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An Evaluation of Insecticidal Trunk Injections for the Control of the European Cherry Fruit Fly *Rhagoletis cerasi* L. (*Diptera: Tephritidae*)

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Abstract: Cherry productivity is challenging in Europe due to the presence of the cherry fruit fly (*Rhagoletis cerasi*). Trunk injection is an alternative method of insecticide delivery that may improve pesticide performance in controlling pests. In our study, four pesticides (abamectin, acetamiprid, flupyradifurone, and cyantraniliprole) were investigated for trunk injection against *R. cerasi*. Acetamiprid trunk injection in a minimum dose of 0.56 g/tree was suitable for protection against the pest, the efficacy of the control was over 95%, and the pesticide residue concentrations in the fruits were below the maximum permissible limit in each experiment. The trunk injection and foliar spray of the same dose of acetamiprid were equally effective. In the case of the other three active ingredients (abamectin, flupyradifurone, and cyantraniliprole), the trunk injection method did not prove suitable for practical use due to various reasons, which are detailed in the manuscript.

Keywords: cherry; trunk injection; endotherapy; *Rhagoletis cerasi*; acetamiprid; abamectin; cyantraniliprole; flupyradifurone

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Citation: Gyuris, R.; Szabó, Á.; László, A.M.; Gutermuth, Á.; Sörös, C. An Evaluation of Insecticidal Trunk Injections for the Control of the European Cherry Fruit Fly *Rhagoletis cerasi* L. (*Diptera: Tephritidae*). *Horticulturae* **2024**, *10*, 278. https://doi.org/10.3390/horticulturae10030278

Academic Editor: Carmelo Peter Bonsignore

Received: 5 February 2024
Revised: 6 March 2024
Accepted: 10 March 2024
Published: 14 March 2024

1. Introduction

Cherries are an important crop in industrial fruit cultivation in Hungary and are also one of the few fruit species that can be easily grown in home gardens. In 2021, the Hungarian cherry productivity was 2.78 tons/hectare [1], while the rest of Europe grew an average of 4.31 tons/hectare. The largest cherry-growing countries in Europe are Poland, Spain, Italy, and Germany [2]. In Hungary, the cherry fruit fly, *Rhagoletis cerasi* (Linnaeus, 1758, *Diptera: Tephritidae*), is the most important plant-protection problem in cherry cultivation and is the main pest of cherries in Western and Central Europe [3]. The larvae feed on the inside of the fruit, causing rotting and loss of the cherry. The damage can lead to significant economic losses of up to 100% [4–6]. Even a small proportion of cherries infested with larvae are unacceptable for the consumer and processing industries [7].

Summer management of *R. cerasi* (and *R. cingulata*) involves multiple spraying sessions due to the long development cycle of the larvae (more than a month); hence, a minimum of 2–5 foliar treatments with contact or systemic pesticides are required for one season [8]. However, using this method, an estimated 45% of pesticides are lost to drift, which risks non-target exposure [9,10]. Indeed, it has also been reported that as little as 0.4% of pesticides contact the target pest [11]. Various techniques have been developed to minimize environmental pollution during pesticide use. Trunk injections of insecticides or endotherapy are a promising alternative to overcome this problem [11,12]. This method uses the tree's vascular transport capacity, which allows active ingredient (a.i.) translocation and subsequent distribution into the canopy where protection is needed. It targets pests that damage trees [13–16] and can be used for numerous tree species, including orchards, forests, or urban areas where chemical spraying is prohibited or cannot be used [17]. Trunk injection can be used for endotherapy treatment, which has several advantages over conventional spraying: (1) the technique requires less pesticide and water; (2) compounds directed at feeding larvae are directly accessible; (3) the active ingredient has no toxicokinetic difficulty (such as cuticle penetration or abiotic degradation); (4) the closed xylem system provides long-lasting protection for the pesticide against external influences, such as sun radiation; and (5) it is safe for non-target organisms [15,18–20].

Endotherapy treatment is often used in urban plant protection for deciduous trees. Several experiments have demonstrated the successful use of trunk injection against different pests [21–25]. Walnut trees have been effectively treated with abamectin against the walnut husk fly (*Rhagoletis completa*) [24]. The tiger longicorn beetle (*Xylotrechus chinensis*) is a pest to Mulberry (*Morus* sp.) trees, living inside the trees and carving exit holes in the trunk. This pest was also controlled successfully with a trunk injection of abamectin [21]. A single injection of azadirachtin provided season-long control, while abamectin provided two seasons of control against pear psylla [26].

