FIELD STUDY RELIABILITY: A HONEY BEE & NEONICOTINOID PERSPECTIVE

Dr. Cynthia Scott-Dupree
School of Environmental Sciences
University of Guelph

UNIVERSITY of GUELPH

ses
school of environmental sciences

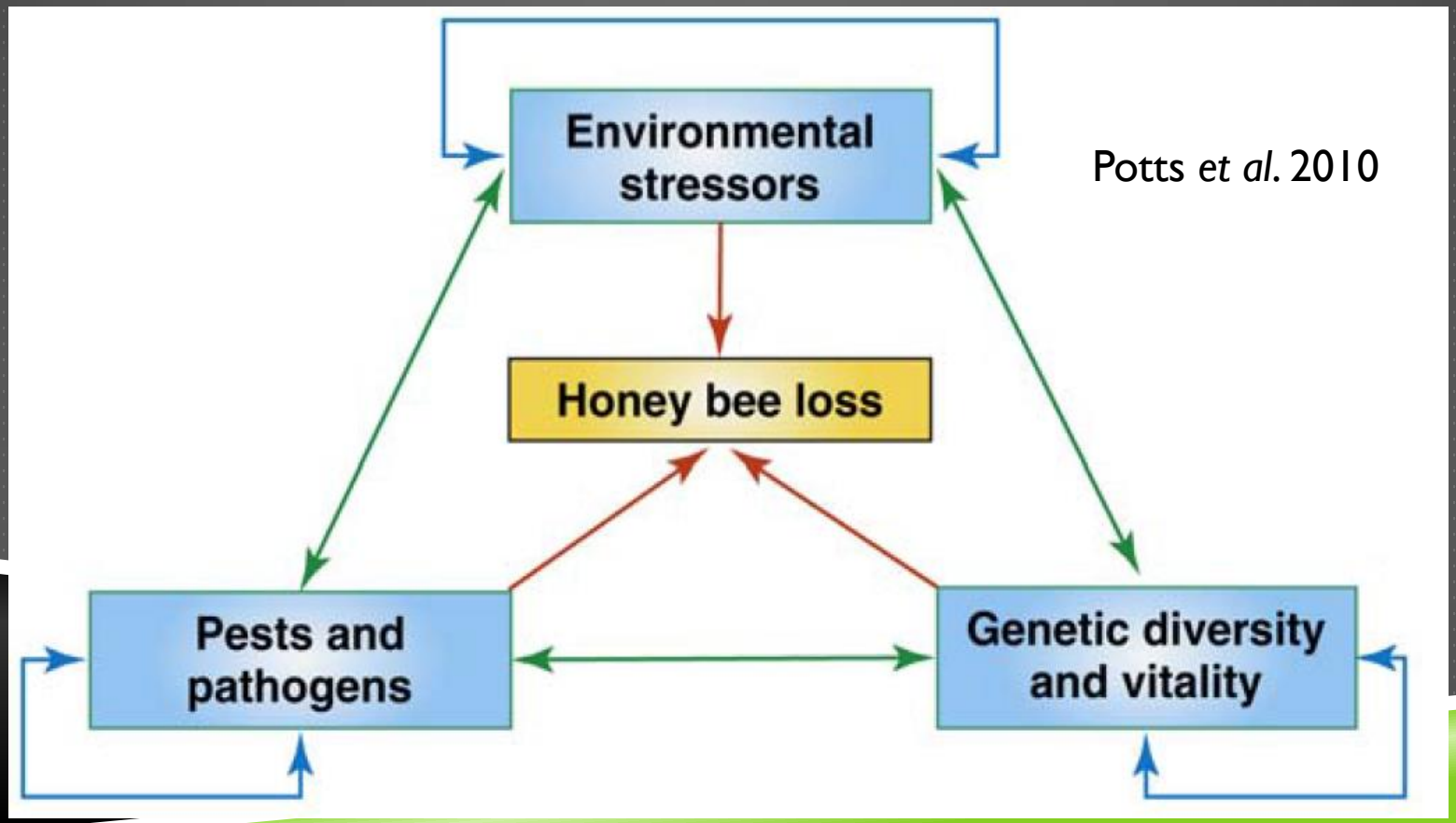
COLONY COLLAPSE DISORDER

Dr. Jeff Pettis (USDA-ARS Research Scientist)

"…..CCD has been a 1,000-piece jigsaw puzzle, the best I can say is that a lot of pieces have been turned over. The problem is that they have almost all been blue-sky pieces – frame but no center picture."

CCD OR BEE DECLINE

MANY AGREE THIS IS THE PROBLEM



Potts *et al.* 2010

BEE DECLINE – LIKELY CAUSES

Multi-factorial:

- Pests and diseases
- Varroa mites and associated viruses
- Feeding methods
- Nutrition
- Monocultures
- Management – migratory beekeeping
- Queen source and genetic diversity
- Chemical medicants placed in colonies by beekeepers
- Pesticides – neonicotinoid seed treatments

LAB TO FIELD STUDY LINKAGES

- Laboratory-based studies garnering lots of media attention
- Field-based studies provide estimates of exposure that are more realistic

Tier 1 → **Tier 2** → **Tier 3**

Models/Lab

Strict lab tests
Well defined
Individual bees

Semi-field

Tunnel (enclosed) tests
Well defined, 'quasi-real'
Whole colonies (small)

Field

Realistic conditions
Hard to control, higher variability
Whole colonies (large)

CANADIAN FIELD STUDY HISTORY…

- Scott-Dupree and Spivak – 2000 study, canola
- Cutler and Scott-Dupree 2005 – GLP study canola
  - Long-term impacts of clothianidin seed-treated canola on honey bees; J Econ Entomol 100:765-772 (2007)
  - No effects on colony honey yield, colony weight gain, adult or brood production, dead bees
  - Clothianidin in 21 samples, BUT… some control (3) nectar samples contained clothianidin
  - Some questioned the validity of the experiments; EPA later determined the study to be supplementary
- Cutler, Scott-Dupree et al. 2012 – GLP study canola

LARGE SCALE FIELD STUDY EXAMINING HONEY BEE EXPOSURE TO CLOTHIANIDIN SEED-TREATED CANOLA IN ONTARIO (2012)

Dr. Cynthia Scott-Dupree (Univ. of Guelph)

Dr. Chris Cutler (Dalhousie Univ.)

