



## The effectiveness of the neem product TreeAzin® in controlling *Cameraria ohridella* (Lepidoptera: Gracillariidae: Lithocolletinae)

ANDREJ GUBKA<sup>1</sup>, MILAN ZUBRIK<sup>1</sup>, SLAVOMÍR REL<sup>1</sup>, NICOLE GAREAU<sup>2</sup>, TARRYN GOBLE<sup>2</sup>, CHRISTO NIKOLOV<sup>1</sup>, JURAJ GALKO<sup>1</sup>, JOZEF VAKULA<sup>1</sup>, ANDREJ KUNCA<sup>1</sup> and RHODA DEJONGE<sup>2</sup>

<sup>1</sup> National Forest Centre, Forest Protection Service, Lesnícka 11, SK-96901 Banská Štiavnica, Slovak Republic; e-mails: andrej.gubka@nlcsk.org, zubrik@nlcsk.org, rel@nlcsk.org, nikolov@nlcsk.org, galko@nlcsk.org, jozef.vakula@nlcsk.org, kunca@nlcsk.org

<sup>2</sup> Lallemand Plant Care/BioForest, 59 Industrial Park Crescent, Unit 1, Sault Ste. Marie, ON P6B 5P3, Canada; e-mails: ngareau@lallemand.com, tgoble@lallemand.com, rdejonge@lallemand.com

**Key words.** Gracillariidae, Lithocolletinae, *Cameraria ohridella*, horse-chestnut leaf miner, *Aesculus hippocastanum*, tree injection, systemic insecticides, systemic applications, azadirachtin

**Abstract.** Infestation by invasive horse-chestnut leaf miner, *Cameraria ohridella* Deschka & Dimić, permanently lowers the aesthetic and cultural value of horse-chestnut in Central Europe. In 2017–2018, in urban zones in the cities Parchovany and Strážske in the eastern part of Slovakia, we assessed the efficacy of systemic applications of TreeAzin®, an azadirachtin-based product, in controlling *Cameraria ohridella* in trials in which it was microinjected into tree trunks. A total of 16 *Aesculus hippocastanum* trees were treated with 3 ml of TreeAzin® per centimetre diameter at breast height [DBH] and another 17 were treated with 5 ml of the same product per centimetre at DBH, at two study plots. In total, 18 trees were left untreated as controls. In this field experiment, we confirmed significantly higher efficacy in the year of application and the following season. Statistically significant differences were found in the average leaf damage caused by *C. ohridella*, between treated (4.2–24.5% avg. leaf damage) and untreated trees (75.5–94.3% avg. leaf damage). At the end of the first growing season, 81.2–95.0% of the untreated control tree crowns were defoliated while defoliation of the treated trees was 19.2–31.6%. Both the 3 and 5 ml/cm doses were equally effective in terms of crown and leaf damage; no statistical differences were found in average leaf and crown damage between trees treated with doses of 3 ml/cm and 5 ml/cm. Similar results were also obtained the following year. Leaf damage of treated trees was 40.4–16.8% and of untreated trees 67.9%. Crown damage of treated trees was 49.7–59.8% and of untreated trees 78.8%. During the period of this study, the crowns of all the treated trees were statistically and visually healthier and fuller than those of untreated trees. Thus, the efficacy of this systemic insecticide in controlling *C. ohridella* in Europe is very promising and provides a suitable treatment for reducing the incidence of this invasive pest.

## INTRODUCTION

The horse-chestnut leaf miner, *Cameraria ohridella* (Gracillariidae: Lithocolletinae), was described by Deschka & Dimić (1986) from Macedonia where it was discovered defoliating European horse-chestnut trees, *Aesculus hippocastanum* L., near Lake Ohrid. This pest has been recorded in many other European countries since its initial discovery. While many of these new identifications may represent populations that have been present, but undiscovered, for many years, there is also strong evidence to suggest that *C. ohridella* can quickly disperse into new areas (Gilbert et al., 2005). Horse-chestnut leaf miner can disperse over long distances aided by human transport and shorter distances by flight (Zúbrik et al., 1999; Gilbert et al., 2004). This insect was recorded in Austria near the city of Linz in 1989 and around Enns in 1990 (Pschorn-Walcher, 1994). It was discovered in Italy in 1992 (Hellrigl, 1998),

Germany in 1993 (Butin & Führer, 1994), then Hungary (Szabóky, 1997), Czech Republic (Liška, 1997) and Slovakia in 1994 (Sivicek et al., 1997; Hrubík & Juhásová, 1998; Juhásová et al., 1998). It was identified on the Balkan Peninsula within this same time frame. First, in Serbia and Montenegro in 1986 (Dimić & Mihajlović, 1993; Dautbašić & Dimić, 1999), then Albania in 1989 (Hellrigl & Ambrosi, 2000), Bulgaria in 1989 (Prelov et al., 1993), Croatia in 1989 (Maceljski & Bertić, 1995), Bosnia and Herzegovina in 1993 (Dautbašić & Dimić, 1999), Slovenia in 1995 (Milevoj & Macek, 1997) and Greece in 1996 (Avtzis & Avtzis, 2003). *Cameraria ohridella* is now found throughout Europe, reaching as far west as Great Britain (Pschorn-Walcher, 1994; Gilbert et al., 2004, 2005) and only absent in the extreme northern, southern and western parts of this continent (Straw & Tilbury, 2006; Valade et al., 2009).

The European horse chestnut (*A. hippocastanum*) is the primary host tree for *C. ohridella* on this continent (Deschka & Dimić, 1986; Pschorn-Walcher, 1994; Dimić et al., 2005). The moth can also attack and develop on other species of the genus *Aesculus* (Tomiczek & Krehan, 1998; Freise et al., 2004; Dimić et al., 2005). However, there is evidence to suggest that the closely related *Aesculus pavia*, which is frequently planted in the same environment, is strongly resistant to *C. ohridella* (Kobza et al., 2011). It is occasionally reported developing on maple trees (*Acer pseudoplatanus* and *A. platanoides*) in which case damage levels may be as high as on horse-chestnut (Pschorn-Walcher, 1997; Hellrigl, 1998; Freise et al., 2004). To date, there is little evidence that *C. ohridella* represents a major risk for *A. pseudoplatanus* (Péré et al., 2010).

European horse chestnut is frequently planted in Europe along roads, in urban parks, large gardens and forest environments (Zúbrik et al., 2006). For several reasons, its cultivation in Central Europe was very popular during the 18<sup>th</sup> and 19<sup>th</sup> centuries, where it was obtained from the Balkan Peninsula (Adam, 1997).

Mining by *C. ohridella* has the potential to significantly reduce a tree's leaf area by midsummer. Heavy infestations decrease the aesthetical value of trees, resulting in branches dying and repeated sprouting and flowering in autumn (Percival et al., 2011). Defoliation has also an effect on seed quality (Thalmann et al., 2003). However, widespread dieback of horse chestnut trees has so far not been observed (Butin & Führer, 1994; Kenis & Forster, 1998).

