

Development and density of lime leafminer *Phyllonorycter issikii* (Kumata, 1963) (Lepidoptera: Gracillariidae) on lime trees

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We have studied some bioecological peculiarities of the lime moth under conditions of the Kharkiv region: the density of mines of the lime leafminer population pieces/leaf, and the dynamics of flight, the population of lime tree leaves. The density of mines of the lime leafminer population was close to 2 pieces/leaf. The flight of butterflies after overwintering began in the first decade of May, of the second generation – in the second – third decades of June. The maximum mines density was registered in the second decade of June and the second decade of August. The maximum leaf population by lime leafminer reached 50% in Danylivsky experimental State Forestry Enterprise and Forest Park.

Keywords: leaf-mining insects, lime leafminer, biological peculiarities, harmfulness, deciduous trees, lime tree, green plantations.

Introduction

The lime leafminer was first described in Japan in 1963 (Kumata, 1963). In 1977, it was discovered on Southern Primorye territory and 1983 – on the Korean Peninsula (Ermolaev, 1977). In 1985, the lime leafminer appeared in Moscow (Gninenko & Kozlova, 2006), in 1987 – in the Voronezh region, Samara, Ufa, and Kyiv (Orlinskij, 2006), in 1999 – in Udmurtia, in 2002 – in St. Petersburg (Ermolaev & Zorin, 2010). In Poland in 1996, it was discovered in the Baltic states in 1997, in Germany in 1998, in the Czech Republic, Austria, and Hungary and in 2000, in Finland in 2002 (Antyuhova & Meshkova, 2011). It is proposed to introduce this pest in the list of internal quarantine objects in Russia (Ermolaev, 2011). In 2007, we found massive damage to lime trees' leaves by the lime leafminer in the forest park area of Kharkiv and single mines on trees in street plantations. In 2008, lime leafminers' prevalence and leaf damage intensity increased noticeably (Meshkova & Mikulina, 2010).

The lime leafminer overwinters at the imago stage in cracks in the bark of lime trees. After wintering, the butterflies sit on the trunk, mate, and then move to the crown. Females lay their eggs one at a time on the lower surface of lime tree leaves, which have fully blossomed out by that time. Eggs develop in 10–14 days (Matosevic, 2007).

The first mines appear in the first decade of June. The caterpillar bites into the leaf tissue and gradually forms a lower-sided mine, first threadlike in shape and then spot-folded. Due to the high density of plant settlement by the leafminer, individuals can form upper-sided mines. The appearance of the first mines can be detected as early as the first decade of June. Due to the high population density, some individuals of the second generation form mines on the lower and the leaves' upper side. Mines are large, elliptical, almost without folds, green – pale brown on the leaf's underside, often between the central and other large vessels (Izhboldina, 2008). Caterpillar pupation occurs in mines, usually at the end of June. The pupal stage lasts 7–9 days. Before the butterfly goes out, the pupa breaks through the mine and extends out for most of its length (Sefrova, 2002). The first butterflies of the new generation fly out in early July, and the first to fly out are longer-winged individuals (Izhboldina, 2008). Butterflies of lime leafminers that fly out in summer are found on trunks from mid-July to early August. They mate and lay their eggs on the leaves.

Under favorable conditions, the caterpillars of the second generation develop in August. Pupae are found at the end of August, and imago passes the winter – in September (Izhboldina & Zorin, 2008). In Udmurtia, second-generation caterpillars often do not have time to complete development. The second-generation imago flight occurs in the first decade of September. Butterflies of the first generation leave for wintering in early August, the second – in mid-September. First, they gather on tree trunks and then move into deeper cracks in the bark, cracks in fences, and buildings (Izhboldina, 2008). In Japan, in the south of the Far East and Voronezh region, the lime leafminer has two generations (Orlinskij, 2006), on the territory of Moscow and Moscow Region under favorable conditions – an incomplete third (Bednova & Belov, 1999), in the Czech Republic – three (Sefrova, 2002).

