Biological studies of the European leafminer *Scaptomyza flava* (Fallén) (Diptera: Drosophilidae)

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Biological studies of the European leafminer *Scaptomyza flava* (Fallén) (Diptera: Drosophilidae)

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Abstract

The European leafminer, *Scaptomyza flava* (Fallén), is an important pest of brassicas, peas and gypsophila in New Zealand. Information on its response to abiotic environment and on feeding, oviposition and mating behaviours is essential for the development and implementation of monitoring and control tactics. The present study reveals that the provision of honey is not desirable for its cost effective rearing as the females of this species have similar fecundity when provided with host plant, and host plant + honey solution. Feeding and oviposition activities are rhythmic in this species and maximum feeding and oviposition occur during the first six hours of the photophase. Therefore, early hours of the day are the optimal time for scouting its population. Mass rearing of this species for use in biological control or sterile insect technique (SIT) programmes should be carried out between 20° and 25°C where maximum fecundity occurs. Female *S. flava* create significantly more feeding punctures and lay significantly more eggs on the four- and six-leaf stage Chinese cabbages than on younger stages, suggesting that the cabbages of four- to six-stages are more susceptible to *S. flava* infestation. My results show that adult females prefer vigorous to water stressed plants for both feeding and oviposition and their larvae perform better in vigorous plants, supporting both preference and performance propositions of plant vigour hypothesis. In practice vigorous plants should thus be provided for *S. flava* adults to obtain flies of high quality and quantity. Female *S. flava* prefer to feed and oviposit on mature leaves where they perform better while their offspring's performance is similar in both young and mature leaves. This result suggests that adult rather than offspring performance is shaping host preference pattern for oviposition in this species, supporting the optimal foraging theory which predicts that females prefer to feed and oviposit on hosts best satisfying their own nutrition requirements. *Scaptomyza flava* males perform courtship displays to females before mounting and mating occur. Males confined in isolation are significantly more likely to achieve mating than males reared in groups, suggesting that the mass reared males to be used in SIT should be kept in isolation before release. Furthermore, mated males are significantly more likely to display courtship behaviour and achieve matings, suggesting that learning may play a role in higher mating success. Sexual harassment by males decreases *S. flava* female feeding, longevity and offspring production. Finally, my study indicates that males of this species prefer to mate with females having larger abdomen and longer ovipositor while females prefer males with longer antennae and fore-tarsi. These morphological traits are likely associated with feeding and reproductive fitness.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Phytophagous insects can be broadly categorized as ectophagous (chewing and sap sucking) and endophagous (leaf mining, galling and stem boring) depending upon the ways in which they consume the host plant (Sinclair & Hughes 2010). There are approximately 10,000 described leafminer species (Connor & Taverner 1997), belonging to four insect orders: Lepidoptera, Diptera, Coleoptera and Hymenoptera (Rott & Godfray 2000). In Diptera, leaf-mining flies are mainly concentrated in Agromyzidae, Anthomyiidae, Drosophilidae and Ephydridae (Hespenheide 1991).

Some leafminer females oviposit externally on leaves (Moore 1966) while others lay eggs beneath the epidermis (Wallace 1970). Their offspring feed on parenchymous or epidermal tissues within leaves (Parrella et al. 1985), hollowing out a mine that is visible as an area of discoloration (Rott & Godfray 2000). Mines act as both habitat and food for the larvae (Sinclair & Hughes 2008). Leafminer larvae usually remain within the mines throughout their life. However, in some species the larvae may spend a part of their life outside the mines, for example, in *Bucculatrix pathenica* (Lepidoptera: Bucculatricidae) larvae emerge from the mines after two instars and then feed externally (McClay et al. 1990).

Leafminer damage is mainly caused by the larvae feeding on both palisade and spongy sections of the leaf mesophyll (Spencer 1973), resulting in aesthetic damage and yield reduction (Björksten et al. 2005). In addition, feeding and oviposition punctures made by adult females reduce photosynthetic capacity and general plant vigour (Johnson et al. 1983; Parrella 1984), and provide entry for pathogens such as *Alternaria alternata* (Deadman et al. 2000). Many dipterous leafminers such as *Liriomyza huidobrensis, L. trifolii*, *L. bryoniae* and *L. staivae* are important pests of various agricultural crops throughout the world (Weintraub et al. 1995; Hawthorne et al. 1992; Hendrikse et al. 1980; Musgrave et al. 1980; Çikman et al. 2008; Hernández et al. 2010; Mujica & Kroschel 2011; Gitonga et al. 2010), and cause significant economic loss (Bueno et al. 2007; Gitonga et al. 2010).

1.2 European leafminer *Scaptomyza flava*

Scaptomyza flava is an exotic species, probably of European origin (Bock 1977). It is a polyphagous species that has been recorded from 10 plant families (Maca 1972; Martin 2004). For example, it has been reared from mustard *Brassica juncea*, rocket *Eruca vesicaria*, wavy bitter cress *Cardamine flexuosa*, hairy bitter cress *C. hirsute*, water cress *Rorippa nasturtium-aquaticum*, sea rocket *Cakile edulata*, twin cress *Coronopus didymus*, dame's violet *Hesperis matronalis*, wild radish *Raphanus raphanistrum*, sea radish *R. maritimus*, hedge mustard *Sisymbrium officinale*, beans *Phaseolus coccineus*, *Tropaeolum majus*, potato *Solanum tuberosum*, and onion *Allium cepa* (Martin 2004).

Cumber & Eyles (1961) conducted a survey of 80 brassica fodder crops in 1959 and did not find any dipterous leafminers in the North Island of New Zealand. *Scaptomyza flava* was first detected in New Zealand in 1964, causing considerable damage to pea crop in Ohau, Levin (Manson 1968). Within three growing seasons it reached Auckland and Gisborne (Martin 2004). It is now considered a key leafminer pest of Asian brassica crops in New Zealand and occurs throughout the country (Walker et al. 2009; MacDonald et al. 2011; Martin 2012). It can also be a significant pest of peas and gypsophila (Martin 2004; MacDonald et al. 2011).

This pest has been reported to be present throughout the year in Auckland, with populations peaking in mid-summer (Martin 2012). It does not enter diapause although under dry conditions its pupae may remain quiescent for 300 days (Maca 1972). Suitable hosts for emerging flies are present all year round in New Zealand (Martin 2004).

1.3 Measures used to control *Scaptomyza flava* in New Zealand

In New Zealand, an integrated pest management (IPM) programme has been developed for European brassica vegetable crops (Berry 2000) where *S. flava* is rarely a problem (Martin et al. 2006). However, the frequent applications of insecticides for controlling this pest on gypsophila and Asian brassica crops have made implementation of IPM in these crops difficult (Martin 2004). According to Martin et al. (2006), several commonly used insecticides such as Bt aizawai, pymetrozine, carbaryl and pirimicarb are not effective for the control of this pest. Therefore, an IPM programme for *S. flava*

that is compatible with control tactics for other pests on Asian brassicas should be developed (Martin et al. 2006).

Several hymenopteran parasitoids have been reared from *S. flava*, for example, *Dacnusa scaptomyzae*, *D. temula* and *Halticoptera smaragdina* in North America, Czechoslovakia and Sweden, respectively (Gahan 1913; Maca 1972; Graham 1969) and *Proacris* sp., *Asobara persimilis* and *Opius* sp. in New Zealand (Martin 2004). Currently, the efficiency of *A. persimilis* for the control of *S. flava* in Canterbury and North Otago is being evaluated as part of the ongoing development of IPM tools for seed, forage and vegetable brassica crops grown in the South Island (MacDonald et al. 2011).

1.4 Importance and relevance of this study

Knowledge on a plant pest's responses to abiotic environment, and its feeding, oviposition and mating behaviour is essential for the development and implementation of innovative monitoring and control measures for the pest (Boake et al. 1996; Tsai & Liu 2000; Sinclair & Hughes 2010). Such information is also vital to optimising insect mass-rearing programmes for research, food for natural enemies, and pest control with sterile insect techniques (Tsai & Liu 2000). So far, the biology of *S. flava* is largely unknown, making it difficult to develop effective control measures for this pest.

1.4.1 Biological studies and pest management

1.4.1.1 General biology

Understanding how temperature affects the growth, development and reproduction of an insect is important for the prediction of population dynamics in the field (Pedigo 2004), mass-rearing of laboratory colonies (Chong et al. 2008) and determination of appropriate time and method for control actions (Chatterjee & Laskar 2010). Prior to the present study nothing was known about the effect of temperature on *S. flava*.

Circadian rhythms occur in most important processes of insect life, such as feeding, mating, oviposition and adult emergence (Jonušatte & Buda 2002), and enable organisms to adapt to ambient environmental conditions by coupling behavioural and

physiological events to cyclic factors in the environment (Kumar et al. 2006). Understanding circadian activity patterns is vital to the development of methods for monitoring and control of pest populations (Reznik et al. 2009). So far, circadian activity rhythms for *S. flava* have not been studied.

Production of a large number of natural enemies for release against the target pests is an important component of biological control programmes (Duan & Oppel 2012). Considering the prospective development of biological control programmes for *S. flava* (MacDonald et al. 2011), it is important to understand how to mass-rear this species in an efficient and cost effective way. Furthermore, constant supply of *S. flava* of high quality and quantity is essential for my PhD studies. However, mass-rearing method for this species was not available for this species. To develop a method of such I need to understand the nutritional requirements of this species.

1.4.1.2 Feeding and oviposition

Modern pest management strategies rely upon an understanding of the host selection and oviposition behaviour of insect pests (Cunningham et al. 1999). Studies indicate that leafminer adults prefer large leaves for feeding and oviposition (Hileman & Lieto 1981; Simberloff & Stiling 1987) as their offspring feed and complete the development on the site of oviposition (Gripenberg et al. 2010). Such oviposition preference may result in heavy larval infestation on preferred plants and reduce their yield significantly. Two theories have been proposed to explain host selection for feeding and ovipositon: optimal oviposition theory - females select oviposition sites which are the best for the development of their offspring (Jaenike 1978), and the optimal foraging theory - females prefer to feed and oviposit on hosts best satisfying their own nutrition requirements (Price et al. 1999; Scheirs et al. 2001).

The plant stress hypothesis proposes that phytophagous insects prefer stressed plants for feeding and oviposition (Mattson & Haack 1987b) because stressed plants have increased levels of free amino acids (Brodbeck & Strong 1987). Some of the previous studies support this hypothesis (White 1969, 1974, 1984 & 1993; Mattson & Haack 1987b) while others do not (Wagner & Frantz 1990; Mopper & Whitham 1992; Huberty & Denno 2004). An alternative hypothesis, plant vigour hypothesis, has also been proposed which predicts that phytophagous insects prefer vigorous plants (Fritz et

al. 2000). This indicates that feeding and oviposition preference differs in different herbivorous species. Knowledge on this aspect of biology in *S. flava* would contribute to the development of its control measures.

1.4.1.3 Mating behaviour

Knowledge on mating behaviour of insect pests can help in the sterile insect technique (SIT) by producing males with sexually selected traits and thus higher mating success (Rodriguero et al. 2002). It is also useful in pest management using sex pheromones as it may help determine the time of the day when the released pheromones are likely to be effective (Cardé & Minks 1995; Baoke 1996).

Males' persistent courtship and attempts to copulate with reluctant females (Clutton-Brock & Parker 1995) impose costs to females (Sakurai & Kasuya 2008). This may be associated with operational sex ratio (OSR). For example, in male-biased OSR sex harassment and superfluous matings may increase (Sih & Kurpa 1996; Wigby & Chapman 2004; Smith & Sargent 2006). Knowledge on these aspects of a plant pest can contribute to effective mass-rearing, sex pheromone applications and SIT. So far, mating behaviour of *S. flava* has not been studied.

1.5 Aim and objectives

The aim of this research was to provide critical information on the biology of *S. flava*, with the following objectives:

1. To investigate the general biology of *S. flava*,
2. To investigate the feeding and oviposition preference in *S. flava*,
3. To investigate the mating behaviour of *S. flava.*

1.6 Hypotheses

The following hypotheses were tested in the present study:

1. Variation in adult diet affects fecundity and longevity in *S. flava*;

2. *S. flava* has characteristic circadian rhythms of emergence, mating, feeding and oviposition;
3. Temperature affects the developmental time and population growth of *S. flava*;
4. *S. flava* has varied feeding and oviposition preference for Chinese cabbage leaves and plants of different growth stages;
5. *S. flava* has varied feeding and oviposition preference for water stressed and vigorous plants;
6. Confinement conditions and previous male mating experience affect the subsequent mating behaviour of *S. flava*;
7. Sexual harassment by males has fitness costs for *S. flava* females, and
8. Mate choice for particular traits occurs in both sexes of *S. flava*.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

This chapter reviews the current knowledge on general biology, ecology, host preference and mating behaviour relevant to my PhD studies. Special references are given to known biology and *S. flava*.

2.1.1 Classification of *Scaptomyza flava* (Fallén)

This species was first described by Fallén in 1823 as *Drosophila flava* Fallén (Lastovkai & Jan 1978). In 1849 Hardy placed it in the new drosophilid genus *Scaptomyza* (Whiteman et al. 2011). Now this species is known as *Scaptomyza flava* (Fallén) Hardy (Watabe et al. 1993). *Scaptomyza flaveola* (Meigen) and *S. apicalis* (Hardy) are the synonyms of *S. flava* (Martin 2004). The present classification of this species is:

Order: Diptera

Family: Drosophilidae

Subfamily: Drosophilinae

Tribe: Drosophilini

Genus: *Scaptomyza*

Subgenus: *Scaptomyza*

Species: *flava*

2.1.2 Worldwide distribution

The genus *Scaptomyza* originated in Hawaii (O'Grady & DeSalle 2008). *Scaptomyza flava* is the only *Scaptomyza* species known to occur in New Zealand. It has been recorded in Azores and Madeira Islands in North Atlantic Ocean, Canary Islands near the north-west coast of Africa, East and West Siberia, Europe, Afghanistan, Uzbekistan, north-west China (Xinjiang), Mongolia, Japan (Dubatolov 1998), Iran,

North America, Australia (Seraj 2000; Björksten et al. 2005) and New Zealand (Martin 2004).

2.2 The evolution and significance of leaf-mining

The earliest fossil records of leaf-mining are known from the Triassic period of the Mesozoic era, 248-206 million years ago (Rozefelds & Sobbe 1987; Rozefelds 1988; Labandeira 2002). Leaf-mining thus appears to have arisen after other common forms of insect herbivory such as skeletonising (approximately 260 mya), galling (approximately 300 mya), margin feeding (approximately 310 mya) and boring (approximately 400 mya) (Labandeira 2002). Three hypotheses have been proposed to explain the adaptive significance of the leaf-mining habit: 1) enemy avoidance, 2) environmental regulation, and 3) avoidance of plant defences (reviewed in Connor & Taverner 1997).

2.2.1 Enemy avoidance

It has been suggested that the mine provides protection to the leafminer from natural enemies (Hering 1951). There is, however, some evidence which shows that leafminers are more susceptible to the natural enemies than external-feeding insects (Hawkins & Gross 1992), and success rates of biological control programs based on the release of parasitoids are higher for leaf-mining than for external-feeding insects (Gross 1991, Hawkins & Gross 1992). For example, the oak leafminer *Phyllonorycter messaniella* Zeller was successfully controlled after the release of two wasp parasitoids, *Apanteles circumscriptus* Nees and *Achrysocharoides splendens* Delucchi (Thomas & Hill 1989). These findings appear to be inconsistent with the hypothesis that the mine provides protection to the leafminer from the natural enemies.

2.2.2 Environmental regulation

The mine may act as a refuge from the adverse effects of UV radiation and desiccation (Connor & Taverner 1997). Contact insecticide sprays have proved to be ineffective against leaf-mining sawflies (Loch et al. 2004), suggesting that the mine buffers leafminers from external environmental factors. Leafminer population was also

found to be not affected by the rainfall (Sinclair & Hughes 2008). Evidence available so far supports the hypothesis that the mine provides protection from the adverse environmental effects.

2.2.3 Avoidance of plant defences

Scaptomyza flava and many other dipterous leafminers such as *Liriomyza trifolii* (Burgess), *L. huidobrensis* (Blanchard), *Phytomyza ilicicola* (Loew) and *Agromyza frontella* feed on mesophyll tissue of the leaf (Parrella et al. 1985; Kimmerer & Potter 1987; Guppy 1981; Weintraub 2001). Because leafminers only feed on certain tissue types within the leaf, it is possible that they can avoid both external defences (such as waxy cuticles and spines) and some internal defences such as secondary metabolites (Connor & Taverner 1997). For example, the jarrah leafminer chews oil glands on jarrah but manipulates the oil to avoid ingestion (Mazanec 1983). Eucalypts have tough leaves (Cooper 2001) with high levels of chemical defences (Cooper 2001; Neilson et al. 2006), and yet at least 45 eucalypt species are mined (Sinclair & Hughes 2010). These findings also support the hypothesis that the leafminers can avoid the internal and external plant defences.

2.3 General biology of *Scaptomyza flava*

Scaptomyza flava is holometabolous, and its life cycle includes four stages: egg, larva, pupa and adult (Fig. 2.1). Because very little was known about the biology of this species, all photos and measurements in this section were taken by me during my PhD studies.

2.3.1 Eggs

Most fly eggs are elongate (Chapman 1998). The whitish, translucent egg of *S. flava* is deposited through the adaxial or abaxial leaf surface into the puncture made by the ovipositor. The average egg length is 0.36 ± 0.0083 mm (mean ± SE), and width 0.15 ± 0.0036 mm (n = 25) (Fig. 2.1b). Eggs are laid singly but often in close proximity to each other. As the eggs develop they gradually become opaque, and the yellowish brown cephalopharyngeal skeleton of the first instar larvae can be seen.

2.3.2 Larvae

The larva is cylindrical and maggot-like, with tapering anterior end (Fig. 2.1c). After eclosion the neonate larva starts feeding on mesophyll tissue within the leaf, resulting in narrow translucent linear mines (Fig. 2.2a). Typically drosophilids have three larval instars (Sharma et al. 1995; Carson 1974). As the larva grows it consumes leaf more voraciously, creating a blotch mine (Fig. 2.2b). When larvae are forced to compete for leaf resources because of crowding, they may tunnel into leaf stalks. When a larva is ready to pupate, it cuts a slit in the leaf surface and comes out of the leaf. It moves on the leaf with peristaltic locomotion, and then with rolling motion falls on to the ground for pupation. Larvae mostly pupate outside the leaf but occasionally pupation may occur within the leaf or in the leaf stalk. Mature larvae are 4.22 ± 0.03 mm long (n = 35).

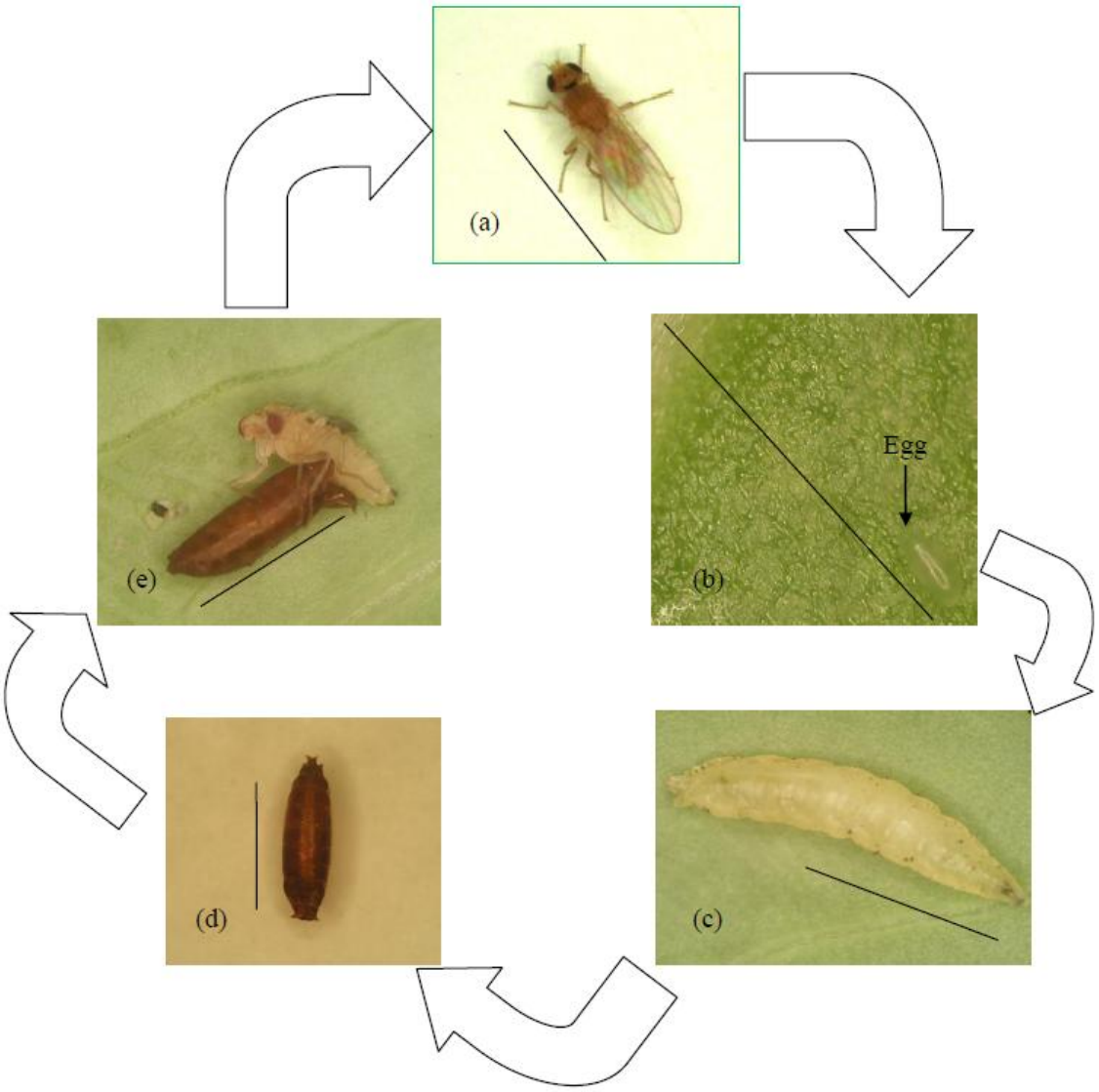

Fig. 2.1 Life cycle of *S. flava*: a) adult, b) egg, c) larva, d) pupa, and e) adult emerging from pupa (Bars = 2 mm).

2.3.3 Pupae

When ready to pupate, the larva becomes shorter and thicker. The final instar skin forms the puparium. The puparia are first light yellow and then within 24 hours become brown (Fig. 2.1d). The pupal length is 3.33 ± 0.23 mm (n = 104), and the male and female pupal weights are 12 ± 0.04 mg (n = 40) and 17 ± 0.04 mg (n = 64), respectively.

2.3.4 Adults

Scaptomyza flava adults have four longitudinal rows of acrostichal setae in front of anterior dorsocentral bristles (Seraj 2000) (Fig. 2.1a). Females are larger than males (n = 30). The hind tibia length in females is 0.91 ± 0.02 mm, compared to 0.79 ± 0.02 mm in males. The hind wing length in females is 3.30 ± 0.04 mm, compared to 2.91 ± 0.04 mm in males. Both males and females mate multiply. Females puncture the leaf with their ovipositors and feed on sap exuding from the puncture. They make feeding punctures on both upper and lower leaf surfaces.

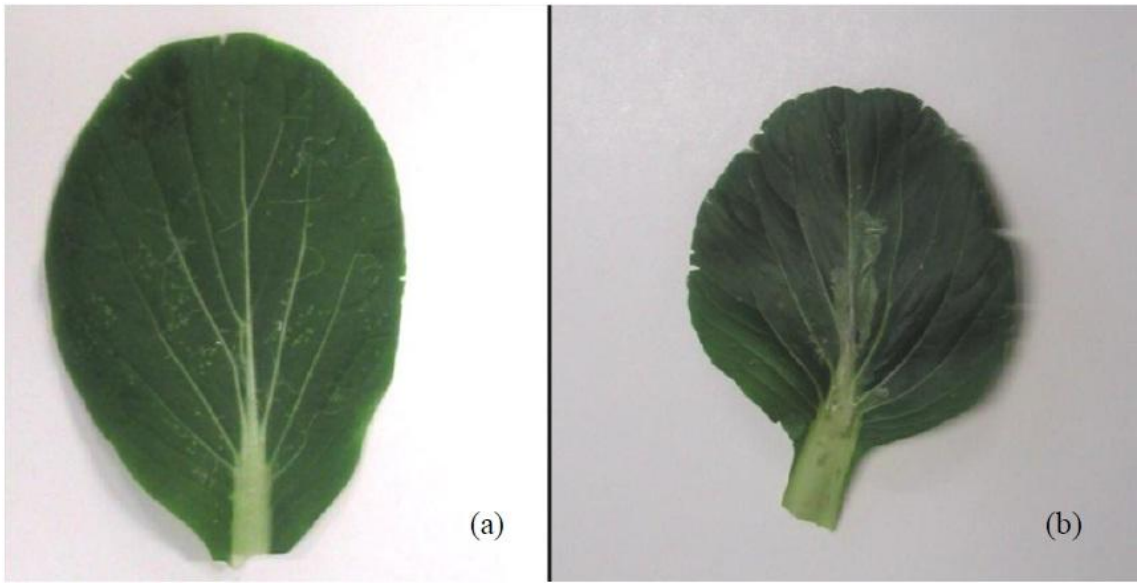

Fig. 2.2 Chinese cabbage leaf with a) linear mines b) a blotch mine.

2.4 Effect of environmental factors on insect biology

2.4.1 Impact of diet on adult longevity and fecundity

For many insects fecundity and longevity depend on the acquisition of food during the adult stage (Hespenheide 1991), and starvation in adults results in reduced longevity and fecundity (Visser & Ellers 2008; Boggs 2009). For example, American serpentine leafminer *Liriomyza trifolii* (Burgess) females live significantly longer when provided with tomato leaves compared to when they are starved (Zoebisch & Schuster 1987), and in Rutherglen bug, *Nysius vinztor* Bergroth (Hemiptera: Lygaeidae), starvation results in reduced fecundity (Kehat & Wyndham 1972). If both larvae and

adults feed but their food is different, the effects of food on adult life-history traits differ depending on which stage experiences restricted feeding (Boggs 2009). For example, in the butterfly *Speyeria mormonia* (Edwards), food stress in the larval stage affects adult survival whereas food stress in the adult stage reduces the fecundity (Boggs & Freeman 2005).

In insects carbohydrates primarily provide energy for somatic maintenance, locomotion, and also contribute to ovigenesis over the reproductive life of synovigenic females (Jervis et al. 2007). For example, *Homona coffearia* Nietner (Lepidoptera: Tortricidae) females need sugar in order to produce eggs (Benz 1991). Feeding on carbohydrate is important for the attainment of reproductive maturity in the Oriental armyworm *Mythimna separata* Walker (Lepidoptera: Noctuidae) (Han & Gatehouse 2009), and in *Phyllonorycter blancardella* Fabricius (Lepidoptera: Gracillariidae) higher fecundity and greater longevity are recorded when females are provided with sugar solution compared to water only (Pottinger & LeRoux 1971). Similarly, in *L. trifolii* higher fecundity and longevity are recorded when honeydew is provided along with tomato leaflets compared to when only tomato leaflets are provided (Zoebisch & Schuster 1987). In tephritid fruitflies *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi) and *C. capitata* Wiedemann (Diptera: Tephritidae), maximum longevity is recorded when the flies are provided with sucrose along with yeast hydrolysate (Manrakhan & Lux 2007). Knowledge about the nutritional requirements of an insect species is the key to its successful rearing (Grenier 2009). Knowledge on insect diet can help in simplification of the rearing technique and reducing the cost of production, which is desirable for mass production of insect pests for utilization of parasitoid or predatory insects in the biological control programmes (Grenier 2009).

2.4.2 Daily and lifespan activity patterns

Circadian rhythms are important regulatory processes (Sandrelli et al. 2008), which enable the organisms to anticipate and to prepare for predictable changes in their environment (Jürgen-Stelzer et al. 2010). Organisms track time in their local environment by entraining their circadian clocks to the light-dark (LD) cycles, a phenomenon that is of paramount importance for their survival under fluctuating environments (Daan 1981), and provides a physiological mechanism that enables them

to anticipate and prepare for seasonal changes in their environment that are often far into the future (Bradshaw & Holzapfel 2010). There are two major rhythms of the biosphere, a daily rhythm of 24 hours and an annual rhythm of 12 months (Bradshaw & Holzapfel 2010). Daily temporal organization by the circadian clock is important for the integration of hundreds of metabolic events on a daily basis (Matsumoto 2006), and seasonal temporal organization is important for maintaining synchrony of life historical events with changing seasonal exigencies and opportunities (Bradshaw et al. 2004; Bradshaw & Holzapfel 2007).

