Corn Dust Research Consortium (CDRC) Preliminary Report

Initial Findings for 2014

July 2015

Final

Reviewed and approved by the CDRC

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Executive Summary

In 2014 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 2014 tests of the seed lubricant conducted in Ohio did not show a significant difference in the effectiveness of the Bayer lubricant compared to the farmer's choice, but these data were affected by weather and a small sample size. 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.

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?

Findings and a summary report of the 2013 study year can be found at http://www.pollinator.org/CDRC.htm. In 2014 the CDRC revisited these two research questions with an additional RFP solicitation. 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.

The goal of the consortium in addressing these two questions is to utilize data from research conducted in during the 2013 and 2014 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.

Nearly 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 is a summary of the approaches used by each of the four research institutions for Question 1 (Ohio State University, University of Guelph, (Bee Alter Technology) University of Montana, and Iowa State University) and the approach to Question 2 used by one institution, Ohio State University. It should be noted that researchers at each of the four institutions took their own approach to the questions. 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.

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Methods – Question 1

Bee Alert Technologies

Experimental Design

Study Sites. Because of our familiarity with the region and past cooperation with growers, we elected to perform our sampling in Nebraska. We selected an area south of Lincoln in Lancaster and Gage counties that encompassed approximately 36 square miles. Corn cultivation mixed with soybean is the principal agricultural activity in the area chosen. Sufficient fallow, natural or riparian areas existed within the selected area to allow the two staged sampling protocols delineated in our proposal. Planting typically begins in this region about mid-April when soil temperatures rise above 45°F, so we performed our initial survey and site selection during the week of 1 to 6 April, 2014. At that time, weather conditions were wet and cool. Plant growth was minimal and no flowering was evident in waste or natural areas. Eight sites were selected (Figure 1). Four (termed “Field” sites) were fallow margins of fields scheduled for corn planting; the remaining four (termed “Yard” sites) were associated with uncultivated or pasture areas near water and natural cover that encompassed enough area to accommodate a typical apiary of 50 to 100 colonies. We set a preferred spacing between sites of 2 miles (3.3 km), but because of grower preference and planting plans, two sites were 1 mile (1.6 km) from their nearest neighbor.

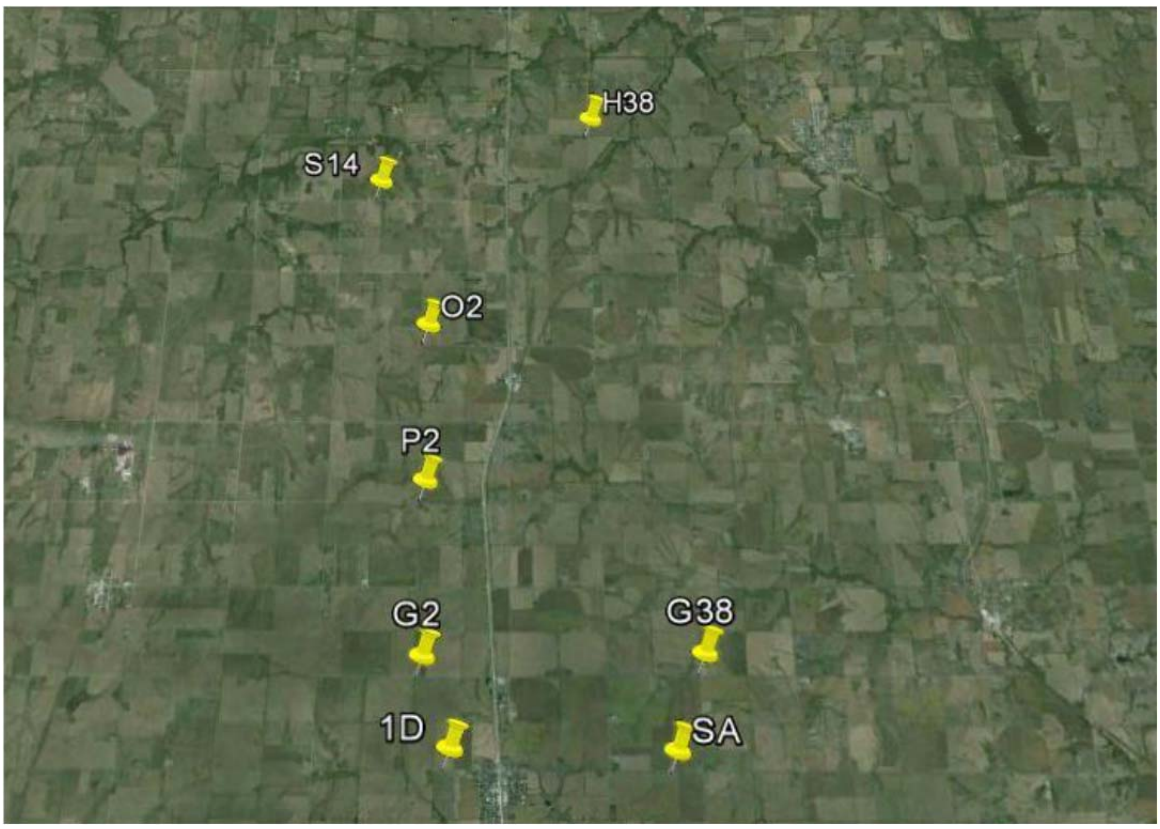


Figure 1. Study site locations and configuration in Lancaster and Gage Counties, NE.

Materials and Methods

Honey Bee Colonies. The colonies used in our study were received on 3 April, 2014 from a commercial bee keeping operation located in Guide Rock, Nebraska approximately 138 miles from the research sites. Sixteen honey bee colonies rented and dispersed among the study sites, two per site. The sixteen colonies averaged eight frames covered with bees at time of receipt. They were all queen right and all stages of brood were present. Brood nests covered at least two frames. All hives had some honey stores and were free of pests and diseases. Each hive was composed of two hive bodies providing these hives with room for their populations to grow throughout the experiment.

Figure 2. Example of colony placement and dead bee trap construction and placement.

Upon placement, the hives were fitted with one 2x3 foot dead-bee trap which was screened top and bottom to prevent loss or predation of collected bees. A few days after colonies were placed pollen traps were affixed and left open to allow bees to acclimate to them. From the outset, colonies were fed one gallon of 2:1 sugar to water syrup mixture every 48 hours. They were concurrently fed Ultra Bee Patties (a pollen substitute) purchased from Mann Lake LTD. Colonies were fed syrup and patties continuously through the course of the study.

Vegetation, bee-collected pollen, and bee sampling.

As outlined in our study plan, we marked a transect extending from each study site for purposes of sampling honey bee and native bee occurrence and to measure floral resource availability. We took advantage of new technology to map those transects onto aerial images of the landscape surrounding the study sites and transects. An application named GeoCam (http://sitis.mobi/) was used to mark and map transect overlays onto Google Maps images (Figure 3 and 4). These maps were used in conjunction with Google Earth Pro to quantify foraging habitat types.

Pollen was collected in traps at hive entrances. The last collection occurred on 19 May, three days before colonies were removed from the study site. All collections occurred over a 48 hour period. Samples were bagged separately and immediately refrigerated in a portable DC cooler that maintained temperatures below -20° F. Samples were kept in this cooler until shipment, packed on dry ice, to the lab at Missoula, MT, and stored in a temperature monitored freezer at < - 20o F. In the laboratory, pollen samples were composited from the two colonies at each site,

tumbled to thoroughly mix, and split into samples for chemical and for pollen diversity analysis. Samples for chemical analysis were shipped under dry ice to the NSL laboratory at Gastonia.

Pollen diversity was assessed by analyzing a composited sample from each of the 8 sites.

This gave us 24 data points, three for each site, representing the Pre-Planting, Planting, and Post-Planting periods. One gram samples of the mixed and composited pollen pellets were first sorted by color and weighed.

The representative colors from each sample were then prepared on a slide and photographed at 10 random locations at 400x magnification. The pollen was identified to family (or genus where possible) by comparing it to known pollen images. When identification was unclear, we used collection date and local phenology data, as well as our own observations and plant pollen specimens collected in the field during the April-May field study. This method gave us a clear phenology over time during the study period.

Dust Sample Collection

We constructed dust collection devices as described in our work plan. Clean glass microscope slides were coated with vacuum jar grease and affixed to the devices to collect vertical and horizontal dust deposition samples at the colony sites, field margins and uncultivated habitats at each sample site. Intermittent heavy rains occasionally washed samples away, but every attempt was made to collect samples before rains occurred and to replace slides immediately following rain events.

For dust deposition analysis, 30 fields of view from the deposition for each site for the three sampling periods were imaged and pictures taken for data archiving using a digital microscope camera. The images were saved and labeled for later counting.

Once all sites and sample period slides were photographed, the dust particles were counted using a gridded transparency sheet and dry erase marker. The grid sheet was placed over the computer screen and each dust particle was counted, and then marked with the dry erase marker to ensure that double counting did not occur. On images with extensive deposition and an even distribution of particles, half the image was counted and multiplied by 2 to get an estimate of the dust particles for the entire image. All counts were recorded into an Excel spread sheet and are included in the data package provided to the CDRC.

Dead Bee Collection

The number of dead bees collected in the screened traps placed in front of each hive was counted and samples taken daily, or as frequently as weather permitted. We experienced both rainfall and snow during the experimental period. Following a Pre-Planting mortality incident at sites G2 and 1D, we collected, bagged in new plastic bags, and froze all dead and dying bees from both the traps and from the bottom boards of the impacted hives. All collected bee samples were frozen upon collection, held in a -20 degree freezer until inspection. Subsamples of these bees were shipped under dry ice to the NSL laboratory for broad spectrum pesticide analysis. Bee samples from these two mortality incidents were washed in alcohol to detect varroa mites and dissected and examined under a microscope to quantify any *Nosema* spores. Bee samples were also submitted to BVS, Inc. for IVDS analysis in order to screen for the presence of viruses.

Figure 3. Example of GeoCam application used to identify sample sites and to indicate location of vegetation and bee sampling transects.

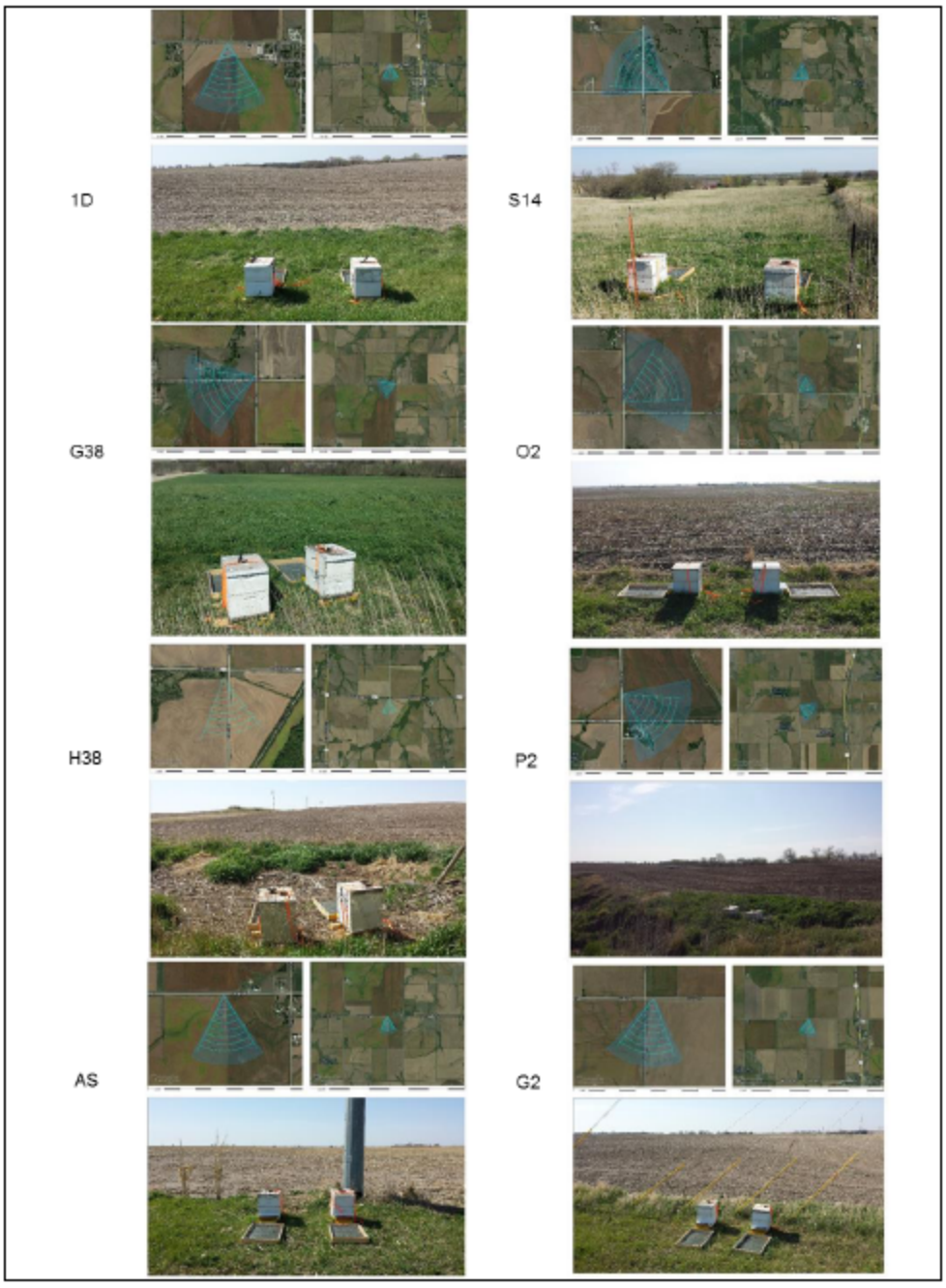


Figure 4. GeoCam images of eight study sites with colony and transect placement mapped onto Google Earth aerial images of surrounding landscape.

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 to 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).

Methodology

Experimental setup

The study was conducted at six sites, at least 15 km apart, in the field- crop-dominated agroecosystem of Central Ohio (Figure 1). The study sites consisted of three Ohio State University experimental apiaries that were previously used in the 2013 CDRC project and three private apiaries managed by experienced beekeepers.

The OSU apiaries were populated with four honey bee colonies, including one overwintered colony and three colonies started from 3-lb packages of bees plus two additional frames of open brood (Figure 2) in mid- to late-April 2014. WB and FSR each had one additional colony started from a package that was not used. The private apiaries contained 6 – 13 overwintered colonies, from which four strong colonies were selected for the experiment. Each of the experimental colonies was equipped with a bottom-mounted pollen trap (Sundance I) that could be turned on, to collect corbicular pollen from returning foragers, or turned off, to allow pollen into the colony for the bees' sustenance. A drop-zone dead-bee trap (40" x 20") was placed in front of each colony. Pollen traps were emptied and turned on and off every 2 days during corn planting and every 3 – 4 days after planting, alternating between colonies, so that pollen was always sampled from two colonies at each site. All dead bees in the dead bee traps were counted and removed on the same day when pollen samples were collected. Sampling at most sites continued through June 9. At this time, 95% of corn in Ohio had been planted (USDA-NASS, 2014) and, based on our observations, most corn fields in west-central Ohio had been planted approximately two weeks earlier.

Corn planting in 2014 occurred in three episodes separated by rainy weather conditions. Highly sporadic planting activities were observed or reported by landowners (OSU sites) on April 25 – 26. Planting was stopped by heavy rains at the end of April that saturated soil and prevented planter operation until May 5 – 10, during which most corn planting occurred throughout west and central Ohio. The remaining fields were planted, or in some cases replanted, between May 22 – 28 (concurrent with soybean planting). According to the USDA-NASS Crop Progress Report, statewide corn planting in Ohio was at 9% on May 5, 40% on May 11, 69% on May 25, and 88% on June 1.

Landscape Analysis

Honey bees encode spatial information about their foraging behavior using their famous "dance language" (von Frisch, 1967). This dance language can be readily decoded to map a colony's spatial foraging patterns.

To estimate the appropriate spatial scale at which to study the relationship between landscape composition and the exposure of honey bees to seed treatment dust, we set up an observation hive at WB and video-recorded one hour of honey bee dances on each sunny day between May 6 and May 12. We then extracted twelve one-minute segments from each one-hour video and analyzed these segments using ImageJ software (Rasband, 1997-2014). Finally, we decoded and mapped each of the analyzed dances using the Bayesian probabilistic method described by Schürch et al. (2013) and implemented in R (R Core Team, 2013).

The overwhelming majority of foraging activity occurred within 1.5 km of the hive (Figure 3), suggesting that, during the corn planting period, floral resources were sufficiently abundant within that 1.5 km radius to give the bees little reason to forage farther. Dandelion (*Taraxacum officinale*), which is highly attractive to honey bees, was in full bloom during the period of dance recording, and there was a large patch of *T. officinale* in the immediate vicinity of the observation hive. These results indicate that a 1.5 km radius was a more appropriate scale at which to conduct landscape analysis than the 3 km scale commonly used (CDRC, 2013).

We defined the total landscape of each apiary as the area contained by a 1.5 km radius centered on the location of our hives. Using a combination of aerial photo analysis, visual ground-truthing, and GIS software, we quantified the composition of each landscape in terms of the following elements: crop fields, forest tracts, grassy fields and strips, and residential lots (Figure 4). WB, CH, and FSR had relatively simple landscapes dominated by corn and soybean fields. The landscapes of TR, MB, and NC were more complex, having relatively large proportions of forested, grassy, and residential habitats in addition to corn and soybean fields. NC was unique in that its landscape included a large complex of orchards. Together, our six sites comprised a gradient of corn land cover ranging from 43% (WB) to 9% (MB) of total landscape area.

On April 29 and 30, immediately before the start of most corn planting, we visually assessed the abundance of bee-attractive blooms in all accessible crop fields. Each field was assigned a qualitative bloom level of 0-2: 0 = virtually no blooms, 1 = scarce blooms, and 2 = abundant blooms. On July 8 and 9, we returned to each site and determined which fields had been planted with corn.

Assuming that honey bee exposure to seed treatment neonicotinoids occurs primarily through the deposition of seed dust onto bee-attractive flora, two spatial categories of exposure risk can be defined: (1) *primary risk zones*, where flowering weeds are present *within* cornfields during planting and subject to direct dust deposition, and (2) *secondary risk zones*, where flowers *outside* of cornfields are contaminated by drifting dust. We determined primary risk zones at each of our sites by identifying all corn fields that were characterized as having abundant flowering weeds during our bloom level survey (Figure 5). To determine secondary risk zones, we used GIS software to create a 100 meter “drift zone” around the perimeter of all cornfields (CDRC, 2014). We then measured the intersection of this drift zone with habitat types that were generally found to have abundant bee-attractive flora during ground surveys. These habitat types included weedy agricultural fields, forest patches, grassy roadsides and field margins, and residential yards. The intersections of these habitats with the drift zone surrounding cornfields comprised the secondary risk zones for each of our sites.

The concentration of seed treatment insecticides to which honey bees are exposed in pollen should be proportional to amount of foraging that occurs in contaminated vs. uncontaminated habitat. Accordingly, we predicted that insecticide exposure would be positively correlated with (1) ratio of primary risk area to total forage area and (2) ratio of total risk area (primary + secondary) to total forage area.

Spearman’s correlation coefficient was non-significant ($p > 0.9$) for both prediction 1 and prediction 2. Moreover, we found no significant relationships by partitioning risk zones according to herbaceous and woody foraging habitat ($p >> 0.1$) We did, however, find a significant positive correlation ($p = 0.033$) between peak seed treatment insecticide exposure and total corn area (Figure 6).

All landscape analysis were performed using QGIS (QGIS Development Team, version 2.2), and statistical tests were performed in R (R Core Team, 2013).

Figures

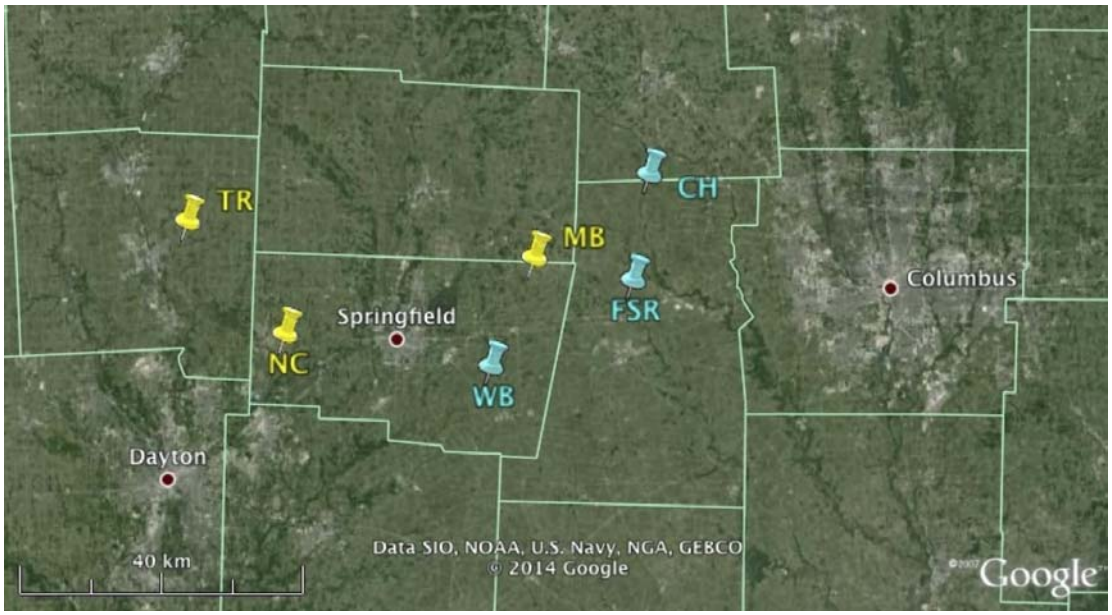


Figure 1. Apiary locations. Yellow markers represent sites added to the study in 2014. Blue markers represent sites studied in both 2013 and 2014.

Figure 2. Apiary setup of OSU sites. Each colony was equipped with a pollen trap fitted between the bottom board and the hive body, and a dead bee trap in front of the entrance. Photo was taken at CH on May 11, 2014.

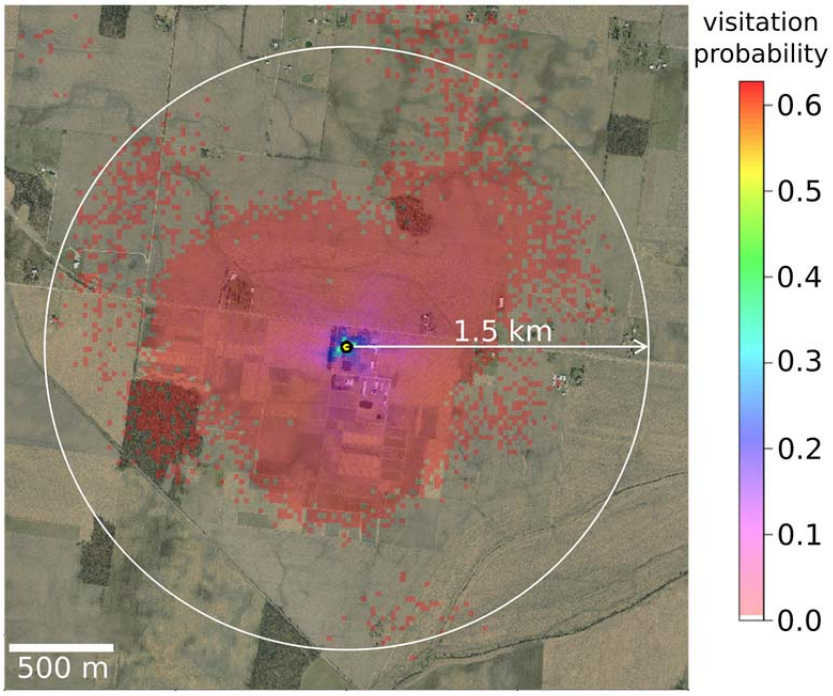


Figure 3. Honey bee foraging activity occurred mainly within 1.5 km of the hive (yellow dot). Pixel colors describe the probability that foraging occurred within the area covered by each pixel based on Bayesian probabilistic modeling of decoded dance data. Foraging activity was especially concentrated on a patch of *T. officinale* located in the immediate vicinity of the hive (blue).

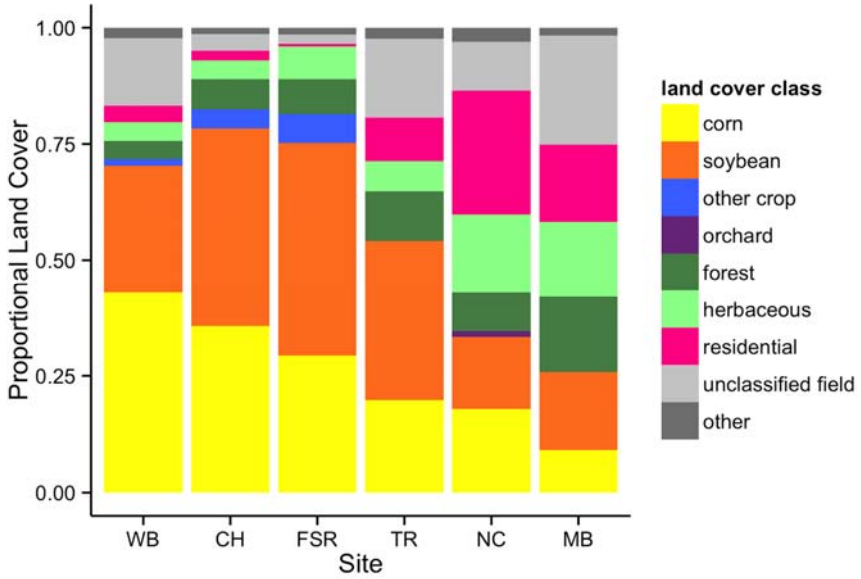


Figure 4. Landscape composition across 6 sites. Corn prevalence ranged along a gradient from 43% (WB) to 9% (MB) of total landscape area. Other field crops, including wheat and alfalfa, were scarce or absent at all sites, and only NC had orchards. Forested areas, consisting of forest tracts and tree-lines/windbreaks, comprised 4-16% of total area at each site. Grassy areas, including

uncultivated fields, roadsides, and field edges, were most prevalent at MB (16%) and NC (17%) and ranged from 4-7% at the remaining sites. Residential land was very scarce at WB (4%), CH (2%), and FSR (1%), moderately scarce at TR (9%), and moderately abundant at MB (17%), and NC (27%). Fields that were not accessible to ground inspection and not easily identifiable by aerial imagery were left unclassified. Other land cover not specified included roadways and miscellaneous undetermined features.

