EFFECT OF HOST PLANT TYPE ON FEEDING, OVIPOSITION AND SURVIVAL OF *Liriomyza huidobrensis* (BLANCHARD), *Liriomyza sativae* (BLANCHARD) AND *Liriomyza trifolii* (BURGESS) (DIPTERA: AGROMYZIDEA) LEAFMINERS IN KENYA

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this is my original work and has not been previously presented in this or any other university for the award of a degree.

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Recommendation

This thesis has been submitted with our approval as the university supervisors.

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DEDICATION

To my parents,
Mr. John Nyabande
and
Mrs. Everlyne Okoth

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ABSTRACT

Liriomyza huidobrensis **(Blanchard),** ***Liriomyza sativae*** **(Blanchard) and** ***Liriomyza trifolii*** **(Burgess) are multivoltine flies, which are highly polyphagous, prolific and invasive. They are currently important pests in areas where horticultural crops are grown in Kenya. Both the adult flies and larvae cause considerable damage to crops. Infestation results to reduced photosynthetic potential of plants, which translates to reduction of the expected yield and loss of the aesthetic value. In addition, these pests are of quarantine importance and stringent measures have been put in place to prevent movement of infested plant materials in the export markets. Controlling these agromyzid pests is difficult. Most of the chemicals available locally are ineffective and highly toxic to natural parasitoids. Economically important crops in Kenya such as French beans, snow peas, brassicas, tomatoes, potatoes among others, are at potential risk of infestation by these agromyzids. In an attempt to contain the** ***Liriomyza*** **menace, a study was conducted to determine the effect of host type and cropping (choice) system on survival, development, feeding and oviposition of** ***Liriomyza huidobrensis*****,** ***Liriomyza sativae*** **and** ***Liriomyza trifolii*****. Egg staining technique using lactophenol-acid fuchsin solution was done to enable observation of the leafminer eggs.** ***Liriomyza huidobrensis*** **preferred** ***Lycopersicon esculentum*** **M. (producing feeding punctures per cm^2 of as high as 11.59±1.36),** ***Vicia faba*** **L. and** ***Phaseolus vulgaris*** **L. for feeding and oviposition, while** ***L. sativae*** **and** ***L. trifolii*** **favoured** ***P. vulgaris*** **and** ***L. esculentum*****. Results showed that all the three** ***Liriomyza*** **leafminer species followed the Hopkins' Host Selection Principle (HHSP), but with no consistency. Correlation analyses showed lack of consistencies both regarding the association between punctures and eggs. The study further revealed the potential of mixed cropping as a way of controlling** ***Liriomyza*** **leafminer infestations and faba bean as a promising crop for host diversification. Highest numbers of offsprings were produced from** ***V. faba*** **for** ***L. huidobrensis*****, and from** ***P. vulgaris*** **and** ***V. faba*** **for** ***L. sativae*** **and** ***L. trifolii*****. Survival**

was at its lowest on *L. esculentum* for *L. sativae* and *L. trifolii*, and on *P. vulgaris* and *L. esculentum* for *L. huidobrensis*. The results of this study are discussed in the context of their relevance for sustainable management of *Liriomyza* leafminers in subsistence agriculture.

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LIST OF ACRONYMS

ANOVA: Analysis of Variance
CABI: Centre for Agriculture and Biosciences International
CRD: Completely Randomized Design
DNA: Deoxyribonucleic Acid
EPPO: European and Mediterranean Plant Protection Organization
EPZA: Export Processing Zone Authority
EU: European Union
GLM: General Linear Model
GOK: Government of Kenya
HCDA: Horticultural Crops Development Authority
HHSP: Hopkinôs Host Selection Principle
LMF: Leafminer fly
IPM: Integrated Pest Management
PCR-RFLP: Polymerase Chain Reaction- Restriction Fragment Length Polymorphism
SNK: Student-Newman-Keuls
TUKEY'S HSD: Tukeyôs Honestly Significant Difference
UoC: University of California

DEFINITION OF OPERATIONAL TERMS

Fecundity:	The number of eggs produced by an organism
Longevity:	The duration of time an adult leafminer can remain alive
Oviposition:	Egg deposition by female leafminers
Performance:	Ability in terms of survival and development
Preference:	Selection of leafminers between host plants and the possibility of ranking of plants regarding feeding and oviposition

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Horticultural crops are important for nutrition, income generation and employment (Weinberger and Lumpkin, 2007). In 2008, it earned Kenya KES 73.7 billion, making it the countryôs biggest foreign exchange earner (Horticultural Crop Development Authority, 2009). French bean (*Phaseolus vulgaris* L.) and snow peas (*Pisum sativum* L.) are some of the important horticultural crops in the export market. Several yield enhancing innovations (fertilizers and crop varieties) have been developed by scientists but farmers continue to realize sub-optimal yields because of pest infestations. Traditionally, the major insect pests attacking these crops have been flower thrips, aphids, African bollworm, cutworm, red spider mite and root knot nematodes (Export Processing Zone Authority, 2005). Lately, species of *Liriomyza* Mik (Diptera: Agromyzidae) leafminers have achieved pest status in Kenya. Gitonga *et al*. (2010) recorded *Liriomyza* leafminers as the most damaging pests on snow peas.

Liriomyza huidobrensis Blanchard, *Liriomyza sativae* Blanchard and *Liriomyza trifolii* Burgess, are highly polyphagous, prolific and pesticide resistant thus becoming the most serious leaf mining species (Suryawan and Reyes, 2006). The three leafminers are threatening the production of potato, tomato, brassicas, beans, snow pea and ornamentals such as *Chrysanthemum spp*. *Liriomyza trifolii* was introduced in Kenya in 1976 (Spencer, 1985). Information regarding when *L. huidobrensis* and *L. sativae* were introduced is limited.

Damage is caused when adult females (using their ovipositors) penetrate the epidermis of host plant leaves, feed and lay eggs, resulting in punctures/stipples that look like white spots (Wei *et al*., 2000). *Liriomyza* leafminersô larvae mine the palisade and spongy mesophyll cells producing serpentine mines. The resultant losses include aesthetic damage, reduced yield following a decline in photosynthetic capacity, plant health, and entry of pathogenic organisms such as *Alternaria alternata* (Bjorksten *et al*., 2005). Infestation by *L. huidobrensis* led to 30% and 100% loss in potato yield in Peru and Western Europe respectively, and 60-70% vegetable loss in Indonesia (Milla and Reitz, 2005). Information about crop losses associated with *Liriomyza* flies in Kenya is however scanty.

Plate 1.1 Tomato leaf showing damage by *Liriomyza huidobrensis*

Control of leafminers is problematic because adults rapidly develop pesticide resistance while larvae develop inside the leaves and pupate in the soil, making them inaccessible to many pesticides. The only effective insecticide, abamectin and cyromazine, are expensive and not locally available.

The polyphagous nature of the *Liriomyza spp*. enables them to feed and reproduce on alternative host plants when the preferred hosts are not available. Female leafminers determine the kind of food their offspring will eat since the latter restrict their feeding to the leaf/plant from which they hatch. Information about the biology of these insects on selected host plants is important for understanding the risk levels and the preference-performance relationships. In addition, the incorporation of host diversification in mixed cropping (choice tests) system is a promising control strategy. The information could aid in developing control strategies.

1.2 Statement of the problem

The horticultural sector is an important revenue generator in Kenya. Productivity of most of the horticultural crops is however threatened by pests that reduce yield, quality and marketability. *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* are highly invasive, prolific and polyphagous leafminers that are gaining importance. Damage caused by leafminers includes delayed plant development, decline in yield, reduced market value and transmission of plant diseases (Bjorksten *et al*., 2005; Durairaj *et al*., 2010). Although observed to reach up to 100% in Europe, little information is available regarding the amount of damage in Kenya. Moreover, their polyphagous nature, development of resistance to chemical pesticides, coupled with the fact that early stages of their development occur inside leaf tissues and pupariation in the soil makes the control of these pests difficult. Leafminersô attacks (both adult and offspring) vary with host plants, their origin (history of previous experience) and cultural practices. Even though the problem of leafminers is currently due to restrictions at the export markets, they may at any time become sufficiently numerous to prove injurious following their prolific nature and diverse host range.

1.3 Objectives

1.3.1 Broad objective

To contribute to increased yield of horticultural crops through controlling *Liriomyza* leafminers.

1.3.2 Specific objectives

1. Quantify the feeding and oviposition of the *Liriomyza* leafminers on selected horticultural crops.
2. Determine the effect of the rearing history on the selection and performance of *L. huidobrensis*, *L. sativae* and *L. trifolii.*
3. Determine the effect of a simulated cropping system on feeding and oviposition of *L. huidobrensis*, *L. sativae* and *L. trifolii.*
4. Determine the effect of host type on development and survival of *L. huidobrensis*, *L. trifolii* and *L. sativae*.

1.4 Hypotheses

1. There is no difference in feeding and oviposition of the *L. huidobrensis*, *L. sativae* and *L. trifolii* on selected host plants.
2. Rearing history has no effect on the selection and performance of *L. huidobrensis*, *L. sativae* and *L. trifolii.*
3. Mixing crops does not have any effect on feeding and oviposition of *L. huidobrensis*, *L. sativae* and *L. trifolii*.
4. There is no significant influence of host type on development and survival of *L. huidobrensis*, *L. trifolii* and *L. sativae*.

1.5 Justification

A majority of the current Kenyan population, estimated at 38.6 million people, live below the poverty line (Government of Kenya, 2010). Horticultural crops play a significant role in the Kenyan economy by contributing about 13% of the Gross Domestic Product and employing over 4 million people both on- and off-farm (FPEAK, 2006; 2007). Moreover, research showed that households relying on horticulture were better off than the non-horticultural households in both urban and rural areas (McCulloch and Ota, 2002). Vegetables such as

French beans and snow pea, tomatoes, potatoes, cowpea among others, and important ornamentals which earn the country good revenue in export markets.

Invasion of the polyphagous and highly prolific leafminers (*L. huidobrensis*, *L. sativae* and *L. trifolii*) is however threatening the benefits. Gitonga (2009) noted that 59.9% of yield losses in snow pea were as a result of leafminer infestations. The leafminers are currently of quarantine concern, meaning infested produce is not allowed in export market. Leafminers therefore, increase food insecurity, reduce family income and amount of revenue earned due to foreign export.

Development of resistance has however rendered insecticide use ineffective. Besides, the pesticide toxicological and ecological hazards, the financial outlay incurred as farmers desperately make various concoctions and frequent sprayings, overruns the returns. The natural parasitoid complex that is based on the pest-parasite balance is disrupted by loss of essential synchronism in emergence times of a parasite and its host or reduction in the parasite population due to disease or unfavorable climate, followed by build-up of the agromyzid populations. Applications of broad-spectrum insecticides such as methomyl are also thought to result in a decline in parasitism. Hence alternative control measures to pesticides have to be sought. In order to effectively control the insect pests, it is important to understand the suitability of selected crops regarding adult preference, offspring performance and using mixed cropping for development of a control strategy.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy and morphology

Liriomyza huidobrensis, *L. sativae* and *L. trifolii* belong to the Order Diptera and Family Agromyzidae. The Agromyzidae is a highly diverse dipteran family of phytophagous species. It consists of about 2300 species in 27 genera (Centre for Agriculture and Biosciences International, 2001), where leaf mining is the most widespread feeding behaviour. *Liriomyza* adults are small (about 1-3mm long) and appear black with a yellow scutellum from above (European Union, 2000)while the larvae are preponderantly leafminers that form either linear or blotch mines (Spencer, 1985).

Liriomyza huidobrensis is commonly referred to as the pea leafminer or the South American leafminer (Head *et al*., 2002; Martin *et al*., 2005). *Liriomyza trifolii* is however commonly referred to as the serpentine, American serpentine or chrysanthemum leafminer (CABI/European and Mediterranean Plant Protection Organization, 2001; Civelek and Weintraub, 2003; Facknath, 2005) and *L. sativae* as vegetable leafminer (Sutherland, 1978; Zhang *et al*., 2000).

Weintraub and Horowitz (1995) and Roques (2006) described *L. huidobrensis* adult as measuring 2.1±0.2mm long. Adults of *L. trifolii* and *L. sativae* are small and compact with body length of 1.3-2.3mm. Some important morphological features of the three adult *Liriomyza* leafminers are illustrated in Plate 2.1 and summarized in the Table 2.1.

The pea leafminer, *L. huidobrensis* egg is whitish and translucent measuring about 0.3 x 0.1 mm (Weintraub and Horowitz, 1995), while those of *L. trifolii* and *L. sativae* are off-white and slightly translucent with the sizes varying from 0.2-0.3mm x 0.10- 0.15mm (CABI, 2001; EPPO, 2001). The larvae are headless maggots with *L. huidobrensis* measuring 3.2x1mm in length when fully grown while those of *L. sativae* and *L. trifolii* measure 3mm in length. On hatching, the first instars are colourless, before changing to pale yellow-orange then to yellow-orange. The larvae have a pair of posterior spiracles shaped like a cone, each opened by three pores (CABI, 2001; EPPO, 2001).

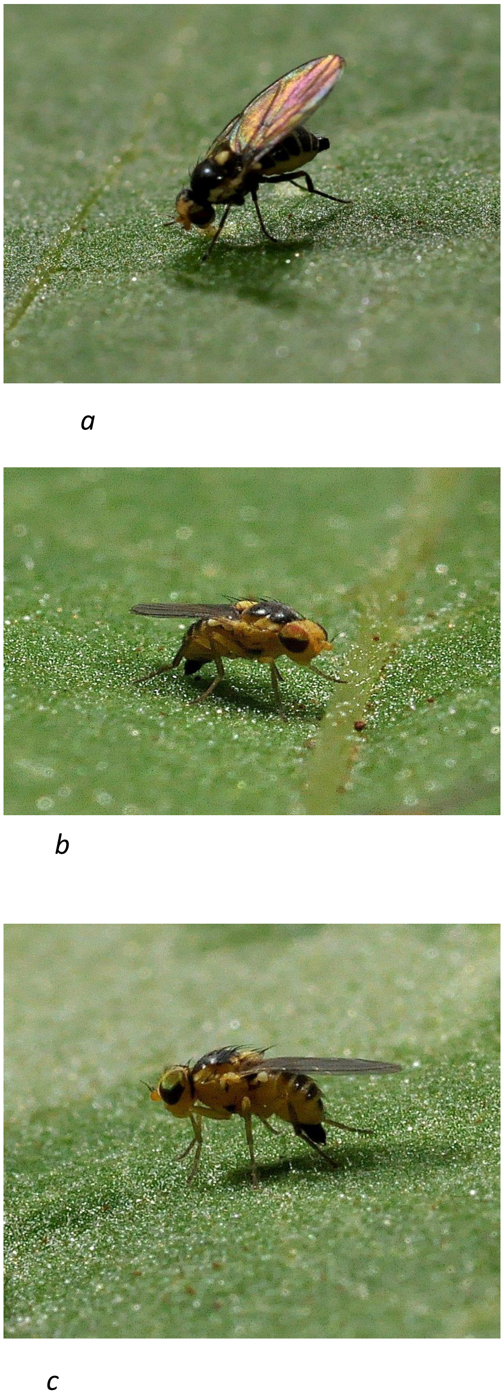

Plate 2.1 *Liriomyza* leafminer adults (a) *Liriomyza huidobrensis* (b) *Liriomyza trifolii* (c) *Liriomyza sativae*

Table 2.1 Morphological features of *Liriomyza huidobrensis*, *Liriomyza trifolii* and *Liriomyza sativae*

Morphological features	**Pest type**		
	Liriomyza huidobrensis	***Liriomyza trifolii***	***Liriomyza sativae***
Mesonotum	Black, matt	Matt black with grey undertone	Black, shining
Wing length	1.7-2.25mm	1.3-1.7mm	1.3-1.7mm
Femur	Yellow, variably darkened with black striations	Yellow	Bright yellow
Third antennal segment	Slight enlarged, usually darkened	Small, yellow	Small, yellow
Frons and orbits	Frons are yellow, generally more orange than pale lemon-yellow, Upper orbits slightly darkened	Frons and orbits yellow slightly brown straitions	Frons and orbits bright yellow
Anepisternum	Yellow with variably black patch generally across the lower three-quarters	Yellow, small blackish grey mark at front lower margin	Predominantly yellow, with dark area varying in size from a small bar along the lower margin, to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin
Male distiphallus	Two distal bulbs meeting only at their rims	One distal bulb with marked constriction between lower and upper halves	One distal bulb with a slight constriction between lower and upper halves
Vein Cu 1 A	a 2.0-2.5 times length of b	a 3.0-4.0 times length of b	a 3.0-4.0 times length of b
Vertical setae	Both vertical setae on black ground	Both vertical setae on yellow ground	Outer vertical seta on black ground which may just reach inner vertical seta which otherwise is on yellow ground

Source: Spencer (1973) and EPPO (2005)

The puparia of *L. sativae* and *L. trifolii* are oval, slightly flattened ventrally in shape and measure 1.3-2.3 x 0.5-0.75mm, while those of *L. huidobrensis* measure 1.6-3.25 x 0.7-1.1 mm. The puparium vary from pale yellow to orange, then darkening to golden brown apart from those of *L. huidobrensis* which vary from light brown to almost black (CABI/EPPO, 2001). The very dark puparia have a longer pupal stage, compared to the lighter ones (Weintraub and Horowitz, 1995). The similarities and small sizes render the larvae morphologically cryptic, making identification using morphological features difficult. A number of procedures including DNA sequence data, and polymerase chain reaction combined with restriction fragment-length polymorphism (PCR-RFLP) analyses have been used for identification (Scheffer *et al*., 2001).

2.2 Host plant types and damage

Liriomyza huidobrensis, *L. trifolii* and *L. sativae* are polyphagous pests with a wide host range (Table 2.2). While host plants range has been identified, information about the feeding and egg laying behavior of the pests is limited.

2.2.1 *Liriomyza huidobrensis*

Liriomyza huidobrensis is a serious pest of ornamentals and vegetable crops. It is a highly polyphagous pest of economical importance across the world (Cisneros and Mujica, 1998; He *et al*., 2002). The highly invasive nature, and the amount of damage caused to a wide range of economically important host plants have led to quarantine measures being taken to prevent the movement of this pest through plants and plant products (Bartlett, 1993; Head *et al*., 2003). . It has been reported to be responsible for major crop losses particularly in potatoes (Prijono *et al*., 2004). The larvae mine through the spongy mesophyll, depositing frass in a thin, broken to continuous line down the middle of the mine (Weintraub and Horowitz, 1997; Steck, 2004). The mines begin on the upper leaf surface and then move to the lower surface after a few millimeters of feeding by the larvae (Parrella and Bethke, 1984). This results in loss of photosynthetic surface area. This insect pest has generally transformed from a sporadic state (that is easy to control) to being present throughout the vegetable growing period (Chaney, 1995), hence making control difficult.