Previously, no scientific articles were published on the combined study of trunk injection and the cherry fruit fly; our work fills a gap in this case. The above-mentioned data indicate that trunk-injection technology can be an effective solution to protect the fruit-growing sector. The amount of applied plant protection agents can be reduced altogether if pests can be reached in a more targeted way. We aimed to investigate the suitability of trunk injection for plant protection in cherry cultivation. In this study, we injected acetamiprid, abamectin, cyantraniliprole, and flupyradifurone and compared their protection against cherry fruit flies during the entire vegetation period. Pesticide residue determination was also conducted at harvesting time, which was compared to spraying.

2. Materials and Methods

2.1. Experimental Sites

Trunk injections were performed during 2021 and 2022 at the same location in Budapest, Hungary (GPS: 47.398334, 19.147432). In both trials, injections were made on 12 May; air temperature was 20 °C, respectively, wind speed was less than 1.5 m/s, and relative humidity was 50% with zero precipitation. In 2022, the average daily temperature was 17.2 °C in May, 22.1 °C in June, and 23.4 °C in July (average: 20.9 °C). In 2023, the average daily temperature was 16.2 °C in May, 19.6 °C in June, and 22.8 °C in July (average: 19.5 °C). Weather data were obtained from a weather station (METOS Hungary) that is within 1 km of the experimental site; this is where the data came from. The size of the plantation was 5 ha, respectively. In both years, the experiments were conducted in the same orchard but in different trees in a randomized complete-block design. No other plant protection treatments were used in the orchard, and no irrigation was used during the whole vegetation period. Trees had an average of 5 m high and a 12–15 cm trunk diameter. The trees stood 5 × 2 m from each other. The cherry tree variety was Prunus avium 'Vera' on Prunus mahaleb rootstocks.

2.2. Trunk Injection Method

Every tree was drilled with four holes, each measuring 3–4 mm wide in diameter and 40 mm long. The injection points were 20 cm above ground level. A trunk injector (Figure 1) was used to inject the liquid into the xylem with a maximum pressure of 12.6 bar. The injection time required was approximately 5 min per tree and person. The cherry trees were easily injected, and the average pressure was around 8 bar. In 2021, 10, 20, or 40 mL pesticides were injected into the trees and evenly distributed into the drilled holes.

For 10 mL volume, 2.5 mL/hole, for 20 mL, 5 mL/hole, and for 40 mL, 10 mL/hole. In 2022, the same amount (40 mL) was injected into every tree but with different pesticide dosages (Table 1). After the injections, the wounds were closed with tree gel (a water-based, air-drying paste for tree wounds; FAGEL, Fenylakk Kft.). In both years, the control trees were injected with 40 mL of water. The injection treatments occurred on 12 May in sunny weather for both years (BBCH 73).

Figure 1. Trunk injector (2022, Budapest).

Table 1. Detailed information about injections and samplings.

Location and Date of Injection	**Active Ingredient**				**Sampling Date (DAT: Days after Treatment)**	
	Name	**Dose**	**g/Tree**	**mL/Tree**	**Fruit Samples**	**Leave Samples**
Budapest, 12 May 2021 (efficacy test)	Abamectin (ABA)	small medium high	0.18 0.36 0.72	10 20 40	23 June 2021 (DAT 42)	- - -
	Acetamiprid (ACE)	small medium high	0.2 0.4 0.8	10 20 40	23 June 2021 (DAT 42)	- - 23 June 2021 (DAT 42)
	Cyantraniliprole (CYA)	small medium high	0.2 0.4 0.8	10 20 40	23 June 2021 (DAT 42)	- - -
	Flupyradifurone (FLU)	small medium high	0.2 0.4 0.8	10 20 40	23 June 2021 (DAT 42)	- - 23 June 2021 (DAT 42)
	Injection control	-	Water	40	23 June 2021 (DAT 42)	-
Budapest 12 May 2022 (dosage evaluation)	Acetamiprid (ACE)	dose 1 dose 2 dose 3 dose 4	0.056 0.56 1.12 2.25	40 40 40 40	15 June 2022 (DAT 34)	- - - 15 June 2022 (DAT 34)
	Flupyradifurone (FLU)	dose 1 dose 2 dose 3 dose 4	0.33 0.66 1.21 3.96	40 40 40 40	15 June 2022 (DAT 34)	- - - 15 June 2022 (DAT 34)
	Injection control		Water	40	15 June 2022 (DAT 34)	-
Budapest 12 May 2022	Spray Control		A. I. (g/tree)		Fruit Sampling	Leaves Sampling
(spray control)	Acetamiprid (ACE)	spraying	1	-	29 June 2022 (DAT 17)	29 June 2022 (DAT 17)
	Flupyradifurone (FLU)	spraying	0.6	-		

The following four insecticidal formulations were used for the 2021 experiment: Mospilan 20 SG containing 200 g/kg of acetamiprid (ACE) as an a.i. (provided by SumiAgro), Vertimec 1.8 EC containing 18 g/L of abamectin (ABA) as an a.i. (provided by Syngenta), Benevia containing 100 g/L of cyantraniliprole (CYA) as an a.i. (provided by FMC), and Sivanto Prime containing 200 g/L of flupyradifurone (FLU) as an a.i. (provided by Bayer). Two repetitions of injections were conducted in 2021 (Table 1).

