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A low environmental impact method to control horse chestnut leaf miner *Cameraria ohridella* (Deschka & Dimić)

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Abstract

The horse chestnut (*Aesculus hippocastanum* L.) is an important feature in parks and avenues in many European cities. Over the last decade, the leaves of chestnut trees have been increasingly attacked by large larval populations of an insect of unknown origin – *Cameraria ohridella* (Deschka et Dimić). The attacked chestnuts cease to function as urban greenery and are often defoliated as early as late August. Therefore, it is of utmost importance to develop appropriate measures to control this pest. Foliar treatment of chestnuts with insecticides involves risks for humans, domestic animals and environmental contamination with insecticides, but these risks can be avoided by using endotherapeutic methods of insecticide application. This paper presents the first-in-city testing results of three different endotherapeutic methods of insecticide injection: controlled pressure injection, gravity (diffusion) injection and soil injection. Two different insecticides were applied in these trials – Confidor SL 200 with active ingredient imidaclopride and Vertimec 018 EC, a.i. abamectin. Trials were conducted in Zagreb, Croatia, during the period from 2003 to 2007. Each year, a minimum of 16 chestnut trees (15-25 m high) were included in four replications trials. Injection of insecticide by diffusion or gravitational methods usually takes too long (up to several days), which makes it unsuitable for public areas. Injecting the insecticide into the root zone in the soil with the use of the Shell Soil Fumigant Injector Model HI is also inappropriate, as it requires a very large dosage of insecticide to achieve successful control, which makes it too expensive and presents a greater risk of groundwater contamination. The most successful *C. ohridella* control was achieved with the application of Vertimec 018 EC and Confidor 200 SL using the controlled pressure application technique (Intus, Arbosan III Steel and injector constructed on Faculty of Agriculture, Zagreb). In practice, the application of imidaclopride was difficult with a pressure lower than 4.0 bars. The use of higher pressures is not recommended because of the risk of damaging the conductive tissue of the plant. Application with a pressure lower than 4.0 bars is easy to use with abamectin.

Key words: Abamectin, *Aesculus hippocastanum, Cameraria ohridella*, endotherapeutic injections, environment protection, horse chestnut, imidaclopride, insecticide efficacy, pest control, pesticide application, urban greenery.

Introduction

Urban greenery improves human urban living. It cleans the air, mollifies temperature extremes, reduces noise and has other beneficial effects for humans. The most common tree species in parks and alleys of many Central European cities are the horse chestnut, plane, maple, poplar, acacia, birch, etc. Horse chestnut *Aesculus hippocastanum* (L.) is widely used as urban greenery in almost all European cities [1-4]. It is also a common ornamental plant in parks, avenues and private gardens in almost every large European town [5].

Wide ranges of different biotic and abiotic factors affect urban greenery. Numerous pests (insects, mites and nematodes), diseases (fungus, bacteria and viruses) and contamination resulting from human activities lead to urban greenery damage [4]. The large populations of horse chestnut moth miner *Cameraria ohridella* (Deschka et Dimić) that were observed all over the Europe in 1990s and 2000s [6-26] resulted in increased public attention to problems associated with plant protection and the preservation of urban greenery. Horse chestnut moth miner causes the foliage to turn yellow and results in defoliation as early as August. This type of damage is readily noticed by the general public. This is the main reason for increased recognition and attention to other phytomedicine problems in urban situations [4].

Leaf mines were first observed on horse chestnut leaves in the Ohrid lake region (Macedonia) in 1985 [6]. The insect that caused the damage was described as *C. ohridella* (Lepidoptera, Gracillariidae) in 1986 [7]. From this region, the insect spread all over Europe and is present today in almost all areas where the horse chestnut is planted. The new species has adapted well to all the areas it has invaded. Because *C. ohridella* had no significant natural enemies in these areas, its population has increased rapidly and caused substantial damage to the horse chestnut trees [27].

