Thiamethoxam Seed Treatments Have No Impact on Pest Numbers or Yield in Cultivated Sunflowers

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ABSTRACT The use of neonicotinoid seed treatments is a nearly ubiquitous practice in sunflower (*Helianthus annuus*) pest management. Sunflowers have a speciose pest complex, but also harbor a diverse and abundant community of beneficial, nontarget organisms which may be negatively affected by pest management practices. Here, we investigate how the foliar and subterranean arthropod pest communities in sunflower fields were affected by a thiamethoxam seed treatment over three site years (two years on one farm, and another year at an additional field in the second year). Thiamethoxam and its metabolite clothianidin in leaf tissue were quantified throughout the growing season, and yield differences between treatments were measured. Across site years, foliar herbivores and key pests of sunflowers were unaffected by the seed treatment. Likewise, subterranean herbivores were unaffected. Thiamethoxam was measurable in leaf tissue through the R1 plant stage, while its metabolite clothianidin was detected throughout flowering (R6). No difference in sunflower yield was observed between treatments across site years. This research suggests that neonicotinoid seed treatments in sunflowers do not always provide economic benefits to farmers in the form of pest reductions or yield improvements. Future research should focus on sunflower integrated pest management strategies that limit nontarget effects of agrochemicals, while providing greater economic returns to farmers.

KEY WORDS *Helianthus annuus*, herbivore, neonicotinoid, metabolite, clothianidin

Producers commonly use neonicotinoid seed dressings to control early-season herbivory on a wide variety of crops (Elbert et al. 2008). Neonicotinoids are a class of water-soluble insecticides which, when applied as a seed treatment, are present systemically through the plant's roots (Van Rozen and Ester 2010), green tissue (Sur and Stork 2003), nectar (Schmuck et al. 2001), and pollen (Laurent and Rathahao 2003). The insecticide gradually becomes less concentrated as the plant grows and as the insecticide is metabolized (Nault et al. 2004, Moser and Obrycki 2009). Metabolites of neonicotinoids applied as seed treatments can be found at measurable levels within treated plant tissue (Nauen et al. 2003). These chemicals often display insecticidal qualities similar to the active ingredient used to treat the plant (Benzidane et al. 2010), but are often overlooked in risk-assessment studies focused on the environmental fate of the active ingredient. Upon consumption of treated plant-tissue by an insect, neonicotinoid toxins compete with naturally occurring neurotransmitters for the same binding sites on a target cell, causing unregulated nerve excitation (Matsuda et al. 2001, Meijer et al. 2014). This overstimulation of a target cell can cause paralysis and eventually lead to the death of the insect (Goulson 2013).

Pests of sunflowers (*Helianthus annuus*; Asterales: Asteraceae) are challenging to manage in North America for several reasons. First, many wild varieties of sunflower are native to this region, and so have coevolved with a diverse suite of indigenous pests (Charlet et al. 1992, Rogers 1992). For example, there are 20 insects of concern recognized in the northern Great Plains (Knodel et al. 2010). Many of these pests feed inside the sunflower's large head and stem which provides physical refuge for an insect, making the timing and placement of insecticide a challenge (Knodel et al. 2010). Managing these head-feeding pests is further complicated because many varieties of sunflowers, although self-compatible, still experience a yield benefit from insect pollination (Jyoti and Brewer 1999), and so insecticide application needs to be balanced against pollination services (Rogers 1992). Finally, there is very low tolerance for seed-damaging pests in sunflowers destined for certain markets (e.g., the confection market; Brewer and Schmidt 1995). In general, insecticides are the primary tool that farmers use to manage pests in their sunflower fields (Rogers 1992, Prasifka and Hulke 2012). It is not uncommon for producers to use multiple applications of broad-spectrum insecticides during a single growing season. In addition to insecticidal sprays, conventional producers very frequently use neonicotinoid seed treatments to control early

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season sunflower pests. The extensive adoption of neonicotinoid seed treatments use has created a situation where interested producers have difficulty finding sunflower seeds that have not been treated (European Academies Scientific Advisory Council [EASAC] 2015).

