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Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit

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Summary

1. Neonicotinoid insecticides are routinely used as seed treatments on most grain and oilseed crops in the USA, yet the extent and likelihood of spread of insecticide residues during planting has not previously been quantified.

2. Honey bees, *Apis mellifera*, are highly mobile and highly sensitive to neonicotinoid residues, presenting an opportunity to estimate non-target exposures to neonicotinoids in mobile insects. We measured neonicotinoid dust drift during maize sowing and used sites of maize fields, apiary locations and honey bee foraging radii to estimate likelihood of forager exposure. We performed a concurrent multi-year field assessment of the pest management benefits of neonicotinoid-treated maize.

3. Our results indicate that over 94% of honey bee foragers throughout the state of Indiana are at risk of exposure to varying levels of neonicotinoid insecticides, including lethal levels, during sowing of maize. We documented no benefit of the insecticidal seed treatments for crop yield during the study.

4. *Synthesis and applications*. We demonstrate movement of neonicotinoid residues well beyond planted fields occurs during maize sowing in Indiana. Based on locations of maize fields and apiaries in the state, the likelihood of neonicotinoid exposure for foraging honey bees is high. Other non-target organisms are also likely to encounter neonicotinoid residues; we conservatively estimate that deposition of neonicotinoid residues on non-target lands and waterways will occur on over 42% of the state of Indiana during the period of maize sowing. However, we also demonstrate that the risk to pollinators and other non-target organisms may be rapidly and dramatically reduced without yield penalties, by aligning use rates of neonicotinoid insecticides with pest incidence.

Key-words: crop yield benefit, honey bee, insecticides, maize, neonicotinoid, pest management, pollinator, risk

Introduction

The neonicotinoid insecticides include the most widely used insecticides in the world (Jeschke *et al.* 2011; Van der Sluijs *et al.* 2013). Neonicotinoids are most often applied as seed treatments to a variety of important oilseed and grain crops, with an estimated 60% of neonicotinoids used globally in this way (Jeschke *et al.* 2011; Simon-Delso *et al.* 2015). One often touted benefit of utilizing these seed treatments is the potential for reducing pesticide drift (Ahmed *et al.* 2001; Koch *et al.* 2005); however, as the use of seed treatments has grown, so too have concerns regarding the wider environmental impacts of this pesticide delivery strategy. Some key pitfalls associated with the use of neonicotinoid seed treatments include the movement of active ingredients into aquatic systems, unintended effects on managed and wild pollinator species (both acute and chronic toxicity), and the potential for contamination of untreated areas during seed sowing (Hladik, Kolpin & Kuivila 2014; Main *et al.* 2014; Pisa *et al.* 2015; Van der Sluijs *et al.* 2015). Contamination in non-target areas around fields during sowing of maize and oilseed rape have been documented in Italy, Slovenia, Germany, the United States, and Canada (Bortolotti *et al.*

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2009; Forster 2009; Pistorius *et al.* 2009; Van der Geest 2012; PMRA, 2013; Krupke & Long 2015; Xue *et al.* 2015). In these instances, honey bee mortality was the primary indicator of neonicotinoid drift. In addition to this direct impact upon non-target organisms, planter-emitted dust may also land upon vegetation surrounding crop fields (Krupke *et al.* 2012; Stewart *et al.* 2014), increasing the risk of secondary exposure for insects that live on or visit these areas, where risk is defined as a parameter that includes both exposure and toxicity (Solomon 2010).

The importance of neonicotinoid drift as an exposure route for non-target organisms remains the subject of debate, as the degree of risk has not been quantified and work to date has focused upon measuring residues on honey bees after exposure (Tapparo *et al.* 2012; Girolami *et al.* 2013). Estimates of the likelihood of non-crop lands being contaminated with dust that arises during planting represent a critical step in safeguarding pollinators and other non-target organisms. A recent publication that reported reduced queen egg-laying as a function of dietary exposure to the neonicotinoid imidacloprid recommended that risk mitigation efforts for pollinator exposure to neonicotinoids focus on early spring, while colony buildup is occurring (Wu-Smart & Spivak 2016); this is the period when neonicotinoid treated seeds are sown. Recently, the state of Minnesota, under a directive from the governor, implemented new guidelines for use of neonicotinoid treated seeds that include demonstration of need (State of Minnesota, Executive Department 2016). At the federal level in the USA, the Fish and Wildlife Service has discontinued the use of neonicotinoid insecticides on its properties, effective 1 January 2016 (US FWS, 2014). However, there is no guidance, in terms of the degree of risk to non-target lands, for the use of neonicotinoid treated seeds planted on the vast majority of arable land in the USA. Using honey bees as a model, we assess the degree of risk of exposure during the planting of neonicotinoid treated maize, while simultaneously assessing the benefits to crop protection and yield offered by this pest management approach. This empirical work can be used to offer guidance to help counterbalance exposure risk with the benefits offered by neonicotinoid treated seeds.

Materials and methods

During the maize planting periods of 2012 and 2013, we conducted experiments to quantify the risk posed to foraging honey bees during the planting of neonicotinoid treated seeds in 12 fields by measuring the concentration of neonicotinoid residues deposited in areas around fields during planting. Using these data, we constructed distribution curves of neonicotinoid insecticides for each individual maize field we sampled. We then used the deposition-distance relationships we observed to estimate and map pesticide deposition around all maize fields in Indiana using land use data from the USDA-NASS database (USDA-NASS, 2014). We used a publicly accessible online database to determine the locations of apiaries and individual beehives in the state of Indiana and used these data to assess the deposition risk to hives, and the honey bees foraging from them. This approach is summarized in Fig. 1. We also assessed the performance of neonicotinoid-treated maize seed in comparison with untreated maize seeds in replicated field trials at multiple locations over a 3-year period, 2012–2014.