While there are a number of control options for *C. ohridella*, chemical control measures for the control of this pest are commonly used in urban forest environments (Blümel & Hausdorf, 1996; Zúbrik et al., 2006). Foliar sprays of synthetic and highly toxic insect growth regulators, such as diflubenzuron, triflumuron and fenoxycarb are the most popular insecticides; however only diflubenzuron consistently results in a high level of control (Blümel & Hausdorf, 1996; Gilbert et al., 2003; Głowacka, 2005a; Głowacka et al., 2009). Mechanical control methods such as removing dead leaves, in which pupae overwinter and burning or composting them, remains the most environmentally friendly method used in urban parks and several small cities. This method is recommended by many authors (Kehrli & Bacher, 2003; Pavan et al., 2003; Głowacka, 2005b; Kukula-Mlynarczyk & Hurej, 2007). Classical biological control against *C. ohridella* also has some potential, but the natural enemy spectrum of this pest is rather small and not very effective (Kenis et al., 2005; Tóth & Lukáš, 2005; Ferracini & Alma, 2007). Systemic insecticides have also been successfully used to control it in the past with good results (Feemers, 1997; Labanowski & Soika, 2003; Pavla & Bárnet, 2005; Ferracini & Alma, 2008; Kobza et al., 2011). There is also the possibility to use glue bands and/or liquid glue on tree trunks (Percival, 2016). Another method is the attract-and-kill technique in urban environments using baited pheromone traps, but results indicate low efficacy in the case of *C. ohridella* (Sukovata et al., 2011).

In an effort to identify more environmentally acceptable control options that are effective for use against invasive insect pests in Europe, we have concentrated our focus on TreeAzin®. TreeAzin® Systemic Insecticide is a proprietary formulation of the natural botanical insecticidal group of compounds referred to as azadirachtins. Formulations prepared from neem seed extracts adversely affect a variety of defoliating and wood-boring pests and are rapidly taken up and translocated following stem injection (Grimalt et al., 2011). Azadirachtin is a botanical insect growth regulator and because of its structural resemblance to the insect moulting hormone ecdysone, azadirachtin inhibits PTTH thereby inhibiting moulting, metamorphosis and development of the female reproductive system. Immature insects exposed to azadirachtin (mainly by ingestion) may moult prematurely or die before they complete their development (Rembold & Sieber, 1981). Those insects that survive treatment are likely to develop into deformed adults, incapable of feeding, dispersing or reproducing (Mordue et al., 2000). Besides the well-known insect growth regulating activity, azadirachtin is also a strong antifeedant for many insects (Schmutterer, 1990). Azadirachtins are non-persistent both within trees and in the environment generally and also exhibit relatively low toxicity to mammals, birds, bees and other non-target invertebrates (Kreutzweiser et al., 2011). By the time of senescence, essentially all azadirachtin residues have dissipated from tree leaves (Grimalt et al., 2011), and therefore no negative impacts to detritivores have been observed when fed treated leaves (Kreutzweiser et al., 2011). The azadirachtin-based systemic insecticide (TreeAzin®) is being widely used for managing invasive insect pests in Canada and the United States. TreeAzin® has been proven effective against and is registered for use on a variety of lepidopteran, coleopteran, hemipteran and hymenopteran insect pests. Thus, it was thought that TreeAzin® could be effective against the horse-chestnut leaf miner, *C. ohridella*, and was used in this study. The aim of this research was to assess the efficacy of two doses (3 ml and 5 ml/cm DBH) of TreeAzin®, (5% azadirachtin) injected into tree stems to control *C. ohridella* in field trials. We predicted that TreeAzin® will offer a high, dose-dependent measure of control of *C. ohridella*.

## MATERIAL AND METHODS

### Study plots

Two plots in Eastern Slovakia, Parchovany (48°44'51.0"N, 21°42'18.4"E, 110 m a.s.l.) and Strážske (48°52'21.8"N, 21°50'03.0"E, 130 m a.s.l.) were selected for assessment. These villages are situated in the eastern part of Slovakia in middle Europe. Predominant type of soil is fluvisoil. Both were in urban areas of the above-mentioned villages. One alley of trees more than 100 m long borders a road built in 18<sup>th</sup> and 19<sup>th</sup> century, near each village.

### Trees selected for experiments

At Parchovany 24 *A. hippocastanum* trees were selected and at Strážske 27 trees. These were numbered and marked using forest marking spray. A total of 16 trees were treated with 3 ml/cm of TreeAzin® at breast height (DBH), 17 others with 5 ml/cm of the



**Fig. 1.** Photograph of the trunk of a tree showing the positions the nozzles and canisters inserted into the trunk for administering the pesticide. When the canisters were empty, they and the nozzles were removed from the trunk.

same product at DBH level, at both sites. In total, 18 trees were left untreated as controls at these two locations.

Trees with little or no visual symptoms of decline in terms of dead branches, small wounds or dead stem wood were selected for experiments. The tree age varied between 105 and 115 years, average diameter at breast height of these was 57.75 cm ( $\pm 5.99$  SD) at Parchovany and from 110 to 120 years, average DBH was 62.9 cm ( $\pm 17.76$  SD) at Strážske. The age of trees was determined based on the city governments' written records. The health status of the trees selected corresponded to their age and some dying branches were present in their crowns.

#### Treatment

TreeAzin® is an botanical injectable systemic insecticide formulated with 5% azadirachtin (lot formulation contains 50.90  $\pm$  4.74 azadirachtin A&B), an extract of the neem tree (*Azadirachta indica* A. Juss.). The main mode of action of azadirachtin is as an insect growth regulator, which reduces insect fecundity and

has anti-feeding properties. The product was applied using the EcoJect® microinjection system, which is a patented technology for the application of systemic insecticides in urban forests and ecologically sensitive areas. Trunk injections were done using a 12-volt battery operated drill with a 15/64" (5.95 mm) drill bit to create a number of holes around the trunk of a tree. Holes were drilled, approximately 13–15 cm apart around the tree, spiralling slightly upwards. The number of holes and volume of product required was determined by the DBH. Each hole was drilled at a 45-degree downward angle and drilled to a depth of about 3 cm beyond the bark. After the holes were drilled, a nozzle was placed in the injection hole and a 20 ml or 8 ml canister was mated to the nozzle and left to empty (Fig. 1). Once the canister had emptied, the canister and nozzle were removed from the trunk. Applications were applied in the field from 24 to 28 April 2017 when the average temperature ranged from 6.7°C (April 24) to 16.4°C (April 26), there was no rain, a S to SE wind direction and wind speed of 7 to 20 km/h.

**Table 1.** Experimental design.

Parchovany																													
Block 1					Block 2					Block 3					Block 4					Block 5									
1	–	2	–	C	–	2	–	1	–	C	–	C	–	1	–	2	–	1	–	C	–	2	–	2	–	C	–	1	
Trees 1–3					Trees 4–6					Trees 7–9					Trees 10–12					Trees 14–15									
Block 6					Block 7					Block 8																			
C	–	2	–	1	–	1	–	2	–	C	–	2	–	1	–	C	–												
Trees 16–18					Trees 19–21					Trees 22–24																			
Strážske																													
Block 11					Block 12					Block 13					Block 14					Block 15									
2	–	C	–	1	–	C	–	2	–	1	–	<del>X</del>	–	2	–	C	–	2	–	<del>X</del>	–	C	–	C	–	1	–	2	–
Trees 1–3					Trees 4–6					Trees 7–9					Trees 10–12					Trees 14–15									
Block 16					Block 17					Block 18					Block 19					Block 20									
1	–	C	–	2	–	2	–	C	–	1	–	C	–	<del>2</del>	–	1	–	1	–	2	–	C	–	2	–	1	–	C	–
Trees 16–18					Trees 19–21					Trees 22–24					Trees 25–27					Trees 28–30									



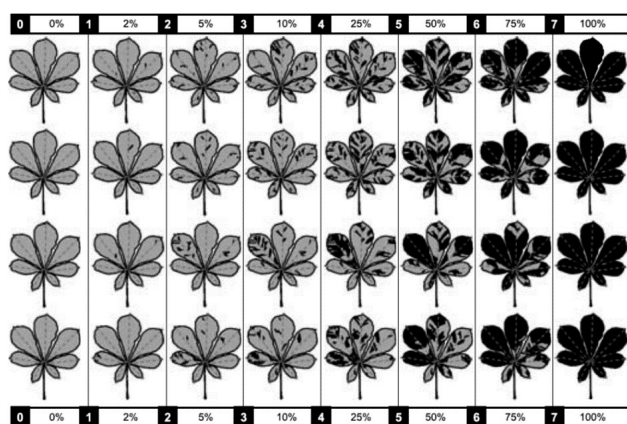


Fig. 2. Leaf damage score card (Gilbert & Gregoire, 2003).

