### Corn Dust Research Consortium (CDRC) ADDENDUM Preliminary Report

- Initial Findings for 2013
- Provisional Recommendations
- Timetable

## **Addendum**

- NEW University of Guelph data (page 27)
- NEW Ohio State University information (page 43)

This document reproduces the original CDRC report on the findings of research conducted in 2013. The original is highlighted in yellow to show the areas where data gaps were anticipated and identified in the original report. Following this original report, starting on page 2, the newly received data and information are made public.

#### <mark>April 17, 2014</mark> FINAL

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#### **Executive Summary**

This preliminary report of the Corn Dust Research Consortium (CDRC), a multistakeholder initiative formed to fund research with the goal of reducing honey bee exposure to dust emitted during treated-seed corn planting, is based on the work of three research teams, led separately by Dr. Reed Johnson of Ohio State University; Dr. Mary Harris of Iowa State University; and Dr. Art Schaafsma, University of Guelph (on behalf of the Grain Farmers of Ontario). It is hoped that the preliminary results and provisional recommendations of the CDRC will inform best practices for the 2014 planting season. Additional research in subsequent seasons will be needed to replicate and substantiate the findings and provisional recommendations.

Two research questions were addressed by CDRC-funded research. The first question sought to develop a greater understanding of the use by honey bees of floral resources in and around corn fields during spring planting. The three research teams took their own approaches regarding this question, incorporating the landscape features, differences in grower practices, and the timing of the planting that varied according to location. Despite these differences, consistencies were observed with respect to honey bee foraging during planting.

The second research question evaluated the effectiveness of a new product developed by Bayer CropScience, *i.e.*, Bayer Fluency Agent (BFA), in comparison to standard lubricants (talc and graphite) on deposition levels of pesticide dust in and around fields when commercially available neonicotinoid-treated corn seed products are planted. This question was studied only by the research team led by Dr. Schaafsma.

With respect to the foraging question, the research found that across all three sites honey bees collected pollen largely from trees and woody plants (apple, hawthorn, willow, maple, *etc.*) during the time of corn planting. A second finding indicated that the highest levels of insecticide residue primarily occurred during the two-week period of peak corn planting. It will be important to replicate this work to ensure that these two findings occur consistently and not just during the 2013 planting season.

In assessing the effect of the alternative lubricant, BFA, as a replacement for talc or graphite to separate corn seeds in the pneumatic planters, the CDRC tests showed that when the BFA lubricant was used, total dust and pesticide load in the dust were reduced while pesticide concentration was increased, when compared to the use of conventional lubricants. Further research is needed to determine the overall effectiveness of Bayer's new lubricant in both reducing dust and dust-borne pesticide.

The CDRC is awaiting final data from one part of the Guelph research. The Guelph researchers received funding from other sources and had a wide spectrum of assessments they conducted. It will be important to have all data in hand to test provisional recommendations and to affirm the results of 2013.

The goal of the CDRC is to be as helpful as possible in influencing the practices of all stakeholders with respect to the 2014 growing season; therefore, several

practical solutions that the research highlighted are offered as provisional recommendations (see page 23). All provisional recommendations are based on one year's data and in some cases, small sample sizes; all will require further testing in the coming year.

Several steps will need to be taken to achieve a reduction in exposure of honey bees to neonicotinoids used to treat seeds. Many contributions toward this goal are needed from every sector involved in this situation – farmers, beekeepers, pesticide and lubricant manufacturers, equipment manufacturers, seed dealers, government agencies and regulators, extension agents, agricultural and commodity organizations, and agricultural media all need to become involved.

The CDRC process involved collaborative oversight of practical research through multiple institutions. It has been complex but extremely rewarding. All stakeholders have shared the responsibility for transparency, open deliberation, and unbiased assessment throughout 2013. They will now begin the tasks of follow-up evaluation, information dissemination, and adaptive management in 2014.

#### 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 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:

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?

The goal of the consortium in addressing these two questions is to utilize data from research conducted in three North American locations during the 2013 planting season to develop best practice guidance for the 2014 corn planting season, 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 Canadian Honey Council Farm Equipment Manufacturers Association 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:

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

The CDRC seeks to be a credible source of information about a very limited segment of pesticide-pollinator interactions. Our initial timeline was presented at the EPA/USDA Pollinator Summit meeting in Crystal City, VA on Tuesday, March 5, 2013. That projected timetable was:

- March 5 March 8, 2013 Evaluate proposals
- March 12, 2013 Deliberations based on proposals
- March 15, 2013 Award grants
- April-May 2013 Spring corn planting
- August 1, 2013 Progress report due
- December 1, 2013 1<sup>st</sup> year final report due from researchers to CDRC
- January-February, 2014 determine possible improvements in corn planting best practices in time for dissemination before spring planting 2014

What follows is a summary of the approaches used by each of the three research institutions for Question 1 and the approach to Question 2 used by one institution, *i.e.*, University of Guelph. It should be noted that researchers at each of the three 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. Despite these differences, consistencies were observed, particularly with respect to honey bee foraging during planting. These are noted in the results section (page 16). Several questions still remain (see page 22), especially since we have only data from one year. These questions and the limits of the data influence the preliminary recommendations (page 23) that are identified as either having come directly from the results of the CDRC study or from common understandings or suggestions supported by other work.

#### Research institutions (in alphabetical order)

#### 1. University of Guelph and Grain Farmers of Ontario (questions 1 and 2)

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#### 3. Ohio State University (question 1)

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#### Iowa State University

#### Methods

#### **Research cooperators and protocols**

Cooperating farmers were located in northwestern lowa providing eight 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 were used. Neonicotinoid treated seeds were planted at 6 no-till sites (4 sites using pneumatic planters and 2 sites using finger-type planters), and untreated seeds were planted with finger-type planters at the remaining 2 sites (these sites were under conventional cultivation). Planter make, model, serial number, seed treatment, planting date and herbicide application date for each site were noted.

#### Hive description and placement

On April 30, 2 bee hives were positioned at each site along the field margin and were fitted with external pollen traps to sample pollen from foraging bees prior to and following planting. Each hive consisted of two 10-frame brood boxes containing a queen, brood, approximately 20,000 workers, honey stores, and a feeding reservoir.

#### **Pollen collection protocol**

Betterbee<sup>®</sup> Anatomic Pollen Traps were externally mounted to the entrance of hives 1 week (May 1) before initial pollen sampling effort to allow the bees to habituate to the presence of the device (figure 2). To collect pollen, traps were closed for 24 hours per sampling effort (forcing returning foragers to enter the hive through the trap mesh which removes ~50% of collected pollen pellets from the bees' corbiculae). In addition, pollen samples were collected from plant species in flower at each site at the time of hive pollen collection.

#### Sampling frequency

Planting dates varied among cooperators between April 29 (site 3) and May 20 (site 6). Flowering plant pollen and bee-collected pollen sampling was completed during the following 24hour time periods:

6-7 May	(pre-planting)	
13-14 May	(post planting)	week 1
17-18 May		week 2
2-3 June		week 4
18-19 June		week 6

Bee-collected pollen sampling was to commence one week prior to planting and continue at one-week intervals for 6 weeks. However, due to the highly variable weather and planting schedules among cooperators, we were unable to sample at site 3 prior to planting. Inclement weather precluded sampling at regular weekly intervals following planting; however, we were able to sample at 1, 2, 4, and 6 weeks after planting. Two additional collections were completed at 13 and 17 weeks post planting.

