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Dispersal and larval hosts of the zigzag elm sawfly *Aproceros leucopoda* (Hymenoptera) in Slovakia, Central Europe

MILAN ZÚBRIK[1]
JURAJ GALKO[1]
ANDREJ GUBKA[1]
SLAVOMÍR RELL[1]
ANDREJ KUNCA[1]
CHRISTO NIKOLOV[1]
JOZEF VAKULA[1]
ROMAN LEONTOVYČ[1]
IVAN LUKÁČIK[2]
IVANA SARVAŠOVÁ[2]
MARTINA ZÚBRIKOVÁ[2]
IVAN ŠPILDA[2]

[1] National Forest Centre – Forest Research Institute Forest Protection and Game Management Banská Štiavnica, Slovakia

[2] Technical University in Zvolen, Zvolen, Slovakia

Correspondence:
Milan Zúbrik
E-mail address: zubrik@nlsk.org

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Abstract

Background and purpose. *The zigzag elm sawfly,* Aproceros leucopoda *(Hymenoptera), originating from Asia is being considered as one of the major pests of elm trees (*Ulmus *spp.) within Europe where it was first recorded in 2003. Recent investigations document the fast spread of this invasive species in many European countries. In Slovakia,* A. leucopoda *was first recorded in 2009 but its occurrence here has not been reported since 2009. Herein, we summarize the data from the two consecutive years about the current distribution of* A. leucopoda *and its hosts in Slovakia, Central Europe, focusing on infestation of elm trees by this successful invader in different altitudes.*

Material and methods. *In 2014 and 2015 the investigations of* A. leucopoda *were conducted at 101 different plots across the country. At each plot, characteristic symptoms of leaf damage, occurrence of larvae, cocoons or adults were recorded along with the geographical coordinates and the altitude, and the host trees of* A. leucopoda *were identified at the species level. The distribution map of* A. leucopoda *was drawn using the field data. The infestation of elm trees along the altitudinal gradient was analyzed with Kruskal-Wallis non-parametric analysis of variance followed by post-hoc Dunn's test for multiple comparisons.*

Results and conclusions. *The zigzag elm sawfly* A. leucopoda *was observed at 31 plots (30.7%, n = 101). In elm trees examined, generally, a low level of infestation by this pest was observed.* A. leucopoda *was found at plots between 107 and 701 m a.s.l. The result show that the infestation level of elm trees was decreasing with increasing altitude. Although we had only a small sample of data, we assume that the altitude has a significant effect on distribution of this pest. The field elm (*Ulmus minor *Mill.) was the most frequently utilized host. Data collected document the establishment of this successful invader in Slovakia.*

INTRODUCTION

Over the last century, a large number of invasive insect species have become established in Europe *(1)*. Many of them cause severe economical and aesthetical damage to trees in forests and/or urban areas. From those species which recently invaded Slovakia we can mention the horse chestnut leafminer *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera: Gracillariidae) *(2, 3)*, the ambrosia beetle *Xylosandrus germanus* Blandford, 1894 (Coleoptera: Curculionidae, Scolytinae) *(4)*, or the box tree moth *Cydalima perspectalis* Walker, 1859 (Lepidoptera,

Pyralidae) *(5)*. The wide spread of the harlequin ladybird beetle *Harmonia axyridis* Pallas, 1773 (Coleoptera, Coccinellidae) in Europe including Slovakia is cause of concern, as it is a threat to native species and biodiversity *(6)*.

In 2007, the zigzag elm sawfly *Aproceros leucopoda* Takeuchi (Hymenoptera: Argidae) was found in Slovakia *(7)* for the first time. This species has an Asiatic origin. It was described from Hokkaido, Japan by Takeuchi *(8)*. It also occurs in China *(9)* and Russian Far East *(10)* but this last record requires taxonomic confirmation *(7)*. In Europe, it was first recorded in Hungary and Poland in 2003. Later on, *A. leucopoda* was found in Austria, Romania and Ukraine *(7)*. In 2009, the sawfly was reported from Italy *(11)*. Its occurrence in Germany *(12)*, Slovenia *(13)*, Croatia *(14)*, Belgium *(15)*, Serbia *(16)*, the Netherlands *(17)*, the Czech Republic *(18)*, Latvia *(19)* and Bulgaria *(20)* was also documented. *A. leucopoda* occurs in the European part of Russia *(21, 22)*.

