



Article

Imidacloprid Uptake and Leaching in the Critical Root Zone of a Florida Entisol

Qudus O. Uthman^{1,2}, Miguel Vasconez³, Davie M. Kadyampakeni^{4,*}, Yu Wang³, Demetris Athienitis⁵
and Jawwad A. Qureshi⁶

¹ Soil, Water, and Ecosystem Sciences Department, University of Florida, 2181 McCarty Hall A, Gainesville, FL 32611, USA; q.uthman@ufl.edu

² Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA

³ Food Science and Human Nutrition Department, Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred, FL 33850, USA; mvasconez@ufl.edu (M.V.)

⁴ Soil, Water, and Ecosystem Sciences Department, Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd, Lake Alfred, FL 33850, USA

⁵ Statistics Department, University of Florida, Gainesville, FL 32611, USA; athienit@ufl.edu

⁶ Entomology and Nematology Department, Southwest Florida Research and Education Center, University of Florida, 2685 SR 29 N, Immokalee, FL 34142, USA; jawwadq@ufl.edu

* Correspondence: dkadyampakeni@ufl.edu; Tel.: +1-863-956-8843

Abstract: Imidacloprid (IDP) products are applied via soil drenching in the citrus critical root zone (CCRZ) at 0–60 cm soil depth. This study aimed to determine the uptake and leaching of IDP in the CCRZ of a Florida Entisol. The treatments include: (1) a control with no IDP applied, (2) 1.6 g of active ingredient (a.i.) per tree ($\times 2$), and (3) 3.2 g a.i. per tree of IDP ($\times 4$). The treatments were applied to two trees within each experiment unit, replicated five times, and completely randomized. The IDP concentration in the Entisol was affected by the amount of water received within the sampling intervals. IDP movement in the Entisol was evident for the field trials in Fall 2021 and 2022, irrespective of the treatment. A total of 10 mm of daily irrigation was the major driver of IDP movement in Fall 2021 (September–December 2021), while 11.7 cm of cumulative rainfall plus 10 mm of daily irrigation were the major drivers for IDP in Fall 2022 (November–December 2022). The IDP uptake level by leaves was relatively low probably because of the relatively low temperature and humidity. More applications of IDP did not result in its higher uptake by citrus leaves in the Entisol. Given the persistence of IDP, there is a possibility of leaching, which could potentially contaminate the groundwater, surface water, and non-target organisms. Therefore, it is crucial to carefully manage the use of IDP in citrus production systems to mitigate the unintended environmental impacts.

Keywords: critical zone; entisol; imidacloprid; sandy soil; solute transport



Citation: Uthman, Q.O.; Vasconez, M.; Kadyampakeni, D.M.; Wang, Y.; Athienitis, D.; Qureshi, J.A.

Imidacloprid Uptake and Leaching in the Critical Root Zone of a Florida Entisol. *Agrochemicals* **2024**, *3*, 94–106. <https://doi.org/10.3390/agrochemicals3010008>

Academic Editor: Qingli Shang

Received: 30 January 2024

Revised: 7 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

Imidacloprid (IDP), a neonicotinoid insecticide, has become the subject of significant interest and concern in agricultural and the environmental fields [1]. IDP's fate and transport in various ecosystems, along with its traditional applications and rates, have been the focus of numerous studies due to the growing apprehensions about its environmental impact, particularly when it is transported off-site [1,2]. The environmental concerns associated with IDP primarily revolve around its potential effects on non-target species, including beneficial insects like bees [3], and its persistence in soil and water systems [1]. IDP belongs to the class of neonicotinoid insecticides [4]. It acts as an agonist to the nicotinic acetylcholine receptor (nAChR) in the nervous system of insects [5]. By binding to these receptors, IDP disrupts the transmission of nerve impulses, leading to paralysis and the eventual death of the insect [6]. There have been reports of resistance development in some insect species to IDP, particularly in aphids and whiteflies [7]. Resistance can arise due to

various mechanisms, such as metabolic resistance where the insect rapidly detoxifies the insecticide, or target-site resistance where mutations in the nAChR reduce the insecticide's effectiveness [8].

IDP is effective against a variety of pests, including aphids, termites, soil insects, Colorado potato beetles, rice hoppers, whiteflies, thrips, and some scale insects (<https://irac-online.org/>, accessed on 17 February 2024). It is used on a wide range of crops, including cotton, cereals, maize, potatoes, rice, vegetables, sugarcane, fruits, and ornamentals. According to the Insecticide Resistance Action Committee, IDP is classified under Group 4A (<https://irac-online.org/>, accessed on 17 February 2024). This group includes neonicotinoids that act as agonists at the nicotinic acetylcholine receptor. The widespread use of IDP and its high efficacy have made it a cornerstone in pest management strategies. However, its usage has also raised environmental concerns, particularly regarding its effects on non-target species like bees [3]. This has led to regulatory scrutiny and restrictions in some regions. As with all pesticides, the judicious and informed use of IDP, in combination with Integrated Pest Management practices, is crucial for sustainable agricultural practices [9]. Other studies have demonstrated that IDP, once applied, can leach into the groundwater, or run off into the surface water, raising concerns about water quality and aquatic life [2,10,11]. The compound's persistence and mobility in soil and water systems depend on various factors like the soil type, climate, and application methods [12].

IDP has been applied in a variety of ways, including soil drenching, seed treatment, and foliar spraying [13]. The application rates vary depending on the crop and pest targeted, but there is an increasing trend towards the use of lower amounts to minimize the environmental impact [13]. The environmental risks of IDP are most pronounced when it is transported off-site, where it can affect non-target organisms [9], particularly concerning its impact on pollinators, with numerous studies linking sub-lethal exposure to IDP to reduced foraging efficiency and colony collapses among bees [3,9]. The key challenge lies in finding a balance that allows for effective pest control without causing undue harm to the environment. Integrated Pest Management (IPM) strategies, which include the judicious use of IDP, alongside other methods, are seen as a way forward in achieving this balance [14].

The management programs practiced by growers in combating citrus greening disease or huanglongbing (HLB) include optimizing fertilization practices and insecticide programs to control its vector, the Asian Citrus Psyllid (ACP) *Diaphorina citri* [15–17]. Soil-applied systemic insecticides integrated with foliar sprays with different modes of action proved effective in reducing the ACP populations, the risk of pest resistance to insecticides, and the incidence of HLB [7]. IDP has shown high ACP suppression levels because of there being no or low resistance for several years [18]. However, rotating this mode of action of this insecticide with others is critical to reduce the risk of pest resistance, which is observed when such measures are not practiced [18]. Entisols are the dominant soils under citrus cultivation in Central Florida [19]. They are sandy in texture, with less than 95% sand with coatings, 0.5% organic matter, and dominated by soil minerals such as quartz and kaolinite [20]. These soils are highly porous and have low capacity to retain water, nutrients, and IDP [10].

