

# The Influence of Parasitoids (Hymenoptera, Eulophidae) on Survival of the Lime Leafminer *Phyllonorycter issikii* (Lepidoptera, Gracillariidae) in Udmurtia

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**Abstract**—The species structure of parasitoids (Hymenoptera, Eulophidae) of the invasive lime leafminer *Phyllonorycter issikii* (Kumata, 1963) (Lepidoptera, Gracillariidae) was reexamined ten years after the latest survey. The work was carried out in 2015 in three previously selected test plots within the city of Izhevsk. Extremely high survival rates (61.7 to 89%) of the leafminer were observed while the parasitoid complex had a negligible impact on its mortality (0.6 to 1.6%). A slight structural transformation of the parasitoid complex was noted, namely a decrease in the number of dominants and a change of the dominant species. In addition, the species structure of the parasitoids collected in 22 populated localities in Udmurtia was studied in 2016. Five new parasitoids of the lime leafminer were found: *Pnigalio pectinicornis* (Linnaeus, 1758), *Sympiesis acalle* Walker, 1848, *Chrysocharis amanus* Walker, 1839, *Ch. pentheus* Walker, 1839, and *Ch. polyzo* Walker, 1839. Two species of parasitoids of *Ph. issikii* were recognized as new to Udmurtia: *Pnigalio agraulis* (Walker, 1839) and *Pediobius saulius* (Walker, 1839).

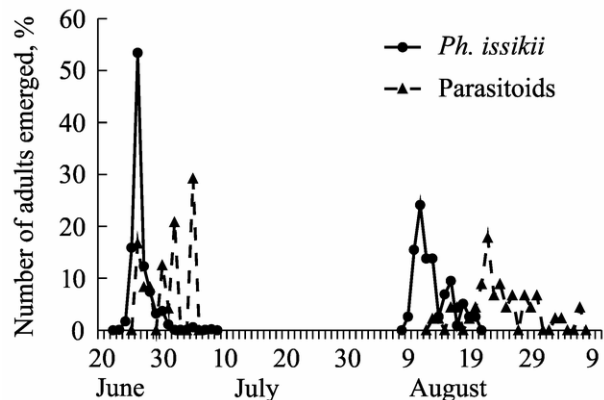
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The primary range of the lime leafminer *Phyllonorycter issikii* (Lepidoptera, Gracillariidae) lies along the Sea of Japan coast. In 1985 the species was first recorded in the landscaped areas of Moscow (Bednova and Belov, 1999). In 27 years, from 1985 to 2011, its distribution area in Europe and West Siberia has increased to 4 086 000 km<sup>2</sup> and now comprises 60.4% of the total range of the native *Tilia* species (Ermolaev and Rubleva, 2017). Under the conditions of West Eurasia, the leafminer passes through three phases of its invasion, namely introduction, establishment, and expansion, in three years. The highest rates of expansion, reaching 80–85 km a year, were observed in the western and eastern directions within the secondary range (Ermolaev and Rubleva, 2017). In Europe *Ph. issikii* damages 8 species and 2 hybrids of the genus *Tilia* (Ermolaev, 2016). According to expert assessments (Gninenko, 2008; Gninenko and Kozlova, 2008), in 2008 the outbreak sites of this miner occupied 1–2 mln ha in European Russia alone. At high densities the lime leafminer negatively affects the productivity and reproductive parameters of the tree,

reducing its growth, the number of flowers and inflorescences, and even sugar content in the nectar. The latter parameter influences the productivity of regional apiculture (Ermolaev and Zorin, 2011).

In the territory of Udmurtia the lime leafminer can produce two annual generations (Ermolaev and Rubleva, 2017). The first generation develops in June, and its adults fly in July. The second generation is formed in August–September and often fails to complete development. As the result of high air temperatures in May–June 2015, the flight of the first and second generations of *Ph. issikii* was observed in Izhevsk 10–20 days earlier than usual: in the third decade of June and the second decade of August, respectively (figure).

Earlier, in 2001–2005, we studied the complex of parasitoids infesting the first generation of *Ph. issikii* in Izhevsk, using three sample plots (Ermolaev et al., 2011). The survival rate of *Ph. issikii* pupae in sample plot 1 varied from 61.5% in 2001 to 79.6% in 2005; that in sample plot 2 varied from 53.2% in 2001 to



Emergence dynamics of *Ph. issikii* and its parasitoids in the field laboratory in 2015 (data for sample plot 3).

