Outline

- What is Nosema apis
  - Biology of the pathogen
- Honey bee division of labor
  - Biology of the honey bee
- The Nosema-JH connection
  - Extended phenotype?
- The test of hypothesis
- Effect of Nosema ceranae on bees

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The Nosema parasite

- *Nosema apis* Zander 1909
- A spore forming microsporadian
- Classification:
  - Protista (now a fungus!): Microsporidia: Microsporea: Microsporida
- Obligatory parasite of epithelial cells of midgut of honey bee adults (all castes)
- A new species (N. ceranae) was described in 1996, initially found in Apis cerana.

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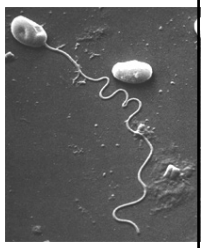
Life cycle of Nosema



- Transmitted by spores
- Spore ingested by bees
- Long, coiled, polar filament everts
- Sporoplasm injected into host cell
- Multiply through vegetative stages
- Spores released when host cells burst
- Spores voided and re-infect other bees or re-infect other midgut cells

Photo: http://www.biol.lu.se/cellorgbiol/microsporidia/proj_descr.html

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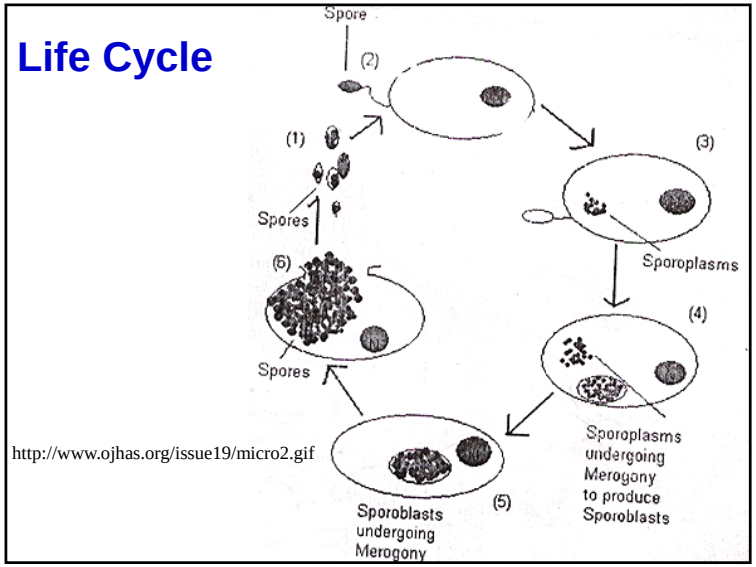
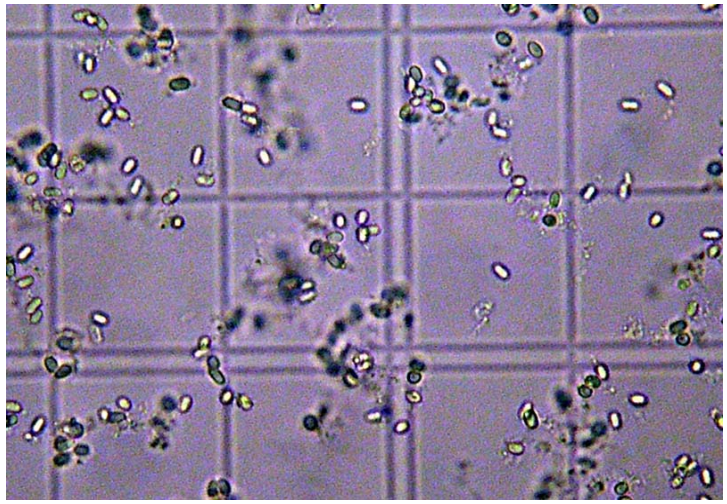
Life Cycle



http://www.ojhas.org/issue19/micro2.gif

Nosema spores (x400)



