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Effect of distribution and concentration of topically applied neonicotinoid insecticides in buffalograss, *Buchloe dactyloides*, leaf tissues on the differential mortality of *Blissus occiduus* under field conditions

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Effect of distribution and concentration of topically applied neonicotinoid insecticides in buffalograss, *Buchloe dactyloides*, leaf tissues on the differential mortality of *Blissus occiduus* under field conditions

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Abstract

**Background:** Neonicotinoid insecticides are generally efficacious against many turfgrass pests, including several important phloem-feeding insects. However, inconsistencies in control of western chinch bugs, *Blissus occiduus*, have been documented in field efficacy studies. This research investigated the efficacy of three neonicotinoid insecticides (clothianidin, imidacloprid and thiamethoxam) against *B. occiduus* in buffalograss under field conditions and detected statistically significant differences in *B. occiduus* numbers among treatments. A subsequent study documented the relative quantity and degradation rate of these insecticides in buffalograss systemic leaf tissues, using HPLC.

**Results:** Neonicotinoid insecticides initially provided significant reductions in *B. occiduus* numbers, but mortality diminished over the course of the field studies. Furthermore, while all three neonicotinoids were present in the assayed buffalograss leaf tissues, imidacloprid concentrations were significantly higher than those of clothianidin and thiamethoxam. Over the course of the 28 day study, thiamethoxam concentrations declined 700-fold, whereas imidacloprid and clothianidin declined only 70-fold and 60-fold respectively.

**Conclusions:** Field studies continued to verify inconsistencies in *B. occiduus* control with neonicotinoid insecticides. This is the first study to document the relative concentrations of topically applied neonicotinoid insecticides in buffalograss systemic leaf tissues.

**Keywords:** *Blissus occiduus*, neonicotinoid insecticides, insecticide degradation, buffalograss

1. Introduction

For more than a decade, neonicotinoid insecticides have provided effective control of a wide array of insect pests. Since the introduction of imidacloprid in the 1990s, these compounds have represented one of the fastest growing insecticide classes in recent history.¹ ² The success of this and other neonicotinoid compounds has been due in large part to their broad-spectrum insecticidal activity, low application rates, systemic uptake and translocation in plants and favorable toxicological profile.³ While crop protection remains the major use for neonicotinoid insecticides,⁴ new markets have emerged for urban, veterinary, turf and ornamental pests.⁵ Neonicotinoid insecticides have been shown effectively to control a variety of turfgrass pests, including the chinch bugs *Blissus leucopterus hirtus* Montandon and *B. insularis* Barber (Shetlar DJ, http://golfdom.com).⁶⁷ Western chinch bug, *B. occiduus* Barber, control has been documented in field studies at the University of Nebraska-Lincoln. In these field studies on buffalograss, significant though inconsistent reductions in *B. occiduus* numbers were observed with imidacloprid and clothianidin.⁹¹¹ Other chemical, environmental or plant physiological factors may have been responsible for the inconsistent results.

*Blissus occiduus* has emerged as a serious buffalograss pest over the past two decades. This chinch bug is widely distributed, from California, Colorado, Kansas, Montana, Nebraska and New Mexico in the United States to Alberta, British Columbia, Manitoba and Saskatchewan in Canada.¹²¹⁴ In Nebraska, two generations of *B. occiduus* occur annually. Adults overwinter within the turfgrass stand. The first generation completes development by mid-June and is present until mid-August. Second-gen-
eration adults appear in late August and remain active until fall temperatures cool.\textsuperscript{14} Chinch bugs injure grasses by withdrawing sap from stolons and plant tissues in the crown area.\textsuperscript{14}

Laboratory bioassays further explored the intrinsic toxicity of the neonicotinoid insecticides. Clothianidin, imidacloprid and thiamethoxam are all toxic to \textit{B. occiduus}, with both topical and systemic applications of these insecticides able to provide significant reductions in chinch bug numbers.\textsuperscript{15} The mortality observed for the systemically applied insecticides could only have occurred if they had been translocated at lethal concentrations through the roots and into the stems and above-ground leaf tissues where chinch bugs feed.\textsuperscript{15}

