

USING THE TECHNIQUE OF VEGETAL ENDOTERAPY AGAINST THE HORSE CHESTNUT'S LEAF MINER (LEPIDOPTERA: CAMERARIA OHRIDELLA DESCHKA & DIMIE)

Margit PÁL, János BÁLINT, Adalbert BALOG

Sapientia University, Faculty of Technical and Human Sciences, Department of Horticulture,
I/C Sighișoarei, Târgu Mureș, Romania Tel/Fax: 004-0265-206211,
E-mail: margitpal62@gmail.com, balintjanos@ms.sapientia.ro

Corresponding author email: margitpal62@gmail.com

Abstract

Described for first in 1986 near Ohrid lake from Macedonia, by Deschka & Dimie, the horse chestnuts leaf miner (*Cameraria ohridella*) a highly invasive moth, in just two decades has spread all over the continent, and caused considerable damages in horse chestnuts populations across Europe. In Romania has been described in 1993. In our climatic conditions develops 3-4 generations per year. Adult moths are transported by wind, anthropogenic transport by vehicles in infested leaf fragments or infested nursery stocks. The most important damage is esthetical, because of fallen foliage but also ecological do to the effect of the reduced active surface of photosynthesis, and progressive decline of trees. Also causes economic losses due to the costs of protect or heal the affected trees. As a consequence different techniques have been adopted in the recent years to control the moth like collection and destruction of fallen leaves where the pupae over-winter or spray of systemic insecticides. They native natural enemies are insufficiently adapted and not able to regulate the leaf miner's population density, even after two decades of moth infestation typical parasitism rates are merely around 0-10%. The chemical control is difficult especially through the application of insecticides; the aerial spraying to treat trees in urban areas requires specialized expensive equipment. Moreover the systemic insecticides may cause side effects on non-target species. The endoterapy technique by introducing pesticides directly inside the trees can be the best solution. Microinjection is a type of trunk injection where small amounts of therapeutic chemicals are introduced directly into the trees body without any contact with the environment. During our experiment a total number of 84 chestnut trees were identified from which 38 were treated with two different active chemical agents: abamectin and imidacloprid. Treatment applications were made in May 2011. A plot of 46 trees where used as control without treatment. The analysis of the collected results shows, that the treated trees of up to 90% are protected with endoterapy against horse chestnuts leaf miner (*Cameraria ohridella*).

Key words: vegetal endoterapy, pest management, invasive species, horse chestnuts leaf miner

INTRODUCTION

Belonging to the Gracillariidae family, the horse chestnut leaf miner *Cameraria ohridella* was observed and described for the first in 1984, Macedonia, near lake Ohrid (Deschka and Dimic', 1986). In 1986 it was observed only in a few places in the southern Balkans, but in a few years later it spread throughout Western Europe causing major damage to the horse chestnut tree populations (Pschorn-Walcher, 1997).

In 2000 appeared in Central Europe, Austria and France, in 2003 was reported from Spain and Denmark while in 2004 from England, Ukraine, Sweden, and Lithuania, and was expected its spread to the border of the east coast of the Scandinavian countries, where the

climatic factors provide favorable conditions for the development of at least one generation per year (Augustin et al., 2004). Natural spreading speed of the species is high, at least 11.8 km per year, but can reach even 37.9 kilometers per year (Augustin and Denux, 2009). In just two decades it was present all around Europe, approaching the necessary living conditions in northern limit. Knowledge of the mechanism of spreading is very important considering the high ability of infestation (Baraniak et al., 2005).

Adult moths are transported by wind, anthropogenic transport by vehicles. Transport of infested leaf fragments or infested nursery stocks can occur frequently.

Adult females attract males with sexual pheromones. In 2002 a Czech team identified

the composition of the leaf miner pheromone as (8E, 10Z) -8,10 - tetradecadienal (8E10Z - 14Al), and first produced a synthetic sample (Franke et al., 2002).

The electron microscopic histological studies identified and described the female moth pheromone glands internal and external morphology. The gland is a modified epidermal cell line, on the membrane surface between the eighth and ninth abdominal sections, without pores. In retracted condition disappears into the body cavity between the two sections.

With the elongation of the abdominal part reveals and facilitate the evaporation of the pheromone molecules (Rasputnig et al., 2003).

Considering the number of generations per year it was observed that under Mediterranean climatic conditions develops more generations than in colder climates. Swarming can be observed almost the entire summer with first peaks in May.

