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POPULATION DYNAMICS OF *LEUCOPTERA MALIFOLIELLA* COSTA IN CHERRY ORCHARD FROM CLUJ COUNTY

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Abstract

Leucoptera malifoliella is a polyphagous leaf miner, which is present across Europe and Asia finding its host plants mostly from the Rosaceae or Betulaceae families. It can cause severe damage in various orchards, especially, pear and apple due to larvae which are feeding on the mesophyll tissue of leaves and cause premature leaf fall and serious yield reduction. Therefore, the main aim of this research was to monitor and investigate the life cycle of the species and the damage caused during 2021 and 2022 on two sweet cherry tree varieties 'Regina' and 'Regina'. The results show that the pear leaf blister moth has three peaks in flight dynamics, which could correspond to the development of three and one generation during the years 2021 and 2022. The flight of the overwintering males (1st generation) began in May and the 3rd generations flew until the 28th of September. The peak of the flight curve for each generation was recorded 2-3 weeks after the start of the flight. The frequency of the attack increased from one generation to another, reaching up to 23.64% in 'Kordia' and 19.04% in 'Regina'. The intensity of the attack increased as well from one generation to another up to 90% at the end of the season. The average number of mines/leaf was over 7 mines, with a maximum of 23 mines for the 'Regina' variety and 28 mines for 'Kordia' leading to a damage level of 17.13% and 21.27% for the two sweet cherry varieties. In 2022 the number of pest generation decreased proving the efficiency of pheromone traps.

***Key words**: pear leaf blister moth, cherry trees, monitoring, AtraScit pheromone, damage.*

INTRODUCTION

In many fruit orchards among the rich complex of pests that attack at the leaf level, the species of mining microlepidopterans are often found. While worldwide over 300 leaf miner species are known, the most frequently reported species are: *Leucoptera malifoliella* (Costa, 1836) (Lyonetiidae), *Lyonetia clerkella Phyllonorycter blancardella*, *Phyllonorycter corylifoliella*, *Callisto denticulella*, *Stigmella malella* (Tešanović, 2011; Płóciennik et al., 2011; Tešanović and Spasić, 2015; Rather and Buhroo, 2015; Kholboevich et al., 2020). Leaf mining moth can easily be recognized primarily, based on the pattern of feeding tunnels they create and the mined leaves which can often contain frass and droppings. The shape of the mine, the pattern of frass deposition and the host plant itself are also important indicators in the determination of these species (Sepros, 1986; Seven, 2006). However, some mining insects are feeding on other parts of the plants such as fruit surfaces or flower petals (Saidov et al., 2018; Lopez-Vaamonde et al., 2021).

Leaf-mining insects are solitary miners whose larvae feed inside the leaves producing concentric galleries, which can reach a diameter of 5-6 mm and are called leaf mines. The mines change their size and colour over time from small and whitish to large with brown spots. Frass may be visible, grouped in dark, concentric circles. In the case of heavy infestation, the leaf surface is severely damaged by the presence of mines affecting photosynthesis and crop production (Nawaz et al., 2021).

In fruit orchards, the dominant leaf-miner species is *L. malifoliella* most commonly known as pear leaf blister moth, pear leaf miner, apple leaf miner or ribbed apple leaf miner (Maciesiak, 1999; Radoslav et al., 2001; Fekete et al., 2005; Molet, 2011).

In Romania, *L. malifoliella* was first reported by Caradja in 1901 in Neamţ County, at Grumăzeşti (Oltean, 2000). Between 1960 and 1970 insect pest outbreaks were observed across the country. Due to the large gene pool of insect populations, their high reproductive rate and the lack of efficient pest management strategies at that time, *L. malifoliella* damaged several orchards including apple, pear and cherry orchards in Arad, Timiș, Hunedoara, Alba, Sibiu, Bihor, Satu-Mare, Maramureş, Sălaj, Cluj, Bistriţa-Năsăud, Mureş, Caraş-Severin, Mehedinţi, Dolj, Olt, Gorj, Argeş, Vâlcea, Vrancea, Buzău, Dâmboviţa, Suceava and Călăraşi counties (Nicolae et al., 2011).

