



**Hungarian University of Agriculture and Life Sciences**

**Effect of Trunk Injection on the Walnut Husk Fly**

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## 1. Background and Objectives

The common walnut (*Juglans regia* L., 1753) is one of the most popular nuts in Europe, yet its production in the region lags slightly behind the global trend. This is attributed to the adverse effects of climate change and the spread of the walnut husk fly (*Rhagoletis completa* Cresson, 1929), whose emergence has caused significant economic losses.

Nowadays, plant protection practices face increasing challenges due to stricter environmental and societal expectations, calling for the adoption of new technologies and sustainable methods. The declining number of active ingredients further complicates pest control, particularly for crops cultivated in smaller area, like walnut. Consequently, there is growing interest in plant protection methods aimed at maximizing the efficacy of available active ingredients while minimizing environmental impacts.

Trunk injection (endothecy) is a plant protection technology that introduces pesticides directly into the tree trunk. This method offers more effective pest control, reduces environmental and human health risks, and aligns better with sustainable agricultural practices.

The objective of this research was to develop an alternative plant protection technology for walnut cultivation that is both effective against the walnut husk fly and safe for the environment. Our investigations focused on addressing key aspects of this technology:

- Selection of active ingredients: identifying compounds with sufficient efficacy for endothecy against the pest.
- Evaluation of technological parameters: assessing the method, timing of treatments and the homogeneity of applications through biological and chemical analyses.
- Assessment of larvicidal effects: determining acceptable economic damage thresholds.

- Characterization of toxicokinetics: analyzing temporal dynamics and the potential for multi-year residual effects.
- Dose determination: balancing plant protection efficacy with food safety requirements.
- Addressing toxicological concerns: investigating potential side effects and environmental impacts inherent to the technology.

## **2. Materials and Methods**

### **2.1. Description of Treatments (Location, Method, Active Ingredients)**

Trunk injection experiments were set up over four years at the following locations: Taksony, Csipkerek, Felsőpáhok, Szelevény. Two different but similarly operating devices were used for the injections. Both devices were directly connected to holes created prior to injection. For the Treenject device, holes with a diameter of 3.5 mm and a depth of 50 mm were drilled in a proportional distribution according to the trunk circumference, with 4-8 holes depending on the trunk size. This device is suitable for injecting smaller quantities (max. 10-20 ml/hole). The other device used was a combination of a multi-layer latex tube and applicator (Ynject GO), in which four 6.5 mm diameter, 50 mm deep holes were created on opposite sides of the trunk. This device is suitable for injecting larger quantities (up to 50-60 ml/hole).

Some of the pesticides used were prepared from crystalline forms, while others were commercially available products. The active ingredients were selected based on (i) their physical-chemical properties and (ii) experiences found in scientific literature. These included abamectin (ABA), emamectin benzoate (EMA), acetamiprid (ACE), flupyradifurone (FLU) and spirotetramat (SPI). The same formulations and active ingredients were tested in basal bark spray applications, to which additives such as Pentra-Bark® (PB) and Invazív® (I) were added to facilitate or increase the penetration through the bark.

Three repetitions were used for each treatment. Two control groups were established for the experiments: in the water control (C aq.), the injection was performed with water only, while in the other control (C no inj.), neither injection nor hole creation was carried out.

## **2.2. Efficacy Evaluation (Larvicidal Effect) and Economic Damage Assessment**

The insecticidal effect was evaluated based on the frequency, specifically the presence of live larvae in the husks. The evaluation was conducted at the natural cracking of the husks. The fruit husks were examined within 1-2 days following collection. A total of 100-150 fruits per tree were collected and classified into two groups based on whether they contained live larvae or not. While this calculated infestation rate indicates the direct insecticidal effect well but does not correspond directly to economic damage. Minor necrotic spots on the husks may not necessarily result in commercially worthless fruit.

The economic damage was estimated through rating, based on the frequency of husks showing black spots and the degree of spotting (the extent of the spot). The economic suitability was always classified according to the stricter parameter. This was based on the fact that damage of walnut husk fly primarily reduces the processability and quality indicators of the husked fruit.

## **2.3. Dose Adjustment**

To set the dosage, emphasis was placed on the trunk diameter and canopy volume of the trees. To calculate the recommended active ingredient amount for the technology, we used the efficacy formula developed by Abbott (1925), which allowed us to set dosage precisely to achieve the desired larvicidal effect.

## **2.4. Toxicokinetic Processes**

To monitor the spatial and temporal distribution of active ingredient content the canopy of the tree was divided by cardinal directions, and 4×15

composite leaves were collected randomly from each quarter. For the analysis of active ingredient content in husks and walnut samples, 100-150 husked fruits were collected from different parts of the canopy of each tree. These samples were then divided into three equal-sized sections for analytical purposes. The goal of the quantitative determination was to observe the movement of the active ingredients in the plant and confirm their practical applicability. To track the temporal changes of the active ingredient content, samples were taken several times during the growing season.

## **2.5. Toxicological Considerations**

The active ingredient content of the walnut kernel was measured for each experimental setup to ensure the safety of the recommended active ingredients for this technology. From treatments that have proven biologically successful (ABA, EMA, ACE), approximately 500 grams of male inflorescences were collected from each tree in the following spring. These inflorescences were collected randomly from all parts of the canopy. The purpose of the evaluation was to determine whether the active ingredient was present in the inflorescence and, if so, in what quantity.

## **2.6. Timing of the Treatment**

The importance of the injection timing was demonstrated through two stages (21 days apart) with treatments using three different doses of ABA. The larvicidal effect and the corresponding active ingredient concentration in the husks were assessed.

## **2.7. Duration Effect**

The duration of the effect of the treatments was monitored over two growing seasons, tracking the concentrations of active ingredients (ABA, EMA) in different plant organs (leaf, husk) and assessing the larvicidal effect.