The maximum density of lime leafminer (up to 10 mines per leaf) is observed in high-density plantations of various compositions, where lime trees take part in the formation of undergrowth or underwood. That is, the lime leafminer is a shade-loving insect (Izhboldina & Zorin, 2008). At the beginning of the settlement, females choose the most suitable places for laying eggs and then – the least suitable ones (Izhboldina, 2008). Lime leafminer prefers the lower third of the crown of a feed plant. The distribution of mines in skeletal branches does not depend on the exposure of branches and plant planting density. Butterflies choose the middle and basal areas of the leaves to lay eggs (Ermolaev & Zorin, 2011). As the settlement density increases, the mines' density in different parts of the leaves evens out (Zorin, 2012). Due to the high density of lime leafminer, the decorative effect of trees is lost (Antyuhova & Meshkova, 2011), their accretion in height and diameter decreases, the intensity of flowering and nectar content in flowers decrease (Zorin, 2012).

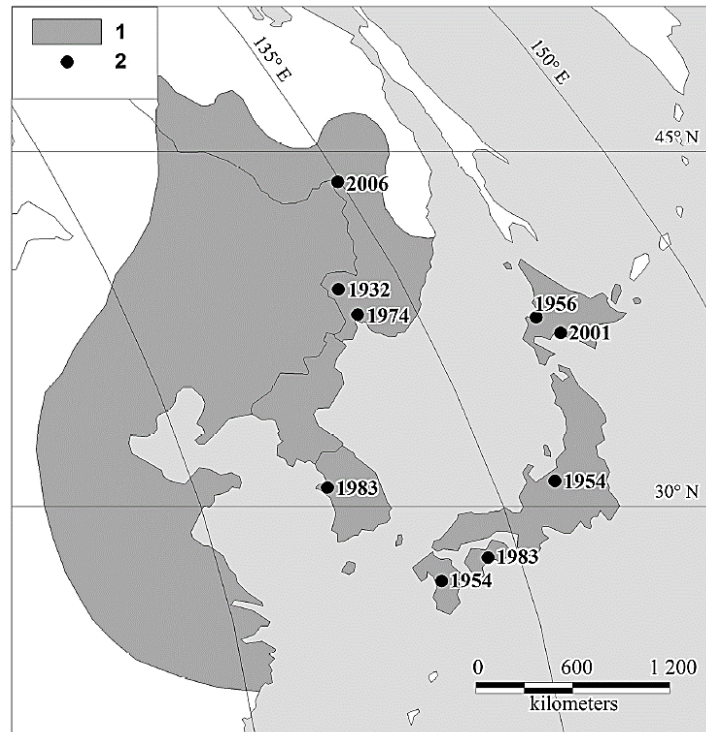


Fig. 1. The area of Japanese lime leafminer *Ph. issikii* in the Far East: 1 – the area of the genus *Tilia*; 2- points of species detection (Ermolaev & Rublyova, 2017).

