

Article

Pesticide Contamination of Pollen in Container-Grown Blanket Flower

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Abstract

Pollinators, particularly bees, are essential for the reproduction of flowering plants, including ornamentals, and for maintaining ecosystem balance, benefitting gardens by supporting plant health and promoting robust flowering. However, pollinator populations are declining, with pesticide exposure recognized as one of several contributing stressors. Thiamethoxam, a commonly used systemic insecticide in ornamental horticulture (and its toxic metabolite clothianidin), has been found in nectar resources at levels harmful to bees. This study evaluated the impact of different thiamethoxam application rates on pollen contamination in blanket flower (*Gaillardia pulchella* L.), a popular source of pollen for bee pollinators. Container plants were drench-treated with low (0.30 g/L) and high (0.64 g/L) rates of thiamethoxam during the mature floral bud stage. The results show that thiamethoxam and clothianidin were present in pollen at both application rates. However, only clothianidin levels significantly increased with the application rate ($p = 0.0009$). When the pesticide concentrations measured in pollen were used to calculate the estimated exposure doses based on pollen consumption, the values exceeded published median lethal doses (LD50) for the common eastern bumble bee (*Bombus impatiens* Cresson) and buff-tailed bumble bee (*Bombus terrestris* L.), indicating substantial ecological risk. These findings underscore the potential threat posed to some pollinator species by thiamethoxam-treated ornamentals.

Keywords: thiamethoxam; clothianidin; pollen; bee pollinators; ornamentals; *Gaillardia pulchella*



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1. Introduction

Pollination is a critical ecological process that facilitates sexual plant reproduction by enabling the transfer of pollen from the male anthers to the female stigmas of flowers, either within the same plant (self-pollination) or between different plants (cross-pollination) [1,2]. This process supports approximately 75% of global crops and 90% of flowering plant species and is essential for both ecosystem stability and food production [3,4]. Animal pollinators (including bees, butterflies, birds, and bats) serve as vital organisms in this process, with insects being the most diverse and abundant group. Among them, bees are particularly important due to their efficiency and high frequency of floral visits [5].

Pollinators are integral to the global food web and economy. They not only influence agricultural yields but also enhance crop quality, with wild and managed bee species contributing significantly to these outcomes [6,7]. For instance, in the United States alone, Calderone [8] reported that pollination services contributed over \$18 billion annually to crop production, with honeybees accounting for about \$12 billion. Additionally, honeybee products provide an extra \$700 million in economic value.

The European/Western honeybee (*Apis mellifera* L., Apidae, Hymenoptera) plays a dominant role globally, fulfilling over one-third of pollination service demands in countries such as the United Kingdom [9,10]. However, other bee species—including carpenter bees, stingless bees, bumble bees, feral bees, and solitary bees—also contribute to effective and diverse pollination outcomes [11]. Despite their ecological and economic significance, pollinators are experiencing steep population declines. Multiple stressors, including malnutrition, parasites, habitat loss, diseases, temperature changes, and pesticide exposure, are contributing to global pollinator declines [12,13]. In North America, over one-fifth of native pollinators face elevated extinction risks. Among insects, bees are the most threatened group, with nearly 35% of species at risk, followed by butterflies, moths, and flies [14]. The widespread use of pesticides in conventional agriculture, particularly systemic insecticides like neonicotinoids, has been strongly implicated in these declines.

Bees use nectar and pollen from flowers as a source of food and energy. During pollination in agricultural and urban settings, bees may be exposed to pesticides, despite not being the intended targets [15]. However, pesticide use in large-scale agriculture and ornamental plant cultivation differs greatly. In agricultural fields, restricted-use pesticides are commonly used for specific pest management needs due to their effectiveness against tough pests. But such pesticides are not available for purchase or use by the general public and require application by trained and certified professionals. As for ornamentals, pesticides are used in home gardens, small ornamental plant collections, urban green spaces, and nurseries. In such settings, pesticides may be applied by non-professional users who do not take into consideration the optimal timing or recommended application practices. Many ready-to-use pesticides can be found at garden supply centers and do not require any special credentials to buy or use.

