Acta entomologica serbica, 2018, 23(1): 25-32

UDC 595.78(497.11)
DOI: 10.5281/zenodo.1213045

CONTRIBUTION TO THE KNOWLEDGE OF *PHYLLONORYCTER ISSIKII* (KUMATA, 1963) (LEPIDOPTERA: GRACILLARIIDAE) IN SERBIA

JOVAN DOBROSAVLJEVIĆ[1], ČEDOMIR MARKOVIĆ[1] and ALEKSANDAR STOJANOVIĆ[2]

1 University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, 11030 Belgrade, Serbia
E-mail: jovan.dobrosavljevic@sfb.bg.ac.rs; Markovicc@ptt.rs
2 Natural History Museum, Njegoševa 51, 11000 Belgrade, Serbia
E-mail: aleksandar@nhmbeo.rs

Abstract

Phyllonorycter issikii (Kumata, 1963) is an invasive leaf-mining moth originating from Asia. It develops on plants from the genus *Tilia*. The first finding of this species in Serbia was in 2013, and since then, the range of the species has spread, and we have found it at five more localities on *T. cordata* and *T. tomentosa*. *Ph. issikii* was studied at two of these localities. The existence of two overlapping generations was determined. No significant differences in the intensity of the attack between them were noticed. Differences existed only between the localities where the research was conducted and between the *Tilia* species on which *Ph. issikii* was found. As regards the lime species, the moth preferred *T. cordata* to *T. tomentosa* at both localities.
Two species of parasitoids were reared from the collected material: *Pediobius saulius* (Walker, 1839) and *Minotetrastichus frontalis* (Nees, 1834).

KEY WORDS: *Tilia*, *T. cordata, T. tomentosa*, lime, moth, Balkan, invasive species

Introduction

Phyllonorycter issikii (Kumata, 1963) is a small moth (wingspan 7-7.5 mm), which develops on plants from the genus *Tilia*. It is the only species from the genus *Phyllonorycter* in the Palearctic that has specialized to develop on lime. It is considered native to eastern Asia (Japan, Korea, Russian Far East), where it feeds on the leaves of *Tilia maximowicziana*, *T. japonica* and *T. kiusiana*. The first finding of this species outside this region was documented in 1985 from *Tilia* plantations in Moscow (Kirichenko *et al.,* 2017). From there it gradually spread, so that today its range covers the whole of central and eastern Europe, extending north to

Finland, west to Belgium, and south to Italy and Bulgaria (Šefrova, 2002, 2003; Wullaert, 2012; Ermolaev, 2014; Szőcs *et al.,* 2015; Buszko, 2016; GBIF Secretariat, 2017; Ermolaev & Rubleva, 2017, Kirichenko *et al.,* 2017). With the expansion of its range, the list of its feeding plants has also expanded. It has started mining the leaves of other *Tilia* species that do not exist in eastern Asia: *T. cordata*, *T. platyphyllos*, *T. tomentosa*, *T.* × *euchlora*, *T.* × *europaea*, *T. sibirica* and *T. americana* (Kirichenko *et al.,* 2017).

Materials and methods

Distribution and host plants

The research was conducted during 2016 and 2017 on the territory of Serbia (67 localities). Trees with leaves mined by *Ph. issikii* were sought in forests and parks where lime trees are present. The mined leaves found were collected and brought to the Entomological Laboratory of the Faculty of Forestry, University of Belgrade. Some of them were herbarized and others were put into photoeclectors to obtain adult moths and their parasitoids. Determination of leaf miners was done by Jovan Dobrosavljević using the following literature: Kumata (1963), Noreika (1998), Šefrova (2002), Csóka (2003), Ellis (2007), Langmaid (2007) and Doorenweerd *et al.* (2012). Determination of parasitoids was done by Aleksandar Stojanović using the following papers: Bouček (1965), Graham (1987), Graham & LaSalle (1991) and Noyes (2017). All the leaf-miner and parasitoid names are compatible with those found on the Fauna Europaea website (de Jong *et al.,* 2014).
Herbarized leaves with *Ph. issikii* mines and the obtained adult moths and parasitoids are deposited in the collection of the Department of Forest Protection at the Faculty of Forestry, University of Belgrade.

