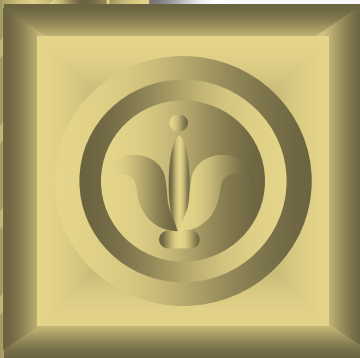


Effect of sublethal exposure to thiamethoxam  
and *Nosema ceranae* infections on  
survivorship and immune response of the  
stingless bee *Melipona colimana*  
(Hymenoptera: Meliponini)



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Bee Research Center

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# Diversity of bees

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



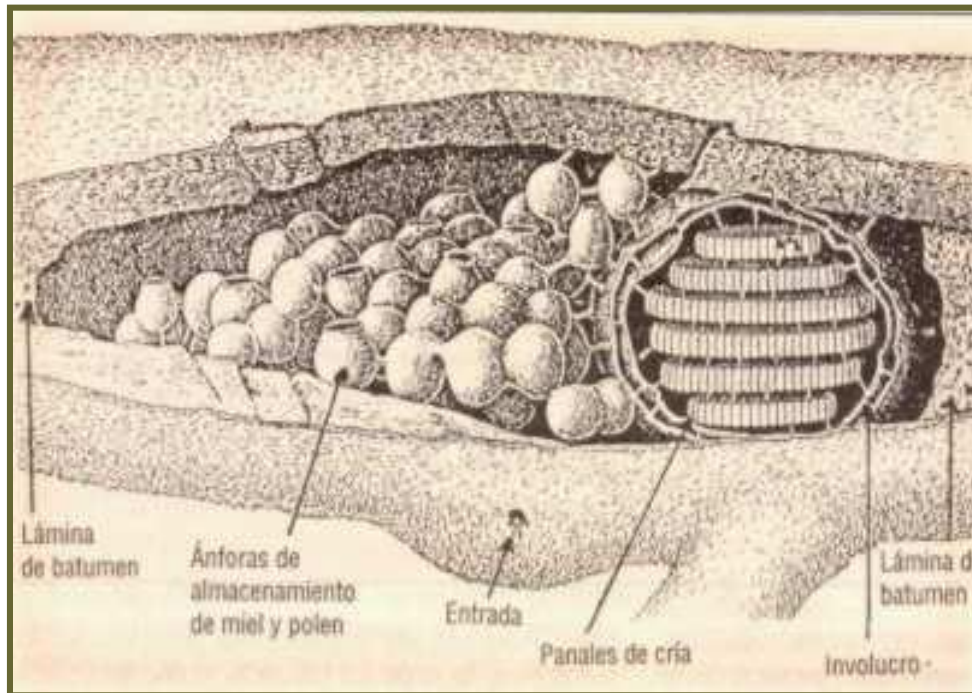
Stingless bees: More than 400 species

- ❁ 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:  Stingless bees = **46 species**

**Jalisco** = **11 species**

↘ Endemic species

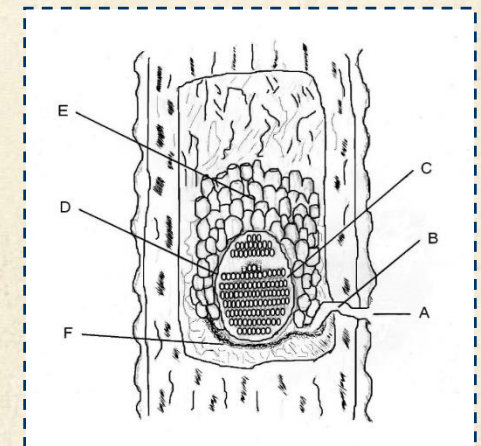
★ *Melipona colimana* (Ayala, 1999)

≡ Nesting sites:



Oak trees (*Quercus laurina*)