In ornamental plant production, pesticides may be applied during blooming and at the higher end of labeled rates, leading to nectar contamination that could potentially impair pollinator behavior and survival [16,17]. Additionally, systemic pesticides are frequently applied to ornamentals at concentrations up to 120-times greater than in food crops to meet aesthetic standards [18,19]. A study by Lentola et al. [20] found 13 different pesticides in pollen samples collected from 18 different plants purchased from retail nurseries. The neonicotinoid thiamethoxam was among the chemicals found in pollen at concentrations coinciding with those known to harm bees. Thus, while ornamental plants may be marketed as pollinator-friendly due to their attractiveness to pollinators, contamination of their floral resources during production may not be taken into consideration [21–23].

Among these chemicals, neonicotinoids—such as thiamethoxam—are widely used insecticides derived from nicotine and known for their systemic activity [24]. The sublethal effects of neonicotinoids on the physiology, behavior, reproduction, and ecology of non-target arthropods have been recently reviewed [25]. Whether applied to the plant or to the soil, thiamethoxam is taken up by the roots, leaves, and flowers of the plant. While foliage applications may immediately contaminate the pollen and nectar, soil applications also lead to contamination through subsequent translocation of the pesticide to the nectar and pollen via the xylem and phloem. Moreover, if treatment is performed at an inappropriate time, pollinating insects visiting the flowers during or shortly after application could be exposed to it.

The characteristics of thiamethoxam that enable its transport via the vascular system of plants include its high water solubility and long soil half-life [26]. These compounds function as agonists at nicotinic acetylcholine receptors (nAChRs), mimicking the action of acetylcholine (Ach) but resisting degradation by acetylcholinesterase (AChE) [27]. This persistence leads to continuous stimulation of neural pathways, resulting in neuronal hyperexcitation and, in severe cases, mortality in exposed insects, including bees [16,28]. Neonicotinoids have also been reported to cause sublethal effects that impair cognitive functions such as olfactory learning and memory [29]. Farooqui [30] proposed that these disruptions may contribute to the development of Colony Collapse Disorder (CCD) in honeybee populations. Importantly, even at concentrations below the LC50, thiamethoxam in nectar and pollen can adversely affect bees during foraging activities [28]. Due to concerns regarding pollinator health, this pesticide is currently facing severe regulatory restrictions globally, including a restriction/ban of outdoor use in the European Union.

Several key factors influencing pesticide residues in floral resources are (1) the method, timing, and rate of application; (2) the chemical properties of the active ingredient; and (3) the plant species [17,31,32]. Given the mounting concerns over pollinator health and pesticide exposure, this study aimed to investigate how different application rates of the systemic insecticide thiamethoxam influence pollen contamination in blanket flower (*Gaillardia pulchella* L.), a model ornamental species of the family Asteraceae. Blanket flower is a summer- and fall-flowering plant, usually forming bicolored yellow and red blooms (Figure 1A). This plant grows up to 24 inches tall, with rounded clumps of pubescent foliage and long-stemmed flowers (Figure 1B). The bright flowers, long blooming season, and drought and heat tolerance of blanket flower make it a popular plant in pollinator gardens [33]. Such knowledge can contribute to a deeper understanding of how pesticide practices in ornamental horticulture may impact pollinator health and pollination services.



Figure 1. Images of (A) blanket flower (*G. pulchella* L.) displaying composite ray and disk flowers, (B) floral abundance of plants situated on a greenhouse bench during the study, and (C) adaptation of a vacuum used to collect pollen.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

Blanket flower plants were grown from seeds collected from a field on the UF campus (Gainesville, FL, USA) and potted into 0.95 L containers filled with a mixture of Pro-Mix HP Mycorrhizae media (Premier Tech Ltd., Quakertown, PA, USA) and 20% sand. Sand was added due to this plant's preference for well-drained sandy soil [33]. The plants were maintained for 4 weeks in an environmentally controlled greenhouse set to cool at 22 °C and heat at 17 °C. The plants were manually fertigated with 20N–8.7P–6.6K water-soluble fertilizer as needed (J.R. Peters Inc., Allentown, PA, USA) and routinely scouted for disease and pests. When necessary, a dedicated spray tank was used to apply non-thiamethoxam/clothianidin-containing pesticides for pest control.

