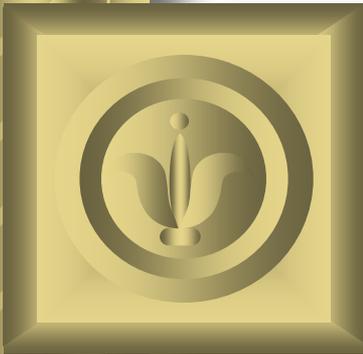


Effect of sublethal exposure to thiamethoxam
and *Nosema ceranae* infections on
survivorship and immune response of the
stingless bee *Melipona colimana*
(Hymenoptera: Meliponini)



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Diversity of bees



Honey bees: 6-7 species. *Apis mellifera*
well known worldwide and widely studied



Stingless bees: More than 400 species

- ❁ 250 in South and Central America
- ❁ 50 in Southern Asia and Malaysia
- ❁ 20 in Australia, Papua and New Guinea
- ❁ 40 in Africa

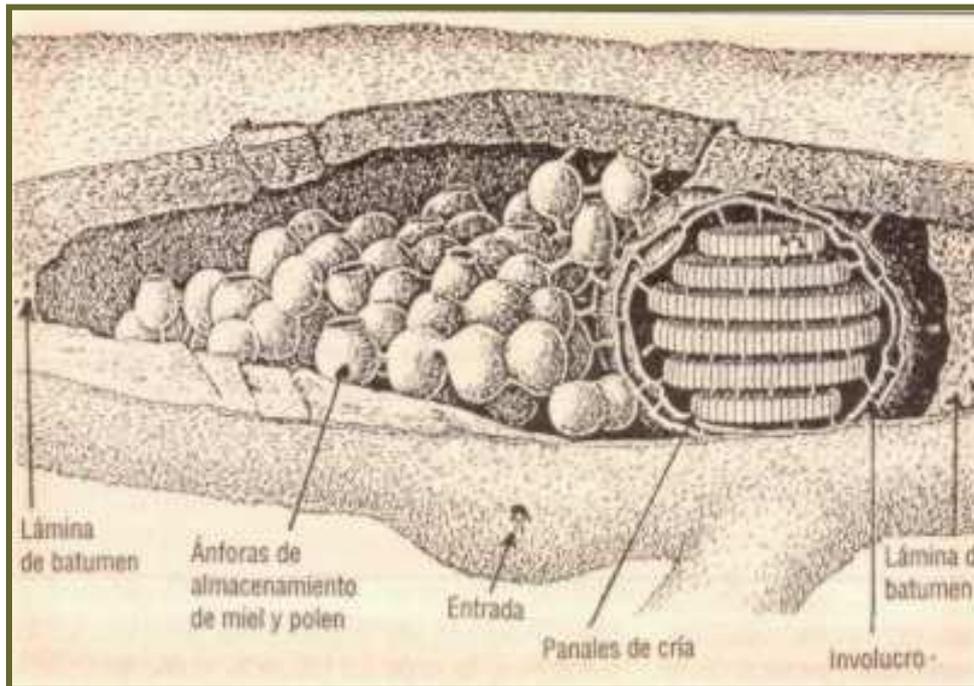




Characteristics



Stingless bees: Horizontal brood combs
Store resources in cerumen pots





