

# Effect of selected prebiotics and probiotics on the parasitic fungus *Nosema ceranae* and on the health of honey bee colonies

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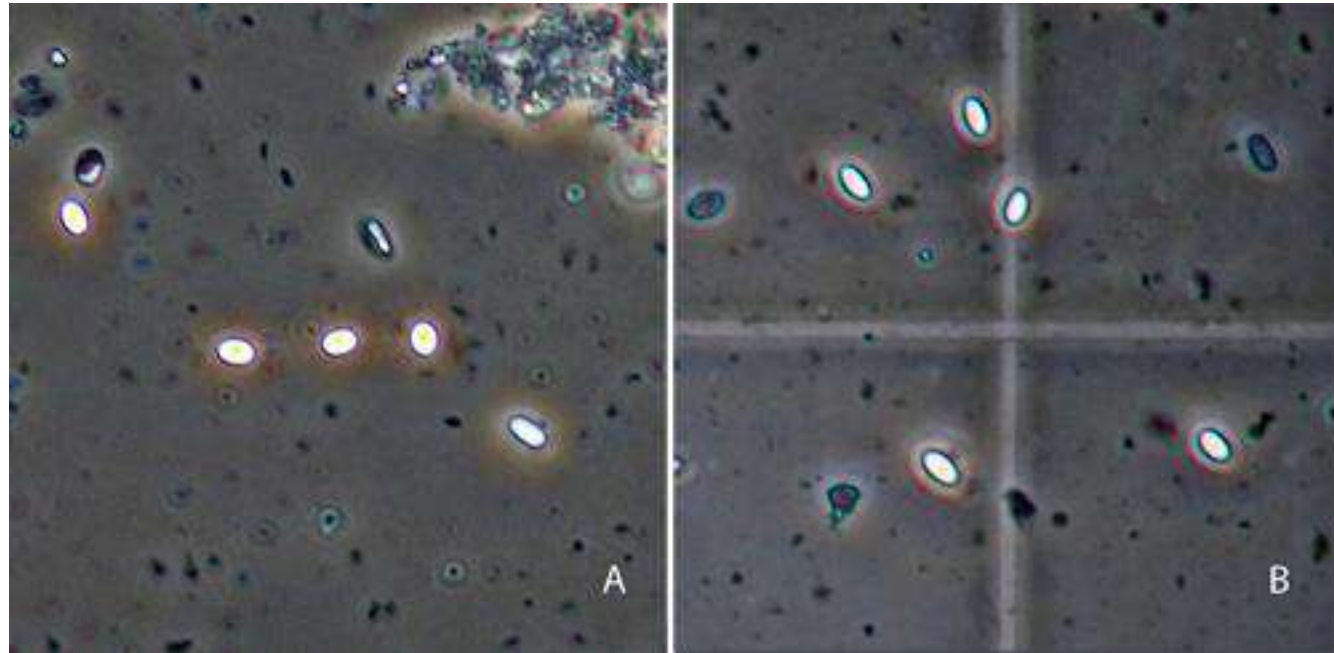
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# *Nosema ceranae*

- Microsporidian parasite
- Destroys gut epithelial lining
- Reduces immune gene expression
- Shorter foraging trips
- Reduces life spans
- Less honey production



<http://www.beeccdcap.uga.edu/documents/CAPArticle12.html>

# Treatment of Nosema Disease

- Only registered option in Canada
- Concerns with antibiotic resistance
- Concerns with contamination of honey and wax



# Alternatives to Antibiotics

- **Prebiotics** are carbohydrates and food ingredients that are non-digestible to the host, in this case, the honey bee.
- **Probiotics** are living organisms that colonize the gut.
- **Essential oils** are concentrated hydrophobic liquids containing volatile aroma compounds from plants.

# Objectives

Determine the effect of essential oils, prebiotics, probiotics and nutraceuticals applied in solid or liquid form to field colonies on bee reproduction, food storage, over-wintering survival and *N. ceranae* infection levels.

