

Article

Distribution, Residue Dynamics, and Insecticidal Efficacy of Trunk-Injected Emamectin Benzoate in Pecan Trees

Zhi Liang ^{1,2,†} , Xi Zhou ^{1,2,†}, Yinlong Li ^{1,2}, Min Zhou ^{1,2}, Xutao Yang ³, Shengnan Zhang ^{1,2}, Jacob D. Wickham ⁴ , Qing-He Zhang ⁵  and Longwa Zhang ^{1,2,*} 

¹ Anhui Province Key Laboratory of Forest Resources and Silviculture, School of Forestry & Landscape Architecture, Anhui Agricultural University, Hefei 230036, China; l17398383165@163.com (Z.L.); zx9090ti@163.com (X.Z.); legendarylyl@163.com (Y.L.); xiaocaizhouzhou@163.com (M.Z.); shengnan@ahau.edu.cn (S.Z.)

² Anhui Province Laboratory of Microbial Control, Engineering Research Center of Fungal Biotechnology, Ministry of Education, School of Forestry & Landscape Architecture, Anhui Agricultural University, Hefei 230036, China

³ Forestry Workstation of Chuzhou City, Chuzhou 239000, China; yangxutao1971@163.com

⁴ A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninsky Prospekt, 119071 Moscow, Russia; jacobwickham@gmail.com

⁵ Sterling International, Inc., Spokane, WA 99216, USA; qing-he@rescue.com

* Correspondence: zhanglw@ahau.edu.cn; Tel.: +86-13-637-082-053

† These authors contributed equally to this work.

Abstract: *Carya illinoensis* (Wangenh.) K. Koch (Pecan), a deciduous tree native to North America, faces significant challenges from pests. This study investigated the insecticidal efficacy and food safety of using the broad-spectrum insecticide emamectin benzoate via trunk injection for potential pecan pest management. Injections were given at dosages of 0.4, 0.8, 1.6, and 2.4 mL/cm of tree diameter at breast height (DBH), and leaf samples were collected at 10, 30, 60, and 100 days post-injection, while fruit samples were obtained during the swelling, ripening, and harvest stages. We established an analytical method for the determination and quantification of emamectin benzoate content in pecans using ultra-performance liquid chromatography–mass spectrometry (UPLC-MS). Leaf emamectin benzoate content was significantly higher compared to nuts ($p \leq 0.036$). The content in leaves following the four dosage treatments decreased over time and at 100 days was 0.1943/0.2799 mg/kg (upper crown/lower crown), 0.1910/0.3957 mg/kg, 0.3663/0.6235 mg/kg, and 1.3988/1.9123 mg/kg, respectively. The pesticide residues of 0.4 mL/cm and 0.8 mL/cm treatment groups in kernels at harvest time were 0.0016 mg/kg and 0.0039 mg/kg, respectively, below the latest European Union Regulation (0.005 mg/kg). All four dosage treatments (0.4, 0.8, 1.6, and 2.4 mL/cm of tree diameter at DBH) in the leaf feeding test caused significant mortalities of the fourth instar *Hyphantria cunea* (Drury) larvae. The mortality rates at 10 days post-injection were 64.7%, 73.3%, 79.3%, and 84.7%, respectively, while at 60 days post-injection, the rates were 26.0%, 47.3%, 53.7%, and 81.7%, respectively. In summary, this study successfully established a sensitive analytical method for the detection and quantification of trunk-injected emamectin benzoate residues in pecans and demonstrates its safety and effectiveness as a chemical control option against foliar pecan pest insects.

Keywords: *Carya illinoensis*; trunk injection; emamectin benzoate; residue; efficacy assessment; *Hyphantria cunea*



Citation: Liang, Z.; Zhou, X.; Li, Y.; Zhou, M.; Yang, X.; Zhang, S.; Wickham, J.D.; Zhang, Q.-H.; Zhang, L. Distribution, Residue Dynamics, and Insecticidal Efficacy of Trunk-Injected Emamectin Benzoate in Pecan Trees. *Forests* **2024**, *15*, 535. <https://doi.org/10.3390/f15030535>

Academic Editor: Chiara Ferracini

Received: 5 February 2024

Revised: 3 March 2024

Accepted: 12 March 2024

Published: 14 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Carya illinoensis (Wangenh.) K. Koch (Pecan) is an economically important nut-producing tree, prized for its nutritious and delicious nuts [1,2]. It is widely cultivated in various regions worldwide, contributing significantly to the global nut industry. Rapid growth of pecan cultivation has been observed in recent years in China, particularly

in Anhui, Yunnan, Zhejiang, and Jiangsu provinces [3,4]. However, like many other crops, pecan trees face numerous challenges, including infestation from a diverse array of pests [5,6]. At present, some of the serious pest insects include the citrus longhorned beetle [*Anoplophora chinensis* (Thomson)], red borer [*Zeuzera coffeae* (Thomson)], yellow peach moth [*Conogethes punctiferalis* (Guenée)] and blackmargined aphid [*Monellia caryella* (Fitch)] [7,8]. Traditional approaches to managing pecan pests use chemical insecticide sprays [9,10]. Despite their short-term effectiveness, these techniques frequently lead to undesirable consequences, such as ecological imbalance resulting from adverse effects on non-target species and the emergence of insecticide resistance within pest communities [11].

In recent years, trunk injection has emerged as a promising alternative to conventional foliar spraying [12,13]. This technique involves directly injecting insecticides or other agrochemicals into the tree's vascular system, allowing for systemic distribution in vascular tissues [14]. Trunk injections have recently been tested in pine, apple, pear, and date trees, offering several advantages, including reduced environmental exposure, targeted delivery to the pest-infested tissues, and prolonged efficacy due to the slow degradation of the pesticide within the tree [15–17]. Commonly used insecticides for trunk injection include imidacloprid, abamectin, emamectin benzoate, thiamethoxam, and others [17–20]. The use of trunk injection technology for delivering insecticides is increasingly being applied to pest and disease control.

Emamectin benzoate, a semi-synthetic derivative of abamectin, is commonly employed for trunk injection [21]. It has been demonstrated as having excellent efficacy against a wide range of insect pests while also highlighting its ecological safety [22]. Trunk injection of emamectin benzoate effectively prevented and controlled red palm weevil infestation in date palm trees for one year, with no residues in fruits detected 60 days after the injection [23]. Trunk injection is more stable from an ecological perspective. For example, the injection of pine trees with emamectin benzoate to control pine wood nematode had no significant adverse effects on the overall community diversity and composition of soil arthropods and flying insects (Hymenoptera) [24]. There is a lack of studies regarding its application in the context of managing pecan pests, particularly the defoliators, and the distribution, residue persistence, and insecticidal efficacy of emamectin benzoate after injection remain unclear. *Hyphantria cunea* (Drury), a significant invasive pest in China, inflicts damage by feeding on the leaves of various tree species, including pecan trees, and typically undergoes three generations per year in Anhui Province. During the summer months, the damage caused by *H. cunea* can be particularly severe, leading to significant economic losses in affected areas [25]. This study primarily focuses on the distribution of emamectin benzoate residues in different pecan tissues after trunk injection and evaluates the insecticidal efficacy of trunk-injected emamectin benzoate against the invasive defoliator *Hyphantria cunea*. Understanding the distribution and persistence of emamectin benzoate in pecan leaves can enhance effective pest control, while understanding its distribution and persistence in pericarps and kernels of pecan nuts is crucial for assessing safety for human consumption.

