LIFE CYCLE OF *Liriomyza huidobrensis* (BLANCHARD) (DIPTERA: AGROMYZIDAE) ON POTATO IN LABORATORY CONDITION IN NEPAL

Binu Bhat and Ajaya Shree Ratna Bajracharya

National Entomology Research Center, Khumaltar, Lalitpur, Nepal.
Correspondence: binubhatchhetri@gmail.com

ABSTRACT

Pea leafminer, *Liriomyza huidobrensis*, is a key pest of potato in mid hills of Nepal causing up to 90% foliar damage. The confirmation of the insect and determination of its life cycle is the basic requirement for development of integrated pest management protocol technology. Considering the fact, life cycle of *L. huidobrensis* was studied at 27 ± 2 ^{0}C temperature and 70 ± 5% relative humidity at the National Entomology Research Center, Lalitpur, Nepal on potato cv. Khumal Seto from March to April, 2023. The insect species was confirmed as *L. huidobrensis* on the basis of morphological similarities of bristles, male genitalia and wing venation with the reference species *L. huidobrensis*. The average duration of pre-oviposition, egg, larval and pupal periods were 2.35, 3.18, 4.47 and 7.88 days, respectively. Adult male and female longevity was 3 days without feeding and 6 and 10 days with honey water feeding for both male and female. The male and female adult emergence ratio was 1:1.41. Eggs were oval and white in colour measuring 0.30 mm long and 0.10 mm. Pupae were coarctate type measuring 2.10 mm in length and 0.90 mm in breadth. Wing span of adult female and male were 1.92 mm and 1.70 mm in length, while width measured 0.78 mm and 0.67 mm, respectively.

Key words : Pea leafminer, distiphallus, *L. huidobrensis*, life cycle, morphological study.

INTRODUCTION

Pea leafminers, *Liriomyza huidobrensis* (Blanchard) are polyphagous insects belongs to the genus *Liriomyza* (Diptera: Agromyzidae) and is considered as pest of solanaceous and many other crop families causing leaf damage (Roert, 1999). *Liriomyza* is the one of the largest genus of order Diptera 1990. Among them, *L. huidobrensis*, *Liriomyza sativae* (Blanchard), and *Liriomyza trifolii* (Burgess) are three important pest species of cereal and vegetable crops. Both adult female and larva cause significant damage in high value crops and ornamental plants through ovipositional puncture and larval mining (Kwon et al., 1995). *L. huidobrensis* causes significant damage to the plants belonging to families: Solanaceae, Cucurbitaceae, Leguminosae, Brassicaceae, Asteraceae, and Compositae (EPPO, 2009; Spencer, 1973, 1990). *L huidobrensis* damages beans, pea, celery, lettuce, pepper, spinach and tomato in the many provinces of Argentina (Caldiz, 2006).

Immediately after hatching, larvae enter into the leaves from lower surface and feed on spongy mesophilic tissues adjacent to the mid and lateral veins and damage the leaves by mining in irregular serpentine pattern (Maharjan, 2014; Spencer, 1973). Larval mining in the palisade parenchyma tissue greatly reduces the photosynthetic capacity of plants which results in the reduction of the potato yield (Heinz & Chaney, 1995; Parrella et al., 1985). According to Johnson et al. (1983) photosynthetic

capacity was found reduced up to 60% due to the insect feeding. Adult feeding of *L. huidobrensis* on leaves can cause secondary infestation as it creates entry sites for pathogens such as *Alternaria alternata* (Fr.) Keissl (Deadman et al., 2000). A Survey was conducted at commercial potato growing areas of mid hills in Lalitpur, Kathmandu, Bhaktapur, Kavrepalanchwok, Sindhupalchwok and Makwanpur districts of Nepal during 2009 and the highest foliar damage was recorded from Lalitpur (89.3%) followed by Kathmandu (82.6%), Bhaktapur (70%), Kavrepalanchowk (46.5%), Makawanpur (27.9%) and Sindhupalchowk (12.1%) (Chiluwal et al., 2012). *L. huidobrensis* is the key pest of main season (winter) potato cultivation at mid hills of Nepal but its study on biology and life cycle is lacking in Nepal.

Outbreak of leafminer caused heavy damage and economic loss of potato as insect changed from potential to key pest due to overuse of insecticides, which negatively affected the population of natural enemies (Vincini & Carmona, 2006).

