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Tran, Dang Hoa
Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu Unviersity

Takagi, Masami
Faculty of Agriculture, Kyushu Unviersity

https://doi.org/10.5109/4650

出版情報：九州大学大学院農学研究院紀要. 50 (2), pp.375-382, 2005-10-01. 九州大学大学院農学研究院
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **50** (2), 375–382 (2005)

Developmental Biology of *Liriomyza chinensis* (Diptera: Agromyzidae) on Onion

Dang Hoa TRAN [1] and Masami TAKAGI*

Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

(Received June 15, 2005 and accepted July 26, 2005)

The stone leek leafminer, *Liriomyza chinensis* (Kato), has become a serious pest on *Allium* spp. in several Asian countries. However, knowledge concerning the biology of *L. chinenis*, which affect development of control programs, is still limited. The biology of the leafminer on onion was investigated at a constant temperature of 25°C and a photoperiod of 16L: 8D. To distinguish different larval instars, some morphological characteristics such as the length of mouth hooks, cephalopharyngeal skeleton, body and mine were measured. The length of mouth hooks and cephalopharyngeal skeleton of first, second and third instars were 0.021 mm and 0.089 mm, 0.054 mm and 0.165 mm, and 0.092 mm and 0.261 mm, respectively. Developmental time for the immature stages was 22.6 days; pupal development lasted slightly longer than the combined egg and larval stages. The females laid a mean of 108 eggs and fed on 1013.9 punctures during an average lifespan of 9 days. Feeding and fecundity peaked at age 5 days. The intrinsic rate of natural increase (r_m) (day^{-1}), net reproduction (*Ro*), and generation time (*T*) (day) were 0.099, 14.3 and 27.1, respectively.

INTRODUCTION

The genus *Liriomyza* contains more than 300 species which are widely distributed in the New and Old Worlds but most occur naturally in the temperate regions (Parrella, 1987). Approximately 23 species of *Liriomyza* have been reported as being economically important (Spencer, 1973). As major pests, *Liriomyza* spp. are the targets of chemical and biological control programs (Murphy and LaSalle, 1999). Biological control of *Liriomyza* leafminers by parasitoids can be very effective and give economic control in some vegetable crops (Waterhouse and Norris, 1987; Minkenberg, 1990; Murphy and LaSalle, 1999). To evaluate the effectiveness of parasitoids for biological control in pre–introduction study, data on the development and reproduction of leafminers and their parasitoids are necessary (Minkenberg and van Lenteren, 1987).

The stone leek leafminer *Liriomyza chinensis* (Kato) has become a serious pest on *Allium* spp. in China, Japan, Malaysia, Singapore, Thailand (Spencer, 1973, 1990; Chen *et al.*, 2003), Korea (Hwang and Moon, 1995), Vietnam (Andersen *et al.*, 2002), and Taiwan (Shiao, 2004). However, knowledge concerning the biology of *L. chinensis*, which affect development of control programs, is still limited. The objectives of the present studies

[1] Laboratory of Insect Natural Enemies, Division of Biological Control, Department of Applied Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

* Corresponding author (E-mail: mtakagi@agr.kyushu-u.ac.jp)

were to determine larval morphology, developmental time for egg, larval, and pupal stages and adult feeding, fecundity, and longevity of *L. chinensis* on onion. The knowledge of the basic biological characteristics of this leafminer is of fundamental importance for development of biological control strategies.

MATERIALS AND METHODS

Insect rearing

Liriomyza chinensis used for the present study was originated from a culture reared by the Fukuoka Agricultural Research Center, Fukuoka, Japan. The leafminer was reared on Japanese bunching onion, *Allium fistulosum* L. Seeds of this plant were sown in a tray (20 cm×60 cm×15 cm). Two months after germination, a single plant was transplanted in a plastic pot (9 cm in diameter). A tray (32 cm×44 cm×6 cm) containing 15 potted plants was placed in a small greenhouse at 20±5 °C and 60±10% humidity.

Six potted plants at 2−3 leaves stage were exposed to 50 mixed sex *L. chinensis* adults in a plastic cage (45 cm×30 cm×25 cm) covered with a fine nylon mesh. After an exposure for 24 h, the flies were removed and these plants were maintained in an environmental chamber at a constant temperature of 25 °C and a photoperiod of 16L: 8D until all leafminer larvae feeding on the plants reach pupae. Before incubation, the upper opening of each pot was covered with a piece of reversed funnel–shaped filter paper (11 cm in diameter) to prevent leafminer larvae from pupating in the soil. The pupae were transferred to petri dishes (9 cm in diameter) containing damp soil and maintained at the same condition to gain adult leafminers.

