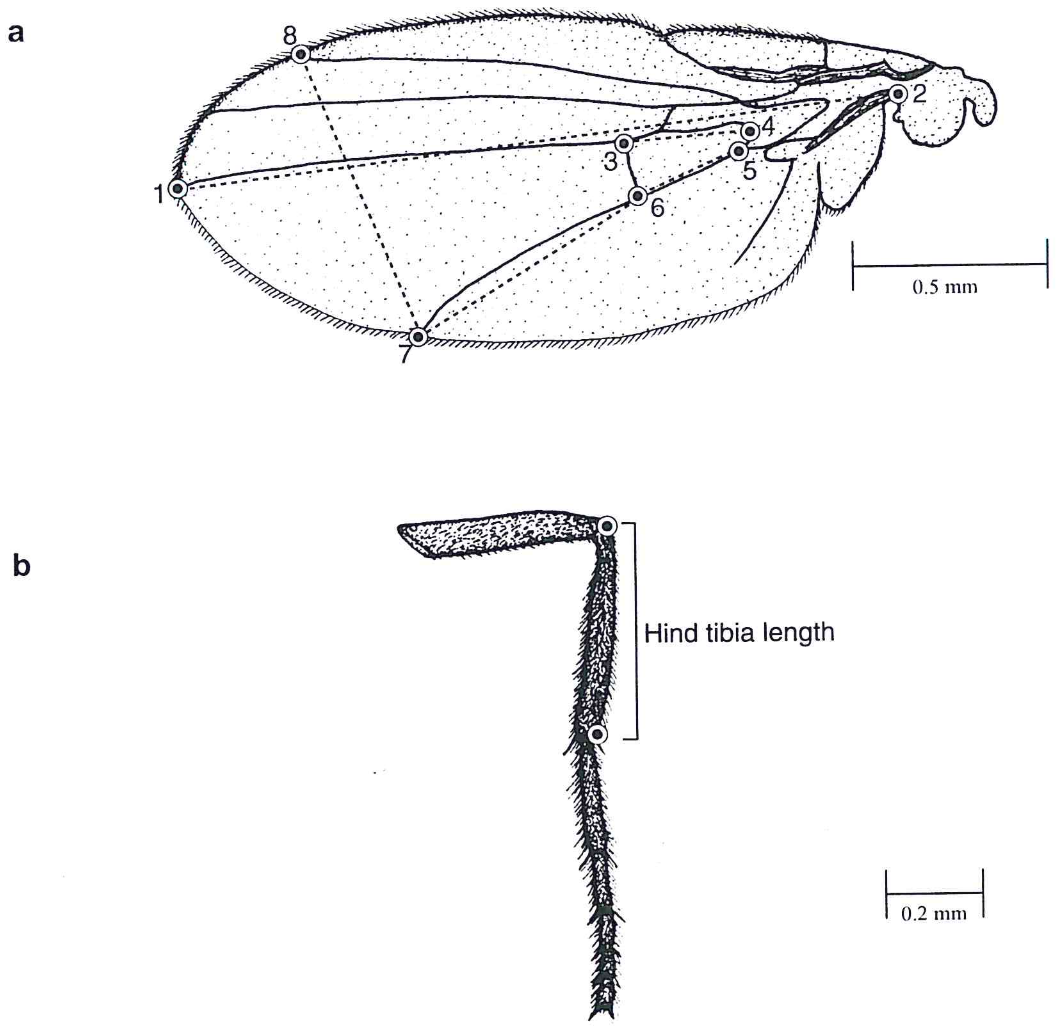

Fig. 2.1. Dissected right wing (verso) of *L. huidobrensis* showing landmarks (1-8) used in morphometric analyses (a) and right hind leg of *L. huidobrensis* (b). Variables consisted of straight-line distances between two chronologically labelled points.

Results

Host plant effect on *Liriomyza* larval density

Across all host plant species, *Liriomyza* larval density was positively and significantly ($r = 0.797$, $P = 0.001$) related to leaf area (Fig. 2.2). However, larval densities did not vary significantly ($F_{3, 29} = 0.910$, $P = 0.448$) among plant species when the plant leaf area ranged between 50-70 cm^2. The overall average larval density across plant and *Liriomyza* species was 0.21 per cm^2 of leaf area (Table 2.1).

Table 2.1. Mean larvae density (± SEM) of *L. huidobrensis*, *L. sativae* and *L. trifolii* on *P. vulgaris*, *P. sativum*, *S. lycopersicum*, and *V. faba* with leaf area ranging between 50-70 cm^2.

Host plant	*Liriomyza* species			*F*-value	*P*-value
	L. huidobrensis	*L. sativae*	*L. trifolii*		
Phaseolus vulgaris	0.247±0.026	0.253±0.080	0.200±0.017	0.340	0.723
Pisum sativum	0.214±0.032	-	-	-	-
Solanum lycopersicum	0.190±0.026	0.190±0.032	0.190±0.020	0.001	0.999
Vicia faba	0.207±0.017	0.203±0.018	0.207±0.027	0.010	0.989
F-value	0.58	0.43	0.15		
P-value	0.6406	0.6694	0.867		

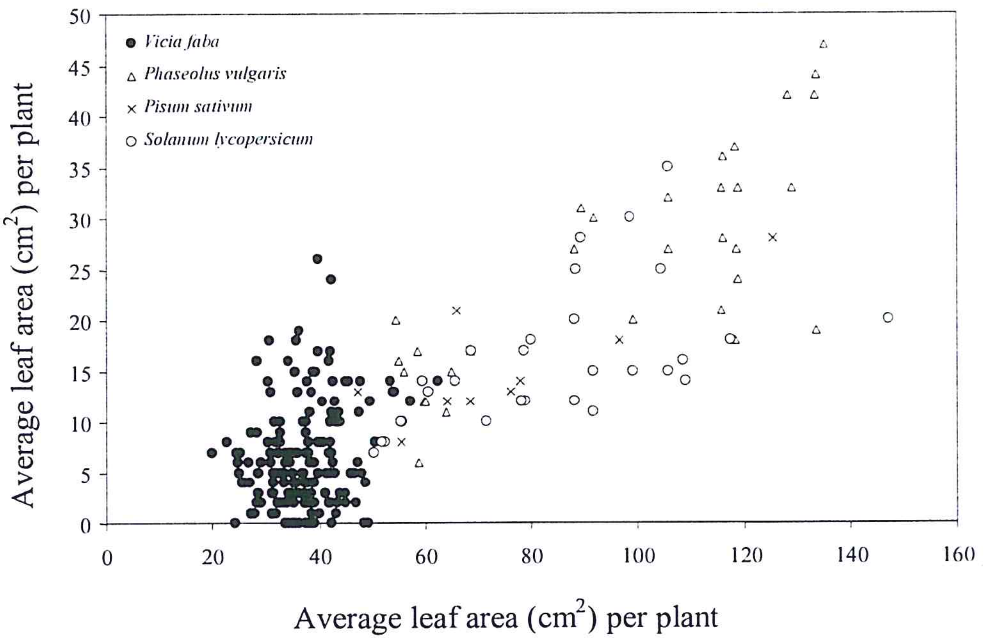

Fig. 2.2 Relationship between plant leaf area (cm²) and *Liriomyza* larval density. Plants with leaf area ranging between 50-70 cm² were used to compare the morphometric characteristics of *Liriomyza* species.

Wing and hind tibia measurements

There were significant species effects on all the measured variables of the three leafminer species reared on the four host plant species (Table 2). *Liriomyza huidobrensis* had the highest mean lengths for wing length (M1), diagonal length of discal cell (M2), length of the penultimate vein (M3), length of M_{3+4} vein (M4), wing width (M5) and length of the hind tibia across all host plant species compared to *L. sativae* and *L. trifolii*. A comparison between *L. sativae* and *L. trifolii* showed significant differences for all variables measured on populations reared on *V. faba* and *P. vulgaris*. For example, the mean wing length for *L. sativae* reared on *V. faba* (1.19 mm) was significantly lower than that of *L. trifolii* (1.39 mm) reared on the same plant species, while *L. sativae* reared on *P. vulgaris* had significantly greater wing length (1.25 mm) than *L. trifolii* (1.17 mm) reared on the same host plant species. There were no significant difference in mean wing length, diagonal length of the discal cell, length of the penultimate vein, length of vein M_{3+4}, wing width and length of the hind tibia between *L. sativae* and *L. trifolii* reared on *S. lycopersicum*.

There were no significant differences in the measured variables for *L. huidobrensis* reared on different host plant species (Table 2). For *L. sativae*, there were significant host plant effects for all variables measured. Wing length (M1), diagonal length of discal cell (M2), length of the penultimate vein (M3), length of M_{3+4} vein (M4), wing width (M5) and length of the hind tibia were smaller on *V. faba* than *P. vulgaris*. There were no significant differences between the variables measured between *S. lycopersicum* and *P. vulgaris* or *V. faba* except for diagonal length of the discal cell when reared on *V. faba*. There were significant host plant effects on measured variables for *L. trifolii*. Measurements of the various variables were larger for *V. faba* than *P. vulgaris* and *S. lycopersicum*; hind tibia lengths were different for all plant species (*V. faba* > *P. vulgaris* > *S. lycopersicum*). Wing length was significantly positively related to hind tibia length (r = 0.773, P = 0.0001) for all the leafminer species reared on the four host plant species.

Table 2.2 Mean (± SEM) linear measurements (mm) of morphometric variables of three *Liriomyza* species reared on different host plant species (n = 57 - 61).

Liriomyza species per morphometric Variable	Host plant species				*F*-value	*P*-value
	Phaseolus vulgaris	*Pisum sativum*	*Solanum lycopersicum*	*Vicia faba*		
Wing length						
L. huidobrensis	1.799±0.021aA	1.786±0.023A	1.759±0.024aA	1.786±0.027aA	0.48	0.693
L. sativae	1.253±0.017bA	-	1.233±0.016bAB	1.188±0.016cB	3.87	0.023
L. trifolii	1.171±0.015cB	-	1.189±0.017bB	1.386±0.012bA	64.26	< .0001
F-value	361.93	-	268.38	239.81		
P-value	< .0001	-	< .0001	< .0001		
Diagonal length of discal cell						
L. huidobrensis	0.333±0.004aA	0.344±0.005A	0.329±0.005aA	0.329±0.005aA	2.60	0.052
L. sativae	0.185±0.003bA	-	0.182±0.004bA	0.167±0.003cB	7.51	0.001
L. trifolii	0.163±0.003cB	-	0.171±0.003bB	0.233±0.003bA	146.03	< .0001
F-value	746.33	-	482.17	437.93		
P-value	< .0001	-	< .0001	< .0001		
Length of penultimate vein						
L. huidobrensis	0.334±0.004aA	0.345±0.004A	0.330±0.006aA	0.335±0.006aA	1.46	0.225

Liriomyza species per morphometric Variable	Host plant species				*F*-value	*P*-value
	Phaseolus vulgaris	*Pisum sativum*	*Solanum lycopersicum*	*Vicia faba*		
L. sativae	0.192±0.003bA	-	0.185±0.003bAB	0.177±0.003cB	5.67	0.004
L. trifolii	0.162±0.003cB	-	0.166±0.003cB	0.229±0.003bA	162.12	< .0001
F-value	752.36	-	445.46	398.76		
P-value	< .0001	-	< .0001	< .0001		
Length of M_{3+4} vein						
L. huidobrensis	0.631±0.008aA	0.606±0.008A	0.604±0.008aA	0.622±0.010aA	2.43	0.066
L. sativae	0.476±0.007bA	-	0.467±0.006bAB	0.451±0.006cB	4.22	0.016
L. trifolii	0.460±0.005bB	-	0.467±0.005bB	0.537±0.004bA	78.86	<.0001
F-value	215.19	-	145.10	152.66		
P-value	< .0001	-	< .0001	< .0001		
Wing width						
L. huidobrensis	0.756±0.008aA	0.770±0.010A	0.750±0.009aA	0.765±0.012aA	0.86	0.465
L. sativae	0.566±0.008bA	-	0.551±0.007bAB	0.535±0.007cB	4.18	0.017
L. trifolii	0.539±0.007cB	-	0.556±0.008bB	0.597±0.006bA	18.65	< .0001
F-value	230.10	-	198.70	181.04		
P-value	< .0001	-	< .0001	< .0001		

Liriomyza species per morphometric Variable	Host plant species				*F*-value	*P*-value
	Phaseolus vulgaris	*Pisum sativum*	*Solanum lycopersicum*	*Vicia faba*		
Hind tibia length						
L. huidobrensis	0.531±0.005aA	0.536±0.006A	0.525±0.005aA	0.531±0.007aA	0.57	0.633
L. sativae	0.414±0.004bA	-	0.406±0.004bAB	0.400±0.004cB	2.90	0.058
L. trifolii	0.384±0.004cC	-	0.401±0.005bB	0.430±0.003bA	28.63	<.0001
F-value	282.29	-	203.42	168.82		
P-value	< .0001	-	< .0001	< .0001		

Within columns for a given morphometric variable (comparison between *Liriomyza* species), means followed by the same lower case letter are not significantly different; within rows for a given morphometric variable and insect species, means followed by the same capital letter are not significantly different (comparison between host plant species) at $P < 0.05$ (Tukey's Studentized Range Test (HSD)).

Morphometric analyses of wings of *Liriomyza* species

Intra- and inter-specific comparisons of leafminer species across host plant species

The projection of the points on the first two canonical axes for *L. huidobrensis* did not distinctly separate the populations reared on *P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*. The amount of variation explained by the first two canonical variates was low, with 17.3 % for canonical variate 1 (CV1) and 4.8 % for canonical variate 2 (CV2) (Fig. 2.2). Tests of dimensionality for the discriminant analysis indicate that both dimensions are statistically significant (Table 2.3). Standardized pooled within-class canonical coefficients show that CV1 for *L. huidobrensis* is negatively correlated to M_{3+4} vein length (M4) and wing length (M1) and positively correlated to wing width (M5) and diagonal length of discal cell (M2) (Table 2.4). The second discriminant dimension (CV2) is dominated by a positive correlation to M5 and length of the penultimate vein (M3), while being strongly negatively correlated to M1 and M2. However, scores were not strong enough to discriminate *L. huidobrensis* populations reared on the four host plant species.

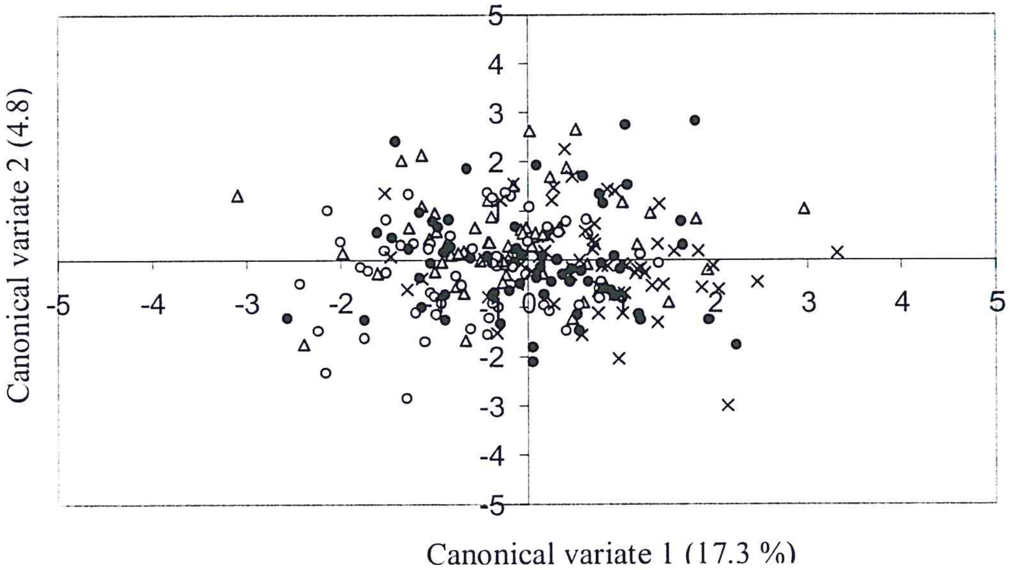

Fig. 2.3. Projection of wing morphometric variables of *L. huidobrensis* reared on *V. faba* (▲), *P. vulgaris* (○), *S. lycopersicum* (●) and *P. sativum* (×). The first two canonical variates cannot separate the populations of *L. huidobrensis* reared on different host plant species.

Table 2.3. Tests of discriminant canonical variates (dimensions) for *L. huidobrensis*, *L. sativae* and *L. trifolii* on host plant species.

Liriomyza Species	Canonical variate	Canonical Correlation	*F*- value	d.f.1	d.f.2	*P*- value
L. huidobrensis	CV1	17.30	4.44	15	644	<0.0001
	CV2	4.80	2.47	8	468	0.0125
L. sativae	CV1	11.60	3.31	10	340	<0.0001
	CV2	6.00	2.73	4	171	0.03
L. trifolii	CV1	79	44.18	10	346	<0.0001
	CV2	8.1	3.85	4	174	0.005

Table 2.4. Pooled within-class standardized canonical coefficients for *L. huidobrensis*, *L. sativae* and *L. trifolii* for the response variables M1-M5 and host plant species.

Morphometric Variable[1]	*Liriomyza* species					
	L. huidobrensis		*L. sativae*		*L. trifolii*	
	CV1	CV2	CV1	CV2	CV1	CV2
M1	-0.90	-1.44	-0.48	-2.33	-1.13	-1.65
M2	1.04	-2.02	2.50	-1.17	0.12	2.68
M3	-0.26	1.26	-0.79	1.92	1.46	-1.98
M4	-1.57	0.02	0.78	0.65	1.37	0.06
M5	1.69	2.13	-1.35	1.58	-1.15	1.36

[1] Morphometric variables M1-5 are wing length, diagonal length of discal cell, length of penultimate vein, length of vein M_{3+4} and wing width, respectively.