2. Materials and Methods

2.1. Reagents and Standards

Emamectin benzoate standard (purity $\geq 95\%$) was obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd., Shanghai, China. A standard stock solution of emamectin benzoate at a concentration of 1000 mg L^{-1} was prepared using acetonitrile and stored at -20°C for calibration curves and method validation. Diluted calibration solutions were subsequently prepared from the stock solution for quantification purposes. To ensure accuracy, matrix-matched calibration solutions of the desired concentrations of emamectin benzoate were freshly prepared for each experiment using extracts obtained from the untreated (control) leaf, pericarp, and kernel samples.

Two percent emamectin benzoate ME was procured from Zhejiang SYNWILL Co., Ltd., Taizhou, China. Acetonitrile and methanol of HPLC grade were procured from Anhui

TEDIA High Purity Solvents Co., Ltd., Anqing, China, while formic acid of the same quality was acquired from Merck Chemicals Co., Ltd. Shanghai, China. Sodium chloride (NaCl) and anhydrous magnesium sulfate (MgSO_4) were purchased from Xilong Scientific Co., Ltd., Shantou, China. The primary secondary amine (PSA) used, with a particle size of 40–60 μm , was obtained from Agela Technologies, Tianjin, China. C18 sorbents (50 mm, 60 Å) were purchased from Macklin Biochemical Co., Ltd., Shanghai, China.

2.2. Field Trial

In 2022, field trials on emamectin benzoate spatial distribution and final residue measurements were conducted in Hefei (117.1° E, 31.5° N), Anhui province. The 10-year-old pecan trees had a diameter ranging from 10 to 15 cm (mean $\approx$ 12.5 cm) at breast height, and the first branch bifurcation was 1.5–2 m above the ground. Two percent emamectin benzoate ME was used for trunk injections during the trial. Injection ports, each measuring 4 cm deep and 6 mm in diameter, were drilled into the xylem tissues of the trunk. Emamectin benzoate was administered on 12 July 2022 in four different doses (0.4, 0.8, 1.6, and 2.4 mL per centimeter of the tree's DBH), corresponding to 5 mL, 10 mL, 20 mL, and 30 mL per tree through these ports [14,17]. The control group did not receive any treatment. One port injection was created approximately 0.5 m above ground level for easier access to the trunk and to ensure effective radial dissipation of emamectin benzoate in the xylem before reaching the branching points. One trunk injection was administered per tree, and twenty replicates (trees) per treatment were injected in a randomized complete block design. Following injection, the holes were plugged with wooden sticks, and residue tests at discrete time intervals were conducted on these trunk-injected trees.

2.3. Sample Collection and Preparation for Determining Emamectin Benzoate Residue

Pecan leaf, pericarp, and kernel samples were collected for residue analysis. Each tree crown was vertically divided into upper and lower sections, and multiple samplings and measurements were taken at discrete time intervals [16]. One set of leaf samples was obtained from each crown section (upper/lower section), and each sample set consisted of 200 leaves collected from all four cardinal directions, resulting in a total of 50 leaves per direction. At each of the specified sampling dates (10, 30, 60, and 100 days after the trunk injection), randomly selected leaf samples were collected from five randomly selected trees from a total of 20 treated trees for each treatment. Similarly, the fruit samples (pericarps and kernels) were collected at three stages of fruit growth and development: the swelling, ripening, and harvest stages (August, September, and October), as indicated in Figure 1. For each sampling date, 20 fruits were sampled from the random set of five selected trees located at the central section of the pecan crown. All collected samples were stored at -20°C for future use.



Figure 1. Three stages of pecan fruit development, along with anatomical details, including swelling, ripening, and harvest stages (from left to right).

2.4. Analytical Methods

2.4.1. Sample Extraction

Frozen samples of pecan leaf, pericarp, and kernel were first cut into small pieces and then ground using a blender [26]. Subsequently, 2 g of each sample was weighed and mixed with 4 mL of water in a 50 mL centrifuge tube. After allowing the mixture to stand for 30 min, 10 mL of acetonitrile was added to the same tube and vortexed for 1 min. Next, 2 g of NaCl was added, and the mixture was vortexed again for 1 min before being centrifuged at $4393 \times g$ rpm for 5 min. A 2 mL volume of supernatant was transferred to another 5 mL centrifuge tube containing 70 mg of PSA, 20 mg of C18, and 200 mg of $MgSO_4$. The mixture was shaken by hand for 1 min and then centrifuged at $13,680 \times g$ for 1 min. Finally, the supernatant was centrifuged at $13,680 \times g$ for 20 min and filtered through a $0.22 \mu m$ pore membrane prior to instrumental analysis [26,27].

2.4.2. UPLC-MS/MS Analysis

An Agilent 1290 Infinity II Ultra-performance Liquid Chromatograph coupled with an Agilent 6460 Triple Quadrupole Mass Spectrometer (Agilent, Santa Clara, CA, USA) in ESI ion source mode with positive ion scanning and multi-reaction monitoring were employed for the analysis of emamectin benzoate. The capillary voltage was set to 3000 V, while the ion source temperature was maintained at $325^\circ C$. The collision gas was argon, and the desolvation gas was nitrogen. The cone and fragment voltages were 25 V and 220 V, respectively, while the nebulizing gas pressure was 35 psi. The drying gas flow rate and temperature were 6.0 L/min and $350^\circ C$, respectively. For emamectin benzoate, the precursor ion was 886.4, and the product ions were 81.9 (qualitative) and 158.1 (quantification) [28]. The grayscale code utilized to depict different concentration ranges of emamectin benzoate in the pecan crown was as follows for the leaves: 25% grey (0.01–0.5 ppm), 50% grey (0.51–1.00 ppm), and 75% grey (1.01–2.00 ppm). Concentrations below 0.01 ppm are represented by white, while concentrations >2 ppm are represented by black in Figure 2 [16].

Emamectin benzoate grayscale code	Concentration range (ppm)
White	0.00
Gray 25%	0.01-0.25
Gray 50%	0.26-0.50
Gray 75%	0.51-1.00
Black	> 1.01

Figure 2. The grayscale code used for depicting different emamectin benzoate concentration ranges in the pecan leaves.

