HARVEST of the MONTH in the CLASSROOM

April

Grades 7-12 • 1 Period

# Investigating the Prevalence of Nosema in Honey Bee Colonies

S.T.E.M.

## OBJECTIVES

Students will gain a deeper understanding of factors that cause honey bee colonies to collapse.

They will estimate the severity of nosema in a hive by predicting outcomes for the hive and discussing solutions.

## ESSENTIAL QUESTIONS

- Why do honey bee colonies collapse?
- How does nosema in a hive play a role in the collapse of honey bee populations?

## MA STATE FRAMEWORK(s)

8.MS-LS1-5
HS-LS2-1
HS-LS2-2

## MATERIALS NEEDED

- honey bees (or access to hives)
- grid paper
- petri dishes
- wet mounts
- pipettes
- compound microscope
- water
- mixing utensils
- hemocytometer

## HANDOUTS

- Diagnosis of Honey Bee Diseases (https://bit.ly/3zmMNIF)
- MDAR Nosema Fact sheet (https://bit.ly/3Zu2OY4)
- Testing for Nosema Spores using Hemacytometer (https://bit.ly/3JYzsLx)

## INTRODUCTION

Nosema are microsporidian parasites found in the digestive tract of honey bees, where they live and grow. Nosema can be initially introduced to a colony via many routes: robbing, swarming, grooming, shared water sources, used equipment, requeening etc. Nosema shortens the lifespan of both worker and queen bees, inhibits development. Infected queens may stop laying eggs, leading to poor colony health ultimately causing the death of the entire colony. By crushing bees and looking at them under a microscope, one can see and count Nosema spores.

In order to conduct this experiment with you can request a sample of bees (½ cup = approximately 300 bees) from several different sources, such as, a local beekeeper, apiary, state apiary inspectors, (https://www.mass.gov/apiary-program-honey-bees), bee club (https://www.massbee.org/links/), or seller of honey products.

The Massachusetts Apiary Program provides free testing kits and other resources and materials to MA residents. To receive kits, send an email to: bees@mass.gov, using the subject line: Bee Kits. Include your name, mailing address, type of kit needed and quantity.

**Lesson Written By:**
Natasha Rausch
The Learning Center for the Deaf-Walden School

## PROCEDURE

### Overview

Introduce and explain the nosema disease, how it spreads, and the impact on colony health.
Review the lab procedure & tools required to perform the lab.
Collect bee samples (collect samples from several different hives if possible, that way students can test and compare results)
Prepare and test bee samples (send extra sample to MD Lab if possible)
Review and discuss results.
Complete lab report

### Wet Mount Procedure

*Materials*

50-100 bees*
grid paper
petri dishes
wet mounts
pipettes
compound microscope
water
plastic baggies
mixing utensils
tweezers
Hemocytometer - optional

Handouts:
How to use a Hemocytometer
(http://bit.ly/40wipHL)

Testing for Nosema with a Hemocytometer
(https://bit.ly/3JYzsLx)

Testing for Nosema Spores
(http://bit.ly/40ObLfH)

**bees must be fresh, decaying bees can skew results*

---

Collect samples of dead bees (fecal samples if using the glass plate method). Bee samples should be labeled with the date and where the bees came from.

Put 10-20 bees into a baggy and crush them. Add 1mL of water per bee. Knead the bag until the bees and water are well mixed, the goal is to get the gut contents released into the water. Use more bees for higher accuracy.

Use a pipette to remove some of the bee liquid and prepare a wet mount slide. Blot excess water carefully. If using a Hemocytometer (this machine makes it easier to count spores, but not necessary) prepare slides following manufacturer instructions.

Put slide under a microscope (x400 minimum) and count visible spores.
# of spores counted / 5 = number of spores in millions per bee. 100 spores / 5 = 20 million.
If using a hemocytometer, follow the instructions for counting spores with the grid. Multiply the number of spores by 25,000 to get the number of spores per bee.

Use a sheet of grid paper to help count the number of spores you can see.

Clean/sterilize all equipment after use.

## Interpret the Results

Nosema spores are elongated ellipses that have a black outline. The infectious dose (the amount of something needed to establish an infection) that infects 50% of the population is 390 spores(N.Apis) and 85 spores(N.Ceranae). The threshold for treatment is the occurrence of at least 1 million spores per bee.

*Option 1: Wet Mount Slide*
Count the number of spores you see and divide by 5. This gives you an estimate of the number of spores per bee, this is not exact but it should be sufficient for management purposes (whether or not to treat).

*Option 2: Hemocytometer* (see linked handout for instructions)

After preparing the slide, count the number of spores in 5 squares.
Multiply the number of spores by 25,000 to get the spore load.
(i.e. 100 spores x 25,000 = 2.5 million spores per bee)

## Factors That Could Affect Results

1. Time of year (are nosema loads higher in spring, summer, or fall?)

2. Type of bee (worker-house, nurse, drone, young, adult). If you're careful and select your bee sample from specific areas of the hive you could get different results. You can also separate bees by category before crushing and compare the results.

## Visual Results Examples

Encyclopedia of Parasitology: Nosema Spores (https://bit.ly/3ZpQFmJ)
Bee Culture: A Closer Look - Impact of Nosema Disease (http://bit.ly/40vdgQm)
Scientific Beekeeping: Simple Microscopy of Nosema for Beekeepers (http://bit.ly/40t6R8k)

## EXTENSIONS & VARIATIONS

Variation 1: An alternative to using dead bees is to use live ones. This can be done by placing glass collection plates at the opening of hives allowing bees to walk/defecate on the glass for some period of time (1-2 hours) before removing to collect the sample.

Variation 2: The abdomens from your bees can be removed and tested instead of crushing the entire bee and having to account for erroneous bee parts in the mix. Page 19 of the USDA Diagnosis of Honey Bee Disease details the procedure for gut/digestive track removal.

Extension 1: Request a FREE Nosema testing kit from MDAR and submit a sample from the same bees for professional testing and have students compare results. Samples are sent to the USDA-ARS Bee Research Lab (http://bit.ly/42RQOCt) in Beltsville, MD. More about the lab and submitting samples can be found here (http://bit.ly/3KmOsWI) . To receive kits, email bees@mass.gov with your name, mailing address, type of kit needed and quantity requested, subject line: Bee Kits.

**EXTENSIONS & VARIATIONS, cont.**

Extension 2: Contact Massachusetts Apiary Inspector Dr. Kim Skyrm (kim.skyrm@mass.gov) for a presentation on nosema and honey bee health in Massachusetts, or any other bee-related topics of interest. Dr. Skyrm is happy to provide workshops and presentations to classrooms and groups of students.

Extension 3: Nosema can be treated with good husbandry practices and an antimicrobial agent called Fumagilin-B (fumagillin). Stay in contact with the original source of your bees and test again following treatment. Compare results. Did treatment affect the amount of nosema present post treatment? 2 weeks? 1 month?