The following two insecticidal formulations were used for the 2022 experiment: Mospilan 20 SG and Sivanto Prime used the same method as the previous year. The product formulations were intended for foliar application. Three repetitions of injections were conducted in 2022.

We sprayed the same pesticide dosage in 2022 to assess the differences between the two technologies, mostly the residual amount of the active ingredient. The spray control occurred on the same orchard on 12 May 2022; however, it was 30 m away from the injection experiment, which had two repetitions.

2.3. Sampling and Evaluation of Insecticidal Effects

Fruit samples were obtained to examine insecticidal effects and pesticide residue analysis. Leaves were also collected for pesticide residue analysis. Fruit samples were collected 42 days after treatment (DAT) in 2021 and 34 DAT in 2022 due to weather differences. During fruit sampling, 100 cherries were collected randomly from each tree, evenly distributed from the four cardinal directions and the upper and lower regions of the trees. We also collected 50 g of leaves from each tree (treated with the highest dose) to check the toxicokinetic behavior of active substances (Table 1). The fruit samples were first examined for insecticidal effects. Fruits were cut, and seeds were removed from the fruit after being examined with a stereo microscope (Zeiss Stemi 2000; ZEISS, Jena, Germany). If a live or dead larva was found inside the fruit, it was declared infected. Abbott's formula was used to evaluate insecticidal effects. Abbott's formula is commonly used to calculate plant protection product efficacy [27].

Abbott's formula calculation:

$$\text{Efficacy (\%)} = (1 - \text{n in T after treatment/n in Co after treatment}) \times 100$$

where n—insect population; T—treated; and Co—control.

After the insecticidal examination, each collected fruit sample was homogenized with a blender. Afterward, the samples were stored in a freezer at −80 °C until chemical analyses of pesticide residue. The collected leaves were also stored in plastic bags in the freezer until chemical analyses.

2.4. Analyses of Pesticide Residue

The pesticide residues in the samples were extracted in accordance with EN 15662:2018 [28] using a citrate-buffered QuEChERS sample preparation method. Fruit samples from cherry trees (without seeds) were collected in both years, and the control trees had 100% damage. According to the regulations, the weight of the fruit pulp must be calculated from the core. An Agilent Ultivo UHPLC-MS/MS system equipped with a triple quadrupole (QqQ) analyzer and mass spectrometry detector was used for the measurements. The system consisted of a high-pressure pump, an autosampler, and a column thermostat unit. The separation was performed on an Agilent ZORBAX RRHD Eclipse Plus C18 (1.8 µm, 2.1 × 50 mm) column, while an ESI ion source (electrospray ionization) was used for molecular ion formation [29]. The four pesticides and a surrogate standard, triphenyl–phosphate, were eluted from the column using (A) 5 mM ammonium formate, 0.1% formic acid in water, (B) 5 mM ammonium formate, and 0.1% formic acid in MeOH mobile phases through a gradient elution program with 6 min of total run time and a flow rate of 0.4 mL/min. The injection volume was 5 µL. The ion-source-dependent conditions were as follows: the gas temperature was 250 °C, the gas flow was 12 L/min, the nebulizer pressure was 40 psi, and the capillary voltage was 3000 V in positive-ion mode. Nitrogen was employed as a desolvation and cone gas. A multi-compound method was developed and validated in-house according to EU SANTE/11312/2021 [30]. The detection limits of abamectin, acetamiprid, cyantraniliprole, and flupyradifurone were 5.0, 0.2, 1.0, and 0.2 ng a.i./g cherry, respectively. The system was used in multiple reaction monitoring modes for target component analyses. The quantified (and qualified) mass transitions of ABA, ACE, CYA, and FLU were 890.5/567.1 (305.1), 223.0/126.1 (90.1), 475.0/444.0 (296.0), and 289.0/245.0 (125.9), respectively. For the measurements, we used matrix-matched calibration methods based on a blind cherry matrix and blind cherry leaves.