Enhanced nutrition programs (ENPs) have been used to mitigate the severity of HLB symptoms, particularly via the foliar application method for micronutrients [21]. Enhanced nutrition programs only maintain the health of the trees and do not control ACPs, which could cause a regional surge in diseases, inoculum sources, and vectors [21]. It is critical to control ACPs, along with nutrient management. Other researchers [21] showed that combined foliar nutrients and insecticides may be the best management practice when the infection covers a relatively small portion of the field. This combination could significantly increase the yield in HLB-affected citrus groves, but the economic threshold for this combination is needed under different environmental and market conditions [21]. Studies have been performed on the foliar spraying of nutrients and insecticides to manage HLB-affected citrus trees on spodosol (sandy soil with no coatings) [21,22]. However, there

is no study on managing insecticides on the coated sand of Entisol. Thus, this study is aimed to determine the uptake and leaching of IDP in a Florida Entisol.

2. Materials and Methods

2.1. Description and Design of the Experimental Site

This study was conducted on 'Valencia' [*Citrus sinensis* (L.) Osb.] on Swingle rootstock (*Citrus paradisi* Macf. $3 \times$ *Poncirus trifoliata* (L.) Raf.) trees spaced at 1.8 m by 4.6 m at the Citrus Research and Education Center (CREC), Lake Alfred, FL, USA (28.09° N, 81.75° W), which were planted in 2019. The soil is classified as Candler sand with a taxonomic class of hyperthermic, uncoated lamellic quartzipsamments and eolian or sandy marine deposits as the parent material [23]. The minimum and maximum temperatures and rainfall within the period of this study were sourced from the Florida automated weather network (FAWN) (<https://fawn.ifas.ufl.edu/>, accessed on 19 June 2023) at CREC, Lake Alfred, FL, USA. All trees were irrigated to meet the daily evapotranspiration demand by 40 L h⁻¹ emitters, with one emitter per two trees having a wetting diameter of about 2 m, with irrigation performed once every day. Fifteen experimental units were used for this study, with a completely randomized design. Each experimental unit had four trees, and the middle two trees received treatments (Figure 1). Soil moisture was monitored using Teros 12 capacitance sensors (MeterGroup, Pulman, WA, USA), at 0–15, 15–30, 30–45, and 45–60 cm depths within a 45 cm radius from the tree in the irrigated zone in each treatment experimental unit for Fall 2022.



Figure 1. Uthman, Qudus. A photo of the field experiment.

2.2. Quantification of Bacterial Titers and Asian Citrus Psyllid (ACP) Counts

Leaf samples of each experimental unit were collected and tested for *Candidatus liberibacter asiaticus* using a quantitative real-time polymerase chain reaction (qPCR) to determine the HLB status of the trees. Leaf samples of a fully expanded new flush were collected, incised, and the midrib was chopped for qPCR analysis following the methods described in [24]. About 50% of the trees tested positive for HLB. A randomly chosen branch of the tree was struck by a 70 cm polyvinyl chloride pipe two times, and the number of ACPs that fell on a laminated sheet held by a clipboard was quickly counted [25,26]. ACP population counts were performed in the Spring 2023 trial because no data were recorded for Fall 2021 and 2022.

2.3. Application of IDP Treatments

IDP (Admire[®] Pro Systemic Protectant, Bayer CropScience LP, St. Louis, MO, USA) was applied using a soil drench at the Institute of Food and Agricultural Sciences (IFAS) at a recommendation rate of 0.56 kg IDP ha⁻¹ [27]. Treatments include the following:

(1) a control plot with no IDP applied, (2) 1.6 g of active ingredient (a.i.) per tree ($\times 2$), and (3) 3.2 g a.i. per tree of IDP ($\times 4$). Treatments were applied on 18 September 2021 and 15 November 2022 for Fall 2021 and 2022, respectively. Potassium bromide (KBr) was applied as a tracer for water movement at a rate of 80 kg Br ha⁻¹ for Fall 2022 (Chemsavers, Bluefield, VA, USA). These treatments were replicated five times, making a total of 15 experimental units. The trees were supplied with 285 L ha⁻¹ of liquid fertilizer (All-Purpose Liquid Fertilizer with Micronutrients (50% Slow-Release Nitrogen), Pendelton Turf Supply, Waterford, WI, USA) three times per year, containing known amounts of nutrients based on IFAS recommendations, as follows: 17% N, 5% P₂O₅, 11% K₂O, 1% Mg, 5.5% S, 0.2% B, 0.5% Fe, 0.05% Mn, and 0.05% Zn [28].

2.4. Collection and Analysis of Soil Samples for IDP

The soil samples were collected four times, once before treatment application and three times after treatment application. The soil samples were collected on days 39, 56, and 74 after treatment application in the Fall 2021 trial and on days 7, 14, and 21 after treatment application in the Fall 2022 trial. The soil samples were collected from the soil surface to 60 cm depth at 15 cm increments to account for IDP movement beyond the 30 cm fibrous rooting zone, where most roots are concentrated [29], within the 30 cm distance to the tree row, at a different spot away from the trees for the four sampling times. The soil samples collected from two trees in the experimental unit were composited for each depth. The samples were stored in coolers, transported to the laboratory, and refrigerated ($-20\text{ }^{\circ}\text{C}$) until further processing. Chemical abstract service (CAS) numbers of the extraction reagents used are provided in enclosed brackets. IDP was extracted from the soil using high-performance liquid chromatography (HPLC) grade 80% methanol (CAS 67-56-1) plus 20% water (CAS 7732-18-5) in 1:1 soil–solution ratio.

The matrix container used for this study was polypropylene centrifuge tubes. They were shaken for 24 h using an orbital shaker and oscillator with an anti-slip pad at an adjustable speed at 0–210 rpm, 110 V (Vevor, Rancho Cucamonga, CA, USA). The samples were centrifuged (Model XC-2450 series centrifuge, C & A Scientific, Sterling, VA, USA) for 20 min at 4000 rpm, and supernatant solutions were filtered using a 0.45 μm pore diameter syringe (Fisher Scientific, Waltham, MA, USA). IDP was analyzed using HPLC (Model 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) with an ultra-violet (UV) detector at a 270 nm wavelength and an LiChrospher reverse phase (RP) select B column (dimensions: 125 \times 4.0 mm; MilliporeSigma, St. Louis, MO, USA). The mobile phase was 40% water and 60% methanol, with a flow rate of 1 mL min⁻¹. IDP analytical standard with 99.9% purity was used for the whole experiment (Sigma-Aldrich, St. Louis, MO, USA). The elution was isocratic, the sample injection volume was 20 μL , and the retention time was about 2 min. The IDP concentration in the soil was determined using a calibration curve of six known concentrations of blank samples and 100 mg L⁻¹ of IDP ($R^2 = 0.99975\text{--}0.99998$). The limit of quantification (LOQ) and limit of detection (LOD) values for IDP were 0.12 and 0.04 $\mu\text{g mL}^{-1}$, respectively. Bromide was extracted using hot water extraction and analyzed using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) at a 154.065 nm detection limit wavelength [4,30,31].

