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

PI:
Reed Johnson, Assistant Professor
Department of Entomology
Ohio State University – OARDC
1680 Madison Ave.
Wooster, OH 44691
Phone: Office – 330-202-3523, Cell – [redacted]
E-mail: Johnson.5005@osu.edu

Co-PI:
Harold Watters, Assistant Professor
Ohio State University Extension
Field Specialist Agronomic Systems
1100 S. Detroit St.
Bellefontaine, OH 43311
Phone: Office – 937-599-4227, Cell – [redacted]
E-mail: watters.35@osu.edu

Co-PI:
Chia-Hua Lin, Postdoctoral Research Associate
Department of Entomology
Ohio State University – OARDC
1680 Madison Ave.
Wooster, OH 44691
Phone: Cell – [redacted]
E-mail: lin.724@osu.edu

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. This aerial transport implies that the body of air above and around the planted field is bears suspended seed treatment particles. **(Section 1)**
- 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 2)**
- 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 3)**
- Despite elevated adult mortality during corn planting, the magnitude of seed treatment exposure is not predictive of colony strength or overwintering success. **(Section 4)**

- Landscape composition, apart from the area of corn fields being planted, is not correlated with pollen contamination 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)**.
    - Use of untreated seeds or seeds treated with an insecticide having lower toxicity to honey bees should be more broadly used.
    - Engineering and quality-control measures to should be explored to ensure seed treatment formulations are well-adhered to seed.
    - Reduced aerial mobility of seed treatment particles may be possible through planter modification or seed treatment reformulation.

OUTLINE

I. **Introduction:** Objectives and study setup
II. **Section 1:** The release of neonicotinoid-laden dust during the planting of treated corn
    A. Dust drift during planting
    B. Qualitative assessment of seed treatment integrity
III. **Section 2:** Neonicotinoid contamination of honey- bee-collected pollen during corn planting
IV. **Section 3:** Elevated mortality of adult honey bees during corn planting
V. **Section 4:** Effects on colony strength
VI. **Section 5:** Routes of exposure and implications for mitigation
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
IX. **Section 8:** Simulation modeling of exposure via floral contamination and its sensitivity to weed suppression
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-framed Langstroth hives on solid bottom boards. Other colonies were overwintered colonies, usually in 10-frame hives, made available by cooperating beekeepers.

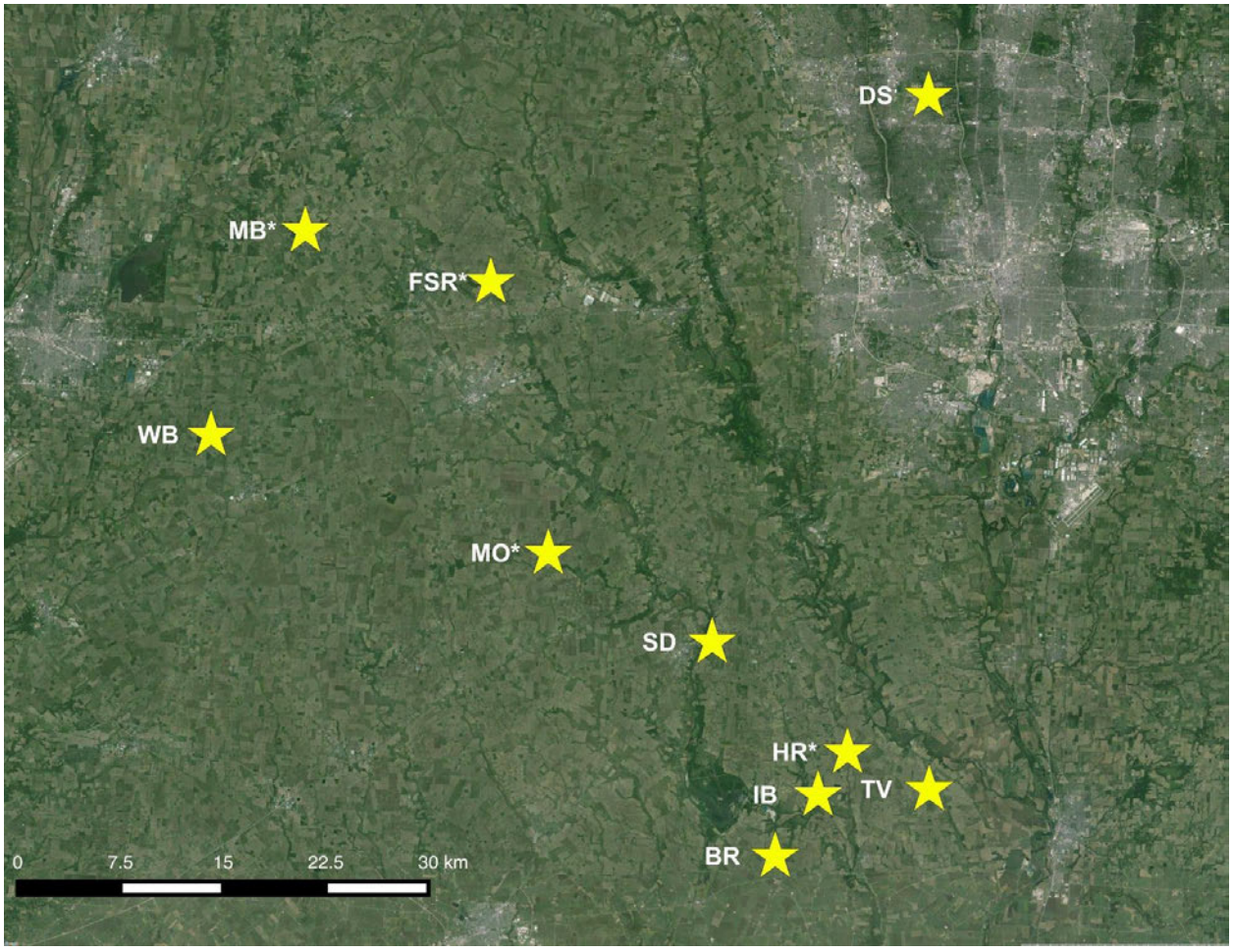


Figure 1. Apiary locations plotted over satellite imagery (Google OpenLayers). Observation hives were installed in four apiaries (asterisked) to record dance activities.

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 4**). 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 5**), 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.

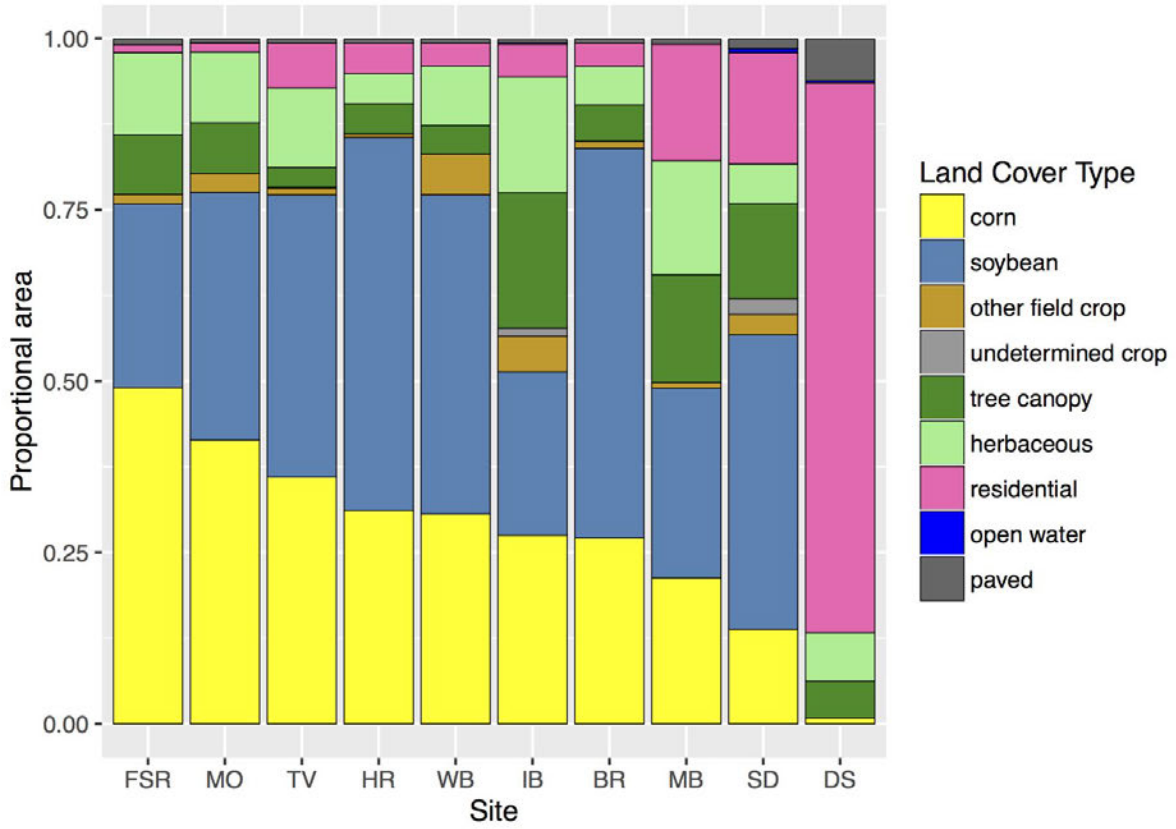


Figure 4: Landscape composition summary within a 2km radius of apiary sites. Corn and soybean were major landscape features at all sites except for the one urban site, DS. Other field crops consisted of wheat, alfalfa and vegetable crops. Some fields visible in satellite imagery could not be surveyed 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.

