Corn Dust Research Consortium (CDRC) Final Report

Reviewed and approved by the CDRC.

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Executive Summary

From 2013-2017 the CDRC assessed the interactions that honey bees were having with pollen and nectar sources during corn planting. In addition to pollen usage and foraging preference, the neonicotinoid residues present in or around potential pollen sources were examined where possible. The effectiveness of an alternative planting lubricant was also examined at one of the four study locations to determine if drift and concentrations of residues were reduced compared to graphite and talc products.

Each of the study centers had different experimental designs and protocols, even when investigating the same question. Because of these differences, which were encouraged by the CDRC, the executive summary illustrates highlights rather than combined conclusions in some instances.

Investigations of pollen usage showed that the majority of pollen collected by honey bees came from woody tree and shrub species, during planting. Salix and Acer species were most commonly used, as were members of the family Roseceae. Herbaceous pollen was less commonly collected. After planting there was a shift in pollen collection to more herbaceous dominated species with clover (Trifolium hybridum) being most common. Honey bees were noted to visit dandelion (Taraxacum spp.) very commonly during this period, although this pollen only accounted for a small amount of pollen returned to the hive, suggesting that they use these flowers as nectar sources.

The concentrations of neonicotinoids within bee-collected pollen during planting were significantly higher than after planting. The data from trees/shrubs and herbaceous plants suggest that the availability of different species of flowering resources relative to the time of corn planting can influence the exposure of honey bees to neonicotinoid insecticides and will vary with the environmental conditions of the year.

Bee mortality results associated with planting activities varied between the replicates, with some showing increased mortality with planting activities (Iowa and Ohio) and some showing no difference in mortality (Nebraska).

Neonicotinoid residues measured using vertical sticky traps and volumetric air samplers downwind during tillage events were much lower than the residues collected during planting (Guelph). The concentration of neonicotinoids captured at the field edge by either method was similar to those at the neighboring field edge downwind. **The CDRC results are not consistent with other research regarding the extent to which synthetic lubricants reduce net emission of dust-borne pesticide during planting of treated seed; however, the CDRC research showed sufficiently significant reductions to warrant use of these synthetic lubricants compared to talc or graphite.**

Efforts to reduce the amount of emitted dust with planter modifications and efforts to place bees in locations that minimize their exposure during planting are recommended.

Introduction and background

Honey bees living near corn fields can have multiple routes of exposure to pesticides. Exposure may be by contact (dust, soil), by ingestion (pollen/nectar/water), or a combination of these exposure routes. The focus of this discussion is exposure via dust from the planting of treated corn seeds.

Corn planting throughout the U.S. and Canada typically occurs from late April to early May when the fields are sufficiently dry to enter with equipment. Corn seeds currently in use by farmers are very frequently treated with pesticide(s). Under humid conditions, treated seeds may become sticky and require a lubricant/fluency agent to move effectively through pneumatic planting equipment; talc and/or graphite are frequently used as seed flow lubricants in the larger pneumatic planters to ensure uniform seed drop. Abrasion of treated seed coatings can result in particles containing pesticide residues mixing with the fluency agents to produce a contaminated "dust" (aka fugitive dust), which can be released by the air exhaust system during planting or subsequent cleaning of the equipment. This "dust" has the potential to be deposited on soil, water, and flowers within and adjacent to corn fields where foraging honey bees, and other pollinators, may be exposed to the pesticide(s).

In 2008, a large number of honey bee colonies in Germany were affected by the drift of dust generated through the abrasion of treated seed during planting. Since that time there has been concern regarding the extent to which one class of pesticides, i.e., neonicotinoid insecticides, can move off-site and represent a route of exposure for bees foraging in the vicinity of fields where neonicotinoid-treated seeds have been planted. Although the incident in Germany was attributed to a combination of factors (i.e., lack of a suitable sticking agent for the pesticide on the seed, seeding equipment that vents upward, dry windy conditions and an abundance of oilseed rape (canola) in full bloom immediately adjacent to the fields being planted), subsequent research (Krupke et al. 2012; Tapparo et al. 2012) has indicated that fugitive dust may still represent a route of exposure even where suitable sticking agents are used and seeding equipment vents downward.

The Corn Dust Research Consortium

The Corn Dust Research Consortium (CDRC) was formed in early 2013 at the request of the Pollinator Partnership, which provides administrative oversight to the CDRC, to explore potential exposure routes of honey bees to seed treatment dust as well as potential options to mitigate exposure. The CDRC secured the funding for and conducted the oversight of research into two specific corn dust/honey bee interactions in 2013 and 2014.

Question 1) What are the flowering resources available to and used by honey bees in and around corn fields during planting?

Question 2) What is the efficacy of a newly proposed fluency agent relative to talc and/or graphite in reducing the abrasion of treated seed coatings within planters during planting and the subsequent levels of pesticide-contaminated dust released into the environment?

Research teams addressing question 1 conducted work in three states (Ohio, Iowa, and Nebraska) and one province (Ontario). Question 2 was addressed by the research team in Ohio. Findings and a summary report of the 2013 and 2014 study years can be found at http://www.pollinator.org/PDFs/July2015CDRCFINAL.pdf. In 2015 the CDRC revisited these two research questions with an additional RFP solicitation and four project areas.

Project 1- Use by Honey Bees of flowering resources in and around cornfields during spring planting, and how this behavior can be effectively managed to reduce exposure to pesticide dust and residues;

Project 2 - The long-term health consequences of exposure of honey bee colonies to dust emitted during planting of neonicotinoid treated corn seeds;

Project 3 – The efficacy of CDRC recommendations in preventing honey bee exposure to corn dust; and

Project 4 - Efficacy of seed lubricant products

In 2015, the research team from Montana addressed project area 2, the team from Ohio addressed all four projects and the team from Ontario addressed project area one. The research team from Iowa did not continue into 2015.

The goal of the consortium in addressing these project areas is to utilize data from research conducted in during the 2013, 2014, and 2015 corn planting seasons across four North American locations to develop best practice guidance for future corn planting seasons, thereby reducing potential exposure of honey bees to fugitive dust during planting.

It was clear from the beginning that the CDRC could not address all aspects of pollinator exposure, and given limited resources and time, the decision was made to be focused in our efforts. The sampling was focused solely on the potential exposure to honey bees

with respect to corn planting. No other species or other crops were considered by CDRC-funded studies.

More than a dozen stakeholder groups that comprise the CDRC invested their time and resources to ensure that the research was conducted and presented in the most un-biased, open, and useful form. The participating stakeholders represent interests from various aspects of this situation and include members from:

American Beekeeping Federation
American Seed Trade Association
American Honey Producers Association
Association of Equipment Manufacturers
Bayer CropScience
BASF
Canadian Honey Council
Farm Equipment Manufacturers Association
Industrial Minerals Association - North America
National Corn Growers Association
Pollinator Partnership
Syngenta
University of Maryland

In addition, reviews of protocols and study results have been provided by the U.S. Department of Agriculture's Agricultural Research Service (USDA ARS), Health Canada's Pest Management Regulatory Agency (PMRA), and the U.S. Environmental Protection Agency's Office of Pesticide Programs (EPA OPP).

The CDRC research was not formed with the intent to address all questions related to potential exposure to a specific class of insecticides, *i.e.* neonicotinoids and their interaction and/or potential effects on honey bees or all pollinators. In fact, the CDRC research is NOT intended as:

- An endorsement of seed treatment, neonicotinoids, or any practice
- A program with a preconceived outcome
- A study involving any pollinator other than honey bees
- An examination of Colony Collapse Disorder (CCD)
- Applicable to any other crop until tested
- An examination of all potential routes of exposure
- An examination of potential additive, synergistic or antagonistic relationships between multiple pesticides (*e.g.*, insecticides and fungicides)

What follows are the final reports for 2016-2017 for the teams from Montana, Ontario, and Ohio, and the final report for 2015 from Iowa. It should be noted that researchers at each of the four institutions took their own approach to the project areas. Their methods and their observations are not identical, nor were they intended to be. The variety of

landscape features and differences in grower practices, as well as the timing of the planting, varied according to location.

JUNE, 2016 FINAL REPORT

Assessing strategies to reduce honey bee exposure to dust emitted during planting of treated corn seeds in Ohio

A research update addressing all four projects outlined in the Corn Dust Research Consortium 2015 request for proposals

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EXECUTIVE SUMMARY

- In the process of planting of corn seed treated with neonicotinoid insecticides (clothianidin and thiamethoxam) particles of seed treatment are released into the environment. Particles are deposited both within and outside the planted field and are detectable at least 100 m from the field edge in the downwind direction. Aerial transport implies that suspended seed treatment particles are present in the air above and around a field during planting. **(Section 2)**
- Honey bees come into contact with seed treatment particles during corn planting. Clothianidin and thiamethoxam residues are reliably detected at elevated levels (8 ppb above background, on average) in honey-bee-collected pollen harvested during corn planting. **(Section 3)**
- The corn planting period is associated with a 2.3-fold increase in adult honey bee mortality. A significant positive correlation was observed between adult bee mortality and the concentration of seed treatment insecticides detected in pollen collected over the same period. **(Section 4)**
- Increased adult mortality is observed even though the level of seed treatment detected in pollen is not at a concentration predicted to cause acute mortality. This suggests that insecticide concentrations measured in bulk pollen serve as a useful indicator of bee exposure, but fail to capture the mechanism of exposure leading to bee death. **(Section 1)**
- Despite elevated adult mortality during corn planting, the magnitude of seed treatment insecticide exposure through pollen is not predictive of colony strength in subsequent months or overwintering success. **(Section 5)**
- Landscape composition, apart from the area of corn fields being planted, is not correlated with contamination of pollen with seed treatment insecticides or adult mortality. The magnitude of seed treatment exposure during corn planting is positively correlated with the total area of corn fields, but not with weed prevalence in corn fields or with the intersection of seed treatment drift and off-field foraging habitat. Adult mortality is not correlated with any landscape variable. This tentatively suggests that honey bee exposure during corn planting occurs primarily by aerial contact with ubiquitously dispersed seed treatment particles, not by the contamination of in-field or off-field flora or by aerial intersection with a localized dust plume. **(Section 7)**
- **Mitigation recommendations (Section 9)**.
    - Engineering and quality-control measures to ensure seed treatments are well-adhered to corn seed.
    - Reduced aerial mobility of insecticide-laden seed treatment particles through planter modification, changes to seed treatment formulation and/or use of fluency agent.
    - Use of an insecticide in corn seed treatments demonstrating a lower toxicity to honey bees.
    - Reduced use of insecticides in treated corn seeds in accordance with the principles of “Integrated Pest and Pollinator Management”.

OUTLINE

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II. **Section 1:** Routes of exposure and implications for mitigation
III. **Section 2:** The release of neonicotinoid-laden dust during the planting of treated corn
    A. Dust drift during planting
    B. Qualitative assessment of seed treatment integrity
IV. **Section 3:** Neonicotinoid contamination of honey- bee-collected pollen during corn planting
V. **Section 4:** Elevated mortality of adult honey bees during corn planting
VI. **Section 5:** Effects on colony strength
VII. **Section 6:** Spatial and taxonomic foraging patterns revealed by dance analysis and pollen identification
VIII. **Section 7:** Landscape as a predictor of exposure and effects
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X. **Section 9:** Conclusions Mitigation recommendations

INTRODUCTION:

Study sites and apiary setup

Apicultural work was conducted at 10 apiaries, at least 3.5 km apart, in Central Ohio **(Figure 1, Table 1)**. Apiaries were either managed by the Ohio State University research team or were managed by experienced private beekeepers.

We selected up to 4 overwintered colonies to be monitored for bee mortality and winter survival at each apiary. Additionally, we installed two new colonies started from packages and one from a 4-frame nucleus colony at six apiaries. All of the new colonies were installed in 8-frame Langstroth hives on solid bottom boards. Other colonies were overwintered colonies, usually in 10-frame hives, made available by cooperating beekeepers.

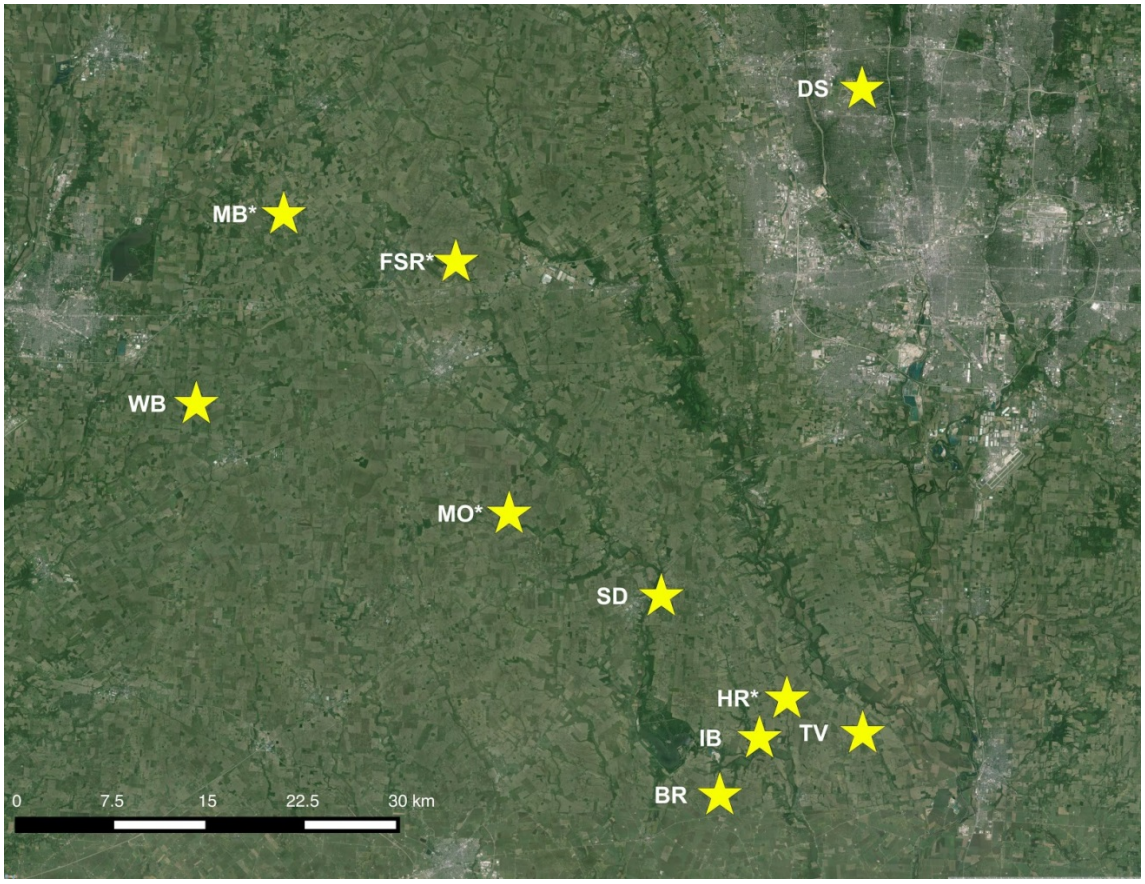


Figure 1. Apiary locations plotted over satellite imagery (Google OpenLayers). Observation hives were installed in four apiaries (asterisked) to record dance activities.

site	% corn (2015 data)[a]	beekeeper	hive equipment[b]	no. hives monitored	new colonies[c]	dance mapping
FSR	49%	OSU	8-f standard	4	yes	yes
MO	41%	private	10-f palletized	6	yes	yes
TV	36%	private	10-f standard	2		
HR	31%	private	10-f standard	6	yes	yes
WB	31%	OSU	8-f standard	4	yes	
IB	28%	private	10-f standard	2		
BR	27%	private	10-f palletized	2		
MB	21%	private	10-f standard	4	yes	yes
SD	14%	private	10-f palletized	6	yes	
DS	< 1%	OSU	8-f standard	2		

a: Percent area of corn fields within 2 km radius from the apiary, calculated by visual groundtruthing and GIS analysis

b: Hive equipment used for the overwintered colonies being monitored. All colonies were housed in 8- or 10-frame Langstroth hives, on standard or palletized bottom boards.

c: Three new colonies, two started from packages and one nucleus colony, were installed and monitored.

Table 1: Apiary information

Corn planting

In 2015, consecutive days of sunny, warm and dry conditions in Central Ohio allowed farmers to complete most of the corn planting quickly in early May. Planting of corn fields near the study apiaries started as early as April 28, although the most intense corn planting activity was observed May 2 - 8. Planting in most corn fields was completed by May 9, but sporadic planting continued through the end of June. Planting in all of Ohio was estimated at 15% complete on May 3 and 55% complete on May 10 (USDA-NASS 2015).

Landscape composition

Based on preliminary dance analysis from 2014 and published data (Couvillon et al. 2014), we chose to define our landscapes using a 2 km radius. This decision was supported by our 2015 dance analysis that showed the bulk of foraging activity (~75%) occurred within two kilometers of the hive.

Landscape methods: Using a combination of visual ground-truthing and satellite imagery analysis (Google OpenLayers), we determined the composition of the landscapes surrounding each of our apiaries in terms the following categories: crop field, forest, treeline, herbaceous patch (e.g. CRP), herbaceous strip (e.g. field margins), roadside, and residential lot. Crop fields were further subdivided according to crop type and pre-planting weed abundance. Pre-planting weed abundance was assessed by visual ground-truthing on April 30 and May 1, immediately prior to the start of corn planting. Fields were assigned a "bloom level" of 0 (no blooming weeds), 1 (scarce blooming weeds), or 2 (abundant blooming weeds).

The crop types in fields were determined initially by ground-truthing in June, 2015 and later, in January 2016, with the updated USDA Cropland Data Layer (http://nassgeodata.gmu.edu/CropScape/). All landscape data were analyzed and visualized using QGIS software (QGIS Development Team 2015).

Landscape results: Our sites represented a wide range of corn abundance, from 49% (FSR) to an urban site with less than 1% (DS) (**Figure 2**). Soybean was the other major field crop at each of our sites. Non-crop land cover (tree canopy, herbaceous, residential) were of relatively low abundance (<25%) at most sites, but were more prevalent at MB (~50%), IB (~40%), SD (~35%), and predominant at the urban DS site (>99%).

The prevalence of blooming weeds in cornfields prior to planting was highly variable (**Figure 3**), presumably due to differences among farmers in herbicide application and tilling practices. At one extreme, FSR had zero cornfield area classified at the highest bloom level. In contrast, the vast majority of cornfield area at HR and SD was classified as bloom level 2. Our estimates of pre-planting bloom abundance might be confounded by herbicide application that occurred in between our ground-truthing trips and the start of planting.

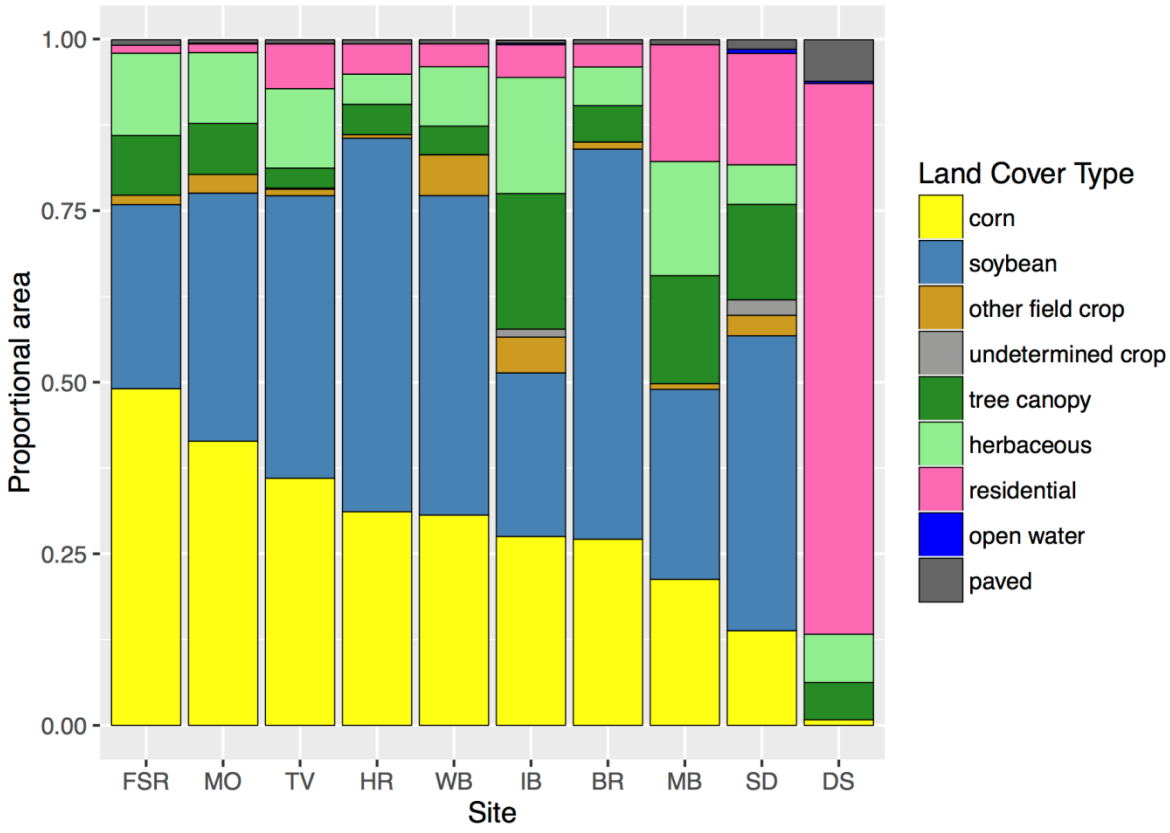


Figure 2: Landscape composition summary within a 2km radius of apiary sites. Corn and soybean were major landscape features at all sites except for the one urban site, DS. Other field crops consisted of wheat, alfalfa and vegetable crops. Some fields visible in satellite imagery could not be surveyed on the ground due to inaccessibility, so these were classified as "undetermined". "Tree canopy" is a combination of all forest, tree line, and orchard land cover, excluding trees on residential properties. "Herbaceous" includes all non-crop herbaceous land cover, e.g. field margins, roadsides, CRP strips, and old fields, excluding herbaceous cover in residential properties. Residential properties were typically characterized by a combination of tree canopy (ornamental/landscaping trees), lawns, gardens, and built structures. "Paved" land cover refers to roadways and industrial/commercial lots.

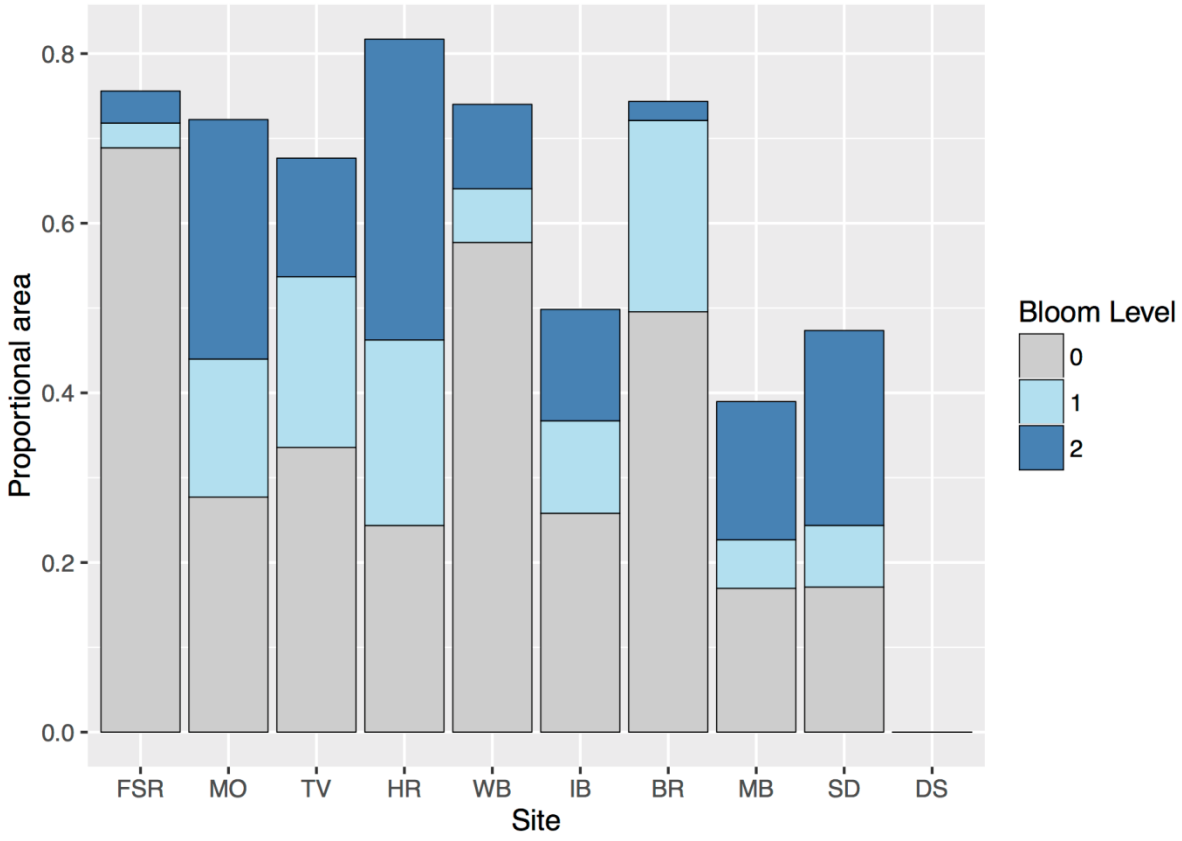


Figure 3: Pre-planting bloom abundance in cornfields. Bloom level 0 fields had virtually no flowering weeds in them, due either to spring herbicide application or fall tillage. Bloom level 2 fields had abundant flowering weeds, with purple deadnettle (*Lamium purpurea*), dandelion (*Taraxacum officinale*), and various mustards (Brassicaceae spp.) being the most common constituents. Bloom level 1 fields had scarce and/or patchy flowering weeds. Given the relative abundance of flowering plants in off-field habitats and bloom level 2 fields, we deemed bloom level 1 fields to be essentially unattractive to honey bees.

SECTION 1:

Routes of exposure and implications for mitigation

Potential routes of exposure. One of the key objectives of our study was to evaluation and discrimination between of the multiple routes of exposure (ROE) that contribute to honey bee mortality during corn planting. While there is evidence for indirect ROE like water contamination and systemic uptake by non-crop flora (Long and Krupke 2015), it is likely that the two most important ROE for honey bees during corn planting are (1) floral contamination by deposited seed treatment particles and (2) aerial contact with suspended seed treatment particles **(Table 2)**.

Floral contamination by deposited seed treatment particles has been the primary focus of CDRC research thus far. Within this ROE, it is important to distinguish two subroutes: (1) the contamination of in-field flora ("weeds") by the immediate settling of seed treatment particles and (2) the contamination of off-field flora by the drifting of seed treatment particles. Seed treatment deposition data generated in our dust collection studies **(Section 2)** show that the magnitude of active ingredient deposited within a planted field is dramatically higher than that deposited even just one meter beyond the field edge. Moreover, the particles that settle immediately within the field are likely larger and less concentrated with active ingredient (Devarrewaere et al. 2016) than the particles that are carried off-field by air currents, potentially causing qualitative differences exposure patterns.

Aerial contact between foraging bees and suspended seed treatment particles has thus far received little attention in CDRC research, but there is evidence suggesting that this ROE may be equally or more important than floral contamination (Girolami et al. 2011, 2013, Tapparo et al. 2012). Again, it is useful to distinguish two sub-routes. First, bees in flight may intersect with the spatially and temporally localized plume of dust emitted from a running planter. The potential for this exposure route to deliver high doses of active ingredient to flying bees has been convincingly demonstrated (Girolami et al. 2011, 2013, Tapparo et al. 2012)□, but distinguishing this route from floral contamination is difficult in a field study such as ours. Bees exposed to a localized plume of dust may die in the field and fail to be detected, and dust adhering to the body of a foraging bee would be groomed into corbicular pollen pellets, which, based on pollen residue data alone, could easily be misinterpreted as evidence of floral contamination. It is also possible, though, that in addition to the threat of localized dust plumes, very fine dust particles may become suspended and widely distributed in the atmosphere, forming a diffuse and persistent route of exposure that extends well beyond the immediate vicinity of a planted field. This route of exposure remains largely unexplored, but it is plausible given the extremely small seed treatment particle sizes (as small as < 1 µm in diameter) we observed with scanning electron microscopy **(Figure 4, Section 2)**. A ubiquitous distribution of fine seed treatment particles could explain the exposure we

detected at our urban “control” site, where corn comprised less than 1% of the surrounding landscape.

Implications for mitigation. It is crucial to discern which of these ROE is/are the principal driver(s) of honey bee poisoning because each interacts differently with proposed mitigation schemes **(Figure 5)**. If, for example, the main ROE is the contamination of off-field flora by drifting seed treatment particles, then the use of a deflector or fluency agent to decrease particle mobility might dramatically reduce honey bee exposure. If, however, the main route of exposure is the contamination of in-field flora by settling of seed treatment particles, the use of a deflector or fluency agent might exacerbate exposure by concentrating emitted seed-treatment particles within the field. Similarly, the suppression of in-field flora might reduce exposure from settling seed treatment particles, but it would do nothing to mitigate aerial exposure, and might even exacerbate aerial exposure by forcing bees to spend more time in flight searching for resources. It is also important to note, however, that multiple mitigation schemes could be combined. For example, a deflector could be used in combination with in-field weed control to keep released seed treatment particles within fields that are free of bee-attractive flora.

In **Sections 6** and **7**, we present data on the spatial and taxonomic patterns of honey bee foraging at our sites during corn planting. We then analyze these data with respect to our data on pollen contamination **(Section 3)** and adult bee mortality **(Section 4)** in an effort to discriminate between the routes and sub-routes of exposure outlined in **Table 2**.

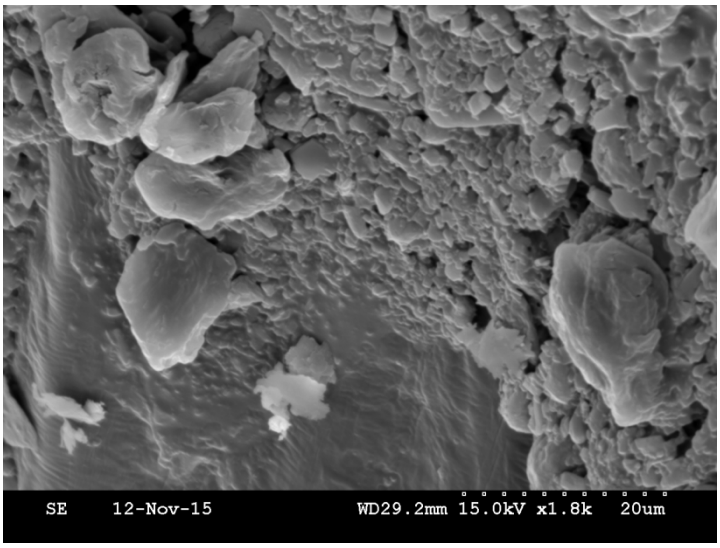


Figure 4: SEM of seed treatment coating illustrating the potential for extremely small articles to be shed. The smallest particles in this image are < 1µm in diameter, suggesting a strong potential for aerial transport.

Table 2: Hypothesized routes of exposure and corresponding predictions of exposure patterns.

Major Route	Sub-route	Predicted patterns of exposure
Floral Contamination	Off-field drift	• Moderate frequency • Low-moderate magnitude • High variability • Strong influence of proximity of foraging habitat to field edge at foraging scale
	In-field settling	• Moderate frequency • Moderate magnitude • Moderate-high variability • Strong influence of weed management in corn fields at foraging scale
Aerial Contact	Localized plume	• Low frequency • High magnitude • High variability • Strong influence of corn area at foraging scale
	Ubiquitous dispersal	• High frequency • Low magnitude • Low variability • Strong influence of corn area, potentially beyond foraging scale

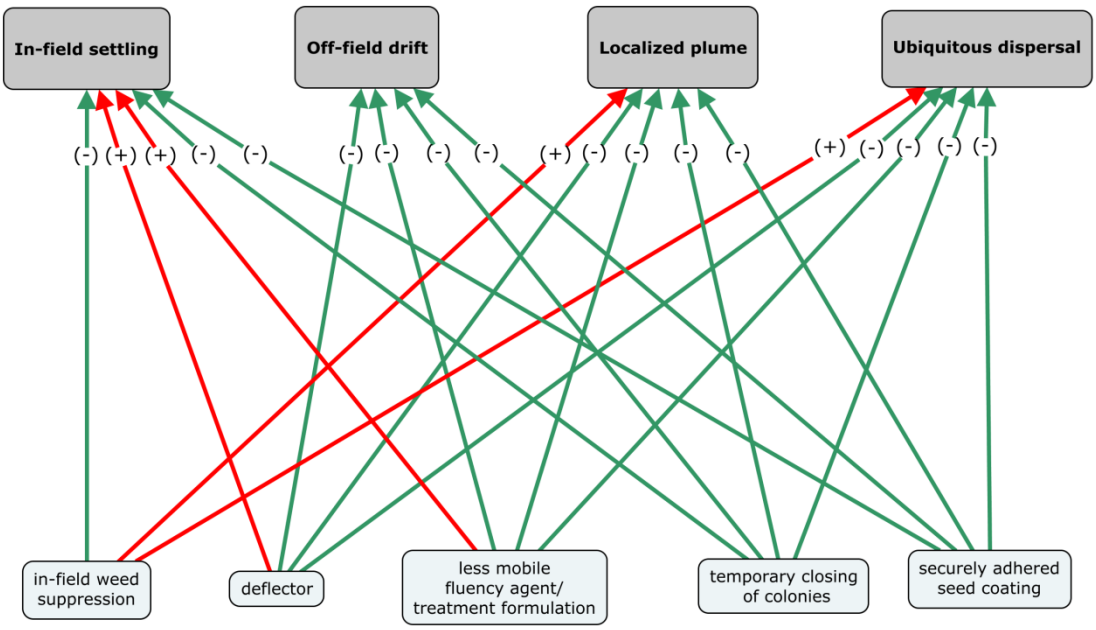


Figure 5: Interactions between hypothesized routes of exposure and proposed mitigation schemes.

SECTION 2:

The release of neonicotinoid-laden dust during the planting of treated corn

Dust drift during planting

Methods. A wide selection of planting equipment was evaluated in 2015 for the release of seed treatment insecticides, with the goal of gaining an overall picture of the variability in seed treatment release in different circumstances. Eight sites were located in central to west central Ohio, with an additional two just across the western border into Indiana.

Planter type–including make, model and serial number–were recorded as well as the type of seed and insecticide seed treatment at each site. A sample of the seed planted was retained for qualitative assessment of seed treatment integrity. During the planting operation, local wind conditions were measured at each field using a handheld wind meter. Wind direction was determined by compass. Relative humidity and temperature were collected at the time of planting from the nearest fixed weather station via the WeatherBug app.

Dust (potentially with insecticide) was collected with a target array arranged under the planter and downwind from the planting activity. The concentration of clothianidin and thiamethoxam in ng/sq cm of the slide was used to determine the relative amount of insecticide-laden dust escaping from the planter. Seed treatment dust traveling downwind was collected using Krupke-style dust collection stations (Krupke et al. 2012). The collection stations were constructed from PVC pipe which held two sets of slide trays: one in a horizontal orientation 30 cm above the ground to estimate dust deposition on herbaceous flowers, and a second in a vertical orientation 2 m above the ground perpendicular to the wind to intercept blowing dust.

The slide tray targets were made up of five microscope glass slides held together by plastic grip strips glued to a piece of cardboard. Stations were held in place by a cleated fence post so that the horizontal and vertical dust collectors would remain fixed at the correct orientation and height (30 cm and 2 m). Slides were attached once the PVC frame was set up and before planting began, then were treated with aerosol Tangle-Trap Sticky Trap Coating to hold dust particles.