2.5. Statistical Analyses

Aligned rank transformation (ART) ANOVA [31,32] was used to compare data from 2021 between two active ingredients (ACE and FLU) for active ingredient residues (n = 12) and between four active ingredients (ABA, ACE, CYA, and FLU) for Abbott efficacy (n = 24) using different injecting doses (small, medium, and high) because residuals of the two-way ANOVA model were not normally distributed. To compare active ingredient residues and the Abbott efficacy measured in 2022 (n = 24) between two active ingredients (ACE and FLU) using four dose groups (1, 2, 3, and 4), a two-way ANOVA [33,34] model was applied. A two-way ANOVA was also used to compare the Abbott efficacy measured in 2022 (n = 10) between spraying and injecting for two active ingredients (ACE and FLU). When the model fit was unsatisfactory (active ingredient residue), Box-Cox [35] data transformation was applied to improve the model fit. The normality of the model residual was checked via skewness, kurtosis, and the d'Agostino normality test [36], in addition to the QQ plot and histogram. The homogeneity of variance was checked using a boxplot and Bartlett's or Levene's test. To compare active ingredient residues and the Abbott efficacy between doses for each active ingredient and between active ingredients via doses for data measured in 2021 ART-C contrast tests [37] and for data measured in 2022, a contrast test followed by a Holm p-value adjustment was run as pairwise comparisons. All statistical tests were two-sided, with the significance level set at 5% (except for the d'Agostino test, where alpha = 0.01). The statistical software SAS Studio (SAS OnDemand for Academics, Release 3.81, SAS Institute Inc., Cary, NC, USA) was applied for analyses.

3. Results

Our objective for the first-year experiment was to select suitable and effective active ingredients for the endotherapy of cherry trees against cherry fruit flies. In the second year, we aimed to determine the minimum effective dosage of the preselected pesticides.

3.1. Efficacy Test in 2021

Protecting fruits against *R. cerasi* larvae was slightly different in terms of active ingredients. We detected a high protection rate (>95%) in trees treated with ABA, FLU, and ACE; however, in the case of CYA, the treatment was ineffective (Figure 2). Based on the protection rate, the three injected pesticides (ABA, ACE in all doses, and the highest dose of FLU) were appropriate for plant protection. It is worth noting that tree leaves treated with FLU showed slight foliar toxicity, and leaf edges started to turn yellow and brown. The infection rate of control tree fruits was 100%; all the collected fruits had larvae inside.

The average variation in Abott's efficacy between the investigated groups was influenced by the active substances and the doses together ($F(6,12) = 6.25$, $p = 0.0036$). Figure 2 shows that the three doses of ABA and ACE resulted in very similar efficacy rates, while in the case of CYA and FLU, the higher the dose, the greater the efficiency. However, the statistical pairwise comparison showed no significant difference between the doses for each active ingredient. We also compared the active ingredients by dose, and in one case, we found a significant difference at the lowest dose between the CYA and ACE pairs ($p = 0.042$).

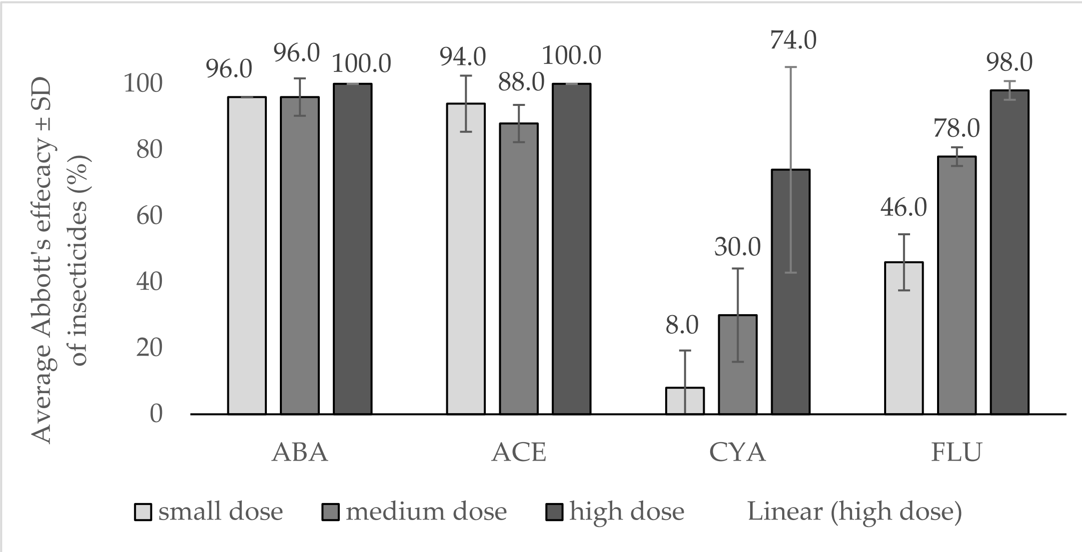

Figure 2. Average Abbott's efficacy for insecticides (%); injected small dose: 0.18 g/tree ABA, 0.2 g/tree ACE, CYA, and FLU; medium dose: 0.36 g/tree ABA, 0.4 g/tree ACE, CYA, and FLU; and high dose: 0.72 g/tree ABA, 0.8 g/tree ACE, CYA, and FLU. SD: standard deviation.