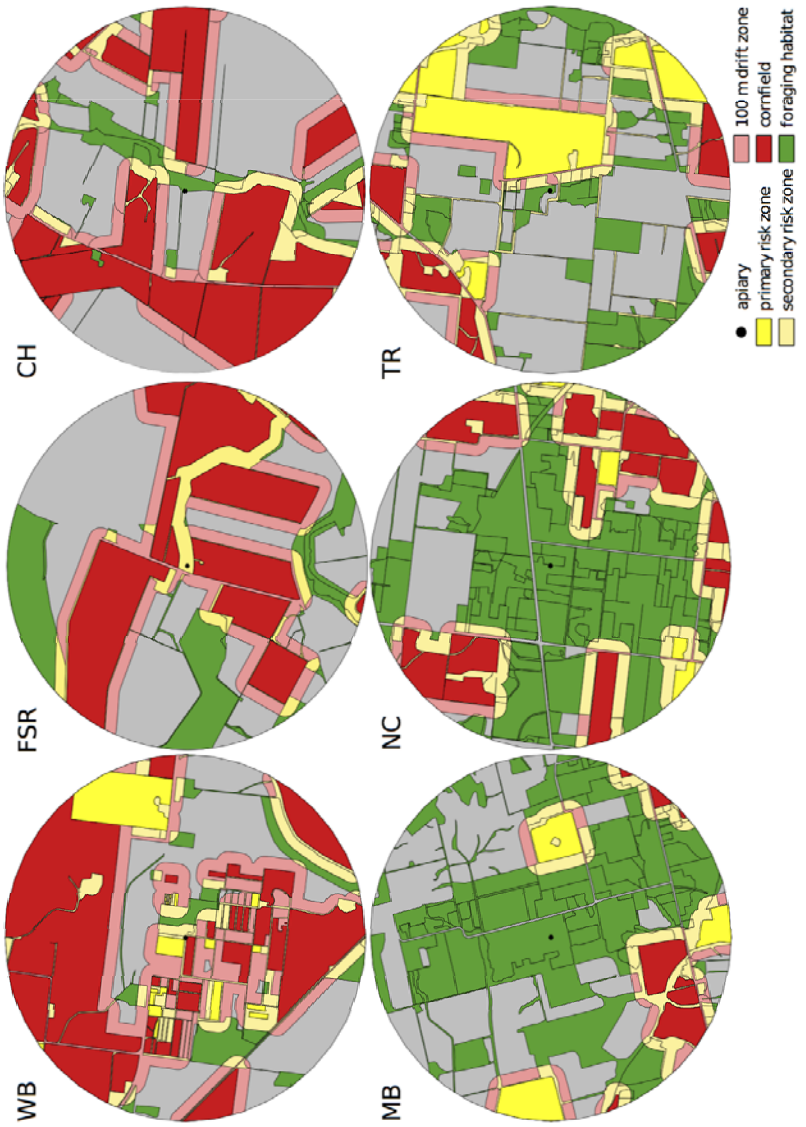


Figure 5. Spatial relationship between predicted dust deposition and foraging habitat. Primary risk zones (deep yellow) occur where blooming weeds are present within cornfields**.** Secondary risk zones (light yellow) are defined as the intersection between foragable habitat and a 100 m drift zone around cornfields.

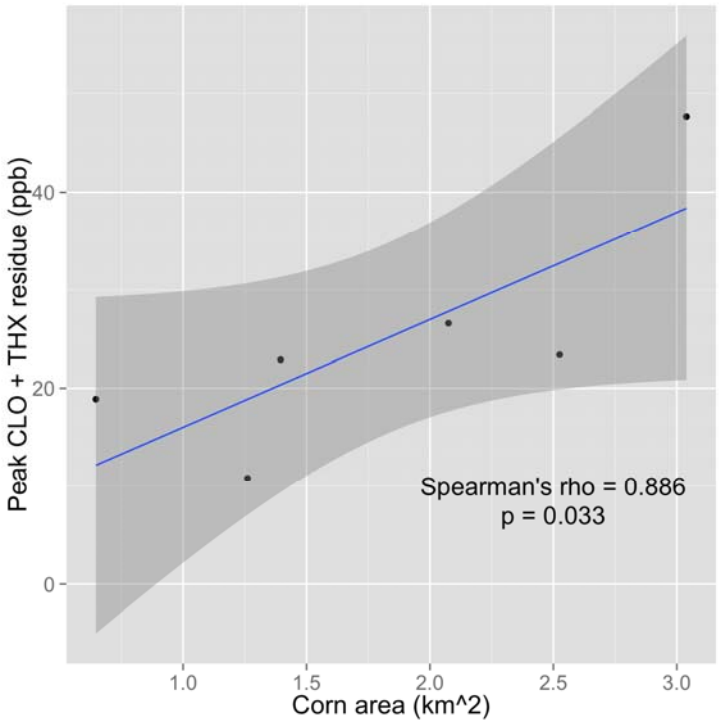


Figure 6. Peak seed treatment neonicotinoid residues in corbicular pollen predicted by total corn area. This relationship should be interpreted cautiously due to our small sample size and the strong variability of residue levels in corbicular pollen.

Study locations

We worked again at 9 locations; each consisting of two commercial fields and one apiary. One of the two fields was the same as used in the 2013 study, and one was a newly recruited corn field. We used as many repeat cooperators from 2013 as possible, plus new recruits as necessary due to rotation to a soybean or other non-corn crop in 2014. Field selection was based on the most suitable orientation to tree lines and bush in proximity (best downwind) of 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, Figure 1). All 9 apiaries studied in 2014 were the same as in 2013, with the exception of apiary 1 which was moved to the newly recruited corn field (1A) and apiary 5 which was placed closer to field 5A.

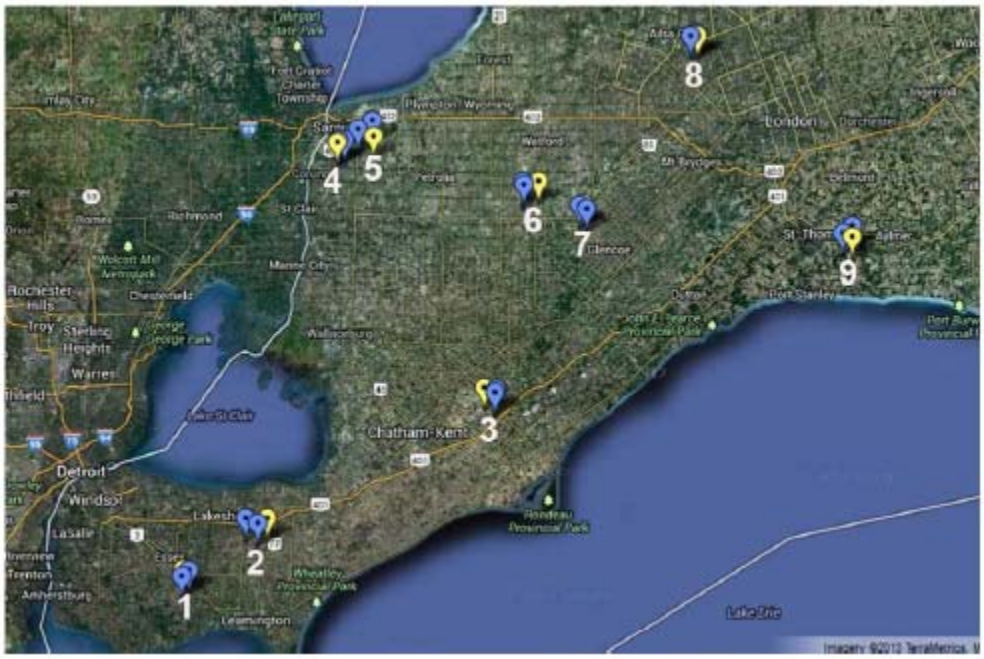


Figure 1. Map depicting locations of corn fields and apiaries studied in southern Ontario in 2014.

Table 1. Experimental fields and associated apiaries studied in southern Ontario in 2014.

Site #	County	Field/Apiary	GPS coordinates	Crop in 2014
1	Essex	1A	42.075947,-82.788867	Corn*
		1B	42.089060,- 82.791718	Soybean
		Apiary 1	42.073875,- 82.786964	Treeline
2	Essex	2A	42.177147,-82.595489	Corn*
		2B	42.197248,-82.625695	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	Lambton	4A	42.896352,-82.389889	Corn*
		4B	42.906519,-82386902	Soybean
		Apiary 4	42.899698,-82.390238	Bush
5	Lambton	5A	42.950829,-82.290901	Corn*
		5B	42.942102, -82.303051	Soybean
		Apiary 5	42.917486, -82.301404	Treeline
6	Lambton	6A	42.836702,-81.943464	Corn*
		6B	42.829001, -81.926401	Soybean
		Apiary 6	42.830101, -81.887554	Bush
7	Middlesex	7A	42.812989,-81.731458	Corn*
		7B	42.776802, -81.767126	Soybean
		Apiary 7	42.821894,-81.758160	Bush
8	Middlesex	8A	43.099319,-81.488125	Corn*
		8B	43.101433, -81.501658	Soybean
		Apiary 8	43.099451,-81.482637	Bush
9	Elgin	9A	42.730421, -81.113945	Corn
		9B	42.744948, -81.093284	Soybean
		Apiary 9	42.724245, -81.089501	Bush

* Newly recruited corn field in 2014.

Table 2. Hybrid, planting date, neonicotinoid seed treatment, and rate of Bayer Fluency Agent used by growers in 2014 corn planting studies.

Corn field	Hybrid	Planting date	Neonicotinoid insecticide used		Rate of Bayer Fluency Agent used (% of recommended[1])
			Active ingredient	Rate (mg a.i./kernel)	
1A	DKC 57-75 RIB	27-May	clothianidin	0.25	50
2A	MZ 4640SMX	24-May	clothianidin	0.25	100
3A	DKC 53-56 RIB	27-Apr	clothianidin	0.50	100
4A	A7270G8	28-May	clothianidin	0.25	100
5A	DKC 48-12 DKC 49-82	31-May	clothianidin	0.25	100
6A	DKC 52-61 RIB	11-May	clothianidin	0.25	100
7A	P0216AM	31-May	thiamethoxam	0.25	100
8A	P0216AM DKC 52-61 RIB	26-May	thiamethoxam clothianidin	0.25 0.25	100
9A	P0216AM	28-May	clothianidin	1.25	100

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

Table 3. Corn planter and tillage used by growers in 2014 corn planting studies.

Corn field	Planter make/model	No. rows	Row width in (cm)	Seeding rate (seeds/ac)	Tillage
1A	John Deere 1680	24	20 (50.8)	33,000	no-till
2A	John Deere 1790 NT	16	30 (76)	34,000	no-till/minimum till
3A	John Deere 1770 NT	16	30 (76)	34,000	conventional
4A	John Deere 1770	12	30 (76)	34,000	no-till
5A	John Deere 1790	24	20 (50.8)	35,000	conventional
6A	Case IH 1240	18	20 (50.8)	34,000	conventional
7A	John Deere 1770 NT	16	30 (76)	33,000	conventional
8A	John Deere DB60 47R15	24	30 (76)	35,000	conventional
9A	John Deere 1770	24	30 (76)	Variable	conventional

Modifications to original protocol and deliverables

The weather in 2013 and 2014 was oppositely exceptional to normal conditions in southwestern Ontario, with 2013 very early, warm and dry, and 2014 very late, cool and wet. In 2014, blossoms of many of the most abundant tree/shrub species (*Acer* spp., *Salix* spp.) in 2013 withered away before it was warm enough for honey bees to forage. Unlike 2013, honey bees were forced to wait and resort to nectar and pollen from flowering herbaceous plants, including dandelion. Trapping of live bees was unsuccessful, so we had to switch to hand collecting. Because corn planting was abnormally protracted and bee activities were asynchronous with corn planting, we conducted our original two trials separately: (1) collected flowering resources around corn and soybean fields, not only at the edges of corn fields; (2) collected the dust generated by corn planting activities in newly recruited corn fields. We were also given full access to a number of portable volumetric air samplers from the Ontario Ministry of Environment which allowed us to quantify soil dust and neonicotinoid residues leaving corn fields during planting. Protocol modifications are outlined in Table 4.

Table 4. Modifications to protocols and deliverables in 2014.

#	Original	Substitute	Reasons for change
1	**Treeline dust:** 18 sticky dust trap samples from the 18 fields at 9 locations	(1) Took the downwind edge of the field as treeline even though there were no trees. (2) Collected 9 sticky dust trap samples from 9 corn fields. (3) Collected 9 volumetric air samples from the 9 corn fields to substitute for the other 9 samples from 9 non-corn fields	(1) It was not always possible to arrange treeline/bush at the downwind edge of the field during corn planting. (2) Some of the fields from last year were not planted to corn fields in 2014 because of crop rotation.
2	**Bush dust:** 18 sticky dust trap samples from the bush downwind of the 18 field of 9 locations	(1) Took the far downwind edge of the neighbouring field as surrogate bush even though there was no bush. (2) Collected 9 sticky dust trap samples from 9 corn field bush surrogates. (3) Collected 9 volumetric air samples from the 9 corn field "bushes" to substitute for the other 9 samples from 9 non-corn fields	Same as above Also to standardize samples across locations, bush locations were not uniform and changed with wind direction on the day of planting.

3	**Tree tissue samples:** The most abundant tree/shrub species adjacent to each field will be sampled to provide composite tissue samples of leaf surface dust, washed leaf tissue, whole blossom surface dust, washed whole blossom tissue, and virgin pollen (anthers) before, during and after planting. (18 fields X 3 tissue sample types X 3 sample periods X 2 sample locations = total 324 samples)	(1) Collected the blossoms of trees/bushes and herbaceous weeds around the fields and bee yards instead of only trees and bushes. (2) Leaf tissue samples were removed from CDRC deliverable following phone discussion with CDRC. Removed leaf samples were substituted with herbaceous weed flower samples. (3) Monitored and collected flower samples from late April to end of June to substitute the three sampling periods of pre-plant, during plant and post-plant.	(1) The season of 2014 was very late in southwestern Ontario, due to abnormally cool and wet weather. Most of the tree/shrub species normally available to honey bees flowered and withered, before honey bees were actively foraging, forcing a wait and shift towards herbaceous resources including dandelion during corn planting. (2) It was difficult to divide the three periods of before, during, and after planting at one location because neighbouring fields were often planted at diverse times. (3) Leaves of many trees/bushes do not emerge during blooming.
4	**Foraging honey bee samples:** before, during and after planting in the sampled trees at the fence line and in the nearest bush, foraging bees will be collected using a malaise trap (18 fields X 2 sample locations X 3 sample periods = total 108 samples)	(1) Collected foraging bees from the flowers of trees/bushes and herbaceous weeds around the fields and bee yards instead of treeline and bush (2) Used vacuum suction trap or sweep net to collect foraging bees. Triple-rinsed the trap/sweep net with alcohol to prevent cross-contamination.	(1) See #3 reason (1) (2) Bee pheromone traps did not work for foraging bee collection. Malaise trap was found to be impractical.

Determine floral resources used by honey bees in and around corn and soybean fields during the corn planting period

Vegetation in bloom was surveyed in and around the 18 study fields from April 22 to June 3 2014 (Fig. 2). 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/m2 for herbaceous weeds or 0-1 observed/100 m2 for trees and shrubs, 2 = 6-10/m2 for weeds, 2-5/100 m2 for trees and shrubs, 3 = 11-20/m2 for weeds, 6-10/100 m2 for trees and shrubs, and 4 = >20/m2 for weeds, >10/100 m2 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 (Fig. 4) or herbaceous plants (Fig. 5). To reflect the exceptionally cool, wet weather and delayed corn planting in 2014 we divided the field survey data into three sampling periods: (1) before planting (6-10 May); (2) during corn planting (11 May - 2 June); and (3) post-planting (10-25 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 bee yards 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. 3). 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. Pollen species identification and proportion were determined by Johanne Parent (Laboratoire BSL, Rimouski, QB).

Figure 2. Collection site of foraging honey bees and blossoms of willow and dandelion near Kingsville, ON, 1 May, 2014.

Figure 3. Anel standard pollen trap.

Determine insect pollinators in and around corn and soybean fields during corn planting season

To determine the important pollinators active in proximity to corn and soybean fields during the time of corn planting, standard 37 cm (15 in.) diameter sweep nets were used to sample insects present on low-lying vegetation around the perimeter of fields. One sampling consisted of 10 consecutive sweeps (180o sweep) completed across the vegetation while moving at a walking pace along the perimeters of the fields. Samples were taken during 10:00 and 14:00 h on sunny days; randomly selecting 5 locations per side of field (50 sweeps per field side). Before corn planting, during the week of 4-10 May, 30 sweep samples were collected in total from 15 of the study fields. After planting, during the weeks of 18-24 May, 25-31 May, and 1-7 June, 11, 9, and 2 samples were collected in total from 6, 5, and 2 of the study sites, respectively. Fewer samples were collected after planting because our focus shifted to collecting foraging honey bees specifically rather than general pollinator sampling. Insects were identified to family and key pollinators were identified to genus and/or species and counted; specimens were preserved and kept at the University of Guelph Ridgetown Campus available for further identification as needed.

Honey bees and dandelions - Diurnal visits

We monitored the study field near Kingsville ON, on 23 May (Fig. 8) for honey bees foraging on dandelions for one day during the period of corn planting using composite sweep net samples collected at approx. 1 hr intervals from morning to evening to observe the diurnal habits of honey bees visiting dandelions.

Figure 8. Kingsville ON. May 23, 2014. Diurnal foraging of honey bees on dandelion. Main photo faces north with farmer-cooperator corn field to the west. Top right inset faces south (notice large bush to the west). Bottom left inset shows honey bees foraging for water in ditch. Top left inset shows the two hives placed by beekeeper cooperator.

All honey bees observed resting on dandelion were captured on the hour with a sweep net while walking a 200-m stretch up and down each side of ditch bank along the field access road between bush and hives (see Fig. 8). Before each sampling, the sweep net was washed in dish detergent and water, rinsed with purified water, followed by a rinse with methanol and then allowed to dry in the sun. Captured honey bees were allowed to move into a clean poly sample bag. Bags were immediately placed in dark picnic coolers with frozen ice packs and out of the sun, until returned to the laboratory. Here they were immediately placed in dark frozen storage at -20°C.

Dandelions seem to open and close in response to light. We used a simple index to indicate the relative openness of dandelion blooms in 10% increments where 1 = 10% and 10 = 100% open. Because honey bees were observed drinking water from the ditch, we also took a 100-mL water sample to be analyzed using our standard sampling, storage and analytical methods validated in the 2013 study. At mid-day 50 dandelion blossoms were sampled and placed into new plastic sample bags using new disposable neoprene gloves and sealed and also placed into picnic coolers as above.

An approximate 100-g sample of soil was taken from below each of ten dandelion plants, from which blossoms were sampled, using a folding shovel. Before sampling the shovel was washed with dish soap and water, followed by rinsing in purified water and again with methanol and allowed to dry in the sun. The soil and roots of the dandelion were dug to a depth of approx. 10

cm and a sample of soil was taken from around the roots using new disposable neoprene gloves. All ten samples were combined in a new and clean plastic utility pail, thoroughly mixed with samples of approximately 500 g placed in sealed plastic bags, which in turn were also placed in to picnic coolers as above.

Honey bees, dandelions and corn stubble

At two locations, St. Thomas and Rodney (Figs. 10 and 11), we had the opportunity to collect honey bees foraging on dandelions in fields with standing corn stubble before (24 and 25 May) any crop planting or tillage was done within foraging range of the fields. Again no field operations had commenced in the region due to the unusually cold and wet spring. The St. Thomas location was one of our study sites and contained a commercial beehive. The Rodney location was found by scouting numerous fields en route to St. Thomas. The Rodney farmer was unaware of any commercial beekeepers or hives in the area, so it was assumed that the hive was feral. We collected composite samples of a minimum 50 honey bees, 50 dandelion blooms and 20 soil sub-samples from around the dandelions randomly from within the corn stubble field. All collections were made, handled and stored as described in Section 5.1. Blossoms were analyzed step-wise. Surface pesticide residues were extracted by first rinsing entire blooms in water. Then disk florets, as a surrogate measure of anther/pollen tissue, were excised and macerated for a separate extraction. This was followed by the maceration and extraction of the entire remaining dandelion bloom tissue. Analytical methods are described in detail in Appendix A. At the Rodney site (Fig. 11 top) and two additional sites near Dutton and Fingal (Fig. 11 bottom) we observed dandelions blooming in standing corn stubble, as well as dandelions blooming in fencerows and/or ditch banks immediately adjacent to these fields. These sites were also sampled for dandelion blooms, and soil in which the sampled dandelions were growing, both in the corn stubble field and in the field perimeter where no crops had been planted (fence line, ditch bank).

Neonicotinoid concentrations in pre-planting and post-planting soil in the seed placement zone

Soil samples were taken immediately before and after planting from each corn field by hand-collecting 500 g from the top 5 cm soil surface at 10 randomly selected subsample sites per field using a new vinyl or latex gloved hand for each sample. The samples were stored at -20^{0}C until residue analysis using LC/MS/MS. PROC MIXED (SAS Ver. 9.4, SAS Institute, Cary, NC) was used to compare the neonicotinoid concentration in the seed placement zone before and after corn planting with sampling time as the fixed effect and field as a random effect. Data were subjected to log10 (x+1) transformation to meet the assumptions of normality.

Neonicotinoid residues in pre-plant surface soil dust produced by the mechanical action of planter units

Surface soil dust samples were collected before and immediately after planting from each field using a modified two-row planter unit pulled through the field by a John Deere Gator™ utility vehicle to simulate dust produced solely from the action of a planter (or other implement) moving through the field (separate from dust produced and exhausted from vacuum planters) (Fig. 12).

Figure 12. John Deere Gator™, two-row mock-planting unit and dust collection device.

Dust was collected by a device attached to the planter unit consisting of a galvanized sheet metal hood, a suction fan and vacuum bag. The collection hood collecting dust-ladened air immediately behind the planter unit was approximately 0.5 m off the ground. The Gator travelled at typical corn planting speed (8-9 km/h), and ran for 500 meters up-wind.air samplers at the field edge and the edge of a neighbouring field during corn planting and tillage

We then compared the total neonicotinoid concentration in dry, soil surface dust mobilized into air mechanically by this method, with that found in the undisturbed 5-cm surface fraction of the soil using PROC MIXED with soil sample type as the fixed effect and field as a random effect. Data were subjected to log10 (x+1) transformation before analysis to meet the assumptions of normality.

Neonicotinoid residues collected from planter exhaust, sticky dust traps and air samplers at the field edge and the edge of a neighbouring field during corn planting and tillage

There were 10 fields where corn was planted in 2014 (Table 13). Seven of these were tilled before planting allowing sampling for soil dust during tillage. We had the opportunity to recruit an additional 4 fields to measure the dust leaving fields during tillage for a total of 11 tillage sites. Of the 10 planting study sites, all were planted using a vacuum planter and the Bayer fluency agent was used at the recommended rate, with the exception of site 1A where only the half rate was used (cooperator's decision). A planter exhaust dust sample was taken at each site planted to corn in 2014 using a pre-weighed vacuum cleaner bag (Electrolux® CB, #635, 53.5 x 37 x 16 cm) attached to the outlet of the exhaust manifold on the day of planting. The bag remained on the exhaust port for one complete round of planting of the field; the distance driven was recorded.

The dust collected was standardized to a100-m distance and a single row (planter unit) to allow comparisons between study locations. Extraction and LC/MS/MS analysis was conducted at the University of Guelph Ridgetown Campus (Appendix A). The extraction and sampling methods used in 2014 were modified from the 2013 study; therefore, the total neonicotinoid recovered from the planter exhaust cannot be directly compared across years.

At all the sites we also collected fugitive dust generated from either tillage and/or planting. Sticky dust traps with vertical panels were used to collect dust (Figure 13). Each dust collection trap 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 the far downwind edge of the neighbouring field (distal). Towers were orientated so that the vertical panels faced directly into the wind. The distance between the proximal and distal traps was

recorded. 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.

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 2014 can eventually be related to those collected in 2013. Extraction and LC/MS/MS analysis were conducted at the University of Guelph Ridgetown Campus. At some locations, the neighbouring field edge was not suitable to set up dust towers because the neighbouring field was also being planted; these data are missing.

Figure 13. Vertical sticky dust trap

Figure 14. Volumetric air sampler

Determine the neonicotinoid residues in dead bees and bee-collected pollen during the corn planting period

At each of the 9 bee yards, dead bees were collected from Drop-Zone Dead Bee traps installed in front of four bee hives during 24 h intervals (Figure 15). Bee-collected pollen that was collected for objective 3 was analyzed for neonicotinoid concentration. There were 3 sampling periods: (1) before planting (6-10 May); (2) during corn planting (11 May - 2 June); and (3) post-planting (10-25 June). Neonicotinoid residues in dead bees and bee-collected pollen were determined by LC/MS/MS.

Figure 15. 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 bee yard as a random effect. Data were subjected to log10 (x+1) transformation to meet the assumptions of normality.

Neonicotinoid residue on and in blossoms foraged by honey bees

Study fields and bee yards were surveyed for plant species that honey bees were actively foraging on prior to corn planting. Blossoms from these plant species were removed using clean pruning shears and new, disposable gloves and placed in brown paper bags. Soil to the 10 cm depth was collected from the base of each plant sampled.

Foraging honey bees found on these plants were collected using clean sweep nets. All plant tissue, soil, and bee samples were stored in complete darkness at -20ºC until processing and testing. *Melilotus* spp. (yellow sweet clover), *Trifolium* spp. (white clover), *Cirsium* spp. (thistle), *Taraxacum* spp. (dandelion), *Salix* spp. (willow), *Acer* spp. (maple), and *Rosaceae* species were sampled. A total of approximately 160,000 complete blossoms of various species were collected processed in this study (See Appendix A for complete analytical methods).

Methods – Question 2 Efficacy of Seed Lubricant Products

Ohio State University

Summary

The most straightforward approach to reduce the exposure of honey bees to seed treatment dust during corn planting is to reduce its emission during the planting process. The simplest way to do this may be through the use of a novel seed lubricant to reduce the abrasion of corn seed and the resulting generation of insecticide-laden dust. The polyethylene-based Bayer seed fluency agent was compared with grower's chosen seed lubricants at three sites in central Ohio in 2014. Dust (potentially with insecticide) was collected with an array of collectors arranged downwind from the planting activity.

Methodology and preliminary results

Three paired sites were located in central to west central Ohio. A fourth site was used for dust collection of the farmer treatment only. Six paired sites were identified before and during the planting season but due to weather related planting delays, two were lost.

Planter type, including make, model and serial number were recorded as well as the type of seed and insecticide seed treatment planted at each paired site. A sample of the seed planted was retained for potential analysis in a Heubach dustmeter. By paired site, the same planter and seed were used for each treatment. The local weather conditions were measured at each field using a handheld wind meter or temperature devices during planting at each site. Wind direction was determined by compass. Relative humidity was collected at the time of planting from the nearest fixed weather station via the WeatherBug app.

Seed treatment dust release and distance traveled were to be determined by using a method similar to the Krupke-style (Figure 1) dust collection procedure. The Ohio collection stations were constructed from PVC pipes 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 one in a vertical orientation 2m above the ground at right angle to the wind to intercept dust blowing by (as shown in the videos).

