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ORIGINAL ARTICLE

Seasonal development of the chestnut leaf miner (*Cameraria ohridella* Deschka & Dimic, 1986) in the eastern forest-steppe of Ukraine

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The authors have studied the peculiarities of seasonal development of horse chestnut leaf miners in green plantations of the Kharkiv region and obtained data were compared with data on the terms of development and temperature conditions in other regions. The sum of effective temperatures required to develop one generation of horse chestnut leaf miner moth was established. Studies of the seasonal development of horse chestnut leaf miners show the overlap of individual generations, which is confirmed by the analysis of the population by stages and ages. It is established that in all years of research, the first maximum density of mines of horse chestnut leaf miner occurred in the period from the third decade of May to the second decade of June, the second – in the I – III decade of July, and the third – in the II – III decade of August. Over the years of research, the density of mines of horse chestnut leaf miners has tended to increase. The highest density of mines of horse chestnut leaf miners was determined in Gorky Park and the Botanical Garden of V. N. Karazin KhNU. The density of butterflies of horse chestnut leaf miner is the highest in the lower part of the trunks.

Keywords: leafminer insects, *Cameraria ohridella*, biological peculiarities, harmfulness, deciduous trees, horse chestnut, green plantations.

Introduction

Horse chestnut, or ohrid leafminer (*Cameraria ohridella* Deschka et Dimić, 1986). Genus Cameraria Champ., 1902 is represented by 80 species and belongs to the subfamily Lithocolletinae, a part of the family of burnet moths – Gracillariidae (Zerova et al., 2007). The horse chestnut leafminer has been causing damage to horse chestnut *(Aesculus hippocastanum* L.) for almost 30 years. It is common in natural forests in the Balkans and is used for landscaping parks, squares, streets, in green plantations along roads (Cebeci & Acer, 2007). This insect was first discovered near Lake Ohrid in the forests of Macedonia on the border with Albania in 1984–1985, which is reflected in its Latin name – "Ohrid leafminer" (Deschka & Dimic, 1986). As a new species, the insect was described by G. Dechka and N. Dymyćh (Deschka et Dimić, 1986). In western Ukraine, the horse chestnut leafminer appeared in 1996–1997, and in 2003 it spread to Kyiv (Zerova et al., 2007). In Kharkiv region, the horse chestnut leafminer was discovered in 2006 by Yu.P. Maksymov and others (Maksimova et al., 2009), in 2007 – by Yu.A. Guglia and A. I. Zinenko (Guglya & Zinenko, 2008) and V.L. Meshkova, I.M. Mikulina (Meshkova & Mikulina, 2008). Now, this pest covers almost the entire territory of Ukraine (Fedorenko, 2009).

According to various authors (Heitland & Metzger, 1997), the horse chestnut leafminer can have 2 to 4, sometimes five generations per year – from 1 in the UK to 4–5 in Greece and Italy. In most countries, horse chestnut leafminer stably develops three generations with an exponential increase in the number from generation to generation (Skuhravy, 1998). Several generations of horse chestnut leafminer overlapping and facultative summer diapause have been observed in Bulgaria (Evans & Oszako, 2007). Therefore, the dates of flight peaks differ by region (IOCB, 2004). According to the data obtained in the Kyiv region (Zerova et al., 2007), during the season under the conditions of Ukraine, 3–4 generations of horse chestnut leafminer can develop. The fourth generation is often facultative; caterpillars develop by the II-IV ages and die in winter, significantly reducing the winter supply of phytophages. The life cycle of the development of the horse chestnut leafminer ends with the leaving of pupae for wintering. In dry July-August and premature leaf aging, a significant part of individuals of the second or third generation (up to 15 %) goes to diapause (Zerova et al., 2007).

Analysis of the rate of the horse chestnut leafminer distribution over the past decades shows that conditions are optimal for its reproduction and distribution in Western Europe and Ukraine. According to Gamanova (2011), the greatest threat to horse chestnut trees exists in the steppe zone, where 4–5 times in 11 years, four generations may develop and 5–6 times – three, and the harmfulness of the phytophage is enhanced by the lack of moisture in the growing season.

In the Forest-Steppe, where the sum of active temperatures ensures the sustainable development of three generations 7–8 times over 11 years and slightly higher humidity of the growing season, the degree of risk and harmfulness of the phytophage is a little lower (Beljaev et al., 2017; Belyaev et al., 2017). In Polissia, Carpathian Foothills, and Transcarpathia, three pest generations most often develop during the growing season. In Sumy, Ternopil, and Khmelnytskyi regions, only two generations can develop 2–3 times over 11 years, and due to the higher level of moisture content of the growing season, the level of harmfulness of horse chestnut leafminer is the lowest (Gamanova, 2011).

