Special Consideration of Cavity-Nesting Bees for Pesticide Exposure

Theresa Pitts-Singer[1], Fabio Sgolastra[2], Natalie Boyle[1], Andi M. Kopit[3] & Jordi Bosch[4]

[1]*ARS Pollinating Insects Research Unit,* [2]*Università di Bologna,* [3]*Utah State University,* [4]*Universitat Autònoma de Barcelona*

An international workshop on pesticide exposure assessment for non-*Apis* bees

- Silvia Hinarejos & Thomas Steeger, Richard Bireley, Jordi Bosch, Natalie Boyle, Wayne Hou, Theresa Pitts-Singer, Rajwinder Singh, & Neal Williams

Routes of pesticide exposure in solitary, cavity-nesting bees

- Andi M. Kopit and Theresa Pitts-Singer

Objectives

- Life history traits relevant in risk assessment.
- Pesticide properties; contribution to risks.
- Exposure routes.
- Bee assay techniques.

Solitary Bees

- Most are temperate species.
- Highest diversity of bees in deserts.
- Nests composed of multiple cells.
- Each cell has pollen-nectar provision upon which egg is laid.

Managed Solitary Bees

Osmia cornuta, Europe

Osmia bicornis, Europe

Nomia melanderi, western U.S.

Osmia cornifrons, Japan, U.S.

Osmia lignaria, North America

Megachile rotundata, Mediterranean, North America

Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

Traits	Honey Bees	Solitary Bees	Expected implications for risk assessment
Fecundity Body Size	~1,500 eggs/day ~100 mg	Usually ≤ 2 eggs/day (total 10-40) 2 - 400 mg	Social colony resilience makes fitness effects from individual to population level hard to assess in honey bees. Easier in solitary bees.

Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

Traits	Honey Bees	Solitary Bees	Expected implications for risk assessment
Nesting substrate, material	Large cavities or hives. Wax and propolis.	Most underground. Others above in cavities. Mud, soil, leaves, resin, floral oil, etc.	Ground-nesters exposed via excavation, dwelling in soil, contact of food with soil. Natural cavities less likely routes, but nest materials may be contamination source to solitary bees.

Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

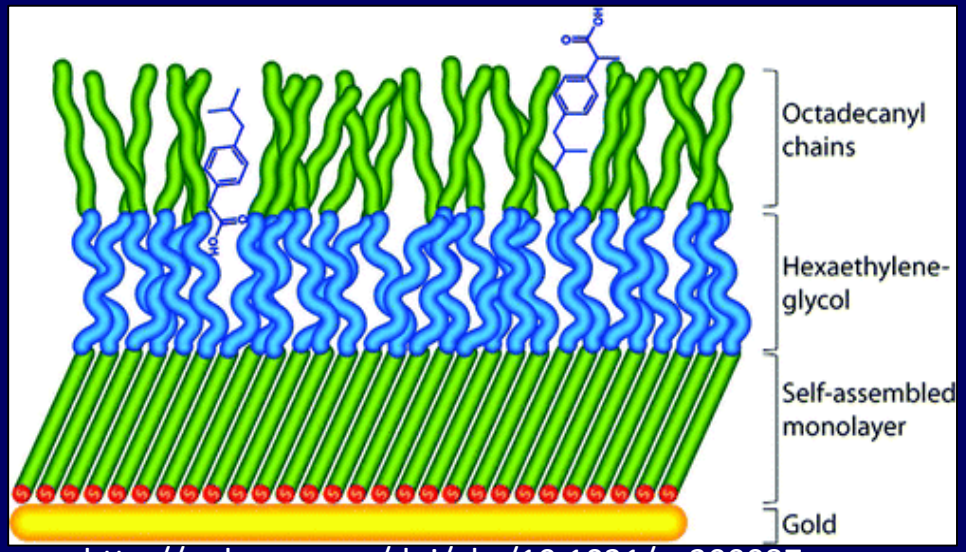
Traits	Honey Bees	Solitary Bees	Expected implications for risk assessment
Nesting period	All or most of the year	Usually 2-3 months in spring or summer.	Honey bees available year-round. Managed adult solitary bees only available for 3-4 months in spring or summer.
Adult food	Nectar + small amounts of pollen. Generalists.	Nectar + small amounts of pollen. Most generalists, but some show preference or specialization.	Solitary bee: nectar/pollen amounts and type varies (most unknown species). Honey bees: prefer nectar high in sugar.

Life History Traits of Honey Bees vs Solitary Bees: Implications for Risk Assessment

Traits	Honey Bees	Solitary Bees	Expected implications for risk assessment
Larval food	Royal jelly, bee bread, and honey.	Pollen mixed with nectar.	Honey bees: larval exposure is “filtered” by nurse bees. Solitary bees: fresh larval food.
Larval food provisioning	Progressive feeding	Mass-provisioning	Honey bees: food for individual larva collected over extended period; food stored and fed later. Solitary bees: larval food collected over short time (1-2 days); feeds within a few days after egg laid.

Chemical Properties of Pesticides

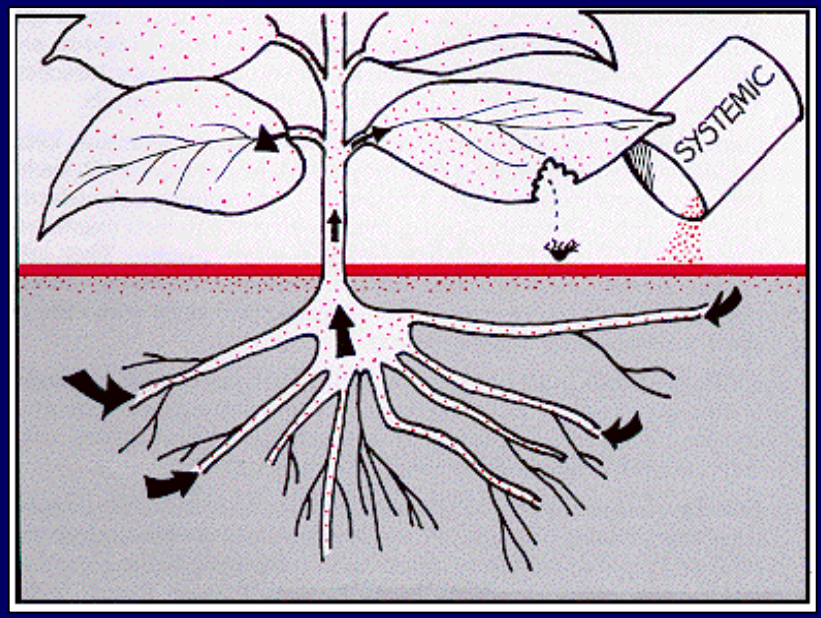
- Contribute to how / where pesticide exist or persist in environment.