Table 2.2 Distribution of host plants of *Liriomyza huidobrensis*, *Liriomyza trifolii* and *Liriomyza sativae*

Insect species	**Vegetable crops**	**Ornamental plants**	**Wild plant hosts**
L. huidobrensis	*P. vulgaris* (French bean), *Apium graveolens* L. (celery), *L. esculentum* (tomato), *Spinacea oleracea* L. (spinach), *Solanum tuberosum* (potato), *Brassica campestris* L. (turnip), *Lactuca sativa* L. (lettuce).	*Dendranthema grandiflora* Tzvel (Chrysanthemum), *Cucubita pepo* (ornamental gourd), Gerbera (Barbeton daisy)	Oxalis (wood sorrels), *Galinsoga parviflora* Cav. (gallant soldier), *Bidens Pilosa* L. (black jack), *Portulaca oleracea* L. (purslane).
L. sativae	*P. vulgaris*, *S. tuberosum*, *Cucumis sativus* (cucumber), *L. esculentum.*	*Dahlia spp.* (dahlia).	*Amaranthus spp.*(amaranth)
L. trifolii	*Capsicum spp*. (pepper), *A. graveolens* L., *L. esculentum*, *S. tuberosum*, *P. vulgaris*, *C. sativus*.	*D. grandiflora* , *Dianthus spp*. (carnations)	*Trifolium spp*. (Clover)

Source: Parrella, 1982; Kotze and Dennill, 1996; Sivapragasam and Syed, 1999; CABI, 2001

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2.2.2 *Liriomyza trifolii*

Liriomyza trifolii is a serious pest that has replaced *L. sativae* in severity on crops such as tomatoes, celery and chrysanthemum (Kotze and Dennill, 1996). The short lifecycle, polyphagy, high fecundity of up to 600 eggs per female and ability to develop pesticide resistance (Kotze and Dennill, 1996), makes it a difficult pest to control. Being polyphagous enables it to use other crops as hosts when a specific plant is not available in season (Zoebisch and Schuster, 1987). It causes considerable yield losses in vegetable estimated by Spencer (1982) at US$ 9 million in USA in 1980. This species was a known vector of plant viruses like those causing *Alternaria* leafspot disease (Durairaj *et al*., 2010). Moreover, the fruits exposed following defoliation as a result of mining activity of this species may become sun-scorched and unmarketable (Kotze and Dennill, 1996). In South Africa, threshold level of six mines for every twenty four leaves is maintained in order to consider chemical control (Kotze and Dennill, 1996).

2.2.3 *Liriomyza sativae*

This *Liriomyza* leafminer is also highly polyphagous and is a serious insect pest to several host plants (vegetable and flower crops (Spencer, 1973, 1990; Johnson *et al*., 1983; Murphy and La Salle, 1999; Tran, 2009). The damage threshold is one active leafminer per three terminal leaflets (CABI, 2001; EPPO, 2001). *Liriomyza sativae* has been recorded as causing up to 70 % yield losses in in tomatoes (Waterhouse and Norris, 1987). In addition, this pest is a vector of plant viruses such as a celery mosaic potyvirus (Zitter *et al*., 1980).

2.3 Origin and geographical distribution

Liriomyza huidobrensis was originally from Brazil (Chavez and Raman, 1987; Weintraub and Horowitz, 1995; Musundire *et al*., 2011), but has rapidly spread to many African (Kenya and North Africa), Asian (Malaysia) and European countries (Cisneros and Mujica, 1998), including, Mexico, Peru and Central America. *Liriomyza trifolii* on the other hand is native to the southern Nearctic (Parrella, 1987). According to CABI (2001) and EPPO (2001), *L. trifolii* originated from North America and spread to the other parts of the world between 1960s and 1980s. *Liriomyza sativae* is however of Nearctic and Neotropic origin and is distributed widely including: South America, North America, Asia, Africa and Central America and Caribbean (CABI/EPPO, 2001) among other places.

Liriomyza trifolii and *L. sativae* were reported to be dominant in lowland areas while *L. huidobrensis* was dominant in vegetables and flowers in higher elevations in areas where they had established (Sivapragasam and Syed, 1999; Rauf *et al*., 2000); In Kenya, however, *L. huidobrensis* was dominant across all the agroecological zones, while *L. sativae* and *L. trifolii* were almost absent in higher altitude areas (Spencer, 1985; Chabi *et al*., 2008; Barmosho *et al*., 2009).

Though it is not clear how *L. huidobrensis* and *L. sativae* were introduced in Kenya, *L. trifolii* was introduced from the United States in 1976 and has since been causing serious damage to chrysanthemum and tomatoes (Spencer, 1985). In 1979, *L. trifolii* had already established on wild plants such as *Bidens pilosa*, *Launaea cornuta* and *Tridax procumbens*and its dispersal was facilitated by the ability to colonize widespread local plants (Spencer, 1985).

2.4 Life cycle

2.4.1 *Liriomyza huidobrensis*

Copulation of *L. huidobrensis* begins one day after adult emergence (Parrella and Bethke, 1984). A single mating is sufficient to fertilize all eggs, but the rate of oviposition is highest four to eight days after emergence (Parrella, 1987; Murphy and La Salle, 1999). At high temperatures like 27^{o}C, the egg stage lasts for 2 to 6 days (Braun and Shephard, 1997). According to Weintraub and Horowitz (1995), eggs are laid singly but in close proximity and on lower leaf surface. On hatching, larvae feed on the spongy mesophyll of the leaf, hence reducing the photosynthetic activity. There are usually three larval instars and as they develop in the leaf, the mines become progressively larger with each molt. A fourth larval instar, the pre-pupa, which lasts 4-5 hours, while larval feeding and pupal stage lasts 3.6 to 10 days and 7.9 to 12.6 days respectively (Weintraub and Horowitz, 1995). Lanzoni *et al*. (2002) estimated the lower threshold temperature for egg, larva and pupa and total development of this species to be between 7.3- 8.1^{o}C. Females live up to 18 days and males 6 days (Weintraub and Horowitz, 1995).

2.4.2 *Liriomyza trifolii*

In *L. trifolii*, copulation takes place 24 hours after emergence and a single mating is sufficient to fertilize all eggs (Parrella *et al*., 1981; Kotze and Dennill, 1996). Multiple mating is however required by females for maximum egg production (Kotze and Dennill, 1996). Most females commence ovipositing within 24-48 hours after emergence with the largest number of eggs laid between the fourth and the tenth day of adult life (Kotze and Dennill, 1996). The female inserts the egg just below the leaf surface, egg numbers vary with temperature and host plant involved. *Liriomyza trifolii* can lay 25 eggs and 400 eggs in celery at 15°C and 30°C respectively, and 493 and 639 eggs in peas and chrysanthemum respectively (Kotze and Dennill, 1996). The hatched larva feeds in the palisade section of the leaf structure until it is ready to emerge from the leaf. There are four larval instars and the mines are observed to increase in diameter and the rate of mining as the larvae develop (Kotze and Dennill, 1996). Larval stage lasts 4-6 days. Pupation occurs externally, on the foliage or in the soil just beneath the surface but is reported to occur in the leaves of onions and lucerne (CABI/EPPO, 2001). Pupal stage lasts 8-11 days, which is 50 % of the total development time (Kotze and Dennill, 1996). Leibee (1982) observed that adult *L. trifolii* emerge 7-14 days from pupariation at a temperature range of 20- 30°C. The adult longevity of this species is between 15 to 30 days, with females living longer than the males. Males usually emerge before the females and most of the studies have shown that peak emergence of adults occurs before midday (Parrella, 1987).

2.4.3 *Liriomyza sativae*

Mating takes place 24 hours after the emergence with a single mating being sufficient to fertilise all the laid eggs (Murphy and La Salle, 1999). According to Parrella *et al*. (1981) about 15 % of punctures made by the *L. sativae* females contain viable eggs. Eggs take between 2 to 5 days to hatch depending on the prevailing temperature. The duration of the larval development is also temperature dependent but will on average take 4-7 days at mean temperatures above 24°C, while the mortality rates of the immature stages rises sharply at temperatures above 30°C.

Pupariation of this species usually occurs externally, either on the foliage or in the soil just beneath the surface. Adults emerge 7-14 days after pupariation, at temperatures ranging from 20°C to 30°C, at lower temperatures, the emergence is delayed (Leibee, 1982). This species is noted to complete its life cycle in California during winter in 24 to 28 days. Adults of *L. sativae* live between 15 to 30 days, with females living longer than the males and the latter emerging before females.

2.5 Control strategies

2.5.1 Biological control

Biological control is the combat of pests using other organisms the natural predators, diseases and plant resistance (Emden, 1989). *Liriomyza* species have rich natural enemy communities particularly in their areas of origin. The natural enemies include parasitoids, entomopathogenic nematodes, predators and entomopathogens (Liu *et al*., 2009). It is an important remediation strategy to combat pest outbreaks, with more than 140 species of parasitoids and a few species of predators (Liu *et al*., 2009).

Kaspi and Parrella (2005) noted that the most promising non-chemical approach for controlling *Liriomyza* leafminers is the augmentative/inoculative releases of the parasitoids such as *Diglyphus isaea* Walker (Hymenoptera: Eulophidae). Moreover, this parasitoid species had been reported to be in Kenya and other African countries (Chabi-Olaye, *et al*., 2008 and Musundire *et al*., 2011). Biocontrol of invasive *Liriomyza* species *per se* involves a complex of local parasitoids acting at the larval and pupal stages (Murphy and La Salle, 1999). Parasitoids include *Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae), *Opius* species, *D. isaea* and in some areas *Gronotoma micromorpha* Perkins (Hymenoptera: Eucoilidae) (Prijono *et al*., 2004). *Dacnusa sibirica* Telenga (Hymenoptera: Braconidae), a parasitic wasp, is effective in the control of the leafminers (Head *et al*., 2003). *Diglyphus isaea* is a larval ectoparasitoid while *D. sibirica* is a larval-pupal endoparasitoid that does not complete its life cycle until the emergence and pupariation of the leafminer larvae (Head *et al*., 2003). Rauf *et al*. (2000) noted that *Liriomyza* parasitoids included eulophids such as *Asecodes* sp. In addition, University of California (2007) indicated that several species of the parasitic wasps, particularly *Chrysocharis parksi* (Hymenoptera: Eulophidae) and *Diglyphus begini* (Ashm.) (Hymenoptera: Eulophidae) when not disturbed, kept the population of the *Liriomyza* flies under control. Jose *et al*. (2003) noted that *D. begini*, is an effective biological control agent for *L. huidobrensis*. This parasitoid has a two-fold effect to control this pest species that is, feeding on the host as well as parasitism. Patel *et al*. (2003) observed that *Diglyphus intermedius* (Girault), an ectoparasitic parasitoid that prefers the third instar leafminers for oviposition, attacked mainly *L. trifolii* species. Besides parasitoids, nematodes are promising leafminer control agents.

Williams and Walters (1994) and Williams and MacDonald (1995) noted that entomopathogenic nematode (*Steinernema feltiae*) used as a foliar treatment against larval instars, could achieve high levels of leafminers control. While the natural biological control is believed to maintain the leafminers population below threshold, activities such as application of broad-spectrum insecticides results in the elimination of naturally occurring parasitoids.

2.5.2 Use of pesticides

a) Synthetic insecticides

Farmers and producers regardless of production scale and crop, commonly use synthetic and natural insecticides for leafminer control. The effectiveness of these insecticides has been reduced by their indiscriminate use, which has adversely impacted on natural enemies and resulted in the development of resistance to several groups of insecticides (Liu *et al.*, 2009).

Prijono *et al*. (2004) and Weintraub and Horowitz (1995) noted that many pesticides, including pyrethroids and organophosphates are ineffective against *L. huidobrensis* because the pest develops resistance towards chemical pesticides. UoC (2007) noted that *Liriomyza* flies had high resistance to organophosphates, carbamates and pyrethroids. Translaminar pesticides like abamectin and cyromazine that are larvicides, are recommended for control of *L. huidobrensis* (Weintraub, 2001; Prijono *et al.*, 2004; Kaspi and Parrella, 2005). Braun and Shepard (1997) also noted that cyromazine (trigard) which is an insect growth regulator that disrupts the life cycle by interfering with the larval stage, as the most specific chemical product effective against leafminers. However, this product can be phytotoxic if applied too often (Braun and Shepard, 1997). Although some chemicals are effective, the potential to develop resistance (Parrella *et al.*, 1984; Weintraub and Horowitz, 1997), availability, financial outlay and ecological toxicity render the system expensive to most farmers in Kenya.

b) Botanical insecticides

Neem insecticides are attractive for use in management programs due to their low contact toxicity and need to be directly ingested by the. Insecticides derived from the seed extract of the neem tree, *Azadirachta indica* (Meliaceae) have negative growth regulating and antifeedant effects on a diversity of phytophagous insects (Weintraub and Horowitz, 1997; Li *et al*., 2003). Parkman and Pienkowski (1990) and Weintraub and Horowitz (1997) further noted that azadirachtin affected the fecundity and longevity of *L. trifolii* adult when treated at the larval stage. However, since azadirachtin is more efficacious as a systematic insecticide and is prone to photo degradation, further testing under field situations is important as recommended by Weintraub and Horowitz (1997). Besides neem, the chinaberry tree (*Melia azedarach* L., Meliaceae) extracts have also been found to be effective in controlling *Liriomyza* leafminers with effect similar to that of neem (Banchio *et al*., 2003).

2.5.3 Cultural control

Cultural control is the deliberate alteration of the production system, either the cropping system itself or specific crop production practices, to reduce pest populations or avoid pest injury to crops (Ferro, 2009). There are four functional mechanisms of cultural control: the creation of adverse biotic conditions that reduce survival of individuals or populations of the pest, impediments to pest colonization of the crop, modifications of the crop in such a way that pest infestation results in reduced injury to the crop and enhancement of natural enemies by manipulating the environment. For leafminers control, possible cultural practices include cold/heat treatment, use of manure/fertilizers, crop rotation and intercropping.

Use of manure, such as vermicompost (an organic fertilizer), results in high tolerance to both pests and diseases (Suryawan and Reyes, 2006). Vigorous plants are less attacked by pests. Rapid healthy plants can reduce pest damage in four ways: rapid growth shortens any susceptible stage thereby inducing resistance against the pest, allows maximum compensation for damage by the plant and promoting uniformity and density of the crop stand since fewer winged immigrants land where less ground is exposed (Emden, 1989).

Crop rotation normally reduces and delays attack rather than giving complete control, although control may be significant within a given field, it is less effective restraint over an area as a whole. It relies on the fact that there are usually a few general feeders among the pest found across the rotation (Emden, 1989). It is an effective pest management tool used as avoiding varieties which are highly susceptible to leafminer infestations (Roques, 2006). Braun and Shephard (1997) reported that hosts such as carrots and beets which are only slightly affected by LMF tend to harbor more parasitoids than other crops, hence could be beneficial when used in crop rotation programmes with susceptible crops such as potato.

Intercropping has also been known to reduce pest infestation. This practice seems to have three main effects on insects, which result in lower pest numbers: host plant finding behaviour of insects may be disrupted by the close juxtaposition of the two species, attracting the pest to a less valuable crop or one where the pest is less serious for some reason, and increasing the impact of natural enemies (Emden, 1989). However, limited work have been done regarding intercropping high value crops with those of low value, but highly preferred by the leafminers for feeding and egg laying. The crops of low value will save the high value ones from the leafminersô attack, hence host diversification as a way of pest management

(Braun and Shephard, 1997). The proposed study uses *Phaseolus vulgaris*, *Pisum sativum* and *Lycopersicon esculentum* as high value crops, and *Vicia faba* as a low value crop.

2.5.4 Plant resistance

Host plant resistance, which involves the use of plant tolerance/resistance as a means of control, is an important method of controlling pest attacks (Schoonhoven *et al*., 1998; Ohta, 2002). In their long association with pests and pathogens, plants evolved an impressive array of defensive tools (Keen, 1999). For example, leaf physical structure, such as thickness, thickness of the epidermis wall, densities of the palisade and spongy tissues, can reduce feeding and ovipositing by female leafminers and mining and development of larvae leafminers (Wei *et al*., 2000). In addition, plant resistance was also observed when an egg laid on growing leaves (upper leaves), then the tissue surrounding the encrusted egg started abnormal multiplication, described as hyper tropic growth of cells, resulting in the egg being pushed out of the palisade to the leaf surface, a process called hypersensitive reaction or induced resistance described by Videla and Valladares (2007) in potato plants. The extruded eggs were exposed to harsh environmental conditions such as dehydration and predation (Cisneros and Mujica, 1998).

Tolerant cultivars have been used in controlling *Liriomyza* leafminers. Chrysanthemum was observed to have negligible damage with only small inconspicuous mines which were less than 0.5 mm (Suenaga *et al*., 1995). Moreover, a watermelon (*Cucumis melo*) cultivar, Nantais Oblong, was shown to be a source of resistance by antibiosis to the leafminer, *Liriomyza trifolii* (Burgess) (Dogimont *et al*., 1999)

Advantages of natural plant resistance include nominal genetic permanency, negligible cost once cultivars are developed, and quite high efficacy. The major bottleneck however, is the reality that selection pressure is placed on pest populations to develop means of overcoming the resistance, thus practically limiting the time of effectiveness (Keen, 1999).

2.5.5 Physical controls

The control aims at reducing pest populations using devices, which affect leafminers physically or alter their physical environment (Emden, 1989). Yellow sticky traps and yellow sticky tapes have been used to monitor and mass trap the agromyzids (Chavez and Raman, 1987). Transparent automobile grease, such as Penzoil 7070L or used motor oil are used in the coating of the yellow traps (Braun and Shepard, 1997). According to UoC (2007) as part of the cultural control, checking the transplants before planting, especially in tomatoes and destroying the infested ones since leafminers reach damaging levels earlier when infestations begin on the seedlings is vital. Molitas-Colting (2002) discovered that using yellow sticky traps with corn, which is a non-host, and carrots, cabbage or beans as intercrops, yielded good results in the management of *L. huidobrensis*.

A few weeks of cold storage at 0^oC has been shown to kill all the stages except the newly laid eggs. It is therefore recommended that cuttings of the ornamental plants be maintained under normal greenhouse conditions for 3-4 days after lifting to allow the eggs to hatch, followed by subsequent storage of plants for 1-2 weeks at 0^oC to kill the larvae that would have emerged (CABI/EPPO, 2001).

2.5.6 Integrated pest management

Integrated pest management (IPM) seeks to provide an effective and economical control strategy that minimizes the disturbance of anthropogenic control measures on the natural components of the agro-ecosystems (Liu *et al*., 2009). It is a pest management system that in the context of the associated environment and population dynamics of the pest species utilizes all suitable techniques and methods in a compatible manner to maintain the pest population levels below those causing economic injury (Dent, 1991). Based on the ecology of leafminer natural enemy communities, more attention needs to be paid in IPM to the enhancement of local enemies (Murphy and La Salle, 1999). Since no single control agent has been proved to be effecient and cost effective, combined use of two of more effective control agents have been developed (Sher *et al*., 2000). For instance, the compatibility of abamectin with *D. isa*ea for the control of *Liriomyza* leafminers in the greenhouses was recommended by Kaspi and Parrella (2005), as a suitable integrated pest management program. Sher *et al*. (2000) also noted the combined use of the eulophid parasitoid wasp *D. begini* and the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) as a suitablecontrol of the leafminer *L. trifolii*. Head *et al*. (2000) reported the potential of using an entomopathogenic nematode *S. feltiae*, in combination with chemical pesticides in an IPM program.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Site description

The studies were conducted in the laboratory and greenhouse at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi. The centre is located at an altitude of 1600 m above mean sea level , longitude of 36^{o}53.8ôE and latitude of 1^{o}13.3ôS. The area experiences average minimum and maximum temperatures of 13.2^{o}C and 25.2^{o}C, 850 mm mean rainfall per annum and relative humidity of 64 %.