## **2.8. Measurement Techniques and Sample Preparation**

Active ingredient measurements were carried out using an Agilent Ultivo triple quadrupole (QqQ) mass spectrometer connected to an UHPLC system, where the components of the samples were separated by liquid chromatography, and the separated components were detected and identified using mass spectrometry (MS/MS).

Samples were prepared using the citrate-buffered QuEChERS method, developed for plant-based food samples (EN 15662:2018) (European Standard – EN 2018).

## **3. Results and Discussion**

### **3.1. Active Ingredient Selection**

The pesticides deemed suitable based on their physicochemical parameters (ABA, EMA, ACE, FLU, SPI) were all able to reach the canopy via the water flow in the xylem. Increasing the dosage resulted in higher detected active ingredient content in plant tissues, with one exception. The SPI formulation was a suspension concentrate (SC), which is not advantageous for trunk injection. Due to the oversaturation of the solutions, higher-dose SPI treatments resulted in lower detected active ingredient levels, likely related to the low solubility of the SC formulation. Another explanation could be that higher-dose mixtures faced increasing toxicokinetic challenges. During SPI injection, the solid suspension particles recrystallized upon re-entering the aqueous medium and could have clogged the cell wall pathways.

Unlike injections, adjuvants play a crucial role in basal bark spray application. This method requires a formulation that allows active ingredients to penetrate through the bark and then behave similarly to injection. Among the active ingredients, ACE and FLU penetrated in notable amounts, while avermectins, due to their large molecular size, were unable to do so. SPI was detected in minimal amounts in the foliage, likely for reasons similar to those

mentioned earlier. This disadvantage could potentially be mitigated by switching formulations. No differences were found in the efficacy of the two applied adjuvants (PB and I) based on the active ingredient concentrations in leaves (Mann–Whitney  $U = 44$ ,  $z = 0.310$ ,  $p = 0.796$ ) or husks (Mann–Whitney  $U = 48$ ,  $z = 0.662$ ,  $p = 0.546$ ).

### **3.2. Efficacy Testing (Larvicidal Effect) and Economic Damage Assessment**

The residual ABA level measured in the walnut pericarp showed a mild positive correlation with the amount of injected active ingredient ( $r_s = 0.478$ ) and a negative correlation with the degree of infestation ( $r_s = -0.455$ ), although these variables were not significantly related ( $p = 0.116$  and  $p = 0.138$ , respectively) (Figure 1). The average ABA content in the husk did not result in significant differences between treatments with varying doses (Kruskal–Wallis  $H = 3.00$ ,  $df = 3$ ,  $p = 0.392$ ).

While the proportion of husks infested with larvae reached 100% in the control group, it was much lower with treatments. The lowest-dose ABA treatment resulted in the weakest insecticidal effect, whereas significantly better larvicidal efficacy was observed in trees treated with higher doses of ABA.

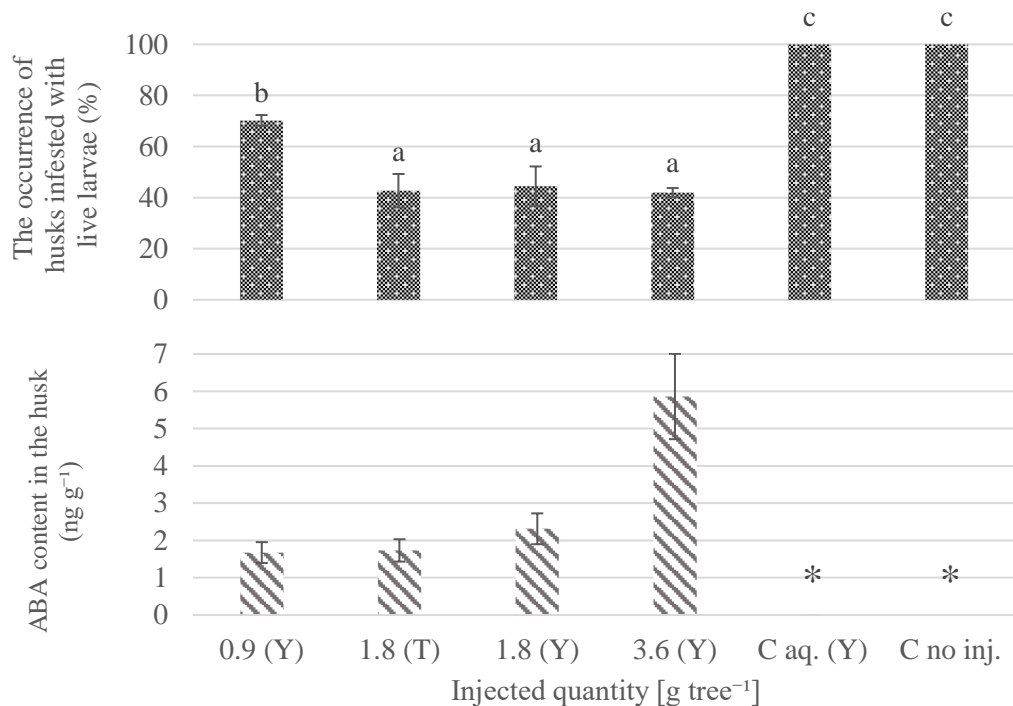


Figure 1. Infestation level (upper) and the active ingredient content (lower) (mean  $\pm$  SE) as a result of different doses of ABA (abamectin) trunk injection. For treatments marked with the same letter, the infestation does not differ significantly based on the Marascuilo comparison ( $p > 0.05$ ). C<sub>aq.</sub>: water control, C<sub>no inj.</sub>: without injection, \* DL (detection limit): 1.2 ng g<sup>-1</sup>.