Population density and biology

The population density research of *Ph. issikii* was conducted in 2017 at two localities in eastern Serbia (in the vicinity of Majdanpek): Ujevac (44°25'N, 21°52'E) and Ravna Reka (44°26'N, 21°59'E). (The names of the localities are given in Serbian without translation to avoid confusion). The localities are about 10 km apart. They have a similar slope (around 20°) and exposition (southwest), but different altitudes (Ravna Reka: 500-530m a.s.l., Ujevac: 270-290m a.s.l.) and stand structure (Ujevac: one-layer stand; Ravna Reka: two-layer stand) (Fig. 1).
Four *Tilia tomentosa* trees were randomly selected at both localities, and four *T. cordata* trees just at the Ujevac locality. Four branches approximately 1 m long were randomly selected from the lower part of the crown (up to a height of 2 m) from each tree. The branches were collected in two sessions, in the period of pupae appearance, for the first time in mid-June, and for the second at the beginning of August. They were then brought to the Entomological Laboratory of the Faculty of Forestry, University of Belgrade. The total number of leaves, the number of mined leaves, and the number of mines per leaf were analyzed on these branches.
The significance of the differences in observed properties was determined by one-way ANOVA, at the level of significance $p<0.05$. StatSoft Statistica 8.0 software was used for statistical analysis.

Results and Discussion

Distribution and host plants

From the 67 investigated localities, leaves with *Ph. issikii* mines were found only at five: Fruška Gora: Iriški Venac – Astali (45°09'N, 19°52'E), Kučevo: Kučajna – Ravnište (44°25'N, 21°37'E), Kučevo: Kučajna – Krst

(44°25'N, 21°36'E), Majdanpek: Ujevac (44°25'N, 21°52'E) and Majdanpek: Ravna Reka (44°26'N, 21°59'E). The mined leaves were discovered on *T. cordata* and *T. tomentosa*. The localities of the findings coincide with the localities where lime tree stands are represented on large areas in Serbia. *Ph. issikii* has never been found in urban environments. Considering that *Tilia* species are represented in Serbia from lowland to mountain habitats, and that they can be found in almost all parks and alleys, it can be assumed that the range of *Ph. issikii* will probably spread in the next few years.

Based on our findings and the findings by Šefrova (2002), Stolnicu & Ureche (2007), Tomov (2007), Matošević (2007), Glavendekić (2014) and Ermolaev & Rubleva (2017), in the Balkans *Ph. issikii* is present only in Serbia, Croatia and Bulgaria. However, as the range of its main host plants, *T. cordata* and *T. tomentosa,* on the Balkan Peninsula extends south to Albania and Greece (Svejgaard Jensen, 2003), it is possible that it will be found in other Balkan countries as well.

Ph. issikii was not the only leaf miner collected on lime trees in Serbia. During this research, mines of *Trachys minutus* (Linnaeus, 1758) (Coleoptera: Buprestidae), *Parna tenella* (Klug, 1816) (Hymenoptera: Tenthredinidae) and *Stigmella tiliae* (Frey, 1856) (Lepidoptera: Nepticulidae) were also found.