For testing, a reverse-phase InfinityLab Poroshell 120 EC-C18 HPLC column ($2.1 \text{ mm} \times 100 \text{ mm}$, $2.7 \mu m$) was utilized as the separation column, which was kept at $40^\circ C$. The flow rate was set to 0.2 mL/min, and the injection volume was 2 μL . The mobile phases A and B were acetonitrile and water containing 0.1 percent formic acid, respectively. The elution program employed a gradient elution method, starting with 78% A and gradually increasing to 88% A at 5 min, 95% A at 6 min, 50% A at 9 min, and finally back to 78% A at 11 min. System balance was then performed. The obtained data were analyzed with the Mass Hunter workstation Version B.03.01 Built 3.1.346.0 software (Agilent Technologies).

All MS data were recorded and exported with AgtQual B.07.00 software. The UPLC-MS chromatogram of the emamectin benzoate standard under the same conditions is shown in Figure 3. For samples with excessively high emamectin benzoate content in the leaves and extremely low content in the kernels, a dilution or concentration method was employed to meet the quantitative requirements.

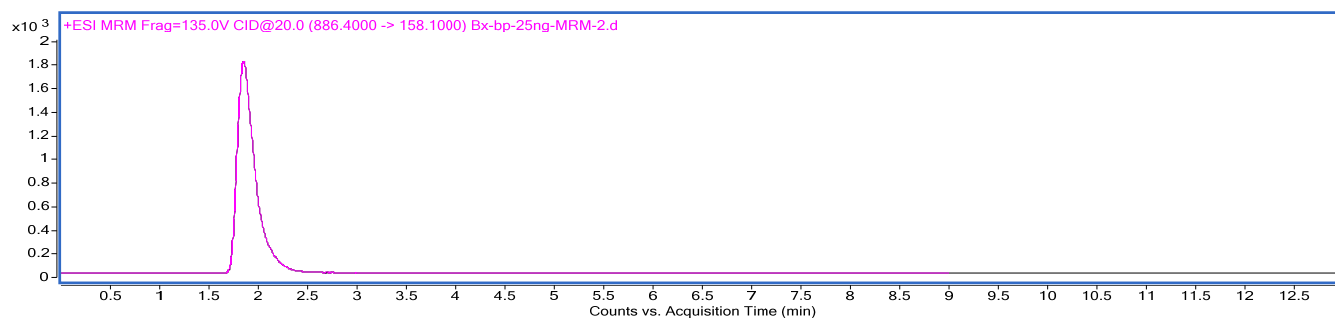


Figure 3. MRM (multiple reaction monitoring) mode chromatogram of emamectin benzoate standard (25 ng/mL), with the parent ion (m/z) at 886.4 and the quantitative ion (m/z) at 158.1 for emamectin benzoate.

2.5. Assessment of Insecticidal Efficacy

To evaluate the effectiveness of emamectin benzoate in controlling leaf-feeding pests following trunk injection, we conducted leaf-feeding trials with *H. cunea* 4th instar larvae on the 10th and 60th days post-injection. The larvae were reared in a controlled indoor environment, with a temperature of 25 °C, relative humidity between 70 and 80%, and a light–dark cycle of 14 h light and 10 h dark. We selected healthy fourth-instar larvae of similar size for our experiments. A total of 200 fresh leaves were obtained from each tree (treated or control), 100 from the upper crown and 100 from the lower crown, with 25 fresh leaves collected from each of the four cardinal directions. These leaves were then used to feed 4th-instar larvae of *H. cunea* in the laboratory [29]. Fifteen larvae were placed into a transparent plastic box (15 × 10.5 × 9 cm) and starved for 12 h. Each experiment was repeated five times ($n = 5$). Subsequently, we fed the *H. cunea* larvae with 15–20 randomly selected pecan leaves for 48 h, counted the number of dead larvae, and calculated the mortality rate.

2.6. Statistical Analysis

The emamectin benzoate concentrations in leaves from the upper and lower crown sections were compared using paired sample *t*-tests. Two-way ANOVA was used to analyze the concentrations of emamectin benzoate in the pecan crowns among different dose treatments and different sampling times. One-way ANOVA was used to analyze the significant difference in the concentration of emamectin benzoate in both the pericarps and kernels at different sampling dates and under different dosages, respectively. The relative area data obtained through UPLC-QQQ-MS/MS were analyzed using AgtQual software, and GraphPad Prism 8 was used for graphing. SPSS (Statistical Package for the Social Sciences) 19.0 was used for data analysis. Error bars show standard error of the mean (SEM) based on sample estimation. Statistical significance is denoted by “***”, indicating a *p*-value below 0.01.

3. Results

3.1. Method Validation

3.1.1. Linear Equation and Matrix Effects

An external standard method within the linear range of measurement was used to quantify the contents of emamectin benzoate in pecan leaf, pericarp, and kernel samples, with each matrix standard curve used for quantification. The standard solution curve

and matrix-matched standard were compared at six concentrations: 0.5, 1, 5, 10, 100, and 200 $\mu\text{g}\cdot\text{L}^{-1}$. The linearity of emamectin benzoate in pecan leaves and fruits was verified, with $R^2 > 0.999$ from 0.5 to 200 mg/L. Calibration curves were constructed by plotting the integrated peak areas (Y) against the concentrations of compound (X). The results of the linear regression and regression equations are shown in Table 1.

Table 1. Calibration curve of emamectin benzoate in different pecan tissues ($n = 5$).

Samples	Linear Equation	Determination Coefficients (R^2)	Slope Ratio (Matrix/Methanol)
Acetonitrile	$y = 1657.63x + 6765.39$	0.9999	-
Leaves	$y = 744.74x + 1656.97$	0.9991	0.4493
Pericarps	$y = 1311.91x + 130.39$	0.9999	0.7914
Kernels	$y = 629.91x + 739.42$	0.9996	0.3800

3.1.2. Recovery and LOQ

To compensate for the matrix effect of the sample, the accuracy and precision of the method were evaluated by spiking emamectin benzoate at concentrations of 0.005 mg/kg, 0.05 mg/kg, and 0.1 mg/kg in pecan leaves and 0.001 mg/kg, 0.01 mg/kg, and 0.1 mg/kg in pecan pericarps and kernels. The spiking was performed with five replicates for each concentration. The average recovery of analyte ranged from 78.6% to 100.7%, in line with the accuracy requirement of the analysis, and the relative standard deviation (RSD) was between 3.34% and 13.05%. The limit of quantification (LOQ) of emamectin benzoate in leaves, pericarps, and kernels was 0.005 mg/kg, 0.001 mg/kg, and 0.001 mg/kg, respectively, determined based on the lower spiked concentration of the analyte that can be considered as LOQ of the method with acceptable precision and accuracy (signal-to-noise ratio ≥ 10). The results indicated that the recoveries of emamectin benzoate were within the acceptable range with good accuracy and repeatability (See Table 2).