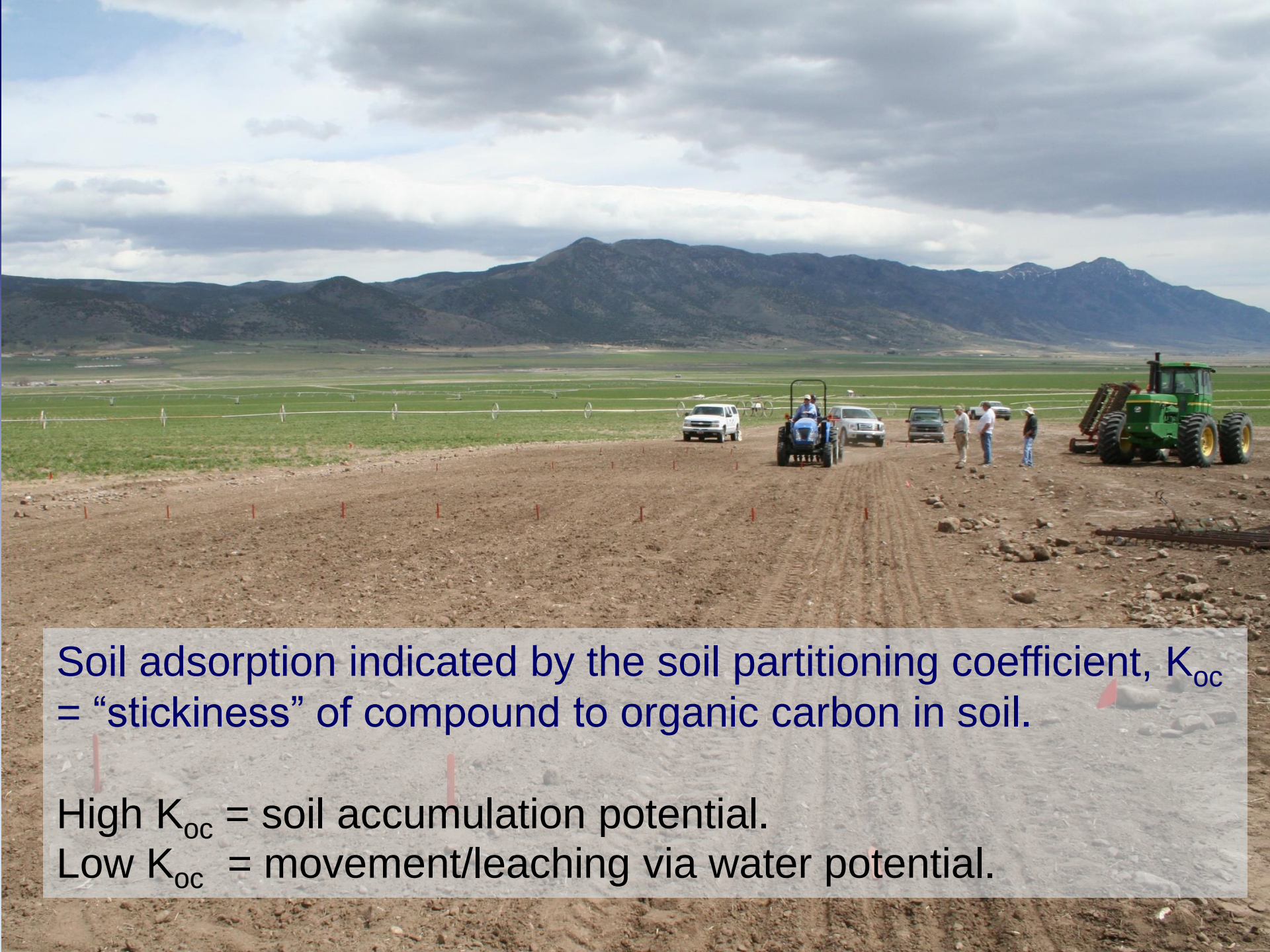
http://pubs.acs.org/doi/abs/10.1021/ac300087z

<u>Octanol:Water Coeffcient</u>

Lipophilicity, High K_{ow}:
a chemical's affinity for lipids.
- allows permeation of plant and insect cuticular lipid layers.



Hydrophilicity, Low K_{ow}:
a chemical's affinity for water.
- effects environmental accumulation & bioavailability for plant uptake; promotes systemic activity.



Soil adsorption indicated by the soil partitioning coefficient, K_{oc} = “stickiness” of compound to organic carbon in soil.

High K_{oc} = soil accumulation potential.
Low K_{oc} = movement/leaching via water potential.

Family/Class	Mode of Action	Active Ingredient	Log K_{ow}	Activity in Environment
Organo -phosphate	Acetylcholine esterase inhibitors	Dimethoate	0.78	Low persistence (microbial degradation), **low biomagnification**; some soluble in water and runoff.
Pyrethroid	Prevents sodium channel closure	Bifenthrin	6.00	**Quick degradation** by UV, water, O_2; **low residuals**; high soil adsorbance; **lipophilic, water insoluble**.
Neonicotinoid	Affects nicotinic acetylcholine receptor	Imidacloprid	0.57	**High water solubility; systemic**; prone to **groundwater leaching**; moderately persistent; does not biomagnify.
Benzoylurea	Chitin biosynthesis inhibitor	Novaluron	5.27	**Translaminar; lipophilic**; low water solubility; strong soil adsorption; low leaching potential; **persistent**.
Fungicide	Succinate dehydrogenase inhibitor	Boscalid, Pyroclostrobin	2.96, 3.99	**Strong soil adsorption; highly persistent**; possible surface run-off with soil particles.

Chemicals in BOB provisions and soil collected from CA almond orchard, March 2016.

Spray application data provided by orchard manager. Analysis by USDA AMS, National Science Laboratories, Gastonia, NC.

In Provision

Chemical	Family/Class	Tim	PPB
Esfenvalerate	Pyrethroid		10.5
Chlorpyrifos	Organophosphate		4.8
Pendimethalin	Dinitroanili	nths	169

We don't know if the presence/amount of pesticides is harmful to these bees at any life stage.

Chemical		Time Since Spray	PPB
Bifenth		8 months	57.4
Es	roid	3 months	35.4
Me	Diacylhydrazines	8 months	248
Oxyf	Diphenyl ether (herbicide)	2 months	301
Pendi ethalin	Dinitroaniline (herbicide)	2 months	1030

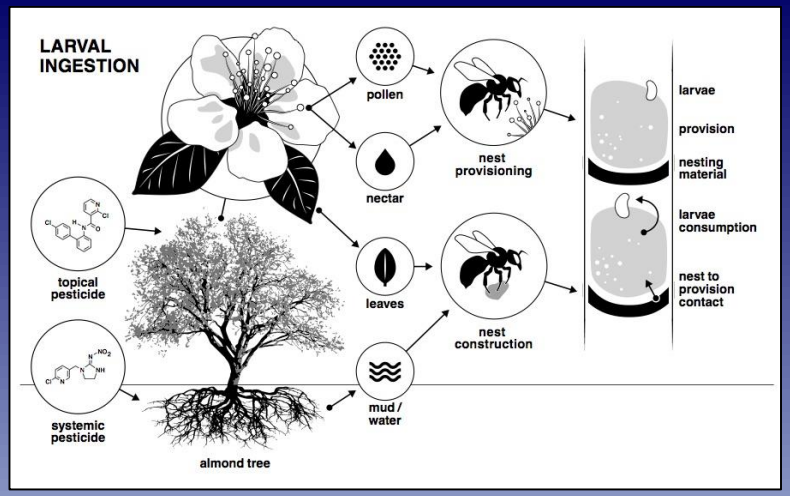
Likely exposure risk for honey bees and solitary bees, based on expert opinion. (compare down columns)

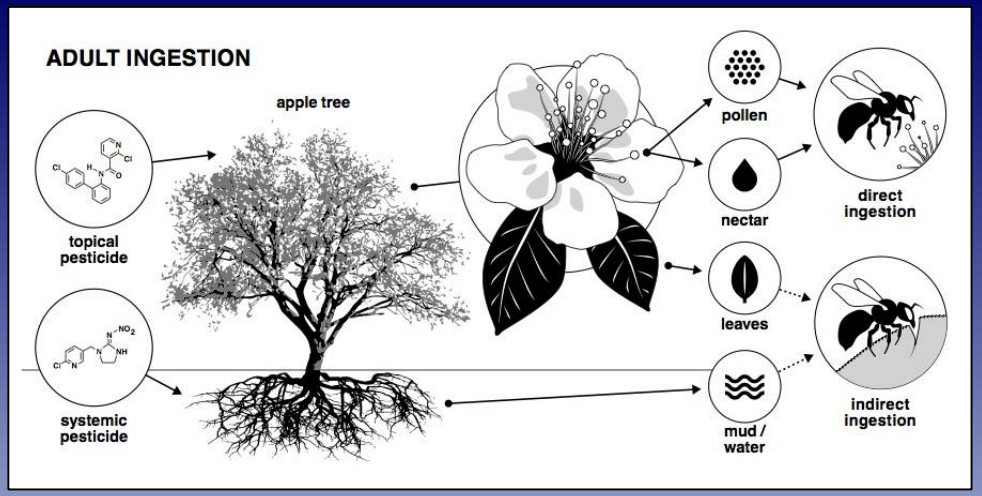
	Adult			Larva		
Routes	Dust/Spray Particles in Air (contact)	Nectar (oral)	Pollen (oral)	Dust/Spray Particles in Air (contact)	Nectar (oral)	Pollen (oral)
Honey Bee	✓			0	✓	✓
***Osmia* spp.**	✓	✓	✓	0	✓	✓
Megachile rotundata	✓	✓	✓	0	✓	✓
Nomia melanderi	✓	✓	✓	0	✓	✓

Likely exposure risk for honey bees and solitary bees, based on expert opinion. (compare down columns)