In the case of EMA, a negative correlation was found between the husk active ingredient content and the infestation rate ( $r_s = -0.867$ ,  $p = 0.002$ ), but there was no correlation between the injected active ingredient quantity and the active ingredient residue ( $r_s = 0.316$ ,  $p = 0.407$ ) (Figure 2). Based on the average active ingredient content in the husks, the treatments did not differ significantly (Kruskal–Wallis  $H = 5.067$ ,  $df = 2$ ,  $p = 0.079$ ). All three doses significantly reduced the number of larvae-infested husks. Compared to the 100% infestation observed in the control, at 1.9 g tree<sup>-1</sup> injected active ingredient, the frequency of larvae-infested husks was 32%, at 3.8 g tree<sup>-1</sup> it was 22%, and at 5.7 g tree<sup>-1</sup> it was 33% (Figure 2).

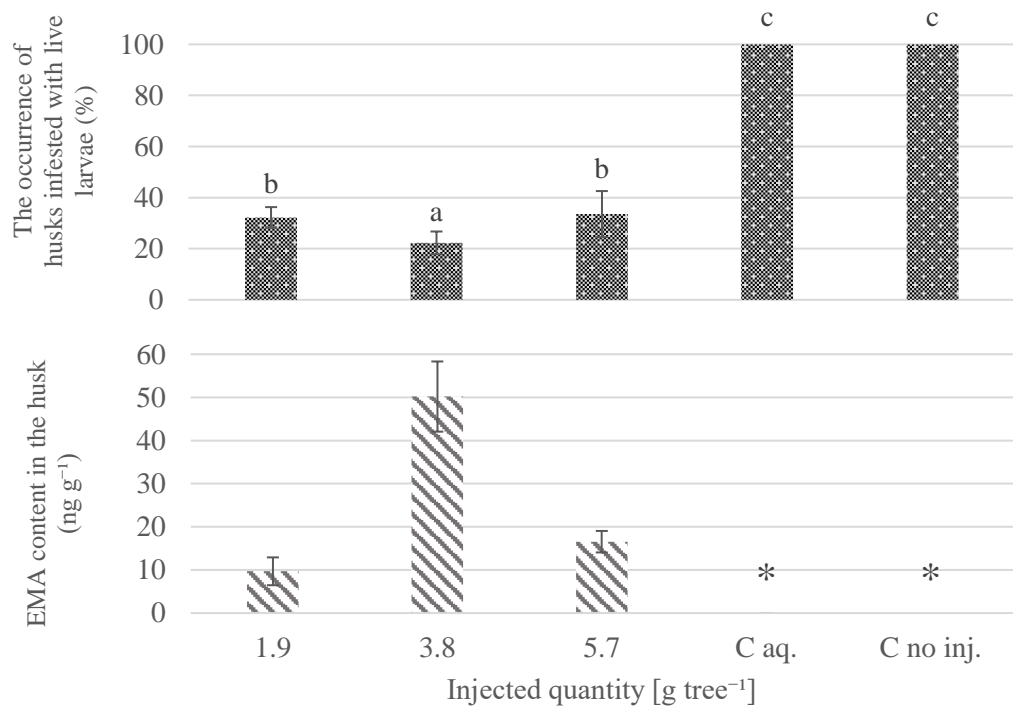


Figure 2. Infestation level (upper) and the active ingredient content (lower) (mean  $\pm$  SE) as a result of different doses of EMA (emamectin benzoate) trunk injection. For treatments marked with the same letter, the infestation does not differ significantly based on the Marascuilo comparison ( $p > 0.05$ ). C<sub>aq.</sub>: water control, C<sub>no inj.</sub>: without injection, \* DL (detection limit): 0,1 ng g<sup>-1</sup>.

In the case of ACE, a negative correlation ( $r_s = -0.829$ ,  $p = 0.042$ ) was found between the husk active ingredient content and the infestation, but no significant correlation between the injected active ingredient quantity and the active ingredient residue content ( $r_s = -0.488$ ,  $p = 0.326$ ) (Figure 3). The active ingredient content in the husks did not result in a significant difference here either (Mann–Whitney  $U = 2$ ,  $z = -1.091$ ,  $p = 0.4$ ).

Both doses reduced the number of larvae-infested husks. Compared to the 100% frequency observed in the control, at 5 g tree<sup>-1</sup> injected active ingredient, the frequency of larvae-infested husks was 31%, and at 10 g tree<sup>-1</sup>, it was 56%. In the case of ACE, the higher dose resulted in lower efficacy (Figure 3).