Materials and methods

We have studied the peculiarities of the seasonal development of horse chestnut leaf miners in green plantations of the Kharkiv region and obtained data compared with data on the terms of development and temperature conditions in other regions.
Obtained data on the terms of development of horse chestnut leaf miner and corresponding temperature indicators in Kharkiv (50°00'00"N 36°13'45"E) we compared with the data obtained in Kyiv (50°27'00"N 30°30'00"E) in 2003–2006 (Zerova et al., 2007) and Tiraspol (46°50'23"N 29°36'23"E) in 2005–2009 (Antyuhova, 2008). Considering that in these regions since the beginning of the year, the authors calculated the sum of temperatures for the dates of emergence of each stage of horse chestnut leaf miner, we determined the differences of the corresponding amounts for the periods of development of individual stages and generations.
The study was conducted in parks and street plantings in Kharkiv (50º00' N, 36º15' E), in particular Shevchenko Park (50º00' N, 36º14' E), Gorky Park (50º01'N, 36º14'E), Botanical Garden of V. N. Karazin KhNU (50º01'N, 36º13'E), street plantings on Pavlovo pole (Dzerzhinski district of Kharkiv) (50º02' N, 36º13' E), Forest Park of Kharkiv (50º03'N, 36º15'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 (49º53'N, 36º27'E), in Danylivske experimental State Forestry of Ukrainian Research Institute of Forestry and Forest Melioration named after H. M. Vysotsky (50º09'N, 36º31'E), in the village of Bereka Pervomaisky district, Kharkiv region (49º28'N, 36º12'E). Simultaneously, several points of accounting were laid in each plantation, which differed in environmental conditions.
Surveys of plantations were performed according to generally accepted methods Vasilev & Livshic, 1984; Meshkova, 2002, Tuzova, 2004, Meshkova, 2006; Zerova et al., 2007, Rubcov & Utkina, 2008; Meshkova, 2009). The dates of Occurrence of phenological phenomena of the studying tree species were recorded using observations twice a week according to the method of M. P. Sakharov (Sakharov, 1961). Calculation of imagoes of horse chestnut leaf miner on the trunks of horse chestnut was carried out using frames measuring 10 × 10 cm (1 dm^2). Once a week, from the beginning of leaves development to their falling (May–September), 100 leaves of the studying species were plucked at each point of accounting, randomly chosen from different parts of the crowns, and placed in separate packages with labels. The volumes of trees and leaves sampling were determined based on the results of previous statistical analysis аналізу (Meshkova & Mikulina, 2008). The population of trees by moth was defined as populated leaves to the total number of analyzed leaves as a percentage.
On model trees, to study the distribution of leaf miner settlements by the stores, leaves were selected from the upper, middle, and lower stores of crowns – at the height of up to 2 m with pruning shears from the middle and upper stories – with lopping shears. On the plots where the planned felling of trees of the studying species was carried out, a complete analysis of the distribution of leaf miners by the crown stores was carried out.
Caught and collected insects were determined in the laboratory using a binocular microscope and determinants (Tarbinskij & Plavilshikov, 1948, Gerasimov, 1952, Zerova et al., 2010) and compared with specimens from the collection of the forest protection laboratory of the Ukrainian Research Institute of Forestry and Forest Melioration named after H. M. Vysotsky and Kharkiv Entomological Society. To determine the species composition of entomophagous, the moths of caterpillars were collected and stored in the laboratory in separate test tubes with labels (Melika et al., 2006). The age of leaf miner larvae was determined by taking into account the length and emergence of mines and the size of caterpillars (Sefrova, 2002, Zerova et al., 2007). The species belonging to the horse chestnut moth was determined with the help of Yu.O. Guglia, a senior researcher at the Nature Museum of V. N. Karazin KhNU. The determination of individual species of the genus *Aesculus* was carried out with the help of V. I. Shatrovska (Botanical Garden of V. N. Karazin KhNU,) and 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 leaf miners on each leaf was determined. In total, about 120,000 individuals of insects were registered and analyzed (reared, determined by species, fertility was counted up).
Given that damage by leaf miner of up to 25% of the leaf surface is compensated by the tolerance of the host plant and has little effect on its productivity. Damage to more than 75% of the surface is catastrophic for plants; we estimated the level of crown damage on a 4-point scale: 0 points – leaves surface is not damaged, 1 point – the area of damaged leaves is from 0 to 25%, 2 points – from 25 to 50%, 3 points – from 50 to 75%, 4 points – the area of damaged leaves exceeds 75% (Antyuhova, 2008).
The area of leaves and mines was measured using the method of pallets. To do this, the leaf was placed on a sheet of millimeter paper. The number of 1 × 1 cm cells that completely fell inside the contour was counted, and half of the number of cells that partially fell inside the contour was added to them. When studying the seasonal development of leaf miner moths, the time of emergence of the first individuals of each stage was recorded. Data from the Kharkiv weather station were used to characterize the temperature conditions of the development of leaf miner moths.
The sum of positive temperatures, the sum of temperatures for a period with an air temperature of more than 10 ºC, and the sum of effective temperatures at a threshold of 10 ºC for the same period was calculated, and the dates of stable transition of the average daily air temperature across 0, 5, 10, 15 and 20 ºC in spring and autumn were graphically and analytically determined (Meshkova, 2009).

Results

The development of the horse chestnut leaf miner referred to the host plant – horse chestnut and the terms and pace of development of both the forage plant and the phytophage depend on the season temperature. According to long-term data of M. P. Sakharov (Sakharov, 1961), in the Kharkiv region, the buds of horse chestnut began to swell on 11 April and open on 23 April. The beginning of covering with horse chestnut leaves was registered in the 50s of the last century on 1 May, complete covering with leaves on 14 May. According to our research, the earliest buds of horse chestnut began to open in 2010 on 1 April; in other years, this phenomenon was registered a little later, the latest in 2011 on 14 April (Table 1). In all years, the dates of this phenomenon are recorded earlier than 50 years ago. We registered the beginning of covering with horse chestnut leaves in 2008 on 9 April, in 2009, 2010, and 2012 – on April 14, 12, and 13, respectively, and the latest in 2011 – on 26 April. The complete covering of horse chestnut with leaves occurred in all the years of our research earlier than 50 years ago – on May 5, 10, and 3 in 2008, 2009, and 2011 and on 25 April in 2010 and 2012. In the 50s of the last century, the yellowing of horse chestnut leaves began on 1 August. Complete yellowing was recorded on 9 October and leaves falling lasted from 19 August to 18 October (Sakharov, 1961).

According to our research, in 2008–2011, yellowing of horse chestnut leaves was recorded on 22 June 2009, 10 July 2010, July 12 and 20, 2011 and 2008, respectively. Twisting of leaves damaged by horse chestnut leaf miner was also detected starting from 29 June 2009, 14 July 2010, July 19 and 25, 2011, and 2008. Damaged leaves fell prematurely in the second (2009–-2011) and third (2008) decades of July. Complete falling of horse chestnut leaves was recorded in the third decade of September (2010) and in the second and third decades of October (2008, 2009, 2011). Premature yellowing and falling of horse chestnut leaves were accompanied by new leaves and autumn flowering, which were registered from September 20 – 27, 2009, and August 13 – 19, 2010.

Table 1. Phenology of horse chestnut (Kharkiv).