3.2 Host plants

Four plant species namely French beans *Phaseolus vulgaris* L. (Juliavariety), snow peas *Pisum sativum* L. (Oregon Sugar pod III variety), tomatoes *Lycopersicon esculentum* M. (Moneymaker variety) and faba beans *Vicia faba* L. (a local Kenyan open-pollinated variety) were used in the study. The first three crops are important horticultural crops in Kenya and are susceptible to the three invasive leafminers. Faba beans on the other hand, were used to test for the potential host diversification as a mechanism of pest control.

Seeds of the above crops, except for tomatoes, were sown directly in plastic pots (10 cm diameter × 10 cm length) containing a mixture of red soil and farmyard manure at a ratio of 7:1. Tomato seedlings were first established in germination trays (60 × 60 cm) before being transplanted into similar sized pots. Temperature and relative humidity were as stated in section 3.1 above.

Three to four week old plants were used in the experiments. At this stage of development, all the plant species were at the seedling level to avoid leaf strata selection by the leafminers. Plants were then chosen on the basis of the same height. For preference experiments, the leaf area of each leaf was determined to correct for the different leaf sizes. This was done through scanning then using adobe Photoshop software to get the areas in pixels which were then converted to square centimetre (cm^{2}).

3.3 Mass rearing of leafminers

A culture of *L. huidobrensis* was established from adult leafminers collected on wild crucifers in the proximity of the ICIPE campus. Colonies of *L. sativae* and *L. trifolii* were established from insects collected from Kibwezi, (02° 15′S; 37° 49′E; 965 m asl), Makindu (02° 16′S; 37° 48′E; 991 m asl) and Masongaleni (02° 22′S; 38° 08′E; 714 m asl) in Eastern Kenya. Adult females of leafminers collected from the field were allowed to feed and oviposit on 4 plant species described in section 3.2 above. The F_1 progenies were tested for species identity by PCR-RFLP analysis in conjunction with morphometric analysis according to Spencer (1973) and Shiao (2004). The procedure for PCR-RFLP was as per Scheffer (2000), Scheffer and Lewis (2001, 2005) and Kox *et al*. (2005). The PCR-RFLP assay was based on the mitochondrial cytochrome oxidase II gene. Amplification was generated with Primer combination P2/P3digested with *ALu*1 in a 2.5 % agarose gel, which allows the discrimination of *L. sativae*, *L. trifolii* and *L. huidobrensis* (Musundire, 2010). The colonies were subsequently mass reared according to their identity, with a photoperiod of 12L: 12D. *Liriomyza huidobrensis* was maintained on *V. faba*, *P. vulgaris*, *P. sativum* and *L. esculentum* while *L. sativae* and *L. trifolii* colonies were maintained on *P. vulgaris*, *V. faba* and *L. esculentum* for five generations prior to the experiments.

Two hundred one-two day-old leafminer flies of both sexes were released in single wooden cages (54 cm length × 51 cm width × 56 cm height) containing 8-10 potted plants. The flies were allowed to oviposit for a period of 24 hrs on the potted plants. The cages were provided with meshed (size of the openings and mesh size) openings on opposite sides (opening diameter of 30 cm and a mesh of 2 x 2 mm size and a finer one in the inner side) for ventilation and a clear glass top for incidence of the light rays. Twenty four hours after oviposition, the potted plants were removed from the oviposition cages, (flies still on the plants were removed using aspirator) and transferred into another similar sized cage to allow for the development of eggs and larvae till pupation. At third instar larval stage (when a larva was observed clearly while still within the leaf tissue), the plants were excised and placed horizontally over the collection trays containing a thin layer of sand as described by Martin *et al.* (2005) to allow the dropping of larvae and their pupation in the sand. The sand was sifted after three days, puparia collected into Petri dishes, labeled and incubated for the emergence of the adults. The three *Liriomyza* leafminer species were maintained separately on the four host plants mentioned above (*L. sativae* and *L. trifolii* were not reared on *P. sativum*, because they did not lay eggs on it).

3.4 Data analyses

Data that was not conforming to normality or homoscedasticity was log-transformed (Gomez and Gomez, 1984) and subjected to the analysis of variance (one-way ANOVA), using the general linear model procedure of SAS (SAS institute, 2008). This was followed by Student-Newman-Keuls (SNK) test as a mean separation procedure, unless otherwise mentioned. Correlation analyses comparing the number of feeding punctures and eggs were also done to examine the associations (positive or negative) among the preference parameters.

CHAPTER FOUR

EFFECT OF HOST PLANTS ON FEEDING AND OVIPOSITION OF *Liriomyza huidobrensis*, *Liriomyza sativae* AND *Liriomyza trifolii*

Abstract

Host plant quality may directly affect oviposition, survivorship and fitness of herbivorous insects. Oviposition preference of herbivorous insects is strongly related with host suitability for their progeny, as they would maximize their fitness by ovipositing on quality plants. Adult feeding and oviposition preference of *Liriomyza huidobrensis*, *Liriomyza trifolii* and *Liriomyza sativae* were examined on *Vicia faba* L., *Phaseolus vulgaris* L., *Pisum sativum* L. and *Lycopersicon esculentum* M. in choice experiments and on only *V. faba* and *P. vulgaris* in no-choice experiments in the laboratory. The effect of each of the above host plants on feeding and oviposition of *Liriomyza* leafminers, effect of mixing crop species and effect of the previous rearing host plant was also assessed. Host plant feeding and oviposition preference varied among respective subpopulations in both choice and no-choice experiments. *Liriomyza huidobrensis* highly preferred *L. esculentum*, *V. faba* and *P. vulgaris* for feeding and oviposition, while *L. sativae* and *L. trifolii* favoured *P. vulgaris* and *L. esculentum*. Choice studies revealed the possibility of mixing plant species to reduce extent of *Liriomyza* leafminers infestation especially in *P. sativum*. The number of punctures made per leaf area ranged between 1.47 ± 0.52 to 23.34 ± 3.35 for *L. huidobrensis*, 0 to 11.75 ± 1.77 for *L. sativae* and 0 to 10.99 ± 1.64 for *L. trifolii*, while eggs oviposited were in a range of 0 to 0.32 ± 0.07 (per cm^2) for *L. huidobrensis*, 0 to 0.33 ± 0.04 for *L. sativae* and 0 to 0.28 ± 0.06 for *L. trifolii*. Correlation analyses showed that the relationship between punctures and eggs was inconsistent. Assessment of previous rearing history (host plant) on feeding and oviposition in a no-choice setting, *L. huidobrensis* preferences were affected while *L. sativae* did not show any rearing history effect on the host plant selection for feeding but for oviposition. *Liriomyza trifolii* selection for both parameters, on the other hand was not affected by the rearing history. In choice setting, however, *L. huidobrensis* and *L. trifolii* showed that rearing history affected their feeding and oviposition preferences, while only feeding was affected in *L. sativae*. This study stressed the need of understanding feeding and egg laying behaviours of the *Liriomyza* leafminers to develop an effective control package. Previous exposure of these pests to a given plant should also be taken into consideration, and there is a potential of incorporating intercropping in an IPM program to control leafminers.

4.1 Introduction

Liriomyza huidobrensis (Blanchard), *Liriomyza sativae* (Blanchard) and *Liriomyza trifolii* (Burgess) are highly polyphagous, prolific, invasive and chemically resistant (Suryawan and Reyes, 2006). They are currently important pests in areas where horticultural crops are grown in Kenya. The three leafminers are threatening the production of most economically important horticultural crops. *Liriomyza* leafminers were first reported in Kenya in 1976 (Spencer, 1985). The pests cause damage when adult females stipple host plant leaves, feed and lay eggs and larvae mine into the leaf tissues (Wei *et al*., 2000). The resultant losses include aesthetic damage, reduced yield among others (Parrella, 1987; Bjorksten *et al*., 2005). Even though crop losses resulting from *Liriomyza* leafminersô infestations have not been well documented in Kenya, Gitonga (2009) reported a yield decline of 59.9% in snow pea due to *Liriomyza* leafminers infestation. Punctures are first made for feeding and later used for oviposition. The number of feeding punctures is an indicator of host feeding selection of female leafminers in no-choice studies (Wei *et al*., 2000). Host selection is affected by various plant factors such as distribution and density of leaf trichomes and trichomes exudates of some plants (Wei *et al*., 2000). Host plant selection has also been reported to be influenced by the previous experience of the pest on a plant, described by Hopkins Host Selection Principle (HHSP) (Jaenike, 1983). The principle states that chemical experience acquired by the larva of an endopterygote insect can be transferred through the pupal stage to the adult. The genetic variation in behaviour and conditioning during the lifespan of an adult insect can contribute to a preference for the host on which an insect developed (Barron, 2001).

Control of leafminers is problematic because adults rapidly develop pesticide resistance while larvae develop inside the leaves and pupate in the soil, making them inaccessible to many pesticides. The only effective insecticide, abamectin and cyromazine, are either expensive or not locally available to farmers. Moreover, the polyphagous nature of the *Liriomyza* spp. enables them to feed/survive and reproduce on alternative host plants when the preferred ones are not available. Accordingly, generation of information about the feeding and oviposition preferences of these insects on selected host plants is important in understanding the risk levels and the preference parameters relationships which may serve as management tools for this pest. Therefore, the aim of this study was to quantify feeding and oviposition of the *Liriomyza* leafminers, determine the effect of the rearing history on selection and performance of the leafminer, and determine the effect of the simulated cropping on feeding and oviposition of *L. huidobrensis*, *L. sativae* and *L. trifolii*.

4.2 Materials and methods

4.2.1 Leafminer assemblage and rearing

The studies were conducted in the laboratory and greenhouse at International Centre of Insect Physiology and Ecology, using the *Liriomyza* leafminers obtained from the colony maintained at the centre. The *Liriomyza* leafminers were reared separately after molecular identification using PCR-RFLP, based on mitochondrial cytochrome oxidase II gene (Scheffer 2000; Scheffer and Lewis 2001 and 2005 and Kox *et al*. 2005). Adult flies were maintained on the specific host plants as described earlier in section 3.1.

4.2.2 Effect of rearing history of *Liriomyza* species and host plant on adult oviposition and feeding preference

Leafminer adults were reared on four different host plants (faba bean, French bean, tomato and snow peas) separately as stated above. The study was set up to investigate whether *Liriomyza* species exhibit preference for the host plant species on which they had developed. The experiment was conducted separately for the three *Liriomyza* species; *L. huidobrensis*, *L. sativae* and *L. trifolii*. For a given *Liriomyza* species, the ability of the fly to feed and oviposit in the subsequent host plants was tested in a choice and no choice tests.

No-choice experiment

Four potted plants of a particular plant species were placed together in a wooden cage of measurements and design described in section 3.3. Twenty three-day-old male and female leafminer flies obtained from the same rearing host plant were released in each cage and allowed to oviposit for 24 hours on the potted plants. Females and males were used together during the bioassays to allow for copulation, in case it did not take place before the females were released in the experimental cages. After oviposition, the potted plants were removed from the oviposition cages, and replaced with four new potted plants for another 24 hours. Infested plants were transferred to another similar sized cage. The experiments were replicated eight times. The number of punctures (feeding and oviposition) was assessed per plant and leaf area (cm^2). Following the differences in the leaf sizes of the plant species, it was thus necessary to determine leaf areas through scanning, for all leaves used in the destructive sampling. Punctures and eggs were counted from the whole leaf then the numbers divided with the respective leaf area.

Choice experiment

The choice experiment was conducted with the four LMF sub-populations reared on selected host plants (faba bean, French bean, tomato and snow peas) as described above. Four potted plants (one each) were randomly placed in a cage similar to the one described above. Cohorts of 3-day-old males and females (20 each) were released in the cage and allowed to oviposit for a period of 24 hours. The potted plants were then removed from the oviposition cages, and flies were provided with new potted plants as above for oviposition for an additional 24 hours. Infested plants were transferred to another similar sized cage. The number of punctures was assessed per plant species and leaf area (cm^2) and the number of eggs laid determined as described above. The experiment was replicated eight times for each *Liriomyza* species sub-population.

4.2.3 Testing of a suitable leafminer egg staining technique

Egg staining technique for the three leafminers; *L. sativae*, *L. trifolii* and *L. huidobrensis* eggs was developed from a modification of methods used independently by Simonet and Pienkowski (1977), Parrella and Robb (1982) and Martin *et al*. (2005). The procedure has been successfully tested and used to clearly count the number of eggs impeded in various host plants tissues. This resulted into a clear improvement of count results, within the leaf tissues in all host plants tested in this study (Plate 4.1). Slight modification was however necessary for the effectiveness of the technique, where, while Parrella and Robb (1982) noted that tomato leaves should not be boiled in the stain, this study found out that eggs were not visible without boiling. Therefore, all leaves in this study, regardless of the plant species type, had to be boiled in the solution for the eggs to pick the stain colour.

The egg staining solution (lactophenol-acid fuchsin) was prepared by mixing the following: one part lactic acid, one part distilled water, two parts glycerine, one part phenol and 0.1 % acid fuchsin powder. Leaves were boiled in the solution for 3 to 5 minutes, then left to stand and cool for 3 to 5 hours. Thereafter, leaves were washed with warm water to remove excess stain, and then put in Petri dishes containing warm water for observation under a dissecting microscope (a digital microscope was used to capture images). This was necessary because the eggs are inserted inside the leaf tissue, coupled with the fact that these leafminer eggs are translucent. After a staining procedure, eggs were counted easily under a dissecting/ digital microscope at a magnification of 20× (Plate 4.1).

4.2.4 Determination of the leaf area

Scanning was done using Canoscan LiDE 50 scanner (1200 x 2400 dpi and 118.11 pixels per cm). The area was converted from pixels to cm^2 (pixels $/118.11^2$ = area in cm^2). The area of each leaf was determined and the counts converted to feeding and oviposition stipples per unit leaf area, respectively. The preference study described above was also carried out using *L. trifolii* and *L. sativae* each reared on all three host plants (*V. faba*, *P. vulgaris* and *L. esculentum*) separately and the number of feeding and oviposition punctures observed and recorded per plant and per leaf as stated above.

4.2.5 Data analyses

Preference data; puncture and egg counts for choice and no choice experiments were analyzed using PROC GLIMMIX (SAS Institute, 2008) with a normal distribution and an identity link function. Following a significant *F* test, means were separated by the SAS PDMIX 800 Macro, using Tukey Kramer procedure of SAS 9.2 (SAS Institute, 2008). Correlation analysis PROC CORR. was used to relate the number of punctures and eggs laid by leafminers on different host plants. Pooled data for choice and no-choice per rearing host plant were analyzed using PROC NPAR1WAY for Kruskal-Wallis test.

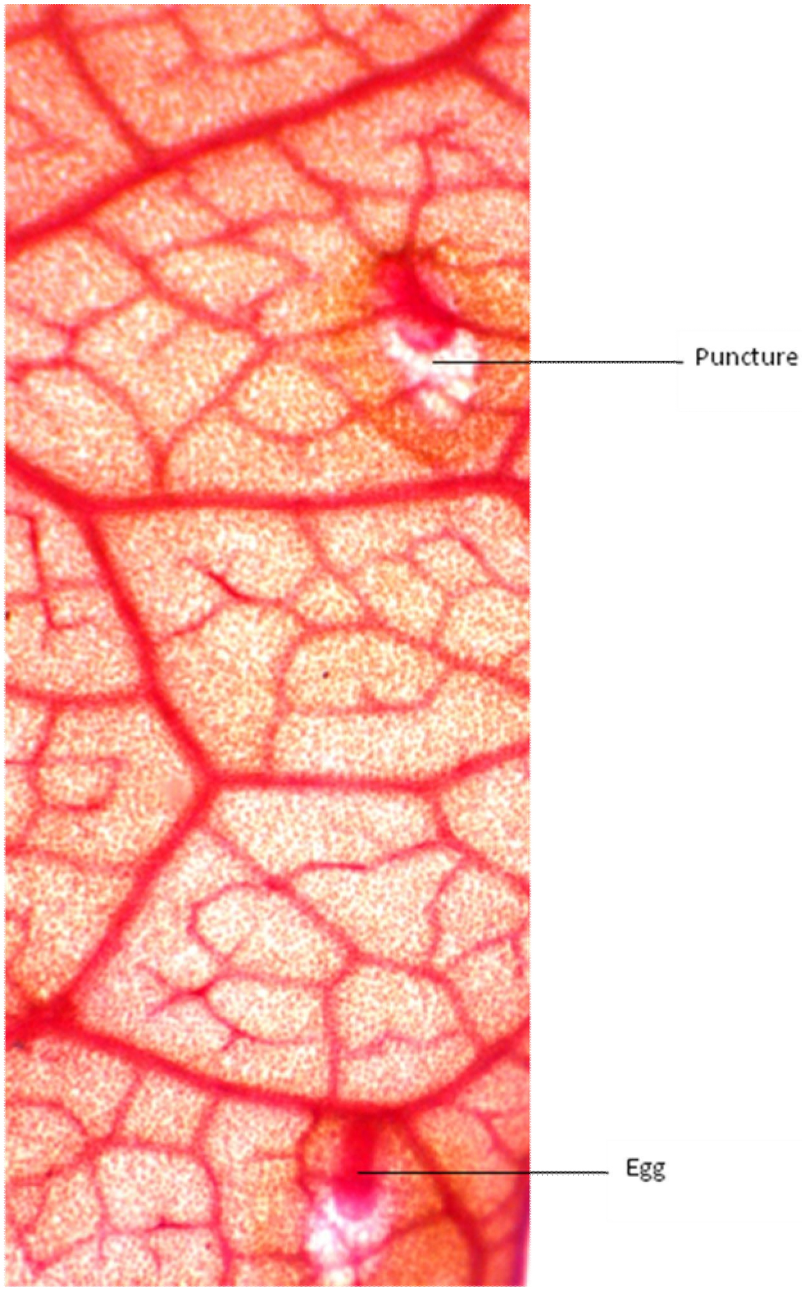

Plate 4.1 Stained leaf showing punctures and eggs

4.3 Results

4.3.1 Feeding and oviposition preference of *Liriomyza* species reared on *Vicia faba*

Punctures made by *Liriomyza* species reared on *Vicia faba*

No-choice experiment. In the no-choice studies, there were significant differences in the number of punctures among the *Liriomyza* leafminers species on all the four host plants species (*V. faba*, $F = 18.70$; d.f. = 2,117; $P < 0.0001$; *P. sativum*, $F = 28.79$; d.f. = 2,117; $P < 0.0001$; *P. vulgaris*, $F = 8.36$; d.f. = 2,117; $P = 0.0004$; *L. esculentum*, $F = 10.51$; d.f. = 2,117; $P < 0.0001$). *Liriomyza huidobrensis* had the highest feeding preference for *V. faba* and *L. esculentum* compared to *L. sativae* and *L. trifolii*. *Liriomyza trifolii* preferred *P. vulgaris* when compared to the other two *Liriomyza* leafminers. Feeding preferences of the three *Liriomyza* leafminer also differed significantly among the four host plants (*L. huidobrensis*, $F = 18.85$; df = 3,156; $P < 0.0001$; *L. sativae*, $F = 18.75$; d.f. = 3,156; $P < 0.0001$; *L. trifolii*, $F = 24.75$; d.f. = 3,156; $P < 0.0001$) (Table 4.1). *Liriomyza huidobrensis* highly preferred *L. esculentum*. *Liriomyza sativae* preferred *L. esculentum* and *P. vulgaris*, while *L. trifolii* preferred *P. vulgaris*, followed by *L. esculentum* and *V. faba* in that order. Rearing the three *Liriomyza* leafminers on *V. faba*, did not influence their feeding preference on *V. faba* exposed (Table 4.1).