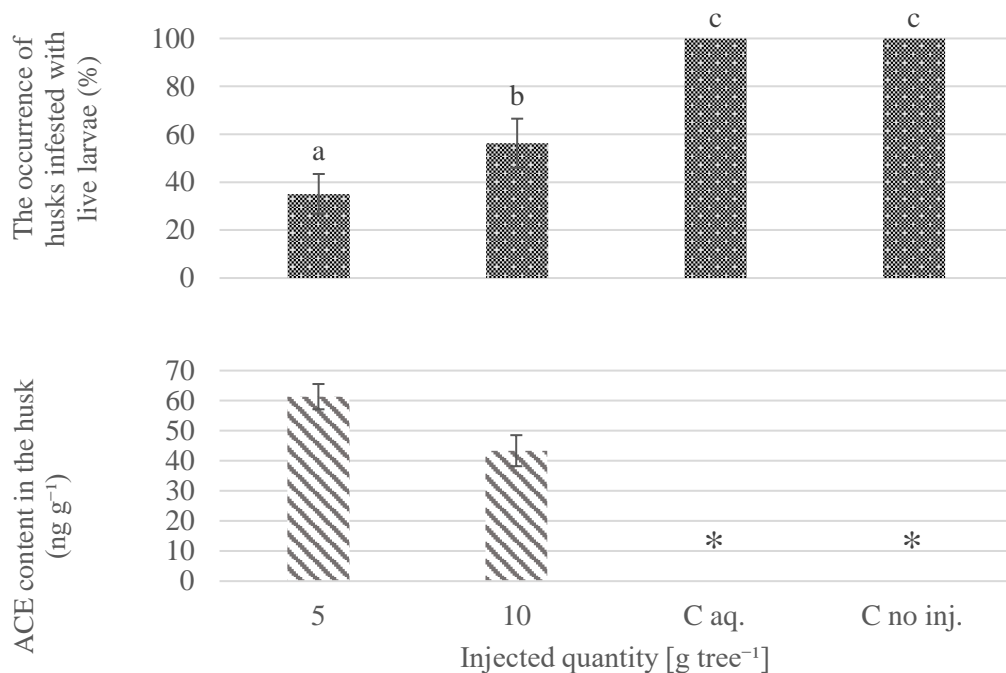


Figure 3. Infestation level (upper) and the active ingredient content (lower) (mean  $\pm$  SE) as a result of different doses of ACE (acetamiprid) trunk injection. For treatments marked with the same letter, the infestation does not differ significantly based on the Marascuilo comparison ( $p > 0,05$ ). C<sub>aq.</sub>: water control, C<sub>no inj.</sub>: without injection, \* DL (detection limit): 0.25 ng g<sup>-1</sup>.

In the evaluation of our experiments, it often happened that some larvae survived the treatment, but the extent of the damage caused was far below the damage observed in the control group. While the frequency of occurrence was increased by the presence of surviving larvae exposed to sublethal doses, the economic damage was minimal. Therefore, husk infestation does not necessarily mean that the crop is damaged and, as a result, impedes processing. Since the level of infestation does not equate to the level of economic damage, we considered it important to also express the extent of economic damage (Figure 4).

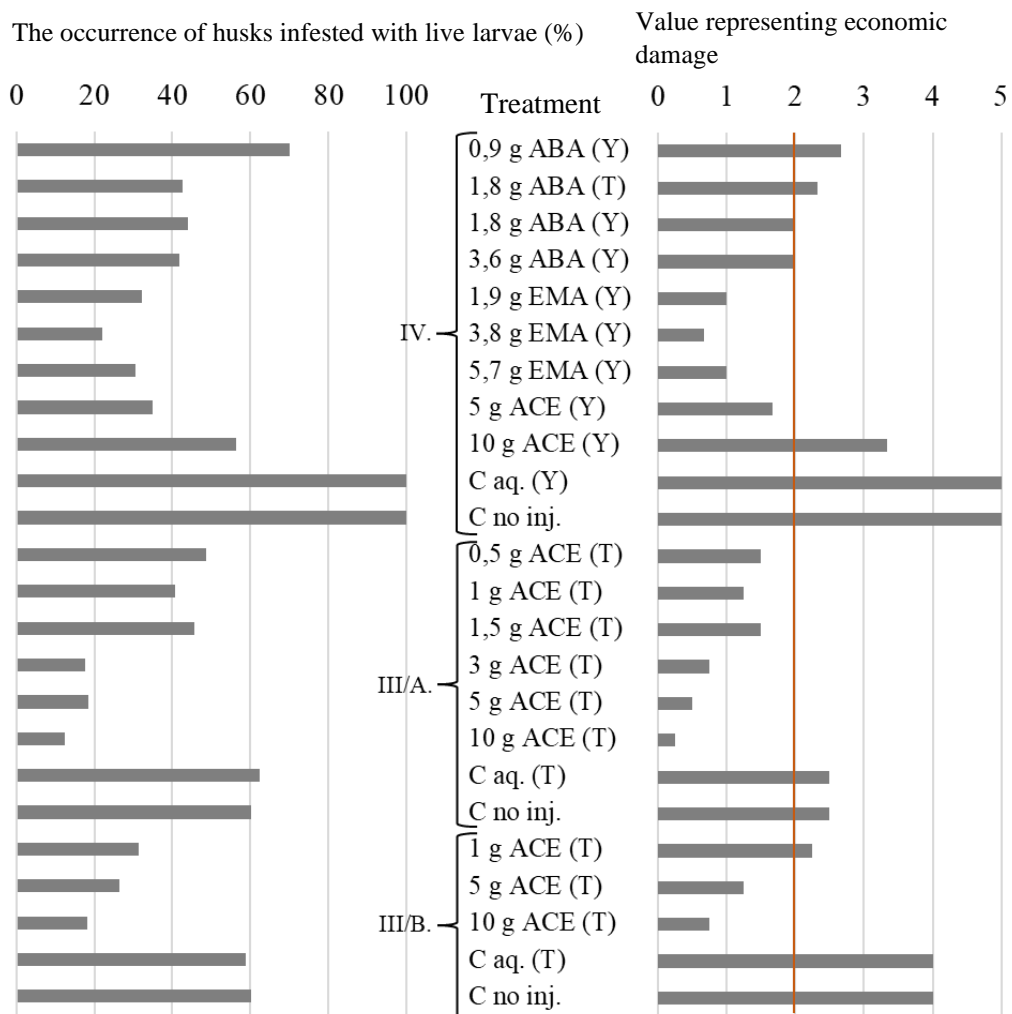


Figure 4. The relationship between infestation and economic damage. ABA: abamectin, EMA: emamectin benzoate, ACE: acetamiprid, C aq.: water control, C no inj.: without injection.

Based on Figure 4, ABA was less effective at keeping the economic damage below the acceptable level characterized by a value of 2, although the higher doses approached this level. From this perspective, EMA was much more favorable by a value of 1, while ACE, with a few exceptions, also resulted in an acceptable level of economic damage.