Collaborators: M. Sultan, A. McFarlane and L. Brewer

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GLP HONEY BEE STUDY 2012

- Objectives:

1. To determine if there are adverse effects on honey bee colonies when they are placed in clothianidin seed-treated canola fields during bloom, an apiary following bloom, and monitored the following spring; and

2. To quantify residues of clothianidin and its toxic degradates in pollen, nectar, honey, beeswax and bees from colonies exposed to clothianidin seed-treated canola fields.

METHOD MODIFICATIONS

2005 GLP STUDY

- Colony numbers - 32 colonies, 8 fields (4 control, 4 treatment)
- Field Size – 1 ha
- Distance between - ≥ 250m
- Pollen analysis – none
- Brood and adult assessment – visual estimated transposed to actual measure
- Apiary after canola – partially isolated
- Size of crew - 4

2012 GLP STUDY

- Colony numbers – 40 colonies, 10 fields (5 control, 5 treatment)
- Field Size – 2 ha
- Distance between – ≥ 10 km
- Pollen analysis – bee collected pollen analyzed to species
- Brood and adult assessment – digital photography, IMAGEJ software analysis
- Apiary after canola – completely isolated
- Size of crew - 18

OTHER ASSESSMENTS

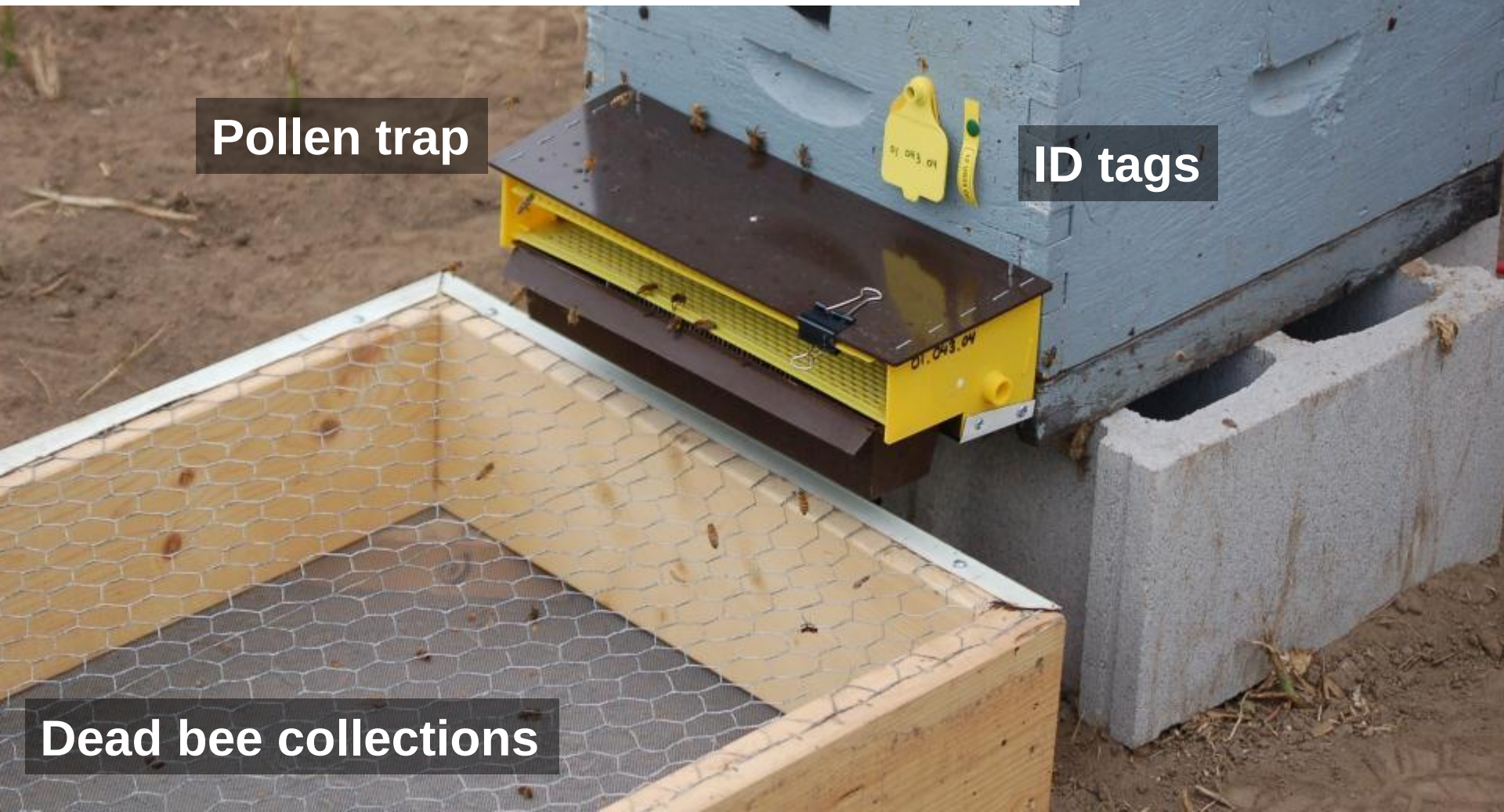
- Weight gain while in canola
- Honey yield – from July to mid-October
- Crop "ground truthing" – by plane (aerial truthing)
- Adult mortality – "Drop Zone" dead bee traps
- Pest, disease and queen assessments
- Nectar, honey, pollen and beeswax samples for residue analysis
- Samples analyzed for clothianidin residues LC/MS-MS –

  USDA National Science Laboratory; reanalyzed BCS

Colony Numbers

Field Size and Location of Colonies

High use of the test sites by foraging bees pollen indentification revealed 88% canola pollen during peak bloom. No other canola available within 10 km.