Choice experiment. *Liriomyza* leafminers differed significantly regarding feeding punctures on the tested plants (*V. faba*, $F = 5.28$; df = 2,93; $P = 0.0068$; *P. sativum*, $F = 7.02$; d.f. = 2,93; $P = 0.0014$; *P. vulgaris*, $F = 27.09$; df = 2,93; $P < 0.0001$; *L. esculentum*, $F = 4.00$; d.f. = 2,93; $P = 0.0216$). *Liriomyza huidobrensis* preferred *V. faba*, *P. sativum* as compared to *L. sativae* which preferred *P. vulgaris* and *L. esculentum*, while *L. trifolii* trailed the two leafminer species. The number of feeding punctures also differed significantly among the host plants for *L. sativae* ($F = 24.81$; d.f. = 3,124; $P < 0.0001$) and *L. trifolii*, $F = 9.14$; d.f. = 3,124; $P < 0.0001$) but not for *L. huidobrensis* ($F = 1.59$; d.f. = 3,124; $P = 0.1959$). *Liriomyza huidobrensis* made equal feeding punctures on all the four host plants. *Liriomyza sativae* had significantly higher feeding preference on *P. vulgaris* as compared to the other three host plants, while *L. trifolii* highly preferred *P. vulgaris*, *V. faba* and *L. esculentum*, then followed by *P. sativum* (Table 4.1). Rearing *Liriomyza* leafminers on *V. faba* affected the selection of *V. faba* for feeding by *L. trifolii* only.

Eggs laid by *Liriomyza* species reared on *Vicia faba*

No-choice. There were significant differences in the number of eggs laid among LMF species on the tested host plants (*V. faba*, $F = 4.11$; d.f. = 2,117; $P = 0.0188$; *P. sativum*, $F = 7.44$; d.f. = 2,117; $P = 0.0009$; *P. vulgaris*, $F = 6.97$; d.f. = 2,117; $P = 0.0014$; *L. esculentum*, $F = 8.64$; d.f. = 2,117; $P = 0.0003$). *Liriomyza huidobrensis* had the highest oviposition level on all plants when compared to *L. sativae* and *L. trifolii*. *Liriomyza sativae* laid significantly more eggs than *L. trifolii* when reared on *V. faba* while the oviposition of *L. trifolii* was significantly higher than *L. sativae* on *L. esculentum* and *P. vulgaris* (Table 4.2). The number of eggs also differed significantly among the host plants (*L. huidobrensis*, $F = 14.88$; d.f. = 3,156; $P < 0.0001$; *L. sativae*, $F = 8.94$; d.f. = 3,156; $P < 0.0001$; *L. trifolii*, $F = 11.25$; d.f. = 3,156; $P < 0.0001$). *Liriomyza huidobrensis* laid more eggs on *L. esculentum*. *Liriomyza sativae* laid more eggs on *V. faba* and *L. esculentum*, while *L. trifolii* laid more eggs on *L. esculentum* and *P. vulgaris*. Rearing *Liriomyza* leafminers on *V. faba* only influenced the oviposition preference on *V. faba* by *L. sativae* (Table 4.2).

Choice experiment. In choice experiment, analysis revealed the existence of significant differences in eggs laid among LMF species (*V. faba*, $F = 11.55$; d.f. = 2,93; $P < 0.0001$; *P. sativum*, $F = 0$; d.f. = 2,93; *P. vulgaris*, $F = 21.01$; d.f. = 2,93; $P < 0.0001$; *L. esculentum*, $F = 12.10$; d.f. = 2,93; $P < 0.0001$). *Liriomyza sativae* showed highest oviposition on *P. vulgaris*, *L. esculentum* and *V. faba* as compared to *L. huidobrensis* and *L. trifolii* (Table 4.2). Eggs laid by *Liriomyza* leafminers also differed significantly among the four host plants. *Liriomyza huidobrensis* ($F = 3.79$; d.f. = 3,124; $P = 0.0121$) laid more eggs on *V. faba*, which were not significantly different from those that were laid on *L. esculentum* and *P. vulgaris*. *Liriomyza sativae* ($F = 9.94$; d.f. = 3,124; $P < 0.0001$) laid more eggs on *P. vulgaris*, *L. esculentum* and *V. faba* as compared to *P. sativum*, while *L. trifolii* ($F = 3.25$; d.f. = 3,124; $P = 0.0243$) laid more eggs on *P. vulgaris*, which were significantly different from those laid on *L. esculentum* and *V. faba* (Table 4.2). . Rearing *Liriomyza* leafminers on *V. faba* only influenced the oviposition preference on *V. faba* by *L. huidobrensis* (Table 4.2).

Table 4. 1 Feeding punctures produced per cm^2 by *Liriomyza* species reared on *Vicia faba* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	4.21±0.46^{bA*}	2.83±0.28^{bB}	1.32±0.2^{cC}
	P. sativum	3.21±0.60^{b}	-	-
	P. vulgaris	6.27±0.74^{bB}	4.81±0.72^{aB}	10.99±1.64^{aA}
	L. esculentum	11.59±1.36^{aA}	5.00±0.74^{aB}	5.81±1.13^{bB}
Choice	*V. faba*	3.32±0.50^{aA}	2.27±0.50^{bAB}	1.31±0.27^{aB}
	P. sativum	1.47±0.52^{aA}	0.12±0.06^{bB}	0.50±0.04^{bB}
	P. vulgaris	2.76±0.04^{aB}	11.75±1.77^{aA}	1.49±0.30^{aB}
	L. esculentum	2.45±0.88^{aAB}	3.59±0.87^{bA}	0.74±0.14^{abB}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species) are not significantly different (Tukey Kramer test, *P* Ò0.05).

Table 4. 2 Eggs oviposited per cm^2 by *Liriomyza* species reared on *Vicia faba* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	0.09±0.02^{bAB*}	0.17±0.04^{aA}	0.07±0.02^{bB}
	P. sativum	0.06±0.02^{b}	-	-
	P. vulgaris	0.21±0.04^{bA}	0.05±0.01^{bB}	0.28±0.06^{aA}
	L. esculentum	0.57±0.11^{aA}	0.14±0.04^{abB}	0.28±0.06^{aA}
Choice	*V. faba*	0.04±0.02^{aB}	0.19±0.03^{aA}	0.05±0.02^{abB}
	P. sativum	-	-	-
	P. vulgaris	0.01±0^{abB}	0.29±0.05^{aA}	0.07±0.02^{aB}
	L. esculentum	0.01±0.01^{abB}	0.22±0.05^{aA}	0.06±0.02^{abB}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species) are not significantly different (Tukey Kramer test, *P* Ò0.05).

Relationship between feeding punctures and eggs deposited

In no-choice bioassays, Table 4.3 shows that feeding preferences were positively correlated with oviposition preferences on *V. faba*, *P. vulgaris* and *L. esculentum* for *L. huidobrensis*, on *P. vulgaris* only for *L. sativae* and, on *P. vulgaris* and *L. esculentum* for *L. trifolii*. In choice studies, however, a significant positive correlation was present only on *P. vulgaris* for both *L. huidobrensis* and *L. sativae*. When *L. trifolii* was used, a positive significant correlation was observed on *L. esculentum* (Table 4.3).

4.3.2 Feeding and oviposition preference of *Liriomyza* species reared on *Pisum sativum*

Punctures made by *Liriomyza* species reared on *Pisum sativum*

No-choice experiment. In no-choice bioassays, analysis showed presence of significant (*F* = 3.80; d.f. = 3,156; *P* = 0.0116) host plant effect on feeding preference by *L. huidobrensis* (Table 4.4). *Lycopersicon esculentum* was the most preferred host plant as compared to *P. vulgaris* and *P. sativum*, which were followed by *V. faba*. Rearing *L. huidobrensis* on *P. sativum* did not influence the feeding preference of *L. huidobrensis* to *P. sativum* (Table 4.4).

Choice experiment. In choice bioassay, on the other hand, there was no significant influence of host plant on the feeding by *L. huidobrensis* (*F* = 2.35; d.f. = 3,124; *P* = 0.076). Rearing *L. huidobrensis* on *P. sativum* did not influence the feeding preference of *L. huidobrensis* to *P. sativum* (Table 4.4).

Eggs laid by *Liriomyza* species reared on *Pisum sativum*

In both no-choice (*F* = 0.55; d.f. = 3,156; *P* = 0.6480) and choice (*F* = 2.13; d.f. = 3,124; *P* = 0.1004) experiments, egg density analyses revealed the absence of significant differences in oviposition preference of *L. huidobrensis* (Table 4.4). Rearing *L. huidobrensis* on *P. sativum* did not influence its selection of *P. sativum* for oviposition (Table 4.4).

Table 4. 3 Correlation matrix between feeding and oviposition preferences of *Liriomyza* species reared on *Vicia faba* and exposed concurrently to various host plants

Experiment	LMF *spp*	Host plant	*r*	*P- value*
No choice	*L. huidobrensis*	*V. faba*	0.197	0.02223
		P. vulgaris	0.372	0.018
		P. sativum	0.104	0.5250
		L. esculentum	0.401	0.0104
	L. sativae	*V. faba*	0.164	0.3130
		P. vulgaris	0.585	< 0.0001
		L. esculentum	0.038	0.8147
	L. trifolii	*V. faba*	0.300	0.0630
		P. vulgaris	0.493	0.0012
		L. esculentum	0.328	0.0385
Choice	*L. huidobrensis*	*V. faba*	-0.084	0.6464
		P. vulgaris	0.418	0.0172
		P. sativum	.	.
		L. esculentum	0.280	0.1204
	L. sativae	*V. faba*	0.171	0.3502
		P. vulgaris	0.502	0.0034
		L. esculentum	0.170	0.3510
	L. trifolii	*V. faba*	0.271	0.1338
		P. vulgaris	0.087	0.6364
		L. esculentum	0.369	0.0376

Table 4. 4 Feeding punctures produced and eggs laid per cm^2 by *Liriomyza* species reared on *Pisum sativum* and exposed concurrently to various host plants

Experiment	Host plant	Puncture	Egg
No choice	*V. faba*	$4.41\pm0.43^{b*}$	0.11 ± 0.03^{a}
	P. sativum	7.68 ± 1.73^{ab}	0.07 ± 0.04^{a}
	P. vulgaris	5.78 ± 0.70^{ab}	0.11 ± 0.04^{a}
	L. esculentum	10.46 ± 1.89^{a}	0.13 ± 0.03^{a}
Choice	*V. faba*	4.89 ± 0.57^{a}	0.05 ± 0.02^{a}
	P. sativum	2.95 ± 0.74^{a}	0.03 ± 0.02^{a}
	P. vulgaris	3.19 ± 0.56^{a}	0.00 ± 0.00^{a}
	L. esculentum	4.24 ± 0.47^{a}	0.01 ± 0.01^{a}

*Means followed by the same letter in a column for a given experiment type are not significantly different (Tukey Kramer test, *P* Ò0.05)

Relationship between feeding punctures and eggs deposited

In no-choice studies, feeding punctures and oviposition by *L. huidobrensis* were positively and significantly correlated with all tested plants (Table 4.5). In choice experiment, the relationship between the two variables was only positive and significant on *V. faba*. There was no relationship between feeding and oviposition on the rest of tested plants (Table 4.5).

4.3.3 Feeding and oviposition preference of *Liriomyza* species reared on *Phaseolus vulgaris*

Punctures made by *Liriomyza* species reared on *Phaseolus vulgaris*

No choice experiment. There were significant differences in the number of punctures among the LMF species for all the tested host plants (*V. faba*, $F = 6.85$; d.f. = 2,117; $P = 0.0015$; *P. sativum*, $F = 44.99$; d.f. = 2,117; $P < 0.0001$; *P. vulgaris*, $F = 29.13$; d.f. = 2,117; $P < 0.0001$; *L. esculentum*, $F = 19.82$; d.f. = 2,117; $P < 0.0001$) (Table 4.6). *Liriomyza huidobrensis* had the highest feeding preference on all the tested host plants compared to the other two *Liriomyza* leafminers. Feeding preference of the three leafminers species differed significantly among host plants (*L. huidobrensis*, $F = 12.15$; df = 3,156; $P < 0.0001$; *L. sativae*, $F = 8.70$; d.f. = 3,156; $P < 0.0001$; *L. trifolii*, $F = 26.40$; d.f. = 3,156; $P < 0.0001$) where *L. huidobrensis* and *L. sativae* highly preferred *L. esculentum* and *P. vulgaris* while *L. trifolii* highly preferred *L. esculentum* (Table 4.6). Rearing the three *Liriomyza* leafminers on *P. vulgaris* seemed to influence selection of *P. vulgaris* for feeding by *L. huidobrensis* and *L. sativae* (Table 4.6).

Choice experiment. In choice bioassay, there were significant differences in the number of punctures among the *Liriomyza* LMF species for all tested host plants (*V. faba*, $F = 3.53$; d.f. = 2,93; $P = 0.033$; *P. sativum*, $F = 17.42$; df = 2,93; $P < 0.0001$; *P. vulgaris*, $F = 3.47$; df = 2,93; $P = 0.0353$; *L. esculentum*, $F = 6.50$; d.f. = 2,93; $P = 0.0023$) (Table 4.6). Even though *L. huidobrensis* had the highest feeding preference for all host plants, *L. sativae* on *V. faba* and *L. esculentum*, and *L. trifolii* on *P. vulgaris* produced significantly similar responses. Significant host plant effect did not exist on feeding preferences for *L. huidobrensis* ($F = 2.25$; d.f. = 3,124; $P = 0.0858$) while *L. sativae* ($F = 13.16$; d.f. = 3,124; $P < 0.0001$) highly preferred *V. faba* and *L. esculentum*, and *L. trifolii* ($F = 19.37$; d.f. = 3,124; $P < 0.0001$) highly preferred *P. vulgaris* (Table 4.6). Rearing the *Liriomyza* leafminers on *P. vulgaris* only influence selection of host plant for feeding by *L. trifolii* but not on the other two leafminers (Table 4.6).

Table 4. 5 Correlation matrix between feeding and oviposition preferences of *Liriomyza* species reared on *Pisum sativum* and exposed concurrently to various host plants

Experiment	LMF *spp*	Host plant	*r*	*P- value*
No choice	*L. huidobrensis*	*V. faba*	0.541	0.0003
		P. vulgaris	0.385	0.0143
		P. sativum	0.582	< 0.0001
		L. esculentum	0.467	0.0024
Choice	*L. huidobrensis*	*V. faba*	0.392	0.0266
		P. vulgaris	-0.148	0.4183
		P. sativum	0.338	0.0581
		L. esculentum	-0.291	0.1067

Table 4. 6 Feeding punctures produced per cm^2 by *Liriomyza* species reared on*Phaseolus vulgaris* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	$5.53\pm0.76^{bA*}$	2.72 ± 0.57^{bcB}	3.05 ± 0.38^{bB}
	P. sativum	11.00 ± 1.64^{bA}	-	-
	P. vulgaris	20.01 ± 2.74^{aA}	4.44 ± 0.44^{abB}	4.71 ± 0.70^{bB}
	L. esculentum	23.34 ± 3.35^{aA}	5.88 ± 1.55^{aB}	7.12 ± 0.85^{aB}
Choice	*V. faba*	3.28 ± 0.51^{aA}	3.37 ± 0.36^{aA}	1.95 ± 0.38^{bB}
	P. sativum	2.55 ± 0.58^{aA}	0.07 ± 0.04^{cB}	0.13 ± 0.06^{cB}
	P. vulgaris	6.85 ± 2.30^{aA}	1.69 ± 0.37^{bB}	4.58 ± 0.60^{aA}
	L. esculentum	5.78 ± 1.21^{aA}	3.41 ± 0.68^{aA}	1.48 ± 0.46^{bcB}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species) are not significantly different (Tukey Kramer test, *P* Ò0.05).

Eggs laid by *Liriomyza* species reared on *Phaseolus vulgaris*

***No-choice experiment*.** In no-choice studies, there were significant differences in the egg densities laid by *Liriomyza* leafminer species on all the host plants except for *L. esculentum* (*V. faba*, $F = 9.39$; d.f. = 2,117; $P = 0.0002$; *P. sativum*, $F = 8.41$; d.f. = 2,117; $P = 0.0004$; *P. vulgaris*, $F = 6.41$; d.f. =2,117; $P = 0.0023$; *L. esculentum*, $F = 1.05$; d.f. = 2,117; $P = 0.3533$) (Table 4.7). *Liriomyza huidobrensis* showed the highest level of oviposition preference on all the four host plants as compared to *L. sativae*, which highly preferred *P. vulgaris* and *L. esculentum*, and *L. trifolii*, which highly preferred *V. faba* and *L. esculentum*. Significant host plant effect was observed on oviposition preferences by *L. sativae*, $F = 25.36$; d.f. = 3,156; $P < 0.0001$; *L. trifolii*, $F = 13.49$; df = 3,156; $P < 0.0001$), but not *L. huidobrensis*, $F = 1.73$; d.f. = 3,156; $P = 0.1639$) (Table 4.7). *Liriomyza sativae* and *L. trifolii* highly preferred *P. vulgaris* and *V. faba,* respectively, while *L. huidobrensis* deposited eggs equally on all the four host plants. Rearing the *Liriomyza* leafminers on *P. vulgaris* only influenced selection of *P. vulgaris* for oviposition by *L. sativae* (Table 4.7).

***Choice experiment*.** There were no significant differences in the egg densities laid by LMF species on *V. faba*, *L. esculentum* and *P. vulgaris* except for *P. sativum* (*V. faba*, $F = 0.31$; d.f. = 2,93; $P = 0.7357$; *P. sativum*, $F = 3.27$; d.f. = 2,93; $P = 0.0424$; *P. vulgaris*, $F = 2.90$; df = 2,93; $P = 0.06$; *L. esculentum*, $F = 3.00$; d.f. = 2,93; $P = 0.0546$). Significant host plant effect was present on oviposition preferences by *L. sativae* ($F = 4.47$; d.f. = 3,124; $P = 0.0051$) and *L. trifolii*($F = 7.12$; d.f. = 3,124; $P = 0.0002$), but not *L. huidobrensis* ($F = 1.24$; d.f. = 3,124; $P = 0.2978$) (Table 4.7). *Liriomyza huidobrensis* showed similar responses on all host plants, while *L. trifolii* laid more eggs on *P. vulgaris* and *L. esculentum*, and *L. sativae* tended to oviposit more on *L. esculentum*, *P. vulgaris* and *V. faba*. Rearing the *Liriomyza* leafminers on *P. vulgaris* only influence selection of *P. vulgaris* for oviposition by *L. trifolii* but not on the other two leafminers (Table 4.7).