The second generation appears in July; with significantly increase of the number of individuals. The third generation occurs in August-September (Czencz and Burges, 1996). The horse chestnuts leaf miner is a daily active, morning and early afternoon flying moth, most active from 20 to 24 ° C (Syryebryennikov, 2008). A female lays 20-80 eggs hatching depending on season and the climate after 4 to 21 days.

The emerging little larva is a flat, white legless larva, which penetrate inside of the leaf blade. The whole developmental period is 20 - 40 days depending on the weather conditions, during this time the larvae lives inside the mine, feed with the leaf blade palisade parenchyma tissue (Sefrova, 2001). The fourth larval stage, cylindrical -bodied larvae pupate in the original mine, that expands along the main veins, up to 4-7 cm under the development.

The final, third, or fourth generation pupa overwinters in fallen leaves.

The population densities annually changes and the annual mortality of larvae beside of temperature is also affected by the intraspecific competition of the last generation (Girardoz et al., 2007a), (Girardoz et al., 2007b).

The horse chestnuts leaf miner damage first visible effect is aesthetic. The park trees ornamental value is seriously reduced because

of countless mines. The trees lose a significant part of leaves reducing the efficiency of internal water balance.

Beside of reduction of the decorative value, the trees are not able to perform its function with the reduced foliage, trees growth slows down the weight and number of fruits decreases gradually. The photosynthetic activity decrease, and is negatively affected their air cleaning ability (Binimelis et al., 2007). The weakened trees die after a few years.

The continuous infection decrease up to 30% of tree life (Salleo et al., 2003). To avoid this, each control option must be used in order to preserve and ensure the survival of the trees, especially in urban areas (Straw and Tilbury, 2006).

Economic impact is also not negligible, the damage reduction requires immediate attention, but the treatment of infected trees is difficult, time - consuming and expensive.

The defense is complicated due the large damaged surface, the great number of mines, where other agents can penetrate easily, so, the leaf becomes over infected with *Guignardia* leaf spot (*Guignardia aesculi*) (Gherghel and Batin, 2008).

Complete removal of leaf litter, in which pupae hibernate, is one effective measure to reduce the damage.

The mechanical control by collecting and destruction of litter, in the autumn, must be supplemented by other measures during all growing seasons.

One of these methods is chemical control, especially in forest stands, where the mechanical method is inefficient, or difficult to be applied (Fora, 2011).

Aerial spraying with systemic insecticide is efficient, but is difficult in urban areas, beside may cause side effects on non-target species.

Over 60 generalist parasitoids have been recorded, however, for biological control a highly specialist parasitoid still needs to be found (Grabenweger et al., 2005a).

A number of natural predators of the larval stages of *C. ohridella* have been recorded. Observations have shown that blue tits (*Parus caeruleus*), great tits (*Parus major*) and marsh tits (*Parus palustris*) feed on the larvae, but they can reduce the number of the larva between 2 and 4% (Grabenweger et al., 2005b). The endoterapy technique by introducing

pesticides directly inside the trees can be the best solution.

Microinjection is a type of trunk injection where small amounts of therapeutic chemicals are introduced directly into the trees body without any contact with the environment.

MATERIALS AND METHODS

During our experiment a total number of 84 chestnut trees were identified from which 38 were treated with two different active chemical agents: abamectin and imidacloprid.

Treatment applications were made in May 2011. A plot of 46 trees were used as control without treatment.

The six groups of trees were selected from different places in Târgu Mureș town, four groups in University of Medicine park each spaced about 300 meters, and other two groups, in the city public area on a 2000 m distance. Some over 100 years old, their trunk circumference in many cases was up to 238 cm. According to their height and trunk girth, during the treatment we bored 4-5 cavities in each tree's trunk.

The 3-5 cm deep holes were prepared, taking into account the vascular bundles' progress. In the prepared cavity was introduced the applicator, and then injected the chemical product into the trunk of tree (Figure 1). After injecting 10 ml of active ingredients in each hole, with the removal of the injector, the applicator closes hermetically, preventing the escape of pesticides into the environment.



Figure 1. The hole on horse-chestnut trunk.