Insects adjust their behavioural rhythms to daily patterns of daylight and conditions that fluctuate in synchrony with light levels (Levine 2004). Timings of several activities such as mating, oviposition, hatching, moulting, pupation, emergence, foraging and flight are rhythmic and occur at a given time of the geophysical day (Jimenez-Perez et al. 2002; Pandey & Rastogi 2009). For example, in Parthenium beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomildae) (Pandey & Rastogi 2009), blood sucking bug *Rhodnius prolixus* Stal. (Hemiptera: Reduviidae) (Ampleford & Davey 1989), and pink bollworm *Pectinophora gossypiella* Saunders (Pittendrigh & Minis 1964), oviposition occurs near the end of photophase while in ladybirds *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae), *C. septempunctata* Linnaeus, *Propylea dissecta* Mulsant (Omkar et al. 2004), and *Cheilomenes sexmaculata* Fabricius (Omkar & Singh 2007), it takes place during the scotophase. The activity patterns of some insect species have two distinct bouts, one centred around dawn (morning) and the other around dusk (evening) (Kumar et al. 2007). For example, *D. melanogaster* flies are active in the morning and in the evening, and show less activity during midday (Rieger et al. 2007) to avoid the heat (Dubruille & Emery 2008).

The information on the rhythmicity in life events such as emergence, oviposition and mating, and the identification of underlying patterns are helpful in enhancing the understanding of the basic biology of any organism (Pandey & Rastogi 2009). Such information also provides the foundation for further studies on the reproductive behaviour, identification of suitable time for population sampling and monitoring, and selection and application of an appropriate control method (Liu et al. 2010; Omoloye & Odebiyi 2001).

2.4.3 Influence of temperature on life history

Abiotic factors like temperature and humidity affect the survival, development and reproductive capacity of insect pests (Iqbal et al. 2010). Temperature is a critical abiotic factor which influences the dynamics of insects, and sets the limits of biological activities such that the low and high temperature threshold and optimal temperature can be estimated for all major life processes (Haghani et al. 2007). Variation in temperature is one of the most important causes of dramatic changes in insect abundance (Cornell & Hawkins 1995). Generally, higher temperature results in higher growth rates and shorter developmental time in insects (Sibly & Atkinson 1994) because of the direct effect of temperature on metabolic rates (Nylin & Gotthard 1998).

Higher temperature has been reported to reduce the longevity and fecundity of several insects such as vegetable leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) (Costa-Lima et al. 2010), mealybug *Maconellicoccus hirsutus* Green (Hemiptera: Pseudococcidae) (Chong et al. 2008), and *Eretmocerus furuhashii* Rose & Zolenerowich (Hymenoptera: Aphelinidae) (Qiu et al. 2007). Variation in temperature has also been reported to affect other life history traits of the insects. For example, in *L. sativae*, the pre-oviposition and oviposition periods decrease with the increase in temperature from 18°C to 32°C (Costa-Lima et al. 2010); in *M. hirsutus*, higher number of female offspring are recorded at 30°C (Chong et al. 2008), and in *E. furuhashii* higher temperature results in reduced larval survival and developmental time, and fewer number of female offspring (Qiu et al. 2007).

Information about the effect of different temperatures on life history parameters of a pest species is useful for the estimation of its intrinsic rate of increase and development of the predictive model for that pest (Xia et al. 2009).

2.5 Feeding and oviposition behaviour

2.5.1 Host plant preference of leafminers

Leaf quality is a major determinant of host choice by leafminers and variations in leaf quality influence their distribution, abundance and survivorship (Cornelissen & Stiling 2008). Several studies have been conducted to gather information about within-plant (Bultman & Faeth 1986a; Jones & Parrella 1986; Valladares & Lawton 1991; Barrett 1994; Facknath 2005; Kharrat & Jarraya 2005), and within-leaf feeding and

oviposition preference of the leafminers (Stiling et al. 1987; Reavey & Gaston 1991; Digweed 2006). Such information can be used to determine sampling location, which can permit a quantitative estimation of the pest population, and help in minimizing crop loss, by the application of management strategies at appropriate time (Torres at al. 2001).

Many leafminer species prefer leaves of certain age for feeding and oviposition (Faeth et al. 1981b). For example, some leaf-mining species on oak trees prefer young leaves while others prefer mid-aged ones (Faeth et al. 1981b); *Nuerostrota guniella* Busck (Lepidoptera: Gracillariidae) (Davis et al. 1991) and *Tinea nectarea* Meyr. (Elliot et al.1998) prefer mature leaves; *Acrocercops plebia* (Lepidoptera: Gracillariidae) (New 1976), *A. laciniella* Meyrick (Common), *Japanagromyza eucalypti* Spencer (Moore 1966), *Perthida glyphopa* Common (Mazanec & Justin 1994), and citrus leafminer *Phyllocnistis citrella* Stainton (Liu et al.1999) prefer immature leaves.

Leafminer females may discriminate between damaged and undamaged leaves for oviposition (Faeth 1986), and their larval survivorship decreases with increased leaf damage (Hespenheide 1991). For example, in *Phyllonorycter harrisella* (Linnaeus) greater larval survival and pupal weights were recorded in undamaged leaves of the oak *Quercus robur* L. as compared to the damaged leaves (West 1985). Leaf nitrogen content is also considered to be an important determinant of leaf quality for herbivorous insects, often affecting host preference (Sinclair & Hughes 2010). However, in the locust leafminer *Odonata dorsalis* (Coleoptera: Chrysomelidae), feeding was not found to be correlated with leaf nitrogen content of *Robinia pseudoacacia* (Linnaeus) leaves (Athey & Connor 1989).

Leafminers may also show preference for leaves in terms of leaf position on the plant. For example, *Bucculatrix cerina* Braun (Lepidoptera: Bucculatricidae) is found exclusively in the upper tree crown (Faeth et al. 1981b), *Stilbosis quadricustatella* Chambers (Lepidoptera: Cosmopterigidae) prefers peripheral leaves (Simberloff & Stiling 1987), and *Cameraria* sp. (Davis) (Lepidoptera: Gracillariidae) prefers the central leaves (Bultman & Faeth 1986b).

2.5.2 Leaf abscission and leafminer mortality

Several studies have shown that leaf abscission is an important and common cause of larval mortality in leafminers (Simberloff & Stiling 1987; Auerbach & Simberloff 1989; Preszler & Price 1993). If abscission occurs before the miner has completed development within the leaf, then the miner may die with the leaf (Stiling et al. 1991; Auerbach et al. 1995). However, miner death is not always inevitable following abscission. For example, Kahn & Cornell (1989) have reported that when the leaves of American holly tree *Ilex opaca* (Aiton) abscise due to mining, *Phytomyza ilicicola* Loew (Diptera: Agromyzidae) is not affected because at the time of abscission the miner has already finished feeding. Similarly, Stiling & Simberloff (1989) reported death due to leaf abscission to be less than 3% for aspen leafminer *Lithocolletis salicifoliella* Chambers (Lepidoptera: Gracillariidae) on quaking aspen *Populus tremuloides* (Michx.).

Leaf-mining by the leafminers may induce leaf abscission (Sinclair & Hughes 2010). Studies have indicated that abscission rates of leaves with mines are much higher than those without mines (Kahn & Cornell 1989; Waddell et al. 2001), suggesting that the damage from the mining process actively induces abscission (Faeth et al.1981a; Kahn & Cornell 1989). Similarly, Stiling et al. (1991) found a positive correlation between the density of mines on an individual leaf and the probability of leaf death resulting from abscission. However, it has also been reported that in some leafminer species, larvae can delay the death of the leaf tissue in and around their mines (Yamazaki 2010).

2.5.3 Host preference, offspring performance and adult fitness

Optimal oviposition theory predicts that females select oviposition sites that are the best for the development of their offspring (Jaenike 1978). The basic premise for this theory is that the offspring of many leafminers have to feed on the oviposition site because of their limited mobility (Jaenike 1978; Thompson 1988; Mayhew 1997). Many studies report the high offspring performance on the hosts preferred by the ovipositing females (reviewed in Gripenberg et al. 2010) while others show a weak relationship between the oviposition preference and offspring performance (Valladares & Lawton 1991; Fritz et al. 2000; Faria & Fernandes 2001; Scheirs & De Bruyn 2002; Scheirs et

al. 2004). Optimal foraging theory predicts that females increase their fitness by feeding on the hosts which best satisfy their own rather than their offspring's nutrition requirements (Jaenike 1986). By feeding on high quality hosts they can allocate higher amount of energy for maintenance and reproduction (Costa et al. 2008). For example, *L. trifolii* (Scheirs et al. 2004), *Chromatomyia nigra* Meigen (Diptera: Agromyzidae) (Scheirs et al. 2000), and *Altica carduorum* Guer. (Coleoptera: Chrysomelidae) (Scheirs & De Bruyn 2002) females have been reported to feed and oviposit on hosts for enhancing their own rather than their offspring's performance.

The relationship between adult host preference and offspring performance is expected to be stronger in the insect like gall-formers and leafminers because their offspring are almost sessile and usually complete their development on the site of oviposition (Gripenberg et al. 2010). For better understanding of the evolutionary adaptation of oviposition preference, attention to both optimal oviposition and foraging should be paid (Lewis et al. 1998; Scheirs et al. 2000; Scheirs & DeBruyn 2002). Adult *S. flava* females feed and oviposit on the same leaf where the larvae feed, and larvae are confined to the mines. Therefore, like the dipterous leafminer *Chromatomyia nigra* (Scheirs & DeBruyn 2002) this species is a good candidate for testing both optimal oviposition and foraging theories.

2.5.4 Effect of plant stress on herbivore's performance

Environmental stress such as water deficit affects plant physiology and development, which in turn influences herbivore performance and population dynamics. In the view of these interactions, two major hypotheses are proposed (De Bryun et al. 2002). The plant stress hypothesis proposed by White (1984) argues that plants experiencing intermediate levels of stress become more susceptible to herbivores (Mattson & Haack 1987b). Improved nutritional quality (Joern & Mole 2005) or reduced production of chemical defences by the stressed host plants results in increased performance by herbivores (Rhoades 1983). A positive relationship between adult feeding preference and plant stress has been found in some insect species (Mattson & Haack 1987b) while Huberty & Denno (2004) reported negative effects of plant stress on the performance of sap-feeding insects.

Plant vigour hypothesis predicts that insect herbivore fitness will be higher on vigorous than on less vigorous plants (Price 1991). Vigour is defined as any plant or plant part that grows rapidly and reaches a larger size in relation to the mean growth rate (Gonçalves-Alvim et al. 1999). Several studies support this prediction (Price & Ohgushi 1995; Stein & Price 1995; Woods et al. 1996; Inbar et al. 2001; Bruyn et al. 2002) while others reject it (Bruyn 1995; Faria & Fernandes 2001; Rehill & Schultz 2001). Price (1991) postulated that those insect herbivores whose offspring development is associated with host-plant growth processes should prefer most vigorous plants where subsequent larval performance is highest.

2.6 Mating behaviour

2.6.1 Mating behavioural sequences

Courtship and copulation behaviours in animals are usually distinctive and characteristic, and their progression is directed by multiple interacting factors (Nicholson 2008). An understanding of mating behaviour of insect pests can lead to successful implementation of sterile insect technique (Hendrichs et al. 2002).

Although little is known about *S. flava*, mating behaviour of many drosophilids has been well studied (Bacigalupe et al. 2008; Wen & Li 2011; Spieth 1952). Drosophilid flies usually display elaborate courtship behaviour before copulation (Bennet-Clark et al. 1980). For example, a *D. melanogaster* male orients his body in the direction of a female, follows her while she moves, and vibrates alternately each of his wings at a 45° angle to his main body axis (Lasbleiz et al. 2006). If she becomes receptive, she reduces her movement and allows him to lick her genitalia with his proboscis, and then he curls his abdomen and attempts to copulate (Lasbleiz et al. 2006). In *Drosophila*, the best known female pre-copulatory behaviour is rejection behaviours, such as wing fluttering, depression or elevation of the tip of the abdomen, decamping, kicking and ovipositor extrusion (Spieth 1974).

2.6.2 Female multiple mating

Female lifetime offspring production is principally a product of female longevity, fecundity and fertility, and all fitness components are known to be affected

by mating (Arnqvist & Nilsson 2000). In some insect species, females obtain enough sperm from a single mating to fertilize their full egg-load (Thornhill & Alcock 1983; He & Miyata 1997). However, females of many species mate more than once in their lifetime (Chapman & Partridge 1996) with different males (polyandry) and/or with the same male (repeated matings). Multiple mating in females is thought to obtain material benefits such as more sperms (Newcomer et al. 1999) or genetic benefits such as avoidance of inbreeding (Tregenza & Wedell 2002), manipulating offspring paternity (Edvardsson & Arnqvist 2000) and production of genotypically attractive sons (Andersson 1994). In nature polyandry appears to be more prevalent in insects because of lack of long-term pair bonds (Choe & Crespi 1997).

Several studies have found a positive relationship between the number of matings and female fecundity (Danthanarayana & Gu 1991; Ward & Landolt 1995; Oberhauser 1997; Wilson et al. 1999) while others do not show such a relationship (Kraan & Straten 1988; Ono et al. 1995; Kawagoe et al. 2001). Multiple mating is common in many drosophilid females in both natural and laboratory populations (Singh et al. 2002). Some drosphilid males may transfer a large number of accessory substances (Pitnick et al. 1991), typically various proteins, with the ejaculate, which may stimulate egg production, egg maturation, oviposition (Arnqvist & Nilsson 2000) and sperm storage (Wolfner 1997).

For females, there are also costs associated with multiple mating such as elevated predation risk (Kronauer et al. 2007), sexually transmitted diseases and metabolic costs of storing more sperm (Sherman et al. 1988; Simmons 2001). Furthermore, male-derived substances may reduce female longevity and receptivity (Fowler & Partridge 1989; Chapman et al. 1998; Eberhard 1996). Females may thus adjust their mating rate according to operational sex ratio, population density and presence of predators (Heller & Helversen 1991; Arnqvist 1992, 1997; Sih & Krupa 1996; Wiklund & Kaitala 1995).

2.6.3 Male multiple mating

Since each mating offers an opportunity to father offspring, males can generally increase their fitness by mating with many mates and high mating rates are thus typically associated with high male reproductive success (Arnqvist & Nilsson 2000).

Some studies suggest that male ejaculate is unlimited and inexpensive and its only function is to fertilise the ova (Thornhill & Alcock 1983). However, male ejaculate also contains male-derived substances that modify the female's behaviour and physiology, and may be used for somatic maintenance or to enhance fecundity (Bissoondath & Wiklund 1996; Gillott 2003). Therefore, production of ejaculate and male-derived substances is costly (Svärd & Wiklund 1986), dynamic in time and space (Weddell et al. 2002), and limited (Royer & McNeil 1993; Giebultowicz & Brooks 1998), and male reproductive investment declines over consecutive copulations (Torres-Vila & Jennions 2005).

Drosophila nigrospiracula (Patterson & Wheeler) males pass no nutritive substance to females in ejaculation, *D. pseudoobscura* (Frolova & Astaurov) males pass such substances in moderate quantity, and *D. mojavensis* (Patterson), *D. mettleri* (Heed), *D. arizonensis* (Patterson & Wheeler) and *D. immigrans* (Sturtevant) males transfer a large quantity of nutritive substances (Markow & Ankney 1988; Pitnick et al. 1991). Nutrients contained in the ejaculate have been found in the soma and eggs of females (Wiklund et al. 1993), demonstrating their importance for the female reproductive output.

2.6.4 Social learning and mating behaviour

Learning is ubiquitous among insects, and various insects rely extensively on learning for all important life activities including feeding, enemy avoidance, aggression and mating (Dukas 2008). In drosophilids the experience gained by the males while courting numerous females could affect and modify their mating behaviour (Dukas 2005 & 2006). Males kept in isolation after emergence may exhibit different behaviour compared to those reared in groups, for example, *D. silvestris* (Carson) males kept in isolation after emergence are less successful in mating compared to those reared in groups (Sene 1977); *D. subobscura* (Collin) males kept in isolation display higher mating frequencies compared to those reared in groups (Maynard-Smith 1956).

Previous experience of courting receptive and unreceptive females also affects the subsequent courtship in drosophilids. For example, *D. melanogaster* males having experience of courting unreceptive, recently mated females exhibit reduced courtship of mated females, and males having experience of courting unreceptive, immature females

exhibit increased courtship of receptive virgin females compared to inexperienced males (Dukas 2005).

2.6.5 Sexual selection

Sexual selection is a major force driving the evolution of diverse behavioural, morphological, and physiological traits (Fedina & Lewis 2008). According to Darwin (1871), "Sexual selection depends on the advantage where certain individuals have over others of the same sex and species solely in respect of reproduction".

In species with conventional sex roles, females are more selective of mates than males (Darwin 1871). The reasons include reduced investment in gametes and parental care by males (Ahnesjo et al. 2001), and higher number of sexually active males to receptive females at a given time (Emlen & Oring 1977). These factors result in intrasexual competition between the males, and stronger selection for traits affecting their competitive ability (Clutton-Brock 2007). In species with sex-role reversed such as the Mormon cricket *Anabrus simplex* Haldeman (Robson & Gwynne 2010), strong sexual selection on females is expected as males provide valuable nutrition or protection to their mates or offspring (Clutton-Brock & Vincent 1991; Gwynne 1991).

Sexual selection may be in the form of pre-copulatory selection, which includes intra-sexual competition and mate choice (Darwin 1871), and/or post-copulation selection via sperm competition (Parker 1970) and cryptic female choice (Birkhead 1998). Mate choice is a process, leading to the tendency of members of one sex to mate non-randomly with respect to one or more varying traits in members of the other sex (Kokko et al. 2003). It occurs in both sexes (Andersson 1994) and is facilitated when there are high reproductive costs, high interaction rates, and large difference in mate quality (Kokko & Johnstone 2002). Mate choice is influenced by the time available for sampling potential mates, the distribution of resources in the environment and the willingness of an individual to invest its resources in a specific mate (Emlen & Oring 1977; Halliday 1983; Alexander et al. 1997; Jennions & Petrie 1997).

Mate choice by females is assumed to provide a fitness advantage to choosy females, either directly (e.g. by improving female fecundity) or indirectly (e.g. by enhancing offspring fitness) (Andersson 1994). Significant heritability of preferred male traits has been documented (Hedrick 1988; Norris 1993; Pomiankowski & Møller,

1995). In many insect species females prefer large males for mating (Gray 1997; Savalli & Fox 1998; Gilburn et al. 1992; Gilburn & Day 1994). However, females in some insect species may mate indiscriminately or prefer to mate with smaller males (Weissman et al. 2008; Parker 2009). Studies have shown that females may prefer to mate with males having certain traits such as longer antennae (Yang & Wang 2004), longer legs (Willemart et al. 2009), and longer wings (Xu & Wang 2010).

Males exhibit mate choice by variation in their intensity or frequency of courtship or copulation attempts to females of varying quality (Bonduriansky 2001). For males in promiscuous mating systems (i.e. multiple mating with relatively brief male-female associations), the key factor in female mate quality is expected to be female fecundity, which they assess through phenotypic correlates such as abdomen width (Bonduriansky 2001). In several insect species males prefer females with larger abdomens for mating (Li et al. 2005; Bussiere et al. 2008; Xu & Wang 2010). In some insects ovipositor is associated with offspring survival (Yang & Wang 2004) and males may prefer to mate with females having longer ovipositors (Mousseau & Roff 1995; Sivinski et al. 2001; Yang & Wang 2004). However, males of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) do not show any preference for females having longer ovipositor (Xu & Wang 2010).

As sexually selected traits are often phenotypic indicators of reproductive fitness (Rodriguero et al. 2002), the study of insect sexual selection helps us understand evolutionary divergence of phenotypic traits in relation to mate choice and reproductive fitness. It may also provide critical information for the development of pest management measures such as sterilized insect techniques (Boake et al. 1996).

2.6.6 Sexual conflict and harassment

Characteristics that are beneficial to the reproduction of one sex may sometimes be detrimental to the other, resulting in sexual conflict (Parker 1979). Sexual conflict is considered to be the third form of sexual selection (Clutton-Brock & Parker 1995; Brown et al. 1997) after male-male competition and female choice (Darwin 1871). A commonly observed conflict relates to the optimal number of matings for each sex (Parker 1979; Arnqvist 1992; Smuts & Smuts 1993). For females, there are many costs associated with mating, such as energetic costs of reproductive behaviour (Lighton &

Feener 1989), risk of injury inflicted by males, time devoted to mating (Martens & Rehfeldt 1989), and less time for foraging (Stone 1995). In most species the optimal number of matings for females is well below that for males, and mated females may refuse to mate further with most soliciting males (Pilastro et al. 2003).

Sexual harassment by males has evolved as male strategy to overcome female reluctance to mate by forcing them to accept matings that are potentially detrimental to their fitness (Clutton-Brock & Parker 1995). It may also constrain the exercise of mate choice by females (McLain & Pratt 1999). Sexual harassment has been reported in many insects whitecrossed seed bug *Neacoryphus bicrucis* Say (McLain & Pratt 1999), solitary bee *Anthophora plumipes* Pallas (Stone 1995), water striders *Aquarius remigis* Say (Watson et al. 1997), and adzuki bean beetle *Callosobruchus chinensis* Linnaeus (Gay et al. 2009).

The evolutionary dynamics of sexual conflicts are often complex because male sexual activity negatively affects a number of other aspects related to female fitness, including fecundity, energy expenditure, probability of disease transmission, foraging efficiency, vigilance and conspicuousness to predators (Martens & Rehfeldt 1989; Magurran & Seghers 1994; Watson et al. 1997; Blanckenhorn et al. 2002; Pratt & McLain 2002). Sexual harassment can affect female foraging efficiency, thus it can be even more costly in species like *Scaptomyza flava* (Fallén), where only females create the feeding punctures on which males rely for feeding.

CHAPTER 3

GENERAL BIOLOGY

3.1 General introduction

Scaptomyza flava is an important pest of brassicas, peas and gypsophilla in New Zealand. However, not much information on the biology of this species is available. This chapter starts with a description of the general methodology applied throughout the thesis, and then reports the investigations into the sexual maturation, fecundity, longevity, feeding and oviposition patterns, circadian rhythms, and the effect of temperature on the development of immature stages and population parameters of *S. flava*.

3.2 General methodology

The materials, procedures, environmental conditions and definitions detailed in this section were used throughout the thesis.

3.2.1 Materials

Plants: The Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) seeds were sown in 60 cell plastic trays with each cell (4.5 cm diameter × 4.5 cm high) filled with potting mix having 15% N, 8.4% P and 10.8% K. Seeds were watered once every two days and when the seedlings emerged, they were watered once every three days. Two-week-old plants were transplanted in plastic pots (8 cm basal diameter and 12 cm high). Two- to 2.5-month-old plants were used for the experiments (Fig. 3.1).

Fig. 3.1 Chinese cabbage plants used for rearing *S. flava*.

Leaves: Middle leaves (10-15 cm long) from 2- to 2.5-month-old Chinese cabbage plants were used for experiments.

Plastic trays: Plastic trays (45 cm × 30 cm × 8 cm) were used for collecting pupae.

Petri dishes: Pupae were maintained in Petri dishes (5.5 cm diameter × 1.3 cm high) having moist sand until adult emergence (Fig. 3.2).

Fig. 3.2 *S. flava* pupae maintained in a Petri dish having moist sand.

Cages: Perspex cages (30 cm × 30 cm × 30 cm) with fine metal screen (aperture diameter = 0.2 mm) on top for ventilation were used for maintenance of breeding colony (Fig. 3.3).

Fig. 3.3 Cages for rearing *S. flava*.

Small test cylinders: Transparent plastic cylinders (8.5 cm in diameter × 10.5 cm in height, LabServ, Auckland) with gauze-covered hole on the top (5 cm in diameter) for ventilation were used for experiments on feeding and oviposition behaviour.

Large test cylinders: Transparent plastic cylinders (17 cm in diameter × 25 cm in height, LabServ, Auckland) with six gauze-covered hole on sides (2 cm in diameter) for ventilation were used for experiments on oviposition behaviour.

Glass tubes: Glass tubes (1.5 cm in diameter × 5.0 cm in height) covered with one layer of nylon mesh (aperture diameter = 0.2 mm) were used for experiments on mating behaviour.

Microscope: A dissecting microscope (Olympus, GH, Japan) equipped with transmitted light and a micrometer eyepiece was used for measurements and counting the number of eggs and feeding punctures.

Electronic scale: A Mettler Toledo AG 135 (Switzerland) scale with 0.00001 g accuracy was used for weighing pupae.

Oven: An oven (Watvic, Watson Victor Ltd., NZ) was used for drying the Chinese cabbage plants before determining their dry weight on electronic scale.

Pressure chamber: Scholander pressure chamber (Model 3005, Soilmoisture Equipment Corp., USA) was used for measuring water status of Chinese cabbage plants (Scholander et al. 1964).

Leaf area meter: A leaf area meter (LI-COR Model 3100, Nebraska, USA) was used for measuring leaf area after the leaf was detached from the plant.

Graph paper: A graph paper was used for measuring leaf area when the leaf was not detached from the plant.

3.2.2 Environmental conditions

Unless stated otherwise, colonies were maintained and experiments conducted under the following conditions: 20 ± 1°C and 60 ± 5% RH, with a photoperiod of 16:8 h light:dark (lights on from 09:00 to 01:00 and off from 01:00 to 09:00). Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

3.2.3 Procedures

Breeding colony maintenance: A Chinese cabbage plant was exposed to 50 *S. flava* adults with a sex ratio of 1:1 in a cage (Fig. 3.3) for 24 hours. The infested plants were maintained for 11 days in the open air. When larvae made blotch mines on leaves, and were about to drop out of the leaves to pupate, leaves were cut at the stalk and put in the plastic trays with about 20 leaves in each tray. When larvae came out of leaves and pupated in the tray, they were collected and put in Petri dishes with about 100 pupae in a dish. When adult flies emerged in these dishes, they were transferred to the perspex cages for oviposition.

Insects for experiments: Pupae were collected and weighed individually on the electronic scale. These pupae were then individually put in the glass tubes. When the adults emerged in these tubes, their sex was identified according to Seraj (2000). Females can be distinguished from males as they have dentate ovipositor at the posterior end of their abdomen, which can be seen through the ventral view of their abdomen.

Fertility assessment: Two-d-old eggs were observed under the dissecting microscope to determine egg fertility. Eggs with black dots (larval heads) were recorded as fertile (Thyssen & Linhares 2007).

3.2.4 Definitions

Fecundity was defined as the total number of eggs laid.

Fertility was defined as the total number of fertilised eggs laid.

3.2.5 Statistical analysis and reported values

All analyses were made using SAS 9.2 (SAS Institute, Cary, NC, USA). Rejection level was set at $P > 0.05$. Unless stated otherwise, all reported values are means ± SE.

3.3 Adult feeding, fecundity, longevity and pupal weight

3.3.1 Introduction

In many insects fecundity and longevity depend on the quality of food acquired during the adult stage (Hespenheide 1991), and feeding on carbohydrates increases their fecundity and longevity. For example, provision of honey along with the host plant increases fecundity and adult longevity in the dipterous leafminer *Liriomyza trifolii* (Burgess) (Charlton & Allen 1981). Starvation during adult stage reduces fecundity and longevity of insects (Visser & Ellers 2008; Boggs 2009). For example, it reduces longevity in *L. trifolii* (Zoebisch & Schuster 1987), and fecundity in *Nysius vinztor* Bergroth (Hemiptera: Lygaeidae) (Kehat & Wyndham 1972). However, in the cowpea weevil *Callosobruchus maculates* Fabricius (Coleoptera: Bruchidae), it reduces fecundity but increases longevity (Messina & Fry 2003).

Understanding the nutritional requirements of *S. flava* adults is important for the development of effective mass-rearing method if large numbers of high quality insects are required, for example for the production of its natural enemies or for use in sterile insect tachnique (Grenier 2009). The objectives of the present study were to determine the effect of different foods on adult longevity and fecundity. Fecundity and longevity are positively correlated with pupal weight in many insects (Danthanarayana 1975; Jones et al. 1982; Hough & Pimentel 1978; Cheng 1972); therefore, I used adults emerging from average weight pupae to minimize the effect (if any) of pupal weight on adult fecundity and longevity.

3.3.2 Materials and methods

3.3.2.1 Insects

The pupae were weighed individually on the electronic scale and individually placed in glass tubes until adult emergence. When the adults emerged in these tubes, they were sexed. Adults emerging from average weight pupae were used for experiments.

3.3.2.2 Daily and lifetime feeding and fecundity of *S. flava* adults

This experiment consisted of two treatments with 20 replicates each: i) host plant, and ii) host plant + 10% honey solution. Newly emerged males and females were transferred to the large transparent plastic cylinders with a single pair in each cylinder. In the first treatment a Chinese cabbage plant was provided for 24 hours, which was then replaced with another similar plant every 24 hours. In the second treatment, 5 ml of honey solution was also smeared on the inside of the plastic cylinder with a dropper at a 12 hour interval.