Larval instar distinguishing

Six potted plants were caged and exposed to 50 mixed sex *L. chinensis* adults in a plastic cage (45 cm×30 cm×25 cm) covered with a fine nylon mesh for an oviposition access period of 2−4h. After oviposition access period, the plants were removed and held in an incubator at a constant temperature of 25 °C and a photoperiod of 16L: 8D. To distinguish the three larval stages occurring within leaves, some morphological characteristics such as the length of mouth hooks, body and mine were measured. The measurement of the length of the mouth hooks and cephalopharyngeal skeleton was made in the same manner as described by Petitt (1990). Few days after oviposition, when the mines became visible on the plants, larvae were collected from primary leaves every 12h and preserved in a 70% ethanol: water mixture until examination. A total of 201 larvae were dissected and examined under a stereomicroscope (SMZ1000, Nikon Intech Co., LTD., Japan).

Immature development

Six plants were infested with *L. chinensis* using the method described above and subsequently placed in an environmental chamber at a constant temperature of 25 °C and a photoperiod of 16L: 8D. Eggs, which became clearly visible after a few days, were individually located and marked by circling that area of the leaf with a felt–tip pen under a microscope. Egg hatch was determined by inspecting the leaves with the microscope every 12 hours. Only the larvae that had hatched at the same time were monitored for

larval development time calculation. Larvae that hatched at different time were killed with an insect pin. Larval instars were distinguished using the method described above. The end of larval development was assessed by collection of pupae from the plants at a 12 hours interval. The pupae were individually placed in petri dishes (6 cm in diameter) containing damp soil and maintained at the same experimental conditions. Adult emergence for each pupa and its sex were daily recorded to determine mean development time.

Longevity, feeding and fecundity

Pupae were randomly removed from the insect rearing cages and placed singly in petri dishes (6 cm in diameter) containing damp soil and maintained in the environmental chamber. On the day of adult emergence, one female and two males were released into a plastic cage (35 cm×20 cm×25 cm) containing one onion potted plant. The cages were made of transparent plastic with openings covered with a fine nylon mesh for air circulation. Honey was not provided to the flies. These were kept in environmental chambers at a constant temperature of 25 °C and a photoperiod of 16L: 8D. Plants were exchanged daily and dead males were replaced with newly emerged males until the females died. The number of feeding punctures and visible eggs were daily counted, and longevity of females was determined.

Rate of population increase

The plants daily exposed to female flies were maintained in the environmental chambers until all leafminer larvae reach pupae. The pupae were individually placed in petri dishes (6 cm in diameter) containing damp soil and maintained at the same experimental condition. The fly offspring emerged for each female at a defined age were daily recorded and sexed. The net reproduction rate (Ro), mean generation time (T) and intrinsic rate of natural increase (r_m) were calculated according to the equations given by Birch (1948):

$$Ro=\sum l_x m_x\,;\ T=\sum x l_x m_x / \sum l_x m_x\,;\ \sum (\exp(-r_m x) l_x m_x)=1$$

where, x is female age, l_x is the proportion of females surviving to age x, m_x is the expected number of daughters produced per female alive at age x.

RESULTS

Distinguishing between larval instars

Distinctive separation among the three larval instars of *L. chinensis* was possible using their morphological variables such as length of mouth hook, cephalopharyngeal skeleton, body and mine (Table 1). The mean length of mouth hooks and cephalopharyngeal skeleton of first, second and third instars were 0.021 mm and 0.089 mm, 0.054 mm and 0.165 mm, and 0.092 mm and 0.261 mm, respectively. There was no overlaps in length of mouth hook and cephalopharyngeal skeleton among three instars (Fig. 1)

Immature development

The developmental time for immature stages was 22.6 days (Table 2). The eggs

Table 1. Length (mm, Mean±SE) of mouth hooks, cephalopharyngeal skeleton, body and mine of *L. chinensis* larvae at 25 °C.

Larval instar	First	N	Second	N	Third	N
Mouth hook	0.021±0.0003 (0.018-0.026)[a]	42	0.054±0.0004 (0.042-0.064)[a]	103	0.092±0.0012 (0.076-0.113)[a]	56
Cephalopharyngeal skeleton	0.089±0.0014 (0.065-0.114)	42	0.165±0.0018 (0.126-0.203)	103	0.261±0.0037 (0.213-0.314)	56
Body	0.685±0.023 (0.425-0.97)	34	1.429±0.039 (0.831-2.35)	105	2.61±0.066 (1.68-3.63)	61
Mine	6.4±0.59 (2.3-13.9)	21	12.2±0.89 (5.2-19.2)	21	37.2±2.68 (10.8-63.9)	21

[a](Range)