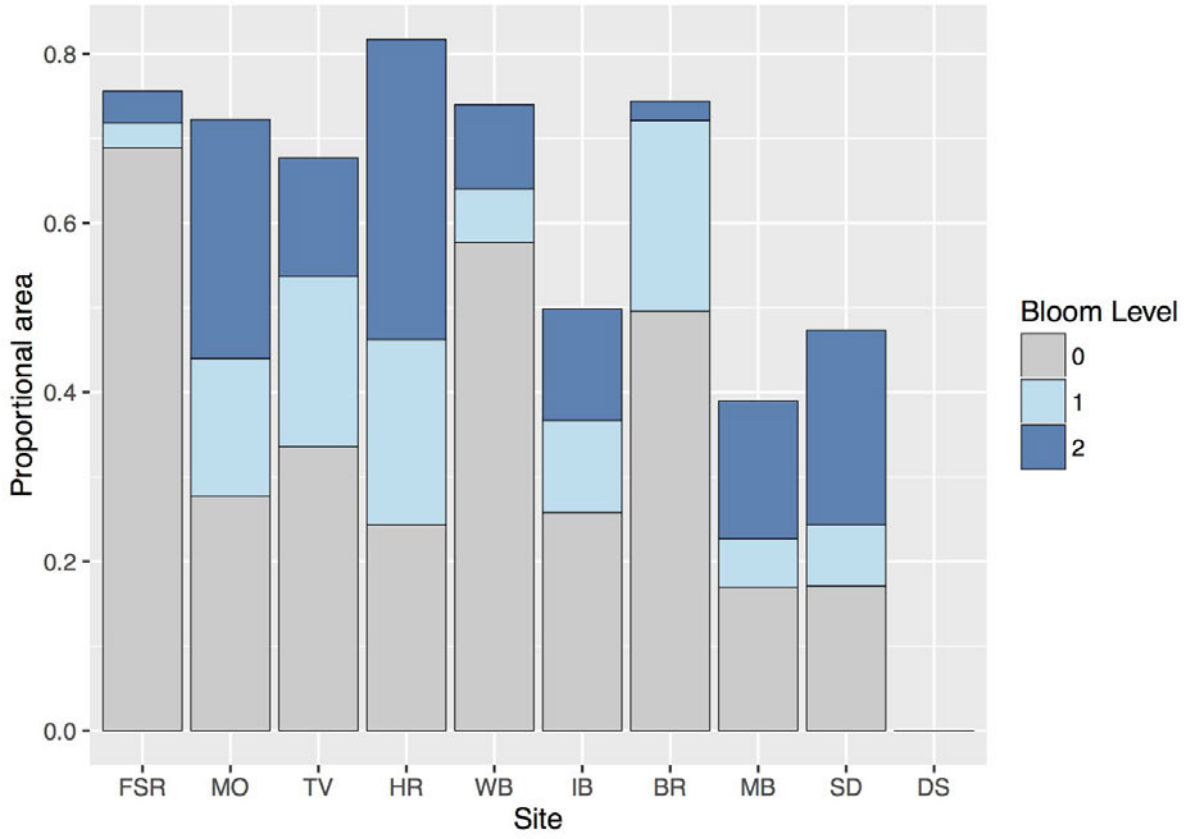


Figure 5: Pre-planting bloom abundance in cornfields. Bloom level 0 fields had virtually no flowering weeds in them, due either to spring herbicide application or fall tillage. Bloom level 2 fields had abundant flowering weeds, with purple deadnettle (*Lamium purpurea*), dandelion (*Taraxacum officinale*), 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:
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 °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 2. 201 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 3. 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 1/2 rate John Deere Talc, BFA Pre treated seed
7f	Nevada	John Deere 7200 Conservation	Row unit vacuum	Poncho 1250/ clothianidin	Beck's	
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-Ferguson/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

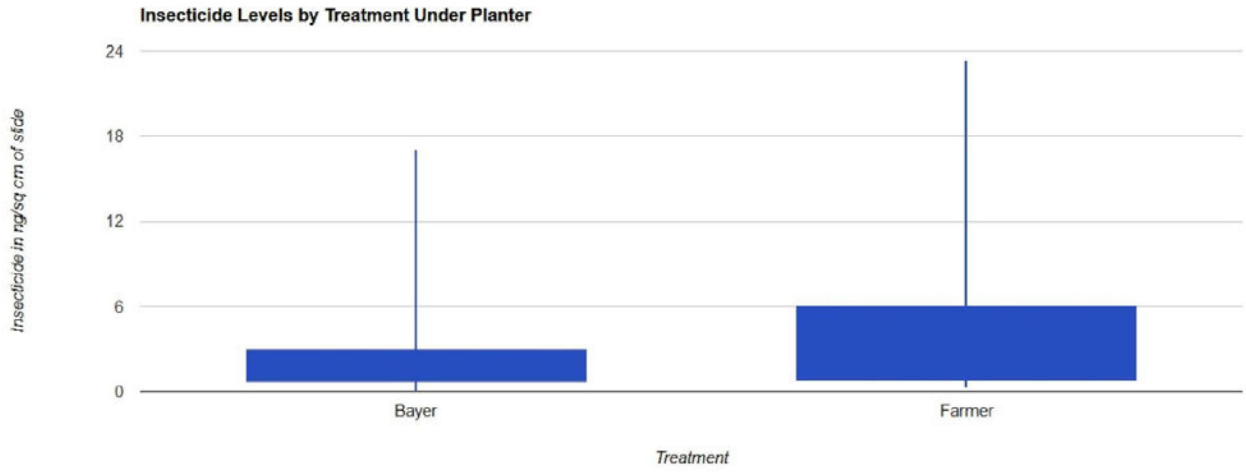


Figure 6: Comparison of insecticide levels by seed lubricant from under planter targets, 2014 and 2015.

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. An attached appendix includes site by site dust analysis values.

As seen in **Figure 6** and discussed in the 2014 report, there is little evidence that the level of insecticide differs between the two types of lubricant shown – the Bayer fluency agent vs. the farmer choice under the planter. While a broader range of insecticide occurred with the farmer chosen seed lubricant, much overlap between the two treatments is apparent.

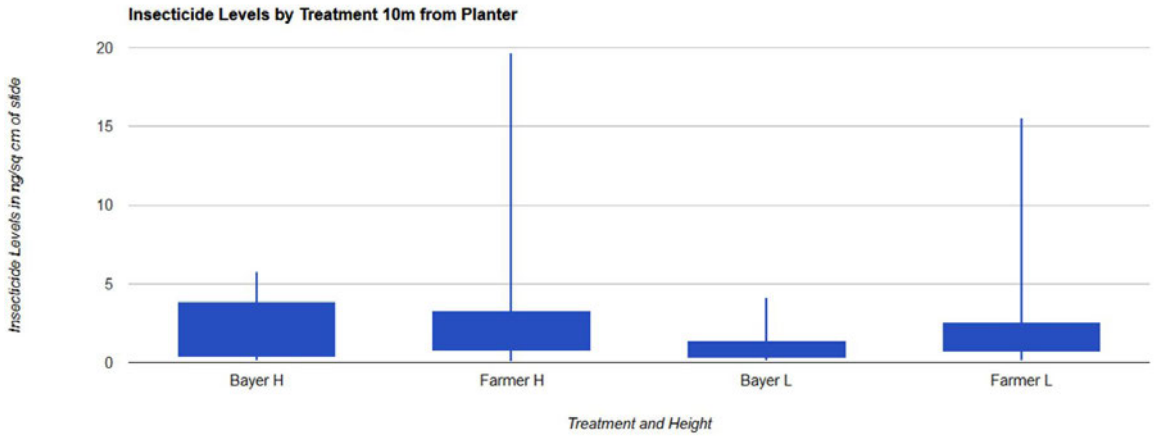


Figure 7: Comparison of insecticide levels by seed lubricant from ten-meter targets, from the high (2m) and low (0.3m) targets for 2014 and 2015.

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 7** 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.

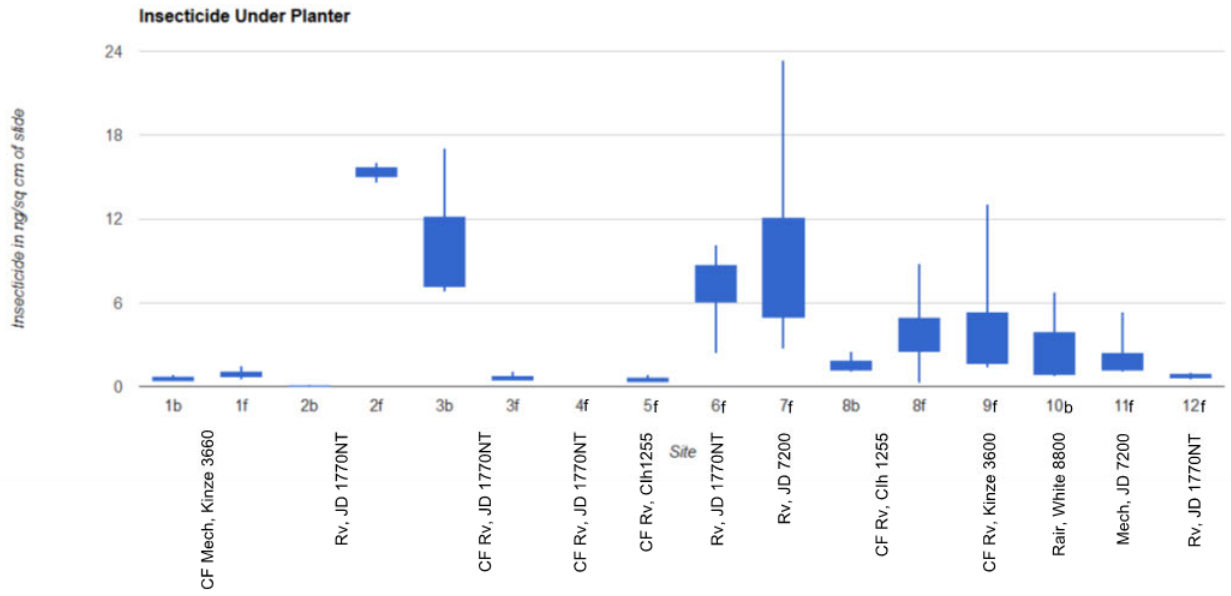


Figure 8. Comparison of insecticide levels by location and planter from under planter targets, 2014 and 2015.

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.

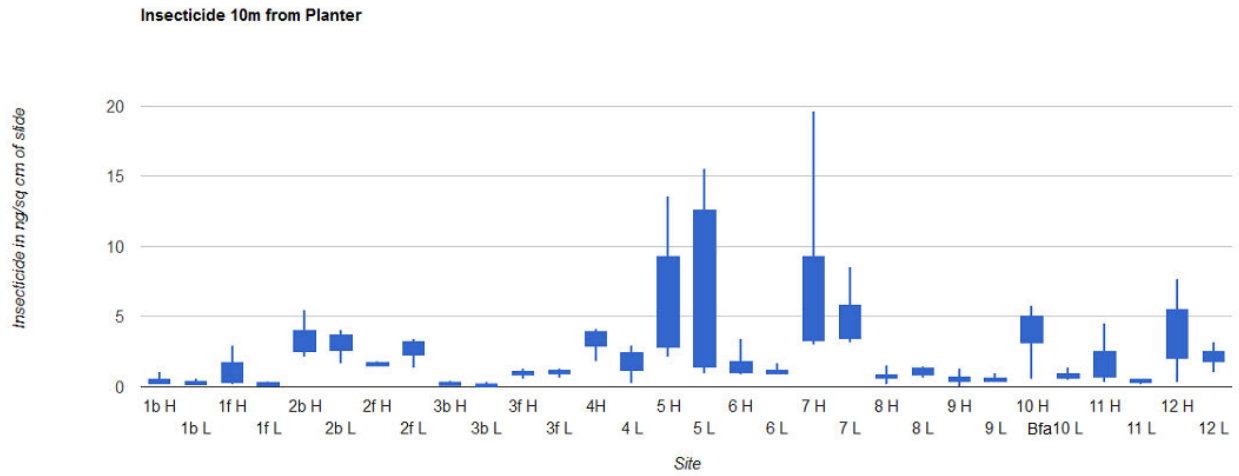


Figure 9. Comparison of insecticide levels by site from ten-meter targets, from the high (2m) and low (0.3m) targets for 2014 and 2015.

