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Exposure of native bees foraging in an agricultural landscape to current-use pesticides

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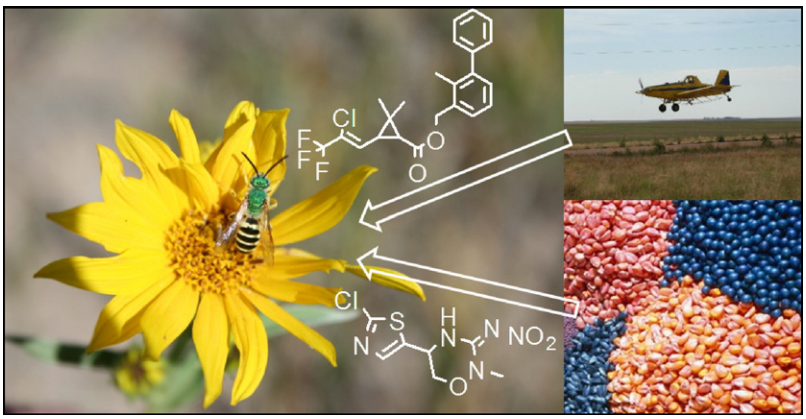
HIGHLIGHTS

- 19 current-use pesticides and degradates were detected in native bees.
- Neonicotinoid insecticides were some of the most frequently detected pesticides.
- Detected other insecticides (pyrethroid, organophosphate), fungicides and herbicides
- Surrounding land cover influences pesticide detections.

GRAPHICAL ABSTRACT



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ABSTRACT

The awareness of insects as pollinators and indicators of environmental quality has grown in recent years, partially in response to declines in honey bee (*Apis mellifera*) populations. While most pesticide research has focused on honey bees, there has been less work on native bee populations. To determine the exposure of native bees to pesticides, bees were collected from an existing research area in northeastern Colorado in both grasslands (2013–2014) and wheat fields (2014). Traps were deployed bi-monthly during the summer at each land cover type and all bees, regardless of species, were composited as whole samples and analyzed for 136 current-use pesticides and degradates. This reconnaissance approach provides a sampling of all species and represents overall pesticide exposure (internal and external). Nineteen pesticides and degradates were detected in 54 composite samples collected. Compounds detected in >2% of the samples included: insecticides thiamethoxam (46%), bifenthrin (28%), clothianidin (24%), chlorpyrifos (17%), imidacloprid (13%), fipronil desulfinyl (7%; degradate); fungicides azoxystrobin (17%), pyraclostrobin (11%), fluxapyroxad (9%), and propiconazole (9%); herbicides atrazine (19%) and metolachlor (9%). Concentrations ranged from 1 to 310 ng/g for individual pesticides. Pesticides were detected in samples collected from both grasslands and wheat fields; the location of the sample and the surrounding land cover at the 1000 m radius influenced the pesticides detected but because of a small number of temporally comparable samples, correlations between pesticide concentration and land cover were not significant. The results show native bees collected in an agricultural landscape are exposed to multiple pesticides, these results can direct future research on routes/timing of pesticide exposure and the design of future conservation efforts for pollinators.
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1. Introduction

Pollinator services provided by commercial honey bees (*Apis mellifera*) and native bees are essential for modern agricultural practices. Today approximately 75% of crop species worldwide benefit from insect pollination (Klein et al., 2013) but farmers typically rely on the honey bee to provide these services worth approximately $200 billion to food production (Gallai et al., 2009; USDA, 2015a). However, due to loss in abundance and diversity of habitat (i.e., flowering plants) and exposure to pesticides and parasites (e.g., varroa mites [*Varroa destructor*] in honey bees), bee populations are on the decline (Goulson et al., 2015). Native bees have the potential to provide pollination services as a number of biotic and abiotic factors have decreased healthy honey bee colonies worldwide (Dainat et al., 2012). However, to provide these services, an abundance of florally diverse areas within flight distance is necessary for sources of pollination and nectar (Garibaldi et al., 2011; Kremen et al., 2002). How the contribution of native bees to pollination is influenced by land management practices is not well understood and continued modification of the landscape (agricultural and natural) can have negative effects on the benefits provided by these species.

Native pollinators foraging in grasslands and crop fields provide ecosystem services at a local scale, but it is unclear how the widespread use of pesticides may affect native bees as they move across the broader agricultural landscape. Studies have shown impacts to honey bees from exposure to pesticides, including neonicotinoid insecticides and certain classes of fungicides, but the effects of these compounds on native pollinators at the field scale are largely unknown. Neonicotinoids are the most widely used class of insecticides worldwide (Jeschke et al., 2011), and their use is increasing as seed treatments become more prevalent (Douglas and Tooker, 2015). Environmentally relevant concentrations of neonicotinoids have been reported to cause a variety of effects to native bees including reduction in population densities and reproduction, impairment of foraging success and development, and increased susceptibility to disease and parasites (Lundin et al., 2015; Rundlof et al., 2015; Sandrock et al., 2013; van der Sluijis et al., 2013). The agricultural use of fungicides has increased dramatically over the past decade to control fungal outbreaks (USGS, 2015). Fungicide use both as seed treatment and foliar application throughout the growing season increase the chance of potential exposure to pollinators. Although fungicides are not considered acutely toxic to honey bees, a recent study observed an increased probability of parasitic fungal infection in bees that consumed pollen with high fungicide loads (Pettis et al., 2013). Fungicide exposure, could in turn, reduce the biodiversity and richness of native pollinators and the ecosystem services they provide.