Forty-two days after injection, fruit and leaf samples were collected from the trees, and the residue content was determined using a chemical analytical method (Figure 3). Interestingly, ABA and CYA resulted in lower concentrations in all the collected fruit samples than the detection limit. The average ACE content in the fruit samples was between 19.6 and 57.8 ng/g, while the FLU values were between 48.6 and 432.8 ng/g.

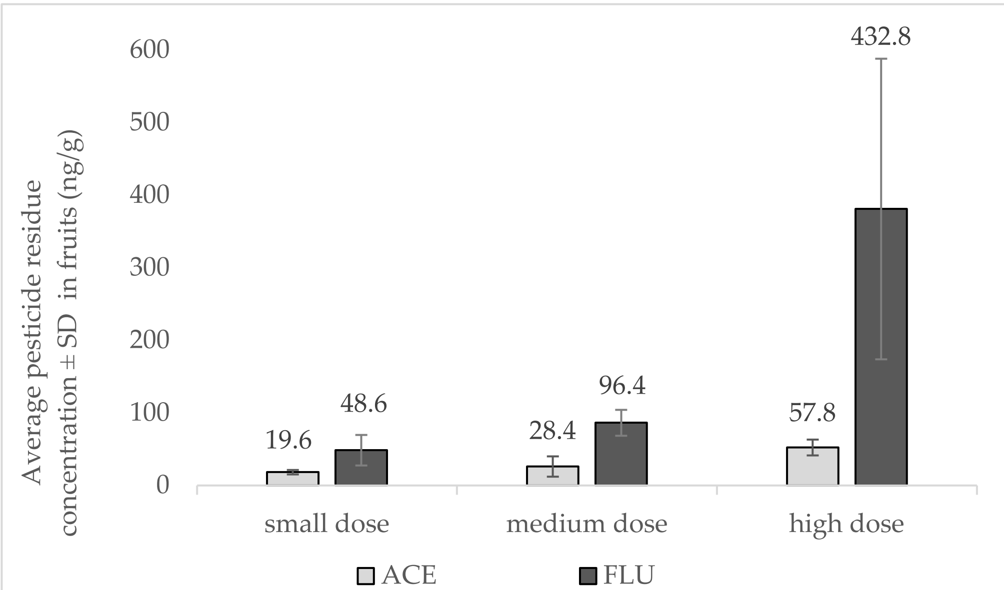

Figure 3. Average pesticide residue concentration in fruits (ng/g). Injected small dose: 0.18 g/tree of ABA, 0.2 g/tree of ACE, CYA, and FL; medium dose: 0.36 g/tree of ABA, 0.4 g/tree of ACE, CYA, and FLU; 0.72 g/tree of ABA, 0.8 g/tree of ACE, CYA, and FLU. SD: standard deviation.

The dose and the active ingredient together influence the development of the average pesticide residue (F = (2,6) = 6.24, p = 0.034). In the case of both active ingredients, the higher dose resulted in a higher a.i. residue concentration in the fruit. As shown in Figure 3, except for the highest dose of FLU, the pairwise statistical test (ART ANOVA) showed no significant difference in either case ($p > 0.05$). Since we had limited data available in the first year of the experiment, we used three replicates in the following year.

Leaf samples were also collected from trees injected with the highest dose (0.8 g/tree) of ACE and FLU. We experienced much higher concentrations of pesticide residues in the leaves compared to fruits. The average values were 1126 ng/g and 2854 ng/g, respectively. In the case of ABA and CYA, leaf samples were not collected.

3.2. Dosage Evaluation 2022

Based on the first-year experiments, we concluded that dose trials of two promising active ingredients, ACE and FLU, are worth continuing. CYA was not effective enough, while ABA is unauthorized for cherry cultivation in Central Europe. Indeed, based on EU Regulation 515/2023, the license to use abamectin was limited to greenhouses. In the second year of the experiment, four increasing doses of ACE and FLU were injected into the trunk of cherry trees. Figure 4 shows that the two higher doses of both pesticides showed an efficiency above 95%. The highest efficiency was obtained with the highest dose of FLU, which was 99%.