There are two different pesticide application methods used to control *C. ohridella*: endotherapeutic methods and foliage spraying. Foliage spraying usually involves sprayers and mistblowers that distribute insecticide solutions onto leaves. This method is limited by the vertical range of the applicators (6-10 m), which are usually shorter than the trees. According our experience, it is likely possible to achieve good protection of horse chestnuts by controlling the first generation of insects. *C. ohridella* females lay their eggs on the leaves of the lower third of the tree crown, which is within the range of mistblowers. If the first generation of *C. ohridella* is controlled with insecticides, the next two or three generations would be predicted to be unable to achieve the population levels necessary to cause higher leaf infestations and significant damage. Good chestnut protection would likely require application of insecticides to all trees in a given area.

The main disadvantage of the foliage spraying method is the risk of exposing and contaminating humans and animals. Such risks can be minimized by using enodotherapeutic methods, which are based on the ability of some insecticides to translocate into the plant vascular system. Endotherapeutic methods include soil injections, plant irrigations (with pesticides), bark smearing with insecticides and trunk injections. After the application, the insecticide is translocated to the higher parts of the trees through the xylem. Once the insecticide reaches the higher parts of the tree, it protects the tree for a period of time. Even taller trees can be protected from pests after endotherapeutic application of insecticide [4, 28]. Endotherapeutic methods are also used to protect fruit trees [29]. Trunk injections are considered to be the most effective method for protecting coconuts and other palms [30]. Trunk injections have the potential to cause serious physical damage to the plants and allow infection, especially if the injections are repeated from year to year. Phytotoxicity on leaves could also result from using pesticide dosages that are too high[10].

The optimal period to apply pesticides in order to control *C. ohridella* is during April and May, when the horse chestnut is in blossom. Unfortunately, pesticide application during this period leads to the risk of poisoning bees and other beneficial insects [25].

Most of the horse chestnut and plane trees in Italy are protected from pests using one of the endotherapeutic pesticide application methods [15]. Pesticide injections, a form of endotherapy, is used to protect chestnut against *C. ohridella* [31-33], and similar injection paradigms have been used to protect against the sycamore lace bug *Corythuca ciliata* in planes [34-36] and *Ceratocystis ulmi* in elms [30].

The insecticide acetamiprid (g l^{-1} of active ingredient or a. i.) was used in Austria for diffusion injection into healthy chestnuts against *C. ohridella* at the end of April, 2003. Trees approximately 20 m high were injected with 200-400 ml of liquid through holes with a diameter of 5 mm. The number of holes depended on tree crown size. In normal conditions, trees absorbed 100 ml of liquid over 25 hours. After injecting, the holes were closed with corks or artificial bark. Acetamiprid mostly accumulates in the top of the crown, so it does not provide good protection against *C. ohridella*. Usage of higher doses by previous researchers led to phytotoxicity and plant wilt. However, this method could potentially still be suitable for *C. ohridella* control if a more suitable insecticide was used [37].

Research on this subject was conducted in a nursery in Regensburg, Germany [38]. Horse chestnuts were injected with the systemic insecticide Confidor WG 70 (700 g l^{-1} of active ingredient imidaclopride) at a concentration of 80 mg ml^{-1}. Four young horse chestnuts (4-5 m high, 40 cm trunk diameter) with 3-8 mines per leaf made by *C. ohridella* larvae were injected on June 24, 1996. Trunks were injected 20 cm above the soil with 6 ml of insecticide per tree. Three additional trees were used as untreated controls. It was difficult to inject such small trees. Initially, there was no difference between injected and untreated trees. Eleven weeks after injecting, however, no mines were registered on treated trees, while the untreated trees showed 20-36 larvae per tree [38].