Larger assemblages of grasslands within agriculturally dominated landscapes contribute permeability through the surrounding matrix (Cane, 2001), providing refuge for native pollinators (Park et al., 2015) and acting as a source of healthy bee populations. The Food, Conservation and Energy Act of 2008 introduced language recognizing the importance of pollinators and allowed for measures to address targeting the conservation of pollinator habitat. US Geological Survey scientists have been monitoring native pollinator habitat, diversity, and richness in the Conservation Reserve Program (CRP) fields in eastern Colorado to evaluate the extent to which CRP grasslands provide floral food sources for native pollinators in large-scale agroecosystems (NPWRC, 2015). Management strategies to benefit pollinators include planting strips or fields with pollinator-friendly plants or hedgerows in and around crop fields to improve floral diversity and nutritional options for pollinators (Hannon and Sisk, 2009). These efforts, as well as grassed corners of center pivot irrigation fields, roadsides, and fallow fields may also provide refuge and ideal nesting substrate for native bees. In agricultural landscapes dominated by row crops not meeting the nutritional demands of bees as well as monoculture grasslands lacking floral diversity, there may be a cost-distance tradeoff for the bees where they incur greater chemical exposure as they seek floral resources outside their habitat. Ongoing research has focused on the value grasslands provide for native bees, but little has been done on broader landscape comparisons involving intensively farmed landscapes interspersed with grasslands. Native bees are limited in maximum foraging distance (typically <1000 m) (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010) and frequently have spatially separated nesting and foraging habitats. Access to suitable nesting and habitat resources necessitates flight between the two, often across a fragmented landscape (Cane, 2001) that includes grassland and cropland.

The objective of this study was to understand which current-use pesticides native bees are exposed to within their foraging range in an agriculturally dominated landscape in northeastern Colorado, USA. It is hypothesized that native bees collected from areas with a greater percentage of surrounding cropland will be exposed to more pesticides than those residing in areas with a higher percentage of grassland. Determining the exposure of native bees to pesticides is the first step in understanding the benefits of conservation efforts on the landscape to increase pollinator habitat in areas of intense crop production and how these efforts may or may not influence pesticide exposure.

2. Experimental

2.1. Site information and field collection

Native bees were collected from fields in Logan County in northeastern Colorado, USA (Fig. 1). The exact locations of the grasslands and wheat fields are proprietary and written permission was obtained from the landowners prior to the start of sampling. Fields were located in the transitional zone between the western Great Plains and the central high tableland regions. Precipitation occurs as high-intensity rainfall from spring through early autumn (average 455 mm) but fluctuates widely across the region. Between 93% and 97% of the land in this region is privately owned cropland and grassland. Dryland winter wheat is the primary crop and typically grown in a wheat-fallow rotation. Native bees were collected in four grassland sites in 2013 and 2014 (sites Grasses 1–4). In 2014, native bees were also collected from an additional six sites located in wheat fields (sites Wheat 0–5). Springstar™ blue vane bee traps were deployed bi-monthly from May to September in all fields from each land cover type. Traps were set at a fixed location at each site from morning until early afternoon (0800–1300) and were collected the following day (0800–1300), for a total of 24 h per trap. Each vane trap was attached to a conduit pipe and moved to the appropriate height level of the nearest vegetation (Stephen and Rao, 2005). Trapped bees were collected in individual labeled bags and put on ice for transport back from the field. In the lab, bees were separated and grouped by body size. Native bee abundance had an average ($\pm$standard deviation) of 22 $\pm$ 3 genera per field and were similar between 2013 and 2014 (22 $\pm$ 2 and 22 $\pm$ 4, respectively) while wheat fields had an average of 18 $\pm$ 2 genera per field. About half of the traps deployed (48%) had enough bees collected during each trapping for pesticide analysis (see Table SI-3). Bees were stored frozen at −20 °C and held for no longer than 9 months prior to extraction. Field locations were mapped in geographic information systems (GIS) using the USDA CropScape-Cropland Data Layer (USDA, 2015b), the buffer radius for each sampling location was set based on known foraging distances of captured native bees (<200 m, <500 m, <1000 m); the 1000 m radius was selected for final interpretation of the data.

2.2. Sample extraction

For each individual sample (total of 54 samples) approximately 10 bees were composited (actual numbers per composite ranged from 4 to 15 in 2013 and 6 to 10 in 2014). Species of bees were not identified for this portion of the study and all bees were composited as whole samples to include residues on external as well as internal parts of the bees