Indicators	Dates					
	N.P. Saharov, 1961	Year				
		2008	2009	2010	2011	2012
Buds swelling	11.04	–	–	–	–	–
Buds opening	23.04	4.04	10.04	1.04	14.04	2.04
The beginning of covering with leaves	1.05	9.04	14.04	12.04	26.04	13.04
Complete covering with leaves	14.05	5.05	10.05	25.04	3.05	25.04
Emergence of inflorescences	–	30.04	20.04	18.04	30.04	22.04
The beginning of flowering	12.05	5.05	7.05	3.05	8.05	27.04
The end of flowering	27.05	20.05	26.05	18.05	25.05	16.05
The beginning of fruit growth	–	26.05	31.05	22.05	30.05	20.05
The end of fruit ripening	–	04.10	27.09	24.09	28.09	–
The beginning of leaves yellowing	1.08	20.07	22.06	10.07	12.07	–
Twisting of damaged leaves	–	25.07	29.06	14.07	19.07	–
Complete yellowing of the leaves	9.10	–	–	–	–	–
The beginning of leaves falling	19.08	27.07	15.07	15.07	17.07	–
Complete leaves falling	18.10	16.10	20.10	26.09	08.10	–
The beginning of covering with new leaves	–	–	20.09	13.08	–	–
Autumn flowering	–	–	27.09	19.08	–	–

The flowering of horse chestnut in the years of research by M. P. Sakharov (Sakharov, 1961) occurred from 12 May to 27 May; he did not register the terms of fruit ripening. In the years of our research, the beginning of horse chestnut flowering was registered in the first decade of May 2008 – 2011, and 2012 – on 27 April. The end of horse chestnut flowering was also registered in 2008 – 2011on similar dates, which do not differ much from the data of M. P. Sakharov (Sakharov, 1961). This confirms the well-known conclusion about the low variability of summer phenomena by years and geographical coordinates (Meshkova, 2009).
The growth of horse chestnut fruits occurred from the third decade of May in all years, and fruit ripening occurred both in the presence of leaves and in their absence at the beginning of the third decade of September in all years except 2008; this phenomenon was registered on 4 October.
Pupae of horse chestnut leaf miner overwinter in fallen leaves. According to our research, the flight of butterflies from overwintering sites began in the first decade of May and 2012 – in the third decade of April (Table 2).
In all years, the mass flight began 4–7 days after its beginning.

Table 2. Phenology of horse chestnut leaf miner (Kharkiv).

Indicator	Dates by years				
	2008	2009	2010	2011	2012
The flying out of imagoes after overwintering	5.05	4.05	28.04	4.05	22.04
The mass flight of imagoes after overwintering	10–26.05	10–23.05	3–16.05	11–25.05	26.04–10.05
Emergence of mines of I generation	29.05	23.05	19.05	20.05	10.05
The emergence of pupae of I generation	19.06	14.06	9.06	15.06	–
The flying out of imagoes of II generation	26.06	25.06	18.06	23.06	–
The mass flight of imagoes of II generation	29.06–15.07	1–15.07	22.06–6.07	29.06–19.07	–
The emergence of mines of II generation	14.07	8.07	30.06	8.07	–
Emergence of pupae of II generation	28.07	24.07	17.07	25.07	–
The flying out of imagoes of III generation	6.08	4.08	27.07	4.08	–
The mass flight of imagoes of III generation	9–24.08	6–19.08	3–17.08	10.08–24.08	–
The emergence of mines of IV generation	20.08	21.08	15.08	16.08	–
Emergence of pupae of IV generation	27.09	25.09	14.09	28.09	–
The emergence of mines of IV generation on the leaves of autumn shoots	–	27.09	20.09	–	–

According to A.V. Antyuhova (Antyuhova, 2008), in Tiraspol, the first butterflies of horse chestnut leaf miner flew out on April 13–16, according to data from the Kyiv region (Zerova et al., 2007) – 24 April–4 May, and the mass flight was recorded on May 12–27. Thus, the beginning of the flight of horse chestnut leaf miner butterflies from overwintering sites in Kharkiv was close to Kyiv's data and later than in Tiraspol. The obtained data are related to the peculiarities of the temperature regime of the regions under

consideration since the development of wintering pupae began after a stable temperature transition across 10 ºC, and the temperature in the overwintering sites determined the duration of their development.
Thus, during the years of research by A.V. Antyuhova (2008) in Tiraspol, a stable transition of air temperature across 10 ºC occurred on April 7–12, and across 15 ºC on May 3–11. In Kharkiv, during the years of our research (2007–2012), a stable transition of air temperature across 10 and 15 ºC occurred on April 10–23 and May 5–24, respectively.
The average air temperature at the beginning of the flying out of butterflies from overwintering sites in Kharkiv ranged from 11.5 to 18.4 ºC; the average long–term temperature was 13.5 ºC (Table 3).
The sum of positive temperatures at the flying out the date of butterflies of horse chestnut leaf miner in Kharkiv was 339.6 – 569.5 °C (average 422.7 ºC), the sum of active temperatures at the threshold of 10 ºC – 173.3 – 320.9 ºC (224.8 ºC), the sum of effective temperatures at the threshold of 10 ºC – 23.3 – 70 ºC (49.8 ºC). The first two indicators were the highest in 2008 and the lowest in 2010. The third indicator was also the lowest in 2010 but the highest in 2011.

Table 3. Characteristic of temperature at the dates of flying out of butterflies of horse chestnut miner from overwintering sites (Kharkiv).