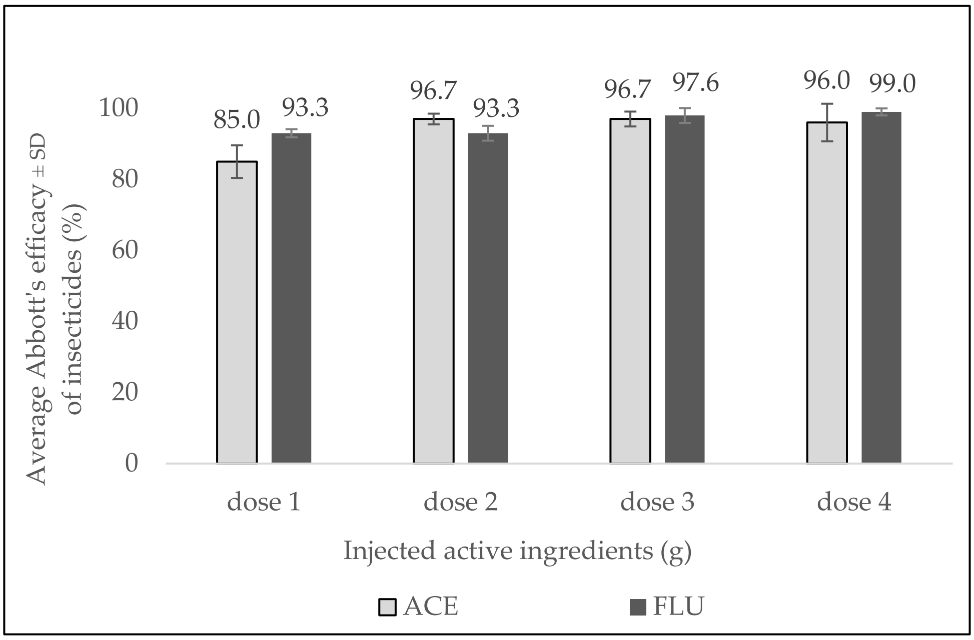

Figure 4. Average Abbott's efficacy of insecticides (%). Dose 1: 0.056 g/tree of ACE and 0.33 g/tree of FLU; dose 2: 0.56 g/tree of ACE and 0.66 g/tree of FLU; dose 3: 1.12 g/tree of ACE and 1.21 g/tree of FLU; and dose 4: 2.25 g/tree of ACE and 3.96 g/tree of FLU. SD: standard deviation.

The average variation in Abott's efficacy between the investigated groups was influenced by the active substances and the doses (F = (3,16) = 4.22, $p = 0.022$). Figure 4 clearly shows that Abbott's efficacy of the first dose is lower among ACE values. The statistical test for ACE showed that the lowest dose was significantly different from the other three doses ($p < 0.01$). The two middle doses of ACE (0.56 g/tree and 1.12 g/tree) provided the highest protection against cherry fruit flies, while in the case of FLU, dose 3 and dose 4 (1.21 g and 3.96 g) provided the best protection. Between the two pesticides, there was a difference only in the smallest dose, where Abbott's efficacy of ACE was significantly lower than FLU ($p = 0.035$). The infection rate of control tree fruits was 100%, as all the collected fruits had larvae inside.

Figure 5 shows the active ingredient concentration in the collected fruit samples. Average ACE content was between 6.6 and 170.0 ng/g, which was much lower than the MRL (maximum residue level), which is 1500 ng/g in Europe [38]. The highest acetamiprid concentration in the fruits (treated with dose 4) was 257.2 ng/g. FLU had the highest residue value during endotherapy treatment, with average concentrations between 51.4 and 400.4 ng/g (Figure 5). The MRL of FLU is 10 ng/g for cherry fruits; none of the injected FLU doses met this expectation [39]. The highest flupyradifurone concentration in the fruits (treated with dose 4) was 484.0 ng/g.