L. malifoliella is a polyphagous species and has many hosts but the most preferred host plants are members of the Rosaceae family (Béguinot, 2017). It occurs in temperate climates causing severe damage in fruit orchards as well (Koç et al., 2014). Among fruit trees this pest prefers the most *Malus domestica* Borkh, *Pyrus sativa* Lam., *Prunus avium* L., *Cydonia oblonga* Mill., *Prunus domestica* L., *Prunus persica* L., *Prunus armeniaca* L. (Bajec et al., 2009; Barić et al., 2010), and *Mespilus germanica* L. (Janick, 2008). This first attack of *L. malifoliella* in a cherry orchard was first reported in 2004 by Balazs and Jenser, followed by other authors in the next years (Nicolae et al., 2011; Mishchenko and Prakh, 2016). Besides fruit trees, this pest finds hosts in different trees and shrubs as follows: *Crataegus monogyna* Jacq., *Clematis vitalba* L., *Cornus mas* L., *Cotoneaster melanocarpus* Lodd. (Kirichenko et al., 2017; Hellers, 2019; Fichtner and Wissemann, 2021) and even in *Alnus, Betula, Quercus* and *Salix* spp. (Celli and Burchi, 1983). Ecevit et al., in 1987, reported this species as a new pest of hazelnuts.

The species is multivoltine, developing multiple generations (1-5) each year depending on the geographical location (Mey, 1988; Sáringer and Csontos, 1998; Tălmaciu et al., 2008).

A warm climate and the increasing number of generations of this species can be extremely dangerous, especially in areas where large orchards of various fruits are located and from where they could be spread toward other locations (Maciesiak and Olszak, 2006; Béguinot, 2017). In some cases, favourable climatic conditions and unsuitable use and application of pesticides can stimulate more the outbreak of this pest.

According to previous research results, in fruit orchards, the frequency and intensity of the attack of *L. malifoliella* is influenced first by the host plant (Andreev et al., 2001; Jenser et al., 2001; Enzsoly and Kuroli, 2003; Tešanović and Spasić, 2010) and then, by pest monitoring methods and pest detection. Due to the very small size of this pest and its hiding behaviour are the main reasons why this pest is not observed in time and treatments are applied too late which could lead to even worse results (Ivanov, 1976; Šubić, 2015a). The average size of the mine is 0.88-1.04 cm², depending on the attacked plant and variety (Baufeld and Freier, 1992). The number of mines on one leaf differs and it is correlated with the population dynamics of the species generally (Górska-Drabik, 2006). A large number of mines cause heavy premature leaf loss during August or early September (Bajec et al., 2009). Early foliage drop adversely affects fruit size, quality and total yield. Heavy leaf loss also influences the differentiation of flower buds and the maturation of the shoots (Šubić, 2015b).

Various studies from the scientific literature revealed it is that 28 insects were reported to attack the final larval instars; most of them belonging to the Eulophidae, Pteromalidae and Braconidae families.

Previous studies also show that the rate of parasitism is low in the first generation of the pest but increases during the second generation (Mey, 1993).

Regarding chemical control management, first and foremost is very important to take into account the phenological stages of the pest in order to identify its sensitive stages and apply the pesticide needed. Several insecticides have already been tested to overcome pear-leaf blister moths (Injac, 1981; Injac and Dulic, 1983; Maciesiak, 1996), but the results showed that their effectiveness is strongly correlated with the moment of treatment application and the coverage area (Pénzes, 1985; Marinkov, 1986).

In this regard, field monitoring and forecasting remain the most reliable methods to use to follow the phenological stages of *L. malifoliella* and apply effective treatments. Pheromone traps 5.9-Dimethylheptadecane became of great importance providing accurate information about the pest population dynamics (Francke et al., 1987; Ciocan-Tarta et al., 1998; Liang et al., 2000) showing good attractiveness and selectivity (Kutinkova and Andreev, 2002). These traps are used for population monitoring and to indicate the best time to apply (Koutinkova et al., 1999; Nikolic et al., 2011; Rosu-Mares et al., 2021) chemical treatments (Nicolae et al., 1996; Drosu, 2010; Grünig et al., 2021).

