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ORIGINAL ARTICLE

# Diversity and distribution of leaf mining insects in deciduous tree plantations. A review.

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The authors present a general description of leaf mining insects of deciduous trees of green plantations. Biological peculiarities of adventitious leaf mining moth are highlighted. Their economic significance, biotic factors of decrease in population, tree species resistance to settlement by leaf mining insects, entomophagous of leaf mining moth are indicated. In particular, we have noted protection measures of trees from leaf mining insects. It is established that adventitious leaf mining moths from the order Lepidoptera (Lepidoptera) of the Gracillariidae family have recently spread in the green plantations of Ukraine: on horse chestnut – horse-chestnut leaf-miner *Cameraria ohridella* Deschka & Dimic; on black locust – locust leaf-miner *Parectopa robiniella* Clemens and *Phyllonorycter robiniella* Clemens; on the lime tree – lime leaf-miner *Phyllonorycter issikii* Kumata. These noted leaf mining moths cause significant damage to plantations, manifested in the loss of decorative effect, decreased photosynthetic surface, and the weakening of trees. In the Kharkiv region, these species have spread over the past decade. Today, detailed studies of the peculiarities of their distribution and development have not been conducted. Among the measures to limit the number of leaf mining insects in urban environments, attention should be paid to resistant forms and hybrids, raking and composting fallen leaves, using pheromone traps, spraying crowns, and injecting insecticides into tree trunks.

**Keywords:** leaf mining insects, biological peculiarities, harmfulness, deciduous trees, horse chestnut, lime tree, black locust, green plantations.

# Introduction

Green plantations in cities play an essential role. The leaves of trees absorb carbon dioxide, release oxygen, and purify the air from dust and emissions from industrial facilities and vehicles (Levon et al., 2008). Simultaneously, urban green plantations are weakened by pollutants and are more susceptible to insect damage. In the city, there is almost no vegetation on which entomophagous can provide additional feeding.

The presence of houses, fences, utility rooms, and other structures provides suitable conditions for the successful wintering of many harmful insects, contributing to the growth of their numbers in cities (Koricheva, 1998). Trees growing in cities have sparse crowns and smaller leaves compared to forest trees. Due to dust cover, the intensity of trees' photosynthesis in the city is several times less than in the forest. Under such conditions, damage to leaves by insects in cities can have more significant consequences for trees' viability (Kozlov, 1991).

In cities, leaf miners are most commonly spread compared to other groups of insects, characterized by a secretive lifestyle and therefore are more resistant to the adverse effects of weather factors and air pollutants than leaf beetles (Myeshkova et al., 2009). Research on leaf mining insects' biology and ecology is relevant due to their spread in cities and the need to develop environmentally-friendly measures to reduce their numbers.

# Results

## General characteristics of leaf mining insects of deciduous trees in green plantations

Leaf mining insects live inside parts or organs of plants and gnaw out long passages or "mines" in them (Belov, 2011). Forming passages-mines in plant tissue, which contains chlorophyll, as a way of phytophagous insect feeding, was found in representatives of Lepidoptera, Hymenoptera, Diptera, and Coleoptera (Belov, 2011). The population density of many leaf minings is stable over the years, and they do not threaten the state of plantations (Belov, 2011). In particular, some species, particularly the lower-sided poplar burnet moth (Phyllonorycter populifoliella Tr.), are capable of mass reproduction (Tarasova

et al., 2004). Simultaneously, some species, even at a low population density, can significantly reduce the decorative effect and resistance of individual trees and plantations (Baranchikov & Ermolaev, 1998).)

Obligate leaf minings carry out the entire larva development cycle inside a single leaf, while facultative leaf minings develop in mines only during several larva ages. In all cases, larva's first ages pass inside plant tissues (Hering, 1951).

Among the leaf minings, oligophages predominate, which damage species of the same or similar genera of woody plants, which is usually reflected in their names – "poplar", "maple", "willow" (Dovnar-Zapolskij, 1969). Most leaf minings develop a single larva in a mine, and when the mines merge, the youngest larvae die. At the same time, in some species, several larva individuals live in a mine. Thus, during the horse chestnut leaf-miner development, it is possible to merge mines and develop two larvae in one mine if it has sufficient dimensions (Akimov, 2003).

Most leaf minings pupate outside the mine, sometimes individuals of the first generation pupate outside the mine, and the second – in mines (*Chromatomyia lonicerae* Robineau-Desvoidy: Diptera). Some species can pupate both inside and outside the mine, in particular *Acrocercops brongniardella* Fabr. (Lepidoptera) (Zerova, 2001).

Most leaf minings overwinter at the pupal stage, but some overwinter at the larval stage (followed by continued feeding or direct pupation after wintering) or imago (Baranchikov & Ermolaev, 1998). At the imago stage, leaf minings spend the winter in cracks in the bark on tree trunks, and in the city, they can spend the winter indoors. At the larval stage, leaf minings can overwinter on the soil surface and pupate next spring (*Zeugophora subspinosa* Fabricius: Coleoptera), winter in coleoptile on branches (*Coleophora sp.*:Lepidoptera), in mines in fallen leaves (*Tischeria ekeblandella* Bjerkander: Lepidoptera). At the pupal stage, leaf minings can winter in the soil (genus *Stigmella*: Lepidoptera), in mines in fallen leaves (genus *Phyllonorycter*: Lepidoptera), in cracks in the bark of trunks (*Leucoptera malifoliella* Costa: Lepidoptera) (Hespenheide, 1991).