# Previous Work

- Daniel Borges (MSc 2015) and Pegah Valizadeh (PhD 2016) had promising results using caged honey bees in incubators for:
- two prebiotics: naringenin (flavone from citrus fruits), and chitosan (derived from the shells of crustaceans)
- one probiotic: Ef (a commercial formulation of *Enterococcus faecium*)
- one essential oil: eugenol (active component of clove oil)

# Treatment Groups

- Positive Control
- Negative Control
- Fumagillin
- Eugenol S
- Eugenol P
- Ef S
- Ef P
- Naringenin S
- Naringenin P
- Chitosan S
- Chitosan P

(P=protein patty, S=sugar syrup)



# Colony Treatments

- Treated 2 times/year (spring and fall)
- Treatments applied in sugar syrup or in a protein patty.





# Colony Treatments



# Monitoring Colonies

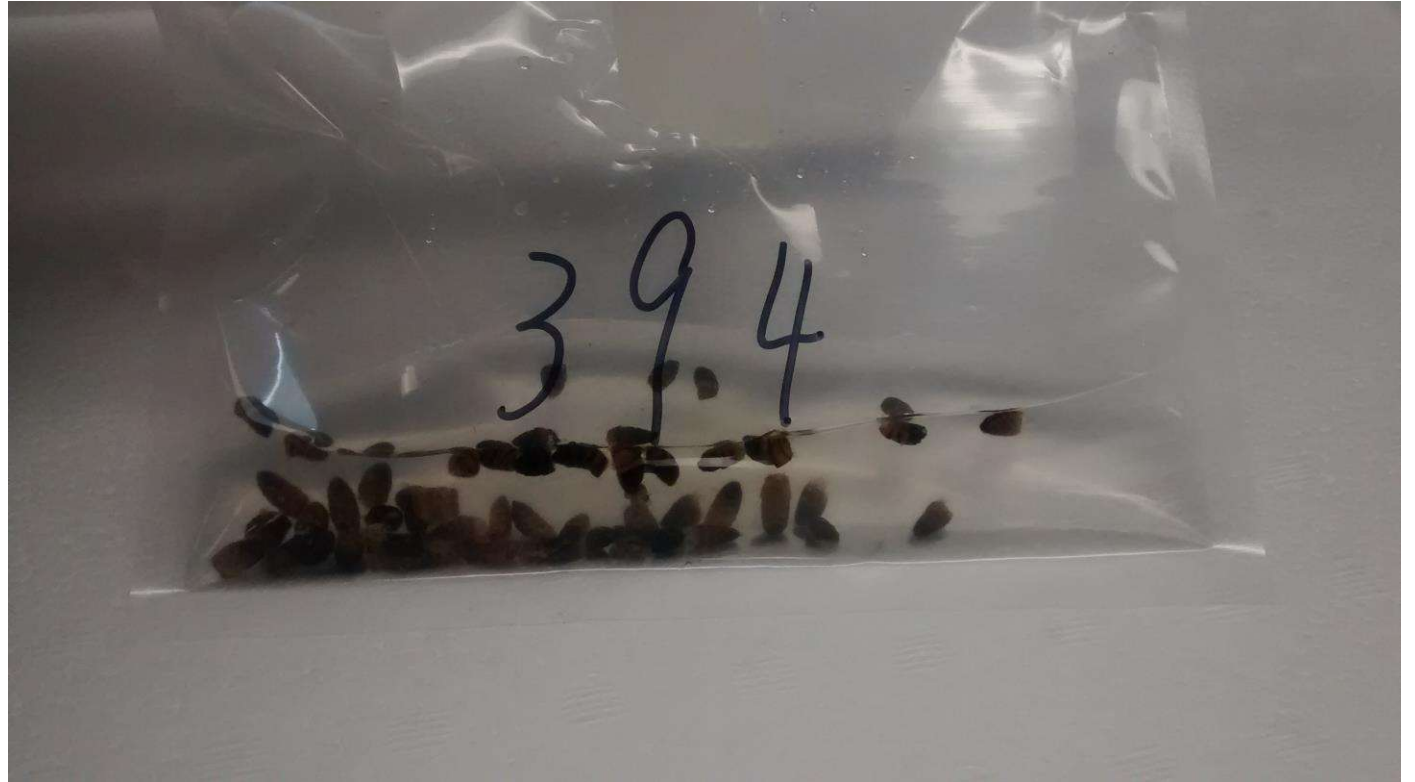
- *Nosema* infection levels
  - Spores per bee
- Population
  - Adult bees
  - Brood area
- Honey production
- Winter survival