2.5. Collection and Analysis of Leaf Samples for IDP

The leaf samples were collected on days 39, 56, and 74 after treatment application in the Fall 2021 trial and on days 7, 14, and 21 after treatment application in the Fall 2022 trial. The citrus leaf sampling technique in [28] was adopted to collect the leaf tissue samples by sampling 4-to-6-month-old leaves from four quadrants of the tree in the northwest, southeast, northeast, and southwest directions and combining the sample per plot for a representative sample. Although it was a young, 3-year-old tree with fewer leaves and branches, tender shoot leaves were sampled for IDP analysis. After collection, the leaf tissues were transported from the field to the laboratory in a cooler, wrapped in an aluminum foil, and refrigerated ($-20\text{ }^{\circ}\text{C}$) until further processing.

IDP analysis of the leaves was performed using a modified procedure from [32]. A standard stock solution of IDP was prepared in methanol for calibration standards and spiking tests. The leaves were cut perpendicular to the leaf stem into 2 mm wide pieces. About 24 g of 4.8 mm diameter beads and 5 mL of methanol were mixed together in a 50 mL tube, alongside 0.5 g of cut leaf tissue and 40 μL of the internal standard solution, consisting of 10 mg L^{-1} of the deuterated analyte, IDP-d4 (Sigma-Aldrich, St. Louis, MO, USA). The tubes were placed in a Bullet Blender, model 50-DX from Next Advance (Troy, NY, USA). A disruption step for maximum speed for 12 min was run, which generated a white paste. Another 5 mL of methanol was added, and the extraction step was run at maximum speed for 12 min for actual extraction, making 10 mL extraction volume in total. It was then diluted into 1 mL aliquots in 1:1 0.1% formic acid in methanol and filtered with 0.22 μm size non-sterile nylon syringe filters (Fisher Scientific, Fair Lawn, NJ, USA) [32].

LC-MS/MS analyses were carried out using an Ultimate 3000 LC system coupled to a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The analyte was separated using a Gemini 3 μm NX-C18 110 \AA chromatography column (2 \times 150 mm, particle size 3 μm) from Phenomenex (Torrance, CA, USA) at a column temperature of 25 $^{\circ}\text{C}$ using gradient elution with 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B). The gradient profile was as follows: 0–4 min 5% eluent B, 4–12 min from 5% to 60% B, 12–13 min from 60% to 100% B, 13–20 min 100% B, 20–20.5 min from 100% to 5% B, and 20.5–26 min 5% B to re-equilibrate the column using the initial composition of mobile phase. The flow rate was 0.25 mL min^{-1} , and the injection volume was 4 μL . The mass spectrometer was equipped with an electrospray ionization (ESI) interface, operating in positive ionization mode. The parameters were as follows: spray voltage, 3500 V; ion transfer tube temperature, 325 $^{\circ}\text{C}$; vaporizer temperature, 300 $^{\circ}\text{C}$; sheath gas, 40 Arb; aux gas, 12 Arb; and sweep gas, 1 Arb. The MS/MS detector was operated using selective reaction monitoring (SRM) mode. The MS/MS parameters for IDP were optimized using the flow injection analysis of individual standards, with the precursor m/z at 256 and 260 for IDP and IDP-d4, respectively, and product transitions at m/z 175, 209 and 212 for IDP and 179 and 213 for IDP-d4. The retention time for the analyte was 10.6 min. Collision energy was optimized at 18, 16, and 10 V for each IDP transition, respectively, and at 19 and 17 V for each IDP-d4 transition. The RF lenses were 53 V for IDP and 147 V for IDP-d4. The concentration of IDP was calculated from the ratio of the analyte area and the internal standard area using calibration curves of known concentrations with a range of 0.5–500 $\mu\text{g L}^{-1}$. The calculated LOD was 4.3 $\mu\text{g L}^{-1}$, and the LOQ was 13 $\mu\text{g L}^{-1}$. The calibration curve was prepared on untreated extracted matrix to account for suppression or enhancement effects and had an $R^2 = 0.9997$ over the tested range.

2.6. Statistical Analysis

The combinations of soil depth (soil samples) or trees (leaf samples), treatments, and experimental unit were used for subject identification. Subject identifications were designated as random effects, and measurements were taken repeatedly on the soil and collected leaf samples four times, and treatments represent fixed effects. The experimental design necessitated the use of a linear mixed effect design with repeated measurements, and the statistical model is given as follows:

$$Y_{ijkl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + (\alpha\gamma)_{ik} + \tau_l + (\alpha\tau)_{il} + (\gamma\tau)_{kl} + \epsilon_{jkl(i)} \quad (1)$$

$i = 1 \dots 3; j = 1 \dots g; k = 1 \dots d; l = 1 \dots t; \beta_{j(i)} \sim N(0, \sigma_{\beta}^2)$

where Y_{ijkl} = observed measurement; $\beta_{j(i)}$ = subject identification effect; μ = population mean; α_i = treatment effects; γ_k = IDP effects; τ_l = time effect; $\epsilon_{jkl(i)}$ = error term; σ_{β}^2 = variance in the subject ID random effect. Four different covariance structures were used, namely compound symmetry, autoregressive of order 1, and heterogeneous and unstructured covariances to account for correlation of measurements over time within the subject [33]. Means were separated using Tukey's honest significant differences (HSD) at

the 0.05 significance level. Statistical analysis and data visualization were performed with ggplot2 package using R statistical software packages in RStudio version 4.3.2 [34]. Data collected on each subject before treatment application (time zero as baseline) were deducted from the data collected after treatment application. The generalized linear mixed model (glmer) function from lme4 package was used for the analysis of ACP population counts using a Poisson loglinear model with random intercepts with each tree [35].

3. Results

The results show that the percentage reduction in population count increased over time after the application of IDP, but the treatments were not statistically different ($p > 0.05$) from each other (Figure 2), implying that increase in the IDP application rate does not cause an increase in the percentage reduction in the ACP population. The percentage reductions in ACP were 9% and 4% for the $\times 2$ and $\times 4$ treatments, respectively, after 7 days (Figure 2). Additionally, 10% and 4% reductions for every subsequent week were observed for both the $\times 2$ and $\times 4$ treatments, respectively (Figure 2).