Between 1985 and 2000, *L. malifoliella* was identified in the fruit-growing basin of Cluj County as a pest that caused severe damage, especially in apple orchards. After this period a significant decrease of the pest population density was reported. In 2019, the species was first reported in cherry orchards in Cluj County and in the following year, a significant pest outbreak was detected in an urban cherry orchard (Steluța farm) in the city of Cluj-Napoca.

Therefore, the main aim of this research was to monitor pest population development, its' density and the intensity of the attack of this species upon the urban sweet cherry orchard in the climatic conditions of Cluj-Napoca. Furthermore, this study aimed also to serve as a forewarning for fellow farmers to help them develop efficient integrated management strategies against this yield-devastating pest.

MATERIALS AND METHODS

The current research has been carried out in an urban sweet cherry orchard (a commercial family orchard) situated in Cluj-Napoca (46°8'1" N, 23°6'0" E) established in 2010 laying out on five hectares. The cherry cultivars were grafted on Gisella 5. All the fruit trees were planted in high density system at 4 x 1.5 m. The inter-rows were grassed and maintained through frequent mowing combined with herbicide strips along the tree rows. The orchard is equipped with a drip irrigation system and protection covers against rainfall and hale.

L. malifoliella (Figure 1) was detected first in 2021 attacking 'Regina' and 'Kordia' sweet cherry cultivars. In order to monitor pest population pheromone traps were installed in the orchard. To determine the exact time of trap placement on the 4th of March in both experimental years. For orchard monitoring and mass capture, 20 AtraSCIT pheromone traps/ha were installed in the orchard to detect and monitor pear blister moth activity (Figure 2a).

Figure 1. *Leucoptera malifoliella* adults

a B

Figure 2. AtraSCIT pheromone traps in the sweet cherry orchard (a) the monitoring population of *L. malifoliella* by counting the males caught in the trap (b)

The monitoring period of this pest started in May and ended in September. During this period 10 readings of the pheromonal traps were carried out, recording the number of males caught in the installed traps (Figure 2b). Based on the data recorded, the flight pattern was determined. To define the frequency and intensity of the attack leaves from 'Regina' and 'Kordia' sweet cherry tree leaves were collected at two different moments during the growing season. The first set of leaves was harvested on the 23rd of July, while the second set was on the 1st of September. In total, 100 leaves/trees were randomly picked from the bottom, middle and upper parts of the tree and transferred to the laboratory for further analysis. To determine the frequency of the attack the following formula was used:

$$F\ (\%) = \frac{\mathrm{n\ x\ 100}}{\mathrm{N}}$$

where:
n is the number of affected leaves;
N is the total number of leaves examined.

After the calculation of the frequency of the attack, the number of mines was recorded. Afterwards, the leaves were further investigated using WinDIAS 3 Leaf Image Analysis System to determine the intensity of the attack by scanning the leaves and measuring the damaged area of the leaf area.

Regarding the intensity of the attack and damage level, these were determined based on the number of mines recorded on the leaves and the damaged area of the leaves and expressed as percentages. Therefore, the results of the analysis carried out on the leaves harvested on the 23rd of July, split the data into four groups based on the number of mines recorded per leaf (Figure 3).

‘Kordia’ ‘Regina’

Figure 3. Damaged leaves of ‘Kordia’ and ‘Regina’ varieties by *L. malifoliella*

Based on the data recorded, the intensity of the attack was calculated using the following formula:

$$I\ (\%) = \frac{\sum(ixf)}{n}$$

where:
i is the damage score for the sample leaf;
f is the no. of leaves scored;
n is the total number of affected leaves examined.

The damage level of the attack was then calculated according to the following formula:

$$Damage\ level\ (\%) = \frac{\mathrm{F\ (\%)\ x\ I\ (\%)}}{100}$$

In cases when the mines were joined due to overlapping of the larvae activity, the number of the mines was counted separately based on the imprint of the larval excrement.

The data were analyzed using the analysis of variance (ANOVA) to detect significant differences between the means at $p<0.05$. When the null hypothesis was rejected, Duncan’s multiple range test was performed to indicate significant differences between the means. Pearson’s correlation was also assessed to measure the linear associations between the variables under study.