Table 2. Recovery and repeatability of emamectin benzoate in pecan leaves, pericarps and kernels ($n = 5$).

Samples	Spiked Level (mg/kg)	Recovery (%)	Average Recovery (%)	RSD (%)
Leaves	0.005	84.9, 91.6, 88.9, 89.7, 93.7	89.8	3.65
	0.05	95.2, 92.6, 95.4, 97.9, 83.5	92.9	5.98
	0.1	80.2, 102.5, 95.8, 110.6, 102.6	98.3	11.58
Pericarps	0.001	82.6, 79.4, 93.7, 95.7, 108.3	91.94	12.51
	0.01	88.3, 95.9, 109.1, 91.6, 94.2	95.82	8.31
	0.1	100.5, 100.1, 85.7, 78.2, 89.5	90.8	10.55
Kernels	0.001	75.3, 99.7, 93.1, 77.6, 77.4	84.62	13.05
	0.01	85.2, 77.7, 77.2, 73.4, 79.7	78.64	5.49
	0.1	80.6, 81.0, 84.2, 79.9, 72.4	79.62	5.48

3.2. Distribution of Emamectin Benzoate in Pecan Crowns

The content of emamectin benzoate decreased gradually over time (Figure 4). At 10 days post-injection, emamectin benzoate was detected in all four dose treatments (0.4, 0.8, 1.6, and 2.4 mL/cm), with its concentrations being 0.3626/2.5837 mg/kg (upper crown/lower crown), 2.3107/2.5428 mg/kg, 1.6201/5.1288 mg/kg, and 6.9798/7.8615 mg/kg, respectively. Emamectin benzoate content was not significantly different between the upper and the lower leaves at 30 days, 60 days, and 100 days post-injection ($t_{(8)} = -2.246$, $p \geq 0.05$). Two-way ANOVA indicated that dosage was the primary influencing factor on the concentration of emamectin benzoate ($F = 4.551$, $\text{df}_{\text{time}} = 3$, $\text{df}_{\text{dosage}} = 3$, $p = 0.033$), and there is a significant interaction between time and dosage ($F = 3.253$, $\text{df}_{(\text{time}, \text{dosage})} = 9$, $p = 0.001$). Specifically, we observed significant differences in emamectin benzoate content between the treatment

with a dosage of 2.4 mL/cm and the lower dosages, with p -values of 0.008, 0.024, and 0.022, respectively. Analysis of emamectin benzoate content in the lower crown indicated that the content in leaves at 10 days post-injection was significantly higher than those at 30 days, 60 days, and 100 days post-injection. The main effect variable was time since injection ($F = 16.729$, $df_{\text{time}} = 3$, $df_{\text{dosage}} = 3$, $p = 0.001$). One hundred days after the trunk injection, the average residues of emamectin benzoate in leaves from different dosage treatments (0.4, 0.8, 1.6, and 2.4 mL/cm) were 0.1943/0.2799 mg/kg (upper crown/lower crown), 0.1910/0.3957 mg/kg, 0.3663/0.6235 mg/kg, and 1.3988/1.9123 mg/kg, respectively.

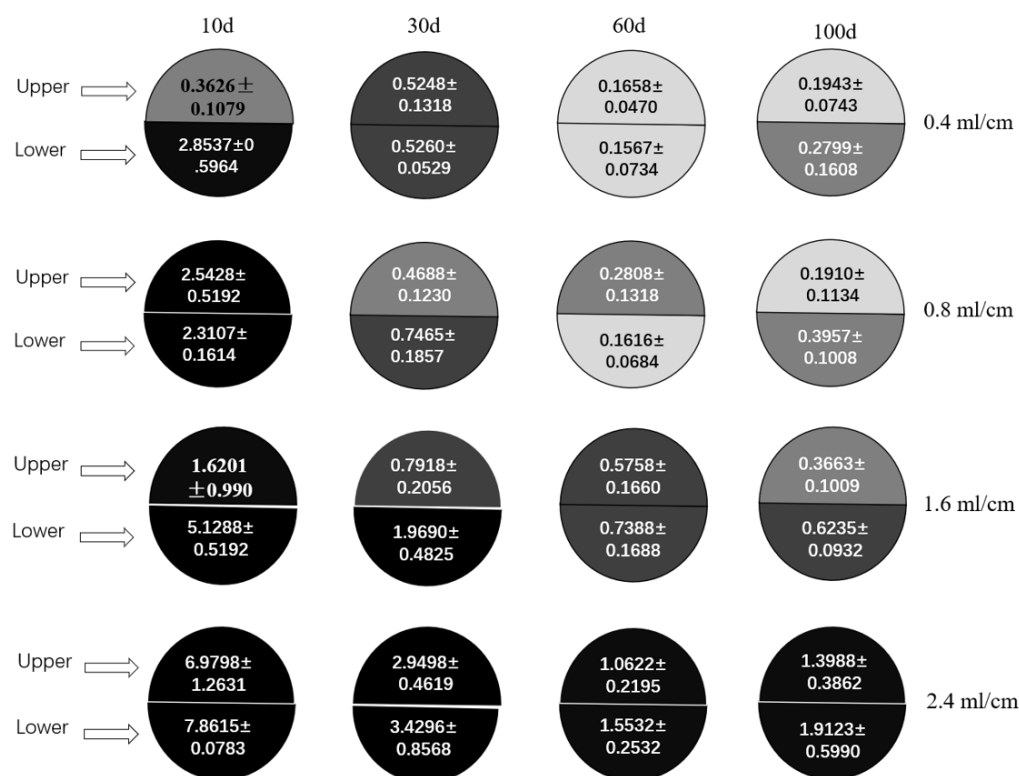


Figure 4. The concentrations of trunk-injected emamectin benzoate (mg/kg) in pecan leaves of upper and lower crowns at 10 to 100 days after the injection (DAI): 10 DAI, 30 DAI, 60 DAI, and 100 DAI.

3.3. Final Residues of Emamectin Benzoate in Pecan Pericarps and Kernels

The concentration of the active ingredient, emamectin benzoate, was found to be much lower in the pericarps and kernels than in the leaves. Furthermore, significant differences in emamectin benzoate residue in the pericarps were observed among different development stages ($F_{2,12} = 3.565$, $p \leq 0.041$). For the treated trees, the measured emamectin benzoate content in the pericarp samples ranged from 0.0146 to 0.2057 mg/kg during August. There were significant differences in emamectin benzoate content in the pericarps between the 2.4 mL/cm treatment group and the 0.4 mL/cm and 0.8 mL/cm treatment groups ($F_{3,16} = 2.836$, $p \leq 0.033$), as shown in Figure 5A. However, its contents in pericarp samples in September and October were lower than that in August, ranging from 0.0059 to 0.0246 mg/kg in October. Emamectin benzoate was not detected in the control samples.

