



A Brief History of Neonicotinoid and Bee Research

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Recent Pollinator Declines



Honeybee Declines

USA, Canada, + Europe- higher rates of colony losses

- 2012-2013: 49.4% average loss
- 2014-2015: 51.1 % average loss

Bumblebee Declines

U.S.A.- 4/8 species

U.K. 6/16 species (3 extinct)

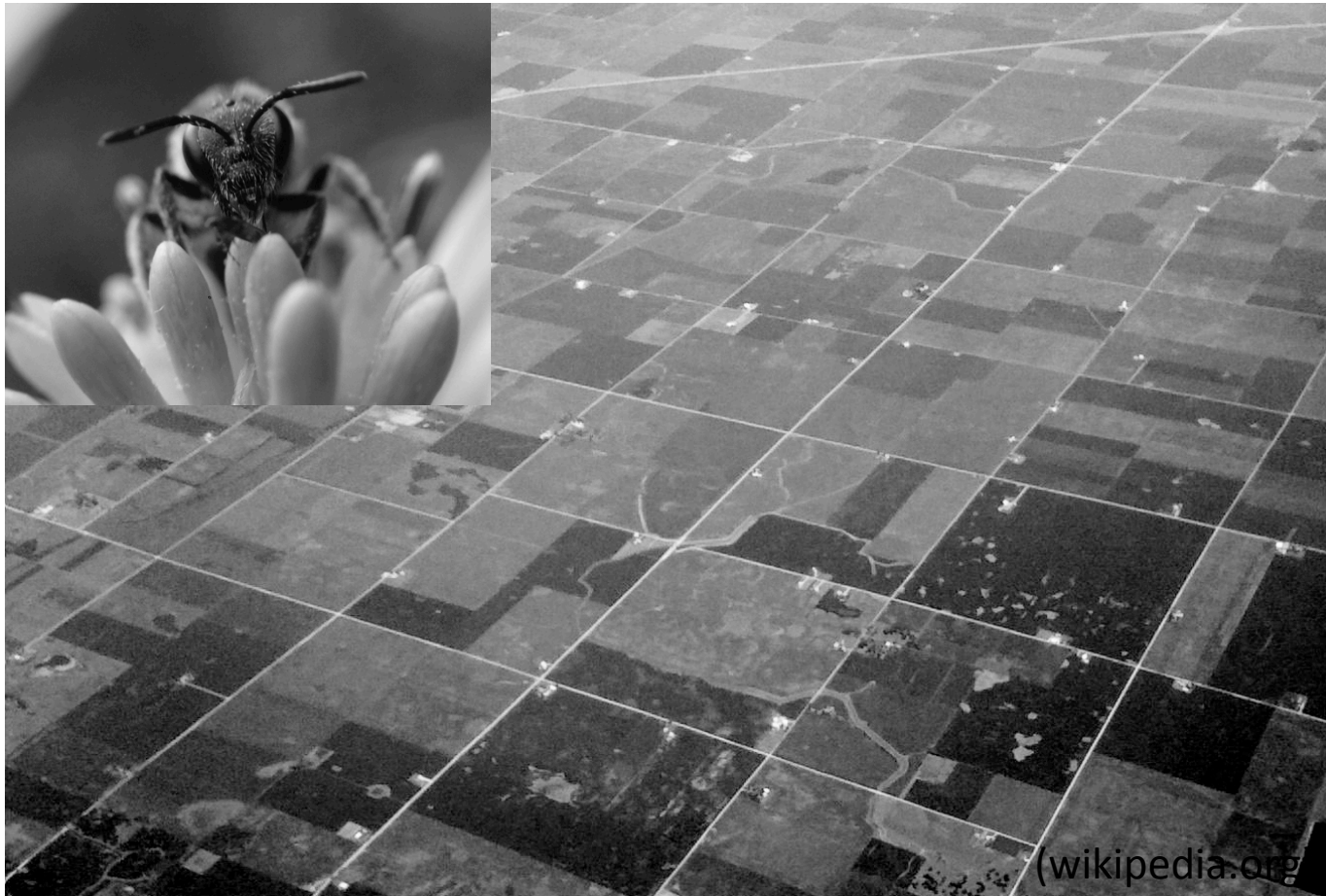


Cameron et al. 2011, Goulson et al. 2008

Other Pollinator Declines

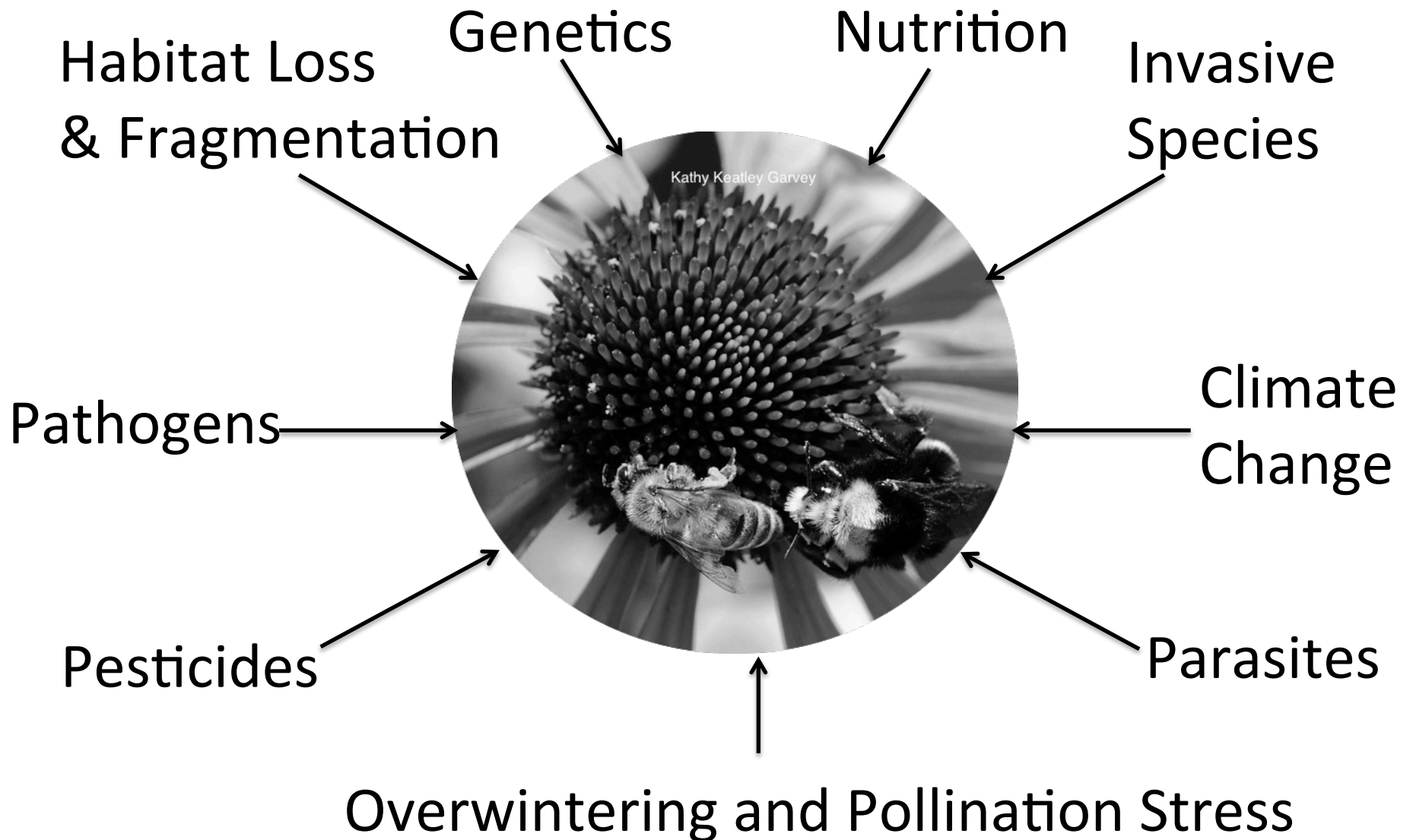


Other Pollinator Declines(?)



Winfree et al. 2009

Causes of Decline



Causes of Decline



Pesticides
(neonicotinoids)

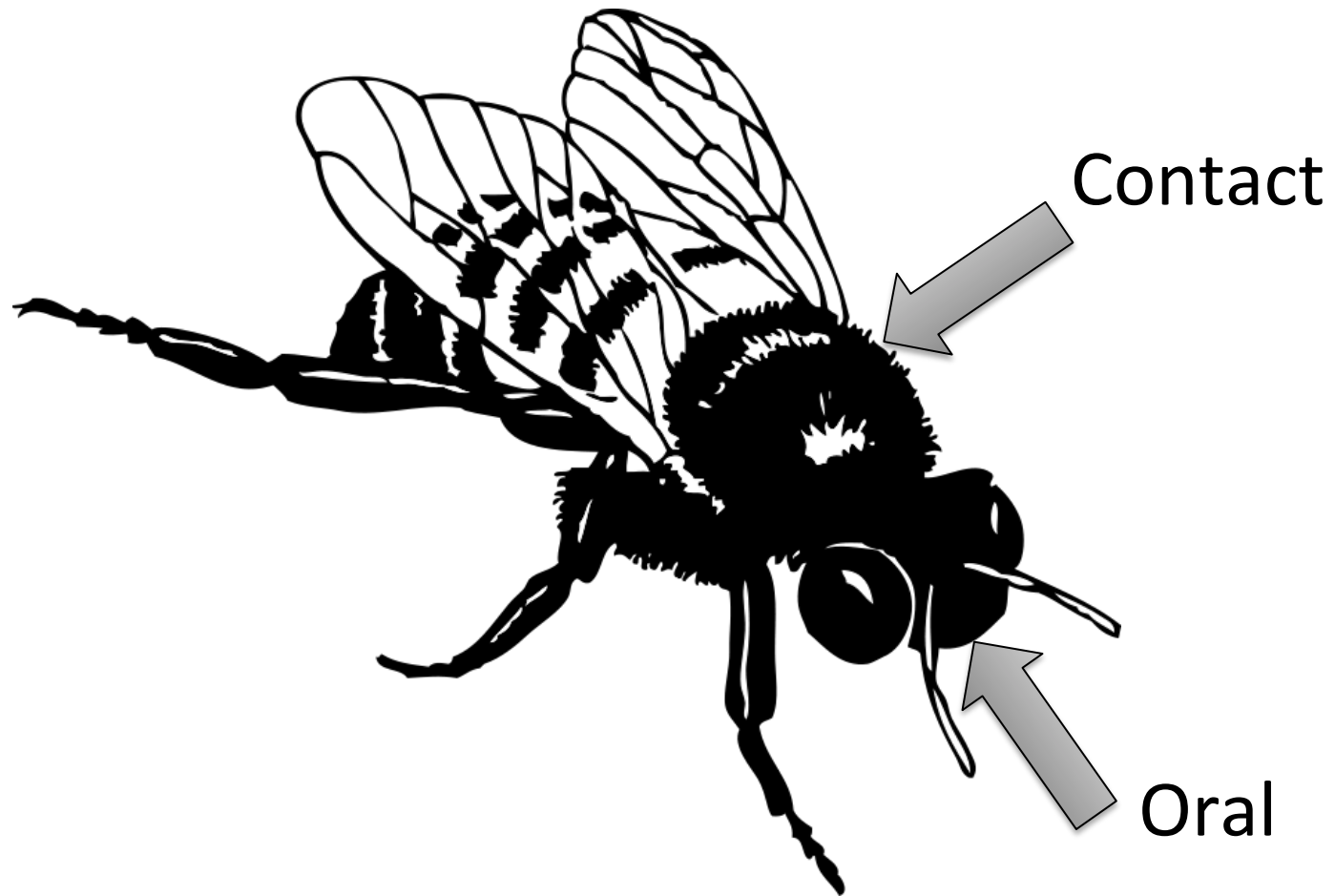
2013- European Commission Restricts Neonicotinoid Use