MEASUREMENT OF PLANTING DUST DURING SOWING OF TREATED SEEDS

Information regarding field locations, seed treatment (insecticide and fungicide active ingredients), and hybrid were recorded for all planted seeds, as well as the model number of the tractor and planter used to sow seeds (Table S1, Supporting Information). A total of 12 fields were evaluated for drifting pesticide residues during the planting of neonicotinoid-treated maize during April and May of 2012 (Table S2) and 2013 (Table S3). Maize fields were 3–48 ha in size and were located in the state of Indiana, USA. All seeds used were commercial hybrids appropriate for the region. Prior to the planting of treated seed, dust collection stations (i.e. dosimeters) were placed along transects in each of the four cardinal directions (N, S, E, W) at distances of 0 m (field edge), 10, 50, and 100 m from the edge of the field. Therefore, each field was surrounded by a maximum of 16 dust collection stations which are composed of glass microscope slides covered oriented both horizontally and vertically to capture dust in both dimensions. These are described in detail in Appendix S1, and are similar to those described in a study by Xue *et al.* (2015). In some cases, dust collection stations were omitted or set at a different distance because roadways or waterways interfered with the placement of collectors. Additionally, because it is common agricultural practice in North America to add seed lubricants such as talc, graphite, or a mixture of both to seeds prior to planting, we evaluated the level of pesticide active ingredients in the 'used' seed lubricant remaining behind in the planter following seed sowing.

ANALYSIS OF DEPOSITION DATA

Analyses were carried out on horizontal and vertical dosimeter data separately. A modified QuEChERS protocol was used to quantify pesticide residues (Stoner and Eitzer 2016) and is described further in Appendix S1. Coordinates of all dosimeters were adjusted so that 0 radians was upwind, that is, transects were rotated so that 360° was directly into the wind. To test the influence of wind we used the adjusted coordinates and calculated a mean distance-weighted concentration using circular statistics (Fisher 1995) and plotted the points and mean distance-weighted concentration with package plotrix in R (Lemon 2006) (Fig. 2a,b). This mean concentration was a vector in the direction of the greatest concentrations, and was analogous to a centre of mass. With a significant influence of wind on the deposition of neonicotinoids, this vector would be long and point downwind. We tested the significance of the vector by creating a null distribution of vector magnitudes with 9999 randomizations of the concentrations. All analyses were carried out in R (R Core Team 2012).

To visualize the plume of neonicotinoids around a field we used kriging on the wind-adjusted coordinates to extrapolate a complete surface using package geoR in R (Ribeirojr & Diggle 2015). We assumed a standard 490 × 490 m field (c. 24 ha, or 60 acres—a representative field size in our study area), with adjusted

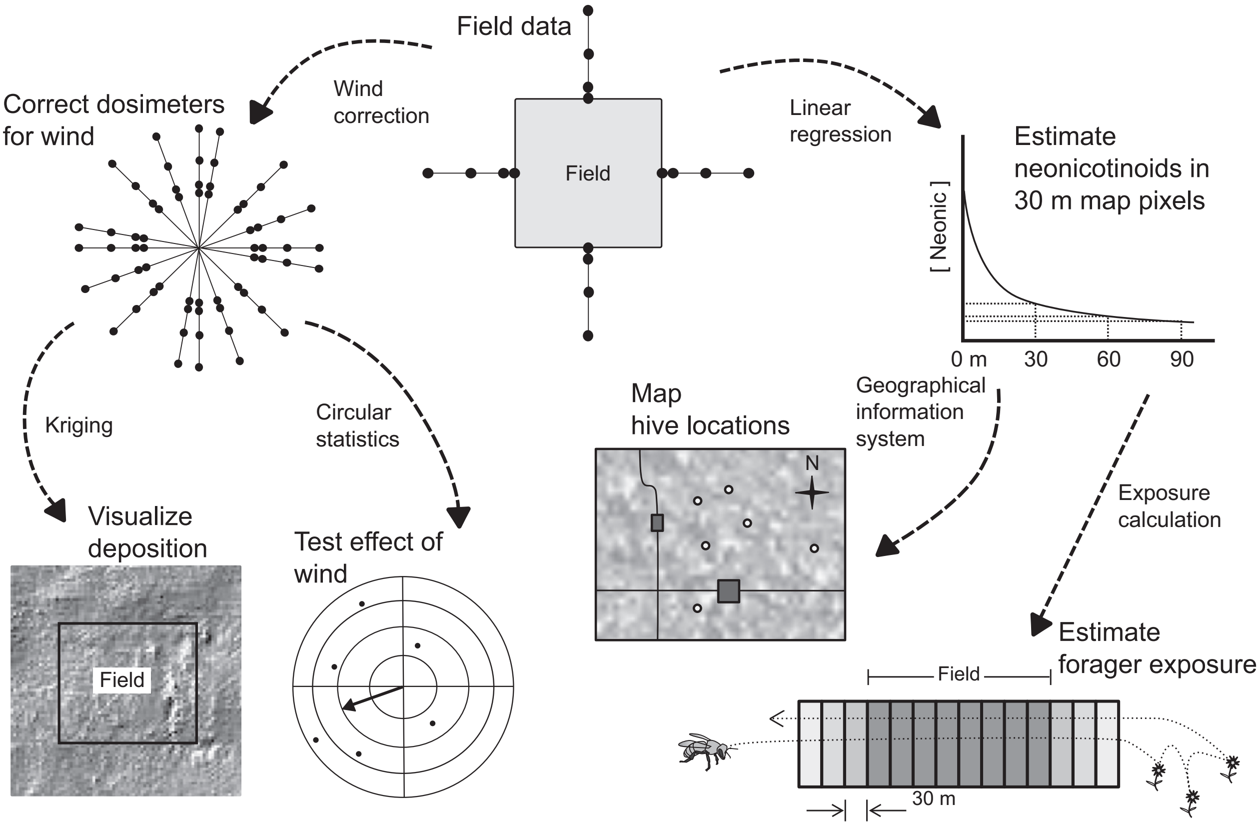

Fig. 1. Schematic summary of methods used to estimate planter dust deposition and assess potential exposure for foraging honey bees.