Two different insecticides were used, Vertimec 1.8 EC and KOHINOOR200SL. The EC Vertimec 1.8 is an acropetal effect systemic insecticide, acaricide, nematocide, digestive poison, the active ingredient is abamectin

(C₄₈H₇₂O₁₄, avermectin B1, major component, and C₄₇H₇₀O₁₄, avermectin B1b, minor component), is a colourless or pale yellow water soluble and organic solvents-soluble crystal, sensitive to strong acids and bases, and by exposure to UV radiation can be easily decomposed.

The KOHINOOR 200 SL, acropetal effect systemic insecticide, digestive poison, the active ingredient is imidacloprid (C₉H₁₀ClN₅O₂), a colourless, mild, characteristic odour, water-soluble crystal. Treatment applications were made in May 2011.

Data Analyses

Altogether a number of 5376 pictures were made to screen the density of the mines on leaves in both treated and untreated trees. All photography was taken weekly from 4 sides of each tree in the next 4 months after the treatment.

The number of mines was counted from pictures by selecting randomly five leaves by picture.

When the mine numbers increased and overlapped the surface covered by mines were estimated by using codifications: Codes 0, for 0 – 10%, 1, for 11 – 20%, 2 for 21 – 40%, 3 for 41 – 60%, 4 for 61 – 80%, and 5, for 81 – 100% damages. All counted data were registered in counter pages.

Data were analyzed with the Kruskal-Wallis test from the PAST statistical suite, supplemented with the Mann-Whitney test.

RESULTS AND DISCUSSION

According to endotherapy treatments all trees became highly protected against horse chestnut's leaf miner and very low or no damages have been observed on trees.

In contrast on control trees the first and second generations' damage was high and were observed all over the year.

The statistical data analysis shows that a significant difference can be detected between the treated and control trees.

The effect of the only one treatment in May was significant and conferred whole protection during the vegetation season even for plants in close proximity to control, untreated plants (medical University park) (Figure 2a, b, c, d).

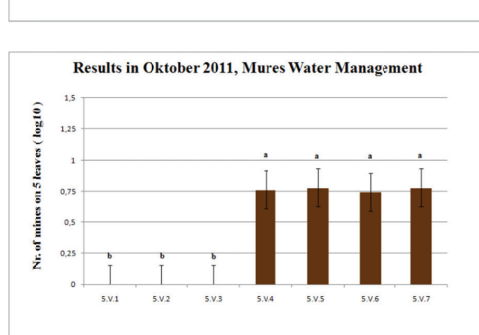
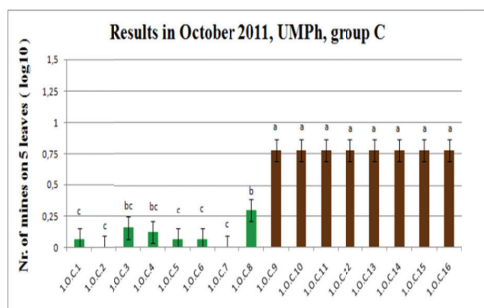
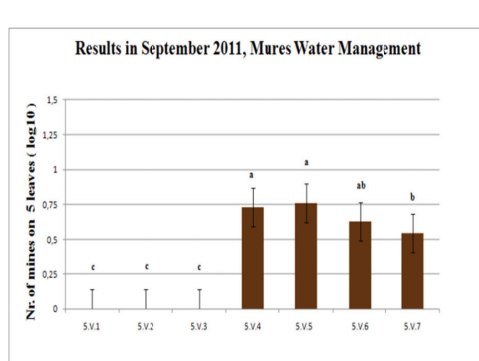
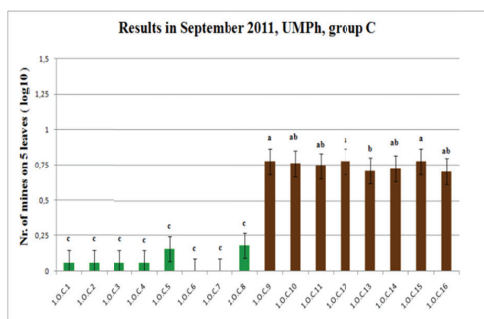
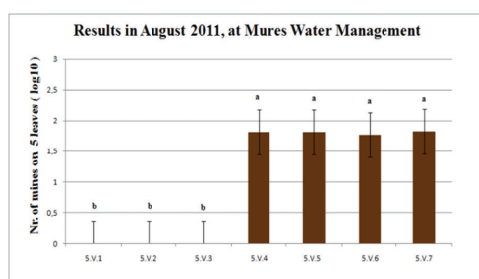
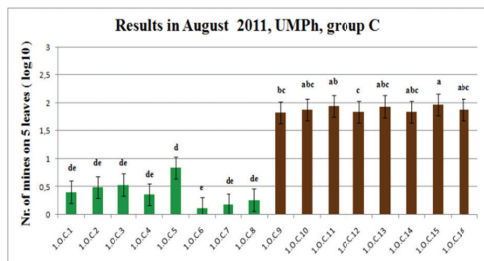
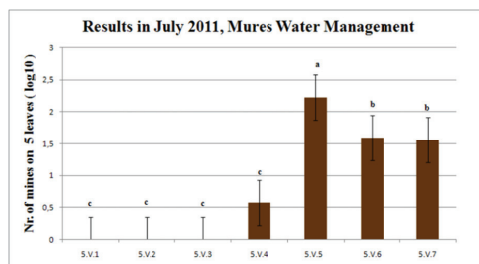
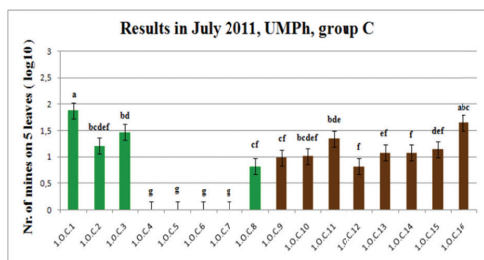