- 2 year restriction on certain applications of:
 - Clothianidin
 - Thiamethoxam
 - Imidacloprid
- Allow time for scientific research

Outline

- Routes of exposure
 - Colony level effects
 - Foraging, learning, and memory
 - Interactions with pathogens
 - Interactions with other pesticides
 - Other pollinators?
-
- Lethal effects
- Sublethal effects

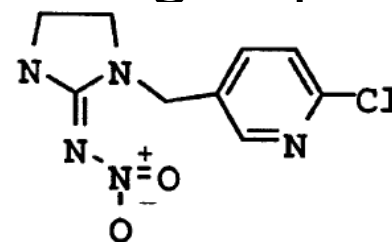
Routes of Exposure



Honey Bee Contact Toxicity (24 hr LD₅₀)

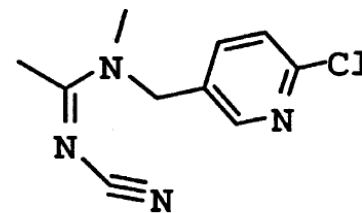
- Imidacloprid (18 ng/bee)
- Clothianidin (22 ng/bee)
- Thiamethoxam (30 ng/bee)
- Dinotefuran (75 ng/bee)
- Nitenpyram (138 ng/bee)
- Acetamiprid (7000 ng/bee)
- Thiacloprid (15000 ng/bee)

Nitro-group




imidacloprid

Cyano-group



acetamiprid

Honey Bee Contact Toxicity (24 hr LD₅₀)

- **Imidacloprid (18 ng/bee)**  78% of studies
- Clothianidin (22 ng/bee)
- Thiamethoxam (30 ng/bee)
- Dinotefuran (75 ng/bee)
- Nitenpyram (138 ng/bee)
- Acetamiprid (7000 ng/bee)
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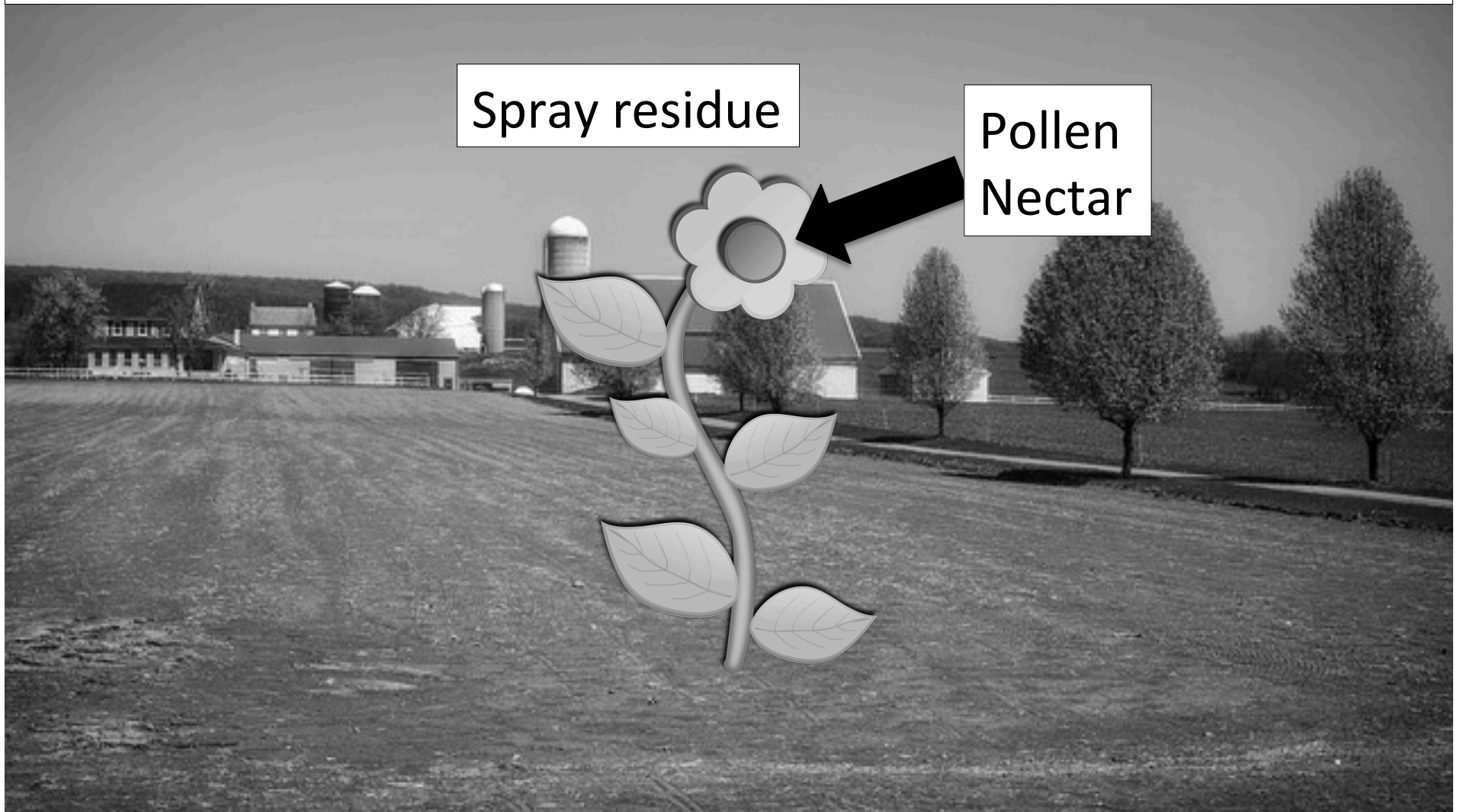
Oral Toxicity

High variability between studies

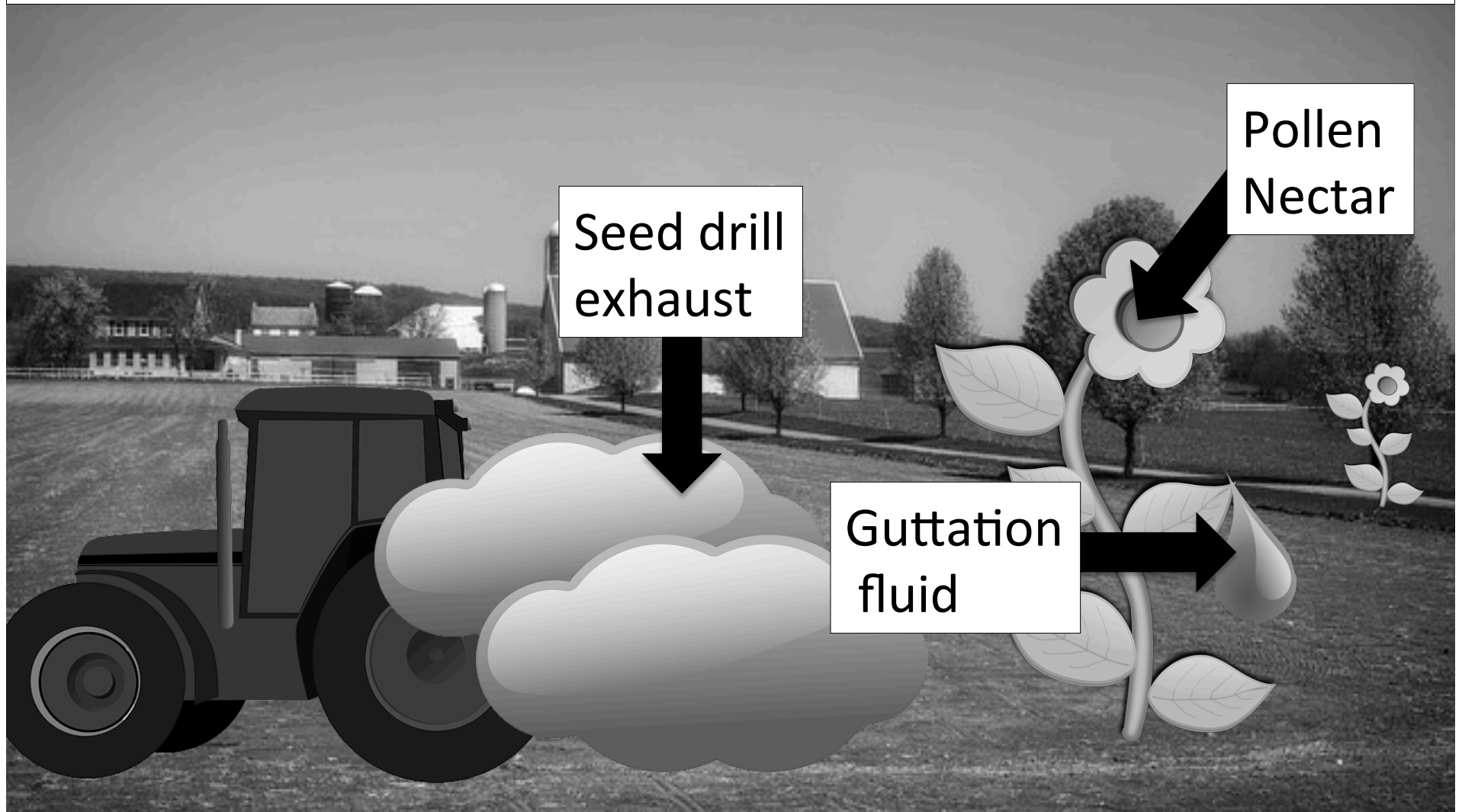


Blacquiere et al. 2012

Pesticide Exposure (Foliar Application)



Pesticide Exposure (Seed Treatment)





Pollen and Nectar

- Field-realistic range of imidacloprid in nectar 0.7-10 ug/L (Cresswell 2011)
- Average maximum of 6.1 ug / kg in pollen, 1.9 ug/L in nectar (Godfray et al. 2014)
- Well below acute and chronic toxicity levels



Guttation Fluid





Guttation Fluid

- Fed honey bees guttation fluid from seed-treated corn seedlings
- Very high peak concentrations
 - nearing concentration foliar spray
 - >200,000 ug /L imidacloprid



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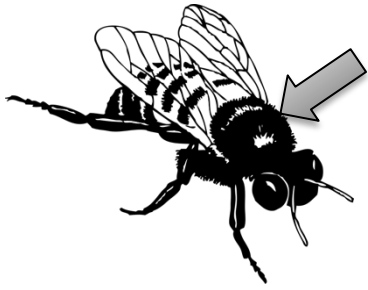
Nectar
concentration:
 $0.7-10$ ug / L
(Cresswell 2011)



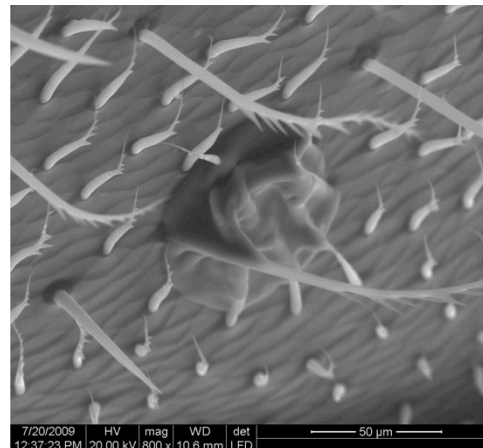
Guttation Fluid

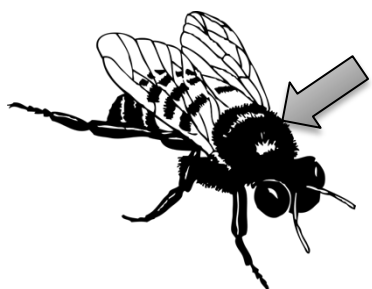
- Fed honey bees guttation fluid from seed-treated corn seedlings
- Very high peak concentrations
- High mortality in treatment groups
- No mortality in negative controls