### Design of the field experiment

The experimental design (Table 1) was based on the EPPO Bulletin on Efficacy Evaluation of Plant Protection Products: Design and analysis of efficacy evaluation trials (OEPP/EPPO, 2012). Trees were assigned to either the low dose (3 ml/cm DBH) or high dose group (5 ml/cm DBH) of TreeAzin® or to the untreated control group. These particular dose rates were chosen based on their efficacy in previous defoliator trials (Bioforest Technologies, 2004, 2005a, b, c). Untreated (blank) trees between treated and control trees were not involved in experiments and served as a barrier between the experimental trees. Trees were randomly arranged in blocks: at Parchovany (blocks 1–8) and at Strážske (blocks 11–20). Some selected trees were later excluded from the experimental design because during treatment their health status was found to be unacceptable (indicated by an X in Table 1).

### Assessment of pre-treatment trees

A few days before insecticide applications, 4–5 branches were cut from each tree, 2–3 leaves per branch, at each of the four cardinal points of the tree (north, east, south and west) in the lower canopy (where the first generation usually occurs), i.e. 8–12 leaves per tree were used to assess the numbers of eggs and larval galleries in the pre-treatment populations. Branches were put into labelled bags and brought to the laboratory to count the number of eggs and galleries on the upper surface of the leaves using a binocular microscope, Leica M205 C.

### Assessment of post-treatment damage

Assessments of post-treatment damage were undertaken five times (May 16, July 11, August 2, August 30 and September 20) in 2017. At every assessment, three leaves at each of the cardinal points (12 leaves per tree) were collected and used to estimate the number of galleries, or the percentage of leaf damage caused by *C. ohridella* (Fig. 3). For estimating leaf damage, we used the scorecard published by Gilbert & Gregoire (2003) (Fig. 2).

Table 2. Number of galleries at Parchovany and Strážske assessed on May 16, 2017.

Plot		N	No of galleries	Average $\pm$ SD gal./leaf	Galleries (min.)	Galleries (max.)
Parchovany	3 ml/cm	96	1396	14.5a $\pm$ 1.6	1	63
	5 ml/cm	96	1898	19.8b $\pm$ 16.3	1	107
	Control	96	1996	20.8b $\pm$ 14.2	2	73
Strážske	3 ml/cm	96	127	1.3a $\pm$ 2.5	0	19
	5 ml/cm	108	193	1.8a $\pm$ 2.4	0	12
	Control	120	197	1.6a $\pm$ 2.1	0	11

N – no. of leaves; SD – standard deviation; averages with different letters differ significantly at a  $P \leq 0.05$  level.

The following season, in 2018, two assessments of the health of the trees were carried out at Parchovany, one on July 23 and second on September 10. The same method of damage assessment was used as in 2017. The Strážske plot was not included in the 2018 assessment, because trees were unexpectedly treated with another insecticide by the city government.

### Statistical analysis

For analyses, nonparametric statistical Kruskal-Wallis tests and pairwise multiple comparisons of mean ranks for particular  $p$ -values were carried out using STATISTICA 10 (StatSoft).

## RESULTS

### Assessment of pre-treatment trees

At Parchovany there was only an average of 0.9 ( $\pm 1.62$  SD) eggs per leaf (103 eggs on 119 leaves) and at Strážske, 0.6 ( $\pm 1.59$  SD) eggs per leaf (69 eggs on 118 leaves). These results indicate that treatment occurred at the beginning of the oviposition period of the pest. There was no significant difference in the density of eggs laid on trees at both plots (Parchovany:  $p = 0.9698$  and Strážske:  $p = 0.5280$ ). There was a significantly lower egg density ( $p = 0.0532$ ) of *C. ohridella* at Strážske than at Parchovany.

### Assessment of post-treatment damage

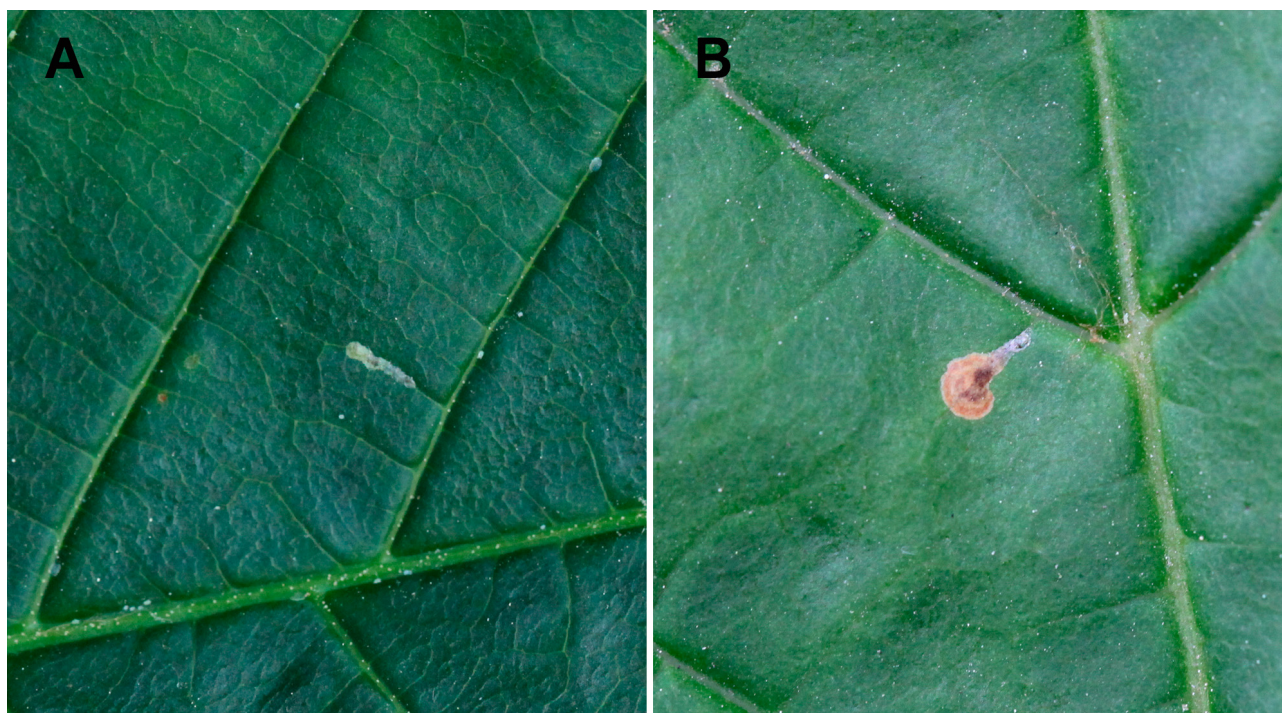
On the first day sampled in the year of treatment (May 16, 2017), we counted the number of larval galleries caused by the youngest, first instar larvae. Most of them look like a simple 2 mm long tunnel or a 1.5–2.0 mm patch at the end of short petiole (Fig. 3). They were very frequent at Parchovany, showing that infestation by *C. ohridella* at this locality was very high, whereas at Strážske it was significantly lower (Table 2).

The level of leaf damage recorded on treated and untreated trees on all the collection dates (July–September) were

Table 3. Average percentage leaf damage with standard deviation recorded at Parchovany and Strážske in 2017.