After removed from the cylinder, the leaves of the infested plant were cut and observed under the microscope to count the number of feeding punctures and eggs. Cylinders were observed at a 24 hour interval for recording male and female mortality. When a male died while the female was still alive, that was replaced with another similar male, so that the female may have mating opportunities. When a female died while male was still alive, that female was replaced with another similar female because *S. flava* males could not puncture leaves to feed on cell sap. Data on adult male and female longevity, and the number of feeding punctures made and eggs laid by adult females during each day were recorded.

3.3.2.3 Effect of food treatments on *S. flava* longevity

Newly emerged males and females were kept individually in the glass tubes and were subject to following treatments with 20 replicates each: i) No food, ii) water, and iii) 10% honey solution. In the second treatment 5 ml of water, and in the third treatment 5 ml of honey solution was smeared on the inside of the glass tube with a dropper at a 12 hour interval. Glass tubes were checked for male and female survival at a 24 hour interval. Data on male and female longevity in each treatment were recorded. These data were also compared with adult male and female longevity when they were provided with only host plant, and host plant + honey solution (Section 3.3.2.2).

To investigate whether host plant feeding throughout life was essential for the survival of male and female *S. flava*, newly emerged male and female flies were transferred to the large transparent plastic cylinders with a single pair in each cylinder and were provided with a Chinese cabbage plant (n = 35). The plant was replaced with a similar plant every 24 hours. When the flies were 9-d-old, they were deprived of host

plants, and provided with 4 ml of honey solution as mentioned above. Data on the number of days adult *S. flava* lived after host plant deprivation were recorded.

3.3.2.4 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on adult lifetime feeding and fecundity were subject to one way ANOVA followed by a Tukey's studentised range (HSD) test. Data on pupal weight, adult longevity on different food treatments, and daily feeding and fecundity were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test followed by the Dunn's procedure for multiple comparisons (Zar 1999).

3.3.3 Results

3.3.3.1 Average pupal weight

Average female pupal weight was 16.99 ± 0.5 mg, which was significantly heavier than average male pupal weight of 12.00 ± 0.4 mg (χ^2 = 32.4; *DF* = 1; *P* < 0.05).

3.3.3.2 Daily and lifetime feeding and fecundity of *S. flava* adults

Each female created 1312.3 ± 17.91 and 1271.8 ± 16.66 feeding punctures in her lifetime when provided with only host plant and host plant + honey solution, respectively. There was no significant difference in the number of feeding punctures created between the treatments (*F* = 2.74; *DF* = 1, 38; *P* > 0.05). In both treatments significantly greater numbers of feeding punctures were created from the 5^{th} to 10^{th} days after emergence, and then the number of feeding punctures significantly decreased (χ^2 = 471.09 and 467.17 for host plant and host plant + honey solution, respectively; *DF* = 24; *P* < 0.05; Fig. 3.4).

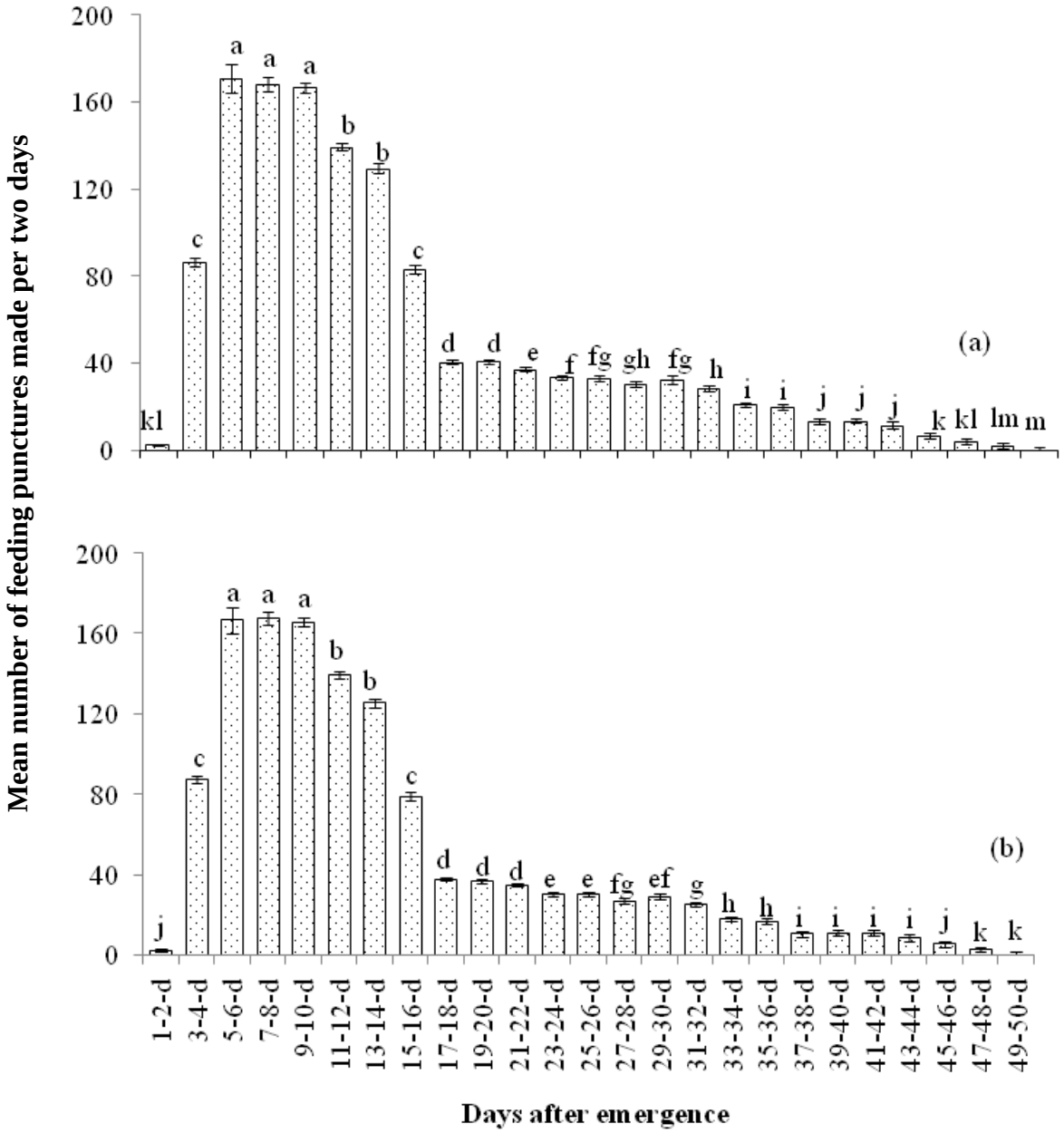

Fig. 3.4 Daily number of feeding punctures made in females' lifetime when provided with (a) host plant, and (b) host plant + honey solution. Bars with different letters are significantly different from each other ($P < 0.05$).

Each female laid 139.5 ± 1.95 and 133.68 ± 2.68 eggs in her lifetime when provided with host plant and host plant + honey solution, respectively. There was no significant difference in the number of eggs laid between the treatments ($F = 3.56$; $DF = 1, 38$; $P > 0.05$). In both treatments significantly greater number of eggs were laid from the 5th to 10th days after emergence ($\chi^2 = 412.21$ and 407.38 for host plant and host plant + honey solution, respectively; $DF = 24$; $P < 0.05$; Fig. 3.5).

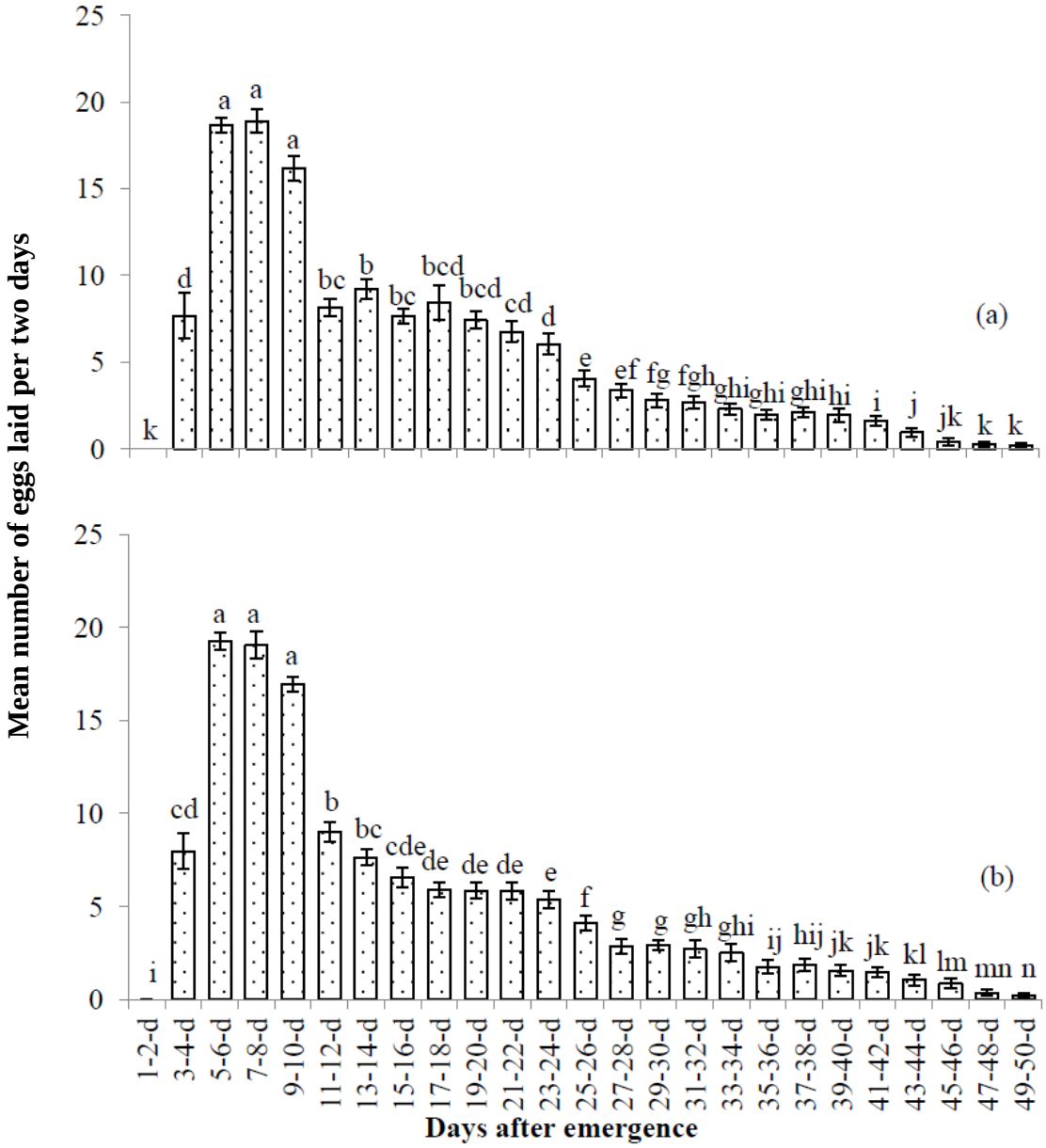

Fig. 3.5 Daily number of eggs laid in females' lifetime when provided with (a) host plant, and (b) host plant + honey solution. Bars with different letters are significantly different from each other ($P < 0.05$).

3.3.3.3 Effect of different food treatments on *S. flava* longevity

Adults lived significantly longer when they were provided with host plant and host plant + honey solution for lifetime compared to other treatments (χ^2 = 90.07 and 89.69 for males and females, respectively; *DF* = 4; *P* < 0.05; Fig. 3.6).

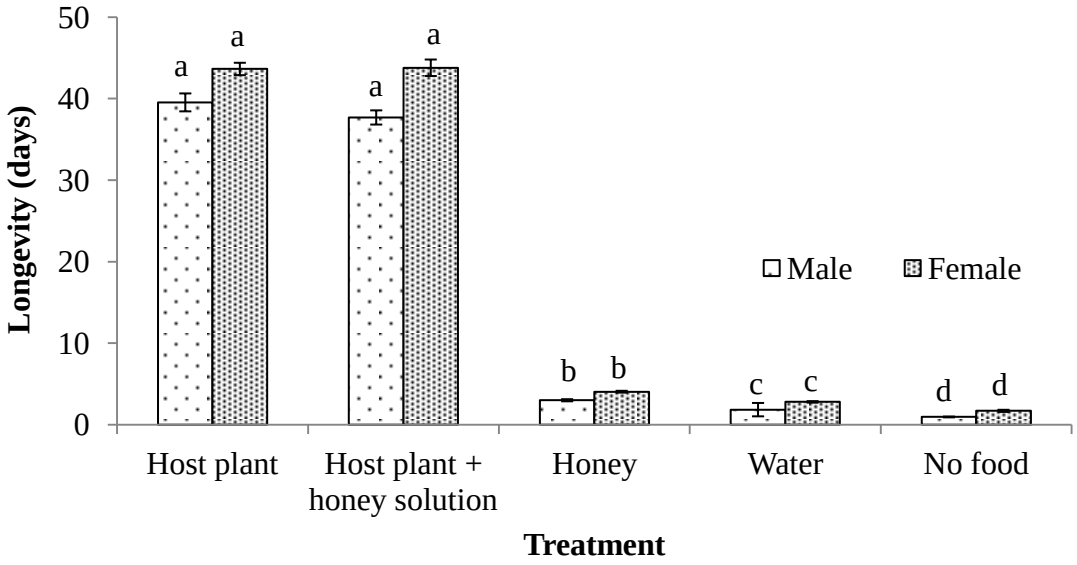

Fig. 3.6 Effect of different food treatments on adult *S. flava* longevity. Bars with different letters are significantly different (*P* < 0.05).

When 9-d-old adult males and females were deprived of host plant and provided with only 10% honey solution, they died 1.4 ± 0.09 and 1.2 ± 0.08 days after host deprivation, respectively.

3.3.4 Discussion

The present study shows that the provision of honey along with host plant did not increase fecundity and longevity in *S. flava*. These findings suggest that *S. flava* are capable of deriving all the required nutrients from the host plant, and the provision of honey is not desirable for cost effective rearing. I also found that post emergence host plant deprivation significantly reduced their longevity. These results indicate that host feeding is essential for *S. flava* survival. Similar results have been reported in jarrah leafminer *Perthida Glyphopa* (Common) (Mazanec & Justin 1986), Mediterranean

fruitflies *Ceratitis capitata* (Wiedemann) (Maor et.al. 2004), and many Drosophilids (Sevenster & Van Alphen 1993).

Adult nourishment is profoundly important for oogenesis in many insects (Wheeler 1996). For example, in fruit flies females need to feed on a source of protein to mature their eggs (Yuval et al. 2007), and ovipositing *Pyrrhocoris apterus* (L.) females have higher feeding requirements than non-ovipositing ones and their food intake peaks during the peak oviposition period (Socha & Zemek 2007). Our studies indicate that in *S. flava* maximum feeding and oviposition take place simultaneously from the 5^{th} to 10^{th} days after emergence. It is suggested that adult feeding is essential for oviposition in this species. These results help identify the appropriate age of *S. flava* females i.e., 5 to 10 day-old, for further biological studies regarding feeding and oviposition.

When 9-d-old *S. flava* adults were deprived of the host plant and then provided with honey solution, they died within 2 days after host deprivation, suggesting that host feeding throughout adult life is crucial for *S. flava* survival. These findings also have implications in the management of this pest, as unavailability of host plants may affect the adult *S. flava* survival. For example, in Swede midge *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), a serious pest of cruciferous plants, successful control was achieved by crop rotation (Chen et al. 2009).

3.4 Circadian rhythms of adult emergence, feeding, mating and oviposition

3.4.1 Introduction

Circadian rhythms are intrinsic to virtually all life forms ranging from bacteria to humans (Wagner-Smith & Kay 2000). These rhythms occur for most important processes of insect life, such as feeding, mating, oviposition, pheromone release, migration and adult emergence (Jonušatte & Buda 2002), and enable organisms to adapt to ambient environmental conditions by coupling behavioural and physiological events to cyclic factors in the environment (Kumar et al. 2006). Synchronising such events maximises an organism's potential to survive under fluctuating environmental conditions (Kumar et al. 2006). Moreover, variation in circadian rhythmicity can reduce direct competition between species that share the same resources, and synchronise mating activities to ensure genetic isolation of sibling species (Saunders 1982).

Understanding circadian activity patterns of insect pests is important for the development of methods for monitoring and controlling pest populations (Reznik et al. 2009). For example, the knowledge about the peak feeding period of adult insects of a species helps in determining the appropriate time of the day for taking samples for estimating its population (Lam 2007). So far no information on circadian rhythms of *S. flava* is available. In the present study, the circadian rhythms of adult emergence, mating, feeding and oviposition of *S. flava* were investigated in the laboratory. This information will enhance our ability to better manipulate *S. flava* population and provide a vital foundation for further investigations on its feeding, oviposition and mating behaviours.

3.4.2 Materials and methods

3.4.2.1 Insects

Pupae were weighed individually on the electronic scale, and then placed individually in glass tubes until adult emergence. When the adults emerged, they were sexed. For experiments on sexual maturation and daily mating rhythm, a 3-4 cm long Chinese cabbage leaf was daily provided to each female in the glass tube while males were provided with similar leaf having 50 punctures made by a dissecting needle until they were used for the experiments.

3.4.2.2 Circadian adult emergence

To observe the adult emergence pattern, two bioassay rooms were set up. The photophase was set during 0900-0100 h in one room (normal-light regime) and during 1330-2130 h in the other room (reverse-light regime). A Chinese cabbage plant was exposed to adult flies for 24 h in the rearing cage in each bioassay room. After the completion of egg and larval development in each light regime, pupae were collected and kept individually in glass tubes in the same light regime. Two hundred and fifty pupae were collected and observed for adult emergence in each bioassay room. Observations on adult emergence were made hourly from 0800 to 0100h (one hour before lights on to the end of the photophase) in the normal-light regime room, and from 1330 to 2130 h in the reverse-light regime room.

3.4.2.3 Sexual maturation

To determine the sexual maturation period of adult flies, I set up two experiments, each with five treatments. In the first experiment 48-h-old virgin females were paired with 0-(newly emerged), 8-, 16-, 24- and 48-h-old virgin males, and in the second experiment 48-h-old virgin males were paired with 0-(newly emerged), 8-, 16-, 24- and 48-h-old virgin females. A virgin male was paired with a virgin female in a glass tube having no food. Twenty pairs were established for each treatment. After paired in glass tubes, each pair was observed for one hour to determine whether they mated or not. When a pair did not mate one hour after paired no further observation was made and such pairs were discarded from the experiment.

3.4.2.4 Daily mating rhythm

For the experiment on daily mating rhythm 48-h-old adults were used. One virgin male was paired with one virgin female in a glass tube 2 h before the lights were on. Twenty pairs were setup, and observed once every one minute during the entire 18 h (2 h before photophase and 16 h photophase). There were two observers for this experiment. Mating during the dark period was observed under red-light conditions. The number of pairs mating at a specific time, frequency of mating for each pair and mating duration were recorded.

3.4.2.5 Daily feeding and oviposition rhythms

To estimate the number of eggs laid and feeding punctures made during the scotophase and different hours of the photophase, 20 5-d-old average weight pairs were randomly selected from the rearing cage, and transferred to the large transparent plastic cylinders with one pair in each cylinder, at the onset of the photophase. Five-d-old females were used for the experiment because maximum feeding and oviposition take place from the 5^{th} to 10^{th} day after emergence (Section 3.3.3.2). A 2-month-old Chinese cabbage plant was exposed to each pair, and replaced with a new similar plant every two hours until the end of 16 h photophase period. Only one plant was exposed to each pair during the entire 8 h scotophase period. Leaves of each exposed plant were cut and the number of eggs laid and feeding punctures made during each 2-h bout of the photophase, and eight hours of the scotophase were counted under the dissecting microscope. Data were collected over a period of five consecutive days.

3.4.2.6 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on feeding and oviposition during different hours of the photophase were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test followed by a Dunn's procedure for multiple comparisons (Zar 1999). Data on the difference in feeding between the photophase and scotophase were analysed using a paired t-test. Data on the number of pairs mating once, twice and thrice were analysed using the Marascuilo's non-parametric procedure (U-test) (Daniel 1990). One way ANOVA was used to analyse the difference in mating duration between the first and second matings.

3.4.3 Results

3.4.3.1 Circadian adult emergence

Adult emergence peaked during the 2^{nd} and 3^{rd} h into the photophase with 57% adults emerging during this period, after which time adult emergence sharply decreased, and no fly emerged 12 h after lights on (Fig. 3.7).

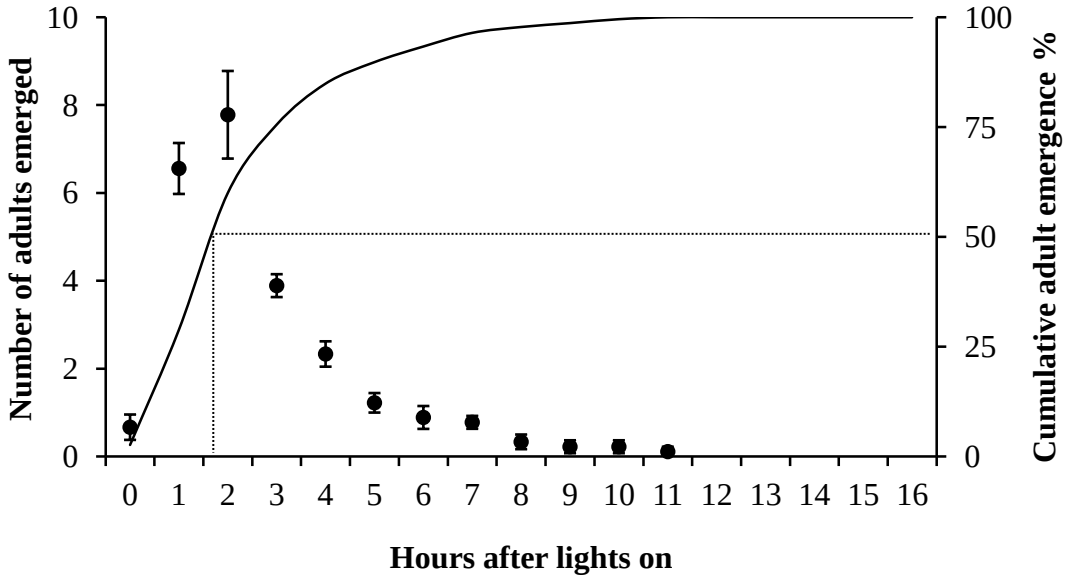

Fig. 3.7 Hourly (number/hour) and cumulative (%) adult emergence of *S. flava* during the photophase.

3.4.3.2 Sexual maturation

Newly emerged, 8-, 12- and 16-h-old males and females did not mate. Ninety percent of males and 85% of females mated when they were 24 h old, and 95% of males and females mated when they were 48 h old. As more than 80% of both sexes mated when they were 24 h old, 24-h-old *S. flava* males and females were considered as sexually mature.

3.4.3.3 Daily mating rhythm

No mating was observed during the scotophase. The first mating was observed 10 min after lights on, and then mating incidence quickly increased and peaked about 30 min after lights on; 90% of pairs were observed to have mated once within 60 min after lights on (Fig. 3.8). Multiple mating occurred in this species, with significantly more adults mating once (100%) or twice (65%) than those mating thrice (5%) ($U = 24.32 > \chi^2_{2,\ 0.05} = 5.99$, $P < 0.05$). No mating was observed 4 h after lights on. When insects mated twice on the same day, the mating duration was significantly longer in the first mating (19 ± 2 min) than in the second mating (12 ± 1 min) ($F = 7.11$; $DF = 1, 31$; $P < 0.05$).

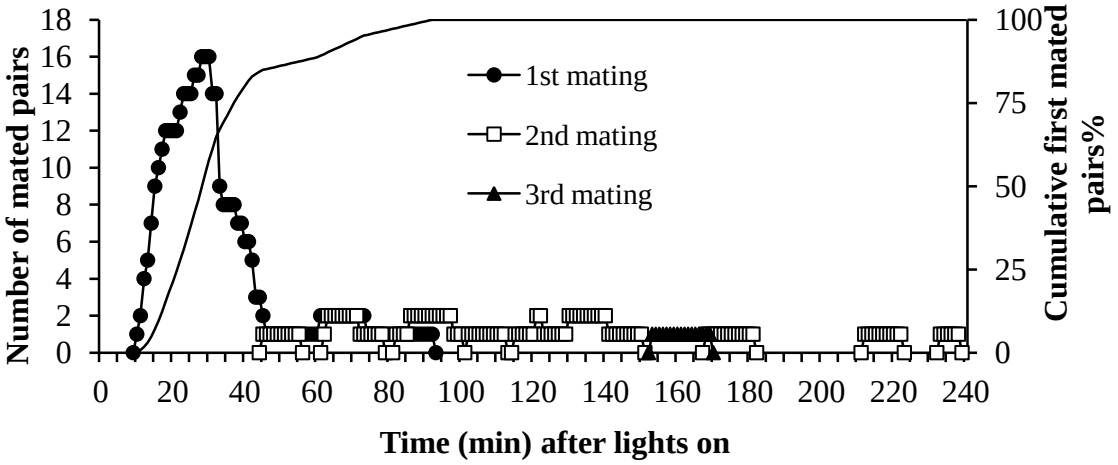

Fig. 3.8 The number of *S. flava* pairs that had mated once, twice or thrice during the photophase. The cumulative proportion of pairs that had mated once is also indicated.

3.4.3.4 Daily feeding and oviposition rhythms

Significantly greater number of feeding punctures was created during the photophase with very few feeding punctures being made during the scotophase (paired t-test: t = 31.72, 57.88, 33.53, 37.61 and 60.37 for 5-d-, 6-d-, 7-d-, 8-d- and 9-d-old, respectively; *DF* = 19; *P* < 0.05; Fig. 3.9).

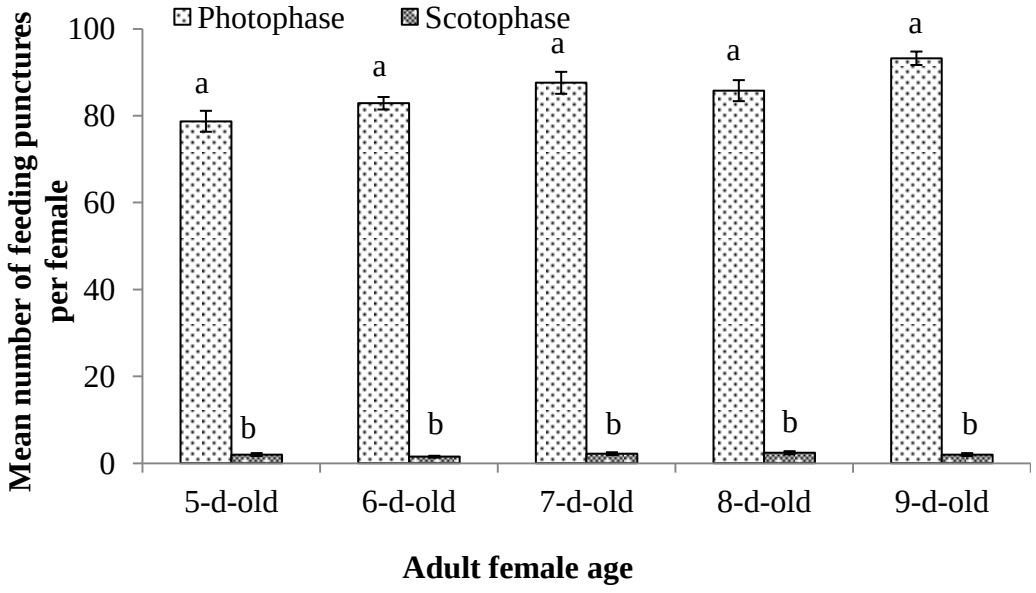

Fig. 3.9 The number of feeding punctures made during the 16 h of photophase and 8 h of scotophase on a Chinese cabbage plant. For each age, bars with different letters are significantly different (*P* < 0.05).

Significantly more feeding punctures were made during the first six hours of the photophase, with 54.65% occurring during this period (χ^2 = 102.82, 111.05, 106.81, 94.38 and 111.20 for 5-d-, 6-d-, 7-d-, 8-d- and 9-d-old, respectively; *DF* = 7; *P* < 0.05; Fig. 3.10).