The number of generations of leaf mining insects depends mainly on the ambient temperature. So horse chestnut and lime leaf-miners in Moscow have the incomplete third generation, and poplar leaf-miner – the second incomplete (Belov, 2011). In Moscow green plantations, D. A. Belov (2011) identifies three leaf-miners' phenological groups. The spring-summer group includes representatives of Nepticulidae and Gracilariidae, whose caterpillars damage the leaves in late May and early June. The summer group includes birch leaf-miner sawflies, birch burnet moths, linden midget moths, linden, and elm leaf-miner sawflies. Representatives of the third group feed throughout the season since they have two or more generations or a long development period. These are, in particular, lime burnet moth and horse chestnut leaf-miner. Due to leaf minings' ability to migrate by air currents, the foci of their mass reproduction along transport routes are often linear (Sefrova & Lastuvka, 2001).

Most female leaf minings lay one egg per leaf, which reduces the likelihood of competition between larvae of the same species for food resources (Hirao & Murakami, 2008). Yu.M. Baranchykov (Baranchikov & Ermolaev, 1998) divides the dynamics of leaf minings populations into horizontal and vertical ones. Horizontal ones act on the same trophic level as leaf minings, while vertical ones influence from the side of other trophic levels. An example of horizontal factors is the competition between individuals of the same population and other types of phytophages; among vertical factors the influence of feed plants, pathogens, predators, and parasites.

The direct effect of abiotic factors on leaf minings has not been directly studied, but they are found mainly in the temperate zone (Zerova et al., 2007; Yevtushenko & Grama, 2011). These are the destruction of mines under the influence of wind and precipitation, the effect of frosts on the viability of various stages of leaf minings (Antyuhova, 2010). Under the influence of low temperatures, the synchronicity of leaf minings larvae and feed suitability dynamics may be disrupted. Then younger larvae cannot complete development due to the transverse leaf veins (Tarasova et al., 2004). On the other hand, favorable abiotic conditions can create prerequisites for leaf-mining foci appearance. Thus, due to the late fall of oak leaves in Northern Virginia (USA), the viability of the second generation of *Cameraria hamadryadella* Clemens increased (IOCB, 2004).

The volume of leaf mass limits the food base of a leaf mining on the tree, so an increase in population density leads to increased competition, an increase in larval mortality, a decrease in pupae's mass, size, and imago fecundity (Csóka & Hirka, 2011). A leafmining can die if it destroys the entire parenchyma before it finishes development or if the leaves fall prematurely (Csóka, 2003). By the nature of nutrition, mono- and oligophages predominate among leaf minings. According to the nature of leaf tissue damage, there are four types of mines. The first group includes mines with a completely eaten leaf parenchyma and limited by the undisturbed upper and lower epidermis. In selective damage to the palisade or spongy parenchyma, mines are classified as upper-sided or lower-sided, respectively. If only the epidermis of a leaf is damaged, the mine belongs to the epidermal type. The depth and shape of mines depend on the type of insect (Hering, 1951). The development of leaf mining insects occurs much faster than in free-living insects since leaf minings do not use leaf tissues that are difficult to digest as food: epidermis, cuticle, and tissues of vascular bundles. Caterpillars make mines only in tissues with a low content of protective substances (Hering, 1951).

Many leaf minings do not feed at the imago stage. The survival and fecundity of offspring directly depend on the choice of egglaying sites by females. When searching for a feed plant, leaf minings orientate on the chemicals secreted by the plant. Females choose leaves of a certain age, size, and area of the leaf blade for laying eggs (Baranchikov & Ermolaev, 1998). Especially dangerous are leaf mining moths, which have relatively recently expanded their natural habitat – adventitious species (Isaev et al., 2001). These species include lime leaf-miner (*Phylonorycter issikii* Kumata, 1963) (Gninenko & Kozlova, 2006), horse chestnut, or ohrid leaf-miner (*Cameraria ohridella* Deschka et Dimić, 1986) (Tregub & Pleskach, 2009), locust digitate leaf-miner (*Parectopa robiniella* Clemens, 1859) and black locust leaf-miner (*Phyllonorycter robiniella* Clemens, 1859) (Sefrova, 2002). A.V. Antiukhova (Antyuhova, 2010) has found in the green plantations of Transnistria, apart from noted species, a juniper moth (*Dichomeris marginella* F., 1775) and plane tree burnet moth (*Ph. platani* Staudinger, 1870). She has studied the biological and morphological signs of local populations of leaf minings of ornamental introduced species, the harmfulness, prevalence of leaf minings, peculiarities of seasonal development, species composition of their entomophagous, and the range of feed plants.

## Biological peculiarities of adventitious leaf mining moths

<u>The horse chestnut leaf-miner or horse chestnut leaf-mining moth.</u> 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 leaf-miner 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 Macedonia's forests on the border with Albania in 1984–1985, which is reflected in its Latin name – "Ohrid leaf-miner" (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 leaf-miner 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, 2010). Now, this pest covers almost the entire territory of Ukraine (Fedorenko, 2009).