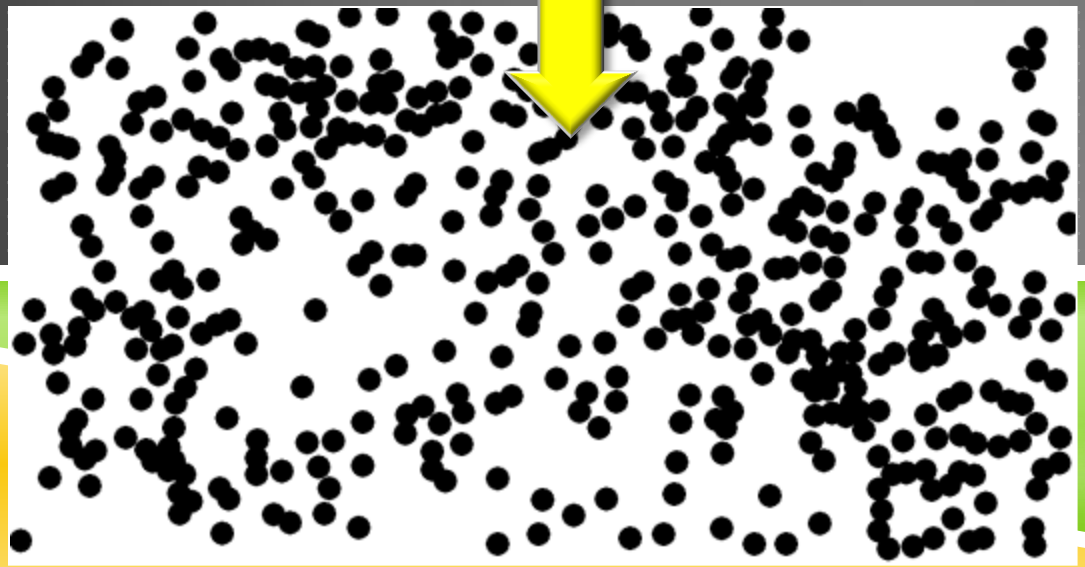


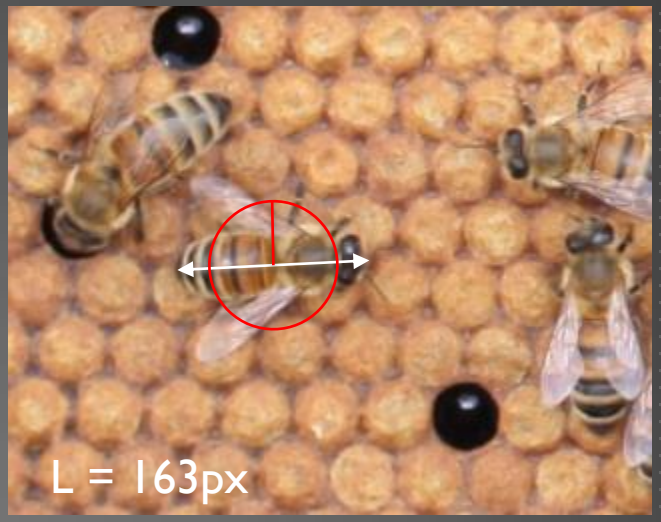
Adult and Sealed Brood Assessments

- Digital photography
- IndiCounter software

Adult Measurements – Image Processing

Automatic Processing using IndiCounter©





Area covered by bee:

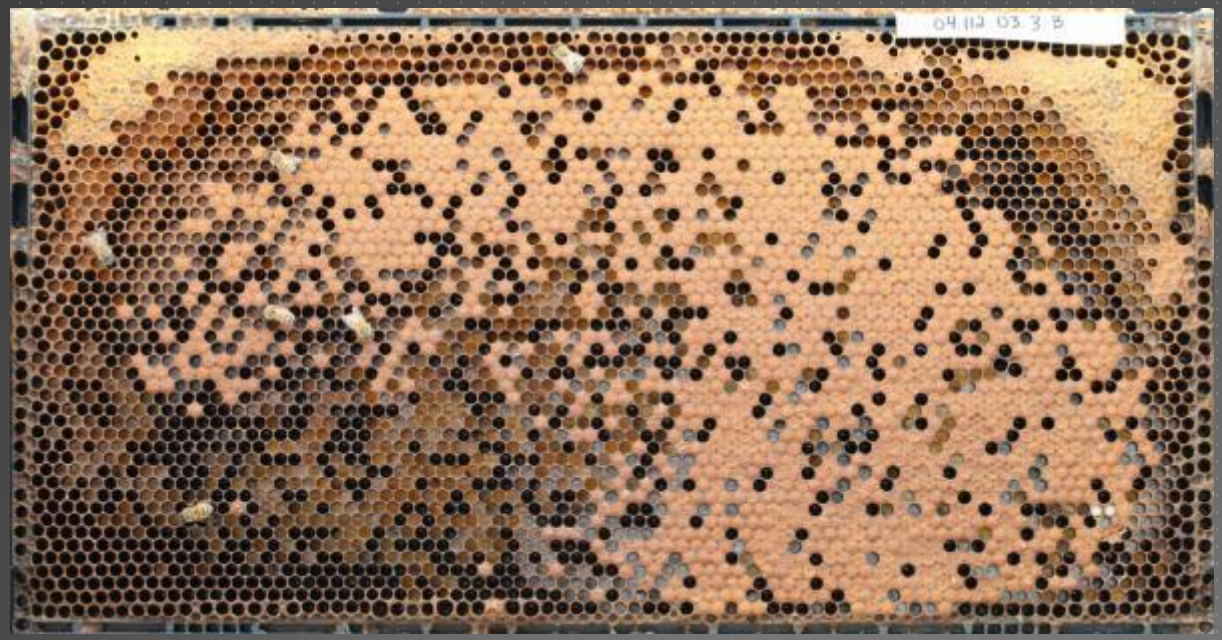
$A = \pi \times (F \times L)^2$

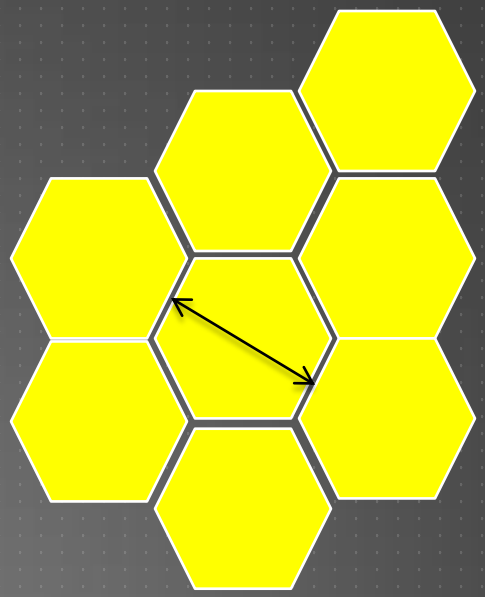
where F = a correction factor (ca. 0.35)
and L = mean length of bee in pixels*

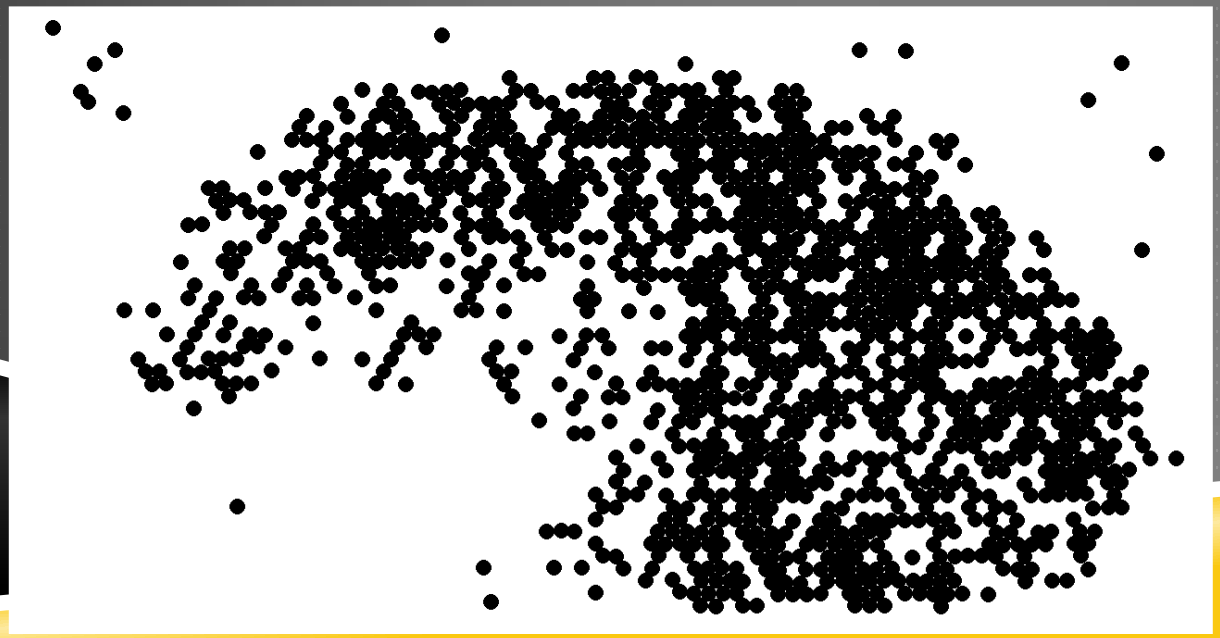
*based on ImageJ measurements of 100 randomly selected bees from 10 randomly selected images