A. leucopoda is an oliphagous pest which feeds on elm trees (*Ulmus* spp.). The larvae were collected from *U. pumila* and *U. japonica* in Japan *(7, 8)*. Within Europe, *A. leucopoda* feeds on native (*U. glabra*, *U. laevis*, *U. minor*) and few cultivated species of elm trees *(7, 11, 20)*. In European part of Russia larvae develop mainly on Siberian elm *U. pumila (21, 23)*.

The populations of elm trees (*Ulmus* spp.) within Europe have declined considerably since the 1920s. The spread of the vascular wilt disease of elm trees, the Dutch elm disease, resulted in two massive and very destructive pandemics killing most of the European elm trees. The first pandemic, caused by the fungus *Ophiostoma ulmi* (Buisman) Nannf., occurred between the 1920s and the 1940s. The second (current) pandemic is caused by *Ophiostoma novo-ulmi* Brasier which has been spreading to regions previously affected by *O. ulmi (24, 25, 26)*. These fungi, transmitted mainly by bark beetles of the genus Scolytus (Coleoptera, Curculionidae, Scolytinae), are responsible for the decline of elm trees in Slovakia too *(27, 28)*. As a result of Dutch elm disease, during the last century elms suffered major losses worldwide, with the near-total disappearance of adult trees in many European, Asian and North American areas *(29)*.

Observations from Japan show that *A. leucopoda* is a parthenogenetic species; no males were recorded in the population of this pest *(7)*. During pest monitoring in Italy also only females were collected *(11)*. In Hungary, adults occur from the middle of April till early September *(7)*. In Italy, the first females appeared in the second half of April (from 22 April) and cocoons were found at the beginning of May (from 6 May). The Italian *A. leucopoda* completes the first generation in mid May, in about 28 days, and the species is multivoltine. In northern Italy the species develops four generations per year, or more *(11)*. Females lay eggs into the edge of leaves. Larvae hatch after 4-8 days and young feeding larvae excavate a very characteristic „zig-zag“ pattern on leaves. Feeding galleries lead from the leaf edge to the main vein and usually do not cross side veins *(7, 30)*. Larvae make either loosely spun cocoons, or more solid, compact cocoons build in the field in the litter or soil. They overwinter in the more solid cocoons. In loosely spun cocoons eonymphs pupate during 2-3 days and adults emerge after 4-7 days. In Europe, *A. leucopoda* has four generations per year *(7)*. High number of generations, asexual reproduction and high fertility of females (one female can lay as many as 49 eggs) *(7)* makes this species a potentially serious pest (defoliator) of elm trees within Europe.

In 2007 and 2009, *A. leucopoda* was first recorded in Slovakia, Central Europe *(7)*. Since then its occurrence has not been reported from this country at all. This is why we studied the current distribution and hosts of *A. leucopoda* in Slovakia with emphasis on infestation of elm trees by this pest in different altitudes.

MATERIALS AND METHODS

Recording, collecting and rearing of *A. leucopoda*

In 2014 and 2015, from May to August, *A. leucopoda* was recorded on elm trees growing singly or in small groups at 101 plots along the roadsides and rivers, at the forest edges, inside the forests and in urban areas. The records of the pest at plot distant at least 500 m from each other were considered as separate observations. For each plot the geographical coordinates were taken and data about the altitude were gathered. Elm species were identified according to Pagan and Randuška (1988) *(31)*. At each plot, characteristic symptoms of leaf damage, occurrence of larvae, cocoons or adults of *A. leucopoda* were recorded.