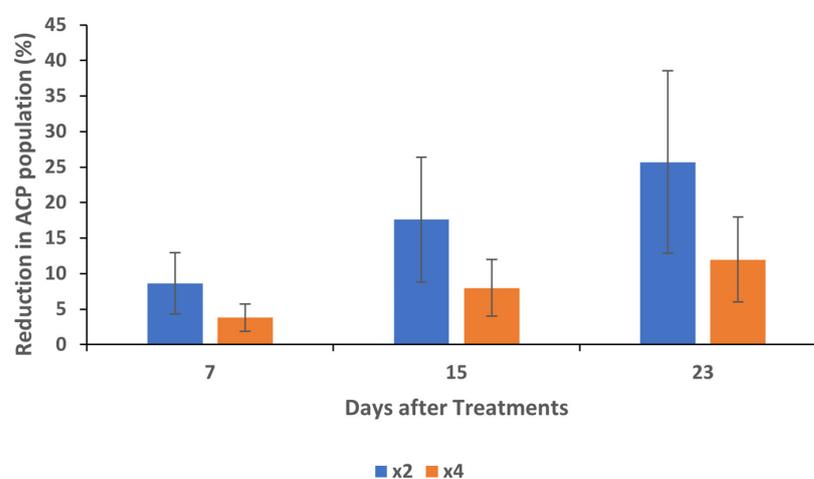


Figure 2. Percentage reduction in Asian Citrus Psyllid (ACP) per tap after treatment application (1.6 g of active ingredient (a.i.) per tree ($\times 2$) and 3.2 g a.i. per tree of imidacloprid (IDP) ($\times 4$)) in spring 2023. The standard error is 50% probable error range.

The double standard treatment of IDP ($\times 2$) showed that the concentration of IDP in soil remained the same at 39 and 56 days, but decreased at 74 days after the treatment application (Figure 3). This could be explained by the cumulative amounts of rainfall plus irrigation (11.4 cm, 36 cm, and 55 cm at 39, 56 and 74 days after treatment application, respectively) after IDP application for Fall 2021 (Figure 4). The quadruple standard treatment of IDP ($\times 4$) caused a significant ($p < 0.001$) higher IDP concentration in the soils than double standard treatment application ($\times 2$) for Fall 2021 (Figure 3; Table 1). The decrease in the concentration of IDP at 74 days after the quadruple standard treatment application could be attributed to factors such as cumulative rainfall and irrigation (55 cm) received during this period (Figures 3 and 4). This significant amount of water might have potentially leached IDP beyond the root zone or led to uptake by the plants, contributing to the observed decrease in concentration.

The results showed that the concentration of IDP in the soil at 7, 14, and 21 days after the double standard treatment ($\times 2$) of IDP remained the same for Fall 2022 (Figure 3). The concentration of IDP in the soil after the quadruple standard treatment ($\times 4$) was significantly ($p < 0.001$) higher than that after the double standard treatment ($\times 2$) and decreased over time for the quadruple standard treatment (Figure 3; Table 1). It seems that rainfall plus irrigation (7.2 cm, 14.6 cm, and 22 cm at 7, 14, and 21 days after treatment application, respectively) could explain the movement of IDP beyond the citrus critical root

zone (Figure 4). Nevertheless, the soil moisture was above the field capacity and never reached saturation at all the depths (Figure 5).

Table 1. Analysis of variance for imidacloprid (IDP) in soils as affected by variable rates of IDP (treatment), soil depth, and time for Fall 2021 and 2022 experimental studies.

	Numerator DF	F-Value	Fall 2021		Fall 2022	
			p-Value	F-Value	p-Value	
Mean (Intercept)	1	111.08	<0.0001	96.64	<0.0001	
Treatment	2	42.77	<0.0001	34.36	<0.0001	
Soil depth	3	13.17	<0.0001	7.44	0.0001	
Time	2	12.90	<0.0001	4.79	0.0097	
Treatment × Soil depth	6	4.45	0.0004	2.60	0.0199	
Treatment × Time	4	5.61	0.0003	2.81	0.0277	
Soil depth × Time	6	0.89	0.5075	0.30	0.9344	
Treatment × Soil depth × Time	12	0.83	0.6181	0.20	0.9982	
Denominator DF	144					

DF means degrees of freedom. F-value means Fisher’s value.

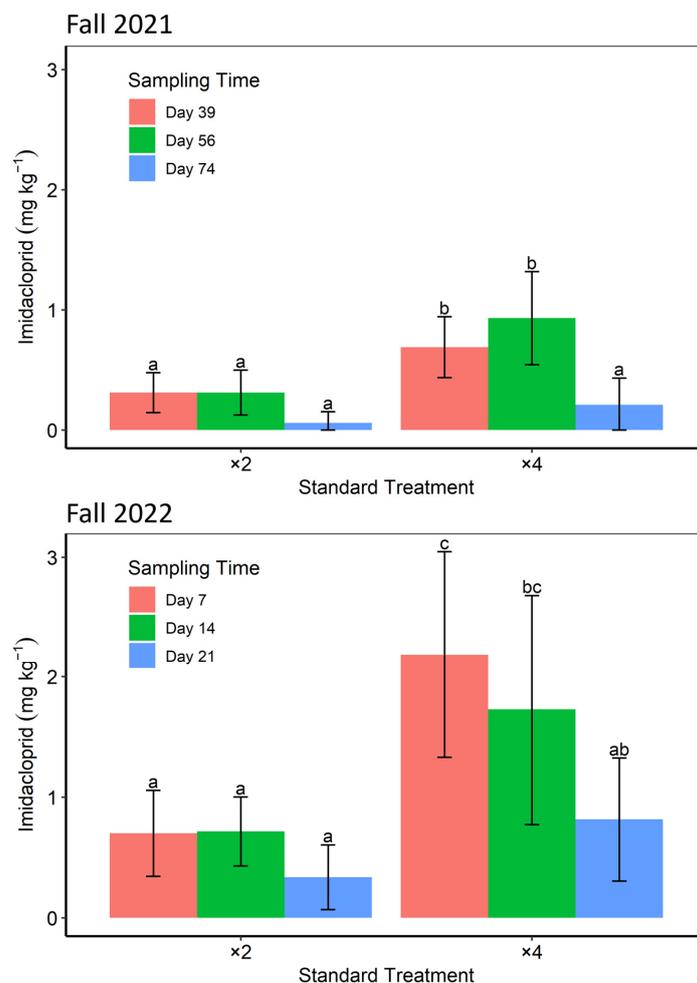


Figure 3. Effect of variable imidacloprid (IDP) rates with repeated measurement of IDP in soil over time for Fall 2021 and 2022 (number of observations = 20). Sampling times are the number of days when soil samples were collected after treatment application. Standard treatment is 0.56 kg IDP ha⁻¹; ×2 and ×4 are 2 and 4 times of standard treatment applications. The upper and lower bars are 95% confidence limits.