In the case of *L. sativae*, the projection of points onto the first two canonical axes did also not distinctly separate populations reared on *P. vulgaris*, *S. lycopersicum* and *V. faba*. The first two canonical variates contributed 11.6 % for CV1 and 6.0 % for CV2 to the total variation (Fig. 2.3). Tests of dimensionality for discriminant analysis indicate that both dimensions (CV1 and CV2) are statistically significant (Table 2.3). Pooled within-class standardized canonical coefficients for canonical variates show that CV1 is strongly dominated by positive correlation with variable M2 while strongly negatively correlated to M5. CV2 is dominated by positive correlation with variables M3 and M5 while strongly negatively correlated to M1 and M2 (Table 2.4). However, the scores were not strong enough to discriminate *L. sativae* populations reared on the four host plant species.

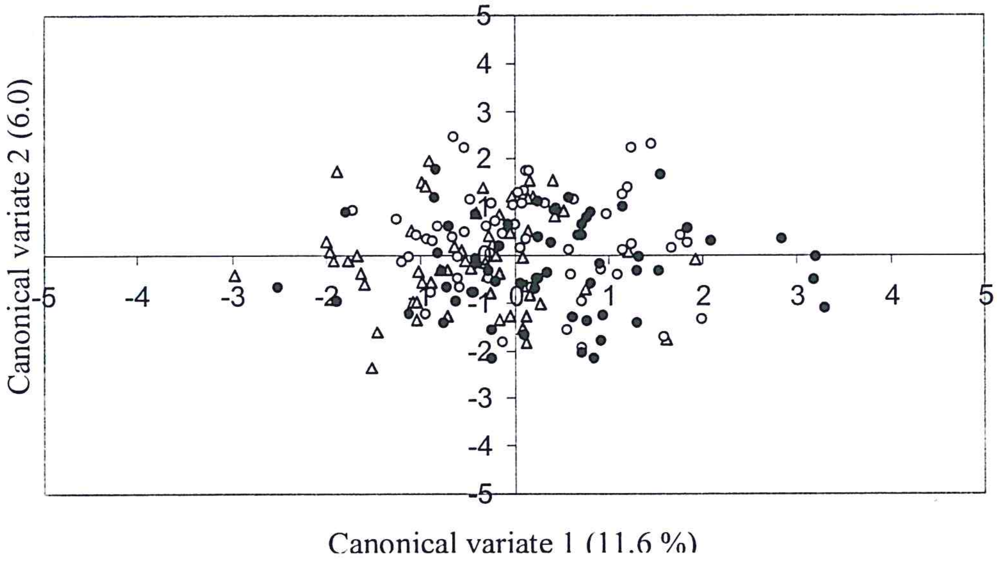

Fig. 2.4. Projection of wing morphometric variables of *L. sativae* reared on *V. faba* (▲), *P. vulgaris* (○), *S. lycopersicum* (●) and *P. sativum* (×). The first two canonical variates cannot separate the populations of *L. sativae* reared on different host plant species.

In contrast to the previous two species, the projection of the points onto the first two canonical axes for *L. trifolii* distinctly separated the population reared on *V. faba* from those reared on *P. vulgaris* and *S. lycopersicum*. However, it did not separate the populations reared on *P. vulgaris* and *S. lycopersicum*. The first canonical variate had a high contribution (79.0 %) and the second a low contribution (8.1 %) to total variation (Fig. 2.5). Tests of dimensionality for discriminant analysis indicate that both dimensions (CV1 and CV2) are statistically significant (Table 2.3). Pooled within-class standardized canonical coefficients for both canonical variates indicate that CV1 is strongly positively correlated to variables M3 and M4 while negatively correlated to variables M1 and M5. CV2 is strongly negatively correlated to variables M1 and M3 while strongly positively correlated to variables M2 and M5 (Table 2.4).

The first canonical variate, CV1, also shows that the linear combination of wing variables measured (CV1 = -1.13 × M1 + 0.12 × M2 + 1.46 × M3 + 1.37 × M4 -1.15 × M5) separates *L. trifolii* populations most effectively, that is, the population reared on *V. faba* separated from the populations reared on *P. vulgaris* and *S. lycopersicum* (Table 2.4).

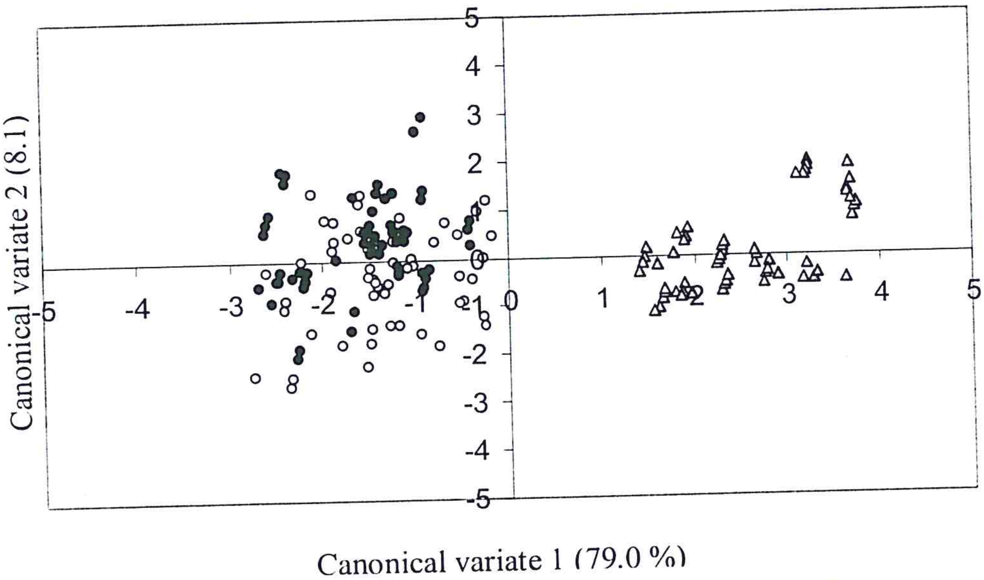

Fig. 2.5. Projection of wing morphometric variables of *L. trifolii* reared on *V. faba* (▲), *P. vulgaris* (○), *S. lycopersicum* (●) and *P. sativum* (×). The first two canonical variates showed separation of *L. trifolii* populations reared on different plant species into two clusters *V. faba* (▲), and *P. vulgaris* (○) and *S. lycopersicum* (●).

Classification of *Liriomyza* plant populations from discriminant analysis with cross validation analysis

A summary of classification of *Liriomyza* populations reared on different plant species from discriminant analysis with cross validation for *L. huidobrensis* shows that the variables wing width (M4) and hind tibia length (M5) contributed most to the discrimination of the species populations reared on different host plant species (r^2 = 0.12 and 0.07, respectively; Table 2.5). The percentage of plant populations of *L. huidobrensis* correctly identified with the cross validation model was estimated at 42.2 %. For *L. sativae* plant populations only two variables, the diagonal length of discal cell (M2) and length of penultimate vein (M3), were discriminatory (r^2 = 0.08 and 0.06, respectively; Table 2.5). The overall correct observations with cross validation for *L. sativae* plant populations were 33.9 %. All the variables (M1 to M6) were discriminatory for plant populations of *L. trifolii* (Table 2.5). However, M3 contributed most to species discrimination (r^2 = 0.85), followed by M5 (r^2 = 0.24), and the remainder of variables with $r^2 < 0.17$ (Table 2.5). The percentage of plant populations of *L. trifolii* correctly identified with the cross validation model was estimated at 77.6 %.

The discriminant analysis also correctly associated *Liriomyza* species with the two different groups recognized above (group 1, *L. huidobrensis* and group 2, *L. sativae* and *L. trifolii*), within and across all host plant species tested (Fig. 2.6). All variables contributed to the discrimination of species, but M2 and M3 were frequently ranked first within and across host plant species (Table 2.6).

Table 2.5. Summary of classification of *Liriomyza* plant populations from discriminant analysis with cross validation.

Morphometric Variable (M)[1]	Partial R-square	F-value	*P*-value	Cross validation[2]	
				N	% Correct
L. huidobrensis				83	42.2
M1	0.02	1.83	0.1432		
M2	0.03	2.6	0.0528		
M4	0.12	10.36	<0.0001		
M5	0.07	6.08	0.0005		
L. sativae				59	33.9
M2	0.08	7.51	0.0007		
M3	0.06	5.1	0.007		
L. trifolii				58	77.6
M1	0.09	8.89	<0.0002		
M2	0.05	4.4	0.0137		
M3	0.85	162.12	<0.0001		
M4	0.16	16.53	<0.0001		
M5	0.24	28.51	<0.0001		
M6	0.11	10.38	<0.0001		

[1] Morphometric variables M1-6 are wing length, diagonal length of discal cell, length of penultimate vein, length of vein M_{3+4} vein, wing width and length of hind tibia, respectively; [2] Overall correct observations with cross validation = 50 %.

Table 2.6. Summary of classification of *Liriomyza* species reared on the same plant species from discriminant analysis with cross validation.

Morphometric variable (M)[1]	Partial R-square	F-value	*P*-value	Cross validation N	Cross validation % Correct
Phaseolus vulgaris				31	96.8
M2	0.20	21.46	<0.0001		
M3	0.90	752.36	<0.0001		
M5	0.16	16.61	<0.0001		
M6	0.03	2.98	0.0536		
Solanum lycopersicum				28	85.7
M1	0.05	4.19	0.0167		
M2	0.84	482.17	<0.0001		
M3	0.16	16.3	<0.0001		
M4	0.04	3.47	0.0334		
M5	0.21	23.52	<0.0001		
Vicia faba				37	89.2
M1	0.22	24.74	<0.0001		
M2	0.83	437.93	<0.0001		
M3	0.25	30.08	<0.0001		
M4	0.12	11.74	<0.0001		
M5	0.05	4.08	0.0185		
M6	0.09	8.23	0.0004		
Across plants[2]				200	85
M1	0.05	16.04	<0.0001		
M2	0.82	1322.69	<0.0001		
M3	0.11	35.16	<0.0001		
M4	0.10	33.12	<0.0001		
M6	0.02	5.52	0.0042		

[1] Morphometric variables M1-6 are wing length, diagonal length of discal cell, length of penultimate vein, length of M_{3+4} vein, wing width and length of hind tibia, respectively. [2] Overall correct observations with cross validation = 95.4 % for *L. huidobrensis*, 74.2 % for *L. sativae* and 80.4 % for *L. trifolii*.

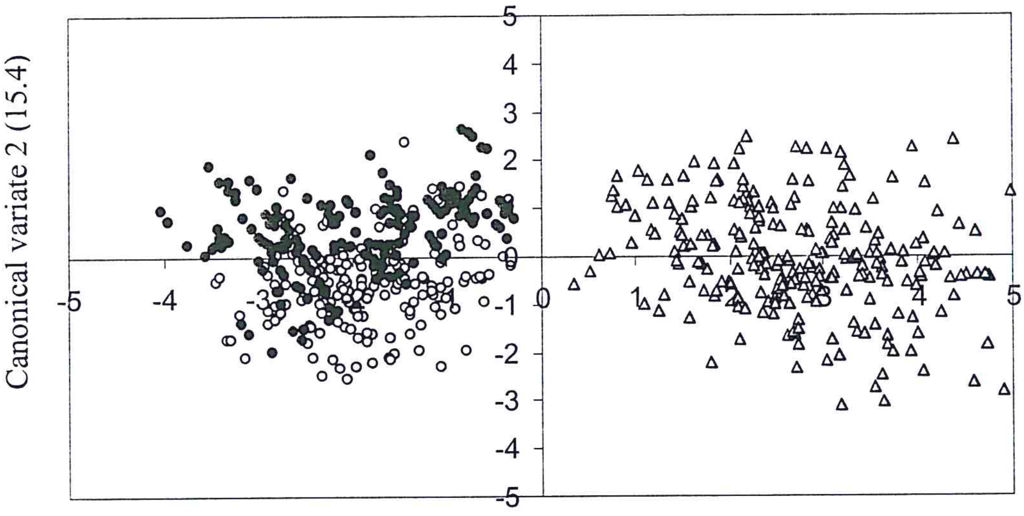

Fig. 2.6. Projection of wing morphometric variables of *L. huidobrensis* (▲), *L. sativae* (○) and *L. trifolii* (●) reared on *P. vulgaris, P. sativum S. lycopersicum* and *V. faba*. The first two canonical variates showed separation of *Liriomyza* species into two clusters.

Discussion

Liriomyza huidobrensis had the highest mean lengths for the wing and hind tibia variables for populations reared on the four host plant species compared to *L. sativae* and *L. trifolii*. This is consistent with the observation by Spencer (1973) that *L. huidobrensis* is generally larger compared to the other two leafminer species. The classification of *Liriomyza* plant populations from discriminant analysis with cross validation analysis in this study also confirmed this result. Two distinct leafminer morphospecies groups were recognized across all plant species. The first group represented *L. huidobrensis* and the second *L. sativae* and *L. trifolii*. These morphospecies groups concur with *Liriomyza* species groups based on male genitalia (OEPP/EPPO, 2005) and grouping based on mitochondrial cytochrome oxidase I (COI) sequence information (Scheffer *et al.*, 2006).

Adult body size of insect herbivores including agromyzid species is, among other factors, determined by the nutritional quality of host plants during immature development (Parrella *et al.*, 1983, Parrella, 1987, Awmack & Leather, 2002). Generally, there is a positive relationship between body size and performance in insects (Clutton-Brock, 1988, Honék, 1993). Videla *et al.* (2006) observed a positive link between performance and adult body size of *L. huidobrensis*, as leafminers were more abundant in the field on *V. faba* on which they attained a larger body size and which they preferred for oviposition compared to *P. vulgaris*. In a study on host-plant-related larval performance, Martin *et al.* (2005) reported heavier pupae of *L. huidobrensis* on celery (*Apium graveolens* L., Apiaceae) than on lettuce (*Lactuca sativa* L., Asteraceae) and cucumber (*Cucumis sativus* L., Cucurbitaceae), but no differences in pupal weight on pea (*P. sativum*) and potato (*Solanum tuberosum* L.). In the present study, there were no differences in body size for *L. huidobrensis* reared on different host plant species. The differences in host plant – body size relationships could be related to differences in *L. huidobrensis* populations and plant species or cultivars used. In the absence of other host plant species, and at the larval densities used in this study, our results suggest that *P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba* are equally suitable for *L. huidobrensis* based on the size of the resulting offspring.

Unlike *L. huidobrensis*, *L. sativae* and *L. trifolii* differed in adult size when reared on different host plants in the present study. Adult offspring of *L. sativae* tended to be larger on *P. vulgaris* compared to *V. faba,* although no distinct grouping according to host plants was detected based on multivariate analysis. In contrast, adults of *L. trifolii* were larger when reared on *V. faba* compared to *S. lycopersicum* and *P. vulgaris,* and fell into two distinct groups. The results indicate differential suitability of host plant species for the two leafminer species; *P. vulgaris* appeared more suitable for *L. sativae* while *V. faba* seemed more suitable for *L. trifolii* based on adult size of offspring.

The performance, e.g. reproductive potential, sex ratio and development time, of parasitoids of agromyzid leafminers frequently depends on the body size of their hosts (Heinz & Parrella, 1989, Spencer, 1990, Salvo & Valladares, 1995, Ode & Heinz, 2002). Hence leafminer size can have a strong influence on population traits at the third trophic level. However, host size is not necessarily an indicator of parasitoid performance. For example, studies by Salvo and Valladares (2002) and Tran *et al.*

(2007) have shown that host plants apart from host size can influence parasitoid performance. Thus, predicting parasitoid performance based on host size alone is limited and further studies are needed to determine how host size differences in the current study influence parasitoid performance.

The size differences observed in the current study are unlikely to be attributable to plant dry mass, leaf area or larval densities, because, leaf areas were standardized to avoid differences in larval densities for all *Liriomyza* species for the four plants species used. In addition, the larval density was kept low to avoid competition.

The length of the hind tibia was highly correlated with wing length in all leafminer species examined across all host plant species. Future studies involving measurement of adult body sizes of *L. huidobrensis*, *L. sativae* and *L. trifolii* can thus use either wing length and hind tibia measurements or their ratios as reliable indicators of the actual leafminer body size. This will considerably save on experimental time associated with measurements of many parameters of the wing.

However, in traditional morphometric analyses, linear distances (landmarks) are measured between pairs of apparently homologous morphological points (Marcus, 1990, 1993). These inter-landmark distances are then analyzed using multivariate statistics. One limitation of this traditional approach, however, is its assumption that these distances, too, are homologous across taxa (Bookstein, 1982, 1990). A second limitation is the partial redundancy of information about shape provided by any two distances, yielding weaker statistical tests of morphological differences (Strauss & Bookstein, 1982). In future studies, describing two- and three-dimensional forms by capturing and analysing the Cartesian coordinates of homologous landmarks (Bookstein, 1989, 1991; Rohlf & Marcus, 1993) is suggested.

In conclusion, host plant effects on body size of the three leafminer species were variable and depended on host plant and *Liriomyza* species. Rearing *L. huidobrensis* on the four different host plant species did not result in differences in size of adult progenies. However, differences in host plant suitability were observed for *L. sativae* and to a larger extend in *L. trifolii*. Therefore, the spartial variation observed in the field for *L. huidobrensis* on these four host plant species could be due to other factors other than performance preference. However, this observation together with the preference of *L. sativae* on *P. vulgaris* and *L. trifolii* on *V. faba* require further investigation through field studies that consider assessment of body sizes of field collections of adults reared on these four host plant species. Further, this study acts as an important background for further tritrophic interaction studies between, host plants, *Liriomyza* species and natural enemies at the third trophic level.

In addition, across all plant species, wing length strongly correlated with hind tibia length and both parameters can reliably be used to determine adult size of the three *Liriomyza* species for studies on host-plant related body size.

