Detection and Management of Microsporidia and *Ophryocystis elecktroscirrha* (OE) in Insect Rearing Laboratories

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Diseases of Insects Caused by Microbes

- Insects are just like any other animal species in that they are vulnerable to an assortment of diseases caused by microbes such as: viruses, bacteria, fungi, and protozoa. When insects are reared in the laboratory, special care must be taken to detect and prevent diseases caused by microbes using a multi-tactic approach.

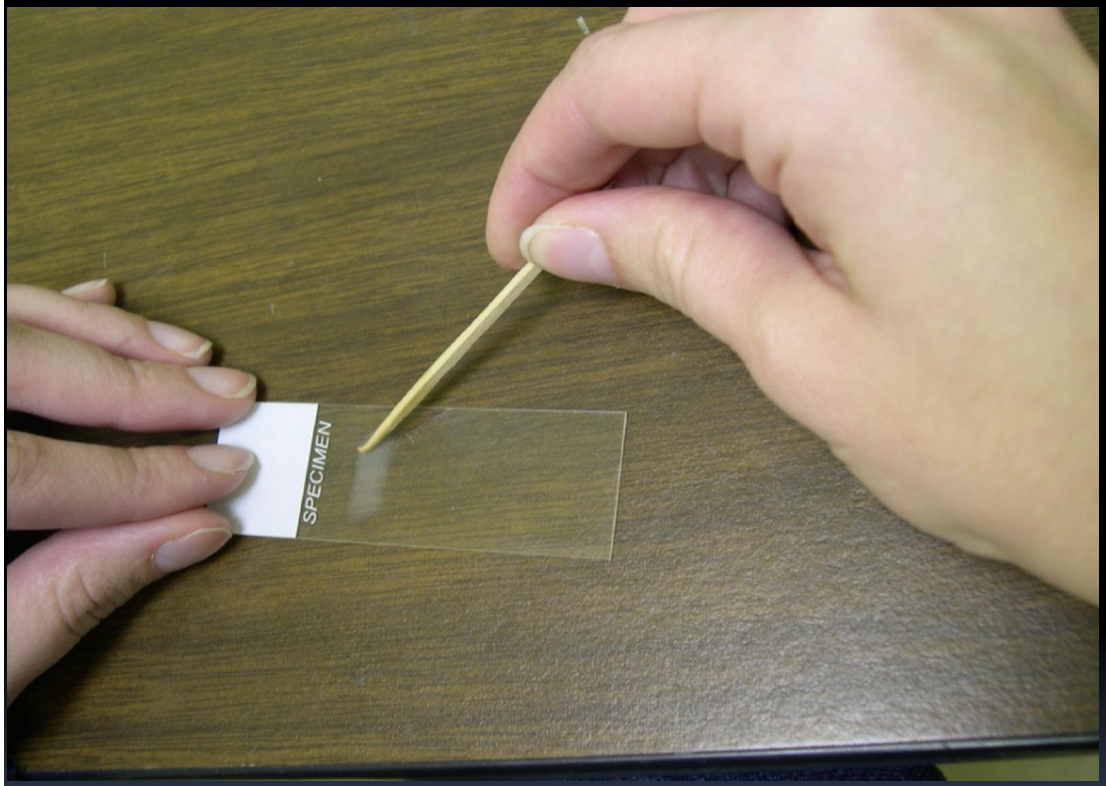
Microsporidia* Detection

* The protozoan Nosema is a Microsporoidia, however there are other types of Microsproidia that affect butterflies.

Remove insect from diet.

Place insect in clean container.

Macerate in distilled water.