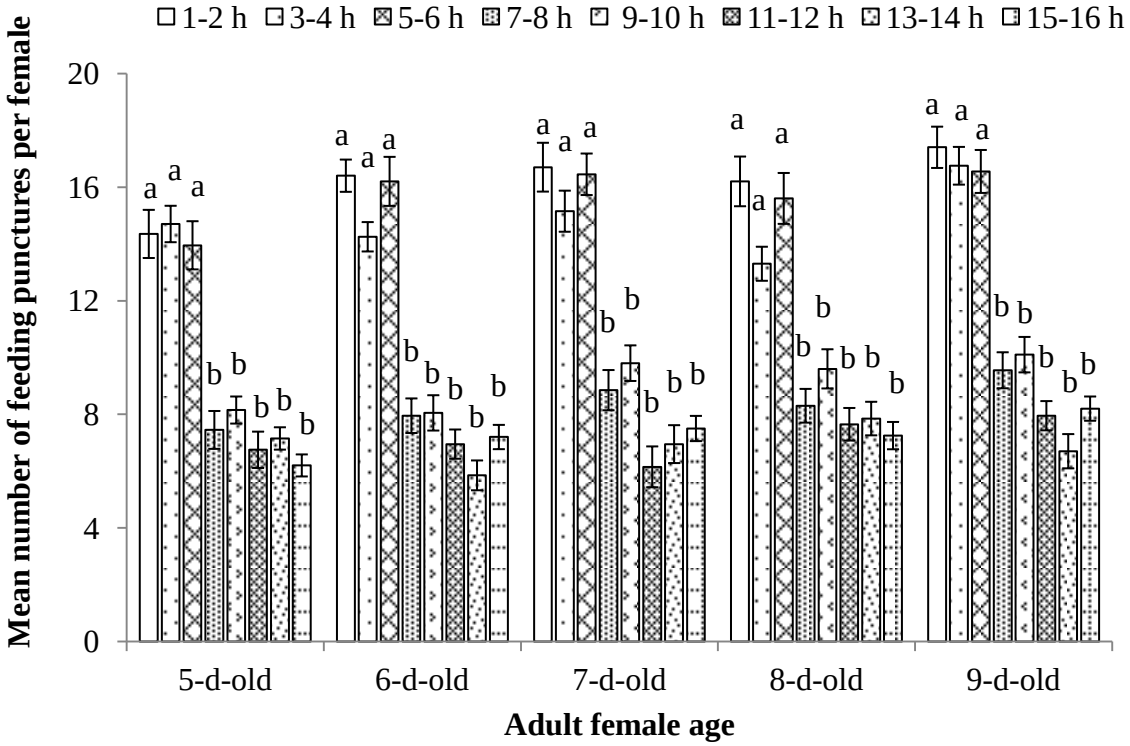

Fig. 3.10 Feeding punctures made during different hours of the photophase on a Chinese cabbage plant. For each age, bars with different letters are significantly different (*P* < 0.05).

Scaptomyza flava females did not lay any egg during the scotophase. Significantly more eggs were laid during the first six hours of the photophase, with 74.91% of eggs laid during these hours (χ^2 = 99.99, 103.0048, 102.8216, 94.353 and 106.21 for 5-d-, 6-d-, 7-d-, 8-d- and 9-d-old, respectively; *DF* = 7; *P* < 0.05; Fig. 3.11).

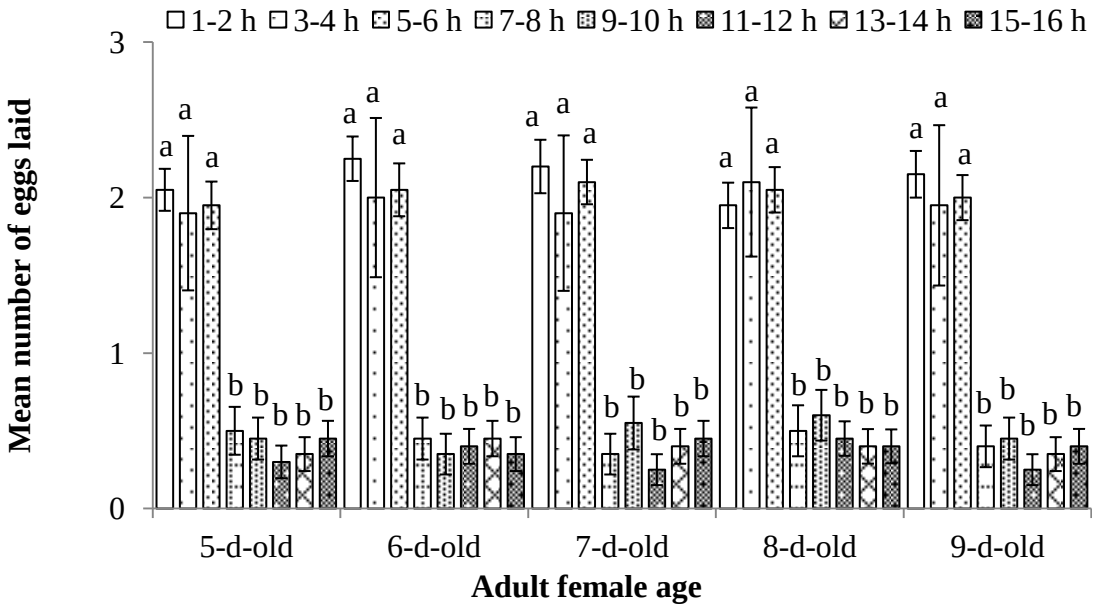

Fig. 3.11 Oviposition during different hours of the photophase. For each age, bars with different letters are significantly different ($P < 0.05$).

3.4.4 Discussion

Circadian rhythm is characteristic of the majority of important processes of insect life, such as adult eclosion, migration, feeding, pheromone release, mating and oviposition (Jonušatte & Buda 2002). The present study shows that in *S. flava* adult eclosion occurred during the day time and peaked in the early morning. It is assumed that emergence during this period has advantages for group activities of flies, such as feeding and mating (Jonušatte & Buda 2002). The pattern detected in this study is similar to that in *Boophthora erythrocephala* (De Geer), *Simulium morsitans* (Edw.), *S. reptans* (L.), *Wilhelmia equina* (L.) and *W. lineata* (Mg.), with adults emerging from pupae just before sunrise (Jonušatte & Buda 2002). The information about adult emergence rhythm is useful for conducting laboratory experiments where a large number of individuals of the same age are required (Jonušatte & Buda 2002).

Wicker-Thomas (2007) states that in the Drosophilidae, visual, acoustic and chemical cues are required for courtship. My current study indicates that in *S. flava* mating mostly occurred soon after lights on, suggesting that visual cues are important for courtship and mating in this species. This pattern of mating during the early hours of the day may also favour the females, allowing them sufficient time for feeding and oviposition during the rest of the day. The present study also reveals that *S. flava* is a

polygamous species, suggesting that one mating may not be sufficient to maximise their reproductive success.

In *S. flava* feeding and oviposition mainly took place during the photophase, indicating that it is a diurnal species. Feeding and oviposition activities were found to be rhythmic with maximum feeding and oviposition during the first six hours of the photophase. These rhythmic activity patterns suggest that the early hours of the day are the optimal time for scouting *S. flava* population (Lam 2007) and probably application of insecticides. This *S. flava* behaviour is similar to that of *Liriomyza* leafminers, which feed and oviposit primarily during the early hours of the day (Parrella 1987).

3.5 Effect of temperature on life history and population growth

3.5.1 Introduction

The level of pest damage is related to the size of its population (Robson et al. 2007). Temperature is a key abiotic factor determining the development, survival (Son et al. 2009; Abril et al. 2010; Rastogi & Pandey 2008), and population size of insects (Kirby & Lindsay 2009) including leafminers (Petitt & Wietlisbach 1994; Schuster & Patel 1985). Information on the effect of temperature on population parameters such as intrinsic rate of natural increase (r_m), net reproductive rate (R_0), and finite rate of increase (λ), is important in the measurement of population growth potential (Southwood 1975), and assessment of the damage potential of a pest species (Zhang et al. 2000).

So far, little is known about the effect of temperature on the developmental time and survival of immature stages, and population growth of *S. flava*. In this section, I investigated its life history and estimated population growth parameters at different temperatures. Such information is important for the development of pest forecasting systems (Palumbo 1995), and of efficient rearing methods for production of natural enemies.

3.5.2 Materials and methods

3.5.2.1 General methodology

This study was conducted at four constant temperatures (15°, 20°, 25° and 30°C). This temperature range occurs within the current geographic distribution regions of *S. flava*. Different bioassay rooms were set to carry out these experiments with each room having one of the mentioned temperatures with 16 h light: 8 h dark, and 60 ± 5% R.H.

3.5.2.2 Development of immature stages

A middle leaf from a 2.5-month-old Chinese cabbage was exposed to the breeding colony in the cage for 1 hour and then was replaced with another similar leaf.

The leaf stalk was dipped in a glass tube having water so that the leaf remained fresh. After removal from the cage infested leaves were immediately observed under the microscope to count the number of eggs. For investigations at a given temperature, these leaves were placed in a bioassay room and maintained at a test temperature. Eggs were observed daily under the microscope and the number of fertilised eggs was counted (n = 100 for each temperature). The leaves were maintained under the same conditions until larvae pupated. Pupae were kept in the glass tubes in the same bioassay room until adult emergence. Data on the duration of egg, larval and pupal stages, and survival of immatures at each stage at each temperature were recorded.

3.5.2.3 Adult longevity, fecundity and population parameters

Newly emerged adults were kept in the same bioassay room where they emerged. They were transferred to large transparent plastic cylinders with one pair in each cylinder and provided with a Chinese cabbage leaf dipped in a glass tube having water for feeding and oviposition, which was replaced with another leaf every 24 hours. The tops of the glass tubes were covered with cotton wool so that the flies could not fall in water and get drowned. The number of eggs laid by a female on each leaf was counted under the microscope. When the male died while the female was still alive, that was replaced with another similar male so that the female still had mating opportunities and *vice versa*. Data on adult longevity and female fecundity were recorded. Infested leaves were then maintained under the same conditions until larvae pupated. Pupae were then individually placed in glass tubes until adult emergence. When the adults emerged their sex was identified. Data on the number of female offspring produced by each female each day at each temperature were recorded.

3.5.2.4 Statistics

The intrinsic rate of increase (female/female/day; r_m) was estimated by solving the Lotka-Euler equation ($\Sigma e^{-r_m x} l_x m_x = 1$) (Birch 1948). Other calculations included the net reproductive rate (females/females/generation; $R_0 = \Sigma l_x m_x$) and finite rate of increase ($\lambda = \exp(r_m)$) (Jervis & Kidd 1996). In these calculations x is the pivotal age, l_x is the proportion of the females surviving to age x, and m_x is the number of offspring produced per female at age x. For each temperature (Table 3.3), a jackknife method (Caswell

2001) was used to estimate the parameters of r_m, R_0 and λ for each individual female. Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on egg, larval and pupal developmental time were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons (Zar 1999). Data on hatching, larval survival and adult emergence rates were analysed using a Chi-square test while the data on fecundity, adult longevity, intrinsic rate of increase, net reproductive rate and finite rate of increase were subject to one way ANOVA followed by a Tukey's studentised range (HSD) test.

3.5.3 Results

3.5.3.1 Development of immature stages

Developmental time of immature stages at different temperatures is shown in Table 3.1. Egg and larval developmental time was similar at 20° and 25°C, while it was significantly longer and shorter at 15° and 30°C, respectively (χ^2 = 241.08 and 221.12 for egg and larva, respectively; *DF* = 3; *P* < 0.05; Table 3.1). Pupal period was significantly affected by temperature, decreasing with the increase of temperature (χ^2 = 196.20; *DF* = 3; *P* < 0.05; Table 3.1).

Table 3.1 Developmental time of immature stages (days) at different temperatures.

Temperature (°C)	Egg	Larva	Pupa
15	4.72 ± 0.048 a	12.47 ± 0.056 a	13.79 ± 0.091 a
20	3.40 ± 0.050 b	7.50 ± 0.053 b	9.46 ± 0.058 b
25	3.27 ± 0.050 b	7.33 ± 0.051 b	8.38 ± 0.054 c
30	2.93 ± 0.027 c	6.68 ± 0.052 c	7.10 ± 0.083 d

Numbers with different letters in columns are significantly different (*P* <0.05).

Similar hatch and larval survival rates were recorded at all test temperatures (for hatch rate: $\chi^2 = 0.17 < \chi_3^2 = 7.82$; for larval survival rate: $\chi^2 = 0.22 < \chi_3^2 = 7.82$; Table 3.2). However, adult emergence rate at 20° and 25°C were significantly higher than at 15° and 30°C ($\chi^2 = 16.53 > \chi_3^2 = 7.82$) (Table 3.2).

Table 3.2 Hatch, larval survival and adult emergence rates at different temperatures (%).

Temperature (°C)	Hatch rate	Larval survival	Adult emergence
15	87 a	93.10 a	48.15 a
20	94 a	94.68 a	93.26 b
25	91 a	95.60 a	93.10 b
30	88 a	93.18 a	45.12 a

Numbers with different letters in columns are significantly different ($P < 0.05$).

3.5.3.2 Adult longevity, fecundity and population parameters

It is indicated that adult longevity significantly decreased with the increase of temperature from 15 to 30°C ($F = 166.93$; $DF = 7, 152$; $P < 0.05$; Fig. 3.12).

My results show that the most optimal temperature for *S. flava* fecundity was between 20 and 25°C ($F = 141.23$; $DF = 3, 76$; $P < 0.05$; Fig. 3.13).

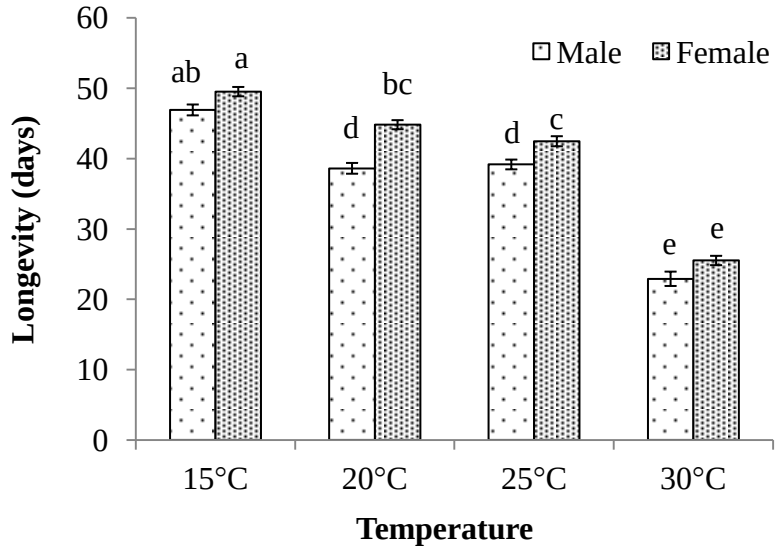

Fig. 3.12 Adult *S. flava* longevity at different temperatures. Bars with different letters are significantly different ($P < 0.05$).

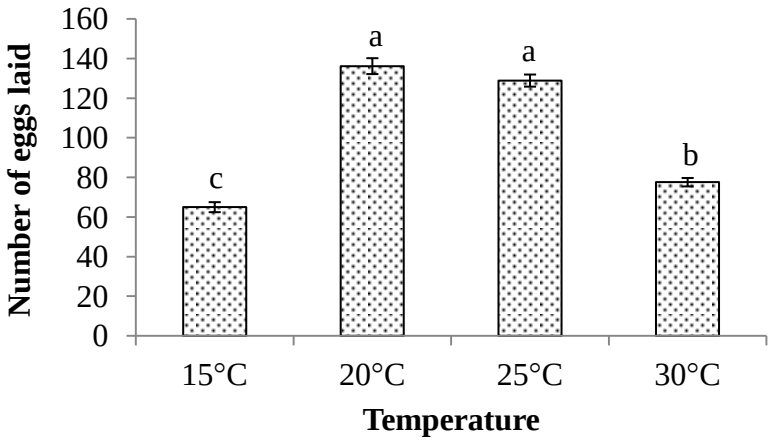

Fig. 3.13 *Scaptomyza flava* fecundity at different temperatures. Bars with different letters are significantly different ($P < 0.05$).

Significantly higher intrinsic rate of increase (r_m), net reproductive rate (R_0) and finite rate of increase (λ) were recorded at 25°C, whereas significantly lower values of all these parameters were recorded at 15°C (F = 1465147.72, 47750.31 and 1456220.45 for r_m, R_o and λ, respectively; DF = 3, 76; $P < 0.05$; Table 3.3).

Table 3.3 Effect of temperature on different population parameters of *S. flava*.

Temperature (°C)	Intrinsic rate of increase (r_m)	Net reproductive rate (R_o)	Finite rate of increase (λ)
15	0.7128±0.000028 d	17.35±0.034d	1.074±0.000030 d
20	0.1231±0.000026 b	35.35±0.040 b	1.131±0.000029 b
25	0.1318 ±0.000013 a	36.00±0.057 a	1.141±0.000015 a
30	0.1225±0.000022 c	23.70±0.032 c	1.130±0.000024 c

Numbers with different letters in columns are significantly different ($P < 0.05$).

3.5.4 Discussion

Temperature significantly affects the developmental time and survival of many leafminers (Issa & Marcano 1991; Leibee 1984; Petitt 1991; Zhang et al. 2000; Lanzoni et al. 2002). The present study indicates that the developmental time of *S. flava* decreased with the increase in temperature. Significant reduction in developmental time with increase in temperature may be attributed to increased metabolism and growth rate at higher temperatures (Tomberlin et al. 2009). Similar report can be found in *Stethorus*

japonicus (Mori et al. 2005), *Coelophora saucia* (Omkar 2006), and *Liriomyza sativae* Blanchard (Zhang et al. 2000).

Suitable temperature leads to optimal metabolic rate and results in higher fecundity (Luciano et al. 2003) and longer life span (Tomberlin et al. 2009). In *S. flava* maximum fecundity occurred at 20° and 25°C. These results suggest that temperature of 20° to 25°C is optimal for *S. flava* oviposition and population growth. Therefore, mass rearing of this species for use in biological control or SIT programmes should be carried out between 20 and 25°C. These results also imply that with decreased fecundity at 15 and 30°C, *S. flava* population growth may be checked when environmental temperature reaches this low and high, respectively. In this regard *S. flava* is different from the tropical leafminer species *L. sativae*, in which maximum fecundity occurs at 28° to 31°C (Zhang et al. 2000).

Significantly lower adult emergence rates occurred at 15° and 30°C, whereas similar hatch and larval survival rates took place at all test temperatures. These results suggest that in *S. flava* pupal stage was more sensitive to temperature than the egg and larval stages. Since *S. flava* eggs and larvae developed inside the Chinese cabbage leaves, the lower mortality in these stages may be due to the difference between ambient air temperatures and leaf temperatures (Leibee 1984). Similarly, in the leafminer *L. sativa*, significantly lower emergence rate takes place at 15° and 30°C, whereas hatching and pupation rates remain similar at these temperatures (Zhang et al. 2000).

Population growth parameters were significantly affected by temperature with higher intrinsic rate of increase (r_m), net reproductive rate (R_0), and finite rate of increase (λ) being recorded at 25°C followed by 20°C. It appears that higher fecundity at these temperatures contributes to the rapid population growth. These results further confirm that the temperature of 20° to 25°C is optimal for *S. flava* population growth. Under such conditions the pest population should be monitored regularly and appropriate control actions should be applied when necessary. These findings may also be valuable for further studies on population dynamics of *S. flava* and eventually for the development of a model to forecast its population density and to implement control strategies.

CHAPTER 4

FEEDING AND OVIPOSITION

4.1 General introduction

Many adult leafminers prefer large leaves for feeding and oviposition (Faeth et al.1981b; Hileman & Lieto 1981; Simberloff & Stiling 1987) as their larvae feed and complete the development on the site of oviposition (Gripenberg et al. 2010). Leaf-mining by the larvae induces leaf abscission (Sinclair & Hughes 2010), slows down plant growth (Wagner et al. 2008), and results in reduction of crop yield (Björksten et al. 2005; Bueno et al. 2007). Like other leafminers, *S. flava* may also have feeding and oviposition preferences in relation to leaf size (Hileman & Lieto 1981; Simberloff & Stiling 1987). Therefore, Chinese cabbage plants of different growth stages having leaves of different sizes and ages may vary in susceptibility to *S. flava* infestation.

Environmental stress such as water deficit affects plant physiology, which in turn affects the performance of phytophagous insects. The plant stress hypothesis predicts that stressed plants have improved nutritional quality (Joern & Mole 2005) and reduced production of chemical defences, resulting in increased performance of phytophagous insects (Rhoades 1983). The plant vigour hypothesis predicts that fitness of phytophagous insects will be higher on vigorous plants than on less vigorous plants (Price 1991). As a result, these insects should prefer most vigorous plants where subsequent larval performance is the highest.

Optimal foraging theory predicts that females increase their fitness by feeding on hosts which best satisfy their own nutritional requirements rather than of their offspring (Jaenike 1986) while optimal oviposition theory predicts that females in order to maximize their fitness, select oviposition sites which are the best for the development of their offspring (Jaenike 1978). The relationship between adult host preference and offspring performance is expected to be even stronger in leafminers because their offspring are almost sessile and complete their development on the site of oviposition (Gripenberg et al. 2010). Investigation into both optimal oviposition and optimal foraging is necessary for better understanding of the evolutionary adaptation of host preference behaviour (Lewis et al. 1998; Scheirs et al. 2000; Scheirs & DeBruyn 2002).

So far, nothing is known about the host preference by *S. flava*. In this chapter I carried out a series of experiments on host preference to test plant stress and vigorus hypotheses as well as optimal foraging and oviposition theories. I also examined the susceptibility of different plant growth stages to *S. flava*.

4.2 Susceptibility of different growth stages of Chinese cabbage to *Scaptomyza flava*

4.2.1 Introduction

Many dipterous leafminers are important pests of various agricultural crops throughout the world (Çikman et al. 2008; Hernández et al. 2010; Mujica & Kroschel 2011; Gitonga et al. 2010) and cause significant economic loss (Bueno et al. 2007; Gitonga et al. 2010). Leafminer damage is mainly caused by larval feeding which reduces photosynthetic capacity, transpiration, and stomatal and mesophyll conductance of the host plants (Chandler & Gilstrap 1987; Foster & Sanchez 1988) and ultimately reduces their yield (Björksten et al. 2005; Bueno et al. 2007).

Studies have revealed that many adult leafminers prefer large leaves for feeding and oviposition (Faeth et al.1981b; Hileman & Lieto 1981; Simberloff & Stiling 1987) as small leaves may not provide enough food for larvae to complete their development (Bultman & Faeth 1986a).

Heavy *S. flava* infestation may reduce crop yield, however, its impact on yield loss has not been determined (Martin 2004). During the present study I recorded the number of punctures and larvae in Chinese cabbage plants of different growth stages at different adult densities. I also investigated the effect of the number of larvae on plant growth.

4.2.2 Materials and methods

4.2.2.1 Insects

Insects were reared as mentioned in Section 3.2.3. For experiments, newly emerged males and females were transferred to a large transparent plastic cylinder and were provided with a Chinese cabbage plant. The plant was replaced with another one every 24 h. Thirty adult flies were maintained in a cylinder with a sex ratio of 1:1. Five-

d-old female *S. flava* were used for the experiment because maximum feeding and oviposition take place from the 5th to 10th day after emergence (Section 3.3.3.2).

4.2.2.2 Plants

Plants were grown as mentioned in section 3.2.1.

4.2.2.3 Susceptibility of different growth stages of Chinese cabbage to *S. flava*

This experiment consisted of 21 treatments with 10 replications each (4 plant stages × 5 female densities + control) which are represented in Table 4.1. Plants having only cotyledon leaves are defined as seedling. Control treatment consisted of the plants that were not exposed to flies.

Table 4.1 Experimental treatments.

Plant stage	Plant age when exposed to females	Female density
Seedling	1 week	One
Seedling	-	Two
Seedling	-	Three
Seedling	-	Four
Seedling	-	Five
Two-leaf	2 weeks	One
Two-leaf	-	Two
Two-leaf	-	Three
Two-leaf	-	Four
Two-leaf	-	Five
Four-leaf	3 weeks	One
Four-leaf	-	Two
Four-leaf	-	Three
Four-leaf	-	Four
Four-leaf	-	Five
Six-leaf	4 weeks	One
Six-leaf	-	Two
Six-leaf	-	Three
Six-leaf	-	Four
Six-leaf	-	Five
Control		

For this experiment, plants were individually exposed to the females in the large transparent plastic cylinders for 24 h. The plants were then removed from the cylinder and the number of punctures on leaves was counted with the help of a hand lens. These plants were then maintained under the same laboratory conditions. When neonate larvae started creating linear mines, the number of mines was counted to have an estimation of the number of larvae, and the larvae were allowed to develop. Plants were observed daily to record leaf abscission due to leaf-mining, and the leaves which were fully mined and then dried and dropped from the plant were considered to be pre-maturely abscised leaves. When the plants used in this experiment were 10 week old, the data on the following growth parameters were recorded: total number of leaves, total leaf area, and fresh and dry weights of each plant (excluding roots). Leaf area was measured by the leaf area meter. Fresh weight was measured by the electronic scale and dry weight was obtained by placing the plant in the oven at 70 °C for 48 h and then weighing it on the electronic scale.

4.2.2.4 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on the number of punctures and larvae in the plants of different growth stages for each female density were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test followed by the Dunn's procedure for multiple comparisons (Zar 1999). Data on the number of larvae and different growth parameters were normally distributed, thus linear regression analyses were used to determine the relationship between the number of punctures and number of larvae in the plants of different growth stages, and the relationship between the number of larvae and different growth parameters of the Chinese cabbage.

4.2.3 Results

At each female density significantly higher number of punctures and larvae per plant was recorded on plants of six-leaf stage followed by four-leaf stage (for punctures: χ^2 = 33.91, 34.06, 33.94, 33.35 and 34.51 for one, two, three, four and five females, respectively; DF = 3; $P < 0.05$; Table 4.2. For larvae: χ^2 = 30.31, 33.28, 33.79, 33.77

and 34.32 for one, two, three, four and five females, respectively; DF = 3; $P < 0.05$; Table 4.3).

With the increase in female density from one to four females the number of punctures significantly increased in plants of all growth stages, however, there was no further increase in the number of punctures after female density increased to four (χ^2 = 40.86, 42.26, 40.56, and 44.90 for seedling, two-, four- and six-leaf stages, respectively; DF = 4; $P < 0.05$; Table 4.2).

Table 4.2 Number of punctures per plant in plants of different stages at different *S. flava* densities.

Plant stage	Adult density				
	1 female	2 females	3 females	4 females	5 females
Seedling	1.1±0.18 cδ	2.4±0.16 cγ	4.4±0.34 cβ	5.7±0.37 cα	5.9±0.31 dα
Two-leaf	1.5±0.17 cδ	2.7±0.21 cγ	4.8±0.33 cβ	5.9±0.23 cα	7.7±0.58 cα
Four-leaf	4.3±0.34 bδ	8.9±0.81 bγ	12.6±0.62 bβ	17.7±1.18 bα	18.5±1.08 bα
Six-leaf	29.9±1.21 aδ	59.6±2.11 aγ	88.2±3.05 aβ	119.8±4.00 aα	129.8±4.71 aα

Numbers with different English letters in columns or Greek letters in rows are significantly different (P <0.05).

In the seedling and two-leaf stages, increasing female density did not result in significant increase in the number of larvae (χ^2 = 1.66 and 0.86 for seedling and two-leaf stage, respectively; DF = 4; $P > 0.05$; Table 4.3). In the four-leaf stage, the increase in female density from one to four females resulted in a significant increase in the number of larvae; however, there was no further increase in the number of larvae after female density increased to four. In the six-leaf stage, the increase in female density from one to five females resulted in a significant increase in the number of larvae (χ^2 = 42.36 and 46.74 for four and six-leaf stages, respectively; DF = 4; $P < 0.05$; Table 4.3).

Table 4.3 Number of larvae per plant in plants of different stages at different *S. flava* densities.

Plant stage	Adult density				
	1 female	2 females	3 females	4 females	5 females
Seedling	0.3±0.15 cα	0.6±0.22 cα	0.5±0.17 cα	0.7±0.26 cα	0.5±0.22 cα
Two-leaf	0.6±0.16 cα	0.7±0.15 cα	0.7±0.21 cα	0.7±0.15 cα	0.8±0.13 cα
Four-leaf	1.3±0.15 bδ	2.1±0.18 bγ	3.2±0.25 bβ	5.7±0.37 bα	6.8±0.44 bα
Six-leaf	5.2±0.33 aε	10.0±0.39 aδ	14.5±0.50 aγ	20.4±0.65 aβ	25.6±0.67 aα

Numbers with different English letters in columns or Greek letters in rows are significantly different ($P < 0.05$).

No leaf abscission was recorded when plants at the seedling and two-leaf stages were exposed to females of different densities. However, when older plants such as those in the six-leaf stage were exposed to females of different densities, higher female density caused greater leaf abscission (χ^2 = 162.86; DF = 20; $P < 0.05$; Fig. 4.1).