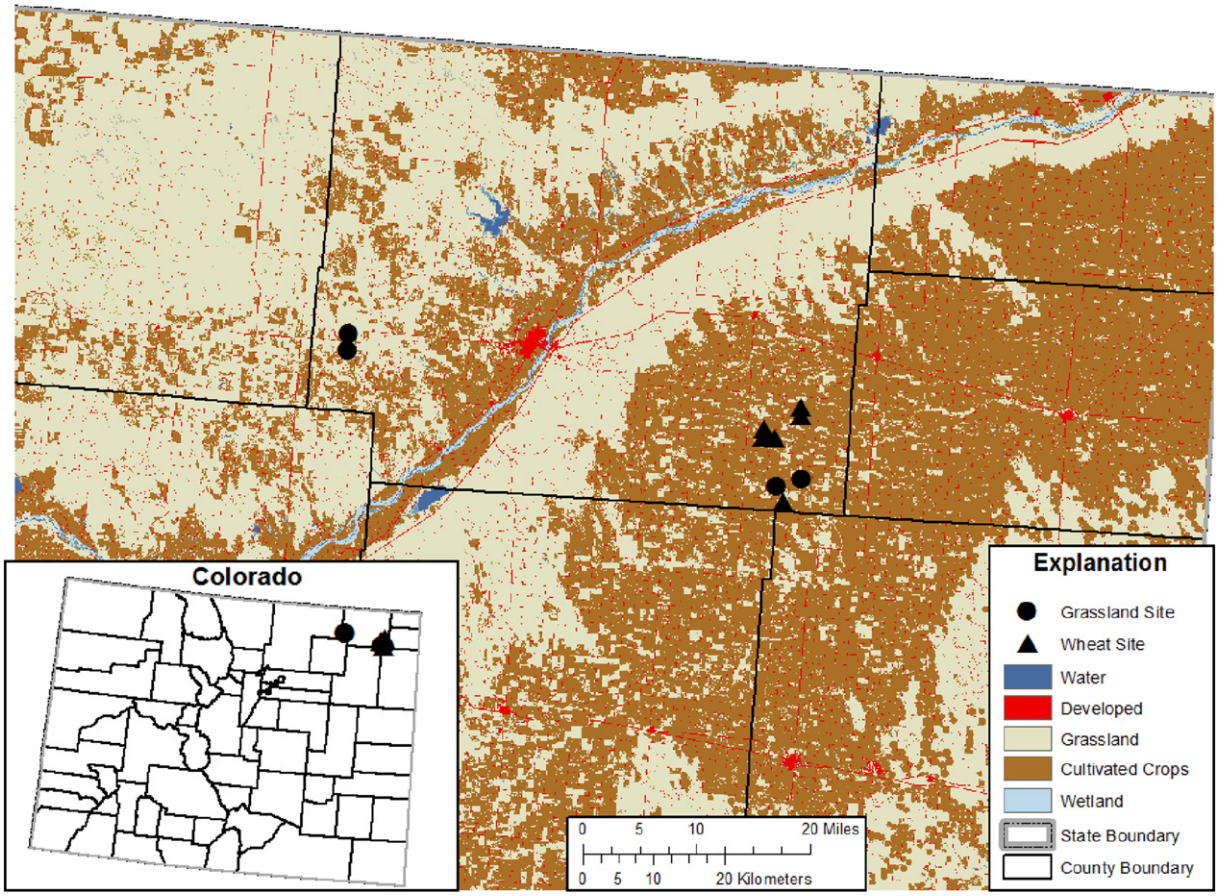

Fig. 1. Location of sites in Logan County, Colorado, USA where native bees were collected from grasslands and wheat fields.

(any noticeable pollen was physically removed before extraction). This reconnaissance approach is intended to represent a field-scale assessment of overall pesticide exposure of native bees. Composite mass ranged from 0.083 to 1.9 g with an average mean (±standard deviation) of 0.69 (±0.32) g. The bees were homogenized with sodium sulfate (Na_2SO_4) using a clean, solvent-rinsed mortar and pestle. Samples were spiked with $^{13}C_{12}$-*p,p′*-DDE, d_4-imidacloprid, $^{13}C_6$-*cis* permethrin, and d_{10}-trifluralin (Cambridge Isotope, Cambridge MA) as recovery surrogates and extracted 3 times with 50:50 acetone: dichloromethane (DCM) using a Dionex 200 accelerated solvent extractor (ASE) at 1500 psi and 100 °C. Following extraction, sample extracts were dried over Na_2SO_4 and reduced to 1 mL. Co-extracted matrix interferences were removed with C18 (Bondesil C18 40 μm; Agilent Technologies, Santa Clara, CA) and Z-sep+ (Sigma-Aldrich, St. Louis, MO) sorbents; samples were loaded onto 1 g C18 solid-phase extraction (SPE) cartridges and eluted with 10 mL of DCM, evaporated to 1 mL and then loaded onto 0.5 g Z-sep+ SPE cartridges and eluted with 10 mL 25:75 methanol:acetone. Samples were again reduced to 1 mL and split into a gas chromatograph (GC) and liquid chromatograph (LC) fraction; the GC fraction was exchanged into ethyl acetate (final volume 100 μL; d_{10}-acenaphthene as internal standard) and the LC fraction was exchanged into acetonitrile (final volume 100 μL; $^{13}C_3$-caffeine as internal standard).

Samples were analyzed for a total of 136 pesticides and pesticide degradates (Table SI-1) using either GC triple quadrupole mass spectrometry (MS/MS) or LC–MS/MS. Extracts were analyzed for 126 pesticides and pesticide degradates on an Agilent 7890 GC coupled to an Agilent 7000 MS/MS operating in electron ionization (EI) mode. Data for all pesticides were collected in multiple reaction monitoring (MRM) mode with each compound having one quantifier MRM and at least one qualifier MRM; further details on the instrument parameters have been previously published (Hladik and McWayne, 2012), the MRM transitions are listed in Table SI-2. Extracts were also analyzed for 10 pesticides and pesticide degradates on an Agilent 1260 bio-inert LC coupled to an Agilent 6430 MS/MS with each compound having a quantifier MRM and one or two qualifier MRMs. Further details on the instrument analysis have been previously published for the LC–MS/MS (Hladik and Calhoun, 2012). The limits of detection (LOD), defined as the value greater than three times the signal-to-noise ratio, were 1 ng/g.

2.3. Quality control

Performance-based quality assurance and quality control included the parallel analysis of procedural blanks, matrix spikes, and replicates. Procedural blanks consisting of 5 g of baked Na_2SO_4 run with every batch of 18 samples did not contain detectable concentrations of pesticides. Mean (±standard deviation) recoveries of $^{13}C_{12}$-*p,p′*-DDE, d_4-imidacloprid, $^{13}C_6$-*cis* permethrin, and d_{10}-trifluralin were 96 ± 13%, 81 ± 11%, 102 ± 14%, and 103 ± 18% respectively. Three matrix spikes (composite of 10 bees at a site that already had an environmental sample) were analyzed and the recovery ranged from 70% to 129% with a mean of 96% (±15%). Replicates (a separate 10 bee composite collected at the same location and date) were collected for 12 samples. Pesticides were detected in both sample and replicate 73% of the time; relative percent differences of pesticide concentrations for the replicate samples (non-detects calculated as 1/2 the LOD) ranged from 9 to 197% with a median of 109% (±64%).

3. Results and discussion

3.1. Pesticide occurrence in native bee tissue

The number of samples analyzed per site and the timing of collection was dependent on the number of bees that were captured per sampling event; of the 112 trap deployments only 54 (48%) resulted in enough bees for pesticide analysis. In the late summer/early fall there were more bees at the study sites so most residue samples were collected between late July and early September (Table SI-3). From our limited data it seems that fewer bees visit the wheat fields right before harvesting (end of June) limiting comparisons to grasslands at this time.

In the 54 composite samples, there were 18 pesticides and 1 pesticide degradate detected (nine of the pesticides were detected in both 2013 and 2014; Table SI-4). Twelve compounds were detected in >2% of the samples including the insecticides thiamethoxam (46%), bifenthrin (28%), clothianidin (24%), chlorpyrifos (17%), imidacloprid (13%), fipronil desulfinyl (7%; degradate); the fungicides azoxystrobin (17%), pyraclostrobin (11%), fluxapyroxad (9%), and propiconazole (9%); and the herbicides atrazine (19%) and metolachlor (9%) (Fig. 2).

