

Resistance of horse chestnut tree (variety ‘Mertelík’) to *Cameraria ohridella* Deschka & Dimić, 1986 (Lepidoptera: Gracillariidae)

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Abstract

Resistance of horse chestnut *Aesculus hippocastanum* ‘Mertelík’ to *Cameraria ohridella* was proven in the trial under semi-field conditions. Seedlings of vulnerable and resistant trees were grown together in rearing cages and were exposed to the strong attack from *C. ohridella* larvae. Significant differences in leaflet injuries were observed between susceptible and insect-resistant trees. Variety ‘Mertelík’ showed almost none or very small damages compared to high defoliation level in case of standard trees. The results confirmed that this resistant clone could be suitable for use under field conditions.

Key words: horse-chestnut leaf miner; resistant clones; defoliation; damages; *Aesculus hippocastanum*

Editor: György Csóka

1. Introduction

The invasive horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić 1986) (Lepidoptera: Gracillariidae) was described from specimens in areas around Lake Ohrid in Macedonia (Dimić & Mihajlović 1993). Since then, it has spread across Europe, causing significant aesthetic damage to the extensively planted horse chestnut trees (*Aesculus hippocastanum* L.) (Hellrigl & Ambrosi 2000; Prelov et al. 2006; Straw & Tilbury 2006). There are different opinions, why these new populations appeared in so many countries during such a short period (Zúbrik et al. 1999; Gilbert et al. 2003).

Horse chestnut (*A. hippocastanum* L.) is indigenous to a Balkan mixed forest (Southeast Europe) (Lack 2002). Planting of horse chestnut trees in Central Europe was very popular during the 18th and 19th centuries, when it was moved from Balkan Peninsula (Adam & Fodder 1997). Thanks to an attractiveness that lasted a whole year, its favourable effect on urban environment and some other positive characteristics, these trees are still frequently planted in Europe both within the cities and as well in forests (Prada et al. 2011; Thomas et al. 2019).

Infestation by *C. ohridella* causes considerable leaf damage to *A. hippocastanum* and significantly lower aesthetic value of the tree. Reducing photosynthetically active tissues results in lower carbon allocation, slowdown of the growth, and decreased leaf life span (Salleo et al. 2003; Takos et al. 2008). Although infestation does not face serious dieback risk, it can negatively affect production of seeds and can increase tree susceptibility to other pests and pathogens (Nardini et al. 2004; Johné et al. 2008).

There are a number of control options for *C. ohridella*, however, chemical means is the most commonly used method in urban and forest environments (Ferracini & Alma 2008; Głowacka et al. 2009). In this situation, developing trees that are resistant to specific harmful organisms may be good approach to integrated pest control (Jacobs 2007; Fraser et al. 2015).

The aim of the study is to state the resistance of the new cultivar was tested in a new way that simulated real conditions in the field end enabled accurate evaluation of this desired property.

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2. Material and methods

2.1. Insect

Leaves infested by *C. ohridella* were collected from intensively attacked standard cultivar of horse chestnut trees in an urban park located in Santovka (Slovakia, 48°09'18.6"N 18°45'45.6"E, 160 m a.s.l.) in autumn 2018. Leaves were raked into paper sacks, transported to the laboratories, and then 20 kg of leaf litter from these trees was put at the bottom of each rearing cage. So we had about 15 cm thick layer of leaves in every cage.

To determine the population density of *C. ohridella* in rearing cages, we took 18 samples of leaf litter (three from every cage), where each one weighted 0.5 kg. Samples were installed in photoelectors to see how many adults emerged from a sample. We did not find a significant difference between the numbers of hatched adults ($P < 0.005$).

2.2. Horse chestnut tree

For test purposes, we used 12 samples horse chestnut trees variety 'Mertelík' and 12 others with standard resistance. Trees were obtained from the Silva Tarouca Research Institute for Landscape and Ornamental Gardening (RILOG) in Pruhůnice, in Czech Republic. They were about 1 m high and well rooted in soil, potted in pots.

2.3. Experimental design

In our external laboratories at National Forest Centre – Forest Research Institute in Banská Štiavnica (Slovakia, 48°27'50.9"N 18°54'17.9"E, 600 m a.s.l.), we used six breeding cages for the experiment. Cages had dimensions of 1.5 m × 1.5 m × 2 m with concrete foundation and were situated under sheltered structure to ensure protection against external weather conditions (hail, heavy rain, etc.) (Fig. 1.).

In the spring 2019 (on April 23), when trees started to grow leaves, two 'Mertelík' and two standard trees were placed in each cage (six cages and four trees by cage = 24 trees). Every tree was linked up to a drip irrigation system.

Meteorological stations were installed near cages where daily temperature and air humidity were measured in height, 2 m above ground (Fig. 2).