Diagrams by M. Sultan

Brood Measurements – Image Processing







Diameter = 65px*

*based on 100 randomly measured cells

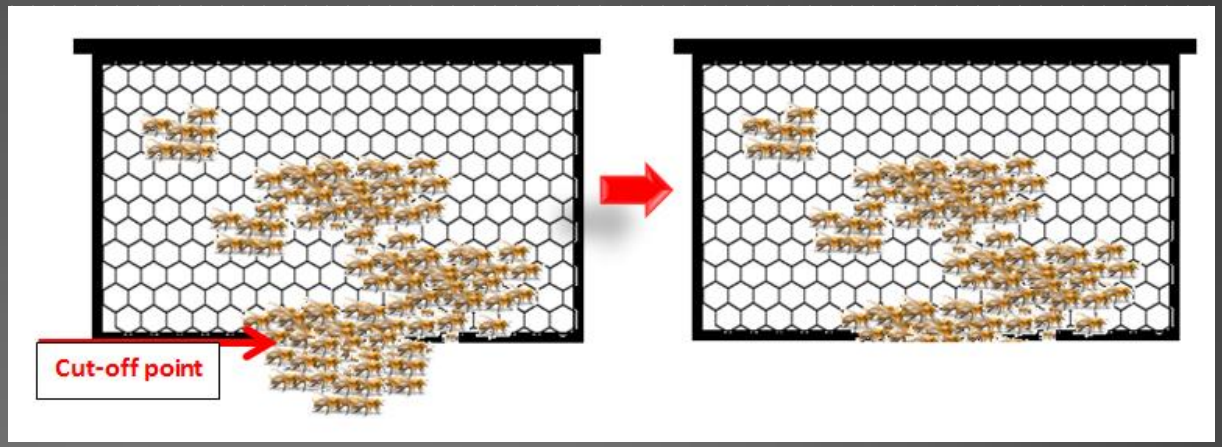
Use of 'Circular Objects' method in IndiCounter©

Diagrams by M. Sultan

Factors Affecting Results

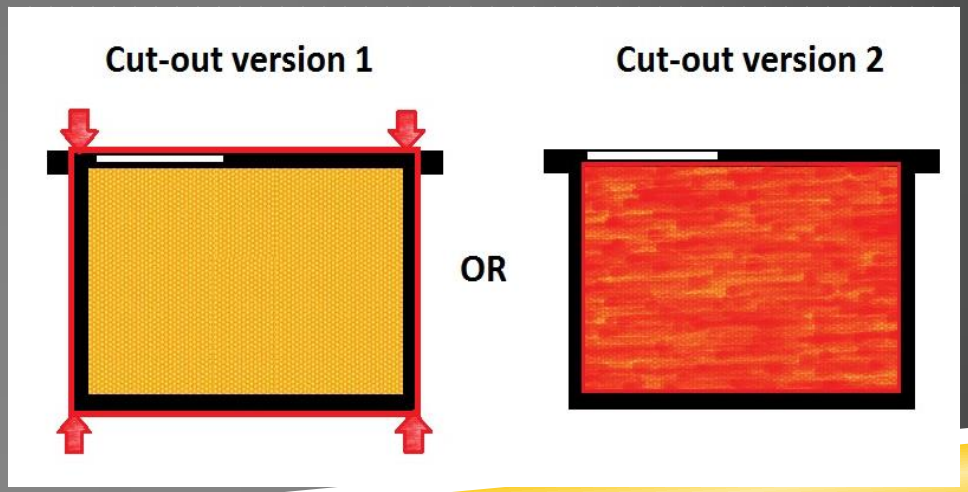
Adults:

- Bees in clusters cut off and excluded from analysis
- Drones
- Heavily populated frames



Brood:

- Reduced counts on wet frames
- Differentiation between capped honey and capped brood cells
- Artificially reduced % coverage as total frame area is increased as per cut-out points



Mean % coverage = (# pixels per cell x # of cells) / # pixels of entire image

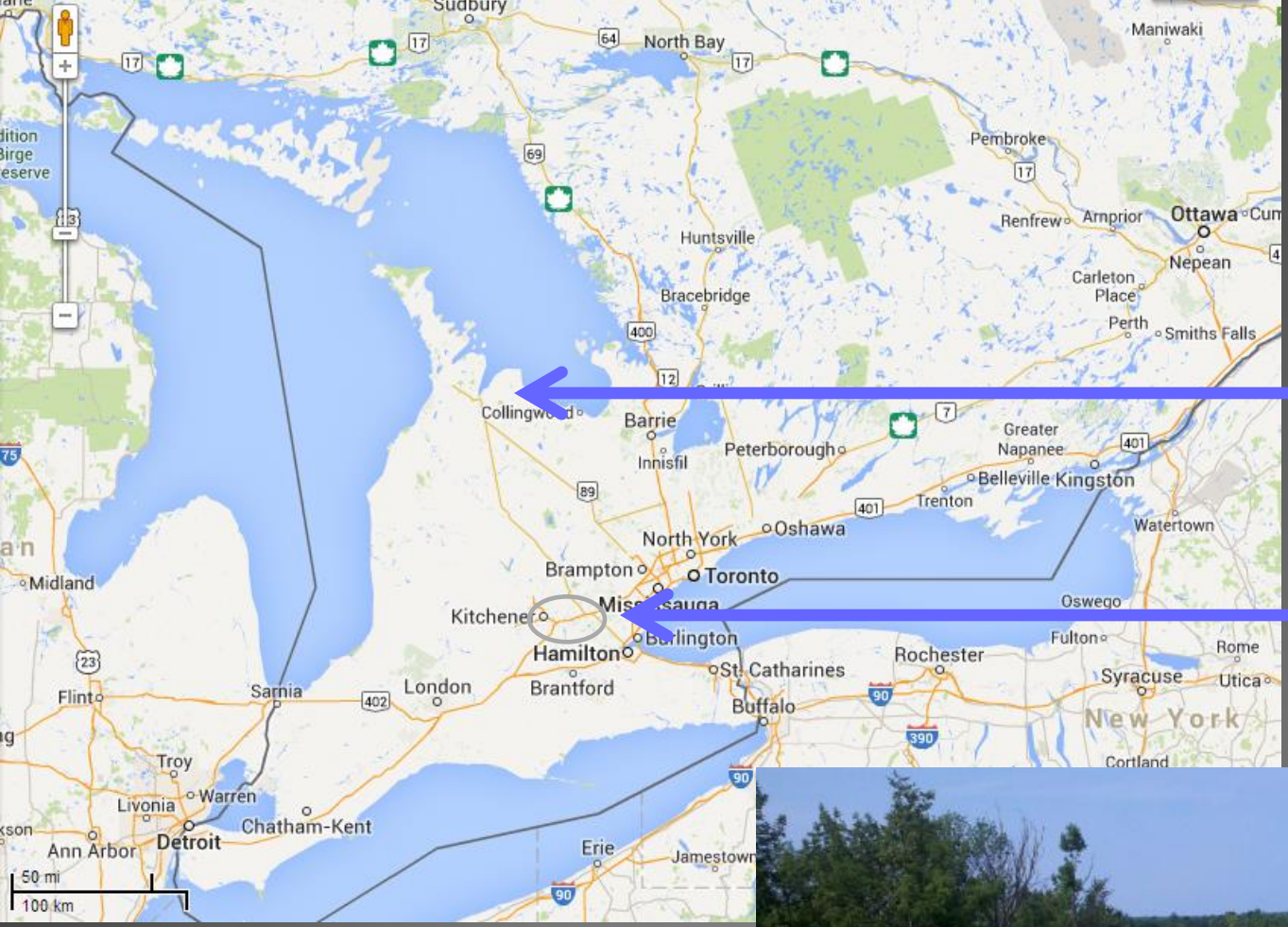
- Hives weighed before and after
- Honey supers weighed before and after