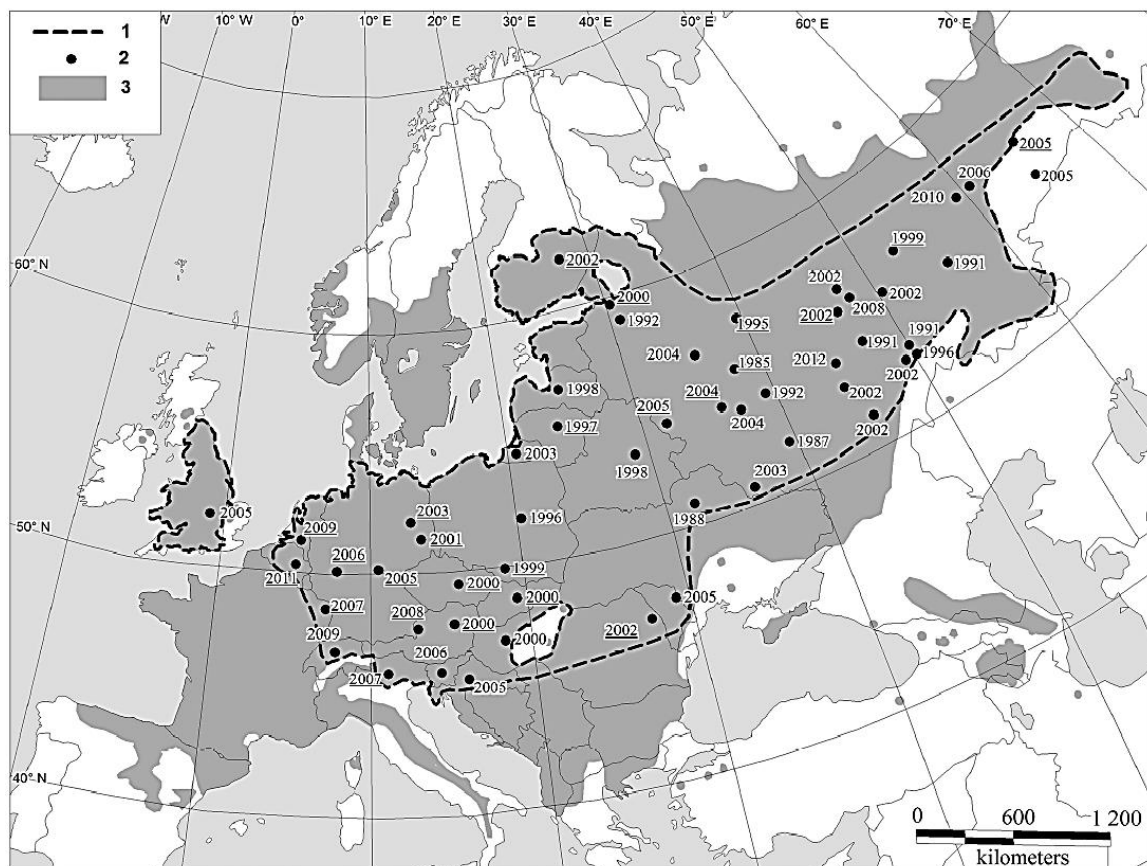


Fig. 2. The area of Japanese lime leafminer *Ph. issikii* in western Eurasia:

1 – boundaries of leafminer distribution as of 2011; 2 – points of species detection; 3 – the area of the genus *Tilia*. Years of species detection in the first phase of invasion are highlighted (Ermolaev & Rublyova, 2017).

Materials and methods

The study was conducted in parks and street plantations in Kharkiv (50°00' N, 36°15' E), in particular in Shevchenko Park (50°00' N, 36°14' E), in Gorky Park (50°01' N, 36°14' E), Botanical Garden of V. N. Karazin KhNU (50°01' N, 36°13' E), street plantations on Pavlovo pole (Dzerzhinsk District of Kharkiv) (50°02' N, 36°13' E), Kharkiv Forest Park (50°03' N, 36°15' E) and in Kharkiv region, in particular near V. V. Dokuchaiev Kharkiv National Agrarian University – in Veterans' Park and Dendrological Park of V. V. Dokuchaiev KhNAU (49°53' N, 36°27' E), in Danylivsky experimental State Forestry of Ukrainian Research Institute of Forestry and Forest Melioration named after H. M. Vysotsky (50°09' N, 36°31' E), in the village of Bereka, Pervomaisky district, Kharkiv region (49°28' N, 36°12' E). At the same time, several accounting points were laid in each plantation, which differed in environmental conditions.

The distribution and biological peculiarities of the lime leafminer were studied on plants of the genus *Tilia* – small-leaved lime (*Tilia cordata* Mill.), large-leaved lime (*T. platyphyllos* Scop.), silver lime (*T. tomentosa* Moench.) or *T. argentea* Desf. ex DC., Amur lime (*T. amurensis* Rupr.), Mongolian lime (*T. mongolica* Maxim), American lime (*T. americana* L.), European lime (*T. europaea* – hybrid between small-leaved and large-leaved lime), Japanese lime (*T. japonica* (Miq.) Simonk), Take linden (*T. taquetii* Schneider), and Siberian lime (*T. sibirica* Bayer).

The entomological object of research was a pest of small-leaved lime leafminer (*Ph. issikii* Kumata, 1963). The survey of plantations was carried out according to generally accepted methods (Meshkova, 2006; Meshkova, 2009; Meshkova & Mikulina, 2010; Mikulina, 2011; Meshkova & Mikulina, 2012).

