Preliminary results about the efficacy of abamectin trunk injection against the walnut husk fly (*Rhagoletis completa*)

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Abstract

The walnut husk fly (*Rhagoletis completa*) is one of the main pests affecting common walnut in both Europe and America. This work examines the effects of abamectin on the development of walnut husk fly larvae by injecting the product Vertimec 1.8 EC (Syngenta) into the trunks of walnut trees in Hungary. In the case of properly injected trees, the infection rate was negligible and the abamectin content in the husk samples ranged between 1.54 and 3.00 ng/g; controls show a very high (> 90%) infestation rate and an abamectin content under the detection limit. Abamectin residue measured in walnut kernel did not exceed the maximum residue limit; moreover, the active ingredient content was below the detection limit (0.0003 mg/kg) in all the collected kernel samples. Our results confirm that trunk injection is a viable method for walnut pest control.

Keywords Abamectin · *Juglans regia* · Trunk injection · Walnut husk fly

Introduction

Walnut husk fly (*Rhagoletis completa*) is one of the main pests of common walnut and can cause significant economic damages not only in Hungary, but all over Europe (Van Steenwyk et al. 2010; Verheggen et al. 2017). This pest is native to the Midwestern USA and north-eastern Mexico. In Europe, it was first detected in Switzerland in 1988, from where it spread (Duso 1991). In Hungary, it was first detected in 2011 in Kőszeg (Voigt and Tóth 2013). The known hosts of *R. completa* are various *Juglans* species, on which the females lay eggs into the pericarp. Larvae hatching can last over 2 months, and feeding on walnut husk

tissues causes brown-purple coloured spots (Guillén et al. 2011). Furthermore, the infected fruit facilitates the penetration of pathogens into the edible kernel as well as the shell surface itself (Duso and Lago 2006). These pathogens (fungi and bacteria) are responsible for considerable yield losses, as they shrink and rot the kernel, after which the infected walnut drops off the tree together with the husks. Damage can occur on 74–91% of untreated orchards, with economic losses as high as 50% (Duso and Lago 2006; Voigt and Tóth 2013).

Protection against the larvae is challenging because it is difficult to access them with insecticides and because adults fly for a long time (Nickel and Wong 1966). Protection should therefore be continuous from early July to mid-September. Another problem is the typical large canopy of walnut trees, where treatment by foliar spray is impractical (Verheggen et al. 2017; Wise et al. 2014). Currently, control of *R. completa* is usually achieved by spraying method; however, trunk injection, a rediscovered plant protection technique, could be an alternative solution to combat this problem (Fettig et al. 2013; Van Steenwyk et al. 2010). With this technique, the pesticide is introduced directly into the vascular system (xylem) of the tree, which then reaches the feeding larvae (Ferracini and Alma 2008; Mota-Sanchez et al. 2009; Roach 1939). Injection techniques have several advantages over conventional spraying, such as (1) lower pesticide amounts are used, (2) the nutrient medium of

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larvae can be directly reached without any toxicokinetic difficulties, (3) closed vessels provide long lasting protection for the pesticide, (4) it is safe for non-target organisms (Acimovic 2014; Burkhard et al. 2015; Doccola and Wild 2012; Halley et al. 1993; Kobza et al. 2011).

Materials and methods

The experimental area was in Taksony, Hungary, where 7 walnut trees were chosen to be injected. The 8-year-old, walnut trees (Alsószentiváni 117) had previously not been treated with any kind of insecticide. The height of the trees is 4.5–5 m, and the planting density is 10×10 m. Yellow sticky traps (Csalomon PALz) were used for the detection and monitoring of the presence of *R. completa* in the field. The first individual was detected on 15th of July, 2019. The trunk injection was performed on the 8th of July, 2019.

Injection

In the case of 5 trees, 10–20 ml of Vertimec 1.8 EC (active ingredient: 18 mg/ml abamectin, Syngenta) was injected into 4 holes of the trunk (Table 1), besides the two control treatments (without injection + water control). A tree injector device was used for injection, which uses a drilled hole of the tree and has a pedal with two springs to provide adequate pressure for the injection of the liquid (Gutermuth 2017). This device can inject the liquid into the xylem with a maximum pressure of 12.6 bar. The injection time requirement is about 5 min/tree for one person. Walnut is a well injectable species, so the pressure was about 8 bar on average. 50 mm deep holes with a 3.5 mm diameter were drilled at the lower/middle/upper third of the trunks of the trees with an electric screwdriver (Table 1). The point of the injection device was put into the holes, and the intended liquid was injected into the xylem system via the holes. The circumferences of

the trees ranged between 28 and 33 cm at a trunk height of 130 cm.

Sample collection

In two cases (4th, 5th trees), leaf samples were collected on the 5th of September in order to check the distribution of the active ingredient in the canopy as a whole. To ensure sampling was representative, 25 composite leaves per tree were collected in three parallels from all parts of the canopy, following which their abamectin content was measured. Walnut fruit samples—all fruits were harvested from each tree—and their husks were collected from all 7 trees (Table 1) on the 19th of September, before their full maturity, which was possible because of the young age of the trees. This allowed us to avoid the natural falling of the walnut fruits and to collect the nuts and their husks together. First, an entomological investigation was performed on the fruits, after which the husks were separated from the nuts to prepare for a chemical investigation in three parallels. All samples were stored in a freezer at −80 °C until chemical investigation.