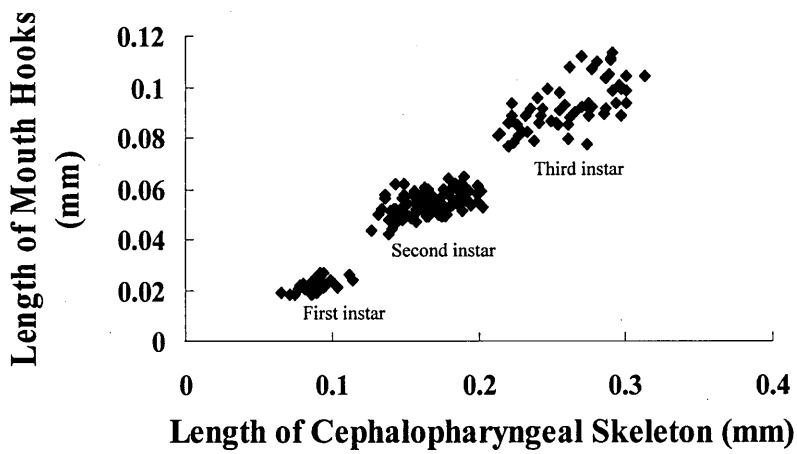

Fig. 1. Relationship between lengths of mouth hooks and cephalopharyngeal skeleton for first, second and third instars of *L. chinensis*.

Table 2. Developmental time (day) of *L. chinensis* at 25 °C.

Stage	Mean±SE	Range	N
Egg	3.4±0.06	2.5-4	50
First instar	1.6±0.08	1-2	35
Second instar	1.7±0.05	1-2	72
Third instar	2.3±0.07	1.5-3	61
Pupa	13.6±0.09	12-16	68
Total	22.6±0.19	20.5-24	35

hatched 2.5 to 4 days after oviposition. The first instar fed on the leaves, and mounted into the second instars after 1 to 2 days. The second instar larvae lasted for 1 to 2 days, then mounted into the third instars. The final instar, after 1.5 to 3 days of its mounting, cut their way out of mines, and finally fell to the ground for pupation. The pupal stage lasted for an average of 13.6 days.

Longevity, feeding and fecundity

The females laid a mean of 108 eggs and fed on 1013.9 punctures during an average lifespan of 9 days (Table 3). While the pre-oviposition period lasted for 2.4 days after

Table 3. Mean feeding (no. punctures), fecundity (visible eggs), longevity (day), feeding rate (no. punctures/day), oviposition rate (viable eggs/day), ratio of eggs and feeding punctures and pre- and post- oviposition periods (day) of *L. chinensis* at 25 °C.

	Mean±SE (range)
Feeding	1013.9±199.1 (191-2592)
Longevity	9±1.1 (4-14)
Feeding rate	104.7±12.1 (47.8-185.1)
Fecundity	108±22.3 (17-281)
Oviposition rate	11.7±1.9 (4.2-25.5)
Eggs/feeding punctures	0.11±0.02 (0.06-0.21)
Pre-oviposition	2.4±0.7 (0-8)
Post-oviposition	0.6±0.2 (0-2)
N	11

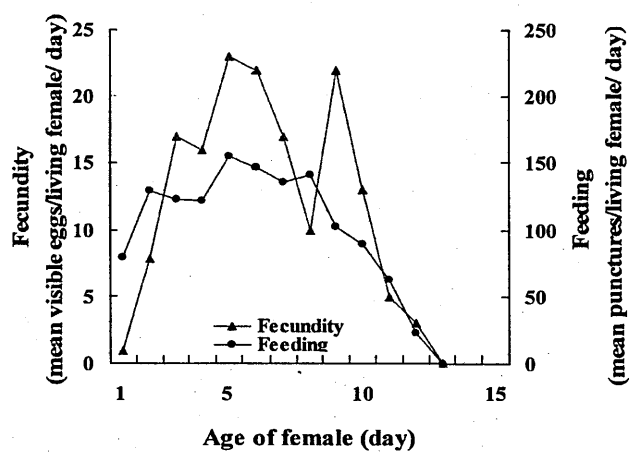

Fig. 2. Gross reproduction and feeding of *L. chinensis* at 25 °C

emergence, the females stopped laying eggs (post–oviposition) just about an average of 0.6 day before death. Mean feeding rate (number of punctures a day) and mean oviposition rate (number of visible eggs a day) were 104.7 and 11.7, respectively.

The oviposition rate showed a slow increase with a peak at day 5, after which a slow decrease followed until females' death (Fig. 2). There was a similar tendency in oviposition and feeding activities. Feeding and fecundity had a correlation coefficient (Fisher's r to z transformation) of 0.58 ($N=11$; $P<0.05$; $Z=1.89$), feeding and longevity of 0.82 ($N=11$, $P<0.01$; $Z=3.26$), and fecundity and longevity of 0.52 ($N=11$; $P>0.05$; $Z=1.64$). Partial correlation between feeding and fecundity was 0.32, and between feeding and longevity 0.74.

