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Biology and larval depiction of an agromyzid leaf miner pest, *Ophiomyia phaseoli* on pea plant (*Pisum sativum*)

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Abstract
Ophiomyia phaseoli (Tryon) (Stem fly or stem fly) is a polyphagous species belonging to the Agromyzidae family. In India, it is a major pest of pea plants, particularly *Pisum sativum*. Female stem fly be apt to place the eggs on the superior (upper) surface of leaves, mostly near the mid rib. The larvae are cortex feeder which feeds exclusively on internal tissues of plant that causes punctures in the seedlings and mesophyll tissues of the leaves. Extensive feeding form a linear serpentine mine on leaf surface known as phyllonomes. Hence, its biological study is necessary for safeguarding of crops from its severe attack and damage. The current study was accompanied on studying the complete biology of stem fly with the objectives of arriving at the crucial conclusion to decrease pest infestation for high potential yield and productivity with the development of new strategies to decrease damage by targeting various phases of stem fly.

Keywords: *Ophiomyia phaseoli*, *Pisum sativum* (pea plant), stem fly, biology, phyllonomes

1. Introduction
Agromyzid, popularly known as "leaf miners" are known to infest a wide range of cultivated crops as well as wild plants. Around 2,500 species of agromyzidae have been reported so far [1, 2]. They are small; with maximum size is 6.5 mm and wing length of 1 mm. They are polyphagous and are major pest of tropical and subtropical areas. A few of them are monophagous but have high degree of host specificity [3].
Agromyzidae larvae are phytophagous and exclusively feed the internal tissue of the plants. Adult females attack on the superior (upper) surface of the leaves while larvae feed inside the plant tissues causing harsh damage to the plants. Severe attack of these leaf miners causes a significant decrease in photosynthetic assimilated food production, that further lead to desiccation and premature fall of leaves. Feeding punctures made by adult females damages the seedlings and young plant tissues. In the past few years, the biology of many species agromyzidae family has been studied [4-6]. Hering, 1951 reviewed the literature on the biology of many flies [7]. Numerous other workers also contributed to the biology of certain species of leaf miners (Table 1).
The common Indian species of Agromyzidae family, *Ophiomyia phaseoli* (Tryon) (stem fly or stem fly) causes severe damage to pea plant (*Pisum sativum*) which is a major cultivation crop in India. The distribution of *Ophiomyia phaseoli* (Tryon) extends throughout tropical and subtropical Africa, Asia, Australia and Middle East [20, 21].
They attack on variety of vegetables, particularly leguminous plants and ornamental crops. The extent of damage varies from crop to crop and season to season, being especially severe to seedlings. If the plant assaulted by stem fly survives, the impact of the injury might be showed later in the grownup plants. In extreme assaults, initially the infested leaves hang down, then shrivel and may even fall. The stems can break and yield is also low. Overall plant growth is stunted and it may die; yield losses in some east-Asian countries can come to 30-50% and even to 100% [22, 23].
But, ominously the complete biology of the *Ophiomyia phaseoli* is very meager in the literature. The larvae of these flies make tunnel in to the leaves of pea plants and start feeding voraciously causing great loss of the crop [24]. Hence, a detailed study of their biology on pea plant and control is greatly needed. The present paper comprises observations on the biology of *Ophiomyia phaseoli* (Tryon) which attacks a variety of garden plants; these observations are made on the host plant *Pisum sativum*.

Table 1: Biology of Some Agromyzid

Species	Author	Year	Reference
Phytomyza articornis	Ahmed and Gupta	1941	[8]
Species of Diptera	Allen	1956	[9]
Liriomyza pictella	Oatman & Michelbacher	1959	[10]
Lomamyia latipennis	Tauber & Tauber	1968	[11]
Phytomyza atricornis	Tandon	1970	[12]
Phytomyza matricariae	Sehgal	1971	[13]
Liriomyza	Beri	1971	[14]
Coleomegilla maculata	Solbreck	1974	[15]
Liriomyza brassicae	Beri	1974	[16]
Chromatomyia horticola	Singh	1982	[17]
Liriomyza trifolii	Schuster & Patel	1985	[18]
Liriomyza sativae	Petitt	1990	[19]

2. Materials and Methods

The field experiment to study insect pest complex of pea was conducted at Department of Zoology, SLS Khandhari Campus, Dr. B. R. Ambedkar University, Agra, Uttar Pradesh, India during Rabi season of 2019-20. The flies used for the present study were bred on *Pisum sativum*.

Sweet seeds of pea were grown in the earthen pots which were kept under cage of transparent cloth respectively. These plants were observed on the biology of adult females and larvae were made under laboratory conditions of 11-27°C temperature and humidity 65- 73%. The observations were made with the help of binocular microscope, hand lens and compound microscope etc. The specimens were identified in Department of Zoology, at Dr. B. R. Ambedkar University, Agra, India.