Clothianidin, imidacloprid and thiamethoxam are in the same insecticide class, but differences in chemical structure can influence arthropod toxicity. Water solubility, for example, can influence insecticidal activity. Thiamethoxam has higher water solubility (4.1 g L\textsuperscript{-1}) than clothianidin (0.30–0.34 g L\textsuperscript{-1}) and imidacloprid (0.63 g L\textsuperscript{-1}), suggesting it should have greater systemic movement and potential to dissolve in the vascular (xylem) tissues.\textsuperscript{5} This may result in greater toxicity at lower chemical concentrations in the plant. Systemicity, however, is only one of many biotic and abiotic environmental factors and plant responses that can influence insecticide toxicity under field conditions.

Insecticide translocation in plants generally occurs more rapidly in younger stem and leaf tissues.\textsuperscript{26} Xylem flow is driven by water transpiration from the leaves, which in turn is regulated by the stomata and varies relative to environmental factors, including light, temperature, wind, etc.\textsuperscript{27} The systemic properties of imidacloprid have been studied in numerous plant species, exhibiting upward movement in the xylem.\textsuperscript{19–21} This systemic movement can be explained in terms of its physicochemical properties. Imidacloprid, a polar and water-soluble compound, typically forms weak bonds with soil particles, making it available in the soil water for uptake by plant roots and transport in the xylem to leaf margins and interveinal spaces.\textsuperscript{21} However, the rate of insecticide uptake is variable among plant species. Soil properties may also influence neonicotinoid uptake in plants.\textsuperscript{22–24}

Currently, little information is available on the movement of systemic insecticide in turfgrasses, especially in buffalograss. This study documented the distribution and concentration of topically applied neonicotinoid insecticides (clothianidin, imidacloprid and thiamethoxam) in buffalograss leaf tissues and correlated these parameters with \textit{B. occiduus} differential mortality under field conditions.

2. Experimental Methods

2.1 Field evaluations

Insecticides were applied to buffalograss in research plots located at the John Seaton Anderson Turf and Ornamental Research Facility (JSA Research Facility), University of Nebraska Agricultural Research and Development Center, near Mead, Nebraska. Soil content at the JSA Facility plots was primarily Tomek silt loam (pH 6.8–7.2). As mentioned, clay content may influence insecticide uptake in plants. Soil content at these sites was unlikely significantly to inhibit insecticide uptake in the plant. Plots were mowed weekly at 7.6 cm (clippings returned with weed content of < 5%), and subsequent irrigation was applied at 2.5 cm month\textsuperscript{-1} to maintain optimal growing conditions. Cumulative rainfall during the studies was 29.7 cm (field study 1) and 20.3 cm (field study 2).

The plots were treated with the highest labeled rates of Arena 0.5 G [clothianidin, (E)-1-(2-chloro-3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine] at 89.7 kg ha\textsuperscript{-1} (0.45 kg AI clothianidin ha\textsuperscript{-1}), Merit 75 WP [imidacloprid, 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine] at 9.6 kg ha\textsuperscript{-3} (0.45 kg AI imidacloprid ha\textsuperscript{-3}) and Meridian 25 WG [thiamethoxam, (E, Z)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro) amine] at 19.1 kg ha\textsuperscript{-1} (0.30 kg AI thiamethoxam ha\textsuperscript{-1}). Talstar One [bifenthrin, 2-methylbiphenyl-3-ylmethyl (Z)-(1R5,9R5)-3-(2-chloro-3,3,3-trifluoropro-1-enyl)-2,2-dimethyl-cyclopropanecarboxylate], a synthetic pyrethroid industry standard, served as a positive control and was applied at 1.6 L ha\textsuperscript{-1} (0.11 kg AI bifenthrin ha\textsuperscript{-1}).