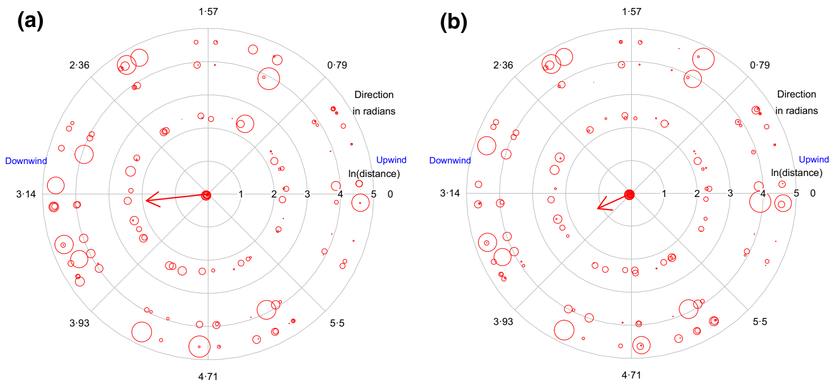

Fig. 2. Radial plot of deposition of neonicotinoid residues on (a) vertical slides and (b) horizontal slides. Distances from central point are ln(m). Vector originating at centre is the mean neonicotinoid displacement. Area within circles are proportional to deposition. [Colour figure can be viewed at wileyonlinelibrary.com]

latitude or longitude coordinates of ±245 m at the beginning of the transects (0 m sample). A spherical model was fit to the log transformed concentration data (Fig. 3a,b).

Neonicotinoid concentration data were natural log transformed to improve normality. A linear regression was fit to the transformed concentration data with the distance from the field as the independent variable. The predicted concentrations at 15, 45 and 75 m were calculated. We used the GRASS geographical information system (GIS) software (GIS; GRASS Development Team 2013) to assign these values to all 30 m pixels located 0–30, 30–60 and 60–90 m, respectively, from any maize field. Maize fields were identified in the 2012 U.S. Department of Agriculture National Agricultural Statistics Service Cropland Data Layer. This spatial dataset has high accuracy (>96%) for identifying maize cover. However, because misclassifications of non-maize area as maize could alter the subsequent analyses, we used a conservative approach and removed all contiguous cells classified as maize if their total contiguous area was 0·54 ha or less (≥6 cells). We then calculated the area of the state of Indiana that was planted in maize in 2012, or was within each of the three distance classes from maize, and therefore predicted to receive the neonicotinoid concentrations projected from the regression model. Cells classified as maize were assumed to contain the same concentration as that predicted for the 0 m from maize cells from the regression analysis.

To examine predicted levels of neonicotinoids at registered hive locations in Indiana, we manually extracted coordinates of hives registered on https://www.driftwatch.org, a website where beekeepers are able to register their hive locations to alert pesticide applicators. We matched these locations to the predicted neonicotinoid concentrations in the GIS map.

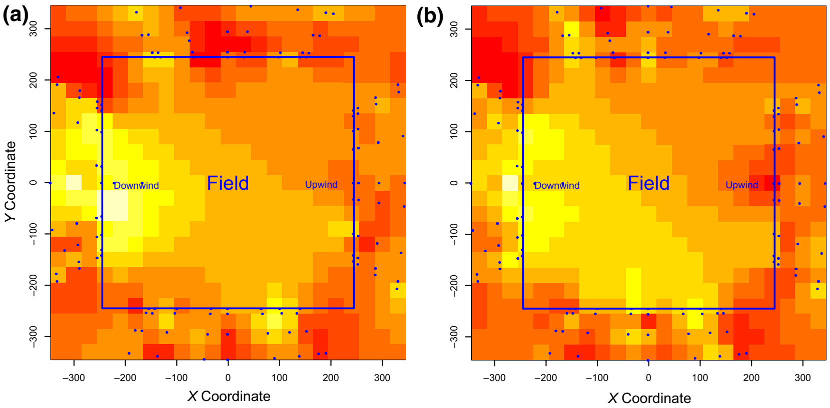

Fig. 3. Heat map of neonicotinoid deposition on (a) vertical collectors around study fields and (b) horizontal collectors (high: white, low: red). Estimated by kriging over values measured adjacent to 12 study fields. Distances are in metres. Locations of samples were first rotated so that the wind comes from the right-hand side. [Colour figure can be viewed at wileyonlinelibrary.com]

APIARY AND HONEY BEE FORAGER RISK

We calculated an LD_{50} for honey bees in our study area by weighting the contact LD_{50} values of clothianidin (21·8 ng per bee) (Iwasa *et al.* 2004) and thiamethoxam (29·9 ng per bee) (Iwasa *et al.* 2004) according to the global proportion detected in our samples. We used the apparent surface area (two-dimensional area viewed dorsally) of a honey bee of 1·05 cm^2 (Poquet *et al.* 2014) to convert our area-wide deposition data into a contact $LD_{50,}$ expressed as ng per bee. This allowed us to calculate the deposition necessary per square metre to achieve a lethal dose on a stationary, resting honey bee. This lethal concentration was compared to the deposition levels generated by our dataset to determine the likelihood that deposition on a stationary honey bee would be lethal. We did not include the wing surface area in this calculation because published LD_{50} values are derived from dorsal applications to the thorax of honey bees (Iwasa *et al.* 2004).