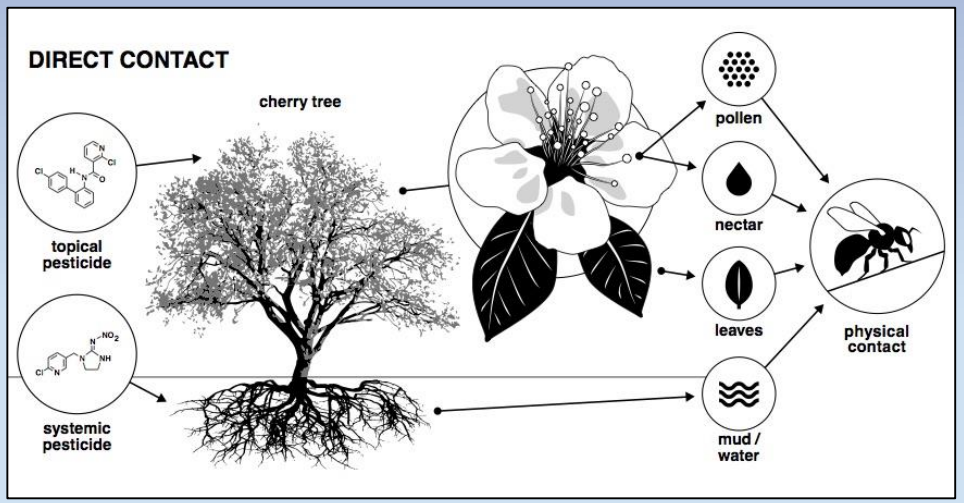
	Adult				Larva			
Routes (via contact)	Mud/ Soil	Wax	Water	Plant Surface (+ oral for M.r.)	Mud/ Soil	Wax	Water	Plant Surface (+ oral for M.r.)
Honey Bee		✓	✓	✓		✓		
***Osmia* spp.**	✓			✓	✓			
Megachile rotundata				✓				✓
Nomia melanderi	✓			✓	✓			

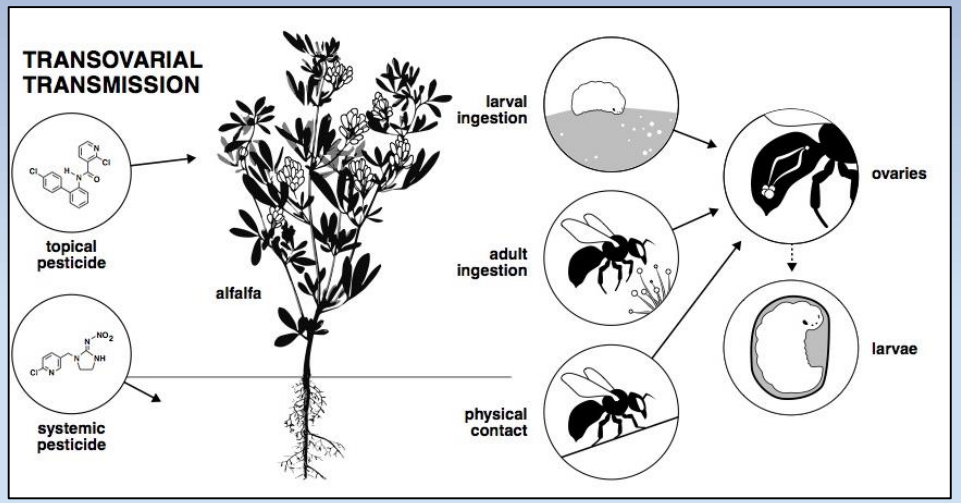
Routes of Exposure for Solitary Cavity-Nesting Bees





1. Larval Ingestion
2. Adult Ingestion
3. Contact
4. Transovarial

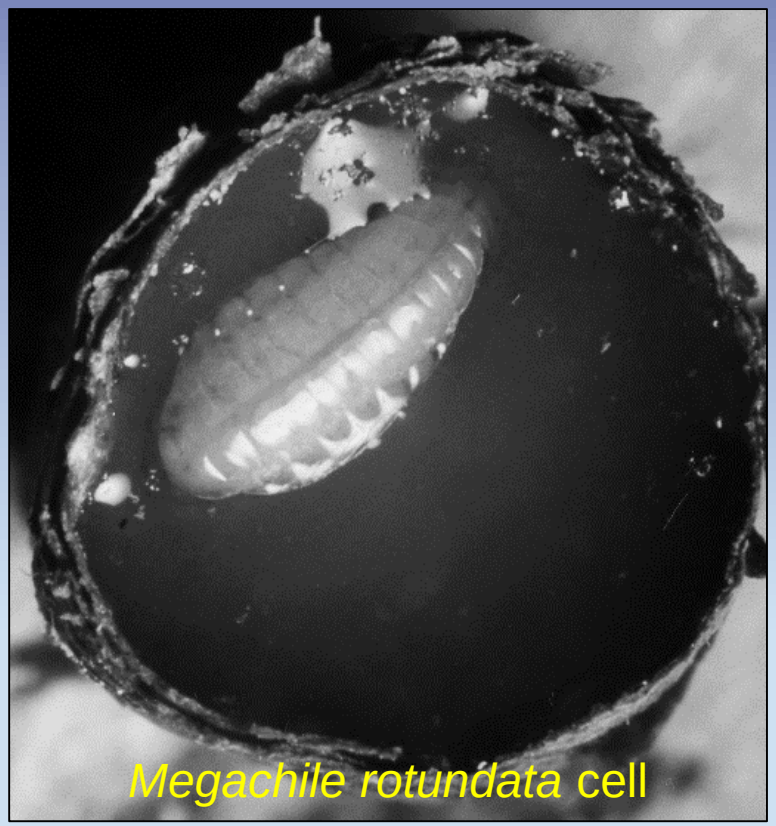




Larval Bee Ingestion Route:

Pollen-nectar mass provision created from natural sources = sole food source.

- detrimental effects on larval development, survival, and/or later adult fecundity.



Megachile rotundata cell

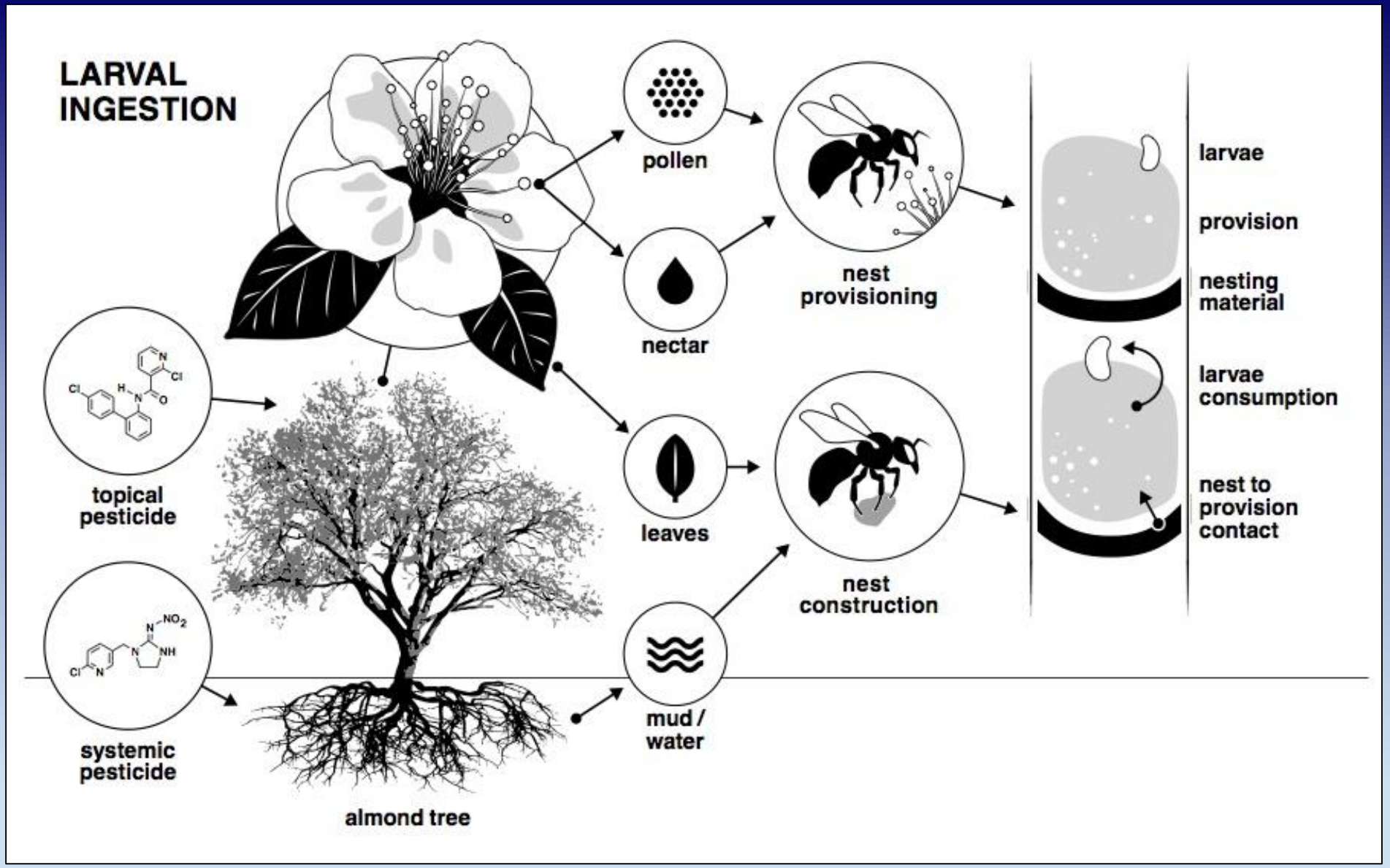
Nomia melanderi cell

Larval Bee Ingestion Route:

Nest-building material contain pesticide residues:

Leaching from material into provision:

- nectar is aqueous, thus could attract hydrophilic chemicals
- pollen contains lipids and proteins, thus could attract lipophilic chemicals.