Girolami et al. 2013, Tapparo et al. 2012



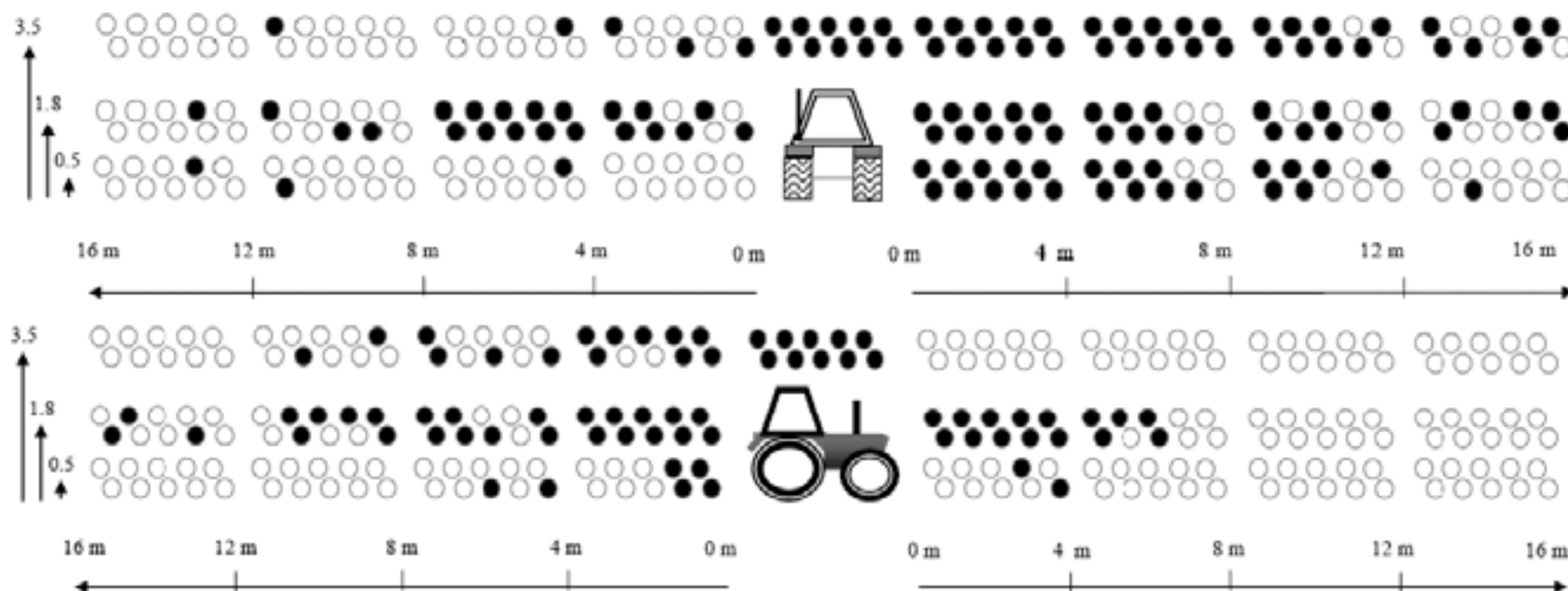
Seed Drilling Particulates





Seed Drilling Particulates

Bee deaths after a single rapid pass
(clothianidin seed treatment):





Seed Drilling Particulates

- Exhaust drift to weedy field margins
- Thiamethoxam, clothianidin detected on flowers in field margins



Kathy Keatley Garvey

Clothianidin
concentration:
1.1-9.4 ug/L

Imidacloprid nectar
concentration:
0.7-10 ug / L
(Cresswell 2011)

Krupke et al. 2012

Conclusions- Routes of Exposure

Neonicotinoid seed treatments present novel mechanisms of exposure to bees (seed drilling dust, guttation fluids)

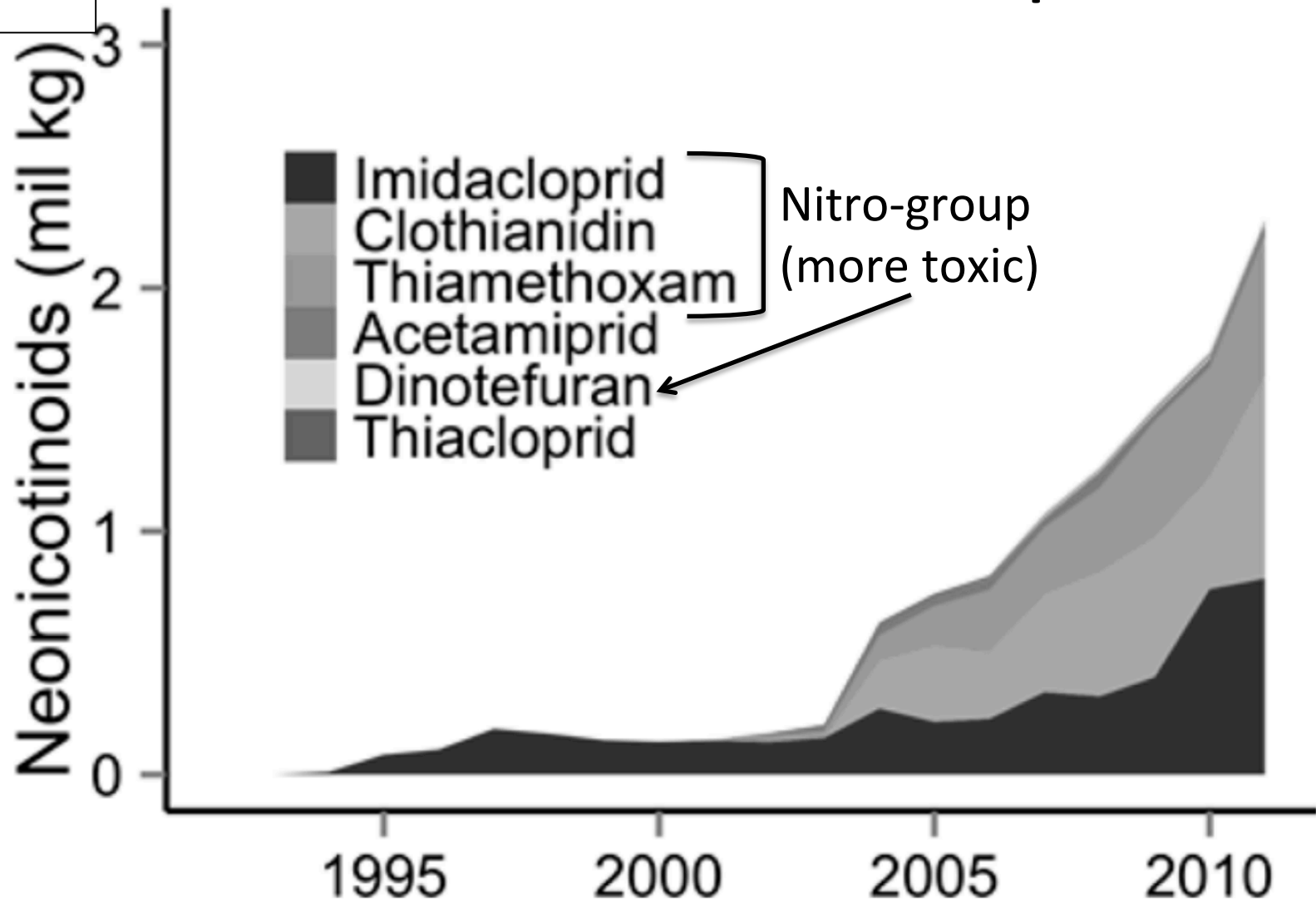
- Depends on seed treatment formulation, type of seed drill used
- Due to types of crops and times of year present, bees unlikely to consume contaminated guttation fluid

Conclusions- Routes of Exposure

Neonicotinoid seed treatments present novel mechanisms of exposure to bees (seed drilling dust, guttation fluids)

Levels of neonicotinoids to which bees are exposed are unlikely to be lethal

Conclusions- Routes of Exposure



Douglas and Tooker 2015

Lethal vs. Sublethal effects

- **Lethal effect**- increased rate of mortality
- **Sublethal effect**- modified individual or colony performance (growth, fecundity, longevity, or behavior)

Outline

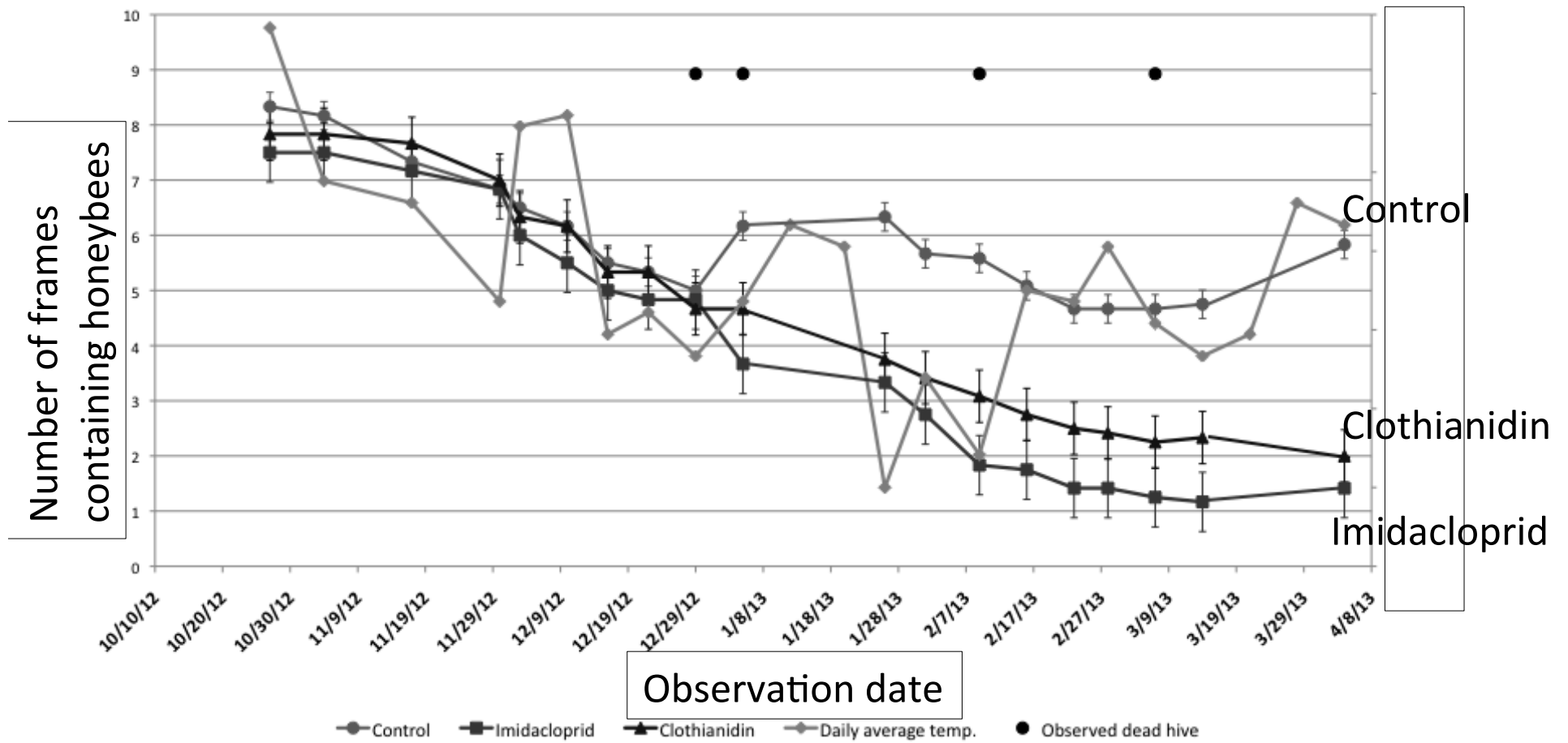
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- Interactions with other pesticides
- Other pollinators?