Temperate and  
humid climate



# Risks for bees

Insecticides



Neonicotinoids

Diseases



*Nosema ceranae*

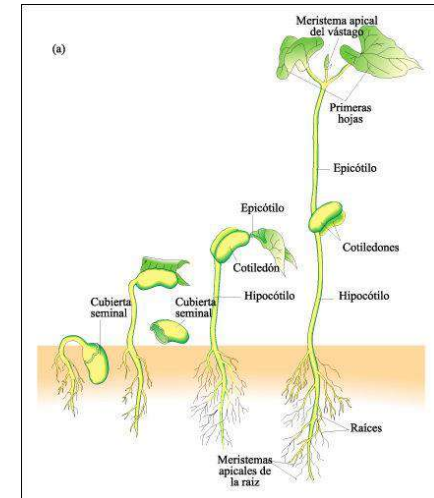
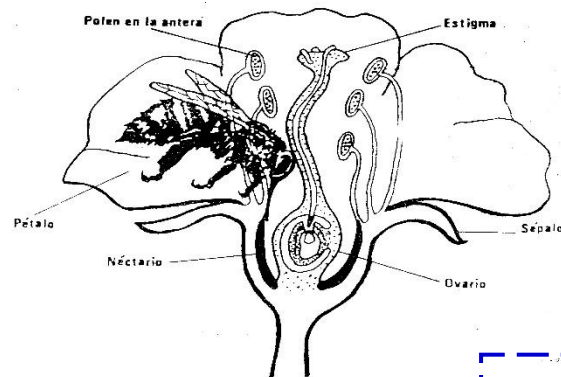




# Neonicotinoids



## Systemic insecticides



Present in nectar and pollen

☠ Colony losses ☠


⚠ Not much knowledge on their impact on stingless bees

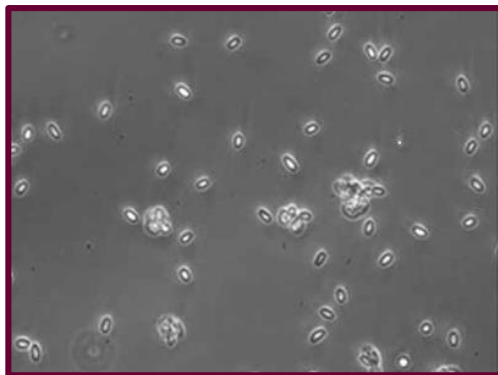
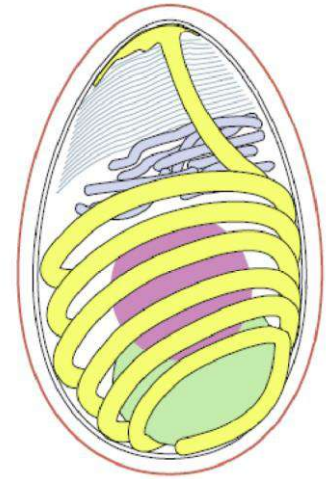




# *Nosema ceranae*



 *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

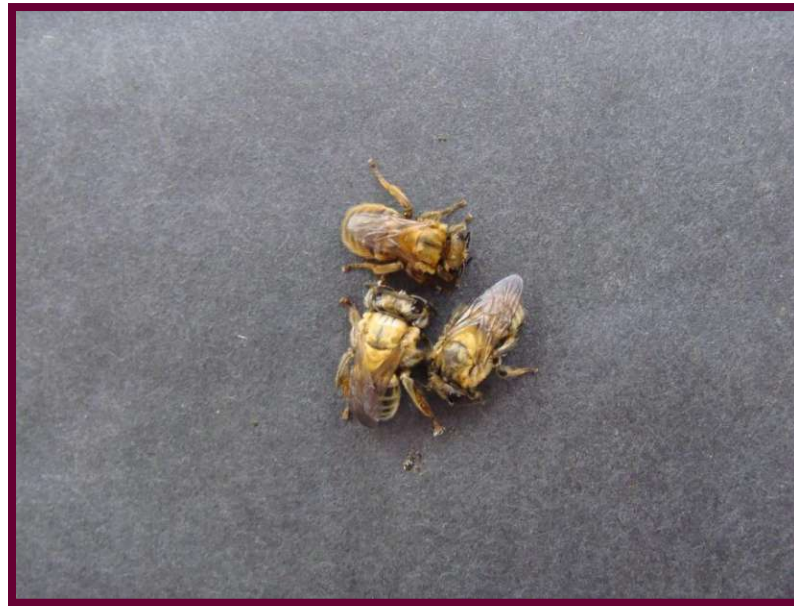
1. Do sublethal exposure to neonicotinoid insecticides affect *M. colimana*'s health?
2. Can *Nosema ceranae* infect and negatively affect the health of *Melipona colimana*?
3. Can the two stressors interact and impact *M. colimana*'s health?