Relationship between feeding punctures and eggs deposited

In no-choice experiment, feeding punctures were positively and significantly correlated only on *P. vulgaris* for both *L. huidobrensis* and *L. sativae*, and *V. faba* for *L. trifolii* (Table 4.8). While in choice study, a positive and significant correlation was found on *V. faba*, *P. vulgaris* and *L. esculentum* for *L. huidobrensis* and *L. trifolii*, and on *L. esculentum* only for *L. sativae* (Table 4.8).

Table 4. 7 Eggs oviposited per cm^2 by *Liriomyza* species reared on *Phaseolus vulgaris* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	0.15±0.03^{aAB*}	0.04±0.01^{cB}	0.25±0.05^{aA}
	P. sativum	0.14±0.05^{aA}	-	-
	P. vulgaris	0.29±0.09^{aA}	0.33±0.04^{aA}	0.06±0.02^{bcB}
	L. esculentum	0.14±0.05^{aA}	0.20±0.04^{bA}	0.12±0.03^{bA}
Choice	*V. faba*	0.08±0.03^{aA}	0.07±0.02^{abA}	0.10±0.03^{bcA}
	P. sativum	0.07±0.04^{aA}	-	-
	P. vulgaris	0.11±0.03^{aA}	0.15±0.04^{abA}	0.25±0.05^{aA}
	L. esculentum	0.03±0.02^{aB}	0.23±0.09^{aA}	0.16±0.05^{abAB}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species) are not significantly different (Tukey Kramer test, *P* Ò0.05).

Table 4. 8 Correlation matrix between feeding and oviposition preferences of *Liriomyza* species reared on *Phaseolus vulgaris* and exposed concurrently to various host plants

Experiment	LMF *spp*	Host plant	*r*	*P- value*
No choice	*L. huidobrensis*	*V. faba*	0.202	0.2113
		P. vulgaris	0.680	< 0.0001
		P. sativum	0.205	0.2051
		L. esculentum	0.010	0.9504
	L. sativae	*V. faba*	0.270	0.0914
		P. vulgaris	0.339	0.0324
		L. esculentum	0.223	0.1659
	L. trifolii	*V. faba*	0.435	0.0050
		P. vulgaris	-0.0313	0.8479
		L. esculentum	0.175	0.2808
Choice	*L. huidobrensis*	*V. faba*	0.405	0.0213
		P. vulgaris	0.430	0.0140
		P. sativum	0.305	0.0891
		L. esculentum	0.652	< 0.0001
	L. sativae	*V. faba*	0.250	0.1685
		P. vulgaris	0.230	0.2062
		L. esculentum	0.564	0.0008
	L. trifolii	*V. faba*	0.580	0.0005
		P. vulgaris	0.505	0.0032
		L. esculentum	0.543	0.0013

4.3.4 Feeding and oviposition preference of *Liriomyza* species reared on *Lycopersicon esculentum*

Punctures made by *Liriomyza* species reared on *Lycopersicon esculentum*

No-choice experiment. In no-choice experiments, analyses showed existence of significant host plant effect on feeding by *L. huidobrensis* (*F* = 3.75; df = 3,156; *P* = 0.0123) (Table 4.9). This *Liriomyza* leafminer made most punctures on *P. vulgaris* and *V. faba* than the other two host plants. Rearing the *L. huidobrensis* on *L. esculentum* did not influence selection of host plant for feeding (Table 4.9).

Choice experiment. In choice bioassay, significant differences existed on the number of feeding punctures among *Liriomyza* leafminers on the tested host plants (*V. faba*, *F* = 23.89; d.f. = 2,93; *P* < 0.0001; *P. sativum*, *F* = 7.82; d.f. = 2,93; *P* = 0.0007; *P. vulgaris*, *F* = 9.90; d.f. = 2,93; *P* = 0.0001; *L. esculentum*, *F* = 7.51; d.f. = 2,93; *P* = 0.0009). Significant host plant effect was present on feeding preferences by *L. huidobrensis* (*F* = 1.24; d.f. = 3,124; *P* = 0.2978), *L. sativae* (*F* = 6.21; d.f.= 3,124; *P* = 0.0006) and *L. trifolii* (*F* = 6.74; d.f. = 3,124; *P* = 0.0003), (Table 4.9). *Liriomyza huidobrensis* highly preferred all the hosts used compared to the other *Liriomyza* leafminers and showed the highest feeding preference on *V. faba*, *P. vulgaris* and *L. esculentum* compared to *P. sativum*. *Liriomyza sativae* highly preferred *V. faba* and *P. vulgaris*, while *L. trifolii* showed highest preference on *P. vulgaris* and *L. esculentum* (Table 4.9). Rearing the *Liriomyza* leafminers on *L. esculentum* was observed to influence selection of *L. esculentum* for feeding by *L. huidobrensis* and *L. trifolii* (Table 4.9).

Eggs laid by *Liriomyza* species reared on *Lycopersicon esculentum*

No-choice experiment. In no-choice test, analysis of egg densities revealed the existence of significant host plant effect on *L. huidobrensis* (*F* = 10.85; d.f. = 3,156; *P* < 0.0001) (Table 4.10). It was observed that more eggs were laid on *P. sativum*, than the other three host plants. Rearing the *L. huidobrensis* on *L. esculenum* did not influence selection of host plant for oviposition by this fly (Table 4.10).

Choice experiment. In choice experiment, there were significant differences in the number of punctures made by LMF species for *V. faba* but not for *L. esculentum, P. vulgaris* and *P. sativum* (*V. faba*, *F* = 10.63; d.f. = 2,93; *P*< 0.0001; *P. sativum*, *F* = 1.00; d.f. = 2,93; *P* = 0.3718; *P. vulgaris*, *F* = 2.31; d.f. = 2,93; *P* = 0.1046; *L. esculentum*, *F* = 3.20; d.f. = 2,93; *P*

= 0.0453) (Table 4.10). *Liriomyza huidobrensis* had the highest preference level for all the host plants tested compared to *L. sativae* and *L. trifolii*, which similarly showed highest preference for *P. vulgaris* and *L. esculentum*. Significant host plant effect was present on oviposition preferences by *L. huidobrensis* (*F* = 1.24; d.f. = 3,124; *P* < 0.0001), *L. sativae*, (*F* = 8.73; d.f. = 3,124; *P* < 0.0001) and *L. trifolii* (*F* = 6.98; d.f. = 3,124; *P* = 0.0002). In the case for *L. huidobrensis*, the highest oviposition preference was on *V. faba*, while *L. sativae* and *L. trifolii* showed the highest preference level on *L. esculentum* and *P. vulgaris* (Table 4.10). Rearing the three *Liriomyza* leafminers on *L. esculentum* only influenced selection of *L. esculentum* for oviposition by *L. sativae* (Table 4.10).

Relationship between feeding punctures and eggs deposited

In no-choice experiment, feeding and oviposition preferences were not significantly correlated on all host plants tested for *L. huidobrensis*. In choice study, a positive significant correlation was observed on *V. faba* and *L. esculentum* for *L. huidobrensis* and on *P. vulgaris* for *L. trifolii* (Table 4.11).

Table 4. 9 Feeding punctures produced per cm^2 by *Liriomyza* species reared on *Lycopersicon esculentum* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	4.94±0.56^{ab*}	.	.
	P. sativum	3.76±1.25^{b}	.	.
	P. vulgaris	7.21±0.72^{a}	.	.
	L. esculentum	3.99±0.50^{b}	.	.
Choice	*V. faba*	9.55±0.94^{aA}	4.47±1.25^{aB}	0.75±0.15^{bC}
	P. sativum	1.86±0.64^{bA}	0^{bB}	0.13±0.06^{bB}
	P. vulgaris	9.07±0.90^{aA}	4.75±0.94^{aB}	3.44±0.96^{aB}
	L. esculentum	11.45±2.93^{aA}	2.35±0.83^{bB}	3.26±0.87^{aB}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species)are not significantly different (Tukey Kramer test, *P* Ò0.05).

Table 4. 10 Eggsoviposited per cm^2 by *Liriomyza* species reared on *Lycopersicon esculentum* and exposed concurrently to various host plants

Experiment	Host plant	*Liriomyza huidobrensis*	*Liriomyza sativae*	*Liriomyza trifolii*
No choice	*V. faba*	0.03 ± 0.01^{b*}	.	.
	P. sativum	0.22 ± 0.05^{a}	.	.
	P. vulgaris	0.10 ± 0.02^{b}	.	.
	L. esculentum	0.05 ± 0.01^{b}	.	.
Choice	*V. faba*	0.32 ± 0.07^{aA}	0.10 ± 0.02^{bcB}	0.05 ± 0.02^{bcB}
	P. sativum	0.02 ± 0.02^{bA}	0^{cA}	0^{cA}
	P. vulgaris	0.01 ± 0.02^{bA}	0.17 ± 0.04^{abA}	0.25 ± 0.07^{aA}
	L. esculentum	0.08 ± 0.04^{bA}	0.23 ± 0.05^{aA}	0.22 ± 0.05^{abA}

*Means followed by the same letter in lower case in a column for a given experiment type (comparison among test host plants) and those followed by the same letter in upper case in a row for a given experiment type (comparison among *Liriomyza* species)are not significantly different (Tukey Kramer test, *P* Ò0.05).

Table 4. 11 Correlation matrix between feeding and oviposition preferences of *Liriomyza* species reared on *Lycopersicon esculentum* and exposed concurrently to various host plants

Experiment	LMF *spp*	Host plant	*r*	*P*- value
No choice	*L. huidobrensis*	*V. faba*	0.249	0.1210
		P. vulgaris	-0.224	0.1644
		P. sativum	-0.050	0.7585
		L. esculentum	-0.049	0.7625
Choice	*L. huidobrensis*	*V. faba*	0.357	0.0451
		P. vulgaris	0.155	0.3969
		P. sativum	0.767	< 0.0001
		L. esculentum	0.042	0.8175
	L. sativae	*V. faba*	0.188	0.3028
		P. vulgaris	0.623	0.0001
		L. esculentum	0.139	0.4478
	L. trifolii	*V. faba*	0.220	0.2268
		P. vulgaris	0.510	0.0029
		L. esculentum	-0.137	0.4542

4.3.5 Comparison between choice and no-choice assays as a simulated cropping system effect on feeding and oviposition behaviour of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* as simulated in choice and no-choice experiments

a. Punctures made by Liriomyza leafminers in choice and no-choice experiments

Experimental setup choice significantly affected feeding preferences of the tested *Liriomyza* leafminers. All the leafminers made more feeding punctures in no-choice experiments than in the choice ones, a part from *L. sativae* that was previously reared on *V. faba* (G^2 = 14.28; *P* =0.0002; no-choice, 4.21±0.37; choice, 4.23±0.64) and *L. huidobrensis* previously reared on *L. esculentum* (G^2 = 0.6502; *P* =0.03; no-choice, 4.97±0.42; choice, 7.98±0.87), which interestingly made most punctures in choice set-up (Table 4.12).

b. Eggs laid by Liriomyza leafminers in choice and no-choice experiments

Analysis showed that experimental set-up choice significantly affected the oviposition preference of the tested *Liriomyza* leafminers (Table 4.13). Tested *Liriomyza* leafminers laid significantly more eggs in no-choice experiments than in the choice ones, a part from *L. sativae* that was previously reared on *V. faba* (G^2 = 1.74; *P* =0.1867; no-choice, 0.12±0.02; choice, 0.18±0.02), *L. trifolii* reared on *P. vulgaris* (G^2 = 1.09; *P* =0.2961; no-choice, 0.14±0.02; choice, 0.13±0.02) and *L. huidobrensis* previously reared on *L. esculentum* (G^2 = 0.21; *P* =0.6502; no-choice, 0.10±0.01; choice, 0.13±0.02), which were not significantly different (Table 4.13).

Table 4. 12 Mean punctures in cm^2 made *Liriomyza* leafminers in choice and no-choice experiments

		Mean ±S.E.			
Rearing Host	LMF	no-choice	choice	G^2	*P*
V. faba	*L. huidobrensis*	6.32±0.50*	2.50±0.31*	53.91	< 0.0001
	L. sativae	4.21±0.37*	4.23±0.64*	14.28	0.0002
	L. trifolii	6.04±0.75*	0.90±0.12*	65.85	< 0.0001
P. vulgaris	*L. huidobrensis*	14.97±1.29*	4.61±0.69*	67.1	< 0.0001
	L. sativae	4.35±0.58*	2.13±0.24*	19.51	< 0.0001
	L. trifolii	4.96±0.41*	2.04±0.25*	56.84	< 0.0001
P. sativum	*L. huidobrensis*	7.08±0.69*	3.82±0.30*	16.2	< 0.0001
L. esculentum	*L. huidobrensis*	4.97±0.42*	7.98±0.87*	4.94	0.03

Table 4. 13 Mean eggs in cm^2 made *Liriomyza* leafminers in choice and no-choice experiments

		Mean ±S.E.			
Rearing Host	LMF	no-choice	choice	G^2	*P*
V. faba	*L. huidobrensis*	0.23±0.03*	0.02±0*	56.49	< 0.0001
	L. sativae	0.12±0.02^{ns}	0.18±0.02^{ns}	1.74	0.1867
	L. trifolii	0.21±0.03*	0.05±0*	29.35	< 0.0001
P. vulgaris	*L. huidobrensis*	0.18±0.03*	0.07±0.02*	9.78	0.0018
	L. sativae	0.19±0.02*	0.11±0.02*	10.19	0.0014
	L. trifolii	0.14±0.02^{ns}	0.13±0.02^{ns}	1.09	0.2961
P. sativum	*L. huidobrensis*	0.11±0.02*	0.02±0*	30.04	< 0.0001
L. esculentum	*L. huidobrensis*	0.10±0.01^{ns}	0.13±0.02^{ns}	0.21	0.6502

4.4 Discussion

In this study, the emphasis was placed on *Liriomyza* leafminer femalesô feeding and oviposition on different host plants. This was necessitated since the success in the development of a practical control of these pests lies in the understanding of biological behaviour; how they select hosts for feeding and egg laying purposes, from host settling, probing, to leaf puncturing, for the selection of the suitable host plants acceptable for feeding and eventual oviposition as stated in Wei *et al*. (2000). The technique for staining the eggs of the three *Liriomyza* leafminers within the leaves of all the four hosts as in Parrella and Robb (1982) was successfully tested, but with slight modification. This sensitive biochemical method improved egg count within the leaves that made this and future related studies much easier. Leaf area measurements were done since the host plants used in this study had varying leaf sizes. This was in line with Scheirs *et al*. (2004) who also noted the significance of determining leaf area when using different plant species.

The results of the laboratory bioassays have shown that all the four host plants were suitable for feeding and oviposition by *L. huidobrensis*, except *L. sativae* and *L. trifolii* which could not feed and lay eggs on *P. sativum*. This confirmed that *L. huidobrensis*, *L. trifolii* and *L. sativae* feed and oviposit on a wide host range (Parrella, 1982; Kotze and Dennnill, 1996; Sivapragasam and Syed, 1999; CABI, 2001). There was, however, considerable variation in the level of preference (feeding and oviposition) among the LMF *spp*. and also among the host plants as shown by the puncture and egg density values, which is in line with the findings of Martin *et al*. (2005) and Videla *et al*. (2006). In all the laboratory bioassays, *L. huidobrensis* was the most superior leafminer with regard to feeding and oviposition in both no-choice and choice experiments. Since a positive correlation was observed between performance and adult body size (Videla *et al*. (2006), it was thought that the bigger size of *L. huidobrensis* as compared to *L. sativae* and *L. trifolii*, contributed to its higher rate of feeding and oviposition (adult performance). Musundire (2010) also noted that the bigger size represents a fitness parameter which is strongly related to the feeding strategy of the insect. This could be a contributing factor to its ability to invade new ecological areas. In Peru, for instance, *L. huidobrensis* was also recorded as the most dominant species at 88 % and only followed by *L. sativae* at 8.6 % (Mujica and Kroschel, 2011). Moreover, Spencer (1985) also reported that *L. trifolii* caused serious damage in the lowlands of Kenya.

Feeding bioassays revealed that *L. huidobrensis* preferred *L. esculentum* in most cases in no-choice bioassays. In the choice bioassays, the pest equally prefered the four host plants. *Liriomyza sativae* and *L. trifolii*, on the other hand, preferred *P. vulgaris* and *L. esculentum* in no-choice and the three host plants; *P. vulgaris, L. esculentum* and *V. faba* in choice bioassays. Generally, for the three *Liriomyza spp*. *L. esculentum* and *P. vulgaris* were the hosts that were mostly fed on, when exposed solely to the *Liriomyza* leafminers. This could probably be because of different leaf thicknesses, and higher densities of the palisade and spongy tissues that may have deterred feeding and subsequent oviposition as stated by Wei *et al*. (2000) and Facknath (2005).

The levels of feeding preferences in choice bioassays by the three leafminers were almost similar on all host plants, except for the case of *L. sativae* and *L. trifolii*, where few feeding punctures were made on *P. sativum*. The numbers of punctures made per leaf area were consistent with the findings by Martin *et al*. (2005) and Videla *et al*. (2006). The slight deviations from the results of the two works mentioned here above and this study could be attributed to the difference in the number of female leafminers used in these experiments. In this study, 20 female leafminers at the age of 3 to 4 days old were used, while Martin *et al*. (2005) used 25 females of one-day old. The other possible cause for the disparities could have been the different plants used as hosts in the two studies.

The three LMF *spp*. oviposited more readily on *L. esculentum*, *P. vulgaris* and *V. faba* when exposed separately. As the case for punctures, leafminers oviposited almost equally on all the host plants, except on *P. sativum* for *L. trifolii* and *L. sativae*. The results showed clearly that even in choice experiments, *L. huidobrensis* laid more eggs on *V. faba*, while *P. vulgaris* and *L. esculentum* were most preferred by the other two leafminers. Again, as with number of punctures, host plant attributes such as leaf sizes, palisade and spongy mesophly part of the leaf that provide physical barrier to female oviposition and selection to provide nutrition for offspring could be some of the reasons behind the observed trend (Wei *et al*., 2000; Facknath, 2005). A distinct oviposition behaviour exhibited by the flies was where feeding punctures were made on host plants that did not have any egg in them.*Liriomyza trifolii* and *L. sativae* punctured but did not lay any egg on *P. sativum*. The study has shown that *P. sativum* was not preferred by *L. sativae* and *L. trifolii*. This is contrary to many findings that reveal a primary preference of the leafminer to the plant (CABI, 2001). A possible reason as to why the two leafminers did not find the plant attractive could have been due to lack of local adaptation. Barmosho *et al*. (2009) similarly found low levels of incidences of the two leafminers at higher altitudes where the crop is grown in Kenya.