Kernel samples collected in August contained emamectin benzoate at a level ranging from 0.0109 mg/kg to 0.1248 mg/kg, while those collected in September showed levels ranging from 0.0023 mg/kg to 0.0113 mg/kg. Additionally, the final residue of emamectin benzoate in pecan kernels at harvest time was 0.0016 mg/kg, 0.0039 mg/kg, 0.0073 mg/kg, and 0.0085 mg/kg, as presented in Figure 5B. The emamectin benzoate content in the kernels varied significantly between different months ($F_{2,12} = 6.907$, $p \leq 0.010$). The final collected kernel samples of the two lower treatment groups (0.4 and 0.8 mL/cm) showed

the active ingredient contents were below the latest detection limit of 0.005 mg/kg [30]. Emamectin benzoate was not detected in the blank control kernel samples.

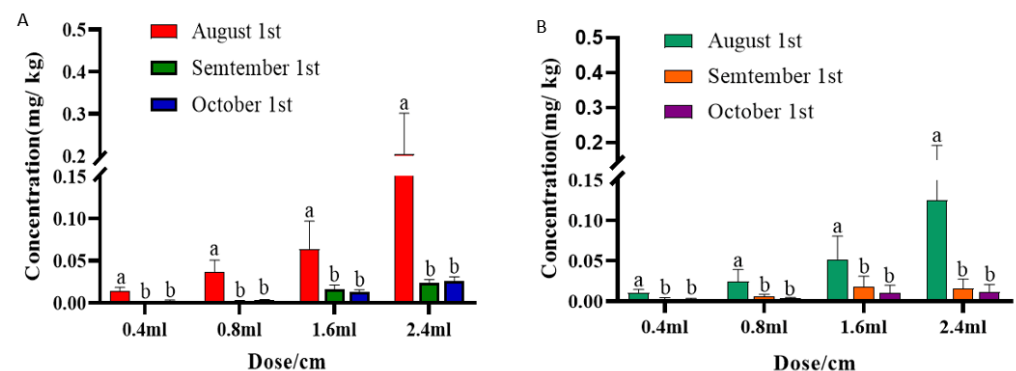


Figure 5. Emamectin benzoate concentrations in pecan pericarps (A) and kernels (B) at three different growth stages; bars with the same letters were not significantly different (LSD test, $p > 0.05$).

3.4. Insecticidal Efficacy of Emamectin Benzoate against *H. cunea* Larvae

Our results showed that trunk injection with emamectin benzoate might have potential as an effective control method against *H. cunea* on pecan trees, as the fourth instar larval mortalities in the treatment groups (dosages) were significantly higher than those in the control group ($F_{4,20} = 3.263$, $p \leq 0.033$). For the leaf samples taken 10 days post-trunk injection, the mortality percentages of fourth instar *H. cunea* larvae were similar among the dose treatment groups for both the lower and upper crown sections ($t_{(8)} = 1.015$, $p > 0.05$, Figure 6A), ranging from 60.0% to 90.7%. For the leaf samples taken on the 60th day post-injection, a stepwise gradient in the *H. cunea* larval mortality rate (%) was seen across the laboratory bioassay for both upper and lower crown sections. The mortality rates were about 24.0%–28.0% in the 0.4 mL/cm treatment group and 81.3%–82% in the 2.4 mL/cm treatment group ($n = 5$, Figure 6B). There were no statistically significant differences in the larval mortality rate (%) between upper and lower crown treatment groups ($t_{(8)} = 0.195$, $p > 0.05$).

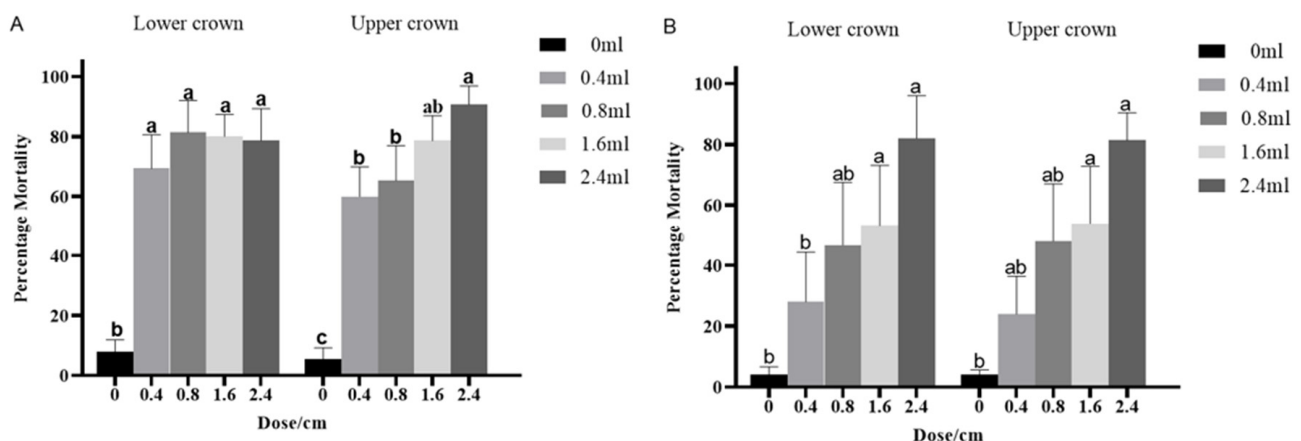


Figure 6. The mortality rate (%) of *H. cunea* larvae fed with leaf samples taken at 10 days (A) and 60 days (B) after the trunk injection. Bars with the same letters were not significantly different (LSD test, $p > 0.05$).

4. Discussion

Trunk injection is an eco-friendly method for delivering substances directly into a tree's trunk to control pests or transport nutrients. It boasts a high pesticide utilization rate and holds significant potential for widespread application in pest and disease management. Our

results provide important insights into the spatiotemporal transportation and distribution of emamectin benzoate following trunk injection in pecan trees and its insecticidal efficacy against defoliator insects. A single injection of emamectin benzoate might have the potential to control *H. cunea* larvae for several months. Meanwhile, we observed an unexpected phenomenon following trunk injection of emamectin benzoate: high concentrations in the leaves and low concentrations in the kernels of pecan trees ($t_{(8)} = 3.251$, $p \leq 0.036$), as depicted in Figure S1. High pesticide residue levels in leaves might indicate a high insecticidal efficacy against defoliating insects, while the low levels in the fruits might represent a high level of food safety.