# 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**

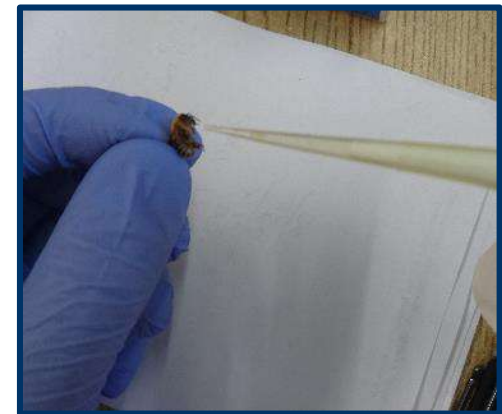
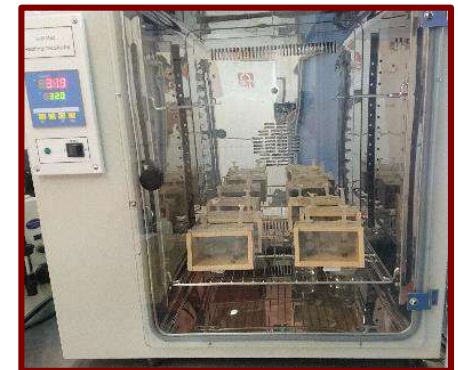
(C).- Control (Only sugar syrup)

(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 ⌚



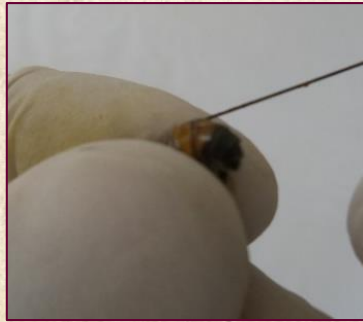
% mortality 14 days



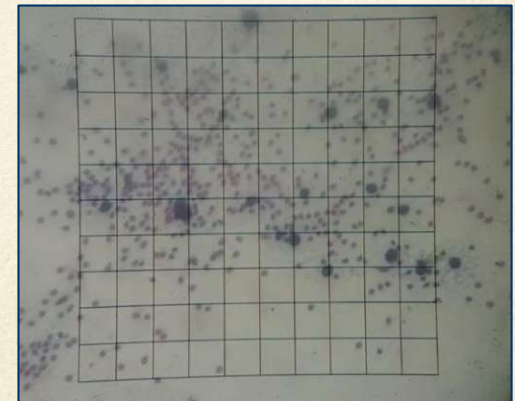
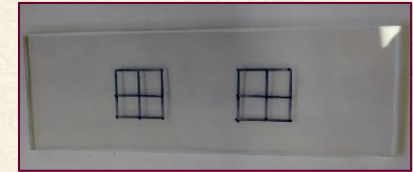