Larvae and adults were collected in the field. Field collected larvae were reared on elm foliage in 2 l plastic rearing boxes. Leaves very changed every second day, together with the absorbent paper lining on the bottom of the box. Leaves were kept in a small plastic epruvetes filled with water to keep them fresh as long as possible. Larvae were kept in the laboratory under room conditions until morphing into pupa. After pupation, elm leaves with cocoons were collected and placed in Petri dishes (15 cm in diameter) until the adults emerged. The larvae and adults were identified using the key by Blank et al. (2010) *(7)*.

Data analysis

At each plot the elm trees were visually examined for presence of *A. leucopoda* symptoms, always by two people. The samples were taken from lower branches from randomly selected trees. Four levels of infestation (IL0, IL1, IL2, IL3) were recognized judging from occurrence of the pest (larvae, cocoons, adults) and/or its feeding traces (marks) on leaves and trees, respectively. The criteria determining the level of infestation are given in Table 1.

Table 1. *Parameters used to determine the level/degree of infestation of elm trees by A. leucopoda. Achievement of at least one of the given criteria decided about the resulting categorization.*

Infestation level (IL)		Presence of *A. leucopoda* (larvae, cocoons and adults) on leaf surface	Presence of zig-zag pattern on leaves of the same tree	Defoliation of trees
Not infested	0	no larva or no cocoon or no adult	Not present	any visible defoliation
Low	1	one larva or one cocoon or one adult	4 or less leaves infested	any visible defoliation
Intermediate	2	more than one larva or more than one cocoon or more than one adult	from 5 to 29 leaves infested	any visible defoliation
High	3	more than 30 larvae or more than 30 cocoons or more than 30 adults	30 or more leaves infested	at least 5-10%

Achievement of at least one of these criteria decided about the resulting categorization of individual observations into one of the four levels of infestation (Table 1). A plot was categorized to the specific category (level of infestation) after 20 minutes of visual inspection of host trees.

The difference in altitude between infestation levels (IL) was analyzed with Kruskal-Wallis non-parametric analysis of variance followed by post-hoc Dunn's test for multiple comparisons. Data were analyzed using R version 3.3.1 *(32)* and 'plot.kw': R function for visually displaying Kruskal-Wallis test's results *(33)*.

The distribution map of *A. leucopoda* was drawn in ArcGIS (ESRI Inc.) using the geographic coordinates of particular plots and the species records.

The average infestation level was calculated as an average of the individual infestation levels of the plots where the pest has occurred.

RESULTS AND DISCUSSION

Distribution of *A. leucopoda* in Slovakia

Over the period 2014–2015, the occurrence of *A. leucopoda* was observed at 101 plots across the whole country. The species was recorded at 31 plots (30.7%). Following the first observations in 2014, the frequency of observations increased in 2015. In most localities, *A. leucopoda* was identified by conspicuous characteristic „zig-zag" feeding larval traces on leaves. However, the larvae or cocoons were also found. The currently known distribution of *A. leucopoda* in Slovakia is shown in Figure 1.

As can be seen from Figure 1, most of the records of this species come from the southwest of the country (Figure 1).

Despite of the records of *A. leucopoda* in eastern Slovakia in 2007 *(7)*, the species was not recorded there again