### 3.3. Efficacy Testing

Manufacturers recommend adjusting the dosage based on the trunk diameter individually measured at a height of 135 cm (Schulte et al., 2006;

Smitley et al., 2010), which, in ideal cases, is a suitable ratio but can sometimes lead to inaccurate dosing. This is because the increase in trunk diameter does not always correspond directly to an increase in dosage if the ratio between the protected canopy and the trunk diameter changes. To avoid this, more accurate dosing could be achieved by considering the canopy volume as well. We compared these two methods of dosage adjustment. In the case of ABA and EMA active ingredients, the measured active ingredient content in the husk and the amount of active ingredient adjusted to the canopy volume showed significant correlations ( $r_s = 0.594$ ,  $p = 0.042$  and  $r_s = 0.833$ ,  $p = 0.005$ ).

### **3.4. Toxicokinetic Processes**

We found no significant differences between the cardinal directions and the active ingredient content in the canopy for any of the studied active ingredients (ABA, EMA, ACE):  $F(3, 24) = 0.391$ ,  $p = 0.761$ ,  $\eta^2 = 0.47$ ;  $F(3, 24) = 0.750$ ,  $p = 0.533$ ,  $\eta^2 = 0.86$ ;  $F(3, 16) = 0.741$ ,  $p = 0.543$ ,  $\eta^2 = 0.122$ . Therefore, the direction of incoming sunlight did not influence the active ingredient content in the canopy.

The active ingredient content detected in the leaf samples showed a decreasing trend over time. Generally, we found a positive correlation between the dosage and the active ingredient content in the leaves. However, in some experiments, this was different, and periodic fluctuations in the active ingredient content were observed. We hypothesize that the fluctuations in the active ingredient content in the leaves are due to two factors: (i) the size of the active ingredient reservoir in the trunk and (ii) the amount of water transported.

### **3.5. Toxicological Considerations**

Based on our experiments, we considered the ABA and EMA to be the most favorable active ingredients from a food safety perspective. The residue levels in the fruits treated with ABA did not exceed the detection limit ( $2.4 \text{ ng g}^{-1}$ ), let alone the MRL ( $10 \text{ ng g}^{-1}$ ), neither in dry nor fresh walnut kernels. In trees treated with the EMA, the active ingredient residue in the walnut kernels

was also below the detection limit ( $0.2 \text{ ng g}^{-1}$ ) in dry kernels. Similarly low residue values were measured in fresh walnut kernels, with one exception, where after a  $0.95 \text{ g tree}^{-1}$  dose, the residue level was  $0.5 \text{ ng g}^{-1}$ , still orders of magnitude lower than the MRL ( $5 \text{ ng g}^{-1}$ ). The residue of ACE exceeded the maximum residue limit (MRL =  $70 \text{ ng g}^{-1}$ ), and even the lowest dose of FLU far exceeded the MRL ( $20 \text{ ng g}^{-1}$ ). Therefore, we set up fewer experiments with these active ingredients because even low-dose treatments resulted in high pesticide residue concentrations, making endotherapy applications unfeasible. The SPI performed well from a food safety perspective (MRL =  $500 \text{ ng g}^{-1}$ ), but due to the solubility issues of the available formulation, it was not preferred for further experiments.

In flower samples collected from trees injected in the previous year, the ABA concentration was below the detection limit, while EMA was detected in very small amounts. No differences were found between the various dosages in any of the experimental setups ( $t(2) = 0.089$ ,  $p = 0.468$ ) and ( $F(2, 6) = 1.044$ ,  $p = 0.408$ ), with similar concentrations observed in the flowers. The ACE content in the flowers was found to be higher in this experiment, but from a bee toxicity perspective, this is not considered harmful, as ACE falls under the "no hazard to bees" category. Different dosages did not result in significant differences in the amount of active ingredient measured in the flowers ( $t(4) = 0.017$ ,  $p = 0.493$ ). The amount of all three active ingredients in the flowers was below concentrations that would cause harmful effects.

### **3.6. Duration Effect**

Over two growing seasons (57–483 DAT), monitoring the active ingredient content in the leaves revealed that the injected ABA and EMA were present in significantly lower quantities in the second year. The quantity of active ingredient injected into the trees positively correlated with the amount detected in the leaves only during the year of application. The measured levels of active ingredients displayed a declining trend over time.

Notably, a significant difference was observed in the rate of change between the two active ingredients. ABA achieved lower concentrations in the year of injection but showed a much slower rate of decline. Conversely, EMA reached higher concentrations during the year of treatment but decreased to ABA's level by the second growing season. This difference in initial concentrations is likely due to the difference in water solubility between the two active ingredients. The greater solubility of EMA allowed the xylem-transported water to extract larger amounts from the reservoir formed around the injection site. Consequently, EMA experienced a significant reduction by the second growing season, as the available reservoir was presumably insufficient to maintain high concentrations in the canopy.

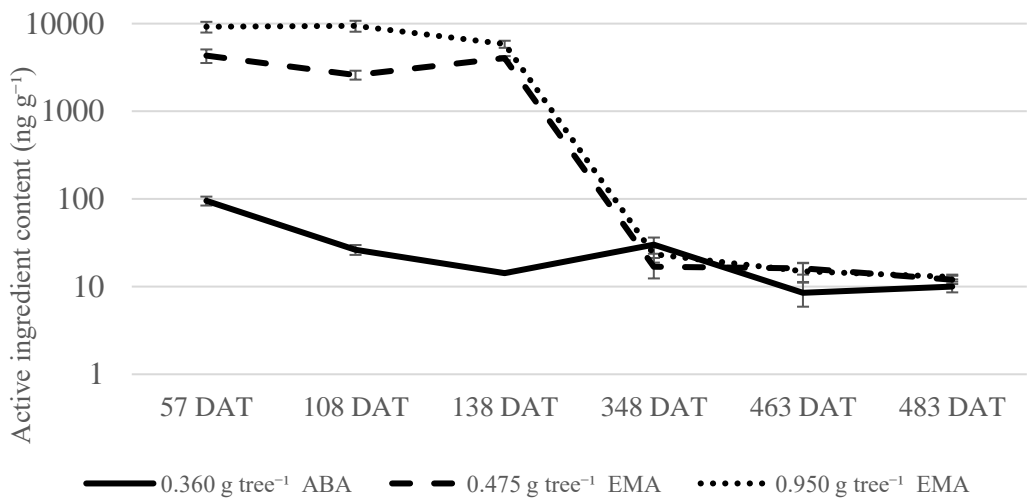


Figure 5. Decrease in leaves active ingredient content over two growing seasons. DAT: days after treatment, ABA: abamectin, EMA: emamectin benzoate