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. For photography and SEM, 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 B**, 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 -20°C for further analysis (e.g. Heubach testing) if necessary.

Results and Discussion. All examined corn seeds showed obvious shedding of seed treatment material, including large bare patches and, in an extreme case, a seed with almost no seed treatment. We noticed no differences between conventional treated seeds and those from companies that advertise a special polymer to enhance sticking. 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.

These results demonstrate that seed treatment material in corn is poorly adhered to the seed surface; its release into the environment in the form of particles would be expected. The observation that visible quantities of seed treatment material are released by gentle handling of small numbers of seeds suggests that large amounts of material may be released during the planting process.

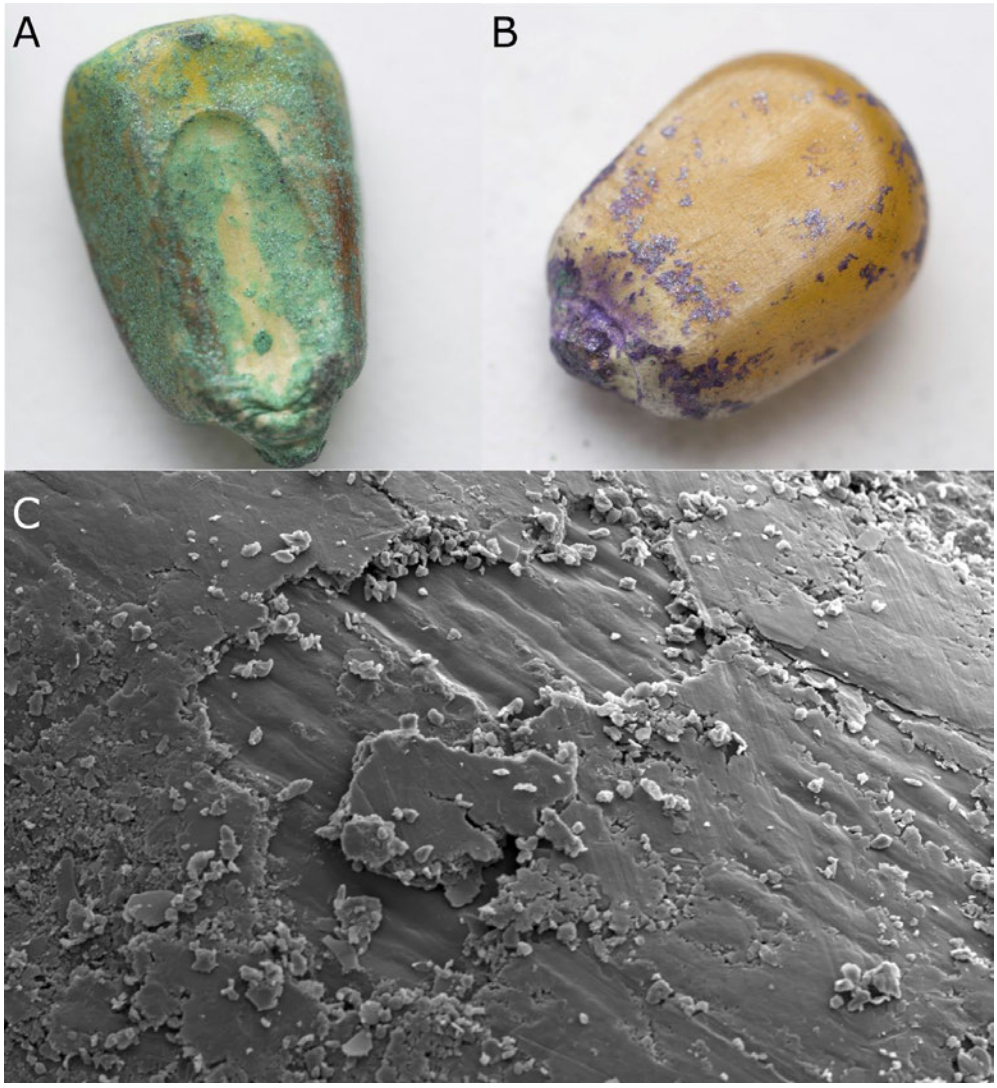
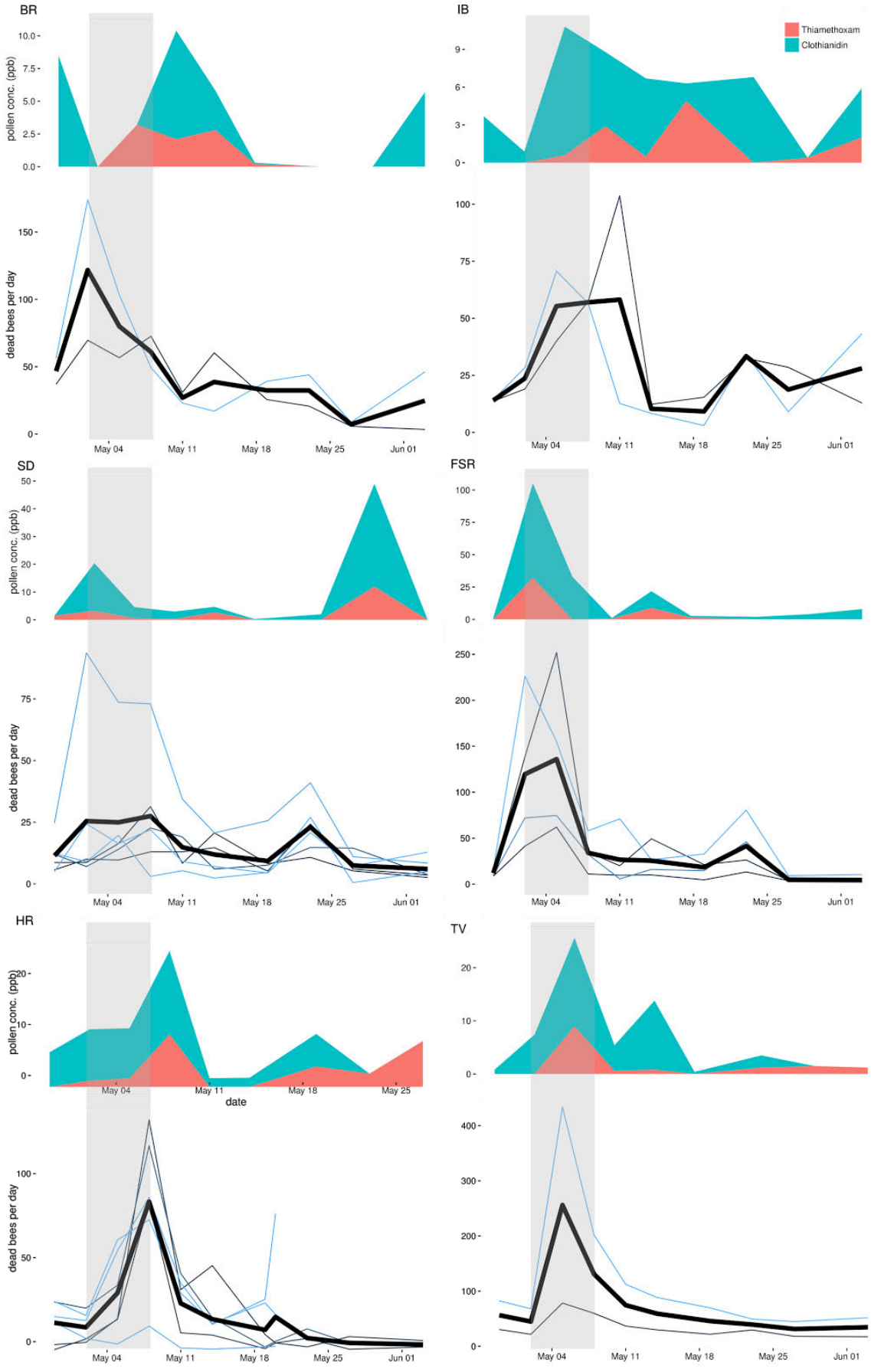


Figure 10: Photographic and microscopic assessment of seed treatment integrity. Typical seeds showed patchy coverage, suggesting either extensive shedding of seed treatment or poor initial coating evenness (A). In an extreme case, almost no seed treatment material remained on the seed (B). Seed treatment easily crumbles away when its structural integrity is compromised, leaving patches of bare seed surface (smoothly striated patch in middle) (C). Shed particles vary widely in size. Photos by M. Spring, SEM by D. Sponsler.

SECTION 2:
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.