Similar studies were conducted in Italy in 2002. In Florence (May 21), the external pressure injection method was used with the "Intus" device, while natural absorption (diffusion) endotherapy was applied in Pistonia (May 23) with the "Ecoiatros" device. In both trials, the insecticide Merit Green was used at a concentration of 188 g l^{-1} of a. i. imidaclopride. The liquid was injected at a concentration of 0.7 g l^{-1}, and the water rate was calculated as 1 ml on 10 cm of trunk circumference. Injecting under 8 bars of pressure was conducted through 3-6 holes (depending upon the trunk circumference) 0.8 cm in diameter and 4-5 cm deep, 1 m above the soil. Diffusion injecting was conducted through 3-15 holes that were 3 mm in diameter and 1 m above the soil. External controlled pressure injecting lasted for a maximum of 30 minutes, while pesticide absorption after diffusion took at least 3 days. A low level of *C. ohridella* control was observed in this trial. Nevertheless, imidaclopride has shown good efficacy against *C. ohridella* in previous studies [39].

Research into the efficacy of azadirachtin injection into tree trunks was conducted in the Czech Republic in 2003. Trees with a diameter of 15-18 cm were injected with concentrated insecticides NeemAzal-T at g l^{-1} and NeemAzal-U at g l^{-1}, both of which contain the active ingredient azadirahtin. All the liquid was absorbed into the tree by diffusion after 60 hours. The efficacy of different doses (0.08, 0.15 and 0.25 g cm^{-1} of trunk circumference) was compared in the trial. Application of 0.08 g cm^{-1} of active ingredient resulted in a 78.6% reduction in the first generation *C. ohridella* larvae and a 68.6% reduction in the second generation. At doses $\geq$0.15 g cm^{-1}, a good horse chestnut protection was achieved for 23 weeks (encompassing the second and third generations). High efficacy (compared to control) was also achieved after application of 0.15 g cm^{-1} of active ingredient: 99.7% for the first generation and 99.1% for the second generation, while the third generation registered only an average of 0.2 mines per leaf. The highest (100% for all three generations) control was achieved after application of 0.25 g cm^{-1} of active ingredient [40].

External pressure injection techniques were investigated in Milan, Italy. The insecticide Mertit Green (a. i. imidaclopride) was used in 2001 and 2002 and Vertimec (a. i. abamectin) in 2003. Injections were conducted through holes of 0.6 mm in diameter and 6-8 cm in depth. The distance between the holes was 30 cm. From 700 to 1000 ml of liquid was injected under a pressure of 4-6 bars.During 2001, 30% of the untreated leaves of *A. hippocastanum* were affected at the beginning of August, 60% at the end of August, more than 70% in September, and almost 100% in October. Simultaneously, damage to injected trees was approximately 15% at the beginning of August, 50% at the end of August and 60% in the middle of September and October. In 2002, damage to injected trees was less than 10% at all time points, while the damage to untreated trees was only a little lower than in previous years. Even better *C. ohridella* control was achieved in 2003 after application of Vertimec. Damage to injected trees was less than 5% during the entire season, while the damage to untreated trees was approximately equal to the previous years [41].

Research into directly injecting horse chestnut trunks to control *C. ohridella* started in July 2000 with the diffusion injector Mauget. The insecticide Imicid was used at g l^{-1} of a. i. imidaclopride in 2000, and a gel for controlling *C. ohridella* and

G. aesculi on the horse chestnut after microinjecting (g l^{-1} of a. i. imidaclopride and g l^{-1} of a. i. tebuconazol) was applied in 2001 and 2002; 2.7 ml was injected per hole. The efficacy of the gel, evaluated during July and August, was 30.0-94.4%. Those results allowed approval for a gel usage license from the Polish Ministry of Agriculture and Rural Development in March 2003 [42, 43].

Different studies throughout Europe have shown that systemic insecticides applied to trees or soil around the tree injection sites protect horse chestnut from *C. ohridella* throughout the season[10]. Best results for *C. ohridella* control were achieved after injecting the active ingredient imidaclopride or abamectin [44, 45]. A good efficacy was also achieved after using the active ingredient dimethoate in previous trials conducted in Germany [46].

The purpose of this research was to investigate possible methods for controlling *C. ohridella* that minimized the risk of human and environment contamination with insecticides.