#### Pollen identification protocol

Pollen pellets from traps were transferred to glass scintillation vials and placed on ice in the field. All pollen was stored at -7°C when not being utilized for identification, to prevent contamination and fungal growth. Pellets were sorted by color and representatives selected randomly from the sorted pollen for imaging.

Slides were made from plant pollen (our reference library) and representative bee-collected pollen samples and photographed in the ISU Light Microscopy imaging facility. All pollen grains were stained with potassium iodide solution and photographed at 40x magnification, and each representative slide of bee-collected pollen was photographed at a minimum of 4 random locations on the slide.

Pollen images were compared to the images of our reference pollen library, which allowed identifications for most pollen types. Further refined identifications were made using collection date, available plant phenology data, location of plants in bloom, color of pollen pellet and comparison to other pollen micrographs. It should be noted that 100% certainty in pollen identification is not possible without electron microscopy.

#### Toxicological analyses

Samples for pesticide residue analysis were sent to the USDA Materials Analysis Laboratory in Gastonia, NC.

#### Hive maintenance

Each hive was inspected monthly to determine if queens were present and brood production was ongoing. Only one super was required and placed on top of the brood boxes of each hive. Honey production levels during the season did not require additional supers. All hives were given supplemental feed (corn syrup solution) to allow build-up of adequate stores of honey for overwintering. The lowa Department of Agriculture and Land Stewardship State Apiarist inspected each hive in late September - early October. One hive (Hive 14) was determined to have only drones and was expected to be lost, and another hive (Hive 6) was missing a queen. The other 14 hives were all queen-right with sufficient honey stores to overwinter.

#### **Ohio State University**

#### Methods and experimental setup

In late April 2013, just prior to corn planting, three apiaries 15 - 34 km apart were set up in areas dominated by field crops in central Ohio. Six honey bee colonies were placed in each apiary, including two new colonies started from packages of bees, two small nucleus colonies, and two large overwintered colonies. Dropzone dead-bee traps (100L x 50W x 14Dcm; or 40"L x 20"W x 5.5"D) were placed in front of four colonies at each site, and dead bees were collected and counted twice per week.

The overwintered and nucleus colonies were each fitted with a pollen trap (Sundance I) that could be turned on to sample corbicular pollen from returning foragers or turned off to allow pollen into the colony for the bees' sustenance. Pollen traps were emptied and turned on and off on a semiweekly cycle, alternating between colonies, so that pollen was always sampled from two colonies at each site. Corbicular pollen collected at each site on each collection date was weighed, then pooled for further analysis.

Concurrently, observational floral surveys were conducted on a weekly basis to determine the diversity and phenology of floral resources in the study area. Voucher specimens and pollen were collected during floral surveys to build a reference collection for pollen identification. Collection of dead bees and pollen continued through June 11, at which point essentially all corn had been planted in Ohio. At this point 3 of the 18 colonies, one at each site, were either dead or weakened to a point where colony failure was considered imminent

Data about the corn planters used by cooperating landowners at each site were collected. Intense local planting of corn occurred between May 3 and May 9, after which rain stalled planting for several days. Planting resumed May 12 and was completed by May 16. According to the USDA National Agricultural Statistics Survey (NASS) Crop Progress Report, statewide cumulative corn planting in Ohio was at 7% on May 5, 46% on May 12 and 74% on May 19 (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID= 1048).

The total landscape of each apiary site was defined as the area contained within a 3-km radius centered on the location of our hives. We quantified the composition of each landscape according to the following categories using a combination of aerial photo analysis and visual ground-truthing: corn, other crops, non-crop fields, tree canopy, residential lots, and margins (including field margins and roadsides). All three sites were dominated by corn and soybean cultivation. Other crops, primarily wheat and alfalfa, occupied the small remainder of cultivated field area. Non-crop landscape elements included uncultivated fields (grazing pasture, fallow agricultural fields, and grassland patches), small forest tracts, scattered residential lots, and marginal strips (field margins and roadsides).

On May 2, immediately before the start of corn planting, the sites were visually assessed for the abundance of bee-attractive blooms in all accessible fields. Each field was assigned a qualitative bloom level of 0-2: 0 = virtually no blooms, 1 = scarce blooms and 2 = abundant blooms. Qualitative floral surveys of non-crop areas were also conducted about twice per week during the period of corn planting. All accessible fields were reclassified on July 4 by crop type, after crops had sufficiently matured to be readily identified. Digital landscape analysis and visualization were performed using Quantum Geographic Information Systems (QGIS) software (QGIS Development Team, version 1.8).

#### **University of Guelph**

#### Methods

Nine farms (2 fields per farm = 18 fields) and 9 bee yards located in 5 key corn growing counties of southwestern Ontario, Canada, were selected for this study. Activities from April to late June 2013 focused on weekly (to Week 6 post-planting) field surveys and sample collection of flowering resources, and pollinators within the field and bordering landscape. Bee yards also were surveyed prior to planting and weekly following planting to Week 6 to collect pollen and dead bees.

Dust generated from pneumatic (negative vacuum) corn planters using either a conventional or novel seed lubricant – Bayer's Fluency Agent (BFA; Bayer CropScience Inc.) was collected during planting using a new vacuum cleaner filtering bag attached to one of the planter's exhaust manifolds, and by sticky dust traps on towers placed along the downwind side of the field during planting. Immediately after planting, whole blossoms of the most common potential forage plant, determined in the study to be dandelions, were collected for residue analysis from the downwind side of the field. All samples collected for residue analysis were placed in coolers with freezer packs for transport back to the lab where they were then stored in a  $-20^{\circ}$ C freezer.

To determine what resources honey bees near experimental field sites were foraging on and whether neonicotinoid residues were transported into the hives via collected pollen, two bee hives per bee yard were equipped with pollen traps one week prior to planting. Pollen samples were collected at regular 24 hr (or at 48 hr if weather conditions were poor for foraging) intervals; 1 week prior to planting, 2 and 6 days after planting and weekly for 6 weeks after planting. Pollen was collected, frozen, and representative samples were sent to the University of Guelph for preparation and then to Johanne Parent (Rimouski, Quebec) for plant species identification.

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

#### Corn fields and apiaries

#### Question 1

Originally, 10 farms and 10 apiaries located in 5 key corn-growing counties of southwestern Ontario, Canada were identified for this study. However, one apiary experienced high levels of unexpected overwintering bee loss and was removed from the study along with the associated farm/corn fields prior to study initiation. Each farm (2 fields per farm) was paired with an apiary that was within 3 km (majority being less than 2 km) of both fields. Fields ranged from 20 to 100 ha in size. During planting, farmers used negative vacuum air corn planters that were 16 to 24 rows wide. One field was planted using standard lubricant (talc,

graphite or a combination talc/graphite product), while the paired field was planted using the BFA. All 18 fields were planted within a ten-day period between May 6 and May 16, 2013, with two fields (Fields 1A and 1B) replanted on May 26, 2013 due to poor emergence.

Vegetation in bloom was surveyed weekly in and around the 18 corn fields from April 29 to June 28, 2013. Field perimeters were divided into 4 sides, with additional zones added based on the landscape and vegetation. Photos of blooming plants, trees and shrubs in the different zones were captured using iCroptrak<sup>™</sup> software (Cogent3D Inc.) for the Apple iPad, and geo-referenced. The plant species and their spatial densities were identified and categorized.