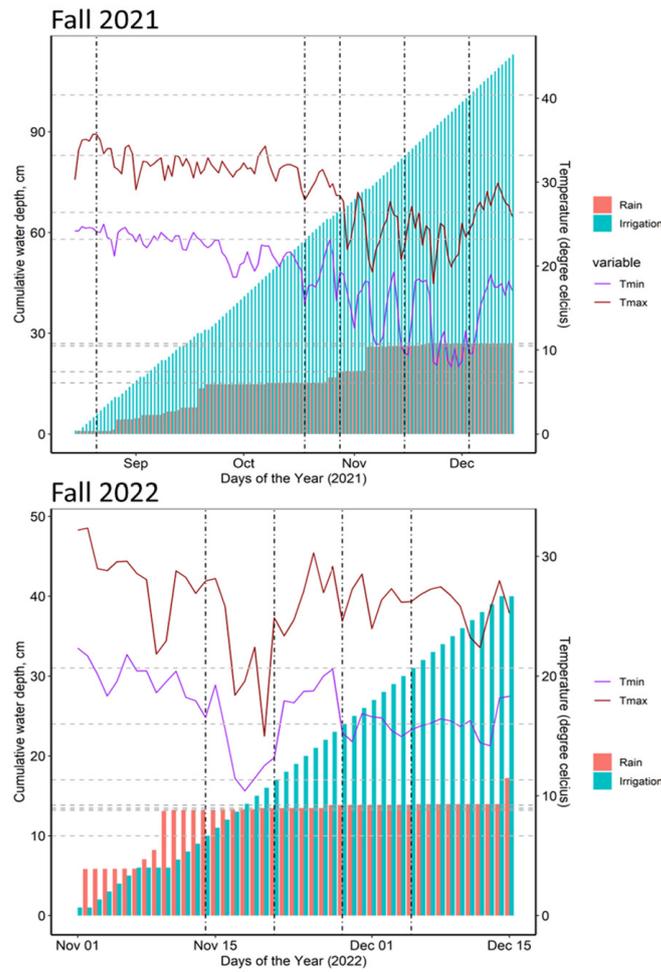


Figure 4. Cumulative rainfall and irrigation during the periods of Fall 2021 and 2022 experimental study trials. Minimum (Tmin) and maximum (Tmax) temperatures during the periods of Fall 2021 and 2022 experimental study trials.

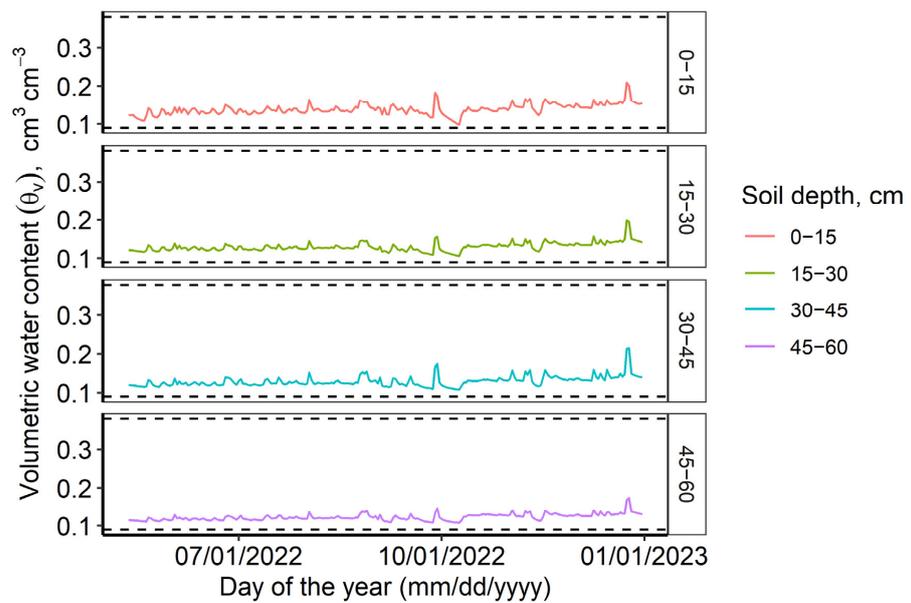


Figure 5. Soil moisture distribution in the 0–60 cm depth at increments of 15 cm. The upper and lower dashed lines for each depth are water content at saturation and field capacity, respectively.

The IDP concentration in the citrus leaves showed that there was IDP uptake, which was not significantly different ($p > 0.05$) from that of the other leaves irrespective of the day after treatment application and amount applied for Fall 2021 (Figure 6; Table 2). Similarly, there was IDP uptake, which was not significantly different ($p > 0.05$) among the samples irrespective of the day after treatment application and amount applied for Fall 2022 (Figure 6; Table 2). The IDP concentration in either the leaves or soils were neither correlated over time within the trees nor soils, but rather minimize the inflation of the standards errors. The low-level uptake of IDP could be because of the low temperatures in Fall that inhibit the high-level uptake of IDP since the Fall temperature range in Central Florida is 13–33 °C (Figure 4) [36].