Materials and Methods

Location, host plant, rearing of C. ohridella and time of application: Trials were conducted from 2003 to 2007 in Zagreb (45º49'N, 16º02'E) in the middle of Croatia. Climate conditions in Zagreb are similar to conditions in many other central European cities. Different methods of pesticide application and insecticide efficacy in *C. ohridella* control were investigated. The trials were created in randomized block systems with four replications and were conducted on *A. hippocastanum* trees with a diameter greater than 100 cm.

Insecticides, dosage and liquid rate: Two systemic insecticides were used in the trials: Vertimec 018 EC and Confidor SL 200. Vertimec 018EC is an emulsifiable formulation of insecticide concentrate with 180 g l^{-1} a. i. abamectin, while Confidor SL 200 is a soluble concentrate with 200 g l^{-1} a. i. imidaclopride. Dosage of both insecticides was calculated as 1 ml of insecticide per 10 cm of tree diameter. The maximum soluble concentration of Vertimec 018 EC and Confidor SL 200 was up to 0.7 ml l^{-1}. After injecting the trees, the holes were treated with Cu-fungicide (at a concentration of 15 g l^{-1}) or carbendazine (5 g l^{-1}). To irrigate the soil with the insecticide Confidor SL 200, three different doses were used: 100, 50 and 25 ml per tree.

Equipment: Two types of injectors were used: a) injectors with controlled external pressure – Arbosan III Steel (Difesa Ambientale S.r.l. Varese, Italy), Intus (Intus S.r.l. Rimini) and ACI (the authors' constructed injector); b) injectors without controlled external pressure – diffusion injector (the authors' constructed injector) and soil injector (Shell).

The Arbosan III Steel injector was constructed of two metal cylinders, interconnected with plastic pipes. The lower cylinder is a compressing chamber that applies up to 10 bars of pressure. The upper cylinder is for the pesticide solution. Pressure is transmitted from the lower cylinder to the upper cylinder through a system of pipes and valves. Pressure from the lower cylinder pushes the solution in the upper cylinder through the tube system, which ends in open needles. The needles are put into previously made holes in the bark. After being forced out, the solution penetrates the tree's vascular system through those holes. When the insecticide solution reaches the plant vascular system, it is transported to the upper parts of the tree, including the leaves, which then acquire insecticide in much the same manner as the leaves being sprayed with the systemic insecticide. The holes in the bark that are necessary for this type of application are 4-10 mm in circumferences and 20-50 mm deep. Using this device, insecticide is injected into the trunk under a controlled pressure of 2-5 bars.

The Intus pressure injector operates in a similar manner. Nevertheless, it is a much more complex device, capable of functioning more accurately by simultaneously registering the application data. It is fitted on a vehicle and has its own operative Otto-engine with an electric generator, computer with proprietary software and three metal vessels (containing clear water, pesticide and a mix of the two). The computer calculates the liquid rate for the application according to the tree diameter, the tree top volume and the tree species. This liquid is injected into the trunk under 2-5 bars pressure. The starting pressure is 0.8 bars, and this gradually increases.

The ACI is a simplified version of the Arbosan III Steel. The diffuse injector consists of a 2000 ml plastic bag and a pipe system similar to the pressure injectors. The plastic bag hangs on the tree bark.

The Shell-Soil Fumigant Injector Model HI was used to inject insecticide into the soil around the roots. It consists of a liquid container, pressure pump, metal hafts and an injector. The pressure pump is used to force inject the pesticide horizontally into the soil. Application dates are presented in Table 1.

Table 1. Application technique and dates of application.

Type of equipment	2003	2004	2005	2006	2007
Arbosan III Steel	5.5.	3.5.	3.5.	3.5.	3.5.
Intus	12.5.	-*	-	-	-
Authors' constructed pressure injector (ACI)	-	3.5.	3.5.	3.5.	3.5.
Diffusion injector	6.5.	3.5.	3.5.	3.5.	3.5.
Shell - Soil injector	6.5.	3.5.	3.5.	3.5.	3.5.

* The application was not conducted in this year.