At each of the 9 apiaries, 4 hives were used for the study, 2 hives were fitted with an Anel-Standard pollen trap ( $39 \times 15 \times 10.5$ cm; Athens, Greece. <u>www.anel.gr</u>. All 4 hives also were fitted with drop-zone dead bee traps ( $100L \times 50W \times 14D$ cm; or 40°L x 20°W x 5.5°D).

Pollen traps were engaged at ca.16:00 h on the day previous to the specified pollen collection date. Pollen samples were then collected from the sites no later than ca. 16:00 h on the day of pollen collection. 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. There were 8 sampling dates: the first within a week prior to planting, then on Days 2 and 6 post-planting, then weekly - during weeks 2, 3, 4, 5 and 6 post-planting. The pollen types and proportion were identified by Johanne Parent, Rimouski, Quebec.

#### Lubricant comparison

#### Question 2

This portion of the study was conducted on the 9 farms described earlier. Each farm had two fields. Each field pair used the same seed treatment product and rate, planting equipment and settings. During planting, one field per grower was planted using the seed lubricant (talc or graphite or combination product of talc/graphite) they normally used, at the rate the grower was accustomed to using (which was not necessarily at the rate recommended by the planter manufacturer), while the other field was planted using the new BFA at the recommended 1/8 cup per unit of seed (1 unit = 1 bushel or 80,000 seeds).

Both fields of each cooperator were planted with the same seed (*i.e.*, same hybrid from the same source, treated with the same seed treatment by the same method). Planter seed hoppers were emptied and the air-flow system operated until no dust was observed escaping (about 5 to 10 min) before a new seed batch and its lubricant were introduced. After the seed was loaded and the lubricant applied, the cooperator planted the headlands of the fields, and the 100-m area along which the dust traps were installed. After two full rounds of planting during the test portion, a sampling bag was installed on one exhaust port for one full round of planting. The sample bag, a new vacuum cleaner filter bag

(Electrolux<sup>®</sup> CB, #635. 53.5 x 37 x 16cm), was fixed and sealed over one outlet of the planter's exhaust manifold using a metal hose clamp and duct tape. The distance per pass was recorded. The dust collected was normalized to a standard 100-m distance and a single row (also called planter unit) to standardize the planter and field pass distance. Field 2A was planted with seed and lubricant that were placed in the planter the previous night, and the planter remained in the field. Due to concerns with humidity overnight which may have affected dust escaping and lubricant performance, we removed the paired data of Fields 2A and 2B during statistical analyses.

The team tested the hypothesis that the quantity of dust escaping from vacuum planter manifolds when using a conventional seed lubricant is similar to that escaping from the same planter when using the BFA. The Statistical Analysis System (SAS<sup>®</sup>; SAS Institute, Cary, NC) procedure PROC MIXED model was used with lubricant as a fixed effect and location as a random effect. Dust weight data were subjected to  $log_{10}$  transformation (trans-dust weight) to meet assumptions for normality, and the model tested was: trans dust weight = lubricant. Similarly the team also tested the hypothesis that the neonicotinoid concentration in the dust escaping from vacuum planter manifolds using a conventional seed lubricant is similar to that escaping from the same planters using the BFA and the hypothesis that quantities of neonicotinoid active ingredient (a.i.) escaping from vacuum planter exhaust manifolds using a conventional seed lubricant are similar to those escaping from the same planters using the BFA.

The CDRC is awaiting final data from two aspect of the Guelph research, the data from the prepared field slides (only slides collected from the sample point nearest the test area have been analyzed and reported) and the data from the remaining pollen trap collections (only samples collected during the first three sample periods were analyzed and are reported). All remaining samples are being analyzed and should be available first week of February. These are delayed as the Guelph researchers received funding from other sources and conducted a wide spectrum of assessments, all of which required analyses. It will be important to have all CDRC-related data to go forward in 2014, to test provisional recommendations, and to affirm the results of 2013.

#### Proprietary data across all research sites

It had been agreed from the beginning that data from each of the three studies would be used by each research team in individual peer-reviewed publications and would be submitted either after this first year of research or after a second year of data collection. These raw data would also be made available on request, but not necessarily before publication in peer-reviewed journals. The results from these studies would be used to develop best practice guidance for the 2014 corn planting season.

#### What was found – trends and concepts among the three studies

#### Question 1 Bee-collected pollen

• The majority of pollen collected by honey bees during planting was from trees and shrub species at each of the three study sites. As an example, at the Guelph site, the most abundant pollen types collected from the bee hives during corn planting weeks were Rosaceae (hawthorn, rose, apple etc, 47.0%), *Acer* (maple, 24.8%), *Salix* (willow, 16.7%), Brassicaceae (mustard, 4.2%) and *Taraxacum* (dandelion, 2.4%). Similar trends were found in Ohio (Figure 1) and Iowa (Table 1) below.

Figure 1: Ohio bee-collected pollen (percent by weight)



#### Table 1: Iowa bee-collected pollen (proportion weight of week total)

Plant	Sampling Date						
Species	5/6	5/13	5/17	6/2	6/18	6/23	8/27
Acer spp.	0.29						
Malus domestica	0.12	.0.72	0.49				
Rosa multiflora		0.16					
Taraxacum officinale		0.1					
Forsythia suspensa			0.12				
<i>Salix</i> sp.			0.33				
Oxalis				0.11			
Rudbeckia hirta				0.22			
Syringa vulgaris				0.52	0.36		
Phlox					0.22		
<i>Trifolium</i> sp.					0.23		
Medicago salvia						0.12	
Melilotus sp.						0.66	
Centaurea sp.							0.34
Helianthus sp.							0.10
Solidago spp							0.34
Trifolium repens							0.11

#### Ohio landscape and bloom observances

Bloom density within cultivated fields was generally very low. At two sites less than 3% of the total field area was classified as having abundant blooms. At the third site, however, approximately 32% of the total field area had abundant blooms. This discrepancy is apparently due to substantial differences in local tilling and herbicide application practices.

Surveys of non-crop areas indicated that in most cases forests, residential lots, and marginal land support the bulk of spring foraging resources for honey bees. This distribution of bee-attractive flora strongly suggests that most honey bee foraging during corn planting season occurs outside of cornfields. This conclusion is corroborated by pollen analysis that identified dandelion, wild mustards, maple, ash, and rosaceous trees--taxa that were observed either primarily or exclusively in residential lots, forest tracts, and marginal land--as the principal pollen sources for our colonies. The significance of these extra-field flora with regard to pesticide exposure should not be overlooked, since many of these critical resources occur in immediate proximity to cornfields, well within the range of drifting seed dust (Biocca *et al.*, 2011; Krupke *et al.*, 2012). There was, however, one site in our study in which dandelions and wild mustards occurred at moderate to high density within corn fields.

In the course of visualizing the three landscapes that were studied in Ohio, it became apparent that the potential exposure of honey bees to seed treatment insecticides may be dependent on the proximity of foraging habitat to the field being planted. The relative rarity of foraging resources within cornfields and the scarcity of foraging habitat outside cornfields create a discrete pattern of theoretical exposure zones where foraging habitat is located adjacent to or (more rarely) within cornfields. Based on a proposed maximum drift distance of 50 m from the cornfield edge (Biocca, 2011), these theoretical exposure zones would comprise only 4-14% of the total landscape area but 29-40% of the total foraging habitat (taken to be the sum of weedy field, forest, residential, and marginal land).