There are no peer-reviewed studies as of yet that evaluate the need for, or effects of, neonicotinoid seed treatments in sunflowers. The persistence of neonicotinoids and toxic metabolites within a variety of treated crops also has not been formally substantiated and requires further investigation (Bonmatin et al. 2015). The present study tested the null hypotheses that thiamethoxam, a commonly used neonicotinoid seed treatment, does not affect yields, crop profitability, or pest populations in oilseed sunflower production systems in central and eastern South Dakota. To further characterize the sunflower agroecosystem, thiamethoxam and its toxic metabolite clothianidin were measured within leaf tissue throughout the growing season across three site years.

Materials and Methods

Sunflower Production and Experimental Design. The experiment was replicated at one location (Brookings) in two successive growing seasons, and at an additional location (Dakota Lakes) in the second growing season. On June 14, 2013, at the eastern South Dakota Soil and Water Research Farm (USDA-ARS) near Brookings, SD (44.3064° N, 96.7881° W), sunflowers (*Helianthus annuus*, Pioneer, Variety: 63M80-N422) were planted in six, 30.5- by 30.5-m fields. Borders (12 m wide) of untreated Sorghum Sudan (*Sorghum* X *drummondii*, Millborn Seeds Inc., Variety: MS9000) grass were planted around and in between the fields to separate them. Three of the fields were planted with untreated seeds while the remaining three were planted with seeds that had been treated with thiamethoxam (Cruiser, Syngenta, Greensborough, NC) at a rate of 0.25 mg a.i./seed. Fields were planted in a randomized block design with a seeding rate of 76,601 seeds/ha.

On May 23, 2014, at the Brookings, SD site there were eight, 24.5- by 36.5-m fields planted to sunflowers (*Helianthus annuus*, Mycogen, var 8H288CLDM) at a rate of 76,601 seeds/ha. Half of the fields were treated with a thiamethoxam seed dressing prior to planting at a rate of 0.25 mg a.i./seed and half of the fields were untreated. Field margins (12 m wide) between sunflower fields were planted with untreated soybeans (*Glycine max*).

On June 6, 2014, the same field arrangement was also established at the Dakota Lakes Research Farm near Pierre, SD (44.3680° N, 100.3364° W) following the same experimental conditions as Brookings, except fields were separated by 6-m untreated-soybean borders. For weed management in Brookings in 2013, fields were sprayed with a mixture of glyphosate (Roundup WeatherMAX 2.34 liter/ha) and sulfentrazone (Spartan; 0.44 liter/ha) the same day that the fields were planted. The same herbicide regimen was used in Brookings in 2014 except the rate of glyphosate was reduced to 1.61 liter/ha. Fields at Dakota Lakes were sprayed with sulfentrazone (0.29 liter/ha) the fall before planting occurred, and in the spring shortly after planting the fields were sprayed with a mixture of glyphosate (rate: 1.17 liter/ha) and pendimethalin (Prowl H2O; 2.92 liter/ha). No fertilizer was applied at the Brookings location in either 2013 or 2014. At Dakota Lakes, nitrogen was broadcasted at a rate of 84.06 Kg/ha on June 1, 2014.

Insect Collections. *Foliar Herbivores.* In 2013, the foliar arthropod communities within sunflower fields were assessed eight times between the V-6 and R-7 sunflower stages (Schneiter et al. 2003) at the Brookings location. In 2014, foliar insect communities were assessed 10 times at Brookings and six times at Dakota Lakes between V-2 and R-6 sunflower stages.