Either a conventional farmer supplied seed lubricant (talc, graphite or blend) or the Bayer fluency agent was used, each added according to directions from the manufacturer. The Bayer fluency agent used consists of ethane, a homopolymer, at a rate of 1/8 cup for every 80,000 seed. On one occasion the grower used Bayer fluency agent treated seed (from the seed provider) but added their own lubricant to it as well. In 2015, a higher priority was given to planter variety than seed lubricant choice.

The stations were placed perpendicular to the orientation of the planting passes and placed at 10-meter downwind of the planter starting point. Four detectors were set and spaced approximately 30 m apart. Planting began after station placement and continued until approximately 100 m of field was planted beyond the starting point. Time of exposure was recorded. Slide trays were also placed under the planter for one pass, to collect dust ejected downward from the planter. The trays were then picked up and stored in a dust free area after that single pass.

Slide trays from the field were removed immediately after planting. Slides were organized and stored in a dust proof cardboard box; taped and sealed. In 2015, when finished with a site slide trays were placed in a dry Coleman cooler, transported within approximately two hours to a secure chest freezer at the Western Agricultural Research Station near South Charleston Ohio, and maintained at 0 degrees C for approximately two weeks.

On June 10, 2015 the center 3 slides from each set of 5 were placed in a 50mL conical tube separated by pipette tips so that their surfaces were not touching. Tubes were labeled and set into a cooler of dry ice to transport to the lab freezer. Processed in batches of 6 to 15 tubes, each tube was filled to the 50 mL mark with acetonitrile. Each tube then received 10 microliters for 2 µg/ml d-4 imidacloprid in acetonitrile as an internal standard. The tubes were resealed and sonicated at room temperature for 1 hour in the dark, then sat in the dark sonicator for an additional 23 hours.

After the 24 hour soaking period, liquid was transferred to another conical tube. New tubes were placed under a nitrogen stream to dry to less than 1.5mL in volume. Drying took between 6 and 15 hours, and remaining liquid was transferred to an Eppendorf tube. Eppendorf tubes were then handed off to the university Mass Spec & Proteomics (http://www.ccic.ohio-state.edu/msp) lab staff to measure insecticide levels.

a. Instrument

i. LC: Dionex UltiMate 3000

ii. MS: Waters Xevo TQ-S

b. LC conditions

i. Column: Waters XBridge BEC130 C18 (1*100 mm, 3.5 µm)

ii. Column temp: 30 ^{o}C

iii. Solvent A: aqueous NH_4COOH (5 mM) with 0.1% formic acid

iv. Solvent B: ACN

v. Flow rate: 100 µL/min

vi. Gradient: 0 min, 5% B; 1 min, 5% B; 5 min, 90% B; 7 min, 90% B; 7.5 min, 5% B; 13 min, 5%B

c. MS channel -- The ion pairs of 256.0/209.0, 250.0/169.0, 292.0/211.0, and 260.0/213.0 are used to monitor the conc. of imidacloprid, clothianidin, thiamethoxam, and internal standard imidacloprid-d4; and the collision energy is 15, 12, 10, and 15 eV, respectively. All other parameters are tuned to give the optimized MRM signal.

Table 3. 2014 Ohio Corn Dust sites

Site	Ohio Location	Planter	Type	Insecticide	Seed Company	Lubricant treatment
1b & 1f	Mechanicsburg	Kinze 3660	Center fill, non-vacuum row	Clothianidin 500	Beck's	Bayer fluency agent or Kinze graphite
2b & 2f	Delaware	John Deere 1770NT	Row unit vacuum	Clothianidin 500	Dekalb/ Monsanto	Bayer fluency agent or John Deere Premium Talc
3b & 3f	London	John Deere 1770NT	Center fill & row unit vacuum	thiamethoxam	Beck's	Bayer fluency agent or Precision Plant E-flow
4f	Kenton	John Deere 1770NT	Center fill & row unit vacuum	Clothianidin 250	Beck's	John Deere Premium Talc

Table 4. 2015 Ohio Corn Dust sites

Site	Ohio Location	Planter	Type	Insecticide	Seed Company	Lubricant treatment
5f	London	Case IH Early Riser 1255 AFS	Center fill, vacuum row	Poncho 1250/ clothianidin	Beck's	Precision Planting E Flow Seed Lubricant 1/2 rate graphite-talc blend
6f	Delaware	John Deere 1770NT	Row unit vacuum	Clothianidin 500	Dekalb/ Monsanto	John Deere Talc
7f	Nevada	John Deere 7200 Conservation	Row unit vacuum	Poncho 1250/ clothianidin	Beck's	1/2 rate John Deere Talc, BFA Pretreated seed

8b & 8f	Ridgeville, IN	Case IH Early Riser 1255 AFS	Center fill, vacuum row	Poncho 1250/ clothianidin	Beck's	Kinze graphite on left, BFA on right
9f	Gettysburg	Kinze 3600	Center fill, vacuum row	Cruiser 250	Master's Choice	Kinze graphite
10b	Ridgeville, IN	White-Massey-Fergusson/Agco 8800	Air pressure row unit	Clothianidin 250	Stewarts	Bayer Fluency Agent
11f	Marion	John Deere 7200 Conservation	No air, mechanical meter	Poncho 1250/ clothianidin	Beck's	John Deere Graphite
12f	Versailles	John Deere 1770NT	Vacuum row unit	Poncho 1250/ clothianidin	Beck's	Precision Planting E Flow Seed Lubricant graphite- talc blend

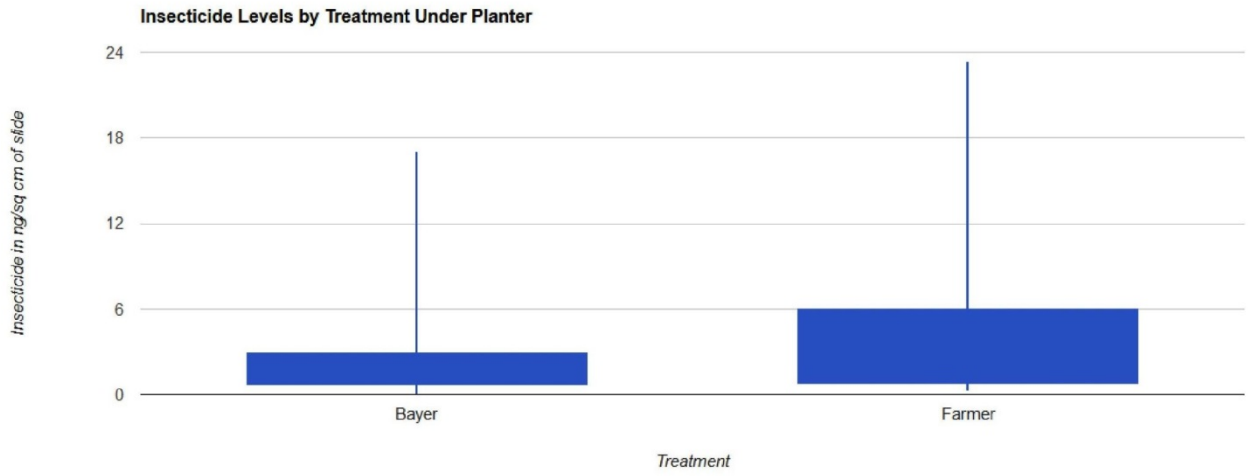


Figure 6: Comparison of insecticide levels by seed lubricant from under planter targets, 2014 and 2015.

Results and Discussion. Comparisons – **Figures 6, 7, 8 and 9** shown indicate the level of insecticide in dust collected on field placed slides. The whiskers represent the min and max values, and the box encompasses the first quartile through the 3rd quartile. The accompanying **Appendix C** includes site by site dust analysis values.

As seen in **Figure 6** and discussed in the 2014 report, any reduction in the level of insecticide differs between the two types of lubricant shown – the Bayer fluency agent vs. the farmer choice – is not large. While a broader range of insecticide occurred with the farmer chosen seed lubricant, much overlap between the two treatments is apparent.

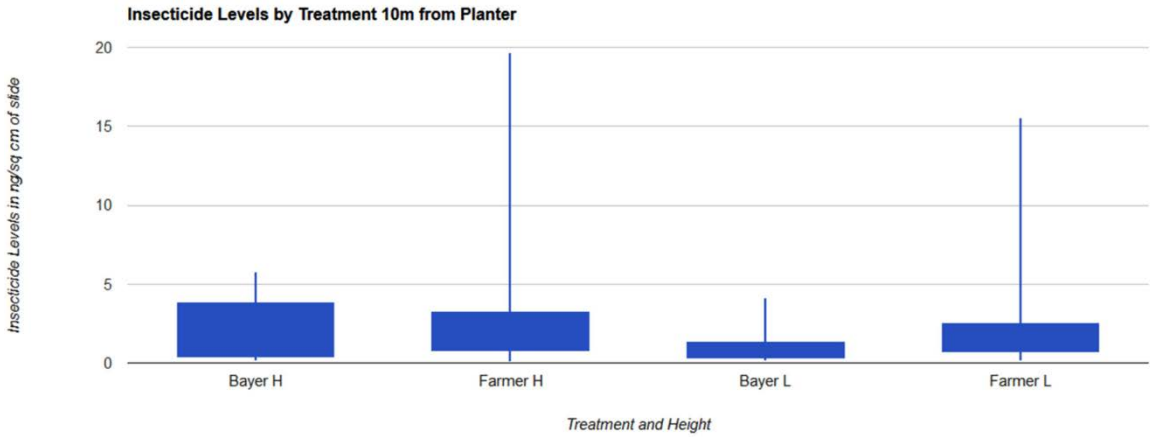


Figure 7: Comparison of insecticide levels by seed lubricant from ten-meter targets, from the high (2m) and low (0.3m) targets for 2014 and 2015.

Shown in **Figure 7** are the comparisons between the Bayer fluency agent and the farmer choice treatment. The 10-meter distance was chosen as the distance for comparison. Detectors were set at a 10-meter distance from the planter on the first pass, then allowed to collect dust as the planter made progress across the field until approximately 100 meters distance was achieved. H indicates the high (2m) target and L the low (0.3m) target.

As seen in **Figure 8** and discussed in the 2014 report there is little evidence that the level of insecticide leaving the planter differs between the two types of lubricant shown – the Bayer fluency agent vs. the farmer choice under the planter. Here at the 10-meter H location the Bayer treatment has a broader range of values, but for the L height the farmer treatment has a wider range – still at both heights at a 10-meter distance there is great overlap between the values.

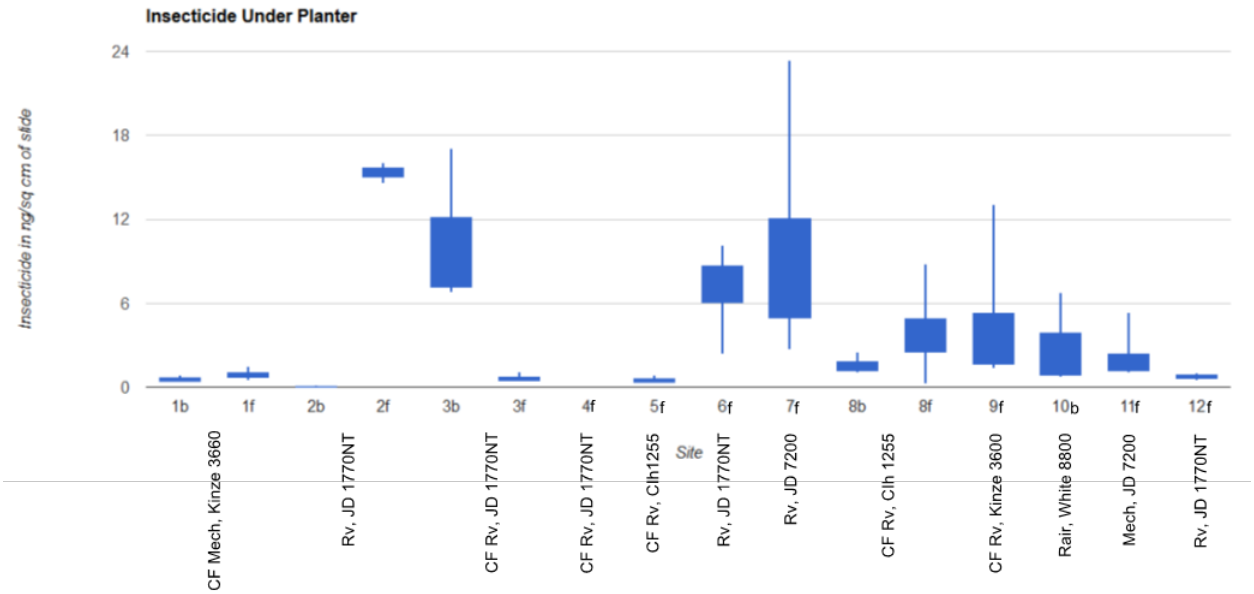


Figure 8. Comparison of insecticide levels by location and planter from under planter targets, 2014 and 2015.

Planter design for seed delivery to the ground uses many methods; center fill hoppers or individual row hoppers, and from pressurized systems to vacuum systems. The sites for 2015 were chosen to broaden the range of planter types and manufacturers beyond the 2014 sites and planters.

Range of planters for 2014 & 2015 –

- Manufacturer
  - John Deere 1770NT (5) and 7200 (2), Kinze (2), CaseIH (2), White/Agco (1). This range approximates the percentage type of planters in use today.
- Hopper type
  - Center fill (CF) – requiring an air system to move seed to the row unit or individual row unit seed box – with no air delivery
- Air system
  - No air at the row unit – meaning a mechanical finger pick up is used (Mech), vacuum at the row unit (Rv), or air pressure at the row unit (Rair)

From 2014, it appeared there may be some planter differences that might lead to a reduction in released insecticide. As shown in **Figure 8**, and across the two years and twelve planters it would appear that planter manufacturer or type does not have a discernable impact on insecticide release. There is some indication that mechanical, finger pickup type row units can reduce dust release but other types on occasion can also meet these levels of release as well. One question from growers during winter meeting discussions was whether the old stand-by finger pick up and row unit boxes with no air or vacuum could eliminate the insecticide loss – but that idea did not help as site number eleven was that type and still had release of insecticide. Lack of gasketing or seals can lead to loss of the seed treatment insecticide through gaps in the assembly.

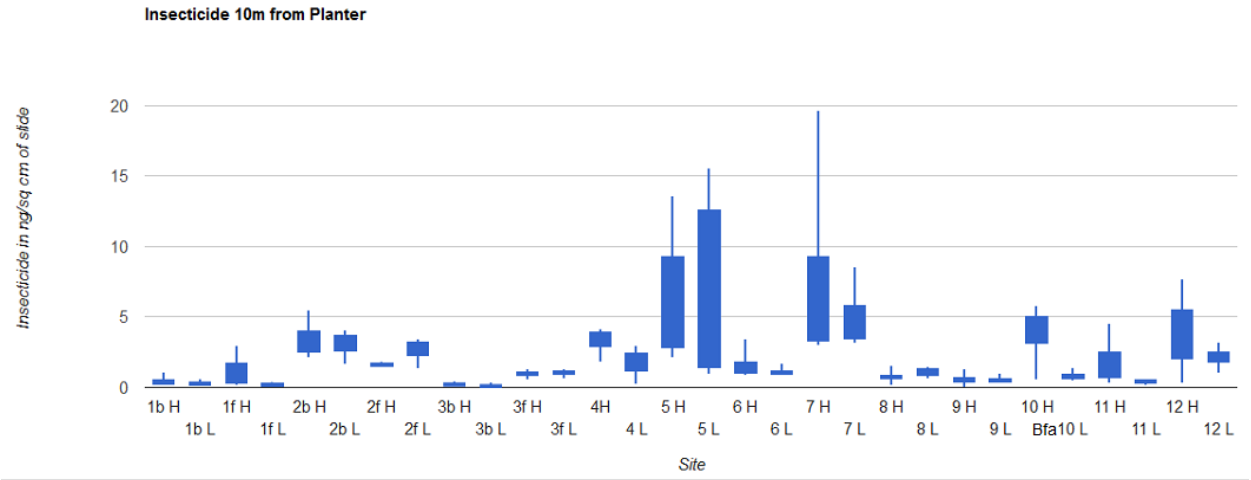


Figure 9. Comparison of insecticide levels by site from ten-meter targets, from the high (2m) and low (0.3m) targets for 2014 and 2015.

Shown in **Figure 9** are the 10-meter site values for insecticide. Generally, the 10-meter incidence follows the under planter target levels for indications of loss from the planter. Exceptions for sites 7 and 12 may be explained by the higher wind speed at the time of the trial – in the range of 10 to 15 miles per hour. Site 3, with the highest level of insecticide loss with the Bayer treatment, had low 10-meter insecticide levels.

A sample of the seed planted was retained for potential analysis in a Heubach dustmeter. This seed was collected during the mid- to late-planting time period for each site. This was after the seed had ample opportunity to bounce and shake in the seed hopper across the field. It was noted and farmers discussed the amount of dyed seed coat debris remaining in the hoppers during seed changes. On at least one occasion the appearance of seed treatment chips were evident on the target slide.

Qualitative assessment of seed treatment integrity

Methods. Color macrophotography and scanning electron microscopy (SEM) **(Figure 10)** were used to perform a qualitative assessment of the integrity of seed treatment material on treated corn seeds. Seed samples were taken directly from the seed units of planters being used by cooperating growers; seed was collected about halfway through the planting process, allowing time for normal seed agitation and abrasion to occur. After collection, seed samples were stored in plastic freezer bags or conical vials at -20°C until further analysis. Photography and SEM was performed in November 2015 (6 months after planting). Seeds were selected at random by shaking a few seeds out of each sample container. An exception to this is the seed pictured in **Figure 10 (B2)**, which was intentionally selected due to its striking lack of seed treatment material. For macrophotography, seeds were arranged on a white background and photographed using a Canon SL1 (crop sensor) camera with a 65 mm MPE Macro lens, a Laowa Twin light flash, and a custom diffuser. For SEM, seeds were mounted on stubs using carbon

sticky pads, coated with platinum, and imaged on a Hitachi S-3500N scanning electron microscope. All seed samples remain stored at room temperature for further analysis.

Results and Discussion. All examined corn seeds showed signs of seed treatment degradation, varying in severity **(Figure 10)**. We noticed no obvious differences between conventional treated seeds and those from companies that advertise a special polymer to enhance sticking, though our analysis was not systematic. Seed treatment material also visibly accumulated on the gloved hands of the investigator who prepared the seeds for photography and microscopy, and the readiness with which the seed treatment material shed from the surface of the seed made it difficult to secure seeds to SEM stubs using standard carbon sticky pads.

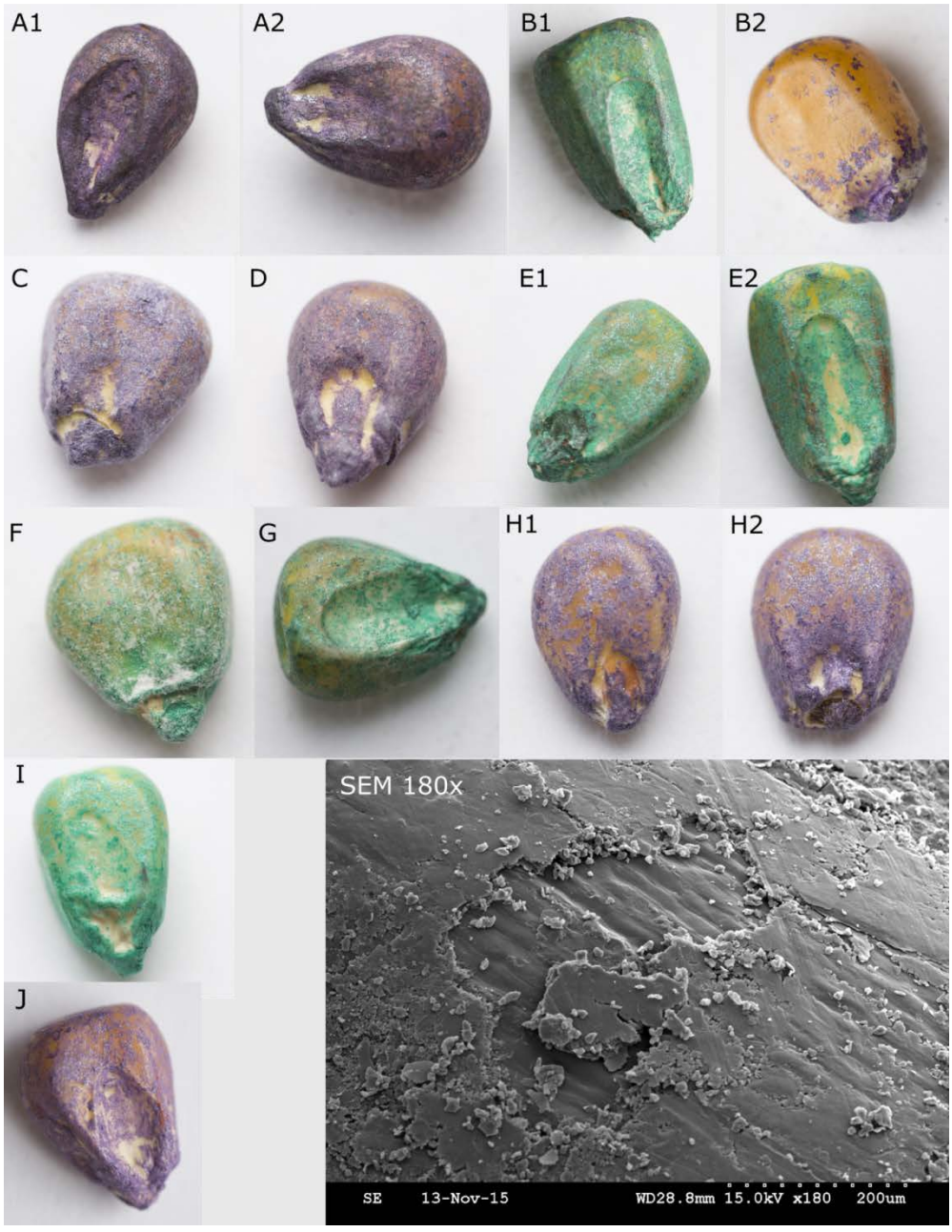


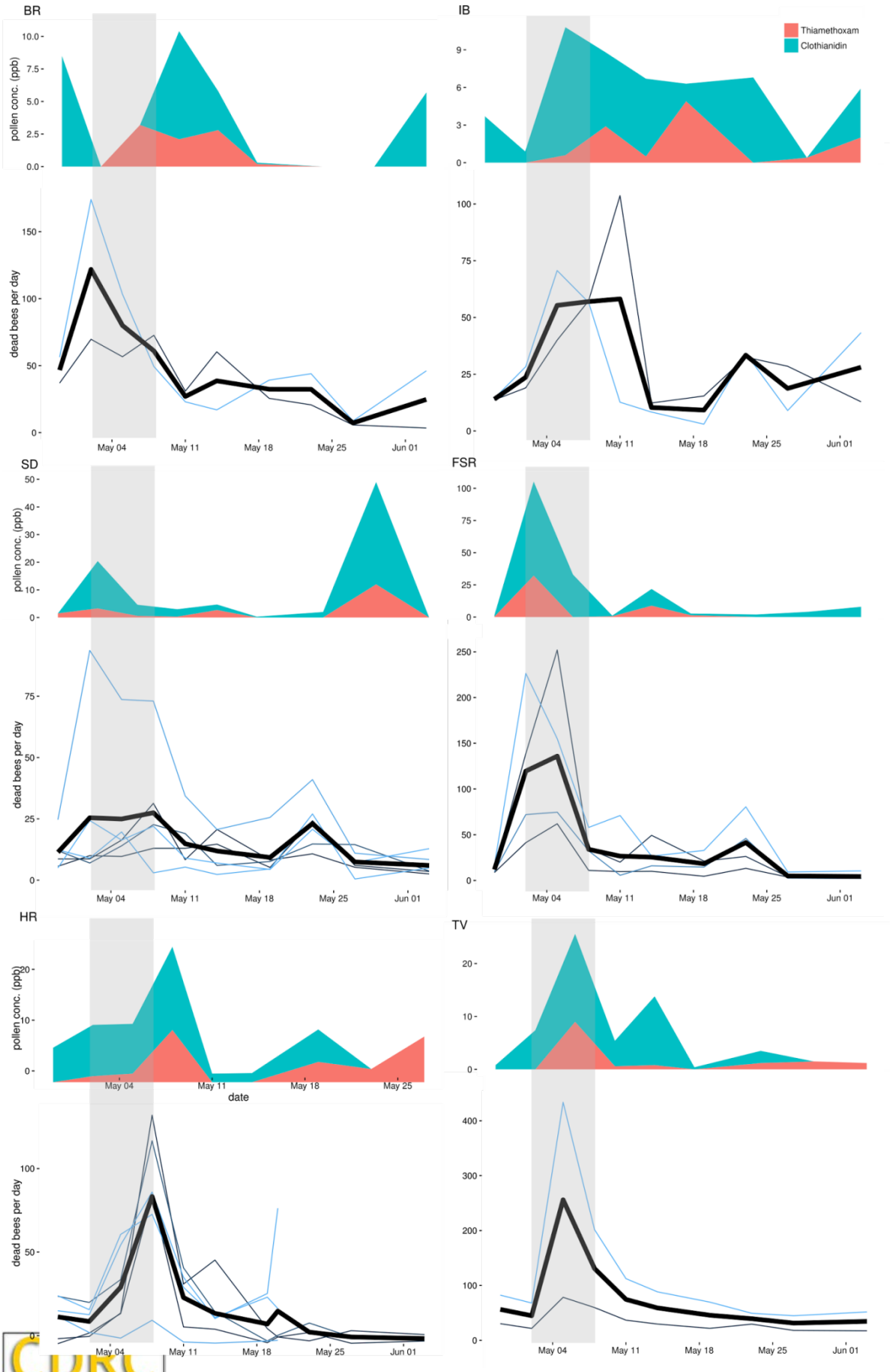
Figure 10: Photographic and microscopic assessment of seed treatment integrity. Seeds varied in the perceived integrity of the coating, but most showed signs particle generation. In an extreme case, we found one seed that had almost no coating left on it (B2), though this was the only example we noticed of such extensive degradation. Seed treatment appears to crumble away leaving patches of bare seed surface (smoothly striated patch in middle) visible by SEM (bottom right). Shed particles vary widely in size. The seeds pictured here came from the same batches of seed listed in Table 4, though they are deliberately not presented in the same order. Photos by M. Spring, SEM by D. Sponsler.

SECTION 3:

Neonicotinoid contamination of honey- bee-collected pollen during corn planting

Methods: A 3 g subsample of the bulk pollen collected from each site every 3-4 days was submitted to the EPA Ecosystems Research lab in Athens, Georgia for quantification of neonicotinoid concentrations. Analysis results for samples collected on May 2 -27 are presented in **Figure 11** and **Appendix A**.

Results and Discussion: Clothianidin and thiamethoxam residues were detected in most samples of pollen collected throughout May. Higher levels were observed May 2 - 8 when most corn was being planted, even at the urban "control" site (DS) . Pollen collected during the peak corn planting window contained significantly more seed treatment insecticide, 8.2 ppb more, than pollen collected at other times (Welch's T-Test, df=33.73, p=0.03). High levels of seed treatment insecticide in pollen observed after the main corn-planting period may be related to late-season planting near the study apiaries. The concentrations observed in pollen (**Appendix A**) are well below the concentrations predicted to cause mortality in nurse bees consuming pollen (predicted thiamethoxam LD_{50} in pollen = 536 ppb; predicted clothianidin LD_{50} in pollen = 389 ppb). See **Appendix B**.


BR
IB
SD
FSR
HR
TV
Thiamethoxam
Clothianidin
pollen conc. (ppb)
dead bees per day
May 04
May 11
May 18
May 25
Jun 01
date

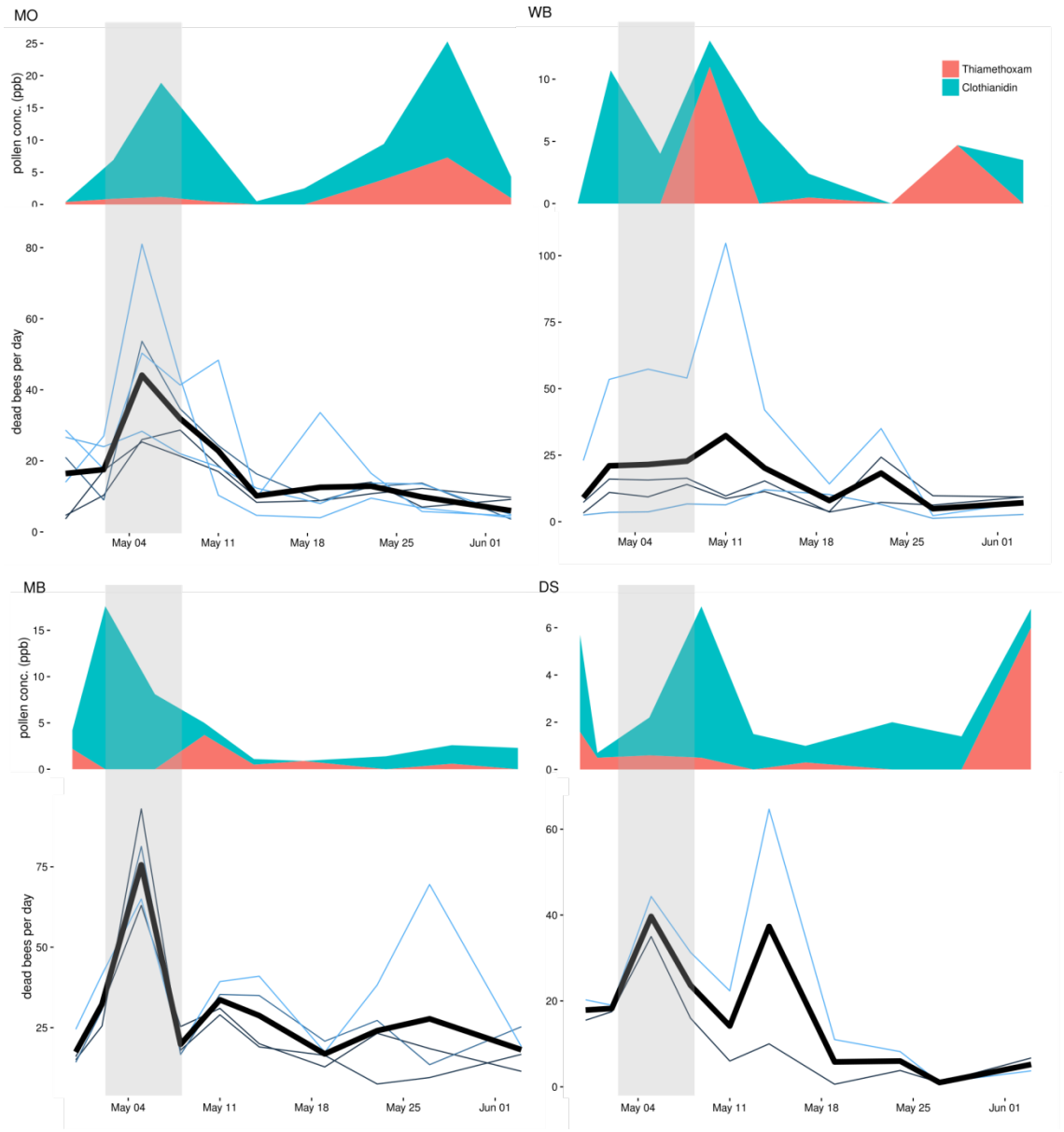


Figure 11: Summary of pollen contamination with seed treatment insecticides (above) and dead bee trap catch (below) for 10 apiary sites. The peak corn planting window (May 2 - 8) is shaded with a gray box. Dead bee trap catches, calculated per day, for individual colonies is indicated with thin lines while the apiary mean is presented in a thick black line.

SECTION 4:

Elevated mortality of adult honey bees during corn planting

Methods. Drop-zone dead-bee traps (40”x20”) were placed in front of each colony being monitored. Dead bees were counted and traps were emptied every 3 - 4 days from April 26 to June 2. Trap catch for each sampling period was standardized to calculate the number of dead bees collected per day. For statistical analyses of the number of dead bees two related approaches were taken. To relate dead bees to insecticide contamination in pollen the dead bees collected from traps at each colony was standardized by day, divided by the mean number of dead bees collected per day over the entire month of sampling, then the natural log was taken of this ratio. The mean of these values was then taken for all colonies at each site for each sample collection period. To relate dead bees to corn planting and landscape measures the mean daily dead bees ejected by each colony was taken for the peak corn planting period and the non-planting period. Each of these values was divided by the average daily dead bees for the whole month, then a natural log was taken of ratio.

Results. There was a 2.3-fold increase (95% CI=2.0 - 2.8) in the number of dead bees collected in dead bee traps during the peak corn planting period relative to non-planting periods (Welch’s Two-sample T-test, t=10.29, df=18, p-value < 0.0001) **(Figure 11)**. There was also a significant positive correlation between the concentration of seed treatment insecticides in pollen and the number of dead bees captured in dead bee traps (Pearson’s correlation, df=86, r=0.34, p=0.001).

SECTION 5:

Delayed effects and long-term recovery

Post-planting colony growth. To evaluate colony-level effects of exposure to corn seed treatment insecticides emitted during planting, we quantified hive parameters including adult bee populations (“seams” of bees, the spaces between frames filled with bees when viewed from above, as well as frame area covered by bees), brood (open and capped), pollen, and honey on the frames of each hive with four complete inspections: April 28 – 30 (before planting), May 20 – 22 (after planting), June 19 - 24, and August 14 - 19. Quantitative changes of these hive parameters between inspections were recorded.

Despite a significant correlation between levels of seed treatment insecticides in pollen and average mortality during the peak planting period (see **Section 4**), we did not detect significant correlation between insecticide levels in pollen and any of the hive parameters in the first inspection interval (April - May, Pearson’s correlations, P > 0.15 for all comparisons). During the second interval (May - June), a positive correlation was found between adult population (seams of bees) and the summed concentration of seed treatment insecticides detected in pollen during peak planting (r = 0.66, P = 0.037). This

increase in adult bees may reflect post-exposure population rebounds or other responses to environmental variations independent of exposure.

Hive parameters in June - August showed no correlation with spring exposure to corn seed treatment insecticides, but increases in pollen and nectar stores were observed at apiaries surrounded by more corn fields (pollen: $r = 0.79$, $P = 0.007$; nectar: $r = 0.67$, $P = 0.038$); this observation may be associated with food sources such as clover and other summer wildflowers grown in grassy fields, roadsides and field margins and blooms of soybean cultivation, which is often planted as a rotating crop along with corn (Sponsler and Johnson, 2015; Lin et al. 2016).

Overwinter survival. Of the 38 colonies being monitored, one colony at MO died in late summer and three colonies at HR were relocated to another location by the beekeeper and were excluded from overwinter monitoring. Therefore, a total of 34 colonies were prepared for overwintering at the end of September. Colonies were checked 3 - 4 times as weather permitted during October - February. Plain baker's fondant and Dantant AP23 winter patties were fed to the colonies as needed and vaporized oxalic acid was applied to all colonies in November to control varroa mites. Thirty one of the 34 colonies (91%) were alive at the end of March, 2016 although one of the surviving colonies was queenless and had developed laying-workers. No significant correlation was detected between overwinter survival and the level of corn seed treatment insecticides in pollen or percent corn area in the surrounding landscape across the 10 sites (Spearman's rank correlation tests, $P > 0.36$ for all tests).

SECTION 6:

Spatial and taxonomic foraging patterns revealed by dance analysis and pollen identification

Honey bees communicate to one another the location of valuable foraging patches by means of the "waggle dance" (von Frisch 1967). Because this dance language can be decoded by human observers, it can provide a unique glimpse into the spatial foraging patterns of a honey bee colony (Couvillon and Ratnieks 2015). These patterns can be combined with the identification of honey- bee-collected pollen to yield both spatial and taxonomic insight into the relationship between a colony and its surrounding landscape. Such insight is central to the question of how honey bees are exposed to seed treatment particles during corn planting, particularly via the floral contamination route of exposure **(Section 1)**.