Stingless bees in Mexico

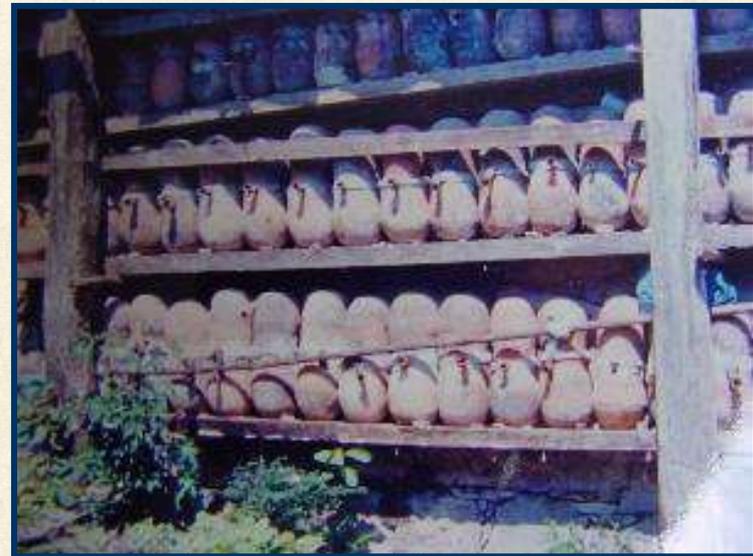
Managed for honey and wax production



Important role as pollinators

⌘ North mountain range of Puebla - Nahuas

⌘ Mayas - Yucatán Peninsula



Mexico: \triangle Stingless bees = 46 species

Jalisco = 11 species

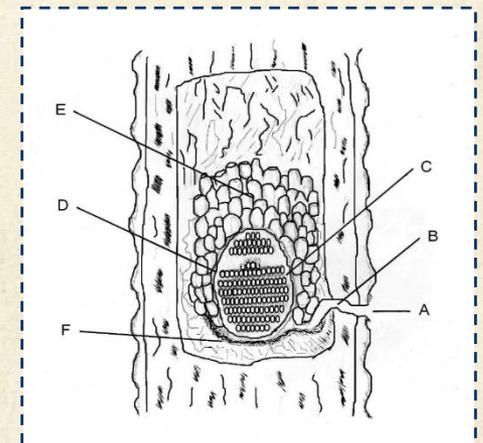
↘ Endemic species

☀ *Melipona colimana* (Ayala, 1999)

🌊 Nesting sites:

🌲 Oak trees (*Quercus laurina*)

Temperate and humid climate

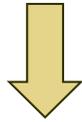




Risks for bees



Insecticides



Neonicotinoids

Diseases



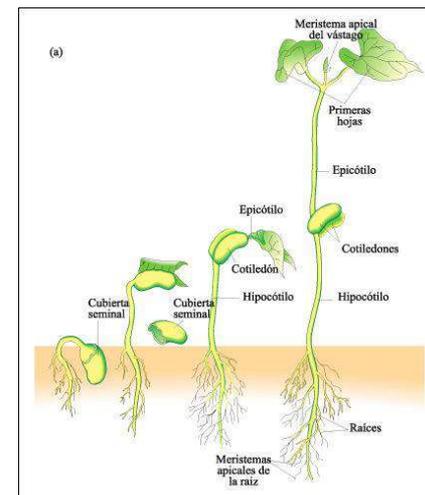
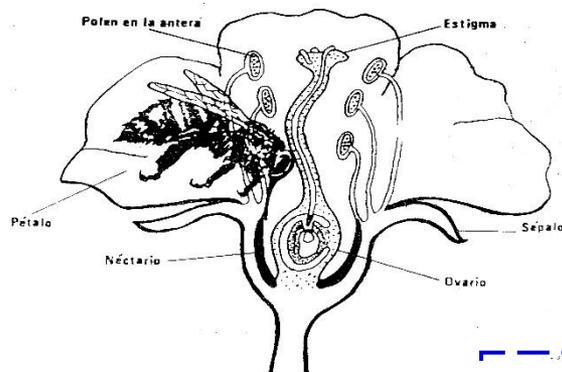
Nosema ceranae