# Methods

## » Cellular immune response



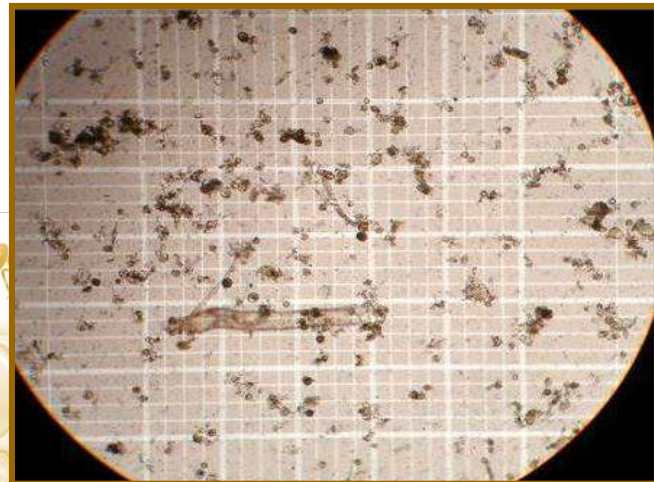
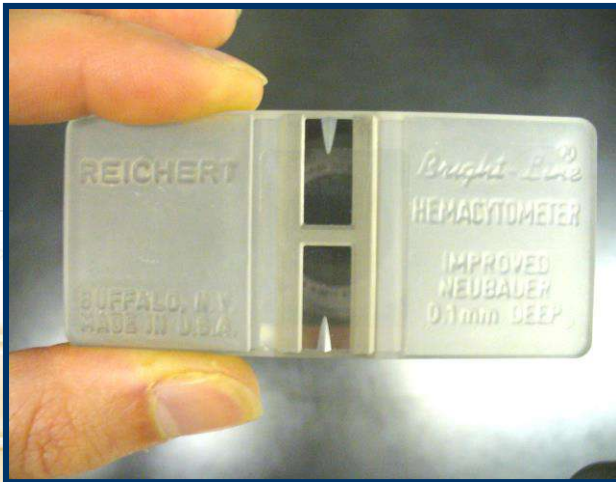
- Hemocyte counts/ $\mu$ L