Afterwards, the plants were repotted into 3.8 L containers using the same media, top dressed with 14 g 14N–6.1P–11.6K slow-release fertilizer (Osmocote Plus; Scotts Co., Maryville, OH, USA) per plant, and then moved to a Quonset-style house covered with shade cloth. A month later, the plants were transplanted into 7.6 L containers to promote growth and flowering. The next month, the plants were moved back into an environmentally controlled greenhouse to protect them from frost and induce flowering. The plants were hand-watered with a hose as needed throughout the experiment. The average temperature in the shade house, measured using an HOBO pendant (Onset Data Loggers Co., Bourne, MA, USA), was 22.6 °C, with a minimum temperature of 13.7 °C and a maximum of 30.7 °C, and the average light intensity was 975.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$, measured using an LI-250 light meter (LI-COR Biosciences; Lincoln, NE, USA).

2.2. Pesticide Treatment

This study used three replicate groups per treatment with 10 plants per group to evaluate the effect of drench application rate (2 levels: low 0.30 g/L, high 0.64 g/L) on the contamination of pollen. In addition, three controls (10 plants/replicate) were included to ensure that the makeup water for the applications did not result in pesticide contamination and to prepare quality control samples (blanks, matrix spikes, and matrix spike duplicates). The plants were assigned an identity number and were then randomly assigned to each treatment group. On the same morning that the treatments were applied, commercially available Flagship 25WG (a water-dispersible granule containing 25% active ingredient thiamethoxam) (Syngenta, Basel, Switzerland) was weighed and dissolved in 4 L bottles of tap water in the lab for each rate. Once the plants started to form buds, drench treatments were applied to half-saturation using a graduated cylinder (500 mL per pot) according to the labeled rates for ornamentals, with care taken not to touch the leaves or stems with the chemical. Half-saturation was measured by watering a fully dry 7.6 L container test plant with a graduated cylinder of water until water leached from the bottom. The total amount of water used was considered full saturation and divided by two to determine the volume of half-saturation. This calculated amount was used as a standard for all pots. Although consistency may not be exact due to variations in plant size and the exact amount of media, this practice aligns with what would be done in an ornamental operation.

2.3. Pollen Sampling

Once each replicate group had about 20 flowers (4 months after pesticide application), pollen samples were collected using a pollen vacuum (Figure 1C), as described in Pioltelli et al. [34], over the span of 4 weeks and consolidated. The pollen vacuum (Honkyob, Denver, CO, USA) sampler tube was assembled with a 5 mL Eppendorf tube, 75 μm stainless steel mesh, laboratory film, and 25 μm pore size filter paper (Quantitative Ashless Filter paper, Tisch Scientific, Cleves, OH, USA). A new tube was used for each sample. After

each sampling day, samples were transferred into a designated Eppendorf microcentrifuge tube and weighed. Sampling continued per replicate until the consolidated sample reached 10 to 20 mg. The sampled pollen tubes were placed in paper bags and stored on ice packs in the field, followed by storage at -80°C until analysis. A new pair of gloves was worn for each plant sampled to prevent possible cross-contamination.

2.4. Pesticide Analysis

Pollen was extracted using a slightly modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, as described by Lentola et al. [20]. Once enough pollen was collected and weighed, the exact amount of the corresponding internal standard was added to the Eppendorf microcentrifuge tube. For the quantification of the pesticides, two different internal standards at 48.4 ppb were used—thiamethoxam (methylene- ^{13}C , 99%; thiazole- $^{13}\text{C}_3$, 99%; ^{15}N , 98%) and clothianidin (methylene- ^{13}C , 99%; thiazole- $^{13}\text{C}_3$, 99%; ^{15}N , 98%). Next, 500 μL of acetonitrile (ACN) with 0.1% acetic acid was added and immediately vortexed, followed by the addition of 400 μL of Optima water. The sample was mixed vigorously in a shaker for 10 min before adding 250 mg of a salt mixture ($\text{MgSO}_4/\text{NaCl}$, 4:1). The sample was then quickly inverted and centrifuged (14,800 RCF, 5 min).