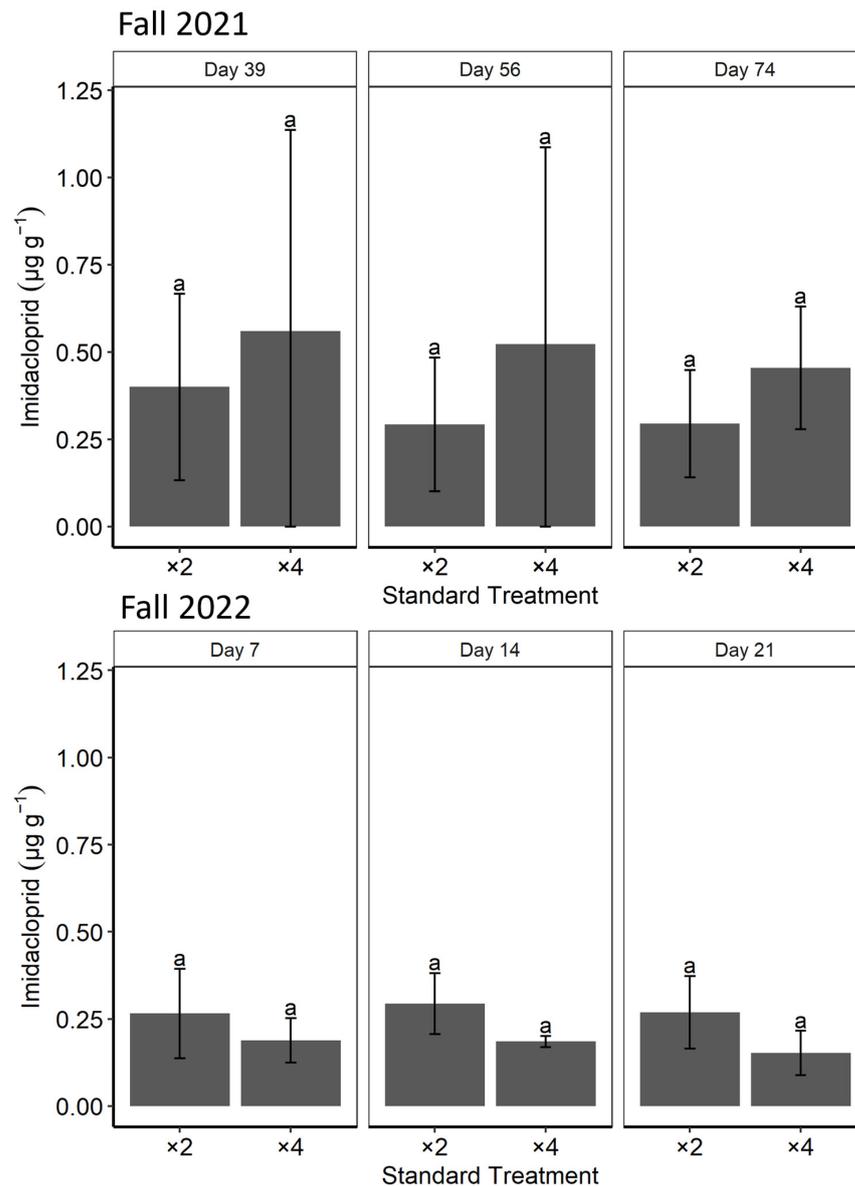


Figure 6. Leaves' uptake of imidacloprid (IDP) as affected by variable IDP rates with repeated measurements over time for Fall 2021 and 2022 (number of observations = 10). Sampling time is the number of days when leaf samples were collected after treatment application. Standard treatment is 0.56 kg IDP ha⁻¹; ×2 and ×4 are 2 and 4 times the standard treatments. The upper and lower bars are 95% confidence limits.

Table 2. Analysis of variance for imidacloprid (IDP) in leaves affected by variable rates of IDP (treatment) and time for Fall 2021 experimental study.

	Fall 2021			Fall 2022	
	Numerator DF	F-Value	p-Value	F-Value	p-Value
Mean (Intercept)	1	55.46	<0.0001	114.26	<0.0001
Treatment	2	1.25	0.2914	2.04	0.1372
Time	2	0.31	0.7364	0.96	0.3877
Treatment × Time	4	0.07	0.9917	1.48	0.2168
Denominator DF	78				

DF means degrees of freedom. F-value means Fisher’s value.

The volumetric water content remained above field capacity of 10% and never reached saturation for Fall 2022 (Figure 5). The water content was in between the field capacity and saturation, which support either the leaching or uptake of IDP (Figure 5). Bromide (Br) demonstrated the movement of water from the upper to lower soil depths since Br does not adsorb or transform in soils (Figure 7). Thus, the evidence of water availability, water movement, and sampling intervals of the soil and citrus leaves had no treatment effect on the uptake of IDP suggest either minimal leaching since saturation was not reached, plant uptake at the field capacity or moisture content below saturation, or another fate of IDP such as degradation.

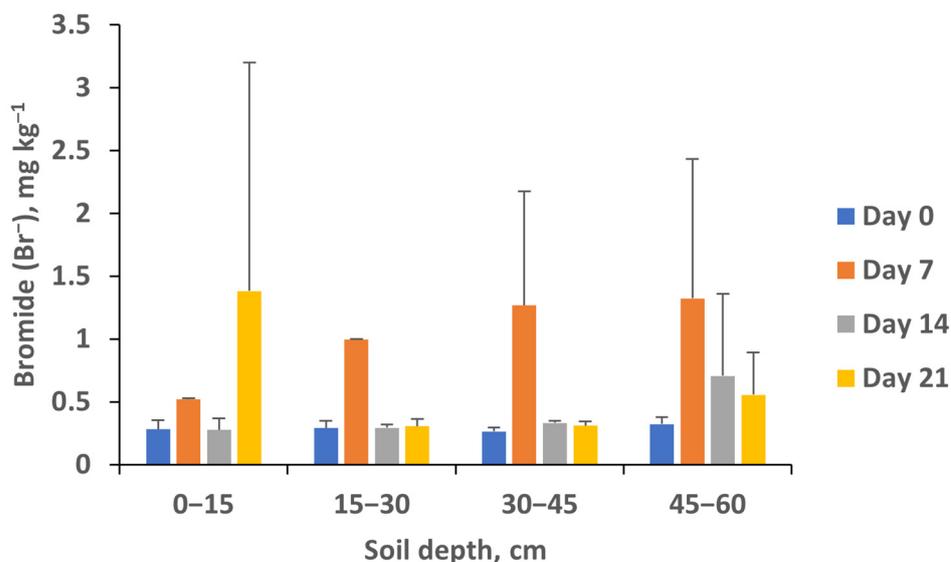


Figure 7. Bromide (Br) distribution in the 0–60 cm depth at an increment of 15 cm before (day 0) and after bromide application as a function of time (days 7, 14, and 21).

4. Discussion

Sandy soils are characterized by large pores, which allow water to move through the soil quickly [37]. This implies that IDP can move through the soil profile quickly and can potentially reach deeper layers of the soil beyond the citrus critical root zone at 0–60 cm, especially under saturated flow. This was evident in the Fall 2021 and 2022 trials after treatment applications of rainfall plus irrigation amounts of 55 and 22 cm, respectively (Figure 4). The field slope can affect the movement of IDP in sandy soils, but the experimental study location site has a slope of 0%. Thus, the downward movement of water and IDP is the only possible direction, and no lateral flow occurred. However, IDP can also be carried by surface runoff in sandy soils during heavy rain or irrigation

events [38,39], but this was not the case because there was no heavy rain or irrigation event that could have caused surface runoff during the period of this study in Fall 2021 and 2022.

To minimize the leaching and water evaporation losses, water management strategies for citrus trees affected by HLB on sandy soils in Florida aim to maintain the water content at field capacity (optimal moisture level) for the 0–60 cm soil depth, which corresponds to the root zone [40]. Conversely, IDP leaching is possible since the water content during Fall 2022 was above the field capacity at the critical root zone. In spite of this, hydraulic conductivity that facilitates water and IDP transport in sandy soils is significantly reduced within the critical root zone, thus minimizing leaching [16].

The peak season of ACP activity is typically from late spring through to early Fall when the temperatures are warm and citrus crops are actively growing [36]. Unfortunately, the ACP populations remained low in our study area and were not observed in both the experiments conducted in the Fall (2021 and 2022). In spring 2023, reductions of no more than 10% and 4% in the ACP population with 2× and ×4 treatments were observed, suggesting that increasing the application rate of IDP did not improve the suppression of ACP populations. However, the previous studies have reported much higher and more significant reduction levels of ACP with IDP sustained from 4 to 8 weeks after drench application [7,41]. Besides the low populations at the study site, another factor that may have contributed to the minimal ACP reduction with IDP in our study is their resistance to the mode of action of this insecticide, which has been observed [18].

IDP uptake is affected by the amount of water available in the soil, as well as the health of the root system [42,43]. Environmental conditions, such as temperature, humidity, and light intensity, can also affect the uptake of IDP [44]. Higher temperatures and humidity levels can increase the rate of absorption, while low light intensity can slow it down [44]. But for both the experimental study trials in Fall, the relatively low temperature and humidity could be the reason for the low-level uptake of IDP when compared with the result in [33], since their study was performed in Spring and Summer on a Florida Spodosol, which exhibits poor drainage.