LARVAL INGESTION
pollen
nectar
leaves
mud / water
nest provisioning
nest construction
larvae
provision
nesting material
larvae consumption
nest to provision contact
topical pesticide
systemic pesticide
almond tree

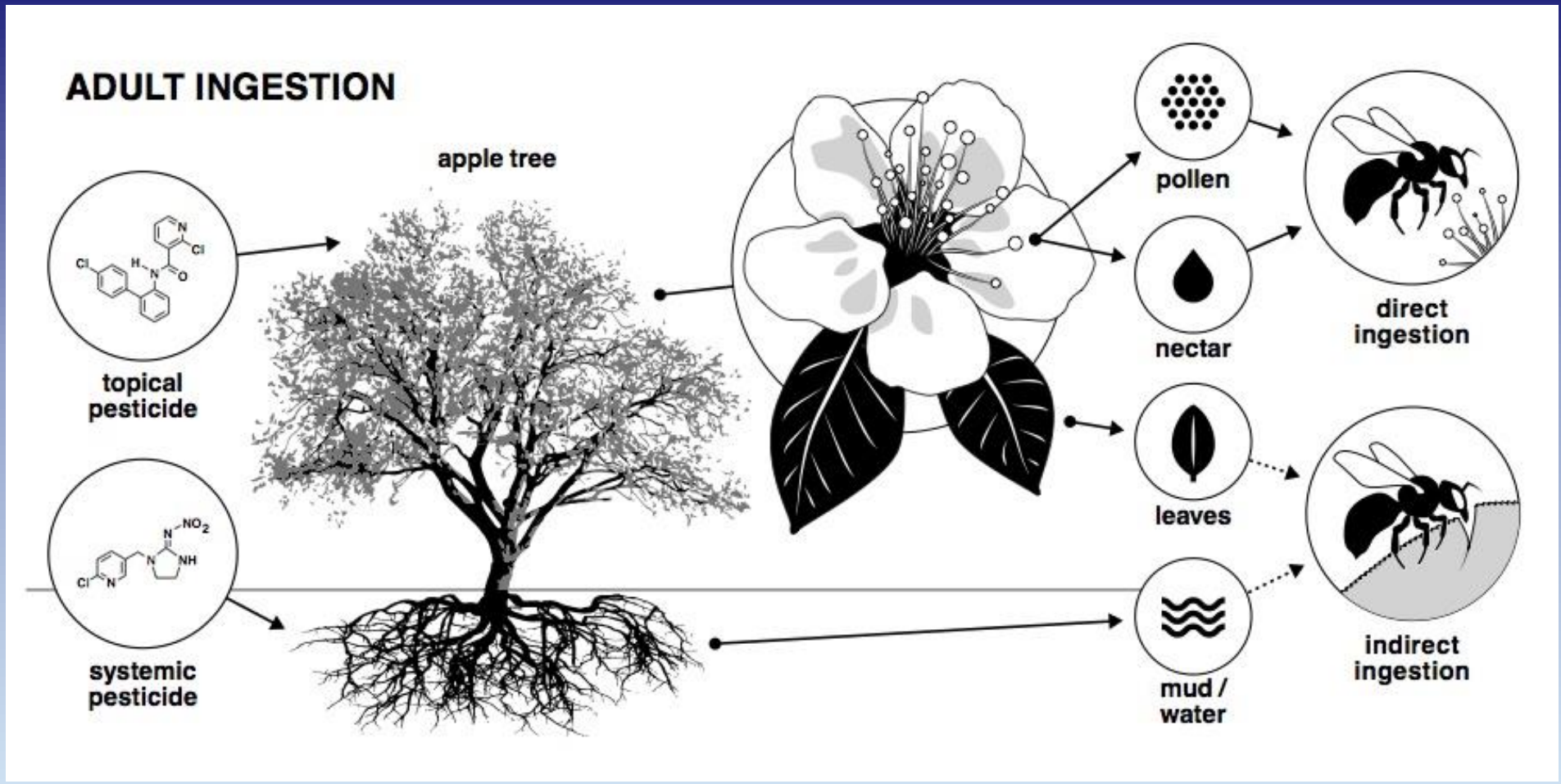
Adult Bee Ingestion Route:

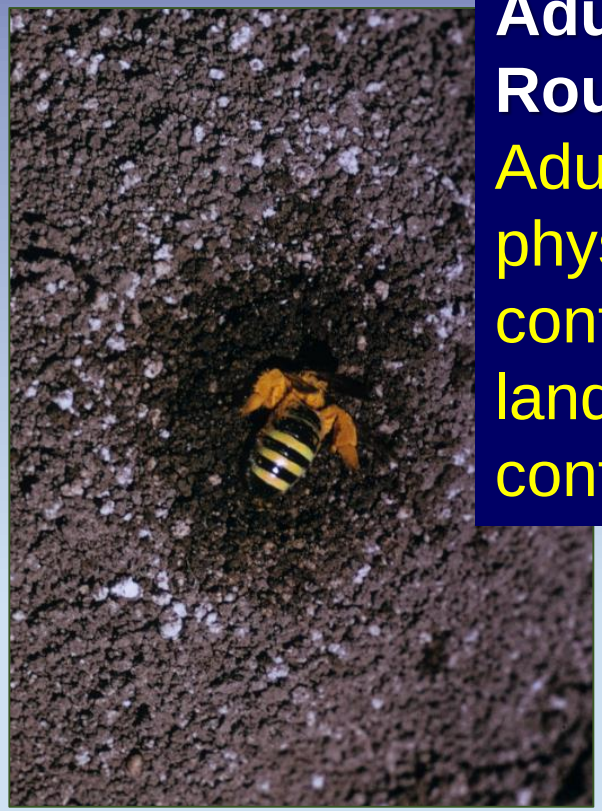
Direct ingestion of contaminated food:

- Nectar for adult energy.
- Pollen for female ovary maturation and egg development.

Incidental Ingestion:

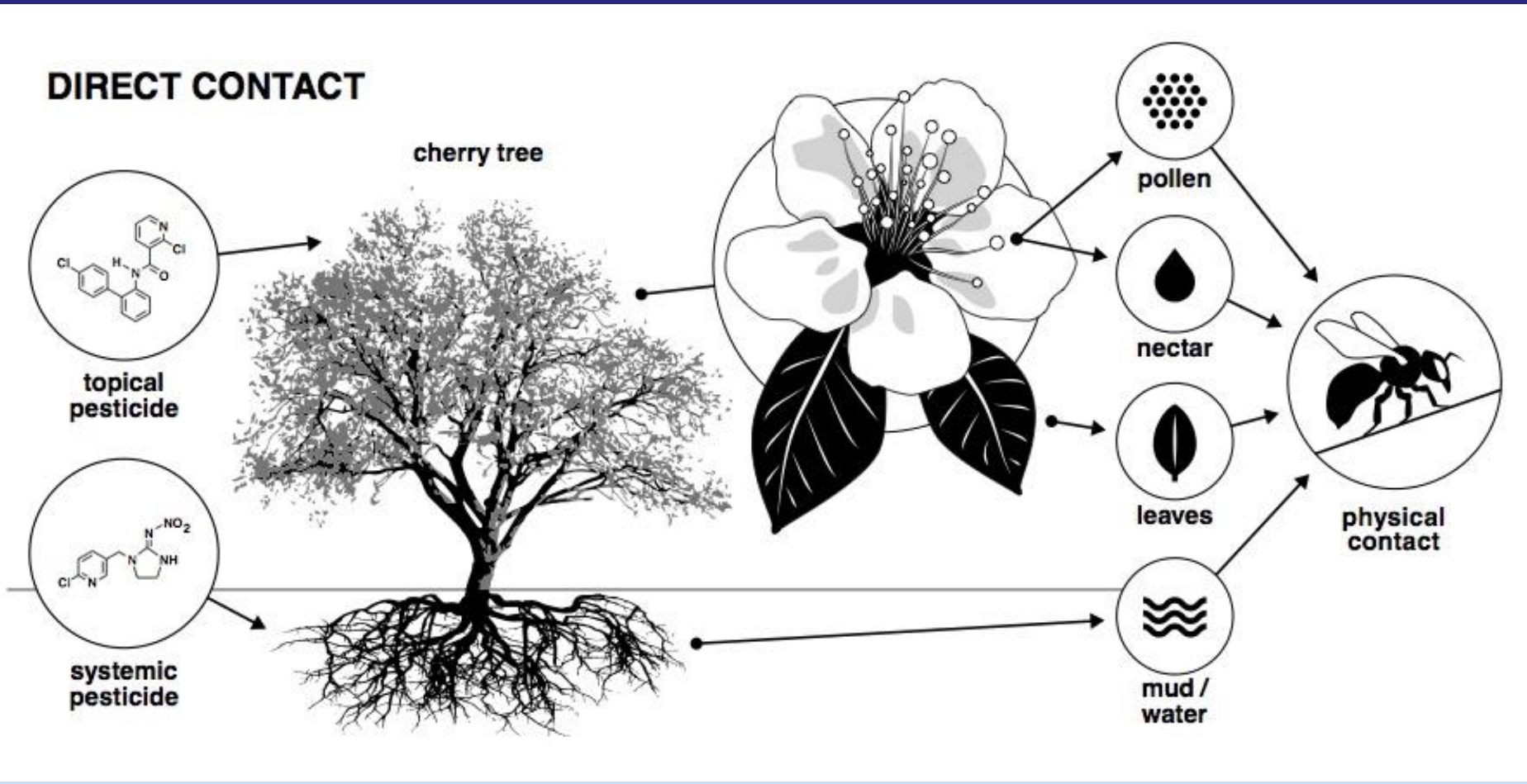
- Grooming of mandibles and tarsi after manipulating / moving contaminated soil and leaf material.


ADULT INGESTION
apple tree
topical pesticide
systemic pesticide
pollen
nectar
leaves
mud / water
direct ingestion
indirect ingestion



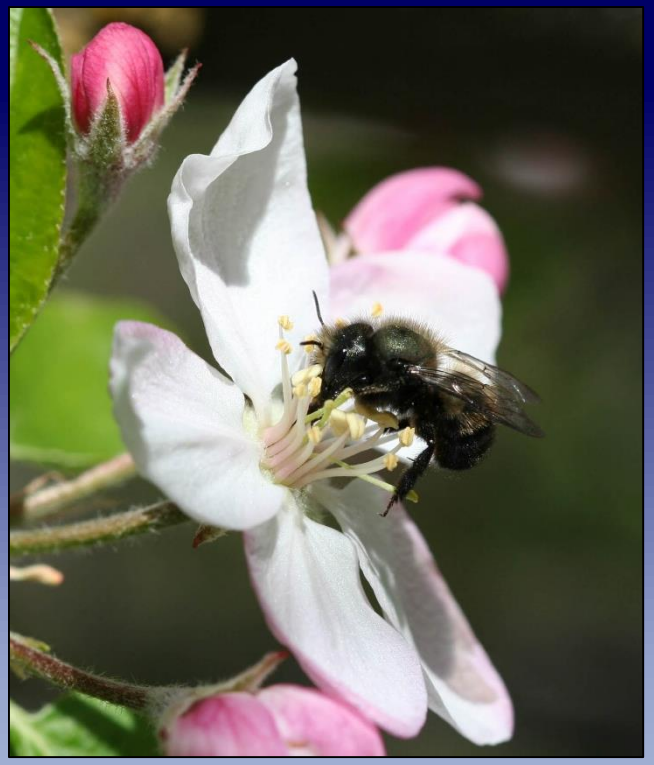
Adult Direct Contact Route:

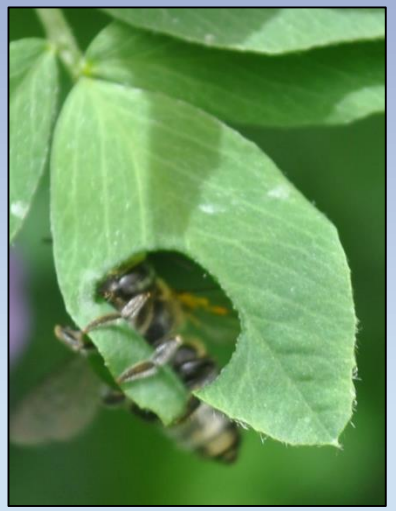
Adult is sprayed or in physical contact with contaminated sources; land on or walk on contaminated surfaces

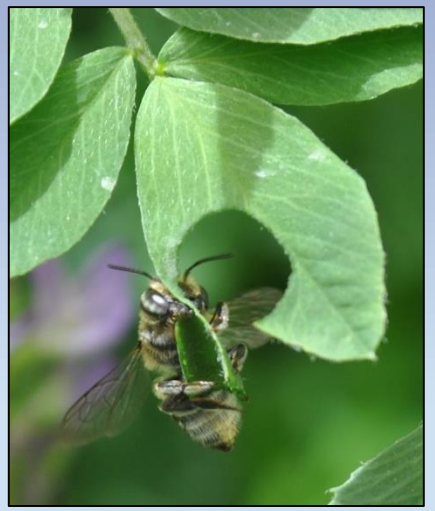

DIRECT CONTACT
cherry tree
topical pesticide
systemic pesticide
pollen
nectar
leaves
mud / water
physical contact

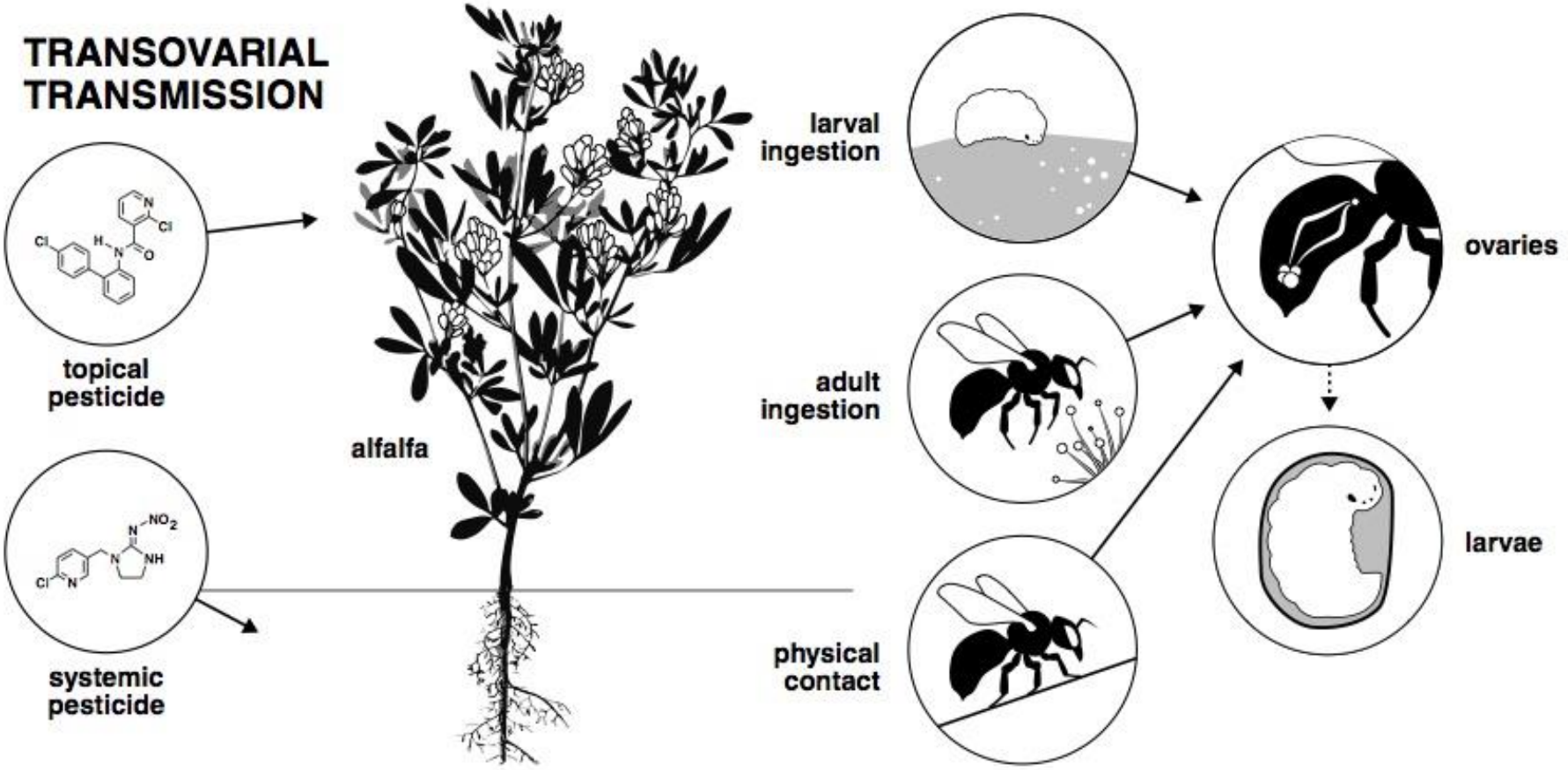
Transovarial Transmission Route:
Results when chemicals are ingested by, or absorbed by, the mother bee.