Honey bee overwintering

Could imidacloprid in high fructose corn syrup fed to overwintering bees lead to CCD-like symptoms?



Honey bee overwintering



Honey bee overwintering

Concentrations of imidacloprid in high fructose corn syrup: 20 – 400 µg/L

Nectar
concentration:
0.7-10 µg/L
(Cresswell 2011)

Bumble bee colony growth, reproduction

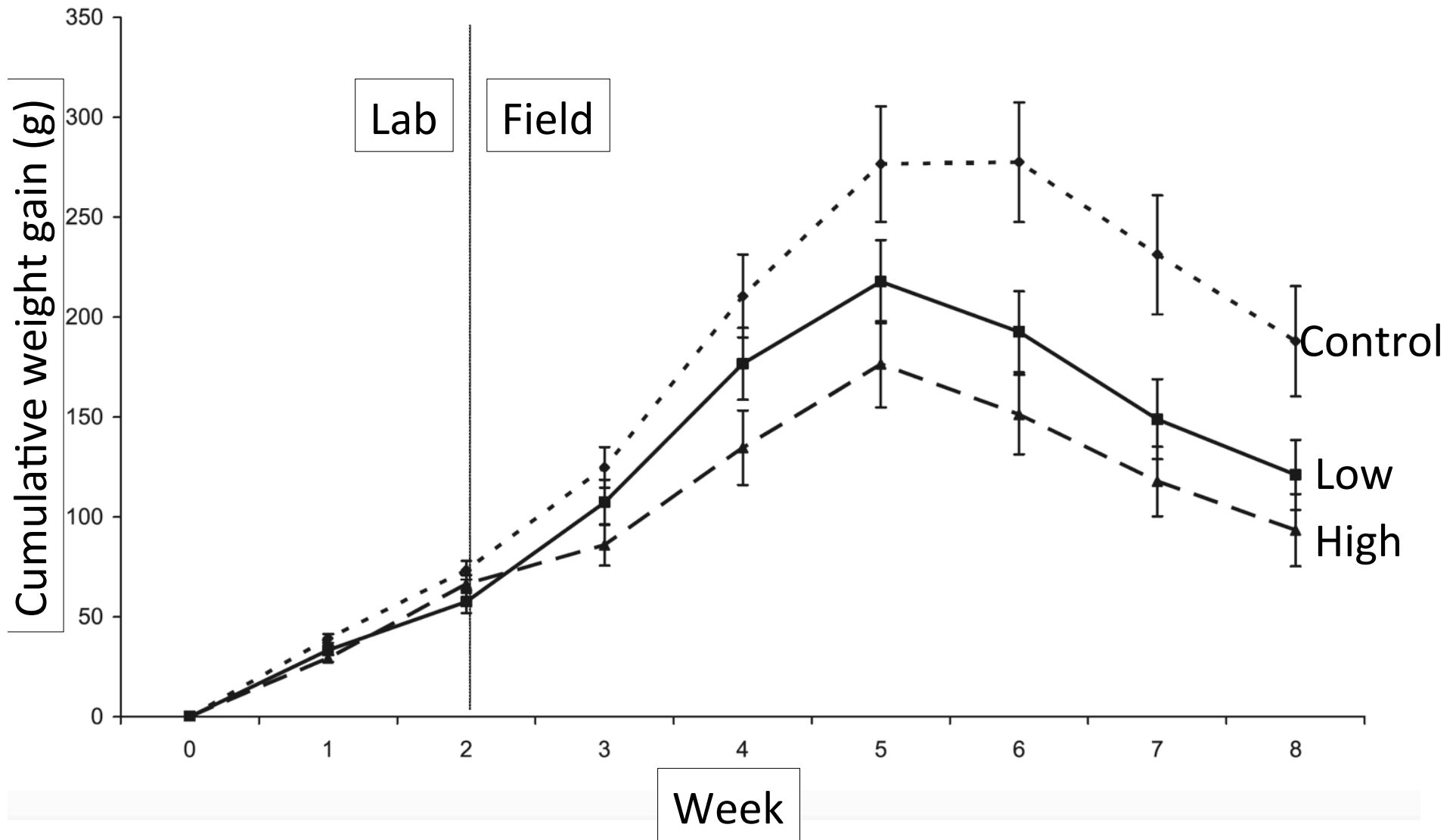
Can trace amounts of dietary imidacloprid contribute to observed bumble bee declines?

- “Low” treatment
 - 6 ug/kg imidacloprid in pollen
 - 0.7 ug/L imidacloprid in nectar
- “High” treatment: double the “low” dose

Nectar
concentration:
0.7-10 ug/L
(Cresswell 2011)

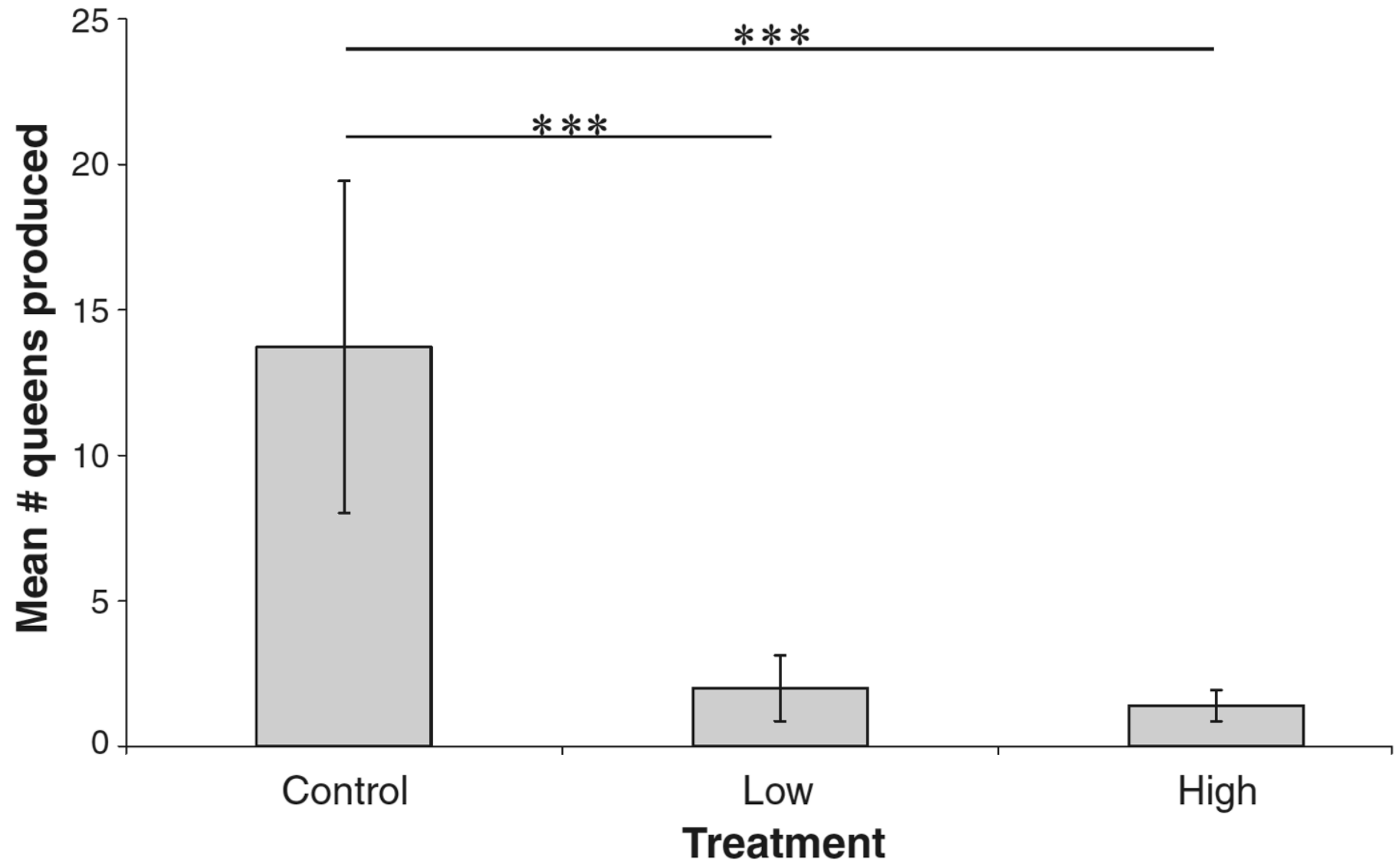
Average maximum
pollen concentration:
6.1 ug/kg
(Godfray 2014)

Bumble bee colony growth



Whitehorn et al. 2012

Bumble bee queen production



Whitehorn et al. 2012

Outline

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Honey bee laboratory studies

- Single acute dose imidacloprid can impair
 - Learning (Lambin et al. 2001; Guez et al. 2001)
 - Motor activity (Lambin et al. 2001; Medzrycki et al. 2003)
 - Memory (Decourtye et al. 2004)
- Chronic sublethal doses imidacloprid can impair
 - Learning
 - Foraging (Decourtye et al. 2003, Han et al. 2010)
- Higher than “field-realistic” doses

A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

Mickaël Henry,^{1,2*} Maxime Béguin,^{2,3} Fabrice Requier,^{4,5} Orianne Rollin,^{2,6} Jean-François Odoux,⁵ Pierrick Aupinel,⁵ Jean Aptel,^{1,2} Sylvie Tchamitchian,^{1,2} Axel Decourtye^{2,6}

Can sublethal quantities of thiamethoxam increase hive death rate through homing failure in foraging bees?

