Seasonal incidence of apple leaf miner (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) in Kashmir, India

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Abstract: The seasonal incidence of apple leaf miner (Lyonetia clerkella [L., 1758], Lepidoptera, Lyonetiidae) was investigated in four districts of Kashmir valley from March 2015 to April 2016. The prevalence of infestation was found higher in Srinagar (70.6 %) and Bandipora (65.3 %) as compared to Pulwama (9.3 %) and Baramulla (6.6 %). Infestation intensity was found at its peak during the month of May (2015) in all the four districts. In Srinagar, the percent infestation intensity during May (2015) was found to be 58.69 % (± 11.46 SD), while as in Bandipora, Pulwama and Baramulla, it was found as 55.71 % (± 12.59 SD), 6.04 % (± 1.97 SD) and 4.27 % (± 1.12 SD) respectively. Infestation of intensity was observed to decline linearly from the first week of June (2015) and disappeared completely with the beginning of winter season. Further, seven generations of L. clerkella were found under laboratory conditions. The1st, 2nd, 3rd, 4th, 5th, and 6th generations completed in 39, 40, 37, 39, 45 and 48 days respectively. However, final generation (7th) was found relatively longer in duration, extended from the 4th week of September (2015) till April of the following year (2016) for about 204 days. In general, the life cycle of L. clerkella is similar to those described for other leaf miner species.

Key words: *Lyonetia clerkella*; prevalence; infestation intensity; seasonal history Sezonsko pojavljanje sadnega listnega zavrtača (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) v Kašmirju, Indija

Izvleček: Sezonsko pojavljanje sadnega listnega zavrtača (Lyonetia clerkella (L., 1758), Lepidoptera, Lyonetiidae) je bilo preučevano v štirih območjih doline Kašmirja od marca 2015 do aprila 2016. Razširjenost okužbe je bila večja v Srinagarju (70,6 %) in Bandipori (65,3 %) v primerjavi s Pulwamo (9,3 %) in Baramullo (6.6 %). Velikost okužbe je dosegla višek v maju (2015) v vseh štirih območjih. V Srinagarju je bil odstotek okužbe, v maju 2015 58,69 % (± 11,46 SD), medtem, ko je bil v Bandipori, Pulwami in Baramulli 55,71 % (± 12,59 SD), 6,04 % (± 1,97 SD) in 4,27 % (± 1,12 SD). Velikost okužbe je linearno upadala od prvega tedna v juniju (2015) in je popolnoma izginila z začetkom zimske sezone. V laboratorijskih razmerah je bilo ugotovljenih sedem generacij sadnega listnega zavrtača. Prva, druga, tretja, četrta, peta in šesta generacija so zaključile svoj razvoj po 39, 40, 37, 39, 45 in 48 dneh. Zadnja, sedma generacija, je imela daljši razvoj, ki je trajal od četrtega tedna v septembru 2015 do aprila 2016, skupno okrog 204 dni. V splošnem je življenski krog sadnega listnega zavrtača podoben tistim, ki so opisani za ostale vrste listnih zavrtačev.

Ključne besede: *Lyonetia clerkella*; raširjenost; velikost okužbe; sezonsko pojavljanje

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1 INTRODUCTION

Kashmir is famous for the deliciousness of its temperate fruits in every part of India (Rather & Buhroo, 2015). Thousands of fruit orchards consisting mainly of apple (*Malus domestica* Borkh) can be seen in many parts of the valley. However, a wide variety of insect pests also occur on apple trees from the onset of foliage till the end of autumn period. Among these pests, apple leaf miner (*Lyonetia clerkella* (L., 1758) is regarded as one of the most common, widespread and destructive in many parts of the valley (Ahmed & Bhat1987; Rather & Buhroo, 2015).