- deleterious effect on offspring.
- low or no survival of eggs or reduced egg production








TRANSOVARIAL TRANSMISSION
topical pesticide
systemic pesticide
alfalfa
larval ingestion
adult ingestion
physical contact
ovaries
larvae

Pesticides Used During Bloom in Alfalfa Seed Fields Against Seed Predator, *Lygus*

Insect Growth Regulator
= Novaluron (called Rimon)

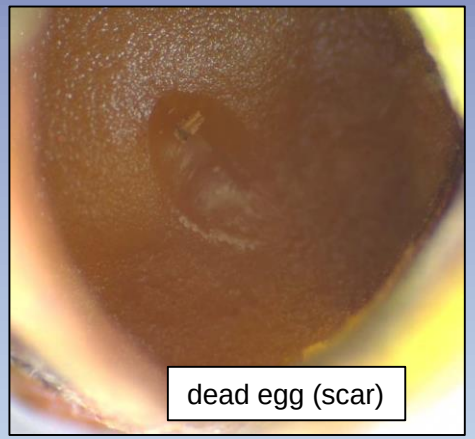
Is novaluron killing eggs or larvae?
If so, how are bees exposed?

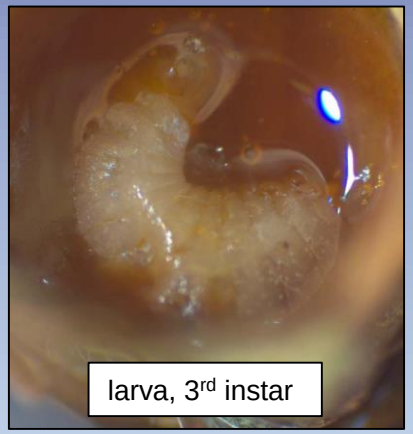
Is novaluron lethal to ALCB eggs if in provision?

ALCB eggs collected from novaluron-free alfalfa seed fields.

Provision doses: 0.5, 1, 2, 10X field rate, water, and blank.

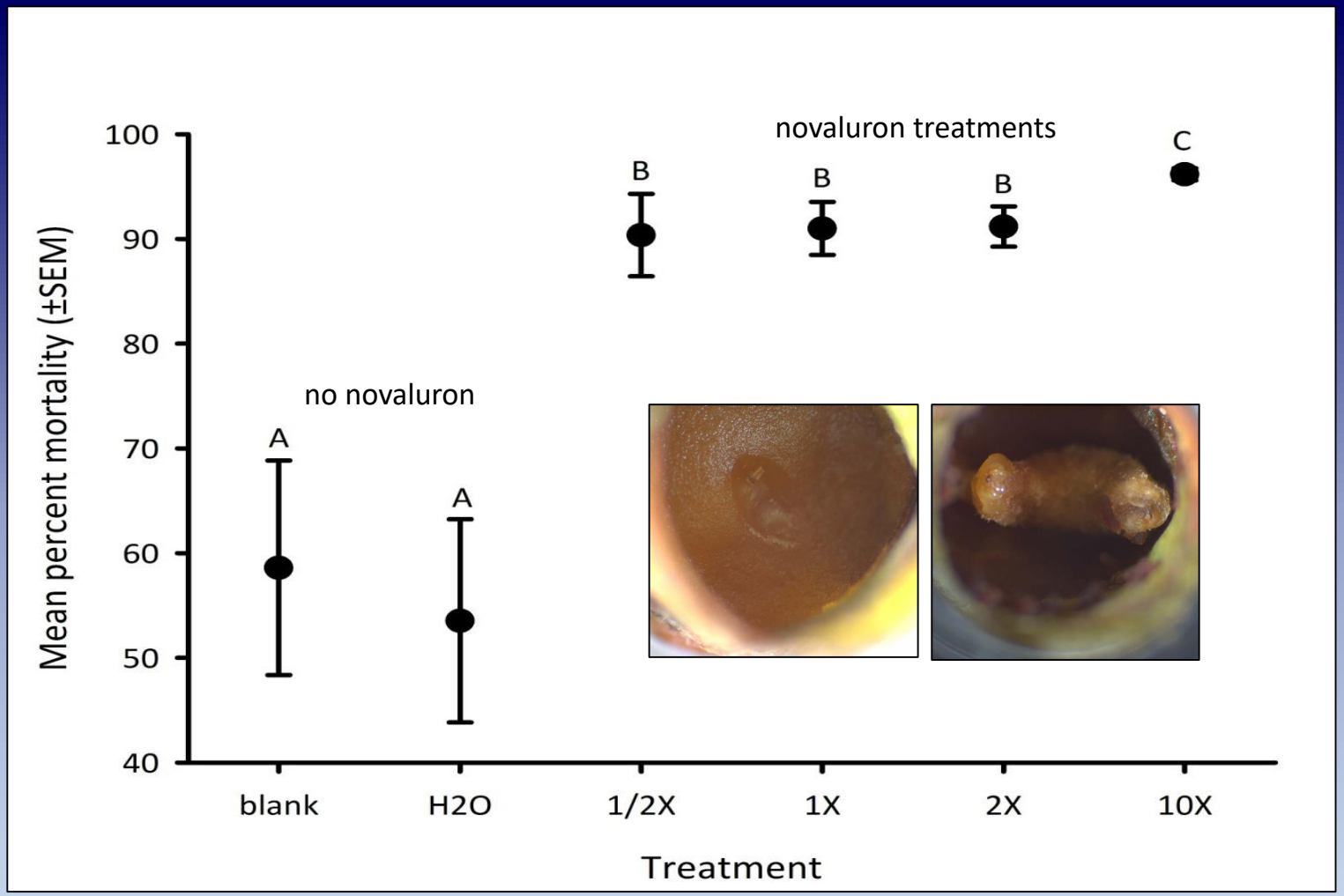
Monitor development of immatures; score mortality.





Hodgson, E.W., Pitts-Singer, T.L. and J.D. Barbour. 2011. *Journal of Insect Science.*

Mean % Mortality



with novaluron: > 90 % mortality, mostly eggs + 1-2 instars

Hodgson, E.W., Pitts-Singer, T.L. and J.D. Barbour. 2011. *Journal of Insect Science.*

Is novaluron lethal to ALCB brood if mothers are directly exposed through nectar-feeding?

