

# Logan Bee Lab Studies Aimed to Improve Alfalfa Leafcutting Bee Management and Sustainability



Ellen Klinger

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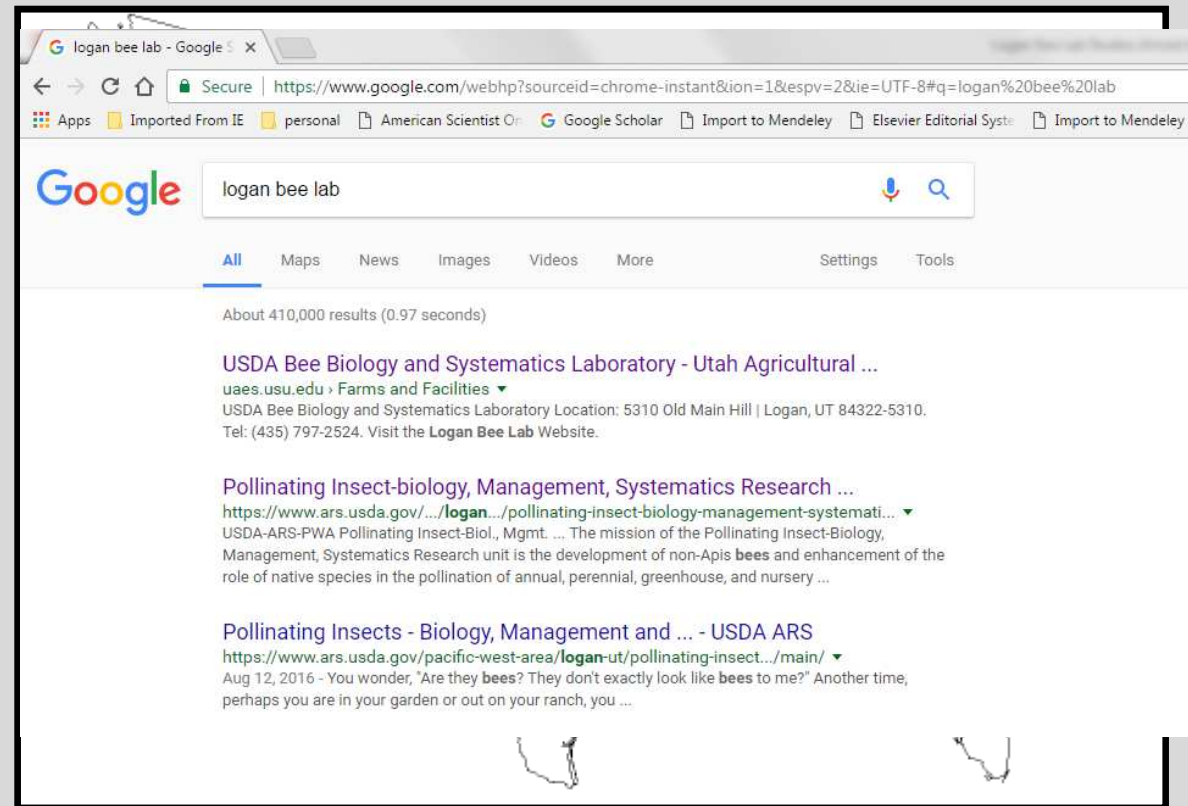
January 10<sup>th</sup>, 2017

ID/OR Alfalfa Seed School





- 5 Research Scientists
- 1 Support Scientist
- 2 post-doctoral researchers
- 5 Permanent technicians
- Graduate, undergraduate students



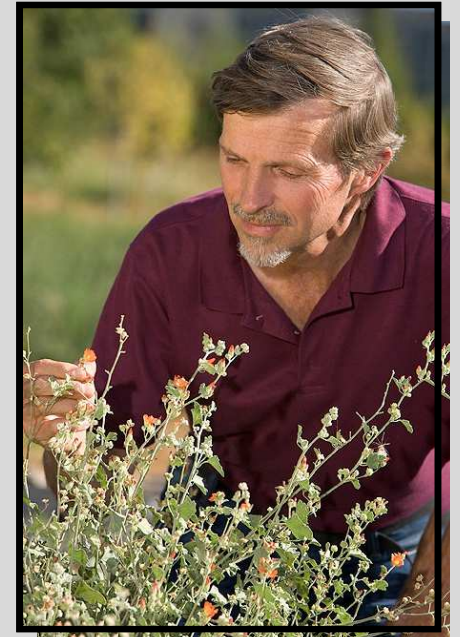
Diana Cox-Foster  
*(bee diseases/toxicology)*



Theresa Pitts-Singer  
*(bee behavior/chemistry)*



Jim Cane  
*(solitary bee nesting biology)*



- Parasite biology and control
- Effect of incubation delays on ALCB survival



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# Parasite biology and control



*Melittobia* sp.



- *Sapyga pumila*

*Tetrastichus* sp.