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CHAPTER THREE

Host plant-related oviposition behaviour and host feeding activities of *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) on *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) (Diptera: Agromyzidae)

Abstract

Diglyphus isaea females oviposit on larger hosts, reject or host-feed on smaller hosts and base sex allocation of progeny on the larval quality of the leafminer host. Host feeding and sex ratio of *D. isaea* on *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* larvae reared on *Phaseolus vulgaris*, *Pisum sativum, Solanum lycopersicum* and *Vicia faba* were determined. In a no-choice test, *L. huidobrensis* had the highest rate of parasitism when reared on *P. vulgaris* (46 %), *L. sativae* when reared on *V. faba* (59 %) and *P. vulgaris* (59 %), and *L. trifolii* when reared on *S. lycopersicum* (68 %). Host feeding was highest on *L. trifolii* reared on *V. faba* (36 %) and lowest on *L. huidobrensis* reared on *P. sativum* (2 %). Results of choice tests showed a significant interaction effect for host plant and leafminer species on host feeding and parasitism. *Liriomyza sativae* reared on *P. vulgaris* had highest rate of parasitism (31 %) while *L. trifolii* reared on *P. sativum* (2 %) had the lowest parasitism. Host feeding was highest on *L. trifolii* reared on *S. lycopersicum* (14 %) and lowest on *L. huidobrensis* reared on *P. sativum* and *S. lycopersicum* (1 %). Results of the study suggest that parasitism by *D. isaea* is host plant and leafminer species dependent and that plant mixtures can have a strong influence on the sex ratio of the parasitoid by causing more female biased *D. isaea* populations compared to sole crops.

Introduction

The leafmining flies *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) (Diptera: Agromyzidae) are economically important pests of a wide range of greenhouse and field-grown plants (Chaput, 2000). These three pests are considered alien invasive species in the Afrotropical region. *Liriomyza trifolii* was first reported in Kenya in 1976, where ten years after its accidental introduction the pest was found to be widespread from the coastal regions to the highlands (Spencer, 1985). The three *Liriomyza* species are highly polyphagous attacking plants in several families (Murphy & LaSalle, 1999). In Kenya they attack snow peas (*Pisum sativum* L., Fabaceae), sugar snaps (*Pisum sativum* L., Fabaceae), French beans (*Phaseolus vulgaris* L., Fabaceae) runner beans (*Phaseolus coccineus* L., Fabaceae), okra (*Abelmoschus esculentus* (L.) Moench, Malvaceae), aubergine (*Solanum melongena* L., Solanaceae), tomatoes (*Solanum lycopersicum* L., Solanaceae) and passion fruits (*Passiflora edulis* Sims, Passifloraceae) and can cause yield losses up to 100 percent (Chabi-Olaye *et al.*, 2008).

Previous studies showed that natural enemies are important in regulating leafminer populations in their native and invaded areas (Johnson, 1993; Shepard *et al.*, 1998; Murphy & LaSalle, 1999; Rauf & Shepard, 1999; Sivapragasam *et al.*, 1999; Thang, 1999; Chen *et al.*, 2003). *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) is a solitary larval ectoparasitoid of agromyzid leafminers including *L. huidobrensis*, *L. sativae* and *L. trifolii* (Ode & Heinz, 2002; Liu *et al.*, 2009). The parasitoid is an effective biological control agent against *Liriomyza* species on a range of crops and ornamental plants (Ode & Heinz, 2002; Liu *et al.*, 2009). The highest success rate has been obtained with augmentative releases (Ozawa *et al.*, 1993; Rodriguez *et al.*, 1997; Ozawa *et al.*, 1999). In Kenya, large-scale mass-production programmes of *D. isaea* have been developed to support biological control of leafminer both within the country and in South Africa (A. L. Owour, Dudutech (K) Pvt (Ltd) (Kenya), pers. comm.).

However, the efficacy of the parasitoids depend on the larval size of *Liriomyza* species (Ode & Heinz, 2002), which in turn can be dependent on the host plant species (Salvo & Valladares, 2002; Chapter 2). Various attributes of host plant species can affect the behaviour of parasitoids such as mate location (McAuslane *et al.*, 1990), oviposition (Powell & Wright, 1992), fecundity (Shukla & Tripathi, 1993), rate of parasitism (Salvo & Valladares, 2002), survival and sex ratio (Hare & Kuck, 1991), and body size (Salvo & Valladares, 2002).

Females of *D. isaea* ingest the contents of host larvae, a process referred to as host feeding (Heinz & Parrella, 1989; Ode & Heinz, 2002). The advantage of host feeding is that nutrients required for egg production and sustenance are readily available precluding the need for searching for foods such as honeydew, nectar and pollen (Kidd & Jarvis, 1989). In the field, these non-host foods are likely to be spatially separated from the host of the parasitoid and highly variable in quality. A foraging parasitoid incurs energy and time losses by searching for non-host foods. *Diglyphus isaea* has been reported to host feed on smaller hosts (Heinz & Parrella, 1989; Ode & Heinz, 2002). Therefore, host-feeding behaviour can indirectly be influenced by the host plant species.

The present study examined attack behaviour, host-killing activity and number of female progeny of *D. isaea* on three *Liriomyza* species reared on *Phaseolus vulgaris* L. (Fabaceae), *Pisum sativum* L. (Fabaceae) *Solanum lycopersicum* L. (Solanaceae) and *Vicia faba* L. (Fabaceae) to determine the implications for biological control using this parasitoid species including mass-rearing of *D. isaea*.

Materials and Methods

Plants

The four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III), *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open-pollinated variety), used in experiments were grown and maintained as described in Chapter 2.

Insect rearing

The leafminer species, *L. huidobrensis*, *L. trifolii* and *L. sativae*, were obtained from the insectary of the International Centre of Insect Physiology and Ecology (*icipe*) (see Chapter 2). The three species were reared at 27 ± 2 °C with a photoperiod of 12L: 12D and relative humidity of approximately 30 %. *Liriomyza huidobrensis* was reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris*. All species were reared on the respective plant species and were of approximately the 18-20 th generations prior to the experiments.

For each treatment, 16 potted plants of each of the four plant species were infested with 50 four-day-old adult male and female leafminers (sex ratio 1:1). Adult leafminers together with the respective potted plant species were kept in ventilated cages (50 cm × 50 cm × 45 cm) and fed on a 10 % sucrose solution while given an oviposition period of 24 hours. Thereafter, plants were removed and transferred to another similar cage free of adult leafminers to monitor the development of larvae until they reached late second to third instar. Infested potted plants with late second- third-instar larvae were used for the experiments.

Diglyphus isaea used in the experiments were supplied by Dudutech (K) Pvt (Ltd) (Kenya) and identity was confirmed C.D. Zhu (Institute of Zoology, Chinese Academy of Sciences).These parasitoids were mass-reared on *P. sativum* under uniform greenhouse conditions. Parasitoids were allowed to mate for a period of 48 hours in ventilated cages (40 cm × 20 cm × 20 cm) and were then given a preoviposition period of 12 hours. Parasitoids were supplied with a 10 % honey solution during the mating and pre-oviposition period.

Effect of host plant on *D. isaea* parasitism and host feeding on larvae of *Liriomyza*

No-choice experiment

Four potted plants of the same plant species infested with the same *Liriomyza* species were placed together in a ventilated Perspex cage (50 cm × 50 cm × 45 cm). Fourty-five pre-mated *D. isaea* females were released per cage for 24 hours. Thereafter plants were removed and placed in a similar-sized cage free of parasitoids to allow for the development of *D. isaea* from parasitized leafminer larvae. The experiment was replicated four times for each host plant species and each *Liriomyza* species.

Choice experiment

Four potted plants comprising one of each of the four plant species, *P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*, each infested with live late second to third instar larvae of the same *Liriomyza* species, were placed in ventilated Perspex cages (50 cm × 50 cm × 45 cm). As in the no-choice experiment, 45 pre-mated *D. isaea* were released per cage for 24 hours. The experiment was replicated 16 times for each *Liriomyza* species.

In the no-choice and choice, experiments were carried out in the laboratory at 27 ± 1°C and approximately 33 % relative humidity. Four days after exposure to *D. isaea*, all mines on leaves were dissected under a Leica EZ4D stereo microscope. The total number of larvae parasitized and host-fed by *D. isaea* as well as unparasitized larvae and larvae dying from causes other than host feeding and parasitism were counted per plant.

Larvae were recorded as host-fed once they became flaccid with black spots on their body as a result of stings of parasitoid females and parasitized when they were found with immatures of *D. isaea* (Minkenberg, 1989).

The mean number of larvae per replicate for the leafminer host plant combinations was: *P. vulgaris-L. huidobrensis*, 43, *P. vulgaris- L. sativae*, 133, *P. vulgaris-L. trifolii*, 33, *P. sativum-L. huidobrensis*, 27, *P. sativum-L. sativae*, 41; *P. sativum-L. trifolii*, 16, *S. lycopersicum-L. huidobrensis*, 24, *S. lycopersicum-L. sativae*, 28, *S. lycopersicum-L. trifolii*, 61, *V. faba-L. huidobrensis*, 106; *V. faba-L. sativae*, 83, *V. faba-L.trifolii*, 33.

Effect of host plant on sex ratio of *Diglyphus isaea*

To determine the sex ratios of *D. isaea* on different host plants and *Liriomyza* species, plants were kept for 7 days after exposure to parasitoids to allow for complete development of both unparasitized leafminer larvae and parasitoids from parasitized larvae. After 7 days, leaves of exposed plants were harvested and incubated in ventilated cages (40 cm × 20 cm × 20 cm) under the same laboratory conditions as in the previous experiments until the emergence of adult *D. isaea* and leafminers.

Three days after the first adult emergence of *D. isaea*, all adults were killed by instant freezing and sexed, using the key developed by Bouček (1988). Adults of *D. isaea* display a sexually dimorphic colour pattern of the hind tibia. The proportions of male and female progenies were determined for all plant and leafminer species in the choice and the no-choice tests. However, due to difficulties in rearing *L. sativae* and *L. trifolii* on *P. sativum*, very few larvae were available for parasitism and hence there were very few or no *D. isaea* progeny recovered from this host plant species. Because of the small sample sizes, *P. sativum* was excluded from some analyses. The mean number of *D. isaea* adults per replicate for the leafminer host plant combinations was: *P. vulgaris-L. huidobrensis*, 30, *P. vulgaris- L. sativae*, 26; *P. vulgaris-L. trifolii*, 25, *P. sativum-L. huidobrensis*, 31, *P. sativum-L. sativae*, 23, *S. lycopersicum-L. huidobrensis*, 39, *S. lycopersicum-L. sativae*, 26; *S. lycopersicum-L. trifolii*, 22; *V. faba-L. huidobrensis*, 25; *V. faba-L. sativae*, 27; *V. faba-L. trifolii*, 29.

Data analyses

Parasitism levels were determined by calculating the number of parasitized leafminer larvae out of the total number of larvae per treatment (total number of larvae - (larvae fed upon + total live unparasitized larvae + larvae dying from causes other than host feeding or parasitism)). Host feeding levels were calculated in the same manner as described above by making the number of larvae fed-upon the subject of the formula above.

In the no-choice test, a logistic regression model was used to evaluate the effect of host plant species, leafminer species and their interaction on proportions of larvae that were either parasitized or host-fed out of the total number of larvae observed per treatment. The analysis was performed using PROC GENMOD (SAS/STAT, SAS version 9.1.3) (SAS Institute Inc., 2002-2003). Mean percentages of parasitized and host-fed larvae were separated with pair-wise χ^2 one-sample test.

In the choice test (where pre-infested plants were mixed), the data were subjected to logistic regression analysis using the same PROC GENMOD. However, because the initial number of larvae per plant in the mixtures was dependent on the host plant cultivar, the initial number of leafminer larvae per plant was expressed as a proportion of total leafminer larvae per replicate, which was then used as a 'weight' variable in the PROC GENMOD. In addition, in this experimental set-up, different host plant cultivars were placed within the same cage. Parasitism and host feeding data for each host plant were therefore not expected to be independent. In view of this, the dispersion parameter in the logistic regression was allowed to be estimated rather than be fixed to a value of 1 to take care of any extra-binomial variations.

The proportions of females out of the total number of parasitoids (males + females) were used as an index of sex ratio. The logistic regression model with logistic link function described above was also used to evaluate the effect of host plants, leafminer species and their interaction on the sex ratios using PROC GENMOD.

Average percentages of parasitized and host-fed larvae as well as sex ratio were separated with pair-wise comparison (χ^2 one-sample test).

Results

Effect of host plant on *D. isaea* parasitism and host feeding on larvae of *Liriomyza*

No-choice experiment

Leafminer and host plant species had a strong influence on the rate of parasitism of leafminer larvae by *D. isaea* (χ^2 = 193.88, *df* = 6, *P* < 0.0001). The highest rate of parasitism of *L. huidobrensis* was recorded on *P. vulgaris*, with a mean of 46 %, followed by *P. sativum* (23 %) and *S. lycopersicum* (20 %), and *V. faba* (9 %) (Table 3.1). *Diglyphus isaea* parasitized a significantly higher number of *L. sativae* larvae on *V. faba* (59 %) and *P. vulgaris* (59 %), compared to those reared on *P. sativum* (35 %) and *S. lycopersicum* (39 %) (Table 3.1). The rate of parasitism of *L. trifolii* was significantly higher on *S. lycopersicum* (68 %) compared to *P. vulgaris* (16 %), *P. sativum* (19 %) and *V. faba* (11 %) (Table 3.1).

The rate of parasitism of *D. isaea* was higher on *L. sativae* compared to *L. huidobrensis* and *L. trifolii* on all plant species except for *S. lycopersicum*, where it was highest for *L. trifolii* (Table 3.1).

The percentage of larvae host-fed by *D. isaea* was generally lower across all the host plant and leafminer species compared to parasitism. It was 0.3 to 3.2 times lower on *V. faba* and *P. vulgaris*, 1.8 to 3.6 times lower on *S. lycopersicum* and 4 to10 times lower on *P. sativum*. Logistic regression analysis showed a highly significant interaction effect of host plant by leafminer species (χ^2 = 25.44, *df* = 6, *P* < 0.0003). The highest host feeding level was recorded on *L. trifolii* reared on *V. faba* (36 %) and lowest on *L. huidobrensis* reared on *P. sativum* (2 %). Host feeding activity by *D. isaea* on *L. huidobrensis* was higher on *P. vulgaris* and *S. lycopersicum* compared to *P. sativum* and *V. faba* (Table 3.1). However, *L. sativae* larvae reared on *P. vulgaris*, *S. lycopersicum* and *V. faba* were equally host-fed (Table 3.1).

Table 3.1. Average percentage parasitism of *D. isaea* on three leafminer species reared on four host plant species in a no-choice test

Liriomyza Species	*Pisum sativum*	*Phaseolus vulgaris*	*Solanum lycopersicum*	*Vicia faba*
% Parasitism of *Liriomyza* larvae by *Diglyphus isaea*				
L. huidobrensis	22.9 ± 1.6bB	46.0 ± 6.2aA	20.4 ± 1.3bC	8.9 ± 0.6cB
L. sativae	34.7 ± 2.8bA	58.9 ± 4.5aA	39.2 ± 2.5bB	59.4 ± 4.9aA
L. trifolii	19.0 ± 4.7bB	16.0 ± 1.4bB	68.4 ± 8.0aA	11.0 ± 3.1bB
% *Liriomyza* larvae host fed by *Diglyphus isaea*				
L. huidobrensis	2.2 ± 1.3bA	14.8 ± 1.5aB	11.4 ± 0.5aB	4.7 ± 1.5bC
L. sativae	5.2 ± 1.9bA	18.6 ± 3.2aB	19.7 ± 1.2aA	18.4 ± 2.0aB
L. trifolii	5.1 ± 3.6cA	33.6 ± 2.1aA	19.0 ± 3.3bA	36.4 ± 4.1aA

Notes: Within columns for a given host plant species, means followed by the same capital letter are not significantly different; within rows for a given leafminer species, means followed by the same small letter are not significantly different at $P < 0.05$ (pairwise χ^2 test).

Choice experiment

Across all *Liriomyza* species evaluated, the proportion of larvae parasitized and host-fed by *D. isaea* varied significantly, for parasitism ($\chi^2 = 22.37$, $df = 3$, $P = < 0.0001$) and host feeding ($\chi^2 = 16.70$, $df = 3$, $P = 0.0008$) (Fig. 3.1a). The highest rate of parasitism of *L. huidobrensis* was recorded on *V. faba* where 28 % of the larvae were parasitized.

The preferred host plant for parasitism of *L. sativae* was *P. vulgaris* (31 %) followed by *V. faba* (16 %) and for *L. trifolii, S. lycopersicum* (29 %) followed by *V. faba* (17 %). Host feeding in general was highest on *L. trifolii* reared on *S. lycopersicum* (14 %) and lowest on *L. huidobrensis* (1 %) reared on *P. sativum* and *S. lycopersicum* (Fig. 3.1b).