Effect of *Nosema* on workers

Protein deficiency due to indigestion
lower protein, lipid and sugar levels in blood

- Earlier regression of food glands: poor nursing
- Earlier onset of foraging and guarding
- Shorter life span (22-44% reduction)

Wang & Moeller, 1970; 1971

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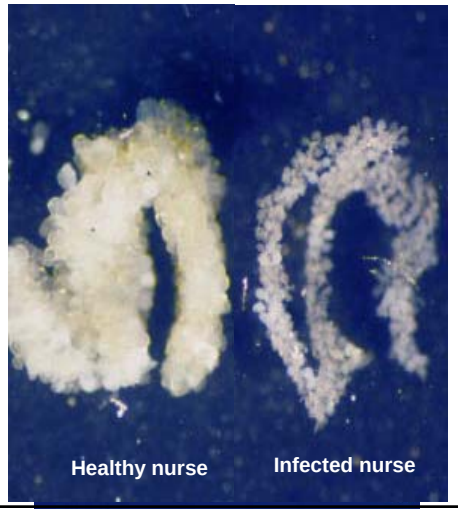
Regression of hypopharyngeal gland



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Worker bees: behavioral development and changes with juvenile hormone

Days since emergence

2 - 10 | 11 - 20 | 21 - 35

Low JH titer → *High JH titer*

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JH affects onset of worker foraging and hypopharyngeal gland development

JH treatment induces

- Earlier regression of hypopharyngeal glands
- Earlier onset of foraging and guarding
- Shortening of life (due to limited foraging life)

Jaycox, 1976, Jaycox et al 1974,
Robinson 1987, Sasagawa, 1989

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Effect of JH on workers

- Earlier regression of food glands: poor nursing
- Earlier onset of foraging and guarding
- Shorter life span

Jaycox 1976, Jaycox et al. 1974, Robinson 1986.

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Nosema → **JH** → **Foraging?**

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R. Dawkins (1982):
"The Extended Phenotype."
All phenotype is a means for replicating genes.

Host manipulation by a parasite
(to increase the fitness of the parasite):

* Caterpillars climb high on grass when dying from virus
* Slugs change color when parasitized
* Honey bees forage early when infected by Nosema
  -- cites a Science paper suggesting Nosema produces JH
    -- infected hemipterans have supernumerary instars
    -- allatectomized animals with Nosema show JH activity

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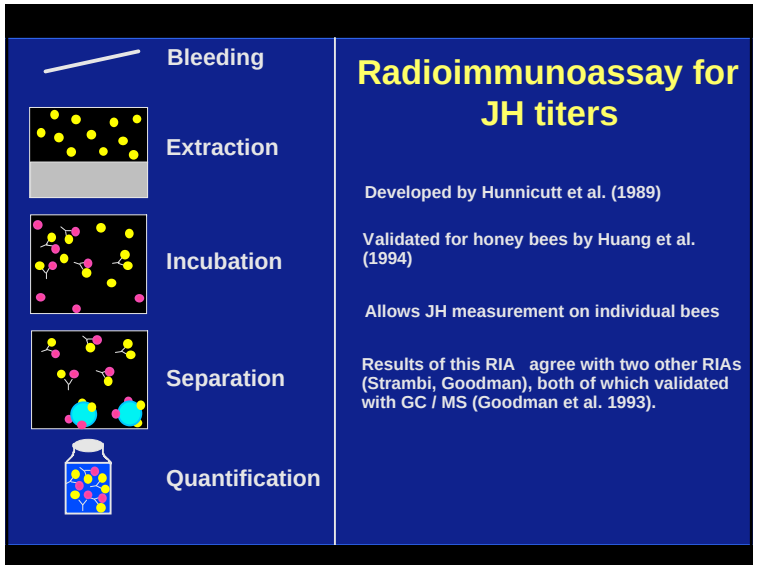