- *Sapyga pumila*



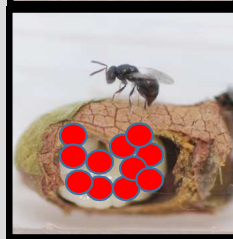
One generation per year



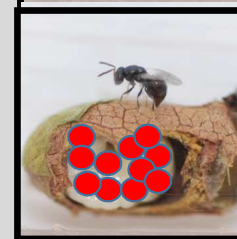
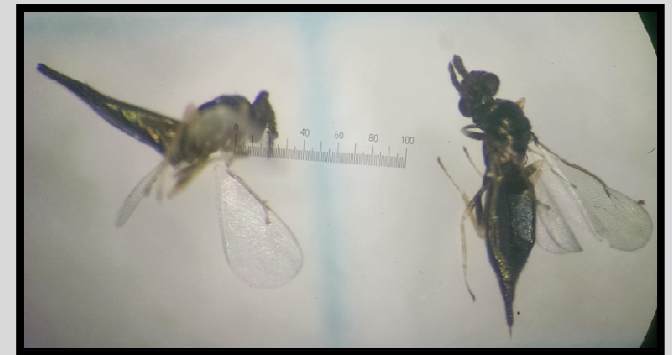
*Tetrastichus* sp.



Multiple generations per year



*Melittobia* sp.



?

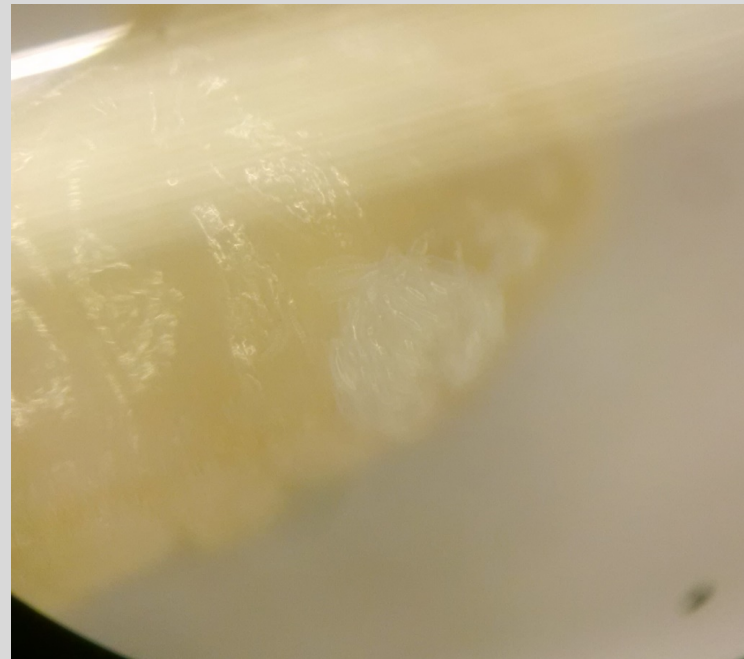


- On day zero, the Melittobia are introduced to healthy Alfalfa leafcutting Bee cells by transferring them with a damp paint brush.
- Through day 1, the wasp inspects the nest cell and selects a location to chew through the cell. The wasp will only make it into the cell within 24 hours if the cell has already been damaged and little work is needed to enter.



- A pin sized entry hole is often located on infected cells.

- By day 2, the wasp can consistently enter the cell. They will also begin to lay eggs on the leafcutter prepupa surface. Often the eggs are laid in a group or groups.

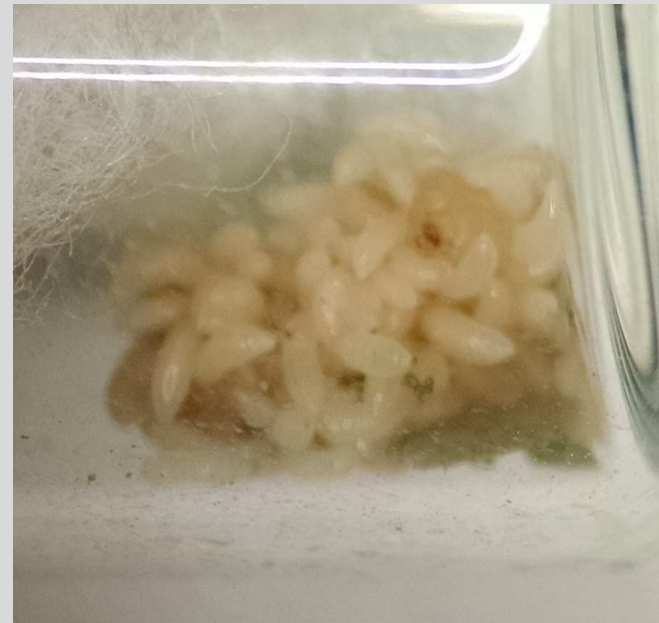






- The eggs will hatch within 24 hours of being laid. The larva start out as a light yellow and darken until day 4.

- On day 4 and 5, the larva will lighten in color and swell by 3-4 times their original size.







- The larva stop growing on day 6 and begin to gray once again. At this time, you can often see frass moving through their body.

- Within 24-36 hours (day 7-8) of becoming gray the wasp will return to the light yellow body color once again. Not long after the larva starts to segment and pupate.