The supernatant was transferred into a tube containing 50 mg of dispersive solid-phase material (PSA/C18/ENVI-Carb) and shaken for 10 min. After centrifuging (14,800 RCF, 5 min), the supernatant was transferred into a spin filter tube (Costar; Salt Lake City, UT, USA), and the dispersive solid-phase extraction sample was extracted again with 200 μL of toluene/ACN (3:1), followed by vortexing. After centrifuging (14,800 RCF, 5 min), both supernatants were combined, evaporated to dryness using a large-volume evaporator (automated Thermo Scientific™ Rocket Synergy™ Evaporator System; Waltham, MA, USA), and reconstituted to 100 μL $\text{H}_2\text{O}/\text{ACN}$ (70:30) using a syringe.

Thiamethoxam and its metabolite clothianidin were analyzed using an Agilent 1290 Infinity II ultra-high-pressure liquid chromatography system (uHPLC) (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a C18 reversed-phase column (Zorbax Eclipse C18, Rapid resolution HD, 100×2.1 mm, $1.8 \mu\text{m}$) and coupled to an Agilent 6495C tandem mass spectrometer for detection. The MS/MS was operated in electrospray ionization (ESI) positive mode with nitrogen used as the source and collision gas. Data were acquired in the multiple reaction monitoring (MRM) mode. The analysis method for thiamethoxam (+clothianidin) used a gradient of solution A (95% Optima LC-MS water, 5% Optima LC-MS ACN, with 0.1% Optima formic acid, 5 mM ammonium formate) and solution B (95% Optima LC-MS ACN, 5% Optima LC-MS water, with 0.1% Optima formic acid, 5 mM ammonium formate) (Table 1). The monitored m/z transitions for thiamethoxam and clothianidin are shown in Table 2. Internal calibration curves were used to determine the concentrations in the samples.

Table 1. Mobile phase gradients developed for analysis of thiamethoxam and clothianidin in the pollen of treated *Gaillardia pulchella* L.

Time (min)	Solvent A (%) ¹	Solvent B (%) ²	Flow mL/min
0	90	10	0.4
1.0	90	10	0.4
2.5	75	25	0.4
3.0	75	25	0.4
7.0	10	90	0.4
7.5	90	10	0.4

¹ Solvent A: 95% Optima LC-MS water, 5% Optima LC-MS acetonitrile, with 0.1% Optima formic acid, 5 mM ammonium formate. ² Solvent B: 5% Optima LC-MS water, 95% Optima LC-MS acetonitrile, with 0.1% Optima formic acid, 5 mM ammonium formate.

Table 2. Multiple reaction monitoring (MRM) transitions (m/z) for identification and quantification of thiamethoxam and its metabolite clothianidin in pollen.

Analyte	Precursor m/z	Quantifier m/z	Qualifier m/s
Thiamethoxam	292.0	211.1 (CE: 13)	181.1 (CE: 25)
Clothianidin	250.0	169.0 (CE: 7)	131.9 (CE: 15)

Method validation was accomplished by spiking two test samples with thiamethoxam (+clothianidin) at a lower concentration within the calibration curve range, followed by extraction and analysis as described previously. Method recoveries were evaluated to rule out possible matrix effects. Blank samples were analyzed in each batch to ensure that no contamination occurred during the extractions. The method detection limit (MDL) was calculated following EPA guidance, and the method quantitation limit (MQL) was the lowest calibration concentration used for quantification (Table 3) [35]. Recoveries of the analytes spiked into pollen samples were also calculated for thiamethoxam and clothianidin (Table 3). While two control samples were free of thiamethoxam, the pesticide was detected in a third control sample (from 1 of 3 consolidations) due to a noted mechanical error in transferring pollen from the low-rate application into a control-labeled tube instead of a low-rate-labeled tube. Clothianidin was not detected in any of the controls.