Dance analysis methods. A glass-walled observation hive (Bonterra Bees, Addison, ME; SV-3TV), housed in a temporary shelter (Suncast Toter Trash Can Shed, **Figure 12)**, was installed at each of four apiaries: MB, HR, MO and FSR. These sites represented a range of corn abundance, consisting of 21%, 31%, 41%, and 49% corn within a 2 km radius, respectively. These sites also varied in landscape complexity, from the mosaic of

small crop fields, residential lots, and uncultivated areas at MB to the more homogeneous crop-dominated landscape at FSR **(Figures 16-19)**. Weedy fields were relatively abundant prior to planting at MB, HR, and MO, but were extremely scarce at FSR, reflecting local differences in tilling and herbicide practices **(Figure 3)**. HR and especially MC had notably more residential habitat than MO or FSR.

Each observation hive consisted of three standard deep frames populated with bees, brood, and a naturally mated queen. Using a wooden diverter at the hive entrance (Seeley 1995), all returning foragers were directed to one face of the bottom frame from which video was recorded using an HD video camera (Canon Vixia HF G20). We recorded dances only on days when weather conditions were favorable for foraging (sunny or partly cloudy with temperature above 65 F). Approximately one hour of bee activity video was recorded at the observation hive on a recording day. We then subsampled one 60-second segment for every 5 minutes of the video (12 segments per hour) and decoded all dances contained in these segments, following Couvillon et al. (2012) adapted for use with FIJI biological image analysis software (Schindelin et al. 2012). Decoded dances were then mapped using the probabilistic method described by Schürch et al. (2013) and in implemented in R software(R Core Team 2015).

Figure 12: Shed housing a 3-frame observation hive.

Pollen identification methods. Pollen was collected every 3-4 days from two healthy queen-right overwintered colonies at each site using bottom mounted pollen traps (Sundance I). Pollen traps remained in the “on” position throughout the study period. Because of the effect that continual pollen trapping may have on colony health we did not collect any other data from the colonies used for pollen collection. Pollen was pooled from the two colonies to provide a single pollen sample for each site, which was weighed, bagged and stored at -20°C. A total of 100 pollen samples (10 sampling dates per site) were collected during April 26 - May 27.

Microscopic pollen identification. Pollen samples were identified by pellet color and by microscopic palynology (Erdtman 1969). Ten grams of pollen from each site and for each collection date were sorted into distinct color categories, and the relative proportion of each color category was estimated by weight. A 10% subsample from each color category was blended in water and four drops of the pollen suspension were mounted separately in basic fuchsin jelly on glass slides for microscopic examination. The pollen type(s) associated with each color category were determined by microscopic comparison with reference pollen collected from fresh flowers.

Molecular pollen identification. The development of novel techniques for the molecular identification of bee-collected pollen has been fruitful and we have published two papers detailing our methods (Richardson et al. 2015; Richardson et al. 2015). While these methods papers have provided a strong foundation for the nascent field of molecular pollen analysis, we are currently working to further improve our method in order to

increase sample throughput capacity, decrease costs and improve our data analysis approach.

Using our current protocol, DNA is first extracted from pollen samples using a bead beater and the QIAGEN DNeasy Plant Mini Kit. Five plant barcoding loci, *ITS2*, *matK*, *rbcL*, *trnL* and *trnH*, are then amplified in separate PCR reactions using primers modified to include the Illumina MiSeq read priming oligo at the 5' end of each primer. At this point, 1 µL of PCR product from each reaction is used in a second PCR to append sample-specific dual indices and the Illumina MiSeq lane hybridization oligo to each amplicon library as in McFrederick et al. (2016)2015. Following this second PCR, 3 µL of product are analyzed using gel electrophoresis to ensure amplification success and 20 µL of the remaining PCR product are purified and normalized for sequencing using the SequalPrep Normalization Plate Kit. Normalized libraries are then pooled in equimolar amounts and the resulting pool is analyzed on a Qubit 2.0 fluorometer and an Agilent 2100 Bioanalyzer to ensure adequate quality and concentration before being sequenced on an Illumina MiSeq platform.

To better analyze our pollen metabarcoding sequence data, we have been working closely with Johan Bengtsson-Palme, author of the Metaxa2 sequence classifier (Bengtsson-Palme et al. 2015), to improve the bioinformatics pipeline used to infer plant species from sequence data. This new approach outperforms other classifiers in terms of both accuracy and sensitivity.

Results. Pollen samples collected at the four apiaries with observation hives have been identified using microscopy and quantified during the corn-planting period **(Figure 13, Table 5)**. Pollen samples collected on April 29, before corn-planting, consisted of 29 – 90% herbaceous plants, predominantly dandelion (*Taraxacum officinale*), mustards (family Brassicaceae) and purple deadnettle (*Lamium purpureum*). The majority (>95%) of the herbaceous pollen sources are weeds found in fields, field margins, roadsides, lawns and uncultivated herbaceous vegetation.

Pollen from herbaceous plants gave way to pollen from trees and shrubs as farmers began planting corn and soybean around May 2. Only 3 – 14% of the pollen collected on May 8 originated from herbaceous plants. During this time, wild and cultivated trees in the Family Rosaceae, such as hawthorn (*Crataegus* spp.), apple (*Malus* spp.), cherry (*Prunus* spp.), and serviceberry (*Amelanchier* spp.) were the most foraged pollen sources by honey bees. After the rosaceous trees, the second most abundant pollen collected by bees was from ash trees (*Fraxinus* spp.). Pollen of other trees including willow (*Salix* spp.), oak (*Quercus* spp.), mulberry (*Morus* spp.), and trees in the family Fabaceae (e.g., redbud, *Cercis canadensis*) were also common in our samples. Bees also collected pollen from honeysuckle (*Lonicera* spp.) and autumn olive (*Elaeagnus umbellata*), which commonly grow near forest edges and along roadways. The phenological switch from field weeds to mass-flowering trees and shrubs occurring from late-April to mid-May is consistent with our 2013 – 2014 pollen collection data.

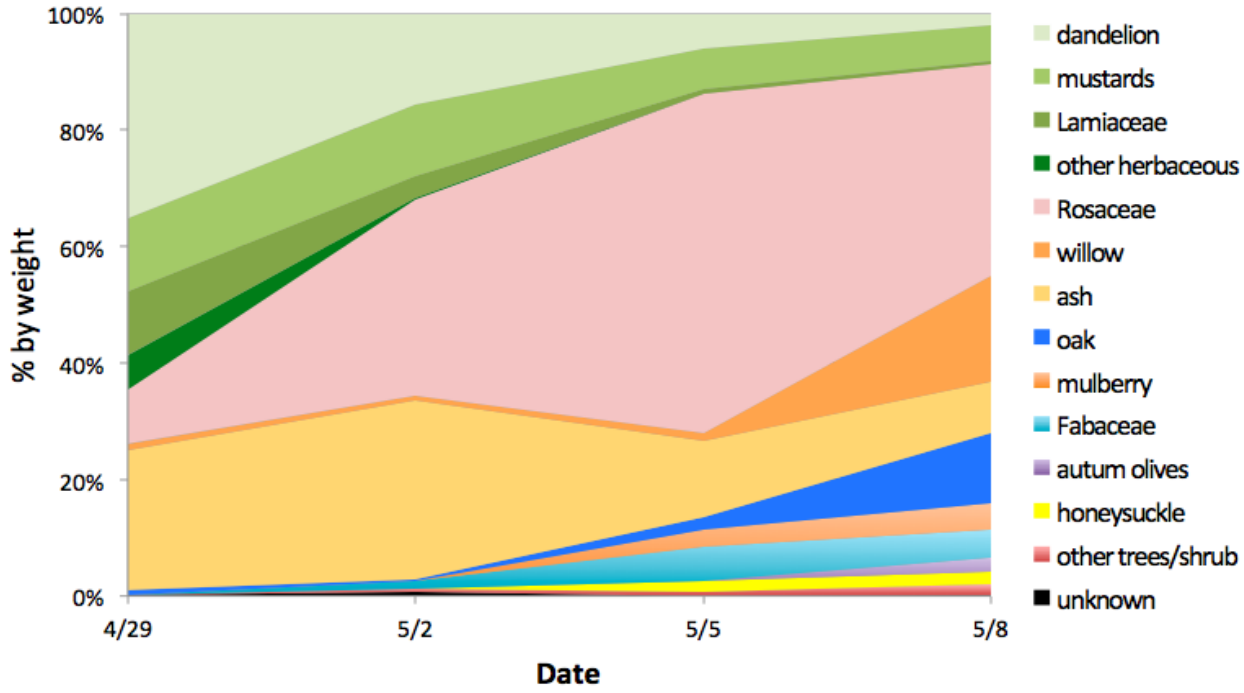


Figure 13. Pollen types collected from four sites (FSR, MO, HR, and MB) from April 29 – May 8. The percent abundance shown for each pollen type is the average of its percent abundance across all four sites.

	Trees and shrubs					Herbaceous plants			
Site	Rosaceae	Ash	Willow	Other	Sum	Dandelion	Mustards	Other	Sum
FSR	42.2%	22.6%	1.2%	7.6%	73.6%	22.6%	1.3%	2.4%	26.3%
MO	25.6%	15.6%	10.6%	4.4%	56.1%	9.7%	27.7%	6.4%	43.8%
HR	23.2%	23.0%	4.2%	18.7%	69.1%	13.1%	6.5%	10.6%	30.2%
MB	53.8%	14.0%	6.4%	16.1%	90.3%	9.6%	0.01%	0.2%	9.7%
Overall	35.4%	19.0%	5.6%	11.6%	71.4%	13.9%	9.3%	5.1%	28.4%

Table 5. Summary of major pollen sources collected from April 29 - May 8.

Dance analysis revealed that foraging activity tended to occur within a 3 km radius **(Figure 14)**, but foraging distances varied between sites **(Figure 15)**. Foraging "hotspots" generally agreed with the phenological changes observed in the assemblages of bee-collected pollen. At MB, HR, and MO, where weedy fields were abundant, dances indicated frequent foraging activity in fields close to the hive on May 4 - 5. This pattern corresponded with the higher proportion of weed pollen collected before May 5 at these sites. As field weeds were removed and fields were prepared for planting, bees increasingly foraged on resources outside crop fields and, consequently, collected less pollen from weeds. Bees returned to forage in fields toward the end of May after most planting had been done and other wildflowers were beginning to emerge. At FSR, where very few floral resources were present in the surrounding fields, bees were forced to travel farther to find floral resources. As a result, bees at this site on average foraged for longer distance and the hotspots were relatively diffuse **(Figure 15)**.

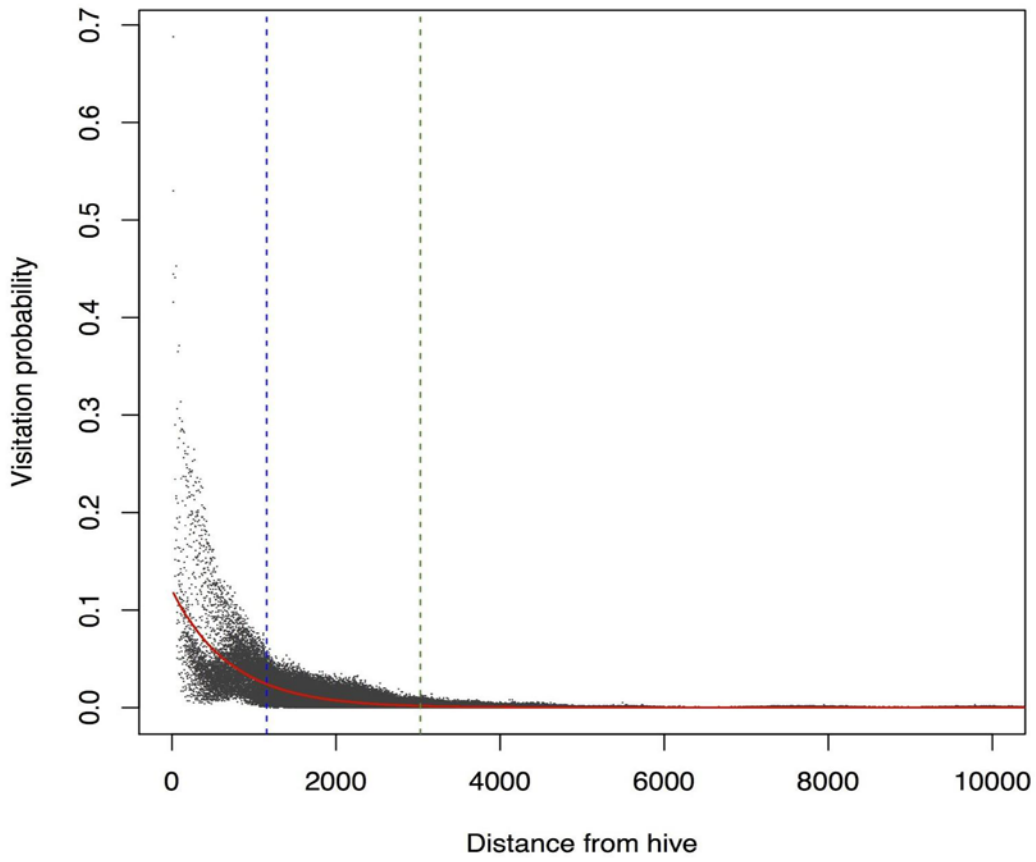


Figure 14: Visitation probability by distance, pooled across all sites and dates. Dashed lines indicate the distances at which 50% (blue) and 95% (green) of total visitation probability were accounted for. Visitation probability can be understood as a proxy of total foraging activity. When data were pooled across all sites, the 50% of

foraging activity occurred within about 1100 m of the hive, and 95% of foraging activity occurred within about 3000 m from the hive. The red curve represents a nonlinear least squares regression of visitation probability on distance.

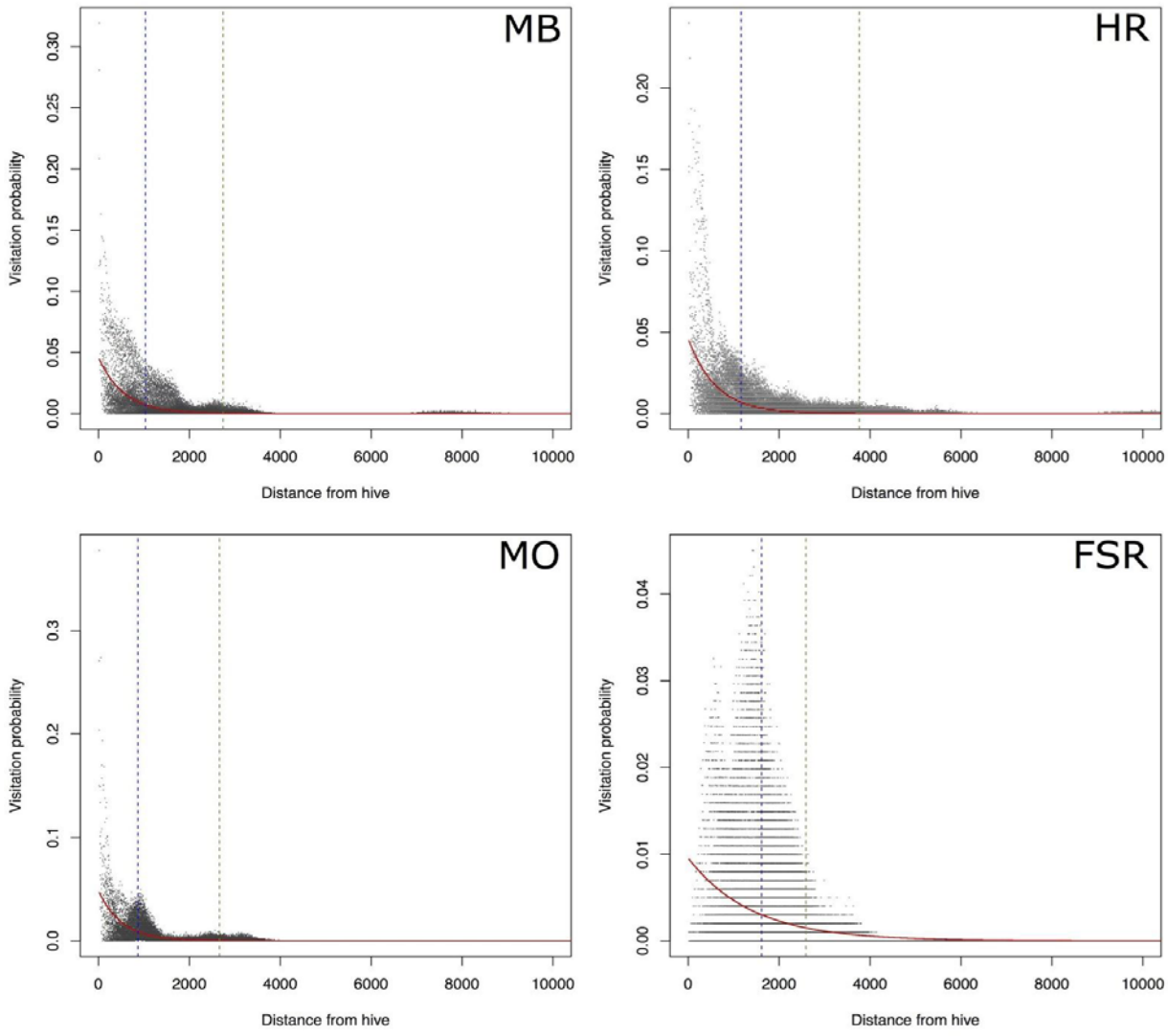


Figure 15: Visitation probability by distance at each site. Dashed lines indicate the distances at which 50% (blue) and 95% (green) of total visitation probability were accounted for. Visitation probability can be understood as a proxy of total foraging activity. The red curve represents a nonlinear least squares regression of visitation probability on distance.

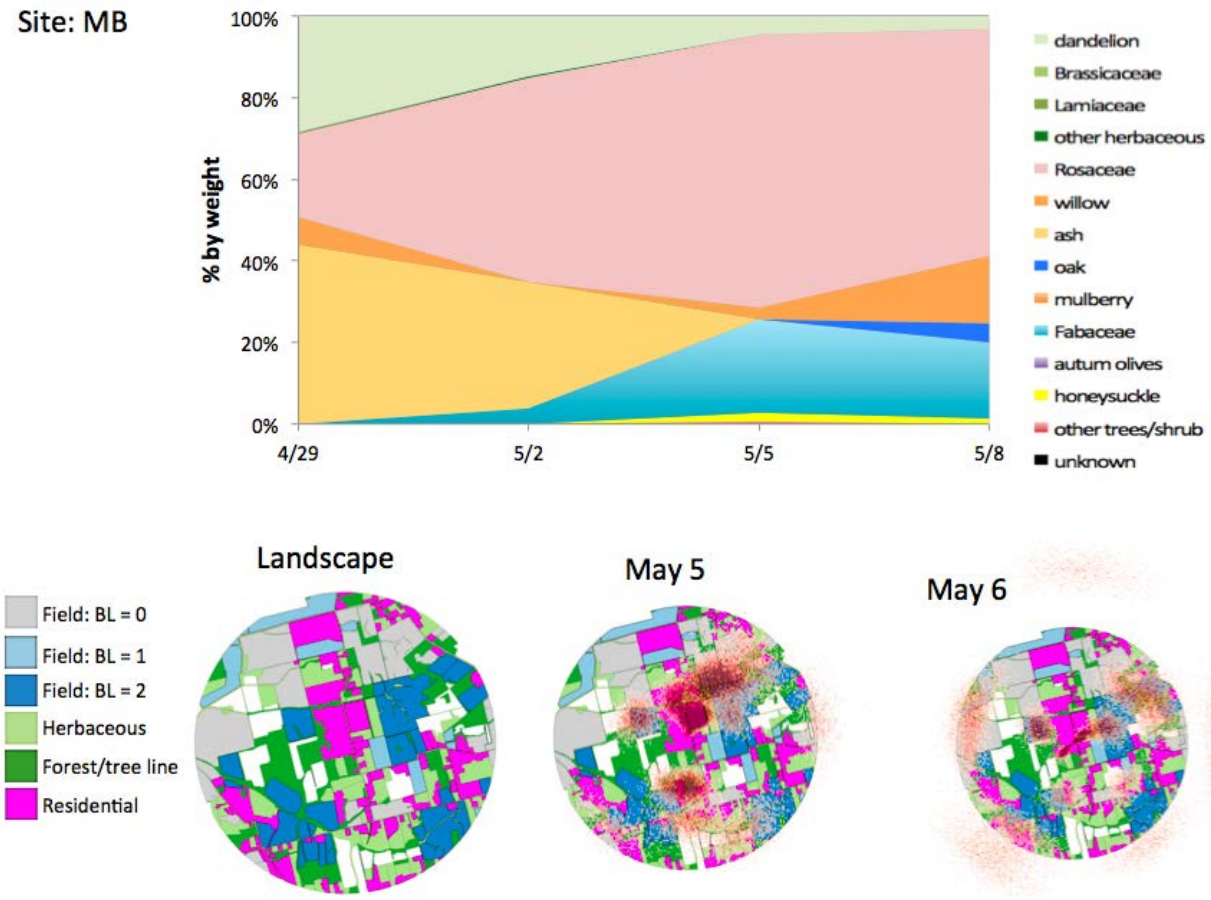


Figure 16. Pollen collected by bees at MB on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). Bees foraged over all land classes, with higher foraging probability over wooded and residential areas. On May 5 (35 dances recorded), foraging was concentrated near residential areas and tree lines close to the apiary. On May 6 (26 dances), activities occurred throughout the 2 km radius area with high concentrations in the residential area where the apiary was located. Some bees were also foraging beyond the 2 km range.

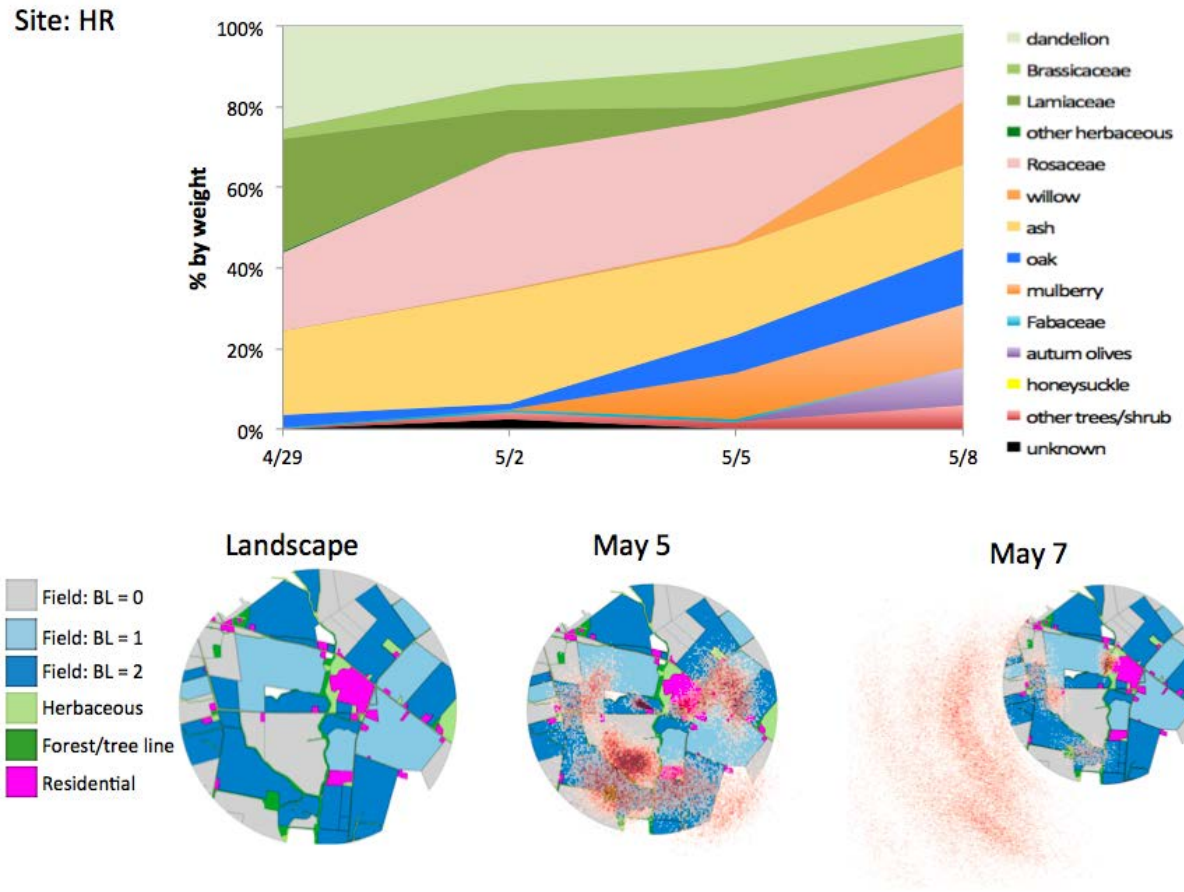


Figure 17. Pollen collected by bees at HR on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). On May 5 (26 dances), bees foraged predominantly in weedy areas and adjacent tree lines; very concentrated activities were observed in the weedy field adjacent to the apiary. On May 7 (33 dances), foraging activity was reduced in weedy fields and became more concentrated along tree lines; bees also foraged beyond the 2 km range to the SW of the apiary. We also noticed farmers applying herbicides to many of the fields here during this time. Removal of field weeds may have driven the bees from fields to forage on trees and travel longer distances for suitable resources.

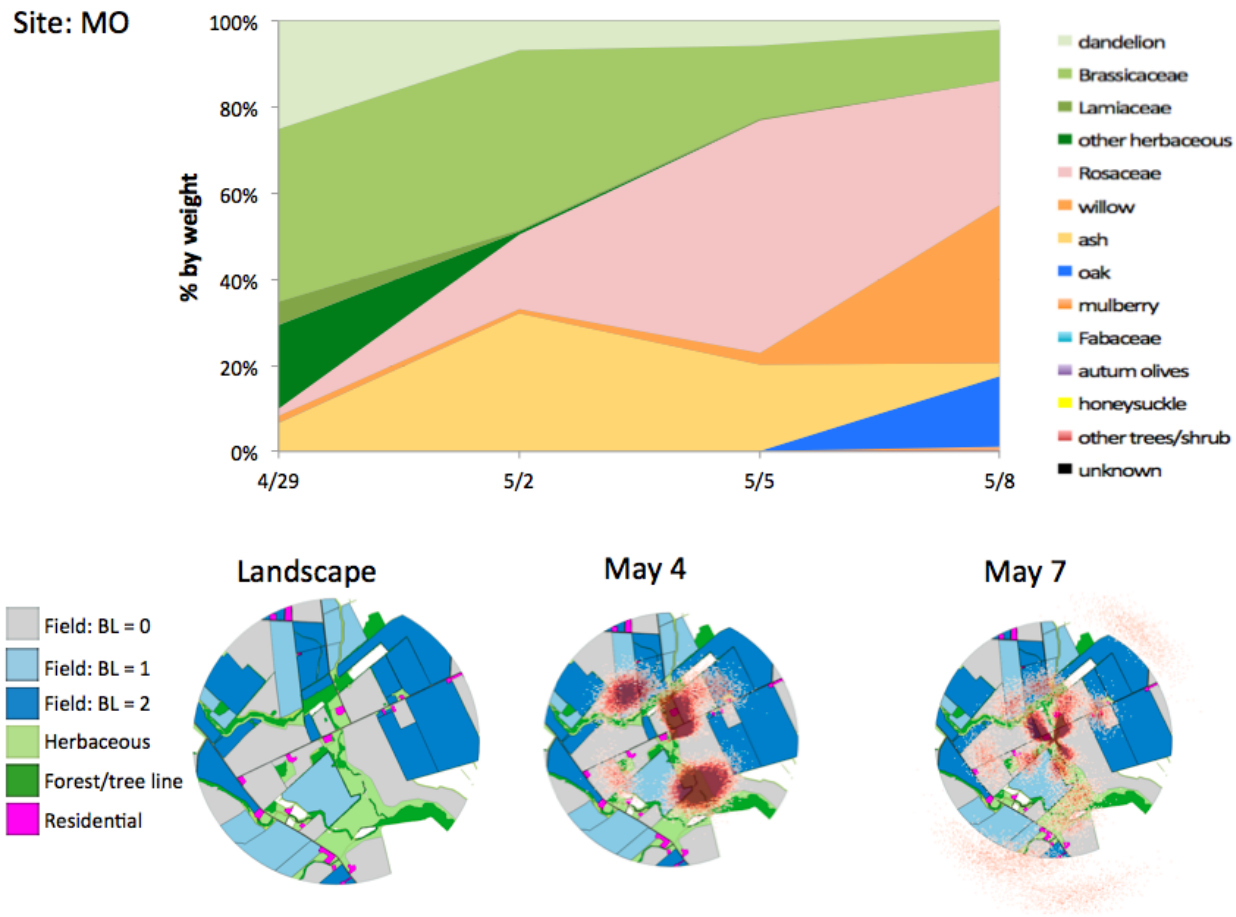


Figure 18. Pollen collected by bees at MO on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). On May 4 (35 dances), dense foraging occurred about 1.5km from the hive in fields, forests and uncultivated herbaceous habitats. On May 7 (27 dances), foraging became less intense in fields and more concentrated in uncultivated areas and at longer distances from the hive.

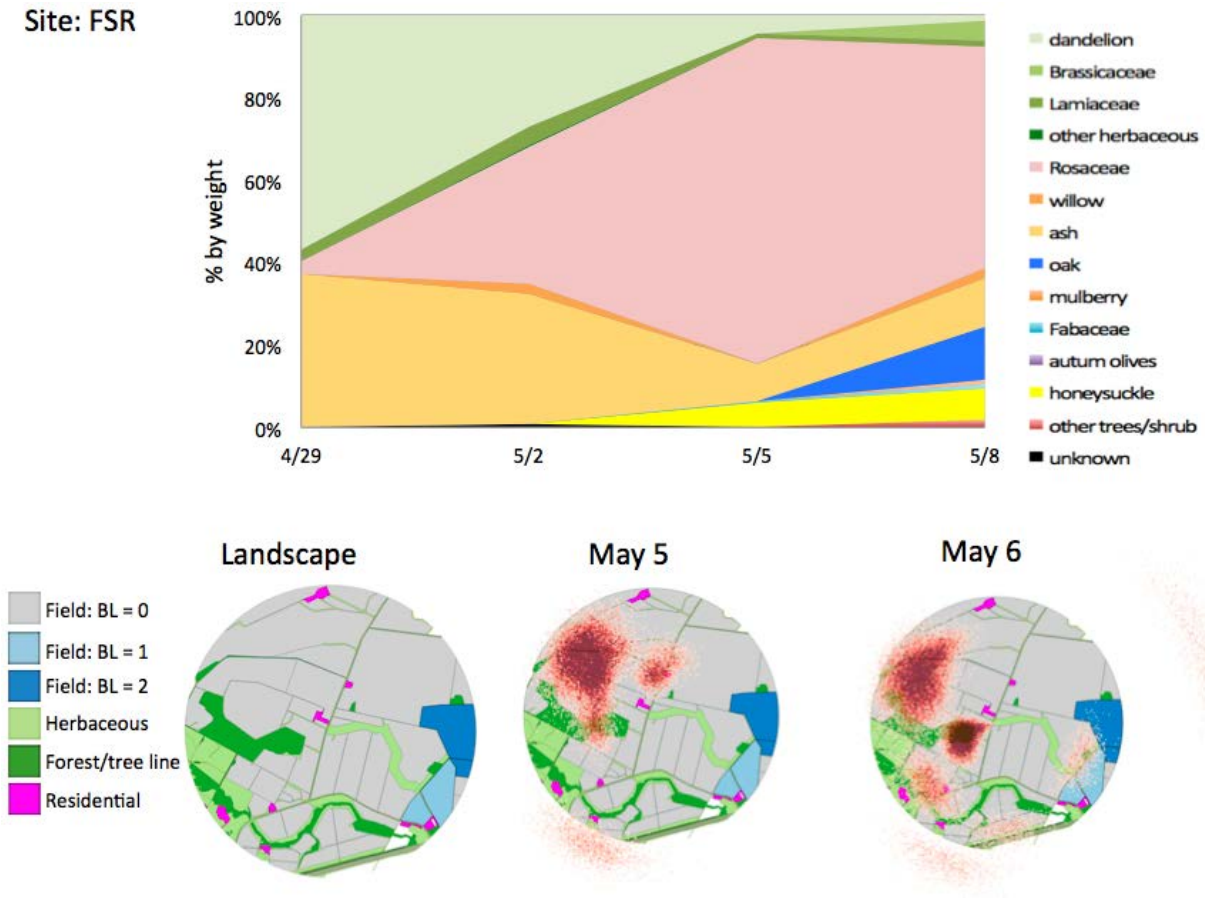


Figure 19. Pollen collected by bees at FSR on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and left). On May 5 (26 dances recorded), bees foraged heavily on the field margins, forest edges, and the residential lot to the west and NW of the hive. Some bees were also foraging approximately 2.2 km on the SW near an interstate highway. Similar resources were being used on May 6 (35 dances), with more concentrated activities near the forested area. Additional activities were observed on the east side along field margins and approximately 3 km outside the mapped landscape.

SECTION 7:

Landscape as a predictor of exposure and effects

Testing predictions of hypothesized routes of exposure. Each of the four routes of exposure (ROE) proposed in **Section 1** implies a distinct prediction about the role that landscape plays in modulating the exposure of honey bees to seed treatment particles (e.g. pollen residues) and the effects thereof (e.g. adult mortality) **(Table 6)**. Thus, the

relationship between landscape and exposure/effects can be used to test the plausibility of each ROE.

Route of exposure	**Predicted relationship to landscape**
Floral contamination: in-field settling	Exposure and effects are a function of the amount of corn field area containing blooming weeds at the time of planting.
Floral contamination: off-field drift	Exposure and effects are a function the amount of intersection between the off-field drift of dust and honey bee foraging habitat
Aerial contact: localized plume	Exposure and effects are a function of total corn area surrounding the colony
Aerial contact: ubiquitous dispersal	Exposure and effects are a function of total corn area surrounding the colony and possibly regional corn prevalence

Table 6: Predictions of each hypothesized route of exposure.

Methods. The landscape surrounding each of our apiaries was first digitized and characterized according to the methods described in the **Introduction**. Further processing was necessary, though, for the statistical testing of hypothesized ROE.

First, the concept of "foraging habitat" had to be formalized in relationship to our landscape classification. Based on the floral surveys we conducted in the field, we chose to classify the following landscape elements as foraging habitat: bloom level 2 crop fields, residential areas, forests/tree-lines, and non-crop herbaceous vegetation (roadsides, field margins, fallow fields).

Next, we rasterized the vector layers of our digitized landscapes and performed a series of functions using the R packages "raster", "rgdal", and "rgeos".

1. Using the rasterized crop layer from each of our landscape, we calculated a new raster layer in which the value of each cell was equal to its distance to the nearest corn field (*corn distance raster*).
2. We fit our 2014 dust drift data with a non-linear function modeling the relationship between dust deposition and distance from corn field edge. We then applied this function to the corn distance raster described above to create a new raster of predicted neonicotinoid concentrations (*contamination raster*).
3. Because honey bee foraging is strongly constrained by distance, a contaminated patch located close to a colony poses greater risk than an equally contaminated patch far from the colony. We formalized the distance-bias of honey bee foraging

by fitting a non-linear function to our pooled dance data **(Section 6)** that models visitation probability as a function of distance from the hive. We then applied this function to a raster whose cell values were equal to the distance of each cell from the hives at the center of the landscape. This resulted in a new raster predicting the probability of each patch being visited based solely on its distance from the hive (*visitation probability raster*).