Currently, there are several reports on the detection and determination of emamectin benzoate in plants. For instance, Wang et al. examined its residue behavior in apples and cabbage [27], while Zhou et al. evaluated its transfer and safety in tea [26]. Deng et al. investigated emamectin benzoate residues in rice and rice-growing environments [28], and Takai et al. explored its distribution in pine tissues [15]. It is noteworthy that these studies utilized various extraction solvents, including acetonitrile, ethyl acetate, and methyl alcohol. In our research, we compared three different solvents for extracting emamectin benzoate from pecan leaves and fruits. Our findings, as depicted in Figure S2, demonstrated that the extraction method using acetonitrile, as described by Zhou et al., yielded the highest efficiency. After verifying the sensitivity, accuracy, and precision of our method adopted from Zhou et al., we concluded that it was suitable and adequate for detecting emamectin benzoate and met the experimental requirements.

The concentration of emamectin benzoate in pecan leaves was significantly higher than in fruits. Within 10 days of trunk injection, emamectin benzoate was detectable in the leaves, indicating its rapid transport into the tree crown and efficient translocation. Simultaneously, there were no notable disparities in pesticide content in leaves between the upper and lower sections of the tree crown at 30, 60, and 100 days post-injection ($t_{(8)} = -2.246$, $p > 0.05$), suggesting a uniform distribution of emamectin benzoate throughout the tree crown within 30 days, with continued uniformity at 60 and 100 days post-injection. Thus, tree trunk injection of emamectin benzoate might be an effective control method against pest insects in various parts of the tree crown. Analysis of emamectin benzoate residue in the pecan fruit samples showed that its contents in pericarps and kernels were higher during the expansion period than during the filling and harvesting stages. The average contents were 0.0016 mg/kg, 0.0039 mg/kg, 0.0073 mg/kg, and 0.0085 mg/kg in kernels during the harvesting stage.

In comparing the European Union (EU) residue standards from 2019 and 2021, it was observed that our experimental results met the Maximum Residue Level (MRL: 0.01 mg/kg) requirements [31,32]. However, in March 2022, the EU Commission Regulation (2022-476) unified the maximum residue limits for emamectin benzoate in various tree nuts, including almonds, cashew nuts, pine nut kernels, and pistachios, to 0.005 mg/kg [30]. Accordingly, the residue levels detected in the 0.4 mL/cm and 0.8 mL/cm treatment groups conform to this latest standard, while those in the 1.2 mL/cm and 2.4 mL/cm treatment groups exceed the updated limit. This underscores the importance of determining the appropriate timing and dosage for trunk injection of emamectin benzoate to ensure effective pest control while meeting food safety requirements.

Two potential reasons for the higher pesticide content in pericarps and kernels during the expansion period than in the later stages can be suggested. First, the pericarp may play a protective role, acting as a barrier that restricts pesticide penetration into the pecan kernels. This concept is supported by research by Yang et al. [33], who found higher pesticide residues in apple skins and pulp compared to the cores and seeds, suggesting a protective function of the skin and pulp against pesticide infiltration. Similarly, Juraske et al. observed an 85% reduction in deltamethrin residues when passion fruit peels were removed [34], and Calvaruso et al. identified higher levels of imazalil and fenhexamid residues in grapefruit peels compared to the pulp [35]. These studies provide compelling

evidence for the potential utilization of pecan pericarps as a protective barrier against pesticide permeation into the kernels.

Another plausible explanation is linked to how pesticides are transported within the tree, which is influenced by nutrient distribution. So far, there is no direct evidence establishing the relationship between pesticide transport and the transport of plant growth nutrients following trunk injection. Some studies suggest that after trunk injection, pesticides are conveyed to the leaves through transpiration pull, resulting in a relatively concentrated pesticide content in the leaves [36]. In July, following the trunk injection, as pecan fruit growth progressed, the pattern of nutrient transport shifted from early vegetative growth to reproductive growth. Nonetheless, due to the rapid internal pesticide transport, a higher concentration of the pesticide remains in the leaves. Subsequently, as nutrient distribution shifts, less pesticide is transferred. Such a distribution pattern shift could contribute to the higher pesticide content in the leaves and lower content in the fruits. Further research is surely needed to determine the specific reasons. Meanwhile, pesticide residues can be detected in fruits, which may be associated with the transportation of pesticides through water into the fruits. Currently, there are some isotope tracing techniques employed for monitoring nutrient transport in plants, primarily focusing on major elements like N, P, K, and various trace elements [37–39]. However, there is limited documentation on isotope labeling for pesticides. In the future, this technology could be applied to investigate the relationship between pesticide residues in leaves and those in fruits.

The insecticidal effectiveness of trunk injection of emamectin benzoate was also evaluated using the *H. cunea* larvae, a serious invasive defoliator that poses a potential threat to the pecan cultivation industry in China. We collected leaves from the treated groups to feed fourth instar *H. cunea* larvae and evaluated emamectin benzoate toxicity. Compared to the control group, larvae that consumed the leaves of treated pecan trees that were collected 10 and 60 days after trunk injection exhibited a significantly higher level of mortality. These results suggest that emamectin benzoate trunk injection in pecan trees is an effective method for killing the defoliating insects (*H. cunea* larvae) with a prolonged insecticidal effectiveness. Studies have demonstrated that trunk injection of emamectin benzoate effectively controls various pest insects, including leaf rollers, Oriental fruit moth, and leafhoppers in semi-dwarf Empire apple trees, while imidacloprid injection on the same semi-dwarf Empire apple trees remains effective in controlling *Empoasca fabae* (Harris) and aphids (*Aphididae*) throughout the growing season [40]. In the case of pear psylla control, abamectin injection has shown superior results compared to foliar spray [17]. These findings collectively emphasize that tree trunk injection is an effective method for pesticide delivery and pest control; however, it may have limited effectiveness against woodboring longhorned beetles such as *Anoplophora chinensis*, whose larvae develop in the lower stem and roots below the 50 cm point where trunk injections are administered.

Our study sheds light on the distribution of emamectin benzoate within different parts of pecan trees after trunk injection and its insecticidal efficacy against *H. cunea*. In addition, the experiment also paid attention to the residue of emamectin benzoate in the pecan kernels, which is of great significance to the food safety of pecan nuts. However, this study has some limitations, such as its focus on the absorption and distribution of emamectin benzoate within the first 100 days following injection, leaving the distribution over longer periods still unexplored, and it is not yet clear how long the insecticidal effect can last after injection. We plan to conduct earlier injections, starting in April or May, to investigate the distribution and residues of emamectin benzoate over an extended timeframe, as well as to assess its insecticidal efficacy against other pest insects in the future.