Table 3. Recovery, method detection limits (MDLs), and method quantification limits (MQLs) for the analysis of thiamethoxam and its metabolite clothianidin in pollen.

	Thiamethoxam	Clothianidin
Recovery (% $\pm$ SD)	109 $\pm$ 4	110 $\pm$ 2
MDL (ng/mL)	1.22	1.56
MQL (ng/mL)	1.26	1.56

2.5. Screening for Ecological Risks

The concentrations of thiamethoxam and clothianidin measured in pollen were used to calculate estimated exposure doses of the pesticide to bees based on their pollen consumption. These values were then compared to published median lethal dose (LD50) values for pollinators, defined as the dose fatal to 50% of exposed bees within an exposed group [36–38]. The exposures of bees to each pesticide through the consumption of contaminated pollen were estimated by multiplying the amount of pollen consumed (from the literature) by the measured pesticide concentration in the pollen (Table 4).

Table 4. Calculation of thiamethoxam exposure and ecological risk for adult *B. impatiens* Cresson and *B. terrestris* L. corresponding to each application rate.

Bee Species	Rate	Pollen Concentration (ng/g)	Exposure ($\mu\text{g}/\text{bee}$)
<i>B. terrestris</i> ¹ 48 h LD50 = 0.005 $\mu\text{g}/\text{bee}$	Low	96.79	0.0059 ²
	High	156.64	0.0095 ²
<i>B. impatiens</i> ³ 48 h LD50 = 0.0012 $\mu\text{g}/\text{bee}$	Low	96.79	0.0205 ⁴
	High	156.64	0.0332 ⁴

¹ [36]; ² 0.0303 g/day $\times$ 2 days $\times$ pollen concentration (ng/g)/1000 (ng/ μg); ³ [37]; ⁴ 0.1060 g/day $\times$ 2 days $\times$ pollen concentration (ng/g)/1000 (ng/ μg).

2.6. Statistical Analysis

Data were subjected to a one-way analysis of variance (ANOVA) using the statistical software JMP Student Edition (SAS Institute Inc., Cary, NC, USA), with significant effects separated using p values. Assumptions for ANOVA were checked, including the normality

of residuals, the homogeneity of variances, and the need for data transformations. The data met the assumptions with no transformations required.

3. Results

3.1. Thiamethoxam

Regardless of the amount of pesticide applied to blanket flower plants, all pollen samples contained thiamethoxam. There was a non-significant effect between the low and high application rates ($p = 0.0547$). When applications were made at the low rate, the concentrations of thiamethoxam in pollen ranged from 84.4 to 106.2 ng/g, with a mean of 96.8 ng/g (Figure 2A). When applications were made at the high rate, the concentrations of thiamethoxam in pollen were higher, ranging from 117.4 to 190.6 ng/g, with a mean of 156.6 ng/g. Although these differences were non-significant, parallels can be made, as the pollen of plants treated with the high thiamethoxam application rate consistently showed high concentrations of this chemical. Based on the mean thiamethoxam concentration in pollen and the average bee's pollen consumption (Table 4), both application rates resulted in estimated exposure doses exceeding the published LD50s for *Bombus impatiens* Cresson (Apidae, Hymenoptera) and *B. terrestris* but not for *A. mellifera*.