Plot	Dose	N	Average leaf damage (%)							
			July 11		August 2		August 30		September 20	
			Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Parchovany	3 ml/cm	96	6.5 a	10.2	22.9 a	32.4	15.9 a	26.4	24.5 a	33.5
	5 ml/cm	96	5.4 a	10.6	29.6 a	35.7	15.3 a	26.0	13.5 a	21.9
	Control	96	25.9 b	13.4	87.5 b	8.3	95.0 b	0.4	94.3 b	8.2
Strážske	3 ml/cm	96	0.8 a	0.8	2.0 a	5.1	6.5 a	11.2	4.2 a	6.7
	5 ml/cm	108	1.3 a	1.8	4.7 a	9.0	7.5 a	10.2	6.7 a	12.5
	Control	120	7.7 b	6.9	24.7 b	21.1	39.2 b	30.0	75.5 b	31.5

N – no. of leaves; SD – standard deviation; averages with a different letter differ significantly at  $P \leq 0.05$ .



**Fig. 3.** Photographs of the galleries in the leaves produced by the first instar larvae of the first generation of *C. ohridella*. They consist initially of a 2 mm long tunnel (left) and then a 1.5–2 mm patch (right) a bit later. In addition, the overall health of the crown (decolourisation and defoliation) was ranked as a percentage of crown damaged at each of the cardinal points for all trees (four data points per tree).

significantly different for both plots in 2017. Average leaf damage was significantly higher on untreated than treated trees. There was no significant differences in the incidence of damaged leaves between trees treated with applications of 3 ml and 5 ml of the pesticide (Table 3).

Overall crown damage increased significantly from the first to the final assessment at both localities in 2017 (Table 4). The difference in the damage to the crowns of treated and untreated trees was statistically significant. There were no significant differences in the results for the 3 ml and 5 ml applications. Variability in the estimates of overall crown damage was not as high as that for damaged leaves.

At the second damage assessment on August 2 an unequal distribution of TreeAzin® to certain parts of the crowns was noticed, with some branches significantly more damaged by *C. ohridella* than others. This was expected due to the age and size of the trees included in this study, in which there may have been areas of dead wood or other vascular damage unknown to us at the time of treatment as

they were not visible on the exterior of the tree; this may have caused the high variability in the measurements of leaf damage recorded on treated trees (Table 3).

At Strážske, the damage caused by *C. ohridella* was lower than at Parchovany as discussed previously. But at both Parchovany and Strážske, significant differences were recorded between treated and untreated trees. There were no significant differences in the incidence of damaged leaves on trees treated with the two doses of the pesticide.

The difference in the damage to the crowns of treated and untreated trees was also clearly visible in the field (Figs 4 and 5). From the middle of the season, leaves on untreated trees were all dry and brown, while those of treated trees were still green; except for some branches that were slightly infested. There was a second flowering and dieback of branches caused by defoliation of the untreated trees.

In the year following the application of TreeAzin®, the trees at Strážske were unexpectedly treated by the local community with a pesticide, so the results for that site in 2018 were not included in the analysis. At Parchovany,

**Table 4.** Average percentage damage to the crowns plus standard deviation of trees at Parchovany and Strážske in 2017.

Plot	Dose	N	Average crown damage (%)							
			July 11		August 2		August 30		September 20	
			Avg.	SD	Avg.	SD	Avg.	SD	Avg.	SD
Parchovany	3 ml/cm	32	4.8 a	3.5	19.9 a	15.3	28.0 a	18.0	31.6 a	17.8
	5 ml/cm	32	5.8 a	5.0	17.8 a	15.9	25.2 a	17.4	28.3 a	17.9
	Control	32	25 b	6.8	88.6 b	2.6	95.0 b	0.4	95.0 b	0.0
Strážske	3 ml/cm	32	0.8 a	1.1	2.3 a	2.1	14.5 a	9.2	19.5 a	8.0
	5 ml/cm	36	1.1 a	1.8	3.9 a	5.0	13.5 a	9.2	19.2 a	8.9
	Control	40	10.3 b	8.0	25.8 b	18.3	48.4 b	27.6	81.2 b	21.4

N – no. of records; SD – standard deviation; averaged with different letters differ significantly at  $P \leq 0.05$ .





**Fig. 4.** Photograph showing mid-season (August 2) crown of an almost completely defoliated untreated trees (right), which is mainly brown with just a few leaves undamaged by *C. ohridella* larvae. Treated trees (left) are still green at this time, with only a few branches attacked by the pest.

leaves from treated trees were significantly less damaged than those from untreated trees in 2018. For both treatments the damage to the crowns of treated trees was significantly less than that of untreated trees. The average leaf damage recorded for trees treated with 3 ml/cm DBH and 5 ml/cm DBH was nearly the same and did not differ significantly (Table 5).

No significant differences were recorded in average leaf and crown damage recorded for the trees treated with 3 ml/cm and 5 ml/cm DBH, which indicates that 3 ml/cm DBH is an acceptable minimum effective concentration of this pesticide for managing this pest. This is also the more cost-effective dose. However, in some cases, better results were recorded for trees treated with 5 ml/cm DBH, although the

differences were not statistically significant (Tables 3 and 5).

## DISCUSSION

Microinjection has been used several times in the past with relatively positive results in Central Europe. In 1997, the systemic insecticide *imidacloprid* was tested against *C. ohridella*. However, good results were obtained in preventing defoliation caused by 2<sup>nd</sup> and 3<sup>rd</sup> generations when applied in July (Feemers, 1997). Krehan (1997), which indicates a much earlier application in April, would prevent defoliation throughout the vegetative season. In 1999, *abamectin* was used in Hungary with good results (Bürgés & Szidonya, 2001) and later on microinjection was used

**Table 5.** Average leaf and crown damage plus standard deviations at Parchovany in 2018.

Plot	Dose	July 23		September 10	
		Average leaf damage (%) ± SD	Average crown damage (%) ± SD	Average leaf damage (%) ± SD	Average crown damage (%) ± SD
Parchovany	3 ml/cm	12.6 a ± 9.0	17.0 a ± 7.6	46.8 a ± 30.5	59.8 a ± 16.4
Parchovany	5 ml/cm	12 a ± 9.2	15.4 a ± 5.6	40.4 a ± 35.4	49.7 a ± 18.4
Parchovany	Control	20.6 b ± 9.9	24.5 b ± 8.3	67.9 b ± 26.6	78.8 b ± 17.0

N – no. of leaves; N<sub>of leaf damage %</sub> = 96, N<sub>of average crown damage %</sub> = 32, SD – standard deviation; averages with different letters differ significantly at  $P \leq 0.05$ .