The female moths of L. clerkella deposit their eggs singly into the leaf parenchyma (Adachi, 1998). The larvae feed on mesophyll immediately after being hatched. Thereafter, the hatched caterpillars form serpentine mines in the upper half of leaves that are visible to the unaided eyes. However, leaf epidermis is not damaged during the mesophyll devouring and larvae occupy mines till the next phase of their life cycle. The mines with variable lengths that range from narrow linear galleries to wide chambers appear as whitish or grey areas on the leaves. Mines excavated by the larvae of L. clerkella were found to be one of the major causes that reduce the photosynthetic capacity of leaves and cause their premature abscission (Spencer, 1973; Parrella, 1987; Parrella & Jones, 1987). The larval tunneling has also been reported to provide a way for the pathogens to enter into the tissues of the plants (Zitter & Tsai, 1977) and decline the annual yield of fruits (Wolfenbarger, 1954; Ledieu & Heyler, 1985; Minkenberg & Van Lenteren, 1986).

The re-emergence of hibernated moths of *L. clerkella* occurs with the flowering of apple trees. As the day temperature rises in spring, male and female population of these moths emerge in about the equal numbers (Faeth,

1985). After arrival, these miner insect pests target the leaves of the host plants. The female moths discriminate and select the suitable leaves for oviposition. Leaf selection is therefore an important aspect as far as insect-plant interactions are concerned (Faeth et al., 1981).

There is no information available on the prevalence and intensity of infestation of this miner pest in the Indian sub-continent. Therefore, the aim of the present study was to investigate the various aspects of *L. clerkella* including prevalence, infestation intensity and the number of generations.

2 MATERIAL AND METHODS

In order to evaluate the infestation prevalence of *L. clerkella* in different districts of Kashmir such as Srinagar (34° 51'.161" N, 74° 47' 50.535" E, Elev. 1,585 m asl) Bandipora (34° 41' 670" N, 74° 68' 69. 425" E, Elev. 2,183 m asl) Pulwama (33° 52' 18.627" N and 74° 53' 57.753" E, Elev. 1,740 m asl) and Baramulla (34° 12' 72.732" N, 74° 20' 53.732" E, Elev. 1,615 m asl) (Fig. 1), three sites in each of these districts were selected.

Fifty host trees (*Malus domestica* 'Red Delicious') at each site were surveyed during the peak season (May 2015) of this pest. Number of infested trees at each site was counted. Percent prevalence of infestation for every district was calculated by the following formula:

For assessing the infestation intensity in the various months of a year (2015), three sites in every district (Srinagar, Bandipora, Pulwama and Baramulla) were

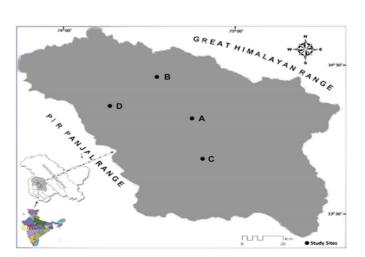


Figure 1: Map showing sampling sites (A = Srinagar, B = Bandipora, C = Pulwama and D = Baramullah)

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selected. The sampling was done as per the method adopted by Adachi (2002). Five infested trees at each site were surveyed randomly at every survey during the final week of every month, from April to December (2015). One branch from each of these host trees at every site was chosen and the total number of leaves (fresh and mined) and mines of this branch were counted. Infestation intensity for each district was thereafter calculated by the following formula:

Total number of infested leaves in three sites Total number of mines they bear x 100

In order to determine the number of generations *L. clerkella* produces under laboratory conditions, methodology adopted by Rather and Buhroo (2015) was followed. Leaves carrying cocoons of this pest were collected from the field and brought in the laboratory. Small sections of these leaves with cocoons were incised and placed inside the 7 litre transparent plastic bottles mounted upon the 2.5 feet apple plants (Figs. 2–3). These bottles had cross ventilations with the dimensions of 10 x 10 cm covered with one layer of nylon mesh for the free circulation of air. About 25–30 cocoons were introduced in each of these bottles. Dead moths of the first generation were collected for preservation and leaves were constantly being observed with the help of torch light (being placed on the underside of the leaves) and high power magnifying glass (Fig. 4). After the appearance of mines, bottles were again mounted on these experimental plants until the formation of cocoons. This process was repeated till the end of final generation. The duration of each generation was thereafter calculated from the time of oviposition till the death of adult moths.