The horse chestnut leaf-miner overwinters at the pupal stage in mines in a soft cradle inside fallen leaves. Butterflies fly out in late April – early May. Imago that did not lay eggs does not fly well and mainly focuses on the shaded side of tree trunks and skeletal branches, making it possible to visually detect the beginning and mass appearance of butterflies (Tribel & Gmanova, 2008). After mating, females spread in the crown of trees, mainly in the low story, and lay eggs atop the leaves, mainly near the lateral ribs, less often near the central rib. The female lays 20-40 eggs; their development, depending on the temperature, lasts 7–14 days (Sefrova, 2001). After hatching, the caterpillar penetrates under the cuticle into the leaf cells' epidermal layer, where it feeds at a younger age. After molting at the IV age, the larva feeds on the tissues of the palisade leaf parenchyma. Unlike representatives of the genus Phyllonorycter, in which the caterpillar of the V age continues to feed and simultaneously weaves a cradle, representatives of the genus Cameraria are characterized by the presence of a different VI age "afaga", in which the caterpillar does not feed, but only weaves a cradle (Gerasimov, 1952). The development of horse chestnut leaf-miner caterpillars lasts 25–36 days, of which 1–3 days account for the first age, 3–5 for II, 4–6 for III, 5–7 for IV, 10–12 for V, 1–3 days for VI (prepupa) (Sefrova, 2001). The caterpillar pupates in a cradle inside the mine. Before the imago flies out, the pupa breaks through the cradle and moves 2/3-3/4 of body from the mine (Sefrova, 2001). According to various authors (Heitland & Metzger, 1997), the horse chestnut leaf-miner 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 leaf-miners stably develop three generations with an exponential increase in the number from generation to generation (Skuhravy, 1998). Several generations of horse chestnut leaf-miner 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 Kyiv region data (Zerova et al., 2007), during the season under the conditions of Ukraine, 3-4 generations of horse chestnut leaf-miner 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 leaf-miner ends with pupae leaving 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).

The distribution rate of the horse chestnut leaf-miner over the past decades shows that conditions are optimal for its reproduction and distribution in Western Europe and Ukraine. According to Kyiv researchers (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. 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 leaf-miner is the lowest (Gamanova, 2011).

Locust leaf-miner. Black locust (*Robinia pseudoacacia* L.) was introduced to Europe at the beginning of the XVII century as an ornamental type, and to Ukraine, it was brought by V. N. Karazin at the end of the XVIII century. It is still considered an exotic species in Europe, although in some countries, particularly in Romania and Hungary, it has become an essential landscape element (Csóka, 2003; Rice, Westerman, & Federici, 2004). This type is used to protect the land from erosion and rehabilitate degraded areas, forest zones, hedges, and wood – in construction, furniture production, parquet (Fodor & Hâruţa, 2009). It is proved (Rice, Westerman & Federici, 2004) that black locust, due to its symbiosis with nodule bacteria, enriches the soil of forest ecosystems with nitrogen, which is favorable for developing oak-pine forests. About 30 years ago, black locust leaf-miner moths spread in Europe, which became dangerous for this plant in many countries (Wojciechowicz-Żytko & Jankowska, 2004).

Locust digitate leaf-miner (*Parectopa robiniella* Clemens, 1863) is a native species of North America, and in the 80s, it spread to Italy, Slovenia, Croatia, Austria, Slovakia, Romania, Hungary, and Ukraine (Melika et al., 2006). Black locust leaf-miner *Phyllonorycter robiniella* (Clemens, 1859) is also of North American origin. In 1983, the black locust leaf-miner was discovered in Switzerland, in 1988 – in France, Germany, Italy, in 1990 – in Austria, in 1992 – in Slovakia, in 1994 – in Slovenia, in 1996 – in Hungary, in 2003 – in Bulgaria (Wojciechowicz-Żytko & Jankowska, 2004). It was first registered in the Netherlands in 1999 (Csóka, 2003). Currently, both types of locust leaf-miners are distributed in 19 European countries. In Ukraine, these species appeared relatively recently and are insufficiently studied. In the south of Hungary, the locust digitate leaf-miner appeared in 1983 and spread throughout the country after 6–8 years. Due to damage to the leaves of black locusts by this insect, they fall off at the end of June (Melika et al., 2006). Locust digitate leaf-miner has 2–3 generations per year, which can overlap each other. This insect feeds only on representatives of the genus *Robinia* (Antyuhova, 2010). Females of the locust digitate leaf-miner lay eggs

on the leaves' underside on the first order's lateral rib at a distance of 0.3–1.0 cm from the central rib. Mines of irregular shape, with branches, include the primary rib. There is always one green larva in the mine.

After hatching, the caterpillars bite into the leaf from the underside. Larvae make a passage along the lateral rib to the central rib, then form a triangular cavity for excrement between the leaf ribs. After that, they gnaw through a small hole in the corner between the ribs on the upper side of the leaf and form a branched mine on both sides of the main rib. The mine on the underside has a hole near the primary rib. Before pupation, the caterpillar leaves excrement near the upper side of the mine, and it spills out through a hole on the lower side. Because of this, the larvae of the last age leave the mines and pupate on the leaf litter (Csóka & Hirka, 2011).