The dates of the beginning of phenological phenomena of studying tree species were recorded by observations twice a week according to the method of M.P. Sakharov (Sakharov, 1961).

Once a week, from the beginning of leaf development to its falling (May – September), 100 leaves of the studied breeds were plucked at each accounting point, having been selected randomly from different parts of the crowns and placed in separate packages with labels. The sample volumes of trees and leaves were determined based on the results of preliminary statistical analysis. The population of trees by moth was defined as the proportion of populated leaves to the total number of analyzed leaves as a percentage.

On model trees, layerto study the distribution of leafminers settlements by layers, leaves were selected from the upper, middle, and lower layer of crowns – at the height of up to 2 m with pruning shears and the middle and upper layers – with a knot cutter. In the areas where the planned cutting in of trees of the studied species was carried out, a complete analysis of the distribution of leafminers in the crown layers was carried out.

Caught and collected insects were determined in the laboratory using a binocular microscope and determinants (Bednova & Belov, 1999; Zerova et al., 2010) and compared with specimens from the collection of the forest protection Laboratory of Ukrainian Research Institute of Forestry and Forest Melioration named after H. M. Vysotsky and the Kharkiv Entomological Society. To determine the species composition of entomophages, caterpillars' moths were collected and stored in the laboratory in separate test tubes with labels (Melika et al., 2006).

The age of leafminers larvae was determined based on the length and appearance of mines and the size of caterpillars (Sefrova, 2002). Leafminers moths were identified with the help of senior researcher of the Museum of Nature of V. N. Karazin Kharkiv National University Yu. A. Huhli, entomophages – with the help of the head of the Department of Entomophagous Taxonomy and Ecological Principals of Biological Method of I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine, Doctor of Biological Sciences A.V. Humovsky. The determination of individual species of the genus *Tilia* was carried out with the help of the head of the Department of dendrology V. I. Shatrovska (Botanical Garden of V. N. Karazin Kharkiv National University) and senior researcher T. V. Orlovska (Ukrainian Research Institute of Forestry and Forest Melioration named after H. M. Vysotsky). During the cameral processing of the material, the number of mines, caterpillars, pupae, and exuviae of leafminers on each leaf was determined.

We are considering that the damage by leafminers up to 25% of the leaf surface is compensated by the tolerance of the host plant and has little effect on its productivity. The damage over 75% of the surface is catastrophic for plants; we estimated the level of crown damage on a 4-point scale: 0 points – the area of the leaf is not damaged, 1 – point the area of the damaged leaf from 0 to 25%, 2 points – from 25 to 50%, 3 points – from 50 to 75%, 4 points – the area of the damaged leaf exceeds 75 % (Antyuhova, 2010).

Results and discussion

According to our research, lime tree buds began to open most early in 2008 – on April 10; in other years, this phenomenon was registered a little later, the latest in 2011 – on April 21 (Table 1).

In all years, the dates of this phenomenon coincided with the dates of a stable transition of air temperature through 10 °C and were earlier than 50 years ago. We registered the beginning of covering lime trees with leaves in 2008 and 2009 on May 4 and 2010 and 2011 – April 24 and 26, respectively. Full cover of lime trees with leaves occurred on May 18 in 2009 and May 3 and 7

in 2010 and 2011. In the 50s of the last century, the yellowing of lime tree leaves started on August 5, complete turning yellow was recorded on October 2, and the fall of leaves lasted from August 22 to October 14 (Sakharov, 1961).

Table 1. Phenology of small-leaved lime (Kharkiv)

Index	Dates				
	Saharov, 1961	by years			
		2008	2009	2010	2011
Opening of the buds	30.04	10.04	16.04	19.04	21.04
The beginning of covering with leaves	05.05	04.05	04.05	24.04	26.04
Full cover with leaves	31.05	14.05	18.05	03.05	07.05
The beginning of flowering	24.06	13.06	12.06	12.06	15.06
The end of flowering	08.07	02.07	01.07	25.06	29.06
The beginning of fruit and seed ripening	04.09	18.07	15.07	25.07	28.07
The end of fruit and seed ripening	22.09	23.08	20.08	25.08	28.08
The beginning of turning yellow of leaves	05.08	20.07	23.07	22.07	26.07
Complete turning yellow of leaves	02.10	28.09	25.09	22.09	21.09
The beginning of leaves falling	22.08	24.07	20.07	29.07	06.08
Complete leaves falling	14.10	20.09	27.09	12.09	18.09