The slide trays are made up of five microscope glass slides held together by plastic grip strips and glued to a piece of cardboard. Stations were held in place by a cleated fence post so that the horizontal and vertical dust collectors were fixed at the correct height above the ground (30 cm and 2 m) and so that no change in direction could occur during the treatments. Slides were attached once the frame was set up and before planting began. Slides were treated with aerosol Tangle-Trap Sticky Trap Coating to intercept and hold dust particles.

The planter was cleaned out by the farmer practice, generally by opening ports, brushing or brooming down the holding tank and seed units; no vacuuming or air blast was used to clean surfaces. Use of either the conventional farmer supplied seed lubricant (talc or graphite) or the Bayer Fluency Agent was randomly assigned, each added according to directions. The Bayer Fluency Agent used consists of ethane, a homopolymer, at a rate of 1/8 cup for every 80,000 seed.

As suggested in the Guelph study in 2013, the first 100 meters of the field was planted and then the collection stations erected in the portion of the field that had already been planted and downwind. This

allowed standardization of the landscape in which the stations were placed and avoided problems with placing collection stations in ditches, roads or on neighboring property.

The stations were placed from the field edge perpendicular to the orientation of the planting passes. Stations were placed at the edge of area to be planted (approximately 0.75 m), as well as 10, 50 and 100 m downwind of the planter passes (Figure 2). Three rows of detectors were set in this configuration and were spaced evenly approximately 30 m apart. Planting began after station placement and continued until 150 m of field was planted perpendicular to the wind direction. The number of passes required varied depending on the size of the planter; 12 passes for the 16-row planters and eight for the 24-row planter.

An addition made for Ohio in 2014 was the placement of three slide trays (by replicate) in the field plus one to ride on the planter. Three slide trays were placed under the first pass of the planter so that dust blown downward was directed toward the slides; the trays were then picked up and stored after that single pass. The slide tray on the planter was held face up and on top of the planter frame for the duration of each treatment.

Slide trays from the field and the planter were removed immediately after planting. Slides were organized and stored temporarily in a dust proof cardboard box. The planter was cleaned and the procedure repeated with the same seed in a different portion of the same field using the second seed lubricant. Once finished with the site, the slide trays were transported in shrink wrapped and tape-sealed cardboard boxes to a commercial cold storage facility in Columbus, Ohio and stored at -20C temperature until July 8th when they were transported to the Wooster Entomology Lab in coolers with dry ice as the coolant.

Figure 1. Dosimeter used for collecting seed treatment dust drift during planting trials. Five microscope slides coated with Tanglefoot adhesive are place in a horizontal orientation 30cm above the surface of the field and in a vertical orientation 2m above the surface of the field.

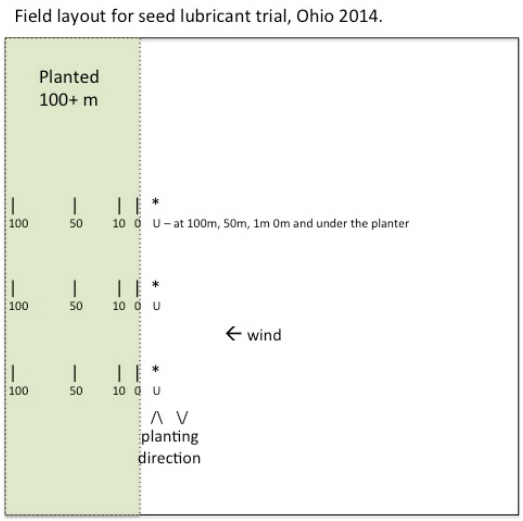


Figure 2. Field layout for placement of dust collection targets. The dust collection slide sets are placed under the planter, and at 0, 10, 50 and 100 meters from the planting start point for each treatment.

2014 Ohio Dust Trials

Overview of Test Sites.

Site #	1	2	3	4
Location	Mechanicsburg	London	Delaware	Kenton
Planting date	7-May	23-May	26-May	31-May
Planter	Kinze 3660	John Deere 1770NT	John Deere 1770NT	John Deere 1770NT
Type	Center fill air seed delivery, mechanical seed unit (non-vacuum)	Center fill air delivery, vacuum seed unit	No center fill (unit fill directly), vacuum seed unit	Center fill air delivery, vacuum seed unit
Field orientation	east-west	east-west	east-west	north-south
Wind direction	from SSW	from NNW	SSW to WSW	ENE
Farmer treatment	1st	2nd	2nd	1st
Bayer agent	2nd	1st	1st	NA
Seed treatment	clothianidin 500	thiamethoxam	clothianidin 500	clothianidin 250

Site 1

The first site planted was on May 7, 2014 near Mechanicsburg, Ohio. Two treatments were completed at the site with the operation in a single field with rows running in the east-west direction. The field was sprayed with the insecticide permethrin on March 18th. Forty-eight acres in total was used at the site. The soil condition was lightly tilled with good soil moisture 1-1/2 inch below the surface.

The planter used was a Kinze 3660 with serial number 0013174, with 16 row units and used an air seed delivery (ASD) system to move seed to the mechanical finger pick up seed unit (no vacuum at the seeding unit).

The hybrid used was Channel 213-59STXRIB. The insecticide on the seed was Clothianidin 500 included in the Acceleron seed treatment product. Other pesticides on the seed were two fungicides (Ipconazole and Metalaxyl) and a nematicide (Baccillus firmus I-1582).

The first treatment occurred from 2:45pm to 6:00 pm using the farmer treatment: Kinze graphite at the rate of 1 pound per hopper load (per 50 units of seed). The temperature was recorded at 79 °F and the relative humidity at 38%. The wind was 7-15 mph from south-southwest.

The second treatment occurred from 6:45 pm to 8:30 pm using the Bayer product. The temperature was recorded at 76 °F, relative humidity was 44% and the wind at 2-10 mph from the south-southwest.

A student assistant recorded video of the start-up of the planting process, which may be deposited in the CDRC Dropbox account.

Site 2

The next site was planted on May 23, 2014 near London, Ohio. Two treatments were completed with the farmer at this field with the planting operation moving in the east-west direction. Approximately 14.5 acres in the same field were used for each treatment for a total to 29 acres. The soil conditions were moist underneath with a dry surface.

The planter was a John Deere 1770 no till that planting 16 rows from a center fill system (CCS) and vacuum at the planter unit. The serial number of the planter is 1A01770Y PDM755556.

The hybrid was United Seed Associates USA1145 RR. The insecticide on the seed was thiamethoxam. The other pesticides that were listed as on the seed included four types of fungicides (fludioxonil, mefenoxam, azoxystrobin, and thiabendaxole).

The first treatment applied from 2:00 pm - 4:30 pm using the Bayer product. The temperature at this time was 68 °F with 46% relative humidity. The wind was variable from 5-8 mph from the north-northwest.

The second treatment was from 5:00 pm - 6:30 pm using the farmer product; Precision Plant E-flow. The product was made up of 80% talc and 20% graphite and the rate used was 1/8 cup for every 80,000 seeds. Temperature recorded was 70°F and relative humidity of 36% with a wind of 8-15 mph from the north-northwest.

Video recordings were made during the planting operation.

Site 3

The third site was planted on May 26, 2014 near Delaware, Ohio. The direction of planting for the test area was west to east. Approximately 35 acres were used during the treatments and both were placed in the same field. Soil at the site was stale, hard packed and dry on the surface with some moisture below.

The planter used on the site was a John Deere 1770 no till with the serial number 1A01770Y KCM745397. The planter had 16-rows that were individually unit-filled with vacuum assistance for seed singulation with Precision Plant meters.

The hybrid was used was Dekalb DKC62-97RIB. The insecticide on the seed was Clothianidin 500 from the Acceleron treatment. The other pesticides that were used on the seed were three fungicides (Ipconazole, Metalaxyl and Trifloxystobin) and a nematicide (Bacillus firmus I-1582).

The first treatment began at 1:30 pm and ended at 3:00 pm using the Bayer product. The temperature at this time was 80 °F and 45% relative humidity. The wind was from the south-southwest with a range of 5-8 mph.

The second treatment started at 4:30 pm and was complete by 6:00 pm using the farmer product on the seed; John Deere Premium Talc at the rate of ½ cup per 80,000 seed. The temperature was 83°F with 50% relative humidity. The wind speed was variable from 6-9 mph from the west-southwest.

The farmer commented during this treatment that the Bayer product reduced the consistency of the seed singulation as shown on the Precision Planting monitor during planting.

Video was taken of the application.

Site 4

The final site was planted on May 31, 2014 near Kenton, Ohio. Only one treatment was applied at the site; field direction being north to south. Approximately 23 acres were used in the field. The field was no till with the soil condition just barely dry enough to plant.

The planter used was a John Deere 1770 no till with serial number A01770 E710352; capable of planting 24 rows with an air delivery center-filled system with vacuum seed handling planter units.

The hybrid was Stewart 7E224RIB. The insecticide on the seed was Clothianidin 250 from the Acceleron treatment. The other pesticides in that mix were three fungicides (Ipconazole, Metalaxyl, and Trifloxystrobin).

The farmer treatment was done from 1:00 pm to 3:00 pm; John Deere Premium Talc at ½ cup per 80,000 seeds. The temperature was 79°F, relative humidity 36%, and the wind 3-7 mph from the east-northeast.

Video was taken of the planting.

What was found – Question 1 – trends and concepts among the four studies

Bee Alert Technologies

Results

Weather

We anticipated that planting would begin on or about 15 April. All colonies were on site and ready for data collection by 10 April, but planting was delayed due to unseasonably cool and wet weather. Rain and snow occurred during the Pre-Planting period; and rain and severe wind occurred during the Post-Planting period. The Planting period was interrupted by rain. Planting started, then got rained out, and resumed the following week, when conditions were warmer, drier, and visibly dustier.

As illustrated below (Figure 5) favorable planting conditions were not present until late in the month. We observed some sporadic planting activity around 15 April but widespread planting began after 22 April. Consequently we divided our sampling periods into a preplanting period from 1 – 20 April, planting period from 21 April – 10 May, and post-planting after 11 May.

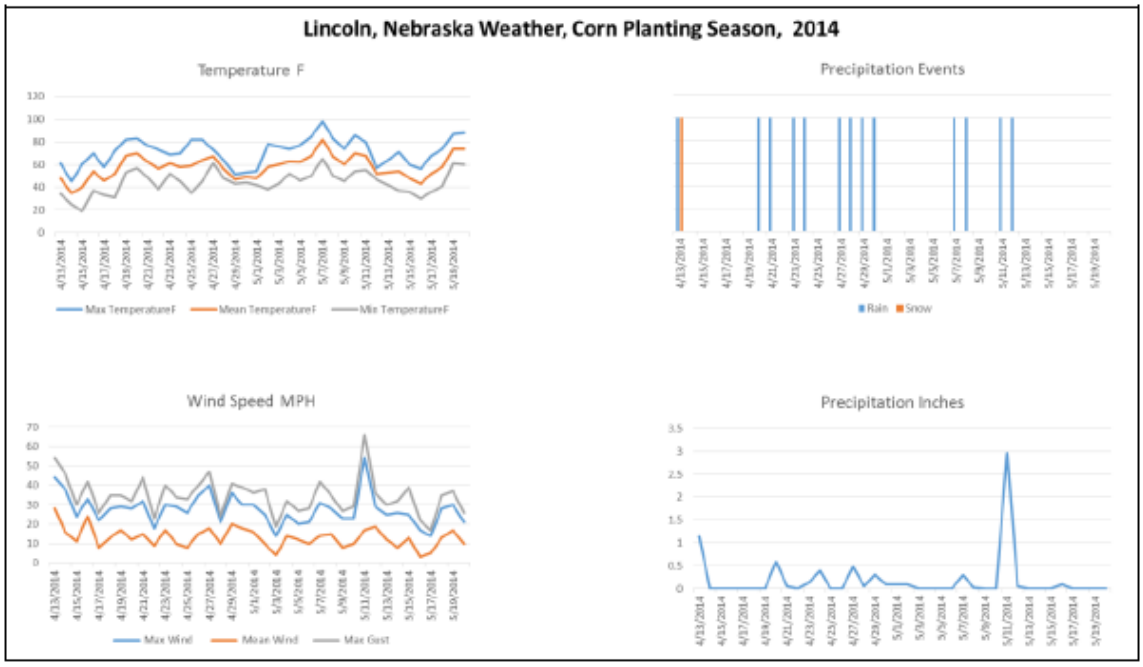
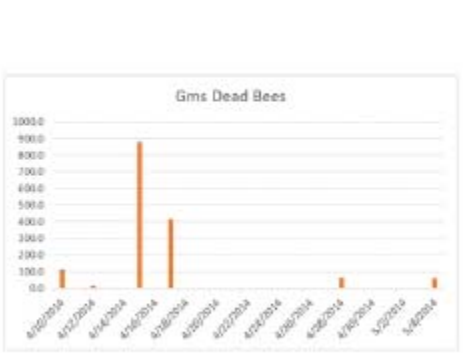


Figure 5. Weather patterns during the field data collection period near Lincoln NE.

Colony Success/Mortality Event

On 10 April, 5 days after placement at study sites, a substantial number of dead bees were discovered in the bee traps at sites G2 and 1D (Figure 6). In addition to bees accumulated in traps, hive inspection revealed more dead bees in the bottom boards of both hives at each site. The dead bees were collected and stored for future chemical residue analysis. No corn planting had begun at this time. No other obvious (i.e., visible) losses comparable to these two events were observed for any other colonies from other sites. Bee populations at these two sites continued to decline for 5 days until frame counts on April 15th revealed that the populations in both hives at both sites had declined to 2 frames covered with bees. After April 15th the colonies were found to be queen right, and despite the worker losses, the colonies began to recover.

We did not observe other severe bee mortality events (Figure 6). Cumulative bee mortality was linear over the course of the study (Figure 7), and Field-Edge site losses were greater than Yard site losses (Table 1).



Location	Grams Dead Bees	Dead Bees
G2	2.6	26
514	10.5	105
G38	11.7	117
A5	65.1	651
G2	724.3	7243
1D	733.7	7337

Figure 6. Grams of dead bees collected by date and site over course of the study.

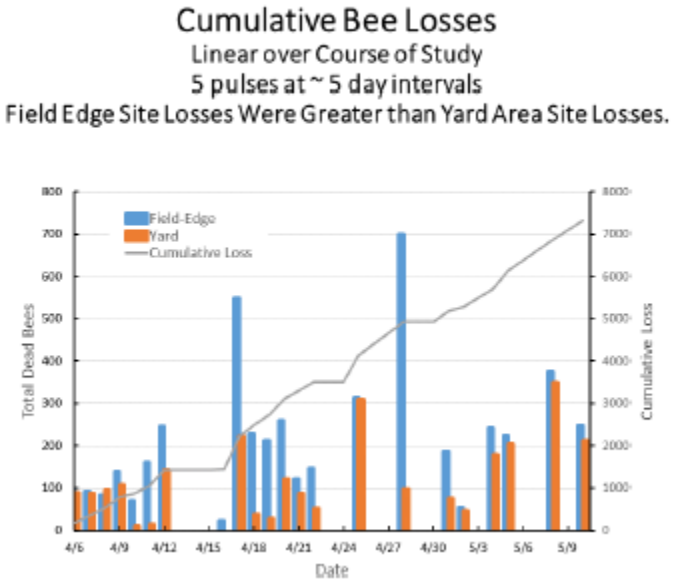


Figure 7. Total dead bees versus culmulative loss of bees over course of the study.

A 2-sample, nonparametric, Kolmogorov-Smirnov test for differences in distribution, where the input variables were Field-Edge versus Yarding site, produced a maximum difference of 0.321, with a two-sided probability of 0.087, which was not significant. The pulsed, linear pattern of adult bee loss was the same for both field-edge and yard areas. However, a Ttest of mean bee loss, using a square transformation of the data to improve normality, based on a paired test with unequal variances, yielded a very significantly higher mean losses of bees in field-edge colonies over the duration of the study (Table 1).

Table 1. Paired T-Test of Mean Bee Losses.

Variable (Square-root)	Mean Difference	Lower Limit	Upper Limit	Standard Deviation of Difference	t	df	p-Value
Field Yard	3.19	1.59	4.79	4.13	4.09	27	0.000

It should be noted, that excluding the mortality incident at G2 and 1D during the pre-planting period, the mean daily adult bee loss for two colonies recorded for each of the eight field edge sites was 17 bees, and for yard sites was only 9 bees. Johansen and Mayer's Pollinator Protection: A Bee and Pesticide Handbook (1990) lists up to 100 dead bees per day in a Todd dead bee trap as a normal die-off, 200-400 as a low kill, 500-900 as a moderate kill, and 1000 or more as a high kill.

Post-Experiment Colony Condition

On May 22nd all colonies were returned to the bee-keeping operation. All colonies were healthy and growing. The four colonies from G2 and 1D that suffered the high initial losses averaged 7.5 frames of bees with a brood nest of more than 3 frames. Unaffected colonies averaged 12 frames covered with bees. All colonies were queen right and all stages of brood were present with brood nests of at least 4 frames in the colonies that did not suffer the bee losses. All hives had increased honey stores and were free of pests and diseases.

Pesticide, Pest, and Disease Analysis of Dead and Dying Bees

Bee samples from sitesG2 and 1D were sent to NSL for broad spectrum pesticide analysis. We expected to see high levels of one or more pesticides in the dead bees. The results showed residues of only two pesticides, atrazine an herbicide, and the insecticide Clothianidin (Table 2).

Table 2. Pesticide Residues (ppb) in Dead and Dying Bees From the Pre-Planting Mortality Incident at G2 and 1D.

Site	Time Period	Detected Insecticides	
Code	Pre-Planting	Clothianidin	Atrazine
G2	√	26.3	30.5
1D	√	8.5	37.5

Subsamples of these bees were also screened for bee pests and diseases. The varroa mite and Nosema results (Table 3) revealed 1 mite in one sample, and 2.6M spores of Nosema in one of the four affected colonies, an amount below recommended treatment thresholds.

Table 3. Varroa mite and Nosema spore counts in Dead and Dying Bees from Pre-Planting Mortality Incident.

Bee Pests and Pathogens in Colonies Affected by Mortality Incident			
Site	Pre-Planting Period		
Code	Colony	Varroa Count	Nosema Count
G2	1	1	N.D.
G2	2	N.D.	2.6 M
1D	1	N.D.	N.D.
1D	2	N.D.	N.D.

Virus Results

Samples of dead and dying bees collected from the four colonies at sites G2 and 1D were submitted to BVS, Inc. for IVDS virus analysis. The BVS report (Figure 8) indicated, as did our microscopic examinations, that the samples had low or no Nosema spore counts. Bees from one of the two colonies at G2 had relatively high Deformed Wing Virus (DWV) and Sacbrood Virus counts. The other three samples had similar viral counts at mid- to high-levels. BVS reported that the sample with high DWV is not typical, and that the other three samples had higher than usual counts for the time of year of collection. The report also notes that the colony with the highest DWV also had the lowest bee weight. A complete BVS laboratory report has been included on the JumpDrive mailed to CDRC.

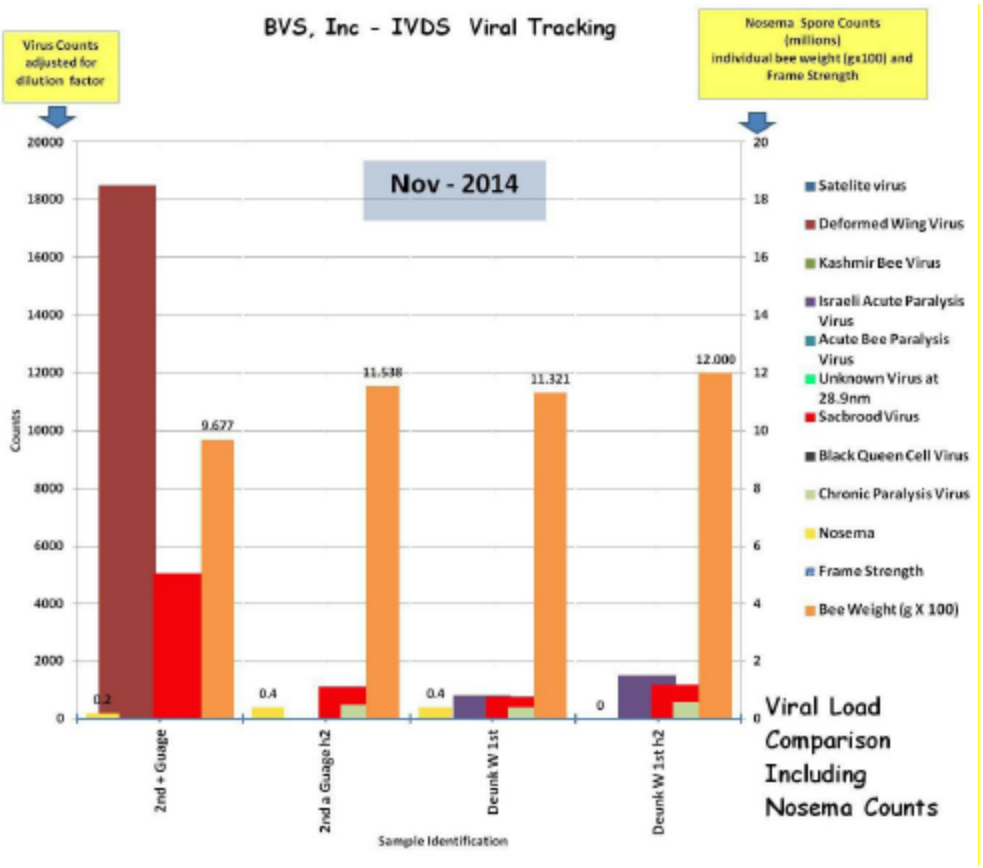


Figure 8. November 2014 report for IVDS Nosema and bee virus analysis with counts of viral prevalence for samples of dead and dying bees collected at sites G2 and 1D, April2014.

Pollen collection

Pollen collection began on 15 April and continued at intervals through the duration of the study. The last collection occurred on 19 May, three days before colonies were removed from the study site. All collections occurred over a 48 hour period. Samples were bagged separately and immediately refrigerated in a portable DC cooler that maintained temperatures below 32° F. Samples were kept in this cooler until shipment, packed on dry ice, to the lab at Missoula, MT.

Midway through the collection period, transit spikes of pollen collected from colonies in the research apiary at Missoula were prepared and shipped to Nebraska where they were placed with the pollen samples. Spikes were prepared to a calculated target of 10 PPB Clothianidin concentration using technical grade material containing 99.2 % A.I. Two spikes and a blank were shipped to Nebraska and a second set of two spikes and blank were kept in the freezer at Missoula.

Ninety pollen samples were collected during the course of the study (Figure 9). The two sites that experienced the large bee kill, 1D and G2, collected less pollen than half the other sites, but were within the range of variation we observed across all sites. Interestingly, pollen collection peaked during the planting period and tapered again post-planting, possibly due to the onset of heavy rains as the study concluded. A Table with Cumulative and Mean Values appears in the attached Powerpoint File.

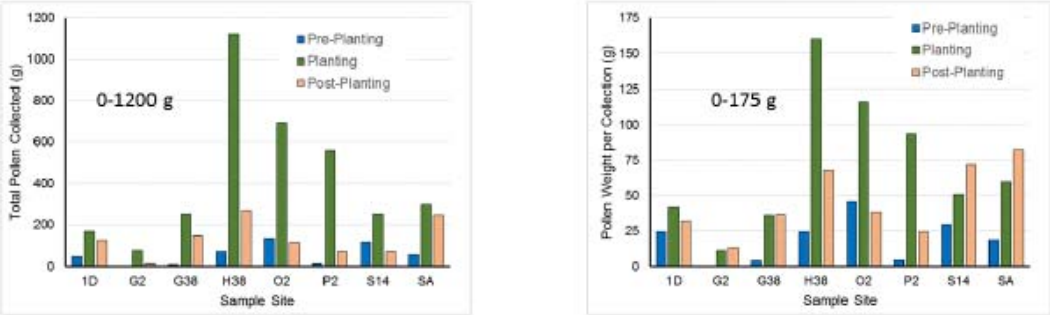


Figure 9. Amount of Bee-Collected Pollen (Total versus Mean for Collection Period).

Residues of Neonicotinoids in Bee-Collected Pollen

Results of chemical analysis for pesticides conducted by NSL appear in Figures 10-11 and Tables 4-6. Unexpectedly high levels of Clothianidin occurred in Pre-Planting pollen samples at three sites, one of which (1D) exhibited a bee-mortality incident. No pollen was brought back to the hives at (G2), as evidenced by the pollen traps. Somewhat elevated levels of Clothianidin were observed for colonies at five of the eight study locations during the Planting period. No Clothianidin was detected in pollen Post-Planting. Low levels of imidacloprid and thiamethozam were observed in pollen at Sites SA and H38 during the Post-Planting period.

Figure 10. Analysis list of neonicotinoids and Clothianidin concentrations in bee-collected pollen by planting period.

Table 5. Other Neonicotinoid Residues Detected in Bee-Collected Pollen By Site and Collection Period.

Clothianidin in Bee-Collected Pollen (PPB)			
Site	Time Period		
Code	Pre-Planting	Planting	Post-Planting
S14	N.D.	N.D.	N.D.
O2	N.D.	30.0	N.D.
P2	N.D.	N.D.	N.D.
G2	—	37.1	N.D.
1D	103.0	N.D.	N.D.
SA	75.1	44.1	N.D.
G38	N.D.	26.8	N.D.
H38	137.0	11.8	N.D.

Table 6. Summary of Clothianidin Residue Concentrations in Bee-Collected Pollen with Site Classification Category Added.

Imidacloprid in Bee-Collected Pollen (PPB)				Thiamethoxam in Bee-Collected Pollen (PPB)			
Site	Time Period			Site	Time Period		
Code	Pre-Planting	Planting	Post-Planting	Code	Pre-Planting	Planting	Post-Planting
S14	N.D.	N.D.	N.D.	S14	N.D.	N.D.	N.D.
O2	N.D.	N.D.	N.D.	O2	N.D.	N.D.	N.D.
P2	N.D.	N.D.	N.D.	P2	N.D.	N.D.	N.D.
G2	—	N.D.	N.D.	G2	—	N.D.	N.D.
1D	N.D.	11.6	N.D.	1D	N.D.	N.D.	N.D.
SA	N.D.	N.D.	3.9	SA	N.D.	N.D.	6.6
G38	N.D.	N.D.	N.D.	G38	N.D.	N.D.	N.D.
H38	N.D.	N.D.	5.0	H38	N.D.	N.D.	9.8

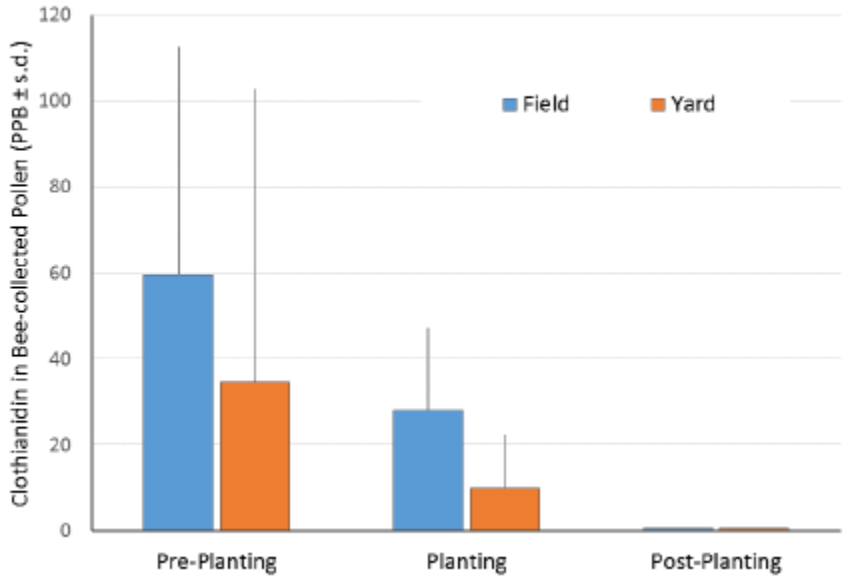


Figure 11. Means and Standard Deviations of Clothianidin in bee-collected pollen betweenField-Edge and Yarding Sites (numerical values are presented in Table in attached Powerpoint File).