Examination of results and statistical analysis: Results were calculated after evaluating the horse chestnut leaves. Mine numbers for individual leaves on each tree were counted and recorded 5 times each year. On each evaluation, 400 leaves (100 leaves per tree × 4 replications) were examined. Statistics were performed using the Agriculture Research Manager 7.0.5 software (GDM Inc.). Variance was stabilized using a log (1+x) transformation. Ballet's Chi-Square and Ballet-Box F test were used to test the homogeneity of variance. If homogeneity was proven, an ANOVA (analysis of variance) was conducted. If homogeneity was not proven, no further analysis was carried out. If significant differences (at the 95% level of confidence) were obtained between objects, a multiple comparison with Duncan's test was executed. Values followed by the same letter are not significantly different ($p<0.05$). The efficacy of different insecticides and application methods was calculated using Abbott's formula.

Results and Discussion

The number of mines registered over time after different insecticide applications were compared to untreated controls, and the results are presented in Tables 2-7. No phytotoxicity was observed for the horse chestnuts included in these evaluations.

Endotherapy has great advantages compared to foliar spraying. Pesticide drift is almost completely avoided by using the bark injection endotherapeutic method. Many conditions need to be fulfilled to achieve good pest control after pesticide application using foliar spraying. Adequate application date, absence of wind and permissions avoid the risk of human and pets contamination etc. All of these conditions are more easily met through the use of endotherapeutic application. Pesticide application using endotherapeutic methods could result in good horse chestnut protection, but it also has the potential of harming the tree trunk. Before the injection, the trunk is damaged by making holes for the needles. Additionally, the injecting pressure used can harm the tree's vascular system. These are the main limitations to using the endotherapeutic method of pesticide application. The risk of harming the tree with high liquid pressure during injection led us to gradually decrease the working pressure used. In the first years of our trials, the pressure used was approximately 6 bars, while we lowered it to 3 bars in the later years.

The control systems evaluated in this research can be divided into three groups. The first group was characterized by the lowest infection rate or the highest efficacy of the pesticide application technique. The second group of techniques did not achieve good results but still held the potential for improvement with future research. The third group consisted of techniques that are not recommended for controlling *C. ohridella*.

The best *C. ohridella* control was achieved after injecting imidaclopride and abamectin with controlled external pressure using the Intus and Arbosan equipment. This method was effective and relatively easy to use, but had the potential risk of damaging the tree's vascular system.

The second group included the diffusion techniques and injection with an external pressure injector that we constructed. It must be emphasized that the main problem with the diffusion technique is a very long application period. This makes it unsuitable for larger public areas where it is quite difficult to ensure that humans and animals do come into contact with the insecticide. The long application period and necessity for preventing human and animal insecticide exposure increase the expenses of this technique. Based on the conducted trials, absorption of abamectin is faster than absorption of imidaclopride, which was the reason for higher efficacy observed after use of abamectin.

In the third group are methods that should not be recommended, such as soil injection (Shell) with different dosages of imidaclopride. This method is easy to use and fast, but has critical limitations. It is not recommendable for three main reasons. First, there is a significant risk of contaminating groundwater with the insecticide. Second, this method is very expensive because of the high dosages of insecticides needed. Third, it is not suitable for all types of tree planting systems (containers, asphalt, etc.). Trials that used the soil injector did not show good efficacy. Again, this technique is not suitable for every type of tree planting and has an inherent risk of polluting soil water with applied insecticides. All these factors have led us to conclude that the soil injection method should not be recommended to control horse chestnut leaf miner or other pests.

Table 2. *Cameraria ohridella* infestation (mines per leaf) in 2003.