Neonicotinoids



Systemic insecticides



Present in nectar and pollen

☠ Colony losses ☠

🚫 Not much knowledge on their impact on stingless bees

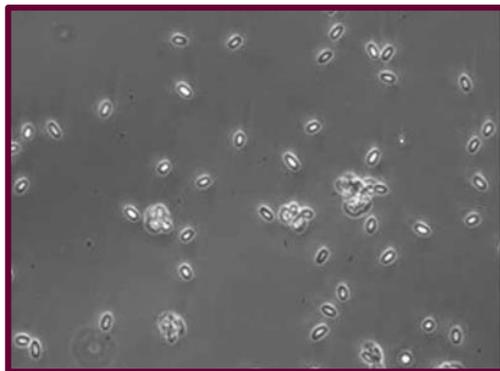
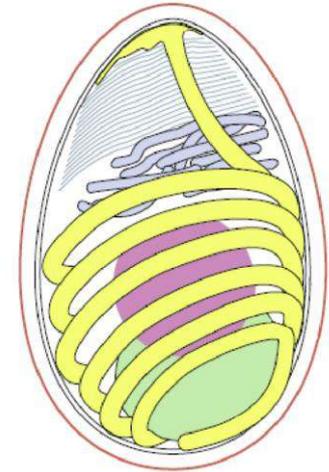




Nosema ceranae



 *N. ceranae* infects honey bees
Other *Nosema spp.* infect *Bombus spp.*





Pathogen spillover



Honey bees and stingless bees share floral resources



= Pathogen spillover =



Research questions

1. Do sublethal exposure to neonicotinoid insecticides affect *M. colimana's* health?
2. Can *Nosema ceranae* infect and negatively affect the health of *Melipona colimana*?
3. Can the two stressors interact and impact *M. colimana's* health?



Objetive

⌘.-Examine the effect of one neonicotinoid insecticide (thiamethoxam) and *Nosema ceranae* on infection development, survivorship and cellular immune responses of the stingless bee *Melipona colimana*



Experimental site



UNIVERSIDAD DE GUADALAJARA
CENTRO UNIVERSITARIO DEL SUR



****** Cd. Guzman,
Jalisco, Mexico.



19° 34' 12" N
103° 38' 00" W
1580 msnm



Methods



Newly-emerged bees



(n=30 per treatment)

Cages in an incubator

(24°C/70 RH)

Treatments

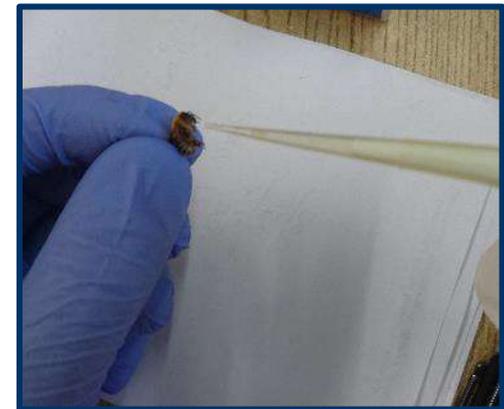
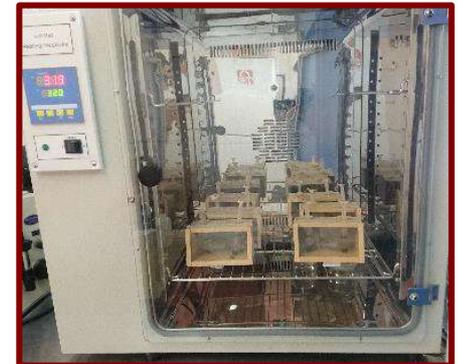
(C).- Control (Only sugar syrup)

(N).- *N. ceranae* (50,000 spores/bee) *

(T).- Thiamethoxam (0.4 ng/bee in sugar syrup)