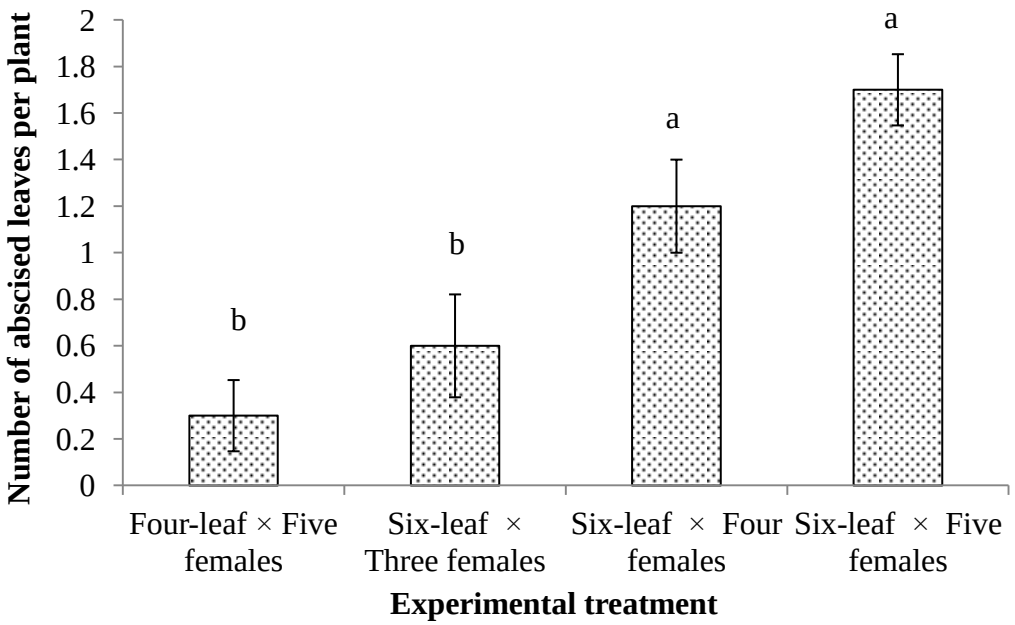

Fig. 4.1 Number of abscised leaves per plant when plants of different growth stages were exposed to *S. flava* females of different densities. Bars with different letters are significantly different ($P < 0.05$).

There was a negative relationship between the number of larvae on a plant and the total number of leaves, total leaf area, and fresh and dry weights of the Chinese cabbage recorded at the age of 10 weeks (F = 431.08, 774.29, 420.38, 430.97 for total number of leaves, total leaf area, fresh weight and dry weight, respectively; DF = 1; $P <$ 0.05; Fig. 4.2).

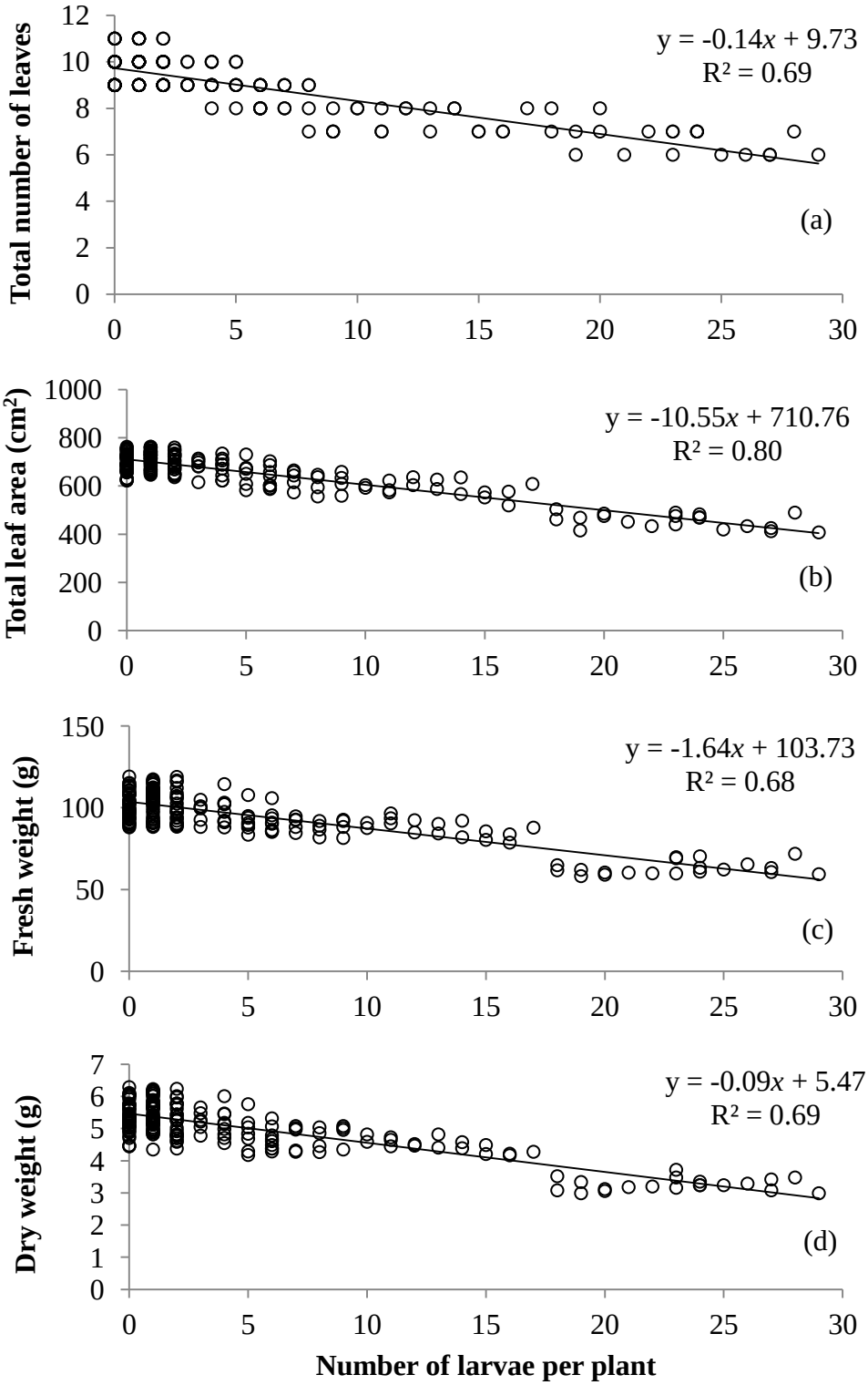

Fig. 4.2 Relationship between the number of larvae per plant and different growth parameters of 2.5-month-old Chinese cabbage plant: (a) total number of leaves, (b) total leaf area (cm^2), (c) Fresh weight (g), and (d) dry weight (g).

4.2.4 Discussion

During this study at each female density significantly higher number of punctures and neonate larvae was recorded in the six- and four-leaf stages compared to the seedling and two-leaf stages. These results suggest that *S. flava* do not prefer plants having small leaves for feeding and oviposition as small leaves may not provide enough food for larvae to complete their development (Bultman & Faeth 1986a). Feeding preference for mature leaves may be attributed to the fact that such leaves have higher nutritive value and more sugars (Bernays & Chapman 1994; Merritt 1996) while emerging young leaves are rich in chemical defences (Lambdon et al. 2003). These results suggest that the four- and six-leaf stage Chinese cabbage is comparatively more susceptible to *S. flava* infestation. This study reveals that increasing female density did not result in a significant increase in the number of larvae in the seedling and two-leaf stage Chinese cabbage while there was a significant increase in the four- and six-leaf stages. These results also suggest that *S. flava* oviposition decisions might be influenced by host leaf size and quality.

Leafminer larval feeding reduces plant's photosynthesis (Johnson et al. 1983; Schaffer et al. 1997) and causes leaf abscission (Nardini et al. 2004). Higher larval density results in increased plant damage (Van Steenwyk & Toscano 1981; Schooler & McEvoy 2006; Weed & Casagrande 2010), slow growth (Wagner et al. 2008; Appleton et al. 1997), and reduced yield (Showers et al. 1983). Current investigation reveals a negative relationship between the number of *S. flava* larvae and the total number of leaves, total leaf area, and fresh and dry weights of the Chinese cabbage. These results suggest that larval feeding causes damage to the Chinese cabbage, and higher number of larvae can significantly reduce their yield. Furthermore, during the present study greater leaf abscission was recorded in the six-leaf stage compared to the younger plants, which may also be attributed to higher number of larvae in these plants.

In conclusion, the present study indicates that *S. flava* can significantly reduce the yield of the Chinese cabbage while the plants having small leaves for example the seedling and two-leaf stage are less susceptible to *S. flava* infestation.

4.3 Adult feeding, oviposition preference and offspring performance on water stressed and vigorous plants

4.3.1 Introduction

Environmental stress such as water stress affects plant physiology and development, which in turn affects feeding and performance of phytophagous insects (Larsson 1989; De Bryun et al. 2002). The plant stress hypothesis (PSH) proposes that phytophagous insects prefer stressed plants for feeding and oviposition (Mattson & Haack 1987b) because stressed plants have increased levels of free amino acids (Brodbeck & Strong 1987) and are less able to synthesize defensive chemicals (Price 1991). Some of the previous studies support this hypothesis (White 1969, 1974, 1984 & 1993; Mattson & Haack 1987b) while others do not (Wagner & Frantz 1990; Mopper & Whitham 1992; Huberty & Denno 2004).

The plant vigour hypothesis (PVH) (Price 1991) has two propositions: a) phytophagous insects prefer to feed and oviposit on vigorous plants (Price 1994), and b) they perform better on vigorous plants (Fritz et al. 2000). Studies have shown that insects whose offspring develop on sites different from the oviposition sites typically show no oviposition preference for vigorous plants (Dodge & Price 1991; Price 1994). However, the leafminers whose offspring develop on the site of oviposition may prefer to oviposit on vigorous plants (Price 1991; Price 1994). Many previous studies provide support to both preference and performance propositions of PVH (Price et al. 1999; Craig et al. 1989; Kimberling et al. 1990; Price & Ohgushi 1995; Stein & Price 1995; Woods et al. 1996). Here performance may be in terms of increased larval survival (Craig et al. 1989; Price & Ohgushi 1995) and/or higher reproduction (Kimberling et al. 1990).

According to PVH, *S. flava* would prefer vigorous plants for feeding and oviposition and their offspring would have enhanced performance on such plants and *vice versa.* The objectives of the current study were to test PVH and PSH using *S. flava* and its host plant, the Chinese cabbage. I investigated the adult feeding and oviposition preference, and offspring performance on water stressed and vigorous Chinese cabbage plants. Information obtained from the current study would help us understand the interaction of *S. flava* with its host plant under water stress and favourable conditions.

4.3.2 Materials and methods

4.3.2.1 Insects

Same procedure was followed as mentioned in section 4.2.2.1.

4.3.2.2 Plants and experimental treatments

Plants were grown as mentioned in Section 3.2.1. Plants were individually applied 60 ml water every 3 days until they were 2 months old and then they were randomly divided into two groups namely vigorous and water stressed. In the vigorous group the plants were maintained as before, i.e., application of 60 ml water every 3 days while those in the water stressed group were not watered anymore. After one week of stopping water application when the leaves of the plants maintained under water stressed conditions appeared slightly wilted i.e., leaves while still green, angled slightly towards the ground (Tyree et al. 2002) (Fig. 4.3), they were considered as water stressed.

Fig. 4.3 Slightly wilted Chinese cabbage plant.

The experiment on adult feeding and oviposition preference consisted of the following two treatments:

a) Water stressed plants
b) Vigorous plants

4.3.2.3 Plant water status and leaf area comparison

Leaf water status of the stressed and vigorous plants (n = 10 for each treatment) was measured. I measured leaf water potential (bar) in the middle leaf (5^{th} leaf from the bottom) using a pressure chamber (Scholander et al. 1964).

4.3.2.4 Adult feeding and oviposition preference

To determine adult feeding and oviposition preference, both choice and non-choice experiments were conducted with 15 replicates each. In the choice experiment, two plants, one from each treatment, were exposed to 10 pairs of 5-d-old *S. flava* for 24 h in the rearing cage. In the non-choice experiment, one plant from each treatment was exposed individually to 5 pairs of 5-d-old *S. flava* for 24 h in the large transparent plastic cylinder. Then the leaves of the plants were cut and observed under the microscope. Data on the number of feeding punctures and eggs on each plant were recorded.

4.3.2.5 Offspring performance

A plant from each treatment was exposed to the breeding colony in the cage for one hour and then was replaced with another plant. After infestation plants were maintained under the same laboratory conditions. When neonate larvae started creating linear mines, the number of mines was assumed to give an estimate of the number of larvae. At least 100 larvae from each treatment were observed for subsequent data recording. When the larvae pupated, the number of pupae was counted and they were individually put in glass tubes until adult emergence. Data on larval survival percentage, adult emergence rate, and offspring developmental time (egg to adult emergence) were recorded.

4.3.2.6 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on plant water status, number of punctures and eggs, and offspring developmental time in water stressed and vigorous plants were not normally distributed even after transformation and thus analysed by using the non-parametric Kruskal-Wallis

test followed by the Dunn's procedure for multiple comparisons (Zar 1999) while the data on larval survival percentage and adult emergence rate were analysed using a Chi-square test.

4.3.3 Results

4.3.3.1 Plant water status

Water stressed plants had significantly lower leaf water potential than the vigorous plants (F = 323.40; DF = 1, 18; $P < 0.05$; Fig. 4.4).

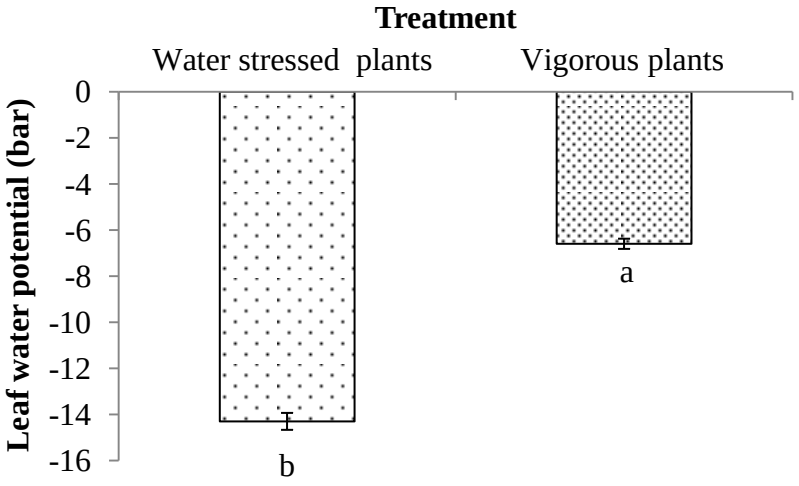

Fig. 4.4 Leaf water potential (bar) of water stressed and vigorous Chinese cabbage plants.

4.3.3.2 Adult feeding and oviposition preference

In both choice and non-choice experiments, significantly lower number of feeding punctures and eggs were recorded on water stressed plants than on vigorous plants (for choice experiment: $\chi^2 = 21.81$ and 22.31 for punctures and eggs, respectively; For non-choice experiment: $\chi^2 = 21.79$ and 21.85 for punctures and eggs, respectively; DF = 1; $P < 0.05$; Table 4.4).

Table 4.4 Numbers of feeding punctures and *S. flava* eggs per plant on vigorous and water stressed plants.

Treatment	Choice		Non-choice	
	Punctures	Eggs	Punctures	Eggs
Water stressed plants	7.40±0.74 b	0.60±0.19 b	23.53±1.27 b	5.13±0.55 b
Vigorous	586.53±11.90 a	55.93±1.35 a	397.80±11.10 a	41.07±1.01 a

Numbers with different letters in columns are significantly different (P <0.05).

4.3.3.3 Offspring performance

Significantly lower larval survival and longer offspring developmental time were recorded on water stressed plants than on vigorous plants (for larval survival and offspring developmental time: χ^2 = 23.12 and 76.93; DF = 1; P < 0.05; Table 4.5). However, there was no significant difference for adult emergence rate between the treatments (χ^2 = 0.07; DF = 1; P > 0.05; Table 4.5).

Table 4.5 *Scaptomyza flava* offspring performance on water stressed and vigorous plants.

Treatment	Larval survival (%)	Adult emergence (%)	Offspring developmental time (days)
Water stressed plants	29.52 b	87.10 a	23.74±0.16 a
Vigorous plants	93.75 a	94.29 a	20.30±0.51 b

Numbers with different letters in columns are significantly different (P < 0.05).

4.3.4 Discussion

Many phytophagous insects are considered to have enhanced performance on water stressed host plants (White 1969; Brodbeck & Strong 1987; Mattson & Haack 1987a, b; Huberty & Denno 2004). However, reports of this enhanced performance are largely based on non-experimental studies (reviewed in Huberty & Denno 2004). Experimental studies have revealed that the impact of plant stress on insect preference and performance is unpredictable and depends on the plant and insect species (Larsson 1989; Inbar et al. 2001). Many studies have indicated that water stressed plants have adverse effects on the performance of phytophagous insects (Larsson 1989; Wagner & Frantz 1990; Mopper & Whitham 1992; Watt 1994; Koricheva et al. 1998; Huberty & Denno 2004).

The development of immature insects is often positively correlated with the amount of water in the leaves (Scriber & Slansky 1981). Water stress modifies the concentration of plant nutrients (Hale et al. 2003), which can negatively affect the performance of phytophagous insects (Slansky & Scriber 1985; Schoonhoven et al. 1998; Hale et al. 2003; Johnson et al. 2011). The current study shows that *S. flava* offspring had lower survival rate and longer developmental time in water stressed plants, suggesting that water stressed plants are less suitable for their development. Though water stressed plants may have higher amount of nutritional compounds (Brodbeck & Strong 1987; White 1993), decreased water contents modify the concentration of these nutrients, which may adversely affect the performance of phytophagous insects (Bultman & Faeth 1987; Hale et al. 2003; Johnson et al. 2011).

This study indicates that adult *S. flava* create fewer number of feeding punctures and lay fewer number of eggs on water stressed plants compared to the vigorous plants, suggesting that adult *S. flava* do not prefer to feed and oviposit on water stressed plants. These results are in contrast to those studies which suggest that phytophagous insects perform better on water stressed plants (White 1969, 1974, 1984 & 1993; Mattson & Haack 1987b), and are in agreement with the studies which indicate that water stressed plants adversely affect adult feeding and oviposition preference (Larsson 1989; Wagner & Frantz 1990; Mopper & Whitham 1992; Watt 1994; Koricheva et al. 1998; Huberty & Denno 2004).

In conclusion, *S. flava* adults prefer vigorous plants for both feeding and oviposition, and their offspring have increased survival rate and shorter developmental time in vigorus plants. These findings support both preference and performance propositions of PVH, and are in agreement with several studies (Price et al. 1999; Craig et al. 1989; Kimberling et al. 1990; Price & Ohgushi 1995; Stein & Price 1995; Woods et al. 1996). Therefore, for constant supply of high quality and quantity *S. flava* for use in laboratory experiments or biological control programmes, vigorous plants should be provided to the adult flies.

4.4 Adult feeding and oviposition preference for different leaves of Chinese cabbage in context of optimal oviposition and foraging theories

4.4.1 Introduction

Leaf selection for oviposition by leafminer females determines where their offspring will feed (Faeth et al. 1981b). Previous studies show that some dipterous leafminer females prefer to oviposit on host they select for feeding; and they select the host to enhance adult performance rather than their offspring, such as in *Chromatomyia milii* (Kaltenbach) (Diptera: Agromyzidae) and *C. nigra* (Meigen) (Scheirs et al. 2000 and 2003). These findings support optimal foraging theory which asserts that females prefer to feed and oviposit on hosts that are optimal for adult performance rather than the offspring (Jaenike 1986), and are in contrast to optimal oviposition theory predicts that females select oviposition sites that are the best for the development of their offspring (Jaenike 1978).

Optimal foraging theory predicts that *S. flava* females would prefer to feed and oviposit on Chinese cabbage leaves that are optimal for their performance rather than of their offspring while the optimal oviposition theory predicts otherwise. Attention to both optimal oviposition and foraging should be paid in order to better understand the evolutionary adaptation of the oviposition preference (Lewis et al. 1998; Scheirs et al. 2000; Scheirs & DeBruyn 2002). In the present study I tested optimal oviposition and foraging theories using *S. flava* and its host plant the Chinese cabbage. I examined the adult feeding and oviposition preference for top (young), middle (mature) and bottom (older) Chinese cabbage leaves, and adult and offspring performance on these leaves. Larvae of some leafminer species may migrate from one leaf to another (Yamazaki 2010). The information on the ability of the larvae to migrate to other leaves in a leafminer species when combined with adult and offspring performance on different leaves, may enable us to show that host preference of the adult females is truly adaptive (Mayhew 2001). Therefore, I also investigated the occurrence of larval migration in this species under resource deficient conditions in a leaf.

4.4.2 Materials and methods

4.4.2.1 Insects

Insects were reared as mentioned in Section 3.2.3.

4.4.2.2 Plants

Plants were grown as mentioned in Section 3.2.1. Two and a half month old Chinese cabbage plants with ten leaves were used for the experiments.

4.4.2.3 Leaf categories

Leaves were divided into the following five categories according to their age and position on the plant (Table 4.6). The first two leaves developed by the plant were considered as the bottom leaves, and the next two leaves after bottom leaves as lower middle leaves and so on while the two youngest leaves on the top were considered as top leaves.

Table 4.6 Leaf categories of the Chinese cabbage.

Leaf category	Leaf number
Bottom	1, 2
Lower middle	3, 4
Middle	5, 6
Upper middle	7, 8
Top	9, 10

4.4.2.4 Adult preference and performance on each leaf category

One-d-old virgin females were individually paired with 1-d-old virgin males in a glass tube and observed to see if mating occured. If no mating occured in one hour after pairing, the male was replaced. If mating still did not occur in the second hour, then the female was discarded. After mating males (n = 50) and females (n = 50) were transferred to the rearing cage and provided with a Chinese cabbage plant for feeding which was replaced with another plant once every 24 h. When these females were 5-d-old they were used for the experiment because in *S. flava* maximum feeding and oviposition take place from the 5^{th} to 10^{th} days after emergence (Section 3.3.3.2).

To determine feeding and oviposition preference for different Chinese cabbage leaves, females were transferred to large transparent plastic cylinders with one female in each cylinder. A 2.5-month-old Chinese cabbage plant was exposed to a female for 24 h in the cylinder (n = 60). After removal from the cylinder, leaves of each plant were cut and observed under the microscope. The total number of feeding punctures and eggs on each leaf of each plant was counted.

To determine female longevity and fecundity on each leaf category, newly emerged males and females were transferred to small transparent plastic cylinders with a single pair in each cylinder. For this experiment, each pair was provided with either a top, middle or bottom leaf throughout their lifetime for feeding and oviposition (n = 25). A hole was created into the lid of these plastic cylinders and the leaf while still intact with the plant was inserted into the cylinder through the hole in the lid. The plant was replaced once every 24 h. When a male died while female was still alive, that was replaced with another similar male so that the female had mating opportunities. Data on female longevity and fecundity on top, middle and bottom leaves were recorded.

4.4.2.5 Offspring performance on each leaf category

A plant having only two leaves, i.e. either top, middle or bottom (all other leaves removed) was exposed to the breeding colony for one hour and then was replaced with another similar plant (n = 20 for each plant category). The rest of the procedure for this experiment was the same as mentioned in Section 4.3.2.5. Data on larval survival, larval developmental time, pupal weight, and adult emergence rate from top, middle and bottom leaves were recorded.

To have an estimation of the longevity of adults that completed their larval development in different leaf categories, males and females were individually transferred to the small transparent plastic cylinders and provided daily with a middle leaf of the Chinese cabbage for feeding (n = 15). Females were provided with the leaf as in Section 4.4.2.4. Males cannot puncture the leaf, therefore, each of them was provided with an artificially punctured middle Chinese cabbage leaf having 50 punctures created with a dissecting needle. Data on adult longevity were recorded.

4.4.2.6 Larval crowding in leaves preferred by adults

First, I determined the average leaf area mined by a single *S. flava* larva. For this experiment, a Chinese cabbage plant was exposed to the breeding colony for one hour and then was replaced with another similar plant. Infested plants were maintained under same laboratory conditions until neonate larvae started making mines. At this stage only one larva in each leaf was allowed to develop and others were killed with a dissecting needle according to Scheirs et al. (2004). When the larvae completed feeding and pupated, the area of the mine created by each larva was measured by a leaf area meter using paper replicas (photocopies of the leaves) following Raimondo et al. (2003). The leaf area mined by a single larva was recorded to be 2.03 ± 0.03 cm^2 (n = 55).

To determine whether oviposition preference for a leaf category can lead to crowded conditions for the larvae, a plant having only three leaves (top, middle and bottom, all other leaves removed) was exposed to the breeding colony for 24 h and then was replaced with a similar plant. Twenty plants were exposed to the breeding colony for these studies. Infested plants were then maintained under the same laboratory conditions. When neonate larvae started making mines, the number of mines on each leaf was counted to give an estimate of the number of larvae in that leaf. The leaves were then cut and their area was measured using leaf area meter, and the average leaf area available for each larva to feed on a leaf (total leaf area/number of larvae) was calculated. As average leaf area consumption of a single larva was recorded to be 2.03 ± 0.03 cm^2, the leaves where less than 2.00 cm^2 leaf area was available for each larva to feed were considered as crowded/resource deficient leaves.

4.4.2.7 Larval migration

To determine whether under crowded conditions *S. flava* larvae could migrate to other leaves, complete their development, pupate and emerge as adults, a plant with only one middle leaf available to *S. flava* for oviposition and all other leaves covered with paper towel to prevent oviposition was exposed to the breeding colony for 24 h and then was replaced with another similar plant. After the plant was removed from the colony, paper towels were removed from the leaves, and the plant was maintained under the same laboratory conditions. The number of mines on the exposed leaf was counted to have an estimate of the number of larvae. Leaf area of the exposed leaf was also

measured with the help of a graph paper. The leaves where less than 2.00 cm^2 leaf area was available for each larva to feed were identified as crowded leaves and larvae in these leaves were allowed to develop. When larvae fully mined the exposed leaves, observations were made to record mines in unexposed leaves and any mine recorded in the unexposed leaves at this stage was considered to have been created by the larvae that migrated from the exposed leaves. Data on the number of larvae that migrated, pupated and emerged as adults were recorded.

4.4.2.8 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on the pupal weights, number of feeding punctures and eggs, larval density, and adult lifetime fecundity on different leaf categories were not normally distributed even after transformation and thus analysed using the non-parametric Kruskal-Wallis test followed by the Dunn's procedure for multiple comparisons (Zar 1999). Data on larval durations, adult longevity, and offspring longevity on different leaf categories were subject to one way ANOVA, followed by the Tukey's studentised range (HSD) test. Linear regression analysis was used to identify the relationship between the number of feeding punctures and the number of eggs on the leaves while the data on larval survival and adult emergence rate in different leaves were analysed using a Chi-square test.

4.4.3 Results

4.4.3.1 Adult preference and performance on each leaf category

Significantly higher number of punctures and eggs was recorded on middle leaves followed by lower middle (χ^2 = 279.62 and 236.98 for feeding punctures and eggs, respectively; DF = 4; $P < 0.05$; Table 4.7).

Table 4.7 Numbers of feeding punctures and *S. flava* eggs per female on different leaf categories of the Chinese cabbage.

Leaf category	Feeding punctures	Eggs
Top	0.80±0.08 e	0.08±0.37 d
Upper middle	6.25±0.20 c	0.45±0.08 c
Middle	59.22±1.31 a	3.62±0.18 a
Lower middle	19.03±0.68 b	2.35±0.13 b
Bottom	3.20±0.20 d	0.10±0.03 d

Numbers with different letters in columns are significantly different (P <0.05).

Regression analysis reveals that the number of eggs in a leaf significantly increased when the number of feeding punctures increased up to about 55 after which they decreased (F = 612.48; DF = 2, 297; P < 0.0001; Fig. 4.5).

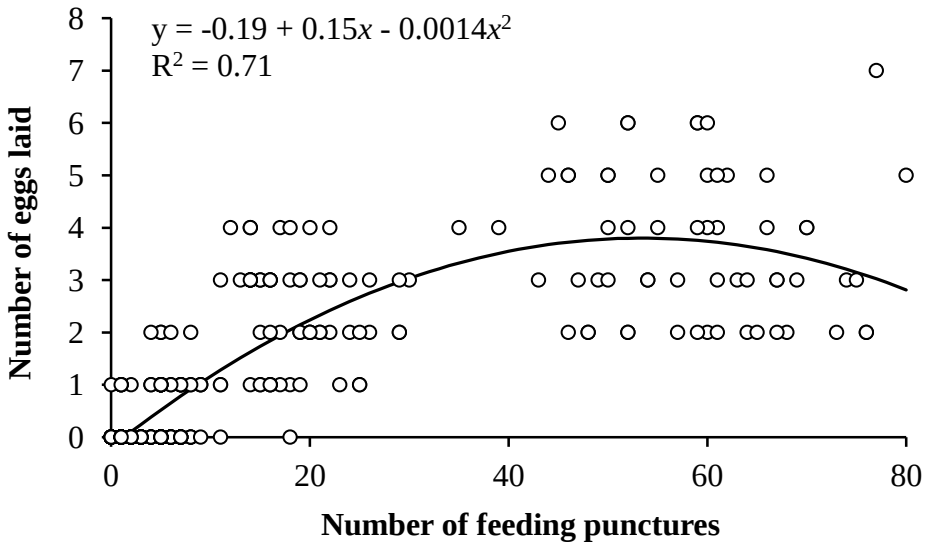

Fig. 4.5 Relationship between the numbers of feeding punctures and *S. flava* eggs in the leaves of the Chinese cabbage.