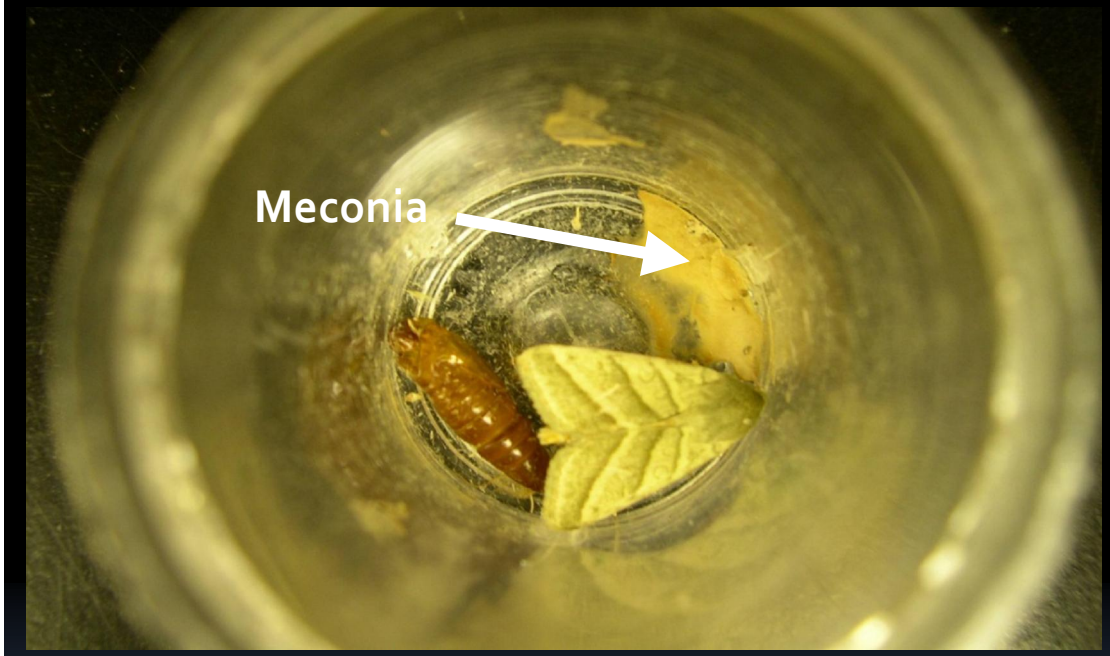
Spread some of the homogenate on a microscope slide.