Thiamethoxam was the most frequently detected compound during the study and was also observed at the highest maximum concentration (310 ng/g) compared to the other pesticides detected (Table 1). The other two neonicotinoids, clothianidin and imidacloprid, were also detected in native bees but less frequently (Fig. 2) and at slightly lower concentrations (maximum concentrations of 87 and 57 ng/g, respectively; Fig. 3). Other insecticides including bifenthrin and permethrin (both pyrethroids), chlorpyrifos (organophosphate) and fipronil (phenylpyrazole insecticide and its degradate fipronil desulfinyl) were also detected (Fig. 2). Bifenthrin was detected in both 2013 and 2014 and was the second most frequently detected pesticide at concentrations generally lower than the neonicotinoids (maximum 19 ng/g; Fig. 3). A variety of insecticides have been documented in honey bees (Mullin et al., 2010), clothianidin was detected in bees from the Midwest, USA (Krupke et al., 2012) and clothianidin, imidacloprid, and thiamethoxam were all reported in bee tissues from various areas in Greece where honey bee deaths had been reported (Kasiotis et al., 2014). In the latter two studies, clothianidin was

Table 1
Pesticides detected in native bee tissue, maximum and average concentration of detections above the limit of detection (LOD); ± standard deviation for locations with >2 detections. Concentrations are in ng/g.

Pesticide	Type[a]	Concentrations (ng/g)					
		2013 grasslands (n[b] = 21)		2014 grasslands (n = 14)		2014 wheat fields (n = 20)	
		Max	Avg (± stdev)	Max	Avg (± stdev)	Max	Avg (± stdev)
Atrazine	H	99	NC[d]	23	9.7 ± 8.9	11	6.8 ± 3.8
Azoxystrobin	F	25	NC	6.6	3.2 ± 3.0	9.1	4.8 ± 2.9
Bifenthrin	I	15	12	18	12 ± 5.5	19	8.9 ± 4.7
Chlorpyrifos	I	ND[c]	ND	55	30 ± 18	26	17 ± 8.2
Clothianidin	I	60	40 ± 28	57	21 ± 21	87	25 ± 35
Difenconazole	F	25	NC	ND	ND	ND	ND
Fenbuconazole	F	ND	ND	1.5	NC	ND	ND
Fipronil	I	3.1	NC	ND	ND	ND	ND
Fipronil desulfinyl	D	4.9	2.8 ± 1.6	ND	ND	ND	ND
Fluxapyroxad	F	10	7.2 ± 4.3	ND	ND	2.6	2.1
Hexazinone	H	ND	ND	27	NC	ND	ND
Imidacloprid	I	82	57 ± 32	1.1	NC	15	6.5 ± 5.7
Metolachlor	H	ND	ND	13	13	11	6.4 ± 3.8
Permethrin	I	ND	ND	ND	ND	120	NC
Propiconazole	F	3.1	NC	3.0	NC	7.0	5.3 ± 2.4
Pyraclostrobin	F	42	NC	37	NC	81	38 ± 35
Tebuconazole	F	3.9	NC	ND	ND	ND	ND
Thiamethoxam	I	56	31 ± 24	310	100 (± 120)	120	26 ± 35
Trifloxystrobin	F	14	NC	ND	ND	ND	ND

[a] I = insecticide; F = fungicide; H = herbicide; D = degradate.
[b] n = number of samples.
[c] ND = not detected; <LOD of 1 ng/g.
[d] NC = not calculated.

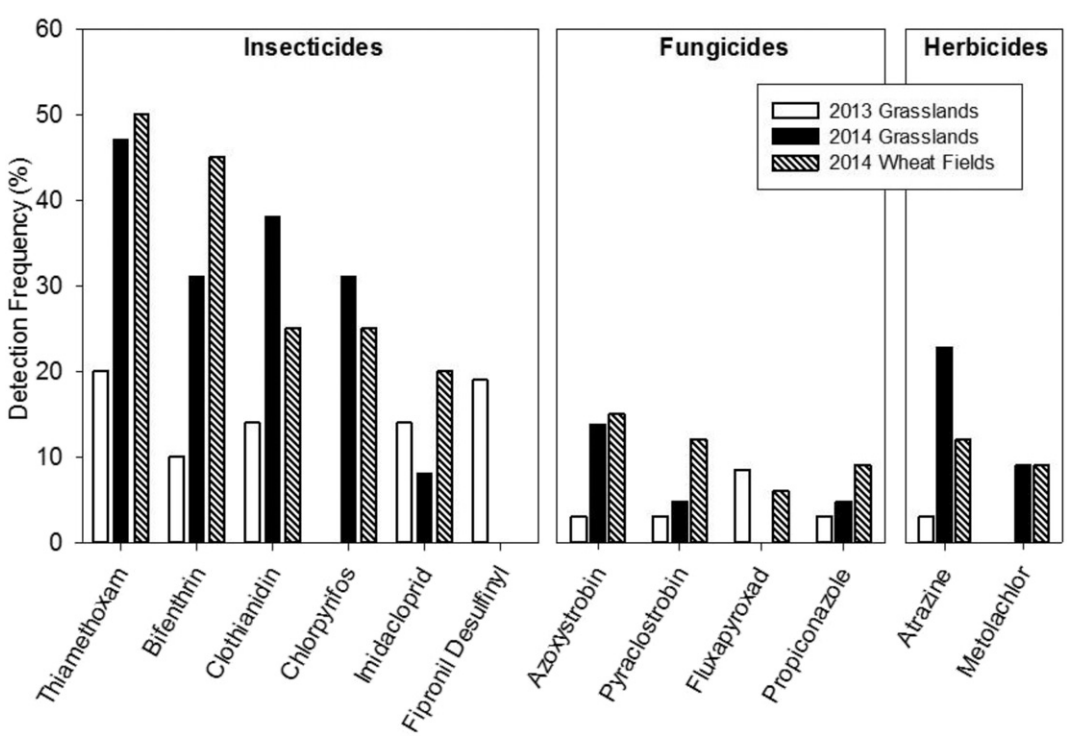

Fig. 2. Detection frequencies of insecticides, fungicides and herbicides in native bee samples collected from grasslands in 2013 (n = 21) and 2014 (n = 14) and from wheat fields in 2014 (n = 20). Compounds detected in only one sample (<2%) are: fipronil and permethrin (insecticides); difenconazole, fenbuconazole, tebuconazole and trifloxystrobin (fungicides); and, hexazinone (herbicide).