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

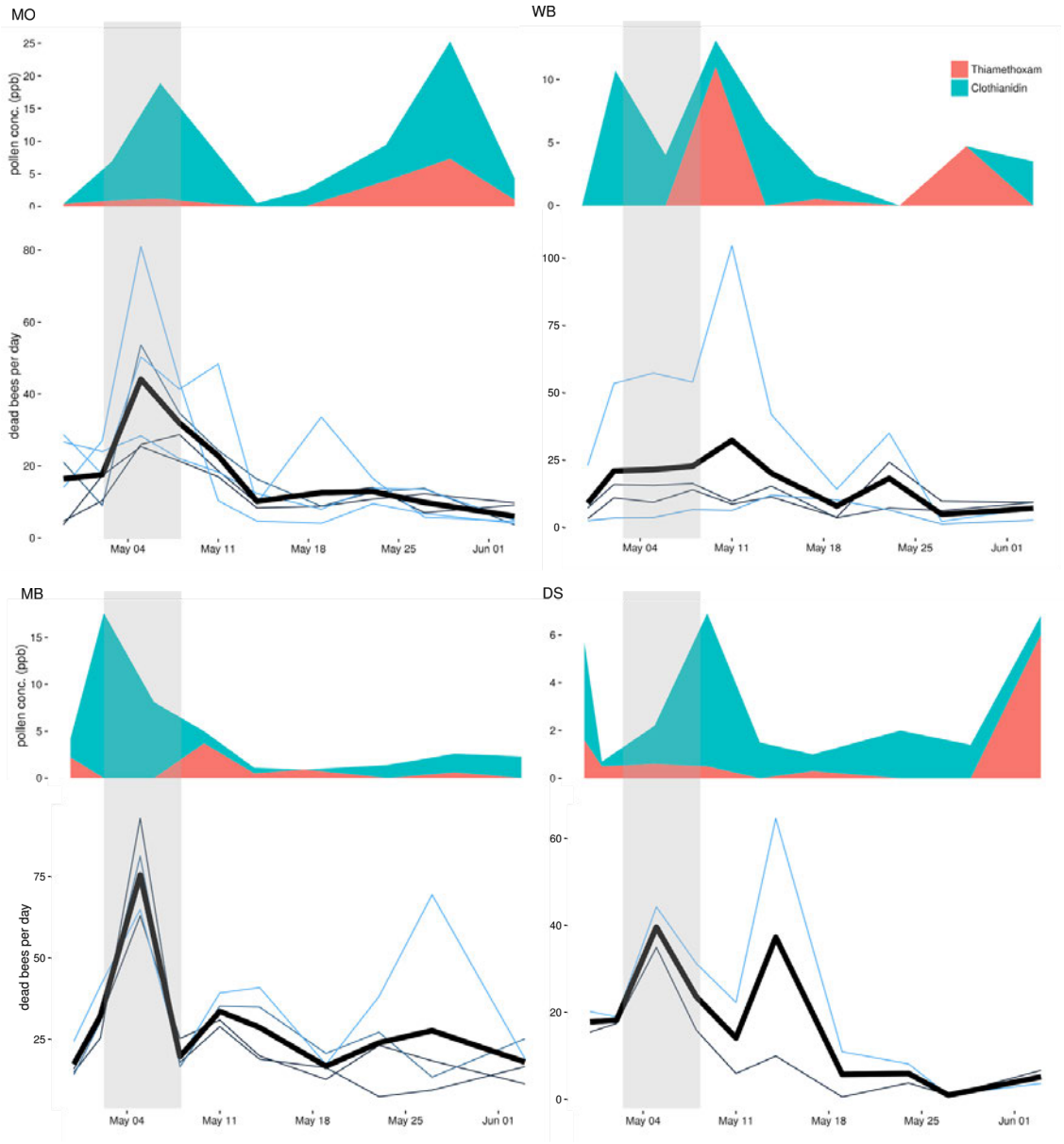


Figure 11: Summary of pollen contamination with seed treatment insecticides (above) and dead bee trap catch (below) for 10 apiary sites. The peak corn planting window (May 2 - 8) is shaded with a gray box. Dead bee trap catches, calculated per day, for individual colonies is indicated with thin lines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

SECTION 3:
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 4:
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 3**), we did not detect significant correlation between insecticide levels in pollen and any of the 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 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 5:
Routes of exposure and implications for mitigation

Potential routes of exposure. The evaluation and discrimination of the multiple routes of exposure (ROE) that contribute to honey bee mortality during corn planting is an ongoing challenge with critical implications for mitigation efforts. 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 4)**.

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 1)** 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 by the exhaust port of 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 hazard that could easily extend 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 12)**. 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 13)**. 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 less mobile fluency agent 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 less mobile fluency agent might actually exacerbate exposure by concentrating emitted 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 synergistically. 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 2)** and adult bee mortality (Section 3) in an effort to discriminate between the routes and sub-routes of exposure outlined in **Table 5**.

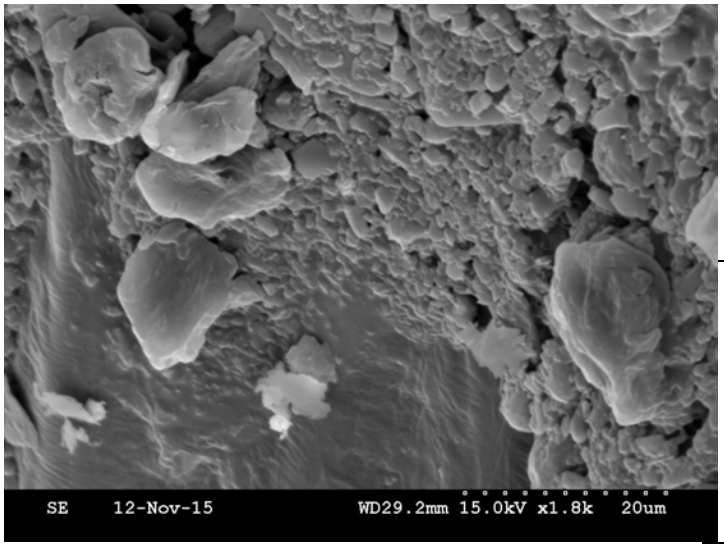


Figure 12: SEM of seed treatment coating illustrating the potential for extremely small articles to be shed. The smallest particles in this image are < 1μm in diameter, suggesting a strong potential for aerial transport.

Table 5: 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

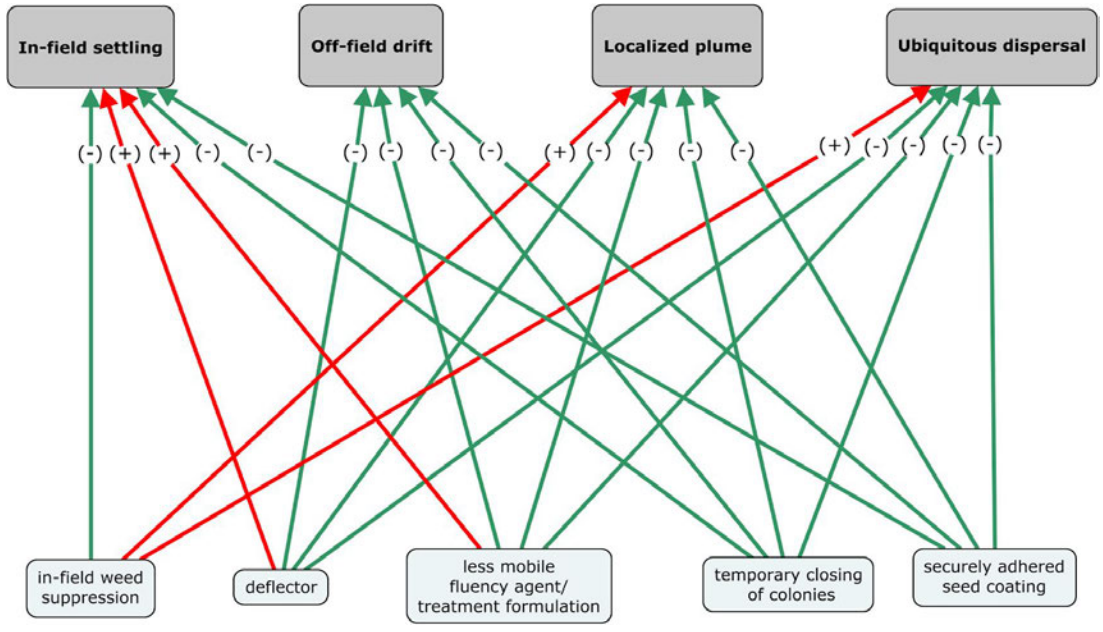


Figure 13: Interactions between hypothesized routes of exposure and proposed mitigation schemes.

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 5)**.

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 14)**, 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 18-21)**. 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 5)**. 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 14: 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 15, Table 6)**. 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.

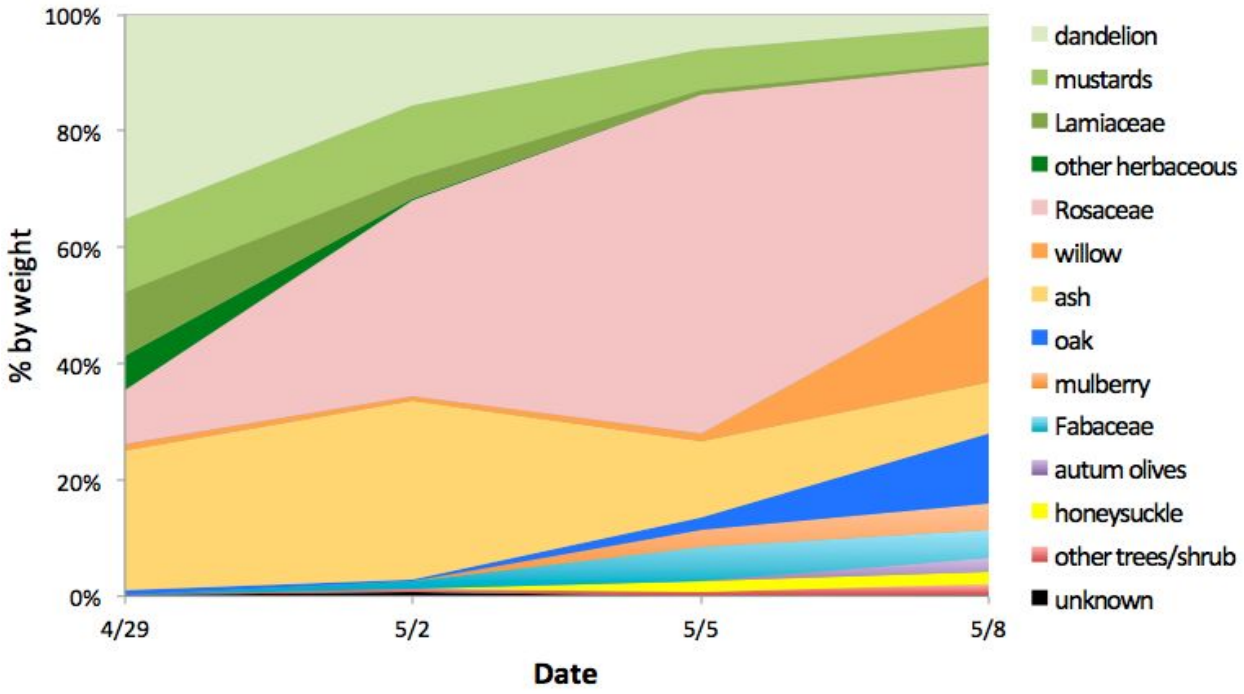


Figure 15. Pollen types collected from four sites (FSR, MO, HR, and MB) from April 29 – May 8. The percent abundance shown for each pollen type is the average of its percent abundance across all four sites.

	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 6. 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 16)**, but foraging distances varied between sites **(Figure 17)**. 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 (**Figure 13)**. As a result, bees at this site on average foraged for longer distance and the hotspots were relatively diffuse **(Figure 17)**.