Females fed in laboratory:
10% sugar-water + novaluron
OR
10% sugar-water

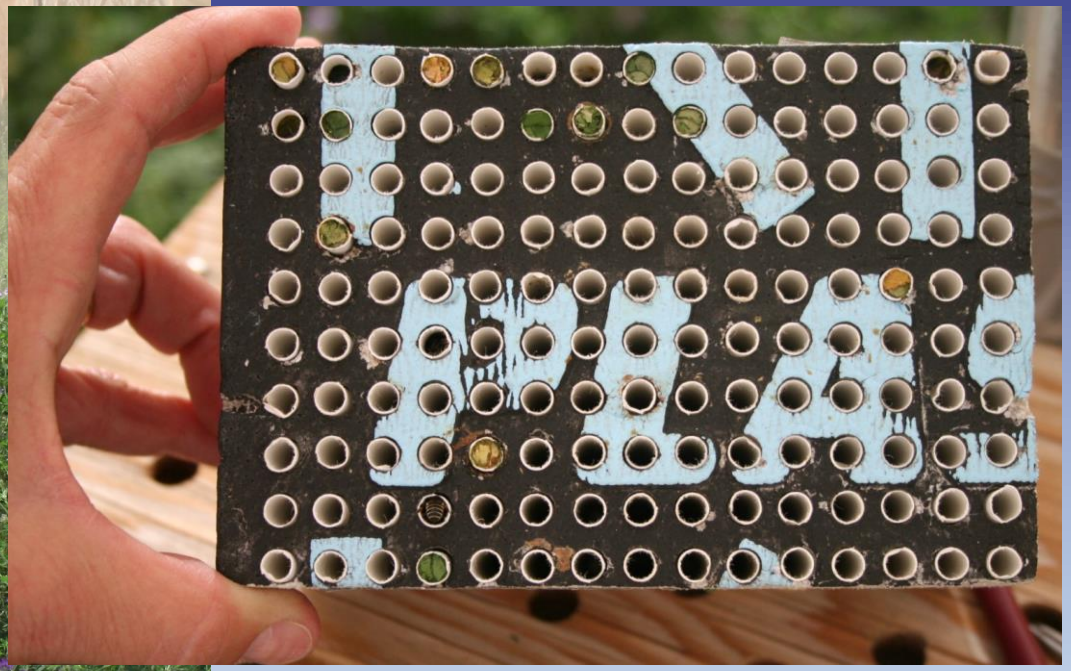
Release in cages for nesting; alfafla never sprayed with novaluron.

Evaluate brood for survival.

Hodgson, E.W., Pitts-Singer, T.L. and J.D. Barbour. 2011. *Journal of Insect Science.*

ALCB Bee Cages in North Logan, UT

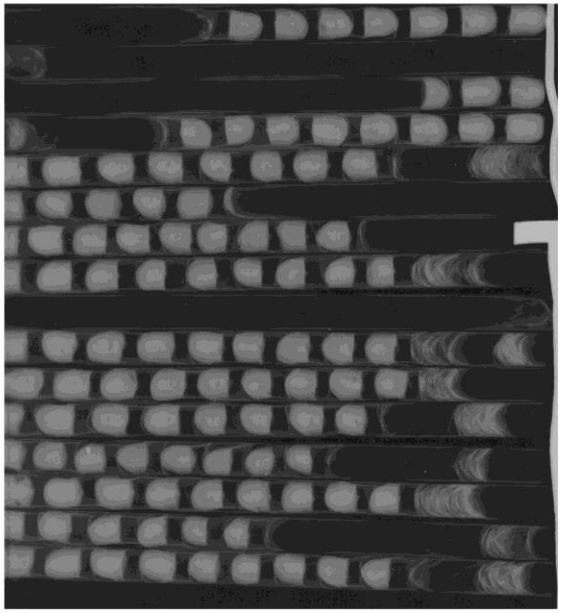
Hodgson, E.W., Pitts-Singer, T.L. and J.D. Barbour. 2011. *Journal of Insect Science.*



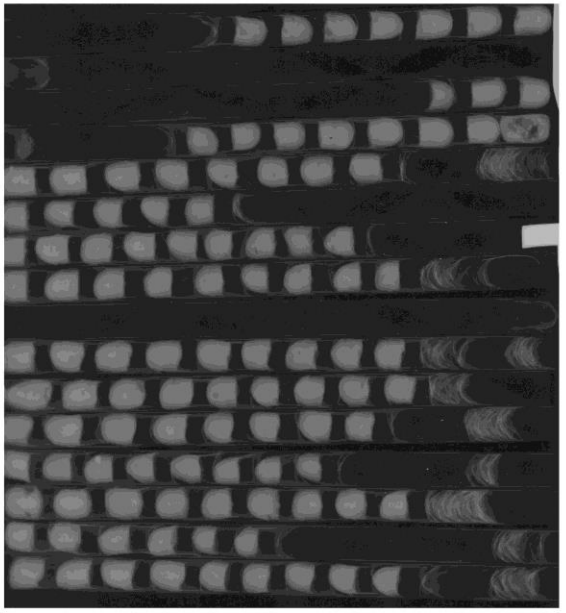
Bees drank sugar + novaluron

Eggs die if mother is fed novaluron.

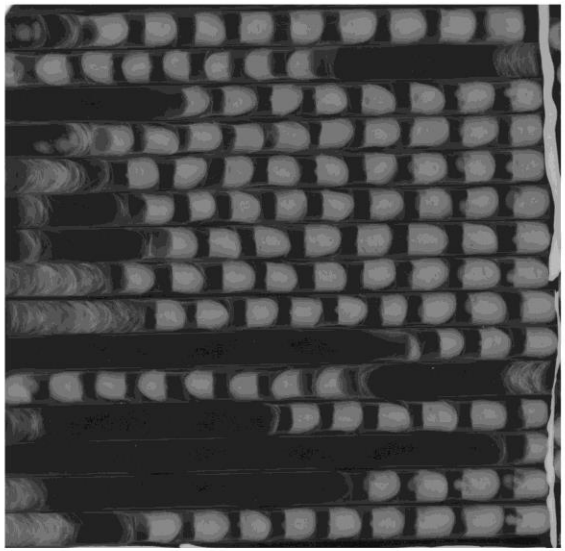
Bees drank sugar only



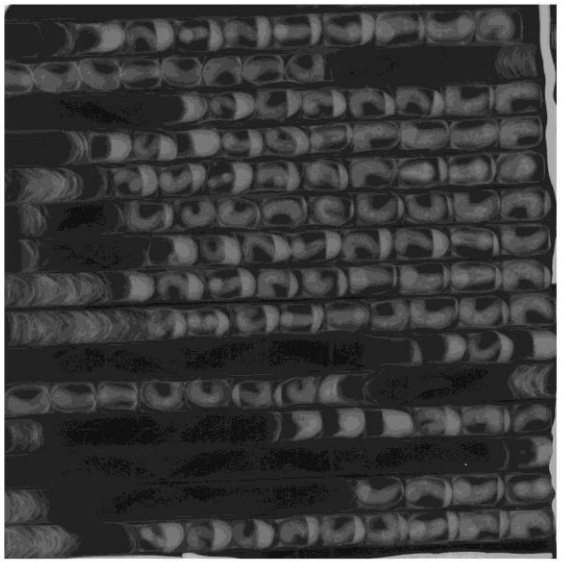
Cage 1, Day 7 for Block 1 (2008)



Cage 1, Day 14 for Block 1 (2008)



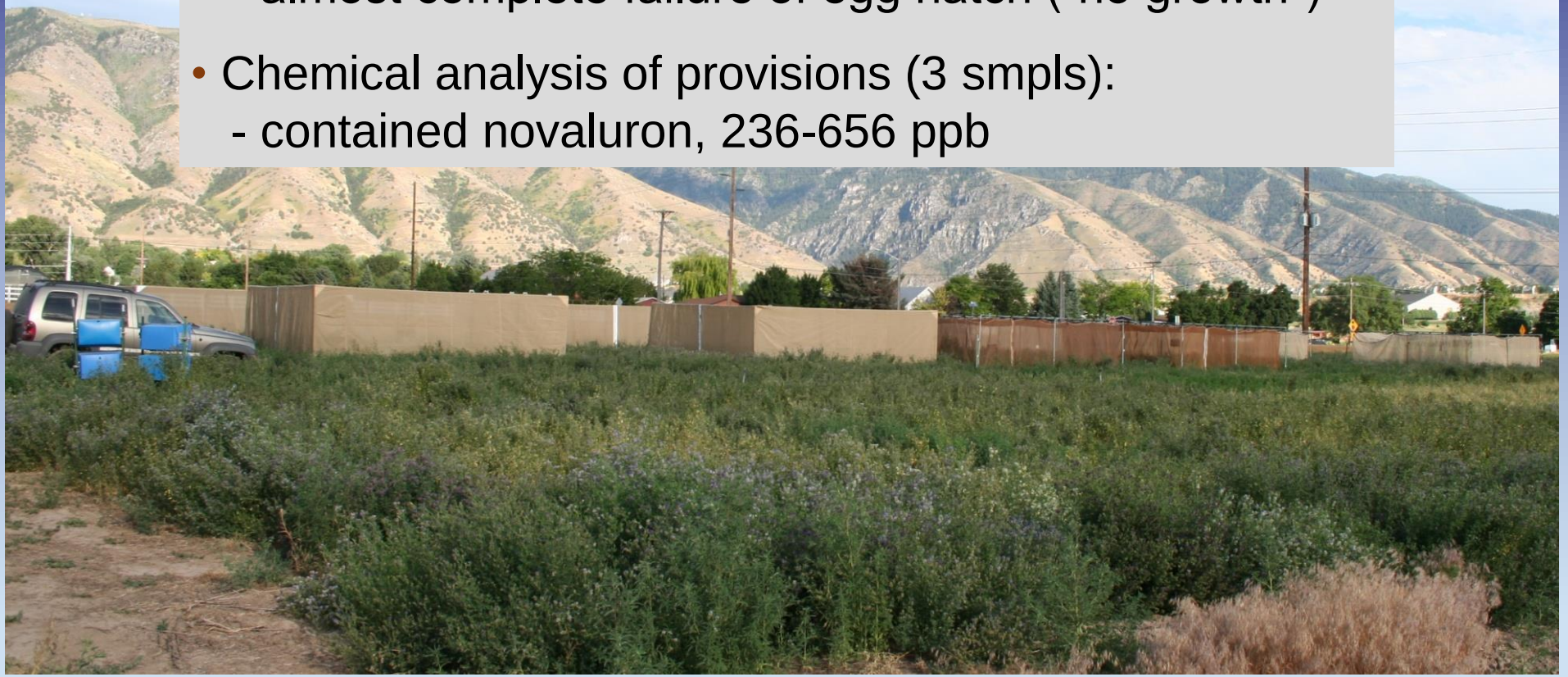
Cage 4, Day 7 for Block 1 (2008)



