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Influence of Thiamethoxam Application Method, Timing, and Rate on Contamination of Floral Resources in Lantana

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Summary

Pollinators are critical contributors to the natural world as well as to humans. However, their population numbers have been rapidly declining, in part due to pesticide exposure. Using the systemic insecticide thiamethoxam and the ornamental species Bloomify™ rose lantana (*Lantana strigocamara* 'UF-1011-2'), this study investigated the influence of application method (drench vs. spray), rate (low, medium, high), and timing (relative to flower bud maturity) on contamination of nectar in container grown plants. Results of nectar analysis showed meaningful differences between treatments. While spray applied thiamethoxam was not observed at quantifiable concentrations in nectar, drench applied thiamethoxam concentrations in nectar ranged from 87.7 to 1163.8 ng/mL, surpassing published LC50's for several bee species even at the lowest application rate. The timing and rate of drench application also affected thiamethoxam concentrations detected in nectar, with concentrations being the highest for applications at the highest rate and at the latest timing. These results provide insight into the development of nursery guidelines to help limit pesticide risk to pollinators before plants go to market.

INTRODUCTION

Pollinators play a critical part in global agricultural crop production and the health of natural ecosystems, their services being of great value ecologically and economically (Siviter et al., 2023). As such, there is marked interest in pollinator friendly gardening and the use of diverse flowering ornamental species to provide important nutritional resources like pollen and nectar for sustained pollinator health (Kalaman et al., 2022). Despite such efforts, pollinator populations are in peril with 40% of insect pollinators highly threatened worldwide, and nearly a quarter of native bee species at risk of extinction in North America (Kopec and Burd, 2017). Pesticide exposure is among a suite of contributors to declining bee populations, especially through necessary practices used by the horticultural industry to control pests and diseases (Halsch et al., 2022). Yet best management practices for the nursery industry in relation to pesticide method, timing, and rate are largely unknown. Prior work by Rostán et al. (2024) showed that drench pesticide applications to container grown indigo spires salvia (*Salvia* × 'Indigo Spires') were recovered in floral nectar samples at levels toxic to bees. To develop comprehensive guidelines for the industry, further studies are necessary to address other flowering species and additional timings and rates. Thus, this study's goal was to characterize the potential contamination of nectar in lantana due to insecticide treatment (thiamethoxam) during container nursery production. A repeat bloomer, Bloomify ™ rose lantana produces many umbel inflorescences throughout the year that attract diverse bee pollinators (Kalaman et al., 2022) and is useful as a model species. Thiamethoxam, the pesticide of interest, is highly toxic to pollinators because it binds to the nicotinic acetylcholine receptors (nAChRs) of insects, affecting the pollinators' ability to function (Simon-Delso et al., 2015). Our specific objectives were to determine the pesticide impacts of 1) spray vs. drench application, 2) timing of applications relative to anthesis (no flower buds, immature flower buds, mature flower buds), and 3) application rates (low, medium or high) on contamination of nectar.

MATERIALS AND METHODS

Plant material and pesticide treatments. Bloomify™ rose lantana plants were purchased as rooted liners (Ball Horticulture Company, West Chicago, IL) and up potted into 4.5-in (11.4 cm) containers filled with Pro-Mix HP Mycorrhizae media (Premier Tech Ltd., Quakertown, PA). Plants were maintained for 8 weeks in an environmentally controlled greenhouse with periodic pruning (3x) to promote branching and control the onset of flowering. Afterwards, plants were repotted into 2-gal. containers using the same media, top dressed with 1Tbs of 14N-14P-14K of slow-release fertilizer per plant and then moved to a Quonset style house covered with shade cloth for the duration of the experiment (**Fig. 1-A**). Plants were drip irrigated twice a week for 15 minutes or as needed throughout the experiment.

Eight replicate plants were randomly assigned to pesticide treatments (including controls), utilizing a 2x3x3 factorial statistical design to explore relationships between pesticide application method (2 levels: spray and drench), timing (3 levels: no flower buds, immature flower buds, mature flower buds) and application rate (3 levels: low- 4.0 oz/100 gal, medium- 6.25

oz/100 gal, and high- 8.5 oz/100 gal). Commercially available Flagship 25WG (a water dispersible granule containing 25% thiamethoxam) was mixed and applied as a soil drench at half saturation (650 mL per pot) according to the labeled rates for ornamentals. Spray treatments were applied using a hand-operated spray bottle. In this case, plants were sprayed to the point where runoff just started to occur, taking care to wet as much foliage as possible. Due to the floral development of lantana, two weeks lapsed in between each pesticide application. The first treatment of plants with no flower buds occurred the day after transplanting. The second treatment occurred on plants where the buds were starting to form, and the last after the flower buds had fully developed on the plant but before they opened.