#### **Pollen contamination**

- Neonicotinoid residue levels found on dandelions downwind of the vacuum planters were positively correlated with residue levels in dust emitted from planters; however, few honey bees visited dandelions for pollen.
- Neonicotinoid residues are associated with dust emitted from vacuum planters, and these residues are presumably associated with abrasion of neonicotinoid-treated seed coatings during planting.
- Levels of neonicotinoid residues in bee-collected pollen ranged widely across all sites. See Iowa (Table 2); Ohio (Table 3); and Guelph (Table 4) below.
- In two of our sites, the contamination of pollen was limited to a two-week period during planting. The results from the complete period of sampling from the Guelph study are not yet available. If this observation remains consistent, then it means that the problem of exposure may exist in a discrete timeframe and opens the possibility of controlling the exposure temporally.

Table 2: Iowa bee-collected pollen contamination levels of clothianidin (clo.), thiamethoxam (thia.) and imidacloprid (imid.)

					Sampli	ng Date					
		5/13				5/17				6/2	
	Mean	Mean			Mean	Mean			Mean		
	(ppb),	(ppb),			(ppb),	(ppb),			(ppb),		
	(no. of	(no. of		Prop.	(no. of	(no. of		Prop.	(no. of		Prop.
	samples)	samples)		sam.	samples)	samp.)		sam.	samples)		sam.
Plant	and range	and range	No.	with	and range	and range	No.	with	and range	No.	with
<b>specie</b> s	clo.	thia.	sam.	neo.	clo.	thia.	sam.	neo.	imid.	sam.	neo.
Malus	40.8 (3)	16.6 (5)			4.7 (3)						
domestica	12.0 - 89.3	9.7 - 23.6			2.9 - 6.0						
Rosa	57.6 (2)	20.8 (3)									
multiflora	41.2 - 89.3	17.9 - 23.7									
Taraxacum		11 3 (1)									
officinale		11.5 (1)	11	1 00			26	0.23		24	0.08
Forsythia				1.00	18.8 (2)		20	0.25		24	0.00
suspensa					15.3 - 22.3						
Salixsn					15 5 (1)	6.6 (2)					
Guin Sp.					10.0 (1)	5.9 – 7.2					
Rudbeckia									26.4 (2)		
hirta									14.2–38.5		

Table 3: Ohio levels of neonicotinoid residues in unsorted, bulk pollen samples collected between April 23 and May 31, 2013

Site	Date	Clothianidin (ppb)	Thiamethoxam (ppb)	Imidacloprid (ppb)
	4/23	0	0	0
	4/29	0	0	0
	5/2	0	0	0
	5/6	11.9	5.4	0
	5/9	18.7	4.8	0
А	5/13	13.3	4.1	0
	5/16	35.1	8	0
	5/20	3.9	0	0
	5/24	0	0	0
	5/27	6.3	0	0
	5/31	0	0	0
	4/23	0	0	0
	4/29	0	0	0
	5/2	0	0	0
	5/6	15	8.4	0
	5/9	35.5	9.1	2.6
В	5/13	7.4	1.6	0
	5/16	4.8	0	0
	5/20	0	0	0
	5/24	0	2.2	0
	5/27	0	0	0
	5/31	0	0	0
	4/23	0	0	0
	4/29	0	0	0
	5/2	0	0	0
	5/6	15.7	0	0
	5/9	10.7	0	0
С	5/13	24.5	2.7	0
	5/16	6.9	0	0
	5/20	0	0	0
	5/24	0	0	0
	5/27	0	0	0
	5/31	0	0	0

Table 4: Guelph neonicotinoid concentration in bee collected pollen

Bee Yard	<b>Pre-planting</b> Neonicotinoid in bee collected pollen (ppb)	Day 2 of planting Neonicotinoid in bee collected pollen (ppb)	Day 6 of planting Neonicotinoid in bee collected pollen (ppb)
1	1.6	4.6	17.3
2	20.9	6.1	5.9
3	2.9	19.1	ND <sup>1</sup>
4	.0.5	6.1	2.5
5	0.9	4.4	4.3
6	0.3	3.6	6.6
7	48.0	25.5	7.1
8	2.0	16.8	ND <sup>2</sup>
9	5.8	17.8	5.0
Mean	9.2	11.6	7.0
Minimum	0.3	3.6	2.5
Maximum	48.0	25.5	17.3

ND<sup>1</sup>: No neonicotinoid data available. Only 0.1g bee pollen was collected. This quantity was below our LC-MS/MS validation limit.

ND<sup>2</sup>: No data available. No bee pollen was collected due to bad weather conditions.

It should be noted that the focus of the CDRC study was floral routes of exposure, and studies were not designed to differentiate whether the residues were a result of dust released from the exhaust manifolds or from pre-existing residues in soils or from sources outside the study area. The study also did not examine potential adverse effects on bees from exposure to residues.

#### **Question 2: Planter dust emissions**

The Guelph research team was the only CDRC group studying the effectiveness of the BFA lubricant. They reported that:

- The BFA (Bayer's alternative lubricant) reduced the amount of dust emitted from vacuum planters compared to conventional lubricants (*i.e.*, talc and/or graphite) by 67.5%.
- The concentration of neonicotinoid residues in the BFA lubricant dust escaping from the vacuum planter was on average 3.7-fold higher than the conventional lubricants.
- The use of BFA at the recommended application rate reduced the quantity of neonicotinoid active ingredient escaping from vacuum planter exhaust by 28% by comparison with the conventional lubricants applied at rates the cooperators were accustomed to using.
- This study compared the BFA fluency powder with what the cooperator used routinely. During this field test, the manufacturer-recommended levels of talc/graphite lubricant (~1 cup per seed unit, defined as 80,000 kernels or 1 bushel) were followed at only one of the 9 test sites. The amount of lubricant in the test was left up to the discretion of the farmer and ranged from 0.06 cup per seed unit to 1.00 cup per seed unit.
- Residues of clothianidin and thiamethoxam were the most frequently detected neonicotinoid residues.

The CDRC is eager to have a second year of data on the comparison of the BFA lubricant to talc and graphite. Also, since only one team made the assessment, CDRC looks to increase the number of research teams assessing this alternative lubricant. Additional teams in the second year will benefit from the experience of the first year. There are several factors in the lubricant report that need to be understood more fully.

The 2013 results for the measurement of exhaust and AI concentration in the lubricants vary widely within the 9 sites. Measurement of planter exhaust is difficult as it requires clean machinery at each measurement and precise collection methods engineered to keep pressure levels constant. Refined methodology is needed so that the exhaust can be measured more consistently.

The evidence and toxicological analysis of dust on the "field panels" will provide data about the concentration as well as the movement of the BFA lubricant vs. talc. The CDRC looks forward to seeing these data.

In applying the research results to practical management steps, the issue of concentration vs. dose can be confusing. With respect to the current study, what is important from a toxicological and risk assessment perspective is the dose applied to the environment and taken up by a species of concern. In pesticide risk assessments the assessor determines the amount of active ingredient

applied per unit area, such as pounds per acre or grams per hectare. What matters is the amount of pesticide active ingredient released and deposited per unit area, and that is reduced with the use of the BFA lubricant. The CDRC anticipates that forthcoming field plate data sets will contribute needed data to illuminate this.

#### **Remaining questions**

1. What kinds of plantings can be added to corn landscapes that would be timed correctly, attractive enough, and sufficiently removed from the exposure area that could provide forage resources for honey bees and other pollinators?