Years	The dates of flying out	The date of stable transition of air temperature across 10 ºC	T °C_{av}	The sum of temperatures, °C		
				positive	active at the threshold of 10 ºC	effective at the threshold of 10 ºC
2008	5.05	10.04	11.5	569.5	320.9	50.9
2009	4.05	23.04	13.3	424.1	205.1	55.1
2010	28.04	15.04	12.7	339.6	173.3	23.3
2011	4.05	22.04	18.4	357.7	200.0	70.0
Average	3.05	18.04	13.5	422.7	224.8	49.8

According to data from the Kyiv region (Zerova et al., 2007), the sum of effective temperatures on the dates of the beginning of flying out of horse chestnut leaf miner imagoes from overwintering sites ranged from 68 to 132 ºC, which is close to the upper value of the indicator obtained in Kharkiv (Table 3). These authors do not provide data on the sums of positive and active temperatures.
According to data obtained in Tiraspol (Antyuhova, 2008), the sum of positive temperatures on the dates of the beginning of flying out of horse chestnut leaf miner imagoes after wintering was 473.4 – 530.8 ºC, and effective temperatures at the threshold of 10 ºC – from 0 to 3.6 ºC. The sum of positive temperatures on the date of flying out of horse chestnut leaf miner is quite close in Kharkiv and Tiraspol, and the sum of effective temperatures in Tiraspol is much lower. The obtained data can be explained by the lack of accurate data on the temperature threshold of development of horse chestnut leaf miner pupae after wintering and the variability of microclimatic conditions of their overwintering sites. In this regard, we consider it appropriate to determine the terms of flying out of horse chestnut leaf miner from overwintering sites by phenoindicators – with a complete covering of horse chestnut trees with leaves and at the beginning of their flowering (Table 1).
Based on the average research data in 2008–2011, a phenological calendar of horse chestnut leaf miner development was constructed (Table 4).

Table 4. Phenological calendar of horse chestnut leaf miner 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
		O														
		+	+	+												
I		•	•	•												
				–	–	–										
						O	O	O								
							+	+	+							
II	O						•	•	•							
								–	–	–	–					
									O	O	O	O				
										+	+	+	+			
III										•	•	•	•			
												–	–	–	–	–
															O	O

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

From the flying out of the first butterflies to the emergence of the first mines on the leaves of horse chestnut passed in different years from 16 to 25 days, the temperature at this time was from 13.9 to 17.9 °C, the sum of positive temperatures was 248.4–376 °C (average 310.2 °C), the sum of active temperatures at the threshold of 10 °C – 239–376 °C (average 294.8 °C), the sum of effective temperatures at the threshold of 10 °C – 78.5–166 °C (average 112.3 °C) (Table 5).
The first mines of this pest were discovered at the beginning of the second decade of May in 2012, at the end of the second decade of May in 2010 and 2011, in the third decade of May – in 2008 and 2009 (Table 2). This phenomenon coincided with the dates of

the ending of horse chestnut flowering (Table 1). In Tiraspol, the first mines were discovered on May 10–15 (Antyuhova, 2008), in Kyiv – on May 5–18 (Zerova et al., 2007), that is, somewhat earlier than in Kharkiv.

From the emergence of the first caterpillars to the emergence of the first pupae in different years it took from 21 to 27 days (average 22 days), the temperature at this time was from 18 to 20.6 °C (average 19.1 °C), the sum of positive temperatures and active temperatures at the threshold of 10 °C was 388.4–473.3 °C (average 420.7 °C), the sum of effective temperatures at the threshold of 10 °C – 176.8–243.3 °C (average 200.7 °C) (Table 5). According to data from the Kyiv region (Zerova et al., 2007), the sum of effective temperatures at the threshold of 10 °C for the development of horse chestnut leaf miner caterpillars was 375–492 °C (average 432.3 °C). According to the data obtained in Tiraspol (Antyuhova, 2008), the sum of positive temperatures for caterpillar development was 258.6–291.3 °C, that is, it was closer to our data.

We identified the first pupae of I generation in the first decade of June 2010 and in the second decade of June 2008, 2009, and 2011 (Table 2). In Tiraspol (Antyuhova, 2008), the first pupae were discovered on May 24–29, in Kyiv – on June 7–17 (Zerova et al., 2007).

Imagoes of II generation were detected in Kharkiv in the third decade of June 2008, 2009, and 2011 and at the end of the second decade of June in 2010. The mass flight was registered until mid – July, only in 2010 – until the first decade of July (Table 2). In Tiraspol, flight of imagoes of II generation was registered in the first decade of June (Antyuhova, 2008), in Kyiv – in the second-third decades of June (Zerova et al., 2007).

From the emergence of the first pupae to the flying out of the first imagoes of II generation in Kharkiv, it took from 7 to 11 days (average 9.5 days) at a temperature of 19.9–22.8 °C (average 21.1 °C). The sum of positive temperatures and active temperatures at the threshold of 10 °C was 119.6–228.6 °C (average 195.5 °C), the sum of effective temperatures at the threshold of 10 °C was 59.6–118.6 °C (average 103 °C) (Table 5). According to data from the Kyiv region (Zerova et al., 2007), the sum of effective temperatures at the threshold of 10 °C for the development of pupae of horse chestnut leaf miner of II generation was 98–122 °C (average 104.5 °C). According to data obtained in Tiraspol (Antyuhova, 2008), the sum of positive temperatures for pupal development was 199.5–320.7 °C, the sum of effective temperatures at the threshold of 10 °C was 109.5–130.7 °C.

Table 5. Sums of positive, active and effective temperatures at a threshold of 10 °C accumulated during the development of individual stages of horse chestnut leaf miner.