Caterpillars of locust digitate leaf-miners of different ages can form new mines after leaving the primary mine. This does not form a cavity for excrement. Primary mines always grow from the leaf base to its top, and secondary mines are often directed in the opposite direction. In the leaf's development process, the caterpillars of the locust digitate leaf-miner go through 5 ages. Prepupa is formed in a cocoon in places of pupation (lvinskis & Rimsaite, 2008). At the beginning of the growing season, mines of the locust digitate leaf-miner are found in the lower parts of the crown, and in subsequent generations – in the middle of the crown in the middle parts of the branches (Sefrova, 2002).

Another species, the black locust leaf-miner (*Phyllonorycter robiniella*), can also develop in 2–3 generations, which overlap between June and October. Depending on the environmental conditions, butterflies start flying from late April to mid-May, after the first leaves appear on black locust trees. Laying eggs by females of the first generation occurs during the flowering period of black locust (the second half of May) and lasts until mid-June. Females lay their eggs on the lower surface of the leaf blade. The egg develops from 6 to 10 days, depending on the ambient temperature (Csóka, 2003). The caterpillar develops in 5 ages: in I–III, the larva feeds only on the spongy leaf parenchyma, and in IV and V – also columnar. After hatching, the caterpillars bite into the leaf from the underside. Initially, the caterpillar forms a narrow passage with a central line of excrement under the leaf's epidermis on the underside; during the development of caterpillars of the II and III ages, it resembles a white spot. Starting from the IV age, the larva tightens the leaf. During the development of V-age individuals, the mine is visible from the upper side of the leaf, and excrement is collected in the center of the mine. Mines do not cross the central rib. Caterpillar development is uneven, lasts 20 to 50 days, depending on the ambient temperature. Mines of the black locust leaf-miner appear a little earlier than mines of the locust digitate leaf-miner, but in both cases, the leaves from intensively populated branches may fall prematurely at the end of June. There may be several mines on the same leaf, which often merge. Caterpillars of the last age pupate in mines in white oval cocoons. Butterflies fly out in 7–10 days in summer and 10–20 days in autumn, depending on the temperature. Full development of individuals lasts 5–11 weeks (Sefrova, 2002).

<u>Lime leaf-miner.</u> The lime leaf-miner was first described in Japan in 1963 (Kumata, 1963). In 1977, it was discovered on Southern Primorye's territory and 1983 – on the Korean Peninsula (Ermolaev, 1977). In 1985, the lime leaf-miner 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 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 leaf-miner in the forest park area of Kharkiv and single mines on trees in street plantations. In 2008, lime leaf-miners' prevalence and leaf damage intensity increased noticeably (Meshkova & Mikulina, 2010).

The lime leaf-miner overwinters at the imago stage in cracks in the bark of lime trees. After wintering, the butterflies sit on the trunks, mate, and then move to the crowns. Females lay their eggs one at a time on the lower surface of lime 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 leaf-miner, 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 not only on the lower but also on 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 a 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 leaf-miners that fly out in the 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 that pass 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 takes place 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 leaf-miner 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 leaf-miner (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 leaf-miner is a shade-loving insect (Izhboldina & Zorin, 2008). At the beginning of settlement, females choose the most suitable places for laying eggs and then – the least suitable ones (Izhboldina, 2008). Lime leaf-miner 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 middle and basal areas of the leaves to lay eggs (Ermolaev & Zorin, 2011). As the settlement density increases, mines'

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density in different parts of the leaves evens out (Zorin, 2012). Due to the high density of lime leaf-miner, the decorative effect of trees is lost (Antyuhova & Meshkova, 2011), their accretion in height and diameter decreases, the intensity of flowering and the nectar content in flowers decrease (Zorin, 2012).

### The economic significance of leaf mining insects

Horse chestnut, locust, and lime leaf-miners mainly do not cause much damage to trees in the first generation. So, on the border of Croatia and Slovenia in June, horse chestnut leaf-miner damages 1% of the leaf surface, in July 3 %, in August 24 %, in September 68%, and in mid-September, the leaves fall off (Milevoj, 2004). Due to the development of mines on the leaf surface, the area involved in photosynthesis decreases. This affects the ability of leaves to retain dust and atmospheric emissions, that is, to perform ecological functions in green areas of cities and the productivity of trees (Pavan et al., 2003).

With a high density of mines, trees' decorative effect decreases, weakens, and becomes more susceptible to pathogens' penetration, and the leaves fall prematurely (IOCB, 2004). Important pathogens of horse chestnut diseases are the fungi *Guignardia aesculi* and *Erysiphe flexuosa*. Both types of fungi spread to Europe from North America in the middle of the last century. With the development of these fungi, leaf necrosis from afar resembles the damage by leaf-miners. At the same time, it is proved that butterflies of horse chestnut leaf-miner lay fewer eggs on leaves with the smell of fungi (Kiss, Vajna & Fischl, 2004).