2. How would the BFA compare to conventional lubricants if they were compared according to the manufacturer's recommended level of lubricant (the current research compared a variety of levels according to the discretion of the farmer)?

3. What impact would removing potentially attractive floral resources in and adjacent to corn fields have on the potential exposure of bees to neonicotinoid residues? What impact would this practice have on the sufficiency and availability of forage for bees over the growing season?

4. Could untested mechanical planter modifications such as foils or deflectors (some of which appear to have had good trials in Europe) have a positive impact in terms of reducing exhaust fan dust generated during planting in North America?

5. Will the implementation of specific drift mitigation measures by the farmer reduce exposure?

6. Contaminated pollen may have a different effect on honey bees than contaminated nectar, and it is unclear how dust may contribute to residues in nectar; how can these differences be taken into account when evaluating ways to reduce exposure routes?

7. How does the implementation of best practices ultimately affect the health of honey bees?

8. Will recommendations from these studies reduce potential honey bee exposure, and what impact will they have on other pollinating species? What impact will they have on floral/forage availability for bees and other pollinators?

9. How will technical solutions that reduce seed treatment insecticide dust emissions and drift distance change the recommended best practices for growers, beekeepers and others?

#### **Provisional recommendations**

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 have been vetted 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.

#### Next Steps

The CDRC's process of collaborative oversight of practical research through multiple institutions has been complex but extremely rewarding. All stakeholders have shared the responsibility for transparency, open deliberation, and unbiased assessment throughout 2013. They will now begin the tasks of follow-up evaluation, information dissemination, and adaptive management.

#### Timetable

1. Review and agree on this report (1/29/14)

2. Disseminate this report through a press release and web site posting (1/30/14) <u>http://www.pollinator.org/PDFs/CDRC\_PR2014.pdf</u>

3. Determine exact questions to be studied during the 2014 planting season (1/30/14)

4. Prepare RFP and/or solicit research proposals from current research teams (1/30/14) <u>http://www.pollinator.org/PDFs/CDRC\_RFP2014.pdf</u>

5. Receive grant applications (2/20/14)

6. Award grants (2/28/14)

#### ADDENDUM Received March 30, 2014

Addendum to CDRC final report including data, statistical analyses and errata not completed when the final report was submitted in December 2013 for the Corn Fugitive Dust Study U of Guelph – 2013

Prepared by: Dr. Art Schaafsma on behalf of Tracey Baute OMAF/RA And Drs. Cynthia Scott-Dupree, Dr. Yingen Xue, Dr. Victor Limay-Rios, University of Guelph

# A) Neonic quantities recovered on dust traps in corn fields during planting, comparing BAYER's new fluency agent and the Growers standard lubricant.

**Results:** A few points about the methods for this study have been debated. The first was around the rate of lubricant used in the study. Some argue that we should have compared the BFA at 1/8 cup per seed unit to the rate of standard fluency agent recommended by the equipment manufacturer. We however chose to compare the BFA at the recommended rate of 1/8 cup per seed unit with the rate of lubricant the producer was accustomed to using with his planter under the conditions of planting that prevailed. We were interested in measuring the reduction of fugitive dust relative to standard practice rather than the recommended practice to ascertain the incremental benefit of using the BFA relative to standard practice in the regions where bee kills have been reported.

Second, the amount of cleaning for each planter that took place between fields was contested. It was impossible to clean the commercial planters to the degree where one could guarantee there was no cross contamination. Our protocol was the same for every field, and the lubricant we started with for each cooperator (two fields per cooperator) was randomized each time. Before planting and after arriving in the field, the vacuum fan on the unit was run at full speed, with the planter empty, until no dust was visible coming from the exhaust. Planter boxes or central hoppers all were filled by auger; with the appropriate lubricant metered in as they were filled. Producers then planted the headlands of the test field and the first 100 m of the field where the dust sticky traps were planned to be installed. These steps allowed us to ensure all the planters were equally conditioned before running our tests. We assume, following this protocol, that the contamination between lubricants on the large scale under which we operated was similar for all tests, but contributed only a small portion to the error. We were not able to test this contribution.

Third, there was considerable discussion about whether data from the two fields near each apiary (one planted using BFA lubricant, and the other using the grower's standard) could in fact be treated as paired treatments, even though they were both planted with the same seed lot, using the same planter, by the same operator. The immense scale of the fields (each 50 to 100 acres in size) made it

logistically impossible to plant both treatments on the same day. Most of the fields were planted on two consecutive days but in some case more than one day elapsed before the second field could be planted. This resulted in uncertainty whether it was appropriate to calculate reduction in fugitive dust based on paired results or by overall means. This debate was most contentious when looking at the data for dust collected from the exhaust port of the vacuum planter.

In employing the BFA lubricant, calculated on the basis of overall means, we reported a 46% reduction in the amount of dust escaping the exhaust port across all tests, a 2.7 fold increase in the concentration of neonic in the dust collected, resulting in a 21% decrease in the amount of neonic active ingredient escaping from the planter exhaust. Using the same data set, calculating differences for each pair of fields, the reduction in dust was 67.5%, there was a 3.7 fold increase in the neonic concentration found in the dust collected, resulting in a net reduction by 28% in the amount of neonic active ingredient escaping the planter exhaust.

Finally, the meaning of the quantity of dust and its concentration collected by the vacuum cleaner bag installed over the vacuum fan exhaust port was questioned. The impact of the filter bag on vacuum pressure, air flow or planter performance was not considered, but was assumed to be the same for each pair of fields because all the planter and operational settings were the same and the only variables that differed were the field, date (planting environment) and the lubricant used. The planter, the sampling bag, how it was affixed to the exhaust port, the planter operator and planter settings were all consistent between field pairs. To our knowledge this is the first attempt to quantify dust and the concentration of neonic active ingredient in this dust, escaping directly from the planter exhaust, which is the point source for the plume of fugitive dust being investigated. This allowed a general approximation by mass balance (Table 1) of the proportion of neonic insecticide intended to be applied to the field, which is escaping the planter in dust, followed by the proportion of dust (19.4 %) escaping the planter that is captured 100 m downwind from the test area on the vertical dust sticky samplers fixed at the 2 m height. We presume this dust was of fine particle size, suspended in the wind, and capable of being carried a considerable distance. This detail is important to consider in light of the findings in other parts of our study which show that an important site of potential exposure to honey bees is in tree forage adjacent to or near the planted field.

	a.i.	recovery
Source	(mg/ha)	(%)
Seed treatment (applied)	20729.3	
dust from planter exhaust (% of applied)	3.1	0.015
dust leaving the field (% of applied)	0.6	0.003

dust leaving the field (% of exhausted)

**Table 1.** Mass balance calculation of neonicotinoids leaving the field during planting. Data for dust leaving field were taken from the 100 m traps shown in Table 3.

We chose to analyze the data using SAS Proc mixed , with lubricant, orientation of dust trap, distance, and wind speed as fixed effects; and growers and humidity as random effects stepwise starting with a full model containing all possible interactions and then removing non-significant terms . The ANOVA for the reduced model is shown in Table 2. All other effects were not significant at P=0.05.