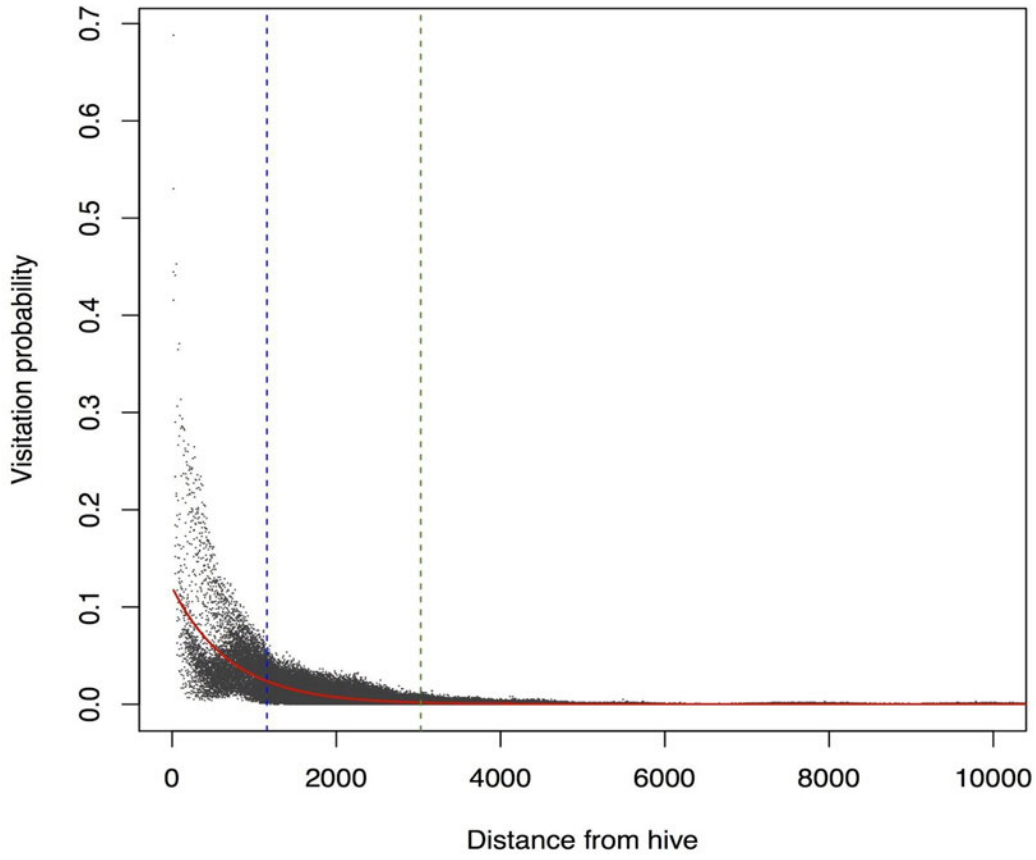


Figure 16: Visitation probability by distance, pooled across all sites and dates. Dashed lines indicate the distances at which 50% (blue) and 95% (green) of total visitation probability were accounted for. Visitation probability can be understood as a proxy of total foraging activity. When data were 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.

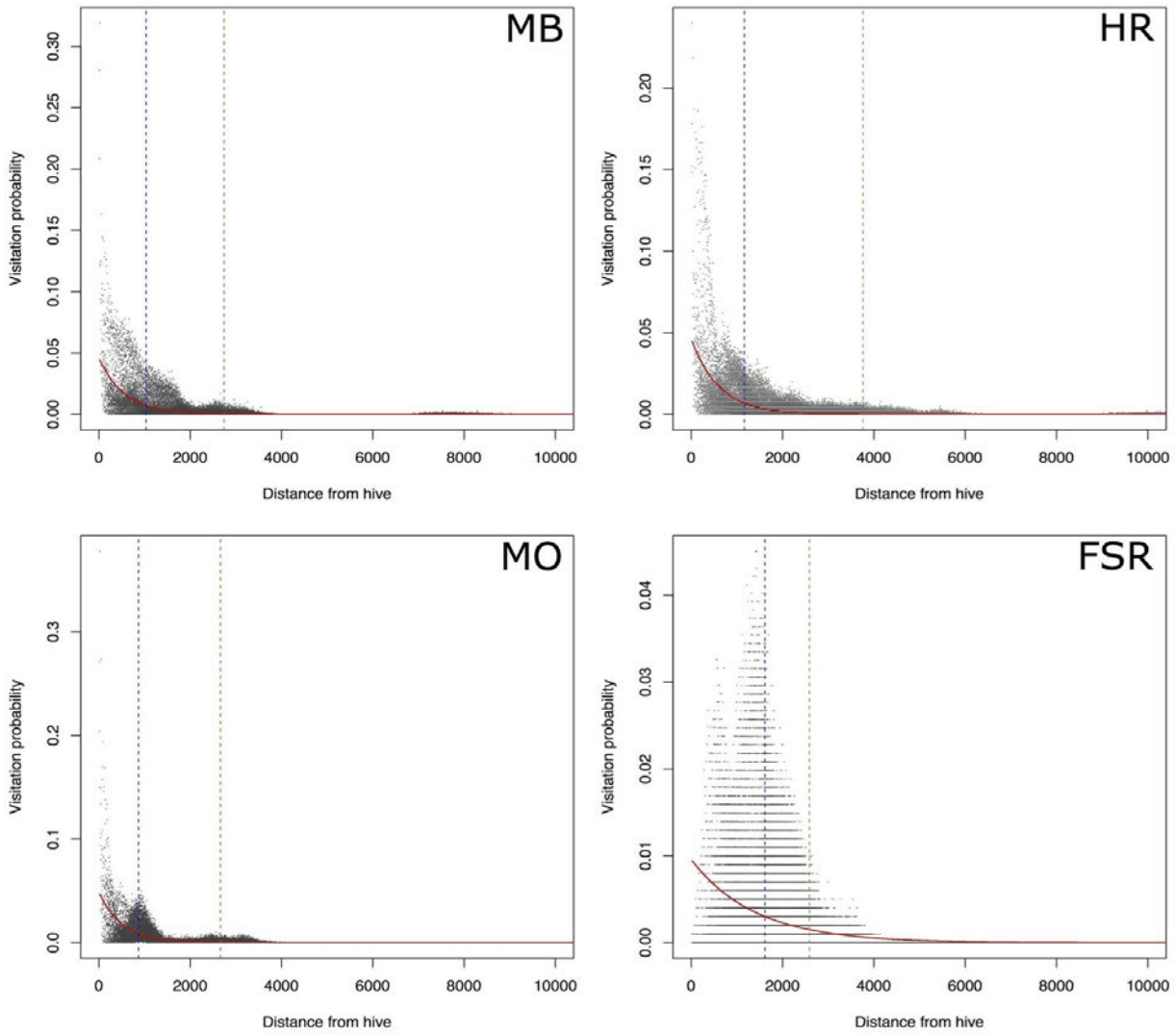


Figure 17: Visitation probability by distance at each site. Dashed lines indicate the distances at which 50% (blue) and 95% (green) of total visitation probability were accounted for. Visitation probability can be understood as a proxy of total foraging activity. The red curve represents a nonlinear least squares regression of visitation probability on distance.

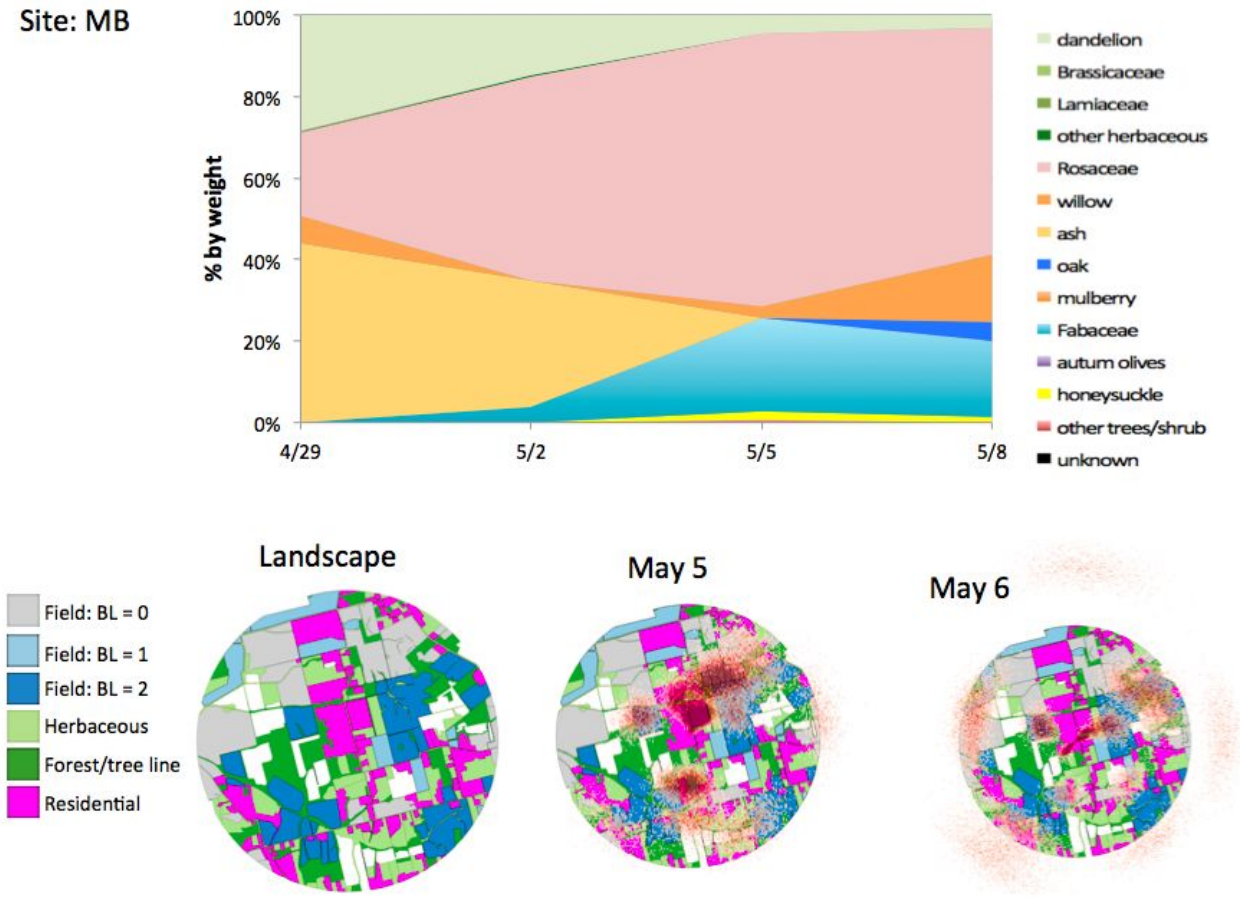


Figure 18. Pollen collected by bees at MB on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). Bees foraged over all land classes, with higher foraging probability over wooded and residential 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.