Figure 6 shows that in the year of injection, both tested doses successfully protected the crop. In the first year, both doses ('a' and 'b') resulted in significantly fewer husks infested with larvae compared to the control ('c'). The average ABA content measured in the pericarp was  $2.27 \pm 0.73 \text{ ng g}^{-1}$  ( $0.18 \text{ g tree}^{-1}$ ) and  $2.41 \pm 0.13 \text{ ng g}^{-1}$  ( $0.36 \text{ g tree}^{-1}$ ), respectively (Figure 6). One year later, while some residual insecticidal effect was still detectable, the infestation rate (64–75%, 'a') was only slightly lower than in the control (90%, 'b'), and the

active ingredient content in the husks could only be detected in the 0.36 g tree<sup>-1</sup> treatment. This reduced larvicidal effect was insufficient to prevent economic damage.

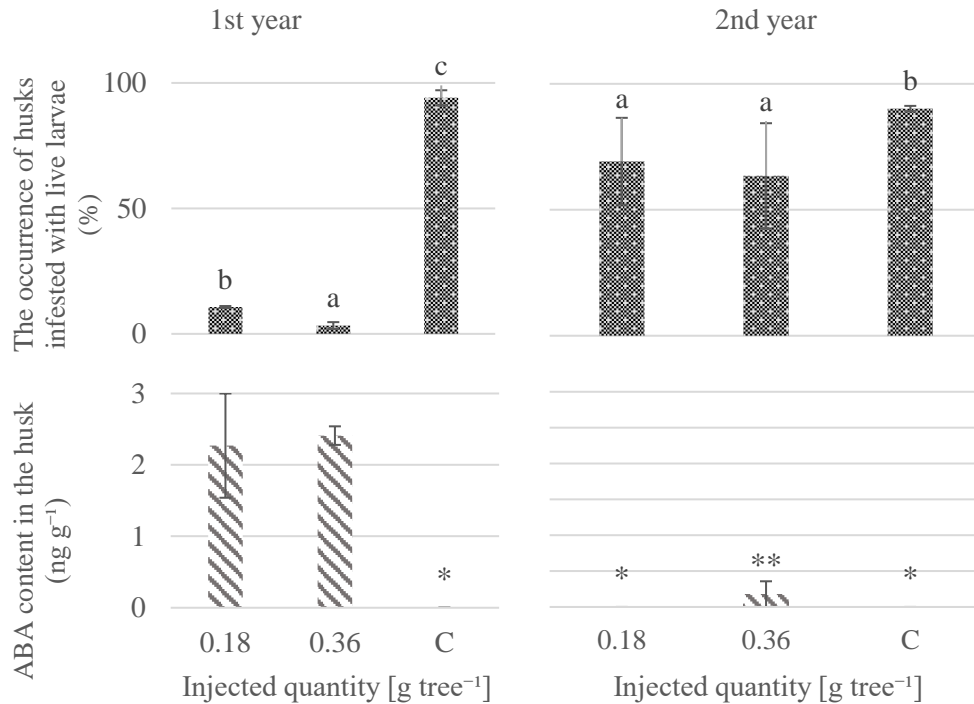


Figure 6. Changes in husk infestation rate and active ingredient content (mean  $\pm$  SE) at ABA (abamectin) trunk injection. Treatments marked with the same letter in the columns do not differ significantly in infestation rates according to the Marascuilo comparison ( $p > 0.05$ ). DL (detection limit): 1.2 ng g<sup>-1</sup>. \*: <DL, \*\*: trace amount, C: control, representing the average of C<sub>no inj.</sub> and C<sub>aq.</sub>

In the year of injection, the number of fruits infested by the walnut husk fly on trees treated with ABA and EMA was significantly lower compared to the control (Figure 7). For the lower dose of ABA, infestation was 35%, and for the higher dose, it was 25%, compared to 85% and 93% observed in the controls. The ABA content measured in the pericarp was 0.65  $\pm$  0.06 ng g<sup>-1</sup> and 1.56  $\pm$  0.18 ng g<sup>-1</sup>, respectively. Treatments with EMA proved to be even more effective, resulting in 14% infestation at 0.475 g tree<sup>-1</sup> and 10% infestation at 0.95 g tree<sup>-1</sup>, with the husks containing 7.65  $\pm$  0.96 ng g<sup>-1</sup> and 16.61  $\pm$  1.55 ng g<sup>-1</sup> of active ingredient, respectively.