OR

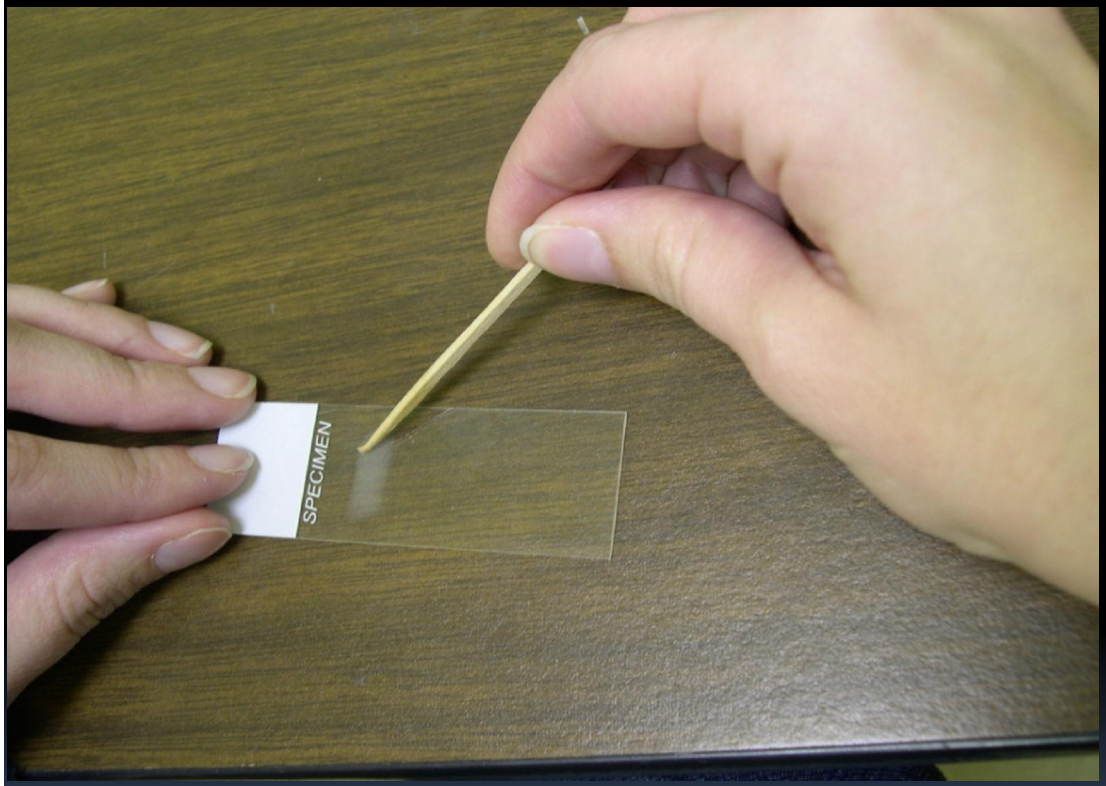
Use Meconia* Method

*Meconia is the initial waste (from pupal metabolism) expelled by the adult upon emergence from the puparium. If the insect was infected with a Microsporidian, the spores will be present in the meconia. This is a non-destructive sampling method.

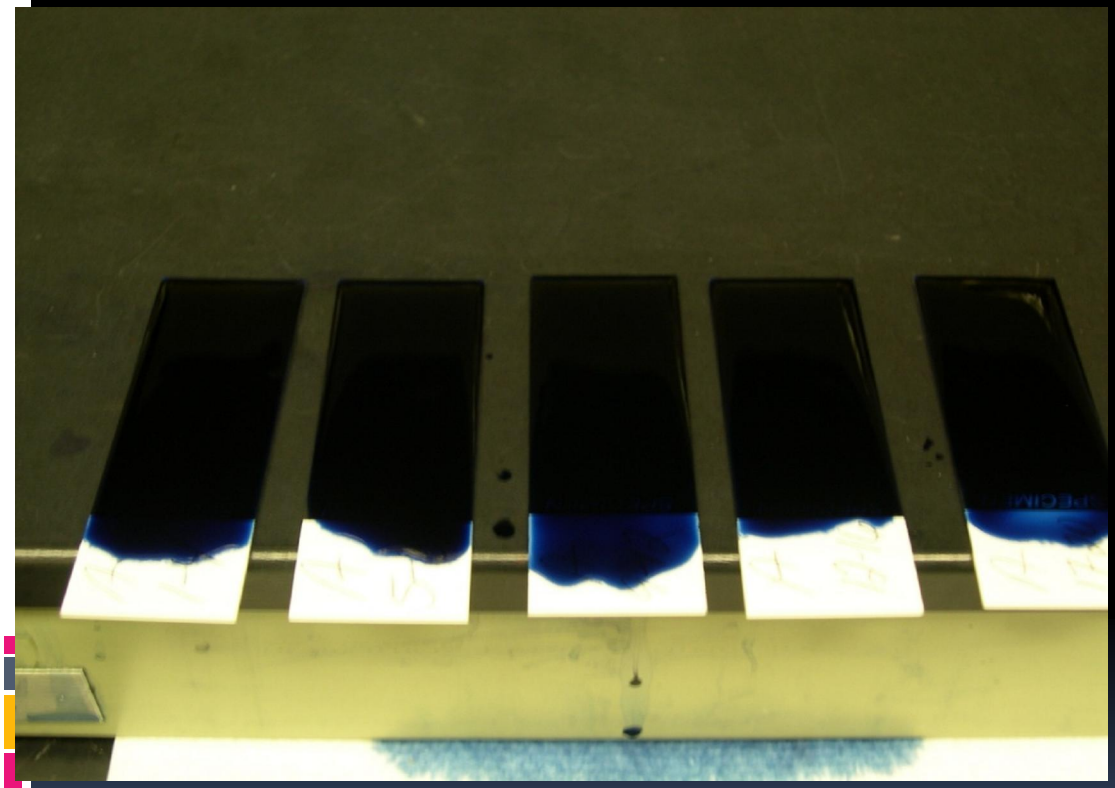
Place pupa in clean cup and allow adult to emerge.



Remove adult from cup. Mix meconia with a couple drops of water.



Smear some of the mixture on a microscope slide.



Stain smeared and air dried slides with Buffalo Black.