Two independent field studies were designed to evaluate the efficacy of these insecticides against first- and second-generation \textit{B. occiduus}. Treatments were applied to third- and fourth-instar chinch bugs in both field studies. Pretreatment estimates were taken prior to each study. Chinch bug numbers in a 0.09 m\textsuperscript{2} area ranged from 10 to 20 (field study 1) and from 35 to 45 (field study 2). Plots were 1.5 m × 1.5 m arranged in a randomized complete block (RCB) design, with five replications. Meridian, Merit and Talstar were applied using a CO\textsubscript{2} sprayer at 276 kPa, with 1627 L ha\textsuperscript{-1} finished spray. Arena 0.5 G, a granular formulation, was uniformly applied using a hand shaker. Lack of product availability necessitated the substitution of Arena 0.5 G for the preferred 50 WG formulation. Fortunately, efficacy trials conducted at the University of Nebraska-Lincoln had shown few differences in \textit{B. occiduus} control between the 0.5 G and 50 WG Arena formulations (Baxendale FB, unpublished). All plots were irrigated with approximately 0.38 cm of water immediately following application to activate the Arena granules, wash the liquid formulations off leaf surfaces and facilitate root uptake of the neonicotinoid insecticides.

Applications to first-generation chinch bugs (field study 1) were evaluated for efficacy at 3, 8 and 106 days after treatment (DAT), while the second generation (field study 2) was evaluated for efficacy 3, 7, 28 and 55 DAT. Two 24 cm diameter areas were sampled (0.09 m\textsuperscript{2} total area per plot) by vacuuming the soil surface with an ECHO Shred ‘N’ Vac (Model No. 2400; ECHO Inc., Lake Zurich, IL).\textsuperscript{23} At the conclusion of each sample date, the vacuum samples were placed individually in Berlese funnels for 48 h. The surviving chinch bugs were collected in 70% ethyl alcohol, sorted by age class and counted using a Leica Zoom 2000 microscope (Leica, Wetzlar, Germany). Treatments were compared with an untreated control for reductions in chinch bug numbers.

2.2 Materials and sample calibration

Individual stock solutions of all neonicotinoid analytes, the surrogate and the internal standard were prepared at concentrations of 5 µg L\textsuperscript{-1} in methanol from analytical-grade clothianidin (99.4% AI), imidacloprid (99.5% AI), thiamethoxam (99.5% AI) (Chem Service Inc., West Chester, PA), terbutylazine (surrogate; Sigma-Aldrich, Milwaukee, WI) and \textsuperscript{13}C\textsubscript{3}-labeled atrazine (internal standard; Merck & Co., Whitehouse Station, NJ). Analyte, surrogate and internal standard calibration spiking solutions were prepared from the stock solutions diluted to 50 ng L\textsuperscript{-1} in methanol. Calibration standard samples were prepared from the calibration spiking solutions in sample matrix obtained from the method extraction of untreated buffalograss. Analytes and surrogate were added to individual calibration samples in amounts of
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250, 1000 and 2500 ng to create a three-point calibration curve. Internal standard (2500 ng) was added to all calibration standards and samples to quantify analyte concentrations on the instrument. Mean percentage recovery of the surrogate from the 47 samples was 109 ± 4%, which has met the acceptance criteria of the United States Environmental Protection Agency. Analyte detection limits were estimated from the instrument signal-to-noise to be 32 ng mL⁻¹ in the final injection matrix, corresponding to an analyte concentration in buffalograss of 0.01 µg g⁻³.

2.3 Extraction procedures

Insecticides were applied to ‘Prestige’ buffalograss refuge plots at the JSA Research Facility, which were independent of the previously described field studies. Application procedures followed the methods used in the field studies. Plots (1.1 m × 1.2 m in size) were treated using previously described insecticide rates. The experimental design was a RCB design with four replications. Plots received a weekly mowing at 6.4 cm (clippings returned with weed content of < 2%) and − 2.5 cm of irrigation and 9.3 cm of rainfall during the course of the 28 day study.