4. We then multiplied the *contamination raster* by the *visitation probability raster* to yield a raster in which contamination values (i.e. hazard) are weighted by distance (i.e. probability of exposure) to yield a *risk raster* (risk = hazard x exposure).
5. This *risk raster* was then constrained in various ways to isolate the components of risk needed to test each hypothesized ROE.
    a. In-field settling hypothesis => *risk raster* constrained to bloom level 2 corn fields
    b. Off-field drift hypothesis => *risk raster* constrained to foraging habitat outside corn fields
    c. Localized plume hypothesis => *risk raster* unconstrained by habitat type
    d. Ubiquitous dispersal hypothesis => *risk raster* replaced with non-distance-weighted corn area

The cumulative risk (sum of all cells in the raster) of each constrainment of the *risk raster* was then used as the explanatory variable in a statistical test of each hypothesized route of exposure **(Table 7)**. In each test, cumulative pollen seed treatment residues during the peak corn planting window **(Section 3)** and the adult mortality ratio **(Section 4)** were used separately as response variables. Because of skew in our data, we used the nonparametric Spearman's rank-order correlation coefficient for all tests. Also, one study site (SD) was omitted from the test of the in-field floral contamination hypothesis because 40% of its corn fields were inaccessible for pre-planting bloom assessment.

Results and Discussion. Pollen seed treatment residues were significantly predicted by unweighted corn area (rho = 0.77, p = 0.01) **(Figure 20)**. All other tests indicate no significant relationship (p > 0.05) between landscape and either pollen seed treatment residues or adult mortality **(Table 7)**. The in-field floral contamination route is refuted by our finding that FSR, which had zero bloom 2 corn field area, also had the highest neonicotinoid residues in pollen samples **(Section 3)**. These findings provide support for the ubiquitous dispersal hypothesis, though this support is weakened by the failure of unweighted corn area to predict adult mortality. It is likely that the influence of landscape is obscured by other drivers of exposure and effects, such as variation in seed treatment quality **(Section 2)** and local climatic conditions.

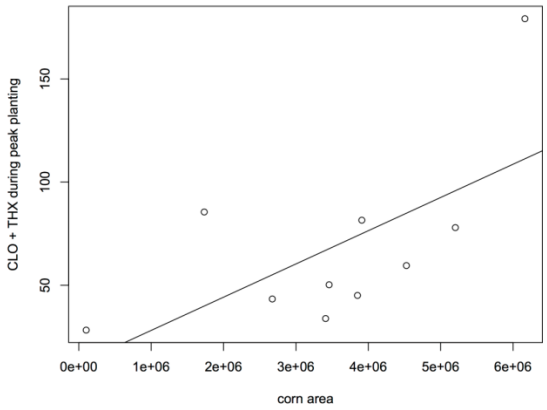


Figure 20: The sum of clothianidin and thiamethoxam residues found in pollen during corn planting was positively correlated with corn area.

ROE	Model	Spearman's rho	p-value
In-field settling	pollen residues ~ risk $_{\text{weedy corn}}$	0.286	0.501
	adult mortality ~ risk $_{\text{weedy corn}}$	0.214	0.620
Off-field drift	pollen residues ~ risk $_{\text{non-corn foraging habitat}}$	0.479	0.166
	adult mortality ~ risk $_{\text{non-corn foraging habitat}}$	-0.248	0.492
Localized plume	pollen residues ~ risk $_{\text{total}}$	0.636	0.054
	adult mortality ~ risk $_{\text{total}}$	0.006	1
Ubiquitous dispersal	pollen residues ~ corn area	0.770	0.014*
	adult mortality ~ corn area	0.430	0.218

Table 7: Statistical models testing hypothesized routes of exposure by relating landscape variables to exposure and effects. Pollen insecticide residues were positively correlated with corn area, but all other models were non-significant.

SECTION 8:

Simulation modeling of exposure via floral contamination and its sensitivity to in-field weed prevalence

In addition to the statistical modeling described above **(Section 7)**, we also approached the problem of honey bee exposure to seed treatment particles from the perspective of simulation modeling. Honey bees, like other motile organisms, experience pesticide exposure as the spatiotemporal intersection of contamination and foraging activity. Conventional models of honey bee exposure assume that honey bee foraging occurs on a single uniformly contaminated crop (Wisk et al. 2014). While such models may be useful for screening-level risk assessment, they provide no mechanistic insight into how exposure occurs under natural foraging conditions where individual bees are exposed a range of doses arising from differentially contaminated habitats. Thus, there is a need for models of honey bee pesticide exposure that capture the key mechanisms of contamination and honey bee foraging activity to generate stochastic and distributional predictions of exposure

Accordingly, we developed a distributional and stochastic model of honey bee exposure to seed treatment particles via the floral contamination route. The goals of our model are to (1) characterize the distribution of exposure levels experienced by colony members on an individual basis and (2) evaluate the sensitivity of exposure to the prevalence of in-field flora.

Methods

Landscape submodel. Simulation environment consists of a 4000 x 4000 array of 1 x 1 meter patches representing an idealized corn/soybean rotation system composed of three habitat types: corn fields, soybean fields, and interstitial strips (roadsides and field margins) **(Figure 21)**.

- Field geometries generated by voronoi tessellation => 25 fields with an average size of 64 ha
- 10 fields (40%) randomly assigned to corn, 15 to soybean (60%), consistent with Ohio crop data
- Linear field borders laterally expanded 5 m into adjacent fields, resulting in 10 m wide interstitial strips representing field margins
- 7/15 soybean fields and a variable number of corn fields set to “weedy”, i.e. containing flowering plants attractive to honey bee foragers; weedy fields and field margins together comprise the “foragable” subset of the simulated landscape

Pesticide drift submodel. Drift of neonicotinoid-laden dust modeled by fitting the field data of Lin et al. (in prep) with a function relating active ingredient deposition to distance from field edge **(Figure 21)**.

Foraging submodel

- Simulated colony draws 10 foraging focal points from the foragable subset of the landscape using a random but distance-biased algorithm. Distance-bias is calibrated to honey bee dance language data generated in the Ohio corn/soybean landscape (Lin et al., in prep) **(Figure 21)**
- 100 simulated "foragers" allocated to each of the 10 foraging focal points, starting randomly within a 250 m radius.
- Foragers proceed in a 10-step random walk; in each step, the forager "picks up" a concentration of pesticide; net exposure = mean concentration over 10 steps
- Colony-level exposure represented as histogram of net exposure experienced by each of 1000 foragers.

Weed control experiment. The presence of flowering weeds in corn fields at the time of planting varies according to tilling and herbicide practices, and may be an important modulator of exposure. We evaluate the influence of weed prevalence in corn fields using the following design:

- Presence of flowering weeds in fields modeled as a binary variable, i.e. presence/absence.
- Soybean fields set to constant 7/15 fields weedy, consistent with typical conditions in Ohio
- Weed prevalence in corn fields set to 0/10, 1/10, 2/10, 5/10, 8/10, and 10/10, respectively.
- Model iterated 3 times at each level of weed prevalence

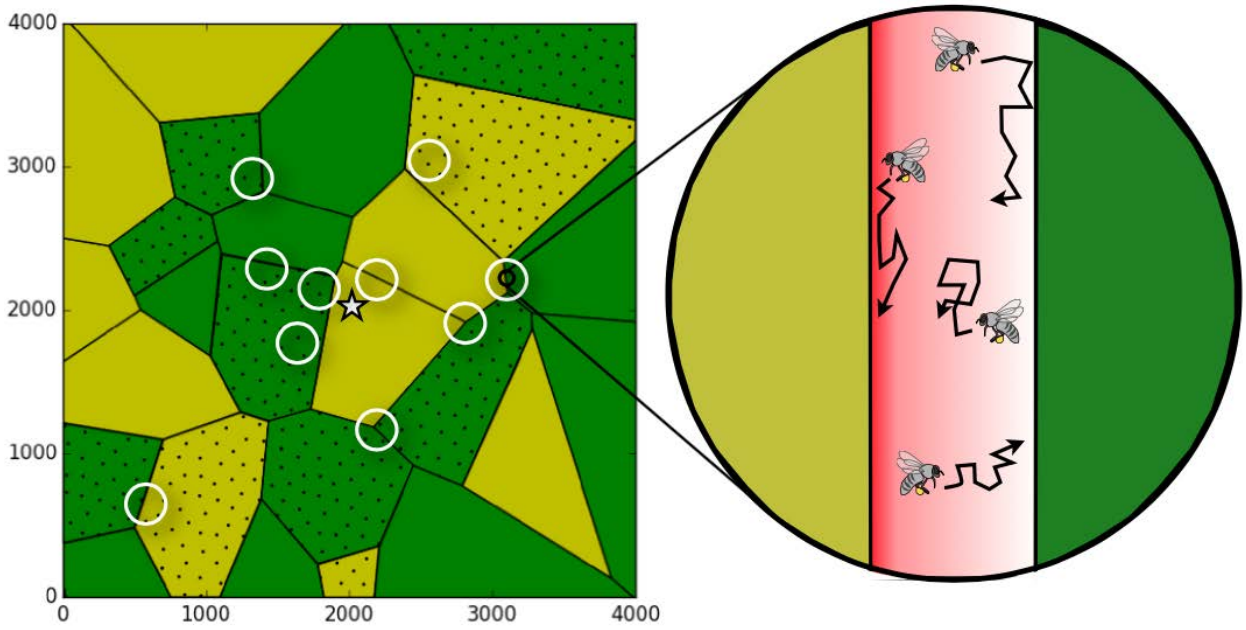


Figure 21: Model simulates a honey bee colony (star) surrounded by corn (yellow) and soybean (green) fields separated by narrow interstices of non-crop habitat. Dust drift

simulated by distributing a contamination gradient according to distance from corn field edge. Weedy (foragable) fields are represented by dotted fill. To simulate foraging, 10 foraging foci are drawn from foragable patches (weedy field or interstitial strip) using a random, distanced biased algorithm. Then, the foraging of individual bees is represented by 100 ten-step random walks distributed within each focal area.

Results and Discussion

Exposure profiles are strongly bimodal, with a mode at zero representing foraging in uncontaminated habitat and a higher mode representing the contamination in and near corn fields **(Figure 22)**. In-field weed prevalence changes not only the magnitude of exposure but also the shape of the exposure distribution. When in-field blooms are absent, foraging is restricted to interstitial strips, resulting in a dispersed distribution of exposure levels. As in-field weed prevalence increases, weedy fields dominate the foraging environment, yielding a tighter distribution around the high mode. The model also exhibits strong stochasticity arising from randomization of landscape geometry, crop and weed assignment, and foraging simulation.

The strong sensitivity of this model to in-field weed prevalence might be interpreted to recommend in-field weed control as an effective means of mitigating honey bee exposure. Our statistical models **(Section 7)**, however, strongly refuted in-field floral contamination as a route of exposure. Interpreted in that light, our simulation model strengthens the conclusions of our statistical models. If in-field floral contamination were an important route of exposure, then our simulation model says that exposure should be highly sensitive to in-field weed prevalence in corn fields. Since exposure was not found to be correlated with weedy corn field area, there is strong evidence in favor of dismissing in-field floral contamination as a major route of exposure for honey bees during corn planting.

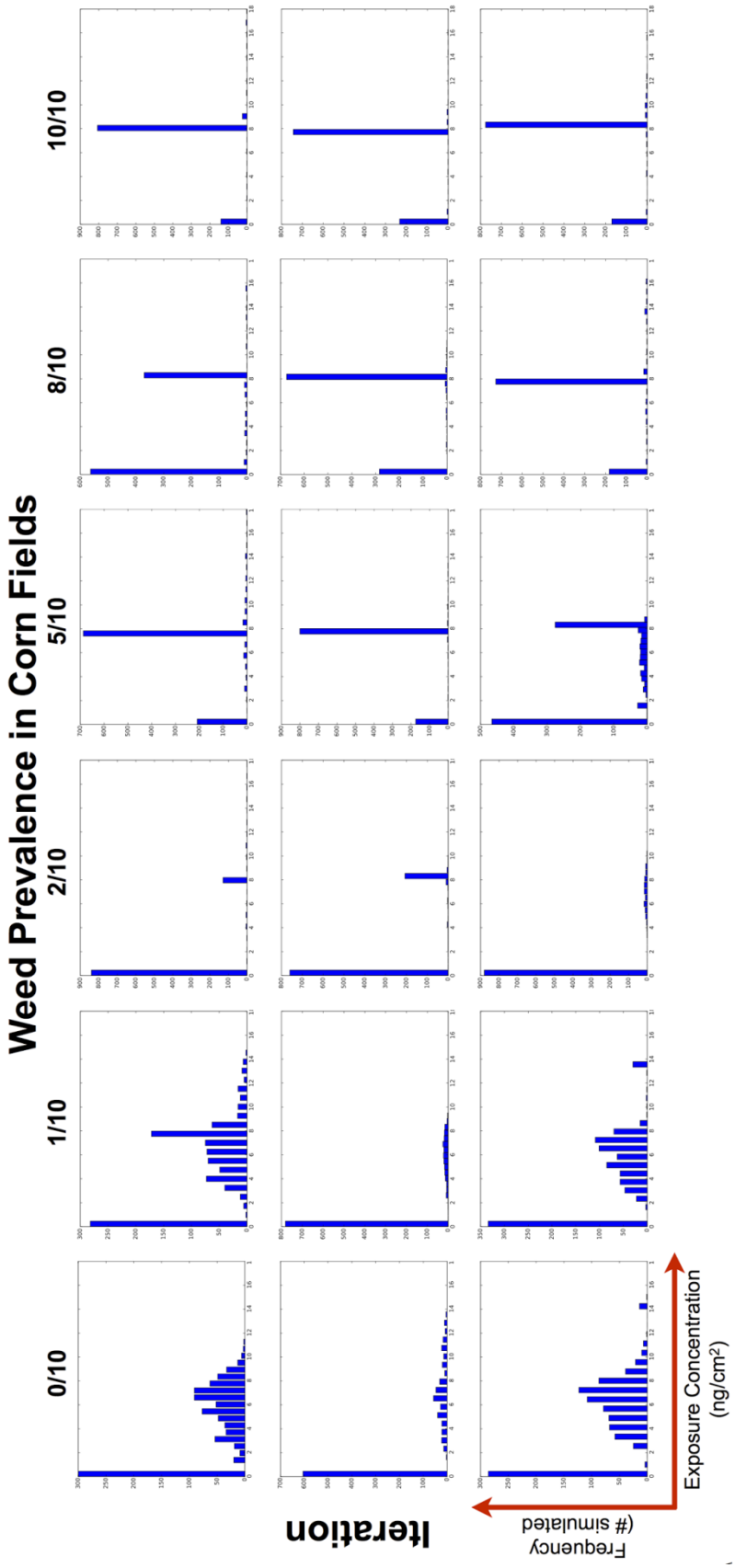


Figure 22. Exposure profiles arising from six levels of flowering weed prevalence in corn fields. Data visualized above represent the output of three iterations (rows) of the model run at each of six levels (columns) of weed prevalence in corn fields: 0/10 fields, 1/10 fields, 2/10 fields, 5/10 fields, 8/10 fields, and 10/10 fields. Each histogram depicts the frequency (y axis) with which 1000 simulated bees received varying levels of net exposure (x axis).

SECTION 9:

CONCLUSIONS and MITIGATION RECOMMENDATIONS

Release, exposure, and effects

Seed treatment insecticides are consistently released during the planting of treated corn. This release appears to be due to the failure of seed treatment material to adhere securely to the seed surface under the stresses of the planting process. Seed treatment active ingredients can be detected both in- and off-field.

During corn planting, seed treatment insecticides are consistently detected in honey- bee-collected pollen. Concurrently, honey bee colonies exhibit a 2.3-fold increase in the number of dead adult hive bees collected in dead bee traps (forager mortality would not be detected by our methods). This increase in mortality had no apparent effect on colony strength in subsequent months, or on The increase in adult mortality, however, would not be expected based on the concentrations of seed treatment insecticide measured in bulk pollen samples.

Route of exposure

While the concentration of seed treatment insecticides in bulk pollen appears to be a reliable indicator of overall exposure, it does not appear to fully represent the exposure that bees receive. The primary route of exposure that drives the mortality of honey bees during corn planting remains uncertain, but landscape analysis and the significant correlation between corn area and insecticide residues in pollen supports the idea that honey bees are exposed via aerial contact with seed treatment particles. We found no support for the hypothesis that honey bee exposure is driven by the contamination of floral resources, either by in-field settling or off-field drift of seed treatment particles.

Mitigating exposure

Because we found no support for the hypothesis that honey bee exposure is driven by the contamination of either in-field or off-field flora, mitigation schemes involving in-field weed suppression or off-field floral enhancement are unlikely to be effective and may be counterproductive. Instead, mitigation efforts should be aimed at preventing the initial release of seed treatment particles through engineering and quality control solutions that ensure seed treatment formulations are well-adhered to the seed**.** To the extent that initial release cannot be prevented, the aerial mobility of seed treatment particles could be minimized through planter modification, seed treatment reformulation or use of a fluency agent. An alternative to these approaches would be to

plant either untreated seeds or seeds treated with an insecticide exhibiting lower toxicity to honey bees.

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Appendix A. Levels of thiamethoxam and clothianidin detected in unsorted pollen samples. Highlighted dates indicate the period when corn planting activity was at its peak in Central Ohio.

Site	Date	Clothianidin (ng/g)	Thiamethoxam (ng/g)	CLO + THI (ng/g)
FSR	4/29	1.1	0.3	1.4
	5/2	73.0	32.2	105.2
	5/5	33.0	0	33.0
	5/8	0.5	0.6	1.1
	5/11	13.0	8.8	21.8
	5/14	1.3	1.4	2.7

	5/19	2.0	0	2.0
	5/23	4.1	0	4.1
	5/27	8.0	0	8.0
MO	4/29	0.0	0.4	0.4
	5/2	6.0	0.9	6.9
	5/5	17.7	1.2	18.9
	5/8	9.3	0.5	9.8
	5/11	0.5	0	0.5
	5/14	2.5	0	2.5
	5/19	5.5	3.9	9.4
	5/23	18.0	7.3	25.3
	5/27	3.3	1	4.3
TV	4/29	0.8	0	0.8
	5/2	7.4	0	7.4
	5/5	16.6	9	25.6
	5/8	4.8	0.6	5.4
	5/11	13.0	0.8	13.8
	5/14	0.4	0	0.4
	5/19	2.3	1.2	3.5
	5/23	0.0	1.5	1.5

	5/27	0.0	1.2	1.2
HR	4/29	6.8	0	6.8
	5/2	10.1	1.2	11.3
	5/5	9.8	1.7	11.5
	5/8	16.4	10.3	26.7
	5/11	1.7	0	1.7
	5/14	1.8	0	1.8
	5/19	6.4	4	10.4
	5/23	0.0	2.6	2.6
	5/27	0.0	9	9
WB	4/29	0.0	0	0
	5/2	10.7	0	10.7
	5/5	4.0	0	4.0
	5/8	2.1	11	13.1
	5/11	6.7	0	6.7
	5/14	1.9	0.5	2.4
	5/19	0.0	0	0
	5/23	0.0	4.7	4.7
	5/27	3.5	0	3.5
	4/29	8.5	0	8.5

BR	5/2	0	0	0
	5/5	0	3.2	3.2
	5/8	8.3	2.1	10.4
	5/11	3	2.8	5.8
	5/14	0.1	0.2	0.3
	5/19	0	0	0
	5/23	0	0	0
	5/27	5.7	0	5.7
IB	4/29	3.7	0	3.7
	5/2	0.9	0	0.9
	5/5	10.2	0.6	10.8
	5/8	5.9	2.9	8.8
	5/11	6.2	0.5	6.7
	5/14	1.4	4.9	6.3
	5/19	6.8	0	6.8
	5/23	0.0	0.4	0.4
	5/27	3.9	2	5.9
MB	4/30	2.0	2.2	4.2
	5/2	17.6	0	17.6
	5/5	8.1	0	8.1

	5/8	1.3	3.7	5.0
	5/11	0.6	0.5	1.1
	5/14	0.0	0.9	0.9
	5/19	1.4	0	1.4
	5/23	2.0	0.6	2.6
	5/27	2.3	0	2.3
SD	4/29	0.0	1.5	1.5
	5/2	17.1	3.3	20.4
	5/5	4.0	0.6	4.6
	5/8	2.7	0.3	3.0
	5/11	2.0	2.7	4.7
	5/14	0.3	0	0.3
	5/19	2.0	0	2.0
	5/23	37.0	12	49
	5/27	0.0	0	0
DS	5/1	4.1	1.6	5.7
	5/2	0.2	0.5	0.7
	5/5	1.6	0.6	2.2
	5/8	6.4	0.5	6.9
	5/11	1.5	0	1.5

	5/14	0.7	0.3	1
	5/19	2	0	2
	5/23	1.4	0	1.4
	5/27	0.8	6	6.8

Appendix B. Visual tool to interpret insecticide residues detected in bees, pollen and surfaces in reference to toxicity of the neonicotinoids clothianidin, thiamethoxam to adult worker honey bees.

Corn Dust Research Consortium 2015

University of Guelph Final Report

December 2015

Reducing honey bee exposure to neonicotinoid dust emitted during planting of treated corn seeds

Addresses Project 1: Use by honey bees of flowering resources in and around cornfields during spring planting, and how this behavior can be effectively managed to reduce exposure to pesticide dust and residues

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Objectives

This project was configured based on the results of 2013 and 2014, with the main purpose to identify the key sites of honey bee exposure to neonicotinoids resulting from the movement of fugitive planter dust. The objectives of this project were: (1) survey of flowering resources in and around corn fields during spring planting; (2) identify species of bee-collected pollen, and determine neonicotinoid residues in bee-collected pollen and dead bees at apiaries associated with corn fields; (3) quantify and characterize neonicotinoid contaminated fugitive dust escaping corn fields; and (4) quantify neonicotinoid residues on/in flowers that are main pollen sources, in honey bees foraging on these flowers, and in the soil these plants are growing in.

Summary of methods

Nine locations were studied, 8 of which were used previously in 2014, and one was newly recruited in 2015. Each location included an apiary and two fields; one corn and one soybean, canola, or corn. Field selection was based on the most suitable orientation to tree lines and bush in proximity (best downwind) of the field with the presence of trees/shrubs of *Salix* spp. (willow), *Crataegus* spp. (hawthorn), *Malus* spp, (apple, wild apple) and/or *Acer* spp.(maple).

Activities from April to June 2015 focused on field surveys and sample collection of flowering resources, foraging honey bees and dust during three sampling periods - the week before, the week during and 2 wk after planting. During each sampling period, blooming vegetation was surveyed in and around the 18 study fields and bee-collected pollen and dead bees were collected from the 9 apiaries. Samples of fugitive dust leaving the study fields were collected using sticky dust collection towers during tillage, planting or wind events. The 18 study fields were also surveyed for plant species that honey bees were actively foraging on during the three sampling periods. The two most abundant flowering plant species were identified from each field and collections of flowers, foraging honey bees, and soil to the 10 cm depth from the base of each plant were made. Representative pollen samples were sent to Johanne Parent (Laboratoire BSL, Rimouski, QC) for species identification. Neonicotinoid residues in dust traps, flowers, soil, dead bees and bee-collected pollen were determined by LC/MS/MS. The α level to determine statistical significance was set at 0.05 for all analyses.

Note: The experiments were designed as separate objectives. The testing of dust drift, while near apiaries, was not designed to allow testing of any direct impacts on honey bees in these apiaries.

Summary of results

Abundance of blossoms in and around cornfields: Before corn planting, the most abundant blossoms available in trees and shrubs were of *Acer spp.* and *Salix spp.* The most abundant herbaceous blossoms were of *Anemone*, *Taraxacum*, and *Viola spp.*. During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Salix, Liliaceae*, *Acer* and *Ulmus spp.* During the planting period between 29 April and 22 May, the most abundant blooming trees and shrubs were of Rosaceae, *Prunus*, *Salix* and *Acer spp*. The most abundant herbaceous blooms were of *Taraxacum*, and *Barbarea*, and *Alliaria spp.* During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Acer*, *Salix,* and Rosaceae *spp*. After planting, beyond 23 May, the most abundant blooming trees and shrubs were of Rosaceae and *Prunus spp*. The most abundant herbaceous blooms were *Brassicaceae*,

Lotus, *Barbarea*, *Asteraceae* and *Viola spp.*. During the same period, bee-collected pollen in associated pollen traps was comprised mainly of Rosaceae, *Salix*, *Rhamnus*, *Brassicaceae* and *Trifolium ssp.*

Neonicotinoid residue in bee-collected pollen and dead bees: The concentrations of neonicotinoids within bee-collected pollen during planting (9.0 ng/g) were significantly higher than before planting (2.2 ng/g) and after planting (2.9 ng/g). Neonicotinoid concentrations in bee-collected pollen during planting in 2015 were similar to that found in 2013 (9.3 ng/g), and 2014 (7.87 ng/g). Neonicotinoid concentration within dead bees increased significantly from before and during planting to after planting. No significant differences in neonicotinoid concentration were found on the surface of dead honey bees among the three sampling periods in 2015. The mean total neonicotinoid residues (those within and on the surface of dead honey bees combined) in 2015 was 1.32 ng/bee, which was similar to that measured in 2014 (1.42 ng/bee) and in the 2013 study (13.9 ng/g, assuming that 10 dead bees = 1 g).

Fugitive dust escaping fields: During corn planting, the mean neonicotinoid concentration captured on sticky traps was 2.16 (0.84 in 2014) and 0.12 (0.42 in 2014) ng/cm^2, for the downwind and neighbouring field edges, respectively. The variation between years may have been a result of the large seed treatment particle size captured by the traps. Using volumetric air samplers, the mean neonicotinoid concentration captured during corn planting at the downwind and neighbouring field edges was 32.58 (21.7 in 2014) and 14.30 (12.2 ng/m^3 in 2014), respectively.

During soybean planting, the mean neonicotinoid concentration captured on sticky traps were 0.11 and 0.10 ng/cm^2, for the downwind and neighbouring field edges, respectively. Using volumetric air samplers, the mean neonicotinoid concentration captured during soybean planting at the downwind and neighbouring field edges were 8.64 and 5.31 ng/m^3, respectively which were much lower than that measured during corn planting.

During tillage events, the mean neonicotinoid concentration captured on sticky traps were 0.01 and 0.04 ng/cm^2, for the downwind and neighbouring field edges, respectively. Using volumetric air samplers, the mean neonicotinoid concentration captured during tillage at the downwind and neighbouring field edges were 3.13 and 2.47 ng/m^3, respectively which were much lower than during corn or soybean planting.

During wind events the mean neonicotinoid concentration captured on sticky traps and volumetric air samplers in the downwind field edge were 0.02 ng/cm^2 and 1.82 ng/m^3, respectively which were much lower than during corn/soybean planting or tillage events.

Neonicotinoid residue on/in flowers, foraging honey bees on the flowers, and soil on the base of the blossoming plants:

Due to difficulty collecting from tall trees, there were not many blossoms of trees and shrubs with foraging bees collected. The trees/bushes included *Salix* (willow), *Acer* spp. (maple), Rosaceae, and *Robinia* (black locust). The anthers and pollen of the blossoms usually had lower neonicotinoid residue than on/in flowers (Table 9). However, the flower surface had

similar neonicotinoid residue as inside the flowers. *Salix* was the most important foraging resource of bees before corn planting (81.7%) (Table 10). During corn planting, *Acer* (35.2%), *Salix* (27.4%) and Rosaceae (27.4%) were the most important foraging resources for bees. The foraging bees collected from *Acer* spp. on May 1 had elevated neonicotinoid residues relative to other sources, which may have been related to fugitive dust at planting.

Similar to our 2014 results, lower levels of neonicotinoid residues were found on the surface of herbaceous flowers compared to inside blossom tissues (Figs. 15 -19). These data suggest that perhaps uptake of neonicotinoids from the soil or from leaf deposition during or after corn planting may result in greater residue levels in the plant. This effect may be less certain for trees which may be generally more distant from the source of neonicotinoid application in space and time, and have different morphology and physiology. Neonicotinoid residue levels found on the surface of foraging honey bees were higher than that inside of bees, which suggest that neonicotinoid exposure is from contact with the residue.

No significant correlation was found between the neonicotinoid concentrations in soil collected from the base of plants with that found in foraging bees or in the anthers/pollen of the plant blossoms (R^2 = 0.006, p = 0.472). Further there was no correlation between concentrations in anthers/pollen of blossoms and in the bees foraging on these blossoms (R^2 = 0.001, p = 0.792).

Site information

Experimental fields and apiaries: We worked again at 9 locations; each one with two fields and one apiary. One of the two fields was a corn field, and one soybean, corn, or canola. Sites 4 and 5 used in 2014 were combined into one site due to beehive accessibility; therefore one site (10) at Ridgetown, ON was added to the study. Field selection was based on the most suitable orientation to tree lines and bush in proximity (best downwind) to the field and presence of trees/shrubs of *Salix* spp. (willow), *Crataegus* spp. (hawthorn), *Malus* spp. (apple, wild apple) and/or *Acer* spp. (maple) (Table 1).

Table 1. Experimental fields and associated apiaries studied in southwestern Ontario in 2015.

Site #	County	Field/Apiary	GPS coordinates	Crop in 2014	Comments
1	Essex	1C	42.075947,-82.788867	Soybean	
		1D	42.101325,-82.790456	Corn	
		Apiary 1	42.101325,-82.790456	Treeline	
2	Essex	2A	42.190045,-82.596593	Corn	
		2C	42.177147,-82.595489	Soybean	
		Apiary 2	42.198964,-82.569236	Bush	
3	Chatham- Kent	3A	42.434817,-81.998893	Corn	
		3B	42.438886,-81.994086	Corn	
		Apiary 3	42.443377,-82.025256	Back yard	
4/5	Lambton	4B	42.906519,-82386902	Soybean	Merged from Site 4 & 5
		5A	42.927426,-82.342670	Corn	
		Apiary 4/5	42.921387,-82.347268	Bush	
6	Lambton	6A	42.827311,-81.727359	Corn	Fungicide only
		6D	42.830091,-81.911898	Soybean	
		Apiary 6	42.830101,-81.887554	Bush	
7	Middlesex	7C	42.812989,-81.731458	Soybean	
		7D	42.821947,-81.765375	Corn	
		Apiary 7	42.821894,-81.758160	Bush	

8	Middlesex	8A	43.101433,-81.501658	Corn	
		8C	43.099319,-81.488125	Soybean	
		Apiary 8	43.099451,-81.482637	Bush	
9	Elgin	9C	42.740948,-81.108048	Corn	
		9D	42.739912,-81.098815	Soybean	
		Apiary 9	42.724245,-81.089501	Bush	
10	Chatham- Kent	10A	42.427791,-81.873907	Corn	New recruit
		10B	42.423072,-81.869027	Canola	
		Apiary 10	42.423072,-81.869027	Farm yard	

Table 2. Hybrid, planting date, neonicotinoid seed treatment, and rate of Bayer Fluency Agent used by growers in 2015 corn planting studies.

Field	Crop	Hybrid	Planting date	Neonicotinoid insecticide used		Rate of Bayer Fluency Agent used (% of recommended[1])
				Active ingredient	Rate (mg a.i./kernel)	
1D	Corn	DKC 57-75RIB	2015-05-22	clothianidin	0.25	100
2A	Corn	DKC 50-78RIB	2015-05-04	clothianidin	0.25	100
3A & 3B	Corn	P0506AM // DKC 57-75RIB	2015-05-01	clothianidin	0.75	100
5A	Corn	A7270G8 // DKC 53-56	2015-05-01	clothianidin	0.25	100
6A	Corn	DKC 53-56	2015-04-29	clothianidin	0.25	100
6D	Corn	DKC 50-78	2015-05-03	None	0	100
7C	Soybeans	N/A	2015-05-25	None	0	0

7D	Corn	DKC 50-78RIB	2015-05-09	clothianidin	0.125	100
8A	Corn	DKC 50-78	2015-05-02	clothianidin	0.25	100
8C	Soybeans	Chikala	2015-05-19	Thiamethoxam	0.122	0
9C	Corn	DKC 50-90	2015-05-13	Clothianidin	0.25	100
9D	Soybeans	P22T69R-KA26	2015-05-20	Thiamethoxam	0.33	N/A
10A	Corn	P0496AMX-KM67	2015-05-04	clothianidin	1.25	100

[1] Recommended application rate for corn: 1/8 cup per 80,000 seeds.

Modifications to original protocol and deliverables

To maximize the samples of fugitive dust escaping corn fields, we combined the 3 sticky traps samples from the 3 replicates for each of the proximal or distal locations in corn fields into one LC/MS/MS injection to allow for additional air sampler samples (original sticky trap deliverables 108; final sticky trap + air sampler deliverables: 80+41 = 121) (Table 3).

Table 3. Number of samples from sticky dust traps and volumetric air samplers collected in 2015.

Field	Crop	Planting date	No. samples during tillage events	No. samples during planting	No. samples during wind events	No. samples from volumetric air sampler
1C	Soybean	after May 31	-	-	-	
1D	Corn	2015-05-22	-	6	-	2 planting
2A	Corn	2015-05-04	-	6	-	2 planting
2C	Soybean	after May 31	-	-	-	1 tillage
3A	Corn	2015-05-01	6	6	-	2 tillage, 2 planting
3B	Corn	2015-05-01	6	6	-	2 tillage, 2 planting
4B	Soybean	missed	-	-	-	
5A	Corn	2015-05-01	-	6	-	2 planting
6A	Corn	2015-04-29	6	6	-	2 tillage, 2 planting
6D	Soybean	2015-05-03	-	6	-	2 planting
7C	Soybean	2015-05-25	-	6	-	2 planting
7D	Corn	2015-05-09	-	6	-	2 planting
8A	Corn	2015-05-02	6	6	-	2 tillage, 2 planting
8C	Soybean	2015-05-19	-	6	-	2 planting
9C	Corn	2015-05-13	-	6	-	2 planting
9D	Soybean	2015-05-20	-	6	-	2 planting
10A	Corn	2015-05-04	-	6	-	2 planting

10B	Canola	Winter canola	-	-	-	
Extra 1	Corn	2015-05-22*	-	-	3	1 wind event
Extra 2	Corn	2015-05-22*	-	-	3	1 wind event
Extra 3	Corn	2015-05-22*	-	-	3	1 wind event
Extra 4	Soybean	2015-05-22*	-	-	3	1 wind event

Methods and results

1. Survey of flowering resources in and around corn fields during spring planting and species identification of bee-collected pollen

1.1. Method

Vegetation in bloom was surveyed in and around the 18 study fields weekly from 28 April to 3 June 2015. For each field and bush, one side of the perimeter with the most abundant blooming species was selected for the survey. Photos of blooming plants, trees and shrubs in the different zones were captured using iPhone and iPad devices and geo-referenced using Photo GPS Extractor free software (http://pge.bvsoft.be/). The plant species and their spatial densities were identified and categorized using an abundance index (AI) of 0-4 where 0 = not observed, 1 = 0-5 individuals observed/m^2 for herbaceous weeds or 0-1 observed/100 m^2 for trees and shrubs, 2 = 6-10/m^2 for weeds, 2-5/100 m^2 for trees and shrubs, 3 = 11-20/m^2 for weeds, 6-10/100 m^2 for trees and shrubs, and 4 = >20/m^2 for weeds, >10/100 m^2 for trees and shrubs. The percentage of occurrences of patches of a blooming plant species was considered its frequency; this was multiplied by the AI value to determine the relative abundance index (RAI) for each plant species which was converted to a percentage of the total vegetation of either trees and shrubs or herbaceous plants. We divided the field survey data into three sampling periods based on the corn planting dates: (1) before planting (before 28 April); (2) during corn planting (29 April – 22 May); and (3) after planting (23 May – 3 June).

