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**BIONOMICS AND HOST RANGE OF  
AMERICAN SERPENTINE LEAF MINER**

*Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera)

By

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**THESIS**

*Submitted in partial fulfilment of the  
requirement for the degree of*

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**Department of Agricultural Entomology**

**COLLEGE OF HORTICULTURE**

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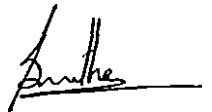
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I hereby declare that the thesis entitled “**Bionomics and host range of American serpentine leaf miner *Lirionmyza trifolii* (Burgess) (Agromyzidae: Diptera)**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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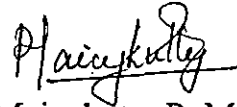
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## CERTIFICATE

Certified that the thesis entitled “**Bionomics and host range of American serpentine leaf miner *Lirionomyza trifolii* (Burgess) (Agromyzidae: Diptera)**” is a record of research work done independently by Ms. Smitha M.K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.



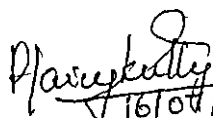
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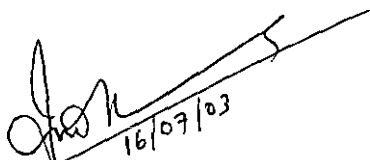
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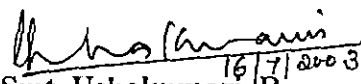
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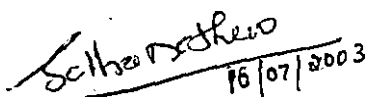
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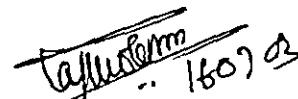
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*In the loving memories of my beloved  
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# *INTRODUCTION*

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## 1. INTRODUCTION

India is the second largest producer of vegetables in the world, next to China, having a production of 87.5 million tonnes from 5.86 million hectares. However, this production is very meagre, considering our requirement. As the scope for increasing the area under vegetable crops is limited, the only way to increase the vegetable production is by improving the productivity of available land. One of the most important constraints felt by the vegetable growers is the menace caused by various insect pests. Insects and plants have co-existed for nearly 400 million years. With the origin of cultivation of plants, insect damage to crops became unacceptable. The intensification of agriculture through the expansion of irrigation facilities, introduction of high yielding varieties and application of increased amounts of agrochemicals during the last three decades have also increased the losses due to insect pests.

The insect pests causing yield reduction in vegetable crops include not only the indigenous pests but also the exotic ones. In earlier days, there were no restrictions on the transport of plants and animals from one country to another. Lack of proper quarantine measures resulted in the introduction of pests from other countries. Cottony cushion scale, wooly aphis and sanjose scale of apple, golden cyst nematode of potato, berry borer of coffee, boll weevil of cotton and spiralling white fly of several crops are some of the exotic pests introduced accidentally to our country. American serpentine leaf miner, *Liriomyza trifolii* (Burgess) is one of the pest introduced in India during 1990's. Now this pest has attained a major pest status.

The serpentine leaf miner is a minute agromyzid fly, which is expanding its distribution throughout the world because of expanded traffic in flower crops. This polyphagous leaf miner is a native of Florida in United States of America and the Caribbean islands (Spencer, 1973) and has become almost cosmopolitan. There are 23 economically important species of *Liriomyza* causing damage to agricultural and ornamental plants by its leaf mining activity. Among these the most serious threat to the world agriculture is *L. trifolii* which is also known as chrysanthemum leaf miner (Poe and Montz, 1982).

This fly possesses considerable economic importance due to the diversity of feeding habits and their role as carriers of disease causing microorganisms. *L. trifolii*

is a serious pest of chrysanthemum since last three decades (Parrella, 1987). The combination of wide host range and high potential for development of resistance to commonly used pesticides makes *L. trifolii* a serious pest of many vegetables and ornamental crops.

This minute fly was suspected to have been introduced to India during 1990-91. It causes severe damage to cotton castor, sunflower, mung bean, vegetables, ornamentals and other crops in Karnataka, Andhra Pradesh, Maharashtra, Gujarat and Delhi (Viraktamath *et al.*, 1993) and nearly 79 species of crop plants were reported as the hosts (Srinivasan *et al.*, 1995) from these areas. The incidence of leaf miner was severe on dicotyledonous plants up to 30 to 45 days.

The mines of the larvae and feeding punctures by the adult flies often lead to severe defoliation of crops. As a vector also it attained serious pest status on most of the vegetable crops especially tomato and cucurbits (Jeyakumar and Uthamasamy, 1998). Pest surveillance studies conducted in Kerala under Kerala Horticultural Development Programme (1998) recorded severe leaf miner incidence on cowpea, ash gourd, bitter gourd and tomato.

Severe damage by the leaf miner will obviously reduce the assimilation by the plant and can lead to desiccation and premature falling of leaves. This type of severe infestation will also reduce the crop vigour or will cause complete destruction of young seedlings. Heavy infestation of *L. trifolii* on ornamental plants not only reduces the vigour but also affects the market value of such ornamental plants. For the better management of *L. trifolii*, it is of utmost importance to make a detailed study on the biology, ecology, host plants, seasonal incidence and natural enemies of *L. trifolii* in Kerala.

Considering the importance of this pest and the severe damage it caused in various vegetable crops of Kerala, the present studies were undertaken with the following objectives

- to study the biology of American serpentine leaf miner on cowpea
- to study the morphometrics of *L. trifolii*
- to study the seasonal incidence in relation to different weather parameters
- to survey for host plants of *L. trifolii*
- to ascertain the natural enemies associated with the leaf miner in Kerala.

# *Review of Literature*

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## 2. REVIEW OF LITERATURE

The American serpentine leaf miner, *Liriomyza trifolii* (Burgess) is a native to Florida in Southern United States and the Caribbean islands (Spencer, 1973). It was reported for the first time from India in castor at Hyderabad in 1991 (Directorate of Oil Seed Research, 1991). This pest was accidentally introduced to India along with the planting materials. Viraktamath *et al.* (1993) reported its incidence in the states of Gujarat, Maharashtra, Karnataka, Andhra Pradesh and Delhi. Srinivasan *et al.* (1995) gave an exhaustive list of 79 host plants of *L. trifolii* based on their survey in Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu. Reghunath and Gokulapalan (1996) reported severe infestation of *L. trifolii* on cowpea in Kerala. Pest surveillance studies in Kerala showed the infestation of *L. trifolii* on cowpea, cucumber, snake gourd, bitter gourd, coccinia and bhindi (KHDP, 1998). The incidence of *L. trifolii* on cucurbits, tomato and cowpea was also reported by Nair (1999).

### 2.1 TAXONOMY AND DISTRIBUTION OF *L. trifolii*

The species "*trifolii*", under the genus *Oscinis* was first described from *Trifolium repens* L., by Burgess in 1880, from United States of America (Spencer, 1973). Later, the species was transferred to the genus *Liriomyza* by de Meijeri in 1925. *L. phaseolunata* (Frost) and *L. alliavora* (Frick) are the synonyms of *L. trifolii* (Frost, 1943; Frick, 1955). It belongs to subfamily Phytomyzinae of family Agromyzidae of Order Diptera.

Till 1970, *L. trifolii* was considered as a major pest of truck crops in Florida. During 1975-76, it got introduced into California (Parrella *et al.*, 1981) and became a major pest of chrysanthemum, tomato, pepper and celery. During the same period the fly got introduced into Kenya probably from Florida, along with chrysanthemum cuttings and from there to European countries (Parrella *et al.*, 1981). Now the pest occurs in Guam (Shreiner *et al.*, 1986), Western Europe, Africa, Mauritius (Trumble, 1981), Taiwan (Wang and Lin, 1988), Korea (Dae, 1996) and India (Lakshminarayana *et al.*, 1992).



Spencer (1973) gave a detailed taxonomic treatment of the polyphagous species of *Liriomyza* including *L. trifolii* and provided a key to distinguish *L. trifolii*, *L. huidobrensis* and *L. sativae*. Electrophoretic studies had been used to distinguish *L. trifolii* from its closely related species of *L. brassicae* and *L. sativae* (Zehnder *et al.*, 1983) and *L. bryoniae* (Menken and Ulenberg, 1983).

## 2.2 NATURE OF DAMAGE

Lediew and Bartlet (1983) conducted studies on the mode of infestation of *L. trifolii*. The female fly made feeding and egg laying punctures on the leaves. These punctures were clearly seen from the upper surface of the leaf as very small bleached spots. The larvae fed inside the leaf making narrow, serpentine mines that appeared whitish when viewed from the upper surface. Thin black trail of excrement were observed on the sides of the leaf mines. Several mines occurred on the same leaf crossing and recrossing often giving the effect of a large blotch mine. Severely infested leaves became shrivelled and dried weakening the growth of the plant.

Parrella (1987) reported the impact of attack by *Liriomyza* leaf miners on crops in six ways (a) by vectoring disease, (b) by destroying young seedlings, (c) by causing reduction in crop yields, (d) by accelerating leaf drop above developing tomatoes, thus causing 'sun burning' of the fruit, (e) by reducing the aesthetic value of ornamental plants and (f) by causing some plant species to be quarantined. These resulted in drastically slowed growth, and reduction in photosynthesis. Other physiological parameters had been measured in vegetable crops but have not been correlated to yield loss. Heavy mining and stippling resulted in the death of young seedlings or transplants. Patil *et al.* (1997) observed that severe infestation of *L. trifolii* in the seedling stage of cotton and castor resulted in reduction in plant height and biomass production.

The leaf miner was found to act as vectors of diseases in crops. Broadbent and Matteoni (1991) observed that both the larvae and adults of *L. trifolii* were able to acquire and transmit *Pseudomonas cichorii*, which caused bacterial leaf spot in chrysanthemum. Laboratory studies conducted in USA by

Chandler and Thomas (1991) showed that the feeding activity of the leaf miner significantly increased the incidence of *Alternaria* leaf blight lesions in musk melon. Kapadia (1995) found that the adults of *L. trifolii* were capable of carrying the spores of the powdery mildew disease of long gourd (*Lagenaria ciceraria*).

### 2.3 BIOLOGY OF *L. trifolii*

Studies on the life history of this species have been made by Spencer (1973). Detailed information on the biology of *L. trifolii* from California was given by Parrella (1987). In India, the biology of this pest was studied by Lakshminarayana *et al.* (1992) at Hyderabad, Viraktamath and Jagannatha (2000) at Bangalore. Nair (1999) provided detailed biology of this pest in Kerala

#### 2.3.1 Egg

Eggs were inserted into the leaf tissues using the ovipositor of the female. Hatching period of egg was 2 days in Florida (Spencer, 1973). Lakshminarayana *et al.* (1992) reported that egg period lasted for 2 to 3 days at Hyderabad. According to Nadagouda *et al.* (1997) the incubation period varied from 2.59 to 3.74 days in Kamataka. Nair (1999) reported that the eggs hatched in 2 to 3 days with a mean value of 2.5 days in Kerala.

The estimated threshold temperature of egg development was 10.0°C on beans and 6.9°C on tomato (Parrella, 1987). Zoebisch *et al.* (1992) studied the difference in the incubation period with respect to temperature. He observed that the mean egg development time was  $1.99 \pm 0.02$  days,  $2.47 \pm 0.15$  days and  $4.08 \pm 0.24$  days at 32°C, 25°C and 20°C respectively. Jeyakumar and Uthamasamy (1996) observed that the number of eggs laid varied with the age of the plant. In cotton, the number of eggs laid on 10 day old plant was 3.4 eggs/leaf and that on 45 day old plant was 1.23 eggs per leaf. Size of the egg varied from 190 to 196  $\mu\text{m}$  in length and 103 to 116  $\mu\text{m}$  in width (Nadagouda *et al.*, 1997).

### 2.3.2 Larva

The larvae began to feed immediately after hatching and continued until it was ready to emerge from the leaf (Parrella, 1987). Different species of *Liriomyza* fed on different sections of the leaf mesophyll. Parrella *et al.* (1985) observed that *L. trifolii* larvae fed mainly on the palisade mesophyll tissues of the leaf.

Parrella (1987) observed four larval instars. The fourth instar occurred between puparium formation and pupation and this was a non feeding stage. So this stage was rarely described by most authors. The fourth instar was also described by Viraktamath and Jagannatha (2000) at Bangalore. According to them, the fourth instar larva came out of the mine and drops to the ground for pupation. The larva completed feeding in 7 to 8 days (Spencer, 1973). The duration of the first, second and third instar larvae was 0.85, 1.23 and 1.42 days respectively on chrysanthemum in Florida (Parrella and Bethke, 1988). Lakshminarayana *et al.* (1992) reported a larval duration of 6 to 9 days at Hyderabad. Jagannatha (1994) recorded a duration of 0.93 to 1.05 days for the first instar, 1.02 to 1.31 days for the second instar and 1.05 to 1.55 days for the third instar on french bean. In Kerala, Nair (1999) reported that the larva became full grown in 7 to 18 days with a mean value of 12.5 days on cowpea. The egg and larval developmental period was 5.4 days on cowpea at Bangalore (Viraktamath and Jagannatha, 2000).

The total larval period varied with the temperature and host plant (Parrella, 1987). The total larval period was  $3.51 \pm 0.06$  days at  $32^{\circ}\text{C}$ ,  $5.02 \pm 0.03$  days at  $25^{\circ}\text{C}$  and  $14.35 \pm 0.06$  days at  $13.9^{\circ}\text{C}$  on tomato (Zoebisch *et al.*, 1992). The estimated threshold temperature for larval development was  $8.9^{\circ}\text{C}$  on *Phaseolus vulgaris* (Saito *et al.*, 1995) and  $10.3^{\circ}\text{C}$  on gerbera (Dae, 1996). In Karnataka, Nadagouda *et al.* (1997) reported that the larval duration varied from 4.05 to 5.02 days at  $25.4^{\circ}\text{C}$  on cowpea.

Tauber and Tauber (1968) observed that the black sclerotised mouth hooks were left within the mine after each moult. These were used to distinguish the duration of the instars as the size of the mouth hook varied in different larval instars (Parrella, 1987). Parrella and Bethke (1988) recorded

the length of mouth hooks of the three instars and it was 0.1 mm, 0.17 mm and 0.27 mm for the first, second and third instar respectively. The studies by Jagannatha (1994), in Bangalore, studied the length of mouth hooks of all the larval instars and observed that it varied from 0.09 to 0.1 mm for the first instar, 0.17 to 0.19 mm for the second instar and 0.26 to 0.30 mm for the third instar.

Jagannatha (1994) reported that on french bean the mine length produced by the first instar larva varied from 15.00 to 20.00 mm and width ranged from 0.24 to 0.28 mm. For the second instar, the mine length and width varied from 35.00 to 45.00 mm and 0.64 to 0.70 mm respectively and that of the third instar was 85.00 to 130.00 mm and 1.68 to 1.84 mm. According to Fagoonee and Toory (1984), the volume of leaf material consumed by the third stage larvae of *L. trifolii* was 643 times greater than that consumed by the first stage larvae and the feeding time was 50 times greater. Both the width of the leaf mine and the rate of leaf mine formation were increased with the development of the larvae.

Parrella (1983) observed an intraspecific competition among the larvae of *L. trifolii*, which affected the survival and size of the larva.

### 2.3.3 Prepupa

After completing the development, the larva which was ready to pupate, cut a semicircular slit on the leaf surface near the end of the mine and fell down to the ground. Occasionally the larvae pupated on leaves or at the base of leaves, stems or stalks (Parrella, 1987 and Lakshminarayana *et al.*, 1992). Parrella (1987) observed that majority of the larvae emerged from the mines during early morning hours, with maximum emergence occurring before 08.00 hours. Prepupal period varied from  $2.67 \pm 0.18$  hours at 35°C to  $4.98 \pm 0.73$  hours at 20°C on celery (Leibee, 1984). Nadagouda *et al.* (1997) recorded a prepupal duration of 62 to 75 minutes on cowpea.

### 2.3.4 Pupa

The pupal period varied from 7 to 11 days in Florida (Spencer, 1973) and 8 to 11 days in California (Parrella, 1987) on chrysanthemum. The same was observed as 6 to 7 days at Hyderabad (Lakshminarayana *et al.*, 1992). The pupal duration ranged from 9.82 to 10.65 days on cowpea in Karnataka (Nadagouda *et al.*, 1997). In Kerala, Nair (1999) reported that the pupal period ranged from 5 to 10 days with a mean value of 7.5 days. Parrella (1987) observed that the pupal duration was also influenced by temperature. It occupied 26.8 days on tomato and 28.33 days on celery at 15°C. Saito *et al.* (1995) worked out the temperature threshold for pupal development as 10.1°C on cowpea. The upper lethal temperature was about 35°C for this stage on cowpea.

Jagannatha (1994) reported that in french bean, the size of the puparia ranged from 1.14 to 2.00 mm in length and 0.68 to 0.92 mm in width. From Karnataka, Nadagouda *et al.* (1997) reported the pupal size as 1.46 to 1.69 mm in length and 0.59 to 0.81 mm in width on cowpea.

### 2.3.5 Adult

#### 2.3.5.1 Behaviour

The process of adult emergence from puparium took five minutes to more than one hour in different species of *Liriomyza* (Oatman and Michelbacher, 1958). Majority of the adults mated soon after emergence. Almost all females mated within 24 hours. Males and females mate more than once and multiple matings were needed for maximum egg production (Parrella *et al.*, 1983).