Entomological survey

The infection rate was recorded in the case of the walnut fruit samples. Each denuded husk and its kernel was visually inspected or, in the cases when it was not enough to determine whether it is infected or not, it was inspected with a stereo microscope (Zeiss Stemi 2000). The inspected fruits, regardless of size, were separated into two groups according to whether they were infected or not. After that, the husks and the nuts were stored at −80 °C until the measurement of their active ingredient content. From the group of intact nuts, a certain amount was stored without freezing with the purpose of imitating traditional walnut processing and storing methods. In line with the methods, we dried and stored these samples by spreading them out at room temperature, carefully protecting them from mould and rancidity.

Table 1 Injection parameters, infestation rate caused by walnut husk fly and statistical analysis of infection and abamectin content

Tree (treatment) tags	Total number of holes	ml/hole	ml/tree ^a	Location of drill holes on the trunk	Infection rate (%)	Marascullo-group (infection rate)	ANOVA-group (a.i. content)
1. (control)	0	0	0	–	98	c	C
2. (aqueous control)	4	2.5	10	Lower third	92	c	C
3.	4	2.5	10	Lower third	10	a	A
4.	4	5	20	Lower third	1	a	AB
5.	4	2.5	10	Lower third	11	a	AB
6.	4	5	20	Lower third	4	a	A
7.	4	2.5	10	Upper third	31	b	B

^a10 ml injection is equivalent to 180 mg a.i. (abamectin)

Analysis of abamectin in samples

The abamectin standard was obtained from Pestanal, Riedel-de Haën, Germany, with a certified purity of 97.1%. The EN 15662:2018 (QuEChERS) sample preparation procedure was used for extracting the active ingredient from the samples (European Standard—EN 2018). The chemicals used for residue measurement are described in Kmellár et al. 2010. In case of kernel samples, a defatting step was also integrated into the procedure, which included freezing out and dSPE cleaning with C18 sorbent. A HPLC–MS/MS instrument was used for determining abamectin content in plant materials, which was performed with an Agilent 1200 LC system coupled to an AbSciex 3200 Q TRAP mass spectrometer equipped with a Turbo V ESI Heated Ion Spray probe operate in positive MRM mode. Regarding the measurement parameters, the Single Residue Method of Abamectin published by the Community Reference Laboratories for Residues of Pesticides was applied (European Commission 2008). In order to achieve a lower quantification limit (QL), a 100 µL injection volume was used with a flow rate of 0.3 ml/min. To prevent signal loss, a 150 °C ion source temperature was used during the analyses. A matrix-matched calibration method was used to quantify the abamectin content of the samples. Expecting a small amount of abamectin content in the samples, method validation was performed for the detection limit (DL), quantification limit, linearity and matrix effect. The method validation was performed in accordance with document SANTE Guidelines (European Commission 2019). DL in the case of husk, leaves and kernel were 0.15, 0.30 and 0.30 ng/g, while the QL values were 0.30, 0.90 and 0.90 ng/g, respectively. The calibration curves were linear up to a concentration of 1000 ng/ml, and the matrix effect calculated with the use of the Matuszewski equation was between 61 and 95% (Matuszewski et al. 2003).

Statistical analysis

Statistical analysis was performed using the mean values of replicates. Differences in the abamectin concentrations of the husks were tested using one-way ANOVA. The Shapiro–Wilk test was applied to the normality of the residuals and homogeneity of variance was checked by applying Levene's test. The multiple pairwise comparison was performed with Tukey's HSD. Marascuilo's procedure was used (Marascuilo 1966) to compare the trees based on their infection rates. The statistical analysis was performed using IBM SPSS Statistics 25 and Excel 2016.

Results

Infection

The infection rate of the husks was very different. We detected a high infection rate in the case of 1st and 2nd trees, but in other cases, with the exception of the tree in the 7th treatment, the number of infected husks was negligible. Based on the infection rate, we can say that the injected pesticide and the tested dose was appropriate for plant protection. In terms of infection, we can consider the 3rd, 4th, 5th and 6th trees to be similar ('a' group), and they were infected to the smallest extent. Of these trees with their low infestation rates, the tree of the 7th treatment is significantly different ('b' group) according to Marascuilo's procedure based on its infection rate ($P < 0.05$, Table 1). The trees with the most significant serious infections were the ones of the 1st and the 2nd control ('c' group). Although eggs were laid and hatched on the injected trees, most of them died in the first larval stage, which was visible on the green shell as some dry spots 1–2 mm in size and had no effects on the development of walnut. Note that the injected abamectin also showed notable efficiency against the mite species that usually occur in walnut cultivars.