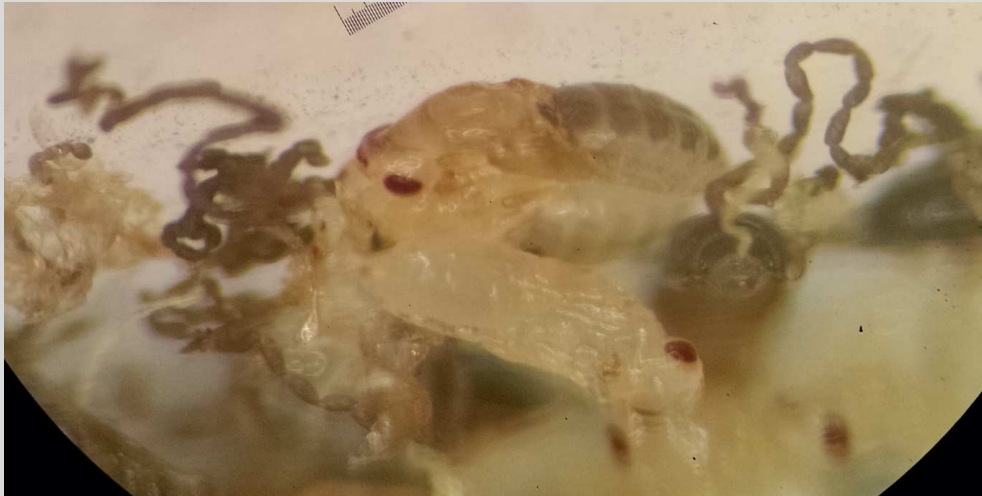




- By the end of day 8, all wasps will have pupated. They will start light yellow or white. Until days 13-16 for males and females, the eyes will get a progressively darker red until the rest of the body darkens with it.

- The males always darken and mature faster than the females. Their pupa is a light tan color and often more narrow.





- Females take longer to pupate than males and will be a pupa between days 8 and 16.

- Females often darken in two phases. They start by turning brown and then continue to turn black. Their bodies are more rounded than the males.





- Males will reach adulthood in 14 days and then start to die within another 8 days, making their lifespan roughly 22 days.

- Males have a light orange to tan head and mesosoma. The metasoma is often a dark brown or black. Their antenna are quite different from the female with a claw-like groove. The wings are short and wrinkled.







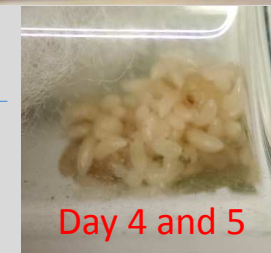
The long wing females very rarely lay eggs on the leafcutter they were born on. Short wing females will lay small clumps of eggs on their birth leafcutter. Short wing females are relatively rare and often unsuccessful with their larva dying as the host leafcutter dies and begins to rot.

Adult females generally die by day 25.

- Females may either be long wing dispersers or short wing non-dispersers. The long wing females have flat, smoother looking wings that extend the entire length of their back. Short wing females have wrinkled, stick like wings similar to the males.







# Parasite trapping

- Effectiveness of trapping is unknown
  - Test various types of trap
  - Test chemical lures (baits)





Black  
Baited No Bait

White  
Bait No Bait



Black  
Baited No Bait

White  
Bait No Bait



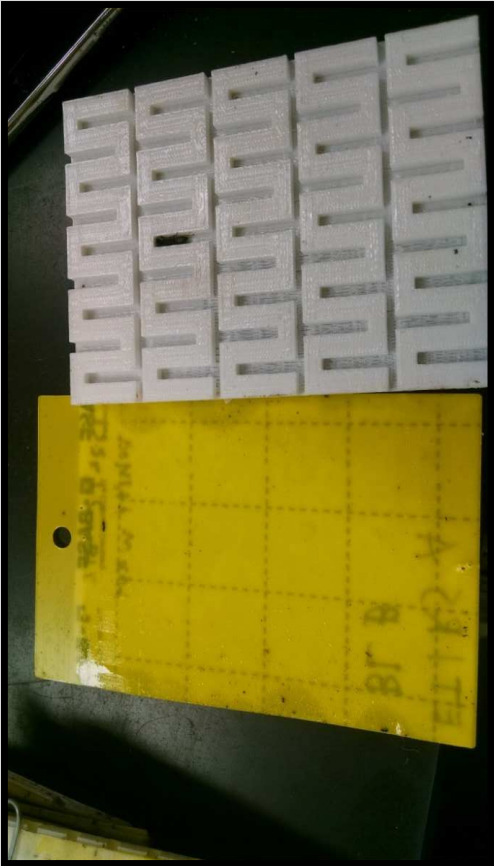
Bait was made by  
extracting  
chemicals from 100  
empty leaf cells