Buffalo Black Stain

- 0.1 g Buffalo Black (Napthol Blue Black)
- 50 ml Methanol
- 30 ml Acetic Acid
- 20 ml water

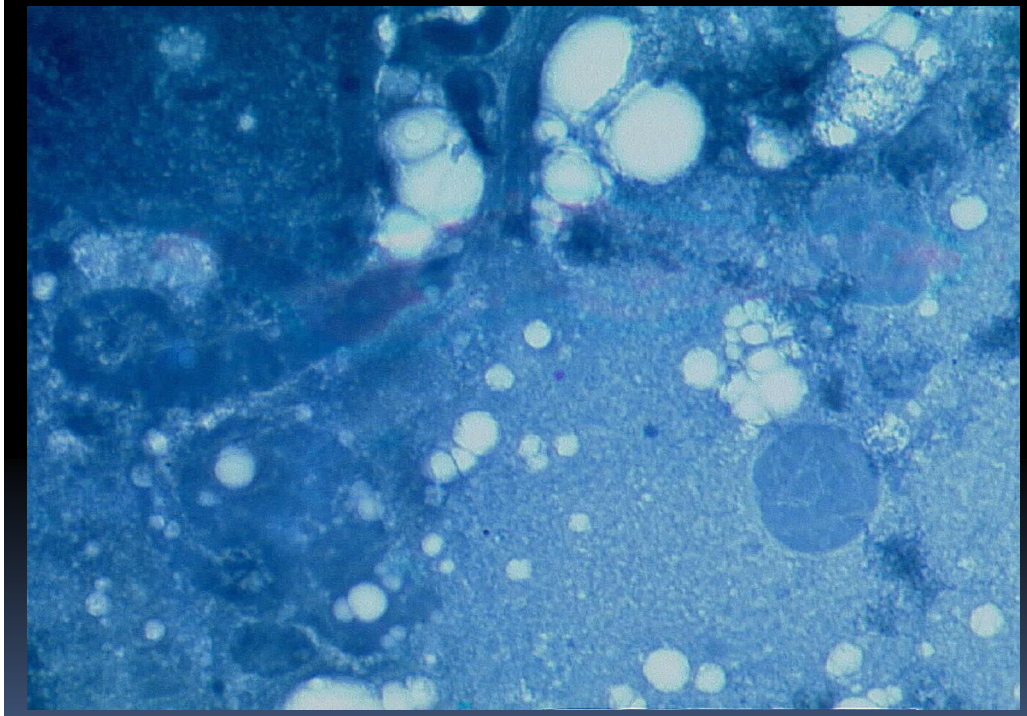
Air dry slides

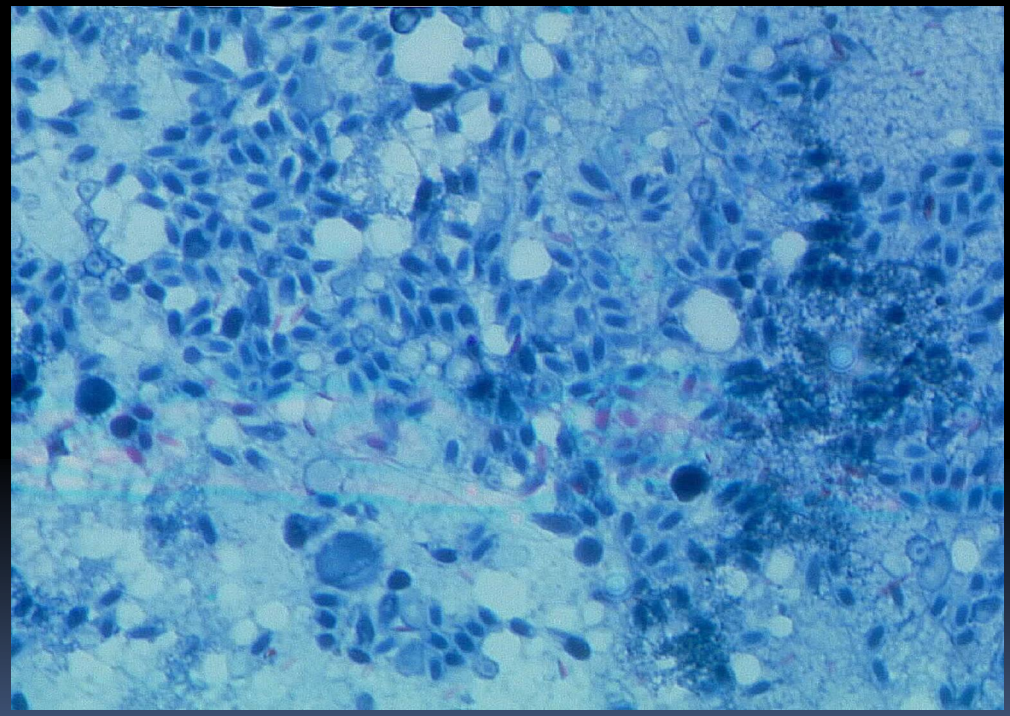
Stain five minutes at 40°c

Rinse stained slides in water.