Figure 1. Images of (A) Bloomify™ rose lantana grown in a shade house during the study with (B) a closeup of the indeterminate inflorescences, and (C) nectar extraction from a single floret using a 20 µL glass microcapillary tube.

Nectar sampling and data analysis. Once all the flowers were blooming (**Fig. 1-B**), nectar samples were collected using 20 µL glass microcapillaries (**Fig. 1-C**). Nectar from each capillary tube was transferred into separate Eppendorf tubes, stored in a cooler on ice, and transported to a -80°C freezer until analysis. The samples were diluted with 180 µL of H_2O:ACN (9:1), then thoroughly vortexed and centrifuged (14,800 RCF, 8 min) before analysis. Thiamethoxam was analyzed using an Agilent 1290 Infinity II ultra high pressure liquid chromatography system (uHPLC) equipped with a C18 reversed-phase column (Zorbax Eclipse C18, Rapid resolution HD, 100 × 2.1 mm, 1.8 µm) and coupled to an Agilent 6495 tandem mass spectrometer for detection. The analysis method used gradient solvents as described in **Table 1** with transitions quantified as shown in **Table 2.** External calibration curves were used to determine the concentrations in the samples. Data were subjected to a three- and two-

way analysis of variance (ANOVA) using the statistical software JMP (SAS Institute Inc., Cary NC) with significant effects separated using Tukey's honestly significant difference test at $P = 0.05$.

Table 1. Mobile phase gradients developed for analysis of thiamethoxam in the nectar of treated lantana.

Time (min.)	Solvent A (%)[z]	Solvent B (%)[y]	Flow (mL/min)
0.00	90	10	0.400
1.00	90	10	0.400
7.00	10	90	0.400
7.50	90	10	0.400

[z]Solvent A: 95% Optima LC-MS water, 5% Optima LC-MS ACN, with 0.1% Optima formic acid, 5 mM ammonium formate.
[y]Solvent B: 95% Optima LC-MS ACN, 5% Optima LC-MS water, with 0.1% Optima formic acid, 5mM ammonium formate.

Table 2. Multiple reaction monitoring (MRM) transitions (m/z) for identification and quantification of thiamethoxam in nectar as described by Rostán et al (2024).

Analyte	Precursor m/z	Quantifier m/z	Qualifier m/z
Thiamethoxam	292.03	211.1	181.1

RESULTS

Significant effects of thiamethoxam application method, timing and rate were observed in the nectar of lantana. The concentrations of thiamethoxam in nectar of spray-treated plants ranged from below detection limits (MDL= 0.1 ng/mL) to 14.42 ng/mL. Only 27 of the 72 spray treatment samples had detectable concentrations, with only three of those concentrations being above method quantification limits (MQL= 0.5 ng/mL). Given the lack of quantifiable concentrations in most samples, this treatment was removed from future analysis. However, drench-applications resulted in significant contamination of nectar with thiamethoxam relative to application rate (P= 0.0001) and timing (P= 0.0007). The interaction between rate and timing for the drench applications was also significant (P= 0.0015). Thiamethoxam concentrations in nectar increased as the flower bud development progressed and as application rate increased. When applications were made before buds had formed, concentrations ranged from 171.4 to 418.9 ng/mL with no

difference between the low and medium application rates (**Fig. 2**). Likewise, applications made to plants with immature buds resulted in higher nectar contamination (ranging from 352.0 ng/mL to 723.4 ng/mL) as rates increased, with concentrations from the low and medium application rates being similar. Applications made to plants when mature buds had formed and just before the florets started to open resulted in the highest concentrations of thiamethoxam in nectar ranging from 276.5 to 951.9 ng/mL, with the concentration of thiamethoxam in nectar for each rate being statistically different from one another.