In order to determine the taxonomic composition and proportion of pollen collected from tree, shrub, and herbaceous plants near corn and soybean fields by honey bees during the spring corn planting season bee-collected pollen was sampled at the 9 apiaries using pollen traps attached to the bee hives (39 cm x 15 cm x 10.5 cm; Anel Standard, Athens, Greece. www.anel.gr.) (Fig. 1). Pollen traps were engaged at ca.16:00 h on the day previous to the specified pollen collection date and pollen samples were removed from the sites no later than 24 hours after trap engagement. When weather conditions were not ideal for bee foraging (i.e. cooler temps or significant rain), pollen traps were left engaged for an additional 24 h. Species identification and proportion of pollen species was determined by Johanne Parent (Laboratoire BSL, Rimouski, QB).

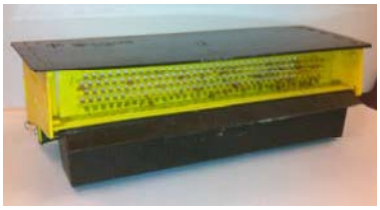

Figure 1. Anel standard pollen trap

1.2. Results

Before corn planting, the most abundant blooming trees and shrubs available were of *Acer.* (relative AI 173.3) and *Salix spp*. (relative AI 66.7) (Fig.2). The most abundant herbaceous blooms were of *Anemone* (relative AI 173.3), Dandelion (*Taraxacum officinale*) (relative AI 66.7), and *Viola spp*. (relative AI 66.7) (Fig. 3). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Salix* (81.7%), Liliaceae (7.6), *Acer* (4.2%) and *Ulmus spp.* (3.5%) (Fig.4).

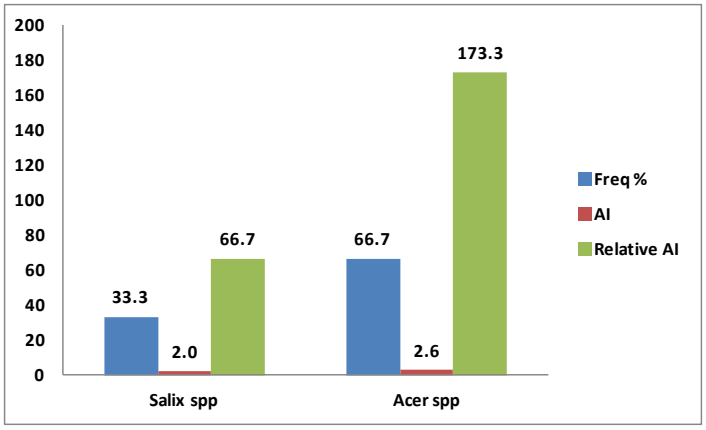

Figure 2. The most abundant blooming tree/shrubs **before** corn planting in 2015.

Legend Notes: Freg. - the percentage of occurrences of the patches of a blooming plant species; AI - Abundance Index, 0-4 where 0 is not observed, 1 is 0-5 individuals observed /m^2 for herbaceous weeds, 0-1 observed/100m^2 for trees and shrubs 2 is 6-10/m^2 for weeds, 2-5/100m^2 for trees and shrubs, 3 is 11-20/m^2 for weeds, 6-10/100m^2 for trees and shrubs), and 4 is >20/m2 for weeds, >10/100m^2 for trees and shrubs. Relative AI is the product of freq (%) and AI.

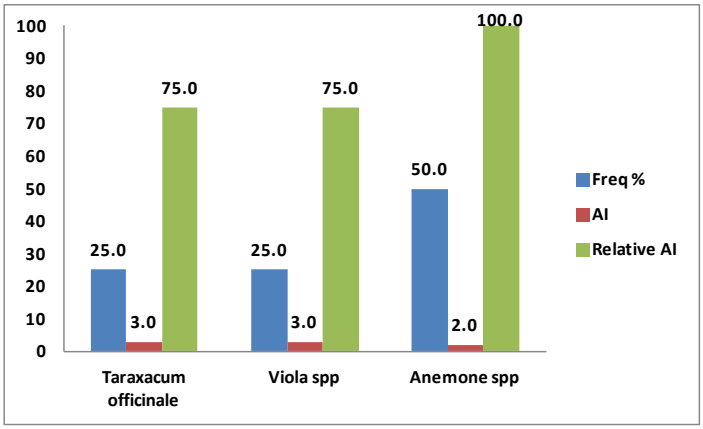

Figure 3. The most abundant herbaceous blooming plants **before** corn planting in 2015. See Fig. 2 for explanation of legend.

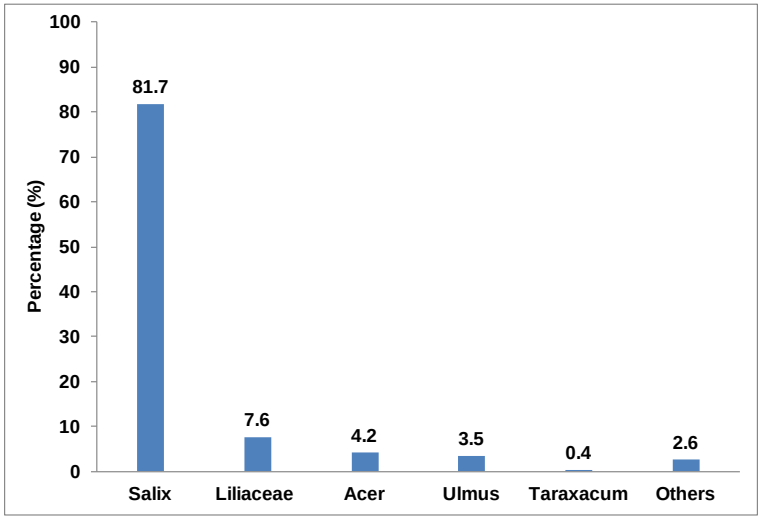

Figure 4. Proportion of bee-collected pollen species **before** corn planting in 2015.

During the planting period between 29 April and 22 May, the most abundant blooming trees and shrubs were of Rosaceae (relative AI 170.4), *Prunus* (relative AI 28.9), *Salix* .(relative AI 18.5), *Acer* (relative 11.1) and *Crateagus spp*. (relative 11.1). The most abundant herbaceous blooms were Dandelion (*Taraxacum officinale*) (relative AI 180.0), *Barbarea vulgaris* (relative AI 90.0), and *Alliaria officinalis* (relative AI 90.0) (Fig. 6). During the same period, bee-collected pollen sampled from associated pollen traps was comprised mainly of *Acer* (35.2%), *Salix* (27.4%) and Rosaceae *spp.* (22.0%) (Fig. 7).

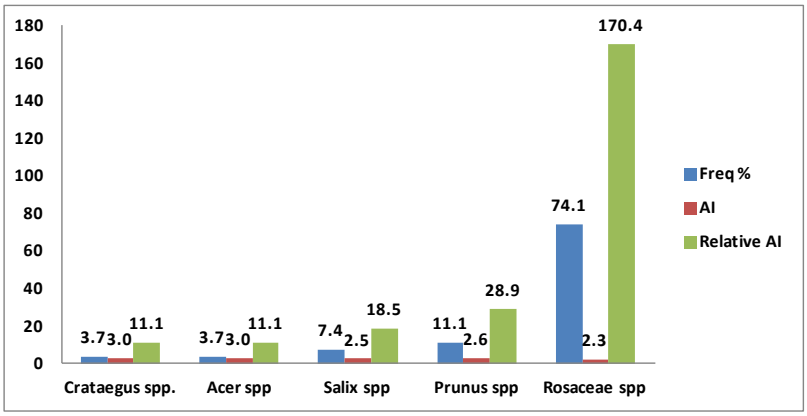

Figure 5. The most abundant blooming trees and shrubs **during** the period of corn planting in 2015. See Fig. 2 for explanation of legend.

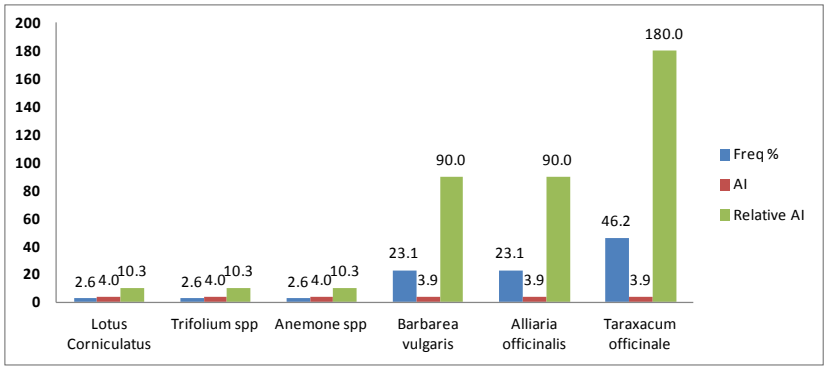

Figure 6. The most abundant herbaceous blooming plants **during** the period of corn planting in 2015. See Fig 2 for explanation of legend.

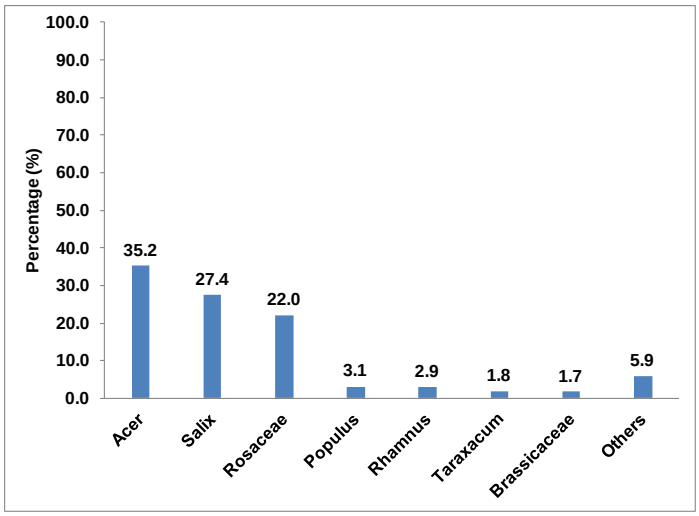

Figure 7. Proportion of bee-collected pollen species **during** the corn planting period in 2015.

After planting, beyond 23 May, the most abundant blooming trees and shrubs were of Rosaceae (relative AI 176.0) and *Prunus spp.* (relative AI 40.0). The most abundant herbaceous blooms were of *Brassicaceae spp* (relative AL 123.0), *Lotus corniculatus* (relative AI 92.3), *Barbarea vulgaris* (relative AI 92.3), *Asteraceae* spp (relative AI 61.5) and *Viola spp* (relative AI 23.1). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Rosaceae* (32.0%), *Salix* (14.0), *Rhamnus* (11.5%), *Brassicaceae* (10.8%) and Trifolium (9.3%) *spp*.

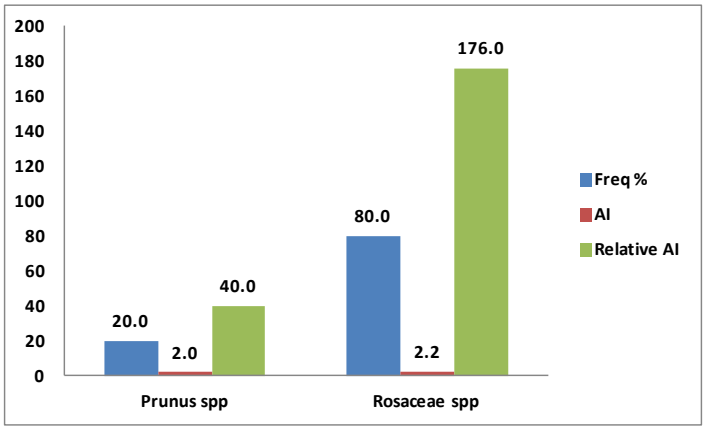

Figure 8. The most abundant blooming trees and shrubs **after** corn planting in 2015. See Fig. 2 for explanation of legend.

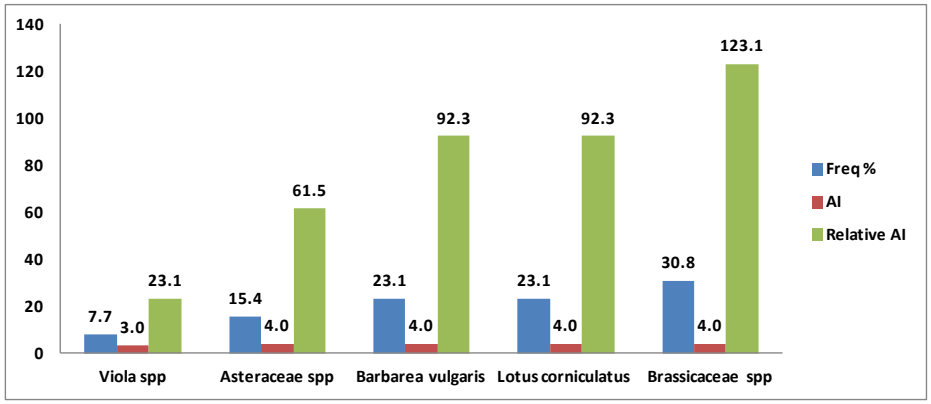

Figure 9. The most abundant herbaceous blooming plants **after** corn planting in 2015. See Fig. 2 for explanation of legend.

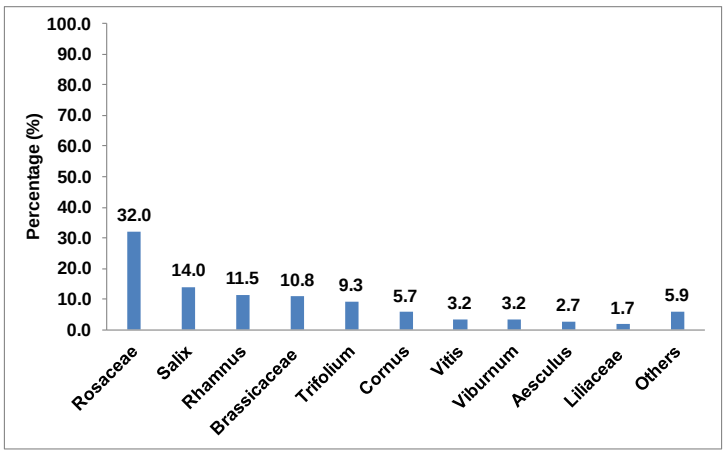

Figure 10. Proportion of bee-collected pollen species **after** corn planting in 2015.

2. Determine neonicotinoid residue in bee-collected pollen and dead bees

2.1. Method

At each of the 9 apiaries, dead bees were collected from Drop-Zone Dead Bee traps (100 x 50 x 14cm) installed in front of four bee hives during 24 h intervals (Figure 11). Bee-collected pollen was collected at the same time for proportional species identification (see Method 1.1). There were three sampling periods: (1) before planting (28 April); (2) during corn planting (29 April – 22 May); and (3) after planting (23 May– 3 June). Neonicotinoid residues in dead bees and bee-collected pollen were determined by LC/MS/MS.

Because clothianidin and thiamethoxam are the most commonly used neonicotinoids in southwestern Ontario and clothianidin is a metabolite of thiamethoxam, we used the total quantity of clothianidin and thiamethoxam to represent the neonicotinoid residues, hereinafter.

Figure 11. Drop-Zone dead bee trap

PROC MIXED was used to compare the concentration of neonicotinoids across sampling periods with sampling period as the fixed effect and apiary as a random effect. Data were subjected to $\log_{10}(x+1)$ transformation to satisfy the assumptions of normality. The α level for statistical significance was set at 0.05 for all analyses.

2.2. Results

The concentrations of neonicotinoids within bee-collected pollen during planting were significantly higher than those before and after planting (Figure 12). Neonicotinoid concentrations in bee-collected pollen in 2015 were similar to those found in 2013 (9.3 ng/g) and 2014 (7.87 ng/g).

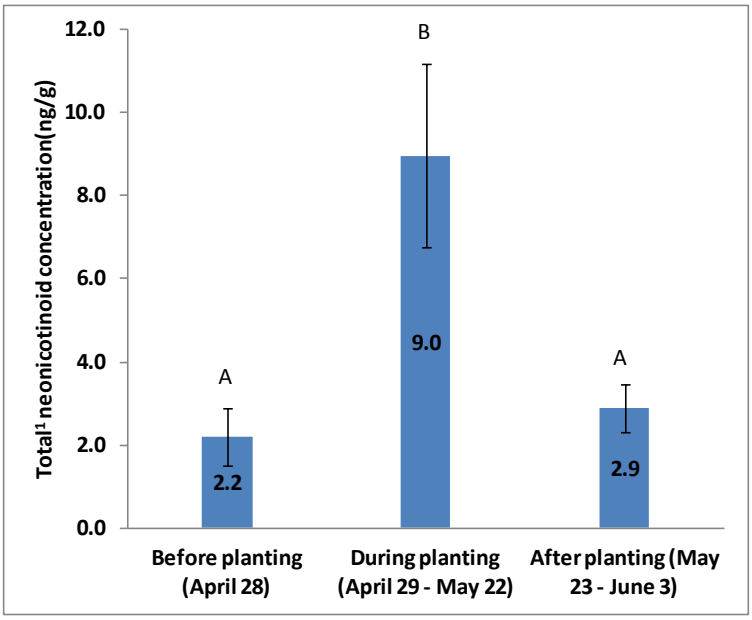

Figure 12. Mean concentrations (±SE) of neonicotinoid residues in bee-collected pollen collected before, during, and after corn planting in 2015. Columns with the same letter are not significantly different (P > 0.05) as determined by PROC MIXED.

[1] Total of clothianidin and thiamethoxam (LOD and LOQ were 0.2378 and 0.5878 ng/g for clothianidin, and 0.0681 and 0.1805 ng/g for thiamethoxam).

The neonicotinoid concentration within dead bees increased significantly from before planting to during and after planting (Table 4). No significant differences in neonicotinoid concentration were found on the surface of dead honey bees among the three sampling periods in 2015 (Table 4). The mean combined total neonicotinoid residue within and on the surface of dead

honey bees in 2015 was 1.32 ng/bee, which was similar to that measured in 2014 (1.42 ng/bee) and 2013 (13.9 ng/g, assuming that 10 dead bees = 1 g).

Table 4. Neonicotinoid residues within and on the surface of dead honey bees collected from dead bee traps at apiaries in 2015. (Data within columns with different letters are significantly different (P < 0.05) as determined by PROC MIXED).

Sampling period	n	Neonicotinoid [1] (ng/bee) (mean ± SE)		
		Within	On surface	Total
Before planting	2	0.16 ± 0.03 a	0.38 ± 0.11	0.54 ± 0.14
During planting	18	0.33 ± 0.03 b	0.36 ± 0.08	0.69 ± 0.09
After planting	7	0.46 ± 0.03 c	2.28 ± 1.34	2.74 ± 1.35

[1] Total of clothianidin and thiamethoxam (For residue within bee, LOD and LOQ were 0.0235 and 0.0667ng/bee for clothianidin, and 0.0078 and 0.0230 ng/bee for thiamethoxam; For residue on bee surface LOD and LOQ were 0.0576 and 0.1631 ng/bee for clothianidin, and 0.0323 and 0.0921 ng/bee for thiamethoxam).

3. Determine the fugitive dust escaping fields

3.1. Methods

Dust leaving the field was collected using sticky dust collection towers (Figure 13) at three sampling times. Dust events such as from tillage, planting, and wind erosion events were targeted as they presented themselves. Each collection tower consisted of one 2-m metal stake with one vertically-oriented panel of microscope slides on a wood frame at a height of 2 m from the ground. The microscope slide panels were coated with Tangle Trap® adhesive (The Tanglefoot Company, Grand Rapids, MI). Three dust collection towers were placed at the downwind edge of the field being planted (proximal) and three at the far downwind edge of the neighbouring field (distal). For the safety reason the dust traps at the downwind edge of the field being planted were located around 2 feet from the planter. Towers were orientated so that the vertical panels faced directly towards the wind. A single volumetric air sampler (Figure 14) was set at 2 m height on the same field edge and far edge of the neighbouring fields as the sticky traps.

Figure 13. Vertical sticky dust trap

Figure 14. Volumetric air sampler

The distance between the proximal and distal traps was recorded. Planting time collections were not made at 2 soybean fields because of extreme delayed planting and at the winter canola field which was already planted in the fall. We also selected 3 extra corn fields (around 1 month after planting) and 1 extra soybean field (1 week after planting) to collect wind event dust samples. All fields for wind event collection were planted with treated seed. Samples collected at each location were all standardized to the time it took to plant or till a 150-m width of each field so data collected in 2015 can eventually be related to those collected in 2013 and 2014. Extraction and LC/MS/MS analysis were conducted at the University of Guelph Ridgetown Campus. To maximize the sampling of fugitive dust escaping corn fields, we combined the 3 sticky trap samples from the 3 replicates of proximal or distal in corn fields into one LC/MS/MS injection, and compensated the deliverables by adding the volumetric air sampler samples (see modification section).

3.2. Results

3.2.1. During corn planting

The mean neonicotinoid concentration captured on sticky traps during corn planting was 2.16 (0.84 in 2014) and 0.12 (0.42 in 2014) ng/cm^2, for the downwind and neighbouring field edges, respectively (Table 5). The variation between the years for field edge may be caused by the large seed treatment particles captured by the traps. Using volumetric air samplers, the mean neonicotinoid concentrations captured during corn planting at the downwind and neighbouring field edges were 32.58 (21.7 in 2014) and 14.30 (12.2 ng/m^3 in 2014), respectively (Table 5).

We expected that neonicotinoid residue concentrations decreased as the distance away from the field being planted increased; however, there were some anomalies at individual locations that showed the opposite. At some locations when samples were taken, other producers were planting corn in the region, which could not have been avoided, and these may have confounded some of our results.

Table 5. Neonicotinoid residues collected at downwind locations during corn planting in 2015 using vertical sticky traps and volumetric air samplers.

Location	Field edge (Proximal)		Neighbouring field edge (Distal)		
	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)	Distance (m)	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)
1D	0.54	7.15	694	0.05	1.61
2A	0.43	6.82	330	0.15	2.42
3A	16.41	95.89	300	0.58	54.76
3B	3.53	175.52	152	0.16	75.95
5A	0.05	8.52	345	0.01	0.19
6A	0.59	6.17	167	0.01	1.84
6D	0.97	4.70	200	0.03	3.65
7D	0.17	13.08	681	0.01	5.70
8A	0.47	11.73	140	0.03	5.60
9C	0.01	0.95	70	0.01	1.20
10A	0.60	27.85	300	0.24	4.42
Min	0.01	0.95		0.01	0.19
Max	16.41	175.52		0.58	75.95

Mean	2.16	32.58	0.12	14.30
SE	1.45	16.42	0.05	7.76

* Data standardized to the area required to plant 150 m of field width. For sticky traps, LOD and LOQ were 0.2712 and 0.9032 ng/cm^2 for clothianidin, and 0.0809 and 0.2694 ng/cm^2 for thiamethoxam; For air sampler, LOD and LOQ were 0.7070 and 2.3544 ng/m^3 for clothianidin, and 0.1061 and 0.3533 ng/m^3 for thiamethoxam).

3.2.2. During soybean planting

The mean neonicotinoid concentration captured on sticky traps during soybean planting was 0.11 and 0.10 ng/cm^2, for the downwind and neighbouring field edges, respectively (Table 6). Using volumetric air samplers, the mean neonicotinoid concentration captured during soybean planting at the downwind and neighbouring field edges was 8.64 and 5.31 ng/m^3, respectively (Table 6) which were lower than during corn planting.

Table 6. Neonicotinoid residues collected at downwind locations during soybean planting in 2015 using vertical sticky traps and volumetric air samplers.

Location	Field edge (Proximal)		Neighbouring field edge (Distal)		
	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)	Distance (m)	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)
7C	0.10	12.89	370	0.03	1.25
8C	0.16	11.14	150	0.21	11.01
9D	0.06	1.88	266	0.06	3.68
Min	0.06	1.88		0.03	1.25
Max	0.16	12.89		0.21	11.01
Mean	0.11	8.64		0.10	5.31
SE	0.03	3.42		0.06	2.93

* Data standardized to the area required to plant 150 m of field width.LOD and LOQ were the same as in Table 5.

3.2.3. During tillage

The mean neonicotinoid concentration captured on sticky traps during tillage was 0.01 and 0.04 ng/cm^2, for the downwind and neighbouring field edges, respectively (Table 7). Using volumetric air samplers, the mean neonicotinoid concentration captured during tillage events at the downwind and neighbouring field edges was 3.13 and 2.47 ng/m^3, respectively (Table 7) which was much lower than during corn or soybean planting.

Table 7. Neonicotinoid residues collected at downwind locations during tillage events in 2015 using vertical sticky traps and volumetric air samplers.

Location	Field edge (Proximal)		Neighbouring field edge (Distal)		
	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)	Distance (m)	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)
2C	n/a	2.65	n/a	n/a	n/a
3A	0.02	4.48	358	0.01	4.47
3B	0.01	1.5	214	0.05	1.04
6A	0.01	3.99	167	0.04	1.15
8A	0.01	3.02	140	0.04	3.22
Min	0.01	1.50		0.01	1.04
Max	0.02	4.48		0.05	4.47
Mean	0.01	3.13		0.04	2.47
SE	0.00	0.52		0.01	0.75

* Data standardized to the area required to plant 150 m of field width.LOD and LOQ were the same as in Table 5.

3.2.4. During wind events

The mean neonicotinoid concentration captured on sticky traps and volumetric air samplers during wind events in the downwind field edge was 0.02 ng/cm^2 and 1.82 ng/m^3, respectively (Table 8). The neonicotinoid residue concentrations were much lower than during corn or soybean planting or during tillage events.

Table 8. Neonicotinoid residues collected at downwind locations during wind events in 2015 using vertical sticky traps and volumetric air samplers.

Location	Field edge (Proximal)	
	Vertical sticky trap (ng/cm^2)*	Air sampler (ng/m^3)
Thamesville 1	0.02	0.65
Thamesville 2	0.04	0.68
Thamesville 3	0.01	4.39
Thamesville 4	0.01	1.55
Min	0.01	0.65
Max	0.04	4.39
Mean	0.02	1.82
SE	0.01	0.88

* Data standardized to the area required to plant 150 m of field width. LOD and LOQ were the same as in Table 5.

4. Determine neonicotinoid residues on/in flowers, foraging honey bees on flowers, and soil at the base of blossoming plants

4.1. Methods

To detect neonicotinoid residues on and in blossoms foraged by honey bees during the corn planting period, the 18 study fields above were surveyed for plant species that honey bees were actively foraging on during the three sampling periods. The blossoms of the two most abundant flowering plant species along with honey bees foraging on them were collected in each field. Soil to the 10-cm depth was collected from the base of each plant sampled for flowers and bees. All plant blossoms, foraging bees and soil samples were stored in complete darkness at -20ºC until processed and tested. There were no foraging bees collected in April, and few foraging bees were found in early May due to unusually low temperatures; therefore, we extended the collection period until 25 June. The trees/bushes with foraging bees included *Salix spp*. (willow), *Acer spp*. (maple), Rosaceae*,* and *Robinia pseudoacacia* (black locust). The herbaceous plants with foraging bees included *Trifolium repens* (white clover) *Melilotus spp*. (yellow sweet clover), *Barbarea vulgaria* (yellow rocket), *Taraxacum spp*. (dandelion), *Cirsium spp.* (thistle), and *Lotus corniculatus* (birdsfoot trefoil). Neonicotinoid residues in flowers (inside, outside and anthers), foraging bees (inside and outside), and in soil samples at the base of the plants were determined by LC-MS/MS.

4.2. Results

4.2.1 Trees and shrubs

Due to difficulties collecting from tall trees, very few blossoms with foraging bees were collected from trees and shrubs in 2015. The trees/bushes sampled included *Salix* spp. (willow), *Acer* spp. (maple), Rosaceae, and Robinia spp.(black locust). The anthers and pollen of the blossoms usually had lower neonicotinoid residues than on/in the flowers (Table 9); however, the flower surface had similar neonicotinoid residue concentrations to those measured inside the flowers. *Salix* spp. were the most important foraging resources of bees before corn planting (81.7%) (Table 10). During corn planting, *Acer* spp. (35.2%), Salix spp. (27.4%) and Rosaceae (22.0%) were the most important foraging resources. Foraging bees collected from *Acer* spp. on 1 May had higher neonicotinoid residue concentrations, which may have been related to corn planting nearby.

Table 9. Total[1] neonicotinoid concentration on/in flowers, foraging honey bees, and soil from the base of blossoming trees and shrubs in 2015.

Tree/bush	n	Soil (ng/g)	Flower (ng/flowers)			Foraging bee (ng/bee)	
			Surface	Inside	Anther + pollen	Inside	Surface
Acer spp. (maple)	1	missing	0.54	0.14	0.06	4.77	18.02
Salix spp. (willow)	1	missing	0.53	0.59	0.17	missing	missing
Rosaceae	5	1.21	0.11	0.05	0.04	0.29	1.22
Robinia spp. (Black locust)	3	13.91	0.06	0.06	0.03	0.44	0.32

[1].Total of clothianidin and thiamethoxam. For soil, LOD and LOQ were 0.1033 and 0.2390 ng/g for clothianidin, and 0.0215 and 0.0448 ng/g for thiamethoxam; For flower surface, inside and anther + pollen, LOD and LOQ were 0.0366 and 0.0977 ng/flower for clothianidin, and 0.0096 and 0.0263 ng/flower for thiamethoxam; For inside of foraging bee, LOD and LOQ were 0.0243 and 0.0808 ng/bee for clothianidin, and 0.0295 and 0.0981 ng/bee for thiamethoxam; For surface of foraging bee, LOD and LOQ were 0.0663 and 0.2208 ng/bee for clothianidin, and 0.0652 and 0. 2171 ng/bee for thiamethoxam).

Table 10. The proportion of pollen from blooming plants observed with foraging bees found in bee-collected pollen before, during, and after corn planting in 2015.

Category	Plant	Proportion of bee-collected pollen (%)		
		Before planting	During planting	After planting
Trees and shrubs	*Acer spp.* (maple)	4.2	35.2	0.0
	Salix spp. (willow)	81.7	27.4	14.0
	Rosaceae	0.0	22.0	32.0
	Robinia spp. (Black locust)	0.0	0.0	0.0
Herbaceous	*Taraxacum* (dandelion)	0.4	1.8	0.4
	Melilotus (sweet clover)	0.0	0.0	0.2
	Lotus (birdsfoot trefoil)	0.0	0.0	0.0
	Barbarea (yellow rocket)	0.0	0.9	1.9
	Trifolum (clover)	0.0	0.0	9.3
	Cirsium (thistle)	0.0	0.1	0.0
	Vicia (cow vetch)	0.0	0.0	0.0

4.2.2. Herbaceous Plants

Similarly to our results in 2014, generally lower levels of neonicotinoid residues were found on the surface of herbaceous flowers relative to what was measured inside blossom tissues (Figs. 15 -19). These data suggest that perhaps uptake of neonicotinoids from the soil or from leaf deposition during or after corn planting may result in greater residue levels in the plant. This effect may be less certain for trees which may be generally more distant from the source of neonicotinoid application in space and time, and have different morphology and physiology. Neonicotinoid residue levels found on the surface of foraging honey bees were higher than that measured inside bees. To be clear we observed in several cases for herbaceous plants the residue levels were greater inside the flower tissue compared with what was found on the flower surface. In many cases the plant leaves would have been unfurled at the time of planting, if flowering coincided with planting and sampling. So in these cases the residues found inside the flower tissue could have come from the soil or from the leave surface after deposition.

***Taraxacum spp*. (dandelion)**

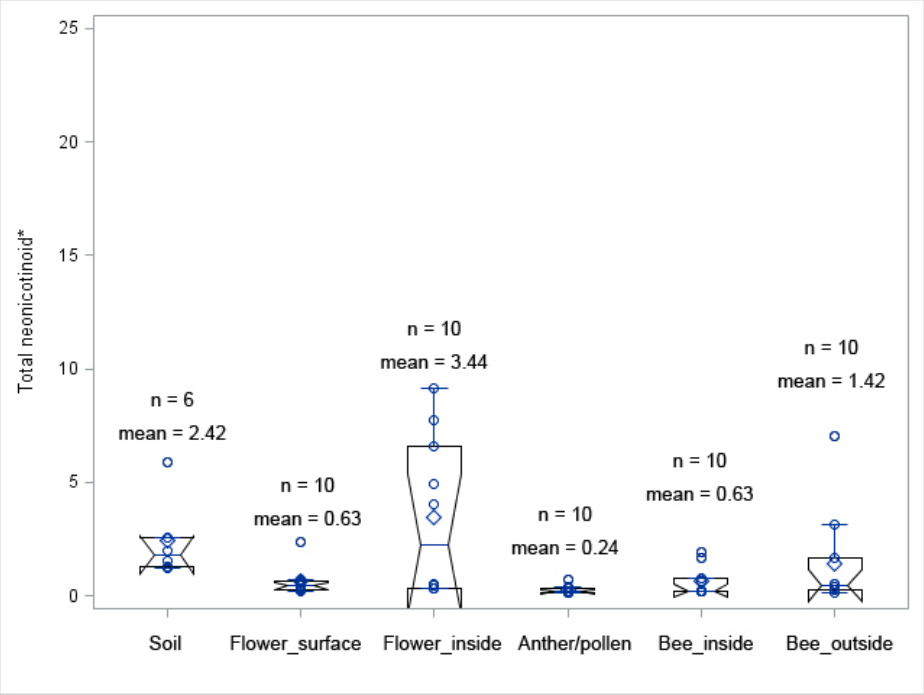

Figure 15. Total[1] neonicotinoid concentration on/in flowers of *Taraxacum spp.* (ng/flower), foraging honey bees (ng/bee) on these flowers, and in soil (ng/g) collected from the base of the same plants. ([1]Total of clothianidin and thiamethoxam) in ON 2015. LOD and LOQ were the same as in Table 9.

***Melilotus spp*. (yellow sweet clover)**

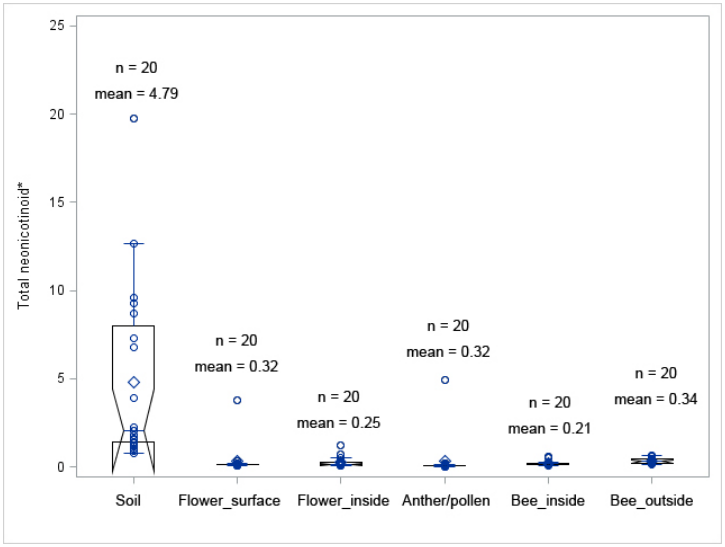

Figure 16. Total[1] neonicotinoid concentration on/in flowers of *Melilotus spp.* (ng/flower), foraging honey bees (ng/bee) on these flowers, and in soil (ng/g) collected from the base of the same

plants. ([1]Total of clothianidin and thiamethoxam) in ON 2015. LOD and LOQ were the same as in Table 9.