81.7% in 2004; that in sample plot 3 varied from 63.9% in 2001 to 76.9% in 2004. The mortality of *Ph. issikii* due to parasitoids reached 3.7% in sample plot 1 (2002), 11.7% in sample plot 2 (2002), and 12.5% in sample plot 3 (2001) (Ermolaev et al., 2011). The previous study revealed 22 species of parasitoids of the family Eulophidae (Table 1). Dominant among them were the primary solitary ectoparasitoids *Pnigalio soemius* (Walker, 1839) and *Sympiesis gordius* (Walker, 1839) and the endoparasitoid *Chrysocharis laomedon* Walker, 1839. The fraction of *P. soemius* in the parasitoid complex of *Ph. issikii* reached 60% (2003, sample plot 3), that of *S. gordius*, 32.5% (2001, sample plot 2), that of *Ch. laomedon*, 30% (2001, sample plot 3).

The goal of this work was to reexamine, ten years after the original survey, the species structure of parasitoids of the invasive lime leafminer *Ph. issikii* in Izhevsk and to estimate the effect of parasitoids on host mortality.

## MATERIALS AND METHODS

The research was carried out in two stages, using the stationary (2015) and route-based (2016) methods.

Stationary assessment of survival of the first generation of *Ph. issikii* was performed in 2015 in three sample plots in the city of Izhevsk. The sample plots “Malinovaya Gora,” “Pitomnik,” and “S.M. Kirov Park” (below referred to as plots 1, 2, and 3, respectively) were marked out in 2000 according to the Branch Industry Standard 56-69-83. The research reported herein was a continuation of the studies carried out in these samples plots in 2001–2005 (Ermolaev et al., 2011). Each sample plot was about 1 ha in area; as recommended by Ilyinsky and co-

authors (1965, p. 55), the plots were selected in such a way that their tree stand structure was not the same. The sample plots were described in detail in our earlier paper (Ermolaev et al., 2011). In each sample plot, 40 model trees of small-leaved lime *Tilia cordata* Mill. were selected. During the pest pupation (the second and third decades of June), 40–50 leaves were collected from the north-facing primary branches of the lower crown layer of each model tree. The mines were cut out with scissors and placed in Petri dishes, separately for each model tree. Emergence of moths and parasitoids was recorded daily in the field laboratory at Siva Biological Station. Altogether, 7634 ind. of *Ph. issikii* and 106 ind. of parasitoids were reared.

The route survey was carried out in 2016. Leaves containing *Ph. issikii* mines were collected in 22 populated localities of Udmurtia (Table 2). As opposed to 2015, the density of lime infestation with *Ph. issikii* in 2016 was generally low, less than 50 mines per 100 leaves. Each sample was a collection of leaves from several (up to 10) trees of *T. cordata*. Emergence of moths and parasitoids was recorded daily in the field laboratory. Altogether, 1704 ind. of *Ph. issikii* and 289 ind. of parasitoids were reared.

In all the cases, the hymenopterans were identified by Z.A. Yefremova.

The following parameters were calculated:

$R = M / L$ , where  $R$  is the density of infestation of an individual model tree with the first generation of the leafminer,  $M$  is the total number of mines on three model branches from the lower crown layer, and  $L$  is the total number of leaves on these branches.

$V = B / N \times 100$ , where  $V$  is the survival rate of the pupae,  $B$  is the number of adult moths reared from the mines, and  $N$  is the total number of mines collected.

$P = W / N \times 100$ , where  $P$  is the rate of infestation with parasitoids,  $W$  is the total number of parasitoids reared, and  $N$  is the total number of mines collected.

The mean and its standard error were calculated in all the cases. Data were statistically processed by the standard methods (Ivanter and Korosov, 2011).

## RESULTS AND DISCUSSION

The density of infestation of lime trees with the leafminer in 2015 was  $900.5 \pm 51.5$ ,  $470.0 \pm 41.7$ , and  $85.1 \pm 9.6$  mines per 100 leaves in sample plots 1, 2, and 3, respectively (Table 3); 40 model trees were examined in each plot.