5. Conclusions

Our research established an effective analytical method for detecting and quantifying emamectin benzoate residues in pecans, with excellent linearity (1–200 µg/L) and efficient extraction (average recovery rate: 79.6–100.7%; RSD: 3.34–13.05%). This method ensures compliance with safety standards and facilitates accurate assessment of emamectin

benzoate levels in pecan tissues. Our findings demonstrate that emamectin benzoate concentrations in leaves were significantly higher than those in fruits post-injection. High emamectin benzoate concentrations in leaves indicate a high insecticidal efficacy, while the low concentrations in the fruits represent a high level of food safety. The residues of emamectin benzoate treatment groups at doses of 0.4 and 0.8 mL/cm (tree diameter at DBH) in pecan fruits were found to be below the EU Regulation limit (2022-476), indicating their safety for consumption. Overall, this study provides important insights into pest control and food safety of trunk injection of emamectin benzoate.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15030535/s1>, Figure S1: Comparison of emamectin benzoate content in leaves and kernels during the harvest stage (*t*-test, **: Significant difference, $p < 0.01$); Figure S2: Comparison of chromatographic peak intensities of emamectin benzoate from extract samples using different extraction methods/solvents; efficiency: acetonitrile > methanol > ethyl acetate.

Author Contributions: Data curation, Z.L., X.Z., M.Z. and L.Z.; Formal analysis, Z.L., X.Z., M.Z. and L.Z.; Funding acquisition, X.Y. and L.Z.; Investigation, Z.L., X.Z., Y.L., X.Y., S.Z. and L.Z.; Methodology, Z.L., X.Z., M.Z., X.Y., S.Z. and L.Z.; Project administration, Z.L., S.Z., J.D.W. and L.Z.; Software, Z.L., Y.L. and J.D.W.; Supervision, Q.-H.Z. and L.Z.; Writing—original draft, Z.L.; Writing—review and editing, S.Z., J.D.W., Q.-H.Z. and L.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Key Research and Development Program (2023YFC2604800), National Key Research and Development Program of Anhui, China (2022i01020010), National Natural Science Foundation of China (32371890), and Anhui Provincial Forestry Science and Technology Innovation Research Project (AHLXCX~2022~8).

Data Availability Statement: The data supporting the findings of this study are available in the Supplementary Materials.

Acknowledgments: We would like to express our gratitude to Tianzi Gu and Mengting Wu from Anhui Agricultural University, China, for their assistance in the experimental design. We also thank Letian Xu from Hubei University, China, for providing valuable suggestions for the manuscript revisions. Additionally, we extend our thanks to Huarong Tan and Ting He from the Biotechnology Center of Anhui Agricultural University for their assistance with residue detection work. Special thanks go to Jun Hu from Anhui SenLü Chenggang Ecological Landscape Technology Development Co., Ltd. for providing the experimental site for this study.

Conflicts of Interest: Author Q.-H.Z. was employed by the company Sterling International, Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Thompson, T.E.; Conner, P.J. Pecan. In *Fruit Breeding*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 771–801.
2. Hall, G.D. Pecan food potential in prehistoric North America. *Econ. Bot.* **2000**, *54*, 103–112. [\[CrossRef\]](#)
3. Mo, Z.; Feng, G.; Su, W.; Liu, Z.; Peng, F. Transcriptomic analysis provides insights into grafting union development in pecan (*Carya illinoensis*). *Genes* **2018**, *9*, 71. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Zheng, J.; Hänninen, H.; Lin, J.; Shen, S.; Zhang, R. Extending the cultivation area of Pecan (*Carya illinoensis*) toward the south in southeastern subtropical China may cause increased cold damage. *Front. Plant Sci.* **2021**, *12*, 768963. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Zhang, R.; Peng, F.; Li, Y. Pecan production in China. *Sci. Hort.* **2015**, *197*, 719–727. [\[CrossRef\]](#)
6. Zheng, X.-R.; Chen, J.-J. First report of leaf spot on pecan caused by *Choanephora cucurbitarum* in China. *Plant Dis.* **2023**, *107*, 2241. [\[CrossRef\]](#)
7. Yang, Y.; Yang, X. Green prevention and control technology of main pests and diseases of *Caray illinoensis*. *Anhui Agric. Sci. Bull.* **2019**, *25*, 95–96.
8. Slusher, E.K.; Cottrell, T.; Acebes-Doria, A.L. Effects of aphicides on pecan aphids and their parasitoids in pecan orchards. *Insects* **2021**, *12*, 241. [\[CrossRef\]](#)
9. Pour, S.A.; Shahriari, M.; Zibae, A.; Mojarab-Mahboubkar, M.; Sahebzadeh, N.; Hoda, H. Toxicity, antifeedant and physiological effects of trans-anethole against *Hyphantria cunea* Drury (Lep: Arctiidae). *Pestic. Biochem. Physiol.* **2022**, *185*, 105135. [\[CrossRef\]](#)
10. Tan, M.; Wu, H.; Yan, S.; Jiang, D. Evaluating the toxic effects of tannic acid treatment on *Hyphantria cunea* larvae. *Insects* **2022**, *13*, 872. [\[CrossRef\]](#) [\[PubMed\]](#)