***Lotus corniculatus* (birdsfoot trefoil).**

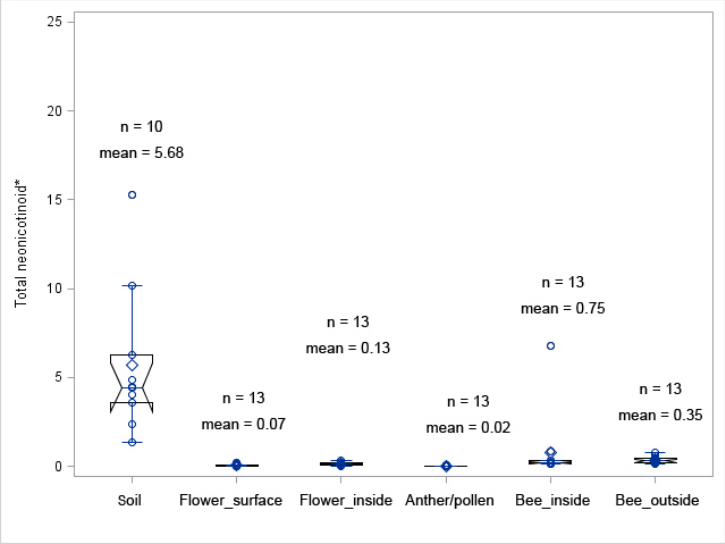

Figure 17. Total[1] neonicotinoid concentration on/in flowers of *Lotus corniculatus* (ng/flower), foraging honey bees (ng/bee) on these flowers, and in soil (ng/g) collected from the base of the same plants. ([1]Total of clothianidin and thiamethoxam) in ON 2015. LOD and LOQ were the same as in Table 9.

***Barbarea vulgaria* (yellow rocket)**

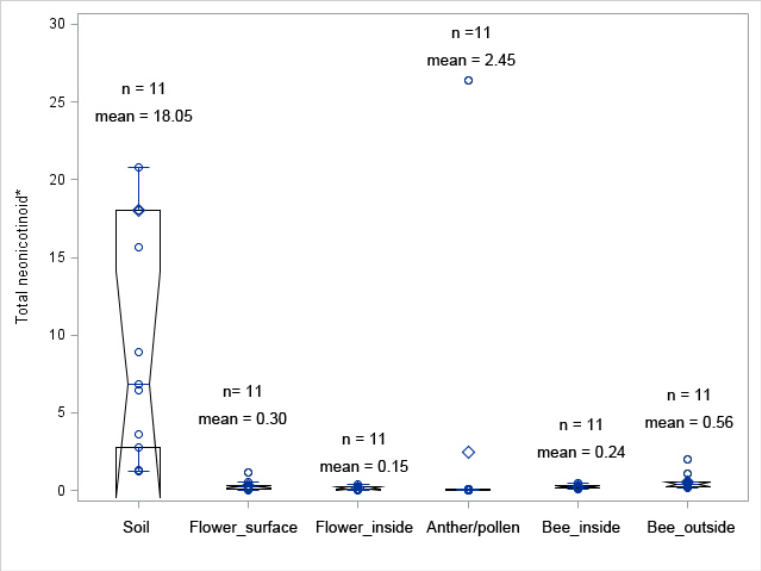

Figure 18. Total[1] neonicotinoid concentration on/in flowers of *Barbarea vulgaria* (ng/flower), foraging honey bees (ng/bee) on these flowers, and in soil (ng/g) collected from the base of the same plants. ([1]Total of clothianidin and thiamethoxam) in ON, 2015. LOD and LOQ were the same as in Table 9.

***Trifolium spp.* (clover)**

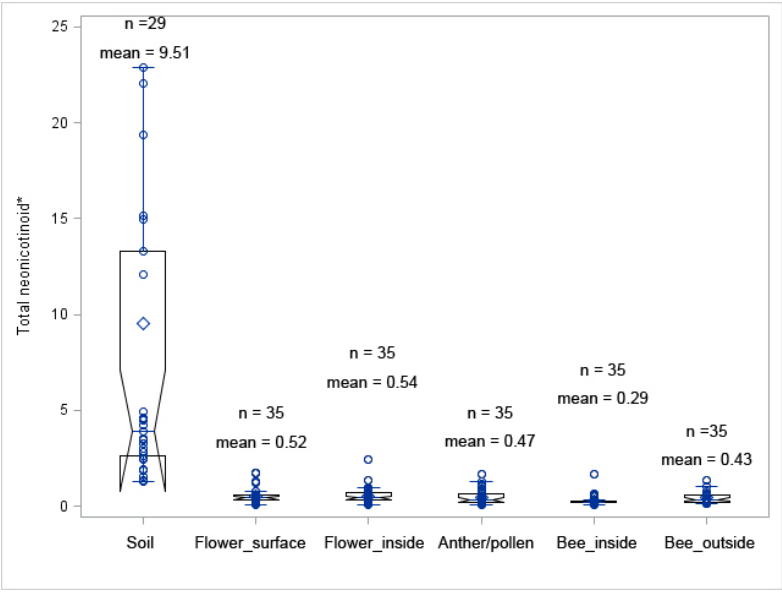

Figure 19. Total[1] neonicotinoid concentration on/in flowers of *Trifolium spp.* (ng/flower), foraging honey bees (ng/bee) on these flowers, and in soil (ng/g) collected from the base of the same plants. ([1]Total of clothianidin and thiamethoxam) in ON, 2015. LOD and LOQ were the same as in Table 9.

Other herbaceous species:

Table 11. Total[1] neonicotinoid concentration on/in other herbaceous flowers (ng/flower), foraging honey bees (ng/bee) on the flowers, and soil (ng/g) on the base of the plants.

Herbaceous	n	Soil (ng/g)	Flower (ng/flowers)			Foraging bee (ng/bee)	
			Surface	Inside	Anther + pollen	Inside	Surface
Vicia (cow vetch)	1	2.06	0.37	0.63	0.47	0.22	0.26
Cirsium (thistle)	4	10.05	0.16	0.22	0.17	0.16	0.27

[1]Total of clothianidin and thiamethoxam. LOD and LOQ were the same as in Table 9.

4.2.3 Relationship between the neonicotinoid concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees.

No significant correlation was found between the neonicotinoid concentrations measured in soil collected from the base of blooming plants and that measured in/on foraging bees or the anthers/pollen of the plant blossoms (R^2 = 0.006, p = 0.472). Nor was there a correlation between the concentrations measured in anther/pollen tissue of blossoms and that measured in/on bees foraging on the blossoms (R^2 = 0.001, p = 0.792).

5. Summary of the level of detection (LOD) and the level of quantification (LOQ)

Table 12. Summary of the level of detection (LOD) and the level of quantification (LOQ)

Matrix	Clothianidin		Thiamethoxam	
	LOD	LOQ	LOD	LOQ
Bee collected pollen (ng/g)	0.2378	0.5878	0.0681	0.1805
Dead bee (inside) (ng/bee)	0.0235	0.0667	0.0078	0.0230
Dead bee (surface) (ng/bee)	0.0576	0.1631	0.0323	0.0921
Sticky trap (ng/cm2)	0.2712	0.9032	0.0809	0.2694
Air sampler (ng/m3)	0.7070	2.3544	0.1061	0.3533
Soil (ng/g)	0.1033	0.2390	0.0215	0.0448
Flower (surface) (ng/flower)	0.0366	0.0977	0.0096	0.0263
Flower (inside) (ng/flower)	0.0366	0.0977	0.0096	0.0263

Flower (anther +pollen) (ng/flower)	0.0366	0.0977	0.0096	0.0263
Foraging bee (inside) (ng/bee)	0.0243	0.0808	0.0295	0.0981
Foraging bee (outside) (ng/bee)	0.0663	0.2208	0.0652	0.2171

The Long-Term Health Consequences of Exposure of Honey Bee Colonies to Dust Emitted During Planting of Neonicotinoid-Treated Corn Seeds

A Research Project Addressing Project 2 of the Corn Dust Research Consortium, 2015 RFP Final Report, June 30, 2016

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The Long-Term Health Consequences of Exposure of Honey Bee Colonies to Dust Emitted During Planting of Neonicotinoid-Treated Corn Seeds

Project Summary

Our project objective was to assess the effect of experimental exposure of whole honey bee colonies to Clothianidin-contaminated dusts and the potential mitigating influence of post-exposure supplemental feeding. Notwithstanding a late date of project approval and restricted availability of colonies, in May of 2015 we successfully secured 44 colonies that we dispersed among three bee yards spaced at a minimum of two-miles separation in the Missoula valley: Fort Missoula (W Missoula, n = 12), Missoula Cemetery (N Missoula, n = 16), and Mount Sentinel (E Missoula, n = 16).

Consistent with our objective; we conducted a balanced, two x two, complete random experiment for pesticide exposure and supplemental feeding. Two frames of bees from half the colonies in each yard (n = 22) were dusted with Clothianidin in chalk dust to produce an estimated contact dose of 0.2 µg Clothianidin per bee. In our laboratory dusting test trials with caged bees, this dose killed half of the exposed bees within the first 24 hours.

Following dusting, half of the exposed colonies and half of the controls received post-exposure supplemental feeding. Colony metrics were collected at pre-dose (July), at two-three weeks post-dose (August), and at a longer-term post-dose period (third week of September), with a final assessment (June, 2016) of all colonies that survived the winter.

We obtained a full set of measurements for colony size and condition metrics: (1) before dosing with Clothianidin, (2) after pesticide exposure, (3) again in September at the end of the 2015 field season with summary metrics for surviving colonies at the beginning of the 2016 field season.

It is noteworthy that during the course of our project, Missoula experienced the second worst drought on record, so nutritional stress in non-supplemented colonies was assured. Then during the last two weeks of August and the first week of September 2015, we experienced persistent heavy atmospheric smoke and ash from extensive forest fires throughout the western U.S. and Canada. Our anecdotal observations suggested that this had some effect on out-of-the-hive foraging activity as well as colony growth.

At the start of the experiment (pre-dosing), frame coverage by adult bees varied from a low of four to a high of 19 frames, with an overall average of 10 frames of bees per colony. Frames of brood varied from a low of 1.63 to a high of 8.75. At the time of Clothianidin dusting, colonies averaged approximately 11 frames of bees and 5 frames of brood. Equalization and proportional assignment to treatment groups kept the within-group range in mean bees and brood within one frame of bees.

As indicated from trapping of dead bees, we successfully induced adult bee kills at levels similar to those we observed at field sites in our previous study in Nebraska in 2014. Clothianidin exposure produced immediate kills in the dusted treatment groups of more than 1,100 bees on average in exposed colonies. After one week, bee mortality dropped to normal levels in the exposed colonies which received supplemental feeding. After two weeks, mortality in the non-supplemented colonies also dropped to normal levels. We observed much variation in colony growth within treatment groups but still measured a trend in colonies exposed to Clothianidin dust to have fewer frames of bees at the end of the season than controls. In August, all colonies were still queen-right, had brood, and had survived the Clothianidin dosing. Frame counts of

bees ranged from 4 to18, frames of brood from 1.5 to 8.75, averaging 11 frames of adult bees. By late September, except for one colony, all colonies were still alive with an average of 10.6 frames of bees for colonies which had both adult bees and brood. One colony had absconded. Another colony had 11 frames of bees but no brood. Curiously, one of these two marked queens (either from the absconded colony or the broodless colony) ended up in another colony, co-existing with that colony's original queen. In sum, 42 of the 44 colonies still had bees and brood. September adult frame counts by colony ranged from 5 to19, brood from 4.25 to 9.

Two-thirds of the colonies were transferred to our fenced apiary at Fort Missoula at the end of September; the third group was moved later. By the end of October, all colonies had been transferred to our fenced apiary at Fort Missoula and placed on elevated stands. Queen-excluders were removed and a quick frame count was conducted to assess the population size of surviving colonies. At that time, 40 of the original 44 colonies were still alive; although brood-rearing had ceased for the season. Colonies then went onto a winter schedule of inspection and feeding (when required). In November, just prior to wrapping, two more colonies had died, bringing the number of surviving colonies down to 40 from 44.

Overall, overwintering colony survival was 50 percent. This is not substantially worse that what is commonly being reported by commercial and hobby bee keepers, with the Bee Informed Partnership reporting 28.1 percent average winter loss and a 44.1percent annual loss nationwide for April 2015 through March, 2016. Given the late start to our project, beginning with package bees near the end of June 2015, and managing them through an unusually dry summer; our 12 month, fifty percent survival rate, especially when half of the colonies were subjected to high dust and dietary doses of pesticides and record-tying drought conditions, was a reasonable outcome.

The importance of extending our project through the winter was illustrated in the final numbers on colony mortality which we recorded in June 2016. *Clothianidin-exposed colonies had a one-third greater risk of death than unexposed colonies, the same as the increased risk associated with the absence of post-exposure food supplementation.*

Although these increases in risk of mortality were not statistically significant, they are in line with our findings from our previous CDRC project. Specifically, we experienced two significant bee kills from pesticide exposure in our previous Nebraska study. Yet, at the end of that study, during in which we provided supplemental syrup and pollen substitute, the final condition of the exposed colonies was nearly equal to unaffected colonies.

Increased post-exposure feeding to pesticide-affected colonies may prove to be an effective way to mitigate colony losses. Relative to other colony attributes, in the 2015 Montana study, colony weights showed only minor differences. Among surviving colonies, queen survival and overall brood success were not greatly affected. However, *we did observe a significant reduction by roughly two-thirds in brood area in pesticide exposed colonies which did not receive post-exposure supplemental feeding*. That effect was not evident during the 2015 summer and fall months following exposure; it only became apparent in the 2016 spring resumption of colony activity. *This suggests that an important potential long-term effect of Clothianidin-bearing dusts occurs the second season following the exposure. Differences in colony vigor reinforce our findings from Nebraska field studies that supplemental feeding of exposed colonies is an important action that can mitigate long-term effects.*

Introduction

Rationale/Background*.* Colonies exposed to Clothianidin-contaminated pollen and dusts in Nebraska during the 2014 planting season were supplemented with nectar (syrup) and pollen (substitute) ad lib. While overall exposure to neonicotinoids was low, two of three sites exposed to high (75.1ppb to 137ppb) levels of Clothianidin in pollen sustained severe losses. Yet by end of trial, all colonies were queen-right. The seriously impacted colonies had recovered to nearly equal population and overall condition of the other colonies. The third site sustained exposure with no obvious acute mortality.

Goals and Objectives. In 2015-2016, we investigated long-term effects of neonicotinoid-contaminated dust exposures (by direct deposition and through contaminated pollen) on honey bees to assess whether the impact can be mitigated by better nutrition and bee management. The persistence of neonicotinoids and other agro-chemicals in corn-growing regions made it necessary not only to document long-term effects of contaminated dust exposure, but also to seek possible interventions which beekeepers can use to sustain bee health.

Project Overview

Measurement Endpoints*.* The experimental design we employed was a balanced, two x two, complete random design that included two factors: Clothianidin dusting and Supplemental feeding. With this design we had controls for each factor and an absolute control that was not exposed to Clothianidin and did not receive supplemental feeding. Measurement endpoints included estimated mortality induced by Clothianidin exposure and changes in population size during the exposure season and the following spring: colony weight (bees plus stores), and reproduction (brood area).

Clothianidin Exposure*.* We exposed bees to Clothianidin by two routes: ingestion of contaminated pollen, and contact with contaminated dust. Dust and contaminated pollen exposures were calculated to approximate those experienced during planting and simulated those measured in our 2014 experiments. Experimental bee colonies were monitored over a full, four-season year; including over-wintering and spring build up. All experiments were conducted in Montana which provides the advantage of a pristine environment, relatively free from agro-chemicals, where we can focus on the effect of Clothianidin with low risk of uncontrolled pesticide exposure.

Colony Maintenance*.* Each hive received normal honey bee colony maintenance. Bee colony maintenance included normal bee medications to control common honey bee ailments and pests throughout the course of the experiments, and monthly inspections monitoring frame counts, honey stores, counts of dead bees in traps, and periodic inspection to ensure that all hives were queen-right. Between exposure events, colonies were treated as 'working' commercial colonies for pollination and honey production; moved and fed as needed.

Over-Wintering*.* In late November/early December 2015, the research bee colonies were prepared for overwintering using the same techniques used by bee keeping operations in similar climates. Over-wintered bees were monitored using thermal imaging, acoustic inspection, and weighing systems. In spring 2016, these colonies were taken out of winter storage, inspected, and all measured endpoints recorded.

Contaminated Pollen and Dust Makeup*.* We used a simulated dust for exposure treatments. We prepared the dust using powdered calcium carbonate chalk and technical grade Clothianidin

(95%) received from Bayer Crop Science. This chalk/Clothianidin mixture was chemically analyzed at the end of the project to verify the final concentration of Clothianidin per gram of chalk used in our pollen and dust treatments. For the contaminated pollen, we mixed Clothianidin directly with Ultra-Bee Pollen Substitute purchased from Mann Lake.

We have previously found that bee colonies readily consume this product and perform well. The objective was for the bees to consume the entire dose over a period of a few days. In Nebraska, we experienced a bee kill associated with pre-planting contaminated pollen that ran its course in about a week.

Application of Contaminated Pollen and Dust Simulants. Contaminated pollen simulant was fed in a single dose, 0.5-pound dry powder, placed on the top bars of the brood nest, inside each beehive. Contaminated crop dust simulant was applied to forager bees using the Atkin's Dust Tower Implosion System.

Target Concentrations. Our objective was to use exposure levels observed in Crop Planting Dust studies. In Nebraska, we observed severe bee mortality in colonies at two sites during the pre-planting period. The measured pesticide residues in the dead and dying bees were 8.5 and 16.3 ppb Clothianidin. Bee-collected pollen displayed the greatest amounts of Clothianidin during the pre-planting period (75-137 ppb at four sites), lower concentrations during planting period (12-44 ppb), and no detectable ppb at three sites pre-planting, three sites during planting, and all sites post-planting. Consequently, we calculated chalk/Clothianidin concentrations so as to achieve concentrations greater than 20 ppb in exposed bees.

Methods

Experimental Design. We employed 44 colonies in a two x two experimental design employing two treatments at two levels with 12 colonies per treatment. Treatments were

☐ Control with no exposure or supplemental feeding.
☐ No exposure with supplemental feeding.
☐ Exposure, no supplemental feeding.
☐ Exposure with supplemental feeding.

We exposed bees from randomly assigned hives to chalk (calcium carbonate) dust mixed with technical Clothianidin at a prescribed concentration.

After exposing bees, we monitored colony progress by measuring classical honey bee population metrics:

☐ Early morning counts of frames covered by bees.
☐ Periodic manual assessment of colony weights.
☐ Passive traps in front of the hives to collect dead and dying bees.
☐ Quantification of brood for reproduction assessments by visual estimates of capped and uncapped brood areas, with photographs taken of a subset of brood frames for calibration of the visual estimates and archival records of brood responses.
o Brood areas were estimated to within ¼ of each brood comb surface.
o Technicians underwent pre-experimental training and periodic calibration against test brood combs to assess, improve, and document observer accuracy.

Chamber Testing and Determination of Dusting Levels. Analytically certified Clothianidin

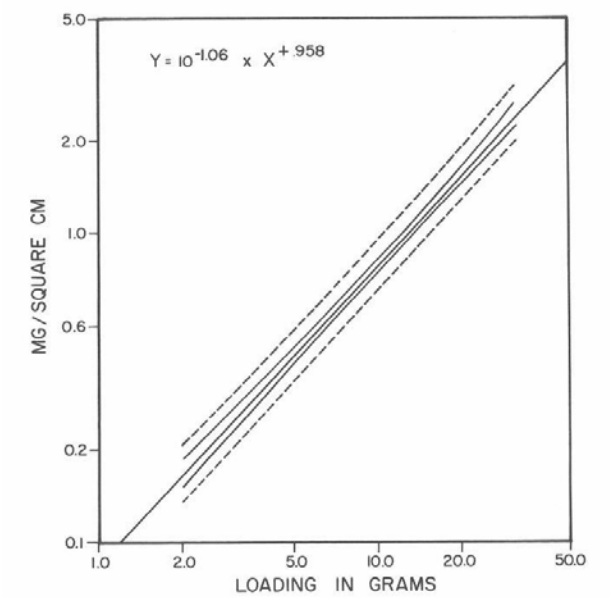

Figure 1. *Calibration curve for dusting chamber; surface deposition of ash results from different application rates. Ash application in mg/cm2.*

technical (99.7%, lot # 2013-003931, ID # 647438) was provided by Bayer Crop Science. We made up the crop planter dust simulant by mixing appropriate amounts of the Clothianidin with chalk dust. We retained subsamples for chemical analysis to verify the final concentration of Clothianidin per gm of dry chalk used in our dust treatments.

Given the concerns of CDRC reviewers about the method of application of the simulated crop dust using a powder coating sprayer, and our own concerns about variable particle size of the simulant, we chose to use a proven dust exposure protocol, equipment, and technique for evaluation of pesticide dusts in toxicological studies of honey bees (Atkins et al. 1954):

☐ Atkins' dust tower, is based on a bell-jar vacuum duster originally described by Farrar (1948), Richards and Murphy (1949), and McCallan (1950). It has been a standard method for determining the toxicity to honey bees of dust-formulated pesticides for US EPA pesticide registration.

☐ We developed and still have a modern, solenoid-controlled, larger version of the Atkins bell-tower dusting system that we constructed for a USDA Forest Service study of the effects of Mount Saint Helen's Ash on spruce budworm larvae. Similar to our proposed study herein, we exposed one set of budworm to treatments of ash in diet and another to a coating with ash dust. The budworm study yielded very different budworm results by route of exposure (Bromenshenk et al., 1987).

☐Based on targets placed in our dusting chamber, our implosion device produced a very consistent coating, as evidenced by the calibration curve below generated for a previous study (Figure 1). As can be seen in the figure, deposition was proportional to loading with reasonable variation among repeated samples at a given loading.

***Table 1**. Chalk deposition as a function of initial quantity in vacuum dosing chamber tests.*

Chalk (g)	Quantity Deposited (g/cm2)	s.d.	CV %
0.5	0.0001	0.00006	57
0.75	0.0004	0.00142	339
1	0.0002	0.00004	23
2	0.0002	0.00017	85
5	0.0008	0.00035	43
10	0.0013	0.00202	157

We improved deposition consistency by using a solenoid-controlled valve which opens instantly and more consistently than a manually-operated valve described by Atkins. When the electronically-controlled solenoid is opened to admit air entry, inrushing air aerosolizes and disperses the dust in a uniform cloud, dusting the bees. Our system is also portable, facilitating exposure in the field at the bee collection site. The dusting chamber is large enough to hold several frames of bees.

We first tested the vacuum dusting chamber in our laboratory to ensure that it worked reliably. Using filter paper deposition targets, we performed a series of tests to establish the type and quantity of dusting powder to be used in the dosing experiment. We explored the use of two inert easily obtainable powders: chalk and talc. Talc proved to be too fine a particle size which affected repeatability and gave us some concern about its tendency to be transported into the tracheal system of bees, causing health effects unrelated to pesticide exposure. Commercially prepared chalk dust of the type used for construction marking purposes was readily and cheaply available. Tests with it proved that it could be satisfactorily dispersed in the vacuum chamber. Chalk dust is commonly used for marking bees for behavioral testing, as well as for studying dust removal
by grooming (Land and Seeley, 2005).

We determined that a 5-gram quantity of powder proved to be easily weighed with precision to 0.001 grams and also produced relatively consistent deposition in the vacuum chamber (Table 1).

Using 5 grams of powder as our dosing quantity we next performed several laboratory trials to determine the dose of Clothianidin in the powder that produced the desired mortality in caged bees. Our emphasis was on producing a bee kill equivalent to the observed severe poisoning incident in Nebraska, not on testing the predicted toxicity of Clothianidin dust.

We collected cages of approximately 200 bees and dosed each at a different concentration of Clothianidin (Table 2). We settled on the lowest concentration that produced significant mortality within 24 hours of exposure for our field dosing. The selected Clothianidin concentration (0.00025 g/g or 250 ppm) weighted by deposition (0.8 mg dust/cm2) yielded an estimated contact dose of 0.2 µg Clothianidin per bee. This dose killed half of the exposed bees within the first 24 hours. Complete kill was observed after 48 hours. That concentration and duration are

also on the same order of magnitude as that which we observed in the bee kill just prior to the 2014 planting season in Nebraska.

Table 2*. Summary of Laboratory dosing experiments to establish field dosing levels of Clothianidin in chalk powder.*

Clothianidin in Powder (g/g)	**Grams Powder**	**Dose (ng/cm2)**	**Observed Effect on Bees**
1.0 x 10-3	5	812.0	rapid kill < one hour
5.0 x 10-4	5	406.0	~75% kill 24 hour
2.5 x 10-4	5	203.0	~ 50% kill 24 hour
1.0 x 10-4	5	81.2	no kill 24 hour
1.0 x 10-5	5	8.1	no kill 24 hour
1.0 x 10-6	5	0.8	no kill 24 hour

Project Chronology

Colony acquisition*.* We had extreme difficulty obtaining 48 packages of honey bees given the drought in California. This was confounded by similar drought conditions which developed in western Montana. Weather conditions in Montana slowed colony growth, even with feeding of syrup and pollen substitute. From the first week of May 2015 until the start of the experiment, we periodically monitored the colonies for population size and visually inspected them for any evidences of pests or diseases. We also medicated all of the colonies with antibiotics.

Our target criteria for colonies to be used in this experiment included absence of disease and pests such as mites, sufficient bee population size (with an overall average size of 8 frames of bees), a laying queen, several frames of brood, and bees in two stories (two deep hive bodies).

We started with 52 colonies in early May 2015. Supercedure of a few of the package queens and removal of two colonies that showed evidence of health problems soon reduced the number of viable colonies to 49. As the drought intensified, by the time most of the colonies reached the target population size, one colony had absconded and a couple of others fell below our minimum target size of at least five frames of bees and three frames of brood, reducing the number of viable colonies to 47.

In order to keep a balanced number of colonies in each of the four treatments; we reduced the number of colonies per treatment from 12 to 11, giving us a total of 44 colonies that met our start-of–the-experiment criteria. We did not want to risk compromising the experiment by including weak, slow-growing colonies that were likely to be marginal in terms of reaching sufficient population size and stores for successful overwintering.

Colony Pesticide Exposure*.* As we described above, we selected 44 colonies that were sufficiently strong to be used in the Clothianidin-contaminated dust exposure experiment. The colonies were dispersed among three sites in the Missoula valley to reduce the risk of floral resource depletion at any one site. We used three registered apiary sites; placing16 colonies on a mountain slope on the east margin of Missoula (MS site), 16 colonies on the north side of Missoula near rolling hills (MC Site), and 12 colonies at our fenced research yard at Fort Missoula (FM Site) on the southeast side of Missoula. This site has access to riparian vegetation along the banks of the adjacent Clark Fork River.

Prior to exposure all colonies were inspected and equalized as needed. Even though we redistributed bees among colonies to equalize them as much as possible prior to exposure, some difference among colonies remained. Consequently, colonies were ranked by relative strength then arbitrarily assigned to different locations and to treatment groups to ensure as balanced a set of colonies at each site as possible.
Within each set, colonies were then randomly assigned to one of four treatment groups as described in our experimental plan.

The dust exposures were all completed between July 23 and July 27, 2015. The vacuum chamber was transported to each site on the day of exposure. All work was coordinated so that bee collection and exposure was completed by approximately 9:00 a.m. to minimize the number of foragers that were away from the hive when bees were collected.

Our target was to expose a sample of bees that approximated the adult forager population or approximately 25% of each colony. As nearly all colonies were 8 frames or greater strong, we settled on two full frames of bees to be exposed per colony. For the weaker colonies some ad hoc adjustments were made to keep the fraction exposed roughly equal. We collected bees from honey/nectar-rich frames to avoid disturbing the queen or developing brood, and also to ensure we collected foragers. Both frames were shaken into a wire mesh cage and dusted with 5 grams of the Clothianidin/chalk mixture. Cages with bees were placed inside a dome-shaped chamber, which had a weighed amount of dry dust on a tray, placed at the central point of the dome. When the prescribed vacuum was reached, the evacuation line was closed, and a release valve opened. After 15 seconds, the bees were removed from the chamber.

After dusting, the cage of bees was returned to the hive. We ensured that bees reentered the hive by placing an empty frame box on top of the colony. The cage was opened and inverted on top of the exposed frames then covered to allow the exposed bees to re-enter the colony. After 15-20 minutes we gently shook any bees remaining in the cage into the colony and removed the box from the top. The photos below (Figure 2) illustrate the field exposure process:

Figure 2. *Dusting of bees using vacuum chamber in the field. Bees were shaken from frames into a screen cage. The cage was placed inside one of the chamber domes. A vacuum was drawn and released, filling the dome and cage with suspended dust. The cage with dusted bees was then returned to the source colony.*

We obtained full colony measurements prior to dusting of the colonies. We then photographed and collected the 24-48-hour bee kills. After 48 hours, we began weekly collection of dead bees from the traps into plastic storage bags which were kept frozen until they were weighed, counted, and subsampled for chemical analysis. Samples were submitted to the USDA AMS National Science Laboratory for analysis for Clothianidin residues.

Photographs of the three apiary sites are shown in Figure 3. Only a portion of the Fort Missoula site is shown. A perimeter fence and adjacent buildings made it difficult to show all 12 colonies in a single photograph. Figure 4 shows 24-hour bee mortality.

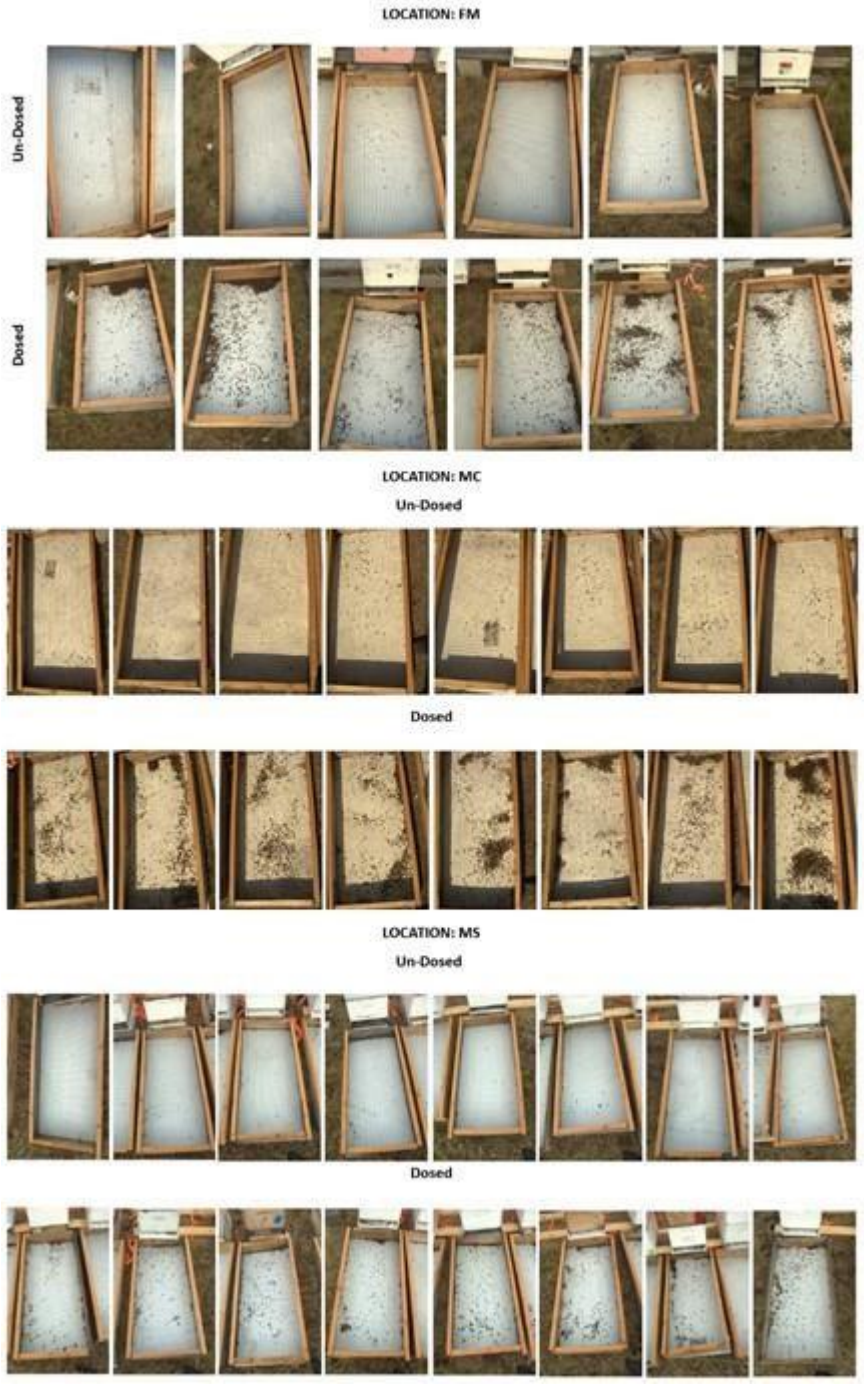

Figure 4. *Photographs of dead/dying bee capture by traps 24 hours after dosing. For each site (FM, MC and MS) the top row depicts the bee loss of control colonies (the bottom row the loss of bees from colonies dusted with Clothianidin in chalk. FM and MC sustained more severe, early loss. MS losses were more evident at 48 hours.*

Figure 5. *(Registered apiary locations), MC (10 colonies), MS (15 colonies), and FM (12 colonies). Note dead bee traps in front of hives and feeder jars on 50% of the colonies, both dosed and not dosed with Clothianidin dust*

In general, bee mortality was readily discernible within 24 hours of dusting, and the bee kill was most severe over the first 48 hours after exposure to the contaminated dust. At MC and FM, the 24-hour dead bee drop was heavier than at MS, where the 24-hour loss of bees wasn't as severe, but after 48 hours all sites exhibited similar total losses.

Post-exposure monitoring. Daily visual observations at all three apiary locations continued through mid-September 2015. After Clothianidin exposure dead and dying bees were collected every 48 hours from all hives for one week. Hive mortality clearly peaked about 48 hours after dosing. One week after exposure, dead bee counts at dosed hives visually appeared to diminish to levels similar to control hives.

After the first week, weekly collections of dead bees were made for two weeks. After three weeks, end-of season feeding protocols were put into effect on all hives to help maintain brood development and foraging in preparation for winter. Collection of dead bees from traps continued on an as-needed basis and tracked in field logs. Hives were surveyed every two days for signs of disease, swarming, or absconding. Consumption of pollen substitute and syrup was recorded. Full colony measurements were taken again the third week of August and in mid-September 2015.

End of season. In Montana, queens typically curtail brood rearing as early as September. Yet in mid-September, except for one colony, all the rest of the colonies were still viable and contained brood. In September we also experienced attempted mammalian predation on several colonies. Two skunks were trapped and removed from the Fort Missoula yard. At Mount Sentinel, a black bear tipped over several hives. As all hives were safety-strapped they remained intact. The affected colonies were righted and the entire apiary moved to Fort Missoula inside the chain-link fenced yard for protection.

Due to the second-worst draught on record for Missoula and extensive forest fires, all of the experimental colonies were exposed to wild fire smoke for a period of approximately three weeks. On some days, when the smoke was heaviest, the colonies exhibited greatly reduced bee flight and foraging. Many also became aggressive and prone to stinging. Despite the predator and smoke stressors, by mid-September at all three apiaries, many of the Clothianidin-exposed colonies had brood areas and frame counts similar to those observed during the pre-dosing inspection.

Smoke and weather conditions cleared significantly by end of September. Colonies appeared to resume foraging on late season bloom, including spotted knapweed bloom, as evidenced by assorted pollens being brought back to the hives by foraging bees.
Weak hives were given entrance reducers, while queen excluders remained in place in between the brood box and honey super in all hives.

Winter preparation. Colonies were wrapped for winter on November 14, 2015 and moved into the fenced yard at Fort Missoula. The fenced bee yard provided a measure of security against vandalism and bears. The elevated stands placed the hives above the reach of skunks.