Examine with a light microscope.

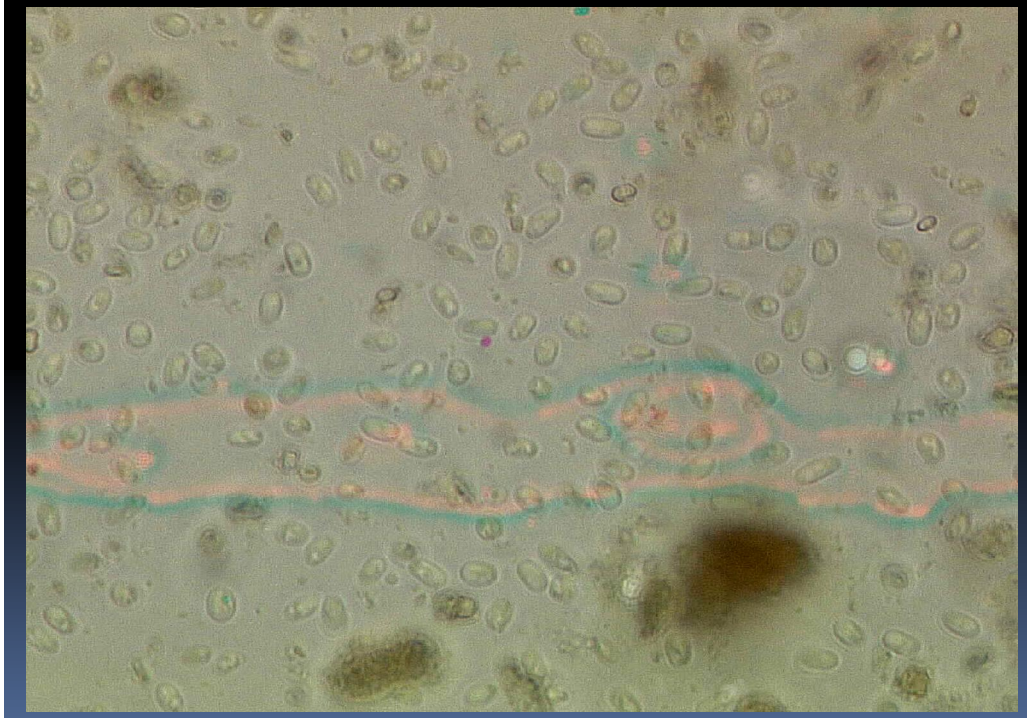
Healthy vs. Infected





Microsporidian spores at 100x magnification

Unstained Slides Showing Microsporidia at 100x and 40x Magnification