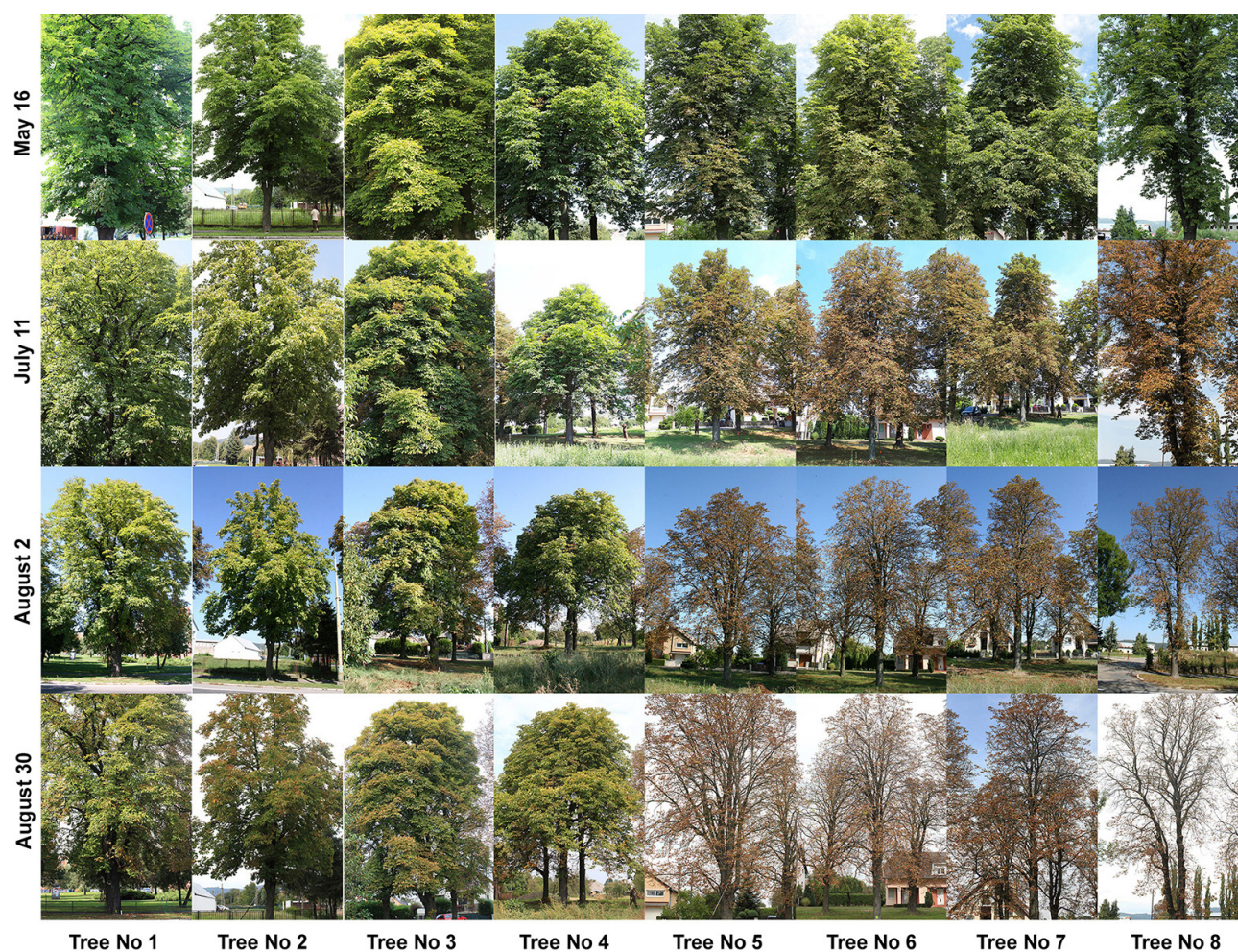


Fig. 5. Photographs showing the progress of defoliation that occurred in 2017 of four treated (No. 1–4) and four untreated (No. 5–8) trees.

to combat *C. ohridella* with mean efficiency of control of between 50% and 95% (Labanowski & Soika, 2003; Pavela & Bárnét, 2005; Ferracini & Alma, 2008; Kobza et al., 2011).

The biological efficacy of the systemic insecticide (in our case, TreeAzin®) was very good in the first year of treatment. At both localities, Parchovany and Strážske, there were significant differences in the average leaf damage caused by *C. ohridella* on treated compared to untreated trees. At the end of the first growing season, 95.0% of the crowns of the control trees were damaged at Parchovany (81.2% at Strážske) and only 19.2–31.6% of the treated trees were defoliated. There was no difference in the average leaf and crown damage recorded for trees treated with 3 ml/cm DBH and 5 ml/cm DBH. In the year of application, the crowns of all the treated trees were statistically and visually in much better condition than those of untreated trees.

Despite a good effectiveness confirmed by the results for some treated trees, sometimes individual branches were infested with *C. ohridella*. A possible reason for this non-uniformity in crown protection is an unequal distribution of TreeAzin® to all branches, which may be associated with the age and overall health of the treated trees.

Like other authors (Pavela & Bárnét, 2005; Kobza et al., 2011), we confirmed that trees older than 100 years can be effectively protected by this pesticide. This is important as many rare old trees growing in parks and gardens have high cultural and aesthetical value and are worth saving. In our experiments, injected trees were not fully protected even though they appeared to be healthy. As translocation often occurs faster in young, healthy trees (Bennett, 1957; Cox et al., 1997, 1998), we assume TreeAzin would more evenly protect the crowns of young trees.

Our results confirm the good efficiency of neem-based systemic insecticide when applied in April. This confirms the need for an early application proposed previously by other authors (Feemers, 1997, excepted), in order to target insects before the swarming period of *C. ohridella* (Krehan, 1997; Pavela & Bárnét, 2005; Ferracini & Alma, 2008; Kobza et al., 2011).

Common criticisms of injecting systemic insecticides include concerns over the potential for: (a) lack of uniform uptake, (b) slow application and (c) wounding of the injected areas (Krehan, 1997). In this trial there was evidence of unequal uptake in some of canopies of treated trees. Despite the overall efficacy, some branches of treated trees were infested with *C. ohridella*. In our study, there were

no concerns about the speed of application, which was viewed as adequate. It was noted that although the speed was dependent on the time of day (e.g. quicker uptake in the morning), weather, or overall health of the tree (as previously demonstrated by Ferracini & Alma, 2008) it was not a slow or onerous process. During a large-scale experiment in 2006 and 2007 involving *abamectin* (VIVID®II) and horse chestnut no phytotoxicity was reported (Pavela & Bárnét, 2005; Juhásová et al., 2007). Scar tissue formed around the shallow holes, which were enclosed in the next growing season. Not all authors experience only positive results (Krehan, 1997; Ferracini & Alma, 2008) and some of them highlight that the influence of the injection (tree's reaction to drilling) on its health should be investigated more deeply (Krehan, 1997). We did not test this aspect of the application method used (injection) as it was not an aim of the study. As far as we can tell by watching the response of trees to drilling, most of the wounds had healed by the end of the first year, or the beginning of the second. However, roughly 10–20% of the injection sites did not produce sufficient callus around the wound, where the tissues appeared to be dead. We believe that healing may be closely related to the sharpness of the drill bit, as dull bits may cauterize holes, preventing healing, but this topic was not investigated here. These factors should be considered, and the injection of trees and its influence on their health should be evaluated more precisely in the future.

## CONCLUSIONS

Our results indicate that the formulation of TreeAzin® tested protects trees for at least two-years. It is likely that the damage caused by microinjection is outweighed by the reduction in harm caused to these trees by *C. ohridella*; and using a biologically based insecticide can provide more benefit to the trees than harm. Indeed, injecting trees reduces the need for spraying the foliage, thereby preventing run-off and spray drift, which may have severe consequences for non-target organisms. On trees treated the previous year the average leaf damage was higher, but still statistically significantly lower than on untreated trees. This is the first report of a two-year efficacy. Trees were only evaluated in the year of injection in other studies (Pavela & Bárnét, 2005; Juhásová et al., 2007; Ferracini & Alma, 2008). In addition, what was encouraging was that this efficacy against *C. ohridella* occurred in very large trees (> 97 cm DBH), which indicates that ancient trees can be protected from attack by this pest.