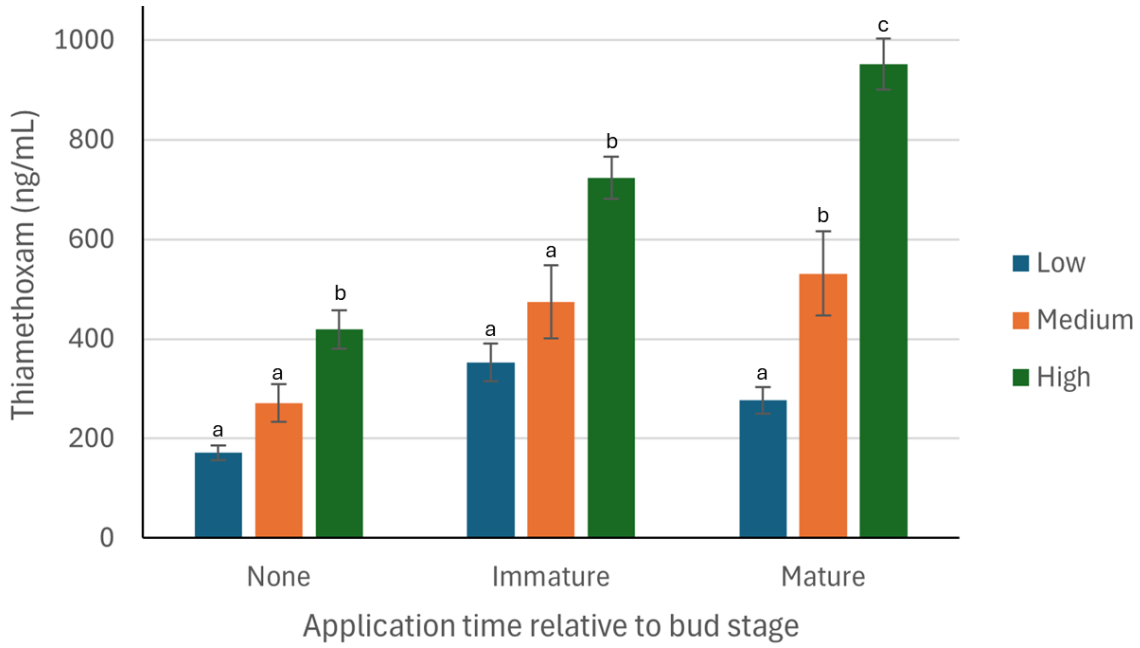

Figure 2. Mean thiamethoxam concentrations (±standard deviation) in nectar associated with three drench application times prior to anthesis (no flower buds, immature flower buds, mature flower buds) and three drench application rates (low, medium, and high). Different letters for each application timing indicate significant responses among application rates at $P \leq 0.05$ confidence.

Comparisons of thiamethoxam concentrations in nectar at each application rate relative to flower developmental stage were also of interest to note from this study. When the low application rate was applied, concentrations in nectar were lowest in applications made before flower buds were present (171.4 ng/mL) compared to applications made when flower buds were immature (352.0 ng/mL) or mature (276.5 ng/mL) (**Fig. 3**). Likewise, applications made to plants at the medium rate resulted in the lowest nectar contamination when made before flower buds were present (271.5 ng/mL) compared to applications made when flower buds were mature (531.2 ng/mL). Most dramatically, applications at the high rate made to plants with mature flower buds (latest timing) resulted in the highest concentrations of thiamethoxam in nectar, followed by the immature flower bud timing and then the earliest timing when plants did not have flower buds.