- Dead bees counted ~ every 4 days

ISOLATED APIARY

Land Forces Central Area – Training Centre, Meaford, ON (Canadian military facility)

Facility size = 6800 ha



Non-agricultural landscape for post-exposure

Field site area

Post-exposure bee yard pasture

RESULTS

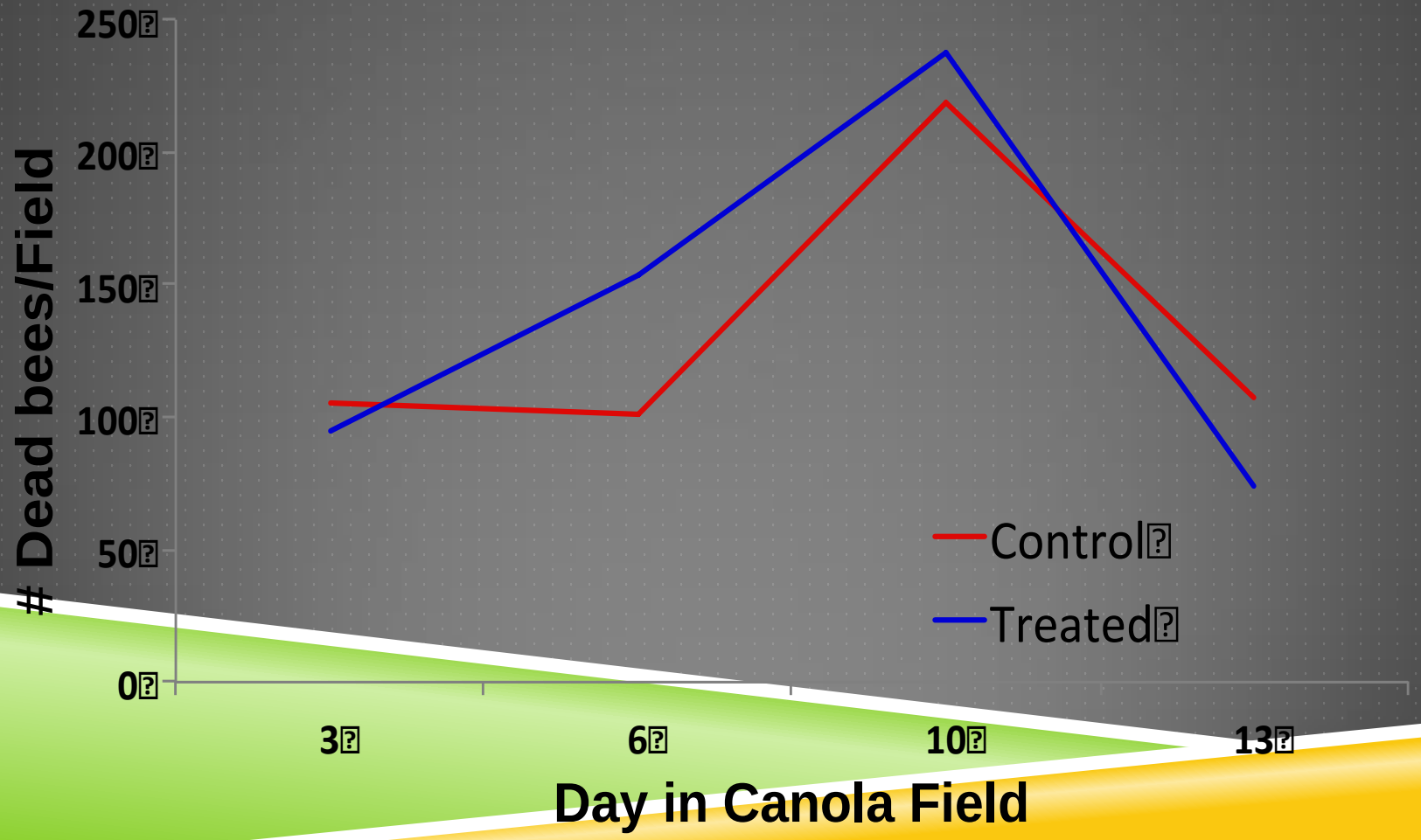
Colony Weight and Honey Yield (kg)