We demonstrated that systemic insecticide TreeAzin® can be used to protect horse chestnut trees against *C. ohridella*. Microinjection has several advantages over traditional chemical methods. For example, small volumes of insecticide are administered, one treatment may protect trees for two years, application is almost independent of weather conditions, it is easy to do, environmentally friendly and precisely targeted (Juhásová et al., 2007; Kobza et al., 2011). Decreasing the damage caused by *C. ohridella* using imidacloprid results in a fast and

long-lasting positive effect on the trees' condition in terms of growth (Jagiello et al., 2019). Along with these advantages, there are also some open questions, especially the side effect of drilling on the health of the trees. The wound response following trunk injection of green ash (*Fraxinus pennsylvanica* Marsh.) has been studied over a period of two years by sectioning tree trunks and collecting data on annual radial growth and rate of healing around injection sites. This revealed that wound closure was positively correlated with tree health measured in terms of annual radial growth (Doccola et al., 2011). This finding supports earlier research indicating minimal damage and effective compartmentalization by trees when wounded by microinjection, particularly when compared with the wounding caused by increment borers (Shigo et al., 1977). Thus, it will be prudent to investigate how European horse-chestnut (*Aesculus hippocastanum*) responds to the wounding associated with trunk injections of insecticide.

Azadirachtin is an important natural pesticide and an alternative to conventional insecticides. It has been successfully used against many insect pests. However, as with any broad-spectrum insecticide, it is not without risk to non-target insects (Oulhaci et al., 2018). For example, azadirachtin is slightly to moderately toxic for honeybees although it did not appear to limit their foraging behaviour and is much less toxic than Imidacloprid, which is also often injected into trees (Challa et al., 2019). There is nearly no negative (lethal) effects of azadirachtin on stingless species of bees (Tomé, et al., 2015). Azadirachtin may induce a significant antifeeding effect or a range of sublethal effects on some stingless species of bees, such as, *Bombus terrestris* or other useful insects. (Barbosa et al., 2015; Gontijo et al., 2015; Bernardes et al., 2017); however, as the risks are minor, azadirachtin is still recommended for use in IPM (Challa et al., 2019). We recommend injecting azadirachtin after flowering in order to limit exposure to spring pollinators. TreeAzin injections pose very little risk to non-target soil-dwelling insects, as there are no residues in the leaves at abscission (Grimault et al., 2011). Soil microbial communities are also not affected by azadirachtin tree injections (Kizilkaya et al., 2012; Suciu et al., 2019). As the active ingredient targets hormones specific to insect moulting, other animals are likely to experience little to no direct negative effects due to azadirachtin. The present findings indicate that TreeAzin is a relatively safe pesticide with very low environmental risk and toxicity.

**ACKNOWLEDGEMENTS.** We are grateful to anonymous reviewers for their constructive comments on earlier drafts of this manuscript. We also thank D. Fournier (Canada) for linguistic and editorial improvements. This work was funded by the Slovak Research and Development Agency under contract No. APVV-16-0031, APVV-19-0116, APVV-19-0119 and the Ministry of Agriculture and Rural Development of the Slovak Republic under contract No. 08V0301 Research and Development for Innovation and Support of the Competitiveness of Forestry. In addition, this work was also funded by the Ministry of Defence of the Slovak Republic.



## REFERENCES

- ADAM T. 1997: Fodder, wood, coffee substitute – or ‘booty for little boys’? The economic utilization of horse chestnut in the 18th and 19th century. — *Forst Holz* **52**: 309–311.
- AVTZIS N. & AVTZIS D. 2003: *Cameraria ohridella* Deschka & Dimić (Lep.: Gracillariidae): A new pest on *Aesculus hippocastanum* in Greece. — *Mitt. Biol. Bundesanst. Land-Forstwirtschaft* **394**: 199–202.
- BARBOSA W.F., DE MEYER L., GUEDES R.N.C. & SMAGGHE G. 2015: Lethal and sublethal effects of azadirachtin on the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). — *Ecotoxicology* **24**: 130–142.
- BENNETT S.H. 1957: The behaviour of systemic insecticides applied to plants. — *Annu. Rev. Entomol.* **2**: 279–296.
- BERNARDES R.C., TOMÉ H.V.V., BARBOSA W., GUEDES R.N.C. & LIMA M.A.P. 2017: Azadirachtin-induced antifeeding in Neotropical stingless bees. — *Apidologie* **44**: 275–285.
- BIOFOREST TECHNOLOGIES INC. 2004: *Control of the Forest Tent Caterpillar with Stem Injections of Azadirachtin in Trembling Aspen in Saskatchewan. 2003. Internal Research Report*. Bioforest Technologies INC, Sault Ste. Marie, Canada, 2 pp.
- BIOFOREST TECHNOLOGIES INC. 2005a: *Efficacy Report for the Control of Gypsy Moth (Lymantria dispar) on Northern Pin Oak (Quercus ellipsoidalis E.J. Hill) and Bur Oak (Quercus macrocarpa Michx.) Using the Neem Based Bio-Insecticide, TreeAzin, in Oconto County, Wisconsin. Internal Research Report*. Bioforest Technologies INC, Sault Ste. Marie, Canada, 5 pp.
- BIOFOREST TECHNOLOGIES INC. 2005b: *Efficacy Report for the Control of Jack Pine Budworm (Choristoneura pinus pinus Freeman) on Jack Pine (Pinus banksiana) Using the Neem Based Bio-Insecticide, TreeAzin, in Bemidji Minnesota. Internal Research Report*. Bioforest Technologies INC, Sault Ste. Marie, Canada, 8 pp.
- BIOFOREST TECHNOLOGIES INC. 2005c: *Efficacy Report for the Control of Spruce Budworm (Choristoneura fumiferana Clemens) on White Spruce (Picea glauca). Internal Research Report*. Bioforest Technologies INC, Sault Ste. Marie, Canada, 4 pp.
- BLÜMEL S. & HAUSDORF H. 1996: Versuche zur Bekämpfung der Roßkastanienminiermotte. — *Gärtner Florist* **10**: 4–6.
- BURGÉS G. & SZIDONYA I. 2001: Injection of horse chestnut trees (*Aesculus hippocastanum*) against horse chestnut leafminer (*Cameraria ohridella*). — *Növényvédelem* **37**: 291–296 [in Hungarian].
- BUTIN H. & FÜHRER E. 1994: Die Kastanien-Miniermotte (*Cameraria ohridella* Deschka & Dimić), ein neuer Schädling an *Aesculus hippocastanum*. — *Nachr. Dt. Pflanzenschutzdienst* **46**: 89–91.
- CHALLA G.K., FIRAKE D.M. & BEHERE G.T. 2019: Bio-pesticide applications may impair the pollination services and survival of foragers of honey bee, *Apis cerana* Fabricius in oilseed brassica. — *Environ. Pollut.* **249**: 598–609.
- COX L., KOSKINEN W.C. & YEN P.Y. 1997: Sorption-desorption of imidacloprid and its metabolites in soils. — *J. Agric. Food Chem.* **45**: 1468–1472.
- COX L., KOSKINEN W.C. & YEN P.Y. 1998: Influence of soil properties on sorption-desorption of imidacloprid. — *J. Environ. Sci. Health (B)* **33**: 123–134.
- DAUTBAŠIĆ M. & DIMIĆ N. 1999: Occurrence of *Cameraria ohridella* Deschka & Dimić in Bosnia-Herzegovina. — *Radovi Šumarskog Fakult. Univ. Sarajevu* **1**: 11–14.
- DESCHKA G. & DIMIĆ N. 1986: *Cameraria ohridella* n. sp. aus Mazedonien, Jugoslawien (Lepidoptera, Lithocolletidae). — *Acta Entom. Jugosl.* **22**: 11–23.
- DIMIĆ N. & MIHAJLOVIĆ L. 1993: Expansion of the range of horse-chestnut leaf miner – *Cameraria ohridella* Deschka & Dimić (Lepidoptera, Lithocolletidae) and its natural enemies. In: *Collection of Summaries, XXI Meeting of Entomologists of Yugoslavia, 17–18.XI*. Entomological Society of Serbia, Belgrade, p. 32. [in Serbian].
- DIMIĆ N., DAUTBAŠIĆ M. & PERIĆ P. 2005: Host plants of *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera, Gracillariidae). — *Entomofauna* **26**: 193–204.
- DOCCOLA J.J., SMITLEY D.R., DAVIS T.W., AIKEN J.J. & WILD P.M. 2011: Tree wound responses following systemic insecticide trunk injection treatments in green ash (*Fraxinus pennsylvanica* Marsh.) as determined by destructive autopsy. — *Arboric. Urb. For.* **37**: 6–12.
- FEEMERS M. 1997: Versuche zur Bekämpfung von *Cameraria ohridella* Deschka & Dimić mittels Stamminjektion (Präparat: Confidor). — *Forstschutz Aktuell* **21**: 24–25.
- FERRACINI C. & ALMA A. 2007: Evaluation of the community of native eulophid parasitoids on *Cameraria ohridella* Deschka and Dimić in urban areas. — *Environ. Entomol.* **36**: 1147–1153.
- FERRACINI C. & ALMA A. 2008: How to preserve horse chestnut trees from *Cameraria ohridella* in the urban environment. — *Crop Prot.* **27**: 1251–1255.
- FREISE J.F., HEITLAND W. & STURM A. 2004: Das Wirtspflanzenspektrum der Roßkastanien-Miniermotte, *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae), einem Schädling der Roßkastanie, *Aesculus hippocastanum*. — *Mitt. Dt. Ges. Allg. Angew. Entomol.* **14**: 351–354.
- GILBERT M., SVATOŠ A., LEHMANN M. & BACHER S. 2003: Spatial patterns and infestation processes in the horse chestnut leafminer *Cameraria ohridella*: a tale of two cities. — *Entomol. Exp. Appl.* **107**: 25–37.
- GILBERT M., GREGOIRE J.C., FREISE J.F. & HEITLAND W. 2004: Long-distance dispersal and human population density allow the prediction of invasive patterns in the horse chestnut leafminer *Cameraria ohridella*. — *J. Anim. Ecol.* **73**: 459–468.
- GILBERT M., GUICHARD S., FREISE J., GRÉGOIRE J.-C., HEITLAND W., STRAW N., TILBURY C. & AUGUSTIN S. 2005: Forecasting *Cameraria ohridella* invasion dynamics in recently invaded countries: from validation to prediction. — *J. Appl. Ecol.* **45**: 805–813.
- GŁOWACKA B. 2005a: The effectiveness of diflubenzuron in the protection of the horse chestnut *Aesculus hippocastanum* L. against the horse chestnut leafminer *Cameraria ohridella* Deschka et Dimić. — *Sylvan* **12**: 12–20.
- GŁOWACKA B. 2005b: Removal of leaves colonized by horse chestnut leafminer *Cameraria ohridella* Deschka et Dimić as a method of protection of horse chestnut *Aesculus hippocastanum* L. — *Leśne Prace Badawcze* **2**: 139–141 [in Polish].
- GŁOWACKA B., LIPŃSKI S. & TARWACKI G. 2009: Possibilities of protection of horse chestnut *Aesculus hippocastanum* L. against horse chestnut leafminer *Cameraria ohridella* Deschka et Dimić. — *Leśne Prace Badawcze* **70**: 317–328 [in Polish].
- GONTIJO L.M., CELESTINO D., QUEIROZ O.S., GUEDES R.N.C. & PICAÑO M.C. 2015: Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae). — *Fla Entomol.* **98**: 59–64.
- GRIMALT S., THOMPSON D., CHARTRAND D., MCFARLANE J., HELLSON B., LYONS B. & SCARR T. 2011: Foliar residue dynamics of azadirachtins following direct stem injection into white and green ash trees for control of emerald ash borer. — *Pest Manag. Sci.* **67**: 1277–1284.
- HELLRIGL K. 1998: On the occurrence of the robinia-leafminer, *Phyllonorycter robiniella* (Clem.) and the horse-chestnut-leafminer, *Cameraria ohridella* Desch. et Dim. (Lep., Gracillariidae).