At 3, 7, 14 and 28 DAT, above-ground leaf material was harvested and stored on ice until being transferred to a −80 °C freezer for later processing. A quantity of 5 g fresh weight of chilled leaf blades (stolons removed) was then randomly selected from the sample, frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The ground tissue was transferred to 50 mL tubes (VWR International, San Diego, CA), and HPLC-grade acetonitrile (30 mL) (Sigma-Aldrich, Milwaukee, WI) was added to the material, which was then stored overnight (ca 15 h) at 4 °C to extract the insecticides.

After extraction, samples were shaken for 30 min at 4 °C on a multipurpose rotator (Model No. 2314; Thermo Scientific, Waltham, MA) and centrifuged at 3500 rpm at 16 °C (IEC Multi-RF; Thermo Electron, Milford, MA) for 20 min. A 10 mL aliquot of the supernatant was mixed with 100 mL of reagent-grade water, and 2500 ng (50 µL of a 50 ng µL⁻¹ solution) of terbutylazine was added as a surrogate. Aqueous extracts were passed through a 200 mg solid-phase extraction (SPE) cartridge (Oasis HLB; Waters, Milford, MA) connected to a vacuum manifold. The Oasis HLB cartridge used for SPE was previously prepared and held for another 10 min to re-equilibrate the column for a bile phase ratio, held for 12 min, then returned to a 90:10 ratio and held for another 10 min to re-equilibrate the column for a total run time of 30 min. The flow rate was 0.3 mL min⁻¹ and the sample injection volume was 25 µL. The LCQ mass spectrometer was operated in atmospheric-pressure chemical ionization (APCI) mode with the vaporizer temperature at 350 °C, the discharge current at 5.0 µA, the sheath gas at 80 (arbitrary units), the auxiliary gas at 1 (arbitrary units), the tube lens voltage at − 5.0 V, the capillary voltage at 3.0 V, the capillary temperature at 150 °C, the lens voltage at − 36.0 V, multipole 1 offset at − 3.0 V, multipole 2 offset at − 5.0 V and the multipole RF amplitude at 500 V p-p. The daughter ion transitions and percentage collision energies used in the analysis for each analyte were as follows: imidacloprid (m/z = 256 → 210, 30%), clothianidin (m/z = 250 → 169, 25%), thiamethoxam (m/z = 292 → 211, 25%), terbutylazine (m/z = 230 → 174, 35%) and 13C₁₆-labeled atrazine (m/z = 219 → 177, 35%). The isolation width was 3 amu, and the activation time was 30 ms for all analytes. The collision gas was helium.

2.5 Statistical analysis

A generalized linear mixed model with a negative binomial distribution and randomized blocks was implemented in PROC GLIMMIX for the two field studies. Chinch bug numbers in the Talstar One-treated plots were not included in the statistical analysis. Data obtained from HPLC were analyzed using PROC MIXED. In all studies, when significant interactions were detected, t-tests were used for mean separation using Fisher’s procedure (α = 0.05).

3. Results

3.1 Field evaluations

Mean numbers of collected chinch bugs for field studies 1 and 2 are reported in Tables 1 and 2 respectively. For field study 1, analysis of variance suggested a significant one-way interaction for insecticide treatment (F = 9.72; df = 3, 48; P < 0.001) and evaluation date (F = 12.37; df = 3, 48; P < 0.0001). Two-way interactions were not significant (F = 1.52; df = 9, 48; P = 0.1906). As one-way interactions were significant, simple effects were used to determine whether differences existed among treatment means. At 3 DAT, statistically significant differences in the number of chinch bugs were detected for all treatments when compared with the untreated control (clothianidin: t = 2.36, df = 48, P < 0.0222; thiamethoxam: t = 2.27, df = 48, P < 0.0275; imidacloprid: t = 2.19, df = 48, P < 0.0336). The treatments were equally effective at reducing chinch bug numbers, with no significant differences detected (clothianidin–thiamethoxam: t = 0.10, df = 48; P = 0.9239; clothianidin–imidacloprid: t = 0.19, df = 48; P = 0.8520; thiamethoxam–imidacloprid: t = 0.09, df = 48; P = 0.9274) (Table 1).