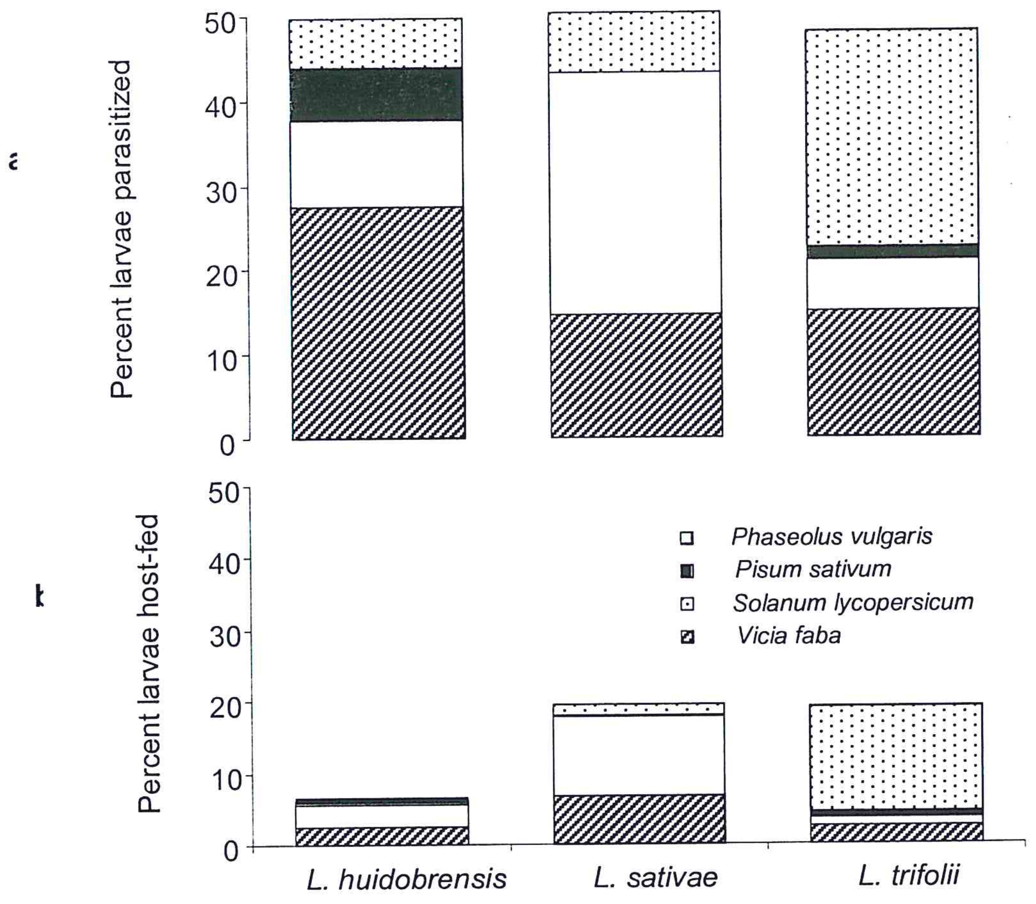

Fig.3.1. Percentage of *Liriomyza* larvae parasitized (a) and host-fed (b) by *D. isaea* in choice experiments

Sex Ratio of *Diglyphus isaea*

In the no-choice test, sex ratios of *D. isaea* varied significantly with host plant ($\chi^2 = 22.26$, $df = 3$, $P < 0.001$) and *Liriomyza* species ($\chi^2 = 14.1$, $df = 2$, $P < 0.001$). No or few *D. isaea* developed into adults from *L. sativae* and *L. trifolii* reared on *P. sativum*. Across the leafminer species, the highest proportion of females was recorded on leafminers reared on *P. sativum* (63 %) (*L. huidobrensis* only) and lowest on when reared on *S. lycopersicum* (25 %) (Table 3.2).

In the choice test, there was a significant interaction effect between host plant species and leafminer species on sex ratios ($\chi^2 = 47.53$, $df = 4$, $P < 0.001$). *Diglyphus isaea* yielded a higher percentage of female progeny from *L. huidobrensis* and *L. trifolii* reared on *V. faba* (71 and 72 %, respectively). Whereas the highest proportion of female

D. isaea from *L. sativae* was obtained from larvae reared on *S. lycopersicum* (50 %), followed by *P. vulgaris* (36 %) and *V. faba* (31 %) (Table 3.2).

A comparison of female proportions of the no-choice and choice experiments showed that, for the same level of larval infestation with leafminers per host plant, the proportion of female progeny of *D. isaea* from *L. huidobrensis* reared on *V. faba* increased from 52 % in no-choice to 71 % in the choice test (Table 3.2). The same pattern was observed for female proportions of *D. isaea* from *L. sativae* reared on *S. lycopersicum* that increased from 25 % in the no-choice to 50 % in the choice test (Table 3.2). For female proportions of *D. isaea* from *L. trifolii* reared on *V. faba*, there was also an increase from 53 % in the no-choice to 72 % in the choice test (Table 3.2).

Table 3.2. Sex ratios (percentage of females) of *D. isaea* obtained in choice and no-choice tests

Host plant	*Liriomyza huidobrensis*		*Liriomyza sativae*		*Liriomyza trifolii*	
	Choice test	No-choice test	Choice test	No-choice test	Choice test	No-choice test
Phaseolus vulgaris	48.3 ± 3.5aBC	53.2 ± 5.4aA	35.5 ± 6.3bA	53.4 ± 4.3aA	34.2 ± 3.8bB	52.0 ± 5.21aA
Pisum sativum	52.7 ± 5.7aB	63.5 ± 4.7aA	-	-	-	-
Solanum lycopersicum	36.3 ± 1.5bC	46.0 ± 3.8aB	49.7 ± 2.5aA	25.4 ± 2.3bC	37.0 ± 1.9bB	30.4 ± 4.4bB
Vicia faba	71.3 ± 3.7aA	52.0 ± 2.4bA	30.6 ± 2.9bB	37.4 ± 2.1aB	72.2 ± 3.2aA	53.4 ± 1.2bA

Notes: Within columns for a given leafminer species and experimental set-up (choice and no-choice), means followed by the same capital letter are not significantly different; within rows for a given leafminer species and experimental set-up, means followed by the same small letter are not significantly different at $P < 0.05$ (pairwise χ^2 test).

Discussion

Females of *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae), a close relative of *D. isaea*, oviposit on larger hosts but reject or host feed on smaller hosts (Heinz & Parrella, 1989, 1990). However, results of the current study suggest that the rate of parasitism does not necessarily depend on host size. For example, although *L. huidobrensis* is the largest of the three *Liriomyza* species (Chapter 2), it did not have the highest rate of parasitism in both the no-choice and choice experiments. The highest rate of parasitism in the no-choice experiment was recorded on *L. trifolii* reared on *S. lycopersicum*, and in the choice experiment was recorded on *L. sativae* reared on *P. vulgris*, hence, suggesting that these leafminer-plant combinations produced the largest leafminer larvae. Although *L. trifolii* adults were largest when reared on *V. faba* (Chapter 2), they together with *L. huidobrensis* had the lowest rate of parasitism for larvae reared on this plant species. Further, *L. huidobrensis* had the highest rate of parasitism on *V. faba* compared to the other three plant species in the choice experiment. Results of Chapter 2 on the other hand suggest that adult size of *L. huidobrensis* was independent of host plant species.

The contrasting results regarding host size suggest a strong influence of host plant species and leafminer species on parasitism by *D. isaea*. Thus, effective biological control may depend on matching this parasitoid species with host leafminer and host plant species. Host plant characteristics have been shown to cause size variation in parasitoids of insect herbivores (Johnson & Hara, 1987; Gross & Price, 1988), which consequently directly or indirectly affects the vulnerability of herbivores to parasitoids (Bergman & Tingey, 1979; Price *et al.*, 1980). Direct effects occur when plant characteristics such as glandular leaf trichomes or gall structure (Cornell, 1983) impede parasitoid searching, or when plant odours attract parasitoids (Elzen *et al.*, 1983). Indirect plant effects on parasitism in the short term occur when plants influence herbivore characteristics such as growth rate (Feeny, 1976) or presence of toxic compounds in the parasitoid (Campbell & Duffy, 1979) that, in turn, affect parasitoid host finding or development. In this study, these factors could not be assessed due to destructive dissections that were made to determine parasitism and host feeding. Further studies are therefore needed to determine the influence of these factors.

Host feeding behaviour in insects has been widely documented (Kidd & Jervis, 1989) and has been reported to be confined to synovigenic parasitoids, i.e. parasitoids whose females emerge with only a fraction of their total egg complement, and which need to feed in order to achieve maximal egg production (Kidd & Jervis, 1989). It is a common occurrence in synovigenic species (Kidd & Jervis, 1989). Jervis & Kidd (1986) separated various types of host-feeding behaviour into concurrent, non-concurrent, destructive and non-destructive host feeding. In concurrent host feeding, female parasitoids use the same host individual for both feeding and oviposition, whereas in non-concurrent host feeding the parasitoid uses different host individuals for the two processes. In non-destructive host feeding, the host survives the feeding encounter, whereas with destructive feeding the host dies as a result of the feeding. *Diglyphus isaea* is a destructive non-concurrent host feeder (Ode & Heinz, 2002). Therefore, the methods used in this study were adequate to assess host feeding effects and parasitism as separate aspects.

Various studies have shown that host feeding contributes considerably to total parasitoid-inflicted mortality (rates ranging from 55 to 78 %) (Legner, 1979; van Driesche & Taub, 1983; Lashomb *et al.*, 1987). Except for the highest host feeding rate of 36 % for *L. trifolii* on *V. faba* in the current study, host feeding levels were lower compared to results of other studies (Legner, 1979; van Driesche & Taub, 1983; Lashomb *et al.*, 1987). One important question that arises is whether this host feeding level for *D. isaea* is an important factor in maintaining equilibrium numbers in *D. isaea* host interactions in the field.

However, despite the relatively low levels of host feeding of *D. isaea* across the host plant species and leafminer combinations evaluated, host feeding still has important consequences for biological control for both inoculative releases and mass rearing of *D. isaea* for inundative releases. A reduced level of host feeding implies a better chance of parasitism that directly leads to increased parasitoid populations compared to host feeding where the mortality due to feeding does not result in population build-up (Ode & Heinz, 2002). On the other hand, despite these apparent low levels of host feeding, from a pest management perspective, host feeding has an additive effect to the total mortality imposed by *D. isaea*. Host feeding in addition to parasitism is, therefore, a benefit to biological control programmes that involve inundative augmentative releases where leafminer larval mortality is of importance.

Host quality is well known to influence sex allocation of progeny by many parasitoid species (Charnov, 1982; King, 1987; Godfray, 1994). Host feeding, and diet in general, might affect parasitoid-host population dynamics by influencing the sex ratio of the progeny of parasitoids. Khafagi (1986) found that the proportion of females in the progeny of *Anteon pubicorne* (Dalman) (Hymenoptera: Dryinidae) increased if the parent was fed on carbohydrate-rich diets such as honey, sugar and pollen, whereas it decreased if the parent was fed on protein-rich food such as peptone. Jervis & Kidd (1986) suggests that diet might influence the sex ratio of parasitoids through a mechanism involving partial resorption of non-fertilized eggs. King (1963) suggested that partially resorbed eggs, if laid, would be less likely to be fertilized, due to a change in their shape and rigidity, and would more likely develop into males. In this study, parasitoids were provided with 10 % honey solution during the pre-oviposition and mating period. Thus, the parasitoids were provided with enough food resources to avoid sex bias towards males due to factors other than those associated with the leafminer host quality.

The proportion of females in any *D. isaea* population in augmentative field releases and mass rearing is important as females are more valuable than males because they are directly responsible for killing pests by ovipostion and/or host feeding (Ode & Heinz, 2002). The highest proportion of females across all the host plant species was observed for *L. huidobrensis*. Although host sizes of leafminer larvae were not measured in this study, results of Chapter 2 suggest that across all the host plants *L. huidobrensis* larvae are bigger sized, suggesting that this species should receive a higher allocation of female progeny compared to larvae of *L. sativae* and *L. trifolii*. In the current study, the proportion of females varied from 25 to 72 % depending on host plant and *Liriomyza* species tested. Except for *D. isaea* populations obtained from *L. trifolii* reared on *S. lycopersicum* and *L. sativae* on *V. faba*, the proportion of females (46 % to 72 %) was higher or comparable to research results obtained in other studies on sex ratios in mass reared parasitoids, e.g. 43 % (Ode & Heinz, 2002) and 47 % (Parrella *et al.*, 1989). The results of the relationships between host plant, *Liriomyza* species and *D. isaea* can be used for adapting mass-rearing procedures for *D. isaea* that are compatible with available resources and circumstances in Kenya. This study utilized varieties of four commercially grown crops in Kenya to rear *D. isaea* as opposed to mass rearing of *D. isaea* on chrysanthemums (*Dendranthema grandiflora* Tzvelev) as is practiced in countries such as the United States of America (Parrella *et al.*, 1989; Ode & Heinz, 2002).

Based on sex ratio results from this study and subject to further studies, the four host plant cultivars used in this study can be used in mass rearing of *D. isaea*. Chrysanthemums are short-day plants requiring a longer period of daylight to avoid early flowering. Rearing *D. isaea* on this host might require additional costs for artificial lighting to manipulate photoperiodic requirements to avoid early flowering (Jerzy & Borkowaka, 2004). Maximum availability of the foliage phase is important for breeding *Liriomyza* species for parasitism by the *D. isaea*. The four host plant varieties used in this study cope well with the natural photoperiod in Kenya; hence, the production of *D. isaea* could be tailor-made to optimize the parasitoid production costs in this country.

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CHAPTER FOUR

Olfactory responses of *Diglyphus isaea* (Hymenoptera: Eulophidae) to *Liriomyza* (Diptera: Agromyzdae) species-induced volatiles of *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba*

Abstract

The role of volatile stimuli in host finding of *Diglyphus isaea* was studied on *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* on four plant species (*Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba*). Two experiments were carried out in the laboratory using a Y-tube olfactometer. In the first experiment four-day-old pre-mated *D. isaea* females were exposed to undamaged and mechanically damaged plants, plants infested with third-instar larvae of one of the three *Liriomyza* species and a blank control. The second experiment compared the behaviour of *D. isaea* females on *P. vulgaris*, *P. sativum* and *S. lycopersicum* infested with third-instar larvae of *L. huidobrensis* against a *L. huidobrensis*-infested *V. faba* control. First and final choices and time spent by parasitoids in arms of the Y-tube olfactometer were recorded. Mechanically damaged and undamaged plants were not more attractive than the blank control. Compared to the blank control, preference of *D. isaea* for *L. huidobrensis*-infested plants was similar for all plant species, while *V. faba* was preferred when plants were infested with *L. sativae* and *P. vulgaris* and *S. lycopersicum* were preferred when plants were infested with *L. trifolii*. Against a *V. faba* control, parasitoids were more attracted to *V. faba* than to *P. sativum* and *P. vulgaris*. However, there were no significant differences in attraction between the *V. faba* and *S. lycopersicum*. This work shows that attraction of *D. isaea* to plant volatiles released by leafminer-infested plants is dependent to a various degree on interactions between *Liriomyza* species and host plants.

Introduction

The agromyzid leafminers *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) are polyphagous and important pests of agricultural crops worldwide (Spencer, 1985; Murphy & LaSalle, 1999; EPPO, 2006; Burgio *et al.*, 2007). *Diglyphus isaea* (Walker) (Hymenoptera, Eulophidae) is used as a biological control agent of *Liriomyza* species in many parts of the world (Heinz & Parrella, 1989; Murphy & LaSalle, 1999; Ode & Heinz, 2002). Although *D. isaea* parasitises several species of *Liriomyza* on different crops, the distribution of parasitoids among crops is uneven, that is, parasitoids are associated with certain host plants and habitats but not with others (Zehnder & Trumble, 1984; Johnson & Hara, 1987; Chabi-Olaye *et al.*, 2008). Occurrences of these patterns indicate the huge challenge that parasitoids encounter before locating *Liriomyza* hosts. In locating their hosts, parasitoids must search for potential hosts living on different plants growing in diverse habitats (Zhao & Kang, 2002).

Host plant and leafminer-larvae related factors that are important in host finding by *D. isaea* include visual cues from the plants (colour of leaves, mine shape, mine size), size of leafminer larvae and volatiles released by the feeding larvae (Finidori-Logli *et al.*, 1996). Among these factors, chemical stimuli (volatiles released due to adult and larval plant damage) act as cues that direct many parasitoids to plant habitats and their hosts in the long to short distance range (Dicke & Minkenberg, 1991). A study by Zhao & Kang, (2002) on *L. sativae* revealed that *D. isaea* was more attracted to volatiles emitted from mined bean (*Phaseolus vulgaris* L.) leaves than from other host plants infested with *L. sativae* larvae. From these studies, it was concluded that herbivore-induced volatile (HIV) components were variable among plants depending on the type of plant damage and important in guiding *D. isaea* to their hosts.

A variety of crops grown in Kenya, for example snow peas (*Pisum sativum* L., Fabaceae), French beans (*Phaseolus vulgaris* L., Fabaceae), tomatoes (*Solanum lycopersicum* L., (Solanaceae), faba bean (*Vicia faba* L., Fabaceae) and cut flowers, which are of high commercial value are prone to high levels of *Liriomyza* attack (Chabi-Olaye *et al.*, 2008). Very little is known about differences in volatile composition of these host plants when attacked by different *Liriomyza* species with different feeding habits, e.g. *L. huidobrensis* mines extend towards the base of the leaf, which tend to sieve leaf segments between the veins (Wei & Kang 2006). Furthermore, *L. huidobrensis* larvae consume mesophyll both in palisade and in spongy tissues (Parrella & Bathke, 1984, Wei *et al.*, 2000), whereas *L. sativae* and *L. trifolii* larvae only feed in palisade tissue (Kang, 1996; Salvo & Valladares, 2004).

In the tritrophic interaction involving host plants, leafminers and *D. isaea*, both the host insects and the parasitoid are generalists (Zhao & Kang, 2002). Considering the wide host range of the three *Liriomyza* species and *D. isaea,* it is critical to establish which host plant-leafminer combinations are suitable for *D. isaea* in biological control programmes. Host plant association of *D. isaea* is poorly understood in Kenyan horticultural systems. As an important step in understanding the role of volatiles in guiding *D. isaea* to plant and leafminer habitats, this study was undertaken to determine the behavioural responses of *D. isaea* to herbivore-induced volatiles of different plants induced by *L. huidobrensis*, *L. sativae* and *L. trifolii*.

Materials and methods

Plants

Four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III) *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open pollinated variety) were used in the experiments. Plants were grown and maintained in a leafminer-free screen house at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya as described in Chapter 2. The average leaf areas of the plants before the experiments were: *P. vulgaris*, 111.68 cm^2, *P. sativum*, 76.65 cm^2, *S. lycopersicum* 98.43 cm^2 and *V. faba* 37.40 cm^2.

Insect rearing

The three *Liriomyza* species, *L. huidobrensis*, *L. sativae* and *L. trifolii*, were obtained from the International Centre of Insect Physiology and Ecology (*icipe*) leafminer rearing facility (see Chapter 2). All species were reared at 27 ± 2 °C with a photoperiod of 12L: 12D and relative humidity of approximately 30 %. *Liriomyza huidobrensis* was reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris*. All species had been reared on the respective host plants for approximately 18-20 generations prior to the experiments.