The herbivorous arthropod communities on entire plants were visually assessed. On each of the sampling dates, randomly selected sunflower plants from each field were examined, and all herbivorous arthropods found on the foliage, stems, and flowers were placed in 70% ethanol to be later curated and identified. At the Brookings location in 2013, 10 plants from each field on each date were examined. In 2014 at the Brookings location 20 plants per field were examined on the first two dates, 15 plants on the second and third dates, and 10 plants per field on all of the remaining dates. In 2014 at the Dakota Lakes location 15 plants per field were examined on the first date and 10 plants were examined per field on all of the remaining dates.

Soil Arthropod Community. In 2013, soil herbivore communities at the Brookings location were assessed on six occasions between planting and the R-6 plant stage. In 2014, soil herbivores were assessed eight times at Brookings and six times at Dakota Lakes between the V-2 and R-6 stages.

In 2013, four soil cores from each field (10 cm diameter, 10 cm deep) were collected using a golf-hole cup cutter from randomly selected locations within sunflower rows. In 2014, three soil cores were taken in each field on every sampling date. On each collection date cores taken within individual fields were pooled and extracted from the soil over 7 d using a Berlese funnel. The arthropods collected in this manner were stored in 70% ethanol, for later curation and identification.

Insecticide in Plant Tissue. *Leaf Tissue Collection.* Leaf tissue was collected on seven dates throughout the growing season in Brookings in 2013, 11 times from Brookings in 2014, and six times from Dakota Lakes in 2014. Two sunflower leaf discs (4.5 mm diameter) were excised using a cork borer from each of five randomly chosen plants in each field. Leaf discs were taken from the latest emerged true leaf (leaf exceeding 4 cm in length), avoiding the leaf's midrib. The growth stages were noted for these plants at time of tissue collection. After leaf discs were cut they were placed on ice in the field and upon returning to the lab where they were frozen at −20°C until analysis with enzyme-linked immunosorbent assay (ELISA).

Insecticide Extraction. Each leaf disc was isolated in a 1.5-ml microcentrifuge tube (SealRite, USA Scientific

Inc., Ocala, FL) and homogenized in 258 µl laboratory-grade water using a plastic pestle (USA Scientific Inc., Ocala, FL). The homogenate was shaken vigorously for 1 h on an orbital shaker and was afterwards centrifuged for 1 min at 10,000 × *g*. The resulting supernatant was isolated and will be referred to as 100% leaf extract hereafter.

Thiamethoxam Quantification. Preliminary tests revealed that an aqueous 10% leaf extract solution provided the lowest background absorbance relative to the strongest signal in positive controls. Therefore, a 10% leaf extract solution was used when completing ELISAs for thiamethoxam quantification. The ELISA kit that was used (Thiamethoxam HS plate kit, lot no. 13014E; Beacon Analytical Systems Inc., Saco, ME) had a reported detection range of 0.05–2.0 ppb. On each plate, two standard curves of purified thiamethoxam (Thiamethoxam PESTANAL, Product number: 37924, Sigma-Aldrich, St. Louis, MO) including concentrations of 0.0, 0.03, 0.06, 0.13, 0.25, 0.5, 1.0, and 2.0 ppb in a 10% solution of untreated leaf extract were generated for each plate. ELISAs were conducted according to manufacturer's instructions, and the optical densities of all the thiamethoxam dilutions were measured at 450 nm using a spectrophotometer (BIO-TEK µQuant; Winooski, VT). For each plant stage that insecticide was quantified, four plants per treated field and two plants per untreated field were tested. (see Fig. 1 for these plant stages).

Clothianidin Quantification. Thiamethoxam is converted to its main metabolite, clothianidin within both insects and plants (Nauen et al. 2003). Clothianidin is a neurotoxin that inhibits signal transduction in nervous systems and is commonly used as an insecticide itself (Jeschke et al. 2010). The sunflower tissue was also examined for clothianidin using an ELISA kit (Product No. 500800, Abraxis LLC, 54 Steamwhistle Drive, Warminster, PA). This ELISA kit binds to both imidacloprid and clothianidin with high fidelity. Imidacloprid had not been used at the study sites in more than 5 yr, and the likelihood of cross contamination is minimal. Methods for sample preparation, standard curve creation, and quantification of clothianidin were the same as those described above, except purified clothianidin (Clothianidin PESTANAL, Product number: 33589, Sigma-Aldrich) was used to spike standard curve dilutions instead of thiamethoxam, and a 25% leaf extract solution was used instead of 10%.