RESULTS AND DISCUSSION

The results of this research revealed that *L. malifoliella* developed three generations in 2021 and one generation in 2022. Regarding the pest dynamics, it was observed that the first overwintering adult month emerged on the 9th of May (Figure 4) and began its flight. The duration of the flight period was approximately 6 weeks, reaching the peak of the flight pattern within 2 weeks from pest occurrence. In total, 6390 individuals were caught in all traps during the entire flight period in 2021 and 4522 in 2022. On the 25th of May, 1528 individuals were counted in the pheromone trap being considered the peak of the flight pattern in 2021 and 2612 in 2022 (Figure 4).

The maximum number of captured adults recorded between two consecutive readings in one trap was 135. The flight of the second generation spanned for approximately one month (from the first decade of July to the first decade of August). The peak of the flight curve was recorded in two weeks, similar to the first generation, with 921 captured individuals on the 20th of July (Figure 4). During the flight period of this generation, 1062 individuals were captured in traps, with a maximum number of 110 adults in a single trap between the two consecutive readings.

The flight of the third generation started at the beginning of the second decade of August and continued until the end of September 2021. The peak of the flight curve was recorded after three weeks, on the 31st of August when 1993 captures were recorded (Figure 4). The total number of males caught in the pheromone traps of this generation was 3001, and the maximum number of captures by a single trap between two consecutive readings was 217. During the 2021 year, the total number of *L. malifoliella* male adults captured in the AtraScit pheromone traps was 6390. The mean number of adults captured in traps was 319.5, while the number of adults captured in one trap ranged between 131 and 517.

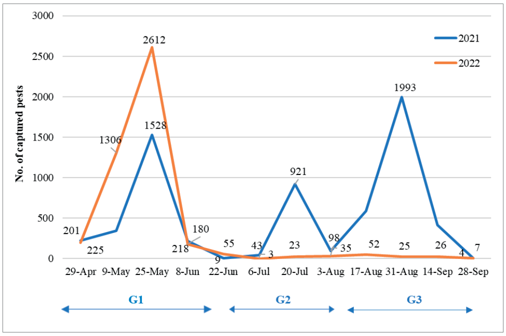

Figure 4. The flight pattern of *Leucoptera malifoliella* in 2021 and 2022. The data presented are the mean values of the 20 traps

The observations made on *L. malifoliella* in the 2022 year, revealed that the highest number of individuals caught in traps was 2612 on the 25th of May and after this date, the number of individuals caught decreased. In this year only two generations were recorded (Figure 4). The total number of *L. malifoliella* male adults captured in the AtraScit pheromone traps was 4522. The lack of the third generation might be explained by the high efficiency of the pheromone traps installed in the previous year which led to the decrease of the *L. malifoliella* population.

The high number of captures during the experimental years demonstrates that AtraScit pheromone traps were suitable not only for pest monitoring but also for mass trapping. Our findings are in accordance with previous research results which enhance the efficiency of this pheromone trap for *Leucoptera scitella* in apple orchards capturing all the existing individuals from the orchard (Rosu-Mares et al., 2021).

To understand better the flight pattern of this species during 2021 and 2022, the number of captured males was correlated with the most important meteorological parameters as follows: minimum, maximum and average temperature, precipitation and wind speed. Therefore, the results of Pearson's correlation indicated a negative linear association between air temperature and the number of captures. As air temperature increased, the number of captures decreased, suggesting that *L. malifoliella* adults preferred lower temperatures, humidity and windy conditions more for their flight and population spread in both years (Table 1).

Table 1. Pearson's correlation coefficients (r) between weather conditions and the number of captured male adults from the 9th of May to the 28th of September 2021 and 2022

Met. Param./Year	Avg. temp. (°C)	Avg. min. temp. (°C)	Avg. max. temp. (°C)	Avg. precip (mm)	Wind speed (km/h)
2021	-0.2982	-0.1097	-0.3256	0.0587	0.0629
2022	-0.1444	-0.1703	-0.2634	0.8202	0.0672

During the monitoring period, it has been observed that pupation of the first and second generations took place predominantly on foliage and fruits (Figure 5) of the sweet cherry trees, but cocoons of the third generation were observed only in more protected (hidden) areas such as under-bark, on stems, twigs and even in the vicinity of flower buds (Figure 6).