Images were taken by Canon EOS 1200-D camera attached with Reynox super macro 250-D lens on a 75–250 mm zoom lens. Analysis of variance (ANOVA)





Figure 2 & 3: 2 Leaf sections with cocoons 3. Bottles mounted on apple plants



Figure 4: Mine observation by placing a torch at the inner side of a leaf

followed by Tukey's multiple comparison test was done in SPSS (Version 20). The significance of differences between means was determined at p < 0.05.

3. RESULTS

3.1 PREVALENCE

One hundred and six (70.6 %) out of 150 host trees in Srinagar were found infested with *L. clerkella* and 98 (65.3 %) trees were found infested in Bandipora (Fig. 5). The situation was quite different in the other two districts. Fourteen (9.3 %) trees in Pulwama and merely 10 (6.6 %) trees in Baramulla were found infested. No significant difference (p > 0.05) in infestation prevalence was found between Srinagar and Bandipora, and between Pulwama and Baramulla. However, this difference was found statistically significant (p < 0.05) between Srinagar–Bandipora and Pulwama–Baramulla districts (Fig. 5).

3.2 INFESTATION INTENSITY

The seasonal variation in infestation intensity (%) in different districts of Kashmir is given in Table 1. The intensity of infestation was found relatively high in May

(2015) than the other months (April and June - December 2015) in all the study areas of the valley, Srinagar, Bandipora, Pulwama and Baramulla. However, the rate of infestation was found much more in intensity in Srinagar and Bandipora districts as compared to other two areas of the Valley (Pulwama and Baramulla). Although the signs of infestation appeared from the third week of April in all these districts, infestation was however found with varying intensities among them throughout the year. In the month of April, the intensity of infestation in Srinagar, Bandipora, Pulwama and Baramulla were calculated to be 7.61 % (± 2.87 SD), 6.74 % (± 2.36 SD), 2.19 % (± 0.61SD) and 1.01 % (± 0.31SD) respectively (Figs. 6-9). The percent infestation intensity of district Srinagar and Bandipora were found statistically similar but different than Pulwama and Baramulla (Table 1).

Infestation intensity was found at its peak during the month of May (2015) in all the four districts. In Srinagar, the percent infestation intensity was found to be 58.69 % (\pm 11.46 SD) while as in Bandipora, Pulwama and Baramulla, it was calculated as 55.71 % (\pm 12.59 SD), 6.04 % (\pm 1.97 SD) and 4.27 % (\pm 1.12 SD) respectively. The percent infestation intensity of district Srinagar and Bandipora are statistically similar but statistically different from Pulwama and Baramulla (Table 1).

The level of infestation intensity was observed to decline linearly from the first week of June till the last week

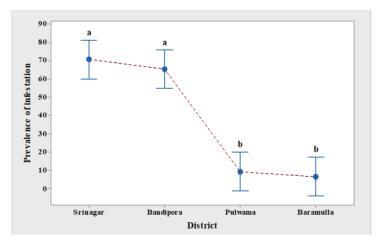


Figure 5: Prevalence of infestation in different districts of Kashmir

Table 1: One way ANOVA showing seasonal variation in infestation intensity (%) in different districts of Kashmir (Mean ± SD)