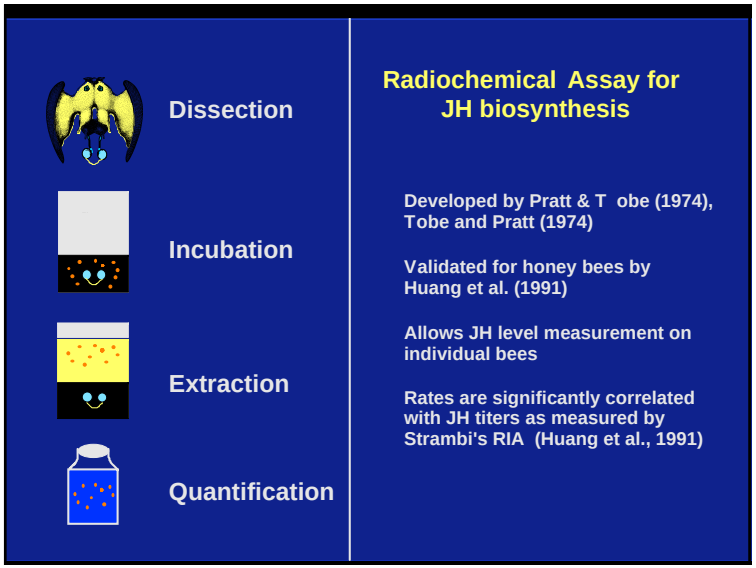

Nosema changes honey bee behavior

but how?

We asked:

1. Do nosema infected workers have higher JH titers?
2. Do they have higher rates of JH production?
3. Do they have lower rates of JH degradation?
4. Does nosema produce JH directly?

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Materials and Methods

- Newly emerged workers obtained in incubator
- Bees paint-marked or individually tagged
- Bees individually fed with Nosema spores
- Bees isolated for 30 min, introduced into colony
- Foraging observation of marked/tagged bees

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Obtaining Newly Emerged Bees

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Tagging bees

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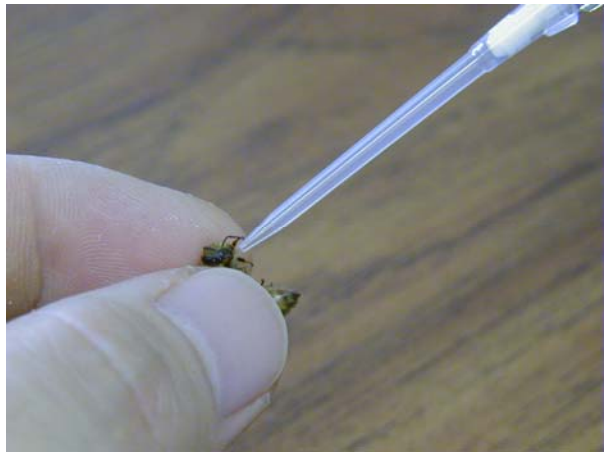
Inoculation



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Inoculation

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Observation

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Sampling Bees

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Armed with bees that respond to Nosema infection, now we can ask the 1st question:

1. Are the earlier foraging in these bees mediated through JH (but not a nosema specific foraging-inducer)

if so, we expect higher JH titers in bees just before they embark on foraging (we did not know if foraging performance itself would elevate JH titers).