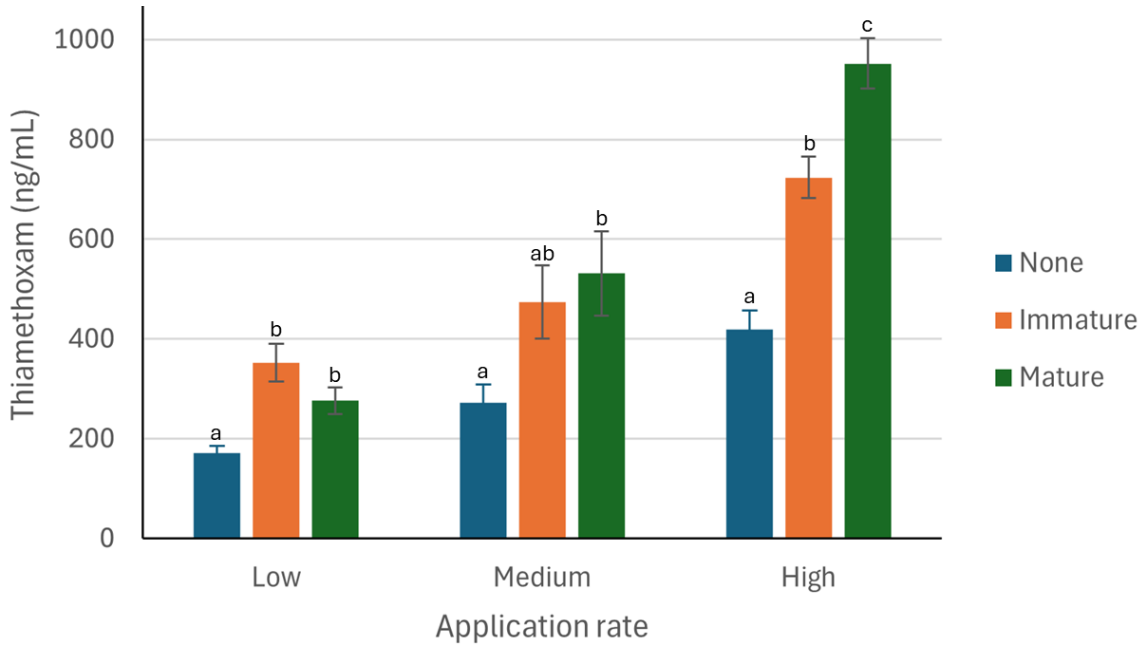

Figure 3. Mean thiamethoxam concentrations (±standard deviation) in nectar associated with three drench application rates (low, medium, and high) and three drench application times prior to anthesis (no flower buds, immature flower buds, mature flower buds). Different letters for each application rate indicate significant responses among application times at $P \leq 0.05$ confidence.

DISCUSSION

Results indicate that pesticide application method, timing, and rate all can influence contamination of nectar with the neonicotinoid insecticide thiamethoxam. As application rates increased, thiamethoxam concentrations in nectar increased from 1.5x to 3.4x depending on the flower bud stage. Likewise, as flower bud maturity increased, contamination of nectar increased by 1.3x to 2.3x depending on the application rate. Interestingly, thiamethoxam was rarely detected in the spray-applied treatments (low concentrations when detected) regardless of the timing or rate; whereas higher and more frequent detections occurred in the drench-applied treatments. These results are counter to those of Rostán et al. (2024) who reported that spray treatments of thiamethoxam applied to indigo spires salvia (*Salvia* × 'Indigo Spires') resulted in detectable concentrations in 100% of nectar samples collected, though concentrations were 1-2 orders of magnitude lower than concentrations from drench treatments. The lack of detections from the spray-applied thiamethoxam in lantana nectar may be attributed to its thick epidermal cuticle and high frequency of both glandular and non-glandular trichomes on the adaxial leaf surface potentially obstructing pesticide absorption (Sultana, 2016). With the high-rate drench treatment, as the application timings approached flowering, concentrations of thiamethoxam in nectar increased significantly. These differences in concentrations between timings likely resulted from less time for biodegradation of thiamethoxam to occur within the plants before sampling (Mach et al., 2018).

In addition, root density was most pronounced at the latest stage (mature flower buds), which would increase interception of the thiamethoxam molecules by the roots and result in higher concentrations within the plants (and presumably the nectar) (Namiki, 2022).

To screen for ecological risks of thiamethoxam, concentrations in nectar were compared to published median lethal concentrations (LC_{50}) in nectar for pollinators. For every drench treatment, thiamethoxam concentrations were found to exceed LC_{50} values of 54.3 ng/mL for the native bee, *Melipona scutellaris*. For every drench treatment except the low rate with no flower buds, thiamethoxam concentrations in nectar also exceeded LC_{50} values of 227 ng/mL for the European honeybee (*Apis mellifera*), indicating significant risks for acute toxicity (Miotelo et al., 2021).

CONCLUSION

The results presented herein indicate care should be taken for this species when drench-applying thiamethoxam as opposed to spray applications that present low risks to pollinators. From a pollinator-protection standpoint, applications should be restricted to the lower rates when possible and pre- to early-bud formation application window. Application of more pollinator-friendly (less toxic) insecticides should be considered if insect control is needed closer to the time when plants will go to market. Additional studies are needed to determine the amount of time it takes for lethal concentrations of pesticide in nectar to dissipate once plants are installed in the landscape.

Future research is being conducted to evaluate other ornamental species and pesticides to aid in the development of best management practices for the ornamental industry.

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