- lariidae) in South Tyrol. — *Anz. Schädlingssk. Pflanzen Umweltschutz* **71**: 65–68.
- HELLRIGL K. & AMBROSI P. 2000: Distribution of the horse-chestnut leafminer, *Cameraria ohridella* Desch. & Dimic (Lepid., Gracillariidae), in the region South Tyrol-Trentino (Northern Italy). — *Anz. Schädlingssk. Pflanzen Umweltschutz* **73**: 25–32.
- HRUBÍK P. & JUHÁSOVÁ G. 1998: Distribution and harmful activity of horse-chestnut leafminer – *Cameraria ohridella* (Deschka) Dimić in Slovakia. — *Acta Hort. Regiotect.* **2**: 21–23 [in Slovak].
- JAGIELLO R., WALCZAK U., ISZKULO G., KAROLEWSKI P., BARANIAK E. & GIERTYCH M.J. 2019: Impact of *Cameraria ohridella* on *Aesculus hippocastanum* growth and long-term effects of trunk injection with pesticides. — *Int. J. Pest. Manag.* **65**: 33–43.
- JUHÁSOVÁ G., HRUBÍK P., ŠAMAJOVÁ O., KULCSÁROVÁ K., IVANOVÁ H. & CHLADNÁ A. 1998: Outbreak of horse-chestnut leafminer – *Cameraria ohridella* (Deschka) Dimić in Slovakia. — *Folia Dendrol.* **24**: 171–179 [in Slovak].
- JUHÁSOVÁ G., KOBZA M. & ADAMČIKOVÁ K. 2007: Treatment of horse chestnut trees with microinjection technology. In Hudec K. & Roháčik T. (eds): *Proceedings of the Second Conference of the Slovak Plant Health Society, 21.–22. November 2007, Nitra*, pp. 70–72 [in Slovak].
- KEHRLI P. & BACHER S. 2003: Date of leaf litter removal to prevent emergence of *Cameraria ohridella* in the following spring. — *Entomol. Exp. Appl.* **107**: 159–162.
- KENIS M. & FORSTER B. 1998: Die Roskastanien-Miniermotte: neu in der Schweiz. — *Pflanzenschutz* **39**: 16–17.
- KENIS M., TOMOV R., SVATOS A., SCHLINSOG P., LOPEZ VAAMONDE C., HEITLAND W., GRABENWEGER G., GIRARDOZ S., FREISE J. & AVTZIS N. 2005: The horse-chestnut leaf miner in Europe – Prospects and constraints for biological control. In Hoddle M.S. (ed.): *Proceedings of the Second International Symposium on Biological Control of Arthropods, Davos, Switzerland, 12–16 September, 2005*. Forest Health Technology Enterprise Team, Morgantown, WV, pp. 77–90.
- KIZILKAYA R., AKCA I., ASKIN T., YILMAZ R., OLEKHOV V., SAMOFALOVA I. & MUDRYKH N. 2012: Effects of soil contamination with azadirachtin on dehydrogenase and catalase activity of soil. — *Eur. J. Soil Sci.* **2**: 98–103.
- KOBZA M., JUHÁSOVÁ G., ADAMČIKOVÁ K. & ONRUŠKOVÁ E. 2011: Tree injection in the management of horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae). — *Gesunde Pflanz.* **62**: 139–143.
- KREHAN H. 1997: Erste Erfahrungen mit Bauminfusionen gegen die Roßkastanienminiermotte. — *Forstschutz Aktuell* **21**: 26.
- KREUTZWEISER D., THOMPSON D., GRIMALT S., CHARTRAND D., GOOD K. & SCARR T. 2011: Environmental safety to decomposer invertebrates of azadirachtin (neem) as a systemic insecticide in trees to control emerald ash borer. — *Ecotoxicol. Environ. Saf.* **74**: 1734–1741.
- KUKULA-MLYNARCZYK A. & HUREJ M. 2007: Incidence, harmfulness and some elements of the horse chestnut leafminer (*Cameraria ohridella* Deschka & Dimić) control on white horse chestnut (*Aesculus hippocastanum* L.). — *J. Plant Protect. Res.* **47**: 53–64.
- LABANOWSKI G. & SOIKA G. 2003: Macro-injection system against the horse-chestnut leafminer (*Cameraria ohridella*) in Poland. — *Hortic. Veget. Grow.* **22**: 512–517.
- LIŠKA J. 1997: Verbreitung der Roskastanienminiermotte in der Tschechischen Republik. — *Forstschutz Aktuell* **21**: 5.
- MACELJSKI M. & BERTIĆ D. 1995: The horse chestnut miner *Cameraria ohridella* Deschka & Dimić – a new dangerous pest in Croatia. — *Fragmenta Phytomed. Herbol.* **23**: 9–18.
- MILEVOJ L. & MACEK J. 1997: Roskastanien-Miniermotte (*Cameraria ohridella*) in Slowenien. — *Nachr. Dt. Pflanzenschutzd.* **49**: 14–15.
- MORDUE (LUNTZ) A.J. & NISBET A.J. 2000: Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. — *An. Soc. Entomol. Bras.* **29**: 615–632.
- OEPP/EPPO 2012: Design and analysis of efficacy evaluation trials. — *OEPP/EPPO Bull.* **42**: 367–381.
- OULHACI C.M., DENIS B., KILANI-MORAKCHI S., SANDOZ J.-C., KAISER L., JOLY D. & ARIPI N. 2018: Azadirachtin effects on mating success, gametic abnormalities and progeny survival in *Drosophila melanogaster* (Diptera). — *Pest Manag. Sci.* **74**: 174–180.
- PAVAN F., BARRO P., BERNARDINELLI I., GAMBON N. & ZANDIGIACOMO P. 2003: Cultural control of *Cameraria ohridella* on horsechestnut in urban areas by removing fallen leaves in autumn. — *J. Arboricult.* **29**: 253–258.
- PAVELA R. & BÁRNET M. 2005: Systemic applications of neem in the control of *Cameraria ohridella*, a pest of horse chestnut (*Aesculus hippocastanum*). — *Phytoparasitica* **33**: 49–56.
- PERCIVAL G.C. 2016: Evaluation of insect barrier glue bands and liquidglue for the management of horse chestnut leaf miner (*Cameraria ohridella*). — *Arboric. J.* **38**: 134–142.
- PERCIVAL G.C., BARROW I., NOVISS K., KEARY I. & PENNINGTON P. 2011: The impact of horsechestnut leaf miner (*Cameraria ohridella* Deschka and Dimić) on vitality, growth and reproduction of *Aesculus hippocastanum* L. — *Urban For. Urban Green.* **10**: 11–17.
- PÉRÉ C., AUGUSTIN S., TURLINGS T.C.J. & KENIS M. 2010: The invasive alien leaf miner *Cameraria ohridella* and the native tree *Acer pseudoplatanus*: a fatal attraction? — *Agric. For. Entomol.* **12**: 151–159.
- PELOV V., TOMOV R. & TRENCEV G. 1993: *Cameraria ohridella* Deschka & Dimić (Gracillariidae, Lepidoptera) – a new pest of *Aesculus hippocastanum* L. in Bulgaria. In: *Proceedings of the National Conference of Forest Protection (Sofia, Bulgaria)*, pp. 95–98 [in Bulgarian, with English abstr.].
- PSCHORN-WALCHER H. 1994: Freiland-Biologie der eingeschleppten Roßkastanien-Miniermotte *Cameraria ohridella* Deschka and Dimić (Lep., Gracillariidae) im Wienerwald. — *Linzer Biol. Beitr.* **26**: 633–642.
- REMBOLD H. & SIEBER K.P. 1981: Inhibition of oogenesis and ovarian ecdysteroid synthesis by azadirachtin in *Locusta migratoria migratorioides* (R. & F.). — *Z. Naturforsch.* **36**: 466–469.
- SCHMUTTERER H. 1990: Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. — *Annu. Rev. Entomol.* **35**: 271–297.
- SHIGO L.A., WALTER E.M. & DALE I.D. 1977: Some internal effects of mauguet tree injections — *J. Arboric.* **3**: 213–220.
- SIVICEK P., HRUBÍK P. & JUHÁSOVÁ G. 1997: Verbreitung der Roskastanienminiermotte in der Slowakei. — *Forstschutz Aktuell* **21**: 6.
- STRAW N.A. & TILBURY C. 2006: Host plants of the horse-chestnut leaf-miner (*Cameraria ohridella*), and the rapid spread of the moth in the UK 2002–2005. — *Arboric. J.* **29**: 83–99.
- SUCIU N., VASILEIADIS S., PUGLISI E., PERTILE G., TOURNA M., KARAS P.A., PAPOLLA A., FERRARINI A., SULOWIC S., FORNASIER F., LUCINI L., KARPOUZAS D.G. & TREVI M. 2019: Azadirachtin and trifloxystrobin had no inhibitory effects on key soil microbial functions even at high dose rates. — *Appl. Soil Ecol.* **137**: 29–38.
- SUKOVATA L., CZOKAJLO D., KOLK A., SLUSARSKI S. & JABLONSKI T. 2011: An attempt to control *Cameraria ohridella* using an attract-and-kill technique. — *J. Pest Sci.* **84**: 207–212.