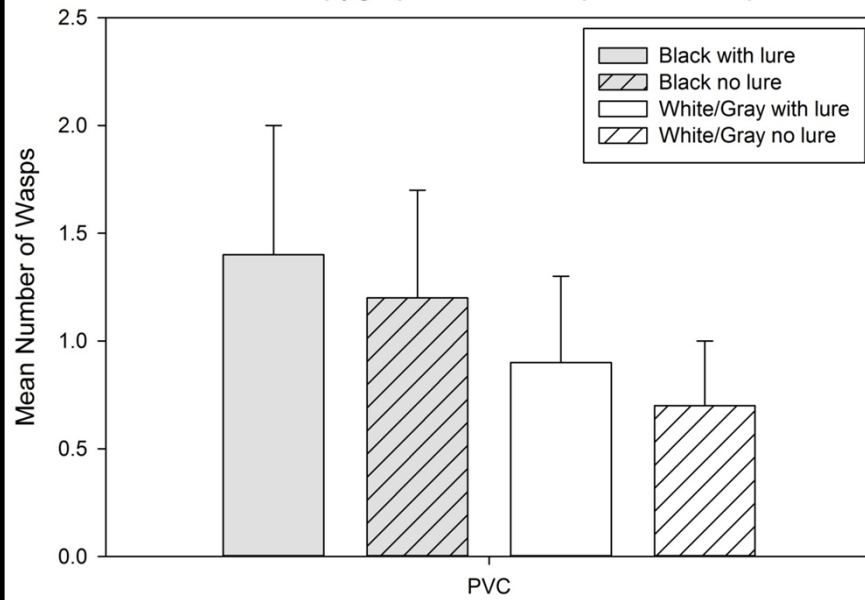




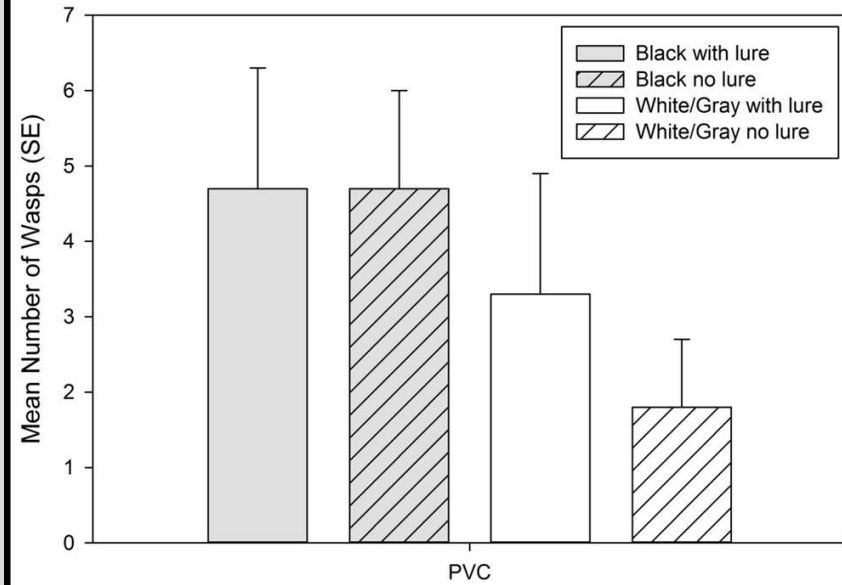




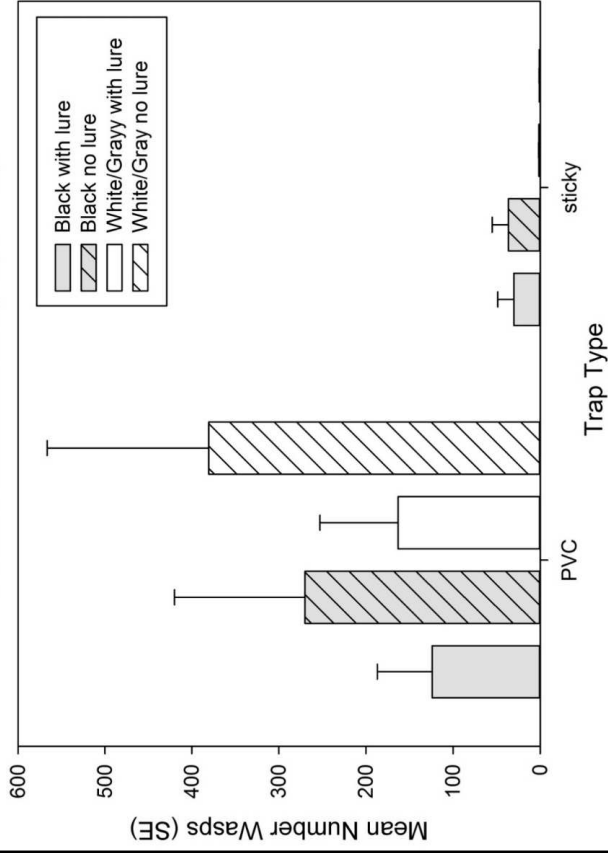
Field 2: *Sapyga pumila* Males per PVC Trap



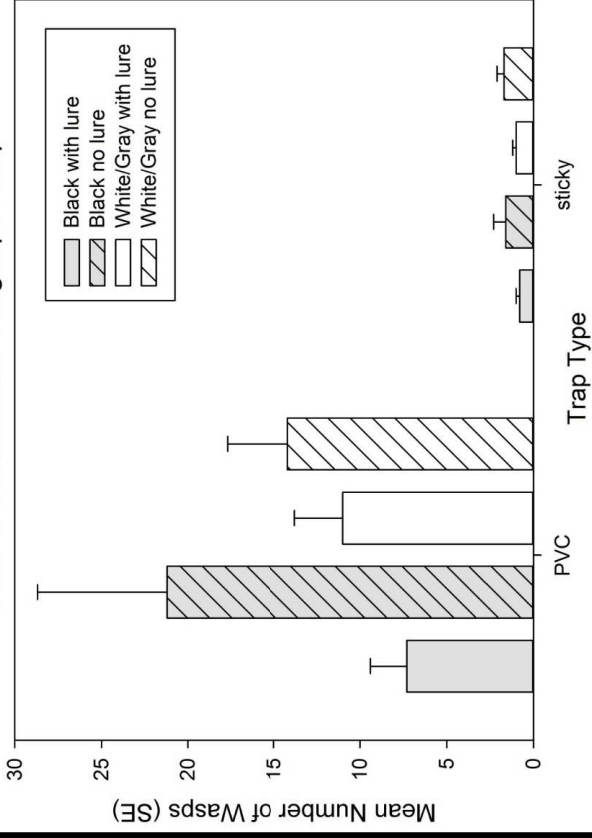
Field 2: *Sapyga pumila* Females per PVC Trap



Field 2: *Melittobia* Caught per Trap



Field 1: *Tetrastichus* Caught per Trap





# Effect of incubation delays on ALCB survival

- Timing of leafcutter incubation must match with alfalfa bloom; however sometimes bloom is delayed
- ALCB require about three weeks of 28-30 °C for emergence
- When is a good time to delay ALCB?
- How long can you delay bees without harming them?