For experimental purposes, three potted plants of each of the four plant species were exposed to 50 four-day-old adult male and female leafminers (sex ratio 1:1) for a period of 24 hours. Plants were then monitored until at least 30 larvae had developed to the third-instar stage. Any damage to the exposed plants was avoided as much as possible to prevent the release of volatiles not associated with the insect damage. The mean number of mined leaves and leafminer larvae per plant species were: FrLH3 (81 larvae/10 mined leaves), SNLH3 (36 larvae/17 mined leaves), TMLH3 (36 larvae/15 mined leaves), FBLH3 (92 larvae/17 mined leaves), FrLS3 (105 larvae/10 mined leaves), TMLS3 (31 larvae/15 mined leaves), FBLS3 (60 larvae/19 mined leaves), TMLT3 (30 larvae/16 mined leaves) and FBLT3 (61 larvae/14 mined leaves).

Diglyphus isaea adults were supplied by Dudutech Pvt (K) (Ltd) (Kenya) where the parasitoid was mass-reared on *P. sativum* under uniform conditions. Parasitoids were received from Dudutech within 24 hours of emergence. They were then allowed to mate for a period of 48 hours in ventilated Perspex cages (40 cm × 20 cm × 20 cm) and thereafter given a pre-oviposition period of 24 hours. The parasitoids were fed on a 10 % honey solution and kept at 25 ± 1 °C, approximately 45 % relative humidity, and a 12h: 12h L: D photoperiod during the mating and pre-oviposition period. After this period, 30 females per treatment were randomly selected and placed singly in a small cylindrical vial (3 cm long, internal diameter 1 cm). All parasitoids used in the experiments were four-day-old naïve female adults, with no previous exposure to either leafminer or plants. Each individual female was used only once.

Y-tube olfactometer behavioural experiments

A Y-tube olfactometer was used to determine the response of *D. isaea* females to different volatiles emitted from the four host plant species (*P. vulgaris*, *P. sativum*, *S. lycopersicum* and *V. faba*) with damage induced by three different leafminer species (*L. huidobrensis*, *L. sativae* and *L. trifolii*). The Y-tube olfactometer was similar to that described by Ngi-Song *et al.*, 1996; Wei & Kang, 2006). It consisted of a 15 cm-long stem, 15 cm-long arms joined at an angle of 60°, an internal diameter of 2.3 cm and an observation arena measuring 95 cm × 60 cm × 45 cm arena (Fig.4.1).

Fluorescent tubes fixed at 1 m above the observation arena provided artificial light while temperatures inside the experimental room were maintained at 25 ± 1 °C. A pump was used to draw air through activated charcoal to purify it from any contaminating odours. The air from the activated charcoal was led to two flow meters, each leading to an arm of the Y-tube via the target (plant treatment).

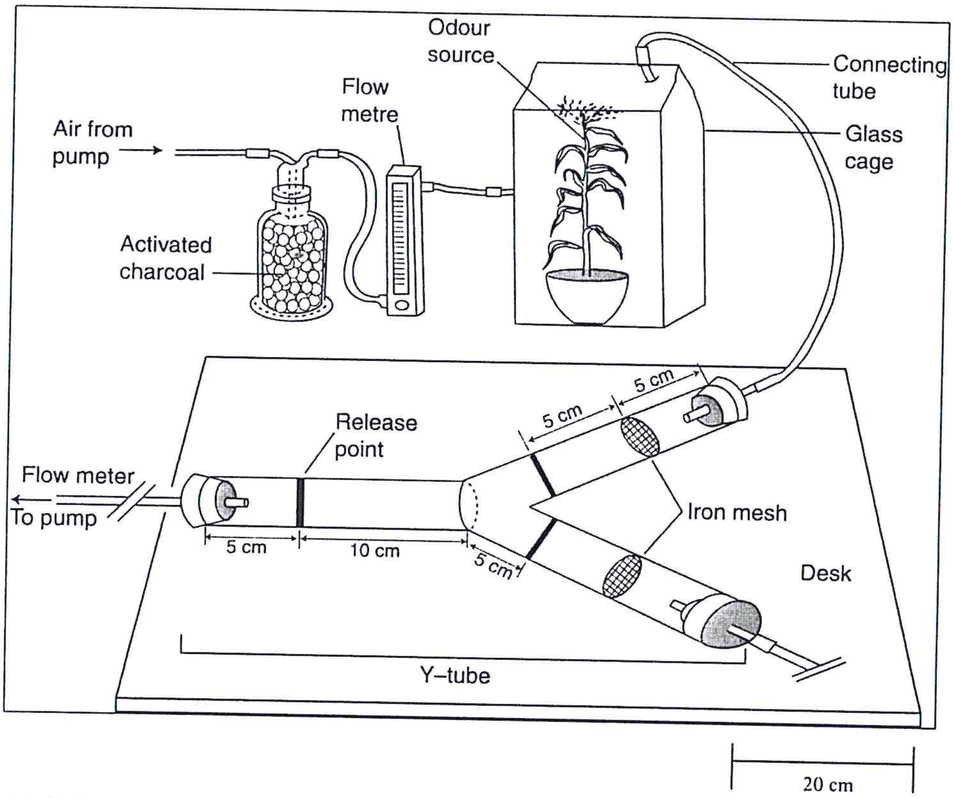

Fig. 4.1. Schematic diagram of the Y-tube olfactometer (Ngi-Song *et al.*, 1996)

The treatments in the first experiment were as follows: (1) blank (control), (2) healthy undamaged plants, (3) mechanically damaged plants (mechanical damage was induced by making four scratch lines along the entire length of eight leaves per plant to simulate larval damage) and (4) plants infested with third-instar larvae of *Liriomyza* species. All plant treatments were compared pairwise with the blank control.

The second experiment was undertaken to determine differences in responses of *D. isaea* to third-instar *L. huidobrensis*-damaged *P. vulgaris*, *P. sativum* and *S.*

lycopersicum plants, each compared against a control consisting of *V. faba* infested with third-instar larvae. *Vicia faba* has been suggested as a good host plant for rearing *Liriomyza* species and parasitoids (Videla *et al.*, 2006). Some commercial insectaries in Kenya have adopted the use of *V. faba* infested with *L. huidobrensis* for mass-rearing *D. isaea* (East Africa Growers Ltd, Kenya, pers. comm.). While *D. isaea* can directly benefit from larger-sized hosts (Videla *et al.*, 2006) and abundance of host larvae, this can only be of advantage if the plants and/or plant-leafminer interactions enable the parasitoid to locate the host. The ability to provide volatile cues that can attract *D. isaea* was therefore tested for *V. faba* against other similarly treated plant species.

Each female parasitoid was introduced individually into the stem of the Y-tube at a distance of 5 cm from the opening of the stem. A parasitoid was considered to have made a choice when it traversed the 10 cm-distance of the stem and an additional 5 cm into either arm (visually assessed by a line marked on both arms) (Du *et al.*, 1996). After release, each parasitoid was allocated 5 minutes in the olfactometer to make a choice between the two treatments. A “no choice” was recorded when a parasitoid failed to satisfy the conditions outlined above.

With the Y-tube olfactometer, short-range responses to volatile compounds can be detected by walking insects in the absence of visual cues (Wei & Kang, 2006). In the current study, the olfactometer was used for a small flying parasitoid. According to Du *et al.* (1996), a legitimate criticism of many Y-tube experiments has been the failure to take into account mistakes made by insects during initial exploration of the apparatus. Therefore, Du *et al.* (1996) suggested recording final choices of insects after a set time period. Wei & Kang (2006) proposed that parasitoids might spend longer time in the arm of an olfactometer containing the preferred odour. Thus, the final choices and the amount of time spent by parasitoids in each arm of the olfactometer were recorded in the current study.

The position of the arms containing plant treatments was reversed to avoid bias after every five parasitoids tested. The Y-tube was replaced with a clean one after 10 parasitoids were tested. The plant treatment was replaced with a similar set of plants after every 10 parasitoids tested. Thirty female parasitoids were tested for each plant treatment. The used-Y-tubes were rinsed with dichloromethane and water and dried for later use in an oven maintained at 100 °C.

Data Analysis

The percentage responses for each of the treatments versus the blank control and the *V. faba* control were calculated. The number of non-responding insects in each set of the experiment was also noted but was excluded from the analyses. Chi-square tests for differences between numbers of parasitoids making choices between plant treatments and controls for both first and final choices were done using the PROC FREQ model (SAS/STAT, SAS version 9.1.3) (SAS Institute Inc., 2002-2003). The paired-sample *t*-test (two tailed) was used to analyse differences between percentages of time spent in each arm of the olfactometer for each test. Non-responding female *D. isaea* ranged between 3 % and 23 % for all the experiments.

Results

Plant treatments vs. blank control

There were no significant differences in first and final choices and time spent between undamaged plants and the blank control for all plant species except for the final choice of parasitoids for undamaged *S. lycopersicum* (70 % vs. 30 % for control) (Fig. 4.2a,b,c). However, as their first choice (Fig. 4.3a), *D. isaea* females significantly preferred the arm leading to host plants infested with *L. huidobrensis* (*P. vulgaris*: 75 % vs. 25 % for control; *P. sativum*: 71 % vs. 29 % for control; *S. lycopersicum*: 79 % vs. 21 % for control; *V. faba*: 80 % vs. 23 % for control). No significant differences ($P > 0.05$) were recorded for *D. isaea* females when *P. vulgaris* (41 % vs. 59 % for control), and *S. lycopersicum* (54 % vs. 46 % for control) infested with *L. sativae* were used. However, *D. isaea* females significantly preferred *V. faba* plants infested with *L. sativae* (70 % vs. 30 % for control). Significant first choice preferences were also recorded for *D. isaea* females for *P. vulgaris* (69 % vs. 31 % for control) and *S. lycopersicum* (66 % vs. 35 % for control) infested with *L. trifolii*. However, there was no significant first choice difference for *V. faba* infested with *L. trifolii* compared to the blank control (37 % vs. 63 % for control).

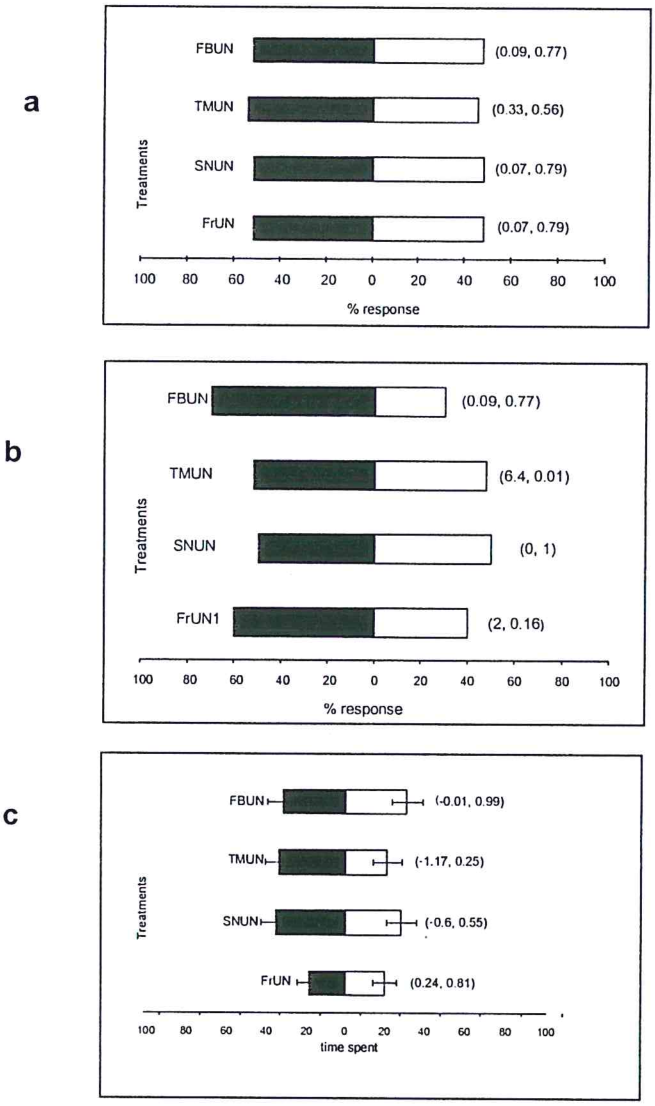

Fig. 4.2. First (a) and final (b) choice of naïve *D. isaea* females in a Y-olfactometer with one of four undamaged host plants versus a blank control. Bars indicate mean percent response for first/final choices of 30 individual females over an observation time of 5 min. χ^2 test statistic for differences between the number of parasitoids in each arm and P-values are given in parentheses. Treatments (y-axis): *P. vulgaris* (FrUN), *P. sativum* (SNUN), *S. lycopersicum* (TMUN), *V. faba* (FBUN).

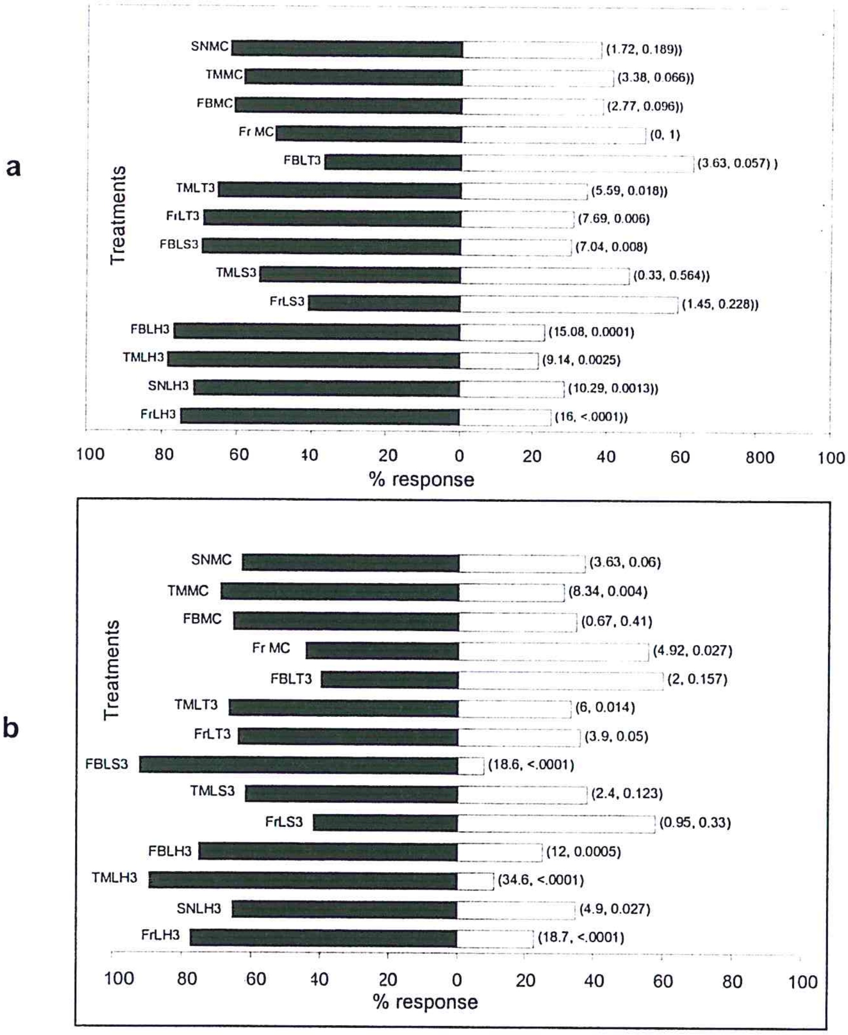

Fig. 4.3. First (a) and final (b) choice of naïve *D. isaea* females in a Y-olfactometer with one of four host plants infested with third-instar larvae of one of the *Liriomyza* species versus a blank control. Bars indicate mean percent response (x-axis) for first/final choices of 30 individual females over an observation time of 5 min. χ^2 test statistic for differences between the number of parasitoids in each arm and P-values are given in parentheses. Treatments (y-axis): plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3), third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3), third-instar larvae of *L. trifolii*: *P. vulgaris* (FrLT3), *S. lycopersicum* (TMLT3), *V. faba* (FBLT3), mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC) and *V. faba* (FBMC)

As their final choice *D. isaea* females significantly preferred *P. vulgaris* (77 % vs. 23 % for control), *P. sativum* (65 % vs. 35 % for control), *S. lycopersicum* (89 % vs. 11 % for control) and *V. faba* (75 % vs. 35 % for control) plants infested with *L. huidobrensis* (Fig. 4.3b). There were no significant difference in final choice compared with blank controls for *P. vulgaris* (42 % vs. 58 % for the control) and *S. lycopersicum* (62 % vs. 38 % for the control) infested with *L. sativae*. However, there was a significant difference in final choice for *V. faba* (92 % vs. 8 % for the control) infested with *L. sativae*. For plants infested with *L. trifolii*, there were no significant preferences for final choices by *D. isaea* female for *V. faba* (40 % vs. 60 % for the control), in contrast to *P. vulgaris* (64 % vs. 36 % for the control) and *S. lycopersicum* (67 % vs. 33 % for the control).

Diglyphus isaea females significantly spent more time in the arms leading to plants infested with *L. huidobrensis* compared to blank controls (*P. vulgaris*: 55 % vs. 16 % for the control; *P. sativum*: 45 % vs. 21 % for the control; *S. lycopersicum*: 55 % vs. 4 % for the control; *V. faba*: 45 % vs. 15 % for the control) (Fig. 4.4). For plants infested with *L. sativae*, there were no significant differences in the amount of time that *D. isaea* females spent in arms leading to either of *P. vulgaris* (17 % vs. 29 % for the control) and *S. lycopersicum* (34 % vs. 22 % for the control) compared to the blank control. However, *D. isaea* females significantly spent more time in the arm leading to *V. faba* (43 % vs. 13 % for the control) infested with *L. sativae* compared to blank controls. For plants infested with *L. trifolii*, *D. isaea* females significantly spent more time in the arm leading to *S. lycopersicum* (44 % vs. 15 % for the control). However, there was no significant difference in the amount of time spent in the arms leading to *P. vulgaris* (23 % vs. 22 % for the control) and *V. faba* (34 % vs. 34 % for the control).