Correlation between feeding and oviposition preferences was positive and sometimes absent. There was no consistency on host plants for the leafminers tested. A positive correlation means that eggs were laid in the punctures (Martin *et al*., 2005). The lack of consistency in the trend implies that the leafminers were able to feed and lay eggs on the tested host plants; an attribute for maintaining polyphagy. Martin *et al*. (2005) similarly noted a positive but inconsistent correlation pattern.

Feeding and oviposition of the tested *Liriomyza* leafminers could have been influenced, in part, by the rearing host plants. Attraction and feeding/oviposition preference in the adults can be explained by the fact that adults emerging normally from leafminers pick up small amount of host plant chemicals from the pupal case (Corbet, 1985). Corbet (1985) further stated that these chemicals may not be sufficiently strong to attract adults to that particular host type, given that all hosts present food/oviposition sites. The potential of population divergence in host preference has similarly been shown for other *Liriomyza* species (Martin *et al*., 2005), where a general principle was proposed that insects that developed on a given host will show greater preference for that host, thus the Hopkinsô host selection principle (HHSP). This study revealed that HHSP appears to operate in these *Liriomyza* leafminers to some extent. The findings of this study agree with Facknath and Wright (2007) who also noted that emerginal experience of a different host type may not modify the innate attraction/preference sufficiently to lead to no clear cut attraction to any particular variety. The effect of the host plant from which a *Liriomyza* leafminer emerge from (rearing host) is an important aspect. Mixing crop species could be used to contribute to the control of insect pests.

Emden (1989) noted that intercropping led to disruption of the host finding behaviour and increasing the impact of natural enemies. This makes the mixing of crop species strategy of great interest in the development of a diversification strategy for *Liriomyza spp*. practical control package. In this study, sole stand cropping system was simulated as no-choice, while intercrop/mixed cropping as choice. Mixing crop species, as simulated in choice experiments, clearly led to reduced feeding incidences/puncturing and oviposition of *Liriomyza spp*. This finding corroborates with that of Midmore and Alcazar (1991), that mixed planting lowered the *Liriomyza* adult populations and feeding punctures in the subsequent cultivars. There were however cases where *L. huidobrensis* and *L. sativae* made most feeding punctures in choice experiments and statistically equal egg counts in no-choice and choice set-up. These scenarios were thought to be partly influenced by the effect of the rearing host plants, since the behaviour was consistent for both punctures and egg counts when *L. huidobrensis* and *L. sativae* were previously reared on *L. esculentum* and *V. faba*, respectively. In addition, Midmore and Alcazar (1991) also found an almost similar case where mixing cultivars resulted to increased number of offspring in the susceptible cultivar. Nampala *et al*. (2002) found intercropping to be an effective tool, has to be part of the integrated pest management system. From this study, the *Liriomyza* leafminers behaviour on the various host plants were very specific, some of which were thought to be of interest.

Different and distinct feeding behaviours were made by the LMF *spp*.; (1) punctures made on the leaf margins of *P. sativum* by *L. huidobrensis*; (2) punctures made evenly on the leaf surfaces of *P. vulgaris*, *V. faba* and *L. esculentum* by the three leafminers and (3) few punctures made by *L. sativae* and *L. trifolii* on *P. sativum*. Probably, the thick cuticle layer on *P. sativum* might have led to the low preference and attack on the leaf margins by *L. huidobrensis*. Besides the effect of local adaptation, it was also thought that for the few cases where *L. sativae* and *L. trifolii* made punctures on *P. sativum*, that this could be an adaptive strategy, probably to protect the offspring from eventual parasitism (Sadek *et al*., 2010).

CHAPTER FIVE

EFFECT OF SELECTED HOST PLANTS ON DEVELOPMENT, SURVIVAL AND FITNESS OF *Liriomyza huidobrensis, Liriomyza sativae* AND *Liriomyza trifolii*

Abstract

Four host plants; *Vicia faba* L., *Phaseolus vulgaris* L., *Pisum sativum* L. and *Lycopersicon esculentum* M. were evaluated for their suitability for the survival and development of three *Liriomyza* (Diptera: Agromyzidae) leafminers; *Liriomyza huidobrensis* (Blanchard) *Liriomyza sativae* (Blanchard) and *Liriomyza trifolii* (Burgess). The *Liriomyza* leafminers were reared on the above mentioned four host plants, unless otherwise stated, and exposed to the host plants separately. Results showed that host plants affected the rate of development of the flies, taking fewer days to reach the adult stage on *P. vulgaris* and *L. esculentum*, and longest on *V. faba* and *P. sativum*. Generally the flies took an average of between 13 to 19 days to complete development depending on the host plant species and the origin of the adult flies used. Most offsprings were produced from *V. faba* for *L. huidobrensis*, and from *P. vulgaris* and *V. faba* for *L. sativae* and *L. trifolii.* Survival was at its lowest on *L. esculentum* for *L. sativae* and *L. trifolii* (21.42±7.6% to 1.90±1.38%), and on *P. vulgaris* and *L. esculentum* for *L. huidobrensis* (46.71±7.57% to 6.05±1.95%). Pupal weight and sex ratios were not affected by the host plant variety. *Liriomyza huidobrensis* adults emerging from *V. faba* and the other two *Liriomyza* leafminers emerging from *V. faba* and *P. vulgaris* were more aggressive, probably owing to their bigger wings sizes, suggesting the suitability of these host plants for rearing of *Liriomyza* leafminers. This study also confirmed that the three *Liriomyza* species are truly polyphagous, with *L. huidobrensis* being most aggressive and able to complete development on most plants. These findings will be helpful in the decision on when a control intervention is to be initiated for an effective practical control measure.

5.1 Introduction

The *Liriomyza* leafminers; *Liriomyza huidobrensis* (Blanchard) *Liriomyza sativae* (Blanchard) and *Liriomyza trifolii* (Burgess) are polyophagous and have been recorded feeding and developing on several host plants (Spencer, 1990). The species are among the most economically important members of the genus *Liriomyza* (Lanzoni *et al*., 2002), seriously damaging vegetable crops and ornamental plants. Adult females insert eggs within the leaf tissue using the ovipositor. Upon hatching, the larvae (first, second and third instars) mine the leaf tissues through their characteristic feeding behaviour (Spencer, 1973). The resultant damage leads to decline of the expected yield and loss of the aesthetic value (Parrella, 1987). Infestations by these pests, besides the stringent quarantine measures at the export points, can lead to upto 100% crop loss (Spencer, 1973). However, information regarding the magnitude of losses incurred following attack by these pests in Kenya has not been fully documented.

Controlling these pests has been complicated by the fact that they develop resistance to chemical pesticides very fast. These *Liriomyza* leafminers have been shown to develop and survive on various host plants at different rates, depending on the type of the host species (Martin *et al.,* 2005; Facknath and Wright, 2007). Information regarding how these pests develop on the different economically important host plants in Kenya is limited. The aim of this study was therefore, to assess the influence of host plants on the development time, number of offspring, sex ratio, survival rate and fitness of *L. huidobrensis*, *L. trifolii* and *L. sativae*. It is hoped that the knowledge generated from this research will be important for future maintenance of the laboratory cultures of these flies for further research purposes and devising strategies for their biological control.

5.2 Materials and methods

5.2.1 Insects assemblage and rearing

Liriomyza leafminer adults were obtained from the colony raised at *icipe* as earlier described in the general materials and method, Chapter 3. The colonies of the various insect species were kept separately on the different host varieties; *V. faba*, *P. vulgaris*, *L. esculentum* and *P. sativum*. It was however not possible to maintain the colonies of *L. trifolii* and *L. sativae* on *L. esculentum* and *P. sativum* hosts.

5.2.2 Effect of rearing history of *Liriomyza* species and host plant on egg hatching, larval development and survival, and adult emergence

5.2.2.1 Host plant effect on the development of the three *Liriomyza* leafminers

Three-days- old adult males and females (20 each) were put in four experimental cages containing four host plants (as in no-choice) in a 2 x 2 matrix and left for 24 hours. This was then repeated for another 24 hours using fresh plants in four replicates. The plants were observed closely after removal from the experimental cages for the emergence of the first instars. The number of emerging instars was recorded daily as well as the egg hatching duration, which was estimated from the day of exposure for oviposition upto the day of emergence of the first instar larvae. The plants were maintained until the emergence of pupae. The number of days taken by each larva from the date of hatching to pupation was recorded as the larval duration. The collected pupae were again observed daily for the emergence of the flies. The duration taken for the adult to emerge was obtained from the date of larval pupation to adult emergence was recorded as the pupal duration.

5.2.2.2 Suitability of the host plants for development of the progeny and survival of *Liriomyza* leafminers

Pupae dropping and emerging adults from various host plants were counted and recorded accordingly. Survival rate was then obtained by determining the percentage (%) of adults that survived from the larval stage.

5.2.2.3 Influence of host plant species on fitness of the three *Liriomyza* leafminers

a) Pupal weight: Pupae in cohort of 10 each were collected and weighed, at approximately 2 days after pupation.

b) Wing length: Wing length measurements were determined by removing the left wings only and using the horizontal orientation for consistency (Plate 5.1). The measurements were taken in millimetres and recorded separately for males and females using LAS EZ software of leica application, suite version 1.4.0).

5.2.2.4 Effect of host plants on the sex ratio of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii*

Adults emerging from the different host plants were put in a deep freezer for 10 minutes, and then observed under a dissecting microscope (20 x) for the presence of ovipositor, in order to easily separate males and females. These were then counted and recorded. Sex ratio was determined as percentage (%) females divided by total number of adults x 100.

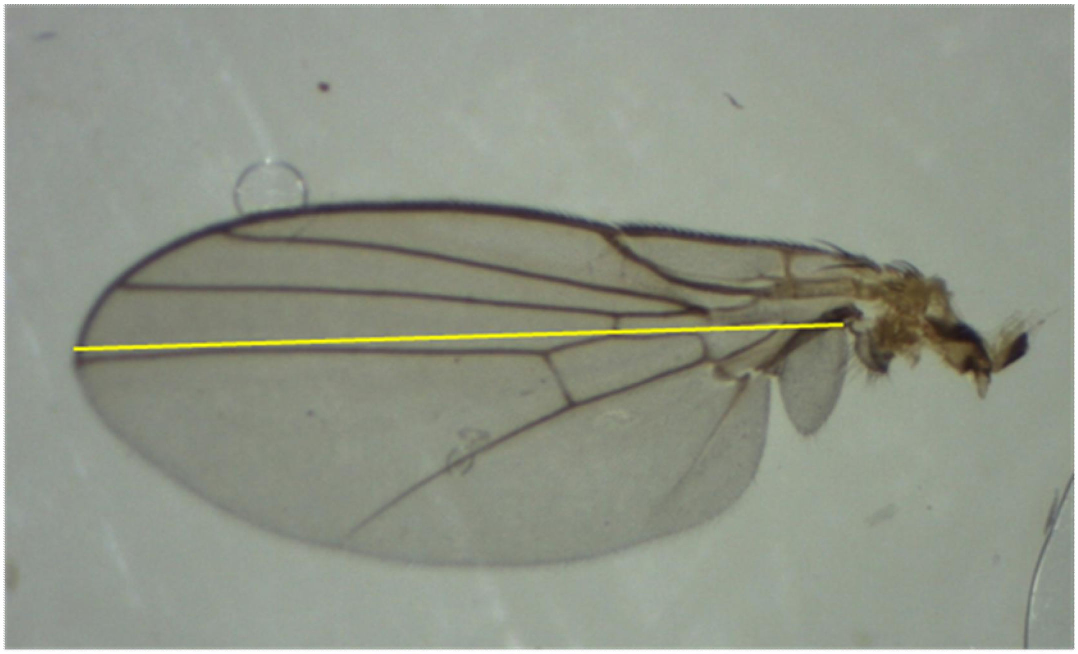

Plate 5.1 *Liriomyza huidobrensis* male left wing (yellow line shows the length measured)

5.2.3 Data analyses

Development time (in days), after homoscedasticity test using PROC UNIVARIATE, were analyzed using one-way analysis of variance (ANOVA) and means separated using SNK procedure. Numbers of pupae and adults emerging from each host plant were log transformed (log (x+1)), subjected to ANOVA and means separated using SNK. Percent adult survival data were arcsine square-root transformed before ANOVA and SNK used to separate the means. Wing length, pupae weight and sex ratios were analyzed using one-way ANOVA and Tukey lines. Analysis of variance was done using PROC GLM at 95% level of significance. All data were analyzed using SAS 9.2 (SAS Institute, 2008).

5.3 Results

5.3.1 Suitability of *Vicia faba*, *Pisum sativum*, *Phaseolus vulgaris* and *Lycopersicon esculentum* for the development of *Liriomyza* leafminers

a) Egg, larval and pupal development time (days) for *Liriomyza spp*. reared on *Vicia faba*

Host plants affected significantly the duration of egg development (For *L. huidobrensis*, *F* = 72.25; d.f. =3,880; *P* < 0.0001; *L. sativae, F* = 378.24; d.f. = 2,371; *P* < 0.0001; *L. trifolii, F* = 719.29; d.f. = 2,730; *P* < 0.0001), larval period (*L. huidobrensis*, *F* = 269.79; d.f. = 3,698; *P* < 0.0001; *L. sativae*, *F* = 373.78; d.f. = 2,250; *P* < 0.0001; *L. trifolii*, *F* = 738.40; d.f. = 2,496; *P* < 0.0001) and pupal period (*L. huidobrensis, F* = 193.88; d.f. = 3,489; *P* < 0.0001; *L. sativae*, *F* = 523.33; d.f. = 2,137; *P* < 0.0001; *L. trifolii*, *F* = 1786.62; d.f. = 2,317; *P* < 0.0001). *Liriomyza huidobrensis* took the shortest period of time (2.95±0.03) days to hatch into first instar larvae on *L. esculentum* and the longest (3.84±0.04) days on *P. vulgaris* (Table 5.1). *Liriomyza sativae* and *L. trifolii* eggs took the least duration (2.39±0.07 and 3.05±0.03 respectively) days on *P. vulgaris* and the longest (4.79±0.06 and 4.87±0.07 days for *L. sativae* and 5.29±0.05) on *V. faba* for *L. trifolii* (Table 5.1).

Larvae of *Liriomyza* took the least number of days on *P. vulgaris* for *L. sativae* (6.28±0.09 days) and on *L. esculentum* for both *L. huidobrensis* (6.38±0.15 days) and *L. trifolii* (6.83±0.14 days). Development was longest on *V. faba* for all the three *Liriomyza* leafminers (Table 5.1).

Pupal duration was shortest on *P. vulgaris* and longest on *V. faba* for *L. sativae* (13.36±0.10 and 18.60±0.12 days respectively) and for *L. trifolii* (13.83±0.06 and 19.15±0.06 respectively).

Liriomyza huidobrensis pupal duration, however, was shortest on *L. esculentum* (14.58±0.23 days) and longest on *P. sativum* (17.43±0.04 days) (Table 5.1).

b) Egg, larval and pupal development time (days) for *Liriomyza* spp. reared on *Phaseolus vulgaris*

Phaseolus vulgaris affected significantly the egg developmental period of *Liriomyza* leafminers (*L. huidobrensis*, F = 118.57; d.f. = 3,419; P < 0.0001; *L. sativae*, F = 45.05; d.f. = 2,460; P < 0.0001; *L. trifolii*, F = 38.61; d.f. =2,496; P < 0.0001), larval duration (*L. huidobrensis*, F = 6.56; d.f. = 3,307; P = 0.0003; *L. sativae*, F = 440.83; df = 2,235; P < 0.0001; *L. trifolii*, F = 151.87; d.f. = 2,319; P < 0.0001) and pupal duration (*L. huidobrensis*, df=3,253; F=6.74; P=.0002; *L. sativae*, df=2,135; F = 733.27; P < 0.0001; *L. trifolii*, F = 928.80; d.f. = 2,189; P < 0.0001). *Liriomyza huidobrensis* took the shortest period of time to hatch into first instar larvae on *P. vulgaris* (2.87±0.06) and *V. faba* (2.96±0.06) and the longest on *L. esculentum* (4.89±0.12), while for both *L. sativae* and *L. trifolii* eggs took less days on *P. vulgaris* (2.61±0.03) and *L. esculentum* (3.39±0.06), respectively (Table 5.2).

Larval duration was shortest on *P. vulgaris* and the longest on *V. faba* for all the tested *Liriomyza* leafminers (Table 5.2). However, pupal development was shortest on the three host plants except on *V. faba* for *L. huidobrensis* and on *P. vulgaris* for both *L. trifolii* and *L. sativae*, in addition to *L. esculentum* for *L. trifolii* (Table 5.2).

c) Egg, larval and pupal development time (days) for *Liriomyza spp.* reared on *Pisum sativum*

Pisum sativum also affected significantly the egg duration of *L. huidobrensis* (F = 100.64; d.f. =3,613; P <.0001), larval duration (F = 147.06; d.f. = 3,402; P < 0.0001) and pupal duration (F = 36.19; d.f. = 3,281; P < 0.0001). Eggs development took less number of days on *V. faba* (2.81±0.05), larvae on *P. vulgaris* (7.76±0.09) and pupae on *L. esculentum* (14.76±0.18) and *P. vulgaris* (14.88±0.11) (Table 5.3).

d) Egg, larval and pupal development time (days) for *Liriomyza spp.* reared on *Lycopersicon esculentum*

Significant host plant effect was present on *L. huidobrensis* egg hatching duration (F = 60.80; d.f. = 3,642; P < 0.0001), larval duration (F = 33.10; d.f. = 3,464; P < 0.0001) and pupal duration (F = 167.10; d.f. = 3,277; P < 0.0001). *Liriomyza huidobrensis* egg development was shortest on *P. vulgaris* (2.76±0.05) and *V. faba* (2.82±0.07), for larval duration on *L. esculentum* (8.04±0.11) and *P. vulgaris* (8.10±0.09) and for pupal development on *P. sativum* (13.83±0.05) (Table5.4).