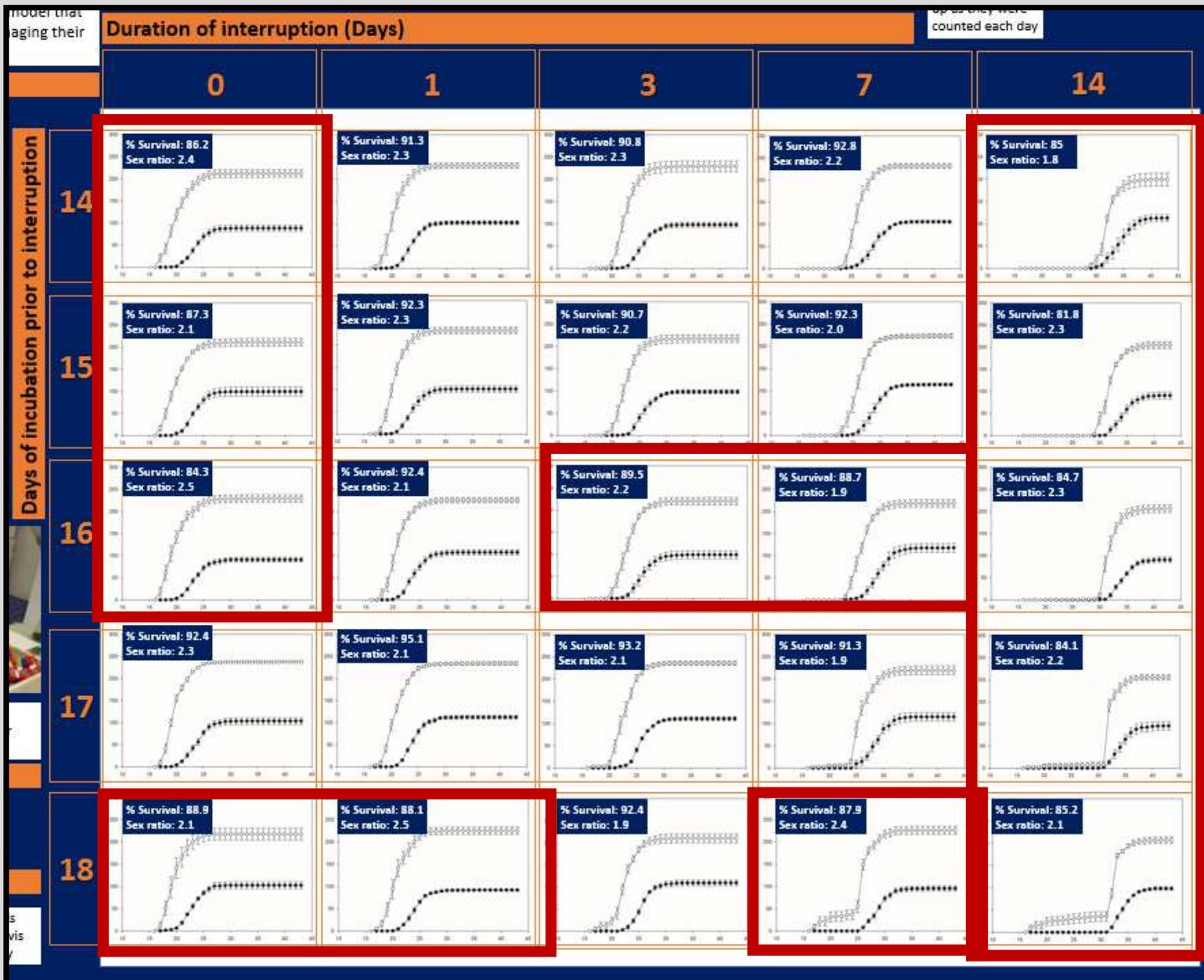


400 cocoons were incubated in separate containers at 29 °C and in 2" deep trays





Bees were collected 2-3 times a day and number of bees and sex of bees were counted



Emergence delays  
of up to 12 days is  
possible

Interrupting bees  
on day 18 was the  
most detrimental

Sex ratio was not  
affected by  
interruption



What is the role of bee nutrition on the development of reproductive structures?