2.4. Bioassay

On June 25 and September 6, assessments of leave damages caused by *C. ohridella* were undertaken, including percent of affected part in every leaflet of leaves on trees separately. Leaflets with less than 5% damage were considered as undamaged (0%). On these, it was possible to find rare signs of an attack, but larvae died at the initial stage of development, so only a small spot was observed (Fig. 3). Data about the leaflet damage by *C. ohridella* were statistically analysed with the use of Welch's t-test in Statistica 10 software.



Fig. 1. Rearing cages used for experiments.

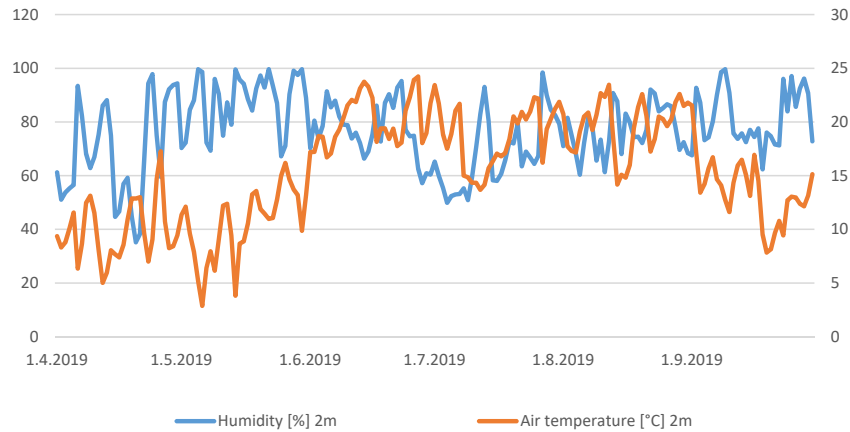


Fig. 2. Air temperature (°C) and humidity (%) near cages, from April 1 to September 30, 2019, measured daily.

3. Results

Population density of *C. ohridella* was determined from samples placed in photoelectors. From that on average, 124.83 ± 6.35 SE adult moths emerged. According to this data, we were expecting about $4,618.71 \pm 254.12$ SE adults of *C. ohridella* in every cage. This represents density of approximately 21 adults per 1 dm^2 .

The first assessment was undertaken in 2019 on June 25. Variety ‘Mertelik’ was significantly ($p\text{-level} = 0.00$) less damaged by *C. ohridella* than in normal trees. Average damage in standard trees was at 45.84 ± 1.02 SE

per leaflet. We found leaflets without any sign of infestation, but also others totally damaged by *C. ohridella*. Most often leaflets were affected at 20% (103 of 618). On variety ‘Mertelik’, they were damaged at only $1.45\% \pm 0.13$ SE on average. Most leaflets were not having any sign of damage (505 of 677); the maximum we noticed was at 30% (Table 1).

The tree mostly affected from variety ‘Mertelik’ had some damage on leaflets at 3.98% on average and the least had only at 0.38%. The standard tree being the most damaged had an average value of 61.52% for its leaflets and the lowest had 31.02%.



Fig. 3. Four trees were placed into six cages, two were resistant and two were normal (control trees).



Fig. 4. Comparison of leaf damage caused by *C. ohridella* on resistant and normal trees under the same conditions.

Table 1. Basic statistical data analysis about leaflet damage caused by *C. ohridella* after the first assessment (on June 25).

June	N	Average	SE	Median	Mode	N Mode	Minimum	Maximum
Standard	618	45.83981	1.020623	40.00000	20.00000	103	0.00	100.0000
'Mertelik'	677	1.449040	0.126725	0.00	0.000000	505	0.00	30.00000

On September 6 (2019), the second assessment was undertaken. One cage (four trees) was excluded because drip irrigation in it was broken and trees were affected by drought. So only 20 (10 specimens belonging to variety 'Mertelik' and 10 standard trees) were assessed in September. Leaflets from 'Mertelik' trees were more damaged than in June, but still significantly less than from standard trees (p -level = 0.00). Leaflet damage on variety 'Mertelik' increase to 9.54 ± 0.63 SE on average. Midpoint was 5%, but most often leaflets were undamaged (270 of 581). In one tree, we also found two leaves (10 leaflets) where this last structure was affected from 70% to 100%. Average damage on leaflets of the standard trees has increased to 67.87 ± 1.59 SE. Most leaflets were damaged at 100% (121 of 406) and midpoint of damage was 80%.

The leaflet damage was at 17.6% on average in most affected 'Mertelik' trees and on those that were the least was at 3.4%. The highest average damage in a standard tree was at 97.89% and lowest at 43.66%.

4. Discussion

The variety 'Mertelik' of the horse chestnut tree is a patented genotype for classical *A. hippocastanum* with claimed cases of behavioural resistance to *C. ohridella*, in the form of a reduced leaf miner due to early larval mortality. Our aim was to test the resistance of *A. hippocastanum* var. 'Mertelik' to *C. ohridella* under controlled, semi-field conditions.