Figure 2. The effect of endoterapy by counting the number of mines on treated (green bars) and control (brown bars) trees located together in park. Different letters represents statistical significant differences (Mann-Whitney test).

Figure 3. The effect of endoterapy by counting the number of mines on treated (green bars) and control (brown bars) solitary trees. Different letters represents statistical significant differences (Mann-Whitney test).

The endoterapy conferred even higher protection for solitary plants all over in the town, with highly significant differences between treated and untreated trees (Figure 3a, b, c, d).

The use of endoterapy method on horse chestnuts trees proved to be effective, the treated trees we reached to keep the pests damage below 10% (Figure 4).



Figure 4. Treated tree in summer.

The control trees in late August and early September have lost their foliage, and blossomed again at the end of September (Figure 5).

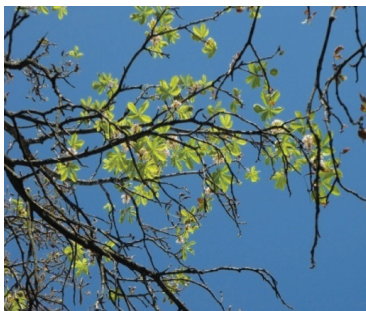


Figure 5. Untreated tree in autumn

CONCLUSIONS

From our perspective, this eco-friendly pest control method provides sufficient internal defense, and is absolute safe for other living beings and environment. The treatment does not depend on weather, can be carried out under any circumstances. This method can be used to protect any high tree that can't be protected with conventional spraying.

The results proved to be satisfactory with both of the used active agents. The effects of plant protection with insecticide containing the active ingredient abamectin (1.8 Vertimec EC) on the basis of subsequent observations is longer, stretches of the year following treatment. The therapy containing imidacloprid like active ingredient (KOHINOOR 200 SL) was less durable in the next year. Evaluating data of the experiment can be said that the suppression of a dangerous and highly invasive pest with using

this technology, this new eco-friendly approach, we have achieved remarkable results. The vegetal endotherapy can be used not only to reduce the horse chestnuts leaf miner damage, can be successfully used for any ornamental or farm trees against pests, fungal diseases, or treat nutrient deficiency.

ACKNOWLEDGEMENTS

I want to express my thanks to Târgu Mureș Municipality 's management, Parks and Green Spaces Administration, Dean's Office leaders of Târgu Mureș Medical and Pharmaceutical University, management of Târgu Mureș "Constantin Brâncuși" High school for their authorization. I am grateful to Mrs Giselher Grabenweger professor of Ökologischer Pflanzenschutz Eidgenössisches Volkswirtschafts departement EVD Forschungsanstalt, for scientific information provided.