Endpoint	**Control**	**Treatment**	***P-value***
Colony Weight (kg)	14.7	14.2	*0.82*
Honey Yield (kg)	51.0	52.8	*0.84*

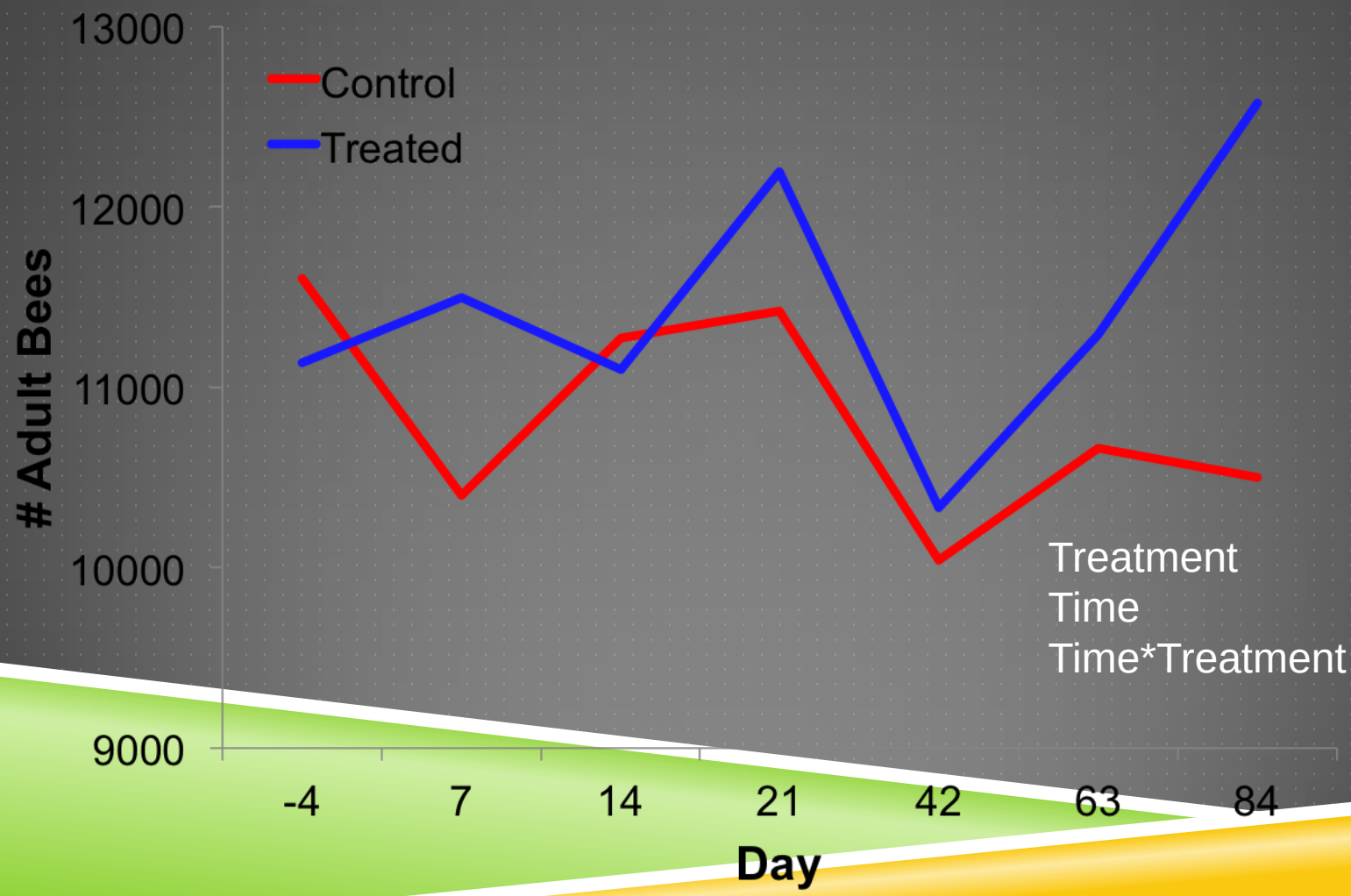
Average Ontario Honey Yield 2012 (6 months) = 37 kg

RESULTS – DEAD BEES

Treatment	$P = 0.80$
Time	$P = \underline{\mathbf{0.013}}$
Time*Treatment	$P = 0.44$