Overall, the trend in Clothianidin chemicals residues is downward over time, with no detections in the Post-Planting interval. There was no statistical difference between Pre-Planting and Planting (Wilcoxon Signed-Ranks test, z = -1.214, P = 0.225), and Planting and Post-Planting (Wilcoxon Signed-Ranks, z = -1.604, P = 0.1090). “Yard” versus “Field-Edge” sites had lower chemical residues over the time periods. There was considerable variation; however, making the difference not significant (Wilcoxon Signed-Ranks, z = -0.943, P = 0.345 for yard versus field-edge colony placement).

A geo-spatial representation of the relative Clothianidin levels by Site and Planting Interval appears in Figures 12-14. The Pre-Planting Figure also shows locations of the mortality incidents.

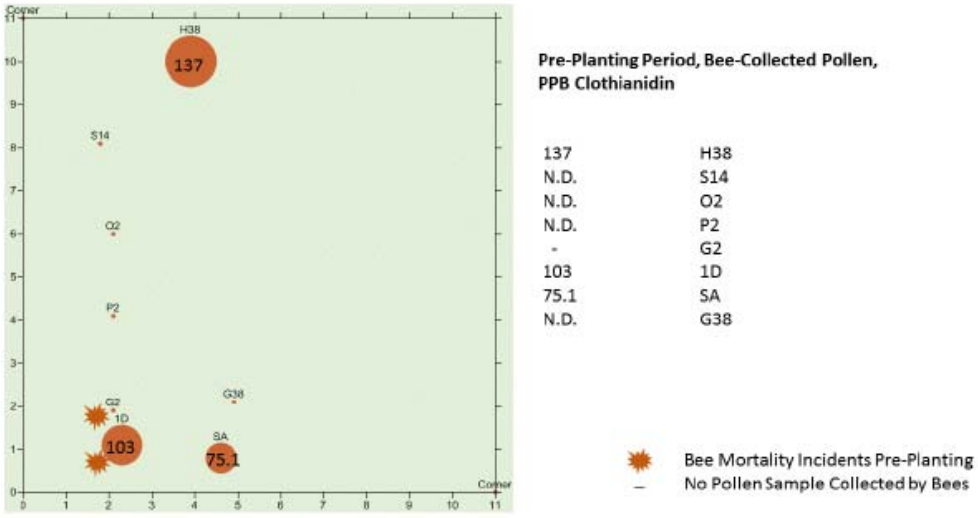


Figure 12. Bee Site Locations, Detected Clothianidin Levels, Locations of Bee Mortality Incident, Pre-Planting Period.

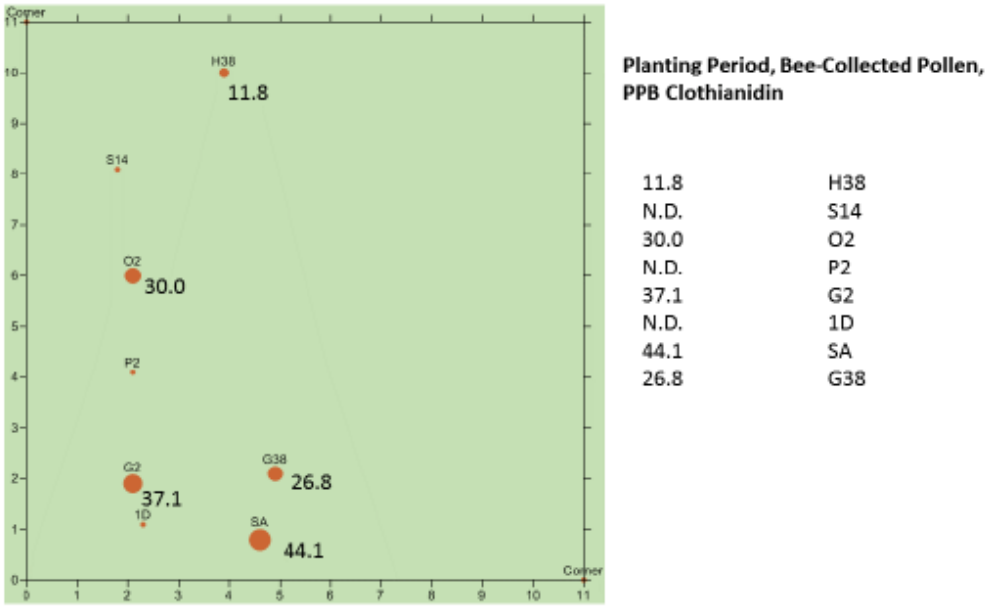


Figure 13. Bee Site Locations, Detected Clothianidin Levels, Planting Period.

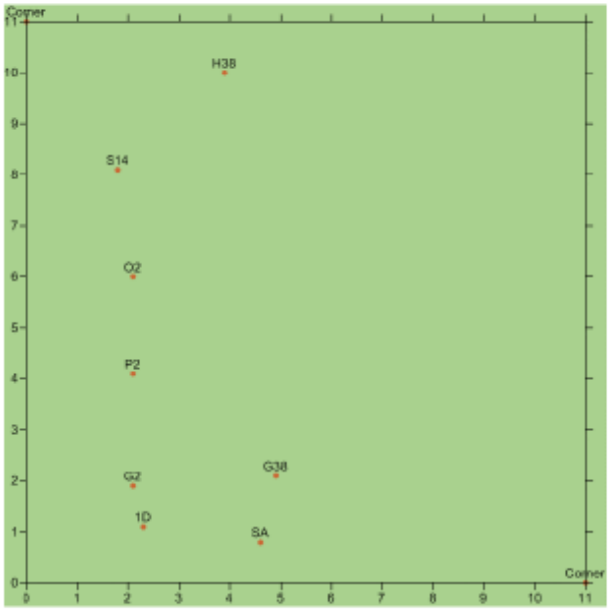


Figure 14. Bee Site Locations, Detected Clothianidin Levels, Post-Planting Period.

Habitat and floral resource use

We quantified relative areas of cultivated and natural areas in a 1 mile radius surrounding the study sites using GeoCam. With that data we are able to describe honey bee foraging behavior during the pre-planting and planting periods (Figure 15).

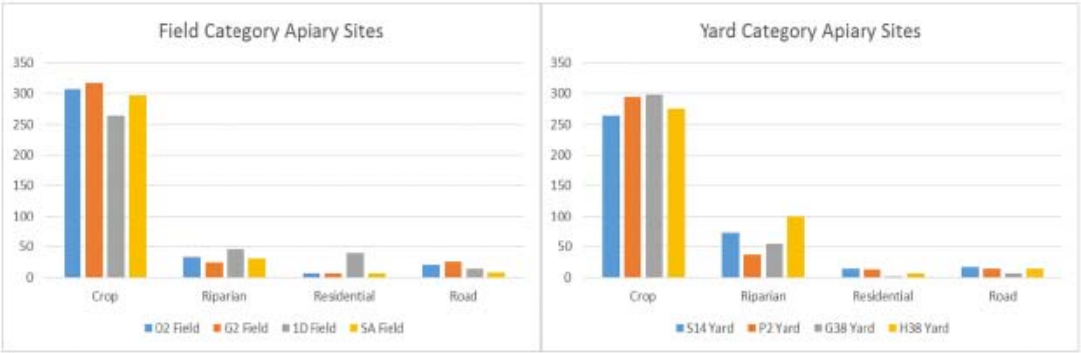


Figure 16. Relative Counts of Habitat and Land Use for Field-Edge and Yarding Sites. The Field-Edge sites had more cropland relative to other resource categories, and the Yarding Sites had more Riparian (tree/shrub) habitat. The largest residential area was closest to the Field Sites 1D and G2 and to the Yarding Site SA.

We compiled a list of floral and tree species in the vicinity of the study sites. Where possible, the pollens were keyed to genus. We constructed a pollen identification key for those species and used microscopic analysis, as described in the Materials and Methods, to assess subsamples of pollen samples to describe floral resource use.

Pollen Diversity Results

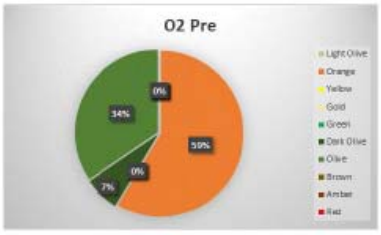
Mixed and composited pollen samples for each site and period were sampled, sorted and weighed by color, and analyzed for pollen diversity. The following pie charts represent the pollen percentage by weight for each site and period. The pollen color represents different flower types utilized by the bees and gives a view of the floral resources available during our study period. Site S14 showed a low diversity with only three different flower types being utilized over the entire period. Site O2 showed six types overall with the highest diversity during the planting period. Site P2 shows five types with the planting period diversity being highest. Site G2 shows low diversity with four types overall and the planting period being highest. Site 1D showed six types overall with the post period having the most diversity. Site SA showed the highest overall diversity with nine different types; the planting period having the highest with six different types. Site G38 had low floral diversity with four types total and the post period being the highest. Site H38 had seven types total with the post period being the highest.

Pollen identification down to the Genus level has begun and should be completed before the end of December. Preliminary identifications include the genera *Malus*, *Taraxacum*, *Ulmus*, *Melilotus*, *Acer*, and *Lamium*, with final verifications and identifications of the three remaining color categories pending. The results from this more in-depth identification could change our current results slightly but we do not expect to see any major changes.


S14 Pre
0%
20%
80%

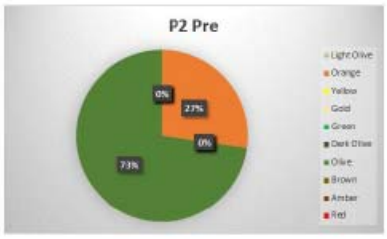

S14 Planting
0%
25%
0%
75%


S14 Post
0%
100%


O2 Pre
0%
34%
59%
0%
7%


O2 Planting
2%
28%
0%
62%


O2 Post
17%
67%


P2 Pre
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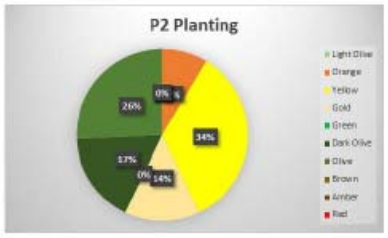
Gold
Green
Dark Olive
Olive
Brown
Amber
Red


P2 Planting
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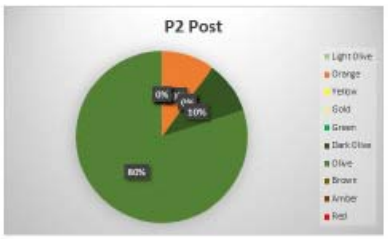
P2 Post
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Amber
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G2 Pre
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Amber
Red


G2 Planting
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Red


G2 Post
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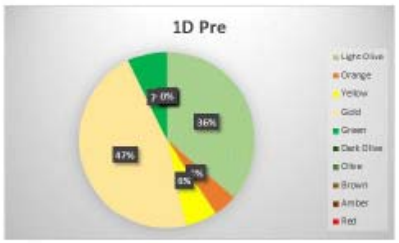
1D Pre
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Yellow
Gold
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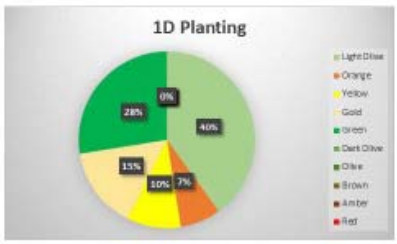
Olive
Brown
Amber
Red
0%
36%
47%
0%
0%


1D Planting
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Amber
Red
0%
40%
28%
15%
10%
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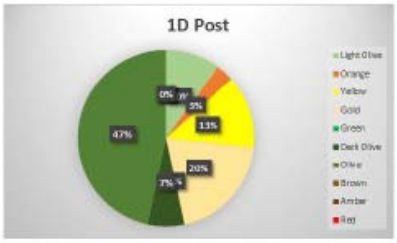
1D Post
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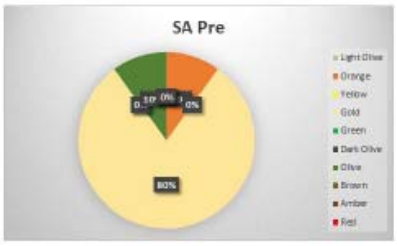
SA Pre
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Olive
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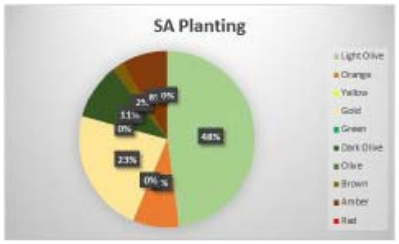
SA Planting
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Olive
Brown
Amber
Red
0%
48%
11%
0%
23%
0%

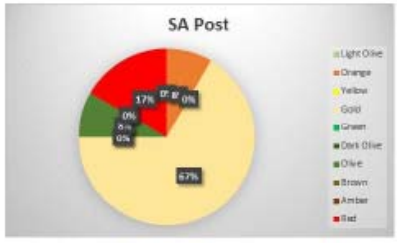

SA Post
Light Olive
Orange
Yellow
Gold
Green
Dark Olive
Olive
Brown
Amber
Red
0%
17%
0%
0%
67%

Figure 17. Pollen Diversity Based on Color and Weight of Bee-Collected Pollen Pellets by Site and Sample Period.

Dust samples

Early and late heavy rains complicated dust collection, but we accumulated 189 dust samples from the colony sites and habitat sites as outlined in our work plan. We consulted with the NSL analytical laboratory on best methods for extracting those samples and prepared them for chemical residue quantification as soon as we completed photographing and quantifying the dust deposition levels on the microscope slides. The chemical analysis results had not yet been received at the time of submission of this report. They will be forwarded when we receive them.

Dust deposition results are presented in Figures 16 and 17. The trend was for increasing dust levels from Pre-Planting through the Planting Period as the weather became warmer and drier, with some reduction during the Post-Planting period when rains resumed. Overall dust deposition was consistently higher for the Field-Edge sites versus the Yarding sites.

Overall, for any individual site, dust deposition was highly variable. The highest dust deposition value was observed at G2 during the Pre-Planting period, when a bee mortality incident was observed at this location.

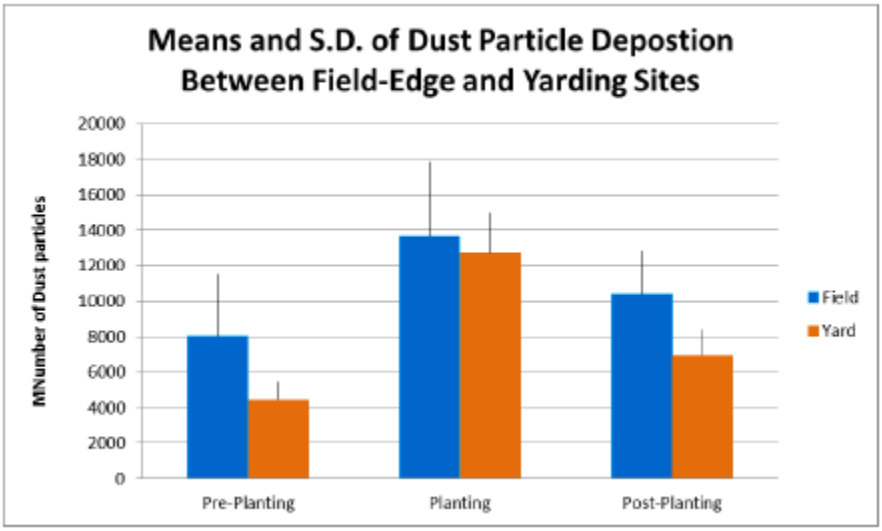


Figure 16. Means and Standard Deviations of Dust Deposition Levels by Site Category and Planting Period.

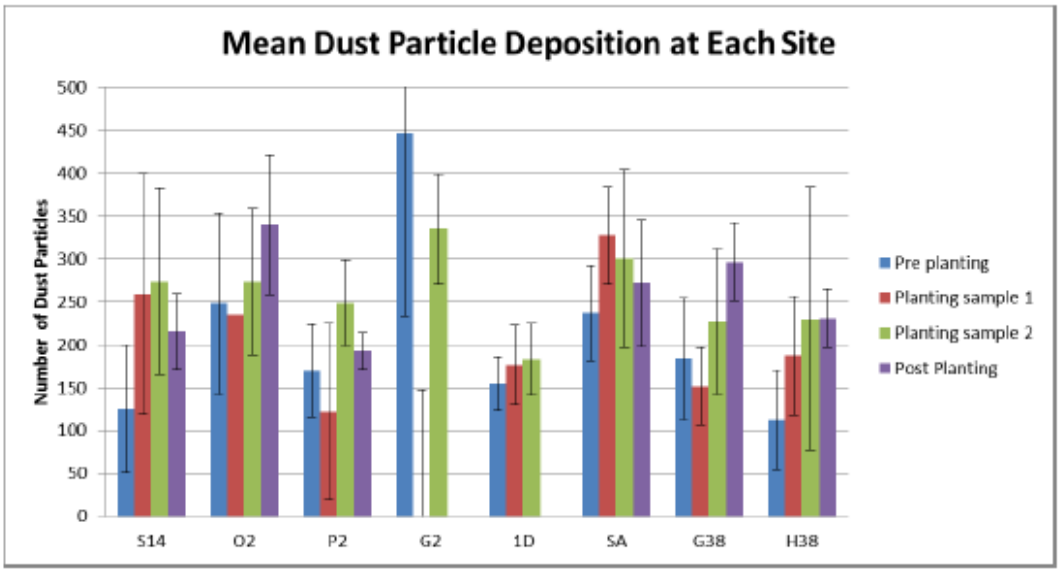


Figure 17. Overall summary of dust deposition. Planting occurred over two weeks, being interrupted by rain between Planting Period 1 and 2. The latter period was warmer and drier. The highest dust density incident occurred at G2 during Pre-Planting.

Discussion and Conclusions

Overall, this study yielded a substantial data set, with clear trends, and statistically significant results. We arrived and set up the colonies and sampling equipment well before corn planting and were able to collect samples and data as the planting season weather unfolded from cool conditions, with snow, changing to rain; then warm and sunny, and back to rain again, culminating with a tornado near the end of the post-planting period. Corn planting occurred in two phases over approximately two weeks. Planting was initiated as soon as soil temperatures reached the planting threshold, but was interrupted by several days of rain. The second week of planting was markedly warmer and dryer.

An unexpected result was an obvious bee mortality incident affecting the colonies at G2 and 1D during the Pre-Planting period. The bee loss was severe enough that dead and dying bees were found in the traps used to collect these bees and on the bottom boards and at the entrances of the hives. The queens and the colonies survived, nearly reaching the size of unaffected colonies by the end of the study period.

Dust deposition levels at G2 during Pre-Planting were higher than for any other site, but dust deposition levels at 1D were amongst the lowest for any site across all periods. The only sample of bee-collected pollen that we were unable to collect was during the Pre-Planting period at 1D – the colonies did not bring back any pollen until planting began. In fact, due to cool weather and snow, many of the colonies only began collecting pollen just before corn planting began.

Chemical analysis of the dead and dying bees from these two sites revealed the presence of only two detectable pesticides, Atrazine, an herbicide, and Clothianidin. Unexpectedly, the Clothianidin levels in honey bees were only 8.5 (1D) and 26.3 (G2) ppb. The Atrazine concentrations were higher at 37.5 (1D) and 30.5 (G2) ppb. Atrazine is reportedly non-toxic to bees, although we found citations that suggest a synergism with insecticides affecting aquatic insects – but no evidence that the same was true for terrestrial insects. Given the severity of the bee loss at these two sites, we expected to see higher levels of Clothianidin.

Given these results, we examined the bees for the presence of varroa mites and Nosema spores, but only found one mite in one colony at G2, and 2.6 M spores of Nosema in another colony at G2. Both were below recommended treatment threshold levels. The viral results revealed what the BVS laboratory reported as mid- to high-level viral loads of Deformed Wing Virus (DWV) and Sacbrood, with one of the colonies at G2 exhibiting atypically high levels of DWV.

Chemical analysis-collected pollen for neonicotinoid insecticides revealed exposure to 103 ppb of Clothianidin at 1D. Since the bees did not bring back any pollen at G2, we don't have any residue results for that site for the Pre-Planting period. The pollen from two other sites showed exposure to Clothianidin at 75.1 ppb at SA and 137 ppb at H38, but there was no evidence of a mortality incident at these two sites.

During the Planting period, bee-collected pollen exhibited detectable ppb levels of Clothianidin, at 11.8 (H38), 26.8 (G38), 30.0 (O2), and 44.1 (SA). The two sites exhibiting excess bee mortality during Pre-Planting had 37.1 (G2) and no detection (1D) of Clothianidin. Bee-collected pollen from the three other sites had no detectable amounts of Clothianidin. These exposure levels were just above, and in one case (H38) below the reported No Observed Adverse Effects = 20 ppb, reported by Schmuck & Keppler, 2003, Bayer- Pflanzenschutznachrichten 56: 26-58)

In our previous Nebraska study, we sampled bee-collected pollen from 30 fields, three times over, during the corn tasseling period. All of the corn was grown from seed treated with Poncho 1250. Our results (presented to Society of Environmental Toxicology and

Chemistry North America - November 21, 2013) yielded a maximum of 4.0 ppb, a mean of 1.2 ppb, with 23% of the pollen samples below the limit of detection for Clothianidin. These results appear to be consistent with the non-detection of Clothianidin in any of the beecollected pollen samples post-planting for our current study. Once planting is finished, overall exposure levels to Clothianidin, even during the tasseling period, are likely to be low.

We should note however, that whereas during the Post-Planting period, none of the beecollected pollen had any detectable Clothianidin, two sites displayed ppb level exposure to Imidacloprid (3.9 at SA, 5.0 at H38) and to Thiamethoxam (6.6 at SA, 9.8 at H38).

Our study design separated our colony placement sites into two categories: 1) Four sites were fallow margins of fields scheduled for corn planting (termed "Field" sites), and 2) Four sites (termed "Yard or Yarding" sites) were associated with less cultivated areas with pasture, natural cover (mainly riparian), and water judged sufficient to sustain a typical apiary of 50-100 colonies.

The data (Figure 15) confirmed that the Field sites tended to have more cropland with less trees and shrubs than the Yarding Sites. In addition, the Field sites consistently demonstrated a trend towards higher dust deposition for all sampling periods (Figure 16).

Statistical analysis showed a significantly higher bee mean mortality rate for the Field sites.This finding seems to reflect the higher dust deposition levels for Field sites (Figure 16) and a consistent trend of higher Clothianidin levels in bee-collected pollen at the Field sites, although due to considerable variation in the chemical exposure levels, the trend was not statistically different. We also noted that the two sites affected by the Pre-Planting mortality event were amongst the lowest of the sites in terms of the amount of pollen collected over the study period.

Pollen diversity results exhibit striking differences by site and sample period. Once we've verified the final pollen identifications, we will more closely examine the pollen results to see if there is any correlation between plant genera and detectable Clothianidin or similarities or differences between Field-Edge versus Yarding sites in terms of pollen diversity. We will then provide an update to CDRC.

What is not readily apparent from the all of the data is why the bee colonies at SA and H38, which experienced exposure to Clothianidin in bee-collected pollen at levels considerably above the NOAEL for honey bees, did not exhibit any obvious bee mortality. Nor is it clear how the colonies at any of the sites experiencing relatively high exposures to Clothianidin were exposed to the chemical before planting commenced. With respect to the two sites demonstrating a mortality incident, there was also evidence of elevated virus levels.

Whether this contributed to the bee loss or was exacerbated by exposure to Atrazine and/or Clothianidin is unknown.

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 nor 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%.

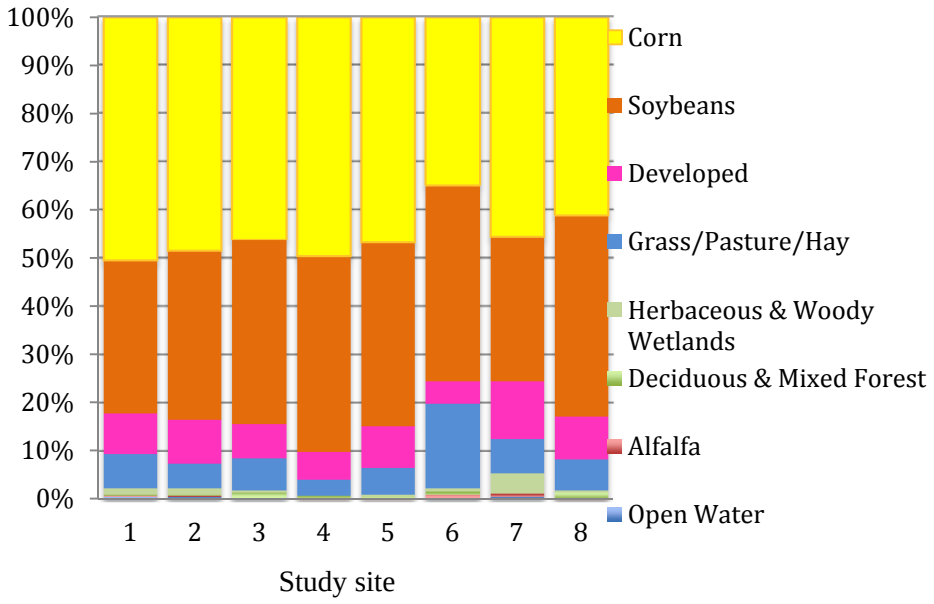


Table 2. Percent landscape cover types potentially providing honeybee forage at each study site. Totals of potential forage cover types ranged between 6 and 12% at all sites except site 7 where these types covered 21% of the landscape.

