

CHAPTER THREE

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

Abstract

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

Introduction

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

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

However, the efficacy of the parasitoids depend on the larval size of *Liriomyza* species (Ode & Heinz, 2002), which in turn can be dependent on the host plant species (Salvo & Valladares, 2002; Chapter 2). Various attributes of host plant species can affect the behaviour of parasitoids such as mate location (McAuslane *et al.*, 1990),

oviposition (Powell & Wright, 1992), fecundity (Shukla & Tripathi, 1993), rate of parasitism (Salvo & Valladares, 2002), survival and sex ratio (Hare & Kuck, 1991), and body size (Salvo & Valladares, 2002).

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

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

Materials and Methods

Plants

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

Insect rearing

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

on the respective plant species and were of approximately the 18-20th generations prior to the experiments.

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

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

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

No-choice experiment

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

Choice experiment

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

released per cage for 24 hours. The experiment was replicated 16 times for each *Liriomyza* species.

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

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

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

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

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

Three days after the first adult emergence of *D. isaea*, all adults were killed by instant freezing and sexed, using the key developed by Bouček (1988). Adults of *D. isaea* display a sexually dimorphic colour pattern of the hind tibia. The proportions of male and female progenies were determined for all plant and leafminer species in the choice and the no-choice tests. However, due to difficulties in rearing *L. sativae* and *L. trifolii* on *P. sativum*, very few larvae were available for parasitism and hence there were very few or no *D. isaea* progeny recovered from this host plant species. Because

of the small sample sizes, *P. sativum* was excluded from some analyses. The mean number of *D. isaea* adults per replicate for the leafminer host plant combinations was: *P. vulgaris*-*L. huidobrensis*, 30, *P. vulgaris*-*L. sativae*, 26; *P. vulgaris*-*L. trifolii*, 25, *P. sativum*-*L. huidobrensis*, 31, *P. sativum*-*L. sativae*, 23, *S. lycopersicum*-*L. huidobrensis*, 39, *S. lycopersicum*-*L. sativae*, 26; *S. lycopersicum*-*L. trifolii*, 22; *V. faba*-*L. huidobrensis*, 25; *V. faba*-*L. sativae*, 27; *V. faba*-*L. trifolii*, 29.

Data analyses

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

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

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

The proportions of females out of the total number of parasitoids (males + females) were used as an index of sex ratio. The logistic regression model with logistic

link function described above was also used to evaluate the effect of host plants, leafminer species and their interaction on the sex ratios using PROC GENMOD.

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

Results

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

No-choice experiment

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

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

The percentage of larvae host-fed by *D. isaea* was generally lower across all the host plant and leafminer species compared to parasitism. It was 0.3 to 3.2 times lower on *V. faba* and *P. vulgaris*, 1.8 to 3.6 times lower on *S. lycopersicum* and 4 to 10 times lower on *P. sativum*. Logistic regression analysis showed a highly significant interaction effect of host plant by leafminer species ($\chi^2 = 25.44$, $df = 6$, $P < 0.0003$). The highest host feeding level was recorded on *L. trifolii* reared on *V. faba* (36 %) and lowest on *L. huidobrensis* reared on *P. sativum* (2 %). Host feeding activity by *D. isaea* on *L. huidobrensis* was higher on *P. vulgaris* and *S. lycopersicum* compared to *P. sativum* and *V. faba* (Table 3.1). However, *L. sativae* larvae reared on *P. vulgaris*, *S. lycopersicum* and *V. faba* were equally host-fed (Table 3.1).