Females lived significantly longer and had significantly higher fecundity on the middle leaves (for longevity: F = 344.04; DF = 2, 74; for fecundity: χ^2 = 54.9357; DF = 2; P < 0.05; Fig. 4.6).

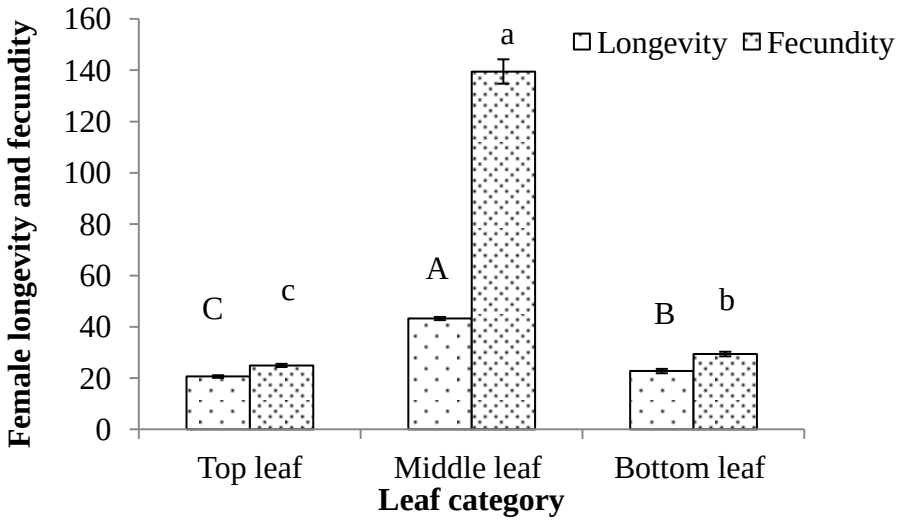

Fig. 4.6 Longevity (days) and fecundity (number of eggs laid) of *S. flava* females provided with different leaves for feeding and oviposition. Bars with different letters are significantly different ($P < 0.05$).

4.4.3.2 Offspring development on each leaf category

There was no significant difference in larval developmental time and pupal weights when the larvae were allowed to develop in the top, middle and bottom leaves of the Chinese cabbage, respectively (for larval developmental time: F = 1.43; DF = 2, 167; $P > 0.05$; for pupal weight: $\chi^2 = 0.3439$ and 1.171 for male and female pupal weights, respectively, DF = 2; $P > 0.05$; Table 4.8).

Table 4.8 *Scaptomyza flava* larval developmental time and pupal weights in Chinese cabbage leaves of different categories.

Leaf category	Larval developmental time (days)	Male pupal weight (mg)	Female pupal weight (mg)
Top leaf	9.29±0.62 a	11.96±0.23 a	17.04±0.31 a
Middle leaf	9.30±0.57 a	11.85±0.44 a	17.64±0.53 a
Bottom leaf	9.17±0.55 a	11.95±0.38 a	17.05±0.32 a

Numbers with different letters in columns are significantly different ($P < 0.05$).

Similarly, there was no significant difference in larval survival and adult emergence rates when the larvae were allowed to develop in the top, middle and bottom

leaves of the Chinese cabbage, respectively (χ^2 = 0.3859 and 0.3676 for larval survival and adult emergence rate, respectively; DF = 2; $P > 0.05$; Fig. 4.7).

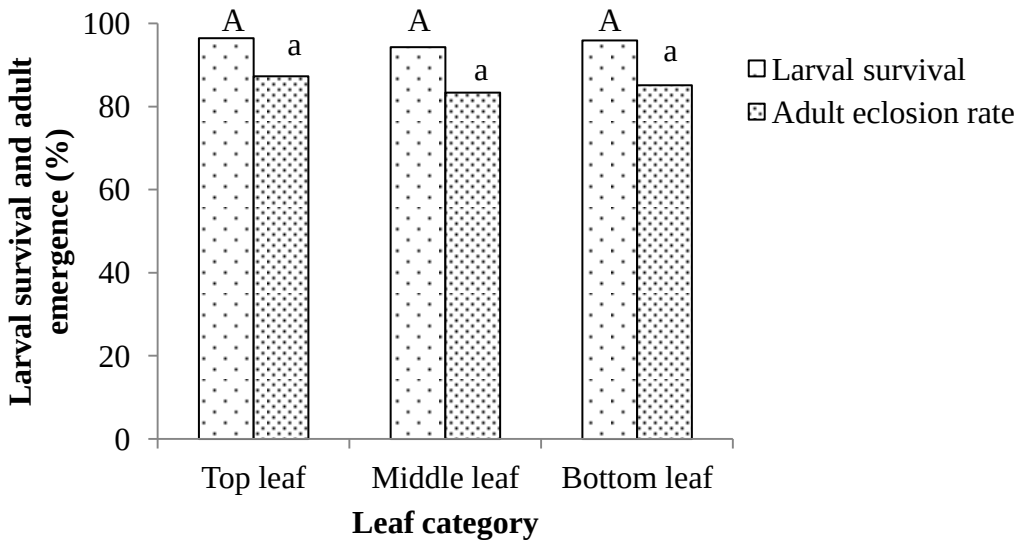

Fig. 4.7 *Scaptomyza flava* larval survival and adult emergence rates from different Chinese cabbage leaves. Bars with same letters are not significantly different ($P > 0.05$).

Adults that developed from larvae living in the top, middle and bottom leaves of the Chinese cabbage had no significant difference in longevity (F = 0.26 and 0.11 for males and females, respectively; DF = 2, 44; $P > 0.05$; Fig. 4.8).

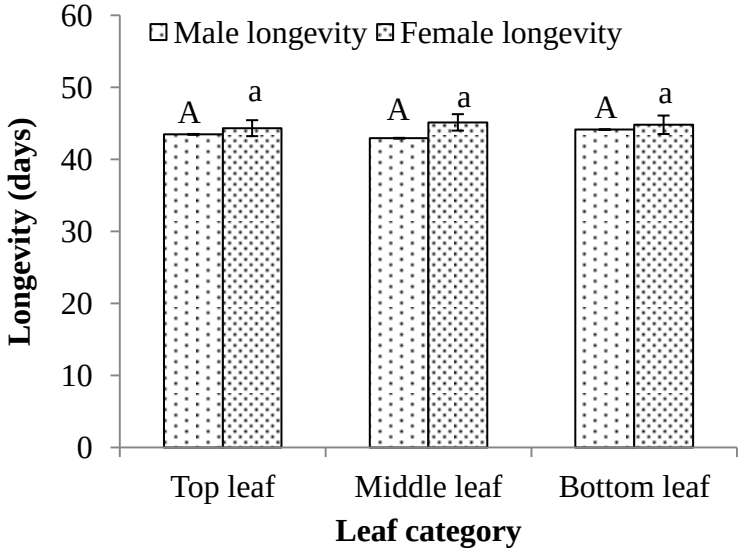

Fig. 4.8 Adult *S. flava* male and female longevity on Chinese cabbage leaves of different categories. Bars with same letters are not significantly different ($P > 0.05$).

4.4.3.3 Larval crowding in leaves preferred by adults

Due to adult preference for oviposition in different leaf categories and resulting variation in larval density, the leaf area availability for larval feeding was significantly different. This result shows that the larval crowding mainly occurred in middle leaves where leaf area for feeding was 1.19 ± 0.04 cm^2, lower than 2.03 ± 0.03 cm^2 a single larva needs for life ($\chi^2 = 37.65$; DF = 2; $P < 0.05$; Fig. 4.9).

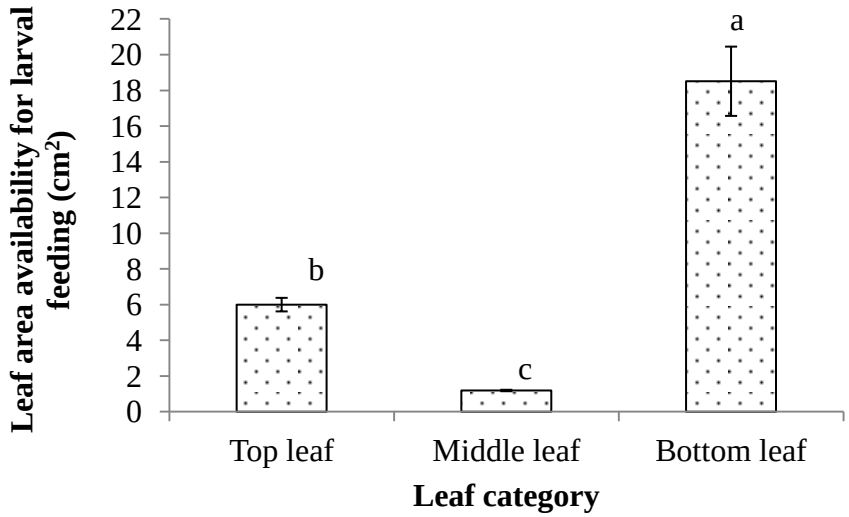

Fig 4.9 Average leaf area availability for *S. flava* larval feeding in Chinese cabbage leaves of different categories. Bars with different letters are significantly different ($P < 0.05$).

4.4.3.4 Larval migration

Initially 227 mines were recorded on leaves exposed to females for oviposition while no mine was recorded on unexposed leaves. Larval migration from exposed to unexposed leaves was evidenced by the fact that 163 mines were recorded on previously unexposed leaves. Pupation (62.56%) and adult emergence (83.80%) were also recorded in these larvae.

4.4.4 Discussion

Although offspring performance may be important in shaping host preference patterns of many herbivorous insects (Price 1994), host preference may also be for

better adult performance (Price 1994; Mayhew 1997). Female fitness is determined by both adult and offspring performance (Reavey & Lawton 1991; Krebs & Davies 1997) and variation in adult performance among different hosts relative to the variation in offspring performance may determine the strategy to be used in order to enhance female fitness (Scheirs et al. 2000).

My study reveals that adult *S. flava* females preferred to feed, oviposit, and had optimal performance on mature middle leaves. Such brassica leaves are rich in nutrients compared to the old senescing leaves (Merritt 1996) and have less defence chemicals compared to the young emerging leaves (Lambdon et al. 2003), and thus may be optimal for the performance of adult *S. flava*. The present study also indicates that different offspring performance parameters like larval developmental time, pupal weight, larval survival and adult emergence rates were similar for the larvae which completed their development in leaves of any category. The reasons for similar offspring performance in different leaves may be that leafminer larvae can manipulate host plant physiology and limit the impact of the initial leaf composition (Whiteman et al. 2011), and can delay tissue death in and around their mines (Yamazaki 2010). These findings about similar offspring performance in different leaves while better adult performance and more oviposition in mature middle leaves suggest that adult rather than offspring performance is shaping host preference pattern for oviposition in *S. flava*. These results also support optimal foraging theory (Price et al. 1999; Scheirs et al. 2001).

Oviposition on preferred leaves may sometimes lead to over-crowded and resource deficient conditions for the developing offspring (Wilson 1991; Scheirs et al. 2004). Though *S. flava* offspring performed equally well in different Chinese cabbage leaves, females preferred mature middle leaves for oviposition. Mayhew (2001) suggested that sometimes the insect behaviour might actually be well adapted but the data and theory are inadequate to show how. Unlike the offspring of some other leaf-mining insects which must complete their development at the site where the egg is deposited (Parrella 1987), *S. flava* larvae could migrate from resource deficient leaves to resource rich leaves and complete their development. Therefore, the information on the ability of these larvae to migrate to other leaves and complete their development, when combined with adult performance (higher fecundity and longevity) on mature middle

leaves, enables us to show that host preference of the adult females is adaptive (Mayhew 2001).

In conclusion, these findings suggest that while offspring performance is similar in different hosts, variation in adult performance in different hosts may shape their preference. The investigation of related factors and mechanisms such as larval migration enables us to show that host selection and preference of herbivorous insects are truly adaptive.

CHAPTER 5

MATING BEHAVIOUR

5.1 General introduction

Courtship and mating behaviours of different animal species are usually distinctive (Nicholson 2008). Mating sequences contain valuable information necessary to understand insects' mating system (Jiménez-Perez 2003). A thorough understanding of mating behaviour of an insect species is helpful for the successful implementation of any behaviour-based control technique such as sterile insect technique (Hendrichs et al. 2002).

Females in many insect species prefer to mate with males having certain morphological traits and *vice versa* (Hanks et al. 1996; Bonduriansky 2001; Weddell et al. 2002; Yang & Wang 2004; Sato & Goshima 2007; Willemart et al. 2009; Xu & Wang 2010). Discovering the sexually selected traits in both sexes provides necessary information for the implementation of behaviour-based control tactics (Boake et al. 1996).

Sexual harassment by males may decrease female feeding efficiency (Pilastro et al. 2003) and reproductive fitness (McLain & Pratt 1999; Wigby & Chapman 2004; Gosden & Svensson 2007). Male-biased operational sex ratio may increase the amount of sexual harassment faced by the females (Wigby & Chapman 2004; Smith & Sargent 2006). Therefore, determination of how sex ratio affects female fitness is useful for the development of insect mass-rearing programmes and understanding of evolutionary significance of sexual harassment.

This chapter reports for the first time the mating behavioural sequences, influence of mating experience on subsequent mating behaviour, costs of sexual harassment faced by females at different operational sex ratios, and sexually selected morphological traits in both sexes in *S. flava*.

5.2 Mating behaviour of *Scaptomyza flava*

5.2.1 Introduction

Mating behaviour comprises the activities and events that take place as the animals seek, identify, win over and appraise, and finally accept partners for reproduction (Lloyd 1979). Many animal species have developed elaborate courtship rituals (Lazareva et al. 2007). The understanding of mating behaviour of a pest species is mandatory prior to establishment of any behaviour-based control technique (Butt 1991). For example, information on the mating behaviour of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), has led to the implementation of the sterile insect technique in pest management programmes (Hendrichs et al. 2002).

Mating behaviour of the Drosophilidae is complex, involving sequential steps of visual, chemical and auditory signals (Petfield et al. 2005), that require interactions between sexes (Bacigalupe et al. 2008). Generally, after orienting and approaching a potential mate the drosophilid male engages in courtship display before attempting to mount and copulate, and such courtships involve species-specific movements of various body parts such as wings, legs, mouthparts and abdomen that occur sequentially (Spieth 1974). For example, in *Drosophila subobscura* upon encountering a receptive female, the male approaches her from the side and enters the 'orientation' phase of courtship, in which he 'taps' the female with his front legs. During orientation the male makes fast flicking movements with both wings. The female may then 'dance' by stepping rapidly from side to side. Either during the dance or when the female stops, the male performs the 'wing display' raising his wings into a 'V' above the body. The male then circles to the rear of the female and attempts to mount. The sequence may break down at any stage and the female may 'decamp' by walking, running or flying away (Steele 1986). Unlike *Drosophila*, the mating behavioural sequences of *Scaptomyza* have not been well studied.

The experience gained by the drosophilid males while courting numerous females could affect and modify their mating behaviour (Dukas 2005 & 2006). For example, *D. melanogaster* males having experience of courting unreceptive, recently mated females exhibit reduced courtship effort on mated females, and males having experience of courting unreceptive, immature females make increased courtship effort

on receptive virgin females compared to inexperienced males (Dukas 2005). Pre-copulatory confinement conditions may also affect the mating success of drosophilid males. For example, *D. silvestris* (Carson) males kept in isolation after emergence are less successful in mating compared to those reared with other males (Sene 1977).

Prior to the present study nothing was known about the mating behaviour of *S. flava*, making it difficult to develop strategies for behaviour-based management programmes for this pest. During this study I observed the mating behavioural sequences, and investigated the courtship behaviour and mating success of virgin and mated males. I also examined the effects of the confinement of males with other males, and previous mating experience on their subsequent mating behaviour.

5.2.2 Materials and methods

5.2.2.1 Insects

Pupae were collected and weighed individually using the electronic scale before being individually placed in glass tubes until adult emergence. A 3-4 cm long Chinese cabbage leaf was provided to each female in the glass tube while males were provided with the leaf having 50 punctures made by a dissecting needle.

5.2.2.2 Mating behavioural sequence

To observe the general mating behavioural sequence, a 2-d-old virgin female was paired with a 2-d-old virgin male in a glass tube (n = 20). The mating behaviours were observed and recorded using a Panasonic SVHS Camcorder (MS-4) (Panasonic, Japan) connected to a Samsung video cassette recorder (DVDV530, Korea) and images viewed on a Panasonic colour monitor (TC-21T1Z, Japan). Probabilities of behavioural events at each transition were calculated as the number of individuals that performed an observed behaviour divided by the number of individuals that performed a previous behaviour.

5.2.2.3 Behaviour definitions

Behavioural interactions between male and female *Scaptomyza flava* were defined as following:

(a) Courtship - a male flapps his wings and taps female's body with his fore tarsi;

(b) Approaching - a male approaches a female with his wings flapping;

(c) Mounting - a male mounts a female;

(d) Mating - a male inserts his aedeagus into a female's genitalia and copulates.

5.2.2.4 Confinement in group versus confinement in isolation

Newly emerged males were kept in glass tubes and were subject to one of the following treatments following Noor (1997) (n = 15): a) kept individually for 2 days prior to pairing with 2-d-old virgin females, b) five males kept together for 2 days prior to pairing with 2-d-old virgin females, c) five males kept together for 2 days and then individually for 1 day prior to pairing with 2-d-old virgin females, and d) kept individually for 2 days and then 5 males together for 1 day prior to pairing with 2-d-old virgin females. For experiments on males reared in groups, one of the five males was randomly selected for pairing with the female. Observations were made from the release of a pair into the glass tube to successful copulation. In cases when a male, after initiating courtship, discontinued it and didn't resume courtship for one hour, no further observations were made. Data on courtship latency and courtship period and mating success in each treatment were recorded. Courtship latency was the period between the release of a male into the glass tube and initiation of courtship while courtship period was the period between initiation of courtship and mating.

5.2.2.5 Previous male mating experience and subsequent mating behaviour

This experiment consisted of two treatments (n = 20 each): a) males having failed mating experience, b) males having successful mating experience. To obtain males that failed to mate, 2-d-old virgin males were individually paired with newly emerged unreceptive females in glass tubes. Those males that courted and mounted the females but were kicked and dislodged by the females either once or multiple times, and

eventually discontinued courting the females and didn't resumed courtship for 1 hour were considered as having failed to mate. To obtain males having successful mating experience, 2-d-old virgin males were individually paired with 2-d-old virgin females in glass tubes and observed for 1 hour. Those males that mated with the females were considered as males having successful mating experience. In both of the above mentioned treatments, females were removed from the tubes with the help of an aspirator and the males were then individually paired with 2-d-old virgin females and the data on courtship latency, courtship period and mating achievement were recorded.

5.2.2.6 Differential mating behaviour of virgin and mated males

A 2-d-old virgin female was maintained with a 2-d-old virgin male and a 2-d-old once-mated male in a glass tube (n = 20). The virgin and mated males were marked randomly on the thorax by different trace colour powders (Magruder Colour Co., Elizabeth, NJ, USA) for identification. Because *S. flava* males become sexually mature when they are 24 hours old (Section 3.4.3.2), I obtained mated males by allowing 1-day-old virgin males to mate with 2-day-old virgin females. Data on the number of virgin and mated males that courted the females, and courtship latency, courtship period and mating success of the virgin and mated males were recorded. Data on the number of virgin and mated males that attempted to disrupt the mating were also recorded. The mating was considered to be disrupted if the second male struck the mating male or female or both with his fore-tarsi, or attempted to mount the mating couple.

5.2.2.7 Statistics

Kolmogorov-Smirnov test was used to test the distribution of data before analysis. Data on the effect of confinement with other males and previous mating experience on courtship latency and courtship period, and on differential mating behaviour of virgin and mated males were analysed using one way ANOVA followed by Tukey's studentised range (HSD) test. Data on the difference in number of courtships, mating achievement and mating disruption between virgin and mated males, and on the effect of confinement conditions and previous mating experience on male mating achievement were analysed using a Chi-Square test.

5.2.3 Results

5.2.3.1 Mating behavioural sequence

The general courtship and mating behaviours are shown in Fig. 5.1. Sexual behaviour started 2-3 min after release into the tube when the male became excited, walked towards the female and started courting. Courtship display by the male included flapping his wings around the female and tapping her body with his fore tarsi. After courtship started, 60% of females and 25% of males moved away. The male approached the female again and resumed the courtship. At this stage most females remained stationary and males walked around them. Females elevated and lowered their abdomen and expanded their wings as an acceptance signal. Finally, 95% of courting males mounted females. Upon mounting, 19% of mounted females rejected males by kicking them. If mating failed to occur, the male again started courting the female. Ninety-five percent of mounted females eventually mated. During mating he grasped her thorax with his fore and mid legs, and her abdomen with his hind legs. After mating *S. flava* males vibrated their wings, and made zigzag movements for about 15 s. Males then did not show any interest in females.

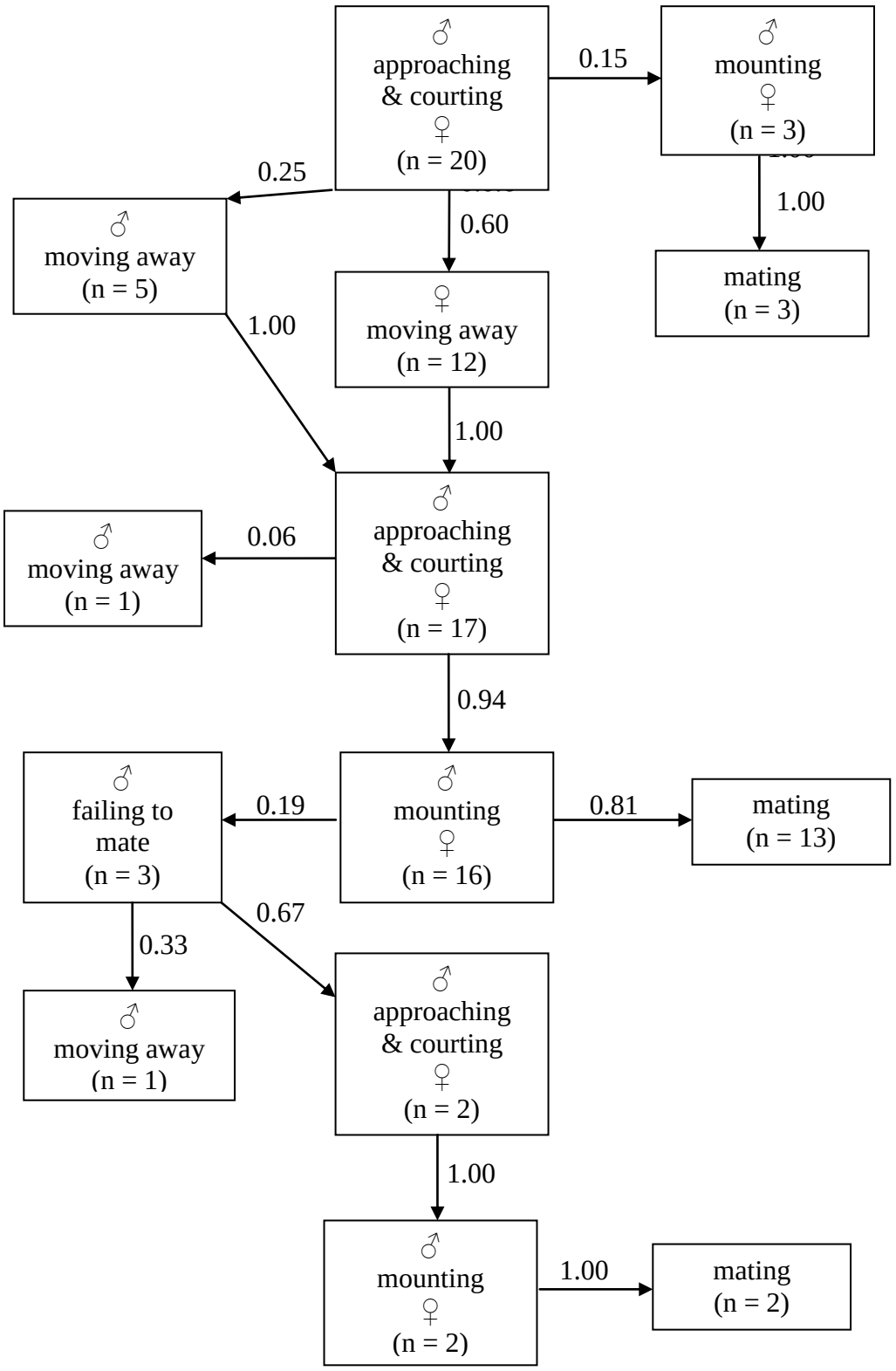

Fig. 5.1 Flow chart of the mating behavioural sequences of *S. flava.* Probabilities of a particular transition between stages are given.

5.2.3.2 Confinement in group versus confinement in isolation

Males confined in isolation initiated the courtship significantly earlier, mated after significantly shorter courtship period and were significantly more likely to achieve mating than males confined in groups (for courtship period: F = 18.20; DF = 3, 56; for courtship latency and mating: χ^2 = 47.51 and 25.89, respectively; DF = 3; $P < 0.05$; Figs. 5.2a & 5.2b). Similarly, males confined in group for 2 days and then confined in isolation for 1 day, courted the females significantly earlier, mated after significantly shorter courtship period and were significantly more likely to achieve mating than those reared in isolation for 2 days and then confined in group for 1 day prior to pairing with females (Figs. 5.2a & 5.2b).

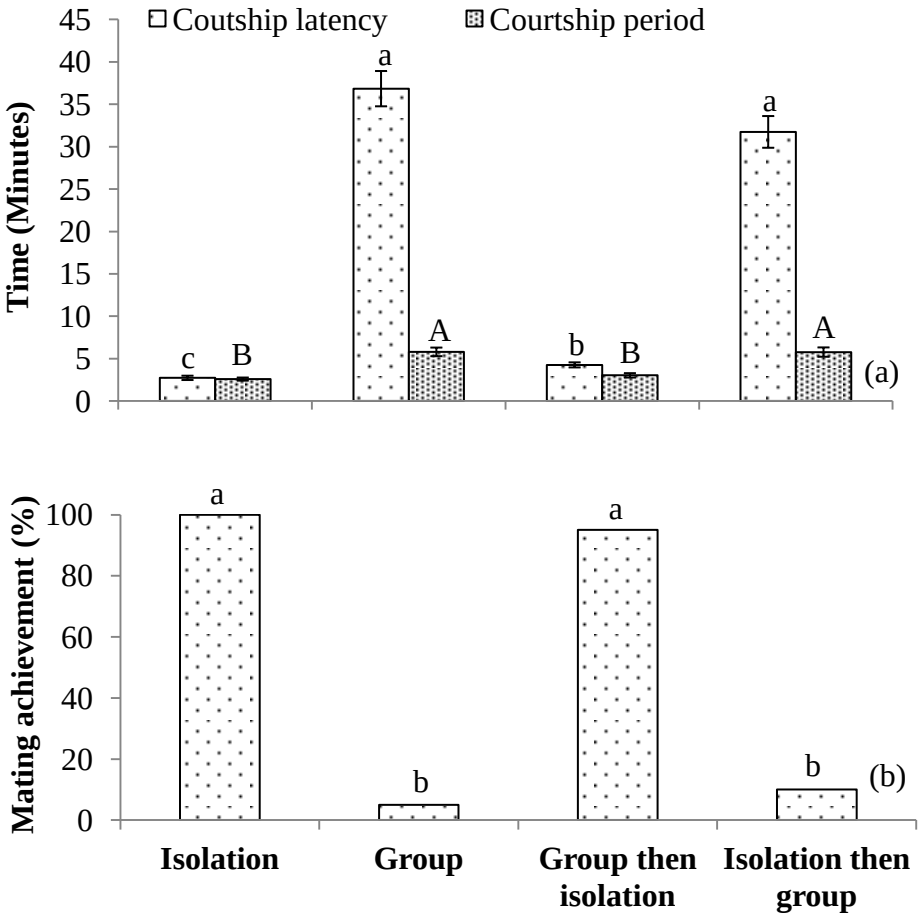

Fig. 5.2 Effect of confinement conditions on male mating behaviour in *S. flava*. For each category, bars with different letters are significantly different ($P < 0.05$).

5.2.3.3 Previous male mating experience and subsequent mating behaviour

Males having previous successful mating experience initiated courtship significantly more quickly and mated after significantly shorter courtship period than did the males having failed mating experience (F = 404.94 and 59.15 for courtship latency and courtship period, respectively; DF = 1, 38; $P < 0.05$; Fig. 5.3a). However, there was no significant difference in mating success between these males ($\chi^2 = 0.28$; $DF = 1$; $P > 0.05$; Fig. 5.3b).