Prior to wrapping, at end of season, another frame count was conducted to assess population size in each surviving colony. Hives placed on stands included all of the trial colonies, whether alive and strong, alive and weak, or weak and expected to die.
Colonies were placed side by side on the stands and pushed tightly together. Styrofoam insulation was place across the top of the hive covers, and the hives then wrapped in 6 ml, black plastic, leaving a small entrance opening. Initial infrared (IR) imaging indicated that the

plastic had to be tightly affixed to the colony front and back in order to detect heat from clustered colonies. Loose plastic interfered with heat transfer from the hive surface to the surface of the plastic wrap.

End-of-season frame counts indicated that some colony populations had decreased in size since the post-dosing collection of data for all measurement endpoints conducted in mid-September 2015. A few colonies had either died or were failing. None of the colony losses appeared to be due to shortage of food. Surviving colonies generally had 100 or more pounds of stores, which appeared to be sufficient for wintering. Nevertheless, we monitored and provided supplemental food if needed to prevent starvation.

IR camera imaging was used from that point forward to periodically monitor colony survival throughout the winter. This provided us with the ability to monitor the population status of each colony without opening the hives and adding cold stress to the bee colonies.

During warm periods beginning in late January 2016 when the bees were taking cleansing flights, colonies were opened and visually examined to verify survival status and to add supplemental food, if needed.

A final colony inspection was made in the spring to collect the full complement of metrics: queen status, brood areas, population size, colony weight, presence of mites, and any evidence of disease.

Results:

Pre-exposure colony condition*.* All colonies were inspected and final adjustments made to ensure all colonies were as consistent as possible at the outset of the experiment. Adult bees (frames), brood (frames) and total colony weight were assessed. Colonies were then randomly assigned to treatments with the constraint that weaker and stronger colonies were proportionally represented in each treatment category. Overall, the number of frames of adult bees ranged from 4 to 19 ($\bar{x}$ = 10.7, s.d. = 3.4, n = 44); number of brood frames ranged from 1.5 to 8.75 ($\bar{x}$ = 5.4, s.d. = 1.7), and weight ranged from 28 to 52 kg ($\bar{x}$ = 39.1, s.d. = 6.1).

After assignment to experimental groups the mean values remained relatively consistent among groups. Mean frames per colony ranged from 10.7 to 11.1 and mean brood frames ranged from 4.9 to 5.6 with groups. (Figure 5). Mean weight ranged from 38.1 to 41.2 within groups (Figure 6).

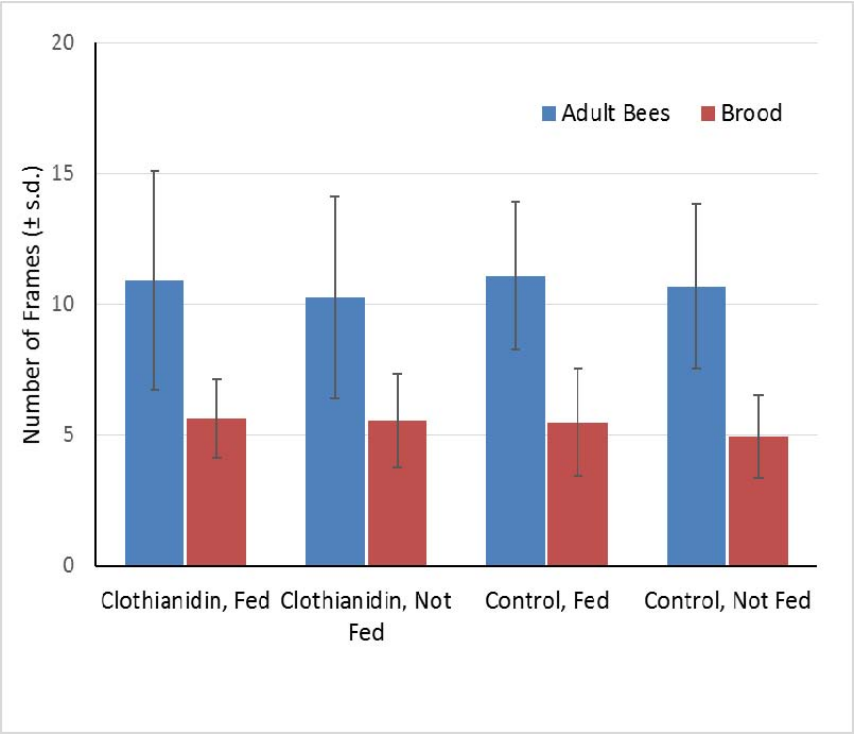

***Figure 5**. Pre-exposure colony strength in frames of adult bees and brood among treatment groups. N=44 or 11 colonies per group.*

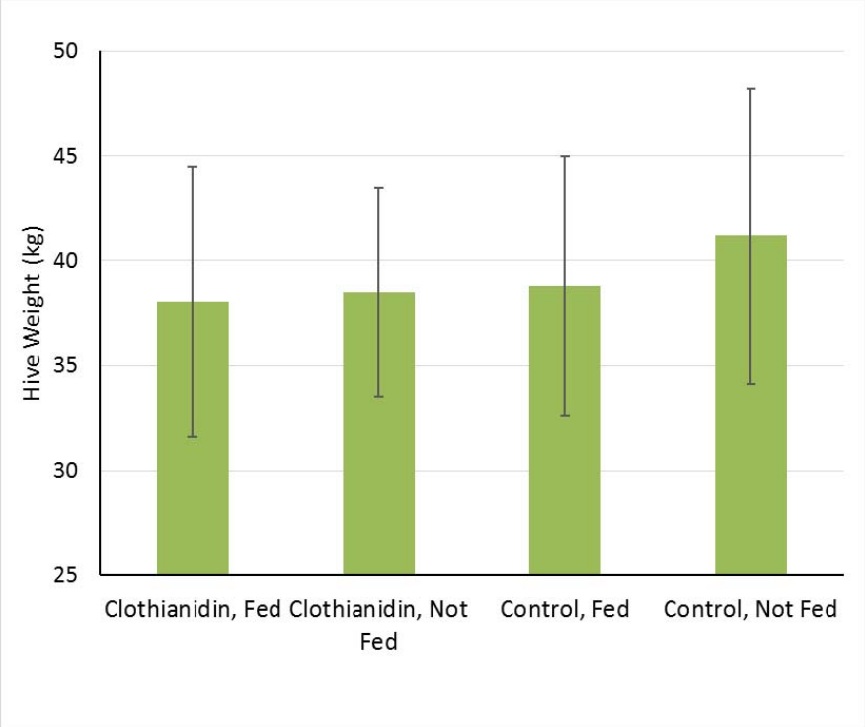

***Figure 6**. Pre-exposure colony weights among treatment groups.*

Post-exposure colony responses. Prior to exposure we observed minimal bee losses in all treatment groups. The mean number of dead bees in traps across treatments was 43 (s.d. = 28, n = 14). Within groups, the mean losses ranged from 24 to 61 bees with no statistical differences among treatment groups. Within the first few days' post-exposure, however, substantial mortality occurred in the treated colonies (Figure 7). A mean of 1,108 and 1,528 dead bees were collected in traps from Clothianidin exposed colonies compared to 113 and 32 from controls. One week after exposure mortality dropped to slightly less than pre-exposure levels in all treatment groups except the treated group which received no supplemental feeding. After two weeks, observed mortality in all groups was near normal and approximately equal.

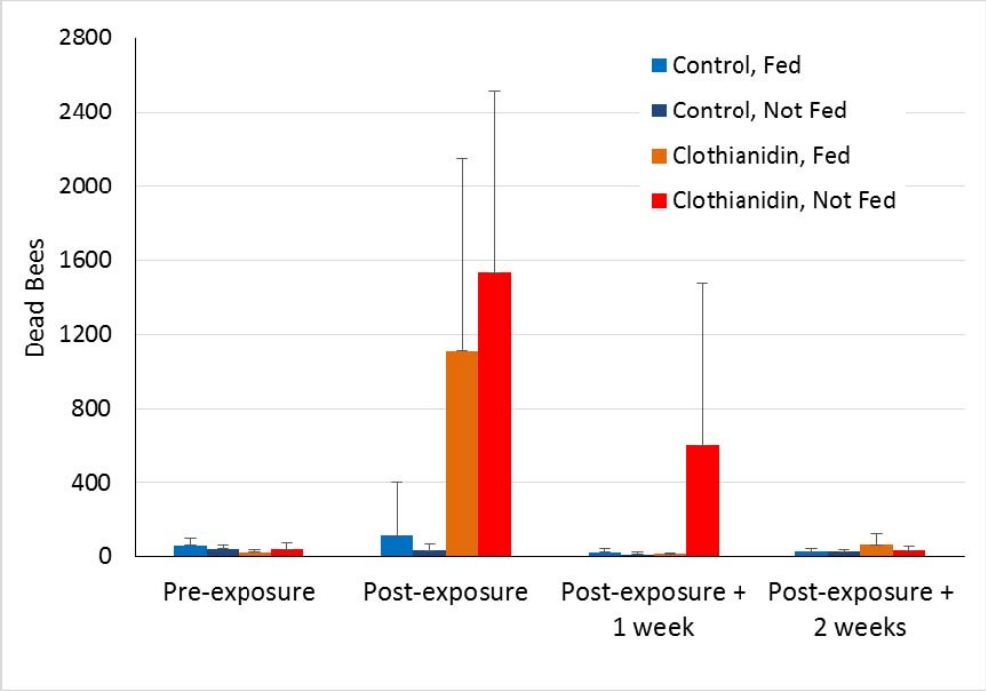

***Figure 7**. Numbers of dead bees collected in traps from the four treatment groups beginning just prior to and up until two weeks following exposure to Clothianidin-contaminated chalk dust.*

Clothianidin residues. The number of bees that died and accumulated in traps following exposure to Clothianidin contaminated dust was dramatic (Figure 7). All dead bees were periodically collected from traps and sorted by treatment and date. Twenty-five bees from each sample were taken from each sample collected during the two weeks following the experimental dusting (July 15-31, 2015). The samples were then pooled into twelve composite samples representing each treatment group and each bee yard used in the study. Analysis of the composite samples was performed by the USDA AMS Laboratory and Testing Division, Gastonia, North Carolina. Samples were tested only for neonicotinoid pesticides and their metabolites.

The returned analyses indicated only Clothianidin in every sample with no other neonicotinoid or metabolites present. Clothianidin residues were present in every composite sample (Table 3). Concentrations ranged from 7.9 to 155 PPB with an overall mean of 58 PPB Clothianidin. Assuming a typical honey bee weight of 0.1 grams, observed concentrations translate to an average Clothianidin residue per bee of 5.8 ng with a range from 0.8 to 15.5 ng per bee.

The only obvious pattern was that *Clothianidin residues were higher in samples collected from colonies in the exposed, unfed treatment group. Residue concentrations in those colonies were more than two times higher than those in the exposed colonies that received supplemental feeding (98.7 vs. 40.4 PPB) or the unexposed colonies (Figure 8).*

Although the difference was large, there was sufficient variation among samples that no statistically significant differences were present. Analysis of variance of square-root transformed Clothianidin concentrations indicated no significant difference in either main effect or the interaction between treatment and supplemental feeding (Table 4).

Table 3*. Clothianidin concentrations in dead bee samples (PPB). Analysis of pooled samples performed by USDA AMS Lab, Gastonia, NC. Limit of detection for Clothianidin was 1.0 PPB.*

	Unexposed by Bee Yard				**Exposed by Bee Yard**				
	FM	**MC**	**MS**	**Sub-Total**	**FM**	**MC**	**MS**	**Sub-Total**	**Grand Total**
Fed	79.6	72.6	7.9	53.4	43.1	66.0	12.1	40.4	46.9
Not Fed	80.7	21.0	17.3	39.7	155.0	88.6	52.6	98.7	69.2
All	80.2	46.8	12.6	46.5	99.1	77.3	32.4	69.6	58.0

Table 4*. Summary of analysis of variance for differences in Clothianidin concentration in dead bee samples.*

	Type III SS	**d.f.**	**Mean Squares**	**F-Ratio**	**p-Value**
Exposure	7.016	1	7.016	0.925	0.364
Supplemental Feeding	5.739	1	5.739	0.757	0.410
Exposure*Supplemental Feeding	14.884	1	14.884	1.963	0.199
Error	60.658	8	7.582		

Post-exposure bee populations*.* The end-of-season inspection indicated that with the exception of the Clothianidin exposed colonies that received supplemental feeding, all colonies increased in numbers of adult bees and population size.

Of the original 44 colonies, 42 survived the dosing. One colony was broodless. Either the queen stopped laying or had been lost. Another colony had absconded. Curiously, we found a marked queen in another colony, which had two queens. Obviously, either the queen from the absconding colony or from the broodless colony had moved into another, queen-right, colony.

The controls grew more than the pesticide dosed colonies, as expected, but substantial variation within experimental groups suggests that the differences are not significant (Figure 9). The change in population size is more evident when the change in frame count from pre- to post-exposure is charted. It is clear that the supplemented, exposed colonies had no increase in population and that the unfed controls increased the most.

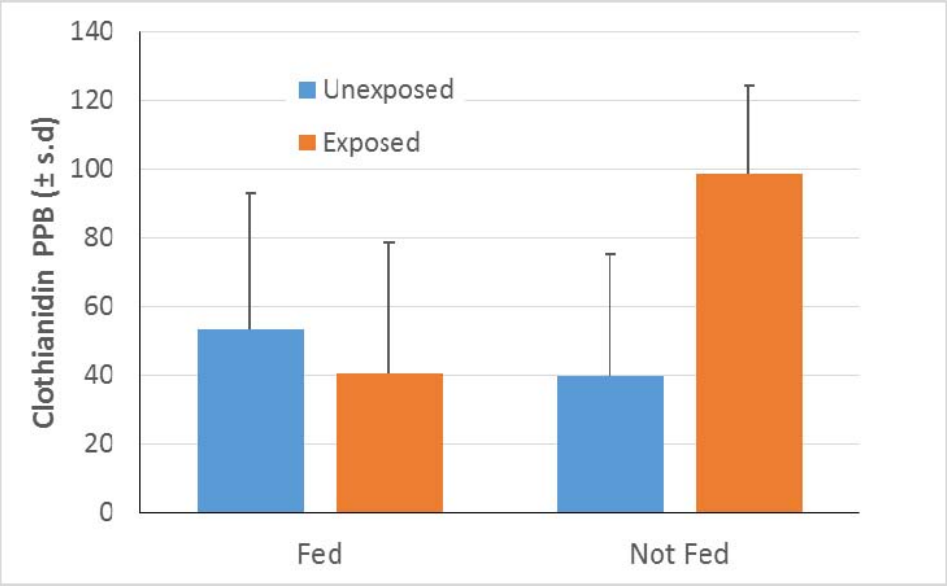

***Figure 8**. Clothianidin concentrations in dead bees collected from traps at colony entrances. Samples were pooled by treatment within separate bee yards before analysis.*

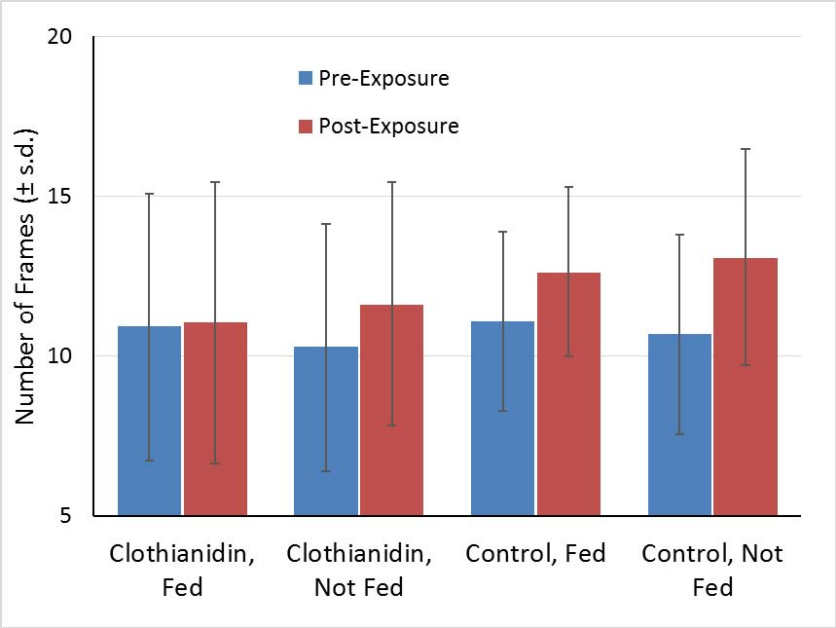

Figure 9. *Frames of adult bees in control and colonies exposed to Clothianidin contaminated chalk dust.*

It is somewhat surprising that the exposed and control groups that did not receive supplemental feeding following exposure increased more in terms of number of frames of bees more than their comparable unfed groups (Figure 10).

Infrared imaging. The IR imaging was done at no equipment cost to CDRC. We had in hand,

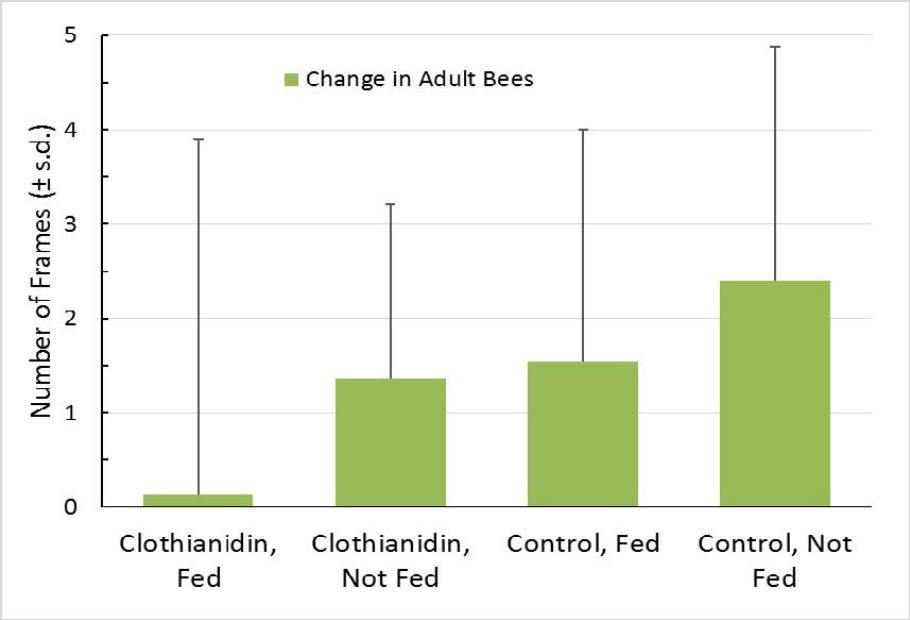

Figure 10*. Change in frames of adult bees among control and Clothianidin-exposed colonies.*

from our own work and a study funded by Project *Apis m* (PAms) several IR cameras, ranging from simple point-and-shoot IR cameras that affix to a cell phone to fully featured, calibrated, professional IR cameras.

For winter checking of the CDRC colonies, we used a FLIR E60 camera to periodically assess colony survival. This handheld camera has 76,800 calibrated temperature measurement points in each image. To avoid any air gap between the hive bodies and the wrapping material, we stretched the plastic wrap tight and secured with staples.

The following discussion and pictures are intended to be illustrative of the setup of the colonies of winter at the FM yard, of the camera, and of the IR images captured.

Figure 11. *Handheld E60 FLIR IR/visible light camera.*

Figure 11 is our best, E60 FLIR camera, priced at about $7,000. It can capture both still and video IR images, and has two cameras – a combined thermal camera and a visible color digital camera. Thus, we get both an IR and digital picture for documentation. Pictures are stored on an SD flash card as radiometric jpegs. A radiometric jpeg saves the actual temperature measurement of each and every pixel. In this case, each picture yields 78,600 calibrated temperature data points. These images can be post-processed using proprietary software from FLIR. We have both the Standard FLIR Tools and the advanced FLIR Research analysis tools software. The first is bundled with the camera; the 2nd is a limited distribution, relatively expensive program for thermographers and researchers. Again, none of the costs for the camera or software were charged to CDRC.

Immediately after wrapping, the hives on one of the stands were imaged (Figure 12) to test the efficacy of the camera. The left picture is a visible color image of one of the sets of colonies on a stand. The end of another set of colonies on a stand appears in the background. The IR image in the picture to the right readily detects the heat from strong colonies, but the image is a bit vague due to taking the picture in daylight and the initial loose fit of the plastic wrap. Colored plastic banners along the bottom edge of the stands indicate overall colony status at time of wrapping.

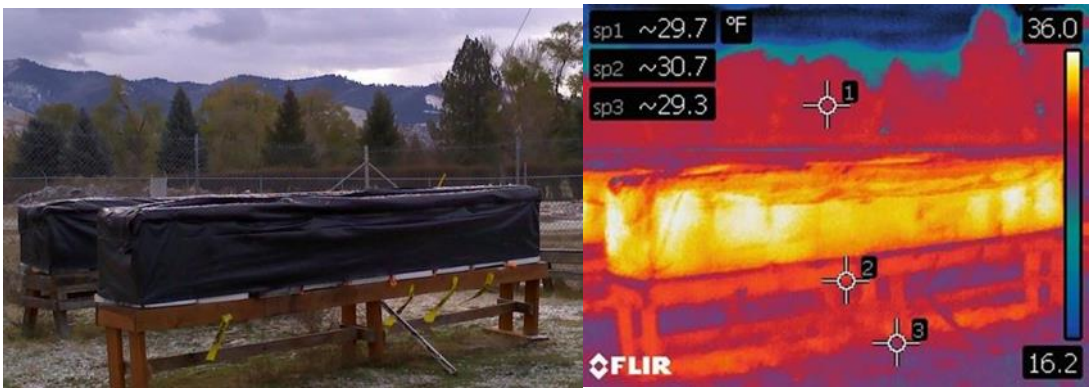

Figure 12. Test photos of wrapped colonies. The somewhat loose wrapping obscures imaging, especially for smaller clusters.

Figure 13 pictures were taken at night after the plastic wrap had been secured more tightly to the hives. The left picture is of four of the hives on a stand, the middle is of a dead colony at the end of a stand, and the right is of a viable, strong (populous) colony.
The cross hairs are spots where the temperatures shown in the upper left corner of each image were taken. The ambient temperature averaged < 28o F. That is why the hive with temperatures ranging from 25.7 to 28o in the center picture is nearly invisible. By comparison, the surface temperatures of the hive with a strong colony range from 29.7 to 36.3o. Securing the black plastic wrap to the hive surface effectively makes the wrap transparent to the thermal image.

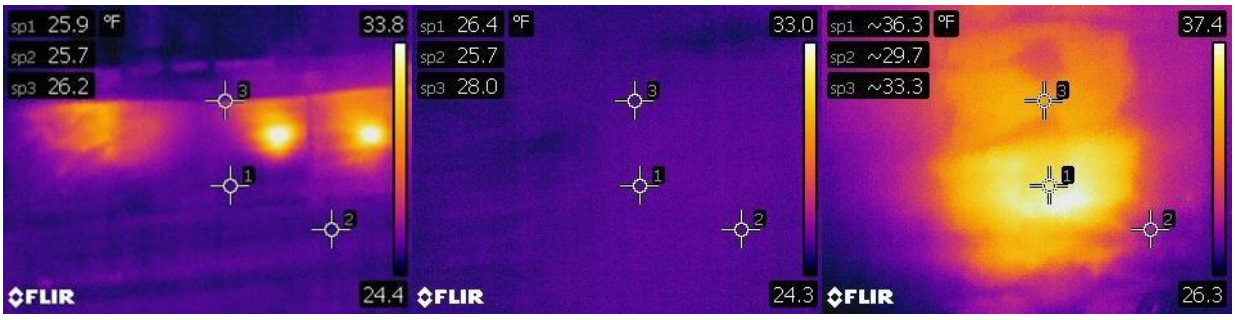

Figure 13. *IR images of wrapped colonies with the plastic tightly wrapped and stapled to hive bodies. The center image is of a dead colony.*

With all colonies stored for winter at the FM apiary for security from bears, vandalism, and theft, periodic checks of colony viability were conducted throughout the winter. Because each month had a few days of above freezing weather, we were able to lift the lid and quickly confirm the IR images of colony viability. A final full-colony inspection and collection of colony metrics was conducted in the early summer of 2016.

Survival of colonies post-treatment. In late September 2015, colonies from the three yards were moved to a common yard and prepared for winter. Colonies were individually wrapped in plastic and placed on stands in a fenced yard. Periodic checks of colonies through the remaining fall and winter were done to check food status and to identify dead colonies. Supplemental food in the form of sugar fondant was added as needed through the winter to

prevent colony death due to starvation. Fifty percent of colonies survived through to spring (Table 5). The general trend was for greater mortality within the exposed colonies where just forty percent survived the winter (Figure 14). Within exposure groups, supplemental feeding had little effect on survival (55% not fed versus 45% fed). Interestingly, more fed colonies survived in the exposed group, while more unfed colonies survived in the unexposed group. Contingency analysis showed that none of the frequency differences were significant (Pearson X2 = 1.73, d.f. = 3, *P* = 0.63).

We calculated the risk that Clothianidin-exposed colonies and unfed colonies would die over the winter period as the relative risk coefficient. Relative risk is a ratio of the risk of two events occurring, in our case the risk of overwinter death in Clothianidin-exposed and unexposed colonies, or the risk of death in fed and unfed colonies. Relative risk for Clothianidin exposure was 1.33, indicating a 33 percent greater risk of winter colony failure after exposure to Clothianidin but that the increased risk was not significant (*z* = 0.903, *P* = 0.37). The same was true for relative risk of failure in colonies that received no supplemental feeding after exposure. Relative risk was identical, 1.33, meaning failure risk was again 33 percent greater in unfed colonies, but not significant.

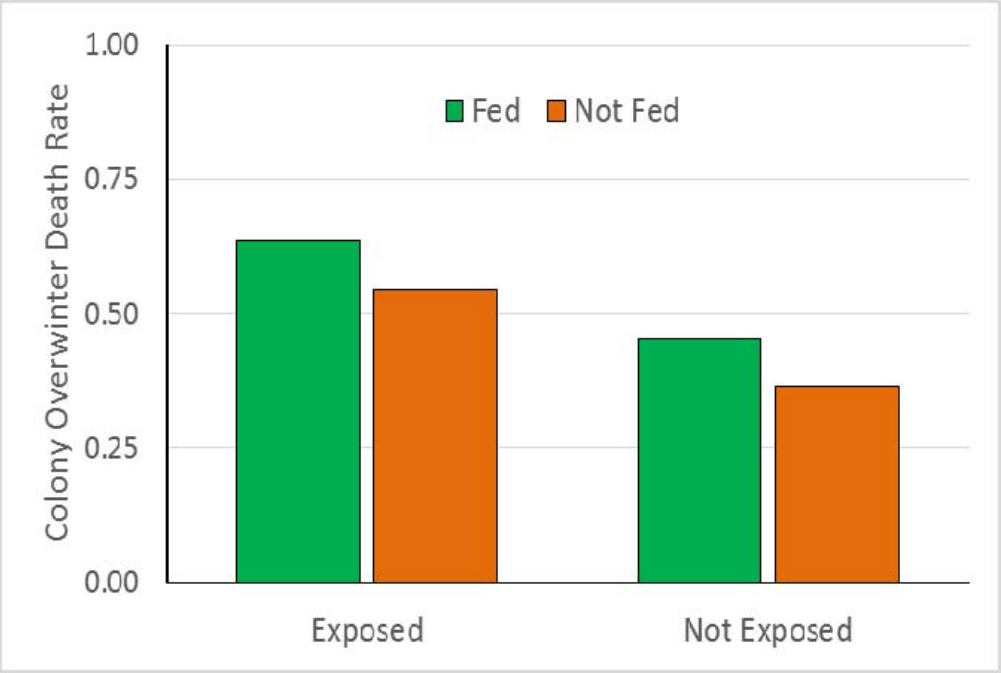

Figure 14*. Comparison of honey bee mortality between exposure and feeding treatments.*

Viability of surviving colonies. Colonies that survived through the winter were generally in good condition. Average weight for all was 39.7 kg (Table 6). Subtracting the average empty box and frame weights of 22.6 kg, bees and stores in the surviving colonies averaged approximately 17 kg (37 pounds).

Table 6. *Weights of surviving colonies taken in June 2016.*

Surviving Colonies	Mean Weight (kg)	s.d.
Exposed	**38.9**	**5.1**
Fed	34.9	3.5
Not Fed	42.2	3.7
Not Exposed	**40.3**	**5.3**
Fed	39.4	2.9
Not Fed	41.0	6.7
All colonies	**39.7**	**5.1**

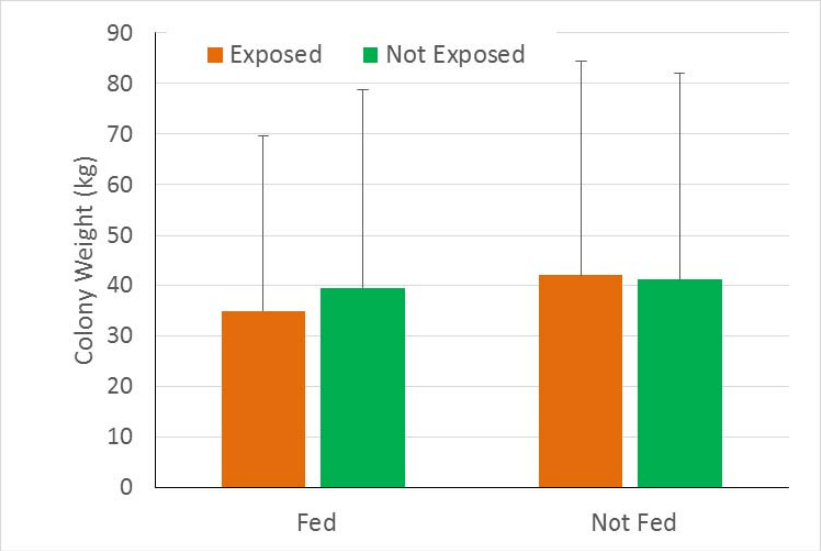

Figure 15. *Colony weights in spring (June) 2016 for surviving colonies.*

Colonies that received no supplemental feeding after exposure were slightly heavier, but none of the differences were significant (Figure 15). Analysis of variance indicated that the only difference approaching significance was the higher weights in unfed colonies (Table 7; F = 4.36;

***Table 7**. Summary of analysis of variance for differences in colony weights of surviving colonies.*

	Type III SS	**d.f.**	**Mean Squares**	**F-Ratio**	**p-Value**
Exposure	14.23	1	14.23	0.618	0.443
Supplemental Feeding	100.34	1	100.34	4.357	0.052
Exposure*Supplemental Feeding	40.85	1	40.85	1.774	0.201
Error	391.48	17	23.03		

d.f. =1, 17; *P* = 0.052).

We inspected the surviving hives and measured the brood area, including a survey for presence of eggs, larvae and pupae. We also searched for presence of a queen in each colony. Only five of the surviving colonies were queenless. Three were in the unexposed colonies; two in Clothianidin-exposed colonies. Four of the five queenless bee populations, however, were in colonies that had no supplemental feeding post exposure. Only one of the queenless colonies had a viable queen cell present.

Only one of the queenless colonies had any brood or pupae present. Total brood area was measured for the remaining 17 colonies (Table 8). The overall mean was approximately 14 frames of brood per colony, and included eggs, larvae and pupae in every case except for one colony that apparently had a newly mated queen. The most important difference that is apparent in the brood data is the three-fold difference in brood between fed and unfed Clothianidin exposed colonies.

***Table 8.** Frames of brood counted in 17 queen right colonies remaining among the 22 colonies that survived to June 2016.*

Supplemental Feeding	**Clothianidin Exposed**		**Not Exposed**		**Row Total**	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
Fed	15.69	1.95	14.90	0.91	15.25	1.42
Not Fed	5.00	7.81	16.65	5.93	12.28	8.59
Column Total	11.11	7.41	15.78	4.10	13.85	5.97

The analysis of variance indicated that although there were no differences in brood area that could be attributed to Clothianidin exposure or to supplemental feeding by themselves, the interaction between exposure and feeding was significant (Table 9; F = 2.9; d.f. = 1,13; *P* = 0.039). Specifically, as pointed out above, Clothianidin-exposed colonies and unexposed colonies that received supplemental feeding post exposure had nearly equal brood areas in our June 2016 inspection. On the other hand, *colonies that received no supplemental feeding post exposure had greatly different mean brood areas—five frames in unfed compared to 15 frames in fed colonies (Figure 16).*

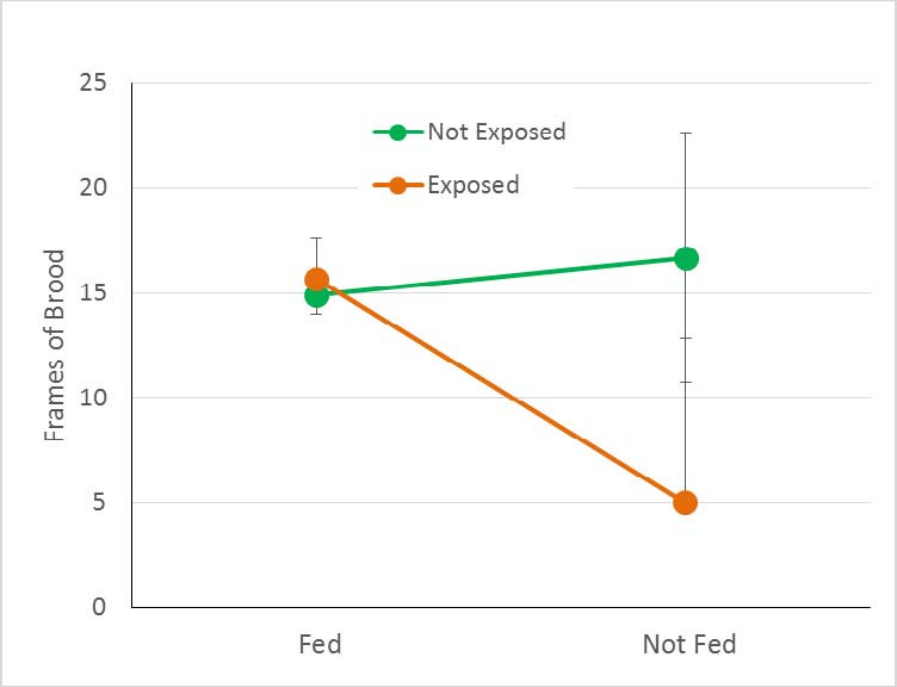

Figure 16*. Interaction plot illustrating the effect of supplemental feeding on spring brood area in Clothianidin-exposed colonies.*

Table 9*. Summary of analysis of variance for differences in brood area.*

Source	Type III SS	d.f.	Mean Squares	F-Ratio	p-Value
Exposure	1.276	1	1.276	2.326	0.151
Supplemental Feed	1.036	1	1.036	1.888	0.193
Exposure*Supplemental Feed	2.892	1	2.892	5.271	0.039
Error	7.132	13	0.549		

General Discussion

Our overall objective was to determine whether supplemental feeding could mitigate some of the adverse impacts of high-dose exposure to dust and food borne Clothianidin.

Our methods and approach included simulating common practices of commercial beekeepers in the USA in terms of colony layout within an apiary as well as typical management practices. We also focused on duplicating the severity of a bee kill that we observed just prior to planting of treated corn seed in Nebraska in 2014.

These are the conditions under which pesticide exposure incidents actually occur. Colonies are placed in yards in numbers ranging from 10 to 200, with 20-30 typical of honey production sites, larger numbers of colonies arranged in groups around fields for pollination, and at times tens of thousands of colonies placed in holding yards prior to shipping. In all cases, hives are set close together to conserve space and to facilitate inspection. Unlike many research designs where colonies are spaced far apart to reduce drift, real, working colonies are packed together, either in rows on single stands, or in groups of four- or six-colonies per pallet.

Whereas it is well known that if colonies are set out in rows where the prevailing wind is parallel to the row, downwind drift to the last colonies in the row may occur, what is not commonly known is that drift is as much, if not more, an innate property of any colony, regardless of its placement or location.