Figure 5. Pupae of *Leucoptera malifoliella* on leaves and fruits (G1 and G2)

Figure 6. Overwintering cocoons of *Leucoptera malifoliella* on woody parts of sweet cherry trees

Thus, in 2021 the results, of the leaf analyses revealed that 48% and 38% of the leaves of ‘Regina’ and ‘Kordia’ varieties respectively, had up to 5 mines/leaf. Leaves with 6 to 10 mines accounted for 29% of ‘Regina’ and 43% of ‘Kordia’ leaves. Between 11 and 15 mines were found in 4% of ‘Regina’ leaves and 9% of ‘Kordia’ leaves. Leaves with 16 and 20 mines were found only on the leaves of the ‘Kordia’ variety. The maximum number of mines on a single damaged leaf was 23 mines in ‘Regina’ and 28 mines for the ‘Kordia’ variety (Figures 7 and 8).

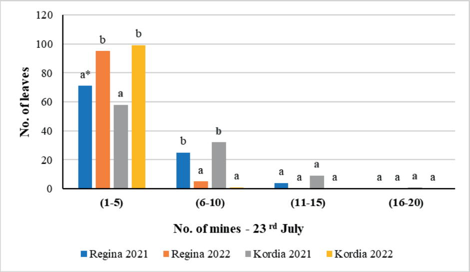

Figure 7. The number of mines per leaf recorded in ‘Regina’ and ‘Kordia’ sweet cherry varieties on the 23rd of July 2021 and 2022. The data shown are means. Lowercase letters above the bars indicate significant differences between the experimental years within the same sweet cherry variety while asterisk denotes significant differences between the varieties from the same experimental year according to Duncan’s multiple range test at P<0.05

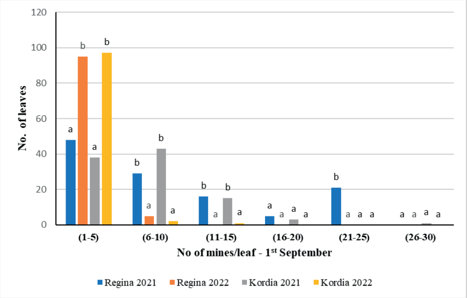

Figure 8. The number of mines per leaf recorded in ‘Regina’ and ‘Kordia’ sweet cherry varieties on the 1st of September 2021 and 2022. The data shown are means. Lowercase letters above the bars indicate significant differences between the experimental years within the same sweet cherry variety. No significant differences were detected between the varieties from the same experimental year according to Duncan’s multiple range test at P<0.05

In 2022, the number of attacked leaves increased by 24% and 41% respectively in July, when 95% and 99% of ‘Regina’ and ‘Kordia’ leaves contained between 1-5 mines/leaf. It has been observed that, as the number of mines/leaf increased the number of attacked leaves decreased. Thus, the maximum number of mines/leaf was 6 and 7 on ‘Kordia’ and ‘Regina’ leaves (Figure 8).

The analyses of the second set of damaged leaves harvested on the 1st of September revealed a slight decrease (2%) in the number of leaves with mines in the ‘Kordia’ variety (Figure 8).

The shape of the mines developed on the attacked leaves can denote certain characteristics depending on the host plant. On most of the attacked plants, the mines of this pest have a circular form. However, the current research suggests that in sweet cherry trees, the shape of the mines is rather elongated and oval-shaped reaching up to 25 mm in length and 5 mm in width (Figure 3).

These results can be explained by the efficiency of the AtraSCIT pheromone traps in the sweet cherry orchard.

These results are similar to previous results reported by Tălmaciu et al. (2008) indicating a more intense attack of *L. malifoliella* during the 3rd generation of the pest.

The intensity of the attack caused by *L. malifoliella* was also analyzed during this research followed by the determination of the intensity of the attack. The results showed that the intensity of the attack in 2021 year of *L. malifoliella* in the urban sweet cherry plantation was very high, ranging between 80-90% in sweet cherry varieties under study.