At 8 DAT, significant differences in the number of chinch bugs were detected in all treatments when compared with the untreated control (clothianidin: t = 3.21, df = 48, P < 0.0023; thiamethoxam: t = 3.06, df = 48, P = 0.0036; imidacloprid: t = 2.92, df = 48, P = 0.0053). However, no significant differences were detected among insecticide treatments, which was consistent with 3 day evaluations (clothianidin–thiamethoxam: t = 0.21, df = 48; P = 0.8361; clothianidin–imidacloprid: t = 0.39;
nymphs and adults. In this case, clothianidin had significantly fewer chinch bugs than the untreated control and provided ≈ 84% control (Arena: \( t = 3.22, df = 64, P < 0.0027 \); imidacloprid: \( t = 1.63, df = 64, P = 0.1073 \)) (Table 2). These results are similar to those of field study 1. Long-term, residual control with thiamethoxim and imidacloprid was low.

### 3.2 Relative quantity of neonicotinoids in treated buffalograss

Mean insecticide concentrations in the buffalograss leaf samples are reported in Table 3. The analysis of variance suggested a significant one-way interaction for insecticide treatment (\( F = 4.31, df = 2, P < 0.0212 \)) and sampling date (\( F = 21.77, df = 3, 35, P < 0.0001 \)). Two-way interactions were not significant (\( P = 0.1000 \)). As one-way interactions were significant, simple effects were used to determine whether significant differences existed.

At 3 DAT, mean concentrations of imidacloprid, clothianidin and thiamethoxim were 14.24 µg g\(^{-1}\) (active ingredient plant material\(^{-1}\)), 6.48 µg g\(^{-1}\) and 6.20 µg g\(^{-1}\) respectively (Table 3). The imidacloprid concentration in the treated buffalograss was significantly greater than the clothianidin concentration (\( t = 3.44, df = 35, P = 0.0015 \)) and the thiamethoxim concentration (\( t = 3.85, df = 35, P = 0.0005 \)). These differences may be due to water solubility, formulation or application rates, but do not explain why clothianidin and thiamethoxim had similar concentrations (\( t = 0.13, df = 35, P = 0.8998 \) when clothianidin was applied at a much higher rate.

<table>
<thead>
<tr>
<th>Treatment (active ingredient)</th>
<th>3 DAT(^c)</th>
<th>7 DAT</th>
<th>28 DAT</th>
<th>55 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clothianidin (Arena)</td>
<td>5.8 ± 1.1 a</td>
<td>1.0 ± 0.4 a</td>
<td>0.4 ± 0.3 a</td>
<td>2.2 ± 0.7 a</td>
</tr>
<tr>
<td>Thiamethoxim (Meridian)</td>
<td>15.6 ± 1.8 a</td>
<td>6.8 ± 1.2 b</td>
<td>2.2 ± 0.7 ab</td>
<td>10.4 ± 1.4 b</td>
</tr>
<tr>
<td>Imidacloprid (Merit)</td>
<td>18.4 ± 1.9 a</td>
<td>9.6 ± 1.4 b</td>
<td>2.8 ± 0.7 b</td>
<td>10.2 ± 1.4 b</td>
</tr>
<tr>
<td>Untreated control</td>
<td>45.8 ± 3.0 b</td>
<td>35.4 ± 2.7 c</td>
<td>8.4 ± 1.3 b</td>
<td>18.2 ± 1.9 b</td>
</tr>
</tbody>
</table>

a. Mean number of chinch bugs per 0.09 m\(^2\).
b. Treatment means within the same column followed by the same letter indicate no significant differences (\( P < 0.05 \)), LSD test.
c. DAT = days after treatment.
Effect of neonicotinoid insecticides in buffalograss on western chinch bug mortality