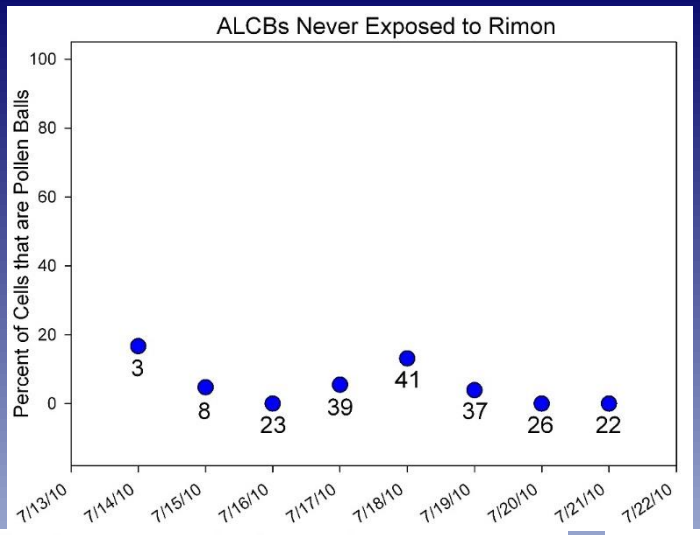
Cage 4, Day 14 for Block 1 (2008)

Back-to-Back Novaluron Sprays on ALCB

- Cages with novaluron-treated alfalfa
- Brood monitored for 1 wk of nesting after each spray:
  - almost complete failure of egg hatch (“no growth”)
- Chemical analysis of provisions (3 smpls):
  - contained novaluron, 236-656 ppb

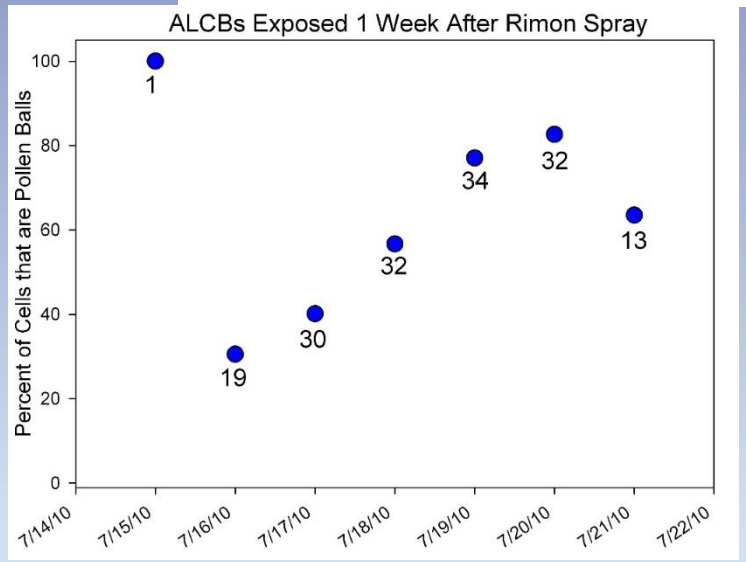


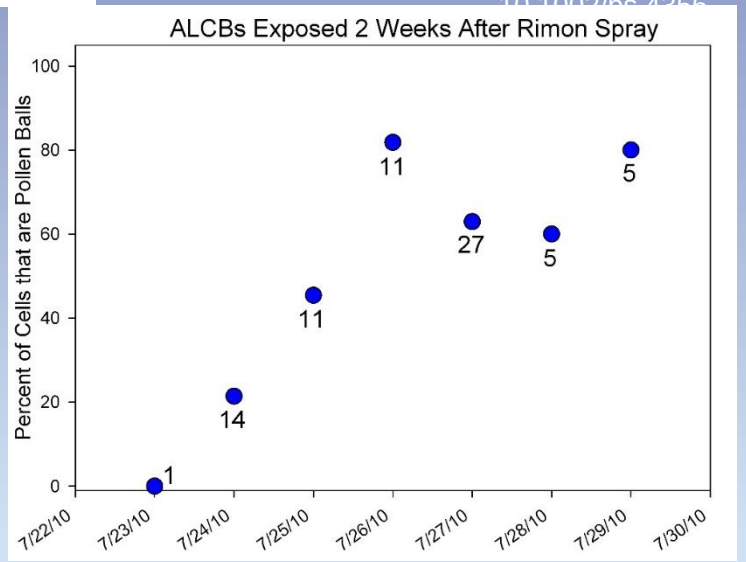
Pitts-Singer T.L. and Barbour, J.D. 2016. Pest Management Sci. DOI 10.1002/ps.4356.



1) Mother bee contaminated internally
   - ingestion or direct contact.
2) Provision contaminated
   - contact with leaf pieces
   - transfer from mother bee's body while making provision.

Pitts-Singer T.L. and Barbour, J.D. 2016. Pest Management Sci. DOI 10.1002/ps.4356





Pitts-Singer T.L. and Barbour, J.D. 2016. Pest Management Sci. DOI 10.1002/ps.4356.

Larval Provision Acquisition and Designing Protocols

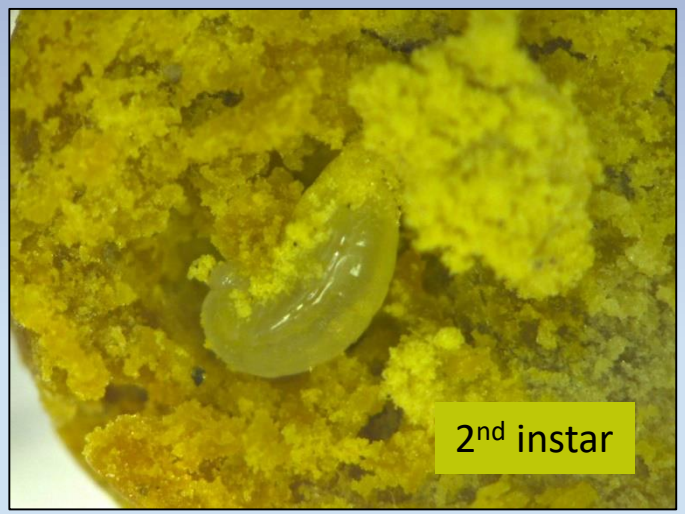
Labor intensive to get natural provisions from nests, make homogenate, and sometimes sterilize it before using in bioassays.

Bee Acquisition and Designing Protocols

Homogenized provision masses in dishes or well plates.

Bee Acquisition and Designing Protocols

Well plates used for pesticide dosing of provisions and rearing of bees from egg to adult.



Conclusion:

More work to do, more decisions to make, more protocols to develop, and more topics to address.

★ How do we verify & quantify exposure routes?

Efforts for conceiving conceptual models (mathematical) done at workshop. e.g., residues on leaves for *M. rotundata*