In the 1990's for several years, we had 27 colonies, all equipped with highly accurate bi-directional bee egress and ingress counters, located at several sites on and near the US Army's Aberdeen Proving Ground – Edgewood.

What we found (Bromenshenk et al., 1996) was that drift was a property of each colony. Whereas for any given apiary, in the absence of exposure to toxins, the daily forager bee return rates were in the mid- to high-90 percent, there were always a few outlier colonies that either consistency lost bees or that consistently gained bees. The colonies that gained bees (i.e., greater than 100% return on a daily basis) were not necessarily at ends of rows or in corners of the apiary, but could occur anywhere, even in the center of a group of colonies. The colony itself, not its position, was the critical factor. We concluded that either these colonies that gained bees were more attractive, possibly emitting higher concentrations of queen pheromone to the atmosphere, or showing less tendency by guard bees to rebuff interlopers. Similarly, we suspect that the colonies that consistently lose bees may have lower titers of queen pheromone which affect ability to recognize their own colony or queen. Thus, we arrayed colonies in rows using commonly found spacing, but we also closely monitored the bee drop into traps for collecting dead bees, kept photographic records, as well as collected, weighed, and counted the number of bees in these traps. These are typical of the conditions that occur within commercial beekeeping operations. Our post-exposure results with respect to numbers of dead bees in each entrance trap and concentrations of Clothianidin in the bees in those traps have relevance to field-sampling of dead and dying bees to determine whether a pesticide exposure has occurred.

Also, when investigating a possible pesticide exposure incident, we remind the investigator that chemical analysis instrumentation has improved to the point that limits of detection are often as low as 1-2 parts per billion, which is a vast improvement over the ppm detection levels of the 1960s and 1970s when bee kills by pesticides first came to the foreground of beekeeper and environmental concerns. The downside of increasingly lower limits of detection (as well as

broad spectrum, simultaneous pesticide determination e.g., 200+ pesticides at one time) is that it is hard to determine what trace levels of one or more pesticides actually mean.

Understandably, there is now a focus on associating small numbers of dead and dying bees in front of beehives which show residues of pesticides with a supposedly confirmed bee kill. As such, it is important to address the question of what constitutes a bee kill. Johansen and Mayer and Atkins spent their entire careers studying, testing, and ranking pesticides and in their seminal book Pollinator Protection: A Bee and Pesticide Handbook, 1990 (available as a recent reprint from Wicca's Press). They point out that in a colony where a queen can lay 1-3,000 eggs per day; one would expect to see some dead and dying bees in front of each hive.

It is excessive numbers of dead bees piling up in front of the hive that is a likely sign of chemical poisoning. For Todd dead bee traps the following criteria are set:

- ☐ > 100 bees per day is a normal die-off,
- ☐ 200-400 is a low kill,
- ☐ 500 to 900 is a moderate kill, and
- ☐ 1000 or more is a high kill.

In other words, a few bees on the ground in front of a hive are not necessarily an indication of a bee kill.

Conclusions

Our objective for this project was to induce an acute honey bee kill, then measure short term and long term effects on colony health. We were particularly interested in the mitigating effects, if any, of post-kill supplemental feeding on colony recovery. We did so in consideration of the outcome of our previous year's research on field exposure to Clothianidin in airborne dusts (Bromenshenk et al., 2014). In that study we generally observed low levels of the pesticide, both in dust and in honey bee gathered pollen.

However, prior to crop planting activity, significant bee kills occurred at two sites, and Clothianidin concentrations in pollen and dust were significantly elevated at three locations. Surprisingly, colonies at one of three high-exposure sites, as evidenced by increased Clothianidin in dust and pollen, did not exhibit a bee kill incident. Colonies at both sites displaying a readily apparent bee pesticide kill event recovered from losses of nearly one-quarter of the colony populations. By the end of the approximately eight-week study period, these colonies were nearly equal in strength to unaffected colonies. Inasmuch as we supplemented all colonies in the 2014 study with pollen substitute and sucrose syrup, we hypothesized that supplemental nutrition may have been the reason the impacted colonies recovered so quickly and so well.

For this current study, our experimental Clothianidin dust exposure mirrored our field observations. Exposing two frames of bees per colony to Clothianidin -contaminated chalk (a surrogate for soil dust) induced heavy kills within a few days of exposure. We observed mean kills of approximately 1,300 bees in exposed colonies which, with an estimated seventy percent recovery efficiency in the dead bee traps we used (Porrini et al., 2002), indicated upwards of 1900 bees per colony were lost in the first few days following exposure. This would be characterized as a high severity kill by Johansen and Mayer (1990). For the eight frame average population size colonies that we used, this represented an acute

loss of between twenty and twenty-five percent of the bee population and was ten-fold higher than losses we observed in control colonies over the same period.

The dust concentration of Clothianidin needed to induce our observed kill, 203 ng / bee, may seem high, but actual contact dose experienced by exposed bees was much lower. Analysis of dead bees indicated only 5.8 ng / bee Clothianidin residue which was well within published LC50 which varies between 2 and 44 ng / bee (Iwasa et al. 2001, EPA 2003, Bailey et al. 2005).

It was initially disconcerting that dead bees collected from traps in control and exposed colonies had similar pesticide concentrations, suggesting that perhaps cross contamination had occurred among treated and control hives. However, dead bee count data do not indicate that any cross contamination occurred and closer examination of the data shows that only three sets of untreated colonies had high Clothianidin levels. It seems more likely that the presence of Clothianidin in dead bees in the traps in front of control colonies is evidence of drift in sick or dying bees.

Several recent studies have suggested that neonicotinoid exposure can effect orientation and memory in bees (Blacquière et al. 2012) which would increase errors in hive fidelity. Sick bees would most likely then be forced out of the hive by guard and housekeeping bees ending up in the traps of otherwise uncontaminated colonies. Regardless, the absence of increased mortality and observable differences in other colony variables make it unlikely that the results of our study are compromised by cross- contamination.

Post-exposure, all colonies increased in number of frames of bees through the season; all were in better condition than pre-exposure counts. Supplement-fed colonies did not increase as much as their unfed counterparts. This observation confused us at first. It may suggest that absence of readily accessible food stimulated increased production of brood in unfed colonies to try to compensate for the difficulty in provisioning the colonies. Through the winter, total colony weights were all skewed in favor of untreated and of fed colonies but by spring, all of the surviving colonies emerged within one kilogram mean weight of each other.

The most important long term effects we observe were that over-winter risk of colony death was 33 percent greater in unfed, exposed colonies. Most importantly, spring 2016 brood counts suggest that the subtle brood effects we observed through the post-exposure flight season in 2015 compounded over the winter to have significant effect when brood rearing resumed the following spring in 2016.

In the spring of 2016, significantly smaller brood area, nearly four frames less, was observed in exposed colonies that did not receive supplemental feeding post-exposure. Depressed brood rearing during the critical early spring period of resumption of foraging would put affected colonies at much greater risk for failure in the second year following pesticide exposure. In the spring, colonies need to replace older, over-wintered forager bees with young, vigorous forager bees as quickly as possible; especially in temperate climates where floral resources become available rapidly and last for a relatively short growing season.

Supplemental feeding effectively negated the second year brood depression; mitigating the first year Clothianidin exposure. So, although hard hit colonies may show apparent rapid recovery from Clothianidin-induced bee kill during the remainder of the foraging season of the year of the exposure incident; it appears that in the absence of supplemental feeding, population growth (reproductive) vigor becomes significantly reduced the following spring. Thus, as we observed in

Nebraska in 2014, post-kill supplemental feeding can negate long-term negative effects, even into the second year.

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Use by Honey Bees of Flowering Resources In and Around Corn Fields, Year 2 (Project 1)

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Summary of Results and Recommendations

The basis of this study was to examine the early season weeds and other flowering plants in and around corn fields from which bees could collect pollen. The objective of this study was to determine best practices for corn production weed management in order to mitigate exposure of bees to insecticide contamination from planting dust. Detectible levels of clothianidin and thiamethoxam were found in samples of all 12 species analyzed and from pollen collected at all study sites. Detection of neonicotinoids was most frequent on May 7 and May 19 corresponding to peak corn planting activities among and adjacent to the study areas. A significant increase in numbers of dead bees was observed as neonicotinoid contamination levels increased. The extended period of localized planting and potential contamination from fields adjacent to each study site may possibly preclude a distinct spike in number of dead bees. Results of this study identify the majority of bee-collected pollen at the time of corn planting in NW Iowa to be from woody plants. Woody vegetation in Iowa does not occur within corn fields or along field margins, but typically is found in farm yards, small woodlots or along water-ways. Therefore weed management changes would do little to reduce the exposure of honeybees to neonicotinoid contaminated forage. Recommendations for mitigating the exposure of bees to neonicotinoid contaminated forage is to discontinue the use of these seed treatments or to improve methodology to reduce discharge of contaminated dust.

Methods, Cooperators and Study Sites Cooperating farmers who participated in 2014 are located in 3 NW Iowa counties and together provided eight corn production sites. Among these sites both pneumatic (4) and finger type (4) planters were employed and no-till (5), strip-till (1) and conventional (2) cultivation used (table 1). The landscape cover was characterized over a 3 km radius centered on the location of study hive pairs for each site (figures 1 and 2). Cover types at all sites were heavily predominated by corn and soybean ranging between 75 and 90%.

Hive pairs were positioned at each site along the field margin. Each hive consisted of two 10-frame brood boxes containing a queen, brood, approximately 20,000 workers, honey stores, and a feeding reservoir. All hives were fitted with an external Betterbee® Anatomic Pollen Trap collect corbicular pollen pellets from returning forager bees prior to and following corn planting. The traps were deployed for 48 hours with the sampling date representing the second 24 hours. Bee-collected pollen sampling commenced in 2014 the day of the earliest planting date among our cooperators (April 21 at site 1). Pollen sampling continued for 5 weeks concluding in late May. Drop-zone dead-bee traps measuring 102 by 51 cm were placed in front of each hive and dead bees collected for the same 48-hour periods the pollen traps were deployed.

Pollen was collected from plant species in flower at each site on each date corbicular pollen pellets were trapped. We used these samples to build a pollen library to utilize in species identification of bee-collected pollen. Bee-collected pollen pellets were sorted by color and representatives selected randomly for imaging. Pollen images were compared to images from our reference pollen library, allowing identifications for most pollen types. Use of collection date, available plant phenology data, location of plants in bloom and comparison to published pollen micrographs allowed identification of samples not represented in our pollen library. Using these methods we were able to identify the species or genus of 98% of 462 samples in 2014.

Selection of pollen samples for chemical analysis was based on sufficient quantity of proportionately important species at the maximum number of sites for each collection date.

Samples were sent to the USDA, AMS, National Science Laboratory, Gastonia, NC for the detection of neonicotinoid contamination. Analyses utilized GC/MS with a limit of detection (LOD) of 1.0 ppb for each of the following neonicotinoids; clothianidin, thiamethoxam and imidachloprid).

Results

Neonicotinoid analyses Important forage species in 2014 included maple (*Acer sp*.) which was particularly important as an early season forage source representing large proportions of total pollen collected over several dates; 72% April 21, 95% April 25, ≥46% May 4 and 7 (figure 3). Furthermore, maple pollen was collected at the majority of the sites among these dates. Another important early season forage species was ash (*Fraxinus sp.*), which represented 34% by weight of total pollen collected May 4. Apple (*Malus domestica)* was of similar importance as a forage source accounting for nearly 60% of all pollen by weight collected on May 19. A pollen species tentatively identified as *Artemisia sp.* was collected at 5 sites on May 16 in representing 48% by weight of all pollen collected that date.

These results identify the majority of bee-collected pollen at the time of corn planting to be from woody plants. Woody vegetation in Iowa does not occur within corn fields or along the margins, but typically is found in farm yards, small woodlots or along water-ways. The landscape analysis of each study site (figure 3) can be used to inform availability and proximity of these important pollen sources (figures 1 and 2). The cover types that could potentially provide woody plant bee forage include developed land, deciduous and mixed forest, and woody wetlands (table 2). Together these landscape cover types account for an average of only 9.71% of the landscape surrounding the hives due to the preponderance of corn and soybean.

Detectible levels of clothianidin and thiamethoxam were found in samples of all 12 species analyzed (appendix A) and from pollen collected at all study sites. Detection of neonicotinoids was most frequent on May 7 and May 19 likely corresponding to peak corn planting activities among and adjacent to the study areas. Planting corresponded to precipitation levels and followed periods of low rainfall. It is possible as well that periods of intense rainfall such as that occurring in the interval between sampling dates of May 7 and 16 can explain some of our observations. None of the analyzed pollen species (4) or samples (11) collected on May 16 contained detectable levels of neonicotinoids. During the preceding interval 5.05 cm (>2 inches) of precipitation was recorded. The effects on pollen contamination may be twofold. First, the rain may rinse any carryover contamination from the exposed flowers and second, newly generated dust contamination was unlikely due to the saturated soils in the fields precluding the use of dust generating planting machinery.

Dead bee counts The extended period of localized planting and potential contamination from fields adjacent to each study site may possibly preclude a distinct spike in number of dead bees. We recorded planting dates for neighboring fields surrounding site 7 that demonstrate the likelihood of a planting period effect instead of a specific planting date effect (figure 4). A significant increase in numbers of dead bees was observed as neonicotinoid contamination

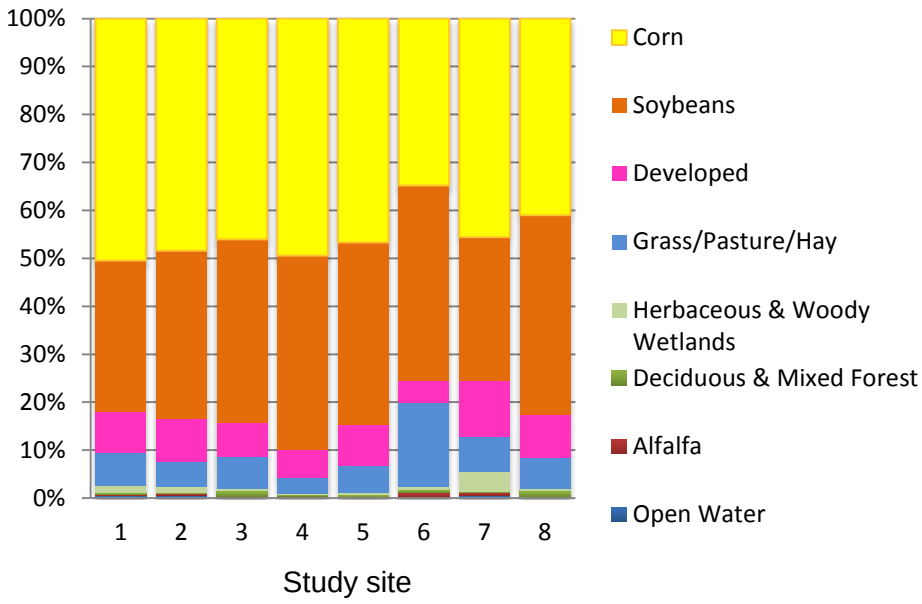
levels increased. Average site ppb clothianidin and thiamethoxam levels and site effects were each highly significant (p<0.0001 and p=0.0006, respectively) (ANOVA; Rsquare=0.65, F Ratio 6.74, Prob>F <0.0001). Furthermore, when days pre/post plant by site was added to the model along with it's square (based on the data distribution) planting date by site was not a significant predictor of number of dead bees (p=0.2751).

Table 1. Cooperator planter make, model and serial numbers, type of cultivation, planting dates and applied seed treatments.

Site	Planter make/model (serial no.)	Cultivation	Planting date (s)	Seed treatment
1	Case 1250, 24 row (Y8S007175)	no-till	23-Apr	clothianidin
2	Case 1250, 24 row (Y8S007175)	no-till	26-Apr	clothianidin,
3	Case 1250, 24 row (Y8S007175)	no-till	21-22 Apr	clothianidin,
4	John Deere 1770NT, 24 row (1A01770ZLBM745110)	no-till	21-Apr	CruiserMaxx250
5	White 6122, 12 row 30" air pressure/disc (607742)	conventional	18- May	none
6	John Deere 7000, 6 row narrow finger (082747A)	conventional	17-19 May	none
7	John Deere 7000 Max-Emerge, 8 row finger (010025A)	no-till	7-May	CruiserMaxx250
8	John Deere 7000, 8 row finger (028220) Kinze 3500, 8 row finger (902925)	strip till	6/16 May	CruiserMaxx250

Figure 1. Land cover types surrounding hives to a 3 km radius at each study site with percent landscape in corn and soybean combined given for each site.

Figure 2. Landscape composition at each study site within a 3 km radius centered on the hive pair. Corn and soybean predominated across all sites ranging between 76 and 90%.



Site	1	2	3	4	5	6	7	8
Alfalfa	0.22	0.30	0.21	0.38	0.98	0.16	0.69	0.05
Deciduous & Mixed Forest	0.37	0.23	1.22	0.34	0.62	0.48	0.06	1.47
Developed	8.43	9.16	6.90	5.77	4.68	8.61	11.79	8.79
Herbaceous Wetlands	1.36	1.13	0.42	0.07	0.75	0.47	4.16	0.37
Herbaceous & Woody Wetlands	1.36	1.14	0.43	0.07	0.76	0.47	4.17	0.37
Total potential forage	12	12	9	6	8	11	21	11

Table 2. Percent landscape cover types potentially providing honeybee forage at each study site. Totals of potential forage cover types ranged between 6 and 12% at all sites except site 7 where these types covered 21% of the landscape.

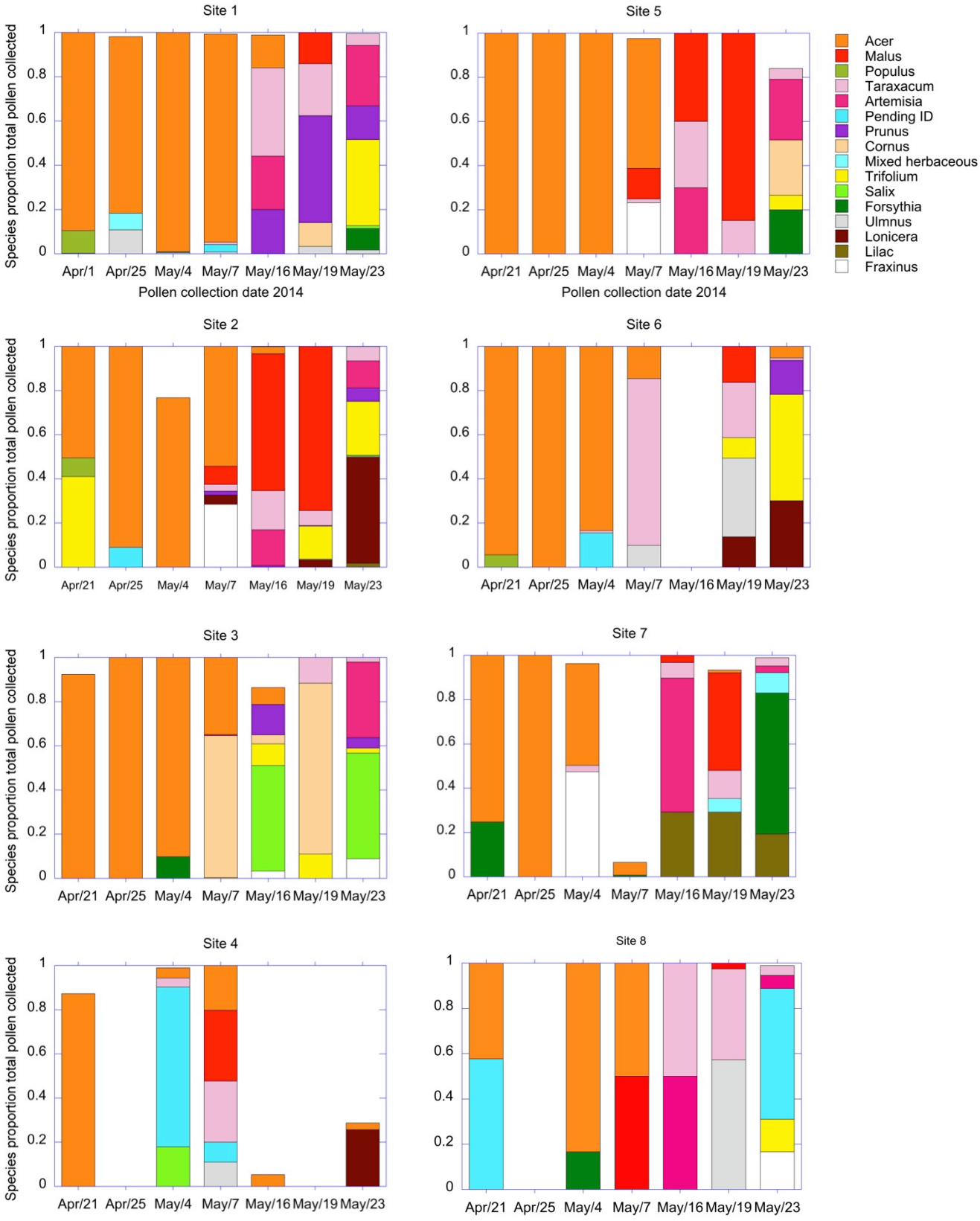

Site 1
Species proportion total pollen collected
Apr/1 Apr/25 May/4 May/7 May/16 May/19 May/23
Pollen collection date 2014
Site 5
Apr/21 Apr/25 May/4 May/7 May/16 May/19 May/23
Pollen collection date 2014
Acer
Malus
Populus
Taraxacum
Artemisia
Pending ID
Prunus
Cornus
Mixed herbaceous
Trifolium
Salix
Forsythia
Ulmnus
Lonicera
Lilac
Fraxinus
Site 2
Site 6
Site 3
Site 7
Site 4
Site 8

Figure 3 (Previous page). A comparison of pollen species proportions at each site across all pollen collection dates

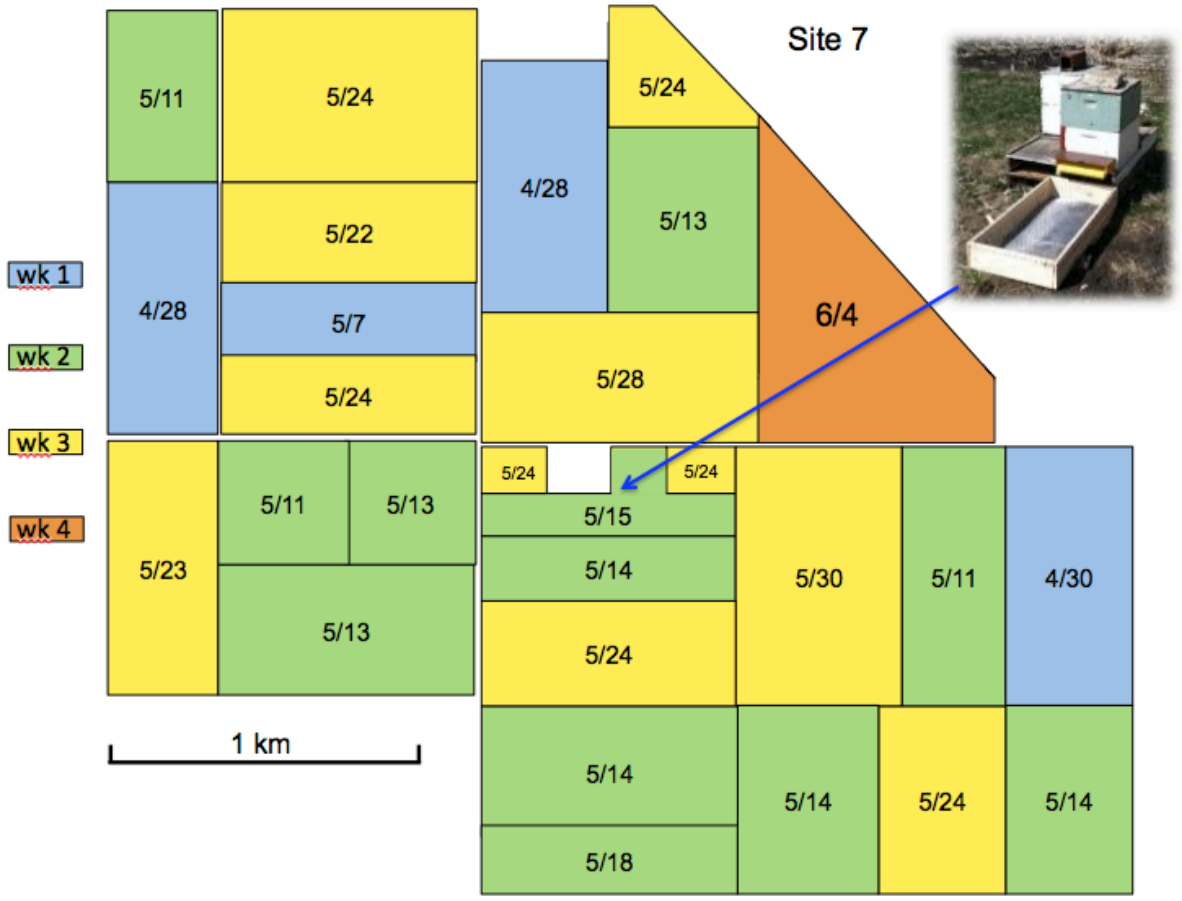


Figure 4: Field configurations and planting dates adjacent to and within foraging distances of study site 7 hives. Planting occurred over a 4-week period between April 28 and June 4 with the majority of fields planted between May 11 and 24.

Appendix A. clothianidin (clo) thiamethoxam (thi)

Date	Pollen species	Clothianidin + Thiamethoxam (ppb) Max.	Min.	Ave.	No. sites	No. samples	% sites with clo/thi	Date ave. clo + thi (ppb)	Precipitation (cm) since previous sample date
4/21	Acer rubrum	48.6	5.9	23.3	3	3	100.0	22.40	-
	Acer sp.	15.2	15.2	15.2	6	6	17.0		
	Populus sp.	26.9	26.9	26.9	3	3	33.0		
4/25	Acer sp.	10.3	8.8	9.6	6	6	33.0	9.60	1.25
5/4	Acer sp.	9.1	9.1	9.1	7	7	14.0	18.53	2.37
	Fraxinus sp.	18.7	8.3	13.5	4	4	50.0		
	Salix sp.	38.0	38.0	38.0	1	1	100.0		
	Taraxacum officinales	0.0	0.0	0.0	2	2	0.0		
5/7	Acer s.	177.1	22.8	60.3	7	13	54.0	61.85	0.00
	Fraxinus sp.	178.1	30.1	77.2	4	4	100.0		
	Malus domestica	215.0	63.8	121.1	4	4	100.0		
	Taraxacum officinales	35.6	5.5	15.4	5	6	100.0		
5/16	Acer spp.	0.0	0.0	0.0	1	1	0.0	0.00	5.05
	Artemisia sp.	0.0	0.0	0.0	5	5	0.0		
	Malus domestica	0.0	0.0	0.0	2	2	0.0		
	Taraxacum officinales	0.0	0.0	0.0	3	3	0.0		

5/19	Lilac	13.1	4.9	9.3	3	3	100.0	43.80	0.00
	Malus domestica	17.0	5.0	11.0	7	7	43.0		
	Taraxacum officinales	268.0	5.5	63.4	8	8	63.0		
5/23	Artemisia spp.	10.5	8.7	9.1	6	6	50.0	9.34	0.02
	Cornus spp.	21.9	6.7	13.0	4	4	75.0		
	Forsythia spp.	9.1	9.1	9.1	7	7	14.0		
	Lilac	9.3	7.6	8.5	5	5	60.0		
	Salix spp.	6.0	6.0	6.0	1	1	100.0		
	Taraxacum officinales	4.0	4.0	4.0	4	4	75.0		

Corn Dust Research Recommendations - 2017

Pollinators provide an important ecosystem service, facilitating the production of foods and thus helping to provide food security. Concerns have been raised about the declining populations of some pollinators, and attempts have been made to identify the factors underlying these declines. Pesticides are among the potential contributors to such declines, and much is known about their toxicity and how they are being used, but there is a need for a better understanding of potential mechanisms of exposure and their consequences.

The CDRC was established to study one potential route of insecticide exposure for honey bees that is associated with the planting of neonicotinoid-treated coated corn seeds. Some of the planters used to plant these treated seeds may result in some abrasion of the coatings, and generation of dust (referred to as fugitive dust or dust-off) containing insecticide residues that can then be dispersed into the environment in and around the planted fields. Research funded by the CDRC has investigated the transmission of fugitive dust, how it can result in exposure to honey bees in the local environment, and what the consequences may be for honey bee colonies. The CDRC recommendations are based on data from three years' work at four separate institutions. The original CDRC goal was to be as helpful as possible in influencing the behaviors of all stakeholders with respect to the corn growing season.

Several steps will need to be taken to reduce exposure of honey bees to neonicotinoids in dust from abraded treated seed coatings that can be released during planting. Contributions are needed from every sector involved – from farmers, beekeepers, pesticide and lubricant manufacturers, equipment manufacturers, seed dealers, seed treatment application operations, government agencies and regulators, extension agents, agricultural and commodity organizations, and agricultural media. **The CDRC recommendations in bold are identified as having come directly from the results of the CDRC research.** Other recommendations are supported by work outside the CDRC research program. All recommendations have been vetted with the members of the CDRC; and, within the group there is general agreement that the recommendations though based on three years' data will benefit from further research. Recommendations are presented as a part of an incremental approach that will need to be tried and tested, monitored and adaptively managed.

RECOMENDATIONS

A. Farmers

- **Use abrasion-reducing lubricants in pneumatic planters during planting to reduce dust. The CDRC results are not consistent with other research regarding the extent to which synthetic lubricants reduce net emission of dust-borne pesticide during planting of treated seed; however, the CDRC research showed sufficiently significant reductions to warrant use of these synthetic lubricants compared to talc or graphite.**

- **All research sites showed that during the corn planting window (approximately two weeks) honey bees foraged primarily on the pollen of woody shrubs and trees including apples, crab apples, hawthorns, maples and/or willow in areas outside of treated fields. These are important foraging sources to honey bees, particularly when sufficiently distant from the planting area to be unaffected by dust but within the foraging range of the honey bee. Bee-attractive woody pollen sources can be vulnerable to drift of pesticides in exhausted dust when corn is planted within 50 meters of such forage.**
- **Remove flowering vegetation within fields through tillage, mowing or use of herbicides where appropriate prior to planting.**
- Follow the principles of Integrated Pest Management (IPM). Get information at http://www.northeastipm.org/ and https://www.epa.gov/managing-pests-schools/introduction-integrated-pest-management.
- Follow all directions on treated seed container labeling and take precautions to reduce dust and drift, especially with respect to wind speed and weather conditions during corn planting. As stewards of the land, farmers play a significant role in the health of pollinators by reducing drift during corn planting. See the Guide to Seed Treatment Stewardship http://seed-treatment-guide.com/.
- Minimize unnecessary use of seed treatment insecticides. Use them only when needed, such as where historic pest infestations are above threshold or high risk factors for pest pressure have been anticipated or determined. While untreated seeds are available, they may need to be ordered in the fall prior to spring planting
- Seeding equipment foils and baffles are available that can help deflect dust downward thereby reducing drift. Please see https://www.iso.org/standard/61136.html for further information, though these standards refer to equipment design, not necessarily foils or baffles.
- Clean and maintain planting equipment regularly and carefully. Keep in mind that dust left in the hopper can still cause harm. In cleaning and maintaining planting equipment avoid generating additional dust and avoid contaminating areas with wash water. The dust needs to be scrubbed or filtered out of the exhaust and placed below ground or properly disposed of.
- Communicate with beekeepers to ensure that they are aware of planting timing and can take appropriate precautions to protect colonies (see below).
- If planting cover crops, choose varieties that are not in bloom during corn planting.

- Properly store and handle seeds by adhering to recommendations on the seed treatment tag. Treated seed should be protected from direct sunlight, extreme heat and moisture and kept in a well-ventilated area.

B. Beekeepers

- **Position hives away from areas where drift of corn dust can settle on herbaceous or woody plants during planting. Prevailing wind direction and wind speed may be helpful indicators for placement.**
- **Supplement the hive with internal feeding during and right after corn planting, and provide clean water to reduce the need for bees to seek water from sources in and adjacent to corn fields that may have been contaminated by fugitive dust.**
- **Protect supplemental food and water from dust drift.**
- Bees that are exposed to fugitive dust can have greatly improved recovery through post-exposure supplemental feeding and access to clean water. This action has the potential to mitigate long-term effects of exposure.
- If possible, reduce foraging of bees on the days of planting by confining bees to colony and/or by providing supplemental feeding source protected from dust drift.
- Communicate with growers/producers when you have hives in the area to be seeded.
- Clearly label hives with your contact information.
- Check hives regularly and report incidents to state/tribal lead agencies and/or EPA. https://www.epa.gov/pollinator-protection/report-bee-kills

C. Pesticide and lubricant manufacturers

- Continue work to improve seed treatments and fluency agents to reduce dust and dust movement at planting to further reduce risk to bees. This includes reduction in the generation and movement of contaminated dust off-field (*e.g.,* improved sticking agents and coatings, and improved fluency agents).
- Ensure the lowest effective labeled rate of neonicotinoid treatment is applied to the seed.
- Offer fungicide-only seed treatment options.
- Avoid/limit post-processing of treated seeds.
- Reach out to farmers, and help make them aware of the potential for pollinators to be exposed to contaminated dust and of the importance of farmers implementing recommended actions to reduce bee exposure.

D. Equipment manufacturers

- **Ensure that equipment users understand the importance of bee protections and the value of using lower-drift lubricants.**
- **Reduce aerial mobility of insecticide-laden particles by directing dust downward through planter design including foils and deflectors in equipment.**
- Provide mechanical means to reduce the movement of dust from fan exhaust during planting using equipment design principles and verification methods established in internationally recognized standards (ref. ISO 17962:2015, Agricultural machinery – Equipment for sowing – Minimization of the environmental effects of fan exhaust from pneumatic systems - https://www.iso.org/standard/61136.html).

E. Seed dealers

- **Adhere to quality control measures outlined in http://seed-treatment-guide.com/wp-content/uploads/2014/12/ASTA-Seed-Guide-Application.pdf**
- Support bee health by providing outreach to producers to make wise seed choices and to follow best seed planting practices.
- Offer untreated seeds as an option for farmers, and make it clear that this option is available.
- Take care in all production, movement, and storage of treated seed before and after it is used in planting. All abrasion of seed or residue from production has the potential to contribute to fugitive dust.

F. Provincial, state and federal government agencies and regulators

- Provide financial and instructional support for maintaining trees and shrubs outside drift areas for bee forage during planting season.
- Provide guidance for the reduction of attractive herbaceous forage in and around corn fields.
- Fully fund governmental provisions to ensure that pollinator forage area enhancement can increase and be sustained.
- Encourage application of the lowest effective labeled rate of neonicotinoid treatment on the seed.
- Ensure that both insecticide-treated and fungicide-only seeds are available, and educate farmers about this option.
- Ensure that IPM practice information is available to the producer.

- Provide a responsive structure for bee-incident reporting and be sure that it is understood and used by beekeepers. Ensure that incident report procedures are adequately funded and operate in a timely fashion commensurate with the urgency of this situation for honey bees and beekeepers.
- Ensure that seed bag labeling is clear and that growers are aware of the potential risk posed by planter dust.
- Dedicate transportation corridor and rights-of-way plantings to support the establishment of pollinator habitat.
- Reach out to farmers, and help make them aware of the situation and of the importance of farmers implementing recommended actions to reduce bee exposure from dust-off.

G. Extension agents, agricultural and commodity organizations, and agricultural media

- Ensure that IPM practice information is available to the grower/producer.
- Educate the beekeeper in practices that will safeguard bees.
- Educate beekeepers on bee-incident reporting.
- Educate growers/beekeepers so that label directions are clearly understood.
- Help agricultural producers, seed dealers and other stakeholders become aware of the situation and encourage them to adopt recommendations from this report to reduce bee exposure.