The females punctured the leaf tissue and fed on the exuding sap. The males fed primarily on natural exudation from the axils of the leaves and also on sap from feeding and oviposition punctures produced by the females (Parrella *et al.*, 1983). Bethke and Parrella (1985) studied the leaf puncturing behaviour on chrysanthemum, the most preferred host and on tomato, the least preferred one. In both the cases, the sequence of leaf puncturing was stereotypic. Once the sequence was initiated two types of leaf punctures were

made, large fan-shaped puncture always without eggs and tubular-punctures with or without eggs. The females fed from both types of punctures, hence all punctures were considered as feeding punctures. The female made fewer, slow abdominal thrusts during oviposition compared to leaf puncturing. The fly fed for a short period and spent less time during the leaf puncturing sequence on tomato compared to chrysanthemum.

The survey conducted by Srinivasan *et al.* (1995) revealed that leaves in the top canopy had fewer mines compared to leaves in middle and bottom canopy. Chin and Chih (1996) found the preference of the female to oviposit and feed on primary leaves. Hammad and Nemer (2000) observed a significantly higher number of larval mines on the lower plant canopy compared to middle and upper canopy of cucumber throughout the cropping season.

The males did not disperse as far away as the females and tend to be slightly aggregated (Jones and Parrella, 1986). Thulasiram *et al.* (1999) reported that the adults of *L. trifolii* were found attracted to baffle type yellow sticky traps on tomatoes in Karnataka.

#### 2.3.5.2 Fecundity

Eggs were laid inside the punctures made on the young leaf. The number of eggs laid by the female during its life varied with the host plant. Fagoonee and Toory (1984) reported that in cowpea only 8 per cent of the leaf punctures contained eggs. Jagannatha (1994) observed that 6.6 to 15.58 per cent of the punctures were used for egg laying in french bean. He also recorded a maximum of 134 to 139 eggs on tomato, 15 to 164 eggs on cotton, 11 to 66 eggs on cucumber and 20 to 47 eggs on okra.

Studies made in California and Florida indicated that the average daily oviposition rate of the female was 38.07 eggs on celery (Leibee, 1984), 100 to 450 eggs on beans (Fagoonee and Toory, 1984) and 17.9 eggs on bell papper (Chandler and Gilstrap, 1987). The daily oviposition rate on *Phaseolus vulgaris* and melon varied from 1.26 to 1.51 respectively in Japan (Ozawa *et al.*, 1999).

Fecundity of the female was influenced by the availability of adult food. Chin and Chih (1996) reported that the egg production strategy was synovigenic and egg size did not increase during development. Studies conducted on tomato showed that the female fly when provided with aphid honey dew laid 78 eggs during its lifetime whereas it laid only 12.4 eggs without access to honey dew (Zoebisch and Schuster, 1987). Minkenberg and Ottenheim (1990) observed a significant increase in feeding and fecundity with increased leaf nitrogen content. Nadagouda *et al.* (1997) worked out the ratio of ovipositional to feeding punctures as 1: 5.37 to 1: 8.55 on different food plants.

The temperature influenced the number of eggs laid by the female (Leibee, 1984). Minkenberg (1988) observed that at 15°C, the fecundity of the female was  $5 \pm 1$  whereas it was  $79 \pm 10$ ,  $64.23 \pm 4.07$  and  $15.63 \pm 2.34$  at 20°C, 25°C and 13.9°C respectively, on tomato.

The oviposition period varied on different host plants. The maximum oviposition period recorded was twelve days on tomato, five days on cotton and three days each on okra, cucumber and cowpea (Jagannatha, 1994). Zoebisch *et al.* (1992) observed the peak oviposition rate on the third day after emergence at 25°C and that at 32°C occurred on the second day after emergence on tomato.

### 2.3.5.3 Longevity

Adult longevity varied with sex and availability of food. Females lived for longer period compared to the males. Spencer (1973) reported that the adults lived for two weeks on caged chrysanthemums in Florida. Parrella (1987) recorded a longevity of 15 to 20 days for females and 10 to 15 days for males in California. Heinz (1996) recorded an average longevity of 9.9 days for male and 14.8 days for female. The longevity recorded at Hyderabad was only 3 to 6 days (Lakshminarayana *et al.*, 1992). Nadagouda *et al.* (1997) observed that the male and female longevity varied from 2.56 to 6.90 days and 4.69 to 9.80 days respectively, on cowpea.

Jagannatha (1994) studied the difference in longevity when the adults were provided with honey solution or water. Adults fed with honey solution

lived for longer period (9.82 to 12.33 days) compared to those fed with water only (1.91 to 2.11 days).

Female longevity was negatively correlated with temperature. Zoebisch *et al.* (1992) reported the longevity of adult female as  $7.5 \pm 0.32$  days at 20°C,  $4.86 \pm 0.21$  days at 25°C and  $3.45 \pm 0.13$  days at 32°C on tomato. When the pupae were exposed to 15 to 30°C the longevity of the adults were reduced at 25°C compared with those at 20°C or 25°C (Suenaga *et al.*, 1999). He also showed that the reduction in longevity of the adults were more at  $\geq 30^\circ\text{C}$  than at 25°C.

#### 2.3.5.4 Sex ratio

Studies regarding the sex ratio of adults emerging from the pupae was indicated as 1:1 or a slight bias in favour of females (Parrella, 1987).

#### 2.3.6 Total life cycle

Spencer (1973) reported that the life cycle of *L. trifolii* was completed in three weeks in Florida. Issa and Marcano (1991) observed that the life cycle took 17 to 74 days at 25.15°C in Venezuela. Viraktamath *et al.* (1993) reported that the total development time was 50 days at 16°C, 39 days at 20°C, 19 days at 26°C and 14 days at 30°C in India. Studies by Chin *et al.* (1996) on *Phaseolus vulgaris* showed that the life cycle took 16.6 days at 25°C in China. The total life cycle ranged from 19.19 to 24.69 days for male and 21.32 to 27.59 days for females in Karnataka (Nadagouda *et al.*, 1997). Ozawa *et al.* (1999) found that the population growth rate of *L. trifolii* was highest at 25°C.

### 2.4 HOST RANGE

The serpentine leaf miner, *L. trifolii* is one of the few truly polyphagous species in the family Agromyzidae. Fifty-nine plants in plant ten families have been recorded as host for this species by Stegmaier (1966) from Florida. The ten families include Compositae (29 spp.), Leguminosae (8 spp.), Solanaceae (7 spp.), Cucurbitaceae (5 spp.), Chenopodiaceae (3 spp.),



Umbelliferae (2 spp.), Zygophyllaceae (2 spp.), Caryophyllaceae (1 sp.), Liliaceae (1 sp.) and Malvaceae (1 sp.). He also reported that this species had preference for the Compositae family. Spencer (1973) reported 18 host plants coming under eight families. These were Solanaceae (4 spp.), Cucurbitaceae (3 spp.), Leguminosae (3 spp.), Chenopodiaceae (2 spp.), Umbelliferae (2 spp.), Liliaceae (2 spp.), Compositae (1 sp.) and Malvaceae (1 sp.). Schuster *et al.* (1991) found that the night shade *Solanum americanum*, spanish needles, *Bidens alba* and Pilewort, *Erechtites hieracifolia* were suitable weed hosts in Florida.

The survey conducted in Andhra Pradesh, Karnataka, Maharashtra, Gujarat and Delhi by Viraktamath *et al.* (1993) revealed its incidence from 55 host plants which included weeds (22 spp.), vegetables (13 spp.), narcotics (6 spp.), pulses (4 spp.), fibre crops (3 spp.), oil seeds (3 spp.), green manure plants (3 spp.) and fodder crops (1 sp.). Survey conducted by Srinivasan *et al.* (1995) for assessing the incidence and severity of the serpentine leaf miner in Andhra Pradesh, Karnataka, Maharashtra and Tamil Nadu revealed its occurrence from 79 host plants. He added 24 new host plants to the list reported by Viraktamath *et al.* (1993). The additional host plants included 14 spp. of vegetables and 10 spp. of ornamentals. These 79 host plants belonged to 16 plant families viz., Leguminosae (17 spp.), Solanaceae (14 spp.), Compositae (14 spp.), Cucurbitaceae (11 spp.), Malvaceae (6 spp.), Amaranthaceae (4 spp.), Chenopodiaceae (3 spp.), Sapindaceae (1 sp.), Tiliaceae (1 sp.), Polygalaceae (1 sp.), Caryophyllaceae (1 sp.), Labiatae (1 sp.), Commelinaceae (1 sp.) and Capparidaceae (1 sp.).

Reghunath and Gokulapalan (1996) reported severe infestation of *L. trifolii* in cowpea, tomato and cucurbits in Kerala. Pest surveillance studies conducted under Kerala Horticulture Development Programme at Kerala Agricultural University showed severe infestation of the leaf miner on cowpea, cucumber, ash gourd, bitter gourd, ivy gourd and pumpkin (KHDP, 1998). The attack of leaf miner was also reported on cowpea, tomato and cucumber (Nair, 1999) and bitter gourd (Reji *et al.*, 2001) in Kerala.

### 2.4.1 Host preference

Host preference studies were conducted by Parrella *et al.* (1983) on chrysanthemum, celery and tomato in California. The mean number of feeding punctures and viable eggs laid per female was 1346 and 298 on chrysanthemum, 986 and 212 on celery and 353 and 35 on tomato respectively. The ratio of oviposition to feeding punctures calculated per female showed that chrysanthemum was the most suitable host followed by celery and tomato.

Srinivasan *et al.* (1995) observed severe incidence of *L. trifolii* on castor (96 mines), cotton (25.9 mines), ridge gourd, (average 22.3 mines), field bean (5.0 mines), potato (4.2 mines), tomato (3.9 mines), okra (3.4 mines). Jagannatha and Viraktamth (1997) found that the female preferred to feed on cucumber and tomato to crops like okra and cotton in a laboratory study at Bangalore. Jeyakumar and Uthamasamy (1998) studied the ovipositional preference of *L. trifolii* among economically important host plants in Coimbatore and found that tomato was the most preferred host. Also french bean, lima bean, bottle gourd and pumpkin were highly preferred by *L. trifolii* (IIHR, 1998).

### 2.5 SEASONAL INCIDENCE

The detailed information about the seasonal incidence of this pest was not available from India. The damage to plant foliage due to larval feeding fluctuated during different stage of the plant. In bell pepper the larval population was highest during the reproductive period of plant growth i.e. from May to June in USA (Chandler and Gilstrap, 1987). Schuster *et al.* (1991) observed a number of weed hosts and volunteer plants acting as alternate hosts of this pest. In cotton and castor, severe incidence was observed during the early stages of the plant (Lakshminarayana *et al.*, 1992). The incidence of leaf miner was severe on dicotyledonous plants upto 30 to 45 days of growth (Viraktamath *et al.*, 1993).

Reports from IIHR (1997) showed its peak incidence during May and September. The pest surveillance studies conducted on vegetables in Kerala

showed that the infestation of *L. trifolii* occurred during the months of October to May with maximum infestation during the months of January, February and March (KHDP, 1998). In Bangalore, *L. trifolii* population showed two major peaks during March to April and August to September on tomato and cucumber, though the population was observed throughout the year (Viraktamath and Jagannatha, 2000).

## 2.6 NATURAL ENEMIES

### 2.6.1 India

Five hymenopteran parasitoids were recorded from India. But no predators were reported so far. Shankar *et al.* (1992) reported a eulophid wasp, *Chrysonotomyia* sp. which parasitised the larvae of *L. trifolii* on cotton in Karnataka. During the survey on the geographical distribution of *L. trifolii*, Srinivasan *et al.* (1995) observed two larval parasitoids i.e., the eulophid, *Hemiptarsenus varicornis* (Girault) and the eucoilid, *Gonotoma* sp.

*Hemiptarsenus varicornis* was a solitary ectoparasitoid. The biology of this parasitoid was studied by Krishnakumar *et al.* (1998) at Bangalore. The parasitised larvae of *L. trifolii* were identified by the presence of pinkish patch towards the posterior end of the host or by the presence of oviposition punctures. There are four larval instars with a total larval period of 4 to 5 days. Pupation was inside the leaf mine. The pupal period was 5 to 6 days. The adult was a small, shiny green coloured insect with 7 to 8 days longevity. The female wasp killed the host by oviposition and host feeding. IIHR (1997) reported maximum parasitisation on the second instar larvae (30 to 32%) while the mean parasitisation observed was 23.95% for the third instar and 9.95% for the first instar larvae. The percentage parasitisation was maximum during July and October in Bangalore.

Men *et al.* (1998) reported the parasitoid *Quadrastichus* sp. from Maharashtra, which checked the population of leaf miner larvae on cowpea.

The field study conducted in Gujarat by Kapadia (1995) also recorded the above mentioned three parasitoids, among which *Chrysonotomyia* sp. was predominant one. A higher rate of parasitism was observed on tomato and cowpea (29.5 to 29.7 per cent). Pawar *et al.* (2001) reported 30 per cent

parasitism by *Chrysonotomyia* sp. on cucumber in Rahuri. He also reported a pteromalid parasitoid namely *Perilampus* sp. causing three per cent parasitisation on cucumber.

## 2.6.2 Other countries

### 2.6.2.1 Predators

Both the larvae and adults were susceptible to predation by a wide variety of general predators, particularly ants. The formicid *Tetramorium caespitum* was found to cause considerable pupal mortality of *L. trifolii* in USA (Keularts and Lindquist, 1989). Arakaki and Okajima (1998) reported the predatory thrips, *Franklinothrips vespiformis* (Aelothripidae: Thysanoptera) from Japan. Both the larvae and adults attacked the larvae of *L. trifolii* on cucumber.

### 2.6.2.2 Parasitoids

Twelve species of parasitoids have been recorded as natural enemies of *L. trifolii* by Stegmaier (1972) from Florida. Eulophids were the predominant group (9 spp.) followed by braconids (2 spp.) and pteromalid (1 sp.). The eulophids were *Chrysocharis* sp., *Closterocerus cinctipennis* Ashmead, *Derostenus agromyzae* Crawford, *Derostenus* sp., *D. variipes* Crawford, *Diglyphus intermedius* (Girault), *D. pulchripes* (Crawford), *Diglyphus* sp., *Mirzagrammosoma lineaticeps* Girault and the braconids were *Opius dimidiatus* (Ashmead) and *Opius* sp. and the pteromalid *Halticoptera patellana* (Dalman). Spencer (1973) also reported all these parasitoids from Hawaii. But the relative importance of the various parasitoids in controlling population of *L. trifolii* was not studied.

Shreiner *et al.* (1986) reported the eulophid parasitoid, *Hemiptarsenus semialbiclavus* (Girault) on yard long beans in Guam. This larval parasitoid was also reported from water melon in Hawaii (Johnson, 1987).

Johnson and Hara (1987) recorded 40 species of hymenopteran parasitoids belonging to four families from the major *Liriomyza* species in North America and Hawaii. Many of these parasitoids belonged to the family

Eulophidae. Of the 19 species found in association with *L. trifolii*, *Diglyphus begini* (Ashmead) and *D. intermedius* were the major parasitoids on celery and chrysanthemum.

The eulophid, *D. begini* was used in inundative biological control programme (Johnson and Hara, 1987). Heinz and Parrella (1990) also recorded this larval parasitoid on chrysanthemum in California. It caused 26.3% parasitisation on tomato in Florida (Schuster and Wharton, 1993).

*Diglyphus intermedius* was an external larval parasitoid of *L. trifolii* (Johnson and Hara, 1987). A maximum of 94.7% parasitism was reported by Parrella *et al.* (1987) on chrysanthemum in California. It preferred to parasitise the third instar larva (Patel and Schuster, 1991). Patel and Schuster (1992) observed hyperparasitism of *D. intermedius* by *Neochrysocharis* sp. Schuster and Wharton (1993) recorded 28.8% parasitisation on tomato in Florida.

Johnson and Hara (1987) observed *Chrysonotomyia* (*Neochrysocharis*) *parksii* (Crawford) from chrysanthemum fields of Florida. It is a larval endoparasitoid of *L. trifolii*. It caused 10.2% parasitisation on water melon in Hawaii (Johnson, 1987).

Another eulophid parasitoid, *Neochrysocharis formosa* Crawford was reported on water melon in Hawaii (Johnson, 1987). Arakaki and Kinjo (1998) reported this parasitoid as the dominant species among the other parasitoids of *L. trifolii* in Japan. *N. formosa* along with *N. pentheus* were found effective for suppressing the *L. trifolii* population on gerbera in the greenhouse of Japan (Ohno *et al.*, 1998).

*Neochrysocharis punctiventris* (Crawford) was reported on leaf miners on water melon in Hawaii. This larval parasitoid caused 45.2% parasitisation during the spring season (Johnson, 1987). Schuster and Wharton (1993) recorded 15.5% parasitisation on tomato in Florida. Other eulophid parasitoids reported by Johnson and Hara (1987) from Hawaii are *Achrysocharella agromyzae* (Crawford), *A. variipes* (Crawford), *Chrysocharis ainsliei* Crawford, *Closterocerus cinctipennis*, *D. pulchripes*, *Mircagrammosoma lineaticeps* and *Zagrammosoma americanum* (Girault).

Johnson and Hara (1987) reported a pupal parasitoid belonging to the family Pteromalidae from Hawaii, namely, *Halticoptera circulus* (Walker). It caused 22.4% parasitisation on water melon in Hawaii (Johnson, 1987).

Parkmen *et al.* (1989) also reported this parasitoid from Florida on castor bean.

Parkmen *et al.* (1989) reported the larval pupal parasitoid, *Opius dimidiatus* (Ashmead) on castor bean from Florida. This solitary braconid parasitoid attacked the leaf miner on the late second instar or third instar larva (Nelson and Roitberg, 1995).