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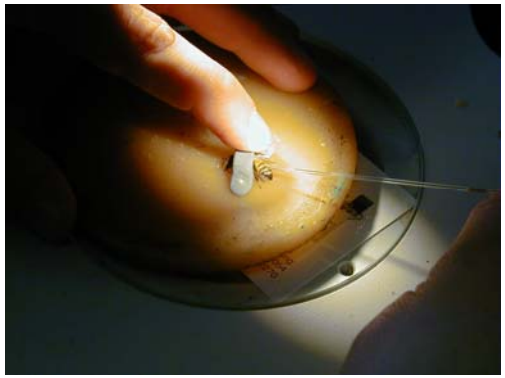
Collecting Hemolymph



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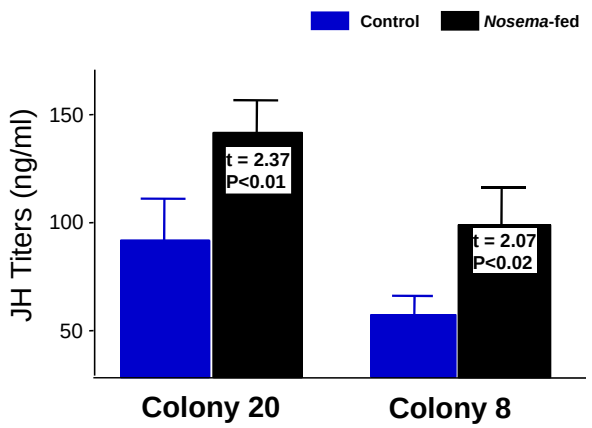
JH titers are significantly higher in Nosema infected bees, even before foraging started (age=12 days)



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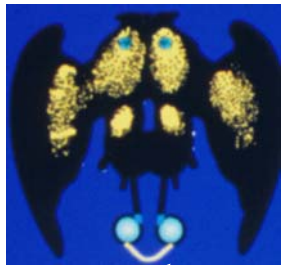
Are the higher JH titers due to higher JH production by infected bees?



Corpora allata (CA)

JH biosynthesis:

remove CA, incubate in vitro with radioactive precursor and measure JH production

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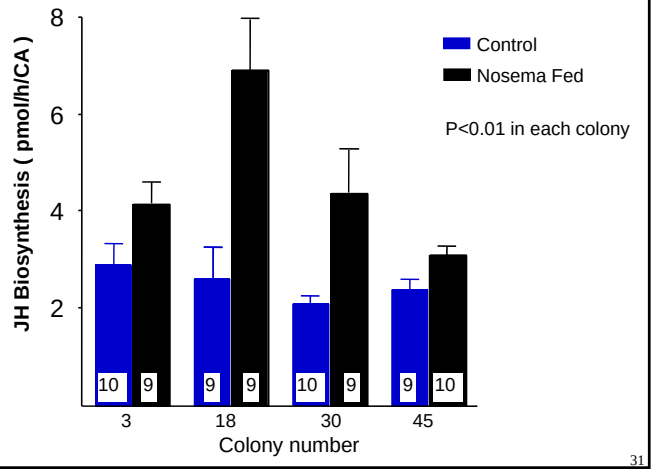
Rates of JH biosynthesis are also higher in young, Nosema infected bees



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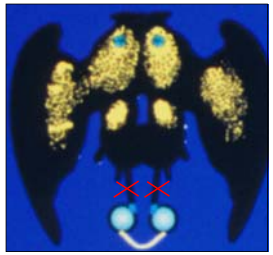
Do bees with corpora allata removed (allatectomized) still forage early?



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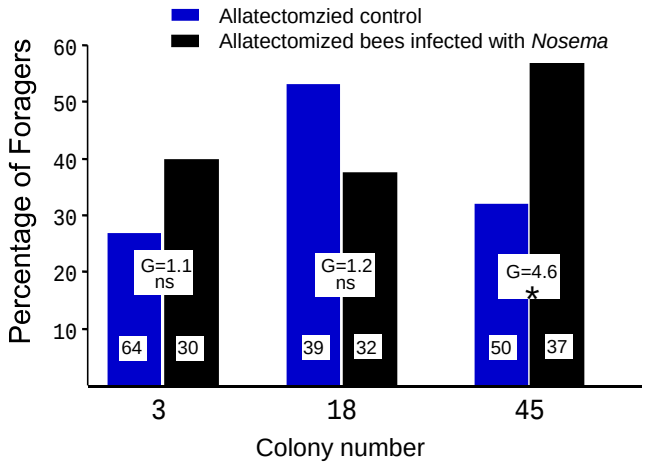
The majority of infected bees did not forage earlier after CA removal



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Evidence that Nosema does not produce JH directly:

1. Allatectomized bees showed no behavioral changes
2. Allatectomized bees contained no JH in their blood
3. No radioactive JH was produced when midgut was incubated with radio-labelled methionine.