**Table 1.** Species structure of the parasitoid complex of the lime leafminer *Ph. issikii* in Udmurtia in different years

Species	2001–2005	2015–2016	Species	2001–2005	2015–2016
<i>Dicladocerus westwoodii</i> Westwood, 1832*	+	0	<i>H. nigritulus</i> (Zetterstedt, 1838)*	+	0
<i>Pnigalio agraulis</i> (Walker, 1839)*	0	+	<i>Pediobius saulius</i> (Walker, 1839)	0	+
<i>Pnigalio pectinicornis</i> (Linnaeus, 1758)*	0	+	<i>Chrysocharis amanus</i> Walker, 1839	0	+
<i>P. nemati</i> (Westwood, 1838)*	+	0	<i>Chrysocharis laomedon</i> Walker, 1839	+ <sup>3</sup>	+
<i>P. soemius</i> (Walker, 1839)*	+ <sup>1</sup>	+	<i>Ch. nephereus</i> Walker, 1839	+	+
<i>Sympiesis acalle</i> Walker, 1848*	0	+	<i>Chrysocharis pentheus</i> Walker, 1839	0	+
<i>S. dolichogaster</i> Ashmead, 1888*	+	0	<i>Ch. phryne</i> Walker, 1839	+	0
<i>S. gordius</i> (Walker, 1839)*	+ <sup>2</sup>	+ <sup>3</sup>	<i>Chrysocharis polyzo</i> Walker, 1839	0	+
<i>S. sericeicornis</i> (Nees, 1834)*	+	+ <sup>2</sup>	<i>Ch. pubicornis</i> Zetterstedt, 1838	+	0
<i>Cirrospilus diallus</i> Walker, 1838*	+	+	<i>Neochrysocharis formosus</i> (Westwood, 1833)	+	+
<i>C. lynxus</i> Walker, 1838*	+	0	<i>N. cuprifrons</i> Erdös, 1954	+	0
<i>C. pictus</i> Nees, 1834*	+	+	<i>Minotetrastichus frontalis</i> Nees, 1834*	+	+ <sup>1</sup>
<i>C. vittatus</i> Walker, 1838*	+	0	<i>Mischotetrastichus petiolatus</i> (Erdös, 1961)*	+	0
<i>Elachertus fenestratus</i> Nees, 1834*	+	+	<i>Oomyzus incertus</i> (Ratzeburg, 1844)	+	0
<i>Hyssopus geniculatus</i> (Hartig, 1838)*	+	+			
			Total number of species	22	18

+<sup>1</sup>, +<sup>2</sup>, +<sup>3</sup>, places among the dominants; \* an ectoparasitoid.

**Table 2.** Collection localities of the lime leafminer *Ph. issikii* in Udmurtia in 2016

Locality	Coordinates	Locality	Coordinates
Yar	58°23' N, 52°11' E	Syumsi	57°10' N, 51°60' E
Glazov	58°13' N, 52°65' E	Uva	56°98' N, 52°17' E
Balezino	57°97' N, 52°99' E	Vavozh	56°77' N, 51°92' E
Kez	57°89' N, 53°70' E	Izhevsk	56°84' N, 53°21' E
Yukamenskoe	57°89' N, 52°25' E	Kizner	56°29' N, 51°52' E
Krasnogorye	57°70' N, 52°50' E	Grakhovo	56°05' N, 51°96' E
Igra	57°54' N, 53°04' E	Alnashi	56°19' N, 52°47' E
Debesy	57°65' N, 53°81' E	Malaya Purga	56°55' N, 52°99' E
Sharkan	57°30' N, 53°87' E	Kiyasovo	56°34' N, 53°13' E
Yakshur-Bodya	57°20' N, 53°15' E	Karakulino	56°01' N, 53°70' E
Siva biological station	56°81' N, 53°89' E	Sarapul	56°47' N, 53°81' E

**Table 3.** Survival and mortality of pupae of the lime leafminer *Ph. issikii* in sample plots in 2015

Sample plot	Infestation density, mines per 100 leaves	Survival, %	Mortality, %	
			due to parasitoids	due to unknown factors
1	900.5 ± 51.5	89.0 ± 1.5	1.6 ± 0.3	9.3 ± 1.4
2	470.0 ± 41.7	86.2 ± 1.8	0.6 ± 0.2	13.1 ± 1.8
3	85.1 ± 9.6	61.7 ± 2.8	0.9 ± 0.4	37.3 ± 2.9

40 model trees were examined in each sample plot.