Intervals between the emergence of individual stages	Years	Duration of period, days	$T°C_{av.}$	Sum of temperatures, °C		
				positive	active at a threshold of 10 °C	effective at a threshold of 10 °C
From the flying out of butterflies after wintering to the emergence of mines of I generation	2008	25	13.9	348.6	305.7	115.7
	2009	19	14.1	267.9	258.5	78.5
	2010	21	17.9	376	376	166
	2011	16	15.5	248.4	239	89
	Average	20,3	15.4	310.2	294.8	112.3
From the emergence of mines to the emergence of pupae of I generation	2008	22	18.0	396.8	396.8	176.8
	2009	23	19.3	424.2	424.2	204.2
	2010	21	18.5	388.4	388.4	178.4
	2011	27	20.6	473.3	473.3	243.3
	Average	23,3	19.1	420.7	420.7	200.7
From the emergence of pupae to the flying out of butterflies of II generation	2008	7	19.9	119.6	119.6	59.6
	2009	11	20.8	228.6	228.6	118.6
	2010	9	22.8	205.6	205.6	115.6
	2011	11	20.8	228.3	228.3	118.3
	Average	9,5	21.1	195.5	195.5	103.0
From the flying out of butterflies of II generation to the emergence of mines of II generation	2008	18	19.6	352.9	352.9	172.9
	2009	13	21.9	284.7	284.7	154.7
	2010	12	24.2	289.8	289.8	169.8
	2011	13	19.5	253.7	253.7	123.7
	Average	14,0	21.3	295.3	295.3	155.3
From the emergence of mines to the emergence of pupae of II generation	2008	14	23.2	185.3	185.3	105.3
	2009	16	24.6	343.9	343.9	203.9
	2010	17	23.8	380.5	380.5	220.5
	2011	17	23.6	329.8	329.8	189.8
	Average	16,0	23.8	309.9	309.9	179.9
From the emergence of pupae to the flying out of butterflies of III generation	2008	9	20.9	460	460	240
	2009	11	22.1	287.2	287.2	157.2
	2010	10	26.1	287	287	177
	2011	10	22.6	338.9	338.9	188.9
	Average	10	22.9	343.3	343.3	190.8
From the flying out of butterflies of III generations to the emergence of mines of III generation	2008	14	26.5	185.8	185.8	115.8
	2009	17	17.8	303.4	303.4	133.4
	2010	19	28.0	532.6	532.6	342.6
	2011	12	23.5	282.3	282.3	162.3
	Average	15,5	24.0	326.0	326.0	188.5
From the emergence of mines to the emergence of pupae of III generation	2008	38	15.7	708.2	642.6	262.6
	2009	35	18.1	596.9	596.9	266.9
	2010	30	18.8	563.2	563.2	263.2
	2011	43	18.4	479.5	479.5	219.5
	Average	36,5	17.8	587.0	570.6	253.1