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

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

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

Choice experiment

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

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

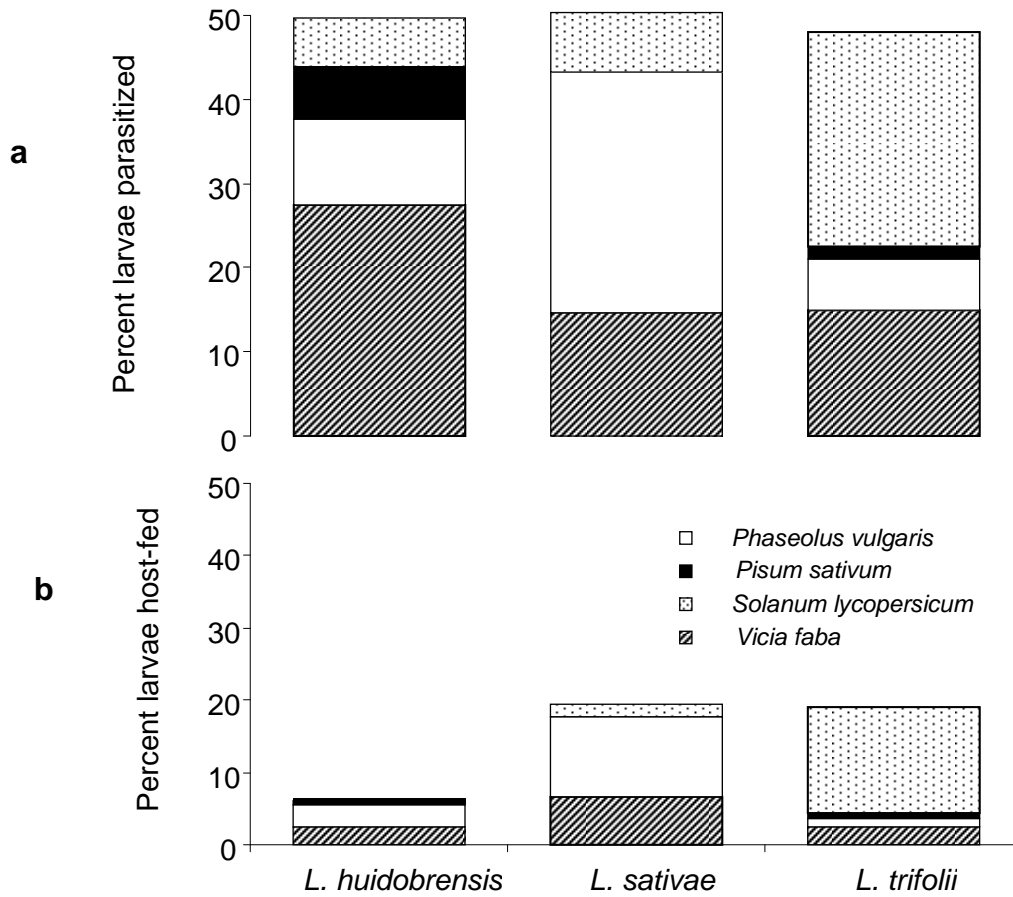


Fig.3.1. Percentage of *Liriomyza* larvae parasitized (a) and host-fed (b) by *D. isaea* in choice experiments

Sex Ratio of *Diglyphus isaea*

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

benefit to biological control programmes that involve inundative augmentative releases where leafminer larval mortality is of importance.

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

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

with available resources and circumstances in Kenya. This study utilized varieties of four commercially grown crops in Kenya to rear *D. isaea* as opposed to mass rearing of *D. isaea* on chrysanthemums (*Dendranthema grandiflora* Tzvelev) as is practiced in countries such as the United States of America (Parrella *et al.*, 1989; Ode & Heinz, 2002).

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

References

- Bergman, J.M. & Tingey, W.M. (1979) Aspects of interaction between plant genotypes and biological control. *Bull. Entomol. Soc. Am.* **25**, 275-279.
- Bouček, Z. (1988) Australian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species. CAB International, Wallingford, Oxon, UK.
- Chabi-Olaye, A., Mujica, N., Löhr, B. & Kroschel, J. (2008) Role of agroecosystems in the abundance and diversity of *Liriomyza* leafmining flies and their natural enemies. Abstracts of the XXIII International Congress of Entomology 6-12 July 2008, Durban, South Africa.
- Campbell, B.C. & Duffy, S.S. (1979) Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science* **205**, 700-702.
- Chaput, J. (2000) Leafminers attacking field vegetables and greenhouse crops. Factsheet Order 00-039. Ontario. Ministry of Agriculture, Food and Rural Affairs.