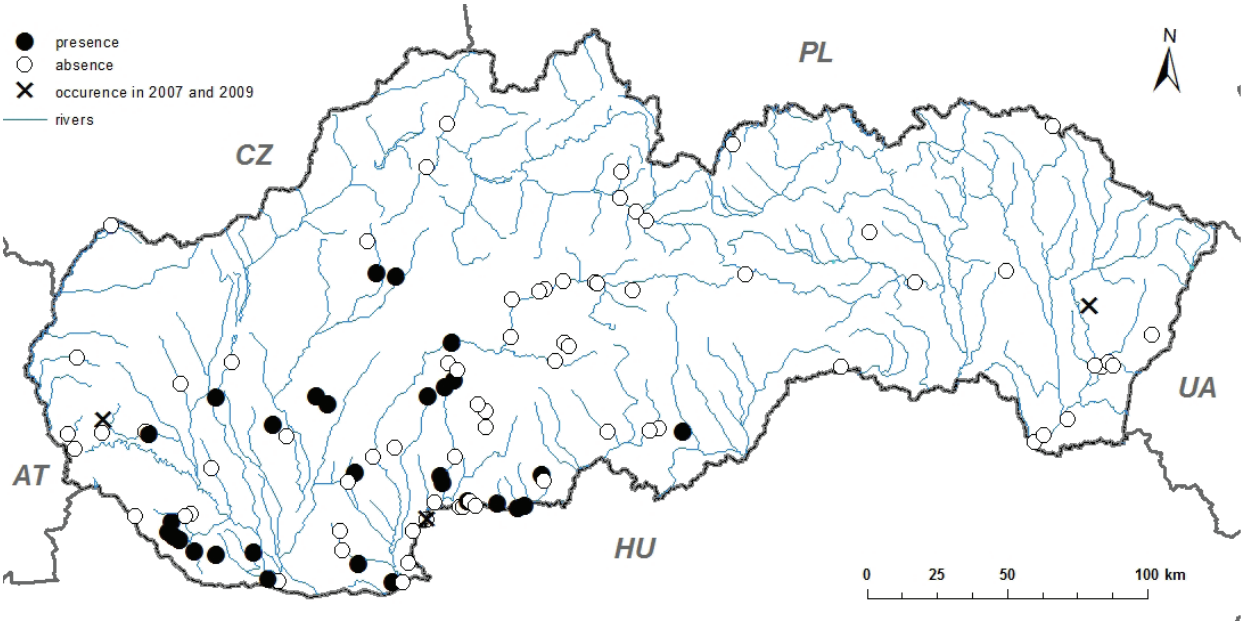

Figure 1. *Distribution of* A. leucopoda *in Slovakia between 2014 and 2015 (presence – filled circles, absence – empty circles) and occurrence of the species in 2007 and 2009 (crosses) (7).*

Table 2. *Plots with occurrence of* A. leucopoda *and their characteristics.*

District	Character of plot	Latitude	Longitude	Infestation level	Tree species	m a.s.l.
Balog nad Ipľom	near road	48.076°N	19.150°E	2	*U. minor*	138 m
Banská Štiavnica	urban area	48.461°N	18.900°E	1	*Ulmus spp.*	619 m
Beladice	near pond	48.349°N	18.303°E	2	*U. minor*	176 m
Bodza	urban area, 150 meter from water canal	47.837°N	17.829°E	2	*U. minor*	111 m
Bojnice	castle park	48.778°N	18.576°E	2	*Ulmus spp.*	312 m
Cabaj – Čápor	forest edge	48.270°N	18.045°E	2	*U. minor*	219 m
Čiližská Radvaň	wet forest	47.842°N	17.729°E	3	*U. minor*	115 m
Dolný Pial	roadside	48.138°N	18.460°E	1	*U. minor*	181 m
Gabčíkovo	forest edge	47.896°N	17.592°E	3	*U. minor*	117 m
Gabčíkovo-Pataš	roadside	47.882°N	17.625°E	2	*U. minor*	115 m
Gbelce	urban area	47.850°N	18.509°E	2	*U. minor*	132 m
Hadovce	open forest near the village	47.776°N	18.092°E	3	*U. minor*	110 m
Hokovce	forest	48.153°N	18.870°E	1	*U. minor*	138 m
Horné Semerovce	castle park	48.127°N	18.882°E	1	*U. minor*	130 m
Jelenec	agricultural land	48.372°N	18.243°E	2	*U. minor*	213 m
Kameničná	marshland	47.857°N	18.010°E	2	*U. minor*	108 m
Koláre	river bank	48.074°N	19.272°E	1	*U. laevis*	138 m
Kráľová pri Senci	roadside	48.205°N	17.458°E	2	*U. minor*	120 m
Kurinec	forest	48.350°N	20.024°E	1	*U. laevis*	208 m
Malý Krtíš	agricultural land	48.180°N	19.355°E	1	*U. minor*	187 m
Nitrianske Rudno	near road	48.781°N	18.482°E	1	*Ulmus spp.*	319 m
Pataš	urban area	47.876°N	17.653°E	3	*U. minor*	113 m
Slovenské Ďarmoty	river bank	48.076°N	19.280°E	3	*U. minor*	137 m
Štiavnické Bane	near lake	48.435°N	18.857°E	1	*U. minor*	701 m
Štúrovo – Železničný Rad	in front of railway station	47.799°N	18.679°E	2	*U. minor*	120 m
Tešmácka Cesta	near road	48.075°N	19.007°E	3	*U. minor*	134 m
Vinohrady nad Váhom	village gardens	48.340°N	17.760°E	2	*U. minor*	160 m
Vrkúň	edge of forest	47.931°N	17.607°E	1	*U. laevis*	116 m
Vysoká	forest near lake	48.401°N	18.777°E	1	*Ulmus spp.*	455 m
Žiar nad Hronom	near pond	48.580°N	18.870°E	1	*U. minor*	248 m
Žibritov	forest	48.389°N	19.022°E	1	*U. laevis*	500 m