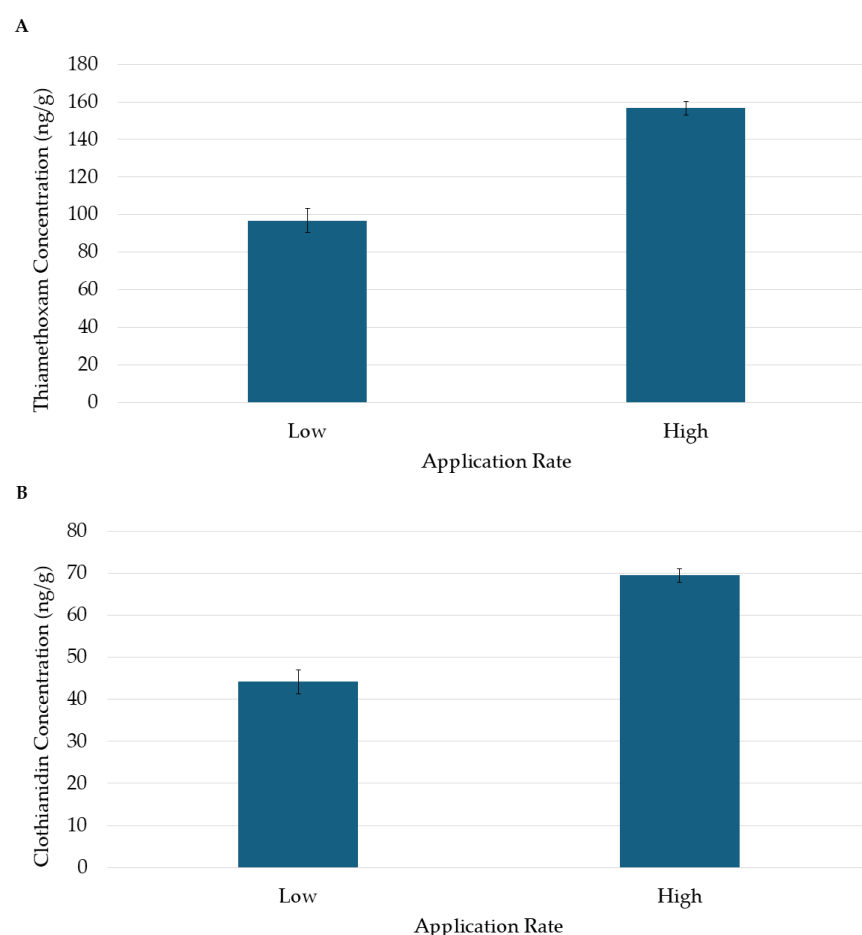


Figure 2. Sampling event means ($n = 3$) of (A) thiamethoxam and (B) clothianidin concentrations ($\pm$ standard error) in pollen associated with two drench application rates (low and high) before anthesis. Pollen was collected from approximately 60 flowers among 10 container plants replicated three times on a greenhouse bench. There was no significant difference between thiamethoxam concentrations ($p = 0.0547$); for clothianidin concentrations, there was a significant difference between the low and high application rates ($p = 0.0009$).

3.2. Clothianidin

Following the application of the thiamethoxam drench treatment to plants, the metabolite clothianidin was also detected in all pollen samples. There was a significant difference between the low and high application rates ($p = 0.0009$). When applications were made at the low rate, the concentrations of clothianidin in pollen ranged from 40.4 to 49.6 ng/g, with a mean of 44.1 ng/g (Figure 2B). When applications were made at the high rate, the concentrations of clothianidin in pollen ranged from 68.3 to 70.4 ng/g, with a mean of 69.5 ng/g. Thus, the mean concentrations of clothianidin in pollen were 1.6 times greater when treatment was carried out with the high rate than when it was carried out with the low rate. Based on the clothianidin concentration in pollen and the average bee's pollen consumption (Table 4), neither application rate resulted in estimated exposure doses that exceeded the published LD50s for *A. mellifera*.

4. Discussion

Our results revealed that thiamethoxam, applied to container-grown blanket flower as a drench, is translocated to its pollen, regardless of the application rate. As the application rate increased, the clothianidin concentration found in the pollen increased significantly. These results are consistent with those of our prior studies assessing pesticide contamination in the nectar of ornamental plants. We applied the systemic insecticide thiamethoxam to Bloomify™ rose lantana (*Lantana strigocamara* R.W. Sanders 'UF-1011-2') and indigo spires salvia (*Salvia longispicata* M. Martens & Galeotti $\times$ *S. farinacea* Benth.), comparing the influence of the application method (drench vs. spray), timing (relative to flower bud maturity), and rate (low, medium, and high) on nectar contamination [17,32]. The results indicated that concentrations in the drench application led to the greatest contamination of nectar with thiamethoxam and clothianidin. Additionally, when applied to plants at the highest rate, the contamination of nectar increased as bud maturity increased, exceeding published median lethal concentrations for pollinators (LC50s).