0.6

19.4

**Table 2.** SAS Proc mixed reduced model, with lubricant, orientation of dust trap, distance, and wind speed as fixed effects; and growers and humidity as random effects. Non-significant terms were removed stepwise from the full model. (lub = lubricant, BFA/conv, dis = distance from test area, 0/10/50/100m, orient = orientation of sampler, vertical/horizontal, wind = wind velocity class, low/med/high)

71				
Effect	Num DF	Den DF	F Value	Pr > F
lub	1	393	6.48	0.0113
dis	3	393	38.46	<.0001
orient	1	393	4.69	0.0309
wind	2	393	14.52	<.0001
dis*wind	6	393	5.77	<.0001
orient*wind	2	393	6.36	0.0019
lub*dis*wind	11	393	2.03	0.0247

#### **Type 3 Tests of Fixed Effects**



Figure1. The main effect of seed lubricant on neonic capture

Over all the samplers deployed there was a significant reduction in ai recovered when the BFA lubricant was used (Fig 1). This comparison is not informative because of the multitude of confounding factors and interactions included in this comparison.

The main effect of trap distance was clear, suggesting that most of the dust escaping was captured near the planter (Fig 2) implying that most of the material has a larger particle size and settles on the planted surface within the field. What happens to this material after planting is unknown.



Figure 2. The main effect of trap distance from planter origin

Over all the samplers deployed, samplers oriented vertically at 2m above the soil surface trapped slightly more neonic residues than samplers oriented horizontally at 30 cm above the soil surface (Fig 3). Assuming vertical samplers at 2 m were more inclined to capture dust carried down wind, and those oriented horizontally low to the ground favoured capturing dust that was settling, there was slightly more dust moving in the wind than settling within the area of sampling.



Figure 3. The main effect of dust trap orientation.



Figure 4. The main effect of wind speed

As expected, wind was an important factor in the movement of fugitive dust (Fig 4), the higher the wind velocity the more dust moved. Likewise the greater the wind velocity the further the dust travelled (Fig 5).



Figure 5. Interaction of trap distance from planter origin and wind speed

At the higher wind velocities more dust was captured by vertically-oriented traps than those oriented horizontally (Fig 6) while under moderate and low wind velocity similar quantities of dust were captured. This observation suggests that, at lower wind velocities, there is seems to be an equilibrium between dust particles moving in the wind and those settling but when wind velocity increases the suspension of particles is favoured.



Figure 6. Interaction of dust trap orientation and wind speed



Figure 7. Interaction of seed lubricant, distance from planter origin and wind speed

At moderate to high wind velocities significantly less neonic ai was observed across both trap orientations (Fig 7) when the BFA lubricant was employed by comparison with the conventional lubricant at the collection points nearest the test area (0 and 10 m). Differences between lubricants disappeared at samplers located beyond 50 m of the test area. These results suggest perhaps the impact of using BFA is on reducing the

amount of larger particles released, and those escaping tend to settle out more guickly. What is troublesome is that there is little impact of employing the BFA on the smaller dust carried by wind, longer distances. Little is known about the impact of this fraction of dust leaving the field on the exposure to honey bees foraging in the trees adjacent to or near planted corn fields. We remind the reader that we measured dust escaping the planter and caught by traps during a defined period that was standardized for all subject fields (the same number of rows were planted in each field during each test). The fields were very large and we did not leave the samplers up waiting for the whole field to be planted. Therefore interpreting the data relative to what the "edge" of the field is should be approached with caution. In the end, about 20% of the contaminated dust escaping from the planter exhaust was captured at 100 m from the test area at the 2 m height on samplers oriented vertically, during the period of planting that we measured. Nothing can be said about how much dust would have been captured after the entire field was planted, nor can anything be said about the dust that landed on the soil surface that might have been re-suspended by wind erosion following planting. Neither of these contributors to exposure was considered in this study.

**Table 3.** Neonic deposition rates  $(\pm$ SD) on sticky traps deployed downwind from corn planting when either BFA or a conventional lubricant was employed. Data were segregated according to wind velocity measured during planting into three classes of low, moderate and high. As a result data from proximal fields were not true pairs.

		Distance	Dust trap orientation				
Wind	Lubricant	(m)		Horizontal		Vertical	
		(11)	n	neonic (ng/cm^2)	n	neonic (ng/cm^2)	
		0	12	0.99±0.20	11	2.10±0.72	
	RΕΔ	10	12	0.39±0.20	12	0.71±0.31	High
	n=95	50	12	0.31±0.12	12	0.62±0.25	>10 km/h
H		100	12	0.15±0.05	12	0.28±0.09	
n=142		0	6	1.92±0.86	6	2.71±0.94	
	Conventional	10	6	0.68±0.21	6	1.78±0.61	
	n=47	50	6	0.43±0.15	6	0.69±0.27	
		100	5	0.18±0.08	6	0.33±0.16	
		0	9	0.59±0.18	8	0.82±0.30	
	RFΔ	10	9	0.23±0.08	9	0.33±0.14	Medium
	n=71	50	9	0.26±0.11	9	0.17±0.08	5-10 km/h
M n_167	7	100	9	0.18±0.09	9	0.27±0.11	
11=107		0	12	1.55±0.49	12	1.58±0.26	
	Conventional	10	12	0.32±0.07	12	0.30±0.07	
	n=96	50	12	0.44±0.23	12	0.19±0.06	
		100	12	0.30±0.15	12	0.30±0.14	
		0	6	0.82±0.33	6	0.34±0.14	
		10	6	0.07±0.02	6	0.06±0.03	Low
	BFA n=48	50	6	0.12±0.05	6	0.27±0.17	<5 km/h
L n_120		100	6	0.03±0.02	6	0.05±0.02	
11=120		0	9	0.24±0.08	9	0.16±0.04	
	Conventional	10	9	0.04±0.01	9	0.08±0.03	
	n=72	50	9	0.04±0.01	9	0.03±0.01	
		100	9	0.07±0.03	9	0.06±0.02	

**Table 4.** Percent reduction in neonic ai trapped on sticky trap dust samplers placed at various distances downwind from where the lubricants were employed during planting.

Wind velocity	% reduction		
	Horizontal	Vertical	Distance (m)
	48.4%	22.5%	0
High	42.6%	60.1%	10
>10 kph	27.9%	10.1%	50
	16.7%	15.2%	100

	% reduction		
	Horizontal	Vertical	Distance (m)
	61.9%	48.1%	0
Medium	28.1%	-10.0%	10
5-10 kph	40.9%	10.5%	50
	40.0%	10.0%	100

The BFA lubricant seemed to be more effective in wind velocities ranging from 5 to 10 kph where the BFA reduced the neonic captured on horizontal traps positioned nearest to the test size (0 m) by as much as 62%. We do not know much, however, about the 20% of the dust escaping from planter exhaust, captured on the vertical trap at the 100 m mark.

**Table 5.** SAS Proc mixed reduced model, with lubricant, orientation of dust trap, distance, and humidity as fixed effects, growers and wind speed random effects. All non-significant terms were removed stepwise from the full model.