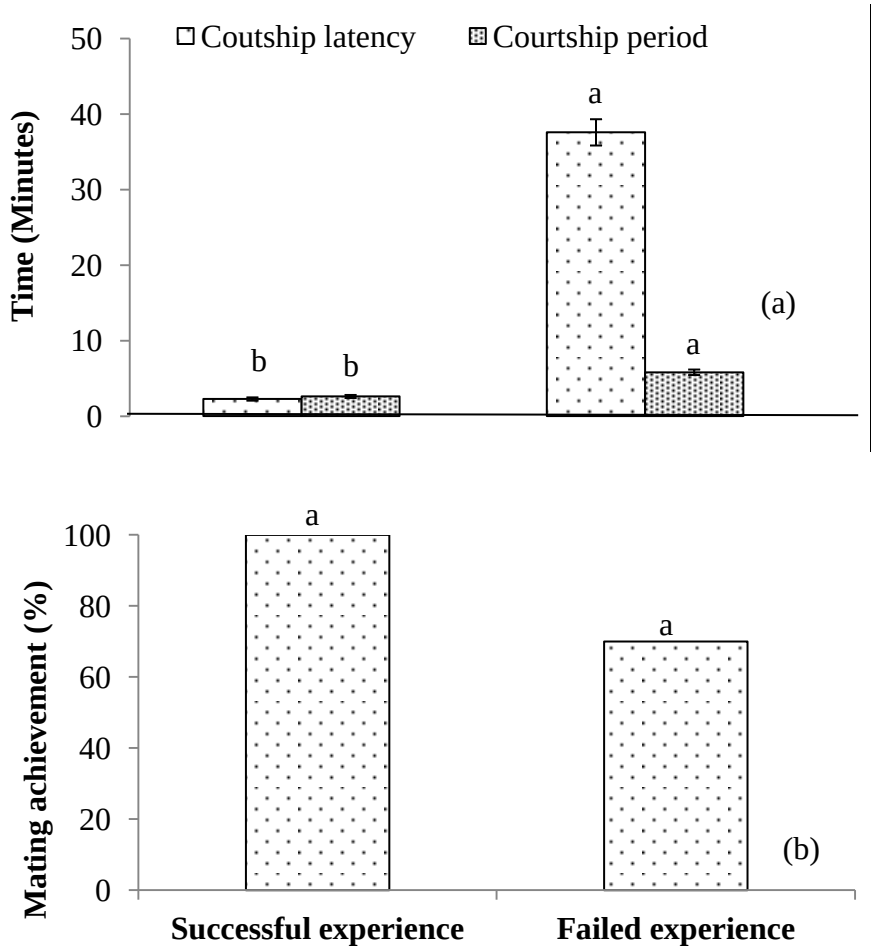

Fig. 5.3 Effect of previous mating experience on male mating behaviour in *S. flava*. For each category, bars with different letters are significantly different ($P < 0.05$).

5.2.3.4 Differential mating behaviour of virgin and mated males

In triad trials, both virgin and mated males displayed similar mating behaviour, and had similar courtship latency (2.93 ± 0.34 and 2.19 ± 0.32 min for virgin and mated males, respectively) and courtship periods (2.14 ± 0.27 and 2.55 ± 0.26 min for virgin and mated males, respectively) (F = 2.52 and 1.21 for courtship latency and courtship period, respectively; DF = 1, 38; $P > 0.05$). However, significantly higher percentage of mated males performed courtship displays and achieved mating than virgin males (χ^2 = 4.17 and 11.74 for courtship and mating, respectively; DF = 1; $P < 0.05$; Fig. 5.4). Both mated and virgin males tried to disrupt the mating couples by striking them with their fore tarsi and attempting to mount the mating couple. However, there was no significant difference in mating disruption by mated and virgin males ($\chi^2 = 0.29$; DF = 1; $P > 0.05$; Fig. 5.4).

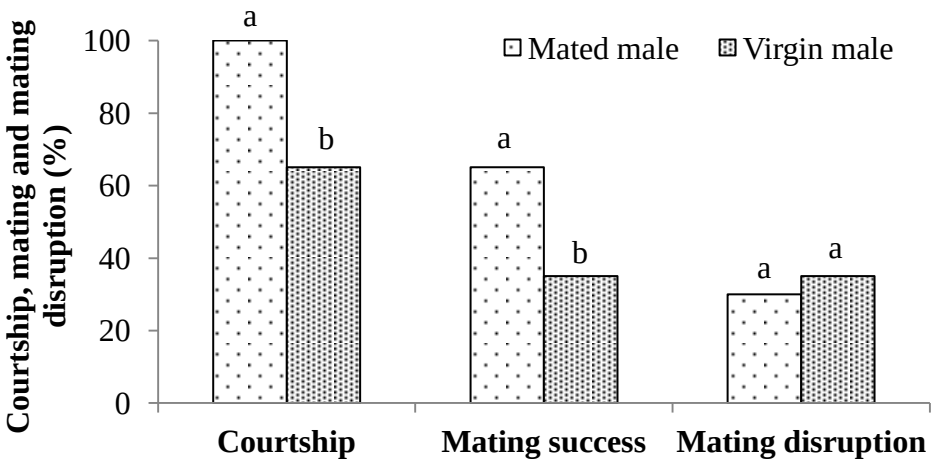

Fig. 5.4 The percentage of mated and virgin *S. flava* males engaging in courtship display, achieving mating success and showing mating disruption behaviour. For each category, bars with different letters are significantly different ($P < 0.05$).

5.2.4 Discussion

My investigation reveals that male *S. flava* performed certain pre-mating behaviour, which is composed of a succession of behavioural sequences typical of the Drosophilidae that can break off at any stage and resume from the beginning. The male approached the female and then performed courtship display, after which time he mounted and mated with her. These results indicate that *S. flava* male mating behaviour

is different from *Drosophila pegasa* where copulation occurs without courtship display (Pruett-Jones et al. 2002) but similar to *D. pseudoobscura* Frolova & Astaurov (Noor 1997) where courtship display is essential to mating success. As the courtship proceeds, *S. flava* females become stationary and expand their wings as an acceptance signal like *D. sechellia* (Tsacas and Bachli) (Cobb & Ferveur 1995). Furthermore, *S. flava* females could dislodge male suitors by kicking them, suggesting that *S. flava* female's acceptance is necessary for copulation.

In many drosophilid species, females release sex pheromones to attract males and elicit their courtship displays (Antony & Jallon 1982; Tompkins et al. 1983; Oguma et al. 1992; Wicker-Thomas 2007; Dickson 2008). Although the sex pheromone of *S. flava* has not been identified, male's approaching and courting females in this species may be stimulated by a female sex pheromone. As reported in some *Drosophila* species (Hoikkala et al. 2000), *S. flava* males perform post-copulation courtship behaviour by vibrating wings and making zigzag movements around females after copulation. However, whether such post-copulation display in *S. flava* serves as post-insemination mate guarding reported in other insects (Allen et al. 1994; King & Fischer 2005) remains unknown.

My study indicates that similar to that of *D. melanogaster* and *D. pseudoobscura* (Van den Berg 1985; Noor 1997), *S. flava* males reared in isolation performed better in mating behaviour than those reared in groups. This may be attributed to fatigue or the inhibitory compounds of other males (Noor 1997). This finding is important in the development of sterile insect technique for *S. flava* control because confinement conditions during mass rearing of *S. flava* males prior to release would influence their mating success. Furthermore, males that had failed mating experience responded to females more slowly than those that had successful mating experience. It is suggested that male mating ability is influenced by previous experience and learning (Greenspan & Ferveur 2000).

No significant differences were found in courtship latency and courtship period between virgin and once-mated males in *S. flava*, supporting Markow et al.'s (1978) study on *D. melanogaster*. However, my results show that compared to virgin males, mated males are significantly more likely to display courtship behaviour and achieve mating success. These results suggest that previous courting experience refines male

courtship behaviour in a way that could increase their mating success. Similar reports can also be found in fruit flies (Tompkins et al. 1983; Dukas 2005).

5.3 Effect of operational sex ratio on female feeding, reproductive fitness and longevity

5.3.1 Introduction

The optimal number of mating is predicted to be lower in females than in males (Clutton-Brock & Vincent 1991) because of the higher mating costs for females (Watson et al. 1997), which leads to sexual conflict (Parker 2006). As a result, males attempt to copulate with reluctant females through coercion and harassment (Clutton-Brock & Parker 1995; Arnqvist & Nilsson 2000). Repeated harassment, males' persistent courtship and attempts to copulate with reluctant females (Clutton-Brock & Parker 1995) impose costs to females (Sakurai & Kasuya 2008), such as energy cost (Watson et al. 1997; Valero et al. 2005), reduced feeding time (Rowe et al. 1996), increased predation risk (Rowe et al. 1994), and disturbance during oviposition (McLain & Pratt 1999).

Male-biased operational sex ratio (OSR, the ratio of sexually active males to receptive females) increases females' encounter rate with males and results in increased harassment and superfluous matings for females (Sih & Kurpa 1996; Wigby & Chapman 2004; Smith & Sargent 2006), reducing their longevity and reproductive fitness (McLain & Pratt 1999; Wigby & Chapman 2004; Gosden & Svensson 2007). For example, in *Drosophila pseudoobscura* (Frolova & Astaurov), *Sepsis cynipsea* (L.) (Diptera: Sepsidae) and *Neacoryphus bicrucis* (Say) (Hemiptera: Lygaeidae), male-biased OSR results in increased harassment (Blanckenhorn et al. 2000; McLain & Pratt 1999; Crudgington et al. 2005) and decreased female reproductive fitness (Martin & Hosken 2003; McLain & Pratt 1999; Crudgington et al. 2005). In *Callosobruchus maculates* (Fabricius) (Coleoptera: Chrysomelidae), increased male harassment results in reduced female longevity (den Hollander & Gwynne 2009), and in mosquitofish *Gambusia holbrooki* (Girard) (Cyprinodontiformes: Poeciliidae), male harassment leads to decreased female feeding efficiency (Pilastro et al. 2003).

The objectives of the present study were to investigate the fitness costs of increased courtship and mating faced by *S. flava* females. Harassment can decrease female foraging efficiency (Pilastro et al. 2003), thus it can be even more costly in terms

of female fitness in *S. flava*, where deprivation from host feeding can significantly reduce adult longevity (Section 3.3.3.3).

5.3.2 Materials and methods

5.3.2.1 Insects

Pupae were collected and weighed individually on the electronic scale before being individually placed in glass tubes until adult emergence. After emergence a 3-4 cm long Chinese cabbage leaf was provided to each female in the glass tube while each male was provided with a similar leaf having 50 punctures made by a dissecting needle.

All females used in the experiment were allowed to mate once before treatments. To obtain mated females, 1-d-old virgin females were individually paired with 1-d-old virgin males in glass tubes and observed for mating to occur. If a female did not mate one hour after paired, the male was replaced. If mating still did not occur in another one hour, then that female was discarded. After mating the males were aspirated out of the tubes. Before release into the small plastic cylinders for experiments, all mated females were marked on the thorax by colour powder (Magruder Color Co., Ellizabeth, NJ, USA) so that they could be easily distinguished from males from dorsal and lateral views during observations.

5.3.2.2 Difference in mating frequency between sexes

Both *S. flava* males and females mate multiply (Section 3.4.3.3). To investigate the male mating frequency, two 1-d-old virgin females were exposed to a 1-d-old virgin male in a glass tube and observed for one hour for the occurrence of mating (n = 15). When the male mated with one of the females during that hour then both females were removed and the male was again provided with two similar females during the next hour of the same photophase and observed for the occurrence of mating. This process was repeated until the male did not mate for two consecutive sessions. The same procedure was repeated to investigate *S. flava* female mating frequency (n = 15).

5.3.2.3 Effect of male density on courtship and mating

This experiment consisted of four treatments. In the first three treatments, a 2-d-old female was released to the following numbers of 2-d-old males: a) one (n = 10), b) five (n = 10), and c) ten (n = 10) in the small transparent plastic cylinder. In the fourth (control) treatment, a 2-d-old female was individually released in the cylinder (n = 10). In order to maintain the male density in the first three treatments until the experiment was completed, I observed the cylinders daily and replaced any dead male with another male.

For recording data on the number of courtships and matings in each treatment, ten observations were made daily on each cylinder at 25 minutes interval until the females died. Average mating time in *S. flava* is 19 ± 2 min (Section 3.4.3.3); therefore, an interval of 25 minutes between two observations was used to avoid counting a mating twice. Observations were started at the onset of the photophase, as in *S. flava* maximum mating occurs during this period (Sections 3.4.3.3). When a male was observed flapping his wings around the female or tapping her body with his fore tarsi, he was considered to be courting her (Section 5.2.3.1). Data on the number of courtship and matings faced by females in each treatment during the observation period were recorded.

5.3.2.4 Effect of male density on female feeding, offspring production and longevity

Each female in the previous experiment was supplied with a middle leaf of a 2.5-month-old Chinese cabbage for 24 h for feeding and oviposition, which was replaced with another similar leaf after that period. To prevent the leaf from drying, leaf stalk was immersed in the glass tube having water. The top of the tube was covered with cotton wool to prevent the flies from drowning. Data on the number of feeding punctures created and offspring produced by each female during the lifetime, and female longevity were recorded. The number of feeding punctures created by each female on each leaf was counted under the microscope while to have an estimation of the number of offspring produced by each female, the number of linear mines created by a neonate larva on each leaf was counted.

5.3.2.5 Statistics

Data on mating frequency, and courtship, mating, feeding and offspring production under different OSR were analysed using non-parametric Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons (Zar 1999).

5.3.3 Results

5.3.3.1 Difference in mating frequency between sexes

Females mated (1.86 ± 0.13) significantly fewer times than males (3.66 ± 0.12) (χ^2 = 22.91; *DF* = 1; *P* < 0.05) during the observation period.

5.3.3.2 Effect of male density on courtship and mating

S. flava females housed with 5 and 10 males faced significantly higher number of courtship events and mated significantly more frequently than those housed with 1 male (for courtship: χ^2 = 20.58, 21.68, 21.12, 14.97 for 1st d, 2nd d, 3rd d and 4th d, respectively, for mating: χ^2 = 10.94, 8.57, 11.59, 10.50 for 1st d, 2nd d, 3rd d and 4th d, respectively; *DF* = 2; *P* < 0.05; Fig. 5.5). However, there was no significant difference in the number of courtship events and matings between male densities of 5 and 10 males (Fig. 5.5).

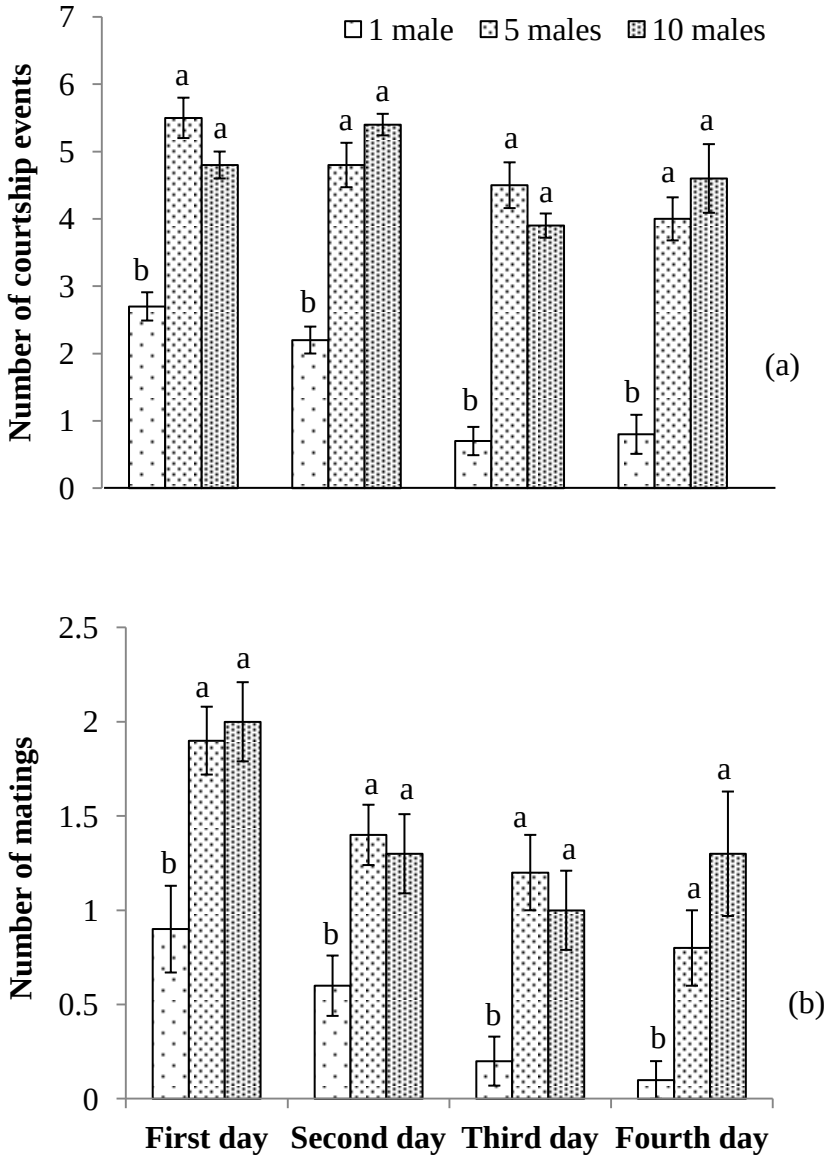

Fig.5.5 Effect of male density on the numbers of: (a) daily courtship events and (b) matings faced by *S. flava* females. For each day, bars with different letters are significantly different ($P < 0.05$).

5.3.3.3 Effect of male density on female feeding, offspring production and longevity

Females housed with 5 and 10 males made significantly fewer number of feeding punctures and produced significantly fewer offspring each day than those housed with 1 male and no male (for feeding punctures: χ^2 = 30.17, 30.67, 30.51 and 18.81 for 1st d, 2nd d, 3rd d and 4th day, respectively, for offspring: χ^2 = 29.59, 26.62, 25.23 and 16.87 for 1st d, 2nd d, 3rd d and 4th day, respectively; $DF = 3$; $P < 0.05$; Fig. 5.6).

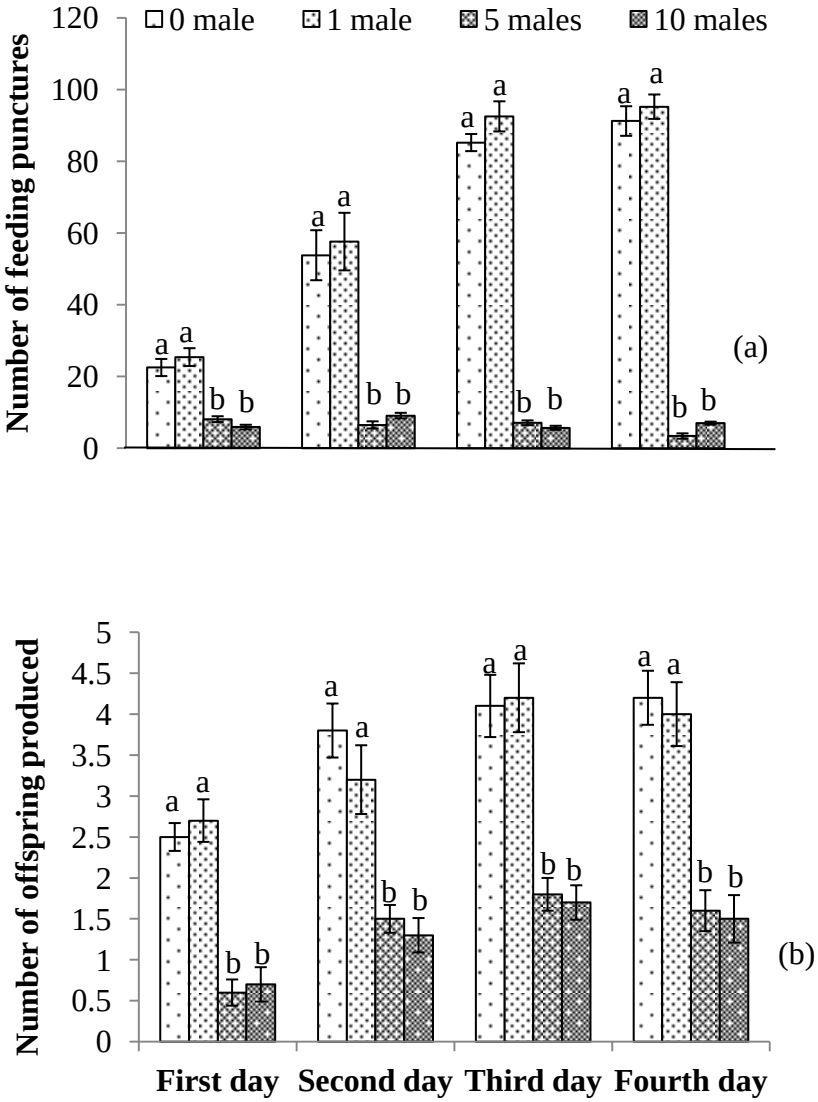

Fig. 5.6 Effect of male density on: (a) feeding and (b) offspring production in *S. flava* females. For each day, bars with different letters are significantly different ($P < 0.05$).

Females housed with no or one male lived significantly longer than those housed with 5 or 10 males ($\chi^2 = 25.15$; $DF = 3$; $P < 0.05$; Fig. 5.7a). Females housed with no or one male made significantly more number of feeding punctures in their lifetime than those housed with 5 or 10 males ($\chi^2 = 29.54$; $DF = 3$; $P < 0.05$; Fig. 5.7b). Similarly, females housed with 5 or 10 males produced significantly fewer number of offspring than those housed with no or one male ($\chi^2 = 33.40$; $DF = 3$; $P < 0.05$; Fig. 5.7c). Females housed with one male for life produced more offspring compared to once mated females housed in isolation (Fig. 5.11c).

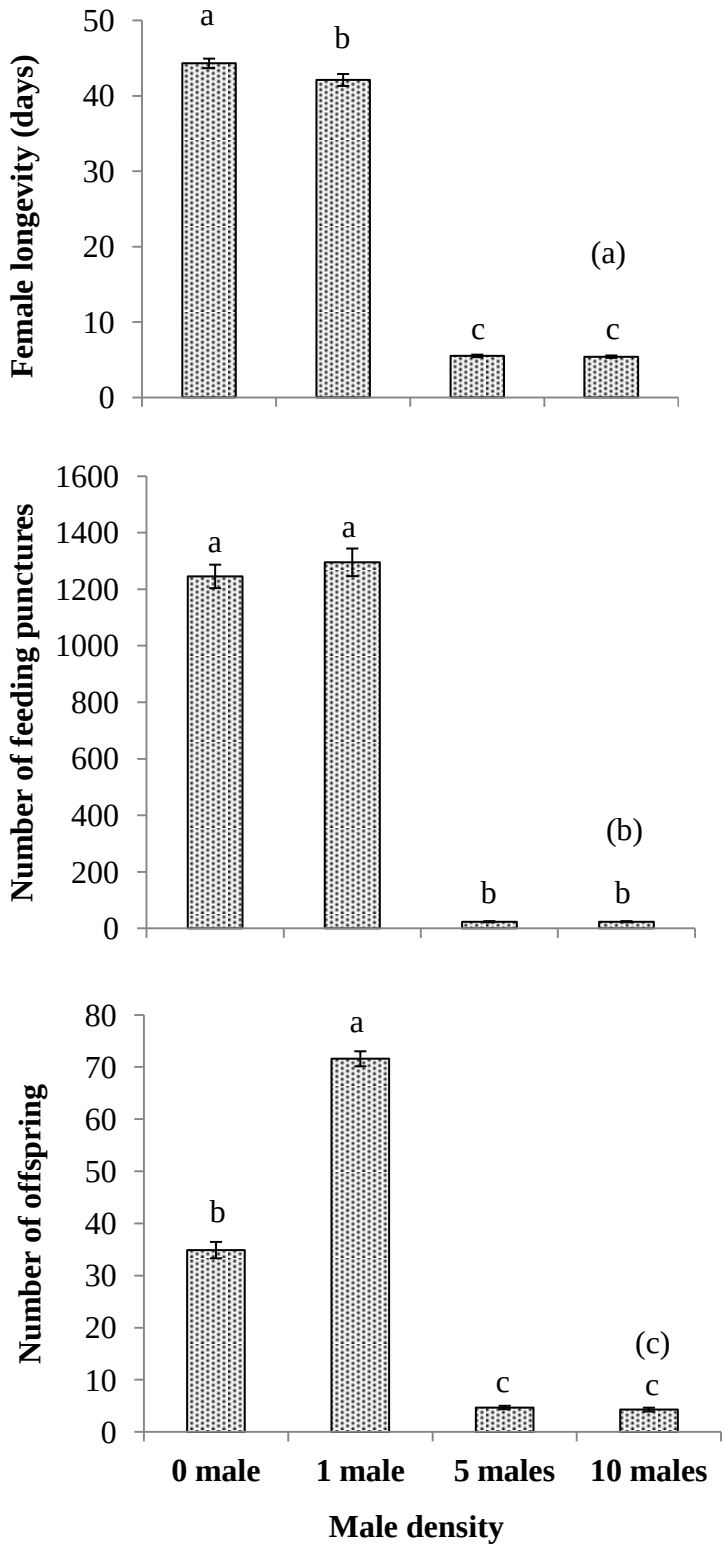

Fig. 5.7 *S. flava* female longevity (a), lifetime feeding (b) and offspring production (c) under different male densities. For each density bars with different letters are significantly different ($P < 0.05$).

5.3.4 Discussion

Sexual harassment by males strongly affects females' behaviour, for example, they usually mitigate males' persistent courtship by leaving that location (Magurran & Seghers 1994; Gay et al. 2009) and avoiding contact with the males (Shine et al. 2005). As a result, sexual harassment hinders females from spending time on activities like feeding and oviposition, reducing their fitness (Magurran & Seghers 1994; Rowe et al. 1996; McLain & Pratt 1999). My results indicate that *S. flava* females housed with higher density of males faced more courtship and mating events than those housed with one male. These females also produced fewer feeding punctures. This suggests that persistent courtship and mating attempts by males may interfere with female feeding activity. This study also reveals that *S. flava* females housed with higher density of males had significantly shorter longevity. Decreased longevity of these females appears to reflect the costs of repeated courtship as reported in tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) (Clutton-Brock & Langley 1997), and probably of less feeding.

Food intake affects immediate and future female reproductive output (Magurran & Seghers 1994). The present investigations show that *S. flava* females housed with higher density of males created fewer feeding punctures and produced fewer offspring. These results suggest that decreased feeding might have contributed towards lower offspring production in these females. Similarly, in *Poecilia reticulata* Peters (Pisces: Poeciliidae) increased harassment results in decreased feeding and lower reproductive output (Magurran & Seghers 1994). Furthermore, lifetime offspring production is also affected by female longevity (Arnqvist & Nilsson 2000). Thus significantly fewer lifetime offspring production in females housed with more males may also be attributed to their significantly shorter longevity. These results suggest that in order to increase *S. flava* population for use in laboratory experiments or biological control programmes, females should not be housed with high male density.

Optimal number of matings maximizes reproductive fitness of dipterous females (South & Lewis 2011). My results indicate that once-mated *S. flava* females housed with no male produced fewer offspring than those housed with a male for life. It is suggested that in *S. flava* one mating is not enough to maximize reproductive fitness. Similar report can be found in the stalk-eyed fly, *Cyrtodiopsis dalmanni* (Diptera: Diopsidae) (Baker et al. 2001).

In conclusion, sexual harassment affects female feeding and fitness in *S. flava*. Higher male density results in increased number of courtship events and matings. It also results in significantly reduced female lifetime feeding, longevity and reproductive output. More than one mating appears to be necessary for females to achieve maximum reproductive fitness.

5.4 Pre-copulatory sexual selection in *Scaptomyza flava*

5.4.1 Introduction

Darwin (1871) used the term 'sexual selection' to explain the evolution of elaborate characters in many animals that confer a mating advantage for the bearer (Clutton-Brock 2007). Sexual selection may be in the form of intra-sexual competition and/or mate choice (Thornhill & Alcock 1983; Blanckenhorn et al. 2000). Mate choice has been associated with body size in many insects (Ward 1983; Gwynne 1984; Simmons 1986; Gilburn et al. 1992; Gilburn & Day 1994; Gray 1997; Savalli & Fox 1998; Preziosi & Fairbairn 2000) including drosophilids (Norry et al. 1994; Sisodia & Singh 2001; Byrne & Rice 2006). However, the association between mate choice and body size could be due to selection on certain traits correlated with body size (Norry et al. 1994; Sisodia & Singh 2001). It is thus important to investigate the relative importance of size related traits that could influence mate choice (Norry et al. 1994).