District	March	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Srinagar	$0.00^{\text{a}} \pm 0.00$	$7.61^{a}\pm2.87$	$58.69^{a} \pm 11.46$	$537.29^{a} \pm 16.19$	$32.61^{a} \pm 9.58$	$27.07^{a} \pm 5.07$	$13.43^{a} \pm 6.28$	$7.92^{\rm a}\pm4.92$	$3.73^{a}\pm1.36$	$0.00^{\text{a}} \pm 0.00$
Bandipora	$0.00^{a} \pm 0.00$	$6.74^{a}\pm2.36$	55.71ª ± 12.59	$47.07^{a} \pm 17.08$	$23.01^{a} \pm 9.86$	$8.17^{\rm b}\pm2.15$	$6.55^{\mathrm{b}}\pm1.10$	$2.75^{\mathrm{b}} \pm 0.99$	$1.22^{\rm b}\pm0.65$	$0.00^{\text{a}} \pm 0.00$
Pulwama	$0.00^{a} \pm 0.00$	$2.19^{\text{b}} {\pm}~0.61$	$6.04^{\rm b}\pm1.97$	$4.85^{b} \pm 0.49$	$4.15^{\rm b}\pm1.09$	$4.16^{\circ} \pm 0.86$	4.49°± 1.76	$1.80^{\rm b}\pm0.81$	$1.63^{\text{b}} \pm 1.30$	$0.00^{\rm a}\pm 0.00$
Baramulla	$0.00^{a} \pm 0.00$	$1.01^{\rm b}\pm 0.31$	$4.27^{\rm b}\pm1.12$	$4.40^{\rm b}\pm0.61$	$3.41^{ ext{b}} \pm 1.09$	$2.45^{\circ} \pm 0.3$	$2.36^{\circ} \pm 1.66$	$2.58^{b} \pm 1.65$	$1.23^{\rm b}\pm0.65$	$0.00^{a} \pm 0.00$

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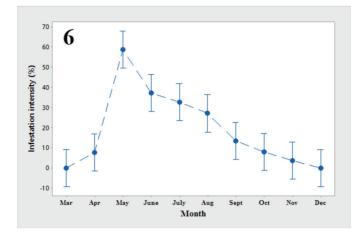


Figure 6: Infestation intensity in various months of a year; Srinagar

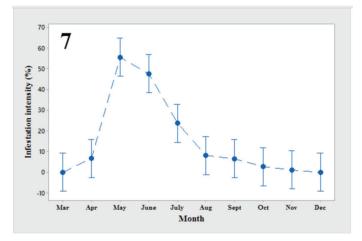


Figure 7: Infestation intensity in various months of a year; Bandipora

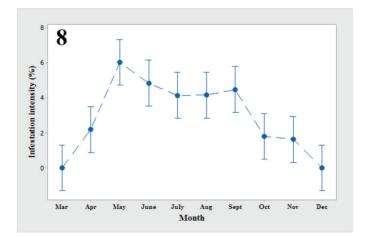


Figure 8: Infestation intensity in various months of a year; Pulwama

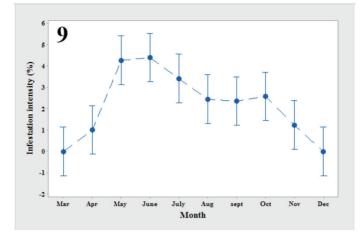


Figure 9: Infestation intensity in various months of a year; Baramulla

of November (2015). The intensity of infestation was found least in the month of November (2015). In Srinagar, infestation intensity in this month was calculated as 3.73 % (\pm 1.36 SD) while as in the other three areas of the valley, Bandipora, Pulwama and Baramulla, it was found to be 1.22 % (\pm 0.65 SD), 1.63 % (\pm 1.30 SD) and 1.23 % (\pm 0.65 SD) respectively. The percent infestation intensity of district Srinagar was found statistically different from all the other three areas.

3.3 SEASONAL HISTORY

Seven generations of *L. clerkella* were found in the laboratory (Fig. 10). First generation started from 12^{th} of April (2015) and ended on 20^{th} of May (2015). Second generation begin from 8^{th} of May (2015) and completed on 16^{th} of June (2015). Third generation commenced from 5^{th} June (2015) and ended on 11^{th} July (2015). Fourth generation begin from 27^{th} of June (2015) and

completed on 4th of August (2015). Fifth generation started from 21st of July (2015) and ended on 3rd September (2015). Sixth generation commenced on 21st of August (2015) and completed on 7th of October (2015). Similarly, 7th generation extended from 24th of September (2015) to mid spring (15th April) of the following year (2016). Moths of the final generation (7th) hibernated during the winter months, from 2nd week of November (2015) till March of the following year ((2016) (Fig. 10).

Thus, the first, second, third, fourth, fifth, and sixth generations of *L. clerkella* completed in 39, 40, 37, 39, 45 and 48 days respectively. However, final generation (7th) was found relatively longer in duration, extended from the 4th week of September (2015) till April of the following year (2016) for about 204 days.