- Charnov, E.L. (1982) The theory of sex allocation. Princeton University Press.
- Chen, X.X., Lang, F., Zhi-hong, X.U., Jun-hua, H.E. & Yun, M.A. (2003) The occurrence of leafminers and their parasitoids on vegetables and weeds in Hangzhou area, Southeast China. *BioControl* **48**, 515-527.
- Cornell, H.V. (1983) The secondary chemistry and complex morphology of galls formed by the Cynipidae (Hymenoptera): why and how? In: Gross, P. & Price, P.W. (1988) Plant influences on parasitism of two leafminers: a test of enemy-free space. *Ecology* **69**, 1506-1516.
- Elzen, G.W., Williams, H.J. & Vinson, S.B. (1983) Response by the parasitoid *Campolitis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: Implications for host habitat location. *Environ. Entomol.* **12**, 1872-1876.
- Feeny, P.P. (1976) Plant apparency and chemical defense. In: Gross, P. & Price, P.W. 1988. Plant influences on parasitism of two leafminers: a test of enemy-free space. *Ecology* **69**, 1506-1516.
- Godfray, H.C.J. (1994) Parasitoids: Behavioural and evolutionary ecology. Princeton University Press, Princeton.
- Gross, P. & Price, P.W. (1988) Plant influences on parasitism of two leafminers: a test of enemy-free space. *Ecology* **69**, 1506-1516.
- Hare, J.D. & Kuck, R.F. (1991) Indirect effects of citrus cultivars on life history parameters of a parasitic wasp. *Ecology* **75**, 1420-1428.
- Heinz, K.M. & Parrella, M.P. (1989) Attack behavior and host size selection by *Diglyphus begini* on *Liriomyza trifolii* in chrysanthemum. *Entomol. Exp. Appl.* **53**, 147-156.
- Heinz, K.M. & Parrella, M.P. (1990) The influence of host size on sex ratios in the parasitoid *Diglyphus begini* (Hymenoptera: Eulophidae). *Ecol. Entomol.* **15**, 391-399.
- Jervis, M.A. & Kidd, N.A.C. (1986) Host feeding strategies in hymenopteran parasitoids. *Biol. Rev.* **61**, 395-434.
- Jerzy, M. & Borkowaka, J. (2004) Photoperiodic response of pot chrysanthemums in twelve all year-round cycles, *EJPAU* 7 (2). Available on:
<http://www.ejpau.media.pl/volume7/issue2/horticulture/art-07.html>.

- Johnson, M.W. (1993) Biological control of *Liriomyza* leafminers in the Pacific Basin. *Micronesica Supplement* **4**, 81-92.
- Johnson, M.W. & Hara, A.H. (1987) Influence of host crop on parasitoids (Hymenoptera) of *Liriomyza* spp. (Diptera: Agromyzidae). *Environ. Entomol.* **6**, 339-344.
- Khafagi, R.M. (1986) The biological relationships of *Macrosteles viridigriseus* (Homoptera) and its parasitoid *Anteon pubicorne* (Hymenoptera). PhD Thesis, University of Newcastle upon Tyne.
- Kidd, N.A.C. & Jervis, M.A. (1989) The effects of host-feeding behaviour on the dynamics of parasitoid-host interactions, and the implications for biological control. *Res. Popul. Ecol.* **31**, 235-274.
- King, P.E. (1963) The rate of egg resorption in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) deprived of hosts. *Proc. R. Entomol. Soc. Lond.* **38**, 98-100.
- King, B.H. (1987) Offspring sex ratios in parasitoid wasps. *Q. Rev. Biol.* **62**, 367-396.
- Lashomb, J., Krainacker D., Jansson, R.K., Ng, Y.S. & Chianese, R. (1987) Parasitism of *Leptinotarsa decemlineata* (Say) eggs by *Edovum puttleri* Grissell (Hymenoptera: Eulophidae): effects of host age, parasitoid age and temperature. *Can. Entomol.* **119**, 75-82.
- Legner, E.F. (1979) The relationship between host destruction and parasite reproductive potential in *Muscidifurax raptor*, *M. zaraptor*, and *Spalangia endius* (Chalcidoidea: Pteromalidae). *Entomophaga* **24**, 145-152.
- Liu, T., Kang, L., Heinz, K.M. & Trumble, J. (2009) Biological control of *Liriomyza* leafminers: progress and perspective. CAB Reviews: *Pers. Agric., Vet Sci., Nutr. Nat. Resour.* **4**, 1-16.
- McAuslane, H.J., Vinson, S.B. & Williams, H.J. (1990) Influence of host plant on mate location by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *Environ. Entomol.* **19**, 26-31.
- Minkenbergh, O.P.J.M. (1989) Temperature effects on the life history of the eulophid wasp *Diglyphus isaea*, an ectoparasitoid of leafminers (*Liriomyza* spp.), on tomatoes. *Ann. Appl. Biol.* **115**, 381-397.