Yield. At the Brookings location in 2013 and 2014 sunflower yields per field were taken using a combine equipped with a scale. Six randomly selected rows from each sunflower field were completely harvested and yields were extrapolated to kg / ha. Due to the absence of a combine at the Dakota Lakes location, 10 sunflower inflorescences were selected at random from each field. Achenes were manually harvested from these inflorescences, weighed, and their mean weights were extrapolated to kg / ha.

Statistics. Treatment comparisons were all performed with the statistical software SYSTAT 13 (Systat Software, Inc., San Jose, CA). To compare treatment effects on insect populations across sample dates, we conducted independent rm-ANOVAs on the mean numbers of herbivores per plant and per m^2 soil (log transformed), that simultaneously examined all 22 sample dates across seasons. In order to examine treatment effects on individual taxa, two-way ANOVAs were used with site year and treatment as the independent variables and seasonal mean individual taxa per plant as the dependent variable.. Likewise, a two-way ANOVA was used in comparing sunflower yields (log transformed) between treatments across site years. Subsequent one-way ANOVAs were used to compare sunflower yields between treatments at individual locations.. Significance level $\alpha = 0.05$ was set for all statistical tests.

Results

Impact of Seed Treatment on the Arthropod Community. The complete foliar and soil community uncovered in sunflowers of this region is presented in Bredeson and Lundgren (2015). The thiamethoxam

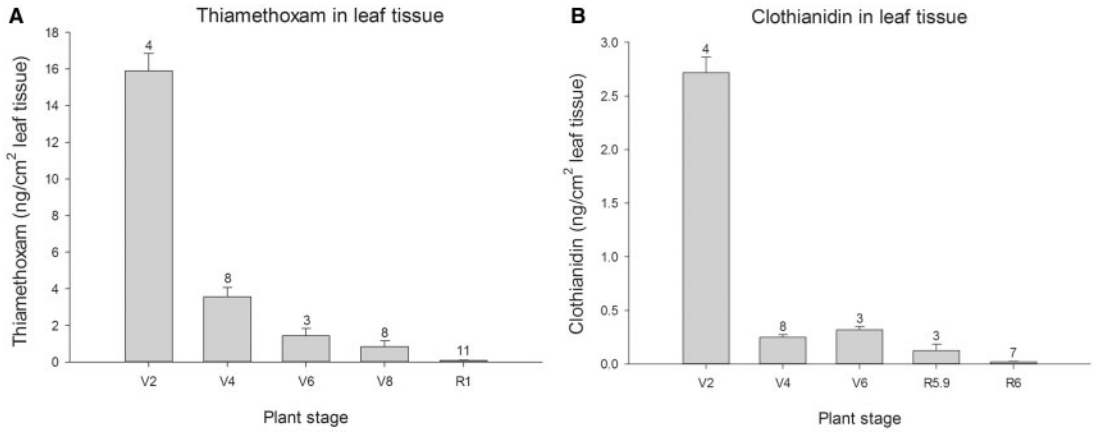

Fig. 1. Quantity of thiamethoxam and clothianidin found in sunflower leaf tissue over the plant's development in eastern South Dakota. Values represent mean ± SEM concentrations per plant found in the newest true sunflower leaf (>4 cm in length). Sample sizes for each treatment and stage are indicated above the error bars.