To estimate levels of exposure to foraging bees we divided each hypothetical foraging trip into two parts: an outbound and return flight across a single field and the surrounding zones of 0–30, 30–60, and 60–90 m from the field margin for a round trip, including foraging on flowers in the non-field area. During the flight across the field and adjacent zones we estimated the neonicotinoids encountered by dispersing the amount in the field and the surrounding zones per square metre throughout the entire planting time and varying potential heights of the dust cloud. Although estimates of foraging flights per day vary widely, and depend in part on whether foragers are seeking pollen vs. nectar, our estimate of 10 flights per day falls into the median range of daily pollen foraging flights summarized in the literature (Winston 1991). We used the planting times recorded at our 12 study fields to calculate the average time per ha to plant maize. This was used to calculate the planting time for our hypothetical 24·3 ha field as 12·6 h. The concentrations of pesticide that accumulated per square metre from the regression within the four zones (planted field, 0–30, 30–60, and 60–90 m) were dispersed through vertical columns of different heights. We do not know the vertical extent of dust that arises from planting operations so we based our calculations on dust clouds extending to 2 and 8 m above the ground. This range also encompasses the range of vertical heights of honey bee foraging flights (Riley *et al.* 2001).

We calculated a weighted deposition within each square metre across the field and surrounding three zones (i.e. 0–30, 30–60, and 60–90 m from field margin) by weighting the deposition according to the relative distance crossed by honey bees flying within each of the zones in a flight perpendicular to the field edge (i.e. across the field to forage on the other side). This resulted in a weighted deposition of 3188 ng m^{-2}. We then dispersed this amount through vertical heights of 2 and 8 m to represent a range of potential concentrations within the dust cloud. We estimated the flight time across the field using a flight speed of foraging *Apis mellifera* of 8·2 m s^{-1} (Heran & Lindauer 1963). The neonicotinoid concentrations encountered in each metre of flight were considered to be a product of the proportion of the 12·6 h of planting time that each 1 m of flight represented. The final estimate for the flight time portion of a day of foraging was the product of the proportion of planting time spent in 10 round trips across a field and surrounding zones, the volume of the air intersected by a forager, or 'flight tube', per square metre (details follow) the distance of 10 round trips, and the concentration of the neonicotinoid for a given dust cloud height.

To estimate the exposure during the flower-visiting portion of the foraging, we used low and high reported values for the numbers of dandelions visited by foraging honey bees. The lower published report for dandelions visited per forager trip is 8 flowers per trip (Ribbands 1949), with 100 flowers per foraging trip as the highest value reported in the literature (Vansell 1942). Dandelions are common in agricultural landscapes in Indiana, have flowering periods that overlap the planting season for maize, and are commonly used by honey bees (Ginsberg 1983). We assumed that the neonicotinoid residues deposited on these flowers would occur in proportion to the depositions calculated for respective areas beyond the planted field, as outlined above. We assumed that the surface area encountered would equal the cross-sectional area of the ventral surface of a honey bee, or 1·05 cm^2 (Poquet *et al.* 2014). We also varied the height of simulated forager flight for this calculation. Therefore, the 'high' estimate of daily exposure risk was calculated using a forager that flew 10 trips through a cloud of 2 m height and foraged on 100 flowers per trip. The 'low' estimate of daily exposure is calculated by a forager making 10 trips through an 8 m high cloud and foraging on 8 flowers per trip.

To obtain the estimate of the volume of air that a single bee flies through during a foraging flight, we measured the frontal aspect of 30 pinned specimens of worker honey bees across two dimensions (described in detail in Appendix S1).

EVALUATING PEST MANAGEMENT AND YIELD BENEFITS OF NEONICOTINOID-TREATED SEED

Location details, sowing and harvest dates for the crop protection and yield components of the study are shown in Tables S2 and S3. Details of plot planting procedures are described in Appendix S1.

Trial design was a randomized complete blocks with four replications. Treatments are as follows: untreated (naked) corn seeds, 0·25 mg clothianidin/kernel + fungicides, and 1·25 mg clothianidin/kernel + fungicides. Plots were 4 rows by 30·5 m, except for the Davis location which were 6 rows by 23 m. Plant populations (i.e. stand counts), root injury due to insect feeding and grain moisture and yield estimates were generated for each treatment – year. An analysis of variance was applied to all data, and means separation was by Fisher's LSD ($\alpha = 0{\cdot}05$). All tests were conducted using SAS version 9·3 (SAS Institute, Cary, NC). The amount of clothianidin applied to seeds used in this phase of the experiment was confirmed using analyses described in Appendix S1.