Site	1	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

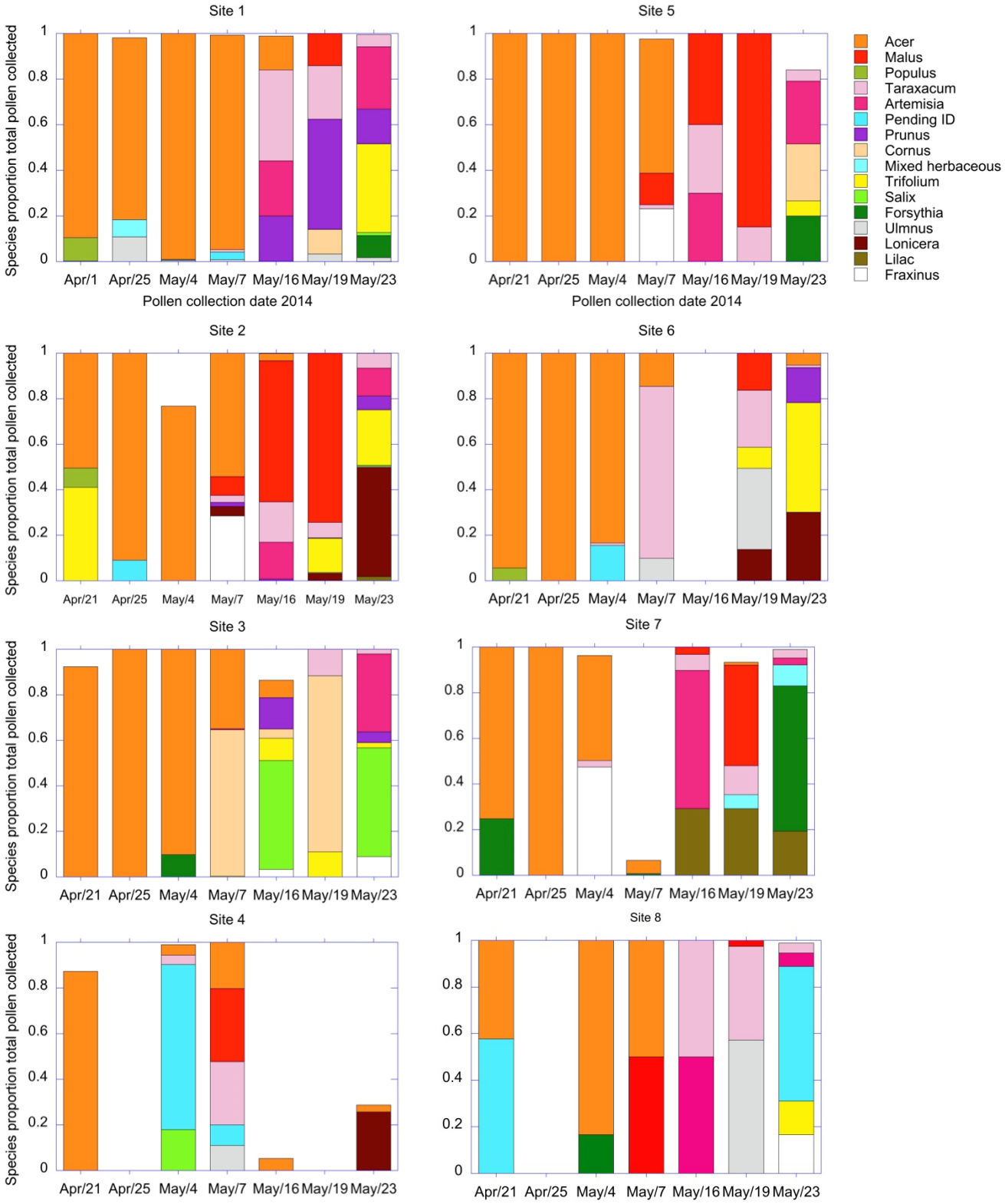


Figure 3. A comparison of pollen species proportions at each site across all pollen collection dates

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		

Results

Honey bee mortality and colony health

Collections from the drop-zone dead bee traps (after log transformation) were significantly different between sites ($p<0.001$) (Figure 7), and between corn-planting and non-corn-planting periods ($p<0.001$; 5/7-5/11 and 5/24-5/30), however, no interaction between site and corn planting was observed ($p=0.395$; Two-factor repeated measures ANOVA).

During the course of the observation two colonies at FSR were diagnosed with chalkbrood disease. One colony at TR went queenless and an egg-laying worker was identified and another colony was observed to suffer from high levels of mite infestation during the study period. The private beekeeper managing the TR site intervened to mitigate the problems and sampling at this site was discontinued on May 20.

Pollen identification

The floral sources of corbicular pollen were identified by pellet color (Figures 8, 9) and by traditional microscopic palynological methods (Erdtman et al. 1969). When available, 10 – 15 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. We subsampled 10 – 15 grams of pollen from each sample because this amount of pollen could be sorted in a reasonable length of time (1 – 3 hours per sample, depending on pollen diversity) while producing at least 1 gram of pollen (minimal requirement for pesticide analysis) for 2 – 3 most abundant pollen taxa in a sample. 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 floral source(s) associated with each color category were identified by comparing corbicular pollen with a reference pollen collection under a compound microscope at 400X – 1000X magnification. If a color class contained pollen of multiple taxa, we counted >1000 pollen grains of that color class to estimate the relative frequency of each taxon, and measured pollen grain dimensions of each taxon from 20 grains. We then used the frequency and grain size data to calculate the proportion of each taxon within a multi-floral color class using the volume formulas described in O'Rouke and Buchmann (1991). The final amount of pollen from each plant taxon, in grams, was determined for each site at each collection date and was corrected for color categories containing multiple taxa.

We have identified and quantified bee-collected pollen sampled during May 1 – 11, 2014 (Figure 10), which covers the period of widespread corn-planting activities in the study area (5/5-5/10; Table 3). The overall pollen sources utilized by bees during this period can be classified to three major types: (1) trees in the family *Rosaceae*, (2) other mass-flowering trees and shrubs, and (3) weedy herbaceous plants. Pollen of rosaceous trees such as cherry (*Prunus* spp.), apple and crabapple (*Malus* spp.), serviceberry (*Amelanchier* spp.) and hawthorn (*Crataegus* spp.) was most abundant, comprising 31 - 80% of the total sample for each site and date. Bees collected pollen from other mass-flowering trees and shrubs increasingly after May 7. However, the quantity of pollen originating from non-rosaceous woody plants, including willow *(Salix* spp.), ash (*Fraxinus* spp.), oak (*Quercus* spp.), and honeysuckle (*Lonicera* spp.), differed greatly among

sites (Figure 11), reflecting the variation in plant assemblages in the apiaries' surrounding landscape. Pollen originated from herbaceous plants was abundant on the earlier sample dates and consisted of primarily dandelion (*Taraxacum officinale*) and very small amount of mustards (family *Brassicaceae*) and other weeds such as *Lamium* spp. and *Ranuculus* spp.. The amount of dandelion pollen declined as hawthorn (*Crataegus* spp.) reached full bloom during the first week of May, even though dandelion flowers were still ubiquitous (Figure 12).

Table 3. Major pollen sources on May 7 – 11. The period during corn planting when corn seed treatment insecticides, clothianidin and thiamethxam, were detected in pollen samples. The two most abundant pollens types at each site are highlighted.

	Trees and shrubs					Herbaceous plants	
Site	**Rosaceae**	***Salix***	**Quercus**	**Fraxinus**	**other**	***Taraxacum***	**other**
WB	73.6%	12.7%	1.2%	7.1%	0.7%	3.9%	0.7%
FSR	76.1%	0.0%	12.4%	1.1%	8.2%	0.8%	1.4%
CH	82.5%	2.8%	0.5%	7.7%	3.9%	1.7%	0.8%
MB	73.0%	10.7%	1.0%	0.0%	4.5%	9.4%	1.4%
NC	40.9%	27.4%	8.2%	10.2%	5.7%	5.5%	1.8%
TR	50.3%	22.9%	4.8%	0.5%	11.9%	8.6%	0.9%
Overall	**65.4%**	**13.3%**	**4.6%**	**4.6%**	**5.7%**	**5.1%**	**1.2%**

Pesticide residue analysis in bulk pollen

Five grams of unsorted pollen from each site and date, 76 samples in total, were submitted to the USDA-AMS lab in Gastonia, NC for pesticide residue analysis of neonicotinoids and neonicotinoid metabolites (Appendix A). The corn seed treatment insecticides clothianidin and thiamethxam were significantly more likely to be detected in pollen during the major corn planting period (5/7-5/11) at levels as high as 21 ppb total neonicotinoid concentration ($p<0.001$, Fisher's Exact Test). No neonicotinoids were detected in pollen after this period of corn planting and only imidacloprid, which is not used as a seed treatment insecticide on corn, was detected prior to corn planting.

Dead bee trap catches (after log transformation) were significantly higher during periods when neonicotinoids were detected in bulk pollen ($p<0.001$, Repeated Measures ANOVA; Figure 7).

Pesticide residue analysis in sorted pollen

In samples collected between May 5 - 13, we isolated the pollen categories that made up over 85% of the total weight collected for each site and date. The sorted pollens were assigned to three groups, determined by habitat types and abundance: *Taraxacum*, rosaceous trees, and other woody species. One to five gram of the each sorted pollen group was submitted to the USDA-AMS lab for quantification of residues (Appendix B). For samples that contained abundant *Salix* pollen, an additional "*Salix*" group was isolated and submitted for analysis.

The frequency of seed treatment insecticide detections in different sorted pollen types (*Taraxacum*, roseaceous and woody pollen) collected during corn planting was not significantly different (p=0.1, Chi-Square Test, X-squared = 4.6, df=2). Pollen from roseaceous and woody species carried similar levels of insecticide contamination (p=0.12, t=-1.98, df=3.8, Welch's T-test), averaging 19.8 ppb.

There were two instances (FSR and CH on 5/11) where neonicotinoids were detected in sorted pollen (21.5 and 23.4 ppb, respectively) but no neonicotinoids were detected in the unsorted samples.

Pesticide residue analysis in bee bread and nectar

Bee bread and uncapped nectar were sampled from the experimental colonies at three different times (5/1 – 5/4, 5/16 – 5/19 for all sites, 6/1 – 6/3 for the OSU sites only). Three grams of homogenized bee bread were submitted to the USDA-AMS lab for analysis of neonicotinoid residues. Only one bee bread sample, collected from MB on May 3, was detected with clothiandin (15.7 ppb), which likely reflects exposure from sporadic corn planting in late April. Although insecticide residues were detected in bee-collected pollen during May 7 – May 11, the absence of insecticide resides in bee bread samples could be explained by a dilution effect. As bees store pollen in compacted layers, bee bread in a cell often contains a variety of pollen collected across different dates (Figure 13).

Approximately 10 g of nectar was collected from the three OSU sites on 5/4 and 5/16 – 5/19 were submitted for the analysis; no detectable levels of neonicotionoids were found in any nectar samples.

Molecular pollen analysis

Our application of ITS2 metabarcoding to pollen in 2013 was successful and we have submitted a manuscript based on last year's results to the journal "Applications in Plant Sciences". For 2014 we pooled pollen samples collected during corn planting (May 5th to May 11th) for each of the six sites and performed sequencing using the same methods with the exception of which barcodes were used. In 2013 we applied metabarcoding using only the ITS2, however, this year we used ITS2 along with the plastid barcodes *rbcL* and *matK.* This allows us to compare three different barcodes in terms of the scope of plant taxa which can be detected and the capacity to infer the relative proportions of pollen types within each sample. We have received final sequence data for this and are beginning our analysis. Our goals are to (1) test the robustness of the metabarcoding approach by comparing samples collected from multiple sites that vary in the abundance of common floral resources, and (2) investigate if any correlation exists between the number of plants identified by metabarcoding and the complexity of surrounding landscapes and (3) validate pollen identification performed using traditional microscopic palynology.

In a preliminary analysis of the sequence data for the site FSR, the ITS2, *matK* and *rbcL* produced alignments to 49, 60 and 196 genera, respectively. Collectively, alignments to 11 distinct plant genera were found across all three barcodes and alignments to 60 plant genera were detected using at least two of the three barcodes employed. Thus, the remaining 173 genera were found in only one of the three barcode libraries. Though many of the taxa being detected in only one library may have resulted from differences in the success of PCR amplification.

To test the ability to infer the relative abundance of different pollen types within the sample, we conducted the Spearmans' rank-based correlation test between the number of paired read alignments and the number of pollen grains per plant taxa, as described above. After conducting Spearmans' correlation tests for each barcode individually, we obtained *rho* correlation coefficients of 0.286, 0.717 and 0.667 for ITS2, *rbcL* and *matK*, respectively. Though none of these correlations were statistically significant, it is apparent that *rbcL* and *matK* are relatively more representative of pollen quantity than ITS2.

Conclusions:

- We observed a significant association between corn planting, the detection of corn seed treatment insecticides in bee-collected pollen, and an increased abundance of dead bees collected near the hive entrance across our 6 sites. These results suggest that insecticide exposure related to corn planting can have detectable effects on honey bee colonies in Ohio.
- Landscape factors are poorly predictive of colony exposure to seed treatment insecticides in pollen. While the area surrounding an apiary that is planted in corn correlates with the peak seed treatment insecticide concentration detected in pollen, this relationship appears to be relatively weak. Even at our least corn-intensive site, MB, with just 9% of the apiary's foraging area planted in corn, we still saw a notable increase in dead bees and the detection of seed treatment insecticides in collected pollen.
- Flower and pollen type is not predictive of colony exposure to seed treatment insecticides. Pollen collected from trees and shrubs, including the preferred roseaceous trees and shrubs, were often contaminated with seed treatment insecticides during corn planting. It is possible that herbaceous flowers could be more highly contaminated during the planting period, given their proximity to planting activity, but bees showed a clear preference for the woody species growing in residential and forested areas to such an extent that we have relatively little insecticide residue data from herbaceous flowers during this period.
- A sequencing approach to pollen identification shows great utility and the promise of semi-quantitative pollen identification using matK and rbcL barcode sequencing. These data will reveal the minor plant species that bees are foraging on during the corn planting period.
- We continue to have a poor understanding of how bees are exposed to corn seed treatment insecticides and how that exposure kills bees over the short-term or affects colony health over the long term. The validity of using average pollen concentrations to determine individual bee exposure is very much in doubt. We observed clear spikes in dead bee trap catches at bulk pollen concentrations as low as 6 ppb. It is unlikely that thiamethoxam or clothianidin evenly distributed in pollen with a mean concentration of 6

ppb could cause acute lethality. Instead, it suggests that bee-collected pollen contains localized insecticide concentrations that are very high, possibly individual seed coating particles embedded in the pollen. But these pockets of very high insecticide concentration are averaged out by the majority of relatively uncontaminated pollen. Some individual nurse bees consume these pockets of insecticide and are immediately poisoned, while most bees in the colony experience very low or no exposure to seed treatment insecticides.

Recommendations:

- Bee exposure to seed treatment insecticides, and the death of individual bees associated with that exposure, could be eliminated through the planting of corn seeds without an insecticide seed treatment. Alternatively, bee exposure could be reduced through use of lower rates of insecticide on corn seeds, improved seed lubricants or changes in planter design.
- Corn planting during the bloom of roseaceous trees and shrubs is not sufficient to protect individual bees from exposure to and death from seed treatment insecticides. Our results do not lend themselves to clear recommendations for changes in land management practices or timing of planting that could reduce bee exposure.
- Locating or moving bees to areas of less intensive corn production may be of limited benefit, but, based on our results, is unlikely to eliminate exposure of bees to corn seed treatment insecticides.
- Bee exposure to seed treatment insecticides occurs over a relatively short period of time -- a matter of a few days. Required notification of beekeepers that corn planting with insecticide-treated seed is about to begin could allow beekeepers to confine their colonies and eliminate foraging during period when collection of corn seed treatment insecticides is most likely.

Results

Abundance of Tree/shrub blossoms

During the period before corn planting, between 22 April and 10 May the most abundant blooms available in trees and shrubs observed were *Acer* spp. (40%), *Salix* spp. (21.2%), *Prunus* spp. (19.7%), and *Crateagus* spp. (12.0%). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Salix* spp. (49.1%) and *Acer* spp. (18.8%) followed by *Populus* spp. (9.4%) and *Rosaceae* spp. (8.4%).

During the corn planting period between 11 May and 3 June, the most abundant blooms in trees and shrubs were of *Prunus* spp. (28.2%), *Cornus* spp. (21.1%), *Crateagus* spp. (16.9%), and *Rosaceae* spp. (15.5%). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Rosaceae* spp. (40.7%), *Salix* spp. (19.3%), and *Rhamnus cathartica* (15.2%). After corn planting, beyond 3 June, the majority of bee-collected pollen from trees and shrubs was of *Rosaceae* spp. (8.5%) and *Salix* spp. (5.8%) (Fig. 4).

Abundance of herbaceous blossoms

The most abundant herbaceous blooms observed before and during corn planting were of *Taraxacum* spp. (66.3 and 64.3%, respectively) (dandelion), *Alliaria* spp. (24.7= and 28.6%, respectively) (garlic mustard), and *Viola* spp. (7.9 and 7.2%, respectively) (wild violet). *Taraxacum* spp., however, only comprised 1.5% of bee-collected pollen from associated bee hives during these sampling periods and no pollen from either *Alliaria* spp. or *Viola* spp. was found in bee-collected pollen.

During the pre-plant period 2.4 and 2.2% of bee-collected pollen was from plants within the *Brassicaceae* and *Liliaceae* families, respectively. During the corn planting period 17.2, 1.9, 1.6% of bee-collected pollen was from *Ambrosia* spp., *Brassicaceae* spp., and *Fragaria* spp., respectively. After planting, 58% of bee-collected pollen was from *Trifolium hybridum* and 12.3, 4.5, and 2.7% was from *Fabaceae* spp., *Vitus* spp., and *Brassicaceae* spp., respectively.

Abundance of other insect pollinators

Before planting, during the week of 4 -10 May, the most abundant pollinating Hymenoptera were *Halictidae*, *Ceratina* spp., *Apis mellifera*, and *Andrenidae*. After planting during the week of 18-24 May *Ceratina* spp., were the only Hymenopteran insects collected in sweep nets and from 25-31 May, 2.3, 2.0, and 1.0 *Apis mellifera*, *Halictidae*, and *Ceratina* spp. were collected per 10 sweeps, respectively; no Hymenoptera were collected in our samples from 1-7 June.

Before planting, during the week of 4 -10 May, the most abundant pollinating Diptera were *Sepsidae*, *Anthomyiidae*, *Scathophagidae*, and *Dolichopodidae*. After planting during the week of 18-24 May, *Anthomyiidae* , *Calliphoridae*, and *Scathophagidae* were the most abundant Dipteran insects collected in sweep nets. From 25-31 May, *Calliphoridae*, *Scathophagidae*, and

Chamaemyiidae were the most abundant Diptera, and from 1-7 June *Anthomyiidae* and *Syrphidae* were most abundant.

Diurnal activity of honey bees on dandelion

Honey bees from the study hive were found foraging on dandelions when temperatures were above 12°C and blooms were open. Foraging in this case seemed to be triggered by a combination of temperature and sunlight, perhaps at the hive level, where the sun and ambient temperature warms the hive. During this same period bees were observed travelling back and forth to the bush and taking water from a nearby ditch which contained less than 0.5 ng/mL total neonicotinoid insecticide residue. We could not distinguish whether bees, captured on dandelions were also taking water and/or foraging in the bush or elsewhere. Bee-collected pollen taken on two sample dates around the day of observation did not contain dandelion pollen, suggesting that dandelion is not a favored pollen source, but perhaps a useful source of nectar.

Honey bees foraging dandelions growing in corn stubble fields

Bees were observed foraging on dandelions at several locations. Bees seemed to avoid the dandelions on the way out of the hive, and it appeared that bees coming back from foraging elsewhere were stopping in on the dandelions. These bees were observed landing and foraging on the central portion of the dandelion blooms where the disk florets are located. Dandelion pollen was only a minor constituent (<0.5%) of the overall pollen collected by bees, suggesting that dandelion seems to serve as a nectar rather than pollen source during this time.

Neonicotinoid residues found in or on dandelion blooms did not seem to correlate with concentrations found in the soil in which the dandelions were. Neonicotinoid residue concentrations in soil samples collected around dandelions growing within the corn stubble fields were approximately 5-fold higher than those found in soil samples collected from those growing outside but adjacent to fields containing corn stubble.

Concentrations of residues on the surface and within dandelion flowers also did not relate well to concentrations found in or on the honey bee samples. Finally, there was no difference in neonicotinoid concentration measured within or on the surface of dandelions collected from either within the corn stubble field or in the non-crop land immediately adjacent. Although the sample size was small, residues found in/on dandelion may be more a function of leaf deposition of contaminated dust/drift than uptake from contaminated soil. Neonicotinoid concentrations determined on the surface of and inside honey bees captured on dandelion blooms from within corn stubble fields were similar for the St. Thomas and Rodney locations averaging about 0.125 and 0.06 ng/bee on the bee surface and inside the macerated body, respectively. This was despite a 5-fold higher concentration in the soil collected around the dandelions at Rodney compared with similar soil collected at St. Thomas; and oppositely, an approximate 3-fold greater concentration in dandelion blooms collected at the St. Thomas location.

Neonicotinoid residues in soils and soil dust in fields planted with corn

The mean neonicotinoid concentration found in the soil sampled to the 5 cm depth after planting was approximately 5 times that found in soil sampled immediately before planting.

The mean neonicotinoid concentration in surface soil dust generated by the action of simulated

planting was more than 17-fold greater than that found in the top 5 cm of soil from the same field prior to planting. These results were similar to those found in 2013.

Fugitive dust from corn fields during planting

Planter exhaust emitted 0.75 mg of neonicotinoid active ingredient per 100 m of row (single row) on average. The mean neonicotinoid concentrations captured on sticky traps during corn planting were 0.84 and 0.42 ng/cm2, for the downwind field edge and neighbouring field edges, respectively. These results were consistent with those found in 2013. Using volumetric air samplers, the mean neonicotinoid concentrations captured during corn planting at the downwind field edge and neighbouring field edges were 21.7 and 12.2 ng/m3, respectively.

For the majority of cases, neonicotinoid residue concentration decreased as the distance increased away from the field being planted. There were some anomalies at individual locations which 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 would have confounded some of our results.

Fugitive dust from corn fields during tillage before planting

Neonicotinoid residues measured using vertical sticky traps and volumetric air samplers downwind during tillage events were much lower than the residues collected during planting. (About 30% of those collected from corn planting on sticky traps and from 6 to 14% in the air samples). The concentration of neonicotinoids captured at the field edge by either method was similar to those at the neighboring field edge downwind.

Neonicotinoid residues in bee-collected pollen and dead bees at hives

The concentrations of neonicotinoids within bee-collected pollen before and during planting were significantly higher than after planting. Mean neonicotinoid concentrations in bee-collected pollen in 2014 were similar to those found in the 2013 study. No significant differences in neonicotinoid concentrations were found within or on the surface of dead honey bees among the three sampling periods in 2014. The mean total neonicotinoid residue (total of within and surface residues) of dead honey bees in 2014 was 01.42 ng/bee, similar to that measured in the 2013 study.

Neonicotinoid residue on and in blossoms foraged by honey bees

- **Trees and shrubs**

  The concentrations of neonicotinoids found on the surface and within flowers of *Salix* spp. were similar in variability. Within *Rosaceae* we found the neonicotinoid concentration on the flower surface and within flowers to be similar. The concentrations of neonicotinoids in the anther and pollen tissue of *Salix* spp. and *Rosaceae* were similar (0.39 and 0.37 ng/flower) however; these species cannot be directly compared due to differences in flower morphology.

  The concentration of neonicotinoids found on the surface of foraging honey bees was higher on bees collected from *Rosaceae* than on *Salix* spp. or *Acer* spp. Bees foraging

on *Rosaceae* were mainly collected during and after corn planting which corresponds with the proportion data of bee-collected pollen from the *Rosaceae* family. The neonicotinoid residue found on the surface of honey bees collected on *Salix* spp. and *Acer* spp. was generally low and less variable; these collections were made prior to corn planting. The concentration of neonicotinoids measured within honey bees across all tree species was consistently low (min=0.02, max=0.42, mean=0.20 ng/bee). The mean concentration of neonicotinoids measured on the surface of honey bees across all tree species was 1.60 ng/bee (min=0.75, max=3.00 ng/bee).

- **Herbaceous Plants**

  Greater variability in neonicotinoid concentration was found within herbaceous flowers than in tree flowers, most notably within the pollen and anther tissue. Surprisingly, lower levels of neonicotinoid residues were found on the surface of herbaceous flowers relative to what was measured inside the same blossom tissues. 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.

  Neonicotinoid residues found on the surface of foraging honey bees were more variable in bees collected from herbaceous plants than on those collected from trees (min=0.25, max=25.00, mean=2.94 ng/bee). Bee-collected pollen data show that foraging on *Meliotus* spp. and *Trifolium* spp. was typically after corn planting. The concentration found within bees was consistently low and similar to that found in bees collected from tree blossoms. The concentration of neonicotinoids measured within honey bees across all herbaceous plant species was consistently low (min=0.02, max=0.94, mean=0.16 ng/bee) and similar to that in bees collected from dandelions (min=0.02, max=0.09, mean=0.09 ng/bee).

  There was a weak positive correlation between the neonicotinoid concentration found in soil and in the anther and pollen tissue when data for all species sampled were pooled (R2=0.0249, p=0.0484). However when correlations were run independently by species we did not find a significant correlation. Combined, 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. In the case of herbaceous plants around corn fields, with the limited data presented, it was surprising to detect lower neonicotinoid residues on the surface of flowers relative to those within flower tissues which suggests that residue uptake by these plants plays a more significant role than simple residue deposition from fugitive dust during or after corn planting.

  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.

Determine floral resources used by honey bees in and around corn and soybean fields during the corn planting period

During the period before corn planting, between 22 April and 10 May, the most abundant blooms available in trees and shrubs observed were *Acer* spp. (40%), *Salix* spp. (21.2%), *Prunus* spp. (19.7%), and *Crateagus* spp. (12.0%) (Fig.4). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Salix* spp. (49.1%) and *Acer* spp. (18.8%) followed by *Populus* spp. (9.4%) and *Rosaceae* spp. (8.4%) (Fig.4).

During the planting period between 11 May and 3 June, the most abundant blooms in trees and shrubs were of *Prunus* spp. (28.2%), *Cornus* spp. (21.1%), *Crateagus* spp. (16.9%), and *Rosaceae* spp. (15.5%). During the same period, bee-collected pollen in associated pollen traps was comprised mainly of *Rosaceae* spp. (40.7%), *Salix* spp. (19.3%), and *Rhamnus cathartica* (15.2%) (Fig. 4). After planting, beyond 3 June, the majority of bee-collected pollen from trees and shrubs was of *Rosaceae* spp. (8.5%) and *Salix* spp. (5.8%) (Fig. 4).