Another braconid parasitoid, *Opius dissitus* (Muesebeck) was reported by Parkmen *et al.* (1989). It was found on castor bean in Florida and caused 37.8% parasitism in chrysanthemum. Schuster and Wharton (1993) reported 51.5% parasitisation on tomato in Florida by this species.

Van der Linden (1990) reported the larval pupal parasitoid, *Chrysocharis oscinidis* (Ashmead) from *L. trifolii* in Netherlands. This was also reported from USA on beans by Kaneshiro and Johnson (1996).

*Diglyphus isaea* (Walker) is a eulophid larval parasitoid commonly used as biocontrol agent of *L. trifolii* of greenhouse tomatoes in Netherlands (Minkenbergh *et al.*, 1991). The adult females caused 100% paralysation of the larvae.

From France, Franco and Panis (1991) reported the larval parasitoid *Epiclerus nomocerus* (Masi) belonging to the family Tetracampidae in tomato greenhouses. Minkenbergh and Lentern (1991) recorded a braconid larval parasitoid *Dacnusa siberica* (Telenga) from Netherlands. This parasitoid was also reported from Japan by Hondo *et al.* (1999).

# *Materials and Methods*

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### 3. MATERIALS AND METHODS

Studies on “Bionomics and host range of American serpentine leaf miner *Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera)” were carried out at College of Horticulture, Kerala Agricultural University (KAU), Thrissur during 2001-2002. The study consisted of field survey to determine the host range, seasonal incidence and intensity of infestation of the leaf miner. Regular surveys were made in the fields of KAU campus, vegetable plots of farmers in the two nearby panchayats viz Nadathara and Pananchery and in a few private nurseries. Biology of the insect was studied in the laboratory of Department of Entomology, College of Horticulture.

#### 3.1 BIOLOGICAL STUDIES ON *L. trifolii*

The biology of *L. trifolii* was studied on cowpea using KAU variety, Lola. Cowpea seedlings were raised individually in small plastic containers (7 cm diameter and 10 cm height) under insect proof condition. Fifteen to twenty day old seedlings of cowpea were used for this study.

##### 3.1.1 Rearing of adults

Infested leaves of cowpea with serpentine mines, containing larvae, were collected from the vegetable plots of Vellanikkara. The leaves were placed in polythene covers with minute perforations to facilitate better aeration. The larvae emerged from the leaf mines pupated within a few hours. The pupae were collected with the help of a camel hair brush and placed in small glass vials covered with muslin cloth. They were kept at room temperature till the adult emergence. The emerged adults were fed with two per cent honey solution and were used for further laboratory studies.

##### 3.1.2 Life cycle

Fifteen to twenty day old cowpea seedlings raised in small plastic pots were used for rearing the flies. Rearing cages were prepared using locked polythene covers of size 22 cm x 28 cm. Two large windows of sizes 6 cm x 10 cm were cut



and replaced by muslin cloth. A single seedling was enclosed in each rearing cage. A pair of freshly emerged adults (one male and one female) were released carefully into this cage for mating and oviposition. Adults were fed with two per cent aqueous solution of honey soaked on cotton. The rearing cage was tightly fixed with the plastic pot using rubber bands and labelled separately (Plate 5). Five replications were maintained. The adults were released usually in the morning hours between 0900 and 1000 hours. Observations on the number of eggs laid, incubation period, number of larval instars, duration of each instar, pupal period and total life cycle were recorded.

The eggs were observed at hourly intervals to record the incubation period. The change in the width and length of mine were observed at intervals of 2 hours to record the developmental time of each instar. Indication about the moult was obtained from the cast off mouth hooks of the larva. The freshly emerged fourth instar larvae which came out of the leaf mines, were collected in glass vials, labelled and kept at room temperature. The date of pupation and adult emergence were recorded to find out the pupal period. Sex ratio was worked out by counting the number of males and females emerged.

### **3.1.3 Fecundity**

A pair of flies (one male and one female) which emerged on the same day, were released on cowpea seedling enclosed in rearing cages for oviposition. The flies were provided with two per cent honey solution on cotton swab. Fresh seedlings were provided at an interval of 24 hours till the female died. Whenever a male of the pair died earlier, it was replaced by another male of uniform age. The number of eggs laid per day and the total number of eggs laid by a female during its life period was recorded.

### **3.1.4 Longevity**

Ten freshly emerged adults of both sexes were kept separately in polythene bottles. These bottles were covered with muslin cloth. Two per cent honey solution was provided for one set of males and females and the second set were fed with cotton soaked in sterile water. A third set was kept as control with out providing any

food. The food was replaced every day and the longevity of the flies in each case was recorded until all flies died.

### **3.1.5 Time of emergence of larvae from the leaf mines**

Heavily infested cowpea leaves were collected from the field. From these leaves, 100 mines containing third instar larvae were separated and divided equally into five lots of 20 each and were kept in polythene bags. Each lot represented a replication. Observations were taken at intervals of two hours starting from 0700 to 2100 hours to record the number of larvae coming out the leaf mines.

### **3.1.6 Morphometrics of various stages**

Measurement on the length and width of the eggs, larvae and larval mouth hooks, pupae and adults were taken, using an ocular micrometer. Ocular micrometer was calibrated with stage micrometer for the different magnifications used.

#### **3.1.6.1 Egg**

Eggs were detected using the staining technique of Parrella and Robb (1982). Cowpea leaves were boiled in lactophenol-acid fuchsin solution for one minute. It was then allowed to cool for 3 to 5 hours. After washing with water, the leaves were placed in petridishes and observed under a dissection microscope. The eggs appeared more darkly stained than the surrounding plant tissues. These were carefully removed from the leaf tissue with a fine needle and placed on a glass slide and measurements were taken.

#### **3.1.6.2 Larva**

The first, second, third and fourth instar larvae were collected and measured the length and width. Measurements of 10 larvae were recorded and average values were worked out in millimetres.

### **3.1.6.3 *Mouth hook***

The mouth hooks of first, second and third instar larvae were carefully recovered from the larval mines and that of the fourth instar larvae was obtained from the pupal case. The length and width of the mouth hook of each instar was measured. For this, 10 larval mines were observed.

### **3.1.6.4 *Larval mine***

Ten larval mines were observed and the length and width of mines produced by first, second and third instar larvae were measured and given in millimetres. The total length of larval mine was also recorded.

### **3.1.6.5 *Pupa***

Length and width of 10 pupae reared from cowpea leaves were measured and size was expressed in millimetres.

### **3.1.6.6 *Adult***

The length and width of ten numbers of one day old flies of both sexes were measured. Length was measured as the distance between the anterior most part of the head to the tip of the abdomen. The greatest width across the mesothorax was recorded as the width of the insect. Length and width are expressed in millimetres

## **3.2 HOST RANGE**

To study the host range, surveys were conducted at fortnightly intervals from June, 2001 to June, 2002 on the various crops raised in the University campus, well managed as well as ignored vegetable plots in farmers fields in two panchayats, namely, Nadathara and Pananchery and few private nurseries. Various host plants showing leaf mines with a trail of black excrement were collected and brought to the laboratory in polythene bags and kept for the emergence of larvae, pupae, adults and parasitoids. The number of mines present on one upper, one middle and one lower

leaf were counted from 10 plants of each host at each time of observation. The common name and scientific name of each host, the date and location of collection etc. were recorded.

### 3.2.1 Intensity of infestation

The intensity of infestation per unit area of the leaf was worked out from leaf area and total mines on that leaf and expressed as mines per cm<sup>2</sup>. It was observed for cowpea, tomato, cucumber, pumpkin, ash gourd and ridge gourd

$$\text{Intensity per unit area} = \frac{\text{Total number of mines}}{\text{Total leaf area}}$$

### 3.3 SEASONAL INCIDENCE

Weather data during the period of survey was noted. Correlation studies were carried out between the intensity of infestation and weather factors. This was used for the determination of seasonal incidence of this pest on various hosts.

### 3.4 NATURAL ENEMIES

Leaves of different host plants, infested with serpentine leaf miner, containing parasitised larvae were placed in finely perforated polythene bags and kept in the laboratory for the emergence of parasitoids. The parasitoids emerged were preserved in 70% ethanol and identified by sending the specimens to experts. Similarly, parasitoids emerged from the pupae were also collected and preserved.

The percentage of parasitisation on *L. trifolii* was worked out for host plants like cowpea, ash gourd, pumpkin and cucumber from September 2001 to April 2002.

$$\text{Percentage parasitisation} = \frac{\text{Number of parasitised larva}}{\text{Total number of larva}} \times 100$$

# *Results*

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## 4. RESULTS

The results of the investigations carried out at College of Horticulture, Vellanikkara during 2001-2002 on the “Bionomics and host range of American serpentine leaf miner *Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera)” are presented here

### 4.1 NATURE OF DAMAGE CAUSED BY *L. trifolii*

The females of *L. trifolii* made series of punctures on the upper surface of the leaf and fed on the exuding plant sap. These punctures were easily located as small white dots when the leaves were held against light (Plate 4). The larvae, immediately after hatching, mined beneath the upper epidermis feeding its way through mesophyll tissue. Initially the mines made by the maggots were very narrow, but gradually, the length and width of the mine increased with increase in size of the larva. The mine was highly contorted, always had a trail of black excrement (Plate 5). Later the mines turned whitish. Under severe infestation, the mines of different larvae criss crossed and the affected leaf turned papery white (Plate 6D). The leaves of severely infested seedlings showed burnt appearance. Severe reduction in photosynthesis adversely affected the vigour of the plant. Later on, these mines got dried up resulting in the immature shedding of leaves. Seedling stage of the plant was found more vulnerable to the leaf miner attack.

### 4.2 BIOLOGY

The biology of *L. trifolii* was studied on cowpea variety Lola in the laboratory during the period from 28-1-2002 to 25-4-2002 and the following observations were recorded.

#### 4.2.1 Egg:

Eggs were elongate, oval, translucent and milky white, measuring 0.2 to 0.24mm (average =  $0.21 \pm 0.017$  mm) in length and 0.12 mm in width (Table 2). Eggs were laid singly in ovipositional punctures made by the female on the leaf

Table 1. The length of larvae, larval mines and mouth hooks in different larval instars of *L. trifolii*

Details of measurement		First instar		Second instar		Third instar		Fourth instar	
		Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	width (mm)	Length(mm)	Width(mm)
a) Larva	Maximum	0.35	0.15	1.34	0.34	1.82	0.52	2.51	0.69
	Minimum	0.25	0.1	0.67	0.22	1.26	0.42	2.17	0.61
	Average	0.31 ± 0.03	0.13 ± 0.17	0.94 ± 0.20	0.18 ± 0.02	1.56 ± 0.15	0.46 ± 0.03	2.35 ± 0.13	0.64 ± 0.02
b) Larval mine	Maximum	15.0	0.25	35.0	0.44	57.0	1.84	-	-
	Minimum	8.0	0.13	16.0	0.24	25.0	1.23	-	-
	Average	12.2 ± 0.24	0.18 ± 0.02	25.9 ± 0.57	0.34 ± 0.06	40.3 ± 0.95	1.72 ± 0.09	-	-
c) Mouth hook	Maximum	0.11	0.04	0.20	0.06	0.27	0.1	0.28	0.09
	Minimum	0.08	0.03	0.14	0.05	0.23	0.06	0.23	0.07
	Average	0.09 ± 0.01	0.03 ± 0.003	0.17 ± 0.01	0.05 ± 0.005	0.25 ± 0.01	0.07 ± 0.01	0.25 ± 0.02	0.08 ± 0.02

**Table 2. Measurement of different stages of *L. trifolii* on cowpea**

Stage	Length (mm)	Width (mm)
1. Egg	0.21±0.017 (0.20 to 0.24)	0.12±0.004 (0.1 to 0.13)
2. Larva	0.31±0.03 (0.25 to 0.35)	0.13±0.017 (0.10 to 0.15)
a) First instar	0.94±0.20 (0.67 to 1.34)	0.26±0.04 (0.22 to 0.34)
b) Second instar	1.56±0.15 (1.26 to 1.82)	0.46±0.03 (0.42 to 0.52)
c) Third instar	2.35±0.13 (2.17 to 2.51)	0.64±0.02 (0.61 to 0.69)
d) Fourth instar	1.66±0.12 (1.46 to 1.78)	0.76±0.09 (0.63 to 0.87)
3. Pupa	1.49±0.07 (1.34 to 1.57)	0.50±0.09 (0.42 to 0.56)
4. Adult	1.70±0.08 (1.6 to 1.82)	0.59±0.03 (0.51 to 0.62)
a) Male		
b) Female		

**Table 3. Duration of different stages of *L. trifolii***

Stage		Duration (days)		
		Maximum	Minimum	Average
1. Egg		3.00	1.50	2.08
2. Larval instar	First instar	1.00	0.98	0.90
	Second instar	1.50	1.00	1.15
	Third instar	1.70	1.20	1.31
	Fourth instar	0.11	0.08	0.10
3. Pupa		8.50	6.80	7.9
4. Adult	Male	5.00	3.00	4.50
	Female	8.50	5.50	7.40



surface (Plate 1A). The incubation period ranged from 1.5 to 3 days (average=2.08 days) (Table 3).

#### 4.2.2 Larva

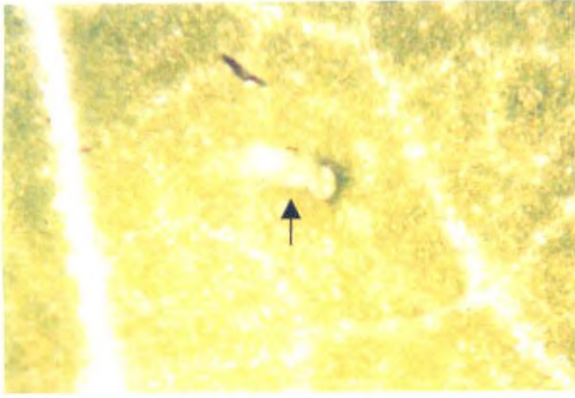
The larva started feeding immediately after eclosion and fed incessantly until it was ready to emerge from the leaf. The larva was cylindrical and apodous. The anterior end was tapered and posterior end was truncated. The larvae moved via peristaltic action of their hydrostatic skeleton. There were four larval instars. Early fourth instar larva, immediately after moulting emerged from the mine and fell to the ground for pupa formation and hence not much studied. The black cephalopharyngeal skeleton (mouth hook) left with in the mine after each moult helped to distinguish the various instars (Plate 2). The size of the mouth hooks was different in different larval instars.

##### 4.2.2.1 *First instar*

Majority of the first instar larvae hatched out during early morning hours from 0600 to 1000 hours. The larvae immediately after hatching, started feeding on the mesophyll tissues and formed the mines. The mines had greenish white appearance. The larva was transparent, greenish-white and minute, measuring 0.25 to 0.35 mm in length and 0.1 to 0.15 mm in width, with minute black mouth hook, which could be seen rapidly scraping the mesophyll region under a microscope (Plate 1B). The length of the mine formed by this instar varied from 8.00 to 15.00 mm and width ranged from 0.13 to 0.25 mm. The mouth hook measured about 0.08 to 0.11 mm in length. The duration of first instar larva varied from 0.98 to 1.00 days (average = 0.90 days) (Table 1).

##### 4.2.2.2 *Second instar*

The second instar larvae were light yellow in colour with size varying from 0.67 to 1.34 mm in length and 0.22 to 0.34 mm in width (Plate 1C). The mouth hook measured from 0.14 to 0.20 mm in length and 0.05 to 0.06 mm in width. The leaf mine produced by this instar varied from 16.00 to 35.00 mm in length



I.A



I.B



I.C



I.D

**Plate 1. Developmental stages of *L. trifolii***

I.A - Egg

I.B - First instar larva

I.C - Second instar larva

I.D - Third instar larva

and 0.24 to 0.44 mm in width (Table 2). The duration of this instar was 1 to 1.5 days (average = 1.15 days) (Table 3).

#### **4.2.2.3 *Third instar***

The third instar larva was golden yellow and its presence could be made out by a slight bulging at the end of the mines (Plate 1D). It fed voraciously on the leaves. The length and width of the mines produced by this instar ranged from 25.00 to 57.00 mm and 1.23 to 1.82 mm respectively. The full grown larvae measured about 1.26 to 1.82 mm in length and 0.42 to 0.52 mm in width. The mouth hook measured 0.23 to 0.27 mm in length and 0.06 to 0.1 mm in width. When the larva was ready to pupate, it cut a semicircular slit on the leaf surface usually, at or near the end of the mine. The width of the mine during that stage varied from 1.5 to 2.0 mm in cowpea. The larvae emerged with characteristic peristaltic motion and fell to the ground for pupation. Larvae occasionally pupated on the leaves or at the base of the leaves, stems or stalks. More than 75 per cent of the larvae emerged from the leaves during the morning hours from 0800 to 1000 hours. The duration of this instar ranged from 0.8 to 1.5 days (average = 1.26 days).

#### **4.2.2.4 *Fourth instar***

The fourth instar larva was also golden yellow coloured (Plate 1E). The larvae measured about 2.35 mm in length and 0.64 mm in width. The moulting occurred immediately after the emergence of the third instar larvae from the mine and puparium formation. The duration of this stage was ranged from 2.0 to 3.5 hours. The mouth hook was seen inside the puparium. The size of the mouth hook was 0.25 mm in length and 0.08 mm in width respectively.

#### **4.2.2.5 *Total larval period***

The total larval period ranged from 2.9 to 4.1 days. Total mine length varied from 46 mm to 94 mm.