Results

ANALYSIS OF DEPOSITION DATA, PLANTING DUST AND FIELD SOILS

As expected, the circular statistical analysis revealed that the mean vector of highest neonicotinoid concentrations was downwind of the planter during seed sowing, and this was significant for both the horizontal ($P < 0{\cdot}001$) and the vertical dosimeters (randomization $P = 0{\cdot}002$). However, there was also considerable variation in the radial deposition of neonicotinoid residues around fields such that deposition of neonicotinoids was detected at some level in all directions in both the horizontal and vertical planes (Fig. 2a,b). This variation in radial deposition was also reflected in the kriged model of the neonicotinoid plume, which indicated that neonicotinoid levels were uniformly >1·4 μg m^{-2} in all directions, up to 100 m from the field edge, with a higher deposition near the downwind edge of the field (Fig. 3a,b). Our data were collected over a range of wind conditions between 0 and 13 km h^{-1} and are not generalizable to greater wind speeds. However, historical trends indicate that this range overlaps the majority of wind speeds encountered during maize planting in our study area. The mean daily wind speed in Tippecanoe County, Indiana during the period of sowing maize seeds (17 April–10 May) from 2003 to 2014 was 14 ± 6 km h^{-1} (data summarized from Indiana State Climate Office website for 40.297°N, 86.902°W, WGS84, http://iclimate.org/index.asp).

Although we detected a significant downwind signal for dust deposition, this explained very little of the variance in neonicotinoid deposition, so we modelled the significant negative relationship (d.f. = 163, $F_{1,163} = 14{\cdot}86$, $R^2 = 0{\cdot}078$, $P = 0{\cdot}0002$) between neonicotinoid-containing dust deposition (N) and distance from the field margin (d) for horizontal dosimeters without regard to wind direction. The results for vertical dosimeters were similar but less strong, so here we focus on the results from the horizontal dosimeters. The relationship was

$$N = e^{1{\cdot}68 - 0{\cdot}0905 \ln d} - 1 \qquad \text{eqn 1}$$

where N is the neonicotinoid concentration in ng per slide, and d is the distance from the field.

Our GIS analysis using eqn (1) leads us to estimate conservatively that 42·4% of the land area within the state of Indiana may be subjected to a pulse of neonicotinoid deposition at levels of ≥1·40 μg m^{-2} during sowing of treated maize. Using our empirical data, predicted deposition levels across the state can be further estimated given the amount of area at increasing distances from maize fields as follows: 25·3% of the state receives 3·81 μg m^{-2} of neonicotinoid residues in the form of planter dust, 6·6% receives 1·70 μg m^{-2}, 5·5% receives 1·49 μg m^{-2}, and 5·0% receives 1·40 μg m^{-2}. These areas correspond with 30 m × 30 m parcels of land that are 0–30, 30–60, and 60–90 m from maize field margins, respectively.

Field soils tested prior to planting in both 2012 and 2013 showed variable levels of the neonicotinoids clothianidin, thiamethoxam and imidacloprid, as well as several other common agricultural pesticides used in field crop production (Tables S4 and S6). Clothianidin, a common maize and soybean seed treatment and breakdown product of thiamethoxam (Simon-Delso *et al*. 2015), was the neonicotinoid detected most frequently prior to planting in 10 of the 12 fields used in our study.

Testing of residual seed lubricant from the planter after seed sowing was completed revealed that the neonicotinoids used to treat seeds were the compounds detected most frequently and at the highest concentrations (Tables S5 and S7). Clothianidin was again the most common neonicotinoid; present in every sample, and often at concentrations several orders of magnitude above the lethal exposure levels for honey bees.

APIARY AND HONEY BEE FORAGER RISK

Although honey bee foraging radii in excess of 10 km have been reported (Gary 1992), foraging most often occurs within 2 km of the colony (Osborne *et al*. 2001). To remain conservative with our analyses, we elected to incorporate an estimate of honey bee foraging at 1400 m. Of the 480 Indiana hive locations available at www.driftwatch.org in April 2014, 28% fell within the areas experiencing depositions of at least 1·40 μg m^{-2}. A foraging risk analysis revealed that only 5·9% of apiaries in the state exhibit a 1400 m honey bee foraging radius with no neonicotinoid exposure risk (i.e. 'safe' hive locations). Note that this analysis includes only a single crop, maize, and assumes no drift of neonicotinoid residues beyond 90 m from fields, due to the limitation of our 100 m transect lengths.

The neonicotinoids detected in the dosimeter samples consisted of 75·0% clothianidin and 25·0% thiamethoxam. We therefore calculated a weighted honey bee contact LD_{50} for these two chemicals of 23·8 ng per bee for our analysis (US EPA, 2003; Iwasa *et al*. 2004). This corresponds with a deposition of 227 μg m^{-2}. Direct deposition, via settling of pesticide-laden dusts, around fields is therefore two orders of magnitude below the contact LD_{50} for stationary honey bees (Fig. 4). The foraging analysis for bees that make 10 round-trip flights across a single average-sized field during planting to visit flowers are predicted to receive a dose of 2·27–28 ng of these neonicotinoid residues, depending on the vertical height of the pesticide cloud (8–2 m, respectively) and the number of flowers visited on each trip (8–100, respectively). Based on our data, the locations of maize fields throughout Indiana at the initiation of our study (USDA-NASS, 2014), and the locations of Indiana apiaries at the initiation of our study (obtained via www.driftwatch.org), we show the proportion of the Indiana landscape predicted to encounter deposition of planter dust up to 90 m from each planted field (Fig. 5).

EVALUATING PEST MANAGEMENT AND YIELD BENEFITS OF NEONICOTINOID-TREATED SEED

Levels of the neonicotinoid active ingredient, clothianidin, on maize seeds in our crop protection experiments aligned with the label rate of mg per seed at both the low (0·25 mg clothianidin per seed) and high (1·25 mg per seed) treatment levels (Table S8). We documented no benefit, in terms of crop yields, of planting neonicotinoid-treated maize over three cropping seasons, including three locations in 2012 and 2013 and two locations in 2014 (Table 1). Measurements were conducted throughout the growing season in each year and included early (plant stand count), mid (root damage ratings) and late season (yield) estimates.