# Type 3 Tests of Fixed Effects Effect Num DF Den DF F Value Pr > F lub 1 411 4.26 0.0397 dis 3 411 38.01 <.0001</td> orient\*hum 3 411 2.30 0.0765

			Dust trap orientation				
humidity	Lubricant	Distance		Horizontal	Vertical		
Training	Lubricant	(m)	n	neonic (ng/cm^2)	n	neonic (ng/cm^2)	
		0	21	0.99±0.15	20	1.41±0.44	
	BFA	10	21	0.32±0.11	21	0.52±0.19	
High	n=167	50	21	0.30±0.08	21	0.36±0.15	
<u>&gt;</u>		100	21	0.14±0.05	21	0.26±0.07	
50%	Conventional n=72	0	9	1.56±0.70	9	0.85±0.38	
n=239		10	9	0.19±0.10	9	0.18±0.09	
		50	9	0.37±0.31	9	0.06±0.02	
		100	9	0.09±0.04	9	0.07±0.04	
		0	6	0.24±0.07	5	0.68±0.21	
	BFA	10	6	0.07±0.02	6	0.16±0.05	
Low	n=47	50	6	0.09±0.06	6	0.50±0.24	
< 50%		100	6	0.11±0.05	6	0.11±0.07	
		0	18	1.01±0.32	18	1.61±0.39	
n=190	Conventional	10	18	0.37±0.09	18	0.74±0.26	
	n=143	50	18	0.28±0.07	18	0.34±0.11	
		100	17	0.26±0.11	18	0.30±0.11	

When data were classified into two groups according to relative humidity recorded at the time of planting (at/above or below 50% RH) there was a slight interaction (p=0.0765, Table 5) between trap orientation and humidity. Total trap deposition of neonic ai at higher humidity was equally distributed between horizontal traps (3.96 ng) and vertical traps (3.71 ng). At p=0.0765, at lower humidity more neonic ai was deposited on vertical traps (4.46 ng) placed at 2 m above the soil surface than on horizontal traps (2.43 ng) placed at 30 cm. These results suggest that more dust is prone to escape from planted fields under less humid conditions. No other interactions were detected

# B) Dead bee counts, concentration of total neonicotinoid insecticide in/on dead bees, and in pollen, collected at nine apiaries associated with the corn fields in A).

**Results:** At the time of the first report, analytical work was complete for only the first two sample periods. The updated complete results are presented in Table 6.

When analyzing bee pollen at the outset, we had difficulty locating a neonicotinoid free source of bee pollen to develop a calibration curve which tended to underestimate the levels at higher concentrations (see section C Errata). A sample of neonicotinoid-free pollen was later obtained allowing the development of a more appropriate calibration curve. All the values in Table 6 for pollen were derived using the new calibration curve. Several values (highlighted in blue) are much higher from the first data report. Two are now reaching ppm. This result is troublesome and we are working with BAYER chemists to verify these results through a double blind interlab study. It is important to note that the two highest values reported coincide wit the two apiaries that PMRA declared as confirmed bee kill sites. Furthermore these purported high levels were recorded from pollen collected before our two test fields were planted. We have no information on the locations and dates of planting for other corn fields planted near these two bee yards.

All beeyards experienced a varying amount of dead bees at the hive entrances throughout the study period (Table 6). No clear pattern of increased dead bee counts related to corn planting was detected (P<0.05), although dead bee counts appeared to drop later in the season by wk11. Neonicotinoid residues were found in all dead bees collected from these traps at all bee yards during all sample periods. Mean neonicotinoid concentrations in these dead bees across all bee yards were higher during the sample periods two and three weeks after corn planting, returning to levels lower than those measured during the week before planting.

Pollen collected from pollen traps placed in all bee yards during all sample periods carried detectable levels of neonicotinoid insecticide residues (Table 6). Unusually high levels of neonicotinoid residues were measured at beeyards 2 and 7 during the preplant sampling period. (See section C Errata). While these two bee yards were the only ones of the nine bee yards monitored declared by PMRA to have suffered a neonicotinoid-related bee kill. Levels in pollen at these two sites were also elevated relative to the other 7 sites samples two days after planting. These two sites are the subject of further interlab confirmation especially for the two samples collected during the first sampling period with unexpectedly high residues. Residues in pollen stabilized to between approximately 4 and 5 ppb at all bee yards beyond the sample period 2 weeks after planting.

Table 6. Dead bees recovered (n/trap), neonicotinoid insecticide concentration in whole dead bees (ppb) and
neonicotinoid concentration in bee pollen (ppb) collected from 2 hives across 6 sampling periods before, during and after
planting of two corn fields near each of nine apiaries in SW Ontario in 2013.

	Number of dead bees					Neonicotinoid concentration						Neonicotinoid concentration									
Bee yard	recovered per trap					in dead bees					in bee pollen**										
	preplant	d2	d6	w2	w3	w6	w11	preplant	d2	d6	w2	w3	w6	w11	preplant	d2	d6	w2	w3	w6	w11
1	37.5	28.3	18.5	18.8	7.8	14.5	8.0	1.9	3.4	6.9	78.7	56.3	1.1	1.7	6.9	37.7	25.5	8.1	2.9	3.1	2.8
2	17.3	29.0	22.5	21.0	15.0	15.8	8.8	2.0	2.5	2.5	3.7	2.9	0.9	1.2	1249.8	41.1	15.7	6.5	8.4	3.3	3.6
3	70.5	48.8	6.3	101.3	16.0	50.8	25.0	2.4	7.8	5.0	7.1	8.8	3.1	0.9	10.9	62.0	$ND^1$	16.6	4.6	3.9	4.3
4	12.8	17.0	40.8	1.3	13.5	3.8	6.3	3.8	4.8	5.0	11.3	2.8	4.5	4.2	5.8	13.8	13.1	9.4	3.5	3.2	8.3
5	12.8	46.0	40.5	18.5	4.3	12.5	2.0	3.2	5.0	1.7	18.2	21.1	0.7	10.8	8.0	13.6	12.0	4.1	2.7	3.0	4.0
6	4.8	0.8	34.5	29.3	64.3	10.5	50.0	1.7	6.1	1.0	44.9	9.8	3.0	2.9	1.9	20.1	28.5	3.3	2.1	14.2	2.7
7	13.8	14.3	22.0	10.5	8.3	2.8	3.8	8.2	17.4	19.8	3.8	3.4	1.2	0.5	816.2	39.5	18.5	3.9	4.4	3.4	3.0
8	17.0	32.0	77.8	34.0	29.0	38.0	15.5	8.2	5.1	4.0	64.7	3.0	0.9	1.2	11.7	38.6	$ND^{2}$	6.0	4.0	2.6	4.5
9	2.5	$ND^3$	35.8	17.0	5.0	13.3	12.0	4.9	$ND^4$	3.5	25.5	4.3	2.3	0.3	7.9	25.8	14.6	5.5	2.5	11.6	3.2
mean	21.0	27.0	33.2	27.9	18.1	18.0	14.6	4.0	6.5	5.5	28.7	12.5	2.0	2.6	7.6	32.5	18.3	7.1	3.9	5.4	4.0
P<0.05*								а	а	a	b	ab	a	a	а	b	b	a	а	а	a
Min	2.5	0.8	6.3	1.3	4.3	2.8	2.0	1.7	2.5	1.0	3.7	2.8	0.7	0.3	1.9	13.6	12.0	3.3	2.1	2.6	2.7
Max	37.5	48.8	77.8	101.3	64.3	50.8	50.0	8.2	17.4	19.8	64.7	56.3	4.5	10.8	1249.8	41.1	28.5	16.6	8.4	14.2	8.3
ND <sup>1</sup> : No neonicotinoid data available. Only 0.1g bee pollen was collected. This quantity was below our LC-MS/MS validation limit.																					
ND <sup>2</sup> :No data available. No bee pollen was collected due to bad weather conditions.																					
ND <sup>3</sup> & ND <sup>4</sup> : No data available. Data sheet recorded 1, 25, 5, and 0 dead bees in the four traps, respectively.																					
However, only one sample bag with one dead bee (0.04g) was found. This quantity was below our LC-MS/MS validation limit.																					
* means within parameters with the same or no letter below are not different (P<0.05, LSmeans Tukey Kramer)																					
** ANOVA using Proc mixed run with preplant values for Beeyards 2 and 7 removed																					