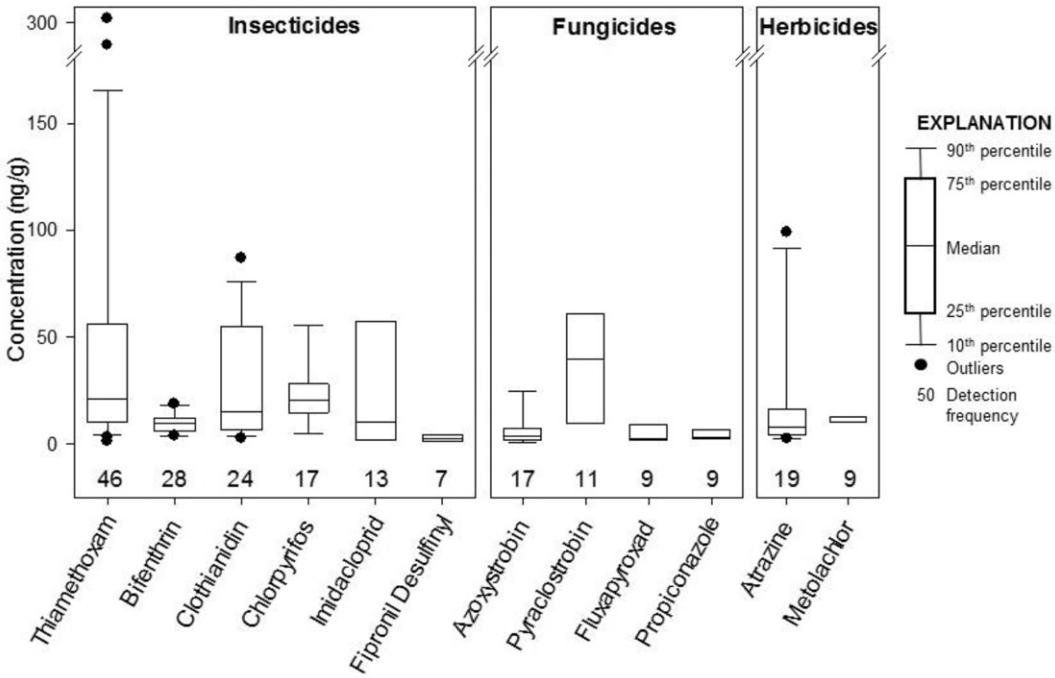

Fig. 3. Concentrations of the most frequently detected insecticides, fungicides and herbicides in native bee tissue (total of 54 samples). Compounds detected in only one sample (concentration in ng/g): fipronil (3.1), permethrin (120), difenconazole (25), fenbuconazole (1.5), tebuconazole (3.9) and trifloxystrobin (14).

detected more frequently in residue samples compared to the other neonicotinoids. Imidacloprid has also been reported in honey bee body residues but at low concentrations compared to other insecticides (Bacandritsos et al., 2010; Chauzat et al., 2011).

Four fungicides (azoxystrobin, fluxapyroxad, propiconazole and pyraclostrobin) were detected in more than one native bee sample collected from the study area (Figs. 2 and 3) with maximum concentrations ranging from 2.6 to 81 ng/g (Table 1). Two herbicides, atrazine and metolachlor, were also detected in multiple samples (Fig. 2), maximum concentrations were 99 and 13 ng/g, respectively (Table 1). Similar to insecticides, several of these fungicides and herbicides have been reported in honey bee samples (Krupke et al., 2012; Mullin et al., 2010), although less frequently than insecticides. To our knowledge this is the first study to document the exposure of native bees to insecticides, fungicides and herbicides.

Estimates of amounts of agricultural pesticides applied (2008–2012) in Logan County, Colorado were summarized for each pesticide detected during 2013 and 2014 (Table 2; Baker and Stone, 2013). Pesticide use changes with variations in pest pressures (e.g., chlorpyrifos total kg fluctuates from year to year) or the introduction of newly registered products in response to new pests, resistance, or technological advancements (e.g., fluxapyroxad was introduced in 2012; USEPA, 2015a). Of the 18 pesticides detected, 17 (94%) have recorded applications in the area in the past; however, data for the years of the study are not currently available. In the study area, herbicides are applied in larger amounts than either insecticides or fungicides (Table 2) but the insecticides and fungicides were detected more frequently (relative to use) in the native bee tissue. Similar results have been documented in honey bees (Mullin et al., 2010) as well as aquatic species such as fish (Smalling et al., 2013) and frogs (Smalling et al., 2015) residing in an agricultural landscape. Understanding the relationship between use patterns and pesticide accumulation in native bees requires more information on the types of species, their foraging distance, detailed pesticide use in the surrounding fields, and metabolism rates of pesticides for each bee species, which was beyond the scope of the current study.

Multiple pesticides were frequently detected in the composite native bee samples. At least one pesticide was detected in 70% of the 54 composite samples, two or more pesticides were detected in 48% of samples. The maximum number of pesticides detected in one sample was nine (Grass-2; Table SI-4). Insecticides were the most frequently detected type of pesticide (at least one insecticide was found in 63% of the samples) while fungicides and herbicides were found in 33 and 19% of the samples, respectively. Insecticides also frequently occurred with herbicides and fungicides; 90% of herbicide and 89% of fungicide detections co-occurred with at least one insecticide. Individual pesticide concentrations in composite bee samples ranged from the LOD of 1.0 to 310 ng/g (Table SI-4). In samples collected from all fields and years, neonicotinoids were generally detected the most frequently and at some of the highest concentrations (Table 1).

The frequency of pesticides detected in composite bee samples varied between site type (grasslands versus wheat fields) and year. For many of the compounds observed in the grasslands, detection frequencies were higher in 2014 compared to 2013 (Fig. 2). However, the

Table 2
Estimated county level pesticide use (E-pest high) data (kg) for 2008–2012 (Baker and Stone, 2013) for the pesticides detected in bee tissue in the current study.