Discussion

Although lethal exposures of honey bees during planting of treated annual crops have been documented in the past, our study is the first to use empirical data to document and describe exposure risk of non-target organisms to neonicotinoid seed treatments across the landscape (Carreck & Ratnieks 2014). Using honey bees as a model organism, our analyses demonstrate that the influence of planting neonicotinoid treated maize seeds is likely to be pervasive; our results indicate that the overwhelming majority of honey bee foragers in our study area are likely to come in contact with neonicotinoid residues from planter dust. The range of estimates that a forager could encounter, from 2·27 to 28 ng per bee, includes the lethal contact dose for clothianidin and thiamethoxam residues, both in terms of previously observed lethal exposures to planting dust residues (Krupke *et al.* 2012; Tapparo *et al.* 2012; Girolami *et al.* 2013) and laboratory assays with topical application of the active ingredients to honey bees (US EPA, 2003; Iwasa *et al.* 2004), indicating that our approach is representative of the range of field exposures that have resulted in mortality of honey bees. While the results of our field studies document the pervasive occurrence of neonicotinoid dust across the landscape during maize planting, our work presents a conservative analysis,

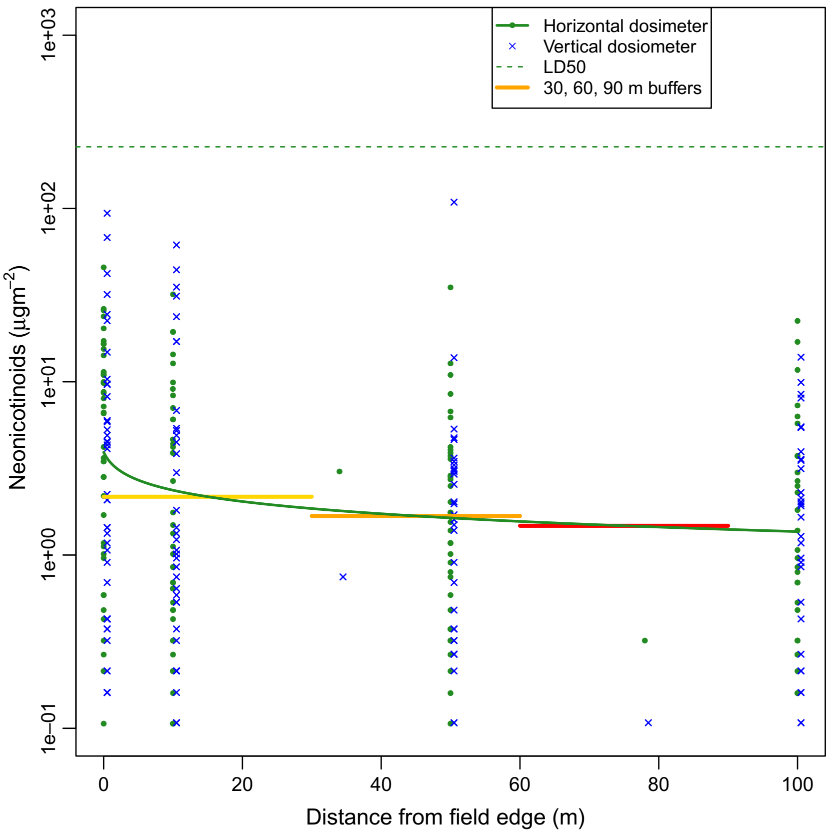

Fig. 4. Direct deposition of dust containing neonicotinoid residues at increasing distances from maize field margins, based on field collections using slides placed at 0, 10, 50 and 100 m from field margins. Neonicotinoid concentrations are shown as total μg m^{-2}. Estimate used for contact LD_{50} of neonicotinoid residues detected in planter dust is 23·9 ng per bee, based upon ratios of seed treatment compounds (thiamethoxam and clothianidin) detected in dust deposited on slides. [Colour figure can be viewed at wileyonlinelibrary.com]