# Colony Assessments with the OBA Tech Transfer Program





# Sampling for *Nosema*

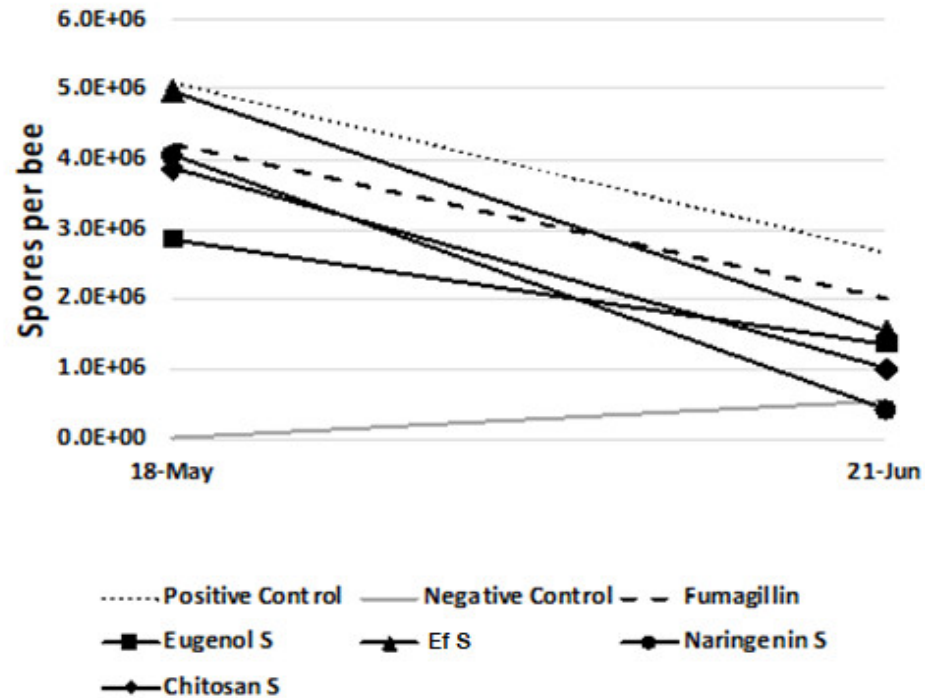


# Honey Production

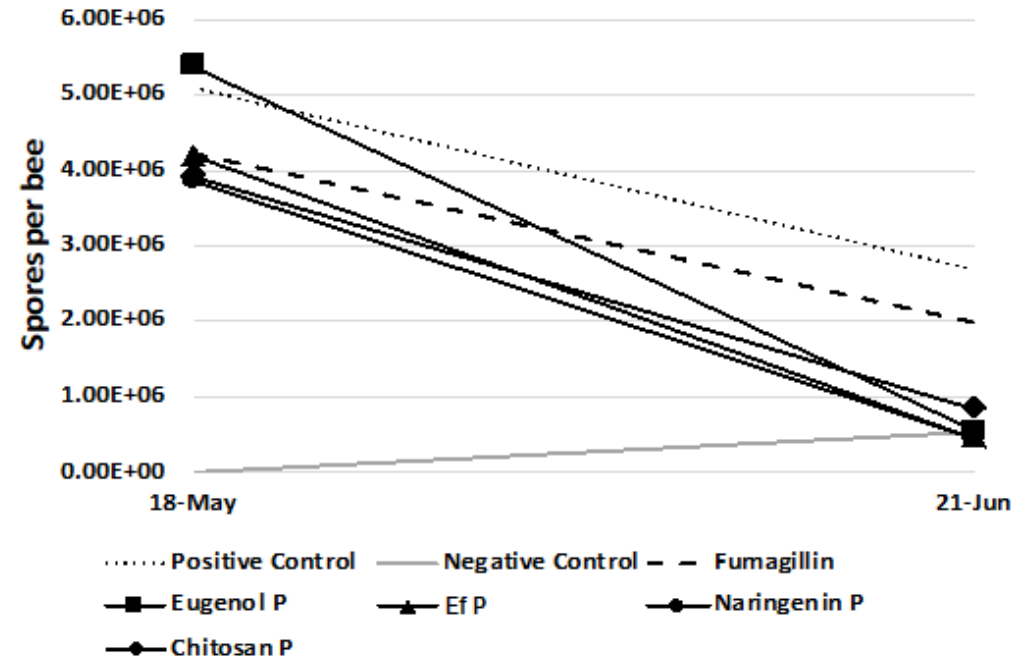