(NT).- *N. ceranae* and Thiamethoxam

*** Bees inoculated individually only in N and NT**



Methods



- Mortality was recorded daily

∞ Survivorship ∞

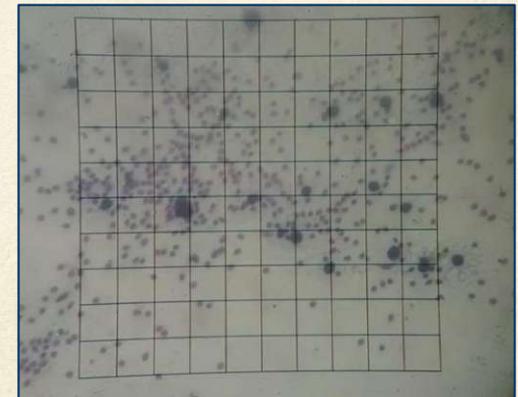
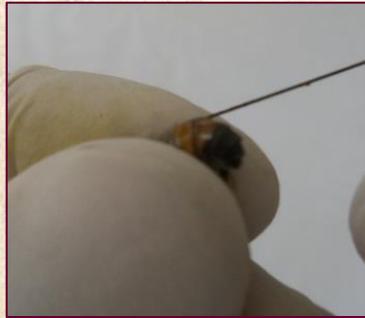


% mortality 14 days

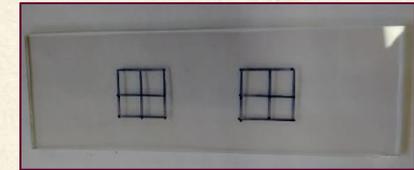


Methods

» Cellular immune response

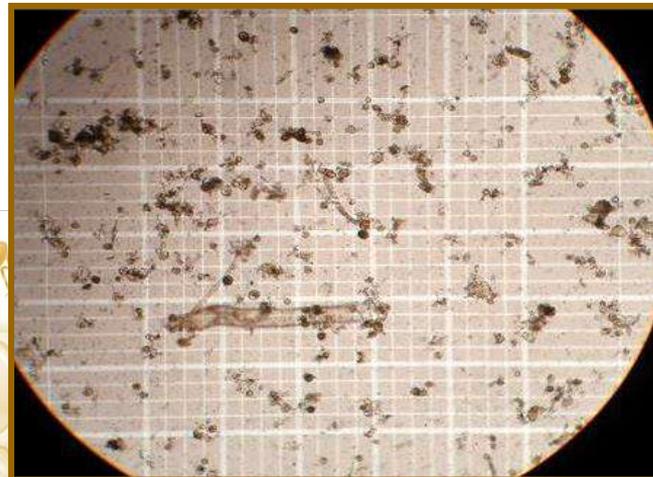
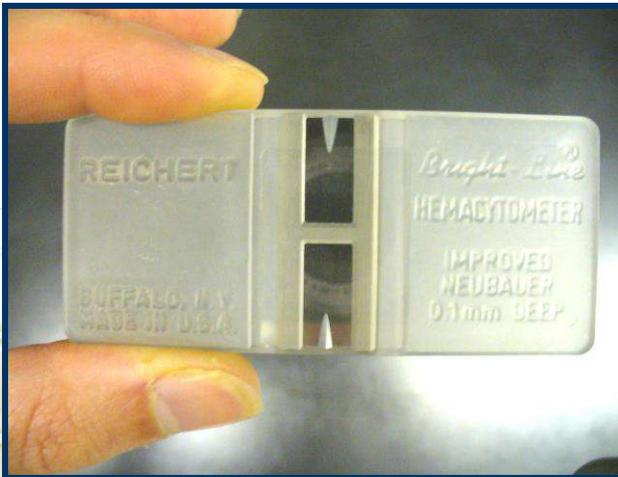