Honey bee foraging

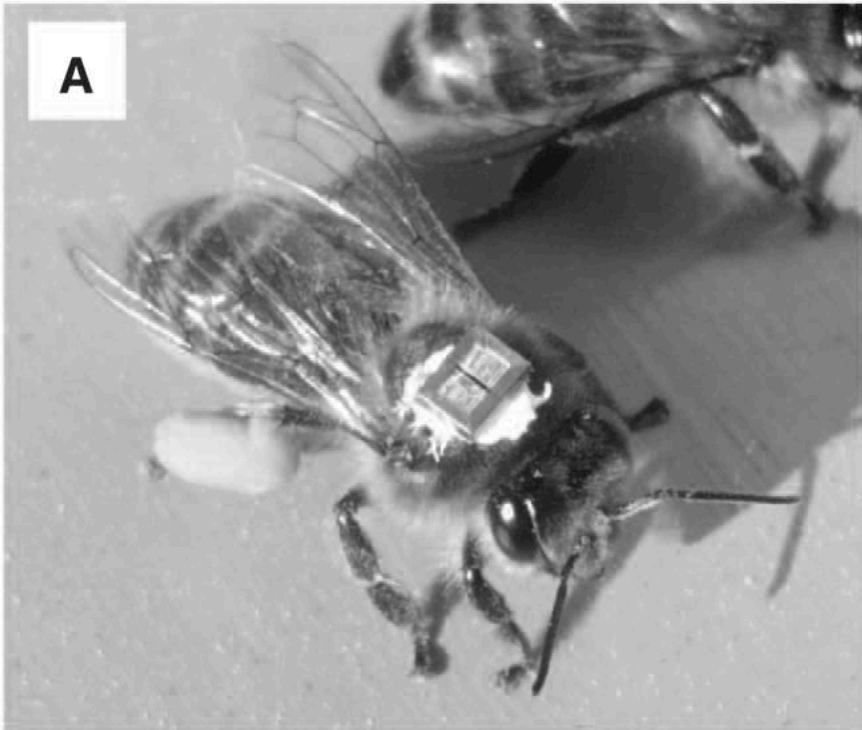
- Fed a single high dose of thiamethoxam
 - 1.34 ng
 - 67 ug/L solution

Average maximum
nectar concentration:
1.9 ug/L
(Godfray 2014)

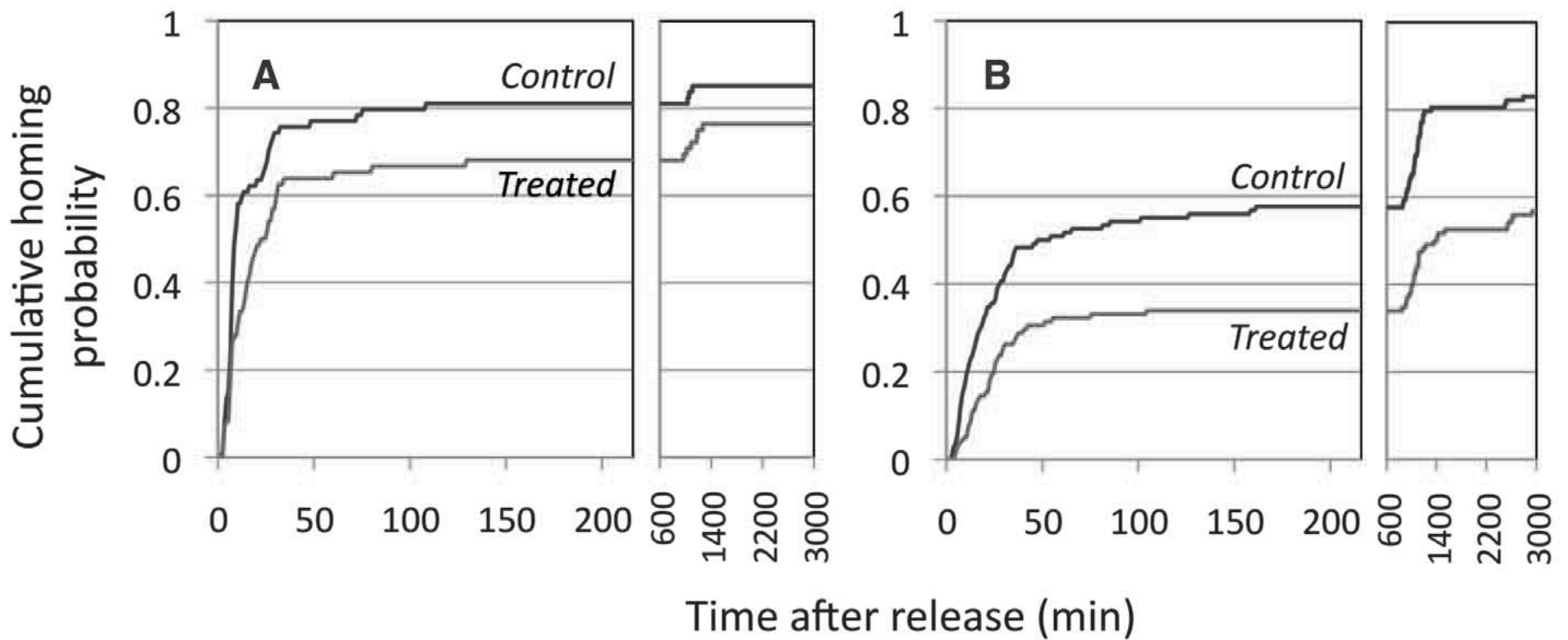
Honey bee foraging

- Fed a single high dose of thiamethoxam
 - 1.34 ng
 - 67 ug/L solution
- Probability of returning to hive monitored after release in
 - Familiar foraging location
 - Random location

Honey bee foraging



Honey bee foraging



Outline

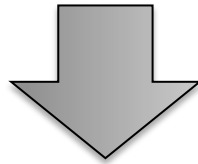
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Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees

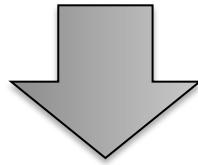
Gennaro Di Prisco^a, Valeria Cavaliere^b, Desiderato Annoscia^c, Paola Varricchio^a, Emilio Caprio^a, Francesco Nazzi^c, Giuseppe Gargiulo^b, and Francesco Pennacchio^{a,1}

^aDipartimento di Agraria, Laboratorio di Entomologia E. Tremblay, Università degli Studi di Napoli Federico II, I-80055 Portici, Italy; ^bDipartimento di Farmacia e Biotecnologie, Università di Bologna, I-40126 Bologna, Italy; and ^cDipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Udine, I-33100 Udine, Italy

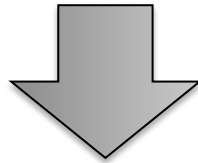
Sublethal amount
insecticide



↑ Protein that inhibits NF-κB
(immune signalling pathway)



↓ NF-κB immune response

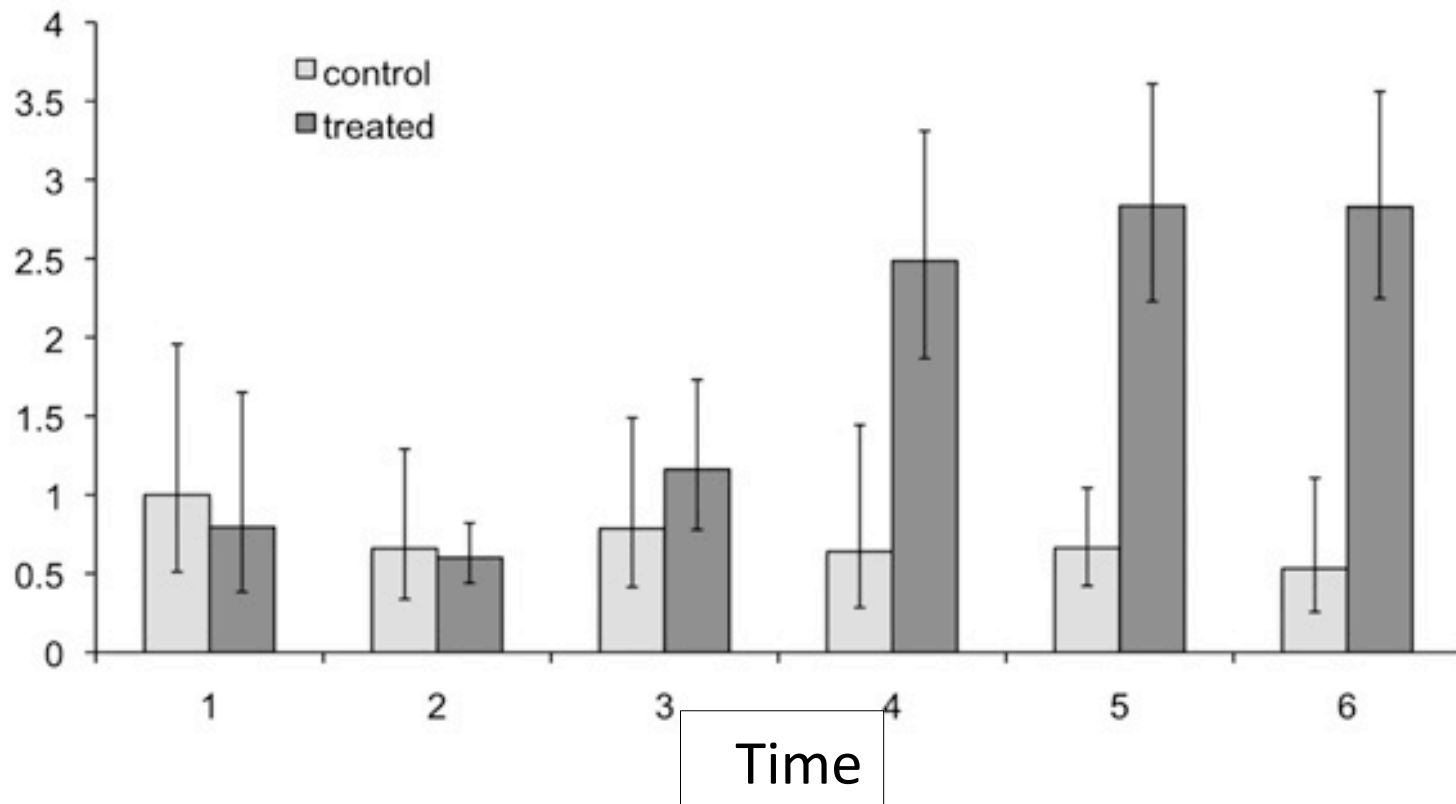


↑ Deformed Wing Virus
proliferation

Change in immunity-inhibiting protein

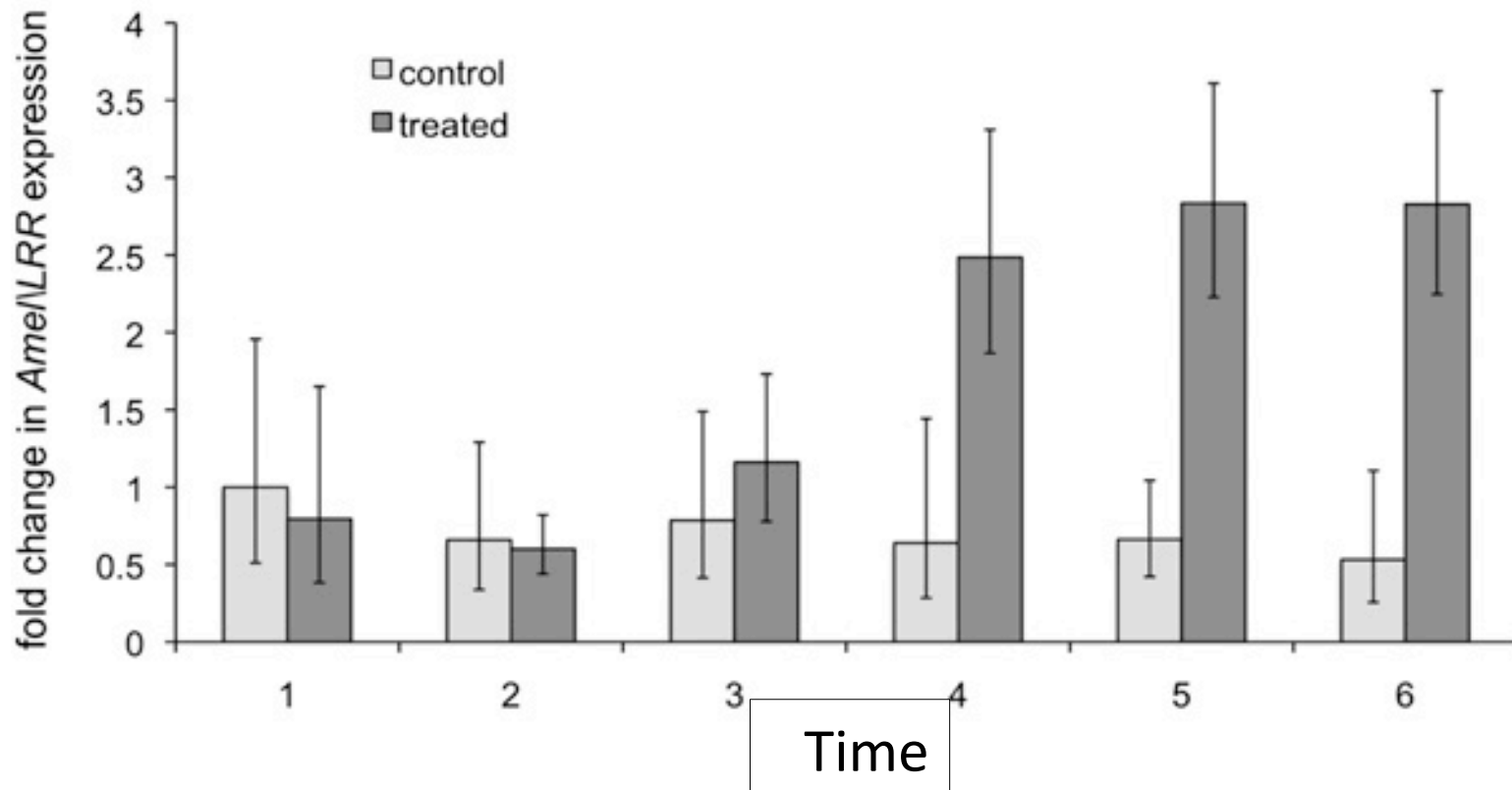
↑ Protein that inhibits NF- κ B
(immune signalling pathway)