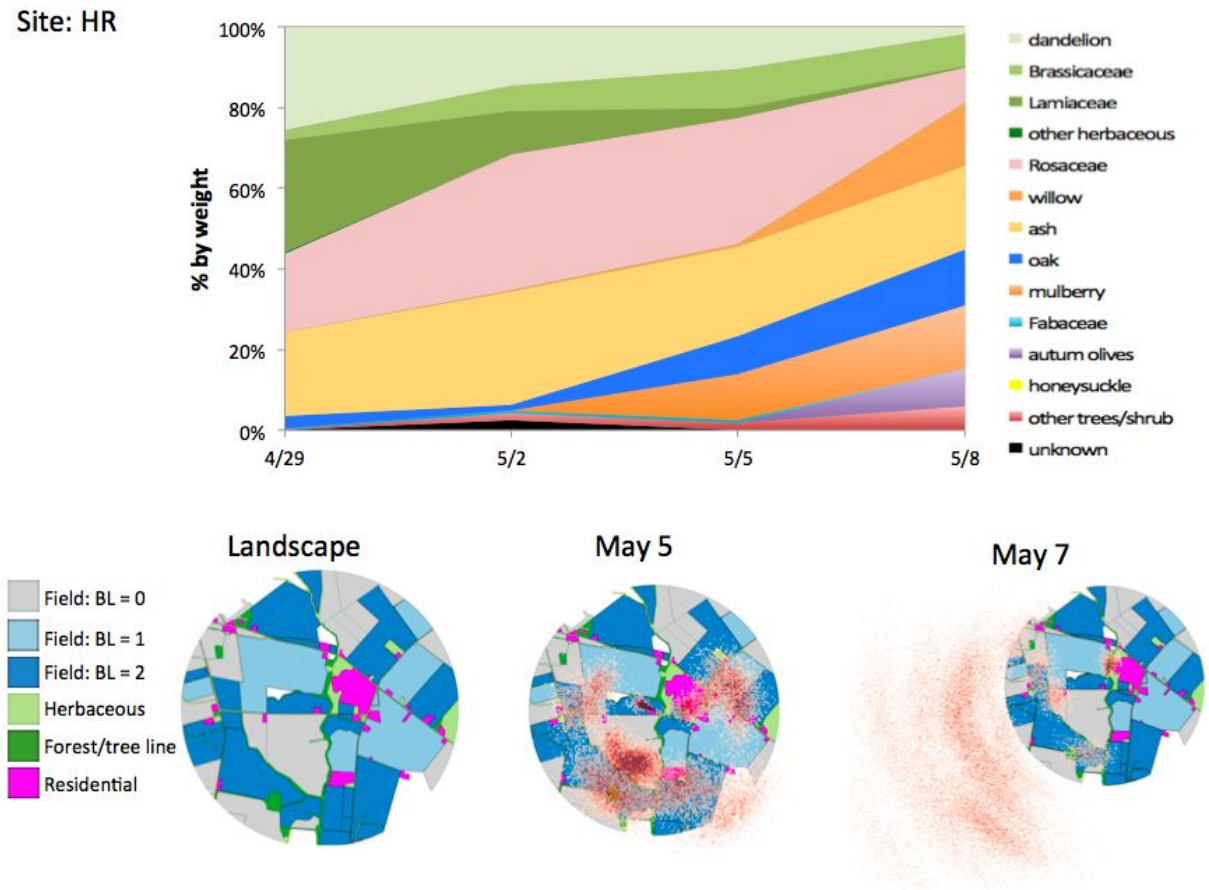


Figure 19. Pollen collected by bees at HR on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). On May 5 (26 dances), bees foraged predominantly in weedy areas and adjacent tree lines; very 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.

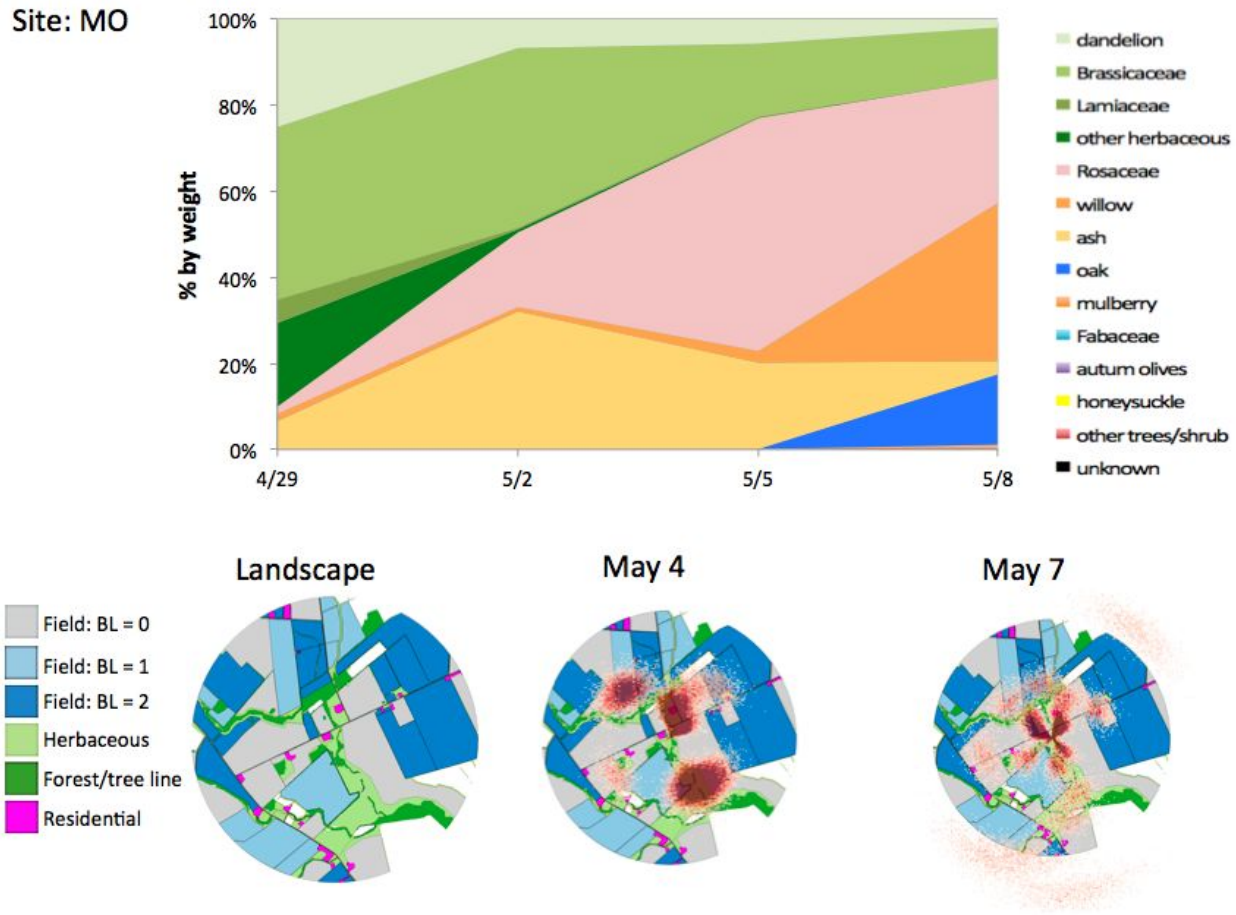


Figure 20. Pollen collected by bees at MO on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and right). On May 4 (35 dances), dense foraging occurred about 1.5km from the hive in fields, forests and 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.

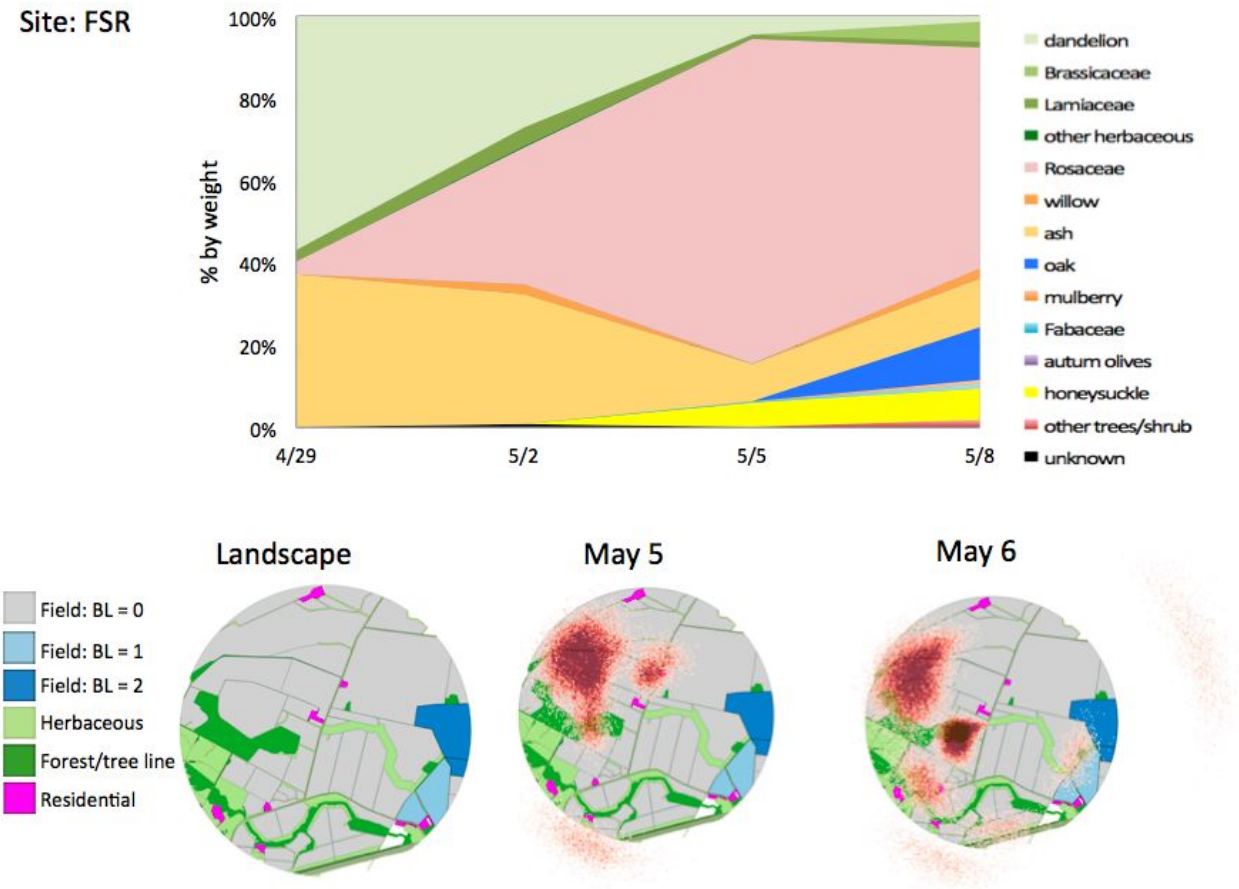


Figure 21. Pollen collected by bees at FSR on April 29 - May 8 (top), landscape with field bloom level before planting (bottom left), and two dance maps recorded during this period (bottom center and left). On May 5 (26 dances recorded), bees foraged heavily on the field margins, forest edges, and the 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 5** 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 7)**. 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 7: 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 8)**. In each test, cumulative pollen seed treatment residues during the peak corn planting window **(Section 1)** and the adult mortality ratio **(Section 2)** 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 22)**. All other tests indicate no significant relationship ($p > 0.05$) between landscape and either pollen seed treatment residues or adult mortality **(Table 8)**. 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.