It is necessary to study life cycle of the pest on potato which is the base for the development of integrated management technology of the pest. Life cycle is the powerful tool for understanding the total life duration of the pest, number of generations per season, their biology and habitat. Similarly, the proper identification of the insect pest is necessary before conducting scientific studies of the pest. Therefore, the present morphological and life cycle studies of *L. huidobrensis* were conducted in a laboratory condition at the National Entomology Research Center (NERC), Khumaltar, Lalitpur, Nepal.

MATERIALS AND METHODS

Studies were conducted at the National Entomology Research Center (NERC) of Nepal Agriculture Research Council (NARC), Khumaltar N 27º39'03.74", E 085º19'38.59", Lalitpur from March to April 2023. The study site was located at 1,308 m above mean sea level. The temperature and relative humidity were maintained at 27 ± 2 ^{0}C and 70 ± 5%, respectively in the laboratory conditions.

Life cycle study

Liriomyza huidobrensis population was collected from the potato field of Tukucha Nala of Kavrepalanchok to maintain the laboratory colony in NERC, Khumaltar.

Infested leaves of potatoes with *L. huidobrensis* larvae were collected from the potato field and kept in transparent zip-lock polybags (40 cm x 30 cm). The infested leaves were transferred into rectangular, transparent, plastic rearing boxes of size 21 cm x 15 cm x 8 cm in length, breadth, and height, respectively, with ventilated lids covered on the top with black muslin cloth. The culture is maintained in the controlled laboratory condition at 27±2 $^{\circ}$C and 70 ± 5% relative humidity. Pupae present on the cultured leaves and dropped pupae from the leaves were collected and transformed into other round plastic boxes (5.5 cm height x 5.2 cm diameter) with aerated lids covered with black muslin cloth on the top and continued until adult emergence. Newly emerged adults were collected and transferred in oviposition cages which contained potato plants of 8-10 leaves.

Potato plants grown from the variety "Khumal Seto" were used for the laboratory experiment, which is also a widely grown potato variety in the mid-hills of Nepal. Individual seed potatoes of approximate size of 50 g were planted in plastic pots (12 cm diameter and 13.5 cm height) filled with

soil and farmyard manure. The plants were raised in controlled laboratory conditions. The plants at 8-10 leaves stage were used for the oviposition of adult flies.

Morphological studies

Insect reared in the laboratory was used for the confirmation of the species. Identification up to the family level was performed by utilizing keys defined by Boucher (2010), Henning, (1958) and Spencer (1987). Similarly, a genus of the insect was confirmed by observing the keys described by Spencer (1976). According to Shiao (2004), the identification of various species of *Liriomyza* is based on "Distiphallus" and "Epiphalus" parts of the male genitalia. Sex differentiation was done by observing "Epandrium" of male and female. Studies on external morphological characters such as setae, head, and wing venation were carried out as suggested by EPPO (2022) and IPPC (2016). For the study of male genitalia, the abdomen of the adult insect was removed from the body and cleared all tissues by using fine dissecting needles. Abdominal tissues were removed by using 10% of potassium hydroxide (KOH). Under Stereomicroscope fine needles were used to dissect the male genital complex and distiphallus and epiphallus were removed and observed. On the basis of male distiphallus and epiphallus, the insect species was confirmed. The whole morphological studies were conducted on 30 insect sample under stereomicroscope (Olympus SZ61) and camera (Bestscope BUC 2B-500C).

Fig. 1. Rearing of *L. huidobrensis* on potato leaves in NERC laboratory.

Life cycle of *L. huidobrensis*

A single potted potato plant with 8-10 leaf stage was placed inside each cage (47.5 cm x 31 cm) (Fig. 1). One pair adult leafminer were released inside the cage. After releasing, leafminers were allowed to feed and reproduce inside the cage plants and to lay eggs on the surface of the leaf, after which

adult leafminers were removed. Data on the development of egg, larva, pupa, and adult with potato as host were obtained. Insects were then removed and observed every 24 hours for egg hatch and larval emergence from leaves, larval duration was recorded as the period from egg hatching to formation of pupa and comes out of mines. Pupae were separated individually in the vial. These were checked at interval of 24 hours for adult emergence, and the sex of each fly is recorded. The newly emerged adult was placed inside the potted cage to observe the adult period with feeding, and a few were placed without feeding to observe the adult period without feeding.