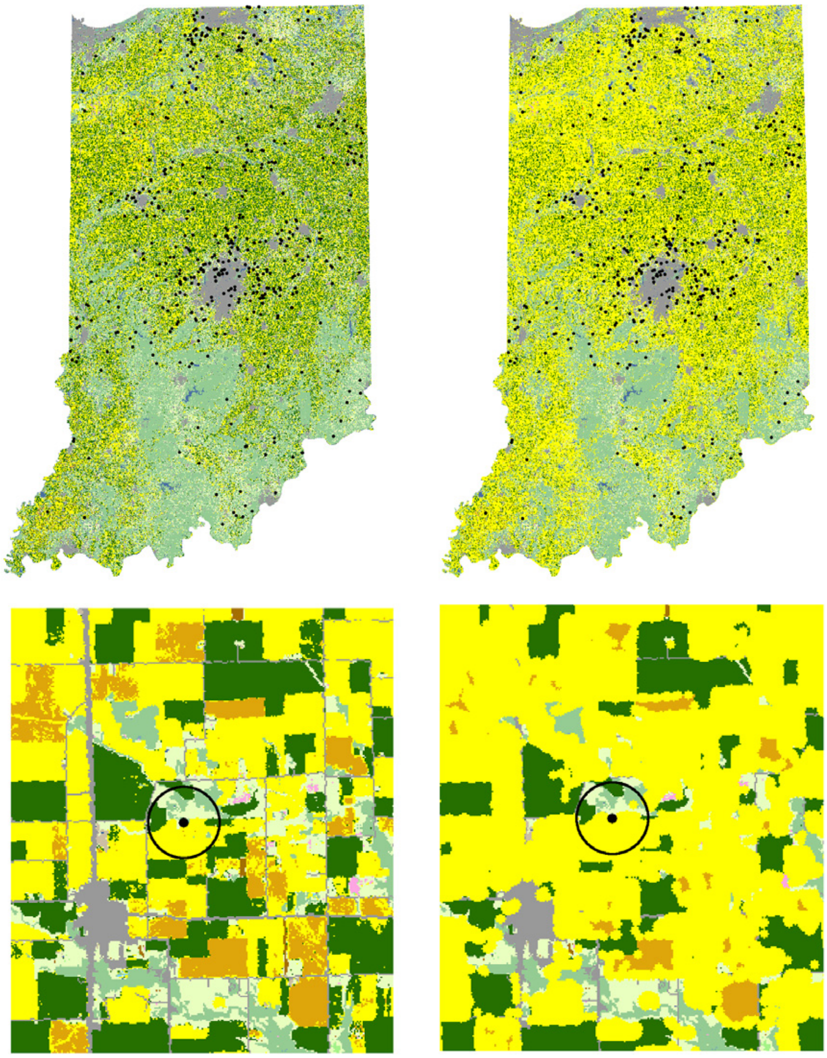

Fig. 5. Maps of Indiana (top) and a representative landscape (bottom) showing spatial extent of neonicotinoid deposition due to maize planting with no drift outside fields (left) and drift to 90 m (right). Black dots show known hive or apiary locations in Indiana, centred in 1400 m radius on landscape maps. Map colours follow standard NLCD data maps and yellow represents areas of deposition. [Colour figure can be viewed at wileyonlinelibrary.com]

and likely underestimates actual exposure for at least three key reasons. First, we consider only maize in our analysis. Other crops, most notably the next most abundant crop planted in both the region and nationally, soybeans, are frequently treated with neonicotinoid insecticides (Douglas & Tooker 2015). Second, we consider no dust drift or deposition beyond 90 m from each field margin. That is, while the distribution of our data demonstrate that deposition beyond 100 m is highly likely (Figs 2 and 3), we do not extrapolate beyond that point. Third, we do not consider static attraction of particles to the honey bee integument (Vaknin *et al.* 2000; Tapparo *et al.* 2012) and use only the forager surface area to calculate intersection between pesticide-laden dust and the bee integument.

Although the landscape that characterizes the production field itself during seed sowing is devoid of flowering plants and unattractive to foraging bees, the magnitude and frequency of crops treated with neonicotinoid insecticides in the form of a seed treatment (including maize, soybeans, wheat and oilseed rape) means that the 'risk zones' generated by planting activities will inevitably overlap with the flight paths of foraging bees (Fig. 5). This is particularly true during the spring bloom period of many important plant species (Ginsberg 1983), which is also when most annual crops are sown. While we have taken steps to remain conservative in our analysis, the results strongly suggest that the risk of exposure for honey bees is high in areas where neonicotinoid seeds are planted extensively. Although we use honey bee foraging radii to generate these risk estimates, the concentration data we generated here provide opportunities to calculate exposure parameters for a range of both mobile and sessile insect species. This may include native pollinators such as ground-nesting bees (Rundlof *et al.* 2015) and sensitive caterpillar species that occupy areas near agricultural fields, such as the monarch butterfly, *Danaus plexippus* (Pecenka & Lundgren 2015).

Three years of field experiments spread throughout the most intensive maize production region of Indiana failed to demonstrate a significant benefit of planting treated maize seeds, which parallels recent reports finding no, or inconsistent, benefits in oilseed rape in the EU (Budge *et al.* 2015), and US soybean production (Seagraves &

Table 1. Average stand counts, root ratings and crop yields in 2012–2014 for maize grown from seed receiving no insecticide or fungicide treatment, a low rate of clothianidin and fungicide treatment, or a high rate of clothianidin and fungicide treatment

