GENERAL INTRODUCTION

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

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

Damage caused by *Liriomyza* leafminers

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

more trimming, cleaning and culling before the produce can be marketed (Capinera, 2001).

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

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

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

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

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

Economic losses caused by *Liriomyza* leafminers

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

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

Description of the leafminer life cycle stages

Adult description

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

Morphological identification of the three-leafminer species is based on the distiphallic structure, a terminal part of the aedaegus (OEPP /EPPO, 2005). The

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

Adult biology

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

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

Eggs

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

Description of larvae

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

Larval behaviour

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

Description of pupae

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

Life cycle

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

Historical profile of pest status of leafminer species in Kenya

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

Robin (1983) reported a similar case in Hawaii (USA) during 1978 and 1979 when watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai (Cucurbitaceae)

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

Current biological control methods in leafminer pest management

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

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

considerable research gaps on the diversity of natural enemies of agromyzids exist which limit our knowledge of conservation biological control techniques of leafminers pests.

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

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

Description of *Diglyphus isaea*

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

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

Female *D. isaea* search for suitably sized host larvae within leaf tissue and sting them through the leaf cuticle. The sting paralyses the host larva within a short time and female wasps then lay one or more eggs alongside the larva (Minkenberg, 1989). The larvae of *D. isaea* feed externally on the paralysed but initially still living host and continue feeding on the paralysed host until it is consumed completely (Ode & Heinz, 2002). Microscopic examination and dissection of the mine will show parasitized larvae to be turgid with a parasitoid larval stage next to it (Fig. 5). Once parasitism occurs, the mine stops growing and the leafminer larvae become discoloured as the parasite develops.

Fig. 4. Adults of *Diglyphus isaea*. The black and yellow patterns on the hind tibia are used to distinguish between males (right) and females (left).

Behaviour of Diglyphus isaea

The amount of food available to each parasitoid larva is variable and is reflected in the variable size of adult parasitoids. Adult female parasitoids are known to be able to control the sex of the eggs they lay. Generally, females will lay male eggs on small host larvae and female eggs on large host larvae (Ode & Heinz, 2002). Thus, the size range of available host larvae can affect the sex ratio of the subsequent adult population. This is important for mass rearing *D. isaea* and the timing of seasonal releases of the parasitoids into crops as it determines the success or failure of establishment of a regenerating population (Ode & Heinz, 2002).

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

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

Life cycle

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

Plant, leafminer species and *Diglyphus isaea* interactions

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

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

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

Thesis organisation

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

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

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