The lime leafminer in Japan (Kumata et al., 1983), in the south of the Far East (Ermolev, 1977), in Transnistria (Antyuhova, 2010), and Udmurtia (Izhboldina, 2008; Zorin, 2012) has two generations, and in Moscow and Moscow Region (Bednova. & Belov, 1999), as well as in the Czech Republic (Sefrova, 2002) develops in two entire generations, but may have an incomplete third generation. In our research region, the seasonal development of the lime leafminer has not been studied yet. Therefore, to effectively reduce the negative impact of this pest on the condition and growth of lime trees, it is necessary to study the peculiarities of seasonal development of this insect and identify the periods of the greatest vulnerability of individuals to protective measures.

The development of the lime leafminer is confined to the host plant – a small-leaved lime tree and the timing and rate of development of both the feed plant and the phytophage depend on the course of the season temperature. According to this, leafminer caterpillars appeared only after the full development of lime tree leaves, and the last individuals completed development before the leaves began to turn yellow.

According to our research, in 2008-2011, the turning yellow of lime tree leaves was recorded in the third decade of July, completely turning yellow — in the third decade of September. The falling of leaves damaged by a lime leafminer began in 2009, on July 20, in 2010 — on July 29, 2011 — on August 6, and complete leaves falling was registered in the third decade of September 2009 and in the second decade of September 2010. Thus, the dates of the temperature transition through 15 °C and further down (see Table 2) could not affect the development of the lime leafminer in any way since turning yellow and leaves falling occurred earlier.

During the years of M. P. Sakharov's research (Sakharov, 1961), the flowering of lime trees occurred from June 24 to July 8, and fruit ripening — from September 4 to September 22. These phenomena also occurred earlier in the years of our research — flowering from mid to late June with a slight variability of dates over the years and fruit ripening — from mid-July to August 28. The timing of lime tree flowering did not differ much by year, like most summer phenomena (Meshkova, 2009), and the ripening of fruits occurred both in the presence of leaves and in their absence.

The butterflies of lime leafminer overwinter in the cracks of tree bark. The flight of butterflies after overwintering began in the first decade of May.

The butterflies mated shortly after flying out. Females laid their eggs one at a time on the lower surface of a leaf (Fig. 3). In all years of our research, the first eggs of lime leafminer were discovered in the second decade of May. At this time, the average decadal air temperature was approaching 15 °C in 2008 and 2009 and exceeded this threshold in 2010 and 2011. At this time, in the years of our research, lime tree leaves have reached their maximum size. In the Czech Republic (Sefrova, 2002), butterflies activate earlier – in the first decade of May, and Udmurtia (Izhboldina, 2008) – in the second decade of May at temperatures above 10 °C.

The development of eggs of lime leafminer lasts from 4-8 days (Sefrova, 2002) to two weeks (Zorin, 2012). It is challenging to trace the development of individual eggs of lime leafminer in natural conditions since the eggs are tiny, and the plucked leaves quickly dry up. According to our research, the period between egg laying by a lime leafminer and the appearance of mines was at least a week. In all years, the first mines of this pest on the lime tree were discovered by us in the third decade of May.

Due to the nonsimultaneous flying out of imagoes after overwintering in different trees and areas, the development of caterpillars and pupae was also stretched over time.

Larvae of I age bite into the mesophyll and form serpentine parts of mines. Mines of larvae of II age are also serpentine. As the caterpillars develop, the mines take on the appearance of spots and an area of 0.5–1.5 cm². By III age, the larva feeds only on the spongy parenchyma of the leaves. Larvae of IV age gradually pull together the leaf, forming an oval lower-sided folded mine, visible on the upper side of the leaf. Larval excrement is gathered in a lump. The larva gnaws out small areas in the palisade parenchyma at the V age, and whitish dots are visible on the upper part of the leaves. The appearance of lime leafminer larvae is shown in Fig. 4.