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Conclusion:

- Nosema does not produce JH directly.
- Nosema-infected workers forage earlier: due to higher JH titers, higher rates of JH biosynthesis, and despite of higher rates of degradation.
- Is this host manipulation? Probably not:
  1. There is genetic variation (some bees do not respond)
  2. Rates of degradation also high (no redundancy)
- Alternative explanations?
  3. Possibly simply higher metabolism due to “stress?”
  4. “Malnutritioned” bees forage earlier because they are not good nurses?

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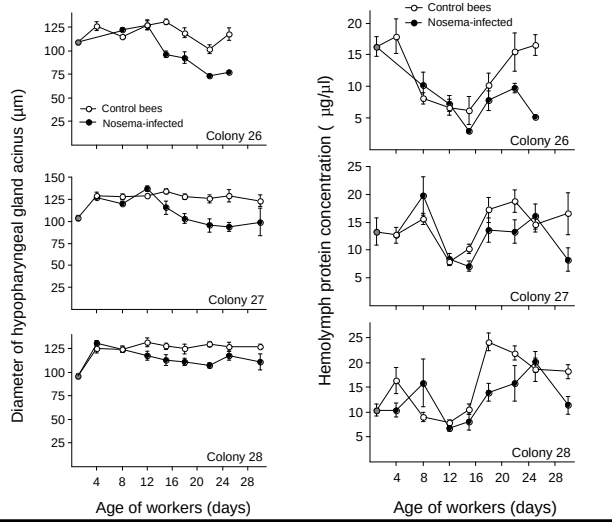
Nosema apis affected food glands and blood protein



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New twist on Nosema

1. The original species was *Nosema apis*.
2. A new species was discovered in 1996 by Ingma Fries, in *Apis cerana*, named *Nosema ceranae.*
3. In 2005 it was reported in our species (*Apis mellifera*) in Taiwan and Europe.
4. Now it seems all the nosema we can found in US is also *Nosema ceranae*.
5. Recent studies in Spain attribute 50% of colony loss to *N. ceranae*.

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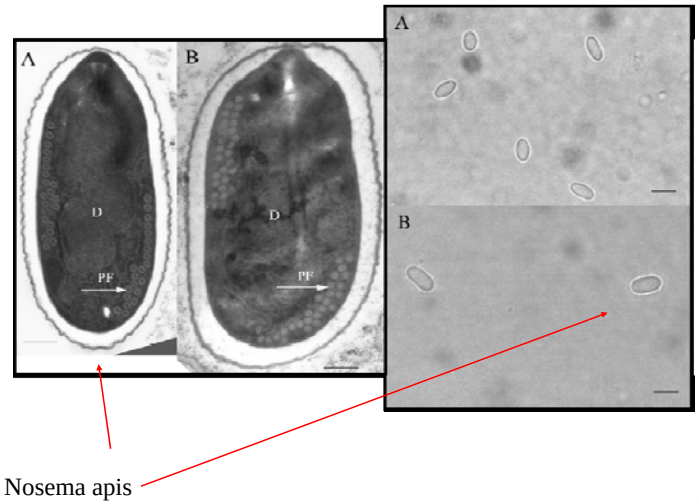