Table 5.1 Mean development period (in days) of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* reared on *Vicia faba* and tested on selected host plants

Liriomyza species	Host plant	Development period (in days)		
		Egg	Larva	Pupa
L. huidobrensis	*V. faba*	3.68±0.03^{b*}	9.86±0.04^{a}	16.19±0.04^{b}
	P. sativum	3.61±0.07^{b}	9.29±0.04^{b}	17.43±0.04^{a}
	P. vulgaris	3.84±0.04^{a}	7.66±0.08^{c}	16.21±0.11^{b}
	L. esculentum	2.95±0.03^{c}	6.38±0.15^{d}	14.58±0.23^{c}
L. sativae	*V. faba*	4.79±0.06^{a}	9.36±0.07^{a}	18.60±0.12^{a}
	P. vulgaris	2.39±0.07^{b}	6.28±0.09^{c}	13.36±0.10^{c}
	L. esculentum	4.87±0.07^{a}	7.08±0.18^{b}	14.80±0.20^{b}
L. trifolii	*V. faba*	5.29±0.05^{a}	10.17±0.07^{a}	19.15±0.06^{a}
	P. vulgaris	3.05±0.03^{b}	7.22±0.04^{b}	13.83±0.06^{c}
	L. esculentum	3.17±0.07^{b}	6.83±0.14^{c}	15.07±0.25^{b}

*Means followed by the same letters in a column for a given *Liriomyza* species (comparison among test host plants) are not significantly different (SNK, *P* Ò0.05)

Table 5.2 Mean development period (in days) of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* reared on *Phaseolus vulgaris* and tested on selected host plants

Liriomyza species	Host plant	Development period (in days)		
		Egg	Larva	Pupa
L. huidobrensis	*V. faba*	2.96±0.06^{c*}	9.13±0.08^{a}	16.27±0.08^{a}
	P. sativum	3.59±0.10^{b}	8.86±0.09^{a}	15.78±0.10^{b}
	P. vulgaris	2.87±0.06^{c}	8.30±0.17^{b}	15.65±0.15^{b}
	L. esculentum	4.89±0.12^{a}	9.06±0.10^{a}	15.88±0.10^{b}
L. sativae	*V. faba*	3.62±0.12^{a}	8.95±0.12^{a}	18.88±0.11^{a}
	P. vulgaris	2.61±0.03^{c}	5.74±0.04^{c}	13.10±0.08^{c}
	L. esculentum	3.03±0.12^{b}	6.88±0.23^{b}	15.00±0^{b}
L. trifolii	*V. faba*	4.02±0.07^{a}	8.85±0.08^{a}	18.70±0.08^{a}
	P. vulgaris	4.04±0.05^{a}	7.07±0.08^{c}	14.05±0.10^{b}
	L. esculentum	3.39±0.06^{b}	7.55±0.07^{b}	14.24±0.06^{b}

*Means followed by the same letters in a column for a given *Liriomyza* species (comparison among test host plants) are not significantly different (SNK, *P* Ò0.05)

Table 5.3 Mean number of development days for of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* reared on *Pisum sativum* and tested on selected host plants

Liriomyza species	Host plant	Development duration (in days)		
		Egg	Larva	Pupa
L. huidobrensis	*V. faba*	$2.81\pm0.05^{d*}$	9.03 ± 0.09^{b}	15.27 ± 0.10^{b}
	P. sativum	3.56 ± 0.05^{b}	10.27 ± 0.09^{a}	16.30 ± 0.10^{a}
	P. vulgaris	3.12 ± 0.07^{c}	7.76 ± 0.09^{d}	14.88 ± 0.11^{bc}
	L. esculentum	3.92 ± 0.04^{a}	8.17 ± 0.07^{c}	14.76 ± 0.18^{c}

*Means followed by the same letters in a column are not significantly different (SNK, *P* Ò0.05)

Table 5.4 Mean development period (in days) of *Liriomyza huidobrensis*, *Liriomyza sativae* and *Liriomyza trifolii* reared on *Lycopersicon esculentum* and tested on selected host plants.

Liriomyza species	Host plant	Development period (in days)		
		Egg	Larva	Pupa
L. huidobrensis	*V. faba*	$2.82\pm0.07^{c*}$	8.93 ± 0.07^{a}	14.72 ± 0.13^{b}
	P. sativum	3.36 ± 0.04^{b}	8.87 ± 0.06^{a}	13.83 ± 0.05^{c}
	P. vulgaris	2.76 ± 0.05^{c}	8.10 ± 0.09^{b}	15.91 ± 0.09^{a}
	L. esculentum	3.92 ± 0.10^{a}	8.04 ± 0.11^{b}	15.90 ± 0.10^{a}

*Means followed by the same letters in a column are not significantly different (SNK, *P* Ò0.05)

5.3.2 Effect of host plants on the number of *Liriomyza* leafminers pupae produced on selected host plants

a) *Liriomyza* leafminers reared on *Vicia faba*

Significant host plant effect on the number of pupae produced was observed for *L. huidobrensis* (df=3,28; F=5.00; *P*=0.0067; Table 5.5), *L. sativae* (df=2,21; F=9.51; *P*=0.0011; Table 5.6) and for *L. trifolii* (df=2,21; F=16.33; *P*<.0001; Table 5.7). Most *L. huidobrensis* pupae emerged from *V. faba* while those of *L. trifolii* had most pupae emerging from *P. vulgaris* and *V. faba* (Table 5.7). *Vicia faba* also had most pupae for *L. sativae*.

b) *Liriomyza* leafminers reared on *Phaseolus vulgaris*

There was significant host plant effect on the number of pupae produced for *L. huidobrensis* (*F* = 3.26; d.f. = 3,28; *P* = 0.0362; Table 5.5), *L. sativae* (*F* = 22.11; d.f. = 2,21; *P* < 0.0001; Table 5.6) and for *L. trifolii* (*F* = 3.80; d.f. =2,21; *P* = 0.0391; Table 5.7). *Liriomyza huidobrensis* produced most pupae from *V. faba*, while *L. sativae* and *L.trifolii* produced most from *P. vulgaris* (Table 5.5, 5.6, 5.7).

c) *Liriomyza* leafminers reared on *Pisum sativum*

No significant host plant effect was observed for the *L. huidobrensis* tested, with regard to the number of pupae produced (*F* = 0.12; df = 3,28; *P* = 0.9458; Table 5.5). Although statistically similar counts were obtained from all the tested host plants, *P. sativum* produced highest number of pupae than the rest of the host plants (Table 5.5).

d) *Liriomyza leafminers reared on Lycopersicon esculentum*

The number of pupae that emerged from *L. esculentum* was not influenced by the type of host plant on which they were raised (*F* = 1.76; d.f. = 3,28; *P* = 0.1774; Table 5.5). Here too, as observed in Section 5.3.2 (c) above, *P. sativum* supported the emergence of higher number of pupae.

5.3.3 Effect of host plants on emergence of adult *Liriomyza* leafminers

a. Emergence of adult Liriomyza leafminers reared on Vicia faba

Significant host plant effect on the number of adults produced was noted for all the three *Liriomyza* leafminers tested (*L. huidobrensis*, *F* = 5.10; d.f. = 3,28; *P* = 0.0061; Table 5.5; *L.*

sativae, *F* = 4.25; d.f. = 2,21; *P* = 0.0281; Table 5.6; and *L. trifolii, F* = 9 .79; d.f. = 2,21; *P* = 0.0010; Table 5.7). The highest number of *L. huidobrensis* adults emerged from *V. faba* and *P. sativum*, while for *L. trifolii* and *L. sativae,* had their most adults emerging from *P. vulgaris* and *V. faba* (Tables 5.5, 5.6 and 5.7).

b. Emergence of adult Liriomyza leafminers reared on Phaseolus vulgaris

There was significant host plant effect on the number of adults produced by *L. huidobrensis* (*F* = 3.07; d.f. = 3,28; *P* = 0.0440; Table 5.5), *L. sativae* (*F* = 16.31; d.f. =2,21; *P*< 0.0001; Table 5.6) and by *L. trifolii* (*F* = 5.90; d.f. = 2,21; *P* = 0.0092; Table 5.7). Most of *L. huidobrensis* adults emerged from *V. faba*, while most *L. sativae* and *L. trifolii* adults emerged from *P. vulgaris* (Tables 5.5, 5.6 and 5.7).

c. Emergence of adult Liriomyza leafminers reared **on** *Pisum sativum*

There was no significant host plant effect recorded regarding adults emergence for the *L. huidobrensis* (*F* = 0.57; d.f. = 3,28; *P* = 0.6374; Table 5.5).

d. Emergence of adult Liriomyza leafminers reared on Lycopersicon esculentum

The number of adults that emerged from test host plants on which they were reared was not influenced by the type of host plant (*F* = 1.09; d.f. = 3,28; *P* = 0.3689; Table 5.5). Statistically equal *L. huidobrensis* adults emerged from all host plants (Table 5.5).

5.3.4 Effects of host plants on percent adult *Liriomyza* leafminers emergence from larval stage

a) Liriomyza leafminers reared on Vicia faba

Significant host plant effects on the survival was found for the three *Liriomyza* leafminers tested (*L. huidobrensis*, *F* = 3.74; d.f. = 3,28; *P* = 0.0224; Table 5.5; *L. sativae*, *F* = 4.15; d.f. = 2,21; *P* = 0.0304; Table 5.6; and *L. trifolii, F* = 9.73; d.f. = 2,21; *P* = 0.0010; Table 5.7). Highest proportion of *L. huidobrensis* adults survived on *V. faba* and *P. sativum* (Table 5.5) while for *L. trifolii* and *L. sativae,* highest survival was observed from *P. vulgaris* and *V. faba*.

Table 5.5 Effect of host plants on pupal and adult survival of *Liriomyza huidobrensis* on test plants

Variable	Host plant tested	Origin of *L. huidobrensis*			
		V. faba	*P. sativum*	*P. vulgaris*	*L. esculentum*
Total pupae/cage	*V. faba*	42.88±11.80^{a*}	14.38±6.69^{a}	15.00±6.09^{a}	15.00±6.36^{a}
	P. sativum	32.63±11.99^{ab}	17.50±7.48^{a}	10.50±2.17^{ab}	23.13±5.17^{a}
	P. vulgaris	10.00±2.94^{ab}	11.25±3.90^{a}	2.88±1.77^{b}	13.88±5.65^{a}
	L. esculentum	2.50±0.73^{b}	7.25±1.93^{a}	8.13±2.29^{ab}	6.13±1.13^{a}
Total adults/cage	*V. faba*	32.13±8.77^{a}	9.00±5.75^{a}	12.25±5.21^{a}	7.75±4.39^{a}
	P. sativum	21.73±8.66^{ab}	7.88±2.67^{a}	9.50±2.11^{ab}	8.75±1.90^{a}
	P. vulgaris	5.13±2.11^{b}	6.00±2.43^{a}	2.13±1.47^{b}	5.63±1.98^{a}
	L. esculentum	1.38±0.46^{b}	2.00±0.60^{a}	7.00±2.17^{ab}	5.25±1.16^{a}
Pupal weight (mg)	*V. faba*	0.63 ±0.05^{a}	0.66±0.02^{a}	0.55±0.02^{a}	0.55±0.02^{a}
	P. sativum	0.53 ± 0.03^{a}	0.45±0.03^{c}	0.52±0.01^{a}	0.49±0.02^{a}
	P. vulgaris	0.61 ± 0.03^{a}	0.54±0.03^{b}	0.58±0.01^{a}	0.55±0.04^{a}
	L. esculentum	0.45 ± 0.04^{a}	0.57±0.04^{b}	0.54±0.05^{a}	0.51±0.02^{a}
Adult emergence (%)	*V. faba*	65.01 ±13.86^{a}	49.09±19.28^{a}	41.85±12.40^{b}	22.94±10.64^{a}
	P. sativum	57.10 ± 22.57^{a}	63.32±27.24^{a}	89.19±12.66^{a}	32.10±5.53^{a}
	P. vulgaris	26.94 ± 9.60^{ab}	45.55±11.09^{a}	9.33±5.48^{c}	24.25±5.95^{a}
	L. esculentum	6.05 ± 1.95^{b}	10.44±3.12^{a}	46.71±7.57^{b}	34.28±8.74^{a}

*Means followed by the same letter in a column for a given variable are not significantly different (SNK, *P* Ò 0.05)

Table 5.6 Effect of host plants on pupal and adult survival of *Liriomyza sativae* on test plants

Variable	Host plant	Origin of *L. sativae*	
	Tested	*V. faba*	*P. vulgaris*
Total pupae/cage	*V. faba*	19.25±2.81^{a*}	5.38±1.76^{b}
	P. vulgaris	10.63±4.68^{ab}	23.38±2.58^{a}
	L. esculentum	1.75±0.80^{b}	1.00±0.42^{c}
Total adults/cage	*V. faba*	10.00±3.36^{a}	4.63±1.56^{b}
	P. vulgaris	6.88±3.27^{ab}	11.38±1.84^{a}
	L. esculentum	0.63±0.50^{b}	0.50±0.27^{c}
Pupal weight (mg)	*V. faba*	0.29±0.01^{a}	0.29±0.01^{a}
	P. vulgaris	0.32±0.02^{a}	0.30±0.02^{a}
	L. esculentum	-	-
Adult emergence(%)	*V. faba*	37.16±10.96^{a}	57.66±24.10^{a}
	P. vulgaris	38.75±13.86^{a}	23.52±3.50^{ab}
	L. esculentum	1.90±1.38^{b}	6.60±3.48^{b}

*Means followed by the same letters in a column for a given variable (comparison among test host plants) are not significantly different (SNK, *P* Ò0.05)

Table 5.7 Effect of host plants on pupal and adult survival of *Liriomyza trifolii* on test plants

Variable	Host plant	Origin of *L. trifolii*	
	Tested	*V. faba*	*P. vulgaris*
Total pupae/cage	*V. faba*	27.25±6.45^{a*}	12.25±2.37^{ab}
	P. vulgaris	31.38±4.88^{a}	14.5±2.26^{a}
	L. esculentum	3.63±1.24^{b}	5.88±1.57^{b}
Total adults/cage	*V. faba*	19.38±5.00^{a}	8.38±1.74^{ab}
	P. vulgaris	17.50±5.99^{a}	12.75±2.19^{a}
	L. esculentum	1.75±0.80^{b}	3.75±1.08^{b}
Pupal weight (mg)	*V. faba*	0.34±0.02^{a}	0.33±0.02^{a}
	P. vulgaris	0.35±0.02^{a}	0.34±0.02^{a}
	L. esculentum	0.42±0.04^{a}	0.38±0.09^{a}
Adult emergence (%)	*V. faba*	58.99±10.36^{a}	55.82±11.29^{a}
	P. vulgaris	33.63±9.61^{ab}	57.15±11.19^{a}
	L. esculentum	6.88±3.12^{b}	21.42±7.62^{b}

*Means followed by the same letters in a column for a given variable (comparison among test host plants) are not significantly different (SNK, *P* Ò0.05)

b) Liriomyza leafminers reared on Phaseolus vulgaris

There was significant host plant effect on the number of adults produced for *L. huidobrensis* (*F* = 9.89; d.f. = 3,28; *P* = 0.001; Table 5.5), *L. sativae* (*F* = 3.35; d.f. = 2,21; *P* = 0.054; Table 5.6) and for *L. trifolii* (*F* = 3.96; d.f. = 2,21; *P* = 0.0346; Table 5.7). Highest proportion of survival of *L. huidobrensis* was from *P. sativum*, while most *L. sativae* and *L. trifolii*, *V. faba* (Table 5.6) and *P. vulgaris* respectively (Table 5.7).

c) Liriomyza leafminers reared on Pisum sativum

No significant host plant effect was found on the survival of *L. huidobrensis* (*F* = 1.62; d.f. = 3,28; *P* = 0.2074; Table 5.5). Statistically, no difference was evident on survival of *L. huidobrensis* on all the tested host plants.

d) *Liriomyza* leafminers reared on *Lycopersicon esculentum*

The number of adults that emerged was not influenced by the type of host plant (*F* = 0.50; d.f. = 3,28; *P* = 0.6873; Table 5.5).

5.3.5 Effect of host plants on fitness of *Liriomyza* leafminers

a) Pupal weight

There was no significant host plant effect on the pupal weight for all the three *Liriomyza* leafminers reared on all the four host plants, except when *L. huidobrensis* that originated from *P. sativum*, where *V. faba* produced heavier pupae followed by *L. esculentum* and *P. vulgaris*. (Tables 5.5, 5.6 and 5.7).

b) Adult wing length measurements

Host plants influenced the fitness of different leafminer species as indicated by the varying wing length measurements. *Liriomyza huidobrensis* was reared and tested on all the four host plants, while *L. sativae* and *L. trifolii* were reared on *V. faba* and *P. vulgaris* and tested on *P. vulgaris*, *V. faba* and *L. esculentum*.

i) Wing length of Liriomyza huidobrensis reared on Vicia faba, Pisum sativum, Phaseolus vulgaris and Lycopersicon esculentum

Liriomyza huidobrensis reared on *V. faba* and tested on the four test plants female (*F* = 16.41; d.f. =3,32; *P* < 0.0001) and male (*F* = 4.82; d.f. =3,31; *P* = 0.0072) flies with significantly different wing lengths (Table 5.8). *Phaseolus vulgaris* as a rearing host, too, produced famales (F = 21.42; d.f. =3,35; P < 0.0001) and males (F = 7.85; d.f. =3,34; P = 0.0004) with significantly different wing lengths(Table 5.8). *Pisum sativum* (famales, F = 4.94; d.f. =3,32; P = 0.0062 and males, F = 13.63; d.f. =3,36; P < 0.0001) and *L. esculentum* (famales, F = 7.68; d.f. =3,36; P = 0.0004 and males, F = 5.63; d.f. =3,36; P = 0.0029) also gave rise to flies with significantly different wing lengths (Table 5.8).

ii) Wing length of Liriomyza sativae reared on Phaseolus vulgaris and Vicia faba

Liriomyza sativae originating from *P. vulgaris* and reared on three test host plants produced males (F = 1.50, d.f. = 2,19; P = 0.2487) and females (F = 0.27; d.f. = 2,19; P = 0.7653) with significantly similar wing lengths (Table 5.9). However, those originating from *V. faba* and reared the same host plants gave rise to flies with significantly different wing lengths only in females (F = 23.55; d.f. = 2,17; P < 0.0001) but not for males (F = 0.84; d.f. = 2,15; P = 0.4515) (Table 5. 9).

iii) ***Wing length of Liriomyza trifolii reared on Phaseolus vulgaris and Vicia faba***

Liriomyza trifolii originating from *P. vulgaris* and tested on three test host plants gave rise to male (F = 11.05; d.f. =2,27; P = 0.0003) flies with significantly different wing lengths but not females (F = 1.52; d.f. =2,27; P = 0.2371) (Table 5.10). On the other hand, those originating from *V. faba* and tested on the same host plants produced both males (F = 4.69; d.f. =2,26; P = 0.0182) and females (F = 7.38; d.f. =2,22; P = 0.0035) with significantly different wing lengths (Table 5. 10).

5.3.6 Effect of host plants on sex ratio of emerging adult *Liriomyza* leafminers

The ratio of the females to males in most cases was balanced at 1:1 for the three *Liriomyza* leafminers tested on all the host plants (Tables 5.11 and 5.12). More *L. huidobrensis* females (74%) than males were recorded when originating from *P. sativum* and reared on *P. vulgaris* or vice versa (34%) when originating from the same plant and reared on *L. esculentum* (Table 5. 11).