Fold change in Amel\LRR expression

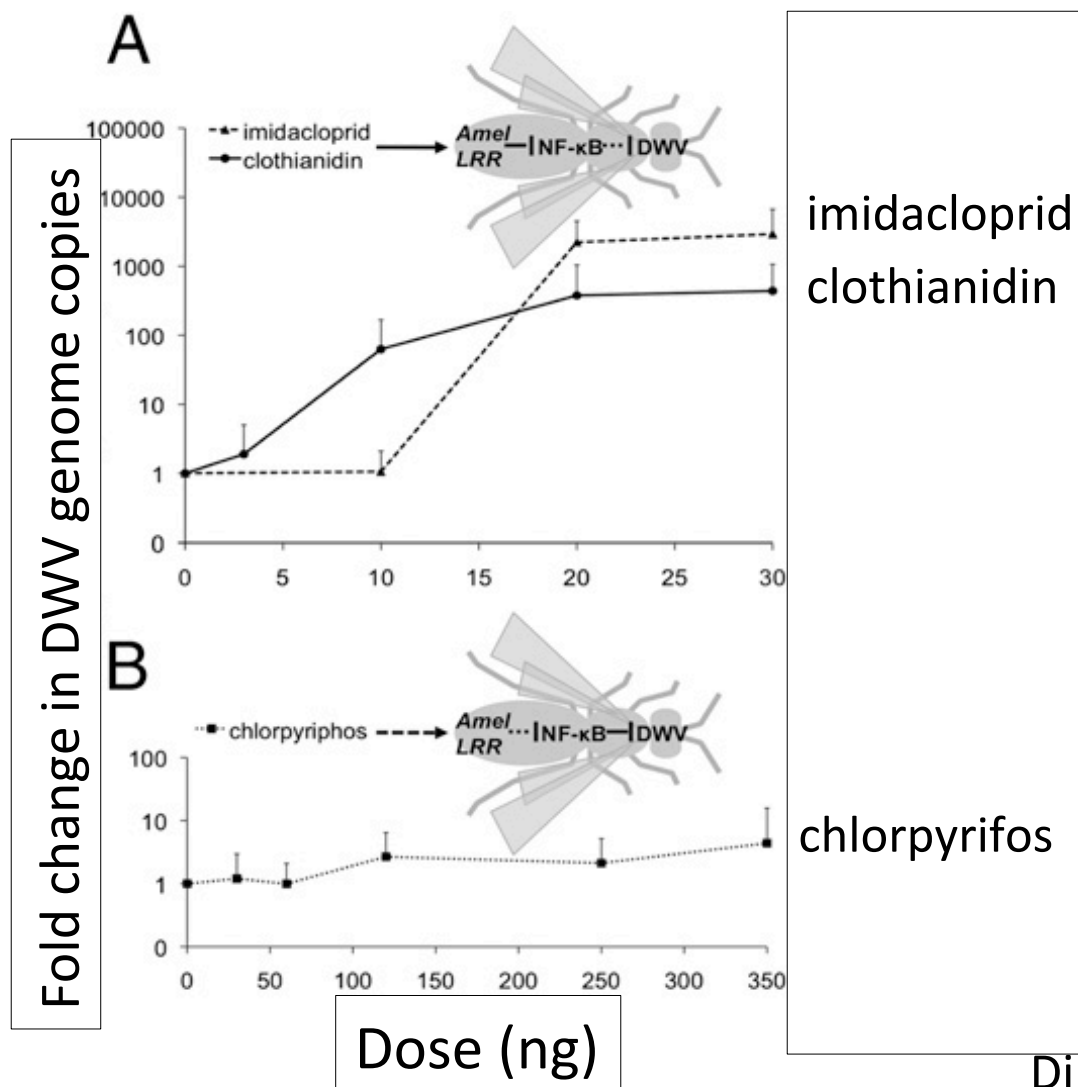


Change in immunity-inhibiting protein

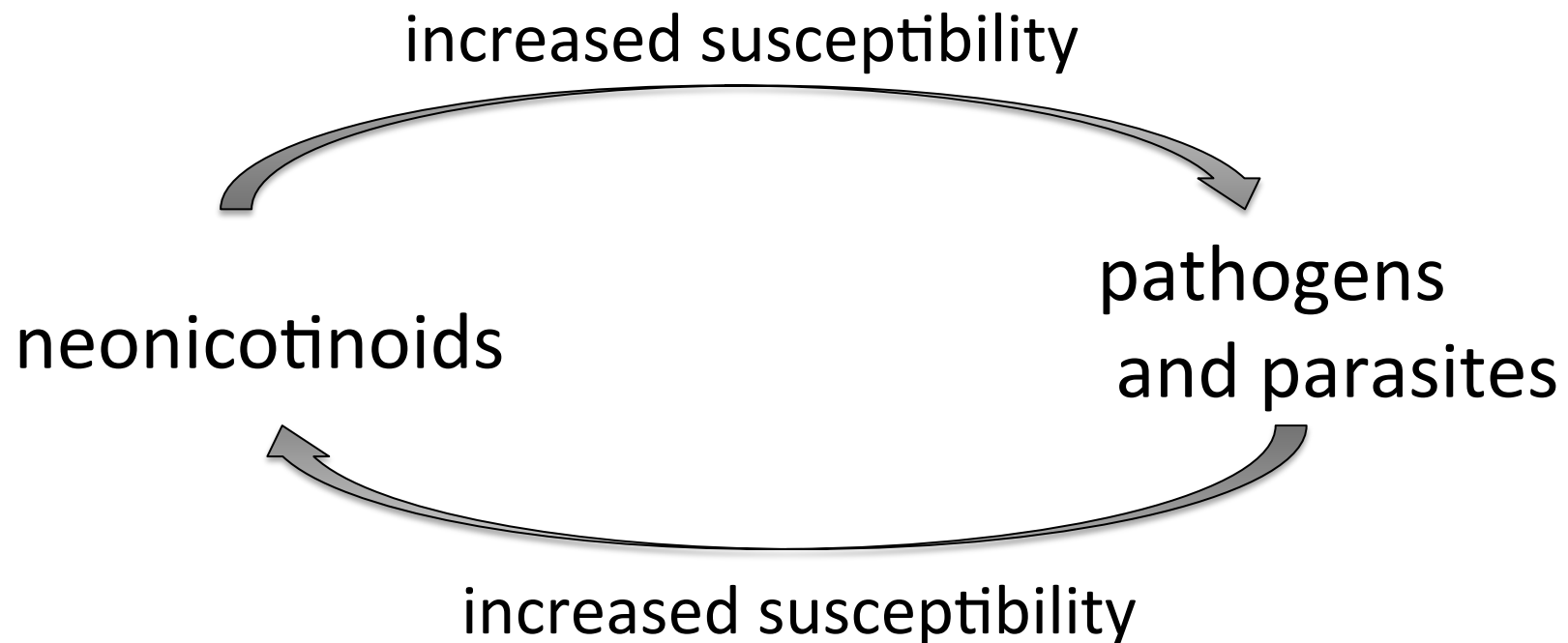
- Clothianidin and imidacloprid altered gene expression
- Chlorpyrifos (an organophosphate) did not



Increase in Deformed Wing Virus



Conclusions- Pathogens and Parasites



Data mostly limited to honey bee studies

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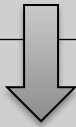
Synergy with DMI-Fungicide Pretreatment

Neonicotinoid	Fungicide	LD ₅₀ (μg/bee)	
Imidacloprid		0.0179	Cyano-group Nitro-group (less toxic) (more toxic)
Imidacloprid	Triflumizole	0.0097	
Acetamiprid		7.07	
Acetamiprid	Triflumizole	0.029	
Thiacloprid		14.6	
Thiacloprid	Triflumizole	0.0128	

Honey bee study

Iwasa et al. 2004

Synergy with DMI-Fungicide Pretreatment

Neonicotinoid	Fungicide	LD ₅₀ (μg/bee)	<div> <div>Cyano-group</div> <div>Nitro-group</div> <div>(less toxic)</div> <div>(more toxic)</div> </div>
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Acetamiprid		7.07	
Acetamiprid	Triflumizole	0.029	
Thiacloprid		14.6	
Thiacloprid	Triflumizole	<div>  0.0128 </div>	

>1,000 x more toxic

Honey bee study

Iwasa et al. 2004

Combined effects on bumble bee foraging

Over 4 week period, exposed *Bombus terrestris* colonies to

- 10 ug/L imidacloprid (fed in sucrose)