There were no significant differences in the first choices made by the female *D. isaea* parasitoids between the mechanically damaged plants and blank controls ($P > 0.05$) (Fig. 4.3a). As their final choices, *D. isaea* females did not show any significant preferences for *P. sativum* and *V. faba* that were mechanically damaged while the only significant preference for the final choice of mechanically damaged plants was for *S. lycopersicum* (70 % vs. 30 % for the control). The parasitoids preferably chose the blank control (56 % vs. 44 %) to mechanically damaged *P. vulgaris* plants. The *D. isaea* females, however, did not significantly spent more time in any of the arms leading to the mechanically damaged plants (Fig. 4.4).

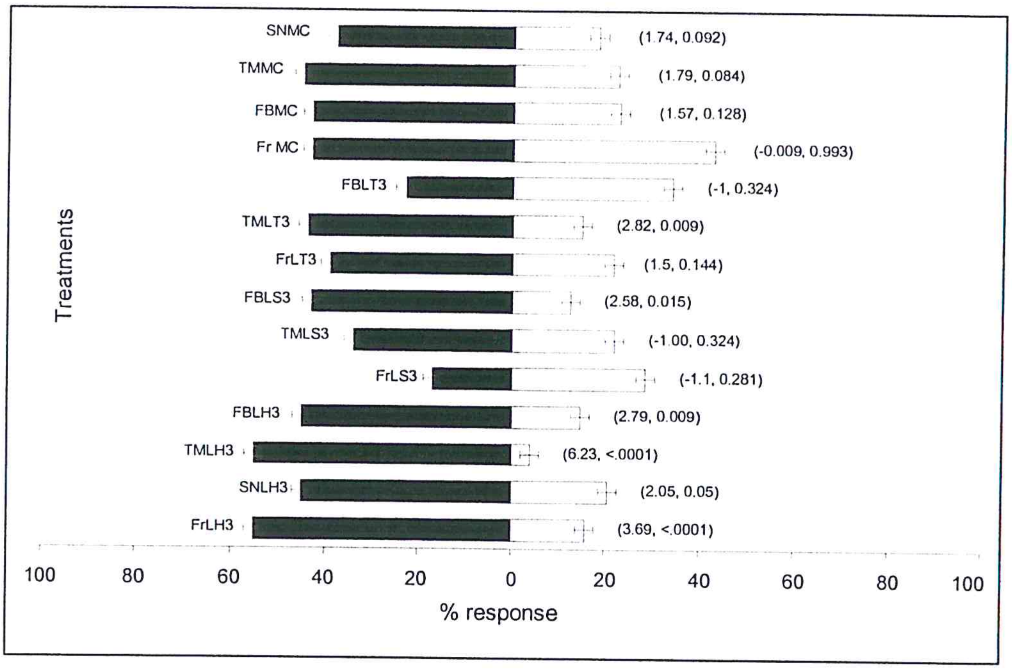

Fig. 4.4. Time spent in each arm by naïve *D. isaea* females in a Y-olfactometer with *P. vulgaris*, *P. sativum*, *S. lycopersicum* or *V. faba* plants infested with third-instar larvae of either *L. huidobrensis*, *L. sativae* and *L. trifolii* (black bars) versus a blank control (white bars) or mechanically damaged plants (black bars) versus a blank control (white bars). Numbers in parentheses represent t-values, P-values (t-value, P-value) for paired-sample t-tests. Bars indicate mean values (± SE) of the percent time spent in each arm by 30 females over an observation time of 5 min. Treatments: plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3), third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3), third-instar larvae of *L. trifolii*: *P. vulgaris* (FrLT3), *S. lycopersicum* (TMLT3), *V. faba* (FBLT3); mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC) and *V. faba* (FBMC).

Plant treatments vs. *V. faba* control

As their first choice, *D. isaea* females significantly preferred the arm leading to *V. faba* plants infested with *L. huidobrensis* compared to *P. vulgaris* (68 % vs. 32 %), and *P. sativum* (68 % vs. 32 %), while there was no significant first choice preference between *S. lycopersicum* and the *V. faba* control (Fig. 4.5a). As their final choice, *D. isaea* females preferably chose the *V. faba* control compared to *P. vulgaris* (78 % vs. 22 %) and *P. sativum* (69 % vs. 31 %). However, there was no significant difference for the final choice between *S. lycopersicum* and *V. faba* infested with *L. huidobrensis* (Fig. 4.5b). *Diglyphus isaea* females spent more time in the arms leading to *V. faba* plants infested with *L. huidobrensis* compared to *P. vulgaris* and *P. sativum* (32 % vs. 12 %) and (43 % vs. 15 %) respectively. However, there were no significant differences in the time spent in arms between *S. lycopersicum* and *V. faba* control (Fig. 4.5c).

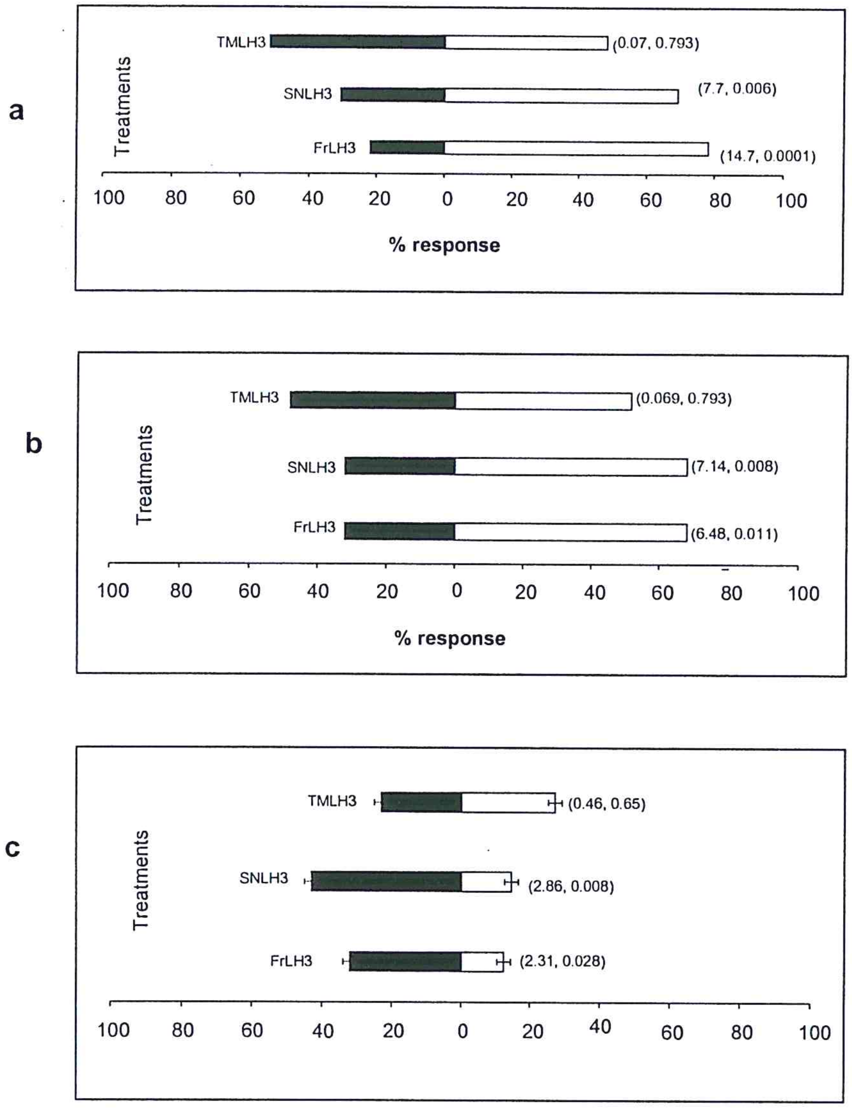

Fig. 4.5. Responses of naïve female *D. isaea* in a Y-olfactometer to whole plants of *P. vulgaris*, *P. sativum* and *S. lycopersicum* infested with third-instar larvae of *L. huidobrensis* (black bars) versus a similarly infested *V. faba* control (white bars). First choice (a), final choice (b) and time spent in each arm (c) were measured. χ^2 test for differences between numbers of parasitoids in each arm and a paired-sample t-test for differences in percent time spent in each arm were calculated. Numbers in parentheses represent t- followed by P-values. Bars indicate mean values (± SE) of the percent time spent in each arm by 30 females over an observation time of 5 min. Treatments: plants infested with third-instar larvae of *L. huidobrensis*: *P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum*. (TMLH3), *V. faba* (FBLH3).

Discussion

Diglyphus isaea females preferred plants infested with third-instar *L. huidobrensis* larvae compared to the blank control in both first and final choices. In addition, female parasitoids spent more time in the arms with plants compared to blank controls. In contrast to plants infested with larvae of *L. huidobrensis*, only *P. vulgaris* and *S. lycopersicum* infested with *L. sativae* and *V. faba* infested with *L. trifolii* were preferred to blank controls. The results suggest that, depending on plant and leafminer combination, plants infested with larvae of *Liriomyza* emitted volatile compounds that were detected by *D. isaea*. For example, Dicke & Minkenberg (1991) showed that *D. isaea* uses a plant volatile emitted from tomato leaves infested by *Liriomyza byoniae* (Kaltenbach) as long-range host location cues. In general, herbivore-inflicted injury has been shown to induce plants to release volatile terpenoids, and natural enemies including parasitoids have been reported to use terpenoids as major cues to locate hosts (Dicke *et al.*, 1990; Turlings *et al.*, 1990; Turlings & Tumlinson, 1992; Ngi-Song *et al.*, 1996; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001; Birkett *et al.*, 2003; Colazza *et al.*, 2004; De Boer *et al.*, 2004; Mumm & Hilker, 2005; Wei & Kang, 2006).

The results of this study show some variation in the response of *D. isaea* females related to leafminer and host plant species. This variation in the response of *D. isaea* females to different host plants attacked by *Liriomyza* species has potentially important implications for biological control programmes. If olfactory cues are important in habitat location, then results from this study can partially explain the variation in spatial distribution patterns and efficacy of parasitoids, including *D. isaea,* among crops in the field (Zehnder & Trumble, 1984; Johnson & Hara, 1987; Schuster *et al.*, 1991; Chabi-Olaye *et al.*, 2008). Results of the current study further emphasize that successful biological control can be achieved by using the right combinations of *D. isaea*, leafminer and host plant (Johnson & Hara, 1987).

Mechanically damaged bean plants released a higher proportion of green leaf volatiles than *L. huidobrensis* and *L. sativae* damaged plants (Wei & Kang, 2006). Zhao & Kang (2002) demonstrated that volatiles from physically damaged bean plants elicited strong electronantennogram (EAG) responses by *D. isaea*. However, in the current study, mechanically damaged plants, including bean plants, did not attract this parasitoid, suggesting that volatile compounds released by mechanically damaged plants do not play a role in attracting *D. isaea*. This would be beneficial because parasitoids can avoid false alarms. However, differences between the current study and Zhao & Kang (2002) and Wei & Kang (2006) may be attributed to the different cultivars used and further studies are needed to determine the role of volatiles from mechanically damaged plants in attracting *D. isaea*.

The current study showed that undamaged plants did not attract *D. isaea*. This result is consistent with findings of Zhao & Kang (2002), where neither undamaged hosts nor non-host plants of *L. sativae* elicited distinctive EAG responses from the parasitoid. Although some parasitoids use volatiles emitted by undamaged plants (Ngi-Song *et al.*, 1996) to locate habitats and possibly the microhabitats of their hosts (Turlings *et al.*, 1990, 1995; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001), it is likely that for *D. isaea* herbivore induced volatiles (HIVs) are more important in host finding than volatiles emitted by undamaged plants.

Overall, the results of the current study indicate that the amount of time spent by *D. isaea* in arms of the Y-olfactometer was consistent with the first and final choices it made. While recording either the first and final choice or time spent may be sufficient based on the results of the current study and would greatly reduce experimental time, all three parameters should be recorded because in one incidence among the experimental treatments (*P. vulgaris* infested with *L. trifolii)* first and final choice and time spent in arms were not in agreement.

Diglyphus isaea used in this study were reared on *P. sativum*. Thus, the results from *P. sativum* could have been influenced by the odour experience the adults were receiving when emerging from dried *P. sativum* plant debris. However, the adults showed strong responses to other host plant species and overcame the bias associated with host plants where they were reared. This therefore makes this parasitoid a good candidate for augmentative biological control.

In the pair wise comparisons between *L. huidobrensis* infested host plants and a *V. faba* control, parasitoids significantly preferred *V. faba* plants compared to *P. vulgaris* and *P. sativum* for first, final choices and the time spent in oflactometer arms. This result emphasizes that volatiles emitted from *V. faba* infested with *L. huidobrensis* were preferred to olfactory cues from *P. sativum* and *P. vulgaris*. However further studies are needed to test if this olfactory preference can be translated into improved parasitism and/or parasitoid fitness of *D. isaea*.

Although *D. isaea* responded actively to whole plant treatments in the current study, the results were obtained under experimental conditions. Each plant was presented separately in the olfactometer, whereas in natural surroundings olfactory stimuli are more complex. To obtain more information on host plant-host-parasitoid interactions, EAG assays are needed to assess the behaviour of *D. isaea* to different herbivore induced plant volatiles. Further, herbivore induced volatiles from the different whole plants (undamaged, mechanically damaged and infested with third-instar larvae of *Liriomyza* species) should be collected, analyzed, identified and quantified and be used in the Y-tube olfactometer assays.

Olfaction is one of the many important factors involved in the search for a host by *D. isaea*. The results of the current study showed that interactions between host plant, *Liriomyza* and *D. isaea* are more complex than previously recognized. To improve biological control of *Liriomyza* species with *D. isaea* the results of the current study should be combined with findings on visual cues, plant chemistry and plant-*Liriomyza* species interactions.

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CHAPTER FIVE

Variation in induced volatiles released from *Phaseolus vulgaris, Pisum sativum, Solanum lycopersicum* and *Vicia faba* in response to *Liriomyza* (Diptera: Agromyzidae) species damage

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Abstract

Plants are known to emit a variety of volatile compounds due to wounding and herbivore damage. *Liriomyza* leafminers can be important pests of horticultural crops worldwide. This study identified volatile components emitted by whole plants of the following species: *Phaseolus vulgaris*, *Pisum sativum*, *Solanum lycopersicum* and *Vicia faba*. The treatments considered were healthy undamaged plants, mechanically damaged and leafminer damaged plants. Three *Liriomyza* species, *viz. L. huidobrensis*, *L. sativae* and *L. trifolii* were used to induce damage to these host plant species. *Liriomyza* damaged plants were used for volatile collection when the larvae had reached the third instar. Volatiles were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). Among several constituents, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, and *n*-nonanal were emitted by *P. vulgaris*, *P. sativum* and *V. faba*. *Solanum lycopersicum* plant treatments resulted in the emission of mainly monoterpenes: 2-δ-carene, sabinene, α-pinene, β-phellandrene and the sesquiterpene (*E*)-caryophyllene. There was considerable qualitative variation within the same host plant species, with different damages mainly for *P. vulgaris*, *P. sativum* and *V. faba*. However, for all *S. lycopersicum* treatments, there were slight qualitative inter-treatment differences. Volatiles identified through reference to the MS library (Adams 2, chemecol and NIST 05a) need to be confirmed experimentally by comparing the spectra obtained with spectra of authentic internal standards and should be quantified. The volatile compounds identified here should also be tested for their behavioural activities in attracting natural enemies of *Liriomyza* spp.

Introduction

Plants have evolved a wide range of defensive (direct and indirect) tactics to protect them from attack by pathogens and herbivores (Mattiacci *et al.*, 2001; Hartmann, 2004; Kang *et al.*, 2009). Indirect defense may involve the recruitment and sustenance of natural enemies to a damaged plant via its induced volatiles (Wei & Kang 2006a, b; Kang *et al.*, 2009). A number of researchers have reported that volatile induction in plants can vary with herbivore species, instar and environmental conditions and have highlighted specificity in plant responses as an important signal to natural enemies for locating their hosts (Turlings *et al.*, 1990, 1995; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001; Gouinguené & Turlings, 2002; Gouinguené *et al.*, 2003; Zhao & Kang 2002a, b, 2003; Rasmann *et al.*, 2005; Röse & Tumlinson, 2005).

The leafminers *Liriomyza huidobrensis* (Blanchard), *L. sativae* Blanchard and *L. trifolii* (Burgess) are polyphagous and important pests of agricultural crops worldwide (Spencer, 1985; Murphy & LaSalle, 1999; EPPO, 2006; Burgio *et al.*, 2007). *Liriomyza huidobrensis* and *L. sativae* have been observed to differ in their feeding habits (Zhao & Kang, 2002a). The larvae of *L. huidobrensis* consume mesophyll in both palisade and spongy tissues (Parrella & Bethke, 1984; Wei *et al.*, 2000), whereas *L. sativae* larvae only feed on palisade tissue (Kang, 1996). Further, *L. huidobrensis* larvae frequently mine along the midribs of leaves, and late instars are usually found mining the lower surfaces of leaves or within petioles. This mining behaviour is distinctly different from the serpentine mines of *L. sativae* and *L. trifolii* on upper leaf surfaces (Parrella, 1987).

The difference in feeding habits of *Liriomyza* species may trigger the emission of different volatile blends. This has been observed in other herbivore species such as leaf-feeding caterpillars, spider mites, stem-boring caterpillars, and sucking insects (Turlings *et al.*, 1990, 1995; De Moraes *et al.*, 1998; Kessler & Baldwin, 2001). Studies by Dicke & Minkenberg, (1991), Petitt *et al.* (1992), Finidori-Logli *et al.* (1996) and Zhao & Kang (2002a, b; 2003) have shown that volatiles emitted from plants with different types of damage can stimulate the behavioural or antennal responses of both leafminers and their parasitoids. Leafminer-induced volatile blends or individual compounds can thus be used to attract natural enemies of leafminers (Kang *et al.*, 2009).