# Effect of spring treatment on spore counts



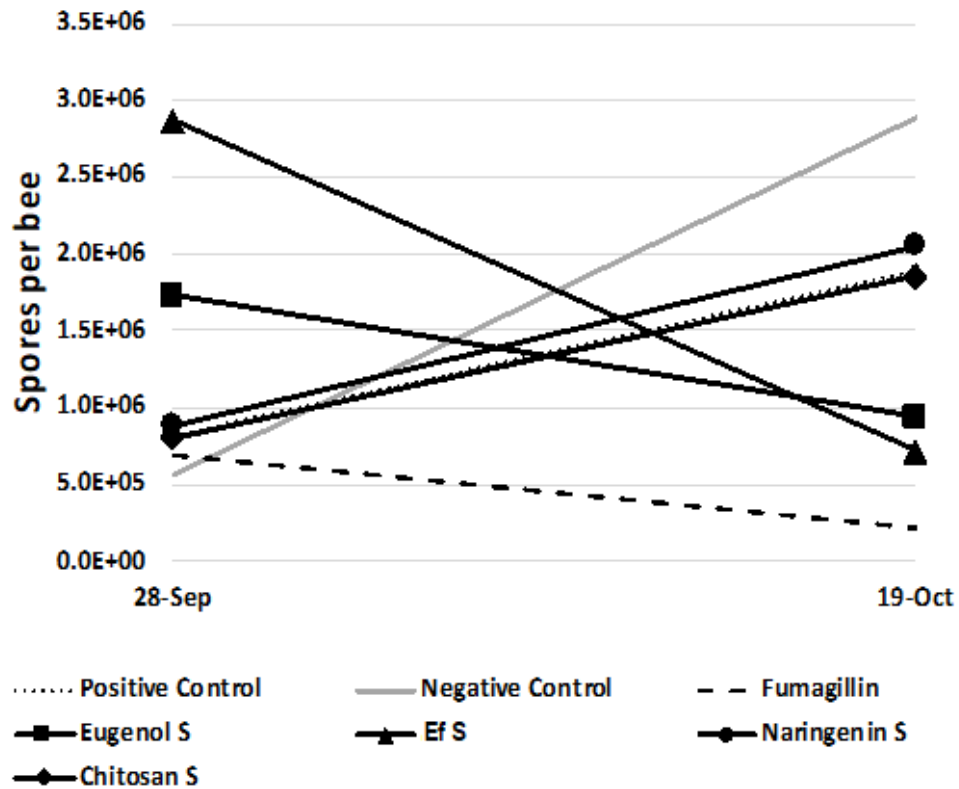
Negative Control increase ( $P < 0.01$ )\*  
 Naringenin S decrease ( $P = 0.03$ )\*