Table 1. The effects of thiamethoxam seed treatments on major pests of and total herbivores in sunflowers in eastern South Dakota

Pest species	Untreated fields	Treated fields	Statistics
Coleoptera			
Curculionidae			
Smicronyx fulvus (LeConte)	0.14 ± 0.05	0.12 ± 0.04	$F_{1, 16} = 0.09$, $P = 0.77$
Smicronyx sordidus (LeConte)	0.03 ± 0.02	0.03 ± 0.01	$F_{1, 16} = 0.04$, $P = 0.84$
Diptera			
Cecidomyiidae			
Contarinia schulzi (Gagné)	0.01 ± 0.00	0.02 ± 0.01	$F_{1, 16} = 2.72$, $P = 0.12$
Tephritidae			
Gymnocarena diffusa (Snow)	0.03 ± 0.01	0.03 ± 0.01	$F_{1, 16} = 0.19$, $P = 0.67$
Neotephritis finalis (Loew)	0.03 ± 0.01	0.03 ± 0.01	$F_{1, 16} = 0.47$, $P = 0.50$
Hemiptera			
Miridae			
Lygus lineolaris (Palisot de Beauvois) (both adults and nymphs)	0.48 ± 0.09	0.36 ± 0.08	$F_{1, 16} = 1.72$, $P = 0.21$
Lepidoptera			
Pyralidae			
Homeosoma electellum (Hulst)	0.03 ± 0.02	0.03 ± 0.02	$F_{1, 16} = 0.12$, $P = 0.73$
Tortricidae			
Cochylis hospes (Walsingham)	0.01 ± 0.01	0.01 ± 0.00	$F_{1, 16} = 0.50$, $P = 0.49$
Total foliar herbivores	7.52 ± 1.13	4.31 ± 0.26	$F_{1, 4} = 2.80$, $P = 0.17$

The focal community presented here is from the sunflower foliage and flowers, and values represent mean ± SEM per plant ($n = 11$ per treatment).

seed treatment did not reduce the overall herbivore population found on sunflower foliage in comparison to untreated sunflowers ($F_{1, 4} = 2.80$, $P = 0.17$; Table 1). Key foliar pests of sunflowers in the Northern Great Plains (Knodel et al. 2010) commonly found on plants (>0.01 per plant), were unaffected by the neonicotinoid seed treatment (Table 1). Likewise, herbivores found beneath the soil surface were also unaffected by the insecticide ($F_{1, 4} = 0.69$, $P = 0.45$) and were found at 92.49 ± 13.41 and 72.42 ± 20.28 per m^2 in untreated and treated fields, respectively. Elaterids were found in 6 of the 22 fields, and were equally abundant in each treatment. During sunflower reproductive development at the Dakota Lakes location, adult *Lygus* sp. reached their economic threshold (>0.11 adult insects per sunflower inflorescence; Knodel et al. 2010) in both treatments (untreated: 0.24 ± 0.09, treated: 0.38 ± 0.09). No other insect pests of concern reached their economic threshold across the three site years.

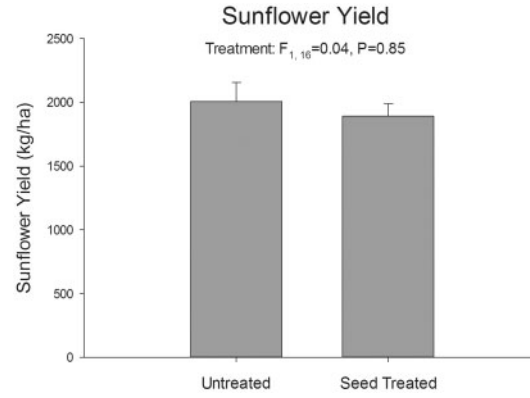

Fig. 2. Sunflower yields in fields treated with a thiamethoxam seed treatment or not in eastern South Dakota. Values represent mean ± SEM kg/ha over three site years ($n = 11$ per treatment).