Studies on herbivore-induced plant volatiles (HIPVs) have received increasing attention since the early 1990s. New developments in the interdisciplinary fields of biochemistry, physiology, and behavioural ecology have resulted in a growing knowledge of chemical ecology of plants, *Liriomyza* species, and their associated parasitoids (Kang *et al.*, 2009). The current study seeks to add knowledge to the already developing trends in this area of research. In this study, the differences that exist in volatile composition from host plant species attacked by three related leafminer species (*L. huidobrensis*, *L. sativae* and *L. trifolii*) with different feeding habits are determined.

Materials and Methods

Plants

The four plant species, *P. vulgaris* (variety Julia), *P. sativum* (variety Oregon Sugar Pod III), *S. lycopersicum* (variety Moneymaker) and *V. faba* (a local Kenyan open-pollinated variety), used in experiments were grown and maintained as described in Chapter 2. The average leaf areas of the plants before the experiments were: *P. vulgaris*, 111.68 cm^2, *P. sativum*, 76.65 cm^2, *S. lycopersicum* 98.43 cm^2 and *V. faba* 37.40 cm^2.

Leafminers

The three leafminer species, *L. huidobrensis*, *L. sativae* and *L. trifolii*, were obtained from the International Centre of Insect Physiology and Ecology (*icipe*) leafminer rearing facility. The colonies were maintained for 22-24 generations prior to experiments. *Liriomyza huidobrensis* was reared on *V. faba* while *L. sativae* and *L. trifolii* were reared on *P. vulgaris*.

Leafminer rearing for experimental purposes

In each experimental set, three potted plants of each of the plant species evaluated were infested with 50 four-day-old male and female leafminers (sex ratio 1:1), previously fed on a 10 % sucrose solution, in a cage for a period of 24 hours to allow for oviposition. The cage (50 cm × 50 cm × 45 cm) was fitted with a wire screen mesh on top for ventilation. Infested potted plants were removed and transferred to another similar cage, free of leafminers, to monitor the development of larvae until the third-instar stage which was used in experiments. Any damage to the exposed plants was avoided as much as possible to prevent release volatiles not associated with the insect damage.

Experimental procedures: volatile collection and analysis

Plant treatments

The following plant treatments were selected for volatile collection and analysis: plants infested with third-instar larvae of *L. huidobrensis: P. vulgaris* (FrLH3), *P. sativum* (SNLH3), *S. lycopersicum* (TMLH3), *V. faba* (FBLH3); third-instar larvae of *L. sativae*: *P. vulgaris* (FrLS3), *S. lycopersicum* (TMLS3), *V. faba* (FBLS3); third-instar larvae of *L. trifolii*: *S. lycopersicum* (TMLT3) and *V. faba* (FBLT3). The following additional plant treatments were used for reference purposes: mechanically damaged plants: *P. vulgaris* (FrMC), *P. sativum* (SNMC), *S. lycopersicum* (TMMC), *V. faba* (FBMC); healthy undamaged plants of all plant species and a blank control (empty trapping bag).

All leafminer-infested plants were obtained as described above while the mechanical damage on the plants was induced by making four straight scratch lines using a sharp needle along the entire length of eight leaves per plant.

Plant volatile collections

A headspace sampling method using a mobile pump was used to collect volatiles from the different treatments. The volatile collection system consisted of an air suction pump (Air Cadet vacuum/pressure station, Cole Palmer Instrument Co., USA), a flow meter (Cole Palmer Instrument Co., USA), Reynolds® oven bag (turkey size 482 mm × 596 mm, Reynolds Kitchens, Richmond, VA) and Super Q adsorbent traps (Analytical Research Systems, Gainesville, FL).

For each volatile trapping process, bags were cleaned by baking them overnight in an oven at 120 °C before use. The oven-cleaned bags were then placed over plants and closed up around the stem of the branch with a strong PVC thread (Fig. 5.1). Airflow into the sampling bag was provided by two Teflon® tubes. One tube pushed air into the bag over the foliage while the other tube pulled air out of the bag through the Super Q adsorbent trap at the end and then through the flow meter at a rate of 265 ml/min. Volatiles were collected for 2 hours in the afternoon between 15:00 and 17:00. The adsorbent trap was removed, sealed with Teflon® tape and stored in a freezer at -20 °C until use. The experiment was replicated between 3 to 6 times per plant and *Liriomyza* species.

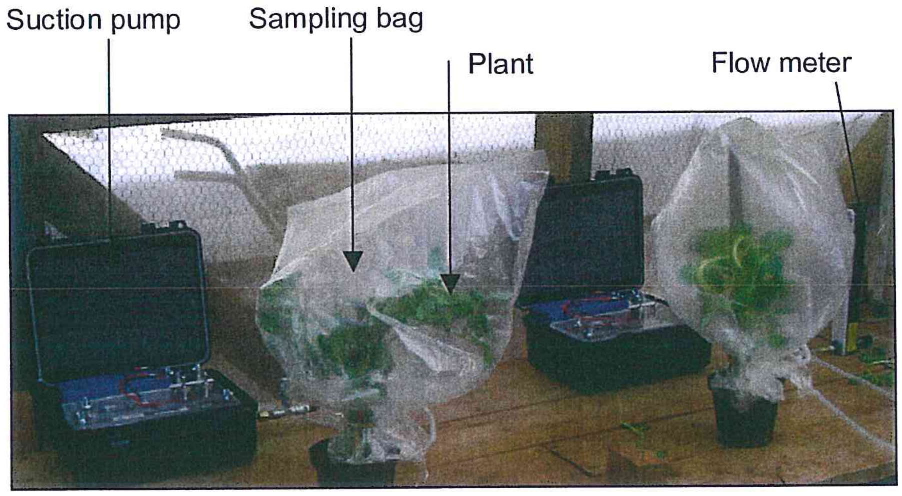

Fig. 5.1. Volatile collection system set-up placed on a bench in the greenhouse

Immediately after collection of volatiles, the number of mined leaves and leafminer larvae per plant were counted. The mean number of mined leaves and leafminer larvae per plant species were: FrLH3 (81 larvae/10 mined leaves), SNLH3 (36 larvae/17 mined leaves), TMLH3 (36 larvae/15 mined leaves), FBLH3 (92 larvae/17 mined leaves), FrLS3 (105 larvae/10 mined leaves), TMLS3 (31 larvae/15 mined leaves), FBLS3 (60 larvae/19 mined leaves), TMLT3 (30 larvae/16 mined leaves) and FBLT3 (61 larvae/14 mined leaves).

Plant volatile analysis

Super Q adsorbent traps were eluted with 200 µl of GC/GC-MS–grade dichloromethane (Burdick & Jackson, Muskegon, Michigan, USA). Fifty microlitres of volatile samples were analyzed by gas chromatography-mass spectrometric methods. The analysis was carried out on an Agilent technology 7890A GC- with 5975C MSD. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and emission current of 34.6µA. The temperature of the source was held at 230 °C (ion source), 150

^{o}C (Quadrope) and multiplier voltage was 1106 V (35 to 280). The pressure of the ion source was held at 7×10^{-6} mBar. The spectrometer had a scan cycle of three scans per two seconds. The mass range was set at m/z 1-1050 and scan range was 38-550 m/z for the compounds. The instrument was calibrated using heptacosa (Perfluorotributylamine) $[CF_3(CF_2)_3]_3N$ (Apollo scientific Ltd. UK). HP5 MS capillary column, 30 m × 0.25 mm (i.d) × 0.25 μm (film thickness) supplied by J & W Scientific was used. The GC-MS was linked to a computer with MS libraries (Adams 2, chemecol and NIST 05a). Identification of chemical components in the volatiles was based on the interpretation of the mass spectral and fragmentation patterns obtained within the data libraries (Adams 2, chemecol and NIST 05a) to obtain preliminary structural assignments.

Results

Across all host plant species treatments (undamaged plants, mechanically damaged plants and *Liriomyza* damaged plants), 52 different plant compounds were identified with the GC-MS analysis. The majority of compounds were produced by *L. trifolii* and *L. huidobrensis*- damaged *S. lycopersicum* and *L. huidobrensis*-damaged *V. faba* plants each with a total of 14, 13 and 13, respectively.

Undamaged *P. vulgaris* plants emitted four compounds (Table 1). Mechanically damaged plants only emitted two compounds, namely (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate. These two compounds were not, however, emitted by undamaged plants. *Phaseolus vulgaris* damaged by *L. huidobrensis* emitted three compounds, namely 2-hexanol, a green leaf volatile, 1,1-dimethyl-3-chloropropanol and 3-methoxy-1-butanol (Table 5.1). 2-hexenol was exclusively emitted in this treatment for this host plant species while the other two were also emitted by *P. vulgaris* damaged by *L. sativae*. *Phaseolus vulgaris* damaged by *L. sativae* emitted the highest number of compounds (11) (Table 5.1) compared to the same plant species damaged by either of *L. huidobrensis* and *L. trifolii*. With the exception of (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, *n*-nonanal, 1,1-dimethyl-3-chloropropanol and 3-methyl-1-butanol, the compounds exclusively occurred in this treatment for this host plant species.

Undamaged *P. sativum* emitted seven compounds (three green leaf volatiles, two ketones and two aldehydes) (Table 5.2). Mechanically damaged snow pea plants emitted three green leaf volatiles and one ketone. The same green leaf volatile compounds were emitted by both the undamaged and the mechanically damaged *P. sativum* plants (Table 5.2). *Pisum sativum* plants damaged by *Liriomyza huidobrensis* emitted two aldehydes and two ketones. All the compounds except (*E*)-2-hexenal were also emitted by undamaged plants. Compounds emitted by mechanically damaged *P. sativum* and leafminer-damaged plants were exclusively different between the two plant treatments (Table 5.2).

Table 5.1. Composition of volatile blends emitted by *P. vulgaris* plants: healthy undamaged (UDFr), mechanically damaged (FrMC), plants with *L. huidobrensis* (FrLH3) and *L. sativae* (FrLS3) feeding damage at third instar; + indicates presence, - indicates absence.

Chemical compound	Plant treatment			
	UDFr	FrMC	FrLH3	FrLS3
Green leaf volatiles				
2-hexanol	-	-	+	-
(*Z*)-3-hexenol	-	+	-	+
(*Z*)-3-hexenyl acetate	-	+	-	+
3-penten-2-ol	+	-	-	-
Octan-3-ol	-	-	-	-
Aldehydes	-	-	-	-
(*E*)-2-hexenal	-	-	-	+
(*Z*)-3-hexenal	-	-	-	+
n-nonanal	+	-	-	+
n-octanal	+	-	-	-
Ketones				
1-cyclopentyl-ethanone	-	-	+	-
3-methyl-2-butanone	-	-	-	+
Dodecane	-	-	-	+
6-methyl-5-hepten-2-one	-	-	-	+
3-hydroxy-3,5-dimethyl-2-hexanone	+	-	-	-
2, 6-dimethyl -4-hepten-3-one	-	-	+	-
3-methyl-6-methylene-octane	-	-	-	+
Pentadecane	-	-	-	+
2-pentanone	-	-	+	+
Other compounds				
1,1-dimethyl-3-chloro-propanol	-	-	+	+
3-methoxy-1-butanol	-	-	+	+
Total number of compounds	**4**	**2**	**6**	**13**

Table 5.2. Composition of volatile blends emitted by *P. sativum* plants: healthy undamaged (UDSN), mechanically damaged (SNMC) and plants with *L. huidobrensis* feeding damage at third instar (SNLH3) and blank control (BL); + indicates presence, - indicates absence.

Chemical compound	Plant treatment			
	UDSN	SNMC	SNLH3	BL
Green leaf volatiles				
2-hexanol	+	+	-	-
(*Z*)-3-hexenol	+	+	-	-
(*Z*)-3-hexenyl acetate	+	+	-	-
Aldehydes				
n-decanal	+	-	+	-
(*E*)-2-hexenal	+	-	+	-
Ketones				
1-cyclopentyl-ethanone	+	-	+	-
6-methyl-5-hepten-2-one	+	-	+	-
2-pentanone	-	+	-	-
Total number of compounds	7	4	4	NIL

Undamaged *S. lycopersicum* emitted 12 compounds, mainly monoterpenes and sesquiterpenes (Table 5.3). Mechanically damaged *S. lycopersicum* plants emitted six monoterpenes and two sesquiterpenes. The same chemical compounds were emitted in the two plant treatments except that *trans*-isolimonene, β-phellandrene, terpinolene and (*E*)-caryophyllene were not emitted by mechanically-damaged plants.

For *S. lycopersicum* plants, *L. trifolii*-damaged plants emitted the highest numbers of compounds, whereas the lowest was from those damaged by *L. sativae*. Undamaged and mechanically damaged *S. lycopersicum* plants also emitted mainly monoterpenes and sesquiterpenes. The compound β-phellandrene was only emitted by undamaged *S. lycopersicum* plants and *L. sativae*-damaged plants. There seemed to be an inverse relationship in emission patterns between the compounds β-phellandrene and sabinene, based on the observation that in treatments where sabinene was emitted (undamaged, mechanically damaged and *L. huidobrensis* and *L. trifolii*-damaged plants), β-phellandrene was not emitted at all (TMMCR, TMLH3 and TMLT3) (Table 5.3). Conversely, for TMLS3, where β-phellandrene was emitted, there were no concomitant emissions of sabinene.

The compounds myrcene, (*E*)-β-ocimene, α-phellandrene, α-terpinene, *ortho*-cymene and (*Z*)-3-hexenol or allo-ocemene were not emitted by either undamaged *S. lycopersicum* plants or mechanically damaged plants. These compounds were only emitted by leafminer- damaged plants (Table 5.3). There were slight qualitative variations in the compounds emitted by leafminer-damaged *S. lycopersicum* plants (Table 5.3).

Table 5.3. Composition of volatile blends emitted by *S. lycopersicum* plants: healthy undamaged (UDTM), mechanically damaged (TMMCR), plants with *L. huidobrensis* (TMLH3), *L. sativae* (TMLS3) and *L. trifolii* (TMLT3) feeding damage at third instar; + indicates presence, - indicates absence.

Chemical compound	Plant treatment				
	UDTM	TMMCR	TMLH3	TMLS3	TMLT3
Monoterpines					
2-δ-carene	+	+	+	+	+
β-elemene	+	+	+	+	+
δ-elemene	+	+	+	+	+
Trans-isolimonene	+	-	+	+	+
Myrcene	-	-	+	+	+
(*E*)-β-ocimene	-	-	+	+	+
α-phellandrene	-	-	-	-	+
β-phellandrene	+	-	-	+	-
α-pinene	+	+	+	+	+
Sabinene	+	+	+	-	+
α-terpinene	-	-	+	-	-
γ-terpinene	+	+	-	-	+
Terpinolene	+	-	+	+	-
Sesquiterpines					
Allo-aromadendrene	+	+		-	-
(*E*)-caryophyllene	+	-	+	+	+
α-humulene	+	+	+	+	+
Other Compounds					
Ortho-cymene	-	-	+	-	+
Allo-ocemene	-	-	-	-	+
Total number of compounds	**12**	**8**	**13**	**11**	**14**

For undamaged *V. faba* plants, only three compounds were emitted, all of which were green leaf volatiles (Table 5.4). Mechanically damaged plants emitted six volatile compounds (Table 5.4). Except for (*Z*)-3-hexenol which was produced in undamaged and mechanically- damaged plants, there were qualitative differences between undamaged plants and mechanically damaged plants.

There were qualitative variations in the volatile compounds emitted by the *V. faba* plants damaged by the three leafminer species. *Liriomyza huidobrensis* damaged *V. faba* plants emitted 13 compounds; *L. sativae*-damaged plants emitted five compounds while *L. trifolii* damaged plants emitted two compounds (Table 5.4).

The majority of compounds emitted by *L. huidobrensis* infested *V. faba* plants were green leaf volatiles and ketones (Table 5.4). Three monoterpenes and two other compounds were emitted by *V. faba* plants damaged by *L. sativae*. β- elemene was the only exclusive emitted compound for this plant treatment.

Overall, across all the different host plant and leafminer species, considerable qualitative variation in the volatiles emitted occurred between *S. lycopersicum* plant treatments and other host plant species treatments. Monoterpenes and sesquiterpenes were largely emitted in *S. lycopersicum* plant treatments as opposed to green leaf volatiles, aldehydes and ketones emitted in the other plant treatments. None of the compounds obtained by trapping from various plant treatments were obtained by trapping from the empty oven bags.

Table 5.4. Composition of volatile blends emitted by *V. faba* plants: healthy undamaged (UDFB), mechanically damaged (FBMC), plants with *L. huidobrensis* (FBLH3), *L. sativae* (FBLS3) and *L. trifolii* feeding damage at third instar (FBLT3). (+) indicates presence, (-) indicates absence.