5. Conclusions

IDP movement in this soil was evident for both the experimental study trials of Fall 2021 and 2022, irrespective of the application rate of IDP. Irrigation was the major driver of IDP movement in Fall 2021, and rainfall plus irrigation was the major driver for IDP in Fall 2022. The frequency of soil and leaf sample collection also affected the amount of water received within the sampling intervals. Increased IDP application had no effect on the citrus leaves uptake of IDP or on ACP suppression under the studied conditions. Given the persistence of IDP, there is a possibility of leaching, which could potentially contaminate the groundwater, surface water, and non-target organisms. Therefore, it is crucial to carefully manage the use of IDP and other chemicals in citrus production systems to mitigate the unintended environmental impacts.

Author Contributions: Conceptualization, Q.O.U. and D.M.K.; methodology, Q.O.U., M.V., Y.W., D.M.K. and J.A.Q.; software, Q.O.U.; validation, Q.O.U.; formal analysis, Q.O.U. and M.V.; investigation, Q.O.U.; resources, D.M.K.; data curation, Q.O.U.; writing—original draft preparation, Q.O.U. and M.V.; writing—review and editing, Q.O.U., M.V. and D.M.K.; visualization, Q.O.U., D.A., D.M.K. and J.A.Q.; supervision, D.M.K. and Y.W.; project administration, D.M.K.; funding acquisition, D.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are especially grateful for the support of UF/IFAS Soil, Water and Ecosystem, Sciences Department for the matching assistantship and the UF/IFAS Citrus Initiative and USDA Hatch Project #006185 for research funds to conduct this research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be available on request from the corresponding author.

Acknowledgments: The authors acknowledge the support of staff from the CREC Water and Nutrient Management Laboratory led by Kadyampakeni and the CREC Food Chemistry Laboratory led by Yu Wang.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Bonmatin, J.-M.; Giorio, C.; Girolami, V.; Goulson, D.; Kreutzweiser, D.P.; Krupke, C.; Liess, M.; Long, E.; Marzaro, M.; Mitchell, E.A.D.; et al. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res.* **2015**, *22*, 35–67. [[CrossRef](#)]
2. Chagnon, M.; Kreutzweiser, D.; Mitchell, E.A.D.; Morrissey, C.A.; Noome, D.A.; Van der Sluijs, J.P. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ. Sci. Pollut. Res.* **2015**, *22*, 119–134. [[CrossRef](#)]
3. Meikle, W.G.; Adamczyk, J.J.; Weiss, M.; Gregorc, A.; Johnson, D.R.; Stewart, S.D.; Zawislak, J.; Carroll, M.J.; Lorenz, G.M. Sublethal effects of imidacloprid on honey bee colony growth and activity at three sites in the US. *PLoS ONE* **2016**, *11*, e0168603. [[CrossRef](#)]
4. Clive, T. (Ed.) *The Pesticide Manual: A World Compendium: Incorporating the Agrochemicals Handbook*; Wiley-Blackwell: Hoboken, NJ, USA, 1981.
5. Moffat, C.; Buckland, S.T.; Samson, A.J.; McArthur, R.; Pino, V.C.; Bollan, K.A.; Huang, J.T.-J.; Connolly, C.N. Neonicotinoids target distinct nicotinic acetylcholine receptors and neurons, leading to differential risks to bumblebees. *Sci. Rep.* **2016**, *6*, 24764. [[CrossRef](#)]
6. Araújo, M.F.; Castanheira, E.M.; Sousa, S.F. The buzz on insecticides: A review of uses, molecular structures, targets, adverse effects, and alternatives. *Molecules* **2023**, *28*, 3641. [[CrossRef](#)]
7. Qureshi, J.A.; Kostyk, B.C.; Stansly, P.A. Insecticidal suppression of Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) vector of huanglongbing pathogens. *PLoS ONE* **2014**, *9*, e112331. [[CrossRef](#)] [[PubMed](#)]
8. Siddiqui, Ali, J.; Fan, R.; Naz, H.; Bamisile, B.S.; Hafeez, M.; Ghani, M.I.; Wei, Y.; Xu, Y.; Chen, X. Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. *Front. Physiol.* **2023**, *13*, 1112278. [[CrossRef](#)] [[PubMed](#)]
9. Hladik, M.L.; Main, A.R.; Goulson, D. Environmental risks and challenges associated with neonicotinoid insecticides. *Environ. Sci. Technol.* **2018**, *52*, 3329–3335. [[CrossRef](#)]
10. Leiva, J.A. *Imidacloprid Fate and Transport in Florida Flatwoods Soils and Plants during Control of the Asian Citrus Psyllid*; University of Florida: Gainesville, FL, USA, 2014.
11. Juraske, R.; Castells, F.; Vijay, A.; Muñoz, P.; Antón, A. Uptake and persistence of pesticides in plants: Measurements and model estimates for imidacloprid after foliar and soil application. *J. Hazard. Mater.* **2009**, *165*, 683–689. [[CrossRef](#)]
12. Hardin, J. *Imidacloprid Persistence, Mobility, and Effect on Ecosystem Function*; East Tennessee State University: Johnson City, TN, USA, 2018.
13. Zhang, C.; Wang, X.; Kaur, P.; Gan, J. A critical review on the accumulation of neonicotinoid insecticides in pollen and nectar: Influencing factors and implications for pollinator exposure. *Sci. Total Environ.* **2023**, *899*, 165670. [[CrossRef](#)] [[PubMed](#)]
14. Pathak, V.M.; Verma, V.K.; Sharma, A.; Dewali, S.; Kumari, R.; Mohapatra, A.; Cunill, J.M. Current status of pesticide effects on environment, human health and its eco-friendly management as bioremediation: A comprehensive review. *Front. Microbiol.* **2022**, *13*, 2833. [[CrossRef](#)]
15. Muraro, R. *Summary of 2008–2009 Citrus Budget for the Southwest Florida Production Region*; University of Florida, IFAS, CREC: Lake Alfred, FL, USA, 2009.
16. Uthman, Q.O.; Atta, A.A.; Kadyampakeni, D.M.; Qureshi, J.A.; Morgan, K.T.; Nkedi-Kizza, P. Integrated Water, Nutrient, and Pesticide Management of Huanglongbing-Affected Sweet Oranges on Florida Sandy Soils—A Review. *Plants* **2022**, *11*, 1850. [[CrossRef](#)] [[PubMed](#)]
17. Vashisth, T.; Kadyampakeni, D. *Diagnosis and Management of Nutrient Constraints in Citrus*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 723–737. [[CrossRef](#)]
18. Tiwari, S.; Mann, R.S.; Rogers, M.E.; Stelinski, L.L. Insecticide resistance in field populations of Asian citrus psyllid in Florida. *Pest Manag. Sci.* **2011**, *67*, 1258–1268. [[CrossRef](#)] [[PubMed](#)]
19. Obreza, T.; Collins, M. *Common Soils Used for Citrus Production in Florida*; UF-IFAS Fact Sheet SL 193; Florida Cooperative Extension Service, University of Florida: Gainesville, FL, USA, 2002.
20. Harris, W.G.; Carlisle, V.W.; Chesser, S.L. Clay Mineralogy as Related to Morphology of Florida Soils with Sandy Epipedons. *Soil Sci. Soc. Am. J.* **1987**, *51*, 1673–1677. [[CrossRef](#)]
21. Shen, W.; Cevallos-Cevallos, J.M.; da Rocha, U.N.; Arevalo, H.A.; Stansly, P.A.; Roberts, P.D.; van Bruggen, A.H.C. Relation between plant nutrition, hormones, insecticide applications, bacterial endophytes, and Candidatus Liberibacter Ct values in citrus trees infected with Huanglongbing. *Eur. J. Plant Pathol.* **2013**, *137*, 727–742. [[CrossRef](#)]
22. Stansly, P.A.; Arevalo, H.A.; Qureshi, J.A.; Jones, M.M.; Hendricks, K.; Roberts, P.D.; Roka, F.M. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. *Pest Manag. Sci.* **2014**, *70*, 415–426. [[CrossRef](#)]