RESULTS AND DISCUSSION

Identification of potato leafminer species on the basis of morphological character was done by examining the genitalia of the male fly under a stereomicroscope. The sex of flies was determined by observing the lobes of epandrium. In female flies epandrium is a tube like structure which were dark and heavily sclerotized with a circular tube opening visible in posterior view at the end of the tube and in male flies it was black and less sclerotized. Similar characteristics was mentioned for identification of male and female leafminer flies (IPPC, 2016). Vibrissae were present on the head region and post vertical bristles were in a divergent pattern (Fig. 2), Wings with coastal break present at the apex of Sc (Sub costa) and wing cell cup wing small–wing veins A_1+ CuA_2 not reaching the wing margin were observed (Fig. 4) which confirmed the family Agromyzidae (Henning, 1958; Spencer, 1987; Boucher, 2010) Genus *Liriomyza* was confirmed by examining following characters Frontal setulae reclinate (Fig. 3), Subcosta fold distally and ends in costa separately, costa extends to vein M_{1+2}, distal cell (dm) small and presence of second (outer) crossvein (dm-cu) (Fig. 4). Similar descriptions mentioned by Spencer (1976), while describing the genus *Liriomyza*. Vein Cu1A in *Liriomyza* species consists of two parts 'a' and 'b' (Fig.4). The ratio of parts (a/b) was found 2.2 in males and found 2.1 in female flies in our present study (Table 1). In species *Liriomyza huidobrensis,* a ratio of such vein parts a and b ranges between 2- 2.5 (EPPO, 2005; IPPC, 2016).

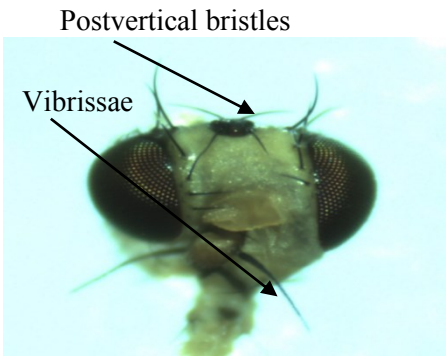

Fig. 2. Postvertical bristles divergent, Vibrissae present.

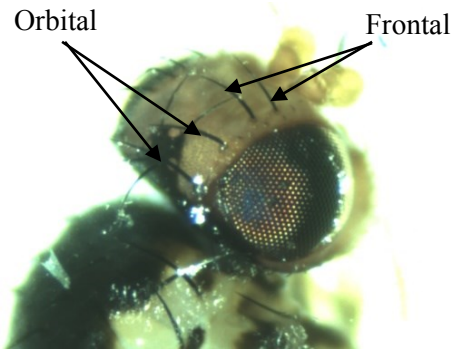

Fig. 3. Fronto-orbital setae reclinate (backward-pointing).

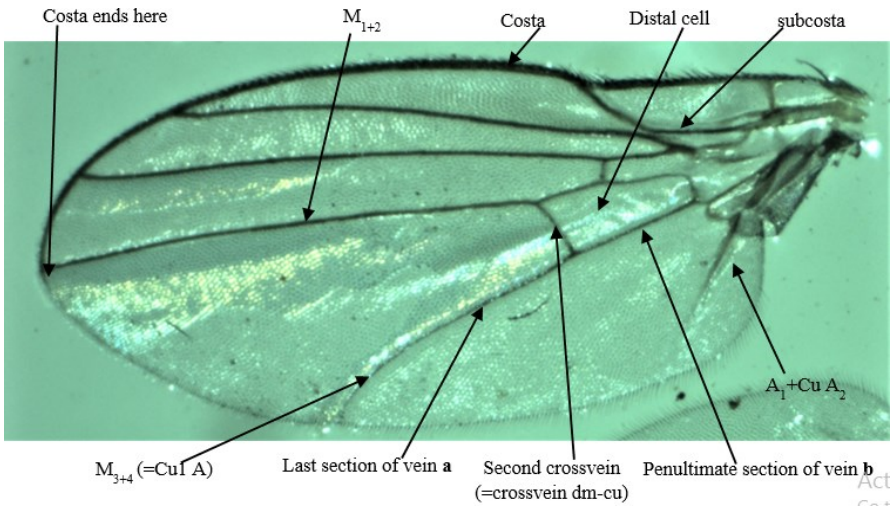

Fig. 4. Wing of *L. huidobrensis* with demonstrating various identification morphological features.

Distiphallus of the insect was in the shape of two distal bulbs, meeting only at their rims and bulbs rim drawn out antero- ventrally (Fig. 5). The apex of epiphallus was found round (Fig. 6). These are the most important characteristics for the identification of *L. huidobrensis* (EPPO, 2005; IPPC, 2016; Shiao, 2004).