Chemical compound	Plant treatment				
	UDFB	FBMC	FBLH3	FBLS3	FBLT3
Green leaf volatiles					
(*Z*)-3-hexenol	+	+	+	-	-
3-hexen-1-ol	+	-	-	-	-
(*Z*)-3-hexenyl acetate	-	+	-	-	-
(*Z*)-3-hexenyl hexanoate	+	-	-	-	-
1-nonen-3-ol	-	-	+	-	-
Octan-3-ol	-	-	+	-	-
Phenyl ethyl alcohol	-	-	+	-	-
Aldehydes					
n-nonanal	-	-	+	-	-
Octanal	-	-	+	-	-
Ketones					
1-cyclopentyl-ethanone	-	-	+	-	-
6-methyl-5- hepten-2-one	-	-	+	-	-
4-octanone	-	-	+	-	-
2-pentanone	-	+	-	-	-
Monoterpines					
β-elemene	-	-	-	-	+
2-δ-carene	-	+	-	+	-
Limonene	-	-	-	+	-
(*E*)-β-ocimene	-	-	+	-	+
β-phellandrene	-	-	-	+	-
Sesquiterpines					
(*E*)-caryophyllene	-	-	-	+	-
Other Compounds					
Decanoic acid	-	-	+	-	-
4-methyl-3-octene	-	-	+	+	-
3,6,6 trimethyl- cyclohex-2-enol	-	+	+	-	-
Total number of compounds	**3**	**5**	**13**	**5**	**2**

Discussion

The number of compounds emitted by *P. vulgaris* was low compared with previous studies involving bean plants. Finidori-Logli *et al.* (1996) identified 15 volatile components emitted by kidney bean (*Phaseolus vulgaris* L., Fabaceae) plants damaged by *L. trifolii.* Wei & Kang (2006a) identified 25 volatiles emitted by healthy, mechanically damaged and leafminer-damaged *P. vulgaris* plants. Although it was expected that different varieties of the same plant species would produce different plant volatile profiles, the current results show very low levels of volatile emission from *P. vulgaris* across all the host plant treatments.

In the current study, the volatile trapping time was shorter (2 hours) than the 10 hours as reported by Wei & Kang (2006a). The volatile trapping duration used in the current study was chosen to avoid trapping physiologically stress-induced compounds resulting from enclosing plants for prolonged periods during the volatile-trapping process.

Of the few volatiles compounds that were identified from mechanically damaged *P. vulgaris* plants in the current study, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate were also identified by Wei & Kang (2006a) on bean plants. 2-hexanol was released exclusively by *P. vulgaris* infested with third-instar larvae of *L. huidobrensis.* This compound seems to be leafminer-induced, as it was not identified in either undamaged or mechanically damaged plants.

The green leaf volatiles (GLVs) always refer to six-carbon-chain-lengths alcohols, aldehydes and esters, while oximes are three to four carbon, nitrogen-containing compounds (Kang *et al.*, 2009). Oximes were not identified from any of the *P. vulgaris* plant treatments nor other plant treatments in the current study. Finidori-Logli *et al.* (1996) observed that the identification of oximes in trapped volatiles obtained from mined and artificially damaged *P. vulgaris* leaves was a rare phenomenon. However, Wei & Kang (2006a) and Wei *et al.* (2007) identified a sizeable number of oximes from leafminer-damaged bean plants. In the current study, oximes might have been emitted by the plants but probably in very small quantities that were beyond detection by the GC-MS analyses. In addition, the *P. vulgaris* variety 'Julia' used in the current study may not have emitted oximes as opposed to varieties used in studies done elsewhere.

The compounds (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate were among the compounds emitted by *L. sativae* damaged *P. vulgaris* plants. Wei & Kang (2006b) observed that these two compounds elicited electroantennogram (EAG) responses by the generalist parasitoid *Opius dissitus* Muesebeck (Hymenoptera: Braconidae) in an olfactometer bioassay. The presence of these compounds in *L. sativae*-damaged plants observed in the current study provides strong evidence of the emission of *Liriomyza* induced volatiles in bean plants.

Few studies have examined the volatile profiles of *P. sativum*, *S. lycopersicum* and *V. faba* in relation to *Liriomyza* damage. In the current study all *S. lycopersicum* plant treatments emitted mainly monoterpenes and sesquiterpenes. Wei *et al.* (2007) demonstrated that *S. lycopersicum* produced many monoterpenes and sesquiterpenes in relatively high concentrations compared to other host plant species. The differences in the volatile profiles of *S. lycopersicum* and other host plant treatments could be related to differences in the larval feeding habits of *Liriomyza* species. *L. huidobrensis* feeds in the palisade and spongy mesophyll (Parella & Bathke, 1984, Wei *et al.*, 2000; Wei & Kang 2006a), whereas larvae of *L. sativae* and *L. trifolii* only feed in palisade mesophyll (Kang, 1996; Salvo & Valladares, 2004).

There was a notable inverse relationship in the emission of the compounds sabinene and β-phellandrene mainly from *S. lycopersicum* treatments. β-phellandrene was only emitted by *L. sativae*-damaged plants and sabinene was absent. β-phellandrene and/or sabinene could have been a by-product of monoterpene synthases. For example, Shimada *et al.* (2004) reported that monoterpene synthase produces specific main products, e.g. β-phellandrene, whereas some synthases may or may not synthesize sabinene as a by-product. Shimada *et al.* (2004) further observed that monoterpene synthase may have unique mechanisms to control specific deprotonation or cyclization of carbocations to produce a wide diversity of monoterpenoids. The results of the current study require further investigation to determine the relationship between damage caused by *Liriomyza* larvae and β-phellandrene and sabinene.

In *S. lycopersicum* plant treatments, the compounds myrcene, (*E*)-β-ocimene, α-phellandrene, α-terpinene, *ortho*-cymene and (*Z*)-3-hexenol or allo-ocimene were likely leafminer-induced as they were not emitted by undamaged and mechanically damaged plant treatments.

Overall, there were qualitative differences in the volatile compounds across all the plant treatments. Although Dicke (1999) observed that differences between volatile blends were greatest between plant species and smallest between individual plants of one species infested by different herbivores, in the current study this conclusion seems only to hold for *S. lycopersicum* plant treatments.

Most of the compounds identified here have been previously mentioned in the literature from undamaged plants. β-caryophyllene, α-humulene, allo-aromadendrene have been reported from undamaged *Dipterocarpus kerii* (Dipterocarpaceae) (Jantan, 1988). α-pinene, limonene, β-pinene, myrcene, β-phellandrene, γ-trepinene have also been identified from leaf essential oils of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux and subsp. *macrocarpa* (Sm.) Ball (Adams, 1999). This suggests that the emission of these compounds by *S. lycopersicum* is not specifically due to wounding or *Liriomyza* feeding damage. However, Loughrin *et al.* (1994) and Röse *et al.* (1996) reported that low molecular weight monoterpenes, myrcene and β-ocimene are formed by *Arabidopsis thaliana* (L.) Heynh. and that these isoprenoids are produced by damaged plant leaves.

Kishimoto *et al.* (2005) studied the expression profiles of genes involved in defense responses upon exposing *A. thaliana* to volatiles. The compounds (*E*)-2-hexenal, (*Z*)-3-hexenol or allo-ocimene were shown to induce the genes expressing chalcone synthase (CHS), caffeic acid-O-methyltransferase (COMT), diacylglycerol kinase1 (DGK1), glutathione-S-transferase1 (GST1) and lipooxygenase2 (LOX2). These genes were also induced by mechanical wounding (Kishimoto *et al.*, 2005). GLVs, including (*E*)-2-hexenal, (*Z*)-3-hexenal and their corresponding alcohols, or esters, are produced from damaged plant tissues as products of fatty acid hydroperoxide lyase (HPL) from 13-hydroperoxides of linoleic or linolenic acid, as one of the branches of phytooxylipin pathway (Hatanaka, 1993). Arimura *et al.* (2001) also showed that (*E*)-2-hexenal, (*Z*)-3-hexenol or (*Z*)-3- hexenyl acetate could induce genes encoding basic pathogenesis-related proteins (PRs), LOX or phenylalamine ammonia lyse (PAL) in lima beans.

Although the above provides evidence for the role of plant volatile emissions in indirect plant responses to wounding and herbivore damage, the evolutionary significance of the production of such compounds in *Liriomyza*-susceptible host plant species is not clear. A susceptible host plant may suffer energy (resource) cost by emitting these compounds that may not be involved in triggering resistance expression genes. Therefore, the most likely reason for the emission of these plant compounds in susceptible host plant species is the attraction of natural enemies of *Liriomyza* species. This should be explored in further studies to test the volatile compounds identified in this study for their ability to attract major natural enemies of *Liriomyza*.

Discussion of the current findings is purely based on the identification of compounds by their MS spectra based on reference to published mass spectra data from (Adams 2, chemecol and NIST 05a) libraries. To confirm the identity of the compounds referred to in the current study, further studies are needed to compare the spectra of the compounds and retention times with those of synthetic standards. The quantitative variations of the compounds also should be taken into account and correlated to levels of leafminer damage.

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GENERAL DISCUSSION AND CONCLUSIONS

The current study forms part of a larger research project on self-sustaining pest management strategies for *Liriomyza* species in Kenyan horticultural systems. It specifically investigated factors related to biological control of agromyzids, specifically *Liriomyza* species, with hymenopteran parasitiods. However, the apparent paucity of information on the Agromyzidae, which include a number of pest species of horticultural importance, and their associated natural enemies in Kenya and the Afrotropical region at large, limits our knowledge concerning biological control approaches. To determine the potential of Afrotropical parasitoids for biological control, data on distribution of agromyzids, host plant records and associated parasitoid species in this region was collated from museum collections, available literature and own observations.

This is the first study in the Afrotropical region that summarizes available literature and provides a snapshot of our current knowledge. The review is critical in advancing biological control research and allows for a more informed approach towards biocontrol projects involving agromyzid species. Agromyzid and parasitoid records (Chapter 1) also provide a framework for collection of additional data on host plant, leafminer, parasitoid associations in the Afrotropical region and can be of great value to agromyzid and biocontrol workers in Africa and elsewhere.

Based on the review in Chapter 1, a wide diversity of agromyzid leafminers has been documented in the Afrotropical region with collection efforts mainly centered on the East and Southern African sub-regions. In contrast to agromyzids, few parasitoid species have been recorded. The number of parasitoid species is approximately 1/6 of the recorded agromyzid species, highlighting either a possible lack of parasitoid diversity associated with agromyzid species or perhaps most likely a lack of sampling effort in the Afrotropical region. There is, therefore, a need for more intensive collaborative research in the afrotropics to identify the causes of the observed pattern.

There are several reports on biological invasions and natural control of *Liriomyza* species from outside the Afrotropical region (Sivapragasam *et al.*, 1999; Shepard *et al.*, 1998; Murphy & LaSalle, 1999; Rauf & Shepard, 1999; Thang, 1999; Chen *et al.*, 2003). According to these reports, local parasitoid species are capable of controlling leafminers in areas they invaded because agromyzid parasitoids, especially eulophids, are generally not host specific (Murphy & LaSalle, 1999). Unlike in regions outside the Afrotropics, only 14 parasitoid species have been recorded from *Liriomyza trifolii* (Burgess), three parasitoid species from *L. huidobrensis* (Blanchard) and only one species from *L. sativae* Blanchard (Chapter 1). Most of these records are likely to be a result of deliberate introduction from other parts of the world, e.g. *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae) from the Palearctic region, for classical biological control programmes or augmentative releases (Minkenberg & van Lenteren, 1986; Neuenschwander *et al.*, 1987; Minkenberg, 1989). On the other hand, a sizeable number of parasitoids have been recorded on *Ophiomyia phaseoli* (Tryon), a widely distributed agromyzid pest of legumes in the Afrotropical region. The relationship between the parasitoid species of *O. phaseoli* and the invasive *Liriomyza* species needs further investigation through more parasitoid collection efforts in this region.

According to previous studies, pest problems with *Liriomyza* species arose because of the use of broad-spectrum pesticides (Hills & Taylor, 1951; Spencer, 1973; Johnson *et al.*, 1980; Parrella *et al.*, 1984; Keil *et al.*, 1985; Macdonald, 1991; Kotzee & Dennill, 1996; Murphy & LaSalle, 1999). According to the limited documentation available on pesticides, mostly broad-spectrum insecticides have been used for control of agromyzids in the Afrotropical region (Abate, 1990; Davies, 1998; Musundire, 2002). It is likely that in this region negative impact on natural enemies of *Liriomyza* species is associated with the indiscriminate use of insecticides in the agro-ecosystems while biological control of leafminers in the natural ecosystems is taking place but escaping notice. In light of this hypothesis, the recommendations made in Chapter 1, that there should be more concerted sampling efforts and capacity building in parasitoid taxonomy, are crucial in advancing biological control of agromyzid pests in the Afrotropical region.

In addition, parasitoid records of *L. huidobrensis*, *L. sativae* and *L. trifolii*, indicate that the presence of some of the parasitoid species is likely a result of deliberate introductions by humans (Neuenschwander *et al.*, 1987), showing a willingness by stakeholders in agriculture to approach invasive *Liriomyza* control in a non-chemical way. Due to the ongoing large-scale disturbance of the natural ecosystems within some parts of the Afrotropical region because of expansion of land under agricultural production, it is unlikely that natural control of invasive *Liriomyza* species by parasitoids attacking sister agromyzid species in natural ecosystems will be realized in agro-ecosystems without human intervention. This provides a firm basis for advocating crop diversification and habitat management in Afrotropical agro-ecosystems while also providing an opportunity for implementing augmentative biological control techniques where parasitoids can be mass-reared and introduced into agro-ecosystems through inundative or inocultive releases.

Conservation biological control programmes have been successful in suppressing *Liriomyza* species to non-economic levels in celery, cucurbits, potatoes and tomatoes whose produce are not directly attacked by leafminers (Johnson *et al.*, 1980; Heinz & Chaney, 1995; Murphy & LaSalle, 1999; Liu *et al.*, 2009). On the other hand, augmentative releases of natural enemies have been successfully applied in greenhouses (van der Linden, 2004; van Linteren *et al.*, 2006). Within the context of the existing large-scale commercial agricultural practices in some parts of the Afrotropical region, augmentative biological control seems suitable as the release of parasitoids can be synchronized with other management strategies within the agro-ecosystems, maximizing the efficiency of parasitoids during periods of their release. However, additional surveys of parasitoid candidates suitable for biological control are recommended to widen the base of parasitoids that can be used as biological control agents.

Production of sufficient and high quality hosts is essential for mass-rearing parasitoids (Liu *et al.*, 2009). One of the requirements for successful rearing of *Liriomyza* species is production of good quality host plants under suitable environmental and nutritional conditions (Liu *et al.*, 2009). The ideal host plants should be easily propagated and maintained, be attractive to females for oviposition and support high numbers of leafminer larvae (Liu *et al.*, 2009). Results of the current study showed differences in host plant suitability for *L. trifolii* and to some extent for *L. sativae* but not *L. huidobrensis,* and as well as differences in host plant – host preference for *D. isaea.* These results highlight the importance of selecting suitable host plant species for mass- rearing leafminers for subsequent mass rearing of parasitoids. This is in accordance with Johnson & Hara (1987) that the best results for field application of *D. isaea* are obtained by matching the parasitoid with suitable host and host plant species. However, under field conditions and for mass rearing of *Liriomyza*, larval density per leaf may well exceed the larval densities/cm^2 leaf area used in this study. There are, therefore, research opportunities to investigate the effect of different larval densities on the size of the resulting leafminer and implications at the third trophic level.

Various host plants have been used to rear *Liriomyza* species including lima beans (Webb & Smith, 1970; Petitt & Wietlisbach, 1994), tomato (Ushchekov, 1994) and cowpea (Jeyakumar & Uthamasamy, 1997). In the current study the underlying factors of host plant preferences of *Liriomyza* species was not determined. Host plant characteristics, e.g. plant chemistry (Isman, 1992; Martin *et al.*, 2005) and nutrition (Minkenberg & Ottenheim, 1990), affect life history parameters of *Liriomyza* as well as parastioid species. Future studies on *Liriomyza* and host plant interactions should involve methods that assess variation in larval size by using measurements of the cephalopharyngeal skeleton (Head *et al.*, 2002) or measurements of pupal lengths (Via, 1984a,b; Via, 1986), combined with analyses of the nutritional content of the host plants.

Results of current study suggest that larval size of *Liriomyza* is not necessarily positively linked with parasitism by *D. isaea* (Chapters 2 and 3). It is likely that plant related factors other than size of the *Liriomyza* larvae influenced parasitism. Apart from indirectly affecting the quality of host larvae, host plants have also been shown to influence the degree of parasitism of *D. isaea* by affecting cues for parasitoids, which include visual, acoustic, contact, taste and olfactory cues (Feeny, 1976; Bergman & Tingey, 1979; Price *et al.*, 1980; Elzen *et al.*, 1983; Visser, 1986; Johnson & Hara, 1987; Gross & Price, 1988; Liu *et al.*, 2009).

Olfaction is one of the many important factors involved in the search for a host by *D. isaea* (Zhao & Kang, 2002). Results from this study (Chapter 4) showed a positive response by parasitoids to all *L. huidobrensis*-damaged plant species evaluated. Although there was some variation in the response by parasitoids to plant species infested with *L. sativae* and *L. trifolii* (Chapter 4), overall parasitoids were attracted to leafminer-damaged plants. In addition, results show that indirect defensive compounds (allomones) were emitted by leafminer-damaged plants (Chapter 5). However, there is no discernable pattern between parasitoid response to damaged plants and parasitism and host feeding on the same plants. This suggests that olfactory preference is not necessarily linked with parasitism.

The apparent discrepancy between attraction of parasitoids to leafminer damaged host plants and parasitism or host feeding in the current study indicates that, while volatile cues are important in successful host location by *D. isaea*, a combination of other signals such as visual, acoustic, gustatory, and touch may be involved in successful parasitism or host feeding by the parasitoid. Therefore, the successful use of *D. isaea* in the field and mass rearing may depend on using suitable host plants for leafminers and parasitoids.

Using plant mixtures for manipulating host feeding, parasitism and sex allocation by *D. isaea* can contribute towards improving biological control of *Liriomyza* species. Firstly, crop mixtures are planted in many agro-ecosystems where *Liriomyza* species pose a problem for subsistence and small-scale farmers. It is, therefore, important to determine the dynamics of *D. isaea* in mixed cropping systems. Secondly, previous studies have shown that *D. isaea* adjusts the rate of parasitism according to the host size encountered previously (Ode & Heinz, 2002). Further research on manipulating *D. isaea* behaviour to maximize its efficiency in agro-ecosystems and improve female biased populations for mass-rearing using plant mixtures should be undertaken.

In conclusion, the current study showed that the suitability of *D. isaea* for controlling *Liriomyza* species is variable and depends mostly on host plant species and leafminer size. A need exists for more intensive regional collaborative research to identify other suitable biological control candidates.

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