**Table 4.** Coefficients of correlation between the density of pupae of the lime leafminer *Ph. issikii* on the host trees and the survival and mortality of pupae in sample plots in 2015

Sample plot	Infestation density, mines per 100 leaves	Survival, %	Mortality, %	
			due to parasitoids	due to unknown factors
1	900.5 ± 51.5	$r = 0.30^*$	$r = -0.28$	$r = -0.27$
2	470.0 ± 41.7	$r = -0.11$	$r = 0.18$	$r = 0.10$
3	85.1 ± 9.6	$r = -0.04$	$r = -0.19$	$r = 0.07$

40 model trees were examined in each sample plot; \* correlation significant at  $P < 0.05$ .

The survival rate of the first generation of *Ph. issikii* was high in all the three plots (Table 3):  $61.7 \pm 2.8\%$  in sample plot 3 and the record values of  $86.2 \pm 1.8\%$  and  $89.0 \pm 1.5\%$  for sample plots 2 and 1, respectively. The leafminer survival in plot 1 was positively and significantly correlated with the density of infestation of model trees (Table 4). The highest survival rate recorded during the previous 5-year monitoring of these sample plots in 2001–2005 was only  $81.7 \pm 0.9\%$  (data of 2004 for plot 2) (Ermolaev et al., 2011).

Mortality of the lime leafminer was mostly determined by unknown factors. The percentage of such cases reached  $9.3 \pm 1.4\%$ ,  $13.1 \pm 1.8\%$ , and  $37.3 \pm 2.9\%$  for plots 1, 2, and 3, respectively (Table 3). In all the three sample plots, the mortality parameters did not depend on the leafminer density on the host trees (Table 4).

The rate of infestation of *Ph. issikii* with parasitoids was low:  $1.6 \pm 0.3\%$ ,  $0.6 \pm 0.2\%$ , and  $0.9 \pm 0.4\%$  for sample plots 1, 2, and 3, respectively (Table 3). No significant correlation with the density of the leafminer on the model trees was revealed in any of the three plots (Table 4). The parasitoid complex included 12 species of Eulophidae: *Pnigalio pectinicornis* (Linnaeus, 1758), *P. soemius*, *Sympiesis acalle* Walker, 1848, *S. gordius*, *S. sericeicornis* (Nees, 1834), *Elachertus fenestratus* Nees, 1834, *Hyssopus geniculatus* (Harti, 1838), *Chrysocharis laomedon*, *Ch. pentheus* Walker, 1839, *Ch. polyzo* Walker, 1839, *Neochryso-*

*charis formosus* (Westwood, 1833), and *Minotetrastichus frontalis* Nees, 1834. The dominant species were *M. frontalis*, *S. sericeicornis*, and *S. gordius*, which respectively comprised 26, 14, and 8% of the total abundance of parasitoids in all the three sample plots.

*Minotetrastichus frontalis* is a gregarious ectoparasitoid developing on larvae and pupae of mining moths (especially *Phyllonorycter* spp.), beetles, and hymenoptera (Graham, 1987). The females of *M. frontalis* lay eggs in the mine of *Ph. issikii*, directly on the host larva or next to it (Yefremova and Mishchenko, 2012). The parasitoid has no specific localization on the host body. From 1 to 5 larvae of *M. frontalis* may develop on one larva or pupa of *Ph. issikii* (Yefremova and Mishchenko, 2012).

*Sympiesis sericeicornis* and *S. gordius* are mostly solitary, primary or secondary (i.e., developing on the primary parasite) ectoparasitoids of larvae and pupae of mining insects, including moths of the genus *Phyllonorycter* (Bouček and Askew, 1968).

Comparison of the monitoring data for three sample plots in 2001–2005 (Ermolaev et al., 2011) and 2015 allows us to conclude that the structure of the parasitoid complex of *Ph. issikii* has been transformed.

First, the diversity of parasitoids of the family Eulophidae developing on *Ph. issikii* has been reduced from 22 to 12 species.

Table 5. Structure of the parasitoid complex of the lime leafminer *Ph. issikii* in different localities of Udmurtia (data of 2016)