- timing of adult pollen diets



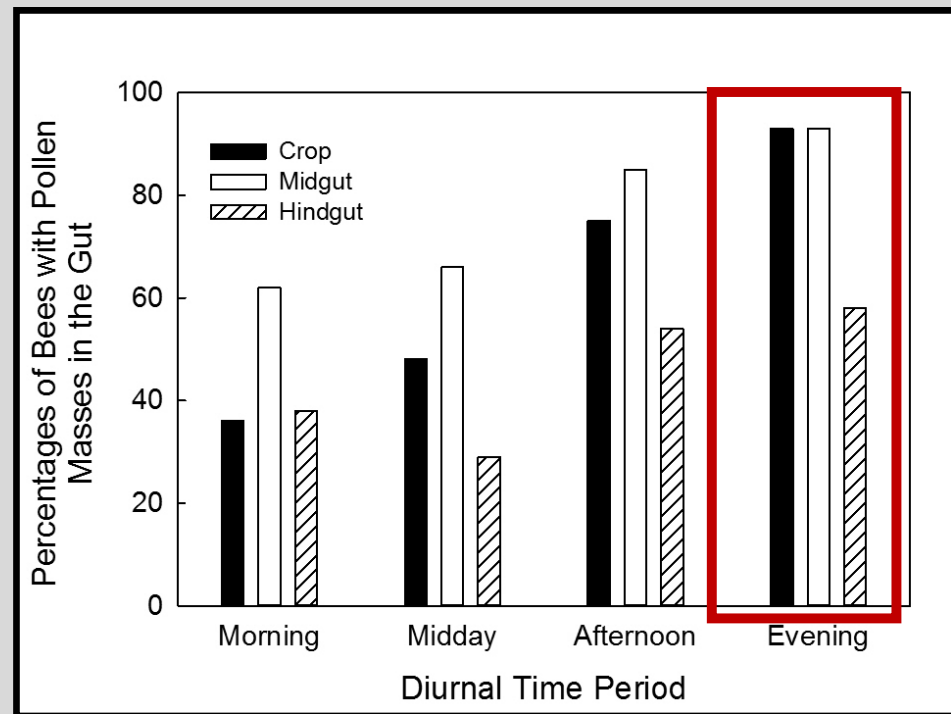
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# Alkali bees and pollen



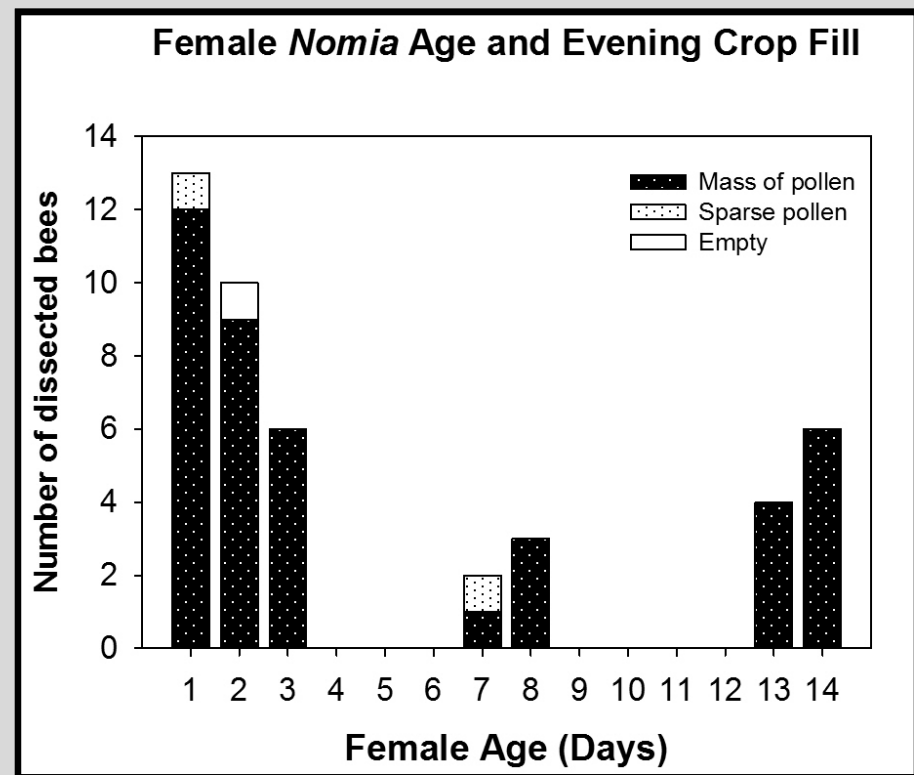
Adult bees eat pollen meals 1-2 times daily



# Alkali bees and pollen



Evening pollen meal  
constant right after  
emergence



# Blue orchard bees and pollen

The pollen meal is critical in blue orchard bees for developing egg laying organs

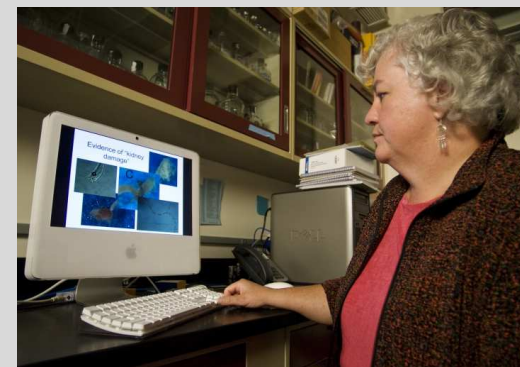


# Pollen and lessons for ALCB

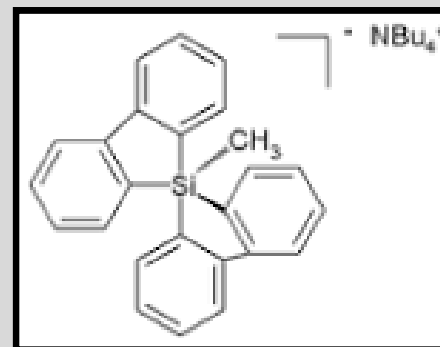
- 1) before nesting, females will need to eat pollen and drink nectar, so females that emerge before bloom won't be progressing toward reproduction
- 2) if they are like *Nomia*, then if all alfalfa bloom is tripped before evening, then females will miss a critical pollen meal and reproduction may be slowed. Don't overstock bees.