Trial variant		Infestation (mines per leaf)				
		Date of examination				
Insecticide	Application technique	24.5.	4.6.	22.6.	24.7.	3.9.
Imidaclopride	Arbosan	0.6a	0.7a	4.5b	10.2a	12.6ab
Abamectin	Arbosan	0.4a	0.6a	4.1b	10.0a	11.2ab
Imidaclopride	Intus	0.4a	0.5a	2.0ab	5.2a	7.0a
Abamectin	Intus	0.4a	0.5a	0.9a	5.0a	6.5a
Imidaclopride	Diffusion injector	23.6*c	25.1c	44.0e	65.1de	90.1e
Abamectin	Diffusion injector	19.3*b	21.4b	34.8d	53.0c	75.6d
Imidaclopride	Shell - 100 ml	19.9b	22.9b	38.8d	60.0d	69.9d
Imidaclopride	Shell - 50 ml	24.2d	28.8c	44.0e	68.6e	84.8e
Imidaclopride	Shell - 25 ml	24.0d	29.0c	44.3e	69.5e	88.6e
Untreated control		24.3d	29.2c	44.2e	69.8e	88.0e
LSD ($p<0.05$)		1.2	1.6	3.2	5.4	6.3

*Injecting stopped after 12 hours; all liquid was not absorbed into the trunk.Values followed by the same letter are not significantly different ($p<0.05$).

Table 3. *Cameraria ohridella* infestation (mines per leaf) in 2004.

Trial variant		Infestation (mines per leaf)				
		Date of examination				
Insecticide	Application technique	25.5.	5.6.	25.6.	25.7.	5.9.
Imidaclopride	Arbosan	0.2a	0.2a	3.8a	9.0a	11.9a
Abamectin	Arbosan	0.1a	0.2a	3.6a	8.1a	11.2a
Imidaclopride	ACI	0.3a	0.7a	12.2c	10.5b	12.6a
Abamectin	ACI	0.1a	0.8a	8.6b	8.2a	13.2a
Imidaclopride	Diffusion injector	12.2*c	22.1*f	40.3f	63.2	80.1d
Abamectin	Diffusion injector	4.3*b	14.3*d	35.2e	50.1d	75.6c
Imidaclopride	Shell – 100 ml	5.2b	14.3d	36.1e	53.3d	80.0cd
Imidaclopride	Shell – 50 ml	20.2d	20.6e	42.1f	63.5f	84.6d
Imidaclopride	Shell – 25 ml	19.8d	20.2e	41.2f	60.0ef	75.6c
Untreated control		20.1d	22.0f	41.6f	61.3f	80.4d
LSD (<0.05)		0.8	1.3	2.3	3.5	4.2

*Injecting stopped after 12 hours; all liquid was not absorbed into the trunk. Values followed by the same letter are not significantly different ($p<0.05$).

Table 4. *Cameraria ohridella* infestation (mines per leaf) in 2005.

Trial variant		Infestation (mines per leaf)				
		Date of examination				
Insecticide	Application technique	20.5.	5.6.	20.6.	20.7.	20.8.
Imidaclopride	Arbosan	0.2a	0.4a	3.5a	9.9ab	11.6a
Abamectin	Arbosan	0.3a	0.3a	3.9a	10.1ab	11.2a
Imidaclopride	ACI	0.3a	0.6a	13.2c	12.5b	12.6ab
Abamectin	ACI	0.4a	0.6a	8.8b	10.5a	13.2ab
Imidaclopride	Diffusion injector	13.2*c	20.1c	44.3e	64.2	80.1e
Abamectin	Diffusion injector	8.3*b	15.3b	35.0d	52.1c	74.6d
Imidaclopride	Shell– 100 ml	16.1d	20.3c	35.1d	60.3d	60.0c
Imidaclopride	Shell – 50 ml	21.2e	20.9c	44.1e	64.5d	84.6e
Imidaclopride	Shell – 25 ml	22.8e	20.8c	44.6e	64.8de	75.6c
Untreated control		22.2e	25.0d	44.5e	65.0e	81.5e
LSD (p<0.05)		0.9	1.2	2.5	4.6	5.0

*Injecting stopped after 12 hours; all liquid was not absorbed into the trunk. Values followed by the same letter are not significantly different (p<0.05).

Table 5. *Cameraria ohridella* infestation (mines per leaf) in 2006.