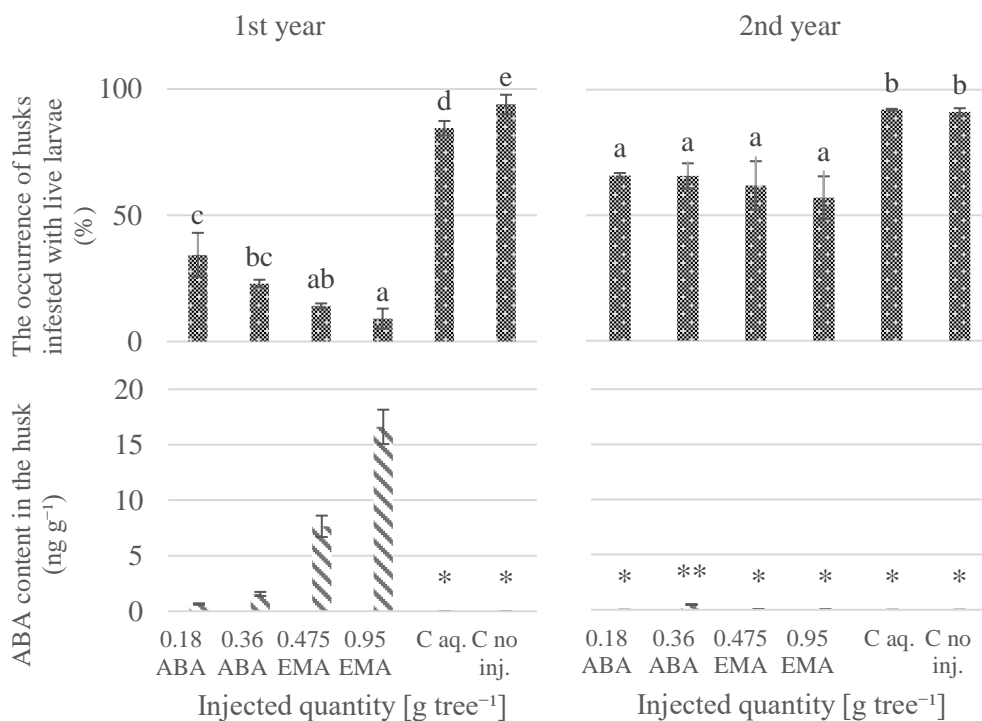


Figure 7. Changes in infestation rates and active ingredient content (mean  $\pm$  SE) of husks following trunk injection with ABA (abamectin) and EMA (emamectin benzoate). Infestation levels within columns marked with the same letter are not significantly different according to the Marascuilo comparison ( $p > 0.05$ ). C aq.: water control, C no inj.: without injection, Detection limit for ABA: 1.2 ng g<sup>-1</sup>, Detection limit for EMA: 0.2 ng g<sup>-1</sup>, \*: <DL, \*\*: trace amounts.

Although several studies have reported the multi-year effects of trunk injection, the behavior of different host and pest species can vary significantly (Eisenbach et al., 2014; Wang et al., 2020). In this case, the insecticidal effect of the injections persisted into the second year, but with reduced efficacy compared to the first year. Infestation rates in the second year were 65% for ABA and 60% for EMA, compared to 92% in the control. This trend was confirmed by a significant reduction in the active ingredient content measured in the husks compared to the previous year.

### 3.7. Timing of the Treatment

The optimal period for trunk injection is considered to be between late May and early June (BBCH 65-73). Compared to this timeframe, ABA treatments

conducted 21 days later resulted in weaker biological effects, even with higher concentrations (Figure 8). This is because the active ingredient must be present in the plant at the appropriate concentration by the time of hatching, as effective control is primarily attainable during the early larval stages.

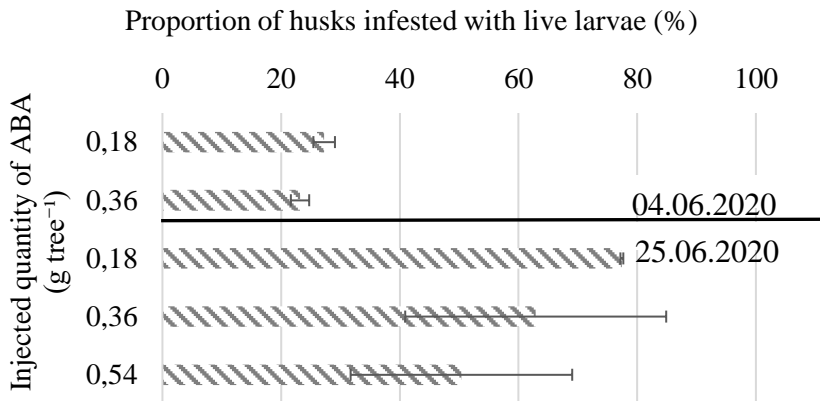


Figure 8. The impact of timing on the efficacy of ABA (abamectin) treatments.

The efficacy of trunk injection is influenced by several factors (Doccola et al., 2011), with the timing of the injection being particularly significant. In general, during leaf flushing, the distribution of dissolved substances within the plant is more uniform and rapid compared to late summer or during dormancy. Therefore, trunk injections are most effective when the trees are actively transpiring (Li and Nangong, 2022).

#### 4. Conclusions and Recommendations

The trunk-injected active ingredients (ABA, EMA, ACE, FLU, SPI) were all detected in parts of tree far from the injection point, including leaves, fruit husks, and in some cases, the kernel and inflorescences. Some active ingredients used in basal bark spray application (ACE, FLU, SPI) were similarly present in the leaves and the husks. While basal bark spray seems a promising technique, it has a more limited range of applicable active ingredients and suitable plant species.

ACE was detected in significant concentrations not only in green plant parts but also in the walnut kernel. Despite its insecticide effectiveness, it is not a viable option for either injection or basal bark spray application due to food safety concerns. From a food safety perspective, ABA and EMA are recommended, as their residue levels in the walnut kernel do not pose risk.

The successful transport of active ingredients (ABA, EMA, ACE) to the husk resulted in causing larval mortality. Dead, desiccated larvae and eggshells were found beneath the exocarp. The small black, dry spots around oviposition sites did not result in quality loss of either the shell or the kernel.

The correct dosage determination should also account for foliage volume, not just trunk diameter. In older orchards or for solitary trees, it is advisable to consider canopy volume, as variations in husk active ingredient content – and consequently, larval mortality rates – can differ between trees with the same trunk diameter but different canopy sizes.