- Hemocyte counts/ μ L





Detection and quantification of *N.ceranae* spores





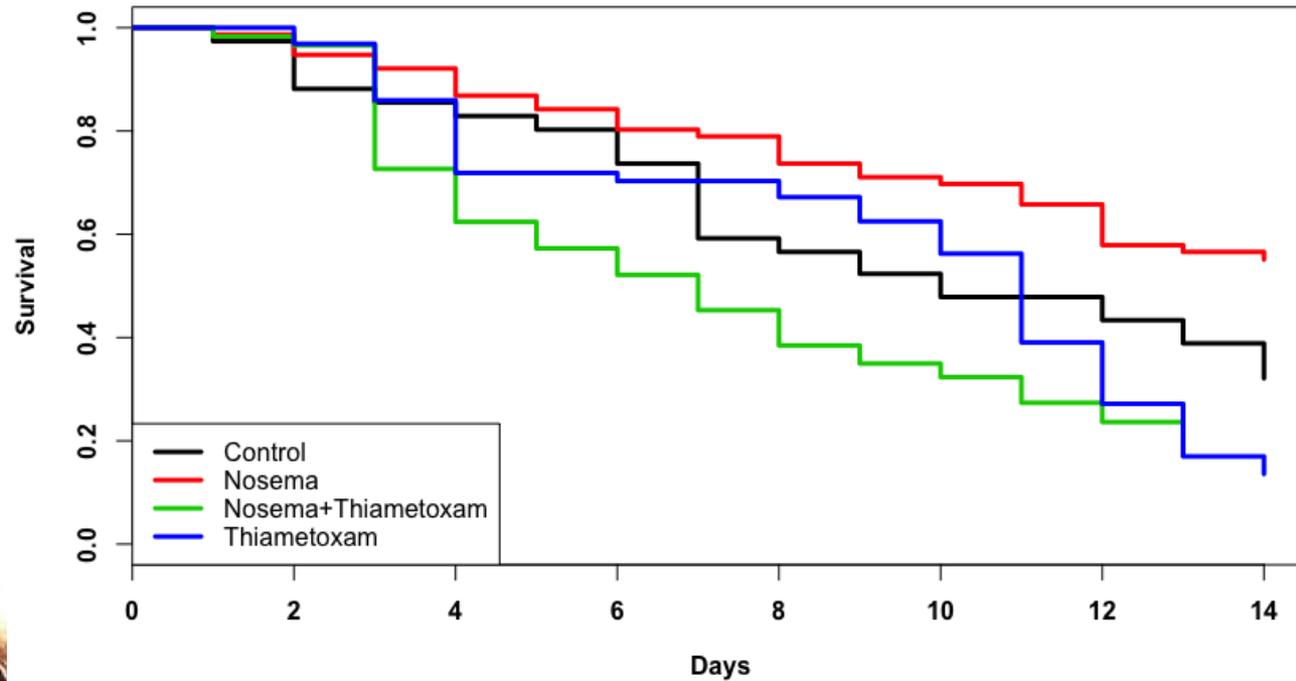
Statistical analyses

- ☀ Mortality data analyzed using the Kaplan-Meier method
- ★ Infectivity was calculated by the % bees where *N. ceranae* infections were detected
- ☀ Data on *N. ceranae* infection intensity (development) were subjected to a Wilcoxon test
- ★ Data on hemocyte counts were log-transformed and analyzed by ANOVA