between 2014 and 2015. It was also not found in northern part of Slovakia. It is a bit surprising that the species was not found practically in whole Eastern Slovakia in spite of the fact that the first record in the country came from there. One from the possible explanations of this may be a misidentification of *A. leucopoda* in 2007 made based on only one female specimen collected from oak leaves *(7).* Other reasons cannot be excluded either. Plots with occurrence of *A. leucopoda* and their characteristics are shown in Table 2.

The records of *A. leucopoda* show that the species mainly occurs in lowlands and river basins and, apparently, spreads along larger Slovakian rivers. In the river valleys it spreads from the south to the north. The records above support Blank et al. (2014) *(34)* who showed that *A. leucopoda* is frequent in floodplain forests along the Danube river separating Slovakia and Hungary. In 2003, the pest was first reported from the village Dejtár (Hungary) only 2.5 km from the border between the two countries *(7).* Also, *A. leucopoda* was often found on the Slovakian side

of the Danube basin, and along rivers flowing into the Danube, namely the river Hron and Ipeľ. These two rivers at least considerably support the northerly spread of this species (Figure 1). *A. leucopoda* occurred in the southern areas more frequently than in the northern (mountainous) ones. The plots Bojnice and Nitrianske Rudno were shown to be the northernmost localities in Slovakia with the documented occurrence of *A. leucopoda*. They both lie only 100 km from the location Dejtár in Hungary where the pest was reported for the first time in Europe (together with one place in Poland) already in 2003 *(7).* The spread of the pest from Northern Hungary into the Slovakia cannot be definitively confirmed by our data, but also cannot rejected especially if frequent occurrence of the pest was found in Danube basin in southern Slovakia, only few kilometers far from Hungarian village Dejtár. There is no information on the occurrence and dispersal of the species from the period between 2009 and 2014, so we do not know how fast and far (and from which points of introduction) it was spreading during this time. The pest had already been reported from many countries, located northwest from Slovakia, before this monitoring program took place *(34)*, therefore the arrival of the sawfly from (also) other directions (i.e. from the north/west to the south/east) may not be excluded either.

At most plots in Slovakia, the population densities of *A. leucopoda* were low, the species caused only insignificant damage to elm trees. Only the slight damage to leaves is also known from Bulgaria *(20)* and Serbia *(16).* In Slovakia, more severe tree defoliations of trees were rarely documented. Higher level of infestation and more serious damage to leaves was observed only near Gabčíkovo and between Šahy and Slovenské Ďarmoty, where defoliation of some *U. minor* trees reached up to 20-30%. In Germany, at most investigated localities only low levels of the pest population were found in 2013–2014, with insignificant damage to the hosts. Serious defoliation reached 50-70%, has been recorded at one plot – Schlieben (Brandenburg) *(34).* Based on the evidence from Hungary, Romania, Italy and Germany, the pest has a potential to cause complete defoliation of elm trees *(7, 11, 23).*

Table 2. *Infestation of elm trees by* A. leucopoda.