Trial variant		Infestation (mines per leaf)				
		Date of examination				
Insecticide	Application technique	21.5.	6.6.	21.6.	21.7.	20.8.
Imidaclopride	Arbosan	0.4a	0.7a	3.7a	9.7a	11.2a
Abamectin	Arbosan	0.2a	0.8a	3.5a	10.0a	11.0a
Imidaclopride	ACI	0.4a	0.9a	9.2b	12.1a	16.6b
Abamectin	ACI	0.3a	0.8a	8.6b	10.0a	16.1b
Imidaclopride	Diffusion injector	13.8*c	19.3c	43.5d	62.2d	76.5cd
Abamectin	Diffusion injector	8.6*b	16.8b	34.1c	51.1c	72.5c
Imidaclopride	Shell – 100 ml	17.0d	20.8c	33.3c	61.3d	80.2d
Imidaclopride	Shell – 50 ml	21.4e	20.4c	43.8d	62.3d	83.6d
Imidaclopride	Shell – 25 ml	21.0e	19.9c	44.7d	64.0de	83.9d
Untreated control		22.0e	25.0d	44.8e	64.2d	83.0d
LSD (p<0.05)		0.8	1.3	2.4	4.5	4.8

*Injecting stopped after 12 hours; all liquid was not absorbed into the trunk. Values followed by the same letter are not significantly different (p<0.05).

Table 6. *Cameraria ohridella* infestation (mines per leaf) in 2007.

Trial variant		Infestation (mines per leaf)				
		Date of examination				
Insecticide	Application technique	20.5.	5.6.	20.6.	20.7.	20.8.
Imidaclopride	Arbosan	0.7a	1.8a	4.9a	10.4a	13.5a
Abamectin	Arbosan	0.5a	1.5a	4.5a	11.3a	13.3a
Imidaclopride	ACI	0.9a	1.6a	5.6a	12.7a	16.4a
Abamectin	ACI	1.0a	1.9a	5.3a	11.9a	14.2a
Imidaclopride	Diffusion injector	15.2*c	22.2c	46.0bc	65.0bc	70.5bc
Abamectin	Diffusion injector	14.0*b	18.6b	43.6b	61.7b	69.8b
Imidaclopride	Shell – 100 ml	17.1d	22.3c	47.1c	68.2cd	76.5cd
Imidaclopride	Shell – 50 ml	23.3e	24.9c	49.1cd	70.7d	82.6d
Imidaclopride	Shell – 25 ml	25.6f	25.8c	49.9d	71.8d	83.6d
Untreated control	-	25.8f	26.0d	50.0d	71.1d	83.7d
LSD (p<0.05)		1.5	1.7	3.4	5.5	6.2

*Injecting stopped after 12 hours; all liquid was not absorbed into the trunk.Values followed by the same letter are not significantly different (p<0.05).

Diffusion injections in Zagreb were stopped after 12 hours, while the trials conducted in Austria with acetamipride [37] lasted a few days. A similar method was used in Pistonia, Italy, where the diffusion-injection period was three days [39]. All three trials failed to achieve adequate control of *C. ohridella*. Better results were achieved with diffusion injections using the active ingredient azadirachtin [40] in the Czech Republic and also in Poland, where the Mauget diffusion injector and the active ingredient imidaclopride were used [42, 43]. Those two trials showed that it is possible to achieve good horse chestnut protection using diffusion techniques, but it requires an application time of a few days. It makes this method unsuitable for use on areas with a frequent influx of humans and animals. Trials conducted in Zagreb showed better *C. ohridella* control with Intus equipment using imidaclopride (200 g l^{-1} of active ingredient) than a trial conducted in Florence with injections of imidaclopride (188 g l^{-1})[39].

In a nursery in Regensburg, trials showed good efficacy in controlling *C. ohridella* by pressure injection with Confidor WP 70 [38]. However, these trials were somewhat limited because the injected trees were (from our point of view) too small for injecting, at only 40 cm in circumference and 4-5 m high, while our recommendation is to use trees not less than 40 cm in circumference. Nevertheless, those results were confirmed in Zagreb. Controlled pressure (4-6 bars) injections of the insecticide Merit Green (a. i. imidaclopride) in Milan achieved a good efficacy and Vertimec (a. i. abamectin) performed even better [41].