Due to premature defoliation, which occurs at a high density of leaf mining insects, the duration of trees' photosynthetic activity decreases, which also weakens the productivity. At the same time, photosynthesis in unsettled parts of leaves does not change, and the anatomy of ribs is not disturbed even in the presence of 90 % mines (Salleo et al., 2003). Trees flowering damaged by leaf minings in late summer and autumn also weakens the trees and deprives them of an opportunity to prepare for the winter cold fully, and such trees often freeze out. In spring, individual branches of such trees dry up, and their susceptibility to harmful insects and pathogens increases. Simultaneously, in regions with mild winters, long-term damage to horse chestnuts by horse chestnut leaf-miner did not lead to mass tree loss and even to a decrease in annual wood increment. This is due to the horse chestnut's ability to form additional wood layers with large holes of conductive fabric throughout the year. Moth-damaged trees in the spring of the following year delay the beginning of vegetation, which to a certain extent protects them from harmful insects (Salleo et al., 2003).

An essential aspect of leaf mining insects s harmfulness is also their impact on the generative organs of damaged trees. It was found (Thalmann et al., 2003) that when 50–70 % of the leaves are damaged, the number of inflorescences on the tree, flowers in the inflorescence, and seeds in the fruit does not change, the size and mass of the fruit decrease. Deterioration of seed quality can negatively affect this plant's reproduction in endemic forests of South-Eastern Europe and the quality of planting landscaping material. In Tiraspol, in 2006, horse chestnut trees heavily settled with horse chestnut leaf-miners re-bloomed in the autumn, which also weakened them. Early frosts damaged autumn-flowering reduced trees' frost resistance and the buds in October and November. The following spring, it was found deformed leaves of a smaller size compared to undamaged trees, and the formation of new buds was disrupted. Trees that were significantly damaged for three consecutive years did not produce fruit, and the lower branches, where the density of mines was highest, withered (Antyuhova, 2009).

Quantitative assessment of lime leaf-miner impact on the productive and generative lime tree organs was carried out only in Udmurtia (Ermolaev & Zorin, 2011). Partial annual defoliation is recorded in lime leaf-miner foci. Due to the high settlement density by lime leaf-miner, the leaves fall prematurely. The primary damage to the leaves by moth caterpillars occurs in June during the host tree's growth period. The effect of lime leaf-miner on the lime tree's generative organs has been proved – by increasing the density of the leaf minings settlement, the number of flowers and inflorescences decreases, which causes damage to beekeeping. The same authors proved that the density of mines of lime leaf-miners increases significantly as the host tree leaves' shading increases. It is the largest in the undergrowth, in the lower parts of the crowns, at the base of lime tree branches (Ermolaev & Zorin, 2008). Considering the more significant role of light leaves in photosynthesis, lime leaf-miners' effects of damage to trees may not always be noticeable. Simultaneously, with an increase in leaf-miners' density, the length of shoots, and the number of formed buds, the increment of earlywood also decreases (Zorin, 2012).

We have not found out quantitative data on locust leaf-miners' harmfulness, although many works indicate their negative impact on black locust's decorative effect (Seljak, 1995).

## Biotic factors of reducing the number of leaf mining insects

The ability to protect trees from leaf minings insects in green areas of cities is limited due to the inadmissibility of using toxic preparation. In this regard, the available research aims to study the biotic factors of regulating the population number of leaf minings, mechanical methods, pheromones (Gilbert et al., 2009).

<u>Species that are resistant to settlement by leaf miners</u>. One of the measures to reduce the damage caused by leaf minings is to use resistant species to the corresponding pests in landscaping. In squares and parks of Europe, hybrid *A. hippocastanum* × *A. pavia* (meat red horse chestnut) – *A. carnea*. On these horse chestnuts, the chestnut leaf-miner caterpillars die in the early stages of development before feeding on palisade tissue (IOCB, 2004). The remaining hybrids are obtained by crossing *A. flava*, *A. glabra*, *A. pavia*, and *A. sylvatica*, the susceptibility of *A. hippocastanum* × *bushii* is temperate, and *A. hippocastanum* × *neglecta* – is stable (Zerova et al., 2007).

It has been shown that the horse chestnut leaf-miner, in the absence of choice, can develop on 21 of the 36 tested species of *Aesculus*, in particular on *A. hippocastanum*, *A. turbinata*, *A. octandra* (= *A. flava*), and *A. pavia*. On *A. chinensis* it does not complete development before imago. On *A. indica*, *A. Californica*, and hybrid *A. carnea*, grafted on *A. hippocastanum*, larvae die in the I–II ages when feeding on sap (Evans & Oszako, 2007). According to other data (Skuhravy, 1998), *A. parviflora*, *A. carnea*, *A. glabra*, and *A. indica*) proved resistant to damage by the horse chestnut leaf-miner, *A. lutea* was slightly damaged, and *A. pavia* – was damaged considerably.