Insecticide Persistence. *Thiamethoxam.* Thiamethoxam was at the highest level in sunflower leaf tissue, as expected, early in the season. At the V2 plant stage the concentration of the toxin per cm^2 of the young tissue was 15.89 ± 0.95 ng. By the next stage tested, V4, the concentration of thiamethoxam was 3.56 ± 0.52 ng/cm^2 leaf tissue. The toxin level gradually fell until the R1 plant stage where the lowest concentration was recorded (0.07 ± 0.04 ng thiamethoxam/cm^2 leaf tissue) before the insecticide became undetectable (Fig. 1A). No thiamethoxam was detected in untreated sunflower tissue.

Clothianidin. The ELISAs performed for quantifying thiamethoxam's metabolite, clothianidin, showed positive occurrences throughout sunflower anthesis (R6). At V2, the earliest life stage tested, 2.72 ± 0.15 ng clothianidin/cm^2 leaf tissue was observed. By V4, the next life stage examined, clothianidin had fallen to 0.25 ± 0.02 ng/cm^2 leaf tissue, and remained at low but detectable concentrations throughout flowering (R6; Fig. 1B). Clothianidin was not present in any untreated leaf tissue samples.

Sunflower Yield

Across site years there was a slight yield reduction in fields that were treated with the insecticidal seed treatment compared to untreated fields, but the difference was not significant (treated yield: 1,891.71 ± 95.22 kg / ha, untreated yield: 2,007.04 ± 147.02 kg / ha, $F_{1, 16} = 0.04$, $P = 0.85$; Fig. 2). There was a marginally significant difference between treatments at Dakota Lakes ($F_{1, 6} = 5.59$, $P = 0.056$) where untreated sunflower fields yielded higher than treated fields (yield: 2,950.77 ± 140.21 kg / ha, and 2,468.10 ± 181.04 kg / ha, respectively), but no differences in yield were observed at the Brookings 2013 or 2014 locations (Brookings 2013: untreated: 1,386.21 ± 154.44 kg / ha,

Table 2. Effects of thiamethoxam seed treatments on sunflower yields in eastern South Dakota

Site year	Untreated fields	Treated fields	Statistics
Brookings 2013	1,386.21 ± 154.44	1,495.32 ± 176.33	$F_{1,\ 4} = 0.04$, $P = 0.86$
Dakota Lakes 2014	2,950.77 ± 140.21	2,468.10 ± 181.04	$F_{1,\ 6} = 5.59$, $P = 0.056$
Brookings 2014	1,528.94 ± 110.60	1,612.60 ± 69.58	$F_{1,\ 6} = 0.24$, $P = 0.64$
Avg.	2,007.04 ± 147.02	1,891.71 ± 95.22	$F_{1,\ 16} = 0.04$, $P = 0.85$

Values presented represent mean ± SEM yield (kg / ha) from sunflower fields ($n = 11$ per treatment) conducted at three site years.

treated: 1,495.32 ± 176.33 kg / ha, stats: $F_{1,\ 4} = 0.04$, $P = 0.86$. Brookings 2014: untreated: 1,528.94 ± 110.60 kg / ha, treated: 1,612.60 ± 69.58 kg / ha, stats: $F_{1,\ 6} = 0.24$, $P = 0.64$; Table 2).

Discussion

The use of systemic insecticides is aimed at protecting plants from herbivory. In this study, however, neither the total foliar nor subterranean herbivores differed significantly between treated and untreated fields. Likewise, none of the major pests of sunflowers in the northern Great Plains, characterized by Knodel et al. (2010), were adversely affected (Table 1). It is conceivable that neonicotinoids may provide protection from pest populations that exceed those observed in our experiments, but the low abundance of key sunflower pests in our study despite our targeting areas with historical sunflower production is noteworthy. Over the three site years in this study, only one pest of concern, adult *Lygus* sp., reached its economic threshold at the Dakota Lakes location in both treated and untreated fields. Although this was the only location with an economically threatening level of pest pressure, yield at Dakota Lakes was greatest of the three replications. Pest populations vary annually in sunflower agroecosystems and do not always reach economic levels. Variance in pest populations can be a result of weather conditions (Barton and Ives 2014), cultural management practices such as planting date (Rogers and Jones 1979), insect migration patterns (Ciss et al. 2014), and other contributing factors. Biological control may also have helped to prevent pest outbreaks here. These findings question the need of neonicotinoid seed dressings as a pest management tool in sunflower production, which violates a fundamental tenet of IPM programs: that the pest should be at economically threatening level to warrant treatment (Stern et al. 1959). Farmers planting insecticide-treated seeds prophylactically guarantees inputs of neonicotinoids into the environment without clear economic benefits of this technology in this system (Goulson 2013). Successful integrated pest management (IPM) programs reduce the risks associated with pesticides and nontarget organisms, and promote natural enemy population health for biological control of herbivores (Landis et al. 2000).