According to studies conducted in Udmurtia (Izboldina, 2008), the lime leafminer has 4 larval ages, according to studies conducted in the Czech Republic (Sefrova, 2002) – 5. According to our research, the appearance and size of mines of V-age caterpillars did not change compared to the appearance and size of IV-age caterpillars' mines, but the size of the caterpillars increased. Pupation of lime leafminer caterpillars occurs in a mine (Fig. 5).

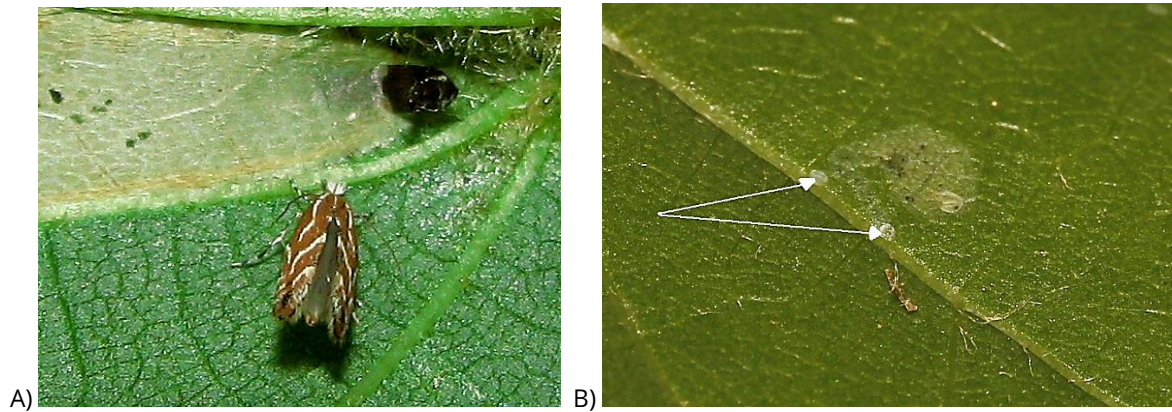


Fig. 3. Imago (A) and eggs of lime leafminer (B) (July 2009, photo by the author)

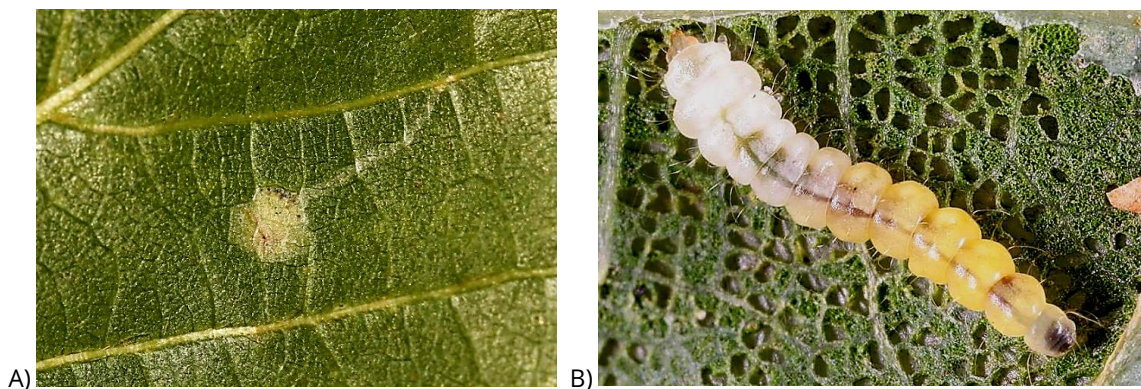


Fig. 4. Larvae of lime leafminer (A – mine with a larva of I age, B – larva of IV age; July 2010, photo by the author)