- SZABÓKY C. 1997: Verbreitung der Rosskastanienminiermotte in Ungarn. — *Forstschutz Aktuell* **21**: 4
- THALMANN C., FREISE J., HEITLAND W. & BACHER S. 2003: Effects of defoliation by horse chestnut leafminer (*Cameraria ohridella*) on reproduction in *Aesculus hippocastanum*. — *Trees* **17**: 383–388.
- TOMICZEK C. & KREHAN H. 1998: The horsechestnut leafmining moth (*Cameraria ohridella*): a new pest in Central Europe. — *J. Arboric.* **24**: 144–148.
- TOMÉ H.V.V., BARBOSA W.F., CORRÊA A.S., GONTIJO L.M., MARTINS G.F. & GUEDES R.N.C. 2015: Reduced-risk insecticides in Neotropical stingless bee species: impact on survival and activity. — *Ann. Appl. Biol.* **167**: 186–196.
- TÓTH P. & LUKÁŠ J. 2005: Parasitic Ichneumonoidea on the horse chestnut leaf miner, *Cameraria ohridella* (Lepidoptera: Gracillariidae) in Slovakia. — *J. Pest Sci.* **78**: 151–154.
- VALADE R., KENIS M., HERNANDEZ-LOPEZ A., AUGUSTIN S., MARI MENA N., MAGNOUX E., ROUGERIE R., LAKATOS F., ROQUES A. & LOPEZ-VAAMONDE C. 2009: Mitochondrial and microsatellite DNA markers reveal a Balkanic origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). — *Mol. Ecol.* **18**: 3458–3470.
- ZÚBRIK M., HELL P. & TURČÁNI M. 1999: A threat called horse-chestnut leaf miner. — *Pol'ovníctvo a rybárstvo* **2**: 14–15 [in Slovak].
- ZÚBRIK M., KUNCA A., TURČÁNI M., VAKULA J. & LEONTOVÝČ R. 2006: Invasive and quarantine pests in forest in Slovakia. — *EPPO Bull.* **36**: 402–408.

Received August 5, 2020; revised and accepted November 10, 2020  
Published online December 10, 2020