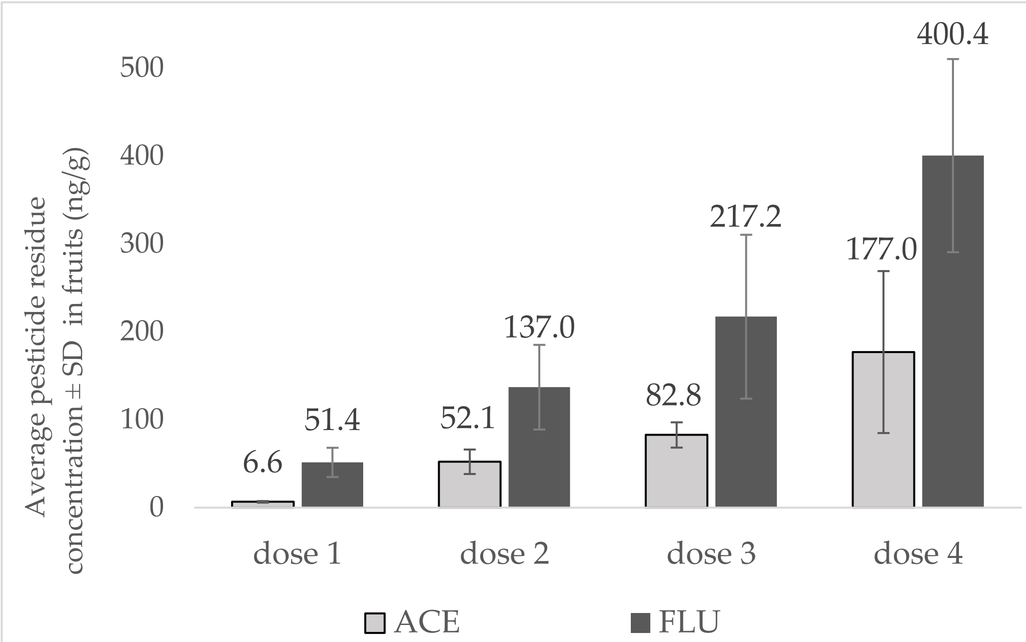

Figure 5. Average pesticide residue concentration in fruits (ng/g). Dose 1: 0.056 g/tree of ACE and 0.33 g/tree of FLU; dose 2: 0.56 g/tree of ACE and 0.66 g/tree of FLU; dose 3: 1.12 g/tree of ACE and 1.21 g/tree of FLU; and dose 4: 2.25 g/tree of ACE and 3.96 g/tree of FLU. SD: standard deviation.

Figure 5 shows a significant increase in terms of ACE and FLU concentrations with increasing doses ($p < 0.05$). A pairwise comparison of ACE and FLU at each dose was also significant ($p < 0.05$), except for dose 2.

To observe the toxicokinetic behavior of the pesticides after trunk injection, residue data were also measured in the collected leaves of the highest injected doses (Table 1). Similar to last year's experiments, much higher accumulation occurred in leaves richly supplied with transport vessels compared to the fruits. The average ACE and FLU residues in leaf samples were between 1281 and 8248 ng/g and 3979 and 20,865 ng/g, respectively.

3.3. Spray Control Comparison

It is important to compare the efficacy of trunk injection with spray treatment, where we must pay attention to the dosage. Injected ACE dose 3 (1.12 g ACE/tree) was compared with sprayed ACE (1 g ACE/tree). FLU dose 2 (0.6 g FLU/tree) was compared with the sprayed dose (0.6 g FLU/tree) (Figures 4 and 6).

Figure 6 shows that spraying had the same effect on the pest as trunk injection. These injected doses resulted in average efficiencies of 96.7% (ACE) and 93.3% (FLU). Spraying with a similar dose of ACE and FLU resulted in an efficacy of 99 and 95, respectively, which were not significantly different from the treatment with trunk injection ($p = 0.111$).

With ACE spray control, the average active ingredient residue in the fruits was 57.4 ng/g, which is lower compared to the injected case (82.8 ng/g, dose 3, Figure 5), whereas it was much higher in the case of FLU: 613.7 ng/g, compared to the injected case (137.0 ng/g, dose 2, Figure 5). For comparison purposes, leaf samples were also collected from sprayed trees. The pesticide residue concentrations were in the same range as the injected case: an average of 1171 ng/g for ACE and 22,448 ng/g for FLU.

Figure 6. Comparison of the average Abbott efficacy of insecticides (%). Dose 3 (1.12 g/tree of ACE) and dose 2 (0.66 g/tree of FLU) were compared with spray doses (1 g/tree of ACE and 0.6 g/tree of FLU). SD: standard deviation.

4. Discussion

This study provides new data, showing that endotherapy can be a suitable solution for controlling *R. cerasi* in cherry cultivation. Several scientific articles were previously published about trunk injection, but nothing concerning the European cherry fruit fly and trunk injection together. Thus, our work fills a gap in the literature.

Our first-year study showed that protecting fruits against *R. cerasi* larvae was slightly different in terms of active ingredients. We detected a high protection rate (>95%) in the case of ABA, FLU, and ACE. Leaves treated with FLU showed a slight foliar toxicity. Based on the first-year experiments, we concluded that dose trials of two promising active ingredients, ACE and FLU, are worth continuing, while ABA is unauthorized in cherry cultivation in Central Europe, and CYA did not show enough protection against the larvae. Indeed, based on EU Regulation 515/2023, the license to use abamectin was limited to greenhouses. In the second year of the experiment, the two higher doses of both pesticides (ACE and FLU) showed an efficiency above 95%, similar to the first year. The highest efficiency was obtained with the highest dose of FLU, which was 99%. Control trees had 100% damage in both years, and all fruits had larvae inside. In the first year, ABA and CYA resulted in lower concentrations in all the collected fruit samples than the detection limit. The average ACE content in the fruit samples was between 19.6–57.8 ng/g and 6.6–170.0 ng/g, while the FLU values were between 48.6–432.8 ng/g and 51.4–400.4 ng/g in the first and second year of the experiment, respectively. Indeed, in both years, ACE residues were under the MRL (1500 ng/g), while FLU residues were higher than the current MRL (10 ng/g).