Results and discussion



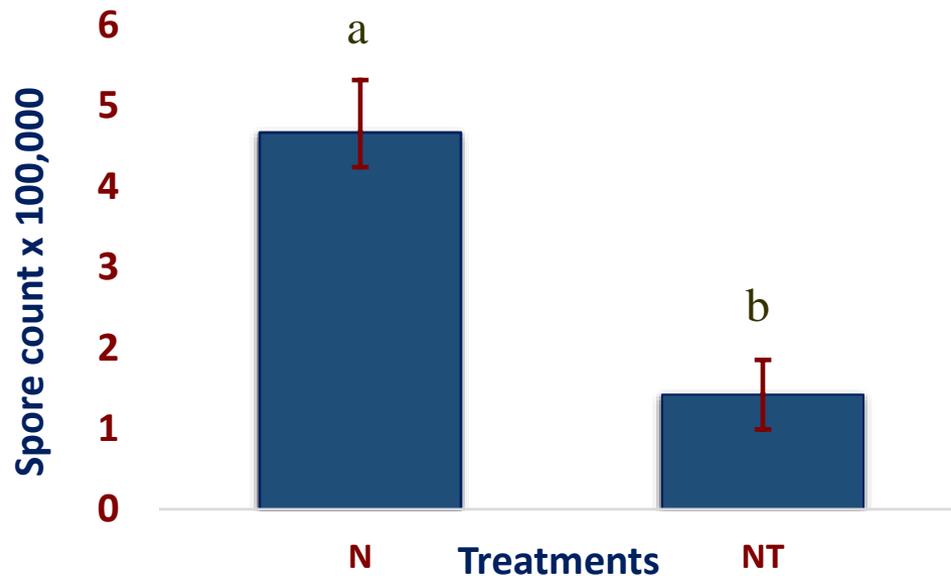
Survivorship



Results and discussion



🦋 Infection development



N. ceranae was capable of infecting $66.2 \pm 16.9\%$ of the inoculated bees.

Bees inoculated with *N. ceranae* only, developed 3.3 times higher infection levels than bees exposed to both stressors.

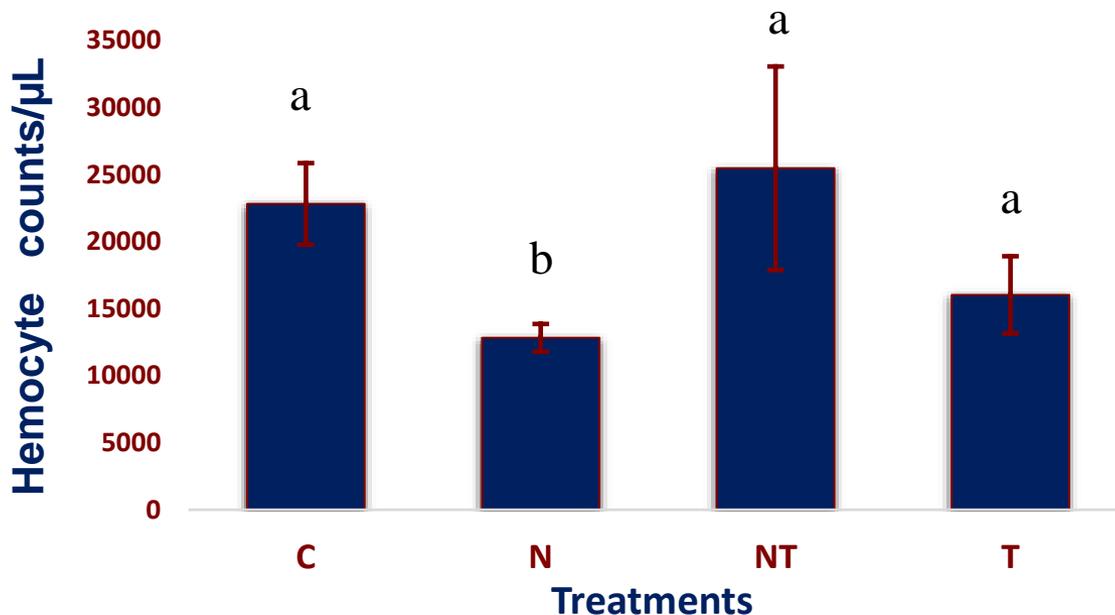
The differences were significant ($W = 507, p < 0.001$).



Results and discussion



» Cellular immune response



Hemocyte counts were significantly lower in N compared with other treatments and untreated bees.

The differences were significant ($F_{3,71} = 5.42, p < 0.01$)



Conclusions



☞ This is the first study that provides evidence that *N. ceranae* may infect and multiply in stingless bees in the Americas and may inhibit their immune cellular responses, which could make them more susceptible to other stressors.



☞ *M. colimana* bees appear to be very sensitive to sublethal exposure to thiamethoxam, which could have negative implications in their populations and in their pollinating services.