Pesticide	Type[a]	Year				
		2008	2009	2010	2011	2012
Atrazine	H	14,000	28,000	14,000	20,000	21,000
Azoxystrobin	F	130	230	240	280	290
Bifenthrin	I	55	55	76	1601	730
Chlorpyrifos	I	770	3900	2600	1900	8300
Clothianidin	I	480	350	510	900	840
Difenconazole	F	1.7	16	49	51	100
Fipronil	I	35	22	55	NA	NA
Fluxapyroxad	F	NA	NA	NA	NA	NA
Hexazinone	H	400	320	220	640	570
Imidacloprid	I	14	89	83	54	110
Metolachlor	H	6000	7000	2900	4900	22,000
Permethrin	I	NA	6.9	2.8	0.3	250
Propiconazole	F	130	62	210	400	96
Pyraclostrobin	F	320	110	560	350	400
Tebuconazole	F	350	1100	30	142	45
Thiamethoxam	I	33	484	300	94	320
Trifloxystrobin	F	44	500	320	590	1500

NA = not available.
[a] I = insecticide; F = fungicide; H = herbicide.

insecticides imidacloprid, fipronil desulfinyl, and the fungicide fluxapyroxad were detected more frequently in 2013 compared to 2014 (Fig. 2). Yearly differences in the grasslands could be related to variability in nearby cropping patterns (fallow versus active acreage), the type and amounts pesticides applied, the species collected, or the timing of collection. For the 2014 samples, pesticide concentrations were similar in the bees caught in grasslands compared to those caught in wheat fields (within one standard deviation; Table 1). Detection frequencies in the wheat fields were similar to the grasslands in 2014 (Fig. 2). Both field types, grassland and wheat field, exist in a greater agroecosystem where pesticide exposure may be influenced by the greater land cover around the sampling location.

3.2. Land cover differences

Although variation in pesticide concentrations were observed throughout the year, there were not enough samples for statistical analysis on a within year temporal basis for the different fields. For discussion of yearly (2013 versus 2014 grasslands) and field type differences (grassland versus wheat fields) the August/September samples were used as these are the dates with the most samples collected (Table SI-3). A 1000 m radius around each trap was selected to maximize the foraging distance for native bees collected (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010) and to incorporate the greatest landscape diversity around the traps.

For the four grassland fields the amount of grassland ranged from 39 to 92% at the 1000 m radius (Table SI-5). The amount of wheat surrounding these fields ranged from 0 to 40% and other crops (corn, millet, sorghum and sunflower) ranged from 6 to 51%. The amount of pesticides detected in the grasslands varied more from field to field in 2013 than 2014 (Fig. 4). In 2013 three sites had maximum detections <15 ng/g while the fourth site had a maximum concentration of 300 ng/g; in 2014 the maximum concentrations ranged from 35 to 130 ng/g. The maximum total pesticide concentrations detected in August/September for each year at each field were compared to the surrounding land-cover to determine if the percent agriculture or percent grasslands affected native been exposure. There were no statistically significant differences ($P > 0.1$ using a Spearman rank correlation; $n = 8$) with maximum pesticide concentrations in native bees and the amount grass, wheat, or other crops (Fig. 4).

Agricultural fields in northeastern Colorado offer the opportunity to compare pesticide residues in native bees collected directly from wheat fields with those from grasslands, the latter of which are considered a more natural or enhanced habitat for pollinators. The wheat fields, similar to the grasslands, had varying land cover at the 1000 m radius. The amount of wheat ranged from 14 to 55% (53–55% at 3 of the 6 fields), other crops (corn, millet, sorghum and sunflower) ranged from 1 to 33% and the percent grassland ranged from 1 to 34%. The maximum pesticide concentrations for the August/September samples from the wheat fields were less variable than the grasslands and ranged from 45 to 230 ng/g (Fig. 5). There were no statistically significant differences ($P > 0.1$; using a Spearman rank correlation; $n = 6$) between maximum pesticide concentrations and the amount of grass, wheat or other crops surrounding the wheat fields (Fig. 5). Although qualitative, native bee