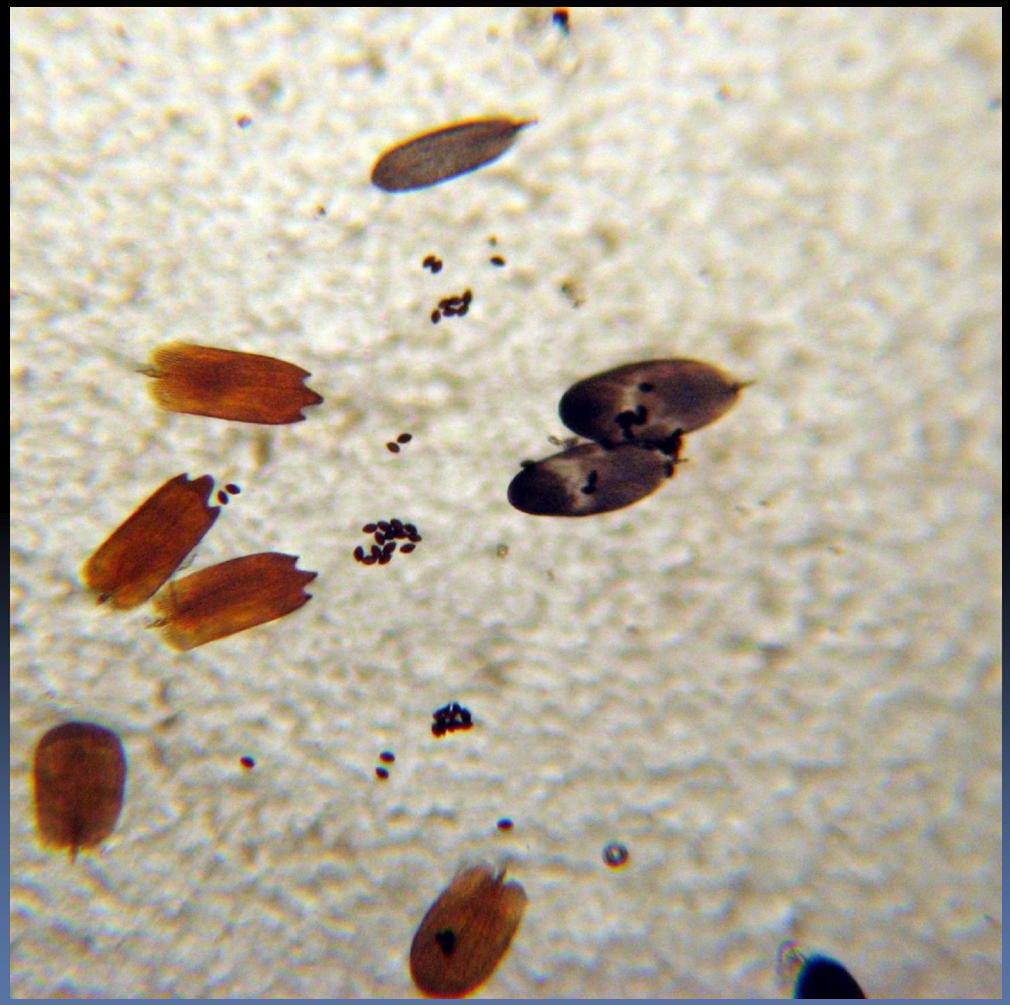
OE Detection

Gently place a piece of tape against the abdomen to extract scales.

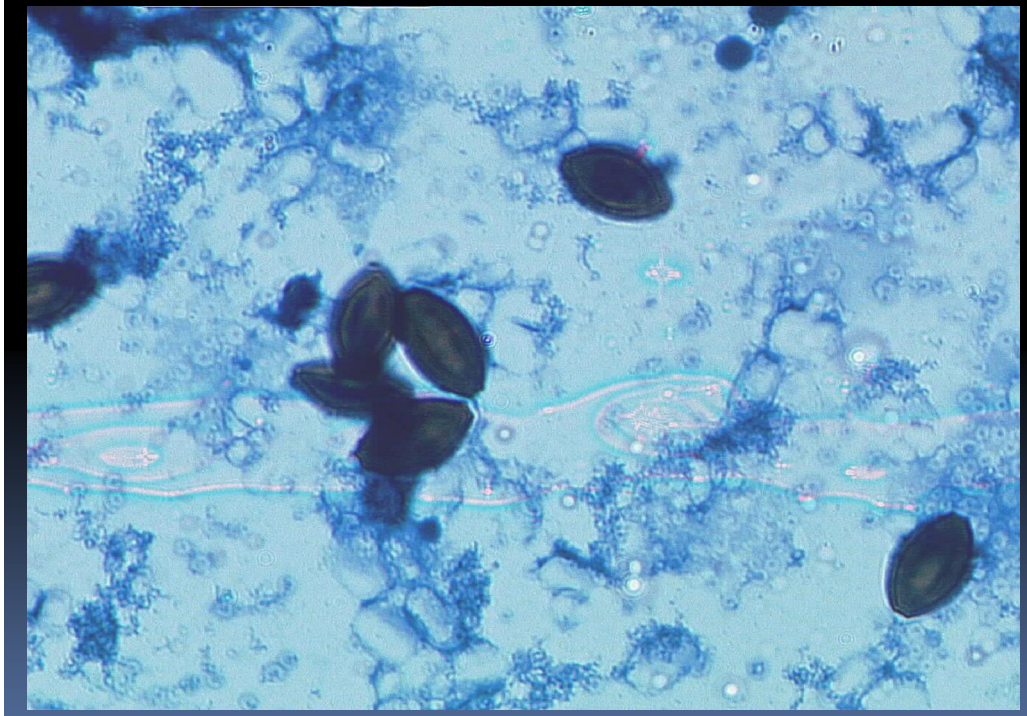
Place the tape on a slide.