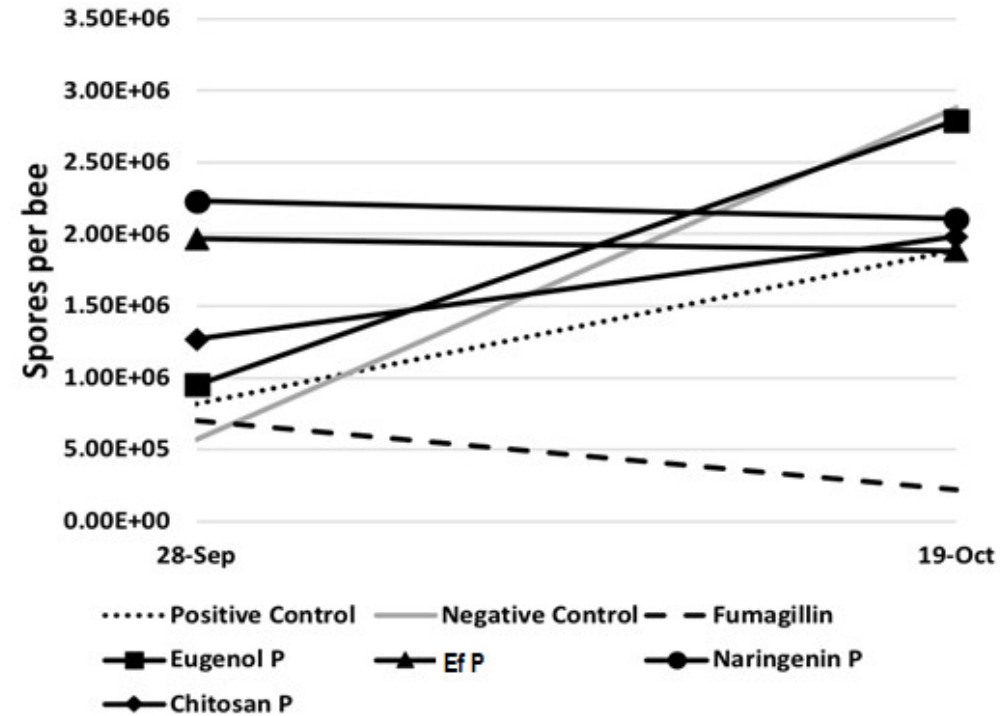
Ef P decrease ( $P = 0.03$ )\*  
 Eugenol P decrease ( $P = 0.06$ )