- Chalkbrood research
- Viruses in ALCB
- How do inerts in pesticides affect ALCB?



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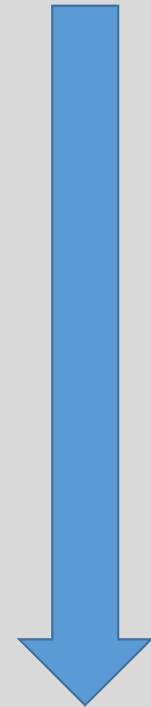
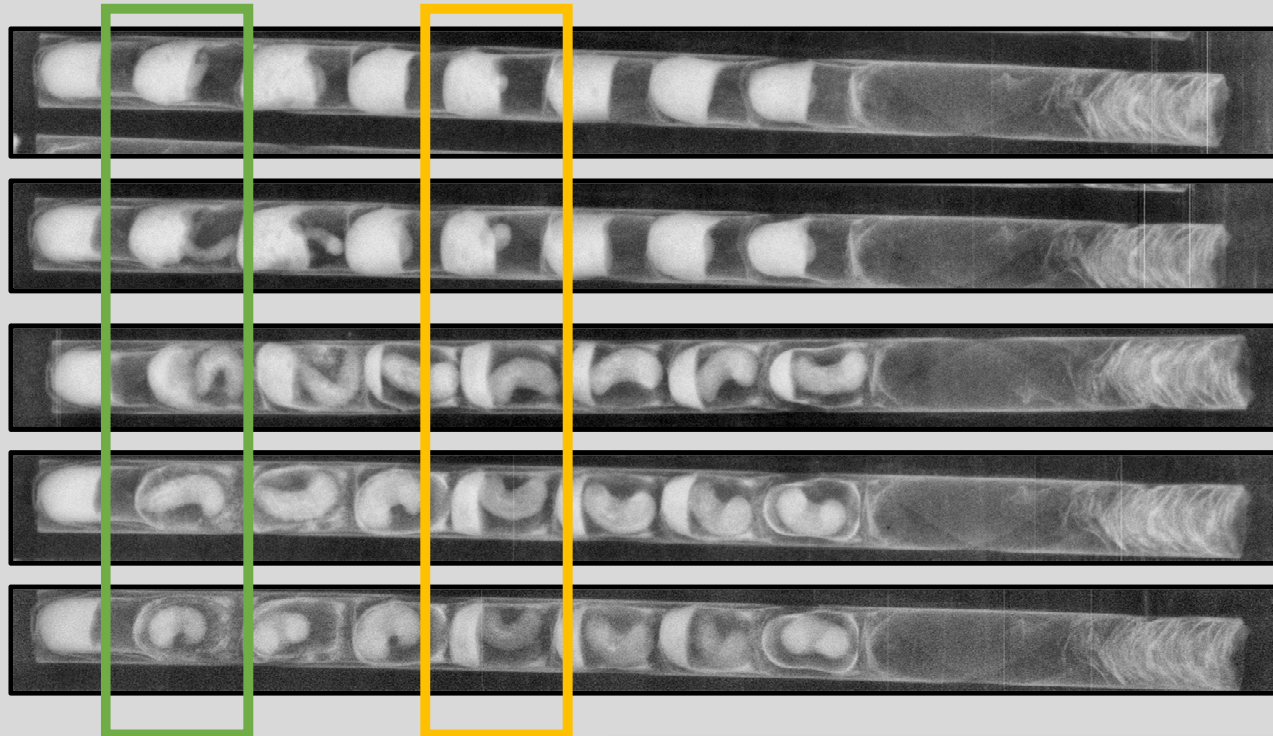




Healthy bee

Chalkbrood infected  
bee

Time



This is what happens with second generation

# Chalkbrood

- Chlorine dioxide fumigation tests (Fargo, ND)

No apparent effect of fumigation  
on chalkbrood germination



- Increased chalkbrood in blue orchard  
bee populations- something to watch in  
ALCB?

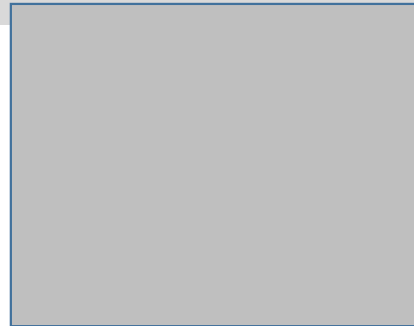
# Viruses in ALCB?

ALCB can be infected  
in the lab with various  
honey bee viruses

Is this causing some  
ALCB death?

Some ALCB fed with  
honey bee viruses

A



B

C



D



E



F



G



H



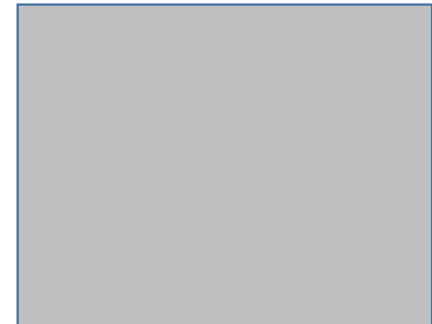
I



J



K



L



# Effect of inert ingredients on bees



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

Current Opinion in  
Insect Science



## Effects of 'inactive' ingredients on bees

Christopher A Mullin

Honey bees are sensitive to widespread co-formulants used in agrochemicals, and evaluation of the role of these 'inerts or 'inactives' in pollinator decline is only in its formative stages. Lack of disclosure of formulation ingredients in major products and lack of adequate methods for their analysis constrain the assessment of total chemical load and agrochemical exposures on bees. Most studies to document pesticide effects on honey bees are performed without the formulation or other relevant spray adjuvant components used to environmentally apply the toxicant. Formulations are generally more toxic than active ingredients, particularly fungicides, by up to 26,000-fold based on published literature. Some 'inactive' candidates for future risk assessment for pollinators include the organosilicone surfactants and the co-solvent N-methyl-2-pyrrolidone.