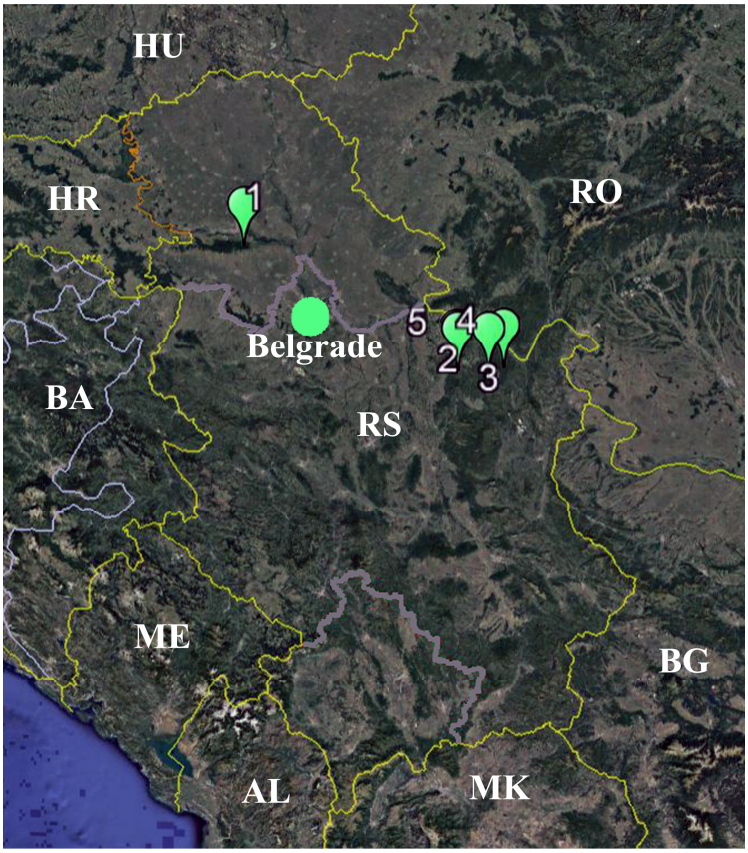

Figure 1. Localities where *Ph. issikii* was found in Serbia; source: Google Earth. 1 − Fruška Gora: Iriški Venac – Astali; 2 − Majdanpek: Ujevac; 3 − Majdanpek: Ravna Reka; 4 − Kučevo: Kučajna – Krst; 5 − Kučevo: Kučajna – Ravnište.

Biology

Ph. issikii develops two generations per year in central Europe. The adults of the first generation appear from the end of May to the middle of July, and the second from mid-August to the beginning of October (Šefrova, 2002; Jurc, 2012; Ermolaev, 2014). Based on the data obtained, *Ph. issikii* develops at least two overlapping generations per year in Serbia. First generation moths were obtained from the material collected in June (only adults of the aestival marked form were reared). Unfortunately, second generation moths were not obtained due to severe drought and high temperatures during the summer of 2017, which caused the death of most of the larvae in nature (only 2 second generation pupae were found in 153 *Ph. issikii* mines analyzed from the collected samples). A similar situation occurred in 2010 in Udmurtia, where extremely hot and dry weather led to the disappearance of lime leaf miners in all lime tree stands (Ermolaev, 2014) (Table I).