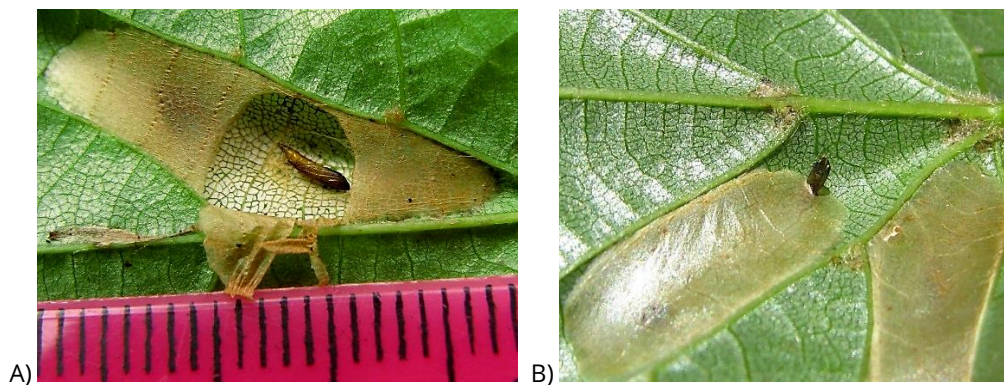


Fig. 5. Pupa of lime leafminer (A) and exuvium in a mine of lime leafminer (B) (June – July 2010, photo by the author)

We discovered the first pupae of I generation in the third decade of May–June. Development of caterpillars continued for two weeks in 2011 and three weeks in other years, depending on weather conditions. According to H. Shefrova (Sefrova, 2002), in the laboratory at a temperature of 25 °C, the development of lime leafminer caterpillars lasts two weeks, and in the forest, it can be more than five weeks. In our research region, the air temperature exceeded 20 °C in the third decade of May 2011, and other analyzing years – in the first decade of June. Mass pupation of caterpillars and the I generation occurred in the second or third decades of June and coincided with the mass flowering of linden trees. Pupae developed for about 10 days. They were found in mines during June and the first decade of July, even in July's second decade in 2011.

Imagoes of the first generation were detected in the second decade of June in 2008 and 2010. They were detected from the third decade of June in 2009 and 2011. According to N. Izboldina (Izboldina, 2008), in Udmurtia, the flight began in early July, and according to H. Shefrova (Sefrova, 2002), in the Czech Republic – in early June. In our region, the air temperature had an intermediate value between these indicators, which was reflected in the development of the lime leafminer.

The development of the second generation of the lime leafminer was even more stretched. Eggs were detected in 2008 and 2010 from the third decade of June to the second decade of July, in 2009 and 2011 — during July.

Mines of the second generation appeared en masse in the second decade of July, and the pupation of caterpillars occurred from the third decade of July to the second decade of August. The mines with pupae discovered at the end of July could have been the last mines of the first generation or the first mines of the second generation.

Pupae of the second generation were detected until the third decade of August in all years except 2011 when the last pupae were discovered in mid-September.

Imagoes were detected throughout the season. Imagoes of the second generation sometimes laid eggs, from which caterpillars of the third generation emerged (II and III decades of August 2008, III decade of August and I decade of September 2010, I and II decades of September 2011). At the same time, due to turning yellow and leaves falling, these caterpillars did not complete development and died.

Imagoes of the second generation that overwintered had a darker color. At the low population density of the lime leafminer and shaded areas where the leaves turned yellow, later, mines were detected even in September.

Based on the average research data in 2008-2011, a phenological calendar of lime miner development was constructed (Table 2).

Table 2. Phenological calendar of lime leafminer development based on average long-term data in green plantations (Kharkiv, 2008-2011).

Generation	Wintering	Months-decades														
		May			June			July			August			September		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
I	+	+	+	+	+											
			•	•	•											
				—	—	—	—									
					O	O	O	O								
						+	+	+	+	+						
II							•	•	•	•						
								—	—	—	—	—				
										O	O	O	O	O		
											+	+	+	+	+	+
												•	•	•	•	•
													—	—	—	—

Reference designation • – egg, — – larva, O – pupa, + – imagoes.

Conclusions

The density of mines of the lime leafminer population was close to 2 pieces/leaf. The flight of butterflies after overwintering began in the first decade of May of the second generation – in the second – third decades of June. The maximum mines density was registered in the second decade of June and the second decade of August. The maximum leaf population of lime leafminer reached 50% in Danylivsky experimental State Forestry Enterprise and Forest Park.

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