The most abundant herbaceous blooms observed before and during corn planting were of *Taraxacum* spp. (66.3 and 64.3%, respectively) (dandelion), *Alliaria* spp. (24.7 and 28.6%, respectively) (garlic mustard), and *Viola* spp. (7.9 and 7.2%, respectively) (wild violet). *Taraxacum* spp., however, only comprised 1.5% of bee-collected pollen from associated beehives during these sampling periods and no pollen from either *Alliaria* spp. or *Viola* spp. was found in bee-collected pollen (Fig. 5). During the pre-plant period 2.4 and 2.2% of bee-collected pollen was from plants within the *Brassicaceae* and *Liliaceae* families, respectively (Fig. 5). During the corn planting period 17.2, 1.9, 1.6% of bee-collected pollen was from *Ambrosia* spp., *Brassicaceae* spp., and *Fragaria* spp., respectively (Fig. 5). After planting, 58% of bee-collected pollen was from *Trifolium hybridum* and 12.3, 4.5, and 2.7% was from *Fabaceae* spp., *Vitus* spp., and *Brassicaceae* spp., respectively (Fig. 5).

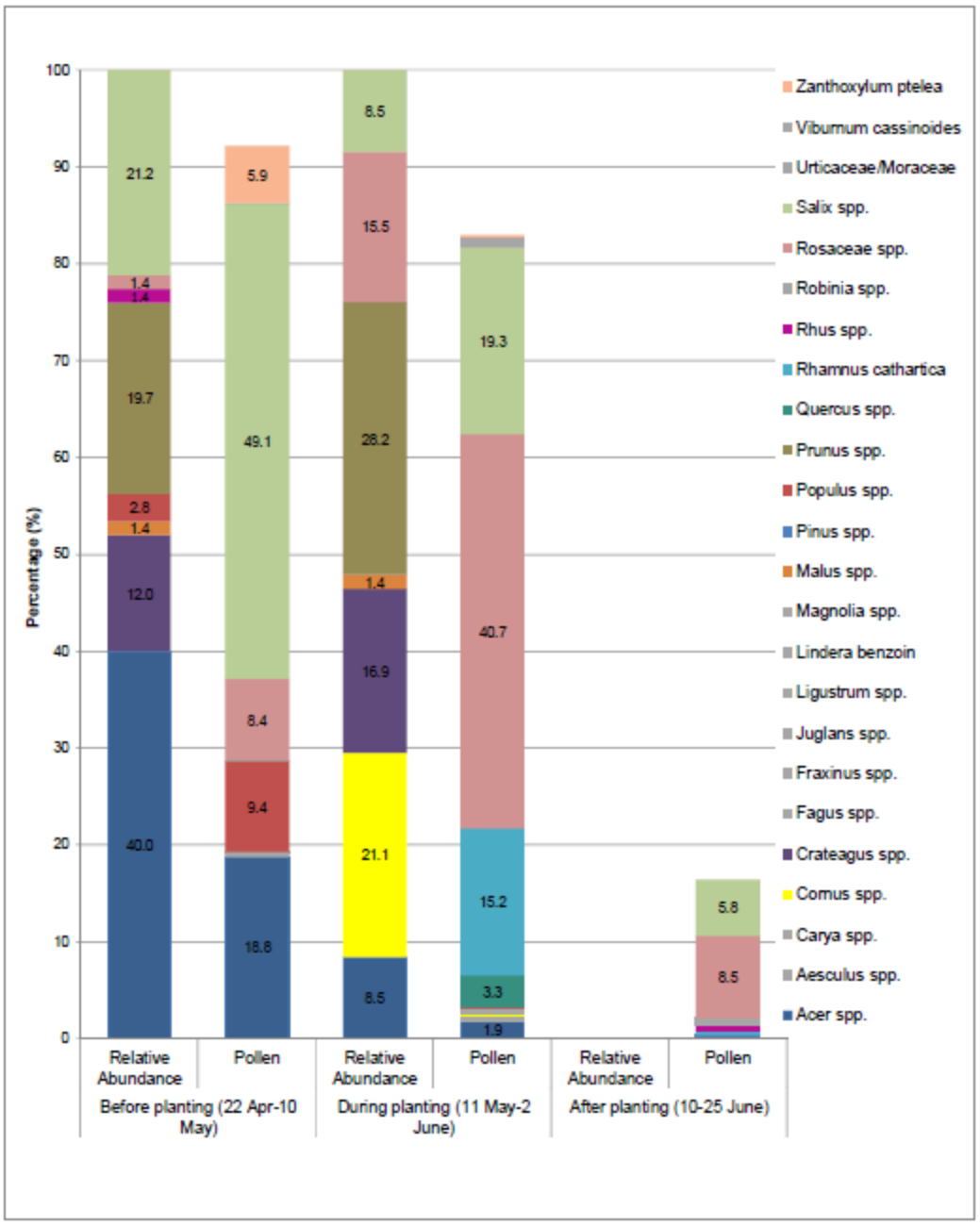


Figure 4. Relative abundance of trees and shrubs around the perimeter of corn and soybean fields studied in 2014 and their proportion in bee-collected pollen before, during and after corn planting in 2014.

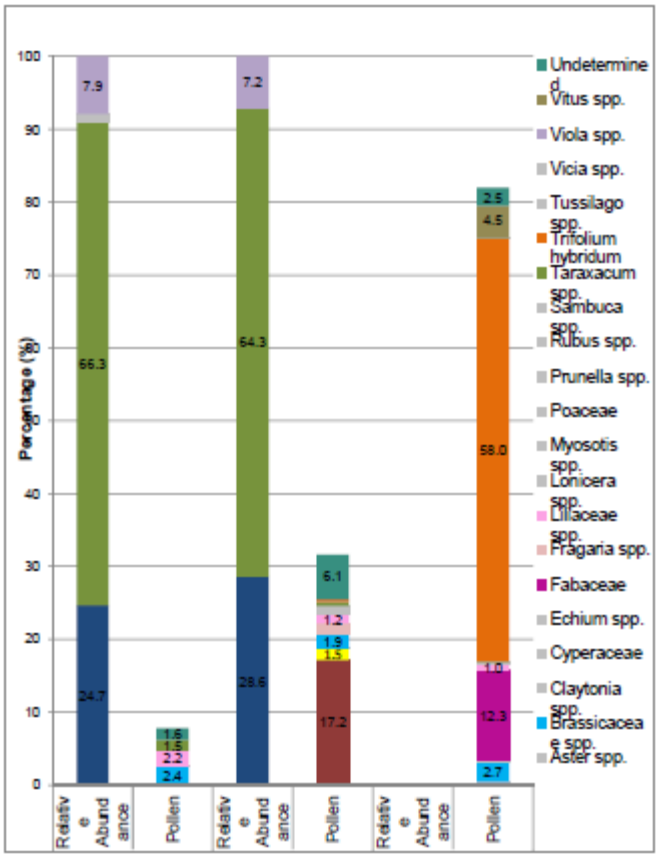


Figure 5. Relative abundance of herbaceous plants around the perimeter of corn and soybean fields studied in 2014 and their proportion in bee-collected pollen before, during and after corn planting in 2014.

Determine insect pollinators in and around corn and soybean fields during corn planting season

Before planting, during the week of 4 -10 May, the most abundant pollinating Hymenoptera were *Halictidae* spp., *Ceratina* spp., *Apis mellifera*, and *Andrenidae* spp. (Fig 6). After planting during the week of 18-24 May *Ceratina* spp., were the only Hymenopteran insects collected in sweep nets and from 25-31 May, 2.3, 2.0, and 1.0 *Apis mellifera*, *Halictidae* spp., and *Ceratina* spp. were collected per 10 sweeps, respectively; no Hymenoptera were collected in our samples from 1-7 June (Fig. 6). Before planting, during the week of 4 -10 May, the most abundant pollinating Diptera were *Sepsidae* spp., *Anthomyiidae* spp., *Scathophagidae* spp., and *Dolichopodidae* spp. (Fig 7). After planting during the week of 18-24 May, Anthomyiidae spp. , Calliphoridae spp., and *Scathophagidae* spp. were the most abundant Dipteran insects collected in sweep nets. From 25-31 May, *Calliphoridae* spp., *Scathophagidae* spp., and *Chamaemyiidae* spp. were the most abundant Diptera, and from 1-7 June *Anthomyiidae* spp. and *Syrphidae* spp. were most abundant (Fig. 7).

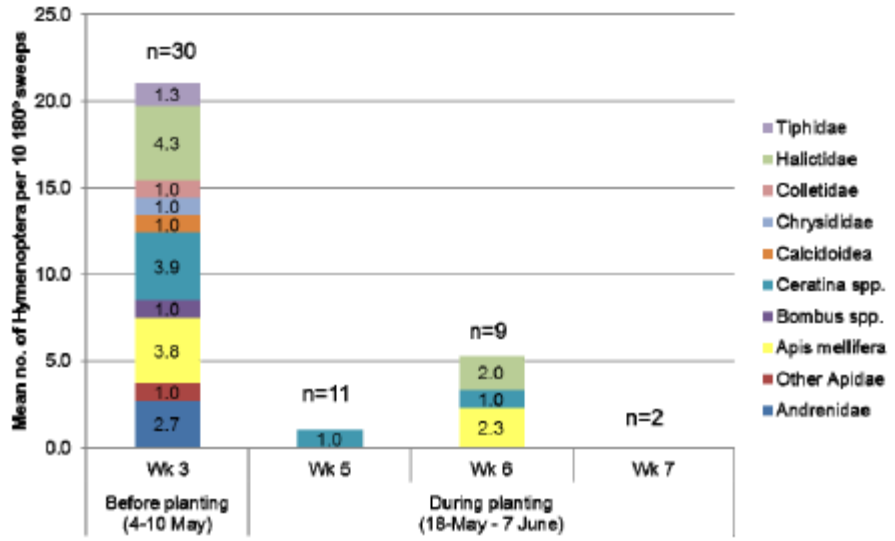


Figure 6. Abundance of Hymenopteran insects on low-lying vegetation around the perimeter of corn and soybean fields sampled using sweep nets before (4-10 May) and during (18 May – 7 June) corn planting in 2014.

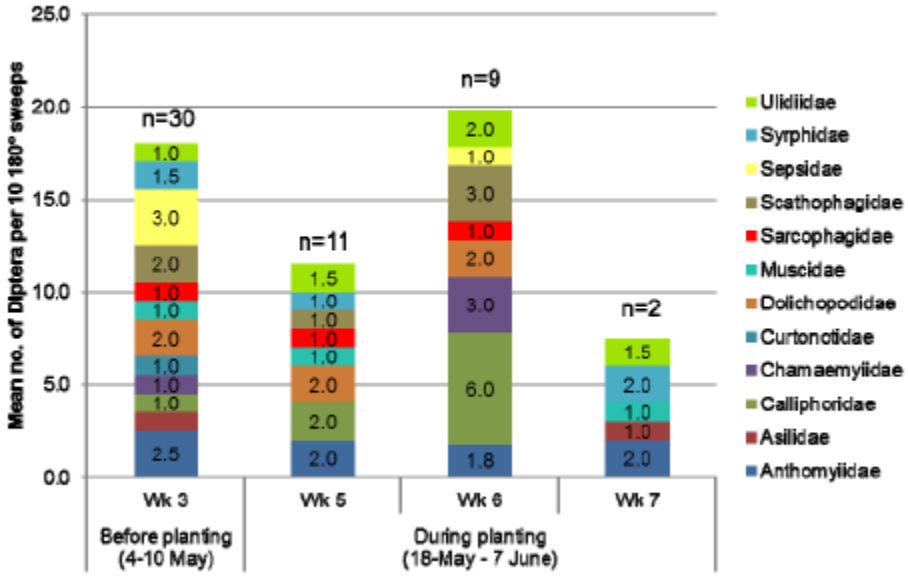


Figure 7. Abundance of Dipteran insects on low-lying vegetation around the perimeter of corn and soybean fields sampled using sweep nets before (4-10 May) and during (18 May – 7 June) corn planting in 2014.

Diurnal visits

Honey bees from the study hive were found foraging on dandelions when temperatures were above 12°C and blooms were open (Fig. 9). The day was cool but mainly sunny with light wind. Foraging began 3 hours after sunrise and slowed again approximately 3 hours before sunset.

During the latter part of the day, temperature was not limiting. Foraging in this case seemed to be triggered by a combination of temperature and sunlight, perhaps at the hive level, where the sun and ambient temperature warms the hive. During this same period bees were observed travelling back and forth to the bush and taking water from a nearby ditch (Fig. 8). We could not distinguish whether bees, captured on dandelions, were also taking water and/or foraging in the bush or elsewhere. Bee-collected pollen taken on two sample dates around the day of observation did not contain dandelion pollen, suggesting that dandelion is not a favored pollen source, but perhaps a useful source of nectar. The ditch water that the bees were drinking contained less than 0.5 ng/mL total neonicotinoid insecticide residues (Table 6). The sampling occurred before any crops had been planted within the foraging range of the subject hive. Crop planting was severely delayed due to the unusually cold and wet spring.

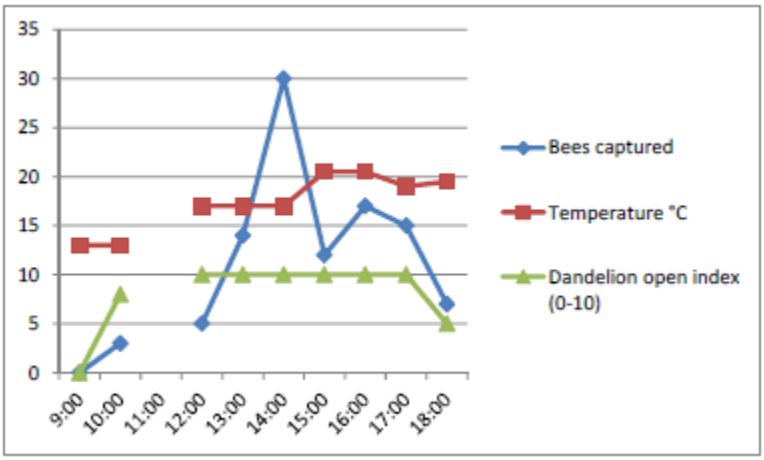


Figure 9. Kingsville ON. 23 May, 2014. Diurnal foraging of honey bees on dandelions. Sunrise and sunset occurred at 06:00 and 20:51 hr, respectively. All bees observed foraging/resting on dandelion were captured with a sweep net while walking a 200-m stretch up and down, along either side of a ditch bank along the field access road between bush and hives (see Fig. 8). Dandelion open index shows relative openness of blossoms in 10% increments where 10 = 100% open.

Table 5. Species composition of bee-collected pollen from samples taken from the hives on the two sampling dates nearest 23 May, the date of foraging bee sampling on dandelions. Kingsville ON, Spring 2014.

Identification	Pollen analysis on 500 grains (%)	
	9 May	28 May
Salix spp.	33.4	17.6
Rosaceae - Fruit trees type	31.4	27.6
Rosaceae (folded)	ND	26.4
Zanthoxylum - ptelea	25.4	ND
Brassicaceae	6.6	ND
Trifolium hybridum	9.6	89.4
Taraxacum spp.	ND	ND

Table 6. Concentration (ng/mL) of neonicotinoid insecticide residues in a ditch water sample from which honey bees were observed drinking on 23 May, 2014, Kingsville, ON.

	clothianidin	thiamethoxam	imidacloprid	thiacloprid	acetamiprid	dinotefuran	nitenpyram	metalochlor	atrazine	imazethapyr
LOQ	0.037±0.007	0.011±0.002	0.031±0.004	0.005±0.001	0.021±0.001	0.0510.004	0.1550.041	0.015±0.002	0.008±0.001	0.0060.0003
LOD	0.017±0.002	0.004±0.001	0.011±0.001	0.002±0.0004	0.007±0.001	0.019±0.001	0.0660±017	0.006±0.001	0.003±0.0004	0.002±0.0001
Ditch water	0.15	0.01	0.05	0.27	0.02	0.00	0.02	0.00	0.10	0.01

Honey bees, dandelions and corn stubble

At the St. Thomas site honey bees were actively traversing the corn stubble to the apple orchard. Bees were observed on both dandelions, which were plentiful, and on the apple blossoms in the adjacent orchard (Fig. 10). After careful observation, bees seemed to avoid the dandelions on the way out of the hive, and it appeared that bees coming back from foraging (most likely in the orchard) were stopping in on the dandelions. These bees were observed landing and foraging on the central portion of the dandelion blooms where the disk florets are located. From the bee-collected pollen data sampled from the commercial hive at the St. Thomas site on the three dates around the observation date, it was clear that dandelion pollen was only a minor constituent (<0.5%) of the overall pollen collected by bees at this hive (Table 9). This followed a similar pattern to that observed at the Kingsville site (section 5.1) suggesting further that dandelion seems to serve as a nectar rather than pollen source during this time.

Neonicotinoid residues found in or on dandelion blooms did not seem to correlate with concentrations found in the soil in which the dandelions were growing (Tables 7 and 8).

Neonicotinoid residue concentrations in samples collected around dandelions growing within the corn stubble fields were approximately 5-fold higher than those found in soil samples collected from those growing outside but adjacent to the corn stubble fields (Table 7). Concentrations of residues in and on dandelion blooms also did not relate well to concentrations found in or on the honey bee samples. Finally, there was no difference in neonicotinoid concentration measured in or on dandelions collected from either within the corn stubble field or in the non-crop land immediately adjacent. Although the sample size is small, residues found in/on dandelion may be a more a function of leaf deposition of contaminated dust/drift than uptake from contaminated soil. These samples are part of a larger data set which is discussed in section 8.

The St. Thomas location had the highest concentration of neonicotinoid residues in/on dandelions of the four sites visited perhaps as a result of its location adjacent to a commercial apple orchard and near other more intensively managed crops (i.e. perhaps greater use of neonicotinoid insecticides). The other three sites were similar to one another in that they had similar surrounding agricultural landscapes.

Finally, neonicotinoid concentrations determined on the surface of and in honey bees captured on dandelion blooms from within corn stubble fields were similar for the St. Thomas and Rodney locations averaging about 0.125 and 0.06 ng/bee on the bee surface and inside the macerated body, respectively (Table 7). This was despite a 5-fold greater concentration in the soil collected around the dandelions at Rodney compared with similar soil collected at St. Thomas; and oppositely, an approximate 3- fold greater concentration in dandelion blooms collected at the St. Thomas location.

Table 7. Mean neonicotinoid residues on the surface and within dandelion flowers and disc florets, foraging honey bees and soil collected in corn stubble from 2013 crop. Samples were collected 24, 25 May 2014.

Location	Dandelion (ng/flower*)				Honey bees (ng/bee)			Soil
	Surface	Within	Disk Florets	Total	Surface	Inside	Total	(ng/g)
Rodney	3.17	0.15	1.20	4.52	0.10	0.07	0.17	5.87
St. Thomas	4.43	3.60	6.38	14.40	0.15	0.05	0.20	1.08

*mean surface area and fresh weight of one complete dandelion flower are 90.2 cm^2 and 0.67 g, respectively. The mean combined fresh weight of disc florets for one dandelion bloom was approximately 0.35 g.

Table 8. Mean neonicotinoid residues on the surface and within dandelion flowers and disc florets and soil collected with and outside of fields containing corn stubble from the 2013 crop. Samples collected 24 and 25 May 2014.

Location	Soil (ng/g)		Dandelion* (ng/flower) in corn stubble field			Dandelion* (ng/flower) outside field		
	In field	Outside field	Surface	Inside	Anthers	Surface	Inside	Anthers
Dutton	5.95	0.57	0.37	0.17	0.67	0.26	0.32	0.95
Fingal	5.22	1.02	0.44	0.20	1.33	1.98	0.75	0.75
Rodney	5.87	0.39	3.17	0.15	1.20	0.40	0.00	0.40

*mean surface area and fresh weight of one complete dandelion flower are 90.2 cm^2 and 0.67 g, respectively, the fresh weight of anthers of one dandelion is 0.32 g.

Table 9. Species composition of bee-collected pollen from samples taken from the hives at the same site on the two sampling dates nearest the date of the foraging bee sampling on dandelions St. Thomas, ON. 24 May 2014.

Identification	**Pollen analysis on 500 grains (%)**		
	10 May	30 May	12 Jun
Salix spp.	77.2	4.0	ND
Acer negundo	15.2	ND	ND
Brassicaceae	3.4	ND	ND
Liliaceae / Iridaceae	1.2	ND	ND
Brassicaceae (other)	1.2	16.6	ND
Rosaceae - Fruit trees type	ND	9.4	43.4
Rhamnus cathartica	ND	51.6	ND
Urticaceae / Moraceae	ND	5.4	ND
Juglans spp.	ND	ND	1.6
Vitis spp.	ND	ND	20.0
Trifolium hybridum	ND	ND	15.0
Rubus spp.	ND	ND	8.2
Liliaceae / Iridaceae	ND	ND	4.4
Rosaceae (folded)	ND	ND	3.2
Taraxacum spp.	0.2	0.4	ND

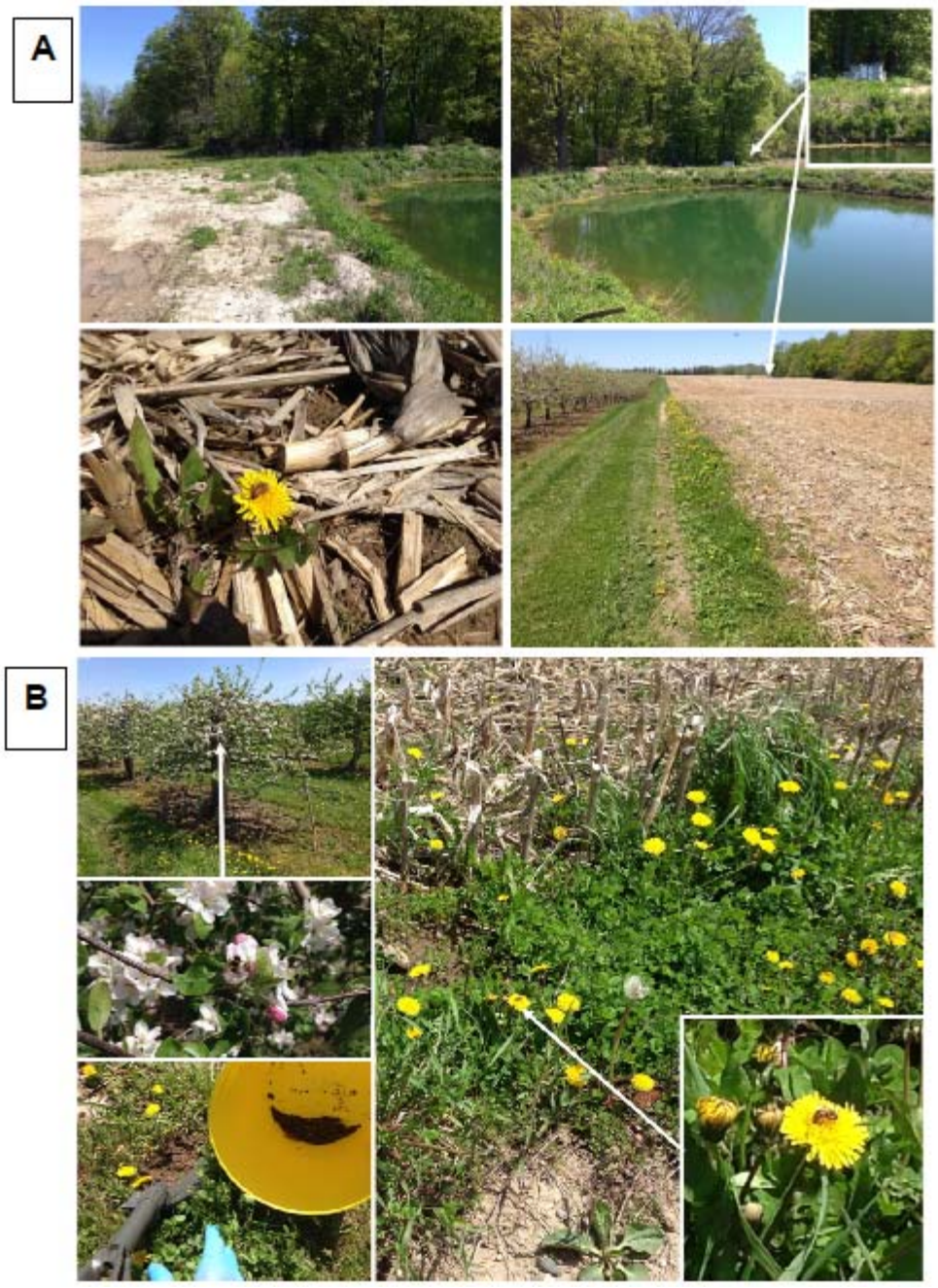


Figure 10. St. Thomas, ON, May 24 2014. Honey bees foraging on dandelion and apple blossoms, adjacent to farmer-cooperator corn field. Collage A shows location of commercial hives relative to corn stubble field and apple orchard. Bottom right inset in A faces west. The top insets in A all face north. Many bees travelling back from apple blossoms stopped to forage on dandelions for what was most likely nectar. Bottom left inset in Collage B shows soil and dandelion sampling.

Figure 11. Dandelions and honey bees in corn stubble. Top collage: Rodney ON. 24, 25 May 2014. Foraging bees on dandelions in corn stubble in region where no corn had been planted within at least 10 km radius due to cool, wet spring; corn planted in 2013 was treated with neonicotinoid at 0.25 mg a.i./seed. (Main photo faces east, left inset also faces east showing the ditch bank between highway and field (sampling location for dandelions and soil outside field). Top right inset faces south showing west side of field). Dandelions sampled from within the field were taken at random areas that clearly had rows of corn stalks. (Lower right inset shows one of the foraging bees sampled from dandelion in the stubble field). Bottom left: Fingal, bottom right: Dutton ON. 24 May, 2014. No honey bees sampled, dandelions and soil sampled in corn stubble and in field perimeter (outside of stubble field).

Fugitive dust escaping corn fields during planting

The mean neonicotinoid concentration found in the soil sampled to the 5 cm depth after planting was approximately 5 times that found in soil sampled immediately before planting ($F_{1,9}=18.29$, $p = 0.0021$) (Table 10).

Table 10. Neonicotinoid concentration in pre- and post-plant soil in corn fields studied in 2014.

Soil	Mean neonicotinoid concentration (ng/g)	SE
Pre-plant soil	6.1 a[1]	1.50
Post-plant soil	30.4 b	10.25

[1]Means within columns followed by the same letter do not significantly differ ($P < 0.05$, LSD) as determined by ANOVA and Fisher's Protected LSD.

Neonicotinoid residues in pre-plant surface soil dust produced by the mechanical action of planter units

The mean neonicotinoid concentration in surface soil dust generated by the action of simulated planting was more than 17-fold greater than that found in the top 5 cm of soil from the same field prior to planting ($F_{1,17}=68.63$, $p < 0.0001$) (Table 11).

Table 11. Total neonicotinoid residues in soil surface dust and in soil from top 5cm in 2014.