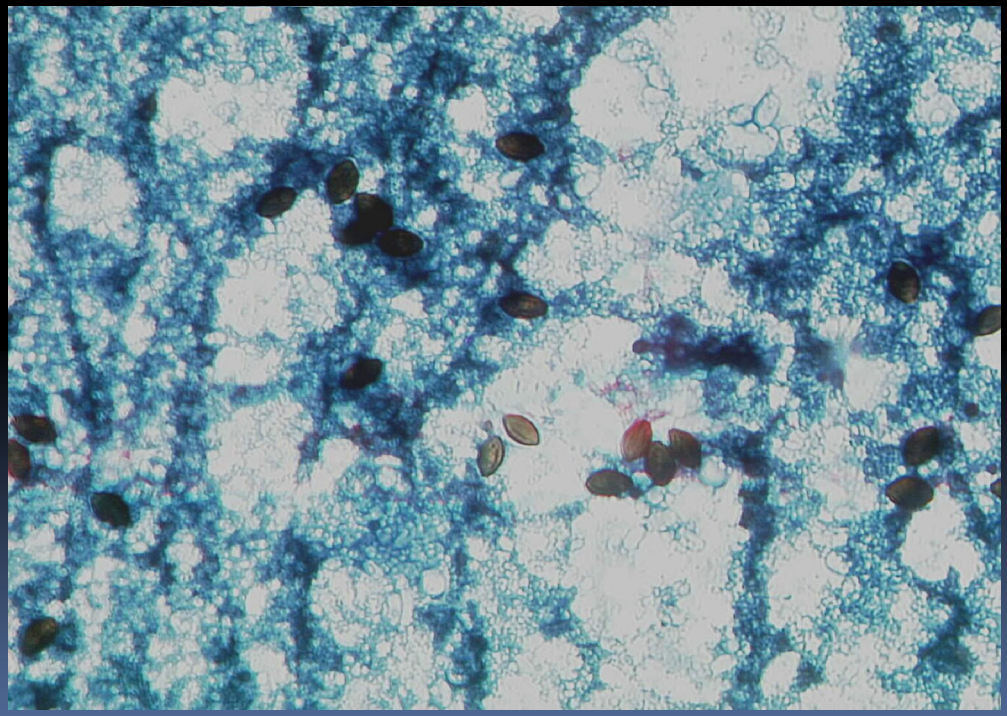
Examine slide with a light microscope.

Scales and Spores at 100x and 40x Magnification



Buffalo Black Stained Slides Showing OE spores at 100x and 40x Magnification





Management Tactics

Establish a disease free colony

This can best be done by using progeny from single pair matings in which the male and the female have been found to be free of disease causing microbes.

Quarantine

Field-collected or insects obtained from another laboratory should be isolated from colony insects until proven that they are free of any disease causing microbes.

Humans are carriers of microbes, especially their hands and clothes. Wearing clean clothes and sanitizing hands between rearing tasks using anti-microbial soaps is essential to managing harmful microbes.

Work bench spaces should be sanitized before and after each rearing task

Floors should be sanitized daily - using such compounds as sodium hypochlorite (common bleach/Clorox) or ammonium chloride products mixed with water.

Egg surface sterilization

Chemical compounds such as formaldehyde and sodium hypochlorite are often used to eliminate microbes from the egg's outer surface.

Concentration of active ingredient mixed in water and time eggs are left in the solution are factors that must be determined for each insect species.

Diet - Natural

Host plants should be surfaced sanitized to remove disease causing microbes such as, OE.