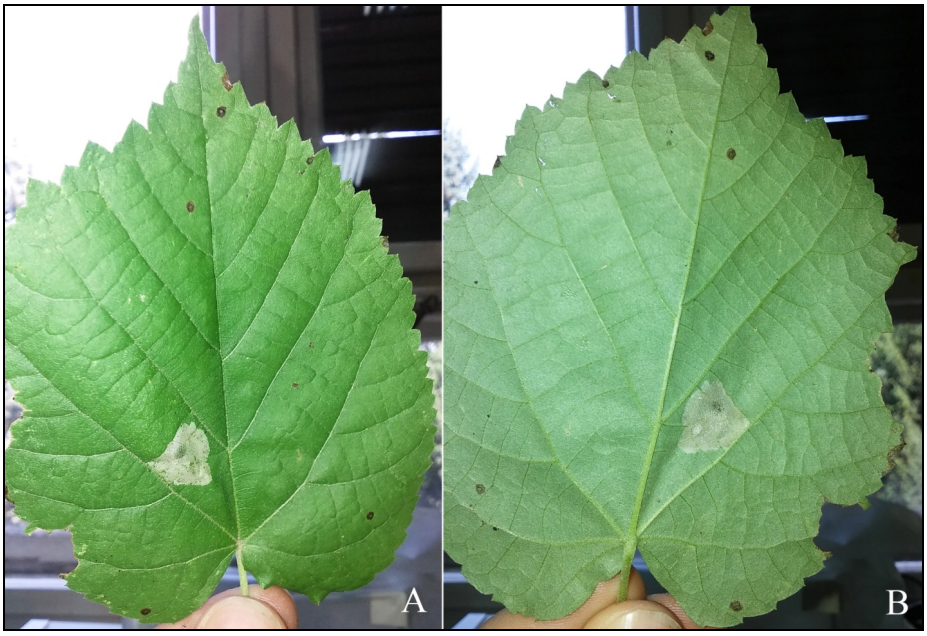

Figure 2. A – Leaf with a *Ph. issikii* mine from the upper side; B – Leaf with a *Ph. issikii* mine from the lower side (Photo by J. Dobrosavljević).

Population density

At the localities where the research was conducted, in both series of samples no statistically significant differences in the intensity of the attack between the investigated trees within the groups were noted ($p>0.05$). Neither did differences exist among the same groups of trees in the two series of samples (Table II). However, if the intensities of the *Ph. issikii* attacks in both series of samples on *T. tomentosa* are compared at the two localities, statistically significant differences can be observed (Table III). This suggests that locality had an impact on the intensity of the attack. Greater population densities were noted at locality Ujevac, at a slightly lower altitude (about 250 m) than locality Ravna Reka. The species of lime also had an influence on the intensity, but only in the first series of samples (Table IV).

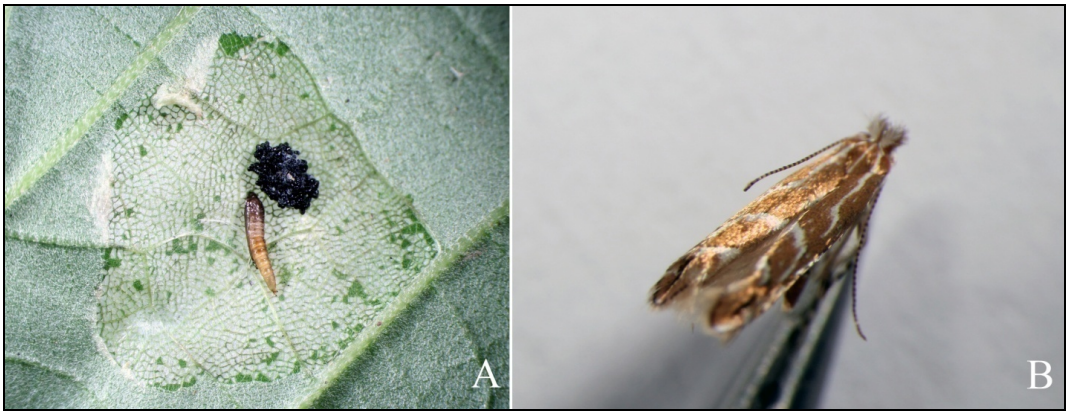

Figure 3. A – Opened *Ph. issikii* mine with a pupa and a heap of excrement; B – *Ph. issikii* adult of the aestival marked form (Photo by J. Dobrosavljević).

Table I. The total number of analyzed leaves and mines found on them.

Locality	Tree species	The total number of	
		Leaves analyzed	Mines found on the analyzed leaves
Ujevac	*T. tomentosa*	1537	69
	T. cordata	1506	417
Ravna Reka	*T. tomentosa*	1306	14
Σ		4349	500

Table II. The influence of the series of samples on the intensity of the attack.

Locality	Tree species	Average number of mines per leaf per tree		ANOVA	
		June	August	F	p
Ujevac	*T. tomentosa*	0.044 ± 0.024	0.190 ± 0.143	0.7526	0.4253
	T. cordata	0.298 ± 0.112	0.272 ± 0.102	4.0346	0.0913
Ravna Reka	*T. tomentosa*	0.010 ± 0.012	0.011 ± 0.010	0.0204	0.8910

Table III. The influence of locality on the intensity of the attack.