The swarms of hibernated moths were first seen from 11^{th} to 20^{th} of April in the year 2015 and the average day temperature of this period was recorded to be 21 °C (Table 2). However in 2016, the swarms of these moths

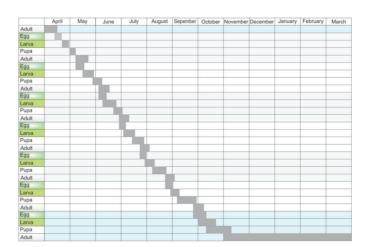


Figure 10: Seasonal history of L. clerkella as seen in laboratory

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were observed from 17th to 25th of April with an average day temperature of 21.4 °C.

4. DISCUSSION

In the present study, the prevalence and infestation intensity was found to fluctuate seasonally in different study areas (Srinagar, Bandipora, Pulwama and Baramulla) of the valley with relatively high level of prevalence and intensity being observed in the month of May. The highest prevalence was found in Srinagar and Bandipora districts (70.6 % and 65.3 %). Similarly, the highest intensity of infestation was found in Srinagar, 58.69 % (± 11.46 SD) followed by Bandipora, 55.71 % (± 12.59 SD) whereas, least infestation intensity was found in Pulwama 6.04 % (± 1.97 SD) and Baramulla 4.27 % (± 1.12 SD). A significant difference in infestation intensity was also observed among these four districts (Table 1). The variations in prevalence and infestation intensity among different districts of the valley (April to November 2015) could be attributed to the factors such as insecticides. parasitism, temperature and food quality as reported by Dixon (1987) and Feeny (1970).

These insect pests resume their activities in spring under varying temperatures at different places of the world (Kholchenkov, 1974). In Azerbaijan, Kholchenkov (1974) reported that *L. clerkella* appear in spring at the temperature of 12 °C. However, in Kashmir, the swarms of hibernated moths of this pest were found to appear in early spring, between 11th to 25th of April at an average day temperature of 21.2 °C (Table 2).

Since L. clerkella is widely distributed in many parts of the world, the number of generations subjected to specific temperatures and elevations were reported varying from place to place. Agata et al. (2007) reported three generations of L. clerkella at the altitude of 200-300 m and 1-2 generations at an elevation of 750-860 m. This pest was found to develop 3-4 generations per year in Ukraine (Kholchenkov, 1974). Furthermore, Shoji & Ueno (1981) reported that the apple leaf miner moths grow for 5 to 6 generations per year in Yamagata Prefecture while as Miyaji (1991) found L. clerkella produced 9 generations per year in Kagoshima prefecture, Japan. Apple leaf miner was also found to produce 5 generations in a year under natural conditions in Kashmir (Rather & Buhroo, 2015). The 7 generations in experimental room as observed in the present study fell within this expected range.

Relatively shorter duration of the first two generations (April to June) could be attributed to the availability of better food quality (Dixon, 1987). In the months of spring and early summer (April to June), the mesophyll content remains soft which enables the caterpillars to feed voraciously as compared to subsequent months when the available foliage dries and becomes relatively tougher due to the deposition of cellulose, hemicelluloses, pectins, and other materials (Feeny, 1970). Seasonal changes in the leaf tannin composition can also have an adverse impact on the generation time as increased tannin concentration negatively affects the growth of lepidopteron caterpillars (Varley & Gradwell, 1962). The ability of tannins to form complexes with proteins enhances defense mechanism of plants and thereby affecting the growth of insects (Feeny, 1970).

The work of many authors indicated that the duration of puparial stage of apple leaf miner insects extends relatively from the onset of autumn period because of reduction in daily temperatures (Kholchenkov, 1974; Kuznetsov & Seksyaeva, 1994; Savkovskii, 1976). In the present study, the puparial stage was also found comparatively longer in duration from the beginning of autumn season because of linear reduction in daily temperatures. The longevity of adult moths of this pest was also found to decrease with the decrease in temperature from the month of September.

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