While bees collect and consume more pollen than nectar, few studies have specifically focused on the relationship between pesticide management during ornamental plant production and the subsequent contamination of pollen [38]. In cottonseed dressed with thiamethoxam, the pesticide was found in 90% of pollen samples, ranging from ND to 14.5 ng/g [39]. Additionally, in oilseed rape dressed with thiamethoxam, Botías et al. [40] reported average concentrations of 3.3 ng/g of thiamethoxam and 2.3 ng/g of clothianidin in the pollen. In maize pollen, Stewart et al. [41] found average thiamethoxam levels of less than 1 ng/g and clothianidin at 3 to 6 ng/g across a range of seed treatments.

Due to the crucial role that pollen plays in the development of bee larvae and the overall health of the colony (through the provision of protein, vitamins, fats, and minerals), more research is needed on how pesticide applications affect pollen contamination. However, not all ornamental plants are suitable for pollen sampling due to the amount needed to quantify pesticide concentrations. While plant families such as Asteraceae are known to produce abundant pollen (Figure S1), species belonging to other families, such as Lamiaceae and Verbenaceae, produce generous amounts of nectar with floral morphology that is easy to access [21,42]. Regardless, the collection of pollen and nectar from flowers can be time-consuming and not possible in certain species, limiting widespread research in this area.

A critical point of this study was the comparison of pesticide concentrations in pollen with known toxicity levels for pollinators. The most relevant measures of exposure are the concentrations in bee-collected plant materials, such as pollen; in bee products like bee bread and honey; and in the bees themselves [21]. Research has shown that 75% of honey samples tested positive for at least one neonicotinoid [43]. In another study, agrochemicals,

including neonicotinoids, were detected in all 55 bee colonies of apiaries located both near and away from corn fields [44]. Bees, which rely on advanced cognitive skills for foraging and navigation, are particularly vulnerable to neurotoxic pesticides [45]. The effects are compounded by atypical climate patterns, which disrupt floral resource availability and increase nutritional stress [46,47].

In a study by Henry et al. [48], individual honeybee foragers were administered an acute oral dose of 1.34 ng of thiamethoxam dissolved in a 20 µL sucrose solution—equivalent to 27% of the established LD₅₀ value. Following administration, the bees were released 1 km from their hives to assess their homing ability. The study found that thiamethoxam-exposed bees exhibited a significantly lower return rate to the colony compared to untreated controls, indicating impaired navigation or foraging efficiency due to the sublethal exposure. Similarly, Whitehorn et al. [49] investigated the effects of dietary exposure to neonicotinoid-treated floral resources on buff-tailed bumble bee (*Bombus terrestris* L., Apidae, Hymenoptera) colonies. Colonies were fed pollen containing 6 or 12 ng/g and nectar containing 0.7 or 1.4 ng/g of neonicotinoids under laboratory conditions for two weeks, simulating a realistic pulse exposure associated with foraging on treated oilseed rape. After subsequent outdoor foraging for six weeks, the treated colonies exhibited significantly reduced growth and produced 85% fewer new queens relative to the control groups, highlighting the potential long-term reproductive consequences of neonicotinoid exposure in wild bee populations.