REFERENCES

- Augustin S., Denux O., 2009. *Cameraria ohridella*: Un nouveau ravageur du marronnier. Société Natl. Hortic. Fr.
- Augustin S., Guichard S., Svatoš A., Gilbert M., 2004. Monitoring the Regional Spread of the Invasive Leafminer *Cameraria ohridella* (Lepidoptera: Gracillariidae) by Damage Assessment and Pheromone Trapping. *Environ. Entomol.* 33, p. 1584–1592.
- Baraniak E., Walczak U., Zduniak P., 2005. Appearance and migration of the horse-chestnut leafminer *Cameraria ohridella* in relation to city size and leaf-raking, using the example of two cities in Western Poland. *J. Pest Sci.* 78, p. 145–149.
- Binimelis R., Born W., Monterroso I., Rodríguez-Labajos B., 2007. Socio-Economic Impact and Assessment of Biological Invasions. In *Biological Invasions*, D.W. Nentwig, ed. (Springer Berlin Heidelberg), p. 331–347.
- Czencz K., Burges G., 1996. The horse-chestnut leaf miner (*Cameraria ohridella* Deschka et Dimic 1986, Lep.: Lithocolletidae). *Novenyvedelem Hung.*
- Deschka G., Dimic' N., 1986. *Cameraria ohridella* sp. n. (Lep., Lithocolletidae) from Macedonia, Yugoslavia. *Acta Entomol. Jugosl.* 22, p. 11–23.
- Fora C., 2011. The efficiency of spraying with insecticide against the invasive moth *Cameraria ohridella*. *J. Hortic. For. Biotechnol.* Volume 15(1), p. 59–61.
- Franke W., Franke S., Bergmann J., Tolasch T., Subchev M., Mircheva A., Toshova T., Svatoš A., Kalinova B., Kárpáti Z., et al., 2002. Female Sex Pheromone of *Cameraria ohridella* Desch. and Dim. (Lepidoptera: Gracillariidae): Structure Confirmation, Synthesis and

- Biological Activity of (8E,10Z)-8,10-tetradecadienal and Some Analogues. *Z. Naturforschung* 57c, p. 739–752.
- Gherghel M., Batin S.M., 2008. Boli ale plantelor lemnoase forestiere (Editura Nico Tg Mures).
- Girardoz S., Quicke D.L.J., Kenis M., 2007a. Factors favouring the development and maintenance of outbreaks in an invasive leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae): a life table study. *Agric. For. Entomol.* 9, p. 141–158.
- Girardoz S., Tomov R., Eschen R., Quicke D. I. j., Kenis M., 2007b. Two methods of assessing the mortality factors affecting the larvae and pupae of *Cameraria ohridella* in the leaves of *Aesculus hippocastanum* in Switzerland and Bulgaria. *Bull. Entomol. Res.* 97, p. 445–453.
- Grabenweger G., Girardoz S., Avtzi N., Kenis M., 2005a. Parasitism of *Cameraria ohridella* (Lepidoptera, Gracillariidae) in natural and artificial horse-chestnut stands in the Balkans. *R. Entomol. Soc. Agric. For. Entomol.*, 7, p. 291–296.
- Grabenweger G., Kehrli P., Schlick-Steiner B., Steiner F., Stolz M., Bacher S., 2005b. Predator complex of the horse chestnut leafminer *Cameraria ohridella*: identification and impact assessment. *J. Appl. Entomol.* 129, p. 353–362.
- Pschorn-Walcher H., 1997. Biology and population dynamics of the horse-chestnut leaf miner, *Cameraria ohridella*. *Forstsch. Aktuell*, p. 7–10.
- Rasputnig G., Schicho R., Stabentheiner E., Magnes C., Stelzl M., 2003. Morphology of female sex pheromone gland in the horse chestnut leafminer *Cameraria ohridella* (Lep., Gracillariidae). *J. Appl. Entomol.* 127, p. 121–126.
- Salleo S., Nardini A., Raimondo F., Lo Gullo M.A., Pace F., Giacomich P., 2003. Trees, Effects of defoliation caused by the leaf miner *Cameraria ohridella* on wood production and efficiency in *Aesculus hippocastanum* growing in north-eastern Italy. In *Trees*, (Springer Verlag), p. 367–375.
- Sefrova H., 2001. Control possibility and additional information on the Horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimic (Lepidoptera, Gracillariidae). *Acta Univ. Agric. Silv. Mendel. Brun.* 5, p. 121–128.
- 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, p. 83–99.
- Syryebryennikov B., 2008. Ecology and control of Horse chestnut leaf miner (*Cameraria ohridella*).