Nosema apis

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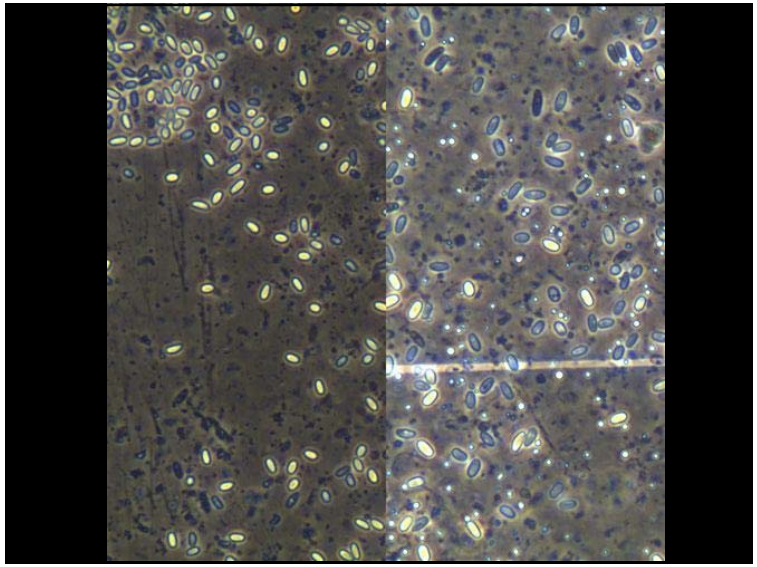
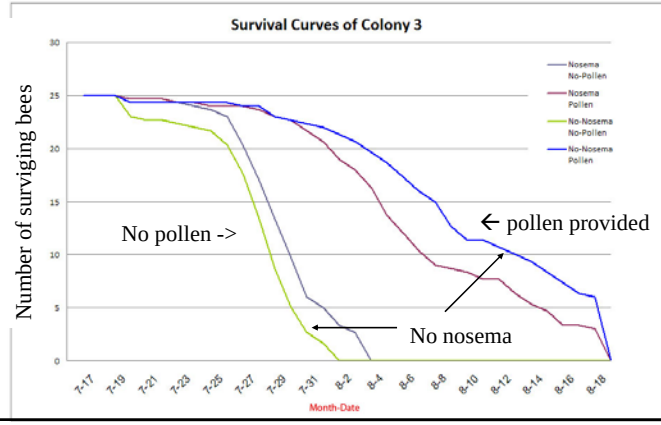
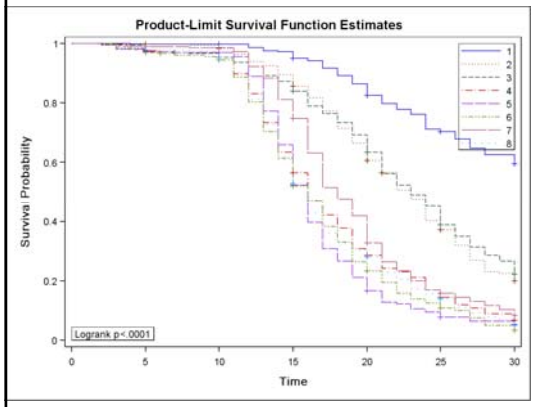


No nutrition x *Nosema ceranae* interaction,
but pollen shows a huge impact on worker longevity.



Co-infected bees die much earlier than single-species-infected-bees



#	Treatment
1	0 spores, control group
2	30,000 *N. ceranae spores*
3	30,000 *N. apis spores*
4	25k *N. c* + 5k *N.a* spores
5	20k *N. c* + 10k *N.a* spores
6	15k *N. c* + 15k *N.a* spores
7	10k *N. c* + 20k *N.a* spores
8	5k *N. c* + 25k *N.a* spores