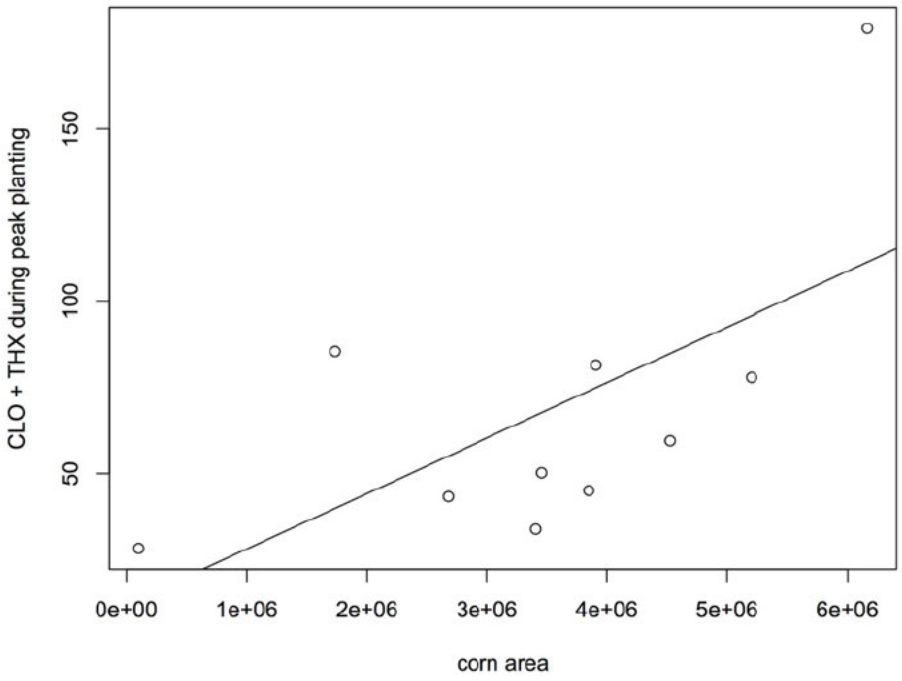


Figure 22: The sum of clothianidin and thiamethoxam residues found in pollen during corn planting was positively correlated with corn area.

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 8: 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 23)**.

- 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 23)**.

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 23)**

- 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

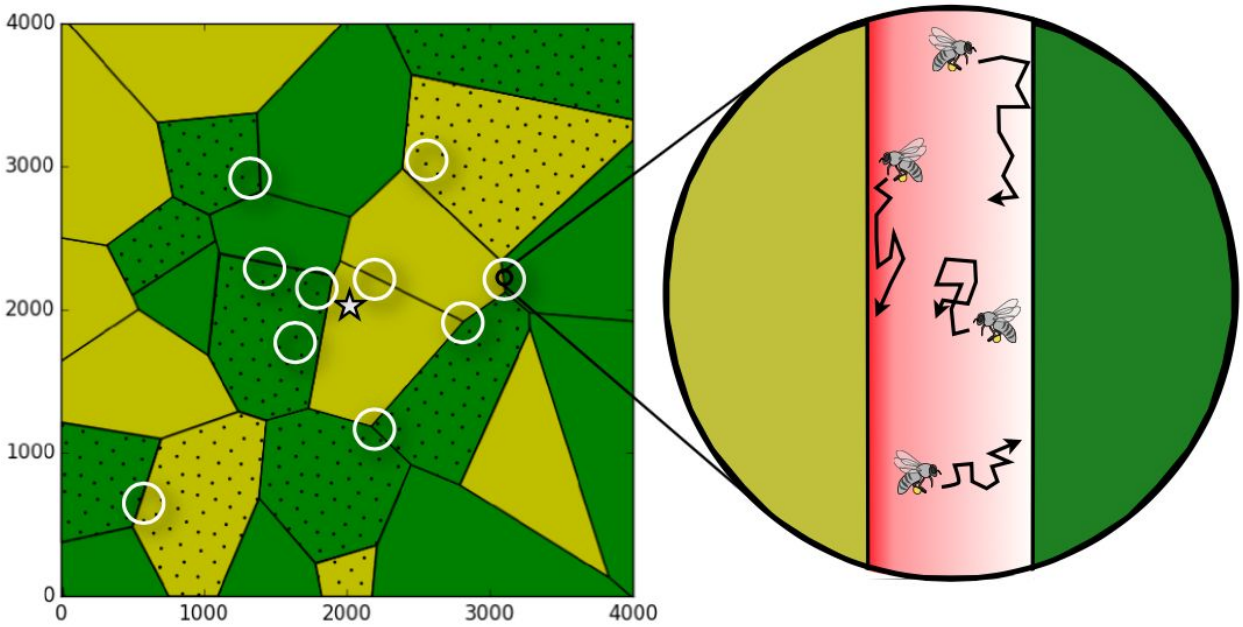


Figure 23: Model simulates a honey bee colony (star) surrounded by corn (yellow) and soybean (green) fields separated by narrow interstices of non-crop habitat. Dust drift simulated by distributing a contamination gradient according to distance from corn field edge. Weedy (foragable) fields are represented 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 24)**. 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.

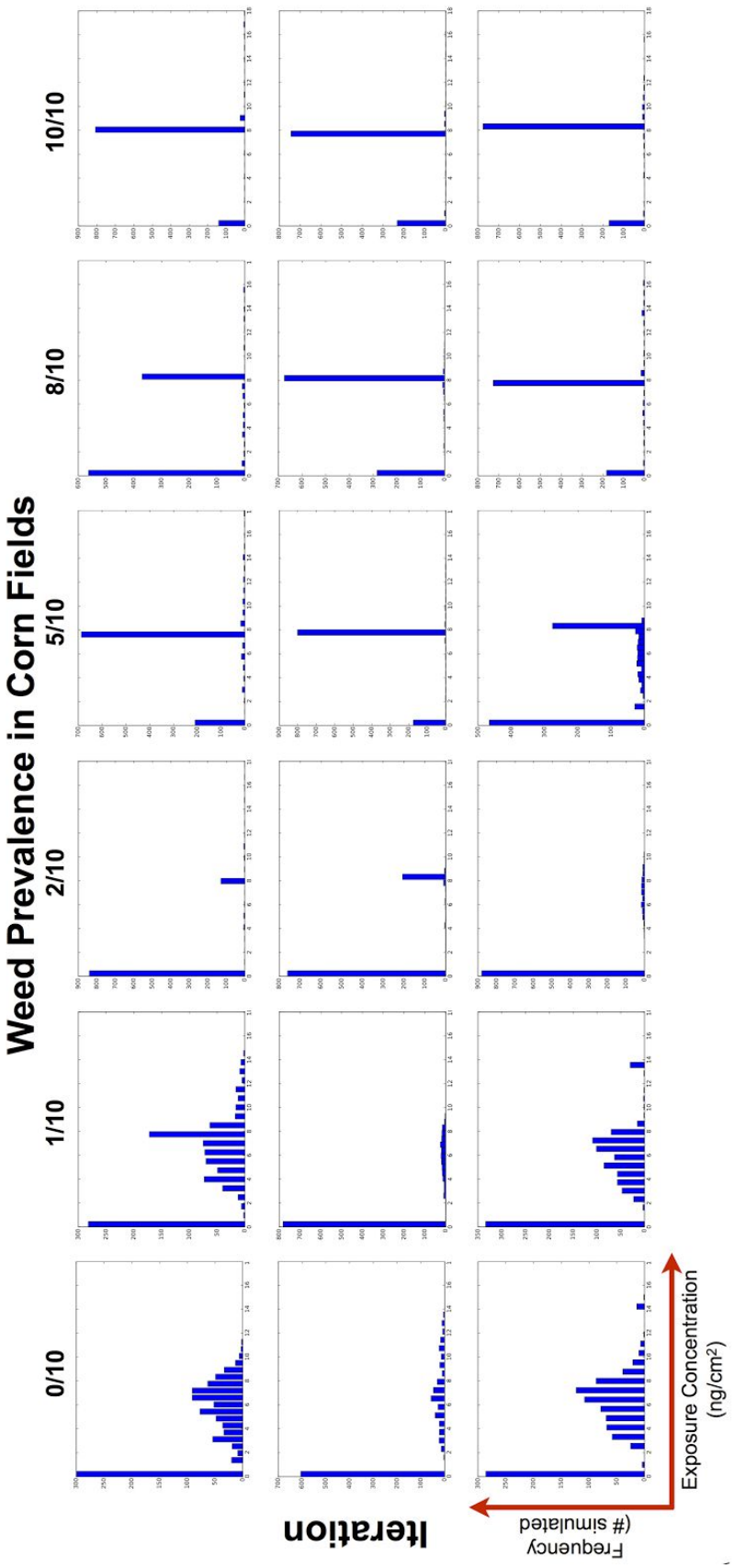


Figure 24. Exposure profiles arising from six levels of flowering weed prevalence in corn fields. Data visualized above represent the output of three iterations (rows) of the model run at each of six levels (columns) of weed prevalence in corn fields: 0/10 fields, 1/10 fields, 2/10 fields, 5/10 fields, 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 is due to the failure of seed treatment material to adhere securely to the seed surface. Seed treatment active ingredients can be detected both in- and off-field, implying aerial transport.