Mines of II generation of horse chestnut leaf miner were discovered in Kharkiv the earliest in 2010 – on 30 June, in the first decade of July 2009 and 2011, and the latest – in 2008 (14 July) (Table 2). In Tiraspol (Antyuhova, 2008) II generation mines were detected on June 9-20, pupae – on July 4–9. In Kyiv (Zerova et al., 2007), mines of II generation were detected on 23 June–4 July, pupae – on July 15–31. The duration of the period from the flying out of butterflies to the emergence of mines of II generation horse chestnut leaf miner in Kharkiv was 12-18 days (an average of 14 days), the average temperature during this period was 19.5–24.2 °C (an average of 21.3 °C). In Tiraspol (Antyuhova, 2008), the average temperature of this period was 21.2–24.4 °C. The sum of positive and active temperatures at the threshold of 10 °C temperatures for the period from the flying out of butterflies to the emergence of mines of II generation horse chestnut leaf miner in Kharkiv was 253.7–352.9 °C (average 295.3 °C), the sum of effective temperatures at the threshold of 10 °C was 123.7–169.8 °C (average 155.3 °C). In Tiraspol (Antyuhova, 2008), the sum of positive temperatures for this period was 183.4–208.6 °C, the sum of effective ones at the threshold of 10 °C – 103.4–108.6 °C, in Kyiv (Zerova et al., 2007) the sum of effective temperatures at the threshold of 10 °C was 114–161 °C.
Pupae were formed in Kharkiv from the second decade of July (2010) to the end of the third decade of this month (2008, 2009, 2011) (Table 2), in Tiraspol (Antyuhova, 2008) – on July 4-9, in Kyiv (Zerova et al., 2007) – on July 15–31. The duration of the development of horse chestnut leaf miner caterpillars of II generation in Kharkiv was 14–17 days (average 16 days) at an average temperature of 23.2–24.6 ◦C (average 23.8 °C), in Tiraspol (Antyuhova, 2008) it took 20–26 days at an average temperature of 21–23.3 °C. The sum of positive temperatures for the development of caterpillars of II generation in Kharkiv was 185.3–380.5 °C (average 309.9 °C), in Tiraspol (Antyuhova, 2008) it were 426.2–602.7 °C. The sum of effective temperatures at the threshold of 10 °C in Kharkiv was 105.3-220.5 °C (179.9 °C), in Tiraspol (Antyuhova, 2008) it was 236.2–352.7 °C in Kyiv (Zerova et al., 2007) – 386-400 °C (average 393.8 °C).
Flying out of the first III generation imagoes in Kharkiv was the earliest registered in 2010 (27 July), in 2008, 2009, and 2011 – on August 6 and 4. The mass flight of III generation imagoes began a few days after the beginning of flying out and lasted until the end of the second decade (2009 and 2010)–the beginning of the third decade of August (2008 and 2011) (Table 2). In Kyiv, III generation imagoes were identified on 22 July–8 August (Zerova et al., 2007), in Tiraspol on 14 July (Antyuhova, 2008). Pupal development continued in Kharkiv for 9–11 days (average 10 days) at a temperature of 20.9–26.1 ◦C (average 22.9 ◦C). In Tiraspol (Antyuhova, 2008) pupal development lasted 10–11 days; in Kyiv (Zerova et al., 2007) it was 7–17 days. The sum of positive temperatures for pupal development in Kharkiv was 287–460 °C (average 343.3 °C) (Table 5), in Tiraspol it was 112.2–225.8 °C (Antyuhova, 2008). The sum of effective temperatures at the threshold of 10 ◦C in Kharkiv was 157.2–240 ◦C (average 190.8 °C), in Tiraspol it was 62.3–126.7 °C (94.5 °C) (Antyuhova, 2008), in Kyiv 56–153 °C (average 109.8 ◦C) (Zerova et al., 2007).
Mines of III generations of horse chestnut leaf miners were found in Kharkiv on August 15 and 16, 2010 and 2011, and a little later (August 20 and 21) – in 2008 and 2009 (Table 2). In Tiraspol (Antyuhova, 2008), mines were detected on July 21–28, in Kyiv (Zerova et al., 2007) – on 28 July–23 August. The average temperature from the flying out of butterflies to the emergence of mines, which lasted 12–19 days, in Kharkiv was 17.8–28 °C (24 °C). The sum of positive temperatures for this period in Kharkiv was 185.8–532.6 °C (average 326 °C) (Table 5), in Tiraspol (Antyuhova, 2008) 153.6–400.5 °C (277.1 °C), the sum of effective temperatures at the threshold of 10 °C in Kharkiv was 115.8– 342.6 °C (188.5 °C), in Tiraspol (Antyuhova, 2008) it was 83.6–260.5 °C (172.05 °C), in Kyiv (Zerova et al., 2007) – 78–229 °C (156.5 °C).
Pupae of III generations were formed in Kharkiv in the third decade of September in 2008, 2009, 2011, and a little earlier in 2010 (14 September) (Table 2). In Tiraspol, they were formed on 30 August–11 September (Antyuhova, 2008), in Kyiv – on 20 August–23 September (Zerova et al., 2007). The development of caterpillars of III generations lasted in Kharkiv 30–43 days (average 36.5 days) at a temperature of 15.7–18.8 °C, in Tiraspol 24–40 days of 25.2–27.4 °C (Antyuhova, 2008). The sum of positive temperatures for the period of development of III generation caterpillars in Kharkiv was 479.5–708.2 °C (570.6 °C), in Tiraspol, it was 928.3–1042.6 °C (985.5 °C) (Antyuhova, 2008), and the sum of effective temperatures at the threshold of 10 °C reached in Kharkiv 219.5–266.9 °C (253.1 °C), in Tiraspol 528.3–592.6 °C (560.5 °C) (Antyuhova, 2008), in Kyiv it was 294–448 °C (375.8 °C) (Zerova et al., 2007).
Most of III generation horse chestnut leaf miner pupae remained in diapause until spring. At the same time, in 2009 and 2010, we discovered IV generation mines in the third decade of September (Table 2) on a leaf that has grown in autumn (Table 1). Due to the presence of autumn frosts, a decrease in the average air temperature in the third decade of October of less than 10 °C and the cessation of vegetation of horse chestnut, the development of individuals of this generation could not be completed.
According to data from the Kyiv region (Zerova et al., 2007), IV generation's imagoes flew out of pupae of III generations in September, and the caterpillars of this generation developed from mid to late September and pupated in October. No individuals of IV generation of horse chestnut leaf miner were found in Tiraspol.
Comparison of different generations shows (Table 5) that the period from the emergence of butterflies from overwintering sites to the emergence of mines averaged 20.3 days at 15.4 °C, II generation – 14 days 21.3 °C, III generation – 15.5 days at 24 °C. The obtained data are coordinated with data on the acceleration of insect development with an increase in temperature. The sum of positive temperatures for the development of butterflies and eggs of these generations was 310.2, 295.3, and 326 °C; that is, it differed little by generations. Simultaneously, the sum of effective temperatures at the threshold of 10 °C in different generations reached 89; 155.3 and 188.5 °C and tended to grow more than twice from I to III generations.
The duration of caterpillar development of I, II, and III generations was 23.3, 16, and 36.5 days at an average daily temperature of 19.1, 23.8, and 17.8 °C. The fastest was the development of II generation caterpillars when the air temperature was the highest. The sum of positive temperatures during the period of development of caterpillars of I, II, and III generations was 420.7, 309.9, and 587 °C, and the sum of effective temperatures was 200.7, 179.9, and 253.1 °C. The development of pupae of I and II generations was 9.5 and 10 days at an average temperature of 21.1 and 22.9 °C. A slightly more extended period of pupae development of II generation compared to I generation, despite the higher temperature during their development, may be associated with an ineviTable overlap of generations. The sum of positive temperatures during the pupae development of I and II generations was 195.5 and 343.3 °C, and the sum of effective temperatures was 103 and 190.8 °C. The increase in these indicators during the development of II generation also indicates the impact of generation overlap.
We calculated the duration of development of individual generations and the corresponding temperature indicators for the dates of emergence of butterflies, mines, and pupae of adjacent generations (Table 6). The duration of the periods between the emergence of wintering butterflies of I generation in Kharkiv was 50–53 days (average 51.5 days), II and III generations – 39–42 days (average 40.5 days). According to data from the Kyiv region (Zerova et al., 2007), this indicator was 55 and 46 days, and in Tiraspol (Antyuhova, 2008) – 46–58 and 34–43 days, respectively.

The duration of the periods between the emergence of mines of I and II generations in Kharkiv was 42-49 days (average 35.8 days), II and III generations – 37–46 days (average 41.5 days) (Table 6). According to data in Tiraspol (Antyuhova, 2008), this indicator was 25– 60 and 31–49 days, respectively.

The duration of periods between the emergence of pupae of I and II generations in Kharkiv was 38–40 days (average 39.3 days), II and III generations – 59–65 days (average 62 days). According to data in Tiraspol, this indicator was 41 and 52–69 days, respectively. In all cases, the development of II generation continued faster than I one due to higher air temperature value during the II generation development (Table 6).

The sum of positive temperatures for the development of one generation, determined by the flying out dates of imagoes of I and II generations in Kharkiv, was 926.4 and 948.4 °C, determined by the dates of the emergence of mines – 911.5 and 979.2 °C, by the dates of the emergence of pupae – 811.8 and 1256 °C, the average for all analyzed intervals – 970.4 °C (Table. 6). According to data obtained in Tiraspol (Antyuhova, 2008), this indicator is determined by butterflies and was 821 and 880 °C, by the dates of emergence of mines – 664 and 961 °C, by the dates of emergence of pupae – 918.6 and 1432 °C, the average for all analyzed intervals – 946 °C.

The sum of effective temperatures for the development of one generation, determined by the flying out dates of imagoes of I and II generations in Kharkiv, was 416 and 525.9 °C, determined by the dates of the emergence of mines – 459 and 559.2 °C, by the dates of the emergence of pupae – 438.2 and 632.4 °C, the average for all analyzed intervals – 505.1 °C (Table 6). According to the data obtained in Tiraspol (Antyuhova, 2008), this indicator is determined by butterflies and was 296.7 and 495 °C, by the dates of emergence of mines – 335 and 561 °C, by the dates of emergence of pupae – 508.6 and 827 °C, the average for all analyzed intervals – 503.8 °C. For the Kyiv region, the average sum of effective temperatures was 505 °C (Zerova et al., 2007).