Sample type	Mean neonicotinoid concentration (ng/g)	SE
Soil surface dust	97.4 b[1]	32.82
Soil from top 5 cm	5.6 a	0.95

[1]Means within columns followed by the same letter do not significantly differ ($P < 0.05$, LSD) by ANOVA and Fisher's Protected LSD.

We compared the results from the 2014 study to a similar study conducted in 2013. No significant difference in neonicotinoid concentration was found between years of the study ($F_{1,2} = 3.33$, $p = 0.0767$), therefore data were combined across years. The mean neonicotinoid concentration within soil surface dust was 14-fold greater than within the seed zone prior to planting ($F_{1,33} = 137.29$, $p < 0.0001$) (Table 12).

Table 12. Total neonicotinoid residues in soil surface dust and in soil from top 5 cm in 2013 and 2014.

Soil	Mean neonicotinoid concentration (ng/g)	SE
Soil surface dust	69.5 b[1]	17.78
Soil from top 5 cm	4.8 a	0.73

[1]Means within columns followed by the same letter do not significantly differ ($P < 0.05$, LSD) as determined by ANOVA and Fisher's Protected LSD.

Neonicotinoid residues collected from planter exhaust, sticky dust traps and air samplers at the field edge and the edge of a neighbouring field during corn planting and tillage – Fugitive dust from corn fields during planting

Planter exhaust emitted 0.75 mg of neonicotinoid active ingredient per 100 m of row (single row) on average (Table 13).

The mean neonicotinoid concentrations captured on sticky traps during corn planting were 0.84 and 0.42 ng/cm2, for the downwind field edge and neighbouring field edges, respectively. Using volumetric air samplers, the mean neonicotinoid concentrations captured during corn planting at the downwind field edge and neighbouring field edge were 21.7 and 12.2 ng/m3, respectively.

For the majority of cases, neonicotinoid residue concentrations decreased as the distance increased away from the field being planted. There were some anomalies at individual locations which 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 would have confounded some of our results.

Table 13. Neonicotinoid residues collected in the planter exhaust and at downwind locations from the field being planted to corn in 2014 using vertical sticky traps and volumetric air samplers.

Location	Planter exhaust total a.i. (mg/100 m-row)	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)
1A	0.565	0.81	4.01	34	0.45	16.27
2A	0.066	1.28	11.98	n/a	n/a	n/a
3A	0.873	0.73	n/a	537	0.28	3.80
3B	n/a	1.16	39.54	355	3.61	n/a
4A	0.777	1.38	10.30	n/a	n/a	n/a
5A	0.944	1.85	60.72	300	0.65	17.33
6A	1.062	4.62	22.04	254	0.31	1.05
7A	0.402	0.68	12.71	n/a	n/a	n/a
8A	1.060	4.46	54.18	104	1.03	22.65
9A	1.000	0.11	1.49	n/a	n/a	n/a
Min.	0.067	0.11	1.49		0.28	1.05
Max	1.062	4.62	60.72		3.61	22.65
Mean	0.750	1.71	21.70		1.06	12.22
SE	0.113	0.49	6.99		0.52	4.17

* Data standardized to the time it took to plant 150 m of field width.

Fugitive dust from corn fields during tillage

Neonicotinoid residues measured using vertical sticky traps and volumetric air samplers downwind during tillage events were much lower than the residues collected during planting (Table 14). They were about 30% of those collected from corn planting (Table 12)on sticky traps and from 6 to 14% in the air samples. The concentrations of neonicotinoids captured at the field edge by either method were similar to those at the neighbouring field edge downwind.

Table 14. Neonicotinoid residues measured on vertical sticky traps and volumetric air samplers downwind of tillage events in 2014.

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)
2A	2.46	2.38	130	1.16	0.70
3A	0.41	0.13	n/a	n/a	n/a
5A	0.46	1.63	300	0.86	3.74
6A	0.24	0.58	254	0.28	1.54
7A	0.47	1.28	n/a	n/a	n/a
8A	0.18	0.16	104	0.22	0.92
9A	0.22	2.95	n/a	n/a	n/a
10	n/a	0.72	n/a	n/a	n/a
11	n/a	0.80	n/a	n/a	n/a
12	n/a	1.19	n/a	n/a	n/a
13	n/a	1.57	n/a	n/a	n/a
Min.	0.18	0.13		0.14	0.70
Max	2.46	2.95		0.50	3.74
Mean	0.63	1.22		0.33	1.73
SE	0.31	0.27		0.09	0.69

* Data standardized to the time it took to till 150 m of field width.

Determine the neonicotinoid residues in dead bees and bee-collected pollen during the corn planting period

The concentrations of neonicotinoids within bee-collected pollen before and during planting were significantly higher than after planting (Figure 16). Neonicotinoid concentrations in bee-collected pollen in 2014 were similar to the average level of 9.3 ng/g found in the 2013 study.

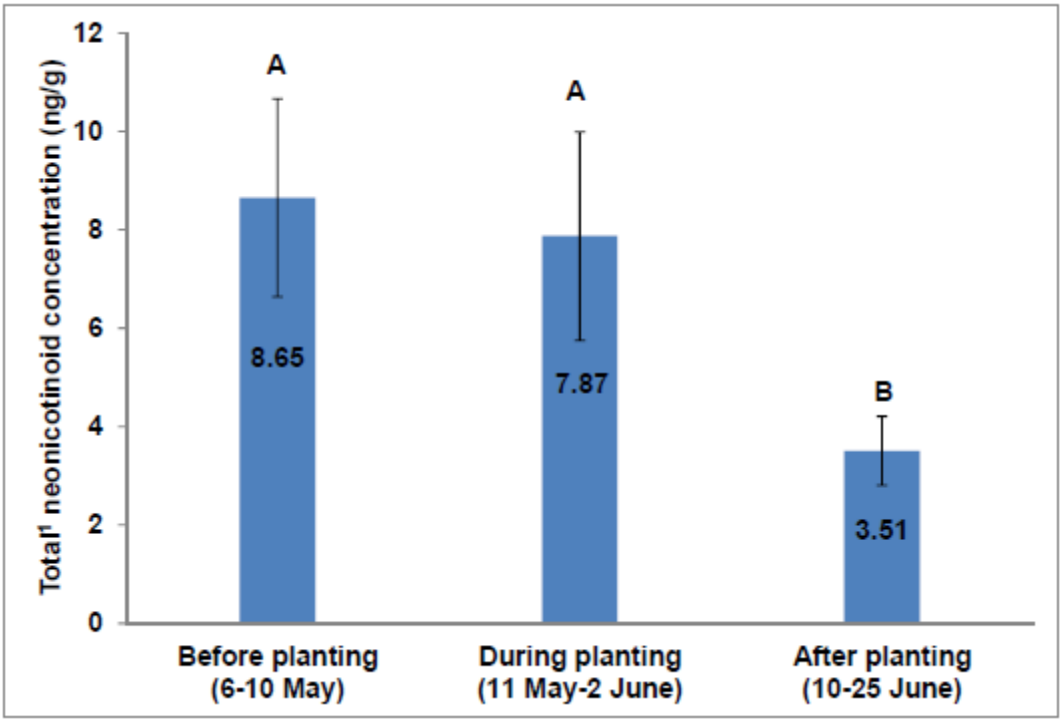


Figure 16. Mean concentration (±se) of neonicotinoid residues in bee-collected pollen collected before, during, and after corn planting in 2014. Columns with the same letter are not significantly differ (P < 0.05, LSD) as determined by ANOVA and Fisher's Protected LSD. 1 Total of clothianidin and thiamethoxam.

No significant differences in neonicotinoid concentration were found within or on the surface of dead honey bees among the three sampling periods in 2014 (Table 15). The mean total neonicotinoid residue within and on the surface of dead honey bees in 2014 was 1.42 ng/bee, which was similar to that measured in the 2013 study (13.9 ng/g, assuming that 10 dead bees = 1 g).

Table 15. Neonicotinoid residues within and on the surface of dead honey bees collected from dead bee traps at apiaries in 2014.

Sampling period	n	Neonicotinoid[1] (ng/bee) (mean ± SE)		
		Within	On surface	Total
Before planting	9	0.31 ± 0.16	2.23 ± 1.27	2.54 ± 1.32
During planting	10	0.25 ± 0.06	0.47 ± 0.13	0.71 ± 0.14
After planting	6	0.23 ± 0.05	0.78 ± 0.37	1.00 ± 0.41

[1]Total of clothianidin and thiamethoxam.

Neonicotinoid residue on and in blossoms foraged by honey bees

- **Trees and shrubs**

  The concentrations of neonicotinoids found on the surface and within flowers of *Salix* spp. were similar in variability (Fig. 17). The sample size of analyzed *Acer* spp. flowers was too small to draw conclusions from (Fig. 19). Within the *Rosaceae* sampled we found the neonicotinoid concentration on the flower surface and within flowers to be similar (Fig. 21). The concentration of neonicotinoids in the anther and pollen tissue of *Salix* spp. and *Rosaceae* was similar (0.39 and 0.37 ng/flower) (Figs. 17 and 21) however; these species cannot be directly compared due to differences in flower morphology.

  The concentration of neonicotinoids found on the surface of foraging honey bees was higher on bees collected from *Rosaceae* than on *Salix* spp. or *Acer* spp. (Figs. 21, 17, and 19). Bees foraging on *Rosaceae* were mainly collected during and after corn planting which corresponds with the proportion of bee-collected pollen from the *Rosaceae* family (Fig. 22). The neonicotinoid residue found on the surface of honey bees collected on *Salix* spp. and *Acer* spp. was generally low and less variable; these collections were made prior to corn planting (Figs. 18 and 20). The concentration of neonicotinoids measured within honey bees across all tree species was consistently low (min=0.02, max=0.42, mean=0.20 ng/bee). The mean concentration of neonicotinoids measured on the surface of honey bees across all tree species was 1.60 ng/be (min=0.75, max=3.00 ng/bee).

***Acer* spp. (Maple)**

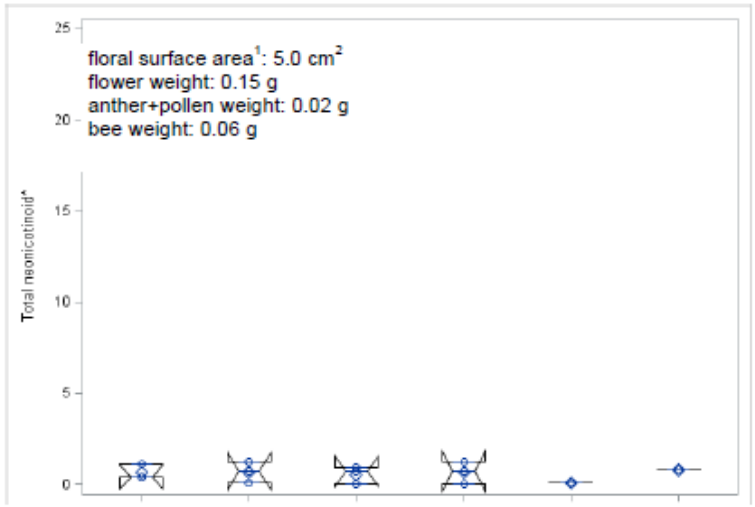


Figure 19. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from *Acer* spp. before corn planting (3 flower samples collected between 8-9 May 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

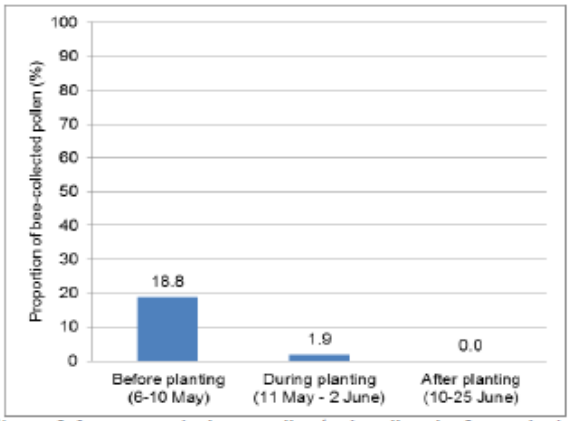
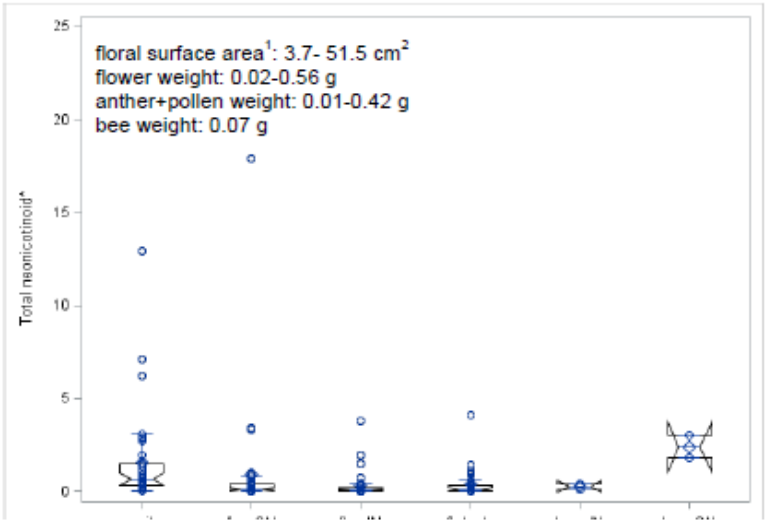


Figure 20. Proportion of *Acer* spp. in bee-collected pollen before, during, and after corn planting in 2014.

Rosacea family: Wild Rose (n=2); Bladdernut (n=2); Chokecherry (n=10); Hawthorn (n=21); Honeysuckle (n=1)



	ng/g in soil	ng/flower			ng/bee	
		surface	inside	anther+pollen	inside	outside+pollen
n	36	36	36	36	2	2
Mean	1.56	0.88	0.30	0.37	0.26	2.38

Figure 21. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from Rosaceae spp. before, during and after corn planting (1 flower sample collected on 6 May, 33 flower samples collected between 13 May and 2 June, and 2 flower samples collected between 9-10 June 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

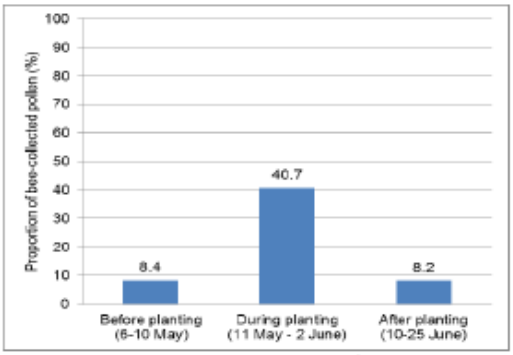


Figure 22. Proportion of Rosaceae spp. in bee-collected pollen before, during, and after corn planting in 2014.

- **Herbaceous Plants**
  Greater variability in neonicotinoid concentration was found within herbaceous than in tree blossoms, most notably within the pollen and anther tissue (Figs. 23, 25, 27, 28). Low levels of neonicotinoid residues were found on the surface of herbaceous flowers relative to what was measured inside blossom tissues (Figs. 23, 25, 27, 28). These data

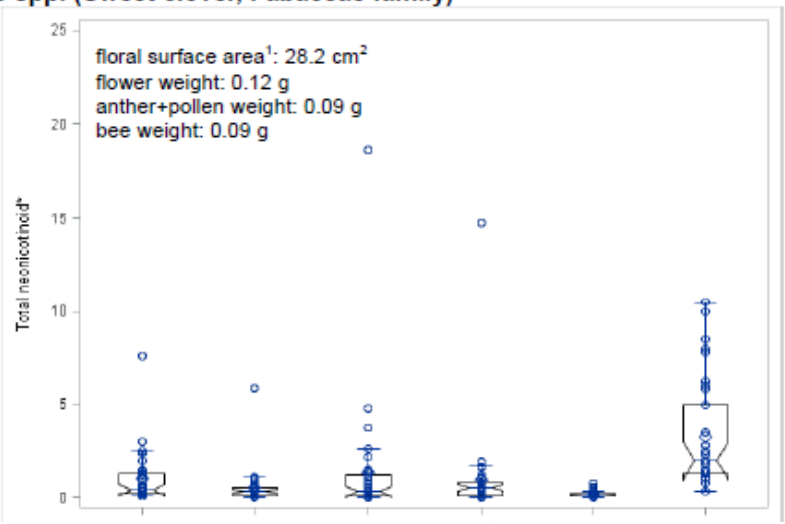
suggest that perhaps uptake of neonicotinoids from the soil or from leaf deposition during or after corn planting may result in greater residue levels in the plant.

Neonicotinoid residue levels found on the surface of foraging honey bees were more variable in bees collected from herbaceous plants than on those collected from trees (min=0.25, max=25.00, mean=2.94 ng/bee) (Figs. 23, 25, 27, 28). Bee-collected pollen data show that foraging on *Meliotus* spp. and *Trifolium* spp. was typically after corn planting as shown in Figs. 24 and 26. The concentration found within bees was consistently low and similar to that found in bees collected from tree blossoms (Figs. 23, 25, 27, 28). The concentration of neonicotinoids measured within honey bees across all herbaceous plant species was consistently low (min=0.02, max=0.94, mean=0.16 ng/bee) and similar to those collected from dandelions (min=0.02, max=0.09, mean=0.09 ng/bee).

There was a weak positive correlation between the neonicotinoid concentration found in soil and in the anther and pollen tissue when all species sampled were pooled (R2=0.0249, p=0.0484) (Fig. 30). When correlations were run independently by species we did not find a significant correlation.

Collectively the data from trees 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. In the case of herbaceous plants around corn fields, with the limited data presented, it was surprising to detect lower neonicotinoid residues on the surface of flowers relative to those within flower tissues which suggests that residue uptake by these plants plays a more significant role than simple residue deposition from fugitive dust during or after corn planting. 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.

***Melilotus* spp. (Sweet clover, Fabaceae family)**



	ng/g in soil	ng/flower			ng/bee	
		surface	inside	anther+pollen	inside	outside+pollen
n	33	32	32	32	33	33
Mean	1.00	0.52	1.35	0.97	0.19	3.24

Figure 23. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from *Melilotus* spp. after corn planting (33 flower samples collected between 6-27 June 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

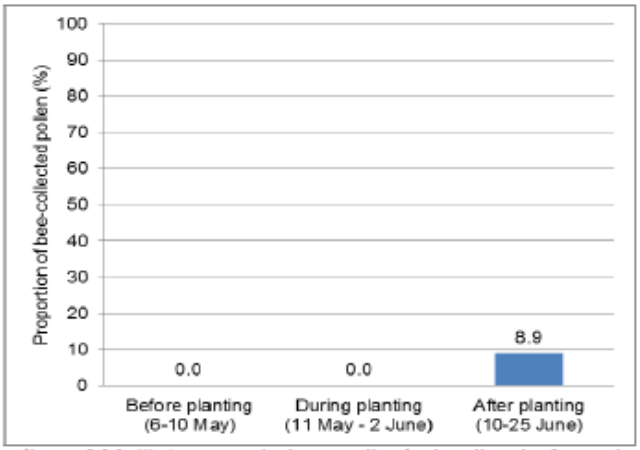
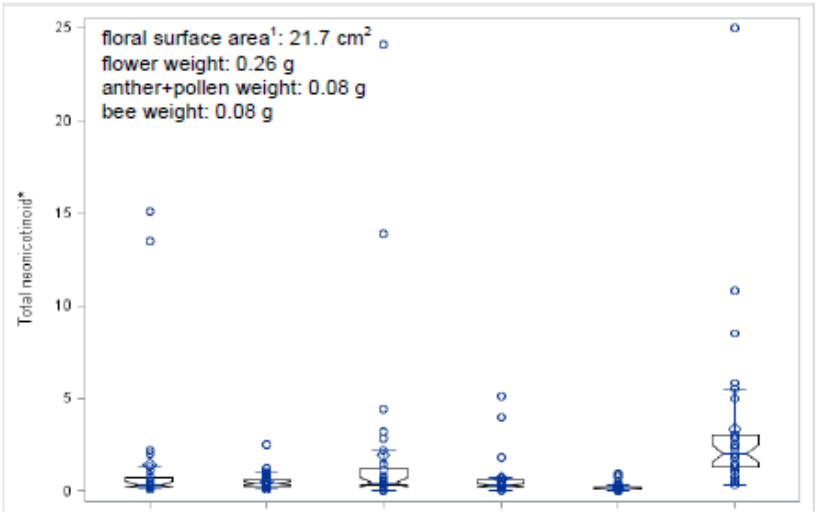


Figure 24. Proportion of *Melilotus* spp. in bee-collected pollen before, during, and after corn planting in 2014.

***Trifolium repens* (White clover, Fabaceae family)**



	ng/g in soil	ng/flower			ng/bee	
		surface	inside	anther+pollen	inside	outside+pollen
n	33	32	32	32	33	33
Mean	1.38	0.52	1.95	0.63	0.15	3.24

Figure 25. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from *Trifolium* spp. after corn planting (33 samples collected between 6-27 June 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

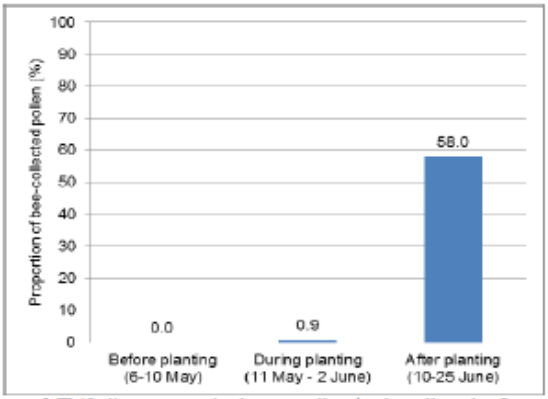
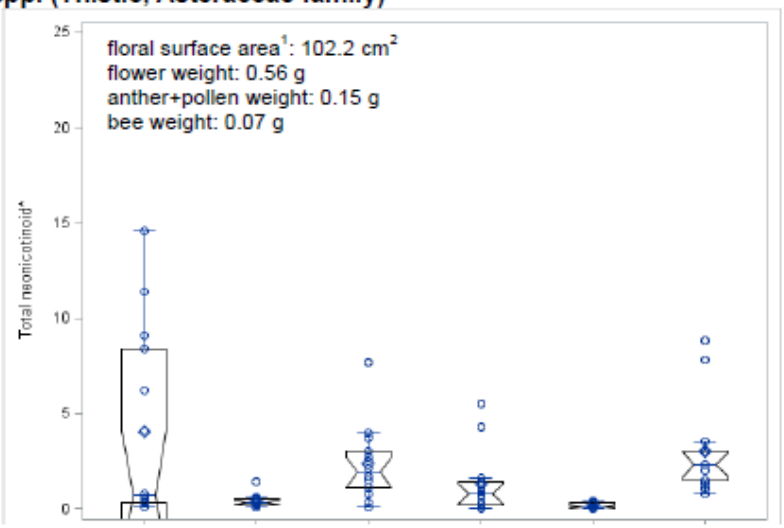


Figure 26. Proportion of *Trifolium* spp. in bee-collected pollen before, during, and after corn planting in 2014.

***Cirsium* spp. (Thistle, Asteraceae family)**

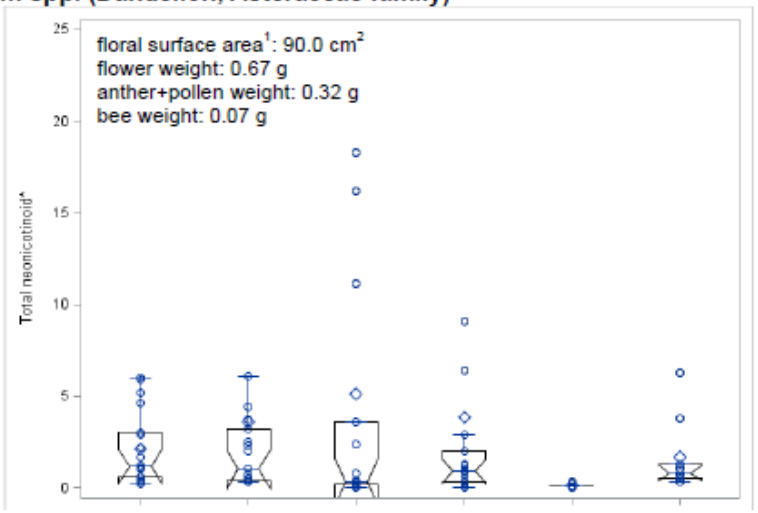


	ng/g in soil	ng/flower			ng/bee	
		surface	inside	anther+pollen	inside	outside+pollen
n	14	14	14	14	13	13
Mean	3.48	0.41	2.36	1.28	0.19	3.02

Figure 27. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from *Cirsium* spp. after corn planting (14 flower samples collected between 19-27 June 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

No thistle pollen was identified in bee-collected pollen in this study.

***Taraxacum* spp. (Dandelion, Asteraceae family)**



	ng/g in soil	ng/flower			ng/bee	
		surface	inside	anther+pollen	inside	outside+pollen
n	18	17	17	17	9	9
Mean	2.11	3.57	5.10	3.82	0.09	1.61

Figure 28. Total neonicotinoid[2] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from *Taraxacum* spp. before, during, and after corn planting (5 flower samples collected between 6-8 May, 11 flower samples collected between 11 and 29 May, and 3 flower samples collected between 9-12 June 2014).
[1]Mean area or weight for individual flower or bee.
[2]Total of clothianidin and thiamethoxam.

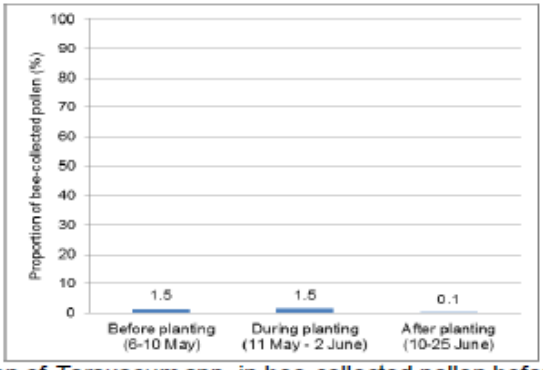


Figure 29. Proportion of *Taraxacum* spp. in bee-collected pollen before, during, and after corn planting in 2014.

Other herbaceous species:

Table 16. Total neonicotinoid[1] concentration in soil, on the surface, inside, anthers and pollen of flowers, and on the surface and inside of foraging honey bees collected from herbaceous plants after corn planting (flower samples collected between 9-16 June 2014).

Species	Site	ng/g soil	ng/flower			ng/bee	
			surface	inside	anthers+pollen	inside	surface
Vicia cracca (Tufted vetch, Fabaceae family)							
	4	0.7	4.1	8.2	1.1	0.1	1.3
	6	2.5	7.7	0.7	8.5	0.2	3.0
	6	0.5	2.7	0.5	6.6	0.4	9.5
Mean		1.2	4.9	3.1	5.4	0.2	4.6
Lotus corniculatus (Birdsfoot trefoil, Fabaceae family)							
	7	0.2	0.1	0.1	0.1	0.1	2.3
	8	2.6	0.1	0.4	0.1	0.2	4.0
Mean		1.4	0.1	0.3	0.1	0.1	3.1
Barbarea vulgaria (Yellow rocket, Brassicaceae family)							
	8	1.4	0.6	0.1	0.3	0.1	2.3
	8	0.2	3.5	1.6	0.9	0.1	1.0
Mean		0.8	2.0	0.8	0.6	0.1	1.6

[1]Total of clothianidin and thiamethoxam.