The impact of *C. ohridella* can be important, not only for particular trees, but also for associated biodiversity and whole ecosystems. Usually, during May and June under conditions in Central Europe, first generation larval feeding of *C. ohridella* results in leaf browning, drying and defoliation (Snieskienė et al. 2011; Weryszko-Chmielewska & Haratym 2011). These changes are easily noticeable by public and reduce aesthetic value of trees (Pocock & Evans 2014). Early defoliation caused by *C. ohridella* also has other negative effects, which are more important, namely the degree of leaf damage on

Table 2. Basic statistical data analysis about leaflet damage caused by *C. ohridella* after the second assessment (on September 6).

September	N	Average	SE	Median	Mode	N Mode	Minimum	Maximum
Standard	406	67.78325	1.589731	80.00000	100.0000	121	0.00	100.0000
'Mertelik'	581	9.542169	0.629569	5.000000	0.000000	270	0.00	100.0000

photosynthetic productivity, as measured by the reduction in net primary production, degradation of chloroplasts, and decrease in leaf conductance and transpiration to water vapour (Raimondo et al. 2003; Konarska et al. 2020). Seeds from affected trees were characterised by lower germinability, and seedlings were shorter and less vigorous than those from non-infested plants (Thalman et al. 2003; Percival et al. 2011). Leaf loss also leads to lower frost resistance of trees (Balder et al. 2004), and other harmful abiotic and biotic factors. However, widespread dieback of the horse chestnut tree (Butin & Führer 1994; Kenis & Forster 1998) has so far not been observed.

Removing dead leaves, in which pupae overwinter, and burning or composting them, remains most environmentally friendly method recommended by many authors (Kehrli & Bacher 2003; Pavan et al. 2003). Classical biological control against *C. ohridella* has some potential too, but natural enemy spectrum of this pest is rather small and not very effective (Kenis et al. 2005; Tóth & Lukáš 2005). Microinjection technology in stem cell research has also a possibility, however, the side effects of drilling are still not fully investigated (Feemers 1997; Ferracini & Alma 2008; Gubka et al. 2020).

There are already some examples in the industry from fruit or ornamental trees when different health problems were solved with disease-resistant plants or insect-resistant cultivars. Cultivar trees with complete or partial resistance are available on the market, for instance the apple and crabapple varieties resistant to apple scab, fire blight or powdery mildew (Thurn et al. 2018), prunes resistant to *Plum pox virus* (Zuriaga et al. 2018), gooseberry cultivars resistant to American gooseberry mildew (Beyer & Roser 1989), disease-resistant roses, etc. Cultivars resistant to insect pests exist as well, such as the Japanese beetle (Thurn et al. 2018). Also in forest protection, there are a few examples; disease-resistant cultivars could be an effective and ecologically acceptable method. We can speak, for instance, about elm trees resistant to Dutch elm disease (Santini et al. 2010) or naturally-occurring ash trees that possess substantial resistance to *Chalara fraxinea* = *Hymenoscyphus fraxineus* (McKinney et al. 2011; Kunca et al. 2013).

In 1998, a mature horse chestnut tree (with working designation HZR1357) with a visible natural resistance to *C. ohridella* was found in East Bohemia. Small, elongated, atypical uneven mines were put together on leaves and sometime with typical larger mines, but the larval development in either was not completed. Typical mines with a complete development were found only sporadically in comparison with heavily attacked trees in surrounding areas (Mertelík et al. 2004a). The scions of HZR1357 were grafted on rootstocks in order to obtain the plant cloning material for experiments. Grafted plants of HZR1357 were tested under conditions of artificial infestation in a greenhouse chamber, and under natural conditions of field infestation in fully grown horse-

chestnut trees in Pruhůnice Park. The resistant behaviour and its stability were verified during the period of 2001–2003. In 2006, the clone HZR1357 with a high level of behavioural resistance to the leaf miner *C. ohridella* was patented as a new genotype of horse chestnut named ‘Mertelík06’ (M06) (Mertelík & Kloudová 2006). Evaluation of ‘Mertelík06’ resistance continued and in 2010, the uniformed plant material from this unique genotype was listed in the Czech Republic as national plant variety called ‘Mertelík’ (Mertelík & Kloudová 2010).

5. Conclusions

We found significant difference in damage caused by *C. ohridella* between standard tree and those of variety ‘Mertelík’. Similar results with this clone were gathered by other authors (Mertelík et al. 2004b; Mertelík & Kloudová 2012). Results from this study confirmed that using of resistant horse-chestnut variety ‘Mertelík’ should be a good addition to protective measures against *C. ohridella*.

Acknowledgements

The authors thank to Dominique Fournier (Canada) for linguistic and editorial improvements. This work received support from the Slovak Research and Development Agency under the contracts No. APVV-19-0116, APVV-19-0119 and APVV-21-0131, from the Ministry of Agriculture and Rural Development of the Slovak Republic based on the item No. 08V0301, from the Ministry of Defence of the Slovak Republic via the Program Integrated Infrastructure for the project FOMON, No. 313011V465 and from the Center of Excellence of Forest-based Industry – LignoSilva (ITMS code: 313011S735), co-financed by the European Regional Development Fund.

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