In Bosnia and Herzegovina, the horse chestnut leaf-miner settled and completed development on horse chestnut and *A. glabra* var. *glabra* and *A. flava* (*A. lutea*, *A. octandra*) chestnuts (Evans & Oszako, 2007). Horse chestnut *A. japonica* was also susceptible to settlement by a moth (Skuhravy, 1998; Ferracini & Alma, 2008). There is information about the complete successful development of horse chestnut leaf-miner on maples (*Acer platanoides* and *A. pseudoplatanus*), but the imago was small, the mortality rate of larvae is high, although the females were fecund (Skuhravy, 1998). Simultaneously, studies in Tiraspol did not reveal the settlement of the leaves of noted maples by horse chestnut leaf-miner (Antyuhova & Meshkova, 2011).

In the Czech Republic, a horse chestnut tree was discovered, which was slightly damaged by a horse-chestnut leaf miner. Larvae in mines did not complete development. Cuttings were taken from the tree for grafting. Moths slightly damaged grafted seedlings during the first two years, and in the third year, their damage increased to 50 % (IOCB, 2004). The black locust leaf-miner *Phyllonoricter robiniella* in Transnistria, according to O.V. Antiukhova (Antyuhova, 2010), is a monophagous. The second species, the locust digitate leaf-miner *Parectopa robiniella*, was not widely spread in the region. Caterpillars of the lime leaf-miner *Phyllonoricter issikii* in Japan damage different species of lime tree and Asian white birch (*Betula platyphylla* Sukaczev), in the south of Primorsky Krai – Amur lime (*Tilia amurensis* Rupr.) and Manchurian lime (*T. mandshurica* Rupr. et Maxim) (Ermolaev, 1977). In areas where this species has spread in recent years, individual lime tree species' resistance has not been studied yet. Analysis of literature sources indicates the possibility of selecting leafminer-resistant hybrids and tree forms. However, it is estimated (Zerova et al., 2007) that replacing 80 % of Berlin's horse chestnut trees with sustainable species will require about 300 million euros. It would be best if we also kept in mind the possibility of adapting insects to new hosts.

<u>Entomophagous of leaf-miner moths</u>. The complex of natural enemies of leaf-miners and other harmful insects includes parasitoids, predators, and pathogenic microorganisms. According to many researchers (Hansson & Shevtsova, 2009), predators' activity has little effect on the number of leaf-miners. Many countries report a decrease in the number of horse-chestnut leaf miners by ants, birds, and spiders (Skuhravy, 1998). Insectivorous birds are most important among predators, but they feed on leaf-miners in the absence of other food (Zerova et al., 2007).

According to the nature of damage to miners, entomophagous are divided into two groups (Grabenweger et al., 2005). The first group includes chickadees, which feed on the moths' caterpillars of older age or pupae and peck mines from above. The second group mainly includes insects that gnaw holes in the lower parts of mines. The southern oak bush cricket *Meconema meridionale* Costa (Saltatoria: Tettigoniidae) effectively reduced horse-chestnut leaf miner caterpillars in laboratory experiments and released them into trees (IOCB, 2004).

Parasitoids of leaf-miners are known more than predators, and about 50 % of individuals can be parasitized (Grabenweger et al., 2007). Parasitoids can enter new places simultaneously as the phytophage; in other cases, local parasitoids adapt to the new host. The latter process is prolonged due to the lack of synchronicity of the entomophagous development cycles and the new host. Thus, the horse-chestnut leaf miner's parasitoids overwinter in dry leaves and leave it when there are no host mines yet, which may explain the low parasitism in the first generation. At the same time, in an experiment with the artificial settlement of *Minotetrastichus frontalis* Nees. the effectiveness of parasitism was also low (Grabenweger et al., 2007). Therefore, despite the significant number of horse-chestnut leaf miner entomophagous species, they do not play a significant role in regulating its quantity (Kuldova et al., 2007). This may also be due to the lack of flowering vegetation in cities for additional entomophagous nutrition and places for their successful wintering.

Only 24 species of Hymenoptera are known to parasitize the horse-chestnut leaf miner in Europe, but the mine infestation does not exceed 10 % (Heitland & Metzger, 1997). These parasitoids are mostly broad polyphages, while mono- and oligophages are absent. In Tuscany (Italy), the activity of horse-chestnut leaf miner entomophagous did not exceed 5 %. It was identified seven species of local polyphages eulophid with the domination of *Minotetrastichus frontalis* and *Closterocerus trifasciatus* Westw. and the braconid *Colastes braconius* Haliday. Sixteen horse-chestnut leaf miner parasitoids are known in Poland, and more than 30 species are known in Macedonia, but they destroy 1 % of individuals (Evans & Oszako, 2007).

In Ukraine, 12 species of horse-chestnut leaf miner parasitoids were identified, and, as in other countries, *Minotetrastichus frontalis* dominated (Zerova et al., 2007). This species is included in the complexes of entomophagous and other leaf-miners of *Phyllonorycter apparella* (aspen pest), *Ph. issikii* (lime tree pest), and *Tischeria ekebladella* (oak pest) (Efremova & Mishenko, 2008). In the Balkans, except *M. frontalis*, in the parasitoids complex of the horse-chestnut leaf miner *Pediobius saulius* (Walker) dominated, which affects up to 30 % of the pest population (Deschka & Dimic, 1986).