### 4.2.3 Pupa

The freshly formed pupa was golden yellow in colour which later turned dark brown. Posterior part of the pupa is narrowed and cylindrical with a pair of lateral cuticular processes (Plate 1F). Majority of the larvae pupated in soil. After the adult emergence, the empty puparium became light amber coloured, rather brittle structure, with a torn out dorsal lid-like part left hung open at the anterior end. The pupae measured about 1.46 to 1.78 mm in length and 0.63 to 0.87 mm in width. The pupal period ranged from 6.8 to 8.5 days (average =  $7.91 \pm 0.41$ ) (Table 3). About 50 per cent of the total developmental time of *L. trifolii* was spent in this stage.

### 4.2.4 Adult

Adult was a small beautifully coloured fly. Head was yellow with dark brown eyes (Plate 1G). The ocellar triangle and back of the head were black. Mesonotum was blackish brown with lateral margins, pleura and halteres yellow. Scutellum was yellow. The spines on the thorax were black. Legs were yellow with tibia and tarsi fuscous. Wings were transparent with a brownish hue. Abdominal terga were brownish with the intersegmental area paler. The abdominal sternum was yellowish. The male genital capsule was blackish and the female ovipositor was black.

The newly emerged fly was grey coloured and the full compliment of colour developed in one to two hours after emergence. The female was comparatively larger than males, measuring 1.70 mm length and 0.59 mm width. The male had 1.49 mm length and 0.5 mm width (Table 2). Adult females emerged from comparatively larger puparia than the males. Males emerged from the puparia before the females. The female had prominent, black and pointed ovipositor at the end of the abdomen whereas the male flies carried a small black spot on the ventral side of last abdominal segment.

The adults emerged during morning hours between 0800 and 1000 hours. The adults started mating immediately after emergence and started egg laying within 24 hours after mating.



1.E



1.F



1.G

**Plate 1. continued..**

1.E - Fourth instar larva

1.F - Pupa

1.G - Adult



Plate 2. Larval mouth hook



Plate 3. Rearing cage

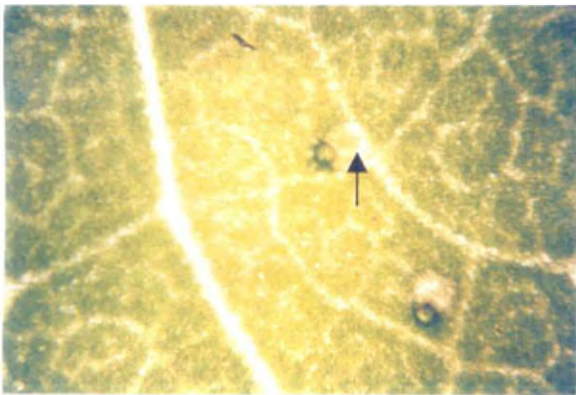


Plate 4. Feeding punctures



Plate 5. Larval mines

#### 4.2.4.1 Feeding

The adult females made oviposition punctures on the leaves by holding the ovipositor perpendicular to the leaf and fed on the exuding sap. The males also used these punctures for feeding. Females laid eggs in these punctures. The proportion of feeding punctures used for egg laying by the female varied from 3.47 to 15.33 (Table 5). The distribution of feeding punctures on the leaf lamina was not uniform. Considerably more number of feeding punctures were found along the margin of the leaf compared to the middle area. The eggs were deposited in tubular leaf punctures. Large fan shaped punctures were also made by the female. Both these punctures were used as feeding punctures by the female. The total number of punctures made by a female varied from 375 to 492.4 (Table 5).

The number of punctures produced by the female increased in the presence of a sugar source. More number of punctures were present on the lower leaves especially on the cotyledons in the case of seedlings. Top most leaves were rarely infested by the leaf miner. But under severe infestation, all the leaves were uniformly attacked. Adults rested on the lower surface of the leaves. They are negatively phototropic.

#### 4.2.4.2 Longevity

Adult longevity varied with the availability and kind of food provided. Adult females survived for an average period of 7.4 days and the males for 4.5 days when honey was provided as food source (2% aqueous solution) (Table 3). When they were provided with water, the males survived for 1.5 days and the female for 2.5 days. When the adults were held in glass vials without food they survived only for one day.

#### 4.2.4.3 Fecundity

The females laid a maximum of 48 to 50 eggs during its life period. The oviposition period was only four days in cowpea. The peak egg laying was noticed on the second day after mating, with an average of 19.6 eggs closely

**Table 4. Emergence pattern of larvae of *L. trifolii* from the leaf mines during different hours of the day**

Time	Total no. of larvae	Number of larvae emerged from the leaves					
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	Mean
0800 hours	20	3	2.5	2.5	4	2.5	2.9±0.58
1000 "	20	6	7.0	9.0	7	7.0	7.2±0.98
1200 "	20	4	4.5	2.0	1	4.0	3.9±1.02
1400 "	20	3	2.5	3.5	1	1.5	2.3±0.93
1600 "	20	2	1.5	0.5	1	1.0	1.2±0.51
1800 "	20	0.5	1.0	1.0	2	0.5	1.0±0.55
2000 "	20	1.5	1.5	0.5	1	1.0	0.9±0.58
2200 "	20	0	0.5	1.0	0.5	0.5	0.7±0.51

R<sub>1</sub> - R<sub>5</sub> = replications

**Table 5. Feeding and oviposition punctures made by the female *L. trifolii*, at different days after mating (Numbers)**

Days after mating	Total no. of punctures / female	No. of punctures with egg	No. of punctures without egg	Per cent of punctures with egg
1	57.75	3.20	54.55	5.79
2	175.80	19.60	157.45	11.21
3	141.20	17.45	123.75	12.35
4	77.27	8.30	68.95	10.74
Total	452.02	48.5	403.52	

(Each value is the average of four replications)



Table 6. Sex ratio of *L. trifolii*

Sl.No.	Total no. of pupae	No. of adults emerged	No. of males	No. of females	Sex ratio (male: female)
1	20	20	9	11	1:1.22
2	20	17	6	11	1:1.83
3	20	18	8	10	1:1.25
4	20	17	7	10	1:1.43
5	20	17	7	10	1:1.43
6	20	14	5	9	1:1.80
7	20	14	7	7	1:1.00
8	20	19	9	10	1:1.11
9	20	18	7	11	1:1.57
10	20	15	6	9	1:1.50
Average	20	-	-	-	1:1.41

followed by the third day. Repeated matings were required by the female for maximum egg production. The average number of eggs laid by the female during different days varied from 3.2 to 19.6 eggs. The details are given in Table 5.

On the first day, the number of eggs laid varied from 2.2 to 5.8 eggs. Only 5.79 per cent of the punctures were used for oviposition. Maximum number of eggs were laid on the second day after mating. It ranged from 15.8 to 22.6. An average of 11.21 per cent of punctures were used for egg laying (Table 5).

The number of eggs laid on the third day was also higher compared to first and fourth day, 15.4 to 21.8 eggs. 12.35 per cent of the punctures were used for oviposition. On the fourth day after mating, the per cent of eggs in feeding punctures was only 10.74. The number of eggs laid varied from six to ten.

#### 4.2.4.4 Sex ratio

Sex ratio was studied from a randomly selected 20 pupae. The sex ratio (male : female) ranged from 1:1 to 1:1.83 (Table 6). More number of females were emerged during December and January months.

### 4.3 HOST RANGE STUDIES

Survey was conducted to find out the host range of *L. trifolii* in vegetable plots of University campus, plots of farmers in two panchayats, namely, Nadathara and Pananchery and a few private nurseries. The incidence and intensity of infestation were recorded during June 2001 to June 2002. In the survey, *L. trifolii* was recorded on 48 species of plants (Table 7) belonging to the families of Cucurbitaceae (10 spp.), Compositae (10 spp.), Leguminosae (9 spp.), Solanaceae (6 spp.), Amaranthaceae (3 spp.), Labiatae (2 spp.), Verbenaceae (2 spp.), Boraginaceae (1 sp.), Capparidaceae (1 sp.), Cariophyllaceae (1 sp.), Euphorbiaceae (1 sp.), Rubiaceae (1 sp.) and Malvaceae (1 sp.). The host plants included vegetables (19 spp.), ornamentals (12 spp.), weeds (10 spp.), pulses (3 spp.), oilseeds (2 spp.) and green manure crops (2 spp.).

The intensity of infestation varied in different plant species. The attack was severe on cowpea (85.9 mines per leaf), ash gourd (85.3 mines per leaf), ridge gourd (72.3 mines per leaf), tomato (62.5 mines per leaf), pumpkin (51.4 mines

Table 7. Host plants and intensity of infestation by *L. trifolii*

Common name	Scientific name	Family	Maximum intensity of infestation (mines/leaf)
<b>a) Vegetables</b>			
1. Cowpea	<i>Vigna unguiculata</i> (L.)	Leguminosae	85.90
2. Ash gourd	<i>Benincasa hispida</i> (Thunb.)	Cucurbitaceae	85.30
3. Ridge gourd	<i>Luffa acutangula</i> (L.) Roxb.	Cucurbitaceae	72.30
4. Tomato	<i>Lycopersicon esculentum</i> Mill	Solanaceae	62.50
5. Pumpkin	<i>Cucurbita moschata</i> D.	Cucurbitaceae	51.40
6. Brinjal	<i>Solanum melongena</i> L.	Solanaceae	44.83
7. Cucumber	<i>Cucumis sativus</i> L.	Cucurbitaceae	32.60
8. Bitter gourd	<i>Momordica charantia</i> L.	Cucurbitaceae	29.50
9. Okra	<i>Abelmoschus esculentus</i> L.	Malvaceae	27.00
10. Peas	<i>Pisum sativum</i> L.	Leguminosae	25.40
11. Snake gourd	<i>Trichosanthes anguina</i> L.	Leguminosae	18.90
12. Coccinia	<i>Coccinia grandis</i> (L.) Voigt	Cucurbitaceae	7.40
13. Water melon	<i>Citrullus vulgaris</i> Schrod	Cucurbitaceae	6.30
14. Sword bean	<i>Canavalia gladiata</i> DC	Leguminosae	4.40
15. Amaranthus	<i>Amaranthus</i> sp. L.	Amaranthaceae	4.30
16. Dolichos bean	<i>Dolichos lablab</i> L.	Leguminosae	3.73
17. Bottle gourd	<i>Lagenaria siceraria</i> (Mol.)	Cucurbitaceae	2.40
18. Chilli	<i>Capsicum annuum</i> L.	Solanaceae	1.10
19. Bell pepper	<i>C. frutescens</i> L.	Solanaceae	0.60
<b>b) Pulses</b>			
20. Soyabean	<i>Glycine max</i> (L.) Merrill.	Leguminosae	2.80
21. Black gram	<i>Vigna mungo</i> L.	Leguminosae	2.50
22. Green gram	<i>Vigna radiatus</i> L.	Leguminosae	2.30
<b>c) Oil seeds</b>			
23. Sunflower	<i>Helianthus annus</i> L.	Compositae	3.10
24. Castor	<i>Ricinus communis</i> L.	Euphorbiaceae	11.50

Contd.

Common name	Scientific name	Family	Maximum intensity of infestation mines/leaf
<b>d) Ornamentals</b>			
25. Dahlia	<i>Dahlia variabilis</i> (Cav.)	Compositae	34.10
26. Marigold	<i>Tagetes indica</i> Cav.	Compositae	32.10
27. Salvia	<i>Salvia azurea</i> Lam.	Lamiaceae	7.00
28. Verbena	<i>Verbena hybrida</i> L.	Verbenaceae	4.30
29. Astor	<i>Callistephus chinensis</i> Nees.	Compositae	3.80
30. Zinnia	<i>Zinnia elegans</i> L.	Compositae	3.00
31. Cock's comb	<i>Celosia argentea</i> L.	Amaranthaceae	2.60
32. Thulsi	<i>Ocimum sanctum</i> L.	Labiatae	2.50
33. Petunia	<i>Petunia axillaris</i> Lam.	Solanaceae	2.40
34. Gerbera	<i>Gerbera jamesonii</i> Bolus	Compositae	2.10
35. Indian Pink (Carnation)	<i>Dianthus chinensis</i> L.	Cariophyllaceae	2.10
36. Butter daisy	<i>Melapodium paludosum</i> L.	Compositae	2.00
<b>e) Green manures</b>			
37. Sesbania	<i>Sesbania grandiflora</i> (L.) Poirel.	Leguminosae	4.40
38. Sunnhemp	<i>Crotalaria juncea</i> L.	Leguminosae	2.60
<b>f) Weeds</b>			
39. Ground cherry*	<i>Physalis minima</i> Linn.	Solanaceae	8.60
40. Narivali*	<i>Stachytarpheta indica</i> Vahl.	Verbenaceae	8.60
41. Thekkala*	<i>Heliotropium indicum</i> Linn.	Boraginaceae	7.90
42. Erippacha*	<i>Spilanthes calva</i> DC.	Compositae	6.60
43. Wild coccinia	<i>Coccinia indica</i> W. & A.	Cucurbitaceae	6.40
44. Wild mustard	<i>Cleome viscosa</i> Linn.	Capparidaceae	3.40
45. Poovamkurunthala*	<i>Vernonia cinerea</i> Less.	Compositae	2.80

Contd.

Common name	Scientific name	Family	Maximum intensity of infestation mines/leaf
46. Moyalcheviyan*	<i>Emilia sonchifolia</i> (L.) DC	Compositae	2.80
47. Tharuthaval* (Malamkoorka)	<i>Borreria hispida</i> K.Schum.	Rubiaceae	2.30
48. Balippovu*	<i>Aerva lanata</i> Juss.	Amaranthaceae	0.70

\* Common names in Malayalam

**Table 8: Intensity of infestation (mines/cm<sup>2</sup>) and width of the leaf mine (mm) on preferred host plants of *L.trifolii***

Host plants	Intensity of infestation per unit leaf area (mines per cm <sup>2</sup> )	Width of the leaf mine just before pupation (mm)
Cucumber	0.36	1.65
Ash gourd	0.40	1.83
Tomato	1.74	2.14
Cowpea	1.14	1.62
Ridge gourd	0.48	2.00
Pumpkin	0.33	1.82

per leaf), brinjal (44.8 mines per leaf), dahlia (34.1 mines per leaf), cucumber (32.6 mines per leaf) and marigold (32 mines per leaf) (Table 7). The observations on the number of mines from top, middle and lower leaves showed that, more number of leaf mines were observed on the middle and bottom canopy and lowest counts were observed on the top leaves in all the crops surveyed. The intensity of infestation per unit area of leaf was maximum for tomato (1.74 mines per cm<sup>2</sup>) (Plate 6G) and minimum for pumpkin (0.33 mines per cm<sup>2</sup>) (Table 8). The severity of damage depended on the age of the plant.

#### 4.3.1 Cowpea

The survey revealed that cowpea plants in the seedling stage were found more susceptible to leaf miner. Plants in the cotyledon stage (7 to 14 days old) were severely damaged by the leaf miner (Plate 6A). Older plants were also infested by the leaf miner. The number of mines in the lower most leaf varied from 0.1 to 85.9 mines per leaf and that of middle and upper leaves were 0.1 to 73.7 mines per leaf and 0 to 10.8 mines per leaf respectively. A maximum of 76 mines was recorded on the top leaf during December 2001 (Table 9). The intensity of infestation per unit area of the leaf was 1.74 mines per cm<sup>2</sup> (Table 8).

#### 4.3.2 Cucurbits

Among the cucurbits, ash gourd, ridge gourd, pumpkin and cucumber were highly susceptible to the leaf miner. The incidence was also found on other cucurbits such as bitter gourd, snake gourd, bottle gourd, coccinia and water melon. Severe leaf miner incidence was reported on ash gourd in the farmers plots of Nadathara panchayat. A maximum of 85.3 mines per leaf was observed on the bottom most canopy during the second fortnight of November (Table 10). The intensity per unit leaf area was worked out as 0.40 mines per cm<sup>2</sup> (Plate 6C).