Species	Yar	Glazov	Balezino	Kez	Yukamenskoe	Krasnogorye	Igra	Debesy	Sharakan	Yakshur-Bodya	Siva biological station	Syumsi	Uva	Vavozh	Izhevsk	Kizner	Grakhovo	Almashi	Malaya Purga	Kiyasovo	Karakulino	Sarapul	
<i>Pnigalio agraules</i>																							
<i>P. pectinicornis</i>																							
<i>P. soemius</i>																							
<i>Sympiesis acalle</i>																							
<i>S. gordius</i>																							
<i>S. sericeicornis</i>																							
<i>Cirrospilus diallus</i>																							
<i>C. pictus</i>																							
<i>Elachertus fenestratus</i>																							
<i>Hyssopus geniculatus</i>																							
<i>Pediobius saulius</i>																							
<i>Chrysocharis amarus</i>																							
<i>Ch. laomedon</i>																							
<i>Ch. nephereus</i>																							
<i>Ch. pentheus</i>																							
<i>Ch. polyzo</i>																							
<i>Neochrysocharis formosus</i>																							
<i>Minotetrastichus frontalis</i>																							
Infestation rate, %	8.6	1.1	12.8	14.0	4.5	6.3	2.1	12.1	2.1	9.4	9.2	3.0	5.8	6.4	1.0	9.3	4.8	11.7	8.0	20.0	7.8	8.4	

Data for Izhevsk are given according to the results of studying three sample plots in 2015.

Second, the dominant species of the complex have changed. *Pnigalio soemius* was clearly prevalent during 2001–2005, whereas in 2015 it was replaced by *Minotetrastichus frontalis*. The latter species is also dominant in the parasitoid complexes of the lime leafminer in Central Europe. For instance, it comprised  $31.8 \pm 2.3\%$  ( $n = 60$ ) of the total abundance of the parasitoid complex in the city parks of Bratislava in 2011–2013 (Ermolaev and Aimbetova, 2016). In the same years, the total fraction of *M. frontalis* in collections from 16 localities in Hungary reached 83.7% (Szöcs et al., 2014). The dominant position of *M. frontalis* in the parasitoid complex of the lime leafminer was also noted in Romania (Andriescu et al., 2016). Besides, in 2006 *M. frontalis* was the second most abundant species in Ulyanovsk Province, where it comprised  $19.3 \pm 3.8\%$  of the whole complex ( $n = 22$ ) (Yefremova and Mishchenko, 2008).

It was shown by the data of sample plot 3 that the second generation of *Ph. issikii* was infested with *Sympiesis acalle*, *S. gordius*, *S. sericeicornis*, *Ch. laomedon*, and *M. frontalis*. Of these, *S. sericeicornis*, *M. frontalis*, and *S. gordius* were prevalent and made up 41.5, 24.4, and 21.9% of the whole parasitoid complex.

The study of parasitoids of *Ph. issikii* carried out in 2016 revealed 18 species of Eulophidae (Table 5) and also some representatives of the families Encyrtidae and Proctotrupidae. Five species were recorded for the first time as parasitoids of the lime leafminer: *Pnigalio pectinicornis*, *Sympiesis acalle*, *Chrysocharis amarus* Walker, 1839, *Ch. pentheus*, and *Ch. polyzo*; two species were recorded for the first time on this host in the study region: *Pnigalio agraulis* (Walker, 1839) and *Pediobius saulius* (Walker, 1839). Considering the results of our earlier work (Ermolaev et al., 2011), a total of 29 species of parasitoids infesting *Ph. issikii* were recorded for the territory of Udmurtia (Table 1). The mean infestation rate of *Ph. issikii* in 22 localities of Udmurtia was  $7.6 \pm 1.0\%$ , varying from 1.0% in Izhevsk to 20% in Kiyasovo. The dominant parasitoids were *Sympiesis gordius*, *S. sericeicornis*, and *M. frontalis* which comprised 26.0, 20.4, and 15.9% of the whole parasitoid complex, respectively. The first two species occurred throughout the whole study region whereas *M. frontalis* was restricted to the nemoral zone and was absent in the districts of Udmurtia positioned to the north of 57°N.

Thus, a repeated study of three sample plots in Izhevsk revealed high (up to  $89.0 \pm 1.5\%$ ) survival rates of *Ph. issikii*. The contribution of the parasitoid complex to the host mortality was still negligible, no more than  $1.6 \pm 0.3\%$ . Low efficiency of the regulatory factors facilitates a rapid population increase and high invasion rates of the lime leafminer (Ermolaev and Rubleva, 2017). Five new parasitoids of *Ph. issikii* were recorded as the result of research in different districts of Udmurtia: *Pnigalio pectinicornis*, *Sympiesis acalle*, *Chrysocharis amarus*, *Ch. pentheus*, and *Ch. polyzo*. Two species, *Pnigalio agraulis* and *Pediobius saulius*, were recorded as parasitoids of the lime leafminer new to the study region. Further studies of the structure of the parasitoid complex of *Ph. issikii* within its secondary range would help to understand the establishment of natural regulators of the pest populations.

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