	Average number of mines per leaf per tree		ANOVA	
Locality	Ujevac	Ravna Reka		
Tree species	*T. tomentosa*		F	p
June	0.044 ± 0.024	0.010 ± 0.012	6.4367	0.0443
August	0.190 ± 0.143	0.011 ± 0.010	6.2025	0.0471

Table IV. The influence of lime-tree species on the intensity of the attack.

	Average number of mines per leaf per tree		ANOVA	
Tree species	*T. tomentosa*	*T. cordata*		
Locality	Ujevac		**F**	**p**
June	0.044 ± 0.024	0.298 ± 0.112	19.482	0.0045
August	0.190 ± 0.143	0.272 ± 0.102	0.8692	0.3872

According to Ermolaev & Zorin (2011), the threshold at which the damage on leaves starts affecting the vitality of lime trees is generally when the average number of mines per leaf exceeds 1 or 2, depending on the climate conditions that year (Fig 2). A greater number of mines results in decreased height and girth increment, smaller numbers of buds, flowers and seeds, a decrease in the amount of sugar in the nectar, and premature falling of the leaves (Ermolaev & Zorin, 2011) (Fig 3). According to Ermolaev & Rubleva (2017), this damage threshold can be crossed in 3 years from the beginning of an invasion, as happened in the Moscow area, Slovakia or Ukraine. If the intensities of attack that we obtained in Serbia during the 2017 (Table II) are compared with the damage threshold specified by Ermolaev & Zorin, we can easily conclude that the population density of *Ph. issikii* is still low here. Consequently, this species does not cause significant damage on lime in Serbia. However, *Ph. issikii* is known to cause serious damage on lime in central Europe and Russia. All the *Tilia* trees in a forest can be infested, and additionally, there can be more than 20 mines on a single leaf (Ermolaev & Motoshkova, 2008; Jurc, 2012). This is why the population density of *Ph. issikii* should be monitored, so that when it reaches the critical point we can react and protect forests if necessary.

Parasitoids

Ph. issikii has a very rich parasitoid fauna. So far, around 50 species of parasitoids have been found in its range (Szőcs *et al.,* 2015; Ermolaev & Rubleva, 2017). From the material collected in the first field session, two species of parasitoids were reared: *Pediobius saulius* (Walker, 1839) (Eulophidae: Entedoninae) and *Minotetrastichus frontalis* (Nees, 1834) (Eulophidae: Tetrastichinae). Both species are very common in Serbia and have been found in other species of leaf miners besides *Ph. issikii* (Stojanović & Marković, 2004, 2005; Marković & Stojanović, 2012). They are already known parasitoids of *Ph. issikii*, but they are certainly not the only ones on it in Serbia. The fact that Szőcs *et al.* (2015) found a much greater number of parasitoids (20 species) in neighboring Hungary leads us to this conclusion. However, according to the data they provided, the influence of parasitoids on the population density of *Ph. issikii* is usually not great.

References

Bouček, Z. (1965). Studies of European Eulophidae, IV: *Pediobius* Walk. and two allied genera (Hymenoptera). *Acta Entomologica Musei Nationalis Pragae, 36,* 5-90.

Buszko, J. (2016). Fauna Europaea: Gracillariidae. In: Van Nieukerken, E. J., & Karsholt, O. (2016). Fauna Europaea: Lepidoptera, Moths. Fauna Europaea version 2.6. Retrieved from www.fauna-eu.org. [Accessed on: December 23rd 2016].

Csóka, G. (2003). *Leaf mines and leaf miners*. Budapest. Agroinform Kiadó és Nyomda. 192 pp.

de Jong, Y., Verbeek, M., Michelsen, V., de Place Bjørn, P., Los, W., Steeman, F., Bailly, N., Basire, C., Chylarecki, P., Stloukal, E., Hagedorn, G., Wetzel, F. T., Glöckler, F., Kroupa, A., Korb, G., Hoffmann, A., Häuser, C., Kohlbecker, A., Müller, A., Güntsch, A., Stoev, P., & Penev, L. (2014). Fauna Europaea - all European animal

species on the web. *Biodiversity Data Journal, 2*, e4034. Retrieved from https://fauna-eu.org [Accessed on: October 18th 2017].