23. Web Soil Survey. Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2011. Available online: <https://websoilsurvey.nrcs.usda.gov/app/> (accessed on 8 March 2023).
24. Irely, M.S.; Gast, T.; Gottwald, T.R. Comparison of visual assessment and polymerase chain reaction assay testing to estimate the incidence of the Huanglongbing pathogen in commercial Florida citrus. *Proc. Fla. State Hort. Soc.* **2006**, *119*, 89–93.
25. Monzó, C.; Stansly, P. Monitoring asian citrus psyllid populations. *Citrus Ind.* **2015**, *96*, 10–12.
26. Qureshi, J.A.; Stansly, P.A. (Eds.) Integrated approaches for managing the Asian citrus psyllid *Diaphorina citri* (Homoptera: Psyllidae) in Florida. *Proc. Fla. State Hort. Soc.* **2007**, *120*, 110–115.
27. Diepenbrock, L.M.; Qureshi, J.; Stelinski, L. 2022–2023 Florida Citrus Production Guide: Asian Citrus Psyllid; CPG ch. 23, CG097, rev. 3/2022; EDIS: Pyeongtaek, Republic of Korea, 2022.
28. Kadyampakeni, D.M.; Morgan, K.T. *Nutrition of Florida Citrus Trees*, 3rd ed.; EDIS: Pyeongtaek, Republic of Korea, 2020; Volume 2020. [[CrossRef](#)]
29. Kadyampakeni, D.M.; Morgan, K.T.; Schumann, A.W.; Nkedi-Kizza, P. Effect of Irrigation Pattern and Timing on Root Density of Young Citrus Trees Infected with Huanglongbing Disease. *HortTechnology* **2014**, *24*, 209–221. [[CrossRef](#)]
30. Adriano, D.; Doner, H. Bromine, chlorine, and fluorine. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; Wiley: Hoboken, NJ, USA, 1983; Volume 9, pp. 449–483.
31. Vtorushina, E.; Saprykin, A.; Knapp, G. Optimization of the conditions of oxidation vapor generation for determining chlorine, bromine, and iodine in aqueous solutions by inductively coupled plasma atomic-emission spectrometry. *J. Anal. Chem.* **2008**, *63*, 643–648. [[CrossRef](#)]
32. Leiva, J.A.; Nkedi-Kizza, P.; Borejsza-Wysocki, W.S.; Bauder, V.S.; Morgan, K.T. Imidacloprid extraction from citrus leaves and analysis by liquid chromatography–mass spectrometry (HPLC–MS/MS). *Bull. Environ. Contam. Toxicol.* **2016**, *96*, 671–677. [[CrossRef](#)] [[PubMed](#)]
33. Littell, R.C.; Pendergast, J.; Natarajan, R. Modelling covariance structure in the analysis of repeated measures data. *Stat. Med.* **2000**, *19*, 1793–1819. [[CrossRef](#)]
34. Wickham, H. ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* **2011**, *3*, 180–185. [[CrossRef](#)]
35. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [[CrossRef](#)]
36. Halbert, S.E.; Manjunath, K.L. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. *Fla. Entomol.* **2004**, *87*, 330–353. [[CrossRef](#)]
37. Juang, C.H.; Holtz, R.D. Fabric, pore size distribution, and permeability of sandy soils. *J. Geotech. Eng.* **1986**, *112*, 855–868. [[CrossRef](#)]
38. Satkowski, L.E.; Goyne, K.W.; Anderson, S.H.; Lerch, R.N.; Webb, E.B.; Snow, D.D. Imidacloprid sorption and transport in cropland, grass buffer, and riparian buffer soils. *Vadose Zone J.* **2018**, *17*, 170139. [[CrossRef](#)]
39. Wang, J.; Chen, L. The effect of hillslope geometry on Hortonian rainfall-infiltration-runoff processes. *J. Hydrol.* **2021**, *594*, 125962. [[CrossRef](#)]
40. Hamido, S.A.; Morgan, K.T.; Ebel, R.C.; Kadyampakeni, D.M. Improved irrigation management of sweet orange with Huanglongbing. *HortScience* **2017**, *52*, 916–921. [[CrossRef](#)]
41. Fletcher, E.; Morgan, K.T.; Qureshi, J.A.; Leiva, J.A.; Nkedi-Kizza, P. Imidacloprid soil movement under micro-sprinkler irrigation and soil-drench applications to control Asian citrus psyllid (ACP) and citrus leafminer (CLM). *PLoS ONE* **2018**, *13*, e0192668. [[CrossRef](#)] [[PubMed](#)]
42. Li, Y.; Yang, L.; Yan, H.; Zhang, M.; Ge, J.; Yu, X. Uptake, translocation, and accumulation of imidacloprid in six leafy vegetables at three growth stages. *Ecotoxicol. Environ. Saf.* **2018**, *164*, 690–695. [[CrossRef](#)]
43. Sétamou, M.; Rodriguez, D.; Saldana, R.; Schwarzlose, G.; Palrang, D.; Nelson, S. Efficacy, and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leafminer, two foliar-feeding citrus pests. *J. Econ. Entomol.* **2010**, *103*, 1711–1719. [[CrossRef](#)] [[PubMed](#)]
44. Farha, W.; Abd El-Aty, A.M.; Rahman, M.M.; Shin, H.-C.; Shim, J.-H. An overview on common aspects influencing the dissipation pattern of pesticides: A review. *Environ. Monit. Assess.* **2016**, *188*, 693. [[CrossRef](#)] [[PubMed](#)]

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.