Rate of population increase

The population growth of *L. chinensis* at 25 °C was shown by the intrinsic rate of increase value (r_m) of 0.099 day^{-1}. The generation time (T) was 27.1 days. A population may multiply 14.3 times per generation.

DISCUSSION

In the present study, some morphological characteristics such as the lengths of mouth hook, cephalopharyngeal skeleton, body and mine were measured to distinguish the three larval stages of *L. chinensis* occurring within onion leaves (Table 1). Comparison of the morphological variables among three instars of *L. trifolii* revealed that the length of mouth hooks was a reliable character to distinguish the larval instars (Minkenberg, 1999). However, a slight overlap in the size ranges of mouth hook structures for three instars of *L. huidobrensis* was recorded, but this potential source of error can be overcome by using measurements of both the mouth hooks and the cephalopharyngeal skeleton to accurately distinguish between instars (Head *et al.*, 2002). Our study identified that no overlap in variation of mouth hook and cephalopharyngeal skeleton lengths occurred among three instars of *L. chinensis* (Fig. 1). Therefore, measuring the sizes of mouth hook and cephalopharyngeal skeleton will enable the three instars to be easily distinguished.

The efficacy of chemical and biological control agents frequently varies between different instars of a target pest (Head *et al.*, 2002). A previous study on insecticide susceptibility of *L. trifolii* suggested that most insecticides were more efficacious against the first instar than the third instar (Parrella *et al.*,1982). Petitt and Wietlishbach (1993) found that parasitization efficiency of *Opius dissitus* (Hym: Braconidae) was higher when second and third instars of *L. sativae* were provided to the parasitoid as compared to the first instars. Therefore, data indicating the developmental time of *L. chinensis* (Table 2) can be used in population sampling record to optimize control decisions by identifying when the maximum proportion of susceptible individuals are present, facilitating improved timing of application of control measures.

The damage caused by *L. chinensis* to onion plants is very similar to other *Liriomyza* species: larvae mine and feed within the leaves, and females produce feeding punctures on the leaves with their ovipositor. The adults feed from all punctures, regardless of whether or not they are used for oviposition (Bethke and Parrella, 1985;

Parrella, 1987). The feeding activity of a female was age–dependent and increased sharply during the early day of her life to a peak at day 5, after which feeding rate declined with age (Fig. 2). Females made an average of 1013.9 punctures during her lifespan (Table 3). Leaf punctures can reduce photosynthesis and may kill young plants (Elmore and Ranney, 1954; Parrella *et al.*, 1985). Therefore, leaf puncturing and feeding by adult *Liriomyza* undoubtedly serve an important role in host plant loss assessment (Parrella, 1987).

The feeding punctures can also serve as oviposition sites. Egg laying begins within 2.4 days after female emergence and peaks at day 5, decreasing thereafter until females' death (Fig. 2). The females laid a mean of 108 eggs during an average lifespan of 9 days (Table 3). Fecundity and longevity of *Liriomyza* females are strongly related to food resources (Parella, 1987). Direct access to honey greatly enhances fecundity and longevity of *L. trifolii* (Zoebisch and Schuster, 1987). However, sugar sources were not likely to present under commercial onion greenhouse conditions, they were not provided to the flies in this study. The present study indicates significant correlation coefficients between feeding and fecundity, and feeding and longevity in *L. chinensis* females. This result is consistent with research in The Netherlands indicating that feeding on plant sap was of importance both for the production of egg and for the prolongation of life span (Minkenberg, 1999).

The average net reproduction rate (*Ro*) suggests that *L. chinensis* population would increase 14.3 times during each generation. The average intrinsic rate of natural increase was 0.099 per individual per day at 25 °C. Although such data are necessary to predict the reproductive potential of *L. chinensis* population in greenhouses and open fields, further experiments at different temperature levels are required, since Minkenberg (1999) showed that the net reproductive rates of *L. trifolii* and *L. bryoniae* were variable with temperatures. To predict population dynamics of *L. trifolii* under field conditions at fluctuating temperatures, interpolation from data measured in the laboratory at constant temperatures is only possible when the life history variables react instantaneously to temperature (Minkenber, 1999)

ACKNOWLEDGMENTS

We thank Mr. Hiroyuki Takemoto (Fukuoka Agricultural Research Centre) for providing *L. chinensis*. This work was supported in part by a Grand–in–Aid from the Japanese Society for the Promotion of Science and Technology (No. 15208007).

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