Tree species	Total plots	Plots with *A. leucopoda* presence	Plots with *A. leucopoda* presence in %	Average infestation level
U. minor	26	23	88.4	1.53
U. laevis	19	4	21.1	0.21
U. spp.	4	4	100.0	1.25
U. glabra	4	0	0	0
Total	53	31	–	–

Host selection

Natural hybridization and planting of non-native hybrids of elm trees in Slovakia make the taxonomy of elm species rather complex and therefore determination of particular species difficult. At 53 plots, elm trees species were identified. In total, three elm species, *U. minor*, *U. laevis* and *U. glabra,* were documented. Considering the performance of *A. leucopoda* on particular hosts, *U. minor* was infested much more (average IL 1.53) than *U. laevis* (average IL 0.21). However, *A. leucopoda* was not found on *U. glabra* at all. *U. glabra* is a sub-mountain/mountain species and cool mountain climate could limit the occurrence of *A. leucopoda* on this elm species, especially in the northern areas of Slovakia (Table 2).

In Europe, *A. leucopoda* larvae may feed on different species of elm trees including a variety of ornamental cultivars. In Romania, average defoliation of individual trees ranged from 74% to 98% and *U. glabra* was infested. In Hungary defoliation of *U. minor* and *U. pumila* trees reached up to 100%, larvae and cocoons were collected also from *U. pumila* var. *arborea*. Larvae or cocoons of *A. leucopoda* were collected from *U. glabra*, *U. laevis* and *U. minor* in Austria (7). Recently, from Hungary is reported heavy infestation of Siberian elm (*U. pumila*) with *A. leucopoda* in 2012 (35). During monitoring of the pest in Germany, larvae, feeding traces and cocoons were frequently found on the native elm species *U. minor* and *U. glabra*, whereas none sighs of presence were detected on *U. laevis* (34). In 2015, various stages of *A. leucopoda* have been found on the *U. minor* in Bulgaria (20). In European part of Russia larvae develop on various species of the genus Ulmus, preferring the Siberian elm *U. pumila* (21). In the Donetsk region in 2014 and in the Middle Vilga Region the same tree species was also heavily defoliated (22, 23). Defoliation by *A. leucopoda* could significantly decrease the resistance of infested trees against fungal pathogens and bark beetles, as in the case of other leaf eating insects (36, 37), as well as their aesthetics value in urban areas.

Occurrence of *A. leucopoda* related to altitude

A. leucopoda and its damages from 2014 and 2015 (n = 31) was recorded in altitudes between 108 m (Kameničná) and 701 m (Štiavnické Bane). Distribution of the infestation levels by altitude is displayed in Figure 2. In Europe, *A. leucopoda* was recorded only rarely above 500-600 m a.s.l. In Austria, it was found between 160–580 m (7, 34), in Italy between 21-678 m (11) and in Bulgaria between 280-680 m a.s.l. (20).

Infestation levels: IL1 (low), IL3 (moderate), IL3 (high infestation). Median, interquartile range and extreme values including outliers (circle).

Figure 2 indicates the negative trend of relationship between the level of infestation and altitude. The mean