In pumpkin, large number of small, compact mines delimited by veins were observed (Plate 6E). On an average 72.3 mines per leaf was recorded on ridge gourd (Plate 6B) where as in pumpkin and cucumber (Plate 6F) it was 35 and 20.17 mines respectively (Table 10). Among other cucurbits, bitter gourd (20 mines per plant) was more susceptible to leaf miner damage, followed by snake

Table 9. Intensity of infestation of *L. trifolii* in cowpea recorded at fortnightly intervals

Month	Fortnight	Intensity of infestation (mines per leaf)			Mean
		Top leaf	Middle leaf	Lower leaf	
June	1 <sup>st</sup>	0	1.2±1.4	3.5±1.36	1.57±1.8
	2 <sup>nd</sup>	0	2.2±1.7	4.5±2.5	2.23±2.4
July	1 <sup>st</sup>	0	2.4±1.6	10.9±1.6	4.33±4.5
	2 <sup>nd</sup>	0	3.4±1.96	9±3.6	4.13±4.03
August	1 <sup>st</sup>	0	2.5±1.5	8.4±2.6	3.63±3.6
	2 <sup>nd</sup>	0.3±0.46	4.8±2.13	9.6±2.8	4.9±4.3
September	1 <sup>st</sup>	0.7±1.27	2.3±1.6	4.4±1.8	2.47±2.2
	2 <sup>nd</sup>	0.6±1.28	2.5±1.8	3.2±1.8	2.1±1.97
October	1 <sup>st</sup>	0	5.6±2.8	8.7±3.6	4.77±4.5
	2 <sup>nd</sup>	0	8.5± 1.3	11.3 ±2.1	6.6 ±3.5
November	1 <sup>st</sup>	4.5±2.3	12.1±4.0	25.6±2.5	14.07±8.3
	2 <sup>nd</sup>	10.8±3.5	40.3±14.3	63.5±19.04	38.2±25.65
December	1 <sup>st</sup>	2.0±1.67	12.4±6.37	19.0±7.5	11.13±9.1
	2 <sup>nd</sup>	76.0±26.9	56.5±17.18	49.0±18.5	61.7±25.1
January	1 <sup>st</sup>	52.9±12.52	73.7±28.0	85.9±36.3	70.83±30.6
	2 <sup>nd</sup>	3.5±3.56	20.5±5.9	25.0±9.4	16.33±11.4
February	1 <sup>st</sup>	5.4±5.7	23.0±5.6	18.6±9.1	15.67±10.2
	2 <sup>nd</sup>	0	9.4±6.0	14.1±5.7	7.83±7.53
March	1 <sup>st</sup>	1.5±0.5	12±0.7	31.5±4.2	15.0 ±8.2
	2 <sup>nd</sup>	3.9±3.2	39.5±20.9	42.1±10.9	27.73±22.8
April	1 <sup>st</sup>	3.1±3.4	43.1±22.1	41.3±22.7	29.17±26.05
	2 <sup>nd</sup>	2.2±2.4	24.1±11.8	33.6±16.1	19.47±17.53
May	1 <sup>st</sup>	0.8±1.7	12.2±8.8	13.5±9.7	8.83±9.5
	2 <sup>nd</sup>	0	0.1±0.3	5.1±2.8	1.73±2.8
June	1 <sup>st</sup>	0.2±0.6	3.8±2.8	6.2±3.9	3.4±3.7
	2 <sup>nd</sup>	0	1.2±0.9	0.1±0.3	0.43±0.76

Table 10. Intensity of infestation of *L. trifolii* in Ash gourd

Month	Fortnight	Intensity of infestation (mines per leaf)			Average
		Top leaf	Middle leaf	Lower leaf	
August	I <sup>st</sup>	0	5.2±1.72	13.6±3.8	6.27±5.13
	2 <sup>nd</sup>	0	4.3 ±0.6	14.3±2.1	6.13±5.0
September	I <sup>st</sup>	2.7±2.41	5.0±3.03	11.1±6.5	6.27±5.60
	2 <sup>nd</sup>	4.9±6.18	12.8±6.18	20.0±12.8	12.56±10.4
October	I <sup>st</sup>	3.2±1.5	23.3±3.2	35.0±7.3	20.5±6.2
	2 <sup>nd</sup>	3.7±2.7	53.1±2.17	58.2±19.9	38.33±29.9
November	I <sup>st</sup>	7.2±2.5	58.3±3.15	62.3±6.5	42.6±12.6
	2 <sup>nd</sup>	10.4±3.4	57.7±10.4	85.3±15.0	51.13±32.7
December	I <sup>st</sup>	3.1±2.0	24.6±5.4	43.2±7.2	23.6±5.4
	2 <sup>nd</sup>	1.5±0.2	28.3±2.7	39.2±4.3	23.0±6.3
March	I <sup>st</sup>	3.1±2.0	14.6±10.7	31.1±15.14	16.26±9.5
	2 <sup>nd</sup>	0	13.5±11.07	13.8±6.49	9.1±9.8



Table 11. Intensity of infestation of *L. trifolii* in other cucurbits

Month	Fortnight	Crop	Intensity of infestation observed on			Average no. of mines/plant
			Top leaf	Middle leaf	Lower leaf	
August	1 <sup>st</sup>	Cucumber	0	4.7±1.2	12.5±1.5	5.6±2.3
		Bitter gourd	0	3.9±1.58	7.3±2.24	3.73±2.58
	2 <sup>nd</sup>	Cucumber	0	9.4±1.0	14.3±0.5	7.5±1.8
		Bitter gourd	0	2.5±1.2	6.8±1.3	3.7±2.1
September	1 <sup>st</sup>	Cucumber	0.5±1.2	3.9±2.26	7.0±1.73	3.8±3.2
		Bitter gourd	0.6±1.28	2.5±1.8	3.2±1.78	2.1±1.97
	2 <sup>nd</sup>	Pumpkin	2.5±1.86	15.4±10.1	22.1±12.27	13.3±12.3
		Bitter gourd	0.3±0.46	6.0±2.76	10.6±3.4	5.53±3.9
October	1 <sup>st</sup>	Cucumber	0	4.5±1.69	4.4±2.37	2.97±2.06
		Pumpkin	2.5±2.25	14.6±10.71	31.1±15.4	17.4±16.7
	2 <sup>nd</sup>	Cucumber	2.7±1.90	17.4±8.82	32.6±14.1	17.57±15.6
		Pumpkin	4.3±2.47	49.3±10.33	51.4±19.87	35±25.34
December	1 <sup>st</sup>	Cucumber	0.2±0.6	8.8±6.27	15.4±9.72	8.13±9.13
	2 <sup>nd</sup>	Pumpkin	1.3±0.6	23.5±6.3	28.5±4.5	17.6±8.5
January	1 <sup>st</sup>	Pumpkin	1.6±2.69	12.7±6.7	7.8±3.84	7.37±6.56
	2 <sup>nd</sup>	Bitter gourd	7.1±4.91	23.4±9.41	29.5±11.39	20.0±13.04
		Snake gourd	2.2±2.56	14.1±7.7	17.1±8.17	11.13±9.25
		Ridge gourd	23.7±8.65	32.1±10.1	21.1±9.3	25.63±6.2
February	1 <sup>st</sup>	Cucumber	7.3±4.69	25.9±11.23	27.3±17.53	20.17±15.33
		Bitter gourd	13.5±11.0	42.1±23.21	24.6±13.36	26.73±20.45
		Ridge gourd	25±13.4	53.4±20.8	72.3±4.7	49.4±8.3
	2 <sup>nd</sup>	Snake gourd	1.9±2.16	17.8±8.99	18.9±8.2	12.7±10.7
		Ridge gourd	13.6±11.9	70.5±15.7	72.3±14.2	52.3±10.3
		Cucumber	2.5±1.3	21.4±4.3	24.6±1.9	16.7±12.3
March	1 <sup>st</sup>	Snake gourd	0	3.1±2.91	2.6±1.5	1.9±2.33
		Cucumber	0	3.6±2.33	7.6±4.65	3.73±4.32
		Bitter gourd	4.5±4.76	27.7±14.17	25.2±10.2	19.13±16.5

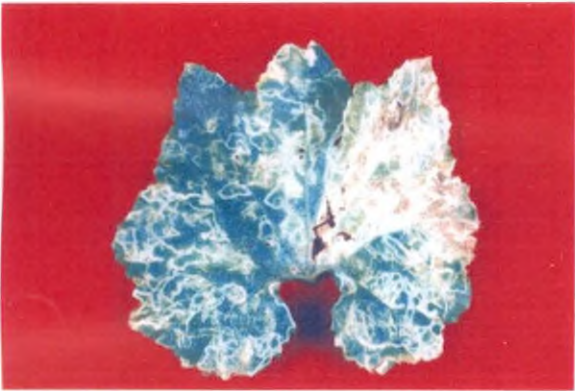
	2 <sup>nd</sup>	Cucumber	0	9.6±7.84	25.4±7.4	11.6±12.1
		Snake gourd	0	1.3±0.5	3.7±1.2	1.7±1.5
April	1 <sup>st</sup>	Cucumber	0	7.3±1.5	14.3±3.9	7.2±4.3
		Snake gourd	3.4±4.59	20.6±13.17	18.2±9.5	14.1±12.3
	2 <sup>nd</sup>	Cucumber	0	3.4±1.28	8.6±4	4±4.29
		Snake gourd	0.3±0.46	3.3±5.6	2.8±1.6	2.17±3.8



6 A



6 B



6 C



6 D

**Plate 6. Symptoms of leaf miner infestation in different host plants**

6.A - Cowpea

6.B - Ridge gourd

6.C - Ash gourd

6.D - Seedlings of Ash gourd

gourd (14.1 mines per plant). The intensity of infestation observed on the top, middle and bottom canopy of various cucurbits are given in Table 11. The intensity per unit leaf area was 0.36 mines per cm<sup>2</sup>, 0.33 mines per cm<sup>2</sup>, 0.48 mines per cm<sup>2</sup> for cucumber, pumpkin and ridge gourd respectively. The larvae within the leaf mines of cucurbits were found to be heavily parasitised compared to cowpea.

#### 4.3.3 Brinjal

Severe leaf miner incidence was observed (74.1 mines per leaf) during the month of February 2002. The incidence of leaf miner was seen only on the middle and bottom leaves of brinjal. On top leaves, the infestation was very less (1 to 2 mines per leaf).

#### 4.3.4 Ornamental plants

Among the ornamentals, severe infestation was observed on dahlia (34.1 mines per leaf) followed by marigold (32.1 mines per leaf) (Table 7). Majority of the ornamentals that were attacked by leaf miner belonged to Compositae family. Among the ornamentals, least preferred host was butter daisy (2 mines per leaf).

#### 4.3.5 Weeds

Weeds were acting as collateral hosts of this insect and thus ensure its survival throughout the year. The weeds, which were associated with crop plants during the peak periods of leaf miner infestation, were found harboured by this pest. The solanaceous weed, *Physalis minima* was severely infested by the leaf miner. Among the 10 spp. of weeds, maximum infestation was observed on ground cherry *P. minima* (8.6 mines per leaf) followed by *Heliotropium indicum* (7.9 mines per leaf), *Spilanthus calva* (6.6 mines per leaf) and wild coccinia (6.4 mines per leaf) (Table 7). Lowest incidence was observed on *Aerva lanata* (0.7 mines per leaf) (Plate 6H).



6 E



6 F



6 G



6 H

**Plate 6. continued..**

- 6.E - Pumpkin
- 6.F - Cucumber
- 6.G - Tomato
- 6.H - Spilanthus



#### 4.4 SEASONAL INCIDENCE

The incidence and intensity of attack by the leaf miner was studied from the first fortnight of June 2001 to the first fortnight of June 2002. Observations made on the incidence of the leaf miner on cowpea and cucurbits during 2001-2002 showed its presence throughout the year. The leaf miner population in cowpea was highest during the first fortnight of January 2001 (85.9 mines per leaf). The population decreased to a minimum of value 0.1 mines per plant during the second fortnight of June 2002. The peak periods of leaf miner infestation were observed from second fortnight of November to second fortnight of April. The average number of mines during that period varied from 7.83 to 70.8 (Table 9).

The leaf miner incidence was recorded on ash gourd from the first fortnight of August 2001 to the second fortnight of March 2002. The peak infestation was observed in the second fortnight of November (51.13 mines per plant). During this period the number of mines observed in the bottom most canopy was 85.3 mines per leaf (Table 10).

In pumpkin, the leaf miner infestation was highest during October (51.4 mines per leaf) (Table 11). In the case of ridge gourd, severe leaf miner incidence was observed during the second fortnight of February (72.3 mines per leaf). In cucumber, peak incidence was observed during October (32.6 mines per leaf) and February (27.3 mines per leaf). A maximum of 29.5 mines per leaf was recorded from the bottom canopy of bitter melon during the second fortnight of January.

Two peak periods of infestation of leaf miner were observed in cowpea. One major infestation was during the first fortnight of November where the average value was 63.5 mines per leaf. The second peak was recorded in the first fortnight of January where the average intensity was 85.9 mines per leaf. Weeds act as collateral hosts for this pest. So when there was no crop, the leaf miner occurred on these weeds. Thus the leaf miner survived throughout the year.

##### 4.4.1 Relationship between weather factors and incidence of *L. trifolii*

The correlation between the incidence of leaf miner on cowpea with various weather factors prevailed at KAU campus, Vellanikkara was worked out and shown in Table 12. The incidence of leaf miner was found positively correlated

**Table 12. Correlation coefficients between weather factors and the incidence of *L. trifolii* on cowpea**

Weather factors	Intensity of infestation			Average no. of mines/leaf
	Top leaf	Middle leaf	Lower leaf	
(a) Wind velocity (km per h)	0.747**	0.674**	0.615**	0.723**
(b) Sunshine hours	0.374 <sup>NS</sup>	0.550**	0.503**	0.511**
(c) Rainfall (mm)	-0.281 <sup>NS</sup>	-0.465*	-0.427*	-0.421*
(d) Evaporation (mm per day)	0.441*	0.610**	0.526**	0.562**
(e) Maximum temperature (°C)	0.040 <sup>NS</sup>	0.449*	0.399 <sup>NS</sup>	0.321 <sup>NS</sup>
(f) Minimum temperature (°C)	-0.344 <sup>NS</sup>	0.118 <sup>NS</sup>	0.133 <sup>NS</sup>	-0.028 <sup>NS</sup>
(g) Relative humidity (%)	-0.416*	-0.553**	-0.475*	-0.515**
(h) Mean temperature (°C)	-0.080 <sup>NS</sup>	0.399 <sup>NS</sup>	0.363 <sup>NS</sup>	0.245 <sup>NS</sup>

\* Significant at 5% level

\*\* Significant at 1% level

NS Not significant



with wind velocity (+0.723), evaporation rate (+0.562), sunshine hours (+0.511) and was significantly negatively correlated with the relative humidity (-0.515) and total rainfall (-0.421). There was no significant correlation between maximum temperature, minimum temperature and mean temperature with the incidence of the leaf miner.

## 4.5 NATURAL ENEMIES

### 4.5.1 Pathogens

Few dead larvae were obtained from the field and the fungus, *Aspergillus* sp. was isolated from the cadavers. But artificial inoculation of the fungus on the larvae did not give any positive results, indicating that the fungus was only a saprophytic one.

### 4.5.2 Parasitoids

The larvae and pupae of *L. trifolii* were parasitised by different parasitoids. The parasitised larvae turned black and could be easily identified in the leaf mines. However, the parasitised pupae were difficult to distinguish.

#### 4.5.2.1 Larval parasitoids

Two species of parasitoids, namely, *Chrysonotomyia rexia* (Eulophidae) and a scelionid wasp, *Gryon* sp. were found to parasitise the larvae during September 2001 to April 2002. Parasitisation was observed on the late second instar larvae of the leaf miner. Among the two species of parasitoids, *C. rexia* was more abundant on cowpea and was also obtained from ash gourd and pumpkin.

#### 4.5.2.2 Pupal parasitoids

Pupal parasitism was lower in the leaf miner. Parasitised pupae were detected its black coloured appearance. But it could not be detected in the early stages. A species of braconid, *Bracon* sp. parasitised the pupae of *L. trifolii* in cowpea.



Table 13. Per cent parasitism on *L. trifolii* larvae on various host plants

Month	Fortnight	Cowpea	Ash gourd	Pumpkin	Cucumber
September 2001	1 <sup>st</sup>	7.08	14.6	15.3	5.1
	2 <sup>nd</sup>	0	0	22.0	2.27
October	1 <sup>st</sup>	0	15.3	12.5	11.1
	2 <sup>nd</sup>	5.78	5.4	25.3	0
November	1 <sup>st</sup>	18.00	16.49		-
	2 <sup>nd</sup>	20.74	29.5	-	-
December	1 <sup>st</sup>	22.00	33.38	-	16.48
	2 <sup>nd</sup>	25.09	35.70	-	0
January 2002	1 <sup>st</sup>	30.69	-	31.5	10.27
	2 <sup>nd</sup>	31.97	-	29.7	20.00
February	1 <sup>st</sup>	31.5	-	-	-
	2 <sup>nd</sup>	30.00	-	-	-
March	1 <sup>st</sup>	21.92	-	-	0
	2 <sup>nd</sup>	19.85	-	-	23.5
April	1 <sup>st</sup>	16.49	-	-	0
	2 <sup>nd</sup>	9.95	-	-	0

#### 4.5.2.3 Parasitisation on *L. trifolii* in different hosts

In cowpea, parasitisation was observed during the months of September to April and maximum was observed during the second fortnight of January (31.97%) followed by first fortnight of February (31.5%) (Table 13). Higher parasitism was observed during the months of December, January and February. The major parasitoid recorded from cowpea was *Chrysonotomyia rexia* from the larvae of *L. trifolii*. The parasitisation of larva by *Gryon* sp. was very low.

In the case of ash gourd the parasitism was studied during September 2001 to December 2002. Maximum parasitisation was observed during the second fortnight of December (35.7%) and the major parasitoid was the eulophid, *C. rexia*. *Gryon* sp. and *Bracon* sp. were not obtained from ash gourd. In the first fortnight of January, *L. trifolii* on pumpkin was found heavily parasitised by *C. rexia* (31.5%). The per cent parasitism was lesser in cucumber (20.5%) than other crops.

## *Discussion*

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## 5. DISCUSSION

Recent years have witnessed progressive changes in the insect pest complex of vegetable crops. Along with the introduction of planting materials from other countries, several exotic pests were also introduced accidentally to our country. Serpentine leaf miner is such a pest. It has become a serious pest of vegetable crops, ornamentals, pulses, oil seeds etc. from 1990 onwards in India. The present study was undertaken to gather information on the bionomics and host range of *L. trifolii* in Thrissur district of Kerala. The work was conducted in the Department of Entomology, College of Horticulture, during 2001-2002. The results of the study are discussed below based on the earlier literature and relevant conclusions were derived from these results.

### 5.1. NATURE OF DAMAGE

The adult female of *L. trifolii* made a series of punctures on the upper surface of the leaf and fed on the exuding sap. The punctures could be seen by holding the leaf against light. The nature of damage observed was similar to that reported by Parrella *et al.* (1981). The larvae after hatching, mined beneath the epidermis feeding its way through the palisade mesophyll tissue. This is in close agreement with the observations made by Parrella *et al.* (1985).

Under severe infestation, the mines of different larvae became criss crossed. The leaves of severely infested seedlings showed burnt appearance. Later on, these mines got dried up resulting in the immature shedding of leaves. Seedling stage of the plant was severely affected by the leaf miner attack. Parrella (1987) reported the various types of damages caused by *Liriomyza* leaf miners on crops. These include destruction of young seedlings, accelerated leaf dropping symptoms and reduction in the aesthetic value of ornamental plants.