4. Discussion and Conclusions

Field studies continue to document previous findings and continue to verify contradictions in chinch bug control with neonicotinoid insecticides. Initial reductions in chinch bug numbers were significant, which may be the result of contact toxicity. Mortality quickly declined, possibly owing to the translaminar and systemic movement of these compounds. Although clothianidin was applied as granular formulation without translaminar systemicity, it continued to be more effective for controlling B. occiduus, especially for season-long chinch bug control. As mentioned, it is unlikely that a WDG formulation would have shown differences in B. occiduus control (Baxendale FB, unpublished).

This is the first report documenting neonicotinoid insecticide concentrations in buffalograss leaf tissues. While statistical comparisons were not made between studies, interestingly, initial concentrations of imidacloprid (at 3 DAT) in the buffalograss leaf tissues were greater than those of clothianidin and thiamethoxam. However, field mortality appeared to be similar, possibly owing to water solubility or other chemical properties that influence the amount of residue on the leaf surface and in the systemic tissues of the plant. Similar findings were documented in laboratory bioassays, which indicated no differences in systemic toxicity among the three neonicotinoids to B. occiduus nymphae. Chemical differences became less apparent in only a matter of days, with the relative persistence of these compounds being similar. In final comparisons, all three insecticides were detected at similar concentrations 28 DAT, while B. occiduus field mortality differed at 106 and 55 DAT (field study 1 and field study 2 respectively). This suggests that the concentration of insecticide in leaf tissues may not be closely correlated with chinch bug mortality under field conditions.

The biochemical and metabolic pathways of the neonicotinoids, especially imidacloprid, have been well studied within treated plant tissues. An olefin derivative has been identified as the predominant metabolite of imidacloprid, with moderate to high insecticidal activity. In the present study, using full-scan MS to characterize transformation products, metabolites of imidacloprid and clothianidin were not detected. However, clothianidin was detected as a metabolite of thiamethoxam, suggesting that buffalograss has the capacity to metabolize thiamethoxam to clothianidin. At the 3 DAT sampling period, the concentration of the clothianidin metabolite was a small percentage of the total active ingredient available in the plant tissues. However, by 14 DAT, clothianidin represented a much higher proportion of the total of both parent

Table 3. Relative quantity of neonicotinoids in treated buffalograss

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean concentration ± SEM</th>
<th>3 DAT</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>28 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>14.24 ± 3.68 a</td>
<td>3.25 ± 0.72 a</td>
<td>0.77 ± 0.17 a</td>
<td>0.20 ± 0.05 a</td>
<td></td>
</tr>
<tr>
<td>Clothianidin</td>
<td>6.48 ± 1.97 b</td>
<td>1.56 ± 0.61 a</td>
<td>0.66 ± 0.17 a</td>
<td>0.19 ± 0.06 a</td>
<td></td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>6.20 ± 0.70 b</td>
<td>0.60 ± 0.29 a</td>
<td>0.16 ± 0.05 a</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

a. Mean insecticide concentration (µg g⁻¹ fresh weight).