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 adult mortality, however, did not translate into measurable reductions in overall colony strength.

Route of exposure

The primary route of exposure that drives the mortality of honey bees during corn planting remains uncertain, but some support was found for the hypothesis that honey bees are exposed via aerial contact with ubiquitously distributed airborne seed treatment particles. We found no support for the hypothesis that honey bee exposure is driven by the contamination of floral resources, either by the 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. 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 should be minimized through planter modification or seed treatment reformulation. An alternative to these approaches would be to plant either untreated seeds or seeds treated with an insecticide exhibiting lower toxicity to honey bees.

REFERENCES

Bengtsson-Palme, J., Hartmann, M., Eriksson, K. M., Pal, C., Thorell, K., Larsson, D. G. J., & Nilsson, R. H. (2015). metaxa2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Molecular Ecology Resources, 15(6), 1403–1414. http://doi.org/10.1111/1755-0998.12399

Couvillon, M. J., Riddell Pearce, F. C., Harris-Jones, E. L., Kuepfer, A. M., Mackenzie-Smith, S. J., Rozario, L. A., Schurch R., Ratnieks, F. L. W. (2012). Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding. Biology Open, 1(5), 467–472. http://doi.org/10.1242/bio.20121099

Couvillon, M. J., Schürch, R., & Ratnieks, F. L. W. (2014). Waggle Dance Distances as Integrative Indicators of Seasonal Foraging Challenges. PLoS ONE, 9(4), e93495. http://doi.org/10.1371/journal.pone.0093495.g004

Couvillon, M. J., & Ratnieks, F. L. W. (2015). Environmental consultancy: dancing bee bioindicators to evaluate landscape "health." Frontiers in Ecology and Evolution, 3. http://doi.org/10.3389/fevo.2015.00044

Devarrewaere, W., Janssen, S., & Foqué, D. (2016). CFD modelling to simulate dust emissions from pneumatic seed drills. Aspects of Applied Biology 132:227-234.

Girolami, V., Marzaro, M., Vivan, L., Mazzon, L., Greatti, M., Giorio, C., et al. (2011). Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. Journal of Applied Entomology, 136(1-2), 17–26. http://doi.org/10.1111/j.1439-0418.2011.01648.x

Girolami, V., Marzaro, M., Vivan, L., Mazzon, L., Giorio, C., Marton, D., & Tapparo, A. (2013). Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. Journal of Applied Entomology, 137(1-2), 35–44. http://doi.org/10.1111/j.1439-0418.2012.01718.x

Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS ONE 7(1): e29268. doi 10.1371/ journal.pone.0029268

Lin, C.-H., P. Monagan and R. Johnson. 2016. Soybeans as a potential nectar source for honey bees. 2016 American Bee Research Conference, Ponte Vedra Beach, FL.

McFrederick, Q. S., & Rehan, S. M. (2016). Characterization of pollen and bacterial community composition in brood provisions of a small carpenter bee. Molecular Ecology, 25(10), 2302–2311. http://doi.org/10.1111/mec.13608

QGIS Development Team (2015). QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at http://qgis.osgeo.org

R Core Team. 2013. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at http://www.R-project.org/.

Richardson, R. T., Lin, C.-H., Sponsler, D. B., Quijia, J. O., Goodell, K., & Johnson, R. M. (2015). Application of ITS2 Metabarcoding to Determine the Provenance of Pollen Collected by Honey Bees in an Agroecosystem. Applications in Plant Sciences, 3(1), 1400066. http://doi.org/10.3732/apps.1400066

Richardson, R. T., Lin, C.-H., Quijia, J. O., Riusech, N. S., Goodell, K., & Johnson, R. M. (2015). Rank-Based Characterization of Pollen Assemblages Collected by Honey Bees Using a Multi-Locus Metabarcoding Approach. Applications in Plant Sciences, 3(11), 1500043. http://doi.org/10.3732/apps.1500043

Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682.

Schürch R, Couvillon M. J., Burns D. D. R., Tasman K.,Waxman D., Ratnieks F. L. W. (2013) Incorporating variability in honey bee waggle dance decoding improves the mapping of communicated resource locations. J Comp Physiol A 199:1143–1152

Sponsler, D. B., & Johnson, R. M. (2015). Honey bee success predicted by landscape composition in Ohio, USA. PeerJ, 3, e838. http://doi.org/10.7717/peerj.838/supp-5

Tapparo, A., Marton, D., Giorio, C., Zanella, A., Soldà, L., Marzaro, M., et al. (2012). Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds. Environmental Science & Technology, 46(5), 2592–2599. http://doi.org/10.1021/es2035152

von Frisch, K. (1967). The Dance Language And Orientation Of Bees. Cambridge, MA, USA: Belknap Press of Harvard University Press.

Wisk JD et al. (2014). Assessing exposure of pesticides to bees. In Fischer D, Moriarty T, eds, Pesticide Risk Assessment for Pollinators. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 45–74.

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
BR	4/29	8.5	0	8.5
	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. Insecticide levels for 2014 and 2015 under planter and 10 meter targets in ng/cm sq.

			2014 Ohio corn dust trials				
Site >	*1b*	*1f*	*2b*	*2f*	*3b*	*3f*	*4f*
On planter for duration of treatment							
Rep#	B-B	B-F	D-B	D-F	F-B	F-F	L-F
1	46.62	33.58	13.62	13.01	17.09	7.35	15.38
Under Planter - on ground for 1 planter pass							
Rep#	B-B	B-F	D-B	D-F	F-B	F-F	L-F
1	0.60	1.51	0.10	16.08	17.09	1.14	
2	0.87	0.49	0.03		7.31	0.49	
3	0.46	0.70	0.05	14.58	6.80	0.45	2.77
10m away, high target							
Rep#	B-B	B-F	D-B	D-F	F-B	F-F	L-F
1	0.14	2.97	2.15	1.71	0.21	0.56	4.21
2	1.10	0.17	2.74	1.85	0.34	1.35	3.89
3	0.21	0.67	5.51	1.75	0.45	0.94	1.85
10 m away, low target							
Rep#	B-B	B-F	D-B	D-F	F-B	F-F	L-F
1	0.64	0.28	3.44	3.16	0.39	1.36	0.24
2	0.22	0.43	1.67	3.45	0.16	1.12	2.01
3	0.23	0.25	4.12	1.31	0.16	0.63	3.00
Insecticide	clothianidin	clothianidin	clothianidin	clothianidin	thiamethoxam	thiamethoxam	clothianidin
Seed Co.	Channel	Channel	Dekalb	Dekalb	USA Seeds	USA Seeds	Stewarts
Manuf.	Kinze 3660	Kinze 3660	John Deere 1770	John Deere 1770	John Deere 177	John Deere 17	John Deere 1770N
Type	CF, non-vac.	CF, non-vac.	non-CF, vacuum	non-CF, vacuum	CF, vacuum	CF, vacuum	CF, vacuum
wind speed	2-5-10mph	7-15 mph	5-8 mph	6-9 mph	5-8 mph	8-15 mph	3-7 mph
City >	Mechanicsburg	Mechanicsburg	Delaware	Delaware	London	London	Kenton
Notes:							
For site "b" indicates the Bayer fluency agent, and "f" the farmer choice.							
Planter - planter mounted slide set - on the top of the tool bar to the end on the right - and for the length of the treatment - non-replicated							
For distance 10 - in meters downwind from first pass of planter. On holder for duration of each treatment.							
For location of trap. H is 2 meters or L is 0.3 meters off the ground.							
For planter type - CF indicates center fill by air delivery to individual units. Vacuum indicates air assist on seed unit.							

clothianidin+thiamethoxam in ng/sq cm of the slide

2015 Ohio corn dust trials

Site >	*5f*	*6f*	*7 b+1/2f*	*8b*	*8f*	*9f*	*10b*	*11f*	*12f*
no on-planter target in 2015									
Under Planter - on ground for 1 planter pass									
Rep#	BO	D	G	L Bayer	L farmer	SG	SR Bayer	WM	WV
1	0.53	2.39	2.65	2.56	3.15	1.34	0.73	1.05	0.45
2	0.61	7.14	8.37	1.00	0.22	13.04	0.81	1.07	0.71
3	0.28	10.14	5.66	1.71	8.82	2.80	2.98	1.49	0.92
4	0.85	8.30	23.36	1.13	3.66	1.69	6.74	5.34	0.99
10 m away, high target - 2 m vertical orientation									
Rep#	BO	D	G	L Bayer	L farmer	SG	SR	WM	WV
1	2.99	0.83	2.98		0.65	0.03	3.88	1.93	4.85
2	7.94	1.01	3.27		0.20	0.61	5.79	0.30	2.57
3	13.61	3.44	5.90		0.78	0.42	4.88	4.56	0.29
4	2.13	1.36	19.69		1.55	1.30	0.53	0.75	7.74
10 m away, low target - 0.3 m horizontal orientation									
Rep#	BO	D	G	L Bayer	L farmer	SG	SR	WM	WV
1	11.75	1.12	3.50		1.50	0.63	1.39	0.13	2.00
2	0.98	0.99	3.12		0.84	1.00	0.94	0.25	2.38
3	15.56	0.96	4.98		0.65	0.43	0.54	0.58	3.22
4	1.45	1.74	8.60		1.35	0.43	0.49	0.65	1.06
Insecticide	clothianidin P1250	clothianidin P500	clothianidin P1250	clothianidin P1250	clothianidin P1250	thiamethoxam C250	clothianidin P250	clothianidin P1250	clothianidin P1250
Seed Co.	Beck's (Pioneer)	Deka b	Beck's (Pioneer)	Beck's	Beck's	Master's Choice	Stewarts	Beck's (Pioneer)	Beck's (Pioneer)
Manuf.	CaseIH 1255AFS	John Deere 1770N	JD 7200 Conservation	CaseIH 1255AFS	CaseIH 1255AFS	Kinze 3600	White/Agco 8800	JD 7200 Conservation	John Deere 1770NT
Type	CF, row vacuum	row vacuum	row vacuum	CF, row vacuum	CF, row vacuum	CF, vacuum	row air pressure	no air, mechanical	row vacuum
Wind speed	10-15 mph	6-8 mph	2-6 mph	4-7 mph	4-7 mph	2-4 mph	8 mph	7-9 mph	14 mph
City >	London, OH	Delaware, OH	Nevada, OH	Ridgeville, IN	Ridgeville, IN	Gettysburg, OH	Ridgeville, IN	Marion, OH	Versailles, OH