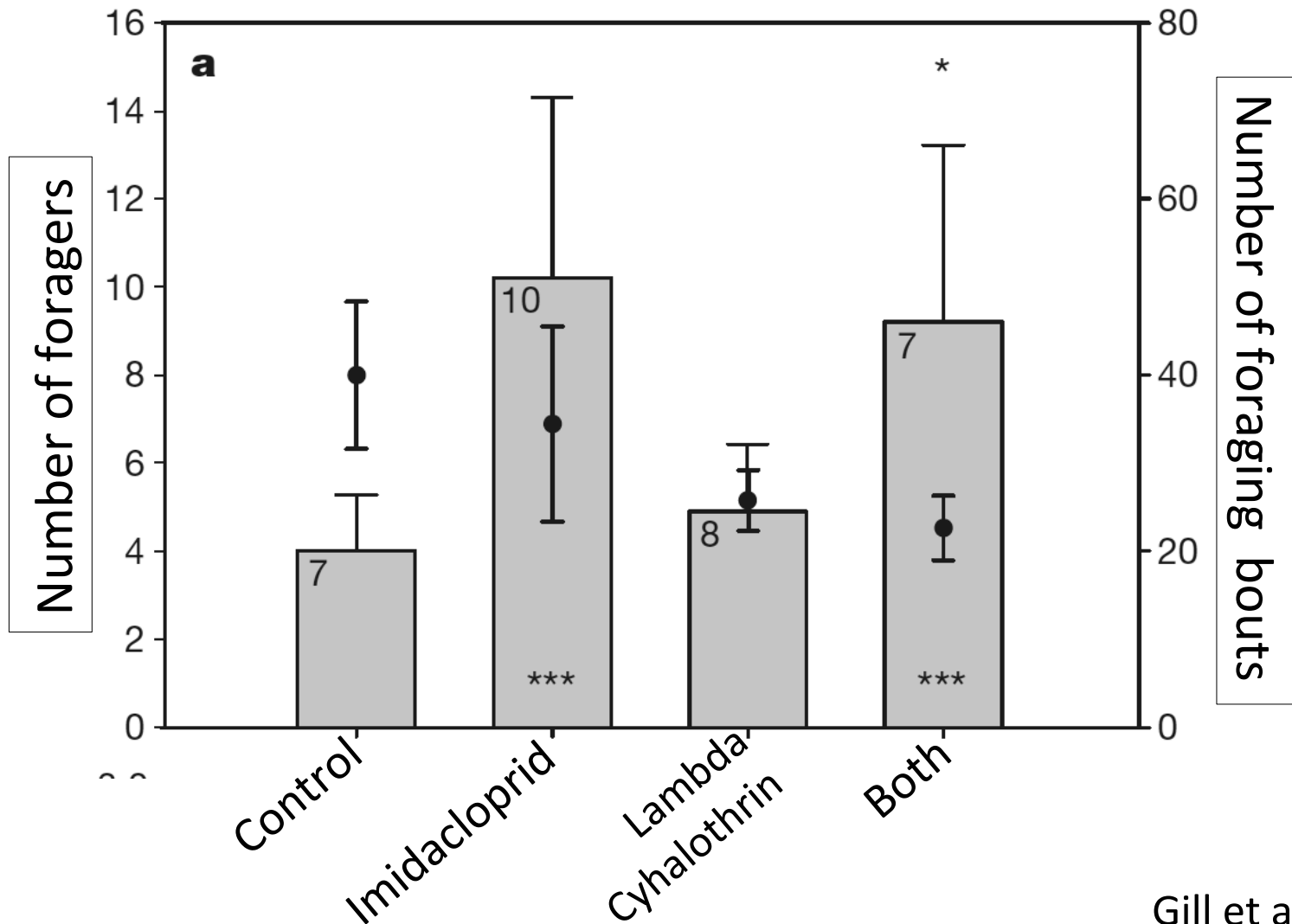
Nectar
concentration:
0.7-10 ug/L
(Cresswell 2011)

Combined effects on bumble bee foraging

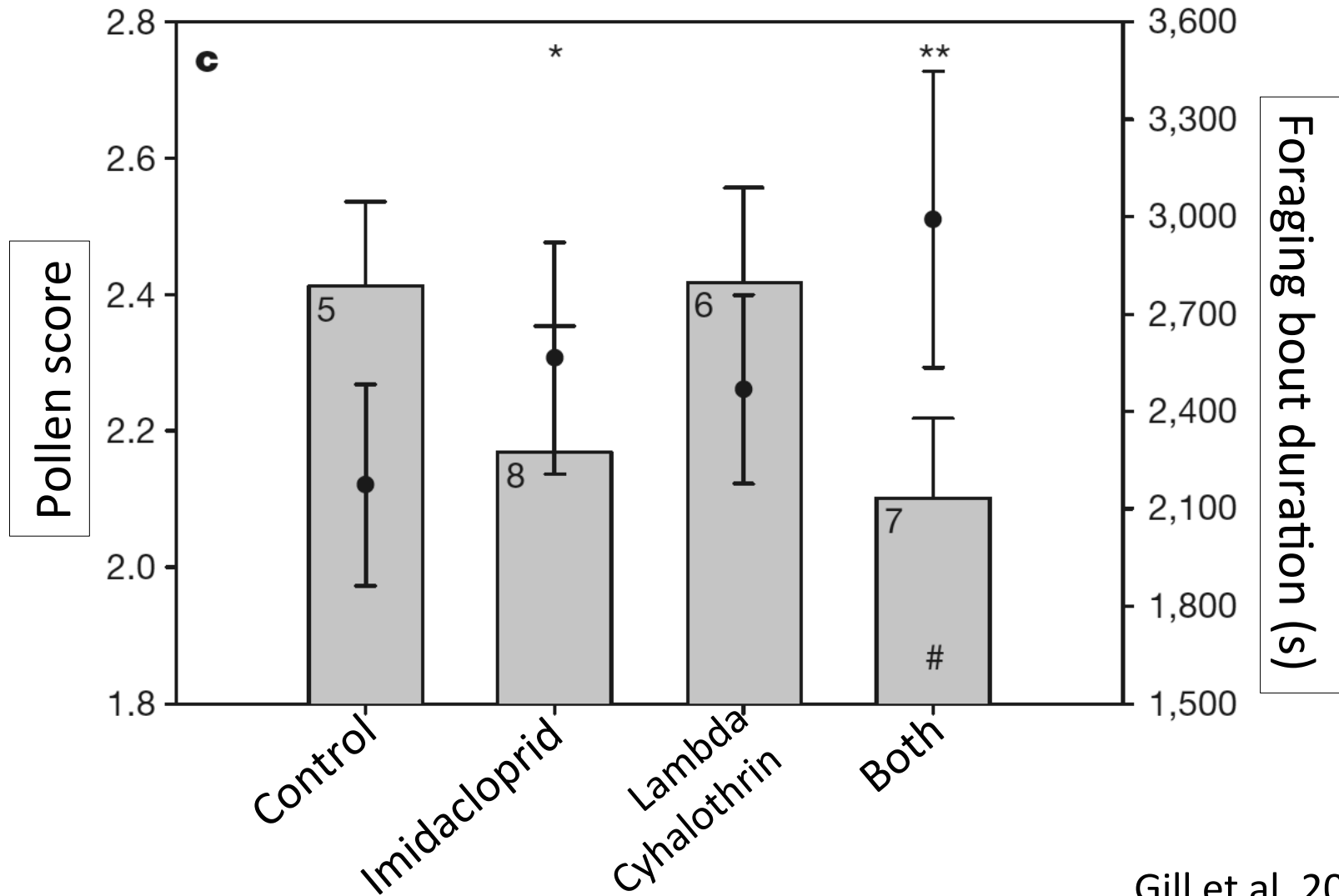
Over 4 week period, exposed *Bombus terrestris* colonies to

- 10 ug/L imidacloprid (fed in sucrose)
- Lambda-cyhalothrin (pyrethroid) sprayed on flowering crops (pollen)
- Both imidacloprid and pyrethroid