3. Results and Discussion

3.1 Copulation

The male and female flies were kept under the normal temperature and humidity in the transparent cloth cage on a healthy plant. Mating was observed only after 8-10 hrs of emergence. Mostly, copulation occurs between 8 am to 5 pm. Oatman and Michelbacher, 1959 recorded copulation in *Liriomyza pictella* within six hours after emergence [10]. Venugopal & Venkatraman (1954) also observed mating once a day after the emergence in *Melanogromyza obtuse* [25]. Ahmad & Gupta (1941) and Ipe (1965) reported mating in *Melanagromyza obtnsa* between 8.00 am to 4.00 pm nine days after emergence while Beri (1974) observed it only up to 10.00 am in *Liriomyza brassicae* [8, 16, 26].

The male fly mounts on the female with the help of its second pair of legs and introduces intromittent organ into the female genital chamber .The copulation continued only for 20 to 30 minutes. Oatman and Michelbacher (1959) in *Liriomyza pictella* observed the duration from 30-180 minutes and 22-45 minutes respectively [10].

3.2 Eggs

The female *Ophiomyia phaseoli* (Tryon) laid the creamy white rounded eggs in mesenchymatous tissues of leaf below the epidermis in apuncture as also reported by Beri (1974) [16]. The eggs on both the ends measured an average 0.28 mm in length and 0.16 mm in breadth. At the time of oviposition, the anterior region of eggs comes out first.

After rupture of chorion, the head region of larvae comes out after hatching the larvae makes its way into mesophyll tissue of leaf forming the mine on the lower and upper surfaces of the leaves. Hering (1951) and Beri (1971) noted that the egg shell is discarded and eaten by the larvae [7, 14]. In the present study, however, the larvae of *Ophiomyia phaseoli* (Tryon) do not eat the egg shell. It is worthwhile noting that the female fly mostly dies after lying of eggs. The egg viability showed most variations with minimum viability recorded 70.58% while the maximum 83.88% and average viability is 78.45% (Table 2).

3.3 Incubation Period

The incubation period under laboratory conditions varied between 36-57 hours & 20 minutes at 11-27°C temperature and 73% relative humidity. The average time period was 45 hours and 11 minutes 1 in accordance with the temperature variations. The higher the temperature, the lesser was the time to hatch. The average viability of the eggs after hatching was 79.42% under the laboratory conditions, during December and January in 2019-20 (Table 2).

Table 2: Incubation Period of *Ophiomyia phaseoli*

No. of Observations	Eggs Laid on (Date/Time)	Eggs Hatched (Date /Time)	Incubation Period	Average Incubation
1	10.12.2019 / 3.15 p.m.	12.12.2019 / 7.55 a.m.	40 hrs 40 min	45 hrs 11 min
2	11.12.2019 / 4.20 p.m.	13.12.2019 / 4.20 a.m.	36 hrs	
3	12.12.2019 / 3.00p.m.	14.12.2019 / 9.00a.m.	42 hrs	
4	13.12.2019 / 9.00 a.m.	15.12.2019 / 2.25 a.m.	41 hrs. 25 min	
5	14.12.2019 / 10.20 a.m.	16.12.2019 / 12.00 noon	49 hrs. 40 min	
6	15.12.2019 / 8.20 a.m.	17.12.2019 / 9.50 a.m.	49 hrs. 30 min	
7	16.12.2019 / 9.00 a.m.	18.12.2019 / 2.10 p.m.	53 hrs. 10 min	
8	17.12.2019 / 7.50 a.m.	19.12.2019 / 8.10 p.m.	48 hrs. 20 min	
9	18.12.2019 / 8.15 p.m.	20.12.2019 / 9.00 p.m.	48 hrs. 45 min	

3.4 Larval Period

Duration of the larval form was particularly 4-5 days, 3 hrs. and 30 minutes with an average of 4 days and 3 hrs. during winters under laboratory conditions. Conversely, in the field conditions, the larval stages lasted from 6-9 days with an average of 7 days and 7.2 hrs. during winters (Table 3). It can be identified in 3 different instars (Table 4).

Table 3: Duration of Larval Period *Ophiomyia phaseoli*

No. of observation	Larva hatched on	Larva Pupated	Duration of larval period	Average duration
1	14.12.2019	20.12.2019	6 days	7 days 7.2 hrs.
2	15.12.2019	23.12.2019	8 days	
3	16.12.2019	23.12.2019	7 days	
4	21.12.2019	27.12.2019	6 days	
5	23.12.2019	29.12.2019	6 days	
6	27.12.2019	02.01.2020	7 days	
7	30.12.2019	08.01.2020	9 days	
8	01.01.2020	10.01.2020	9 days	
9	04.01.2020	11.01.2020	7 days	
10	09.01.2020	17.01.2020	8 days	

Larval period was observed in winters at 11.5°C-27.5°C temperature & 74% relative humidity under field condition.

3.5 First Instar Larva

The first instar larva at the time of hatching was glistening white or creamy. Cephalopharyngeal skeleton was clearly visible at its anterior end through the larval skin. The anterior spiracles were absent in this stage but posterior spiracles were very minute but like in structure, the cephalopharyngeal skeleton was triangular in shape. It works as a grasping organ. The average life span of 1st instar larva was 26 hrs. and 52 minutes.