Studies of the seasonal development of horse chestnut leaf miners show the overlap of individual generations, which confirms the analysis of the population by stages and ages. So, at the end of June 2012, when temperature conditions caused the emergence and development of horse chestnut leaf miner almost two weeks earlier than in 2011, the population was dominated by caterpillars of II generation (77% in total), among which the vast majority were in I and II ages (30.1 and 28.2%, respectively).

Table 6. Sums of positive, active, and effective temperatures at a threshold of 10 °C, accumulated during the development of individual generations of horse chestnut leaf miner.

Periods	Years	Duration of period, days	T°C$_{av.}$	Sum of temperatures, °C		
				positive	active at a threshold of 10 °C	positive
From the flying out of butterflies after wintering to the beginning of the flight of II generation butterflies (development of I generation)	2008	53	16.3	865	822.1	352.1
	2009	52	17.7	920.7	911.3	401.3
	2010	51	19.0	970	970	460
	2011	50	19.0	950	940.6	450.6
	Average	51.5	18.0	926.4	911.0	416.0
From the beginning of the flight of II generation butterflies to the beginning of the flight of III generation butterflies (development of II generation)	2008	41	20.8	998.2	998.2	518.2
	2009	40	22.9	915.8	915.8	515.8
	2010	39	24.5	957.3	957.3	567.3
	2011	42	22.0	922.4	922.4	502.4
	Average	40.5	22.5	948.4	948.4	525.9
From the emergence of I generation mines to the emergence of II generation mines (development of I generation)	2008	46	18.9	869.3	869.3	409.3
	2009	46	20.4	937.5	937.5	477.5
	2010	42	21.0	883.8	883.8	463.8
	2011	49	20.3	955.3	955.3	485.3
	Average	35.8	20.2	911.5	911.5	459.0
From the emergence of II generation mines to the emergence of III generation mines (development of II generation)	2008	37	22.5	831.1	831.1	461.1
	2009	44	21.2	934.5	934.5	494.5
	2010	46	26.1	1200.1	1200.1	740.1
	2011	39	23.2	951	951	541
	Average	41.5	23.2	979.2	979.2	559.2
From the emergence of I generation pupae to the emergence of II generation pupae (development of I generation)	2008	39	20.6	657.8	657.8	337.8
	2009	40	22.6	857.2	857.2	477.2
	2010	38	23.7	875.9	875.9	505.9
	2011	40	21.4	811.8	811.8	431.8
	Average	39.3	22.0	800.7	800.7	438.2
From the emergence of II generation pupae to the emergence of III generation pupae (development of II generation)	2008	61	18.3	1354	1288.4	618.4
	2009	63	18.8	1187.5	1187.5	557.5
	2010	59	23.0	1382.8	1382.8	782.8
	2011	65	20.8	1100.7	1100.7	570.7
	Average	62	20.2	1256.3	1239.9	632.4
Total average		46.6	21.0	970.4	965.1	505.1

Almost half as many individuals were at III age (14.5 %), and only 4.1 % – at IV age (fig. 1). At the same time, I generation was represented by individuals of V age (0.4%), pupae (3.8 %), and exuviae (18.8 %).

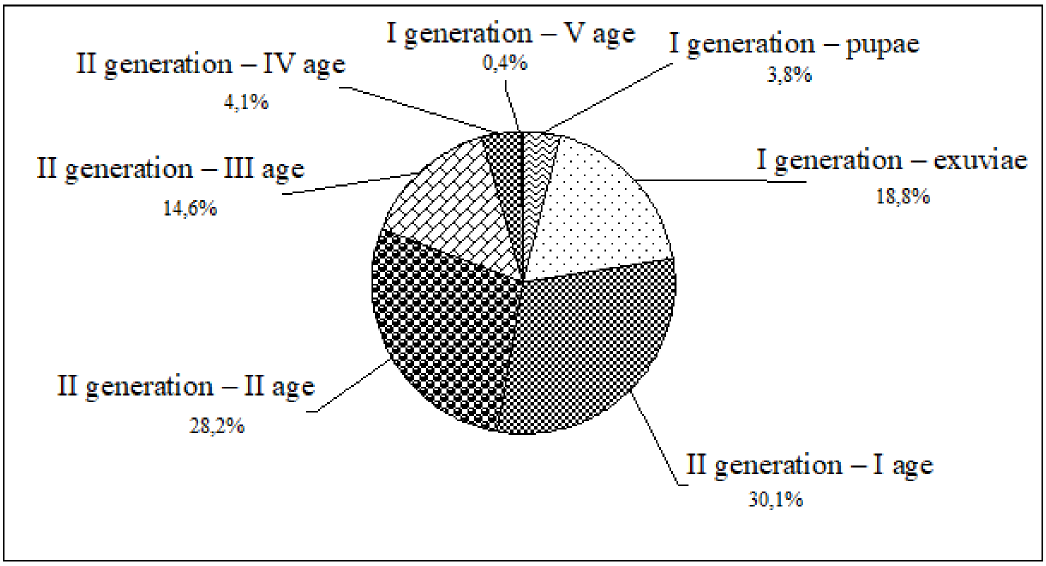

Fig. 1. Distribution of horse chestnut leaf miner individuals by generations and ages in the third decade of June 2012 (mixed sampling from Shevchenko Park and Gorky Park – 5642 mines).