Doorenweerd, C., van Haren, M. M., Schermer, M., Pieterse, S., & van Nieukerken, E. J. (2012). A Linnaeus NG interactive key to the Lithocolletinae of North-West Europe aimed at accelerating the accumulation of reliable biodiversity data (Lepidoptera, Gracillariidae). *ZooKeys, 422*, 87-101.

Ellis, W. N. (2007). Leafminers and plant galls of Europe. Retrieved from http://www.bladmineerders.nl/ [Accessed on: December 18th 2016].

Ermolaev I. V., & Rubleva E. A. (2017). History, rate and factors of invasion of lime leafminer *Phyllonorycter issikii* (Kumata, 1963) (Lepidoptera, Gracillariidae) in Eurasia. *Russian Journal of Biological Invasions, 8*(2), 115-130.

Ermolaev, I. V., & Motoshkova, N. V. (2008). Biological invasion of the lime leafminer *Lithocolletis issikii* Kumata (Lepidoptera, Gracillariidae): Interaction of the moth with the host plant. *Entomological Review, 88*(1), 1-9.

Ermolaev, I. V., & Zorin, D. A. (2011). Ecological consequences of invasion of *Phyllonorycter issikii* (Lepidoptera, Gracillariidae) in lime forests in Udmurtia. *Entomological Review, 91*(5), 592-598.

Ermolaev, I. V. (2014). Biological Invasion of the Lime Leaf miner *Phyllonorycter issikii* Kumata (Lepidoptera, Gracillariidae) in Europe. *Contemporary Problems of Ecology, 7*(3), 324-333.

GBIF Secretariat (2017). GBIF Backbone Taxonomy. Checklist Dataset. Retrieved from https://www.gbif.org/species/1750166 [Accessed on: February 13th 2018].

Glavendekić, M. (2014). New alien insects in forests and urban green spaces in Serbia. In: Kirichenko, N., Roques, A., Augustin, S., Lopez-Vaamonde, C. (Eds.), *"Invasive insects in a changing world" Abstracts of the International Le Studium conference Invasive insects in a changing world,* (p. 15). Orléans, France. Le Studium Loire Valley Institute for Advanced studies.

Google Earth (2017). Locations where *Phyllonorycter issikii* was found in Serbia [Accessed on: November 2nd 2017].

Graham, M. W. R. de V. (1987). A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certain genera. *Bulletin of the British Museum (Natural History) (Entomology), 55*(1), 1-392.

Graham, M. W. R. de V., & LaSalle, J. (1991). New synonymy in European Tetrastichinae (Hymenoptera: Eulophidae) including designation of some neotypes, lectotypes and new combinations. *Entomologist's Gazette, 42*, 89-96.

Jurc, M. (2012). Lipin moljac miner (*Phyllonorycter issikii*) u Sloveniji. *Šumarski list, 136*(3-4), 119-127.

Kirichenko, N., Triberti, P., Ohshima, I., Haran, J., Byun, B., Li, H., Augustin, S., Roques, A., & Lopez-Vaamonde, C. (2017). From east to west across the Palaearctic: Phylogeography of the invasive lime leaf miner *Phyllonorycter issikii* (Lepidoptera: Gracillariidae) and discovery of a putative new cryptic species in East Asia. *PLoS ONE, 12*(2), 1-22.

Kumata, T. (1963). Taxonomic studies on the Lithocolletinae of Japan (Lepidoptera: Gracillariidae), part 1. *Insecta Matsumurana, 25*(2), 53-90.

Langmaid, J. (2007). British leaf miners. Colin Plant Associates (UK) LLP/Consultant Entomologists. Retrieved from www.leafmines.co.uk./ [Accessed on: December 23rd 2016].

Marković, Č., & Stojanović, A. (2012). Parasitoids od *Phyllonorycter platani* (Staudinger) (Lepidoptera, Gracillariidae) in Serbia. *Journal of Plant Studies, 1*(1), 79-84.