By 7 DAT, the concentration of imidacloprid, clothianidin and thiamethoxam in leaf tissues had significantly declined (clothianidin: t = 2.18, df = 35, P < 0.0359; imidacloprid: t = 5.26, df = 35, P < 0.0001; thiamethoxam: t = 2.68, df = 35, P = 0.0112), thiamethoxam had the greatest decline (≈10-fold), with 0.60 µg g⁻¹ of active ingredient remaining in the leaf tissues, as opposed to imidacloprid (3.25 µg g⁻¹) and clothianidin (1.56 µg g⁻¹) with a = 4-fold and = 8-fold decline respectively. Concentrations of imidacloprid and clothianidin significantly declined since the previous sampling date (imidacloprid: t = 0.46, df = 35, P = 0.6497; clothianidin–imidacloprid: t = 1.27, df = 35, P = 0.2137). From 7 to 14 DAT there were no significant declines in mean insecticide concentrations in the treated buffalograss were not significantly different (clothianidin–imidacloprid: t = 0.81, df = 35, P = 0.4243; clothianidin–thiamethoxam: t = 0.46, df = 35, P = 0.6497; imidacloprid–thiamethoxam: t = 2.18, df = 35, P = 0.0939). At 28 DAT, insecticides were still detectable and had not significantly declined since the previous sampling date (clothianidin: t = 0.23, df = 35, P = 0.8226; imidacloprid: t = 0.27, df = 35, P = 0.7889; thiamethoxam: t = 0.07, df = 35, P = 0.9425). The insecticide concentrations were not significantly different (imidacloprid: t = 0.01, df = 35, P = 0.9937; clothianidin–thiamethoxam: t = 0.09, df = 35, P = 0.9322; imidacloprid–thiamethoxam: t = 0.09, df = 35, P = 0.9259); however, thiamethoxam had declined = 20-fold and was less than its method detection limit. Imidacloprid and clothianidin had declined = 4-fold, with 0.20 and 0.19 µg g⁻¹ respectively (Table 3).

Insecticide concentrations declined significantly over the 28 day study (clothianidin: t = 2.79, df = 35, P = 0.0085; imidacloprid: t = 6.72, df = 35, P < 0.0001; thiamethoxam: t = 2.96, df = 35, P = 0.0055). In this 25 day period (3–28 DAT), thiamethoxam concentrations declined 700-fold, whereas imidacloprid and clothianidin declined only 70-fold and 60-fold respectively.

Some metabolites of neonicotinoid insecticides are known to be toxic to arthropod pests. Concentrations of imidacloprid and clothianidin metabolites were below the present estimated detection limit (0.01 µg g⁻¹) and unlikely to contribute to observed chinch bug control. However, clothianidin is a known metabolite of thiamethoxam and was detected in samples from the thiamethoxam treatment at 3, 7 and 14 DAT (0.36 ± 0.15 µg g⁻¹, 0.12 ± 0.05 µg g⁻¹ and 0.07 ± 0.02 µg g⁻¹ respectively).

The biochemical and metabolic pathways of the neonicotinoids, especially imidacloprid, have been well studied within treated plant tissues. An olefin derivative has been identified as the predominant metabolite of imidacloprid, with moderate to high insecticidal activity. In the present study, using full-scan MS to characterize transformation products, metabolites of imidacloprid and clothianidin were not detected. However, clothianidin was detected as a metabolite of thiamethoxam, suggesting that buffalograss has the capacity to metabolize thiamethoxam to clothianidin. At the 3 DAT sampling period, the concentration of the clothianidin metabolite was a small percentage of the total active ingredient available in the plant tissues. However, by 14 DAT, clothianidin represented a much higher proportion of the total of both parent

C. ND = not detected (i.e. less than detection limit).
and metabolite, and may have contributed to the overall effect of the thiamethoxam treatment, especially later in the growing season.

This research provides a better understanding of neonicotinoid toxicity and degradation under field conditions. Neonicotinoids have been shown to photodegrade,35 36 and soil bacteria are capable of metabolizing these compounds.37 38 These factors could limit B. occiduus control with certain neonicotinoid insecticides, and may have important implications for management of other turfgrass insect pests. Additional research is also needed to understand B. occiduus behavior in response to neonicotinoid exposure. Chinch bug probing location has been documented,39 but no studies have explored the specific feeding patterns on neonicotinoid-treated plant tissues. Electrical penetration graphs (EPGs)40 41 would be a valuable tool to document the effects of neonicotinoid insecticides on chinch bug feeding and behavior.

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