The papery white appearance of the mine and the falling of leaves were observed on different crops. Similar symptom was observed by Lediew and Bartlet (1983). Both the adult and larvae caused damage, but damage by the adult is not that much important compared to that of the larvae during its active third instar larvae. Severe infestation resulted in the reduction of photosynthetic area and ultimately affected the yield and dry matter production of the host plant. Later stage

of infestation by *L. trifolii* on cowpea showed rusty appearance on the leaves and leaves became highly susceptible to leaf spot diseases. The disease vectoring behaviour was reported by Broadbent and Matteoni (1991) also, where they found that the larvae and adults of *L. trifolii* were able to acquire and transmit *Pseudomonas cichorii*, which caused bacterial leaf spot in chrysanthemum.

## 5.2. BIOLOGY

### 5.2.1 Egg

The eggs were elongate, oval, translucent and milky white with an average length of 0.21mm and width of 0.12mm. The average incubation period was 2.08 days. The results were in agreement with the findings of Parrella (1987) and Viraktamath *et al.* (1993). However, the size of the egg was slightly smaller than that reported by Viraktamath *et al.* (1993) on french bean. The difference might be due to the host effect, as all the measurements made here were from the eggs laid on cowpea under laboratory conditions. The incubation period was shorter on cowpea in Kerala (2.08 days) compared to that reported by Nadagouda *et al.* (1997) on cowpea in Andhra Pradesh, where they recorded an incubation period of 2.59 to 3.74 days. Spencer (1973) and Nair (1999) recorded the incubation periods as 2.5 days. Zoebisch *et al.* (1992) recorded a duration of 2.47 days at 25°C and 1.99 days at 32°C on tomato. Parrella (1987) reported the hatching period of egg as 2.5 to 4.5 days on chrysanthemum. These differences in the incubation period might be due to the host influence and the temperature prevailed during the study in these countries (Fig. 1).

### 5.2.2 Larva

Larva was cylindrical and apodous with colour varying from transparent white in the first instar to orange yellow in the last instar. The length and width of the mine increased with the instar. The length of mine caused by the first instar was 12.2mm and width was 0.18mm. The second instar produced a leaf mine of 25.9mm length and 0.34mm width and that for the third instar was 40.3mm length and 1.72mm width. But higher mine width and length were recorded by Jagannatha



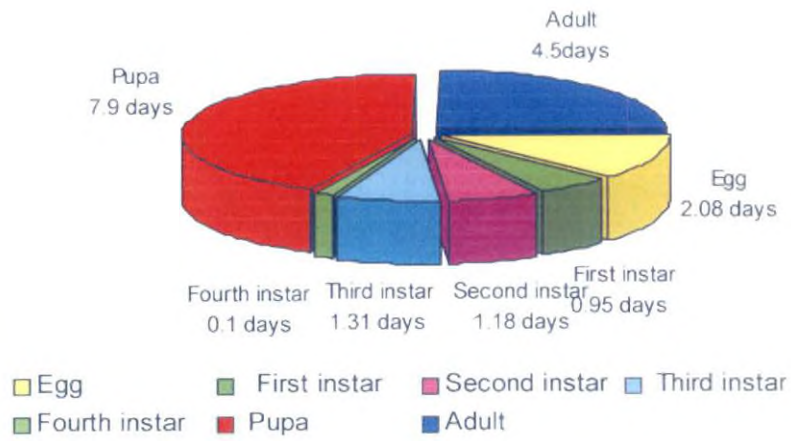


Fig. 1 Duration of different stages of *L. trifolii*

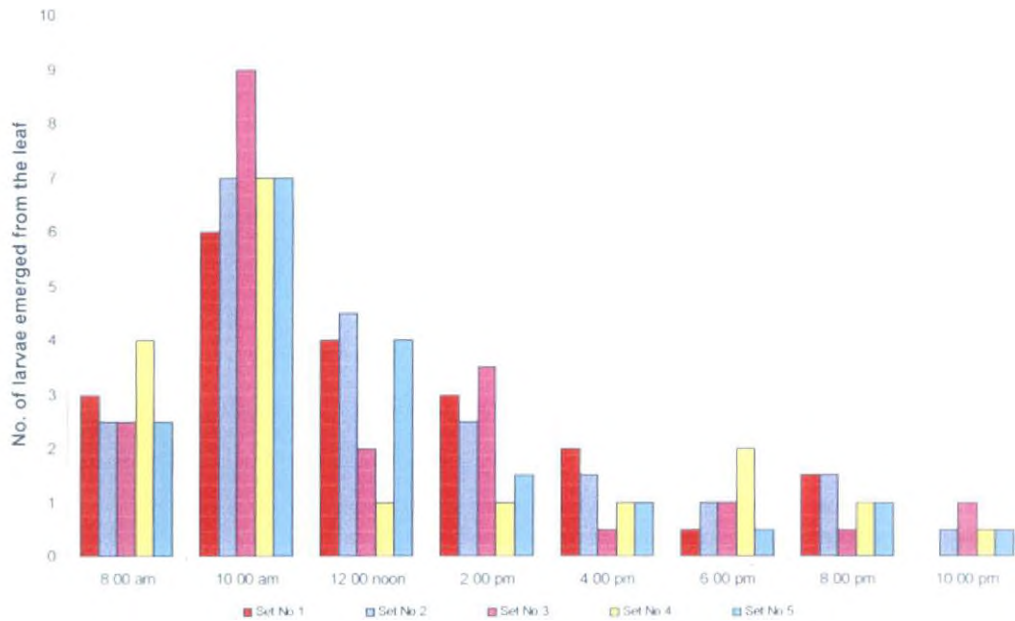


Fig.2 Emergence pattern of the larvae of *L. trifolii* from the leaf mines during different hours of the day

(1994) on french bean. These differences might be due to the effect of host and rearing conditions provided.

Measurement of the length and width of mouth hooks of the larvae gave an indication of the instars. Mouth hook of first instar larva has 0.09 mm length and 0.03 mm width. The corresponding values for the second, third and fourth instars were to 0.17mm, 0.05mm, 0.25 mm, 0.07mm and 0.25 mm, 0.08 mm respectively. Thus distinct variation in the length and width of mouth hooks of the different instars was obtained. These measurements were in agreement with Parrella and Bethke (1988) and Jagannatha (1994).

The third instar larvae after moulting came out of the leaf mines through out the day. But the peak emergence was observed between 0800 and 1000 hours which is in agreement with the finding of Zehnder and Trumble (1984) in Florida (Fig. 2). However Parrella (1987), from California reported that the larvae emerged before 0800 hours which might be due to the differences in the prevailing day length. The emergence pattern of the larvae has much significance in pest management programme. With this knowledge we can adopt spraying of any contact insecticide in the early morning hours preferably just before the peak emergence time of the larvae. This practice will effectively kill the larvae while they crawl on the leaf surface before falling to the ground for pupation.

The duration of the first instar larva was 0.95 days and that of the second and third instar were 1.18 days and 1.26 days respectively. The early fourth instar larva emerged from the leaf mine and fell down to the soil for pupation. The larval duration was observed as 2 to 3.5 hours (average = 2.5 hours). Though several workers studied the biology of *L. trifolii*, except Parrella (1987) and Viraktamath and Jagannatha (2000) no other workers noticed this brief fourth instar stage. But they have reported this as prepupa. Our finding of four instars in this Cyclorrhaphan is against the findings of Richards and Davis (1977), where they mentioned that there are only three larval instars in the Sub.order Cyclorrhapha, to which *L. trifolii* belongs.

The total larval duration was 3.4 days. Parrella and Bethke (1988), Nadagouda *et al.* (1997) and Viraktamath and Jagannatha (2000) also observed similar results. A slightly prolonged larval period of 7 to 8 days was reported by Spencer (1973) on chrysanthemum at Maryland, USA and 7 to 18 days by Nair (1999) on cowpea in Kerala. Zoebisch *et al.* (1992) reported a larval duration of



5.02 days at 25°C on tomato. These results were in close agreement with the above findings as the prevailing room temperature was in the range of 25°C to 28°C during that period. The difference in larval duration was due to the differences in the temperature as well as host plants as reported by Parrella, (1987) where he observed that larval metabolic rate was doubled with every 10°C increase in temperature and so the larval development varied with the temperature and host plants.

In cowpea, the maximum width of the leaf mine was 1.62 mm. The width of the mine just before pupation was 2.4 mm for tomato, 1.83 mm for ash gourd, 1.8 mm for pumpkin and 2.0 mm for ridge guard. Thus the mine width will help to predict the day of emergence of the larva from the leaf mine which will help to plant the day and time for insecticide applications.

The fourth instar larva was reported as prepupa by several workers (Leibee, 1984 and Nadagouda *et al.*, 1997). According to Richards and Davies (1977), the prepupa occurs inside the puparium. Hence the prepupa reported by the earlier workers is the fourth instar observed in the present study.

### 5.2.3 Pupa

During the laboratory studies, it was found that the larvae pupated inside the polythene covers. Parrella (1987) reported that, majority of the larva pupated in the soil. A few larvae pupated in the depressions on the leaves, leaf axils or at the bases of branches as reported by Lakshminarayana *et al.* (1992). Pupa was initially golden yellow in colour but later turned dark brown.

Among the four life stages, the pupal stage was found longer for *L. trifolii*. The pupal period lasted from 6.8 to 8.5 days. Similar result was reported by Spencer (1973), Parrella (1987) and Nair (1999). But a higher pupal duration of 9.82 to 10.65 days was reported by Nadagouda *et al.* (1997). This may be due to the difference in the rearing conditions provided. The pupae measured about 1.66 mm length and 0.76 mm width. A slight difference in the size was recorded by Nadagouda *et al.* (1997) where he reported the pupal size as 1.46 to 1.69 mm in length and 0.59 to 0.81 mm in width .





#### 5.2.4 Adult

The adult is a small, beautifully coloured fly with yellow head region. The back of the head and the ocellar triangle were black and had a yellow scutellum. Similar descriptions were given by Spencer (1973). The adults started mating immediately after emergence and started egg laying within 24 hours after mating. Repeated matings were needed for maximum egg production. The number of eggs laid was considerably reduced or practically nil when the death of the male occurred among the pair of adults. Similar observations were reported by Parrella *et al.* (1983).

Females were comparatively larger (1.7 mm in length) than males (1.49 mm in length). Similar measurements were reported by Parrella (1987) and Viraktamath *et al.* (1993). Adults emerged from the pupae during early morning hours between 0600 to 1000 hours. Parrella (1987) also observed that the peak emergence of adults was during the early morning hours.

##### 5.2.4.1 Feeding

The adults made feeding injuries on the leaves with their pointed ovipositor and from these punctures they fed on the plant juice. Two types of punctures were made by the female, fan shaped punctures for feeding and tubular punctures for oviposition. The females made the wounds in fan shape in order to get maximum plant sap. The oval shaped eggs were correctly fitted inside the tubular punctures. These observations were similar to the findings of Bethke and Parrella (1985). The feeding punctures were also used by the male fly (Parrella, 1987). The ratio of oviposition punctures to feeding punctures was 1: 8.09 to 1: 9.3 which was in agreement with that of Nadagouda *et al.* (1997).

##### 5.2.4.2 Fecundity

A female laid 48 to 50 eggs during its lifetime in cowpea. The number of eggs laid varied in different host plants. Bethke and Parrella (1985) reported 250 eggs on chrysanthemum, 405 eggs on celery (Leibee, 1984) and 100 eggs on beans (Fagoonee and Toory, 1984) during the life period. Since the pest has been recently

introduced to India it may take some years to stabilize and reach a fecundity level reported from its native country (Viraktamath *et al.*, 1993).

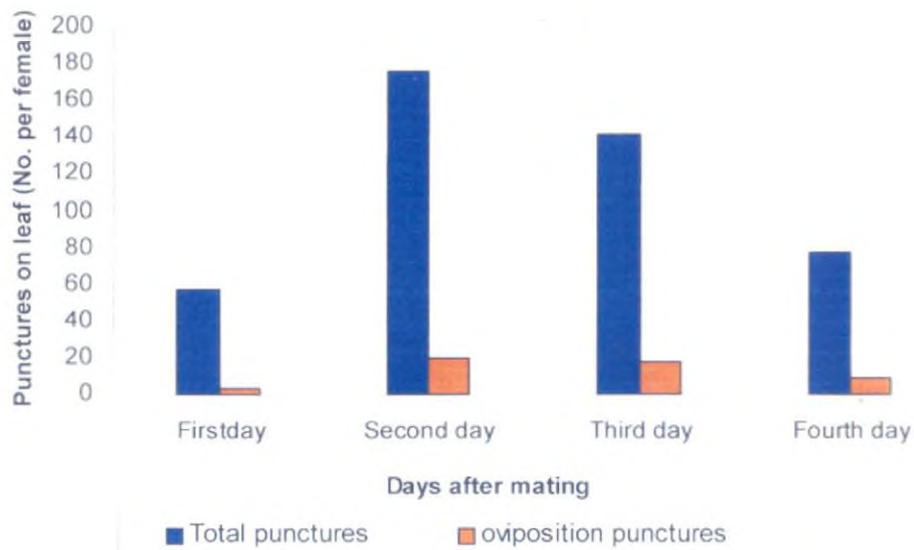
The maximum oviposition period was observed to be four days in cowpea and the peak egg laying occurred on the second day after mating (Fig. 3). Zoebisch *et al.* (1992) observed that the peak oviposition rate was on the third day after emergence at 25°C and that at 32°C occurred on the second day after emergence in tomato. The oviposition period observed in the present study was slightly higher than those reported by Jagannatha (1994) who reported three days on cowpea, 12 days on tomato and five days on cotton.

#### 5.2.4.3 Longevity

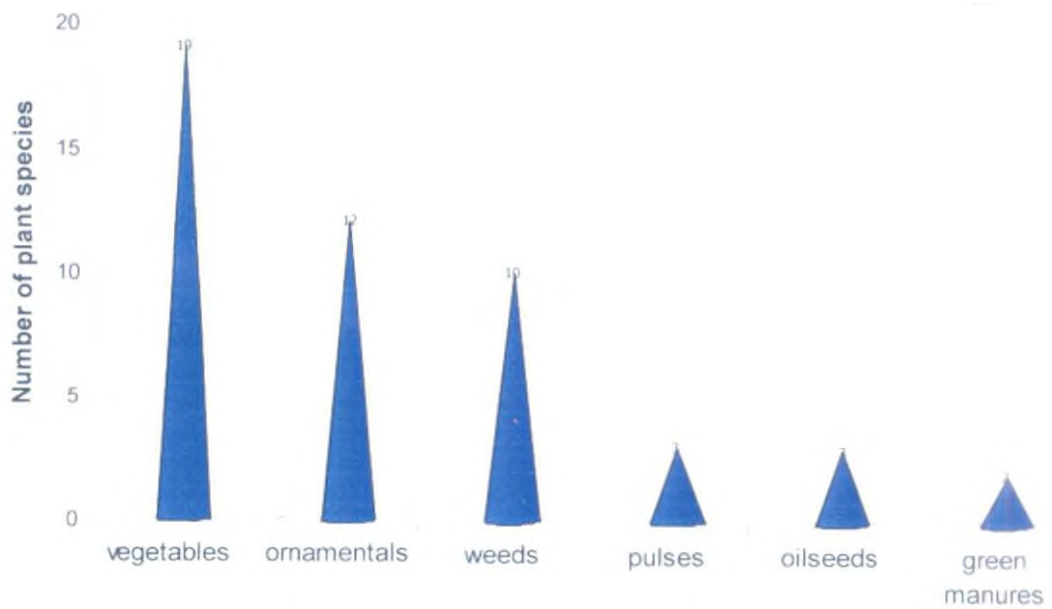
The longevity of the adult varied with sex and availability of food. The female lived for longer period compared to male. Adults provided with honey solution lived for longer period *i.e.*, 4.5 days for males and 7.4 days for females compared to 1.5 and 2.5 days respectively, for those provided with water only. The results were in agreement with Lakshminarayana *et al.* (1992) where they reported the longevity of adults as 3 to 6 days at Hyderabad when provided with honey. Similar observations were reported by Nadagouda *et al.* (1997) where they observed that the male longevity varied from 2.5 to 6.9 days and female longevity varied from 4.69 to 9.8 days in cowpea

The longevity recorded in the present study was, however, shorter compared to the reports of Parrella (1987) and Heinz (1996), where they have recorded an adult life span of about 15 to 20 days for females and 10 to 15 days for males as well as 9.9 days for males and 14.8 days for females, respectively. This might be due to the low temperature during culturing. Parrella (1987) also reported that the longevity of the adult was negatively correlated with temperature. Minkenberg (1988) showed that adults lived longer at 20°C (14.4 days) compared to those at 15°C (6.5 days) or 25°C (5.6 days). The males lived only for 2 to 3 days possibly because they could not puncture the foliage and therefore could feed only less than that of female (Minkenberg and Van Lentem, 1986).