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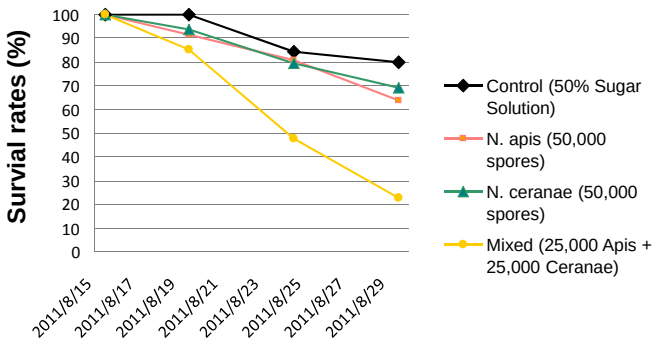
Co-infected bees died earlier in nucs



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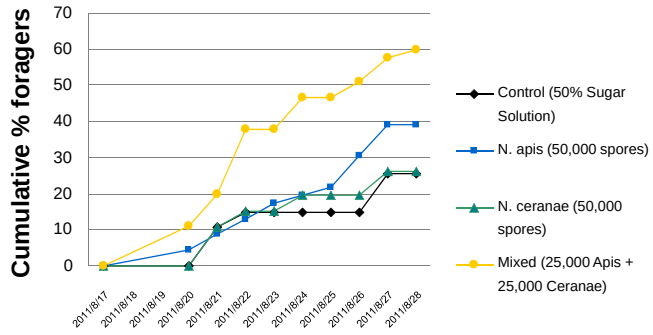
Co-infected bees foraged faster than others



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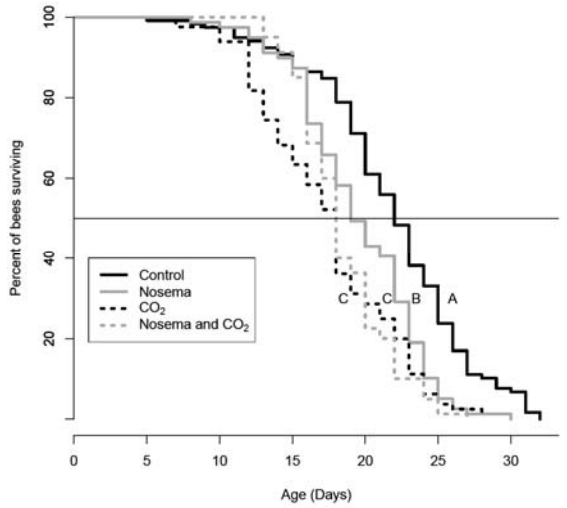
Method of infection makes a difference



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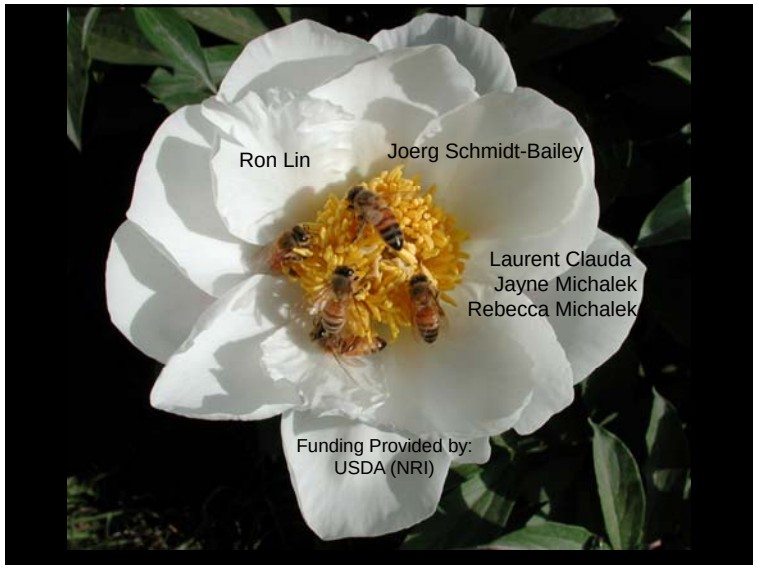
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Thank you for your attention.
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