Males are considered to be more competitive sex having higher variation in mating success (Shuster & Wade 2003); studies on sexual selection thus mostly focus on males (Abell et al. 1999; Norry et al. 1999; Rodriguero et al. 2002; Sciurano et al. 2007). However, evidence shows that males display energetically costly courtship (Judge & Brooks 2001), male ejaculates are costly and limited (Svärd & Wiklund 1986; Cook & Gage 1995), and females differ in reproductive potential (Ghiselin 1974; Sato & Goshima 2007). Therefore, males also exhibit mate choice among available females (Weddell et al. 2002; Bonduriansky 2001; Sato & Goshima 2007).

Empirical evidence suggests that females in different insect species prefer to mate with males having certain traits such as longer antennae (Hanks et al. 1996; Yang & Wang 2004), longer legs (Willemart et al. 2009), and longer wings (Xu & Wang 2010). Males may prefer females with larger abdomens for mating **(**Li et al. 2005; Bussiere et al. 2008; Xu & Wang 2010), and the abdominal size of females is positively correlated with female fecundity (Funk & Tallamy 2000; Bussiere et al. 2008). Males may also prefer females with longer ovipositor as ovipositor is an important organ in insects that helps ensure the maximal survival of offspring (Bradford et al. 1993; Mousseau & Roff 1995; Sivinski et al. 2001; Yang & Wang 2004).

Thorough understanding of sexual selection mechanisms is essential for the successful implementation of the sterile insect technique to manage insect pest

populations (Burk & Calkins 1983). Mass-rearing conditions could be manipulated for improving male and female quality to promote the occurrence of sexually selected phenotypes in a high frequency. Furthermore, directed selection events may be used to select genotypes with high mating success (Rodriguero et al. 2002). The aim of the present study was to examine sexual selection in both sexes of *S. flava*. Here I performed mating experiments on *S. flava* under biased operational sex ratios and measured those traits that may be subject to sexual selection. I then statistically analysed the results to determine how these traits were associated with mating success.

5.4.2 Materials and methods

5.4.2.1 Insects and general methodology

A potted Chinese cabbage plant was exposed to the breeding colony for 24 hours for oviposition and then replaced with another plant. The average leaf area for an *S. flava* larva to complete development was 2.03 ± 0.03 cm^2 (Section 4.4.2.6). When neonate larvae started making linear mines in leaves, I allowed only ten larvae to continue to develop in each of the middle and bottom leaves and killed others with a dissecting needle. These larvae were killed so that the remaining larvae may have enough leaf area available to feed and complete their development.

Pupae were collected and individually placed in glass tubes until adult emergence. Males and females were kept in isolation in the glass tubes until they were used for experiments to ensure virginity. A 3-4 cm long Chinese cabbage leaf was provided to each female in the glass tube while each male was supplied with a leaf of similar size having 50 punctures made by a dissecting needle. For experiments, 2-d-old randomly selected males and females were maintained in glass tubes according to the sex ratios mentioned in sections 5.4.2.2 and 5.4.2.3 during first hour of the photophase, and observed for the mating events until successful mating occurred. Insects were then placed in a freezer at -20°C for 24 h immediately after mating. Morphometric traits were then measured under the dissecting microscope. All measurements of length, width, and thickness reported here were the greatest length, width, and thickness. For example, body length was the length between the frons of the head and the tip of abdomen. Wing length was the length of the left wing from thoracic articulation to the distal tip (Fig. 5.8a). Female abdominal thickness and width were measured at the

middle of the 3rd visible abdominal segment, where thickness was the length between dorsal and ventral sides and width was the length between sides. I also measured the lengths of antenna, ovipositor (Fig. 5.8b), hind tibia and tarsus, and fore tibia and tarsus (male only).

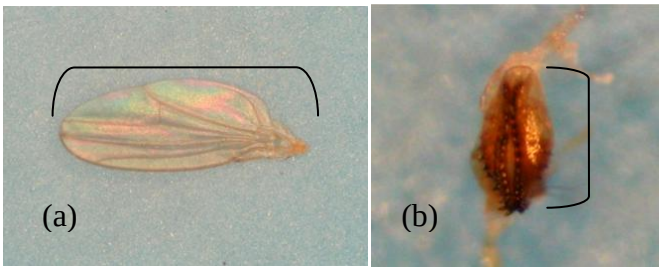

Fig. 5.8 Measurements of *S. flava* morphometric traits made for these studies: a) wing length and b) ovipositor length.

5.4.2.2 Sexual selection on female traits

To determine female traits that might be associated with mating success and to attempt to find traits that may be subject to directional sexual selection, I allowed mating to occur under a female-biased sex ratio (n = 60). For each replicate, I released a randomly selected virgin male and three randomly selected virgin females into a glass tube. Each individual was used only once. All females were randomly marked by different trace colour powder (white, pink, or sunset orange; Magruder Color Company, USA). I made the measurements on above mentioned body parts of both mated and unmated females. Then I dissected all females and counted the number of mature eggs in their ovaries and also measured their ovipositor length. To count the number of mature eggs, the ovaries were separated out and immersed in 1% acetocarmine for 10 s to stain the eggs before being transferred to clean saline solution. The chorion of mature eggs prevents the stain but immature eggs absorb the stain (Edwards 1954). Therefore, stained eggs were classified as immature and unstained eggs as mature. Then I examined the relationship between female abdominal thickness, abdominal width, and the number of mature eggs in the ovaries at the time of mating.

5.4.2.3 Sexual selection on male traits

To determine male traits that might be associated with mating success and to attempt to find traits that may be subject to directional sexual selection, I allowed mating to occur under a male-biased sex ratio (n = 60). For each replicate, I released a randomly selected virgin female and three randomly selected virgin males into a glass tube. Each individual was used only once. All males were randomly marked by different trace colour powder (white, pink, or sunset orange; Magruder Color Company, USA). I made the measurements on above mentioned body parts of both mated and unmated males.

5.4.2.4 Statistics

For estimation of net sexual selection on traits and for identification of direct and indirect effects of selection, I applied standardized bivariate and multivariate selection analyses (Lande & Arnold 1983; Arnold & Wade 1984) where I used morphological traits as independent variables and mating success as a dependent variable. Male and female *S. flava* that succeeded in mating were coded as 1, and those that failed as 0. All of the included independent variables were standardized to mean 0 and unit variance prior to analyses. Because standardizing removes the effects of differential scaling, it allows a comparison (in standard deviation units) of the relative importance of each variable (Gibson 1987).

Directional selection differentials (*s*) estimate the net selection (combined direct and indirect effects via correlated traits) acting on a trait by measuring the relative strength of bivariate relationships (bivariate analysis) between relative mating success and each trait (Pryke et al. 2001). Directional selection gradients (β) quantify the strength of the selection acting on the trait independent of variation in other traits included in the regression model (Lande & Arnold 1983). β values were calculated as partial linear regression coefficients from multiple regressions (multivariate analysis) of relative mating success to the standardized value of the traits (Pryke et al. 2001). A conditional logistic regression using SAS's PROC PHREG procedure (SAS 9.2) was used for the above bivariate (selection differentials, *s*, were estimated by the coefficient of the regression) and multivariate (selection gradients, β, were estimated as partial linear regression coefficients) analyses. *S* and β′ were estimated using the models of

Logit (mating success) = $S_i z_i + \epsilon$, and Logit (mating success) $= \sum_{i=1}^{n} \beta_i' z_i + \epsilon$, respectively, where z_i is the vector of a trait and ϵ the error term (Lande and Arnold 1983). Only trials that significantly affected by the net selection were included for multivariate analysis.

The net selection (directional selection differentials, *s*) significantly acted on four and five traits for females and males, respectively (Tables 5.2 and 5.4), and these traits were significantly correlated. In the multivariate regression, extreme correlations among independent variables (multicolinearity) may lead to an overestimation of the standard errors of the regression coefficients, reducing the power of the analysis (Mitchell-Olds & Shaw 1987). To assess the influence of multicolinearity on the results, I examined the principal component analysis correlation matrix of those standardized variables. The condition numbers (square root of the ratio of the largest eigen value to the smallest) were 2 for both female and male selection, which is much lesser than the critical number of 10 suggested by Fry (1993), indicating that the correlations among those traits were not sufficiently high to adversely affect the significance tests and thus acceptable for the multivariate analyses.

However, because I may have still missed the possibility of stabilizing selection using the above linear analysis, I also tested stabilizing selection in the quadratic term (Norry et al. 1999). Stabilizing selection gradient (γ) measures the forces of stabilizing selection acting directly on the characters, independent of the forces of directional selection, and accounting for the phenotypic correlations between characters. The γ value was estimated by the coefficient of the quadratic term from a multiple regression, using the full model of squared and cross-product terms as the predictor variables (Lande & Arnold 1983). The above mentioned PHREG procedure was used for this analysis.

I also analysed the relationships between female abdominal thickness, width and the number of mature eggs that the individual carried at mating using an analysis of regression to evaluate whether the difference in abdominal thickness really reflects the variance in reproductive fitness.

5.4.3 Results

5.4.3.1 Sexual selection on female traits

Directional selection differentials (*s*) analysis shows that body length, ovipositor length, abdominal width and abdominal thickness were significantly associated with mating success (Table 5.2). Further analysis of these traits for stabilizing selection gradient (γ) reveals that only ovipositor length and abdominal thickness and width were significantly selected in females (Table 5.3). None of the linear coefficients of these variations was statistically significant ($P > 0.05$), and hence the β values are not given.

Table 5.1 Correlation (coefficient, *P* value) of morphological traits of females or males selected for multivariate analysis of sexual selection.

Female	Body length	Abdominal width	Abdominal thickness	Ovipositor length
Body length	1			
Abdominal width	0.4310	1		
	<0.0001			
Abdominal thickness	0.2316	0.4234	1	
	0.0018	**<0.0001**		
Ovipositor length	0.2527	0.5976	0.4892	1
	0.0006	**<0.0001**	**<0.0001**	

Male	Fore-tibia length	Fore-tarsus length	High-tibia length	Body length	Antenna length
Fore-tibia length	1				
Fore-tarsus length	0.0373	1			
	0.6191				
High-tibia length	-0.2329	-0.0573	1		
	0.0017	0.4452			
Body length	-0.0818	0.1992	0.2193	1	
	0.2752	**0.0073**	**0.0031**		
Antenna length	-0.0137	0.1666	0.1182	0.4575	1
	0.8557	**0.0254**	0.1140	**<0.0001**	

Table 5.2 Morphological traits in females that succeeded in mating (n = 60) and those that failed (n = 120).

Trait	Succeeded	Failed	*s*	χ^2	*P*
Wing length (mm)	3.3033±0.0047	3.3050±0.0037	-0.037	0.061	0.8036
Hind tibia length (mm)	0.8316±0.0022	0.8335±0.0016	-0.092	0.394	0.5301
Hind tarsus length (mm)	0.8586±0.0042	0.8590±0.0030	-0.010	0.004	0.9472
Body length (mm)	2.8525±0.0071	2.7912±0.0073	1.16	21.877	0.0001
Abdominal width (mm)	0.2236±0.0015	0.1971±0.0014	1.74	31.041	0.0001
Abdominal thickness (mm)	0.6431±0.0025	0.5961±0.0019	2.66	20.110	0.0001
Antenna length (mm)	0.3332±0.0012	0.3321±0.0009	0.09	0.434	0.5100
Ovipositor length (mm)	0.2949±0.0013	0.2670±0.0012	1.56	37.095	0.0001

Table 5.3 Multiple regression of female morphological traits in relation to mating success.

Trait	γ	χ^2	*P*
Body length (mm)	-0.0396	0.0363	0.8489
Abdominal width (mm)	0.8894	5.6761	0.0172
Abdominal thickness (mm)	1.5284	11.5775	0.0007
Ovipositor length (mm)	1.1510	10.0407	0.0015

The regression analysis reveals that female abdominal thickness and width were significantly positively correlated with the number of mature eggs that the individual carried at the time of mating (Figs. 5.9 & 5.10).

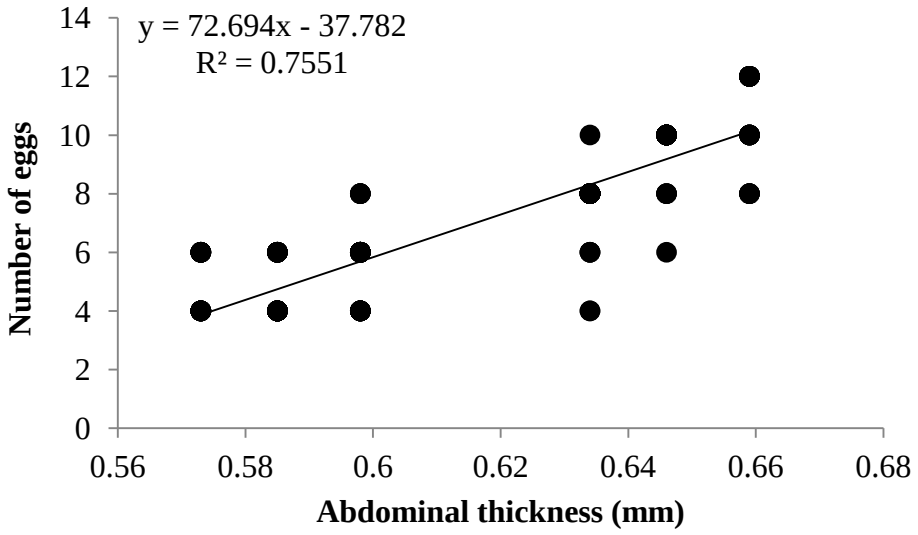

Fig. 5.9 Relationship between the abdominal thickness and number of mature eggs at the time of mating in female *S. flava*.

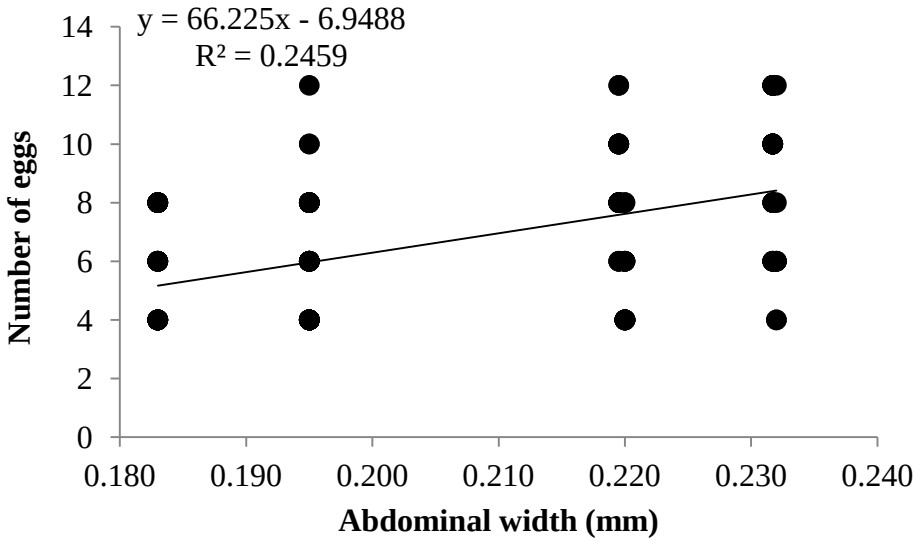

Fig. 5.10 Relationship between the abdominal width and number of mature eggs at the time of mating in female *S. flava*.

5.4.3.2 Sexual selection on male traits

Directional selection differentials (*s*) analysis indicates that fore tibial and tarsal length, hind tibial length, body length and antennal length were significantly associated with mating success (Table 5.4). Further analysis of these traits for directional selection gradient (β) reveals that only fore tarsus and antennal length were significantly directly selected in males (Table 5.5). None of the quadratic coefficients of these variations was statistically significant ($P > 0.05$), and hence the γ values are not given.

Table 5.4 Morphological traits in males that succeeded in mating (n = 60) and those that failed (n = 120).

Trait	Succeeded	Failed	*s*	χ^2	*P*
Wing length (mm)	2.8942±0.0053	2.8399±0.0306	1.71	2.68	0.1016
Fore tibial length (mm)	0.5869±0.0019	0.5929±0.0012	-0.38	6.05	0.0139
Fore tarsus length (mm)	0.6613±0.0015	0.6483±0.0012	1.19	24.76	0.0001
Hind tibial length (mm)	0.7309±0.0016	0.7221±0.0013	0.62	12.85	0.0003
Hind tarsus length (mm)	0.7729±0.0020	0.7717±0.0013	0.07	0.28	0.5963
Body length (mm)	2.4367±0.0070	2.3708±0.0028	1.44	32.21	0.0001
Antenna length (mm)	0.3418±0.0035	0.2833±0.0031	1.73	31.90	0.0001

Table 5.5 Multiple regression of male morphological traits in relation to mating success.

Trait	β	χ^2	*P*
Fore tibia length (mm)	-0.4033	1.1527	0.2830
Fore tarsus length (mm)	0.7975	4.5804	0.0323
Hind tibia length (mm)	0.2516	0.2437	0.6216
Body length (mm)	0.7507	1.6927	0.1932
Antenna length (mm)	1.8310	6.5598	0.0104

5.4.4 Discussion

Mating success in drosophilids is influenced by various factors such as larval food, adult age and pre-copulatory confinement conditions (Noor 1997; Markow et al. 1978; Long et al. 1980; Markow & Sawka 1992). Therefore, during current study I used individuals of the same age and maintained under the same feeding, environmental and pre-copulatory confinement conditions to minimize the variances caused by these factors.

Body size of many drosophilid females is correlated with mating success (Sisodia & Singh 2001), and their fecundity usually increases with body size (Lefranc & Bundgaard 2000; Byrne & Rice 2006). My results show that for females only the variances in abdominal thickness and width, and ovipositor length were sexually selected. Female abdominal size is positively correlated with their reproductive output in the seed bug *Nysius huttoni* (Yang & Wang 2004), the moth *Helicoverpa armigera* (Li et al. 2005), the fly *Rhampholyia longicauda* (Bussiere et al. 2008), and the moth *Ephestia kuehniella* (Xu & Wang 2010), and thus males of these species prefer to mate with females having larger abdomen. In *S. flava* females' abdominal thickness and width were also positively correlated with the number of mature eggs these females carried at the time of mating. These results suggest that males may choose their mates on the basis of immediate reproductive benefit (fertilizing mature eggs in females at the time of mating). Ovipositor length is another sexually selected female trait in this species. These females not only use their ovipositor for oviposition in host leaves but also for creating feeding punctures in the leaves. Therefore, males choosing females with longer ovipositors may benefit from higher survival of their offspring, as ovipositor length may affect egg deposition in the leaf and have impact on offspring survival (Bradford et al. 1993). Furthermore, *S. flava* males cannot create punctures in host leaves and they feed on sap exuding from the punctures created by the females.

Therefore, females with longer ovipositors could possibly create deeper feeding punctures, which may result in more cell sap for males to feed on.

The present study indicates that only longer fore tarsus and antennae were sexually selected in *S. flava* males. These males grasp the female thorax with their forelegs during mating, and tap female body with their fore tarsi during courtship, and also use their fore tarsi to disrupt the mating couple (Sections 5.2.3.1 & 5.2.3.4). *Scaptomyza flava* males may also use their fore tarsi to assess female reproductive fitness by touching her abdomen with their fore tarsi, as reported in *Hoplothrips karnyi* (Hood) (Thysanoptera: Thripidae) (Crespi 1988). Antennae are important olfactory organs in insects (Keil 1999), and antennal length has been associated with mating success in *N. huttoni* (Yang & Wang 2004) and *Phoracantha semipunctata* (Fabricius) (Coleoptera: Cerambycidae) (Hanks et al. 1996). Males with longer antennae in these species can better locate the females for mating and court them at greater rate, which contributes towards their mating success (Hanks et al. 1996; Yang & Wang 2004).

In conclusion, *S. flava* female abdominal thickness and width, and ovipositor length; and male fore tarsal and antennal length contribute towards their mating success and are subject to sexual selection. Furthermore, variation in *S. flava* female abdominal thickness and width appear to indicate the difference in their reproductive fitness. These results provide support to conceptual framework of sexual selection that incorporates the processes leading to the evolution of sexually selected traits in both sexes. The information generated through this study is also helpful for the management of this pest, as the mass-reared sterile males having desired traits may be released for successful mating with wild females (Boake et al. 1996).

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.1 Introduction

The paucity of knowledge on *S. flava* prior to this study provided me an opportunity to explore for the first time its general biology, and feeding, oviposition and mating behaviour, providing an insight into the life history of this species. In this chapter, I summarise and discuss my main findings and their relevance to the development of management strategies and mass-rearing techniques for this pest.

6.2 General biology

My study demonstrates that adult emergence, mating, feeding and oviposition activities are rhythmic in *S. flava*. Maximum adult emergence occurs in the early morning. The emergence during this period is beneficial for group activities such as feeding and mating (Jonušatte & Buda 2002). Such information is important for experiment conduction when a large number of insects of the same age are required (Jonušatte & Buda 2002). Most matings of this species occur soon after lights are on, suggesting that visual cues are important for their courtship and mating. Feeding and oviposition activities also mainly take place during the first few hours of the photophase. These rhythmic activity patterns suggest that the early hours of the day are the optimal time for scouting *S. flava* population (Lam 2007) and probably application of insecticides.

Scaptomyza flava grows and develops significantly faster with the increase in temperature, which may be attributed to increased metabolism and growth rate at higher temperatures (Tomberlin et al. 2009). Pupal stage is more sensitive to temperature than the egg and larval stages with significantly fewer adults emerging at 15° and 30°C. Leibee (1984) suggests that the differential sensitivity to temperature between eggs/larvae and pupae is due to the fact that the former live inside the plants where temperature is more stable.

Optimal temperature leads to optimal metabolic rate and higher fecundity (Luciano et al. 2003). The maximum fecundity in *S. flava* occurs at 20°C and 25°C as

compared to that in the tropical leafminer species *L. sativae* which occurs at 28°C to 31°C (Zhang et al. 2000). This finding has two implications: (1) laboratory colonies of *S. flava* for research or natural enemy food should be maintained be at 20 - 25°C and (2) pest management measures should be considered when the optimal temperature occurs. The significant decrease in fecundity at 30°C suggests that *S. flava* population growth may be checked when environmental temperature reaches this high.

Information about insect diet may help simplify the rearing technique and reduce the cost of production, which is desirable for mass production of the insect pests as food for the natural enemies (Grenier 2009). The present study reveals that *S. flava* adults have similar longevity and fecundity when provided with host plant or host plant + honey solution, indicating that provision of honey is not necessary for their cost effective rearing for research and biological control programmes. Post emergence host plant deprivation significantly reduces adult longevity in *S. flava*. This finding suggests that crop rotation may be practiced for managing the population of this pest (Chen et al. 2009).

6.3 Feeding and oviposition

Optimal oviposition theory predicts that females select oviposition sites which are the best for the development of their offspring (Jaenike 1978). My study indicates that *S. flava* females prefer to feed and oviposit on mature leaves where they perform better while their offspring's performance is similar in both young and mature leaves. This suggests that adult rather than offspring performance is shaping host preference pattern for oviposition in this species, supporting the optimal foraging theory which predicts that females prefer to feed and oviposit on hosts best satisfying their own nutrition requirements (Price et al. 1999; Scheirs et al. 2001).

The leaf nutritional values in *Brassica* plants vary with leaf age (Emden & Bashford 1969; Awmack & Leather 2002; Koukounaras et al. 2007). Preference for mature leaves by *S. flava* adults may be attributed to the fact that mature leaves have higher nutritional values such as more sugars for adult feeding (Bernays & Chapman 1994; Merritt 1996) while emerging young leaves are rich in defence chemicals (Lambdon et al. 2003). The lack of difference in larval performance within leaves of different ages may be explained as (1) leafminer larvae can manipulate host plant

physiology towards their advantage (Yamazaki 2010; Whiteman et al. 2011) and (2) *S. flava* larvae can migrate between leaves to maximise their nutritional needs.

Although water-stressed plants may have higher nutritional values (Brodbeck & Strong 1987; White 1993), decreased water contents adversely affect the performance of phytophagous insects (Bultman & Faeth 1987; Hale et al. 2003; Johnson et al. 2011). In the present study I show that *S. flava* females prefer vigorous to water-stressed plants for feeding and oviposition and their offspring have higher survival rate and shorter developmental time in vigorous plants, supporting both preference and performance propositions of plant vigour hypothesis. It is thus suggested that to obtain flies of high quality and quantity for research and biological control programmes, vigorous plants should be provided to the adult flies for feeding and oviposition.

In the present study I demonstrate that the total number of leaves, total leaf area, and fresh and dry weights of the Chinese cabbage decrease with the increase of *S. flava* larval density. Many previous studies also suggest that higher larval density results in increased plant damage (Van Steenwyk & Toscano 1981; Schooler & McEvoy 2006; Weed & Casagrande 2010) and reduced yield (Showers et al. 1983).

6.4 Mating behaviour

In *Scaptomyza flava* males approach the females and then perform courtship display, after which time they mount and mate with the females. Their mating behaviour is different from *Drosophila pegasa* where copulation occurs without courtship display (Pruett-Jones et al. 2002). After copulation males vibrate their wings, making zigzag movements around females. Whether such post-copulation display by these males serves as post-insemination mate guarding as reported in other insects (Allen et al. 1994; King & Fischer 2005) remains unknown.

Females release sex pheromones to attract males (Antony & Jallon 1982; Tompkins et al. 1983; Oguma et al. 1992; Wicker-Thomas 2007; Dickson 2008) in many species of Drosophilidae. Although the sex pheromone of *S. flava* has not been identified, males' approaching and courting females in this species may be stimulated by a female sex pheromone. Females of this species can dislodge male suitors by kicking them, suggesting that females' acceptance is necessary for copulation in this species.

Scaptomyza flava males reared in groups perform worse in mating behaviour than those reared in isolation. This may be attributed to fatigue or the inhibitory compounds of other males (Noor 1997). This finding suggests that the mass reared males to be used in sterile insect technique should be kept in isolation before release.

Males that have successful mating experience respond to females more quickly than those that have failed mating experience, suggesting that male mating ability is influenced by previous experience and learning (Greenspan & Ferveur 2000). Furthermore, when compared to virgin males, mated males are significantly more likely to display courtship behaviour and achieve mating success. This suggests that previous courting experience refines male courtship behaviour in a way that could increase their mating success

Males' persistent courtship and attempts to copulate with reluctant females (Clutton-Brock & Parker 1995) impose costs to females (Sakurai & Kasuya 2008), such as reduced feeding time (Rowe et al. 1996), and disturbance during oviposition (McLain & Pratt 1999). My study indicates that *S. flava* females housed with higher density of males face more courtship and mating events, and produce fewer feeding punctures than those housed with one male, suggesting that persistent courtship and mating attempts by males may interfere with female feeding activity. Furthermore, females housed with higher density of males also have shorter longevity, suggesting that repeated courtship and resultant decreased feeding may be the reasons for their shorter longevity.

Mating success and fecundity of many drosophilid females is correlated with their body size (Sisodia & Singh 2001; Lefranc & Bundgaard 2000; Byrne & Rice 2006). For *S. flava* females, the variances in abdominal thickness and width are sexually selected, and are also positively correlated with the number of mature eggs these females carry at the time of mating. My findings suggest that *S. flava* males may choose their mates on the basis of immediate reproductive benefit (fertilizing mature eggs in females at the time of mating). Ovipositor length is another sexually selected female trait in this species. These females use their ovipositor for oviposition and making feeding punctures in host leaves. Therefore, males choosing females with longer ovipositors may benefit from more food and higher survival of their offspring, as ovipositor length may affect feeding puncture depth and egg deposition (Bradford et al. 1993).

In *S. flava* males, longer fore tarsus and antennae are sexually selected. These males grasp the female thorax with their forelegs during mating, and tap female body with their fore tarsi during courtship, and also use their fore tarsi to disrupt the mating couple. Antennae are important olfactory organs in insects (Keil 1999), and antennal length has also been associated with mating success in *N. huttoni* (Yang & Wang 2004) and *Phoracantha semipunctata* (Fabricius) (Coleoptera: Cerambycidae) (Hanks et al. 1996). Males with longer antennae in these species can better locate the females for mating and court them at greater rate, which contributes towards their mating success (Hanks et al. 1996; Yang & Wang 2004). Information on sexually selected traits is useful for the management of this pest, as the mass-reared sterile males having desired traits may be released for successful mating with wild females (Boake et al. 1996).

6.5 Future studies

In this thesis, I reported and discussed my main findings on the general biology, and feeding, oviposition and mating behaviour in *S. flava*. This work has provided a much firmer basis of knowledge of this pest that existed hitherto. Such knowledge is vital, as noted in the thesis, to appraising prospects for further investigation on feeding, oviposition and mating behaviour and integrated management of this increasingly important pest.

However, this study is still far from completion. Future research could focus on further understanding of biological features of this pest and developing measures for its management. I hereby recommend the following future studies:

- Impact of varying thermal regimes on life history;
- Feeding and oviposition preference on different host plants;
- Potential damage levels on major vegetable and seed crops in New Zealand;
- Identification of sex pheromones
- Biological control potential of *A. persimilis* in the North Island of New Zealand, and
- Development of an artificial diet for *S. flava*.

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