Maize stand counts	Naked seed Plants per ha (SE)	0·25 mg clothianidin + fungicide Plants per ha (SE)	1·25 mg clothianidin + fungicide Plants per ha (SE)	*F*-test for significant treatment effects
Davis 2012	11 987·1 (503·7)	11 458·1 (380·8)	13 044·6 (259·5)	$F_{2,11}$ = 3·80, *P* = 0·09
Pinney 2012	12 075·1 (333·7)	11 281·8 (589·1)*	13 529·4 (340·4)*	$F_{2,11}$ = 5·00, ***P* = 0·05**
Throckmorton 2012	12 780·2 (50·9)	11 458·1 (374)	11 281·8 (804·6)	$F_{2,11}$ = 2·21, *P* = 0·19
Davis 2013	12 533·4 (202·3)	13 044·6 (149·6)	13 344·3 (243·2)	$F_{2,11}$ = 3·25, *P* = 0·11
Pinney 2013	11 458·1 (187)	10 929·3 (764·2)	12 317·5 (782)	$F_{2,11}$ = 1·61, *P* = 0·27
Throckmorton 2013	10 841·1 (139·4	11 259·8 (184·8)	11 193·7 (107·9)	$F_{2,11}$ = 1·77, *P* = 0·25
Pinney 2014	11 281·8 (160·9)	11 942·9 (220·3)	11 590·3 (84·4)	$F_{2,11}$ = 3·27, *P* = 0·11
Throckmorton 2014	11 546·3 (152·6)	11 898·8 (209·8)	11 546·3 (233·2)	$F_{2,11}$ = 0·86, *P* = 0·47
Maize root ratings	**Node injury rating (SE)**	**Node injury rating (SE)**	**Node injury rating (SE)**	
Davis 2012	0·109 (0·002)	0·115 (0·008)	0·101 (0·006)	$F_{2,11}$ = 1·54, *P* = 0·29
Pinney 2012	0·175 (0·013)	0·204 (0·02)	0·189 (0·03)	$F_{2,11}$ = 0·62, *P* = 0·57
Throckmorton 2012	0·12 (0·012)	0·092 (0·006)	0·077 (0·009)	$F_{2,11}$ = 2·18, *P* = 0·19
Davis 2013	0·057 (0·005)*	0·049 (0·003)	0·047 (0·005)	$F_{2,11}$ = 5·73, ***P* = 0·04**
Pinney 2013	0·98 (0·29)	0·51 (0·16)	0·36 (0·12)	$F_{2,11}$ = 3·79, *P* = 0·12
Throckmorton 2013	0·11 (0·0084)	0·12 (0·0052)	0·11 (0·0039)	$F_{2,11}$ = 0·25, *P* = 0·78
Pinney 2014	2·22 (0·37)	2·42 (0·13)	1·09 (0·33)*	$F_{2,11}$ = 7·32, ***P* = 0·02**
Throckmorton 2014	0·030 (0·0036)	0·021 (0·0038)	0·017 (0·0066)	$F_{2,11}$ = 2·71, *P* = 0·14
Maize yields	**kg per ha (SE)**	**kg per ha (SE)**	**kg per ha (SE)**	
Davis 2012	1213 (47·3)	1196·5 (50·4)	1211·9 (96·6)	$F_{2,11}$ = 0·01, *P* = 0·98
Pinney 2012	1864·7 (79·2)	1770·1 (21·6)	1802 (42·1)	$F_{2,11}$ = 0·79, *P* = 0·50
Throckmorton 2012	1084·5 (53·5)	1197·5 (67·8)	1188·3 (50·4)	$F_{2,11}$ = 0·84, *P* = 0·48
Davis 2013	1766 (58·6)	1735·2 (126·4)	1902·7 (128·5)	$F_{2,11}$ = 2·38, *P* = 0·17
Pinney 2013	1857·5 (36)	1857·5 (24·7)	1939·7 (38·03)	$F_{2,11}$ = 1·42, *P* = 0·31
Throckmorton 2013	2241·9 (82·2)	2199·8 (21·6)	2277·9 (30·8)	$F_{2,11}$ = 0·82, *P* = 0·48
Pinney 2014	2151·5 (53·5)	2140·2 (129·5)	2225·5 (90·5)	$F_{2,11}$ = 0·38, *P* = 0·70
Throckmorton 2014	2141·2 (37·01)	2149·4 (30·8)	2156·6 (53·5)	$F_{2,11}$ = 0·03, *P* = 0·97

P-values shown in bold indicate significant differences within locations ($P < 0.05$).
*Significant differences between treatments at the same location.

Lundgren 2012; US EPA, 2014). These reports and our data suggest that the current use levels of insecticidal seed treatments in North American row crops are likely to far exceed the demonstrable need and our results likely reflect a scarcity of target pests. Most pests that can be effectively managed by neonicotinoid seed treatments of maize in the US are considered ‘secondary pests’ (Douglas & Tooker 2015) and, by definition, are infrequently and sporadically encountered. As a result, current use rates in maize, estimated at 79–100% of seed sown (Douglas & Tooker 2015), are likely to far exceed pressure levels.

This work was conducted in Indiana, which is representative of the most intensive agricultural regions in North America, ranking 5th among US states in the production of maize for grain and 4th in soybean production (USDA-NASS, 2015); seeds of both crops are typically treated with neonicotinoids (Douglas & Tooker 2015). The area sown with these crops, as well as other crops commonly grown from neonicotinoid treated seeds, such as cotton and oilseed rape or canola, is estimated to be over 65 million ha $year^{-1}$ (USDA-NASS, 2015) in the US alone. Our work can be used to inform mitigation and conservation practices for honey bees and other sensitive non-target organisms living near these crops, for example, in development of buffer zones and wildlife corridors in the United States and Canada.

The use of both seed treatments and modern pneumatic sowing equipment is widespread and contaminated dust stands out as an important source of acute exposure to neonicotinoids for honey bees and a wide range of other non-target organisms across areas that far exceed the planted field. However, there is reason for optimism: our work suggests that significant reductions in risks to pollinators and other non-target organisms could be achieved rapidly, and with little or no corresponding reduction in maize production simply by reducing the percentage of maize seed that is treated with neonicotinoid insecticides to levels that more realistically reflect pest pressure.

Authors’ contributions

C.H.K. designed the experiments. C.H.K. and E.Y.L. conducted the field experiments. B.D.E. conducted chemical analyses. C.H.K., E.Y.L., and J.D.H. analyzed data. C.H.K., J.D.H. and E.Y.L. wrote the paper.

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Data accessibility

Data used in this manuscript are available from Dryad Digital Repository https://doi.org/10.5061/dryad.pp127 (Krupke *et al*. 2017).

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Field sites used for planter dust deposition study.

Table S2. Field sites used for treated seed efficacy study, 2012.

Table S3. Field sites used for treated seed efficacy study, 2013.

Table S4. Active ingredients detected in soil at planting sites, 2012.

Table S5. Active ingredients detected in planter lubricant following sowing, 2012.

Table S6. Active ingredients detected in soil at planting sites, 2013.

Table S7. Active ingredients detected in planter lubricant following sowing, 2013.

Table S8. Concentration of active ingredient on maize seeds treated with clothianidin.

Appendix S1. Supporting information.