Matošević, D. (2007). Prvi nalaz vrste *Phyllonorycter issikii* i rasprostranjenost invazivnih vrsta lisnih minera iz porodice Gracillariidae u Hrvatskoj. Radovi - *Šumarski institut Jastrebarsko, 42*(2), 127-142.

Noreika, R. (1998). *Phyllonorycter issikii* (Kumata) (Lepidoptera, Gracilariidae) in Lithuania. *Acta Zoologica Lituanica, 8*(3), 34-37.

Noyes, J. S. (2017). Universal Chalcidoidea Database. World Wide Web electronic publication. Retrieved from http://www.nhm.ac.uk/chalcidoids. [Accessed on: September 15th 2017].

Šefrova, H. (2002). *Phyllonorycter issikii* (Kumata, 1963) - bionomics, ecological impact and spread in Europe (Lepidoptera, Gracillariidae). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 50*(3), 99-104.

Šefrova, H. (2003). Invasions of Lithocolletinae species in Europe - causes, kinds, limits and ecological impact (Lepidoptera, Gracillariidae). *Ekologia (Bratislava), 22*(2), 132-142.

Stojanović, A., & Marković, Č. (2004). Parasitoid complex of *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Serbia. *Phytoparasitica, 32*(2):132-140.

Stojanović, A., & Marković, Č. (2005). Parasitoid complex of *Phyllonorycter robiniella* (Clemens, 1859) (Lepidoptera, Gracillariidae) in Serbia. *Journal of Pest Science, 78*(2): 109-114.

Stolnicu, A - M., & Ureche, C. (2007). Data regarding the presence of the *Phyllonorycter issikii* (Kumata) (Lepidoptera: Gracillariidae) in Romanian fauna. *Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, s. Biologie animală 53*, 103-108.

Svejgaard Jensen, J. (2003). *EUFORGEN Technical Guidelines for genetic conservation and use for lime (Tilia* spp.). Rome, Italy. International Plant Genetic Resources Institute. 6 pp.

Szőcs, L., George, M., Thuróczy, C., & Csóka, G. (2015). Parasitoids of the lime leaf miner *Phyllonorycter issikii* (Lepidoptera: Gracillariide) recorded throughout the area it recently colonized. *European Journal of Entomology, 112*(4), 1-8.

Tomov, R. (2007). Pest status of alien leaf-mining moths (Lepidoptera) in Bulgaria. *Plant Protection, 18*, 79-81.

Wullaert, S. (2012). *Phyllonorycter issikii* (Lepidoptera: Gracillariidae), new to the Belgian fauna. *Phegea, 40*(3), 63-65.

ПРИЛОГ ПОЗНАВАЊУ *PHYLLONORYCER ISSIKI* (KUMATA, 1963) (LEPIDOPTERA, GRACILLARIIDAE) У СРБИЈИ

ЈОВАН ДОБРОСАВЉЕВИЋ, ЧЕДОМИР МАРКОВИЋ И АЛЕКСАНДАР СТОЈАНОВИЋ

Извод

Phyllonorycter issikii (Kumata 1963) је инвазивна врста лисног минера пореклом из Азије. Развија се на биљкама из рода *Tilia*. У Србији је први пут пронађена 2013. године. Њен ареал се од тад проширио, и ми смо је пронашли на још пет локалитета, на две врсте липе: *T. cordata* и *T. tomentosa*. *Ph. issikii* је проучаван на два од тих пет локалитета. На њима је утврђено је постојање две преклапајуће генерације. При том, разлике у погледу интензитета напада између њих нису констатоване. Те разлике су само постојале између локалитета на којима су истраживања обављена и врста липе на којима је *Ph. issikii* пронађен.

Из сакупљеног материјала гајењем су добијене две врсте паразитоида: *Pediobius saulius* (Walker 1839) and *Minotetrastichus frontalis* (Nees 1834).

Received: January 19th, 2018
Accepted: March 31st, 2018