In our study, we found that the estimated exposure doses based on the thiamethoxam concentrations in pollen and pollen consumption per bee exceeded the LD₅₀ for bumble bee species but not honeybees (Table 4). The reported daily consumption rates of bumble bees were 106 mg/day for the common eastern bumble bee (*B. impatiens*) and 26.6 to 30.3 mg/day for the buff-tailed bumble bee (*B. terrestris*) [37,50–53]. As for the western honeybee, *A. mellifera* consumes about 3.4 to 4.3 milligrams (mg) of pollen/day [54]. Concerning lethal doses from the consumption of pollen, the acute oral LD₅₀s for thiamethoxam are 0.0050 µg/bee (48 h) for *A. mellifera*, 0.0012 µg/bee (48 h) for *B. impatiens*, and 0.005 for *B. terrestris* [38–40]. For clothianidin, only the honeybee had a published LD₅₀, 0.0035 µg/bee [40]. Given the rate of pollen consumption and the acute oral LD₅₀ for adult *B. impatiens*, the drench treatments resulted in exposure doses exceeding the toxicity thresholds by 17 to 28 times. Drench treatments resulted in exposure doses exceeding the toxicity thresholds 1 to 2 times for *B. terrestris*.

A limitation of this study was the small number of replicates and amount of time that lapsed (4 months) between pesticide application and pollen collection. This was due to delayed flowering associated with earlier-than-predicted onset of cooler weather. However, the fact that pesticide concentrations were still detected in the pollen after such an extended period demonstrates the persistence of thiamethoxam. More studies with long sampling windows could aid in revealing the long-term exposure risks of pesticides to pollinators. In future studies requiring large amounts of pollen, considerations should be made to ensure that greenhouse conditions prevent winter dormancy to achieve more rapid and profuse flowering in a shorter window of time. In addition, it may be worthwhile to use cultivars as a model crop with more uniform flowering. The seeds in our study were collected from a large local population of blanket flower that was extremely diverse in flower production, time, and size. More uniform flowering would facilitate the pollen sampling process for the inclusion of additional replicates. Future studies examining other routes of pesticide intake for bees apart from nectar and pollen would also be valuable. For example, guttation fluid (the small water droplets exuded by plants) is an overlooked potential exposure pathway for pesticides. After soil or drench pesticide applications, guttation droplets are known to contain elevated concentrations of systemic insecticides, and bees have been

observed collecting guttation water, especially during warm and humid conditions when floral resources are limited [55].

5. Conclusions

The methods developed during this experiment provided a practical technique for collecting pollen in quantities large enough for thiamethoxam analysis. The results indicate that consideration should be taken when applying thiamethoxam as a drench to blanket flower (and possibly other ornamentals). Drench treatments resulted in exposure doses that exceeded the toxicity thresholds for the bumble bee species *B. impatiens* and *B. terrestris*. Thus, from a pollinator protection standpoint, other pesticides should be considered if insect control using drench treatments is needed. Further research is being conducted to evaluate the floral resources of other ornamental species and additional pesticides to aid in the development of best management practices for the ornamental industry. Such information would contribute to a better understanding of the non-target consequences of neonicotinoid use and assist growers in making informed decisions regarding their pest management practices.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12070891/s1>, Figure S1: *G. pulchella* L. anther and pollen microscope images. (A) Single disk flower of blanket flower (*Gaillardia pulchella* L.) with (B) pollen-covered anthers (3× magnification) and (C) spiked, spherically shaped pollen (8× magnification).

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACh	Acetylcholine
AChE	Acetylcholinesterase
ACN	Acetonitrile
ANOVA	Analysis of Variance
CCD	Colony Collapse Disorder
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
g	Gram
H ₂ O	Water
LC50	Median Lethal Concentration

LC-MS	Liquid Chromatography with Tandem Mass Spectrometry
LD50	Median Lethal Dose
mM	Millimole
mm	Millimeter
μL	Microliter
MDL	Method Detection Limit
MgSO ₄	Magnesium Sulfate
mg	Milligram
mL	Milliliter
MRM	Multiple Reaction Monitoring
MQL	Method Quantification Limits
<i>m/z</i>	Mass/Charge Ratio
nAChR	Nicotinic acetylcholine receptor
NaCl	Sodium Chloride
ng	Nanogram
RCF	Relative Centrifugal Force
uHPLC	Ultra-High-Pressure Liquid Chromatograph

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