Unlike leaf-miners of the genus *Cameraria*, the parasitoids complex of the genus *Phyllonorycter* is very diverse. Thus, in Japan, two Eulophidae family species were found among the lime leaf-miner parasitoids, six in Europe and 12 in the Volga Region (Efremova & Mishenko, 2008). Simultaneously, 50 % of lime leaf-miner mines were parasitized in Brandenburg and Saxony (IOCB, 2004), and 0.4–13.5% in Udmurtia (Ermolaev, Izhboldina & Efremova, 2011).

Parasitocomplexes of two locust tree leaf-miners, *Phyllonorycter robiniella* and *Parectopa robiniella*, were studied in Hungary (Melika et al., 2006). Nineteen species of *Ph. robiniella* parasitoids and 12 of *P. robiniella* parasitoids were identified. The species diversity of parasitoids on both types of leaf-miners and their species composition's seasonal dynamics were studied. No correlation was found between population density and *Ph. robiniella* parasitism levels, whereas in *P. robiniella* parasitism levels increased slightly as host density increased. Nematodes *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. feltae*, *S. kraussei*, and bacterial preparations based on *Bacillus thuringiensis* were tested among microbiological agents for reducing the number of leaf-miners. However, these measures did not provide a long-lasting effect (Gilbert et al., 2005).

Infection of leaf-miners with pathogenic fungi and bacteria is rare since the insect forms mines in the host plant's tissues, which contain chlorophyll, and isolates itself from the plant using the natural protection of the feeding plant (Heitland & Metzger, 1997). Besides, some leaf-miner moths remove excrement from mines. On the other hand, damage to plant tissue can cause an increase in the content of tannins and phenols, which have bactericidal and fungicidal properties. On artificially damaged leaves, the mortality rate of leaf-miners decreases (IOCB, 2004).

#### Diversity and distribution of leaf mining

<u>Measures to protect trees from leaf-miner insect</u>. Methods for controlling and limiting the number of horse-chestnut leaf miners are described in many publications (Pop et al., 2008); at the same time, no country has an effective system for protecting horse chestnuts under urban conditions. The simplest and safest measure for the environment to protect green plantations from leaf-miner moths is the cleaning up fallen leaves, which reduces the pesticide load, leads to the destruction of leaf-miner pupae and spores of pathogenic fungi (Pavan et al., 2003). The leaves should be raked without damaging them. So, pupae do not fall out, burned, composted, or kept at a distance of at least 50 m from the nearest trees. Raking leaves also leads to removing valuable organic substances from the soil and the death of parasitoids or predators (Pavan et al., 2003). For leaves cleaning up, garden vacuum rigs are used, which suck them up and steam treatment of them with a temperature of 80 °C, which leads to the death of 90% of pupae. This measure can be effective when reducing the number of moths after wintering, but in subsequent generations, the number of leaf-miners is restored, and to prevent its growth, it is necessary to regularly clean up fallen leaves (Zerova et al., 2007).

Sex pheromones of the horse-chestnut leaf miner and other Gracillariidae family species were separated and synthesized, but they are mainly used to determine the timing of insecticide application (Ferracini & Alma, 2008). There are known studies on the saturation of crowns with high doses of a synthetic pheromone that disrupts females' search by males, the so-called method of disorientation of males. The use of pheromone traps may reduce the density of the population of horse-chestnut leaf miners, but on the one hand, pheromone traps in cities can be destroyed by the inhabitants. On the other hand, any pheromone does not attract all individuals, and the offspring of individuals that are not attracted successfully reproduce (IOCB, 2004).

One of the measures to reduce the number of leaf-miners is sticky traps, to which pheromones and insecticides are added. In experiments, the polyethylene surface was treated with chestnut leaf extract with the addition of glue and various dyes, and it was studied the dependence of the butterflies number caught on the color, location, and orientation of this surface. More butterflies were caught on trunks than in crowns. Yellow color attracted them the most, blue – the least. Simultaneously, traps should be refreshed as the glue dries or fills with insects, spiders, or litter, as they are not selective (Evans & Oszako, 2007).

Many chemical measures to protect horse chestnut from horse-chestnut leaf miners were tested – spraying crowns with insecticides, injecting insecticides directly into the trunk, watering near-trunk circles with insecticides, the imposition of polyethylene film with insecticides on the trunks (Evans & Oszako, 2007). However, given the ban on the use of insecticides under urban conditions, such products are unlikely to be widely implemented. An exception is the protection of individual trees of high value.

Insecticides based on bifenthrin, carbaryl, chlorpyrifos, cyhalothrin, esfenvalerate, malathion, methyl parathion, permethrin, and regulators of insect growth and development, are widely used for spraying chestnuts (Evans & Oszako, 2007). Regulators of insect growth and development that affect egg development should be used during the flight and mating of insects in late April and early May (Gilbert et al., 2009). Preparations of intestinal action should be applied before the larva penetrates the leaf. However, all these preparations do not protect trees from settlement by the next leaf-miners generation. Systemic preparations that have a long-lasting effect are introduced into the soil before leaf development begins, but this can negatively affect other living organisms (IOCB, 2004).