**Fig.3 Egg laying patern of *L. trifolii***



**Fig.4 Host range of *L. trifolii***

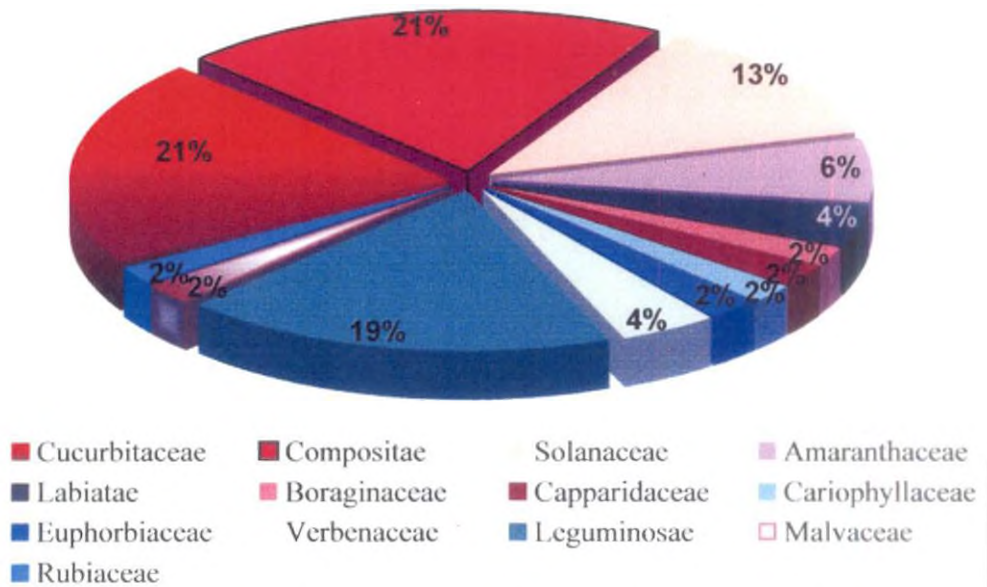
#### 5.2.4.4 Sex ratio

Laboratory studies carried out to study the sex ratio of *L. trifolii* showed the female to male ratio as 1:1. It was found that the sex ratio was altered with increase in severity of infestation. A shift in sex ratio in favour of the females was observed during December and January months. The ratio of 1:1 was in conformity with the findings of Fagoonee and Toory (1984) and Parrella (1987). This increase in proportion of females (1:1.83) during the months of December and January might be due to the weather effect. The increase in pest population is possible only when there is a higher proportion of females since the peak period of infestation of *L. trifolii*, was observed during December and January.

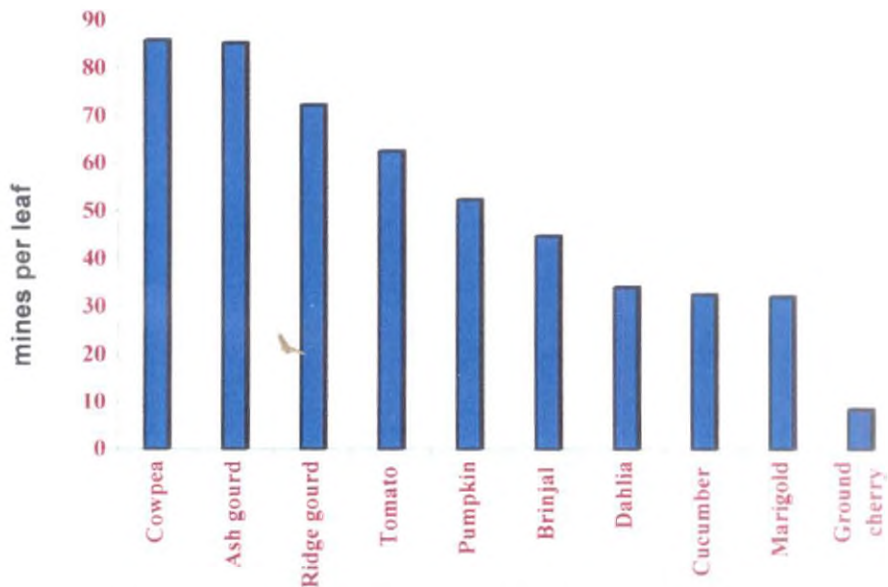
### 5.3 HOST RANGE STUDIES

During the present study, the leaf miner was found feeding on 48 host plants belonging to thirteen plant families (Fig. 4). Earlier workers also reported wide host range for *L. trifolii* (Stegmaier, 1966 (54 host plants); Spencer, 1973 (18 host plants); Viraktamath *et al.*, 1993 (55 host plants) and Srinivasan *et al.*, 1995 (79 host plants)). The infestation of *L. trifolii* was observed on 10 plant families by Stegmaier (1966). In the present study more number of host plants were belonging to the families of Compositae (10 spp.) and Cucurbitaceae (10 spp.). Stegmaier (1966) also reported the majority of the host plants in the families of Compositae. Srinivasan *et al.* (1995) reported that most of the host plants were coming under the family of Leguminosae (17 spp.). However, in the present survey, only nine species of leguminous host plants were reported (Fig. 5). Three new families, namely, Boraginaceae, Rubiaceae and Verbenaceae which were not reported by other workers were also observed in this survey.

Among the 48 host plants recorded during this study, 32 host plants have been already reported by Srinivasan *et al.* (1995), during his survey in Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu where he recorded a total of 79 host plants in 16 plant families. The weed hosts reported by him could not be recorded during the present survey. However, eight species of weeds and four species of ornamental plants were observed as host plants of *L. trifolii* for the first time.



**Fig. 5** Distribution of plant species infested by *L. trifolii* in different plant families



**Fig. 6** Intensity of infestation of *L. trifolii* in preferred host plants



### 5.3.1 Host preference

In Kerala, severe infestation of the leaf miner was observed on cowpea, ash gourd, tomato, ridge gourd, pumpkin, cucumber, marigold, dahlia and bitter gourd (Fig.6, 7 and 9). Pest surveillance studies conducted under Kerala Horticultural Development Programme at Kerala Agricultural University also showed severe incidence of *L. trifolii* on the above mentioned crop plants (KHDP, 1998). Among the cucurbits, ash gourd, ridge gourd and cucumber were highly susceptible (Fig. 8). This is in conformity with reports of Jeyakumar and Uthamasamy (1998). During the survey, it was also understood that, the leaf miner infestation not only affected the vigour of the plants but also the quality or appearance of ornamental plants especially dahlia which resulted in reducing the market value of such crops.

Seedlings of cowpea plants were highly susceptible to leaf miner damage (Fig. 10). Cotyledons were severely damaged. Since the sugar and nitrogen contents were higher in cotyledons, adults were more attracted to these for oviposition. Ananthkrishnan (1992) reported that sugars acted as feeding stimulants and the larvae fed more voraciously on plant parts containing highest concentration of sugars. The chlorophyll content of the cotyledons was also higher (Terman, 1977). Nitrogen is an important component of chlorophyll (Black, 1973). The intensity of infestation was higher on plant parts containing high nitrogen content. Minkenberg and Ottenheim (1990) also observed a significant increase in feeding and fecundity with increased leaf nitrogen content. Addition of nitrogenous fertilizers may increase the susceptibility of the plants to *L. trifolii*. Hence excessive application of nitrogenous fertilizers should be avoided to reduce the infestation by *L. trifolii*.

### 5.3.2 Intensity of infestation

The number of mines per unit area in cowpea was 1.14 mines per cm<sup>2</sup>, during January. However, lower values were reported by Jeyakumar and Uthamasamy (1998) (0.21 punctures per cm<sup>2</sup> with eggs and 0.28 punctures per cm<sup>2</sup> with out eggs). The highest intensity of infestation per unit area was obtained for tomato (1.74 mines per cm<sup>2</sup>) among the different host plants observed. Among cucurbits, the intensity per unit area was highest for ridge gourd (0.48 mines per cm<sup>2</sup>) and the lowest was for pumpkin (0.33 mines per cm<sup>2</sup>). For cucumber, it was worked out as

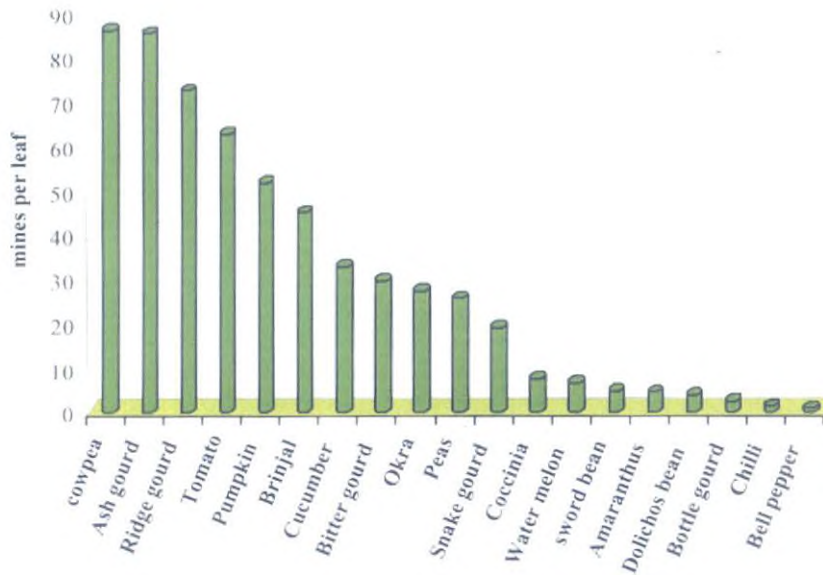


Fig.7 Intensity of infestation of *L. trifolii* on common vegetables

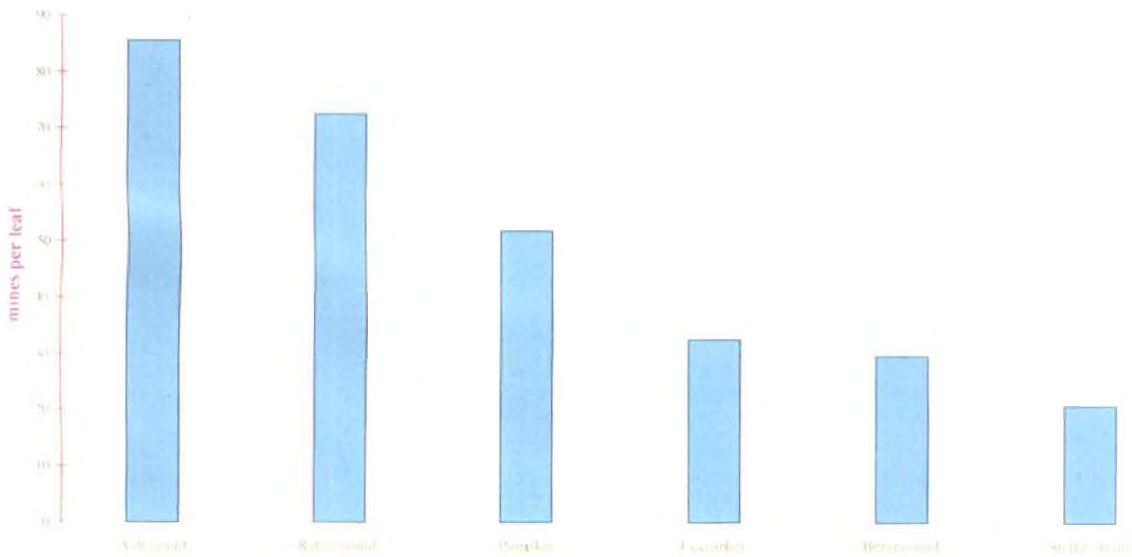


Fig. 8 Host preference of *L. trifolii* among the cucurbitaceous vegetables

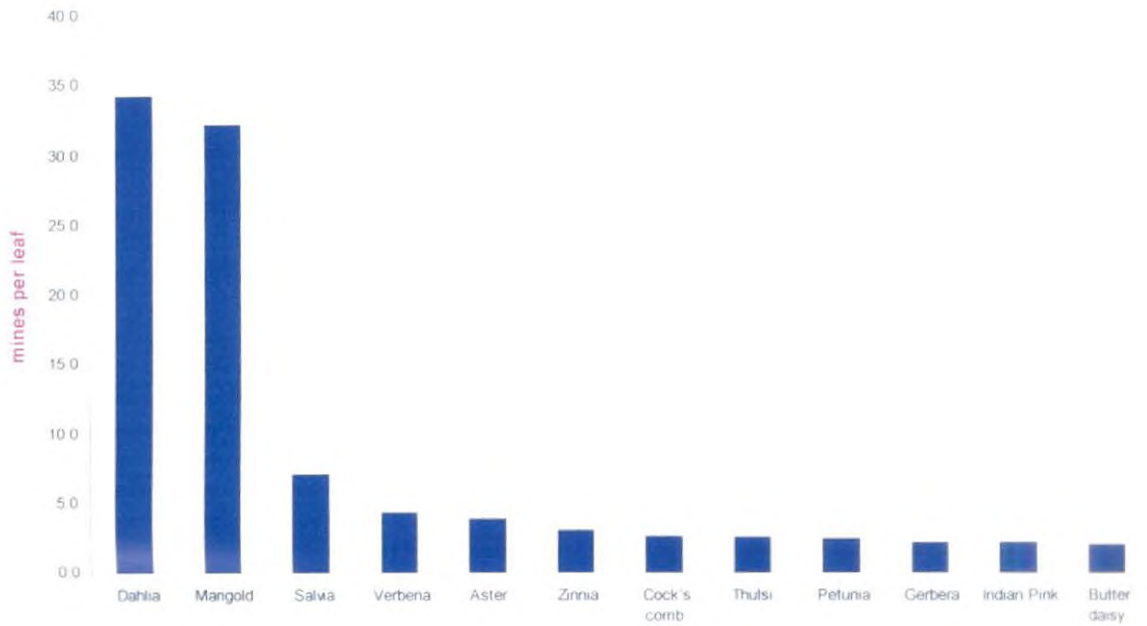


Fig. 9 Infestation of *L. trifolii* in ornamental plants

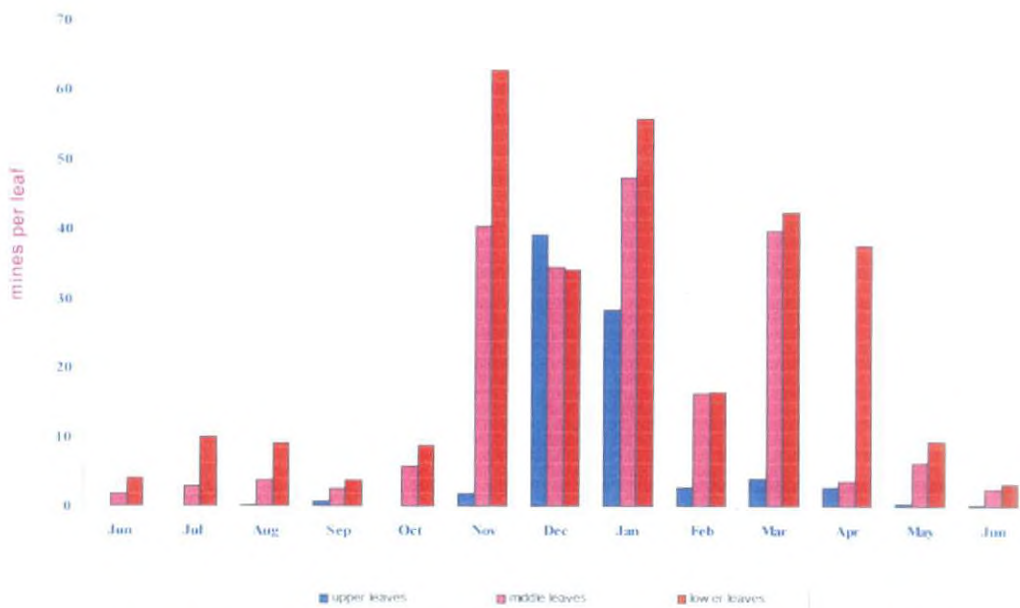


Fig.10 Infestation of *L. trifolii* in the upper,middle and lower leaves of cowpea



0.36 mines per cm<sup>2</sup>. This shows that tomato is the most preferred host plant of *L. trifolii*.

#### 5.4 SEASONAL INCIDENCE

*L. trifolii* infestation was observed through out the period of survey from June, 2001 to June, 2002. Two peak periods of infestation were observed. The first was recorded during the first fortnight of November on cowpea (63.5 mines per leaf). Second peak was recorded in the first fortnight of January (85.9 mines per leaf). The population was minimum during the second fortnight of June (0.43 mines per plant) (Fig.11). Severe infestation of *L. trifolii* was recorded during January, February and March in Kerala (KHDP, 1998). However, Viraktamath and Jagannatha (2000) reported peak infestation during March to April and July to September at Bangalore. This difference might have been due to the difference in the weather conditions prevailed in that area.

Analysis of weather data showed that the leaf miner incidence was positively correlated with wind velocity (+0.723) and sunshine hours (+0.511) and negatively correlated with relative humidity (-0.515) and total rainfall (-0.421) (Fig.12 and 13). Bagmare *et al.* (1995) also observed similar results in Madhya Pradesh. A higher wind velocity and a low relative humidity were recorded during the period from November to March. Hence high intensity of infestation occurred during this period. Temperature did not have any influence on the incidence of *L. trifolii*.

#### 5.5 NATURAL ENEMIES

No predators or pathogens were observed on any of the stages of this pest in the present study. Three hymenopteran parasitoids namely, the larval parasitoids, *Chrysonotomyia rexia* (Eulophidae) and *Gryon* sp. (Scelionidae) and the pupal parasitoid, *Bracon* sp (Braconidae) were recorded on *L. trifolii*. The parasitisation was found during the months of October to April and was maximum on cowpea during the months of December, January and February (Fig.14). The dominant species reported in this study was *C. rexia*.