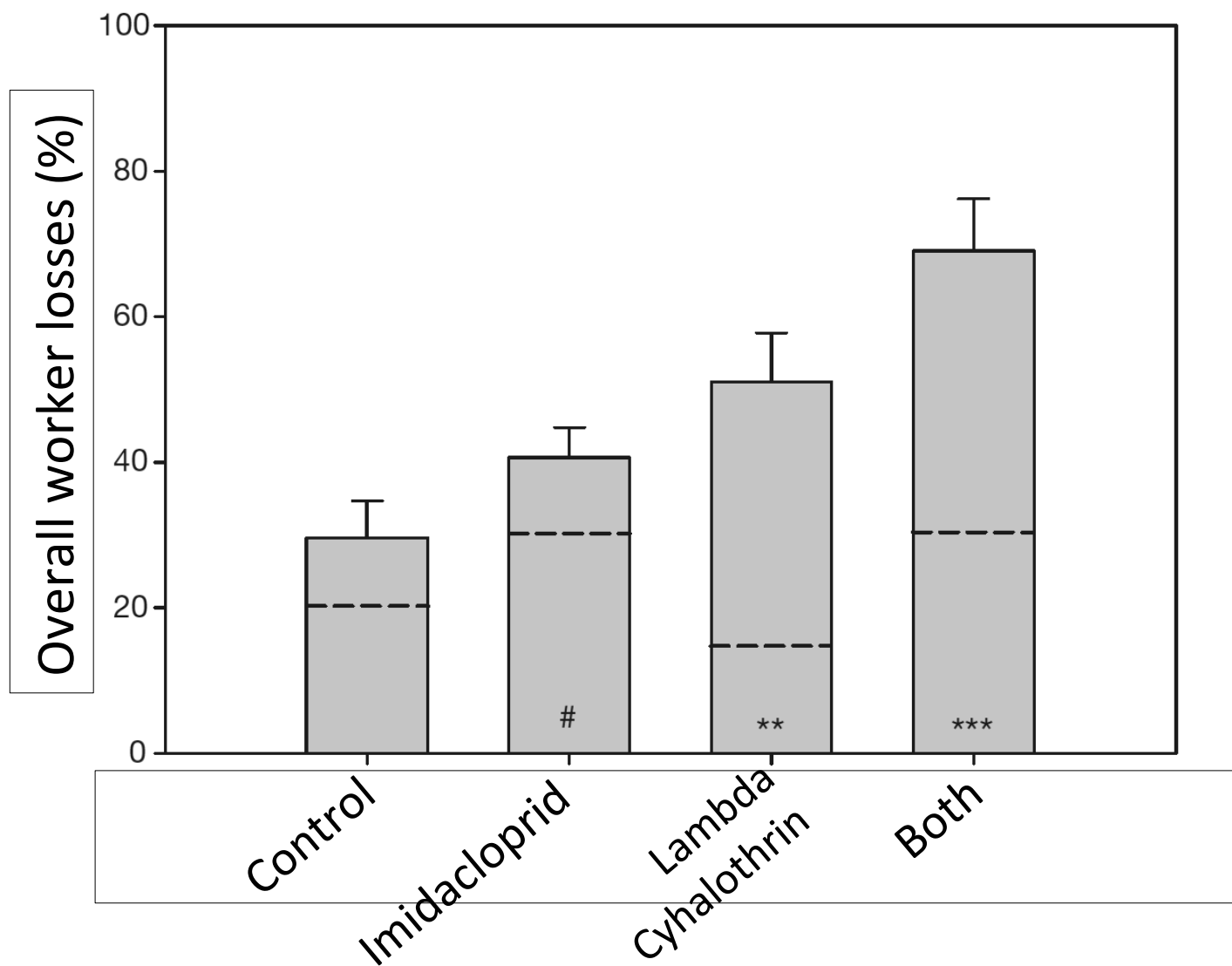
Increased number of foragers



Decreased pollen loads



Increased worker loss

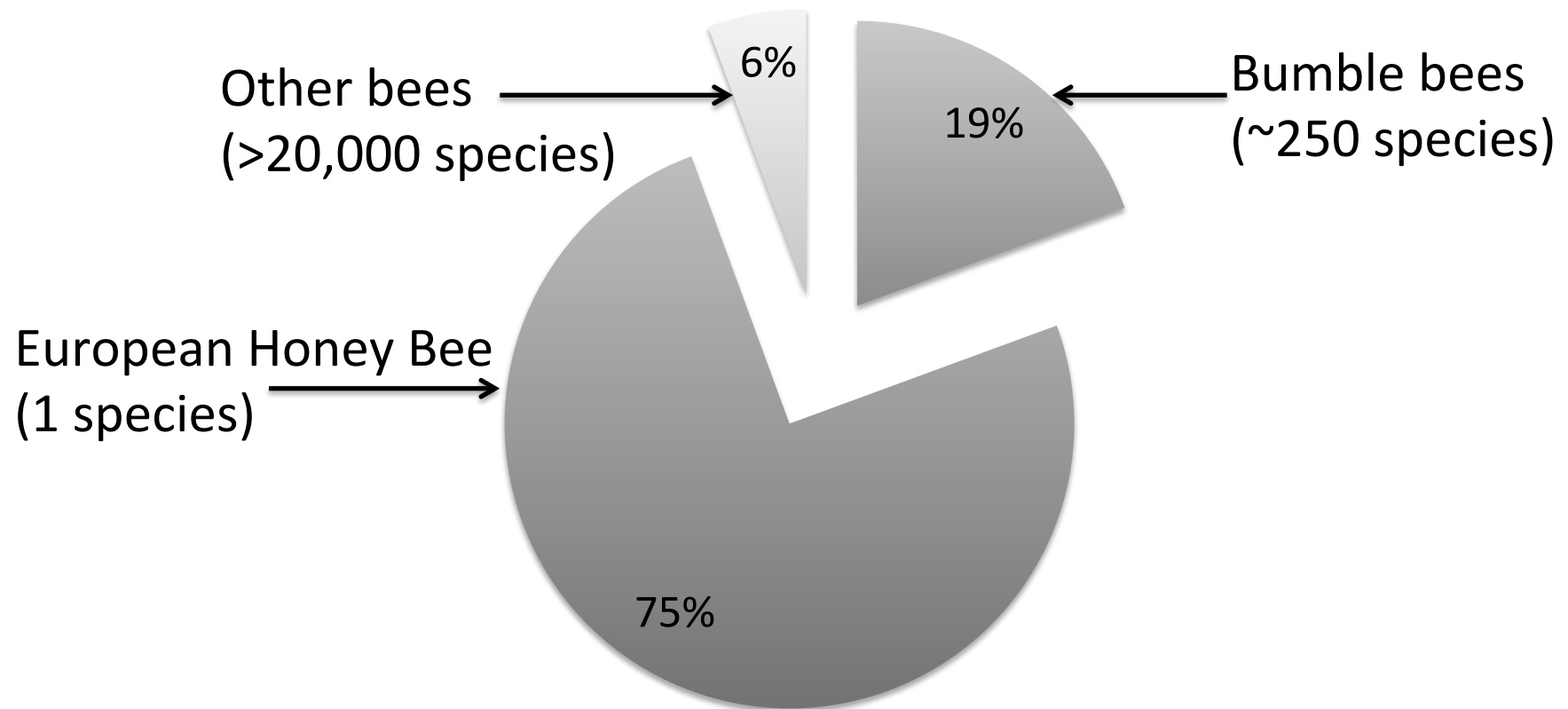


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Limited Information

% of studies of effects of neonicotinoids on pollinators
(by taxon):

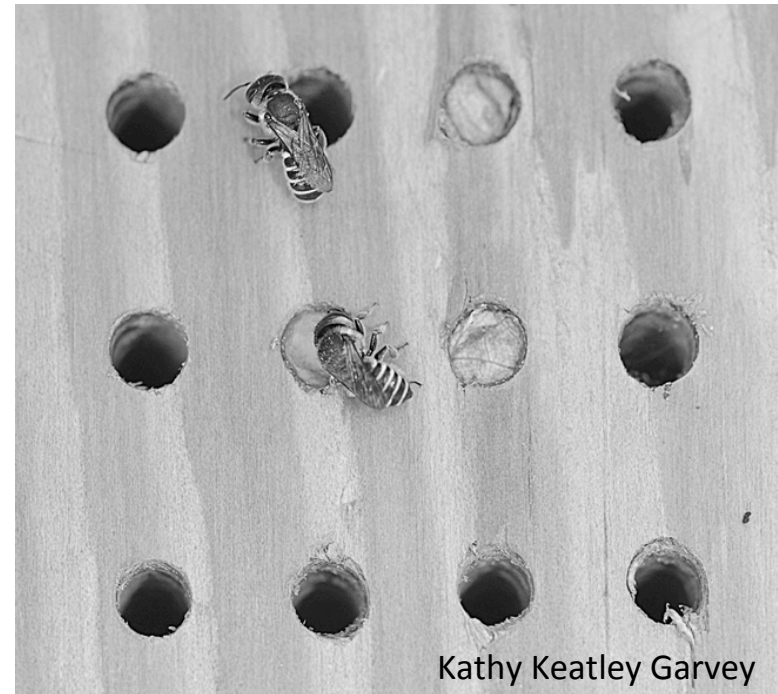


Diversity of Wild, Native Pollinators

- Differences in susceptibility to different neonicotinoids
 - Imidacloprid more toxic than clothianidin to blue orchard bees
 - Clothianidin more toxic than imidacloprid to alfalfa leafcutter bees

Diversity of Wild, Native Pollinators

- Differences in susceptibility to different neonicotinoids
- Differences in nesting behavior



Kathy Keatley Garvey

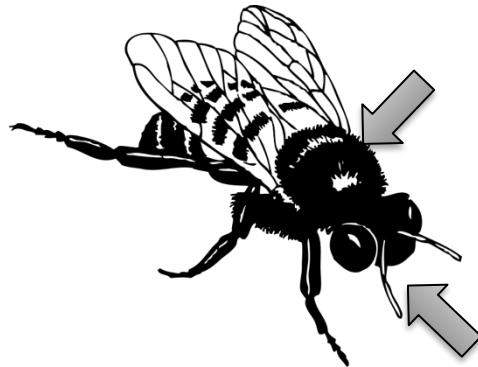
Diversity of Wild, Native Pollinators

- Differences in susceptibility to different neonicotinoids
- Differences in nesting behavior
- Differences in sociality

Conclusions

The effects of neonicotinoids on pollinators will depend on:

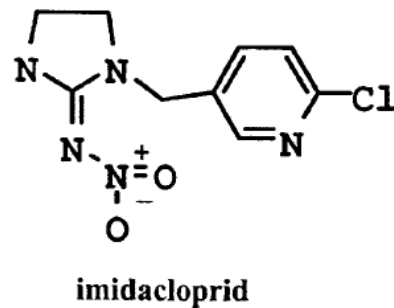
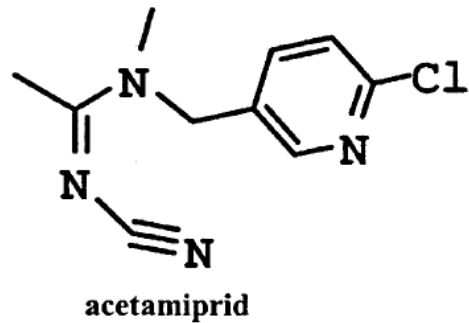
- Route of exposure



Conclusions

The effects of neonicotinoids on pollinators will depend on:

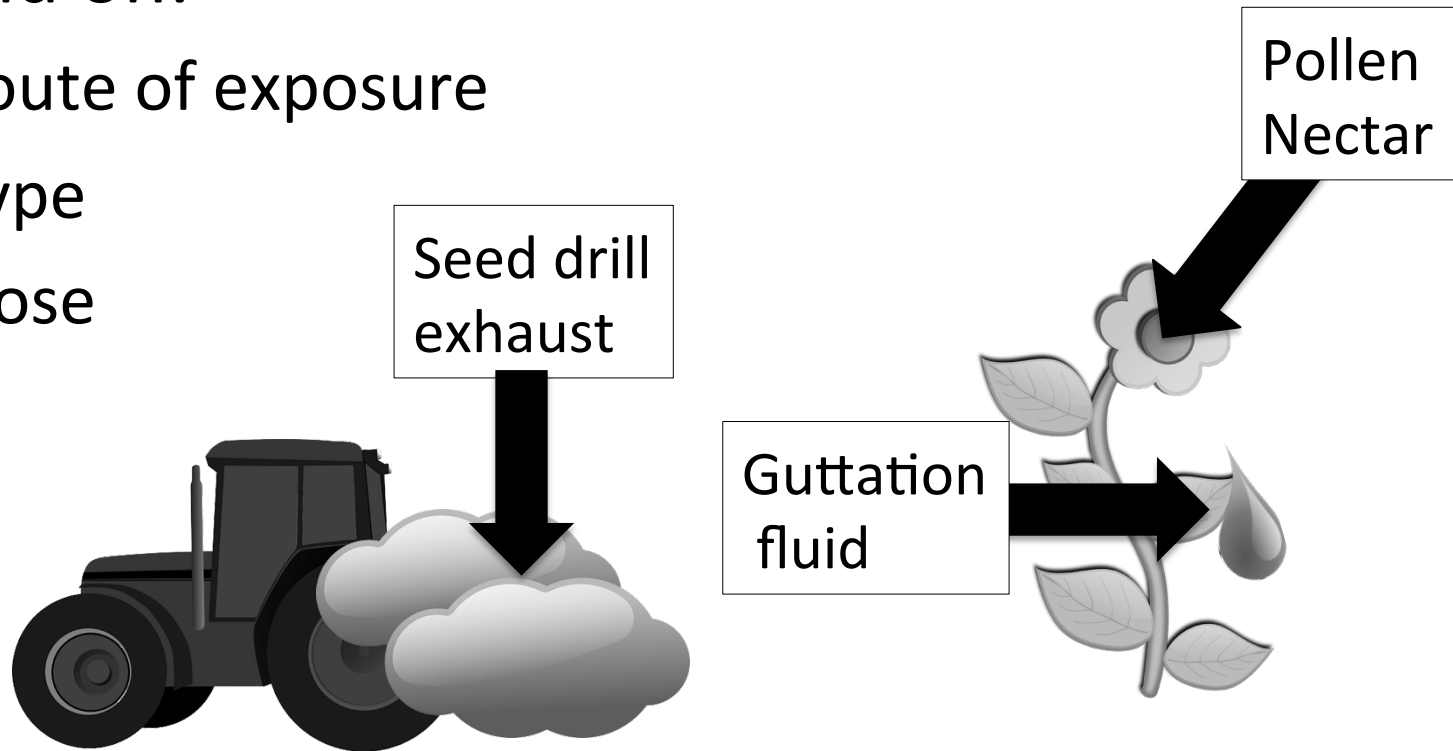
- Route of exposure
- type



Conclusions

The effects of neonicotinoids on pollinators will depend on:

- route of exposure
- type
- dose



Conclusions

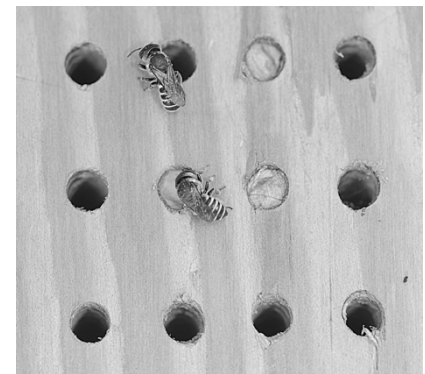
The effects of neonicotinoids on pollinators will depend on:

- route of exposure
- type
- dose
- Interactions
 - Parasites and pathogens
 - Other pesticides

Conclusions

The effects of neonicotinoids on pollinators will depend on:

- route of exposure
- type
- dose
- Interactions
- pollinator species



Conclusions

Pollinators are not likely to experience lethal effects at field-realistic levels of exposure

Sublethal effects have been demonstrated at exposure levels on the higher end of field-realistic

Conclusions

Pollinators are not likely to experience lethal effects at field-realistic levels of exposure

Sublethal effects influence

- Overwintering of honey bees
- Colony growth and reproduction
- Foraging ability
- Immune response



Thank You!