In Italy, chestnut trunks were sprayed in May after flowering with the insecticide Merit Green (17.8 % imidacloprid) in 0.7 % aqueous solution at consumption of 1 ml of mother solution per 10 cm of trunk circumference. The efficiency ranged from 46 to 75.4 % (Ferracini & Alma, 2008). Dimilin SC 48 0.05 % was used for crown spraying in Poland (Gilbert et al., 2009) and the Czech Republic (Najmanová et al., 2006). One hundred twenty-seven days after treatment, 38 % of Dimilin preparation remained in the leaves (Sefrova, 2001). When treating the lower parts of trees with Confidor (imidacloprid) or Calypso (thiacloprid), larvae's development on the treated leaves stopped. Due to insecticides' penetration into tree tissues, miners' development on untreated parts of crowns also partially stopped (Najmanová et al., 2006). Scientists of the I.I.Schmalhausen Institute of Zoology, together with specialists of Kyiv station for the protection of green plantations in the parking area of Kyiv, tested the preparations Insegar 25 WP s.p., Lufox 105 ES k.e. and Lufox 105 ES k.e. with the addition of surfactant Cide Kick II (producer "Syngenta", Switzerland). 6 sprays were carried out per season during the period of mass egg-laying and mass development of caterpillars of horse-chestnut leaf miner of the I – II ages (Zerova et al., 2007). Lufox is a two-component insecticide that contains lufenuron (75 g/l) – an inhibitor of chitin synthesis and the insect growth regulator fenoxycarb (30 g/l). The effectiveness of this preparation was close to 100 %, Insegar – 70–85 %. It was recommended to carry out the first treatment with Lufox in urban plantations in coordination with the Ministry of Environmental Protection Natural Resources of Ukraine at the beginning of the mass appearance of caterpillars of I-II ages of I generation (late May - I decade of June), and the second - during the mass development of caterpillars of I-II ages of III generations (second decade of August) (Zerova et al., 2007).

The method of preparation injection into tree trunks was proposed by the Russian entomologist I. Ya. Shevyriev in 1895, and at the beginning of the last century, it was introduced in Ukraine by S. A. Mokrzhetsky against fungal diseases: on poplar and other species, copper sulfate was injected into tree trunks, and on fruit trees – salicylic acid against moniliosis (Tribel & Gmanova, 2008). The method of toxication with systemic insecticides is effective against sucking and latent pests, in particular leaf-miners. The use of preparations in this way does not depend on weather conditions, can be carried out in advance, and minimizes the environment's negative impact. The fluids injected into tree trunks move through the xylem's conducting vessels and diffuse in the tangential direction. In deciduous trees, fluids rise through conducting bundles to the side branches and leaves. The best time to inject pesticides into tree trunks is at the beginning of the growing season (before sap flow) or after flowering (Tribel, Strygun & Gmanova, 2011).

Imidacloprid-based preparations are used for injection into trunks in Western Europe. In Poland, an injection of a mixture of preparations (12 % imidacloprid and 8% tebuconazole) was used to control horse-chestnut leaf miner. Holes in the trunk were drilled at the height of 1 m at an angle of 45° every 15 cm of the circumference. The holes' diameter is 8 mm; the depth is 70 mm; 2.7 ml of the preparation was poured into the hole. Treex 200 SL (20 g/l abamectin + 180 g/l propiconazole) and Bionim

020 SL (20 g/l azodiractin) were also used (Gilbert et al., 2009). Simultaneously, when injected, the preparations enter the wood to the depth at which the holes are drilled, and in dry urban conditions, they can negatively affect the trees; in particular, embolism of vessels is recorded. There are wounds (up to 25 pieces/tree), rot is detected, wounds grow over with callus only in young trees.

The wood around the holes is wet, discolored due to the trees' protective reaction to wounds. This causes the development of a bacterial infection. The parenchyma is dead around deadwood due to the accumulation of gases that promote exudation from wounds. The wood dries out, and the tree becomes susceptible to infection. Wood turns brown, yeast and mold development, then fungi-destroyers of wood, up to 40 cm above and below the holes and 5 cm to the sides, physiological processes are disrupted (Evans & Oszako, 2007). Carrying out an injection before the beginning of leaf development is convenient, but it is unfavorable for trees' physiology. It is better if growth has begun and substances rise. In many countries, injections in old and weak trees have been discontinued at the experimental stage. It is advisable to introduce this agent only in nursery gardens preventively (Evans & Oszako, 2007).

## Conclusions

In the green plantations of Ukraine, adventitious leaf-miner moths from the order Lepidoptera of the Gracillariidae family have recently spread: on horse chestnut – horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic; on black locust– locust leaf-miner *Parectopa robiniella* Clemens and *Phyllonorycter robiniella* Clemens; on the lime tree – lime leaf-miner *Phyllonorycter issikii* Kumata. These noted leaf-miner moths cause significant damage to plantations, which is manifested in the loss of decorative effect, a decrease in the photosynthetic surface, and the weakening of trees. In the Kharkiv region, these species spread only a few years ago, and studies of the peculiarities of their distribution and development have not been conducted yet.

Despite the rich species composition of entomophagous, they do not significantly reduce the population of adventitious leafminer moths. The use of resistant forms and hybrids, raking and composting fallen leaves, using pheromone traps, spraying crowns, and injecting insecticides into tree trunks suggested to be the most effective measures to limit the number of leafminers in urban conditions.

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