Thiamethoxam was undetectable in leaf tissues after the R1 growth stage, but its metabolite clothianidin remained detectable in the plants through flowering (Fig. 1A and B). Beneficial predators and pollinators visiting sunflowers throughout anthesis would thus be exposed to a plant with low levels of toxins (at least in through the leaves). This also indicates that floral pests would be exposed to the low concentrations of clothianidin, although we saw no adverse effects on pest populations measured in this study. The presence of neonicotinoids in pollen or floral nectar was not examined in this study; however, the translaminar capabilities of these insecticides suggest that their transport to these tissues is possible (Schmuck et al. 2001, Laurent and Rathahao 2003) and should be examined further.

Other investigators have observed that neonicotinoid metabolites, in addition to applied active ingredients, can cause detrimental effects to insects (Kamel 2010), and through changes in chemical properties, can persist longer, and be transported more efficiently in aqueous solutions (Cloyd and Bethke 2011). Depending on the parent insecticide molecule, breakdown products can be of higher toxicity to arthropods than the initially applied insecticide (Benzidane et al. 2010). For example, Iwasa et al. (2004) observed that clothianidin displayed higher contact toxicity to honey bees in field trials compared to thiamethoxam. Similarly, Suchail et al. (2001) performed oral feeding trials by which honey bees were fed either imidacloprid or an imidacloprid metabolite via sugar–water solutions. They reported that an olefin metabolite was more toxic to bees than the parent compound. Treating sunflower seeds with systemic pesticides that persist throughout flowering, and that show toxicity to nontarget organisms either through the active ingredient or metabolites, is a feasible route of exposure to many beneficial insects (Gontijo et al. 2014, Moscardini et al. 2015).

Neonicotinoid seed treatments have been reported to improve yield and stand count in certain cases. For example, Magalhaes et al. (2009) observed a significant yield reduction due to aphid (*Aphis glycines*) feeding in soybean fields lacking a neonicotinoid seed treatment in comparison to fields with treatment. Wilde et al. (2004) reported that corn fields without an insecticidal seed treatment had significantly reduced stand counts, but yields did not differ between treated and untreated fields. The failure of the neonicotinoid seed-treatment to significantly improve yield in any of the site years of this study (Fig. 2) raises the question as to whether or not sunflower producers receive economic benefits through their use. Numerous studies exist where yield increases were not observed from prophylactic use of neonicotinoids in cropland (Environmental Protection Agency [EPA] 2014). For example, in a study determining the nontarget effects of imidacloprid and thiamethoxam seed-treated soybeans on natural enemies,

Seagraves and Lundgren (2012) observed no differences in yield, oil, or protein content between treated and untreated fields. Similarly, yield benefits have been absent, or have not outweighed neonicotinoid seed treatment costs, in oilseed rape (Goulson 2013), winter wheat (Royer et al. 2005), and maize (Wilde et al. 2004) production systems. The use of neonicotinoid seed treatments by producers is expensive, at an estimated $30.00–37.00 (USD) per ha (Seagraves and Lundgren 2012). For the costs of chemical control to not be recovered by yield improvement, as was the case in this study, farmers should perhaps explore other pest management tactics that are more economically rewarding, and which limit the total volume of pesticide entering the environment annually.

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