#### C) ERRATA: Erratum: Recalibration of clothianidin values using an improved matrix-matched calibration curve with deuterium-labeled internal standard

This erratum only refers to the determination of clothianidin in bee pollen. In planta, thiamethoxam is quickly metabolized, with clothianidin being the predominant neonicotinoid found (1). Finding samples with low or no thiamethoxam contamination to be used as a "blank matrix" for analytical purposes is relatively easy thus no correction in the calculated values was needed. Conversely, clothianidin contamination was ubiquitous in the pollen that we sampled thus making it difficult to find an appropriate "blank" sample for analytical determination. From a chemical analysis point of view, the appropriate blank ideally should contain none of the chemical(s) of interest. It only stands to reason that if all the standards are contaminated with significant, unwanted levels of the analyte(s), then estimating concentrations in ordinary samples will be much more difficult (2). Fig 1 (A) shows the original calibration curve used to estimate clothianidin in our original report. The pollen "blank" sample used for calibration was contaminated with chlothianidin [Fig 1 (A)], producing a poor linearity (r=0.3891) resulting in under estimating values, especially those in the higher concentration range. Recalibration was achieved by sourcing a suitable "blank" sample [Fig 1 (B)] and further adjusting for traceable levels of clothianidin still present in the sample. The final calibration curve showed good linearity [(r=0.9956), Fig1 (C)], closer to the linearity reported in our recovery experiment [r=0.9865), Table 2, page 39 in our report]. Total quantity of clothianidin and thiamethoxam were used to represent the neonicotinoid resides in our report thus the values presented in Table 16 page 28 should be corrected as follows:

De e vord	Number c	of dead be	e per trap	Neonicot	inoid conc	entration	Neonicotinoid concentration			
Bee yaru	preplant	d2	d6	preplant	d2	d6	preplant	d2	d6	
1	37.5	28.3	18.5	1.9	3.4	6.9	6.9	37.7	25.5	
2	17.3	29.0	22.5	2.0	2.5	2.5	1249.8	41.1	15.7	
3	70.5	48.8	6.3	2.4	7.8	5.0	10.9	62.0	$ND^{1}$	
4	12.8	17.0	40.8	3.8	4.8	5.0	5.8	13.8	13.1	
5	12.8	46.0	40.5	3.2	5.0	1.7	8.0	13.6	12.0	
6	4.8	0.8	34.5	1.7	6.1	1.0	1.9	20.1	28.5	
7	13.8	14.3	22.0	8.2	17.4	19.8	816.2	39.5	18.5	
8	17.0	32.0	77.8	8.2	5.1	4.0	11.7	38.6	ND <sup>2</sup>	
9	2.5	ND <sup>3</sup>	35.8	4.9	ND <sup>4</sup>	3.5	7.9	25.8	14.6	
mean	21.0	27.0	33.2	4.0	6.5	5.5	235.5	32.5	18.3	
Min	2.5	0.8	6.3	1.7	2.5	1.0	1.9	13.6	12.0	
Max	37.5	48.8	77.8	8.2	17.4	19.8	1249.8	41.1	28.5	

Table 16. Dead bee count an	d neonicotinoid (	concentration i	n dead bee	pollen
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ND<sup>1</sup>: No neonicotinoid data available. Only 0.1 g bee pollen was collected. This quantity was below our LC MS/MS validation limit.

ND<sup>2</sup>: No data available. No bee pollen was collected due to bad weather conditions.

ND<sup>3</sup> & ND<sup>4</sup>: No data available. Data sheet recorded 1, 25, 5, and 0 dead bees in the four traps, respectively. However, only one sample bag with one dead bee (0.04 g) was found. This quantity was below our LC-MS/MS validation limit.

This correction materially impacts only two data points in bee yards 2 and 7 for pollen collected during the "preplanting" collection period. Both of these yards were declared by PMRA as confirmed bee kills due to neonicotinoid exposure. The high level of neonicotinoid insecticide found in the pollen at these two sites before we initiated our study on dust drift in nearby corn fields must have come from a source other than from any of our activities.

References:

- (1) Nauen RE IK U, Salgado VL, Kaussmann M. Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. Pestic Biochem Physiol 2003; 76:55I69.
- (2) Coleman D, Vanatta L. Statistics in analytical chemistry: part 40 blanks. American Laboratory, October, 2010. http://www.americanlaboratory.com/914-Application-Notes/1114-Part-40-Blanks/

**Fig 1.** Standard calibration curves generated for the determination of clothianidin by LC-MS/MS using clothianidin-d3 (N-methyl-d3) as internal standard.



#### Ohio State Update: Update on pollen identification using high-throughput sequencing

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We have developed a high throughput sequencing method capable of detecting some plant taxa present in bee collected pollen. This method involves a DNA metabarcoding approach and utilizes the ITS2 region of the ribosomal sequence in the plant nuclear genome. We used an ITS2-specific primer set which amplifies across a broad spectrum of plant taxa has been developed.<sup>1</sup>Plastid genes are often used in plant taxonomy, but they are unsuitable as plastids are rarely present in pollen grains.

Honey bee pollen was collected with a pollen trap. First, DNA was extracted from the mixed pollen sample using a previously published DNA extraction protocol.<sup>2</sup> Next, ITS2 was amplified using PCR in accordance with the methods of Chen et. al<sup>1</sup> to produce amplicons of approximated 500 bp. After amplification, libraries were constructed and paired ends sequenced on the Illumina Miseq platform. Lastly, sequence data was aligned to a library of ITS2 sequences from Genbank using standalone BLAST. The taxon associated with the best match for each read above a cutoff of e = 1E-20 was assigned. BLAST output was analyzed with MEGAN version 5 to assign taxonomic designations to all reads. *Table 1* below shows the output data for a pollen sample collected in Central Ohio on April 23<sup>rd</sup> 2013

Initial steps have been taken to investigate the accuracy of sequencing identification. For comparison, we measured the presence and relative abundance of different pollen types in our samples by traditional microscopic palynology. Generally, the diversity of morphological features was suitable to identify pollen to family level. Having this morphological data enabled us to compare results of the molecular metabarcoding approach to investigate its accuracy both from a qualitative and quantitative perspective. From this cross validation, we have noted both benefits and drawbacks of sequencing-based pollen identification.

Some families of plants appear to have ITS2 regions which are not amplified using our primer set. Additionally, quantitative estimates of the abundance of plant taxa in pollen are substantially different from morphological determination. From *Table 1* it is clear that the quantities of various taxa detected in the morphological approach correlate poorly with those detected in the molecular approach. This is likely a result of the nature of the plant ribosomal cassette, which contains ITS2, repeats differently across plant taxa. Despite disadvantages, this molecular approach is a powerful tool that does display advantages to the morphological approach. Determining plant species by morphological analysis is quite laborious and is inherently subjective. Molecular analysis provides greater confidence at the genus level, though species designations may be incorrect. *Table 2* shows the species level composition of the April 23<sup>rd</sup> sample. Having such species lists has enabled us to more accurately classify some of the morphologically ambiguous and/or misidentified pollen types in our samples. Lastly, though the molecular method cannot yet be used quantitatively, we now have enough data to make an attempt at calculating correction coefficients at the species level, which would allow us to use molecular data quantitatively in the future.