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Modern agrochemical formulations including seed treatments and spray tank additives comprise an average of 10 ingredients including the active ingredient (AI) and other components termed inerts, co-formulants or adjuvants [8,9] (JD Fowler, abstract ACS AGRO 384, 13th IUPAC Congress of Pesticide Chemistry, San Francisco, CA, August 2014). Numerous studies have found that pesticide AIs elicit very different physiological effects on non-target organisms when combined with their formulation ingredients [7<sup>\*\*</sup>,10]. These formulation surfactants, penetrant enhancers, spreaders, stickers, and co-solvents serve to optimize the pest control efficacy and stability of the AIs. Typical formulations (Figure 1) contain less than 50% AIs, combined with newer technologies including polyethoxylated tallow amines, organosilicone ethoxylates and co-solvents such as N-methyl-2-pyrrolidone (NMP) [7<sup>\*\*</sup>].



Contents lists available at [ScienceDirect](http://ScienceDirect)

Pesticide Biochemistry and Physiology

journal homepage: [www.elsevier.com/locate/pest](http://www.elsevier.com/locate/pest)



## The formulation makes the honey bee poison

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### ARTICLE INFO

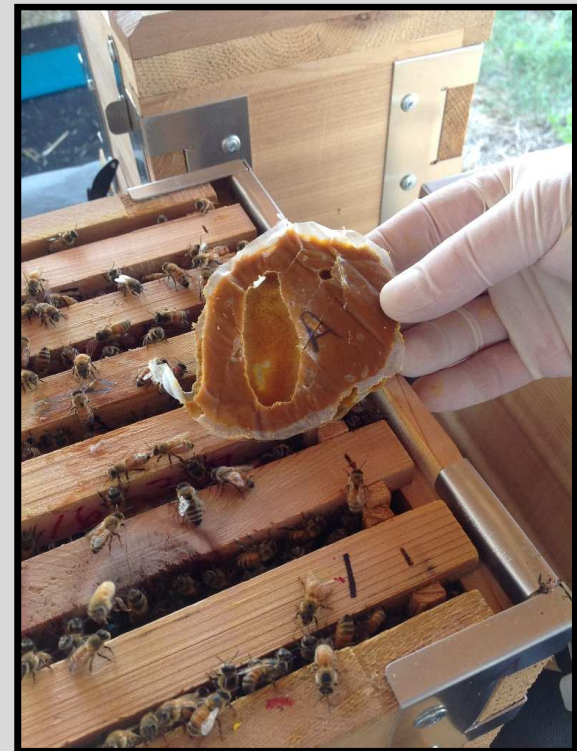
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Surfactants

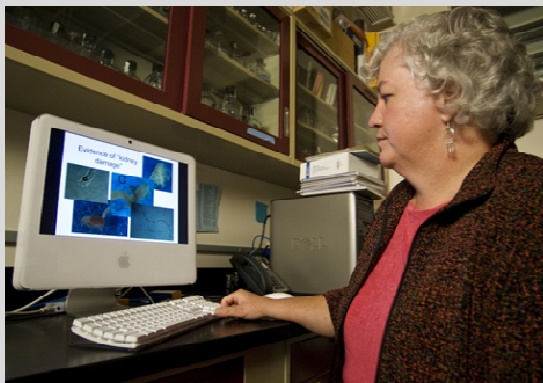
### ABSTRACT

Dr. Fumio Matsumura's legacy embraced a passion for exploring environmental impacts of agrochemicals on non-target species such as bees. Why most formulations are more toxic to bees than respective active ingredients and how pesticides interact to cause pollinator decline cannot be answered without understanding the prevailing environmental chemical background to which bees are exposed. Modern pesticide formulations and seed treatments, particularly when multiple active ingredients are blended, require proprietary adjuvants and inert ingredients to achieve high efficacy for targeted pests. Although we have found over 130 different pesticides and metabolites in beehive samples, no individual pesticide or amount correlates with recent bee declines. Recently we have shown that honey bees are sensitive to organosilicone surfactants, nonylphenol polyethoxylates and the solvent N-methyl-2-pyrrolidone (NMP), widespread co-formulants used in agrochemicals and frequent pollutants within the beehive. Effects include learning impairment for adult bees and chronic toxicity in larval feeding bioassays. Multi-billion pounds of formulation ingredients like NMP are used and released into US environments. These synthetic organic chemicals are generally recognized as safe, have no mandated tolerances, and residues remain largely unmonitored. In contrast to finding about 70% of the pesticide active ingredients searched for in our pesticide analysis of beehive samples, we have found 100% of the other formulation ingredients targeted for analysis. These 'inerts' overwhelm the chemical burden from active pesticide, drug and personal care ingredients with which they are formulated. Honey bees serve as an optimal terrestrial bioindicator to determine if 'the formulation and not just the dose makes the poison'.

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- Inert ingredient fed hives had lower numbers of bees
- Next step: do they have more pathogens?



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