11. Edosa, T.T.; Jo, Y.H.; Keshavarz, M.; Anh, Y.S.; Noh, M.Y.; Han, Y.S. Current status of the management of fall webworm, *Hyphantria cunea*: Towards the integrated pest management development. *J. Appl. Entomol.* **2019**, *143*, 1–10. [\[CrossRef\]](#)
12. Berger, C.; Laurent, F. Trunk injection of plant protection products to protect trees from pests and diseases. *Crop Prot.* **2019**, *124*, 104831. [\[CrossRef\]](#)
13. Byrne, F.J.; Almanzor, J.; Tellez, I.; Eskalen, A.; Grosman, D.M.; Morse, J.G. Evaluation of trunk-injected emamectin benzoate as a potential management strategy for Kuroshio shot hole borer in avocado trees. *Crop Prot.* **2020**, *132*, 105136. [\[CrossRef\]](#)
14. Coslor, C.C.; Vandervoort, C.; Wise, J.C. Insecticide dose and seasonal timing of trunk injection in apples influence efficacy and residues in nectar and plant parts. *Pest Manag. Sci.* **2019**, *75*, 1453–1463. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Takai, K.; Suzuki, T.; Kawazu, K. Distribution and persistence of emamectin benzoate at efficacious concentrations in pine tissues after injection of a liquid formulation. *Pest Manag. Sci.* **2004**, *60*, 42–48. [\[CrossRef\]](#)
16. Aćimović, S.G.; VanWoerkom, A.H.; Reeb, P.D.; Vandervoort, C.; Garavaglia, T.; Cregg, B.M.; Wise, J.C. Spatial and temporal distribution of trunk-injected imidacloprid in apple tree canopies. *Pest Manag. Sci.* **2014**, *70*, 1751–1760. [\[CrossRef\]](#)
17. Wheeler, C.E.; Vandervoort, C.; Wise, J.C. Organic control of pear psylla in pear with trunk injection. *Insects* **2020**, *11*, 650. [\[CrossRef\]](#)
18. Huang, J.; Zhang, J.; Li, Y.; Li, J.; Shi, X.-H. Evaluation of the effectiveness of insecticide trunk injections for control of *Latoia lepida* (Cramer) in the sweet olive tree *Osmanthus fragrans*. *PeerJ* **2016**, *4*, e2480. [\[CrossRef\]](#)
19. Turcotte, R.M.; Lagalante, A.; Jones, J.; Cook, F.; Elliott, T.; Billings, A.A.; Park, Y.-L. Spatial and temporal distribution of imidacloprid within the crown of eastern hemlock. *J. Insect Sci.* **2017**, *17*, 22. [\[CrossRef\]](#)
20. Kou, H.; Sun, Y.; Dong, Z.; Zhang, Z. Comparison between sustained effects of spray and injection thiamethoxam on apple aphids and non-target insects in apple orchard. *Ecotoxicol. Environ. Saf.* **2021**, *207*, 111307. [\[CrossRef\]](#)
21. Roditakis, E.; Skarmoutsou, C.; Staurakaki, M. Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Manag. Sci.* **2013**, *69*, 834–840. [\[CrossRef\]](#)
22. Stavrakaki, M.; Ilias, A.; Ioannidis, P.; Vontas, J.; Roditakis, E. Investigating mechanisms associated with emamectin benzoate resistance in the tomato borer *Tuta absoluta*. *J. Pest Sci.* **2022**, *95*, 1163–1177. [\[CrossRef\]](#)
23. Mashal, M.M.; Obeidat, B.F. The efficacy assessment of emamectin benzoate using micro injection system to control red palm weevil. *Heliyon* **2019**, *5*, e01833. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Ouyang, X.; Fan, Q.; Chen, A.; Huang, J. Effects of trunk injection with emamectin benzoate on arthropod diversity. *Pest Manag. Sci.* **2023**, *79*, 935–946. [\[CrossRef\]](#)
25. Zhang, X.-Q.; Mang, D.-Z.; Liao, H.; Ye, J.; Qian, J.-L.; Dong, S.-L.; Zhang, Y.-N.; He, P.; Zhang, Q.-H.; Purba, E.R. Functional disparity of three pheromone-binding proteins to different sex pheromone components in *Hyphantria cunea* (Drury). *J. Agric. Food Chem.* **2020**, *69*, 55–66. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Zhou, L.; Luo, F.; Zhang, X.; Jiang, Y.; Lou, Z.; Chen, Z. Dissipation, transfer and safety evaluation of emamectin benzoate in tea. *Food Chem.* **2016**, *202*, 199–204. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Wang, L.; Zhao, P.; Zhang, F.; Li, Y.; Du, F.; Pan, C. Dissipation and residue behavior of emamectin benzoate on apple and cabbage field application. *Ecotoxicol. Environ. Saf.* **2012**, *78*, 260–264. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Deng, L.; Chen, L.; Guan, S.; Liu, J.; Liang, J.; Li, X.; Li, Z. Dissipation of emamectin benzoate residues in rice and rice-growing environments. *Molecules* **2020**, *25*, 483. [\[CrossRef\]](#)
29. Yusoff, N.; Abd Ghani, I.; Othman, N.W.; Aizat, W.M.; Hassan, M. Toxicity and sublethal effect of farnesyl acetate on diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Insects* **2021**, *12*, 109. [\[CrossRef\]](#)
30. Commission Regulation (EU) 2022/1343; Official Journal of the European Union; European Union: Brussels, Belgium, 2022.
31. EFSA; Anastassiadou, M.; Brancato, A.; Carrasco Cabrera, L.; Ferreira, L.; Greco, L.; Jarrah, S.; Kazocina, A.; Leuschner, R.; Magrans, J.O. Review of the existing maximum residue levels for emamectin according to Article 12 of Regulation (EC) No 396/2005. *EFSA J.* **2019**, *17*, e05803.
32. EFSA; Bellisai, G.; Bernasconi, G.; Brancato, A.; Carrasco Cabrera, L.; Ferreira, L.; Giner, G.; Greco, L.; Jarrah, S.; Kazocina, A. Modification of the existing maximum residue levels for emamectin in various crops. *EFSA J.* **2021**, *19*, e06824.
33. Yang, T.; Doherty, J.; Zhao, B.; Kinchla, A.J.; Clark, J.M.; He, L. Effectiveness of commercial and homemade washing agents in removing pesticide residues on and in apples. *J. Agric. Food Chem.* **2017**, *65*, 9744–9752. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Juraske, R.; Fantke, P.; Ramírez, A.C.R.; González, A. Pesticide residue dynamics in passion fruits: Comparing field trial and modelling results. *Chemosphere* **2012**, *89*, 850–855. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Calvaruso, E.; Cammilleri, G.; Pulvirenti, A.; Lo Dico, G.M.; Lo Cascio, G.; Giaccone, V.; Vitale Badaco, V.; Ciprì, V.; Alessandra, M.M.; Vella, A. Residues of 165 pesticides in citrus fruits using LC-MS/MS: A study of the pesticides distribution from the peel to the pulp. *Nat. Prod. Res.* **2020**, *34*, 34–38. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Archer, L.; Kunwar, S.; Alferez, F.; Batuman, O.; Albrecht, U. Trunk injection of oxytetracycline for huanglongbing management in mature grapefruit and sweet orange trees. *Phytopathology* **2023**, *113*, 1010–1021. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Silva, L.C.; Salamanca-Jimenez, A.; Doane, T.A.; Horwath, W.R. Carbon dioxide level and form of soil nitrogen regulate assimilation of atmospheric ammonia in young trees. *Sci. Rep.* **2015**, *5*, 13141. [\[CrossRef\]](#)
38. Ding, W.; Li, S.; He, P.; Huang, S. Contribution and fate of maize residue-15N and urea-15N as affected by N fertilization regime. *PLoS ONE* **2019**, *14*, e0210176. [\[CrossRef\]](#)

-
39. de la Puente, L.; Pedro Ferrio, J.; Palacio, S. Disentangling water sources in a gypsum plant community. Gypsum crystallization water is a key source of water for shallow-rooted plants. *Ann. Bot.* **2022**, *129*, 87–100. [[CrossRef](#)]
 40. Wise, J.C.; VanWoerkom, A.H.; Acimovic, S.G.; Sundin, G.W.; Cregg, B.M.; Vandervoort, C. Trunk Injection: A Discriminating Delivering System for Horticulture Crop IPM. *Entomol. Ornithol. Herpetol.* **2014**, *3*, 1.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.