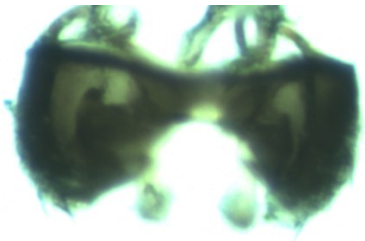

Fig. 5. Male distiphallus

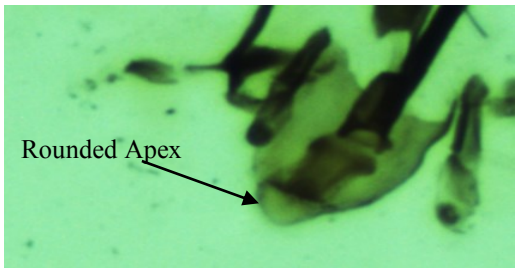

Fig. 6. Rounded apex of Epiphallus (Male)

Table 1. Measurements of parts of wing vein Cu1A of *L. huidobrensis* (n = 30)

Parts of Cu1A	Average length (mm)	Ratio (a/b)
Female		
Last section a	0.63	2.2
Penultimate section b	0.29	
Male		
Last section a	0.53	2.10
Penultimate section b	0.25	

Life cycle of *L.huidobrensis* consists of four development stages viz. egg, larva, pupa and adult (Fig. 7). Adult female flies use their ovipositor to puncture potato leaves that cause wounds. They puncture the leaves to lay eggs and to feed on ooze coming from punctures. Generally, eggs were laid singly on the lower surface of the leaf and are inserted just below the epidermis. Average duration of different developmental stages of *L. huidobrensis* on potato leaves in laboratory conditions is presented in Table 2. The average pre-oviposition period was recorded as 2.35 days. The mean duration of the egg stage in the present study was recorded at 3.18 days (Table 2). Parrella and Bethke (1984) reported the incubation period of *L. huidobrensis* as 3.0, 3.0, and 2.9 days on chrysanthemum, aster, and peas, respectively. The egg period of *L. huidobrensis* was reported to range between 2-5 days by various workers (Wei et al., 2000; Visser 2015; IPPC, 2016). Haghani, Fathipour et al. (2007) reported 3.1 days incubation period of *L. sativa* while studying life cycle at 30 ^{o}C temperature.

Larvae are off-white in color in the early stage and mine the leaf surface through the green palisade tissue, later color changes into pale yellow orange. Due to continuous feeding frass appeared as a broken line on the surface of the leaves. The larval mines were found in irregular shape mainly in serpentine pattern and increased in length as larvae grow. Discoloration of mines occurred with dried brown areas on a later stage. Feeding punctures were found to larger in size than ovipositional punctures which can be observed with naked eyes. Feeding punctures are larger in size due to destruction of large number of cells (EPPO, 2005). Larva fed on the same leaf in which the egg was laid and came out of the leaf mine for pupation in soil or on the leaf surface. Parrella and Bethke (1984) also reported similar behavior of larvae of *Liriomyza* spp. The mean larval period was recorded at 4.47 days in our present study. Parrella and Bethke (1984) reported similar larval period of *L. huidobrensis* recording 4.7 and 4.9 days on chrysanthemums and aster whereas, a shorter larval period of 3.6 days on peas. Hincapie et al. (1993) reported a longer larval period (5.54 days) at 24.7 ^{o}C temperature. Haghani et al. (2007) conducted a study on the developmental period of *L. sativa* and reported a larval period of 4 days.