# Detection and quantification of *N.ceranae* spores





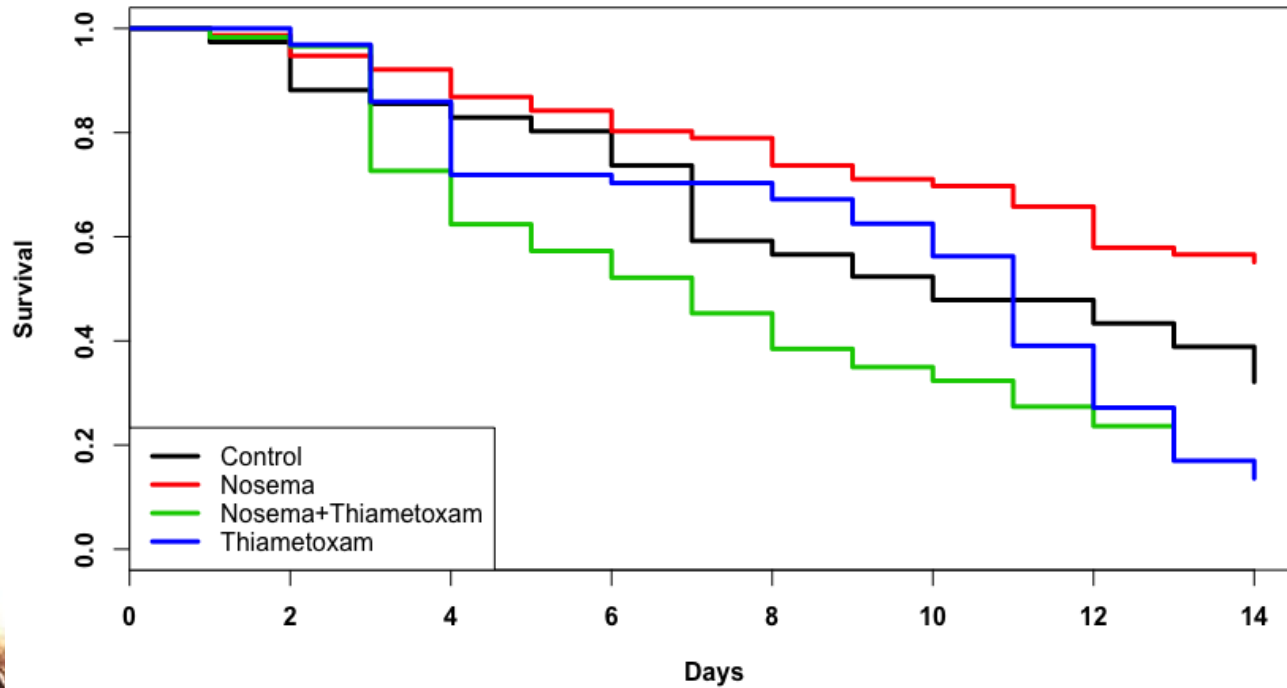


# Statistical analyses

- ☀ 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

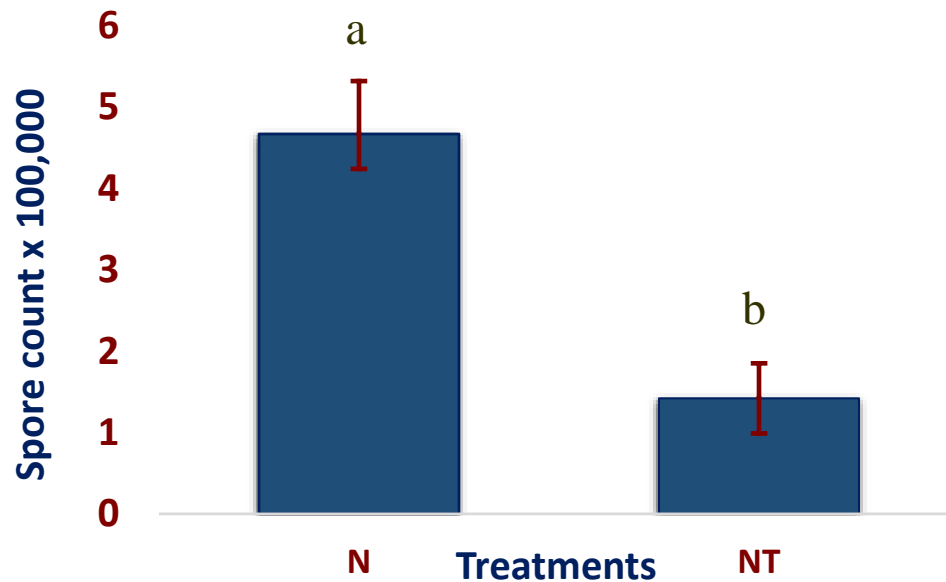
## Survivorship



# 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 ( $W = 507, p < 0.001$ ).

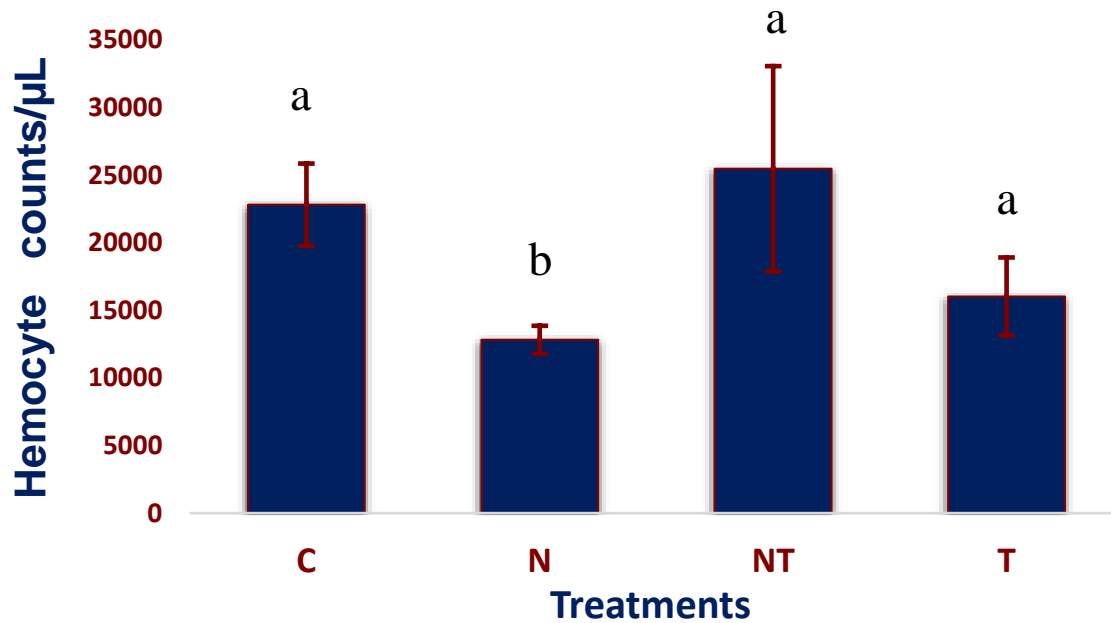




# Results and discussion



## » 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.