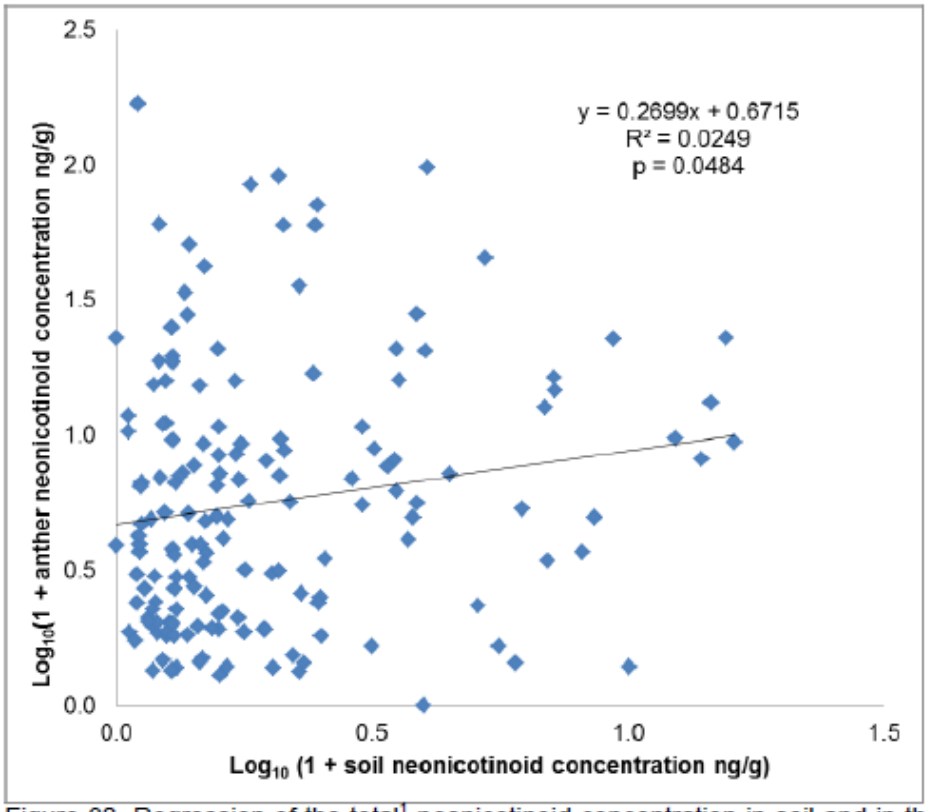


Figure 30. Regression of the total[1] neonicotinoid concentration in soil and in the anther and pollen tissue of all species of flowers collected before, during, and after corn planting in 2014. [1]Total of clothianidin and thiamethoxam.

What was found – Question 2 – trends and concepts among the four studies

Ohio State University

Results

Results of dust slide insecticide appearance and level for the Bayer fluency agent as compared to the farmer's choice material by distance

Table 1. Comparison across three sites and all distances for Bayer fluency agent to farmer choice product. Means are from 3 replicates for all except the "Planter", for which only a single sample was taken. For height of trap: H is 2 meters or L is 0.3 meters off the ground. For "Under" this indicates the slide set that was placed on the ground under the planter for one pass.

	Site	Mechanicsburg		London		Delaware		Kenton	
	Lubricant	Bayer	Farmer	Bayer	Farmer	Bayer	Farmer	Bayer	Farmer
Distance	Location	Mean ng/cm^2		Mean ng/cm^2		Mean ng/cm^2		Mean ng/cm^2	
0	Planter	46.6	33.6	17.1	7.34	7.33	13.0	NA	15.4
0	Under	0.54	0.90	6.49	7.77	12.9	15.3	NA	2.77
1	H	0.38	1.13	1.29	1.55	7.10	3.53	NA	1.37
1	L	1.16	1.39	0.67	1.63	5.34	2.32	NA	2.06
10	H	0.48	1.27	0.33	0.95	3.46	1.76	NA	3.31
10	L	0.36	0.32	0.23	1.04	3.17	2.64	NA	1.75
50	H	0.17	0.19	0.14	1.37	4.08	2.34	NA	0.95
50	L	0.78	0.62	0.32	0.75	3.82	1.91	NA	1.91
100	H	0.46	0.07	0.21	0.51	1.75	0.78	NA	0.33
100	L	0.38	0.66	0.24	0.22	2.62	2.33	NA	0.19

First we analyzed those data that are directly comparable with the previous Guelph study. These data included concentrations taken from dust collections stations at 1,10,50 and 100m, but excluded the under-planter and on-planter values. We used the R package LME4 (comparable to the linear mixed-effects models implemented in SAS Proc Mixed), and also used lubricant,

orientation of dust trap, and distance as fixed effects and grower as the random effect. Neither humidity nor wind speed were included as these were similar for all sites. All possible interaction terms were tested and none were found to be significant. The ANOVA for the main effects terms is presented in Table 1. A higher level of insecticide laden dust is deposited on traps nearest the first planter pass (1), but insecticide levels remain relatively high even out to 10 and 50 meters (Figure 3). There was borderline difference between lubricants (p=0.09) detected. However, if anything the Bayer product made the situation slightly worse (Figure 4).

Table 1. Test of fixed effects in linear mixed effects model with grower as a random effect

Effect	Num DF	Den DF	T value	Pr > |T|
Distance	3	138	-4.03	<0.0001
Orientation	1	138	-0.35	0.72
Lubricant	1	138	-1.66	0.09

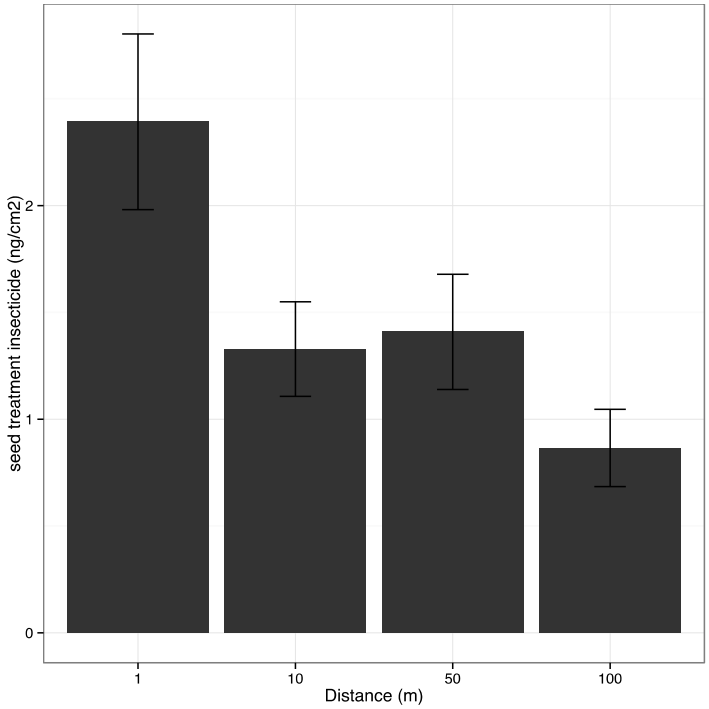


Figure 3. Insecticide laden dust concentration in ng/cm^2 downwind from planter startup.

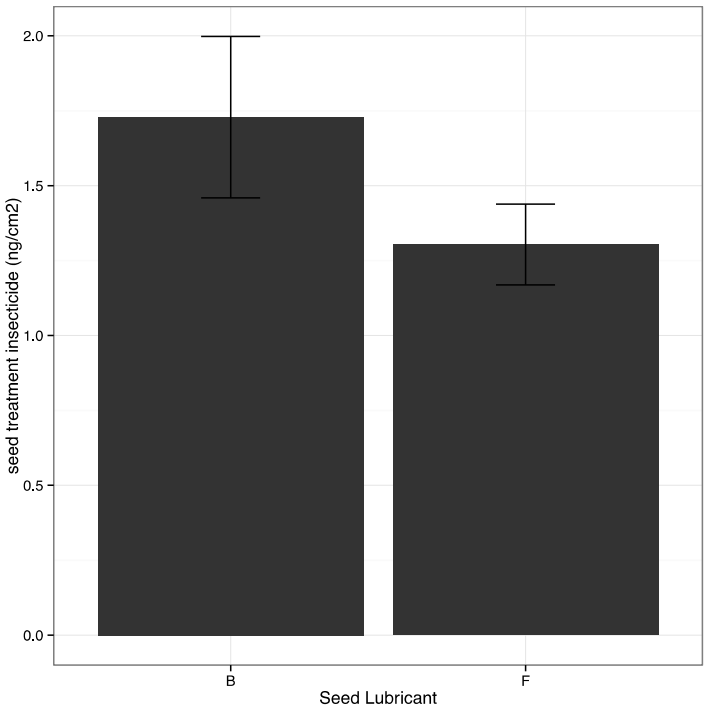


Figure 4. Comparison across three sites and all distances for Bayer fluency agent to farmer choice product for reducing pesticide in dust as determined by insecticide level in ng/cm^2.

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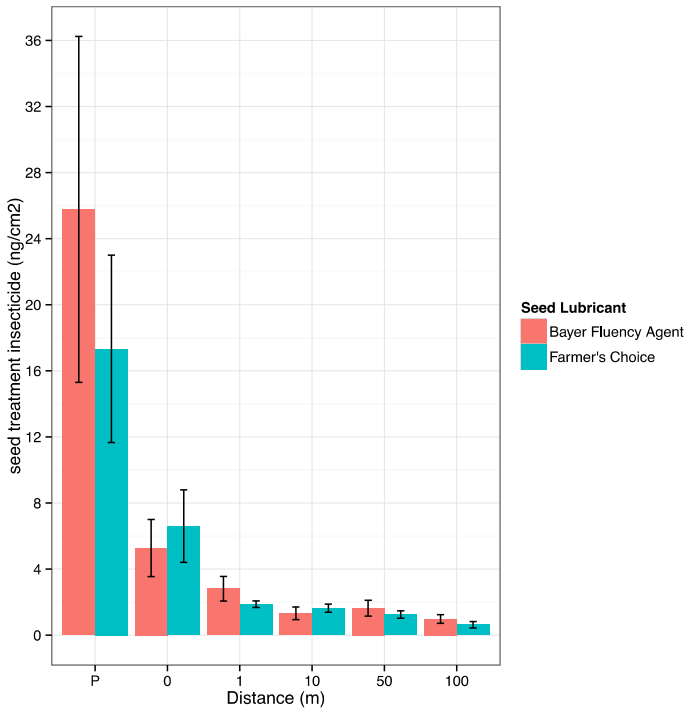

Figure 5. Comparison of effect of lubricant type on insecticide concentrations collected from planter-mounted (P) detectors and detectors that were directly passed over by the planter (0).

The planter (P) mounted slide trays collected very high levels of insecticide (Figure 5), but there was no significant difference between the Bayer product vs. the farmer choice (p=0.25, V=6, paired Wilcoxon test). For the zero (0) distance were the slide trays placed on the field surface, under the planter, for one pass, again no difference in the products here (p=0.36, df=13, t=0.95, linear mixed effects model with grower as the random effect).

These results demonstrate that a high level of insecticide is delivered straight to the ground beneath the planter than moves downwind. It should be noted that the under-planter collection represents one planter pass, after which the dust collector was removed, while other dust collection targets (1, 10, 50 & 100) remained in the field for the duration of the treatment.

Lessons learned:

- Six sites were identified for the 2014 Ohio planter dust trials. Due to planting delays from too-frequent rains during the late April through May period some sites were lost. Four sites were used, with the last planted site using only the farmer chosen lubricant and no comparison there made with the Bayer fluency agent. Clearly, additional sites would improve our ability to draw conclusions and may allow for inclusion of wind-speed into

statistical analyses – a factor that was found to be very important in the Guelph trials in 2014.

    - Three sites did include the farmer chosen vs. Bayer product comparison. We feel this level of replication is sufficient to allow conclusions to be drawn about the efficacy of the fluency agent in reducing insecticide dust emission during corn planting.
    - The cooperators and their planting equipment represented the wide range of air-assisted planters.

- Tillage shortly before planting was limited for the four trial sites. Only the site near London was fully tilled at the time of planting with the others as stale seedbed or no till planting. This is typical for much Ohio corn planting, with a limited amounted of conservation tillage prior to planting.
    - Weather delays (rain induced) also impacted timing of tillage.
- Air assisted planters are many, with air assistance applied at the supply end or the delivery end or both. Again the planters here represent the range found in Ohio.
    - Dust visually seen at planting appeared similar across the planter types, coming from ground engagement of planter units for the most part.
- Dust analysis from the traps determined there were little differences between the Bayer fluency agent and the farmers choice products (talc, graphite or combination product).
    - Our attempt to measure accumulated load with a planter frame mounted slide tray shows that we can accumulate large amounts of insecticide on the planter itself during planting.
    - The slide sets under the planter, with which we hoped to determine the amount of insecticide directly deposited to the soil surface, have higher levels of insecticide as compared to the targets farther away (and above the surface). Directly deposited insecticide is higher at the soil surface than that which moves off site in dust.
- Seed placed insecticides have grower value. But from discussions with entomologists, the current practice of all seed corn receiving an insecticide is a case of over-use. We must determine and share best management practices for use of seed placed insecticide for those few times when they provide the most value.
- The high levels of insecticide collected beneath the planter indicate that weed control in fields is of critical importance to protect pollinators from seed treatment dust exposure
- Very high levels of insecticide were collected across the board at the Delaware site. This suggests that there may be a quality-control issue with seed treatment application to seed used at this site. Samples of seed were saved and could be subject to a Heubach dustmeter test. Rare quality-control issues, as suspected at the Delaware site, could explain the highly sporadic nature of planting-related bee kills.

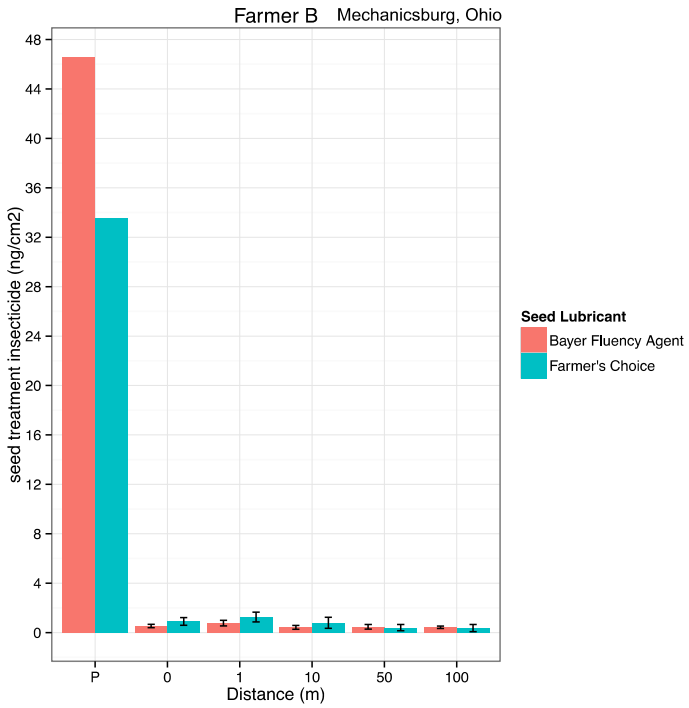

Figure 6. Insecticide levels for Bayer vs. Farmer treatment near Mechanicsburg, Ohio.

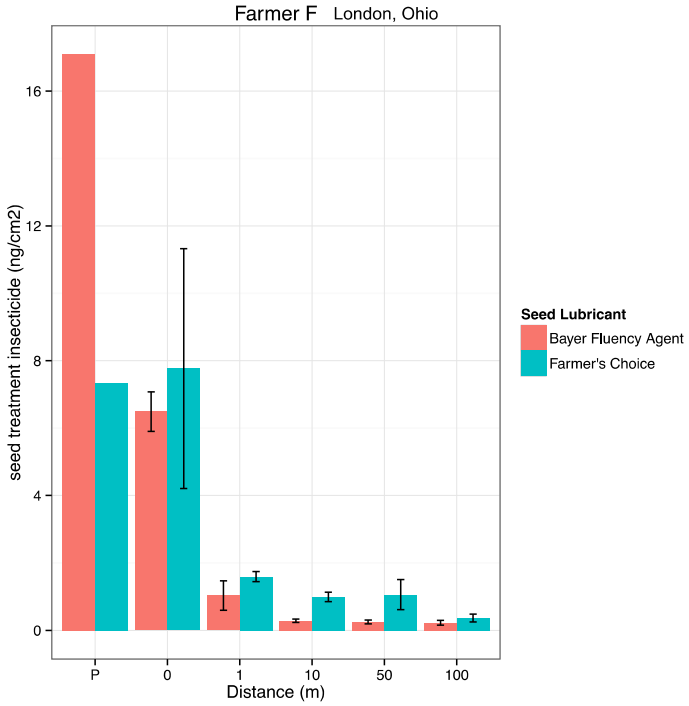

Figure 7. Insecticide levels for Bayer vs. Farmer treatment near London, Ohio.

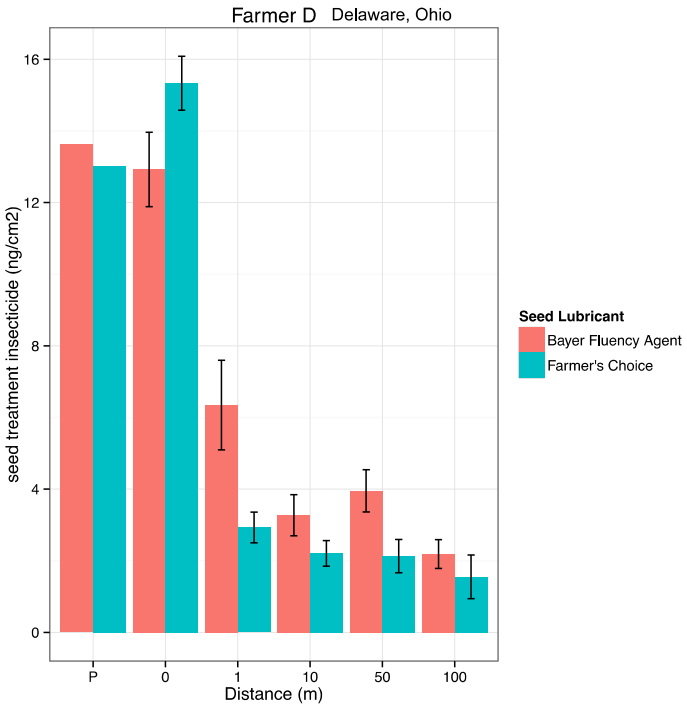

Figure 8. Insecticide levels for Bayer vs. Farmer treatment near Delaware, Ohio.

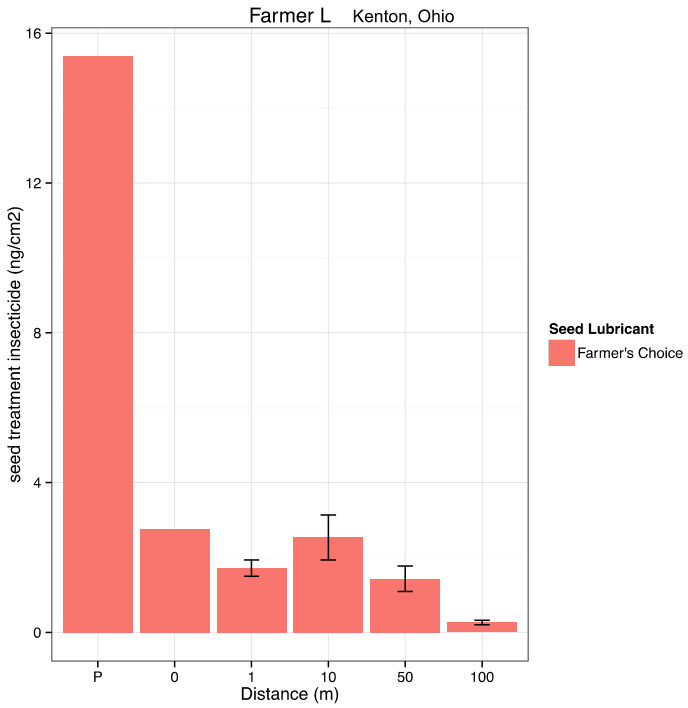

Figure 9. Insecticide levels for Farmer treatment near Kenton, Ohio.

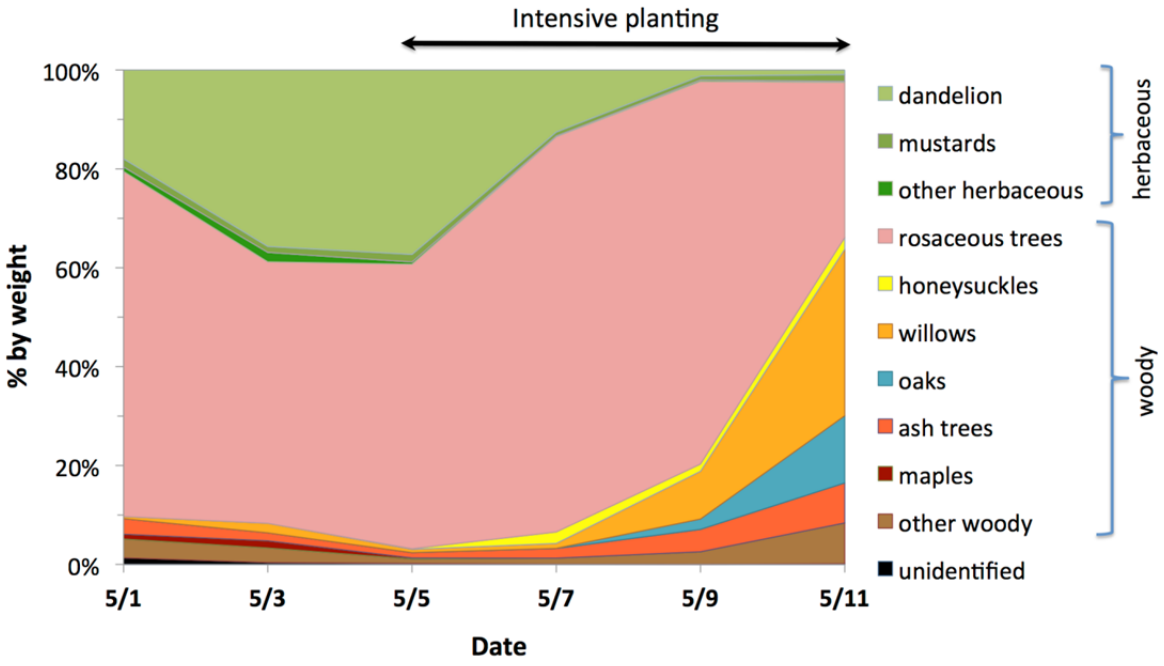

Figure 10. Averaged assemblage of pollen collected from six apiaries during May 1- 11, 2014.

Provisional Recommendations from 2013

A simple, “silver bullet” solution is not the result of these data. The CDRC provisional recommendations are based on small sample sizes and data from one year, and therefore all provisional recommendations require further testing in the coming year. However, the original CDRC goal was to be as helpful as possible in influencing the behaviors of all stakeholders with respect to the 2014 growing season; therefore, some practical solutions from the research are highlighted.

Several steps will need to be taken to achieve a reduction in exposure of honey bees to neonicotinoids used to treat seeds. Contributions are needed from every sector involved in this problem – from farmers, beekeepers, pesticide and lubricant manufacturers, equipment manufacturers, seed dealers, government agencies and regulators, extension agents, agricultural and commodity organizations, and agricultural media. The provisional recommendations in bold are identified as having come directly from the results of the CDRC study. Other recommendations are supported by work outside the CDRC research program. All recommendations have been vetted with the members of the CDRC; however, within the group there is general agreement that the provisional recommendations are, as stated earlier, based on very limited data. They are presented as a part of a building block approach that will need to be tried and tested, monitored and adaptively managed.

Farmers

- Use drift-reducing lubricants during planting to reduce dust. This recommendation comes with a caveat; though the CDRC tests showed that when the BFA lubricant was used, total dust and net pesticide load in exhaust emissions were reduced when compared to the use of conventional lubricants, the concentration of pesticide in the exhausted dust appeared to be higher in these tests. This result may be inconsistent with other tests of BFA elsewhere. Further research is needed to determine the extent to which Bayer’s new lubricant consistently reduces net emission of dust-borne pesticide during planting of treated seed.
- Follow all precautions to reduce dust and drift, especially with respect to wind 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. All research sites showed that this year 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. 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 are particularly vulnerable to drift of pesticides in exhausted dust when corn is planted within 50 meters of such forage.
- Control herbaceous flowers blooming in fields to be planted with corn. This action provides modest benefits to honey bees. Although pesticide residues were detected on cover plants (predominantly dandelions) within seeded fields, the study demonstrated

that honey bees did not forage heavily on these plants, but tended to forage on trees and shrubs.

- 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.
- Follow the principles of Integrated Pest Management.
- Communicate with beekeepers to ensure that they are aware of planting timing and can take appropriate precautions to protect colonies.

Beekeepers

- Protect supplemental food and water from drift dust.
- 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 food to suppress the need for foraging during corn planting, and provide clean water to reduce the need for bees to seek water from sources in and adjacent to corn fields. However, this recommendation is made with the awareness that bees will often seek out any natural pollen before artificial sources.
- Communicate with producers when you have hives in the area.
- Label hives with your contact information.
- Check hives regularly and report incidents.

Pesticide and lubricant manufacturers

- Work to reduce movement of corn dust (*e.g.*, improved sticking agents, improved fluency agency).
- Work to keep all the insecticide on the seed until the seeds are in the ground (*e.g.*, polymer seed coatings).
- Work to reduce abrasion potential of treated seed coatings.
- Ensure the lowest effective labeled rate of neonicotinoid treatment is applied to the seed.
- Offer untreated (fungicide only) seed options.
- 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.

Equipment manufacturers

- Ensure that equipment users understand the importance of bee protections and the value of using lower-drift lubricants.

- 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 under development).

Seed dealers

- 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.

Provincial, state and federal government agencies and regulators

- Provide financial and instructional support for maintaining trees and shrubs outside drift areas for bee forage available during planting season.
- Provide guidance for the reduction of attractive herbaceous forage in corn fields.
- Fully fund governmental provisions to ensure that pollinator forage supports 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
- Ensure that IPM practice information is available to the producer.
- Provide a responsive structure for bee-incident reporting. 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 the establishment of pollinator roadsides for 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.

Extension agents, agricultural and commodity organizations, and agricultural media

- Ensure that IPM practice information is available to the producer.
- Educate the beekeeper in practices that will safeguard bees.
- Educate beekeepers on bee-incident reporting.
- Educate 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.