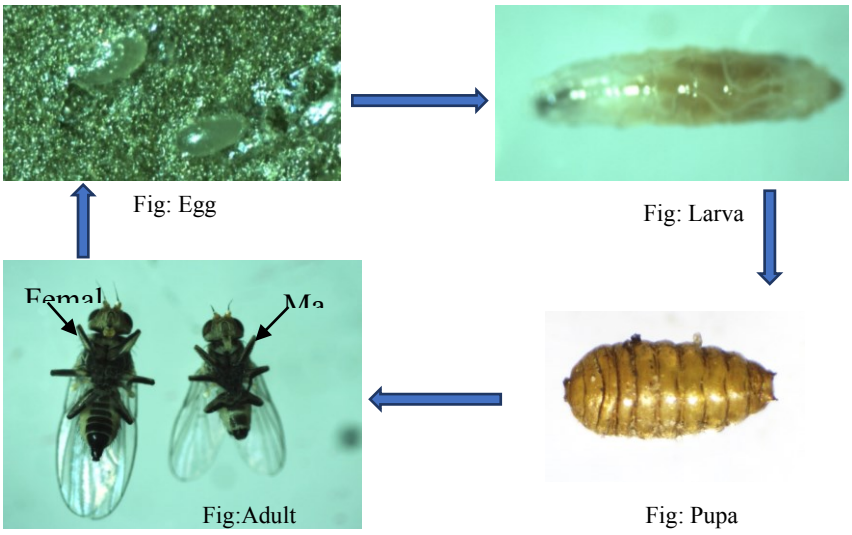

Fig. 7. Different development stages of *L.huidobrensis* (a) Eggs (b) Larvae (c) Pupa (d) adult female and male.

Pupae were coarctate type and the average pupal period was found 7.88 days (Table 2). Parrella and Bethke (1984) reported pupal periods ranged between 8.5 to 9.3 days on chrysanthemum, aster, and pea, respectively. Hincapie et al. (1993) reported a mean pupal period of 8.14 days. Haghani et al. (2007) conducted a study on the development period of *L. sativa* and reported 6.8 days at 30 ºC and 10.2 days at 25 ºC. Our present study was conducted at 27 ºC temperature and the pupal period lay within above findings.

The adult period was varied with and without feeding on 10% honey solution in distilled water. Adult males and female survived for 3 days without feeding. The male survived 6 days and female 10 days with feeding. Female flies were found to live longer than male flies. Hincapie et al. (1993) also reported the adult longevity of females (15 days) was longer than males (7.6 days). The male-to-female emergence ratio was found at 1:41 in the present life cycle study. Similar ratio of 1:1.42 was reported by Hincapie et al. (1993).

Table 2. The average duration of different developmental stages of *L. huidobrensis* on potato leaves in laboratory conditions (n = 30)

Stages	Average Days	Range (Days)
Pre-Oviposition	2.35	2-3
Egg Period	3.18	2-4
Larvae	4.47	3-7
Pupae	7.88	7-10
Adult Male (with feeding)	4.85	1-6 days
Adult Male (without feeding)	1.92	1-3 days
Adult Female (with feeding)	7.17	2-10 days
Adult Female (without feeding)	2.40	2-3 days
Male female ratio	1:1.41	

Morphometrics of egg, pupa, and adult wing of *L. huidobrensis* is presented in Table 3. The eggs were oval in shape and white in color. It measured about 0.30 mm in length and 0.10 mm in breadth. Mugala et al. (2022) recorded egg lengths 0.15 to 0.30 mm and CABI (2018) reported 0.20-0.30 mm length and 0.10- 0.15 mm breadth. Pupae are oval in shape and average length and breadth was 2.10 mm and 0.90 mm, respectively. CABI (2018) reported pupal size of 1.3-2.3 mm long and 0.50-0.75 mm wide. A similar pupal length of 2.0 mm was reported by IPPC (2016). Pupae were brown initially and became black before adult emergence. Female wings measured 1.92 mm and 0.78 mm while adult male wings were about 1.70 mm and 0.67 mm in length and breadth, respectively. A similar wingspan of 1.70 - 2.25 mm in length was reported by IPPC (2016).

Table 3. Morphometrics of egg, pupa, and adult wings of *L. huidobrensis* (n = 30)

Parameters	Average length (mm)
Egg Length	0.30
Egg Breadth	0.10
Pupa Length	2.10
Pupa Breadth	0.90
Female Wings Length	1.92
Female Wings breadth	0.78
Male Wings Length	1.70
Male Wings breadth	0.67

CONCLUSIONS

The leafminer infesting potato plants in Kavrepalnchowk district was confirmed as *L.huidobrensis* of based on a morphological study. The insect had a developmental period of 21.53 to 25.53 days in laboratory conditions of 27 ± 2 ^{0}C temperature and 70 ± 5% relative humidity. Egg, larval, and pupal periods of the insect were 3.18, 4.47, and 7.88 days, respectively. Adult male and female flies survived for 6 and 10 days. Present findings on the life cycle and morphology of *L. huidobrensis* will help in the management and further studies of the insect in the future.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the management team of the Nepal Agriculture Research Council for providing the opportunity and financial support to study. Help from the technician Mr. Hark Bahadur Balayar is highly acknowledged. We would like to acknowledge all the staff of NERC.

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