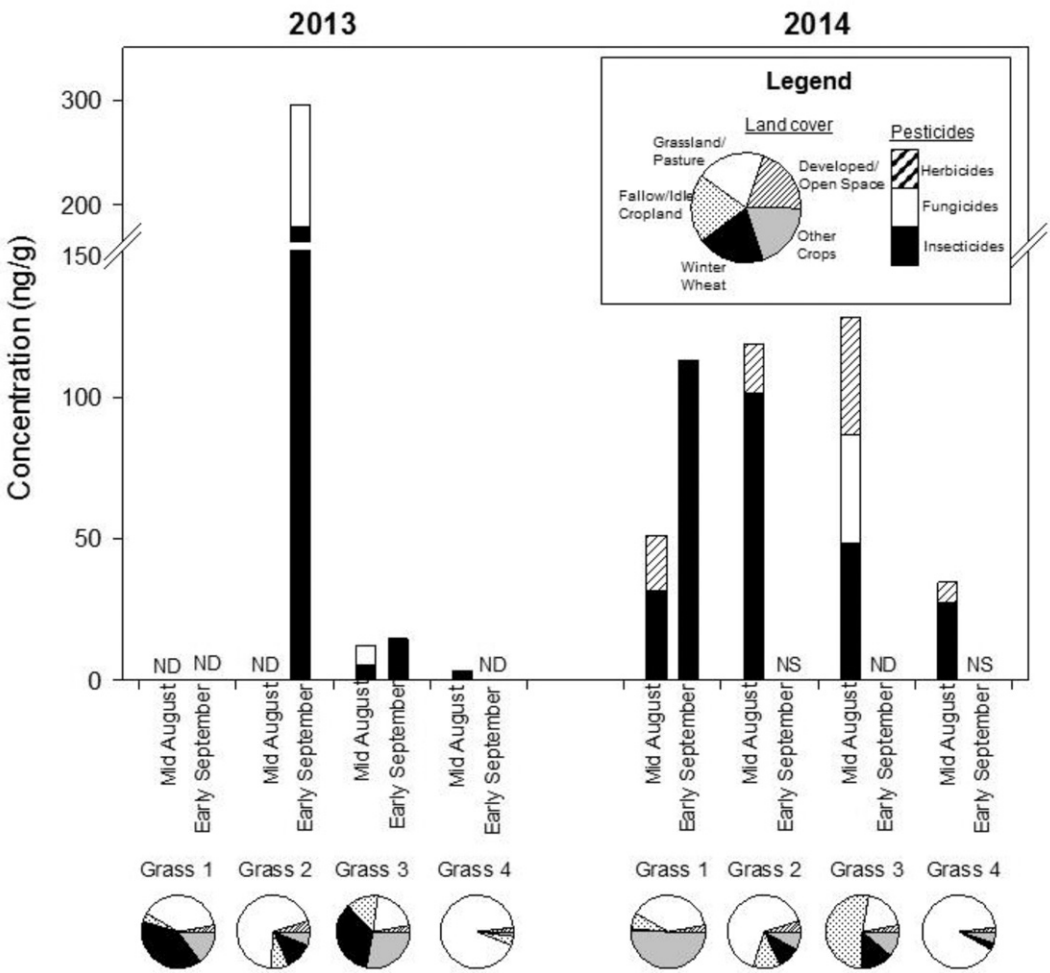

Fig. 4. Concentrations of pesticides detected in native bee tissue for mid-August and early September 2013 and 2014 samples from four different grasslands. The pie charts are the 1000 m land cover radius around the sample collection point. NS = no sample collected and ND = no pesticides detected.

body burdens tended to be higher in fields with a greater percentage of other crops (corn, sorghum and millet) compared to those dominated by wheat. Cropping patterns also have the potential to affect exposure of pesticides to native bees; however, a more robust dataset is needed to further elucidate these impacts on the landscape.

Grasslands, especially those planted with pollinator friendly plants, may provide refuge and a more natural habitat in a landscape dominated by agriculture. Garibaldi et al. (2011) suggested that in order to increase pollinator services from native bees, natural habitats within agricultural areas should be preserved. In the US, the USDA has identified and implemented certain conservation practices that are designed to provide better habitat for pollinators (USDA, 2015c). Natural areas provide increased nesting and foraging opportunities for native bees (Tscharntke et al., 2012) and as the percentage of natural landscape increases so does the abundance and richness of native bees in the agricultural landscape (Park et al., 2015; Watson et al., 2011). Although, grasslands do provide refuge and resources for native bees, the surrounding regional landscape (1000 m) influences pesticide residues in the bees themselves and could affect diversity and abundance. Based on the reconnaissance data presented in the current-study, native bees residing and foraging in a mixed agricultural landscape (less than 89% grassland) have an increased likelihood of exposure to pesticides, indicating position of the natural habitat on the landscape could be important. But to date there are few studies on the exposure of native bees to pesticides and their subsequent effects on native bee abundance in these fields (Winfree, 2010). To better understand the potential impacts of the agricultural landscape on native bees, more detailed studies are needed to assess the effects of temporal and spatial alterations in the landscape and how these influence the uptake of pesticides and the effects on native bee richness and diversity. The relationship between land cover and native bee abundance has been documented in natural landscapes (Morandin et al., 2007) and in comparing organic versus traditional farming practices (Holzschuh et al., 2007; Kremen et al., 2002); however, limited information is available on the level of pesticide residues in native bees in mixed land cover settings and how that impacts pollination services. This reconnaissance study is the first step in understanding the exposure of native bee populations to pesticides in relation to the surrounding landscape.

3.3. Potential effects of pesticides on native bees

Concentrations detected in native bee tissue were also compared against 48 h LD_{50}s for the honey bee (*A. mellifera*) (USEPA, 2015b). Our analytical methods do not allow us to differentiate what fraction of the pesticide is on the surface of bees (contact toxicity) versus inside the organism (oral); therefore, contact LD_{50}s were used as they were available for more of the compounds detected and are considered a primary route of exposure. When a range of contact LD_{50}s was given for a single compound the lowest (most conservative) concentration was used (Table SI-4). To compare residues in the composite sample in terms of toxicity an estimated ng/bee concentration was calculated to facilitate direct comparison to published LD_{50} values. A summation approach was used for all compounds detected in native bee samples. Although not all pesticides operate via the same mode of action, and