The concentration of the active ingredient in the samples

Fifty-nine days after injection, leaf samples were collected from tree 3 and tree 4; the latter was injected in double doses. The abamectin content in the leaves of both trees was as high as 379 and 750 ng/g, respectively. The concentration values correspond to the injected dose (Table 1). On the other hand, the distribution of the pesticide over the whole canopy was uneven. The RSD values were 96% and 42%, respectively. Based on these results, we suspect that this concentration is sufficient for realizing abamectin's plant protection effects. Seventy-three days after injection, fruit samples (husk together with the walnut) were collected and investigated with respect to abamectin content (Fig. 1).

Much lower concentrations were measured in the husk as compared to the leaves. Significant differences were found among the active ingredient contents of the husks ($F(4;10) = 5.70$, $P < 0.05$). In the case of the treated trees, the abamectin content in the husk samples was between 0.29 and 3.00 ng/g (Fig. 1). The homogeneous group separation of the mean abamectin values according to Tukey's HSD and the one of the infection rates by Marascuilo's procedure coincide well. Abamectin was not detectable in the control samples.

Since walnuts have a high nutrition content, which makes them a valuable part of a healthy diet, we measured

Fig. 1 Infection rate and abamectin concentration in the husk samples. DL detection limit (0.15 ng/g in the case of husk). **Measured value (0.29 ng/g) is between DL and QL

the abamectin residue content of the kernel as well. In all the collected kernel samples, the active ingredient content was below the detection limit (0.0003 mg/kg).

Discussion

The main goal of the present study was to provide a preliminary evaluation of the control of *Rhagoletis completa* in walnut cultivars through the trunk injection of a commercial abamectin formula. Although this plant protection technique is not commonly widespread, many of its benefits can be well utilized in fruit production, especially in cases when conventional spraying is not feasible (Schulte et al. 2006; Wise et al. 2014). The effectiveness of trunk injection treatment in walnut trees was evaluated based not only on the infection rate, but also on the active ingredient content of the plant parts. The latter provided direct data on the distribution and appearance of the active ingredient in the walnut husk, which is the target of the investigated pest. Moreover, the relation between the actual infection and the actual abamectin concentration of the husk was also tested. In the case of the control trees, intense infection (>90%) was observed, due to the fact that the active ingredient could not be detected. These results show that the aqueous injection control does not contribute any plant protection effect, nor does the drilling of bores in the trunk induce any biochemical reaction that could result in a lower infection rate. As for the treated trees (trees 3–7), the infection rates were heterogeneous, but in every case they were much lower than in the controls. During our experiment, all five treatments successfully protected walnut trees against *R. completa*.

In our work, Vertimec 1,8 EC was used for injection. Although this product was not specifically developed for this purpose, it provided sufficient results. This outcome is contradictory to some previous observations, when the formulation with oil content was not useable for trunk injections.

Percival and Boyle (2005) examined certain fungicides with various active ingredients and formulations and found that only the water-soluble formulations were suitable for trunk injections while others, including emulsions, were less effective or ineffective. Unlike conventional spray application, the efficacy of injected pesticides is due to uptake and translocation from the injection site to target sites, which in turn depends on pesticide solubility, the health rate and the chemical composition of transport tissues, as well as on the tree species, the transpiration rate, the coal absorption coefficient and the metabolism/degradation of the active ingredient (Acimovic 2014; Doccola and Wild 2012; Ferracini and Alma 2008). This complexity can be the reason for the high pressure and significant time requirement that we experienced during the injection.

Our studies demonstrate that the height of the injection point also plays an important role in the success of the treatment. In the case of trees injected at the lower third of their trunks, the protection was more effective (the infection rate was below 11%) and, accordingly, the active ingredient content was also found to be higher here compared to the tree injected in the upper region. In order to gain a homogeneous and adequate active ingredient level in the entirety of the canopy, a sufficiently low injection height has to be used as in this case the active ingredients have enough time to diffuse along the xylem sheaf before the transport reaches the branches (Fig. 1, trees 5 and 7).

The abamectin content in leaf samples was higher than in the husks. This is attributable to the fact that the injected compounds in the xylem move in the direction of water transport, which corresponds to the degree of evaporation of the different plant tissues (Doccola and Wild 2012; Mota-Sanchez et al. 2009).

The novelty of this work is the use of the trunk injection method in walnut pest control. We investigated the effects of the neurotoxin abamectin on the development of walnut husk fly larvae. By using both insecticidal and

chemical investigations, we proved that the active ingredient was efficiently distributed in the canopy of walnut tree, and the injected dose was enough to provide an insecticidal effect. Abamectin residue measured in the kernel did not exceed the maximum residue limit (0.02 mg/kg) set by Regulation (EC) No 396/2005. This experiment provides insight into the trunk injection technology in walnut plant protection and confirms that trunk injection is a viable method for walnut pest control.

Our future plan is to investigate the effects of the present treatment next season, as residual activity is an already known phenomenon, and to try other forms and active agents (Holderness 1992; Percival and Boyle 2005).

We hope that our work will contribute to plant protection against invasive pests such as *R. completa*, which is an emerging problem threatening the cultivation of walnuts throughout Europe.

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Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval The author warrants that the article is original, written by stated author/s, has not been published before, nor is it currently under consideration for publication elsewhere.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Code availability Not applicable.

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