Table 5.8 Wing length (mm) of *Liriomyza huidobrensis* adult reared and tested on selected hosts

Variable	Host plant	Origin of *L. huidobrensis*			
	tested	*V. faba*	*P. sativum*	*P. vulgaris*	*L. esculentum*
Female	*V. faba*	2.18±0.03^{a}	2.19±0.04^{a*}	2.19±0.03^{a}	2.19±0.03^{a}
	P. sativum	2.08±0.02^{a}	1.96.±0.03^{ab}	2.18±0.06^{a}	1.90±0.06^{b}
	P. vulgaris	2.06±0.03^{a}	2.07±0.11^{ab}	2.14±0.03^{a}	1.83±0.08^{b}
	L.esculentum	1.84±0.04^{b}	1.81±0.06^{b}	1.89±0.05^{b}	1.96±0.05^{b}
Male	*V. faba*	1.77±0.04^{a}	1.82±0.02^{a}	1.71±0.03^{a}	1.78±0.04^{a}
	P. sativum	1.71±0.03^{ab}	1.68±0.03^{bc}	1.62±0.02^{ab}	1.64±0.04^{b}
	P. vulgaris	1.63±0.04^{ab}	1.73±0.03^{ab}	1.67±0.04^{a}	1.67±0.03^{ab}
	L.esculentum	1.57±0.03^{b}	1.59±0.02^{c}	1.54±0.01^{b}	1.59±0.02^{b}

*Means followed by the same letters in a column for a given variable (comparison among test host plants) are not significantly different (Tukey, *P* Ò0.05)

Table 5.9 Wing length (mm) of *Liriomyza sativae* adult reared and tested on selected hosts

Variable	Host plant	Origin of *L. sativae*	
	Tested	*P. vulgaris*	*V. faba*
Female	*V. faba*	1.40±0.06^{a*}	1.34±0.04^{b}
	P. vulgaris	1.44±0.02^{a}	1.49±0.01^{a}
	L. esculentum	1.43±0.04^{a}	1.23±0.01^{c}
Male	*V. faba*	1.27±0.05^{a}	1.21±0.01^{a}
	P. vulgaris	1.18±0.02^{a}	1.17±0.02^{a}
	L. esculentum	1.14±0.12^{a}	1.17±0.02^{a}

*Means followed by the same letters in a column for a given variable (comparison among test host plants) are not significantly different (Tukey, *P* Ò0.05)

Table 5.10 Wing length (mm) of *Liriomyza trifolii* reared and tested selected host plants

Variable	Host plant	Origin of *L. trifolii*	
	Tested	*P. vulgaris*	*V. faba*
Female	*V. faba*	1.46±0.05^{a*}	1.43±0.03^{b}
	P. vulgaris	1.49±0.02^{a}	1.53±0.01^{a}
	L. esculentum	1.41±0.02^{a}	1.45±0.01^{b}
Male	*V. faba*	1.21±0.02^{a}	1.23±0.03^{a}
	P. vulgaris	1.25±0.02^{a}	1.24±0.01^{a}
	L. esculentum	1.11±0.02^{b}	1.16±0.01^{b}

*Means followed by the same letters in a column for a given variable are not significantly different (Tukey, *P* Ò 0.05)

Table 5.11 Sex ratio of *Liriomyza huidobrensis* originating and tested on different host plants

Host plant	Origin of *L. huidobrensis*			
tested	*V. faba*	*P. sativum*	*P. vulgaris*	*L. esculentum*
V. faba	44.37±06.98^{a*}	39.39±09.97^{a}	53.75±17.36^{a}	43.38±07.02^{a}
P. sativum	39.15±11.05^{a}	42.94±09.84^{a}	49.38±05.62^{a}	59.65±06.85^{a}
P. vulgaris	48.98±13.05^{a}	73.79±09.87^{a}	47.22±07.35^{a}	49.65±12.08^{a}
L. esculentum	66.67±16.67^{a}	33.57±14.50^{a}	51.63±11.14^{a}	51.46±06.50^{a}

*Means followed by the same letters in a column are not significantly different (SNK, *P* Ò0.05)

Table 5.12 Sex ratio of *Liriomyza trifolii* and *Liriomyza sativae* originating and tested on different hosts

LMF	Host plant	Origin of *Liriomyza spp.*	
	Tested	*V. faba*	*P. vulgaris*
L. sativae	*V. faba*	54.77±5.71^{a*}	48.91±03.97^{a}
	P. vulgaris	47.50±12.19^{a}	55.60±04.25^{a}
	L. esculentum	75.00±25.00^{a}	66.70±33.33^{a}
L. trifolii	*V. faba*	48.34±03.62^{a}	54.25±10.06^{a}
	P. vulgaris	60.22±07.55^{a}	54.48±03.52^{a}
	L. esculentum	45.00±22.17^{a}	55.56±03.51^{a}

*Means followed by the same letters in a column for a given *Liriomyza* species are not significantly different (SNK, *P* Ò0.05)

5.4. Discussion

The ability of *Liriomyza* leafminers to develop and survive on several host plants have been reported (Minkenberg and Fredrix, 1989; Scheirs *et al*., 2004; Martin *et al*., 2005; Videla *et al*., 2006). The development stages of *Liriomyza* leafminers involve the egg stage, larval (three instars and pre-pupae) and the mature stage, which is the adult fly (Parrella, 1987).

The tested *Liriomyza* leafminers took fewer days to develop on *P. vulgaris*, followed by *L. esculentum*, and most number of days on *V. faba* and *P. sativum*. The results for *L. huidobrensis* are in line with that reported by Videla *et al.* (2006). *Liriomyza* sativae and *L. trifolii* took between 13 to 16 days to complete development, on either *P. vulgaris* or *L. esculentum*, respectively and between 18 to 19 days on *V. faba*. This trend was probably influenced by the succulent attribute of the *V. faba*, thereby providing more food for the offspring, or the possible presence of semio-chemicals that effected a growth delay on the developing offspring for the success of parasitization. Hence, from this study, it is clear that *P. vulgaris* was a promising rearing host for both *L. sativae* and *L. trifolii*, while *V. faba* and *P. sativum* were the best for *L. huidobrensis*.

Most *L. huidobrensis* pupae were produced from *V. faba* when the *Liriomyza* leafminer was previously reared on both *V. faba* and *P. vulgaris,* but generally, this insect produced most pupae from *V. faba* and *P. sativum*. *Liriomyza sativae* and *L. trifolii*, however, produced most pupae from *P. vulgaris* and *V. faba*. A possible explanation for this behaviour could be the nutrient level in the leaves of the pulses coupled with the moisture content (Martin *et al*., 2005). These host attributes meant more food to sustain the offspring development, hence more pupae produced. Another possible explanation could be the effect of previous exposure on the *Liriomyza* leafminer offspring development. It is also important to note that, even though *P. vulgaris* was preferred by *L. huidobrensis* for feeding and egg laying, during pupation, most pupae remained trapped just below the upper leaf layer, thus leading to less pupae recorded from this plant.

Liriomyza huidobrensis had most adults emerging from *V. faba* and *P. sativum* and least from *P. vulgaris* and *L. esculentum*. *Liriomyza sativae* and *L. trifolii* produced most adults from *P. vulgaris* and *V. faba.* This trend too, could be due to reasons as those for pupae above. However, *L. esculentum* supported least development of the three *Liriomyza* leafminers, while *P. sativum* could not support development of *L. sativae* and *L. trifolii*, probably, because it was not preferred for egg- laying in the first place. The number of emerging adults was used to compute the survival percentage. As noted by Parrella and Bethke (1984), successful emergence to adult stage is highly dependent on host plant. Hence, the host plants from which more adults emerged from are suitable for the particular leafminer species.

Liriomyza huidobrensis survived best on *V. faba* and *P. sativum*, except when it was reared on *L. esculentum*, where the proportion that survived was low. Probably, this was so since the adults that arose from the rearing of *L. esculentum* were not fit enough, thus giving rise to offspring that were equally less fit as compared to those that were previously reared on the other host plants. This is consistent with Videla *et al*. (2006) who reported that *V. faba* was a better host that supported higher rate of survival of *L. huidobrensis*. *Liriomyza sativae* and *L. trifolii* on the other hand, had the highest proportion of survival on *V. faba* and *P. vulgaris*. *Lycopersicon esculentum* least supported the survival of these *Liriomyza* leafminers. Even though *P. vulgaris* had high number of *L.* sativae and *L. trifolii* pupae, survival was medium. This lower rate could have been attributed to the fact that pupae from this host plant do not drop easily, hence necessitating harvesting using a camel brush. Sometimes, it is possible that the puparia can get pricked, thereby leading to mortality at the pupal stage, and ultimately lower survival percentage.

Parrella (1983) stated that the puparium size is strongly and positively correlated with the adult vigour. From this study, pupae weight was not affected by the type of the host plant from which the development took place. This finding corroborates that of Parrella (1987) who also failed to establish any host plant effect on the pupal weight. The possible explanation, in this case, was thought to be the fact that those pupae, which were weighed after every two days (in cohorts of 10 each), were not dropping at the same time interval. This meant that pupae with puparia of varying moisture content were weighed after every two days, thus the absence of variability.

Generally, *V. faba* was a suitable host for development of *L. huidobrensis*, and *P. vulgaris* and *V. faba* for *L. sativae* and *L. trifolii* in terms of the number of offsprings produced (pupae and subsequently adults). This was confirmed by the larger wings of adult *Liriomyza* leafminers emerging from these host plants. Wing length can be used to determine adult size of *Liriomyza* species (Musundire *et al*., 2010). The wing lengths recorded from this study, were similar with those reported by Capinera (2005) and Videla *et al*. (2006). *Liriomyza* leafminers with longer wings (larger wings) are thought to be more aggressive, hence, fitter and with higher fecundity. This study also showed that *Liriomyza* leafminer males had shorter wings compared to their female counterparts, a finding that was similar to those of Parrella (1987) and Videla *et al*. (2006).

The largely balanced sex ratio observed in this study implied that the three leafminers are able to continue surviving and reproducing to ensure continuity of future generations on the host plants that they were able to develop on. Parrella (1987) also reported 1:1 sex ratio. This attribute could be a survival strategy by the *Liriomyza* leafminers. There were however, extreme cases where females were as high as 75 %.

This study showed clearly that development time and survival of the three *Liriomyza* leafminers is dependent on the host plant from which the leafminers develop on. Fitness parameters such as pupae weight and wing length are also aspects in determining the suitability of host plants for the development of the flies. This information could be useful in future studies when selecting suitable rearing host plants and also determing when a control intervention should be started to avoid crop losses.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Feeding and oviposition of *Liriomyza huidobrensis* (Blanchard), *Liriomyza sativae* (Blanchard) and *Liriomyza trifolii* (Burgess) were influenced by the host plants tested and to some extent, the rearing host plants. Leafminers employ plant volatiles for host location, feeding, oviposition and mating (Kang *et al*., 2009). Though this work did not involve the isolation of plant compounds from leaves to demonstrate the particular compounds that are significant feeding and ovipositon attractants and deterrents from the host plants. *Lycopersicon esculentum* was generally preferred by the three *Liriomyza* leafminers when they did not have a choice. When they had a choice, *L. huidobrensis*, in most cases, fed and oviposited almost equally on the host plants, and in some cases, found *V. faba* to be more suitable. *Liriomyza sativae* and *L. trifolii*, however found *P. vulgaris* to be more suitable in most cases as compared to *V. faba* and *L. esculentum*. These results were in line with that of Videla *et al*. (2006) and Musundire *et al*. (2010).

Development time was longer on *V. faba* for all the three *Liriomyza* leafminers as compared to *P. vulgaris*, *L. esculentum* and *P. sativum*. Slow development on *V. faba* was thought to be influenced by the need of the feeding insect pest to acquire the nutrient levels that they require for development. Succulent plants are known to have less nutrient levels, hence the longer period taken during feeding. As the leafminers take longer to develop, the parasitoids get more time to parasitize the developing larvae. Therefore, slow rate of development could be described as a delay strategy to maximize parasitism.

Even though previous work have shown that host plant influenced the weight of the leafminer pupae (Martin *et al*., 2005), this study did not find any difference in the weights of the pupae emerging from the four host plants except only when *L. huidobrensis* used originated from *P. sativum* and developed on *V. faba*. The lack of host plant influence on the size of *Liriomyza* leafminers corroborated the finding by Musundire *et al*., (2011). This result showed that the tested host plants were equally suitable for development of the *Liriomyza* leafminers based on the weight of pupae. Wing length can be used to determine adult body size for *Liriomyza* leafminers (Musundire *et al*., 2011). *Liriomyza huidobrensis* that developed on *V. faba* had highest mean wing lengths as compared to *P. vulgaris*, *P. sativum* and *L. esculentum*. Videla *et al*. (2006) also found that *L. huidobrensis* had larger body size when it fed on *V. faba*. *Liriomyza sativae* and *L. trifolii*, however, had highest mean wing length when they developed from *V. faba* and *P. vulgaris* in most cases, which seemed to have been influenced by the rearing host plants. The plants found to support better development that led to larger mean wing lengths probably had better nutritional quality and adequate moisture (Awmack and Leather, 2002). Insect pests that have larger body size perform better than those with smaller body size (Honék, 1993), hence, *L. huidobrensis* reared on *V. faba* and *L. sativae* and *L. trifolii* reared on *V. faba* and *P. vulgaris* were more fitter. This information could be useful in future rearing of these *Liriomyza* leafminers.

6.2 Conclusion

This study confirmed that the three *Liriomyza* leafminers pose serious risk to most horticultural crops and their preference for feeding, egg-laying, development and survival is host dependent. Studies have shown that resistant populations of these flies are fast developing, which has complicated their control. Host plant effect on body size of the three *Liriomyza* leafminers were variable and dependent on test host plants, rearing host plants and *Liriomyza* species.

This study showed a potential for population divergence in host plant selection and confirmed, partly that leafminers showed greater preference for the host plants that they developed on, but this observation was not consistent. *Pisum sativum*, is a good case, which showed almost zero infestation when put in a choice experimental set-up. Lack of correlation between feeding and oviposition preferences could be important attribute of the leafminers to maintain polyphagy as noted by Martin *et al*. (2005).

This study also confirmed that females insects do not always select host plants that are suitable for the development of their offspring. This was so since *L. esculentum* was suitable for feeding and egg-laying by the flies, but had the least offspring survival. This also showed a potential of incorporating deadened hosts in a management programme. The following conclusions and recommendations are drawn from the results of this study.

6.3 Recommendations

1. Further work is needed to determine the specific compounds in *L. esculentum*, *V. faba* and *P. vulgaris* that attract *Liriomyza* leafminers. The compounds, if attractants could be applied on the foliage of low value crops used in an intercrop system with the high value ones, in a IPM programme.
2. Further testing of *V. faba* for preference and performance of leafminers in a large field situation for a possible use as trap crops in mixed cropping with the high value crops.
3. Studies to test various French bean, snow pea and tomato varieties/cultivars for tolerance or the varieties that are less suitable for the leafminers.

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APPENDICES

Appendix I Punctures made by *Liriomyza* leafminers reared on *Phaseolus vulgaris* from Choice test

Procedure:

```
proc glimmix data=nochoice;
class lmf;
model  egg =lmf/dist=normal link=identity;
by host;
lsmeans lmf/pdiff adjust=tukey;
ods output diffs=epp lsmeans=emmm;
ods listing exclude diffs lsmeans;
run;
%pdmix800(epp,emmm,alpha=0.05,sort=yes);
/*proc sort;
by lmf host;
run;
proc glimmix data=nochoice;
class host;
model  egg =host/dist=n link=identity;
by lmf;
lsmeans host/pdiff adjust=tukey;
ods output diffs=epp lsmeans=emmm;
ods listing exclude diffs lsmeans;
run;
%pdmix800(epp,emmm,alpha=0.05,sort=yes);*/
```

Output:

```
The SAS System       11:14 Friday, November 12, 2010
Dependent variable: Punctures

---------------------------- lmf=Lh ---------------------------------
                              The GLIMMIX Procedure

                                  Fit Statistics

                         Pearson Chi-Square          7296.82
                         Pearson Chi-Square / DF        58.85


                           Type III Tests of Fixed Effects

                                Num      Den
                    Effect         DF       DF    F Value    Pr > F
```

```
             host           3      124       2.25    0.0858

-------------------------------------------- lmf=Ls --------------------------------------------
                              The GLIMMIX Procedure

                                  Fit Statistics

                 Pearson Chi-Square           730.22
                 Pearson Chi-Square / DF        5.89


                    Type III Tests of Fixed Effects

                        Num      Den
             Effect        DF       DF    F Value    Pr > F

             host           3      124      13.76    <.0001



-------------------------------------------- lmf=Lt --------------------------------------------
The GLIMMIX Procedure

                                  Fit Statistics

                 Pearson Chi-Square           710.88
                 Pearson Chi-Square / DF        5.73


                    Type III Tests of Fixed Effects

                        Num      Den
             Effect        DF       DF    F Value    Pr > F

             host           3      124      19.37    <.0001
```

```
                          The SAS System      11:14 Friday, November 12, 2010

-------------------------- Effect=host   Method=Tukey (P<0.05)   Set=1 --------------------------

                                    Standard    Letter
         Obs    lmf                  host              Estimate    Error      Group

           1    L. huidobrensis    French bean    6.8506      1.3561      A
           2    L. huidobrensis    tomato         5.7800      1.3561      A
           3    L. huidobrensis    faba bean      3.2822      1.3561      A
           4    L. huidobrensis    snow pea       2.5453      1.3561      A


-------------------------- Effect=host   Method=Tukey (P<0.05)   Set=2 --------------------------

                                    Standard    Letter
         Obs    lmf            host           Estimate     Error      Group

           5    L. sativae     tomato         3.4059      0.4290      A
           6    L. sativae     faba bean      3.3687      0.4290      A
           7    L. sativae     French bean 1.6872      0.4290      B
           8    L. sativae     snow pea       0.07187     0.4290      C


-------------------------- Effect=host   Method=Tukey (P<0.05)   Set=3 --------------------------

                                    Standard    Letter
         Obs    lmf            host              Estimate   Error      Group

           9    L. trifolii    French bean     4.5762      0.4233      A
          10    L. trifolii    faba bean       1.9541      0.4233      B
          11    L. trifolii    tomato          1.4825      0.4233      BC
          12    L. trifolii    snow pea        0.1272      0.4233      C
```

Appendix II Pupae and Adults ANOVA summary

Leafminer fly	Rearing Host	Dependent variable	d.f.	SS	MS	*F* - value	*P* - value
L. huidobrensis	*V. faba*	Pupae	3	22.12	7.37	5.00	0.0067
L. sativae	*V. faba*	Pupae	2	19.79	9.89	9.51	0.0011
L. trifolii	*V. faba*	Pupae	2	22.90	11.45	16.33	<.0001
L. huidobrensis	*P. vulgaris*	Pupae	3	11.14	3.71	3.26	0.0362
L. sativae	*P. vulgaris*	Pupae	2	28.09	14.05	22.11	<.0001
L. trifolii	*P. vulgaris*	Pupae	2	4.25	2.13	3.80	0.0391
L. huidobrensis	*P. sativum*	Pupae	3	0.55	0.18	0.12	0.9458
L. huidobrensis	*L. esculentum*	Pupae	3	7.55	2.52	1.76	0.1774
L. huidobrensis	*V. faba*	Adults	3	24.83	8.28	5.10	0.0061
L. sativae	*V. faba*	Adults	2	10.55	5.27	4.25	0.0281
L. trifolii	*V. faba*	Adults	2	19.03	9.52	9.79	0.0010
L. huidobrensis	*P. vulgaris*	Adults	3	10.75	3.58	3.07	0.0440
L. sativae	*P. vulgaris*	Adults	2	17.92	8.96	16.31	<.0001
L. trifolii	*P. vulgaris*	Adults	2	6.11	3.05	5.90	0.0092
L. huidobrensis	*P. sativum*	Adults	3	2.23	0.74	0.57	0.6374
L. huidobrensis	*L. esculentum*	Adults	3	3.49	1.16	1.09	0.3689