Controlled pressure injecting is easier under pressures higher than 4 bars, but is then potentially harmful to the tree. In trials conducted in Zagreb, the pressure was gradually decreased from 6.0 to 3.0 bars to avoid this risk. It was still possible to accomplish the injections using the reduced pressure, the injection period was greater. This meant that fewer trees could be injected over the same period of time.

Insecticides with the active ingredient dimethoate exhibited

Table 7. Average efficacy (%) of different pesticide application techniques and insecticides for controlling *C. ohridella* (average value±standard deviation).

Trial variant		Efficacy (%, average±standard deviation)				
		Average date of examination				
Insecticide	Application technique	24.5.	4.6.	22.6.	24.7.	3.9.
Imidaclopride	Arbosan	98.2±0.8	97.1±2.4	91.0±1.0	85.1±0.3	85.4±1.0
Abamectin	Arbosan	98.7±0.6	97.4±2.0	91.3±0.6	85.1±1.2	86.1±1.2
Imidaclopride	Intus	98.4±0.0	98.3±0.0	95.5±0.0	92.6±0.0	92.1±0.0
Abamectin	Intus	98.4±0.0	98.3±0.0	96.0±0.0	92.8±0.0	92.6±0.0
Imidaclopride	ACI	98.0±0.2	96.2±0.6	77.3±4.5	81.7±1.0	82.3±2.2
Abamectin	ACI	98.1±0.6	95.9±0.6	82.4±0.6	84.5±1.3	82.8±1.6
Imidaclopride	Diffusion	32.2±16.5	14.1±8.9	3.0±3.1	3.3±4.6	4.7±7.3
Abamectin	Diffusion	53.7±21.9	32.4±4.9	18.9±4.6	19.2±3.9	11.6±4.3
Imidaclopride	Shell - 100 ml	35.2±22.5	21.3±8.1	15.6±7.8	8.6±4.7	11.9±11.2
Imidaclopride	Shell - 50 ml	3.4±4.0	9.4±7.6	0.8±1.3	0.5±2.5	-0.9±3.6
Imidaclopride	Shell - 25 ml	1.1±2.6	9.4±9.1	0.2±0.5	0.4±1.1	2.3±4.0

good results in preliminary tests of *C. ohridella* control conducted in Germany [46], but this insecticide has not been tested in Zagreb.

Conclusions

High populations of *C. ohridella* were registered in Zagreb each year during 2003-2007. The highest average leaf infestation was registered on September 3, 2003, with 88 mines per leaf. One larva or pupa was present in each mine.

Successful *C. ohridella* control was achieved after controlled pressure injection of insecticide. Both tested insecticides (abamectin and imidaclopride) showed good efficacy in controlling *C. ohridella*. The most successful control of *C. ohridella* was achieved with the controlled pressure injections of Vertimec 018 EC (on average 86.1-98.7% with the Arbosan 3 Steel device during vegetation; 92.61-98.12% with an Intus device; and 82.8-98.1% with a device of our own construction) and Confidor SL 200 (on average 85.4-98.2% with the Arbosan 3 Steel device during vegetation; 92.1-98.4% with an Intus device; and 82.3-98.0% with a device of our own construction).

In practice, it was difficult to apply imidaclopride using a pressure lower than 4.0 bars. However, because of the risk of damage to the conductive tissue of the plant, the use of any higher pressure is not recommended.

Injection of insecticide by the diffusion or gravitational method did not achieve satisfactory horse chestnut protection. It also usually takes too long (up to several days), which makes them unsuitable for use in public areas.

The method of injecting the insecticide into the root zone in the soil with the use of the Shell Soil Fumigant Injector Model HI also did not achieve satisfactory *C. ohridella* control. It is also inappropriate for extensive use because it requires a very large dose of insecticide to achieve successful control, which makes it too expensive and presents an increased risk of groundwater contamination.

The controlled pressure injections of abamectin are recommendable for a good protection of horse chestnuts against *C. ohridella*. It is easy to use and effective.

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