On individual trees in the street plantings of Pavlove pole and on the territory of the Botanical Garden of KhNU, individual leaf blades of complex leaves of horse chestnut with the presence of mines of caterpillars of the I – II ages were marked with numbers. Data on the length of mines and the age of larvae in each of them were recorded three times a week (fig. 2).
In I generation, mines of I age caterpillars were found from 3 June to 10 June. Their length increased from 1.5 to 3 mm (on average, 2.2 ± 0.5 mm). Mines of II age caterpillars were detected from 3 June to 13 June and had a length of 2 to 4 mm (3.4 ± 0.2 mm). Mines of III-age caterpillars were detected from 3 June to 18 and ranged in length from 3 to 10 mm (6.1 ± 0.3 mm). Mines of IV age caterpillars from 3 June to 23 June had a length of 8 to 44 mm (on average, 18.8 ± 1.5 mm). Mines of V age caterpillars – from 13 to 20 June had a length of 25 to 45 mm (on average 33.4 ± 1.4 mm), VI age – from 13 to 23 June had a length of 26 to 45 mm (on average 36.4 ± 2.0 mm). Pupae were detected from 15 June to 13 July, imagoes of the new generation – from 26 June to 15 July (Mikulina, 2012).

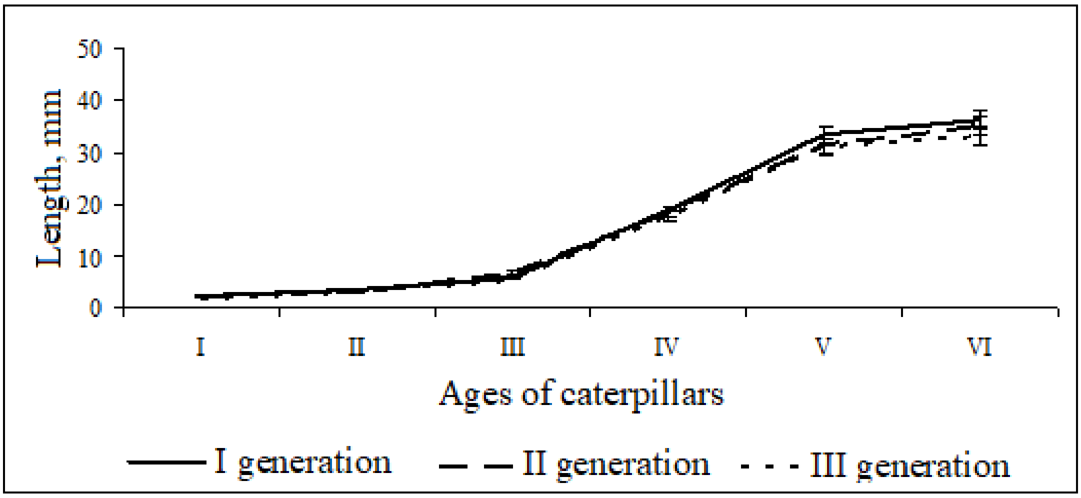

Fig. 2. Dynamics of mines length of caterpillars of different ages and generations.

Mines of larvae of I age of II generation were detected on 17 July (1–3 mm, on average 2.3 ± 0.2 mm), II age – on July 1–19 (2–6 mm, on average 3.3 ± 0.3 mm), III age – on July 17–27 (4–10 mm, on average 6.6 ± 0.3 mm), IV age – on July 17–29 (10–29 mm, on average 18.7 ± 1.2 mm), V age from 19 July to 2 August (20–46 mm (average 31.3 ± 1.6 mm), VI age – from 22 July to 4 August (25–46 mm, average 35.1 ± 2.2 mm). Pupae were detected from 25 July to 15 August, imagoes – from 2 August to 17 August.
Due to the elongated flying out of imagoes from pupae that wintered in sites with different microclimates and the elongated hatching of caterpillars from eggs laid in different parts of the crowns, caterpillars of almost all ages were simultaneously detected during the development of I generation. During the development of individuals of II generation, the overlap of development of individual ages increased, and during the period of III generation development, it was constantly recorded. So, on 26 August, mines of I–III age caterpillars were simultaneously detected, and on 28 September – mines of IV-VI age caterpillars and individual imagoes of the previous generation. In the first decade of October, most horse chestnut leaf miner caterpillars were pupated (Mikulina, 2012).
The increase in the length of mines in different generations did not significantly differ during the development of caterpillars of I–III ages. The length of mines of IV age caterpillars in I generation increased by 12.7 mm, in II and III – by 12.1 and 12.2 mm, respectively. The length of mines of V age caterpillars in I generation increased by 14.7 mm, in II and III – by 12.5 and 13.3 mm, respectively. The obtained data indicate that the most favorable conditions were for the development of I and II generation

caterpillars. During the development of caterpillars of III generations, the leaves of horse chestnuts are significantly damaged, limiting the growth opportunities of mines. Another reason for the differences in the growth of mines of III generations may be a change in the chemical composition of leaves at the end of the growing season (Meshkova & Mikulina, 2013).

Conclusions

1. The sum of effective temperatures for the development of one generation, determined by the flying out dates of imagoes of I and II generations in Kharkiv, and it was 416 and 525.9 °C, determined by the dates of the emergence of mines – 459 and 559.2 °C, by the dates of the emergence of pupae – 438.2 and 632.4 °C, the average for all analyzed intervals – 505.1 °C.
2. Studies of the seasonal development of horse chestnut leaf miners indicate the overlap of individual generations, which confirms the analysis of the population by stages and ages. So, at the end of June 2012, when temperature conditions caused the emergence and development of horse chestnut leaf miner almost two weeks earlier than in 2011, the population was dominated by caterpillars of II generation (77% in total), among which the vast majority were in I and II ages (30.1 and 28.2%, respectively).
3. In all years of research, the first maximum density of mines of horse chestnut leaf miner occurred in the period from the third decade of May to the second decade of June, the second – in the I–III decade of July, and the third – in the II–III decade of August, and in 2009 and 2010, mines of IV generation were found, the larvae in which did not complete development by the end of the growing season and died. Over the years of research, the density of horse chestnut leaf miner mines has tended to increase. The highest density of mines of horse chestnut leaf miners was determined in Gorky Park and the Botanical Garden of V. N. Karazin KhNU. The population of leaves of horse chestnut leaf miners reached 100% only in 2011. The density of butterflies of horse chestnut leaf miner is the highest in the lower part of the trunks.

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