ABA is a frequently used active ingredient for trunk injections, and its use in public spaces is also widespread in Hungary against *Cameraria ohridella*. ABA with quasi-systemic kinetics belonging to the group of avermectins, a nervous system poison. In our experiment, even the smallest treatment dose showed adequate protection against the cherry fruit fly. ABA residues in or on crops are generally low, typically <25 ng/g [40]. This was also experienced in our experiment, as no residue of an a.i. was detected in the fruits. Even though ABA appears to be a promising pesticide for trunk injection, the EU has restricted its use to greenhouses, so further investigation of its use is not justified at this time.

ACE and FLU are active substances belonging to a group of nicotinic acetylcholine receptor (nAChR) agonists. They have systemic kinetics and act rapidly on sucking pests. Their toxicological and ecotoxicological opinions are favorable [41]. Although their chemical structure is similar, binding to the target site may differ; therefore, they are placed in different pesticide chemical groups [42]. ACE is included in neonicotinoids, while FLU belongs to the butenolides, whose chemical differences are also manifested in the toxicokinetic behavior of the two pesticides. In all cases, FLU appeared in higher concentrations in the canopy at the sampling times than ACE. However, this higher residue concentration did not show greater efficiency, except at the lowest injected doses (Figure 4). Dose 2 was the minimum effective dose for ACE. Dose 3 (1.12 g/tree of ACE and 1.21 g/tree of FLU) and dose 4 (2.25 g/tree of ACE and 3.96 g/tree of FLU) resulted in greater than 95% efficacy for both compounds, and there was no difference in efficacy between trunk injected and spray-treated trees. In terms of food safety regulations, it can be concluded that the effective doses of ACE resulted in far lower residue concentrations in the fruit than the MRL. In the case of FLU, we experienced MRL exceedance at all doses; therefore, FLU is not considered suitable for controlling *R. cerasi* using trunk injection at the moment.

All the doses of CYA were ineffective against the pest, with the highest dose (0.8 g) only providing 74% effectiveness. Although CYA has xylem-mobile properties and moderate water solubility (14 mg/L), the translocation was not effective enough in our experiment, as shown by the fruit's undetected residue [43]. Cyantraniliprole is a member of the diamide class of insecticides. It is a ryanodine receptor modulator that kills sucking and chewing pests. The possible explanation of the low mobility of CYA, in our experience, can be the presence of sorption interactions between the a.i. and the xylem wall in the trunk.

Our study proved that endotherapy is a suitable alternative for controlling larvae of the cherry fruit fly. Trunk injection of min. 0.56 g/tree of ACE showed higher than 95% effective protection; the residue concentration was lower than the MRL. The following calculation can be made upon comparing the technology's material requirements with spraying. During the entire season, a minimum of two to three sprays were required to control cherry fruit flies, requiring 50–150 g of an active ingredient per hectare [44,45]. For trunk injections, a suitable dose of acetamiprid could be calculated of a similar magnitude (one injection of acetamiprid into 285 trees (per ha) × 0.56 g a.i./tree = 143 g/ha). In terms of spraying with multiple treatments, we calculated 2500–3000 L of water per hectare, while this amount was only about 10 L for injections. The main advantage of this technology is that the amount of applied water is several orders of magnitude less than spraying, where the entire season can only be protected by several applications of foliar sprays. Injection with multiple active ingredients may be possible in the future, and large-scale cherry farming could be optimized by including robotic technology [46].

Author Contributions: Conceptualization, R.G. and Á.G.; Methodology, Á.S. and C.S.; Software, A.M.L.; Validation, C.S.; Formal Analysis, Á.S.; Investigation, R.G.; Resources, Á.G.; Data Curation, A.M.L.; Writing—Original Draft Preparation, R.G.; Writing—Review & Editing, C.S.; Visualization, R.G.; Supervision, C.S. and Á.S.; Project Administration, R.G.; Funding Acquisition, Á.G., Á.S. and C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This project (KDP-6-3/PALY-2021-1015691) was implemented with the support of the Ministry of Culture and Innovation of Hungary from the National Research, Development, and Innovation Fund, financed under the [KDP-2020] funding scheme.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We would like to thank Gabor Balazs, who kindly made the orchards available to us for the experiments.

Conflicts of Interest: Author Ádám Gutermuth was employed by the company GreenUnit Kft. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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