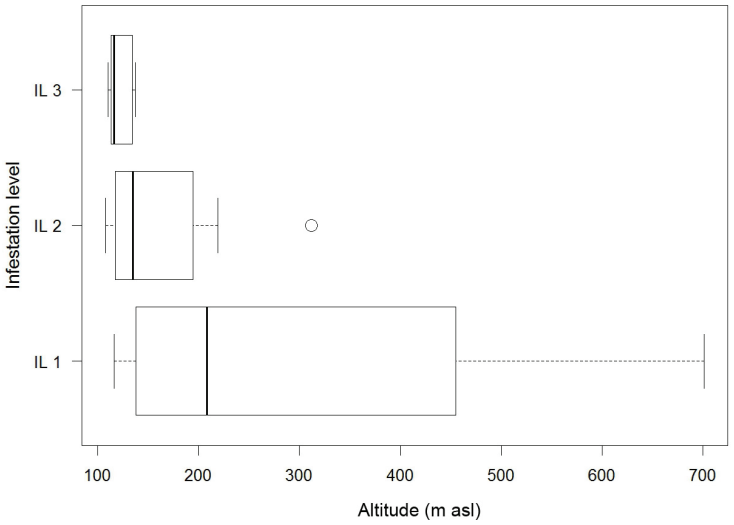

Figure 2. *Infestation of elm trees by* A. leucopoda *by altitude (n = 31).*

altitude of group of highly infested trees (IL 3) was 121 m a.s.l., mean altitude of moderately infested trees (IL 2) was 160 m a.s.l. and mean altitude of the group of low infestation level (IL 1) was 303 m a.s.l. The mean altitude of the plots of taken samples was 239 m.

The Kruskal-Wallis Test (H = 10.843, df = 2, p-value = 0.004) showed there is a significant difference between the IL groups. We used the Dunn's test to determine which group significantly differs from the others. The groups compared by the Dunn's test on a pairwise basis are shown in Figure 3.

The IL groups compared on a pairwise basis are indicated on the left-hand side of the chart. A vertical line indicates the 0.05 threshold.

From Figure 3 it can be seen that there is a significant difference ($p< 0.005$) between the altitude of IL3 and IL1 group. There were no significant differences found between other group comparisons (IL 2 – IL1 ($p< 0.089$) and IL3 – IL2 ($p< 0.532$)). The difference in mean altitude between IL1 and IL3 group was 182 m a.s.l., between IL 1 and IL 2 was 142 m a.s.l. and difference in mean altitude between IL2 and IL3 groups was 39 m a.s.l.

The results suggest that the invasive sawfly *A. leucopoda* has established in Slovakia, Central Europe. The establishment of this successful invader is documented by the records the larval traces on the leaves of elm trees (two species) at 31 different plots and, also, by the records of larvae, pupae and adults, mostly in the southwestern part

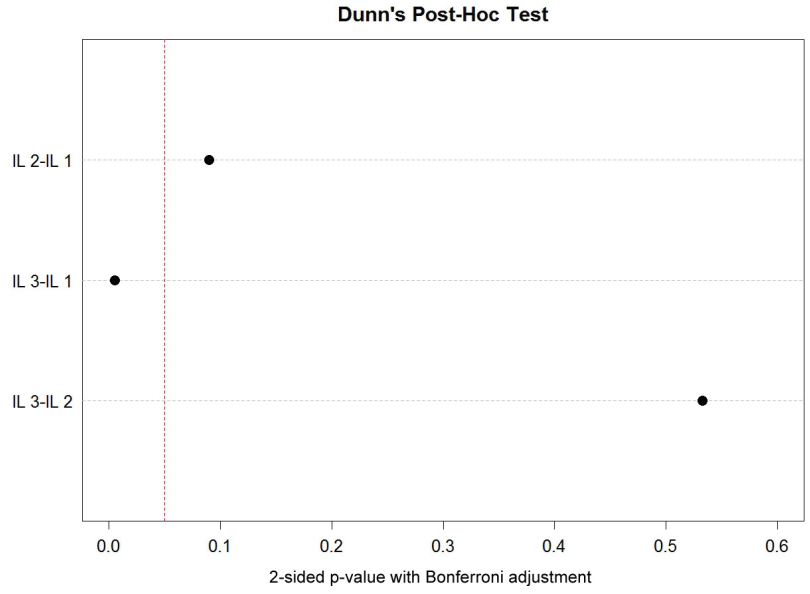

Figure 3. *Dot chart of Dunn's post-hoc test.*

of this country. Although *A. leucopoda* tends to spread further to the north, its occurrence in the northern areas is limited at the present moment. As this invasive pest species has potential as a defoliator of elm trees, relation with fungal pathogens and bark beetle associates of elm trees in forests and urban areas need to be studied in more detail.

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