Based on our results, for setting the appropriate dosage, the recommended active ingredient dose for ABA is 15-20 mg m<sup>-3</sup> canopy (Figure 9), and for EMA,

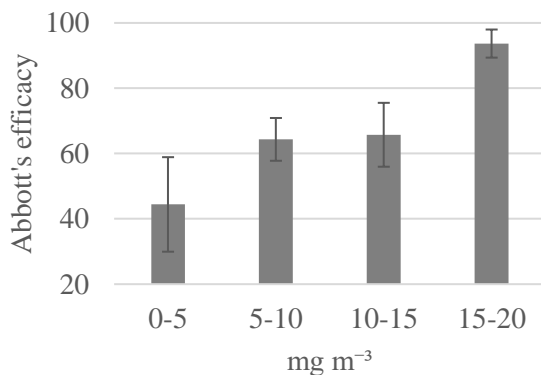


Figure 9. The dosage of ABA (abamectin) when dosed according to the canopy volume.

30-35 mg m<sup>-3</sup> canopy (Figure 10), which should provide an expected efficacy of 90%. The risk of underdosing is higher for ABA than for EMA, where even the lowest quantified dosage range (0-5 mg m<sup>-3</sup>) was associated with nearly 70% efficacy. The efficacy was calculated based on the

occurrence of husks infected with live larvae, which may seem lower than the expected values in pest control practice. However, considering the economic damage threshold, the efficacy of the treatments was acceptable even at the lower dosage ranges in practice.

In the second year after injection lower efficacy was observed concluding that for successful pest control using this type of injection method with ABA and EMA, it is necessary to treat the walnut trees every

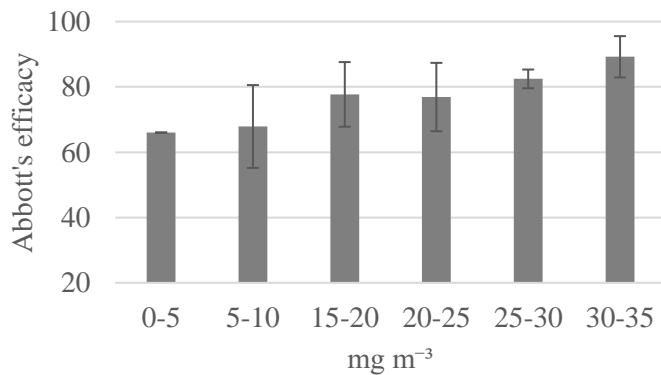


Figure 10. The dosage of EMA (emamectin benzoate) when dosed according to the canopy volume.

year. Although the literature reports multi-year satisfactory effects for endotherapeutic treatments on other plant species (Anulewicz et al., 2009; Holderness, 1992; Percival and Boyle, 2005; Wheeler et al., 2020), our studies indicated that protection against the walnut husk fly was only provided for one growing season.

In our opinion, the application of endotherapy not only provides adequate protection against the walnut husk fly but, by using the right combination of plant protection products, can also give protection against fungal and bacterial infections.

For the successful application of trunk injection, it is crucial to make the plant protection products suitable for endotherapy. When designing these, it is important to consider not only their physicochemical properties but also the species to be treated, the operation principle of the tools used, the injection pressure, as well as the pest resistance or tolerance to the pesticides.

The effectiveness of injection is influenced by several factors (Docola et al., 2011), among which the timing of the treatment is particularly significant. We consider the optimal period to be late May to early June (BBCH 65-73).

The injection-based plant protection technology can achieve similar effectiveness to the modern spraying technology used in larger operations. However, with the latter method, the number of treatments in a normal year can

be around 4-5, which significantly increases the costs of walnut production and the pesticide load on orchards, in addition to the risks associated with timing the treatments. The mechanization of the injection method, and thus its application in orchards, will soon be feasible with the development of robotics (Chengzhang et al., 2019).

In Hungary, a big portion of the walnut production comes from extensive walnut orchards, not to mention the individual backyard walnut trees that form the basis of annual walnut consumption for families living in the countryside. In these areas, plant protection methods similar to those used in commercial walnut orchards are not available. Therefore, trunk injection is recommended for these areas.

## 5. New Scientific Results

- We were the first to confirm that damage caused by the walnut husk fly (*Rhagoletis completa* Cresson, 1929) can be prevented through the endotherapeutic use of abamectin and emamectin benzoate.
- We refined the method of dosing active ingredients based on trunk diameter by emphasizing the necessity of considering canopy volume.
- We determined that on walnut trees (*Juglans regia* L., 1753), endotherapeutic treatment with abamectin and emamectin benzoate provides satisfactory protection against the walnut husk fly, but only in the year of injection, with no significant efficacy in the second year.
- We were the first to confirm that acetamiprid, flupyradifurone, and spirotetramat can reach canopy of walnut trees through basal bark spray, whereas abamectin and emamectin benzoate do not reach the canopy using the same method.
- During endotherapeutic treatment, the residue of abamectin and emamectin benzoate measured in walnut kernels remained below 37%

(DL) and 10% of the MRL values, respectively. Therefore, based on our findings, the technology poses no risk from a food safety perspective.

## 6. List of Publications

Article published in a peer-reviewed journal with impact factor:

- Kiss, M., Hachoumi, I., Nagy, V., Ladányi, M., Gutermuth, Á., Szabó, Á., Sörös, C. (2021): Preliminary results about the efficacy of abamectin trunk injection against the walnut husk fly (*Rhagoletis completa*). *Journal of Plant Diseases and Protection*, 128 (1), 333-338. p. (Q2)
- Kiss, M., Sörös, C., Gutermuth, Á., Ittész, A., Szabó, Á. (2023): Avermectin trunk injections: a promising approach for managing the walnut husk fly (*Rhagoletis completa*). *Horticulturae*, 9(6), 655. (Q1)

Article published in a peer-reviewed journal:

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