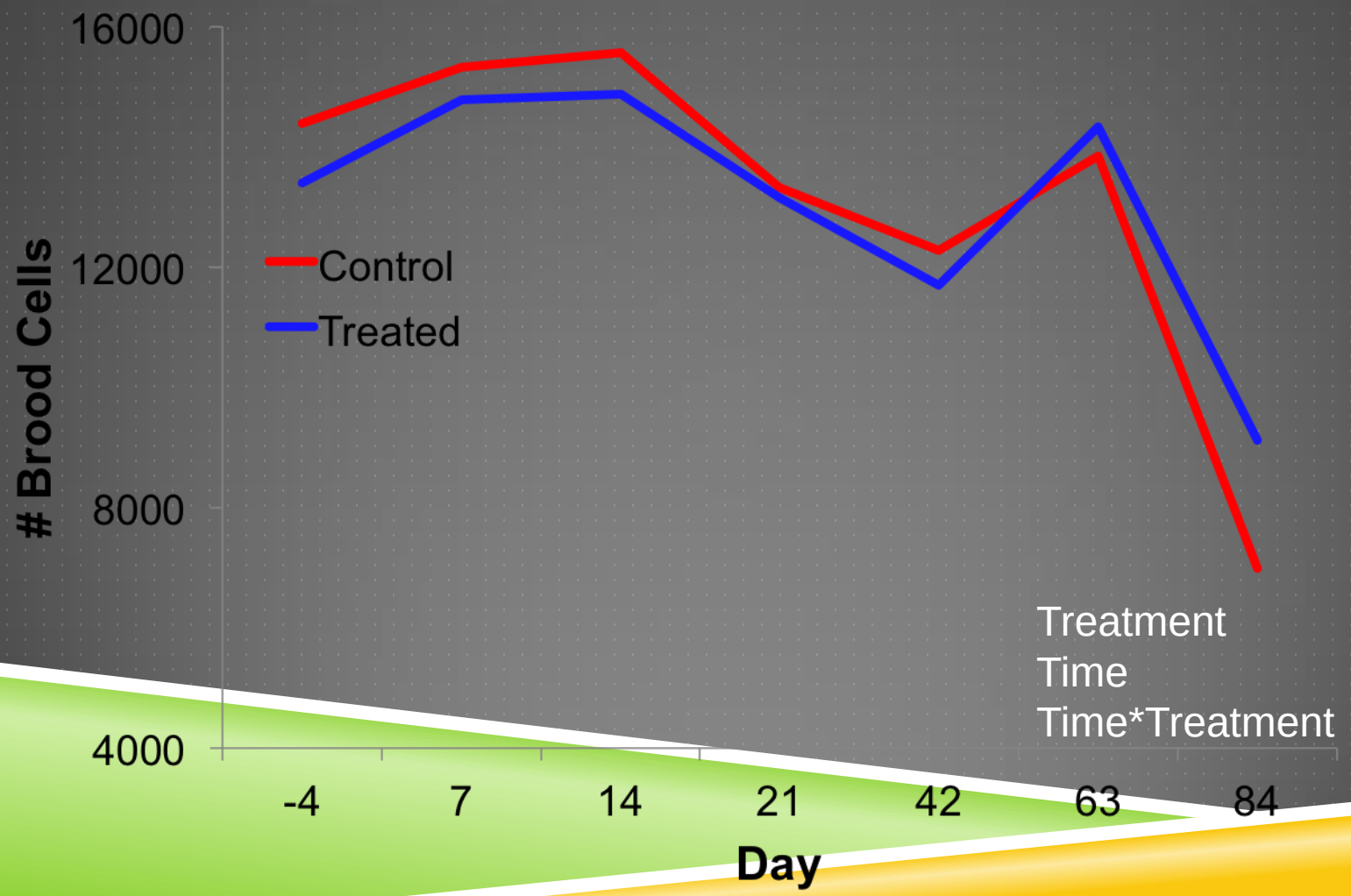


MEAN NUMBER OF ADULT BEES PER COLONY DURING AND AFTER PLACEMENT IN CANOLA



Treatment	$P = 0.20$
Time	$P = 0.26$
Time*Treatment	$P = 0.19$

MEAN NUMBER OF BROOD CELLS PER COLONY DURING AND AFTER PLACEMENT IN CANOLA



Treatment	*P* = 0.92
Time	***P* = 0.047**
Time*Treatment	*P* = 0.66

RESIDUE ANALYSIS

- USDA examined first set of samples
- Few positive samples overall
- 2 control pollen, 2 treatment pollen; 0 nectar detects

Sample No.	Conc. (ppb)	Site (Field)	Week collected	Test Group	% Canola Pollen
PA1004	Trace	5	1	Treatment	89%
PA1008	1.1	8	1	Treatment	95%
PA1017	1.5	6	2	Control	86%
PA1019	Trace	10	2	Control	83%

RESIDUE RE-ANALYSIS - BCS

- LOQ = 0.5 ppb, LOD = 0.35 ppb
- Week 1 pollen samples
  - Control no detections; 0/5 fields
  - Treatment 0.6-1.1 ppb; 5/5 fields
- Week 2 pollen samples
  - Control 0.35-1.3 ppb; 3/5 fields (2/5 ≥ LOQ)
  - Treatment 0.5-1.9 ppb; 4/5 fields
- **At least 10- to 50-fold below the 20 ppb NOAEC**

RESIDUE ANALYSIS – EXPLANATIONS?

- Movement (foraging) of control bees to treatment fields?
  - Unlikely; > 10 km away
- Collection from other neonic treated crops?
  - Possible – some small fields of sweet corn within foraging distance that may have been shedding pollen during canola bloom
  - But, heavy forage on canola when residues detected
- Carry-over in soil from previous years?
  - If an issue, would expect to see in Week 1 control pollen

RESIDUE ANALYSIS – EXPLANATIONS?

- Seed mix up during planting?
  - Easily distinguished and no indication in records/reports
- Contaminated seeder?
  - All control fields planted first
- Contamination/mix-up of samples during collection, processing, analytical prep, etc. ?
  - No indication in records

CONCLUSIONS

- No effects or “poor performance” in treatment colonies
- Follows other lines of evidence
  - Honey bees doing well in canola, soybean, and corn on the prairies and mid-west
  - Recent reviews, monitoring in Europe, risk assessments, etc.
- Overwinter survival comparable to Ontario and Canada
- Few instances of exposure of “control” colonies despite ***extensive*** efforts to isolate treatment and control sites
  - All detections well below the NOAEC

FIELD STUDY RECOMMENDATIONS

- Use only rarely
- Early in the registration process; before the control product in question is utilized widely within agroecosystems
- Potential for "contaminated controls" distorts the optics – gives impression of "uncertainty" which is false
- Tier 2 screening will capture most of the necessary information especially with honey bee
- Once control products are widely used – focus on longterm sentinel monitoring programs especially with honey bees
- Develop methods to look exposure scenarios for non-Apis bees in field studies

QUESTIONS?