Among the three species of parasitoids obtained, *Gryon* species were found parasitising the eggs of *Clavigrella gibbosa*. Five species of *Chrysonotomyia* have

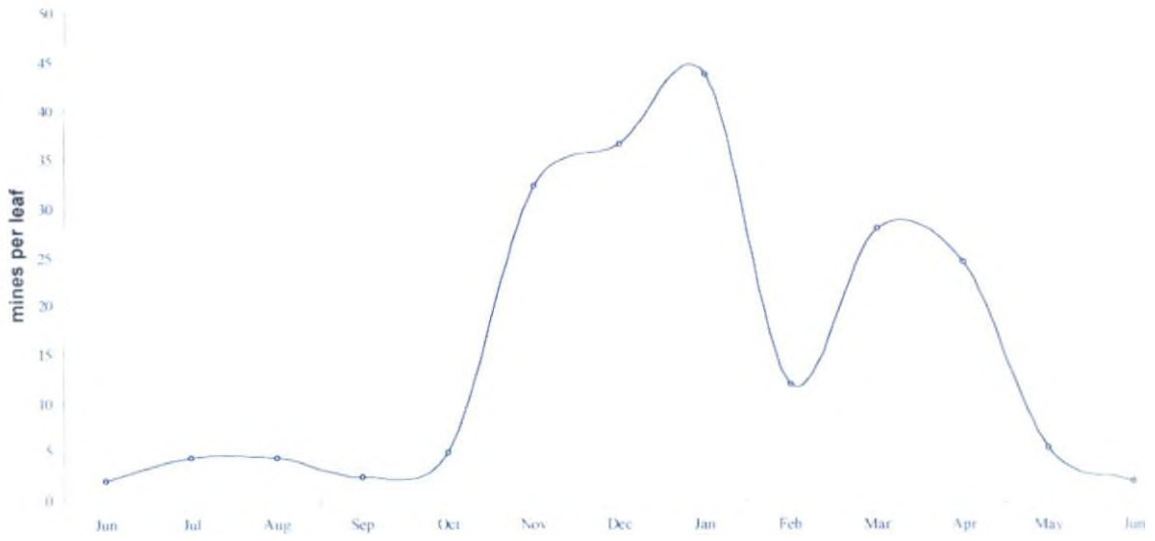


Fig.11 Seasonal infestation of *L. trifolii* on cowpea (June 2001 to June 2002)

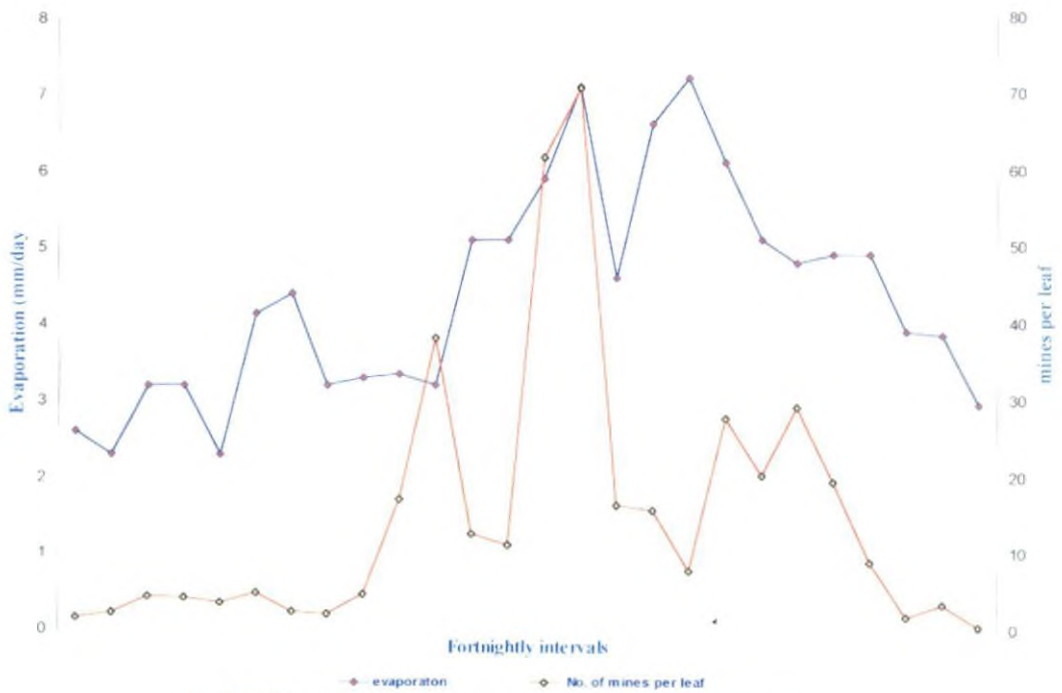


Fig.12 Effect of evaporation rate on the infestation of *L. trifolii*

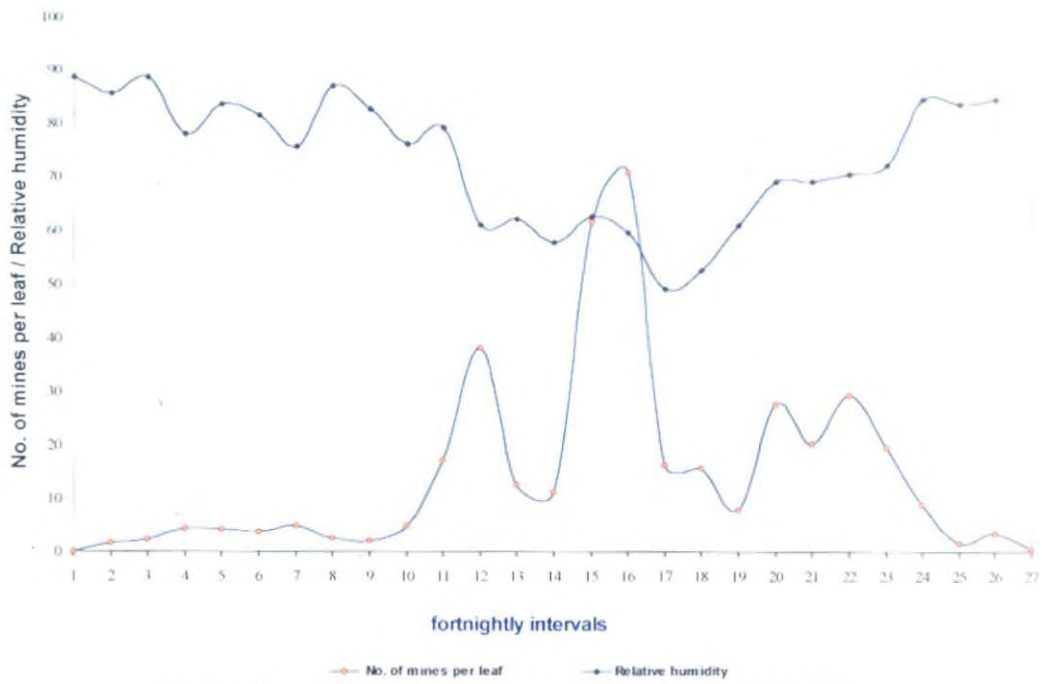


Fig.13 Effect of Relative humidity on the infestation of *L. trifolii*

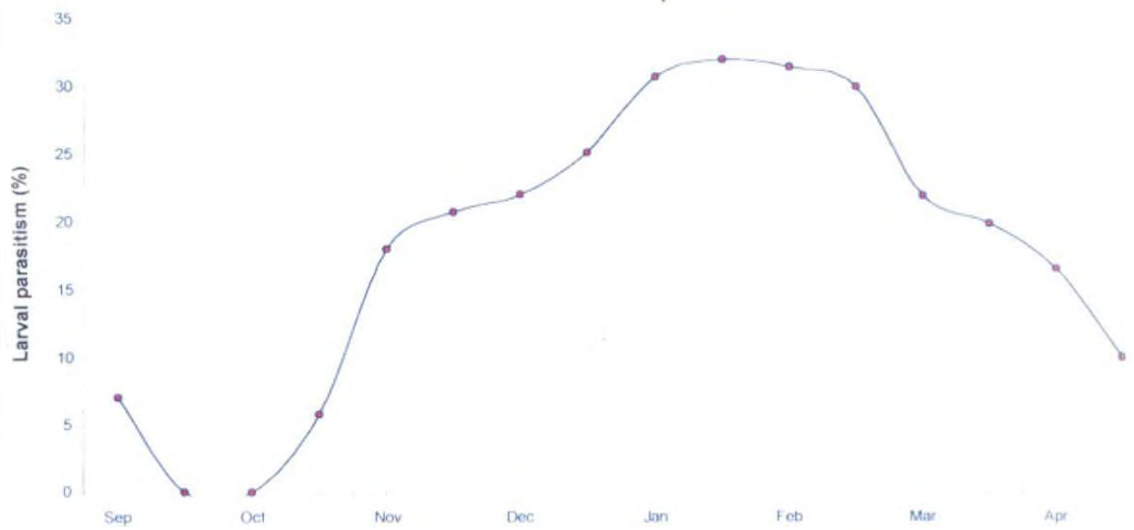


Fig.14 Larval parasitisation on *L. trifolii* on cowpea (Fortnightly intervals from September 2001 to April 2002)

during the months of December, January and February (Fig.14). The dominant species reported in this study was *C. rexia*.

Among the three species of parasitoids obtained, *Gryon* species were found parasitising the eggs of *Clavigrella gibbosa*. Five species of *Chrysonotomyia* have already been reported from *L. trifolii* by several workers from India and abroad. They were *C. punctiventris* from Hawaii on watermelon (Johnson and Hara, 1987; Johnson, 1987) and from Florida on tomato (Schuster and Wharton, 1993), *C. formosa* (Johnson, 1987), *C. pentheus* (Ohno *et al.*, 1998), *C. parksi* (Crawford) (Johnson and Hara, 1987). A species of *Chrysonotomyia* was reported from the India (Shankar *et al.*, 1992; Kapadia, 1995, and Pawar *et al.*, 2001). Also some species of *Gryon* were reported by several workers as egg parasitoids of hemipterans.

Kapadia (1995) reported 29.5 to 29.7 per cent parasitisation by *Chrysonotomyia* on cowpea and tomato. Pawar *et al.* (2001) reported 30 per cent parasitisation on cucumber in Rahuri. A higher rate of parasitisation was observed in cucurbits (35.7%) in December compared to cowpea (31.97 per cent) in January. Hence conservation of the natural enemies can be considered as a component in the integrated pest management of *L. trifolii*.

# *Summary*

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## 6. SUMMARY

The American serpentine leaf miner, *Liriomyza trifolii* (Burgess), introduced from Florida, USA, has become a serious pest of several major crops in Kerala. The incidence of *L. trifolii* was increasing because of its wide host range and the difficulty in managing the pest. In order to adopt various control measures, a thorough knowledge about the biology, host range, seasonal incidence and natural enemies of *L. trifolii* are essential. Hence, the present investigation was undertaken to study the bionomics and host range of American serpentine leaf miner.

The biology of *L. trifolii* was studied under laboratory conditions on cowpea (c.v Lola). The female *L. trifolii* made a series of punctures on the upper surface of the leaf lamina and fed on the exuding sap. The males also used the same punctures for feeding. The larvae, after hatching, fed beneath the epidermis and produced characteristic serpentine mines. The mine was highly contorted and the size increased with the size of the larva. The mining pattern varied with the host plant.

Eggs of the leaf miner were elongate, oval, translucent, milky white and could be detected by staining technique. Eggs measured about 0.20 to 0.24 mm in length and 0.12 mm in width. The incubation period was 1.5 to 3 days (average= 2.08 days).

There were four larval instars. The first instar was very small, transparent, greenish white in colour, the second instar larva was light yellow in colour and the third instar larva was yellow and its presence could be made out by a slight bulging at the end of the mines. The fourth instar larva was also yellow coloured and early fourth instar immediately emerged from the mine and fell to the ground for pupation and hence not much studied by the workers. The duration of the four instars was 0.90 days, 1.15 days, 1.26 days and 0.1 days for the first, second, third instar and fourth instar larvae respectively. The cephalopharyngeal skeleton of the first, second, third instar and fourth instar larvae measured 0.096 mm, 0.17 mm, 0.25 mm and 0.25 mm in length, respectively. The total larval period lasted for 3.4 days. The size of the larvae measured about 0.31 mm, 0.94 mm, 1.56 mm and 2.35 mm in length and 0.13 mm, 0.18 mm, 0.46 mm and 0.64 mm in width for the first, second, third and fourth instars respectively.

There was progressive increase in the length of the mine made by the first (8 to 15 mm long), second (16 to 35 mm long) and the third instar larvae (25 to 57 mm

long). The fourth instar was a non feeding stage. Majority of the full grown larvae came out of the leaf mine before noon and maximum emergence was found between 0800 to 1000 hours.

The pupation took place in the soil. Occasionally, the larvae pupated on the leaf, leaf axils and other plant parts. The golden yellow pupae measured 1.66 mm in length and 0.76 mm in width. The average pupal duration was 7.9 days. The total life cycle from egg to adult occupied an average of 13.4 days in cowpea.

The small, beautifully coloured, female fly was 1.70 mm long and 0.59 mm wide and the male 1.49 mm long and 0.50 mm wide. Majority of the adults emerged during 0600 to 1000 hours. The females made two types of punctures fan shaped and tubular punctures. The fan shaped punctures were used for feeding and the tubular punctures for oviposition. The fecundity was 48 to 50 eggs per female and the oviposition period was four days. The sex ratio was female biased (1:1.41).

During the survey conducted, *L. trifolii* was found breeding on 48 host plants belonging to 13 plant families. The infestation was severe on cowpea, ash gourd, tomato, ridge gourd, pumpkin and cucumber and also on ornamentals like dahlia and marigold. Only dicots were found to be infested by *L. trifolii*.

The pest prevailed throughout the period of observation from June 2001 to June 2002. The peak period of occurrence on cowpea were during the first fortnight of January (85.9 mines per leaf) and November (65.9 mines per leaf). The correlation coefficients were worked out for various weather factors and the larval population and found that a significant positive correlation existed with wind velocity (+ 0.723), sunshine hours (+0.511) and evaporation rate (+0.562). However infestation was negatively correlated with relative humidity (-0.515) and total rainfall (-0.421). Temperature did not have any influence on the population of *L. trifolii*.

Two larval parasitoids and a pupal parasitoid were recorded during the period of study from September 2001 to April 2002. Among these the eulophid wasp, *Chrysonotomyia rexia* was the most predominant parasitoid. The per cent parasitisation varied from zero to 31.97 per cent in cowpea and zero to 35.7 per cent in ash gourd. The percentage parasitisation was highest during the months of December, January and February and zero during June.

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\* Originals not seen

**BIONOMICS AND HOST RANGE OF  
AMERICAN SERPENTINE LEAF MINER**

*Liriomyza trifolii* (Burgess) (Agromyzidae: Diptera)

By

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**ABSTRACT OF THE THESIS**

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## ABSTRACT

The present investigation on the "Bionomics and host range of American serpentine leaf miner, *Lirionomyza trifolii* (Bugess) (Agromyzidae: Diptera)" was undertaken in the Department of Entomology, College of Horticulture, Vellanikkara during 2001-2002. Field surveys and laboratory studies were carried out with the objective of studying the biology, host range, natural enemies and seasonal incidence of *L. trifolii*.

The biology of *L. trifolii* was studied by releasing a pair of one day old adult flies to the rearing cages where cow pea seedlings were kept as host plants and honey as a food source. The female fly inserted its eggs in the tubular punctures made on the leaves with its pointed ovipositor. The oval, translucent, milky white eggs hatched in about 2.08 days.

The larvae mined the upper leaf surface and produced characteristic serpentine mines. There were four larval instars having a total duration of 3.4 days. After the larvae attained full size of 2.35 mm length and 0.64 mm width it made a semicircular cut at the broad end of the leaf mine. Through this cut larva came out of the mine and fall down to the soil for pupation. Inside the soil the larva turned to golden yellow coloured pupa. Female pupa was larger compared to male pupa. Adult emergence took place after 7.9 days.

The adults had a longevity varying from 4.5 days for males and 7.4 days for females where as they were feed with 2 per cent honey solution. Adult females were larger than the males and had black pointed spot on the lower side of last abdominal segment. The fecundity of female varied from 14.6 eggs per day per female. The adult female laid about 48-50 eggs in its life time. The ratio of oviposition of feeding puncture was 1: 8.09 to 1: 9.3. The total life cycle from eggs to adult took 13.3 days.

The host range was studied by conducting surveys at regular intervals. 48 host plants belonging to 13 plant families were reported as host plants of this pest. It is a highly polyphagous insect and majority of the host plants belonged to families of Compositae and Cucubitaceae.

The intensity of infestation of *L. trifolii* on various crops were calculated by counting the number of larval mines per leaf on the upper, middle and lower leaves of the plants. The attack of *L. trifolii* was more on the lower leaves compared to middle and top leaves. Cowpea was found to be the most preferred host plant of *L.*

*trifolii* followed by ash gourd, ridge gourd, tomato, pumpkin and cucumber. Among the ornamentals dahlia and marigold were severely damaged by this leaf miner. The intensity of infestation per unit area was maximum on tomato (1.74 mines per cm<sup>2</sup>) followed by cowpea (1.14 mines per cm<sup>2</sup>). The number of mines per unit area was lowest for pumpkin (0.33 mines per cm<sup>2</sup>).

In the studies on the seasonal incidence of *L. trifolii* two peak periods of infestation was observed, one during November and the second during January. The leaf miner population was observed to be highest from second fortnight of November to second fortnight of April. A significant positive correlation of larval population with wind velocity, sunshine hours and evaporation rate was observed. Relative humidity and total rainfall had negative correlation with infestation of *L. trifolii*.

Parasitisation of *L. trifolii* by larval and pupal parasitoids were observed and the percentage of parasitoids were observed and the percentage of parasitism was maximum during December, January and February months coinciding with the peak infestation periods. These natural enemies can be effectively utilized for the management of *L. trifolii*.