- Murphy, S.T. & LaSalle, J. (1999) Balancing biological control strategies in the IPM of New World invasive *Liriomyza* leafminers in field vegetable crops. *Biocontrol News Inf.* **20**, 91-104.
- Ode, P.J. & Heinz, K.M. (2002) Host-size-dependent sex ratio theory and improving mass reared parasitoid sex ratios. *Biol. Control* **24**, 31-41.
- Ozawa, A., Kobayashi, H., Amano, T., Ikari, T. & Saito, T. (1993) Evaluation of imported parasitic wasps as biological control agents of the legume leafminer, *Liriomyza trifolii* Burgess, in Japan. II. A field test on cherry tomatoes in a plastic greenhouse, in Shizuoka Prefecture. *Proc. Kanto-Tosan Plant Pro. Soc.* 1993. **40**, 239-241.
- Ozawa, A., Saito, T. & Ota, M. (1999) Biological control of the American serpentine leafminer, *Liriomyza trifolii* (Burgess), on tomato in greenhouses by parasitoids. I Evaluation of biological control by release of *Diglyphus isaea* (Walker) in experimental greenhouses. *Jap. J. Appl Entomol. Zoo.* **43**, 161-168.
- Parrella, M.P., Yost, J.T., Heinz, K.M & Ferrentino, G.W. (1989) Mass rearing of *Diglyphus begini* (Hymenoptera: Eulophidae) for biological control of *Liriomyza trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol.* **82**, 420-425.
- Price, P.W., Bouton, C.E, Gross, P., McPheron, B.A, Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* **11**, 41-65.
- Powell, W. & Wright, A.F. (1992) The influence of host food plants on host recognition by aphidine parasitoids (Hymenoptera: Braconidae). *Bull. Entomol. Res.* **81**, 449-453.
- Rauf, A. & Shepard, B.M. (1999) Leafminers in vegetables in Indonesia: surveys of host crops, species composition, parasitoids and control practices. In: Lim, G.S., Soetikno, S.S., Koke. W.H. (eds). Proceedings of a workshop on leafminers of vegetables in Southeast Asia, Tanah Rata, Malaysia, 2-5 February 1999. Serdang, Malaysia; CAB International Southeast Regional Centre, pp 22-25.
- Rodriguez, J.M., Rodriguez, R., Florido, A. & Hernandez, R. (1997) Integrated pest management on tomatoes in Gran Canaria (Canary Islands). *Bull. Int. Org. Biol. Control Nox. Anim. Plants* **20**, 39-44.

- Salvo, A. & Valladares, G. (2002) Plant-related intra-specific size variation in parasitoids (Hymenoptera: Parasitica) of a polyphagous leafminer (Diptera; Agromyzidae). *Environ. Entomol.* **31**, 874-879.
- SAS Institute Inc. (2002-2003). SAS/STAT, SAS version 9.1. Cary, North Carolina, USA.
- Shepard, B.M., Samsudin, K. & Braun, A.R. (1998) Seasonal incidence of *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoids on vegetables in Indonesia. *Int. J. Pest Manag.* **44**, 43-47.
- Shukla, A.N. & Tripathi, C.P.M. (1993) Effect of food plant on the offspring sex ratio of *Diaretiella rapae* (Hymenoptera: Aphididae), a parasitoid of *Lipaphis erysimi* Kalt (Homoptera: Aphididae). *Biol. Hort.* **9**, 137-146.
- Sivapragasam, A., Syed, A.R., LaSalle, J. & Ruwaida, M. (1999) Parasitoids of invasive agromyzid leafminers on vegetables in Peninsular Malaysia. Proceedings of Symposium on biological control in the tropics, MARDI Training center, Serdang, Selangor, Malaysia, 18-19 March 1999, pp 127-128.
- Spencer, K.A. (1985) East African Agromyzidae (Diptera): Further descriptions, revisionary notes and new records. *J. Nat. Hist.* **19**, 969-1027.
- Thang, V.T. (1999) Surveys of leafminers (*Liriomyza*) and their parasitoids on vegetables in Vietnam 1998. In: Lim, G.S., Soetikno, S.S. & Loke, W.H. (Eds), Proceedings of a workshop on leafminers of vegetables in South East Asia, Tanah Rata, Malaysia, 2-5 February 1999. CAB International Southeast Asia Regional Centre, Serdang, Malaysia, pp 42-53.
- van Driesche, R.G. & Taub, G. (1983) Impact of parasitoids on Phyllonorycter leafminers infesting apple in Massachusetts, U.S.A. *Prot. Ecol.* **5**, 305-317.