3.6 Second Instar Larva

The cylindrical body of larva was segmented. The anterior spiracles situated dorsally on the middle of prothracic segment bearing spherical bulbs arranged in a row. The cephalopharyngeal skeleton was better developed from 1st instar larva. The duration of this stage varies from 24-32 hrs. and 10 minutes with an average of 27 hrs. 30 minutes was evidenced. In this stage, larva bore in to deeper layer of mesenchymatous tissues and become more greenish in color. Posterior spiracles were located at the last abdominal segment and maxillary papillae in the head region were distinct.

3.7 Third Instar Larva

The third instar larva was the last stage of larval condition which was the most damaging stage of the agromyzid pest to the host plants. The anterior and posterior spiracles bear 6 and 3 bulbs respectively. The cephalopharyngeal skeleton was moderately sclerotized and the ventral process was less than half of the dorsal process. The life span of third instar varied from 2 days, 2 hrs. to 2 days, 22 hrs. & 5 minutes with an average of 2 days 8 hrs. The 3rd instar is 2.5 mm in length and 0.39 mm in breadth.

The larvae feeds on the parenchymatous tissue of the leaves with the result of extensive feeding a linear mine zigzag in shape is formed (Table 4).

Table 4: Different Larval Stages of *Ophiomyia phaseoli*

Stage	Duration	Average
First Instar	26 hrs. 40 min – 27 hrs. 45 min	26 Hrs.
Second Instar	26 hrs. 9 min – 26 hrs. 15 min	26 Hrs.
Third Instar	2 days 18 hrs. – 3 days 10 hrs.	2 days 10 hrs.
Total Larval Stage	4 days 21 hrs. - 5 days 4 hrs.	4 days 10 hrs.
Total Pupal Period	8 days 1 hr. – 8 days 2 hrs.	8 days 1 hr. 35 min

3.8 Pupal Period

The last instars changed in to the pupa. The body was light yellow, cylindrical and distinctly segmented. The anterior and posterior spiracles lied at the respective extremities. The average duration of pupal period was 8 days at 11-27°C temperature and 73% relative humidity during the month of December and January (Table 5).

Table 5: Duration of the Pupal Period of *Ophiomyia phaseoli*

No. of Observation	Pupated on Date/Time	Emerged on Date/Time	Duration of Pupa	Average Duration
1	25.12.2019 / 8.50 p.m.	02.01.2020 / 9.00 p.m.	8 days 10 min	8 days 1 hr. & 35 min
2	25.12.2019 / 8.55 p.m.	03.01.2020 / 10.55 p.m.	9 days2 hrs.	
3	27.12.2019 / 6.55 p.m.	06.01.2020 / 6.55 p.m.	10 days	
4	27.12.2019 / 11.35 p.m.	05.01.2020 / 1.40 p.m.	9 days 2 hrs. 5 min	
5	28.12.2019 / 5.15 p.m.	06.01.2020 / 5.15 p.m.	9 days	
6	29.12.2019 / 11.40 a.m.	05.01.2020 / 12.00 a.m.	7 days 20 min	
7	31.12.2019 / 8.40 p.m.	08.01.2020 / 9.15 p.m.	7 days 35 min	
8	01.01.2020 / 10.50 a.m.	07.01.2020 / 11.00 a.m.	6 days 10 min	
9	03.01.2020 / 12.15 p.m.	12.01.2020 / 12.15 p.m.	7 days	
10	03.01.2020 / 12.35 p.m.	11.01.2020 / 1.00 p.m.	6 days 25 min	

Pupal period was observed at 11.5 -27.5°C temperature & 73.5% relative humidity.

3.9 Emergence

The adult fly emerged forming a slit and the lateral regions extending from the head to the metathoracic segment and dorsally on the metathoracic region, separating out a flap. For this process, 5 min - 1 hour was consumed. At the time of emergence, the wings remained folded. The head and first pair of legs emerged out first. With the help of fore legs, the remaining body comes out. The emergence occurred mostly

in the evening hours when temperature was low (Table 6). Ipe (1965) observed in the emergence in the morning hours in *Melanogromyza obtuse* [26].

Table 6: Emergence of Adult Fly of *Ophiomyia phaseoli*

No. of Pupa	No. of males	No. of Females	Sex Ratio	
			Male	Female
80	35	45	43.75	56.25
75	33	42	44.00	56.00
62	30	32	48.39	51.61
46	20	26	43.48	56.52
54	22	32	40.74	59.26
36	20	16	55.56	44.44
74	34	40	45.95	54.05
65	32	33	49.23	50.77

4. Conclusion

The consequence of stem flying on the pea plant does substantial damage to the crop. The present chapter involves the biology and stem fly evolution needed to look for alternatives to keep away the flies from crop. In breeding pea plants for stem fly resistance, a crucial approach is to define with a comprehensive screening technique that will provide the plant with constant defense against stem fly populations. The majority of the screening methods were focused on open-field methods, which have their own downsides. Therefore, a reliable methodology must be established that will help to classify resistant lines positively.

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