A common procedure is to wash the leaves in a 10% solution of bleach (active ingredient sodium hypochlorite) in water for 10 to 15 minutes then thoroughly rinse the leaves with clean water to remove the bleach from the leaf surface.

Diet - Artificial

The anti-microbial Fumagillin is often added to the diet to suppress such disease causing microbes as *Nosema*.

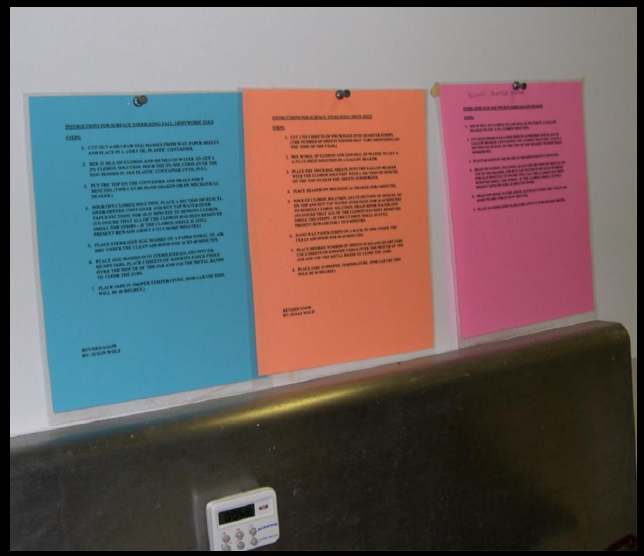
Research needs to be conducted to determine the most effective concentration of Fumagillan in the diet and whether this anti-microbial agent adversely effects the insect before implementing this prevention procedure.

Air Filtration

Microbes can be circulated through out insect rearing facilities in the air on insect scales (prime example OE) and dust particles.

To prevent this from occurring special equipment has been developed to efficiently remove insect scales and dust particles from the air.

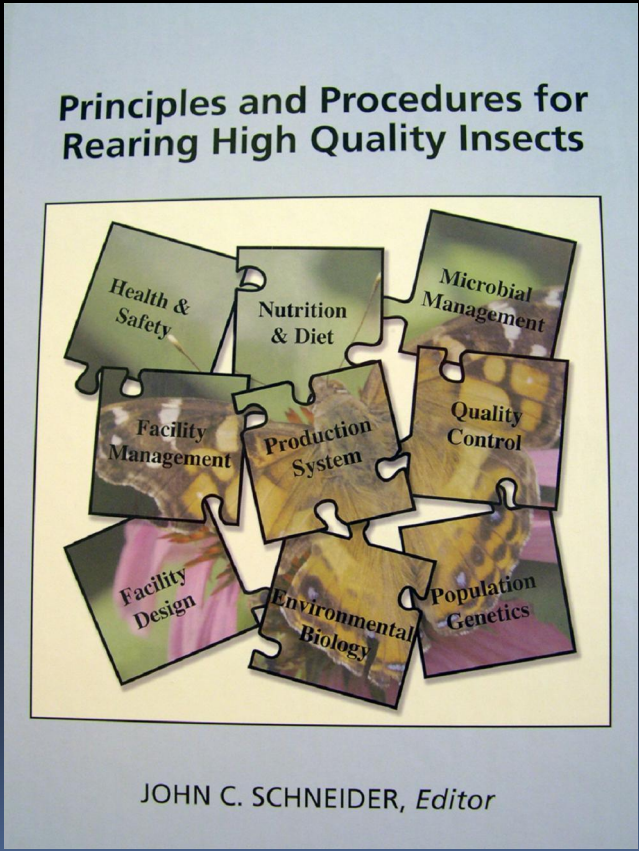
The equipment works by moving air containing the contaminated scales and dust particles through a combination of preliminary and primary filters. This type of equipment would be very useful in preventing OE.



Standard Operational Procedures (SOPs) should be established for each tactic and monitored regularly for compliance.

Staff must be educated as to the necessity for the SOPs and the strict adherence to them.

For Additional Information on Detecting and Preventing Insect Diseases Caused by Microbes



We recommend our book "Principles and Procedures for Rearing High Quality Insects"

View our website (www.irc.entomology.msstate.edu) for more information on the book as well as our Insect Pathology Service.