# Effect of fall treatment on spore counts

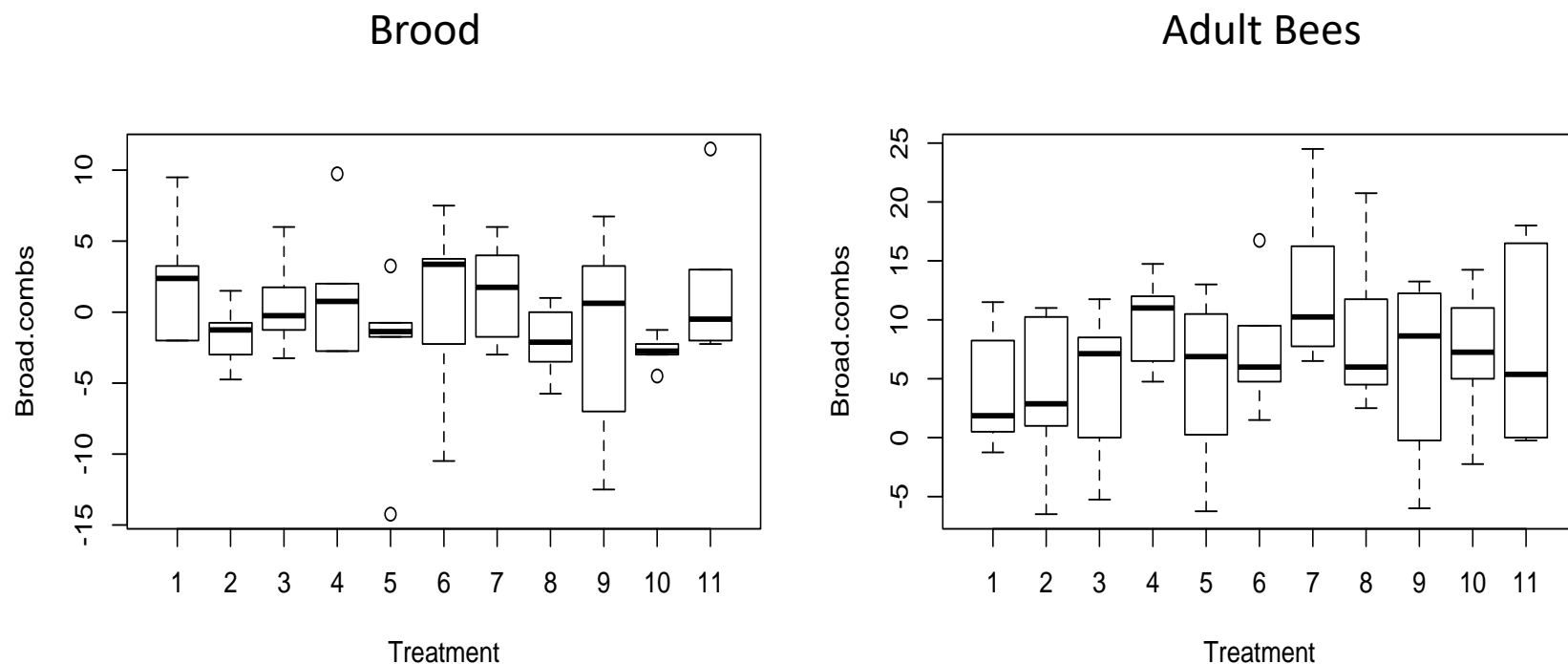


Fumagillin decrease ( $P=0.02$ )\*  
 Ef S decrease ( $P<0.01$ )\*  
 Eugenol S decrease ( $P=0.08$ )