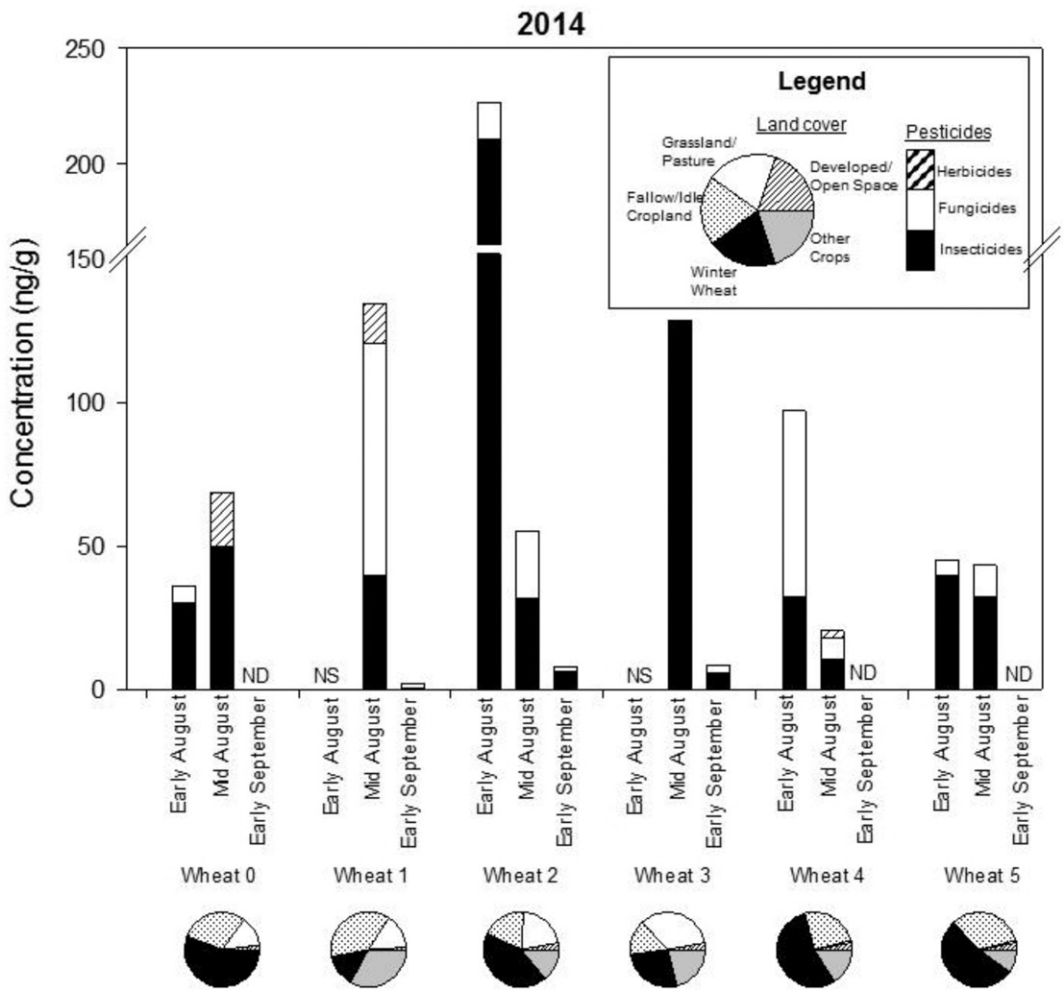

Fig. 5. Concentrations of pesticides detected in native bee tissue for early August, mid-August and early September 2014 samples from six different wheat fields. The pie charts are the 1000 m land cover radius around the sample collection point. NS = no sample collected and ND = no pesticides detected.

toxicity may not be additive, this approach was a simple preliminary step in assessing potential acute toxicity of the mixture of pesticides observed in native bees. In the current study, pesticide concentrations were converted to a toxic unit (TU: defined as the pesticide concentration divided by the LD_{50}, a TU of $1 = LD_{50}$) and summed; the TU values ranged from 0.03 to 0.93 (average 0.14 ± 0.20). All of the ΣTUs were <1, indicating that in the native bees collected none of the concentrations detected were above the cumulative LD_{50} for all compounds. It is also important to note that only bees with sub-lethal concentrations of pesticides were available to sample because lethal concentrations would, by definition, remove those bees from the pool of available samples. Based on a lethality approach, fungicides and herbicides are considered relatively less toxic to bees than insecticides but they could have unknown additive or synergistic effects. Although this TU approach allows the current concentration data in native bees to be put into an ecological effects framework, actual LD_{50} values for the compounds detected in the current study are unknown for native bee species. Furthermore, documented variability in sensitivities to neonicotinoids exist between bee species and one study noted that alfalfa leafcutter bees (*Megachile rotundata*) and blue orchard bees (*Osmia lingnaria*) are more susceptible to neonicotinoid insecticides (clothianidin and imidacloprid) than honey bees (Scott-Dupree et al., 2009).

In addition to lethality, pesticides can have sub-lethal effects. Thiamethoxam has the potential to decrease the foraging success and survival of honey bees (Henry et al., 2012). Recent studies with thiamethoxam and clothianidin on non-Apis bees have documented a reduction in total number of offspring and skewed sex ratios in solitary bees (Sandrock et al., 2013) as well a reduction in wild bee density, solitary bee nesting, and bumblebee colony growth (Rundlof et al., 2015). These studies indicate that sub-lethal effects of neonicotinoids on non-Apis bees are typically expressed in a fitness related context and information from honey bee exposures may not translate directly to native bees. Much of the research to date on the sub-lethal effects of pesticides on bees has focused primarily on neonicotinoids (Blacquiere et al., 2012) and limited attention has been paid to fungicides because they are considered less lethal (Table SI-4) than insecticides. However, exposure to fungicides increased a bee's susceptibility to the pathogen, *Nosema* (Pettis et al., 2013), as well as increasing its sensitivity to acaricides, subsequently reducing the lethal doses (Johnson et al., 2013). Pettis et al. (2013) noted that bees consuming pollen containing pyraclostrobin were three times more likely to become infected after *Nosema* exposure than bees that were not exposed to this chemical. In a controlled experiment the fungicide, chlorothalonil, negatively affected bumble bee (*Bombus impatiens*) colony success through an overall reduction in bee biomass indicating the potential to impact native bee success particularly in agricultural settings (Bernauer et al., 2015). To understand the long-term impacts pesticides have on native pollinators and the services they provide, more studies are needed to design tools and models focused on these more complex sub-lethal effects.

3.4. Summary

This study documented the exposure of native bees collected from an agricultural landscape to pesticides, including multiple insecticides, fungicides, and herbicides. Pesticides were detected in bees from all sites sampled in both 2013 and 2014 and these detections tended to vary with surrounding land cover for bees collected both within grasslands and wheat fields. The neonicotinoid insecticide, thiamethoxam, was the most frequently detected pesticide during the study and was observed in bee tissues throughout the sampling period (May to October). The types of pesticides detected varied with land cover at the sample location and the land cover diversity within a 1000 m radius. No significant differences were observed in this limited dataset between pesticide residues and land cover; additional samples are needed to determine the relative influence of cropping patterns and available grasslands on pesticide exposure. This provides preliminary information on native bee exposure to pesticides and can direct more focused future research. While this study focuses on a specific region in the US, pesticide exposure to native bees is an issue of global concern because many landscapes are fragmented by agricultural activities. Conservation efforts implemented for pollinators are designed to increase pollinator habitat and biodiversity, however, understanding how positioning these efforts on the landscape relate to healthy bee populations is needed for guiding best management practices to optimize pollinator health and enhance sustainability in agroecosystems.

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Appendix A Supplementary data

Supporting information includes tables of analytes measured, multiple reaction monitoring ions for GC–MS/MS analytes, when bee samples were collected at each field, pesticide concentrations measured in each sample, and land cover at the 1000 m radius for each site. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.10.077.

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