Table 2. Average frequency, intensity and damage level of *L. malifoliella* attack in two sweet cherry varieties

Cvs.	Date of capture count	Frequency (%)	Intensity (%)	Damage level (%)
Kordia	23.07.2021	15.02 b	80 b	12.01 b
	23.07.2022	4.51 a	60 a	2.70 a
	01.09.2021	23.64 b	90 b	21.27 b
	01.09.2022	4.13 a	70 a	2.89 a
Regina	23.07.2021	13.64 b	80 b	10.91 b
	23.07.2022	4.10 a	60 a	2.46 a
	01.09.2021	19.04 b	90 b	17.13 b
	01.09.2022	4.66 a	70 a	3.26 a

*Note: different lowercase letters indicate significant differences between the years within the same sweet cherry varieties. No statistically significant differences were detected between the cultivars within the same experimental year according to Duncan’s multiple range test at p<0.05.

These high percentages of intensity indicate that both the 'Regina' and 'Kordia' varieties were extremely vulnerable in front of this pest. The occurrence of this pest was also reported by Ulusoy et al. (1999) in Turkey in sweet cherry and confirmed by Tezcan and Gülperçin (2024) but its frequency was much lower. In Romania, previous research results reveal the occurrence of this pest only in apple orchards. However, the frequency of the attack varied between 9.26-35.22%, it is worth mentioning that the highest frequencies were generated by the 3rd generation of the pest and only in urban areas (Tălmaciu et al., 2008). These results are in accordance with our findings that high frequencies of attack are facilitated by urban areas where the number of natural enemies is reduced. Biological control of various pests is provided by the natural enemies from a certain area, which are controlling the phytophagous pest population below the economic threshold. In cities and in the surrounding areas where green areas are more and more replaced by built-up areas, the natural habitats of natural enemies are diminished and no natural pest control can take place (Korányi et al, 2022).

In order to create an overall image upon pest attack abundance and the vulnerability of 'Regina' and 'Kordia' sweet cherry cultivars, the mean number of mines/leaf was calculated and compared. The results of the statistical analyses show that significant differences between the means existed only in the damage caused during the summer (4.13 mines in 'Regina' and 5.49 mines in 'Kordia') suggesting a slightly higher vulnerability of 'Kordia' variety in front of *L. malifoliella* attack. However, as the number of pest generation increased the damage caused in both sweet cherry varieties increased as well, exhibiting no statistically significant differences between the means at $p<0.05$ (7.36 mines in 'Regina' and 7.46 mines in 'Kordia' as presented in Figure 9. Thus, it has been observed that the intensity of the attack increased by 8.62% in 'Kordia' and 5.40% in 'Regina' by the end of the vegetation period. The level of damage followed a similar pattern; in 'Kordia', an increase of 9.26% was observed, while in 'Regina' the level of damage increased by 6.22% (Table 2). These results indicate that 'Regina' is slightly more resistant to the attack from the beginning until the end of the vegetation period. During the year 2021, the frequency of attacked leaves was 30% caused by the first generation of the pest and reached 90% by the end of the biological cycle.

In 2022, a significant decrease in the number of mines/leaf has been detected in both periods (July and September) compared to the attack that occurred during the experimental year of 2021.

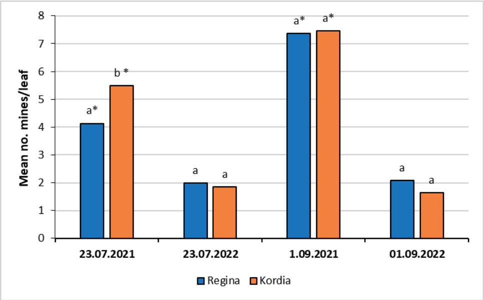

Figure 9. The mean number of mines/leaf recorded in the two sweet cherry varieties under study during 2021 and 2022. The data shown are means. The asterisks indicate significant differences between the means according to Duncan's multiple range test at $P<0.05$

CONCLUSIONS

In summary, *L. malifoliella* can cause severe damage in sweet cherry orchards by destroying up to 70% of the leaf area which leads to a significant decrease in yield. Urban areas seem to be more favorable for pest outbreaks facilitating the development of 2 or 3 generations of the pest. The use of sex pheromone traps proved to be efficient, exhibiting high attractivity and selectivity when placed at the exact time and can be recommended for pest monitoring and mass trapping of male adults for pest management strategies.

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