No significant results

# Effect of spring treatment on brood and adult bee populations

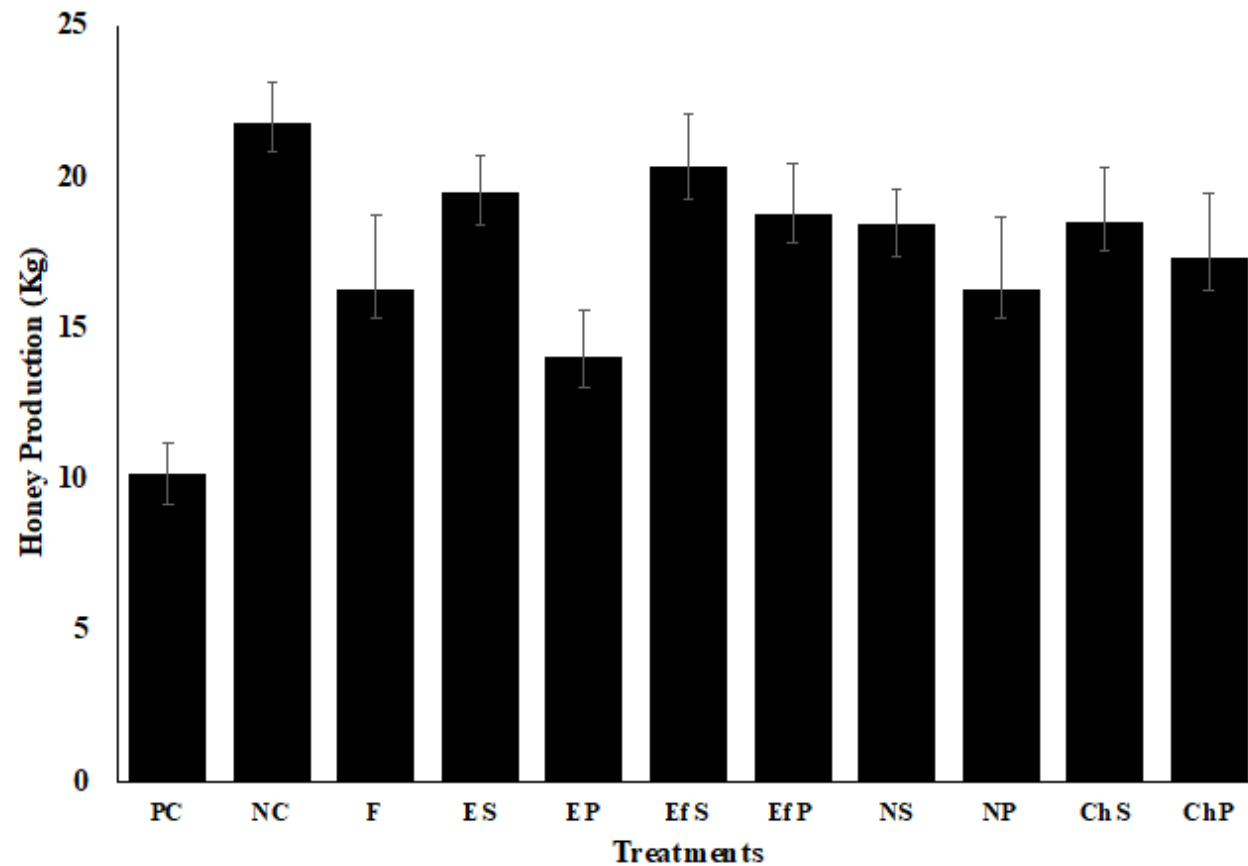


Brood: Naringenin S (treatment 8) decrease (coef.= -2.207, P= 0.03)\*

Brood: Chitosan S (treatment 10) decrease (coef.= -2.913, P=<0.01)\*

Adults: Ef S (treatment 7) increase (coef.=2.367; P=0.02)\*

# Effect of treatment on honey production



- Compared to the positive control:
- Negative control produced more honey ( $P=0.009$ )\*
- Eugenol S produced more honey ( $P = 0.01$ )\*
- Ef S produced more honey ( $P = 0.03$ )\*
- Naringenin S produced more honey ( $P = 0.03$ )\*



# Effect of treatment on winter mortality

Treatment	Number of colonies in the fall	Number of dead colonies	% Winter Mortality
Positive Control	5	2	40%
Negative Control	6	1	16.6%
Fumagillin	5	3	40%
Eugenol S	5	1	20%
Eugenol P	4	1	25%
Ef S	5	0	0%
Ef P	6	0	0%
Naringenin S	6	2	33.3%
Naringenin P	5	0	0%
Chitosan S	6	3	50%
Chitosan P	6	3	50%

**N=6**

# Conclusions

## Ef patty

- Reduced spores per bee after spring treatment (fumagillin did not)
- Had low (0%) winter mortality

## Ef syrup

- Reduced spores per bee and percent infected bees after fall treatment (better than fumagillin)
- Increased the adult bee population (increased life span)
- Increased honey production above untreated bees
- Had low (0%) winter mortality

# Conclusions

## Eugenol patty

- Decreased spore loads in the spring (moderate significance)
- Increased percent positive infected bees in the fall
- Had 25% winter mortality

## Eugenol syrup

- Decreased spore loads in the fall (moderate significance)
- Increased honey production above untreated bees
- Had 20% winter mortality



# Conclusions

## Naringenin patty

- Had 0% winter mortality

## Naringenin syrup

- Reduced spores per bee after spring treatment
- Reduced brood production
- Increased honey production above untreated colonies
- Reduced spores per bee over the winter
- Had 33% winter mortality

# Conclusions

## Chitosan patty

- Decreased percent positive infected bees in the fall
- Resulted in a 50% winter mortality
- Reduced spores per bee over the winter
- Increased percent positive infected bees over the winter

## Chitosan syrup

- Decreased percent positive infected bees in the spring
- Reduced brood production in the spring
- Resulted in a 50% winter mortality
- Decreased percent infected bees over the winter

# ACKNOWLEDGEMENTS



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