

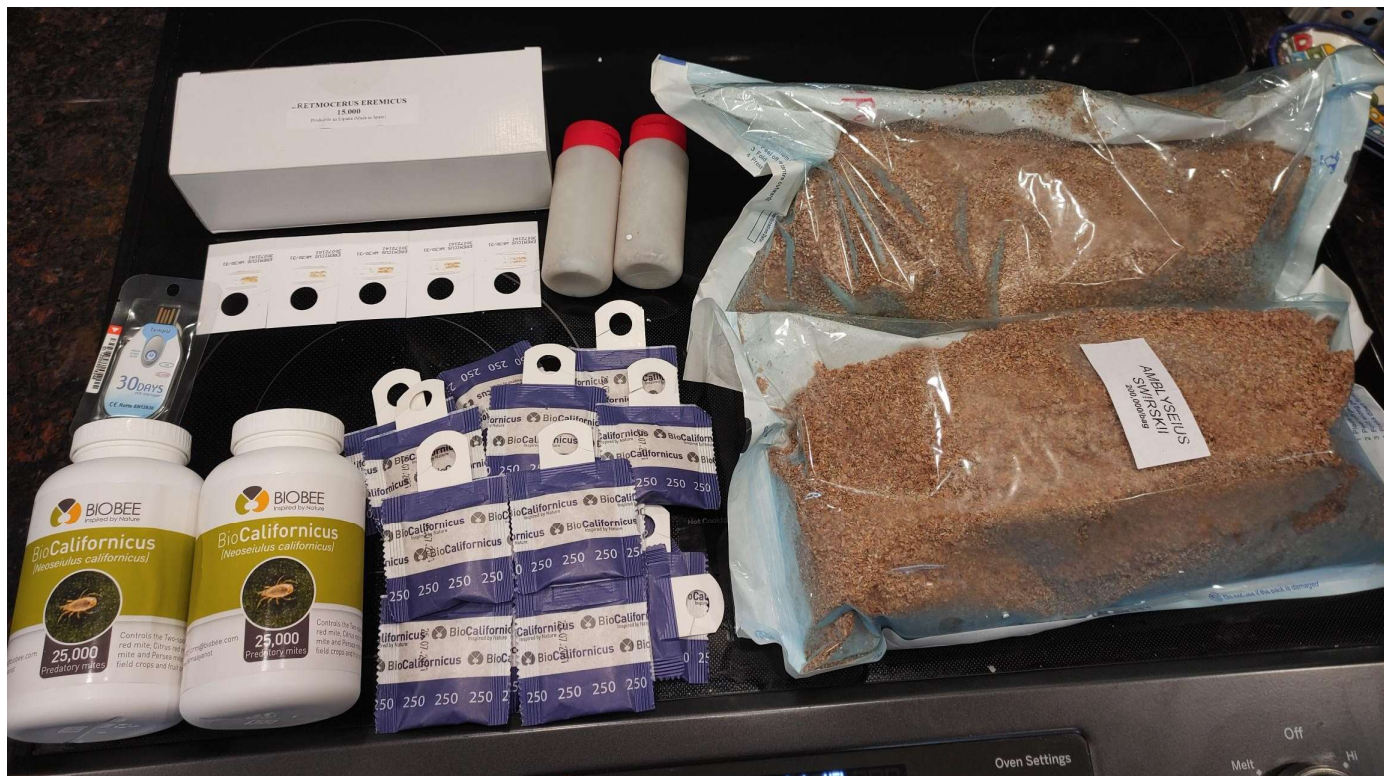
# EVALUATION OF BIOLOGICAL CONTROL PRODUCTS

Workshop and Tour, Greenhouse Biological Control Essentials: Setting Yourself up for Success

2023 Great Lakes Expo, December 7<sup>th</sup> 2023 (steven.arthurs@biobee.us)

## BCA DELIVERY FORMATS

- Bottles, bags, blister packs, cards, cups, capsules, on leaves, slow-release sachets
- Carrier materials – buckwheat or rice hulls, vermiculite, bran, sawdust



## BASIC CHECKS ON ARRIVAL\*

- Ice packs cool? (if included)
- Excess Moisture/Damaged packaging
- Bad odor (fermentation)
- Activity/Dead individuals (up to 10% mortality is not atypical)
- Confirm receipt of the correct BCA species/lifestage(s), label rate and product count

\*Report issues to supplier immediately so they can check issues and if needed replace products ASAP.

Take photos and note batch number and tracking number of shipping carrier.

## USEFUL EQUIPMENT FOR BCA QUANTIFICATION

- Dissecting microscope or at least 10X handlens
- Temperature probe
- Yellow sticky cards (gridded)
- Fine mesh cage for flying BCAs and Insect aspirator
- Hand-held tally counter
- Onion slicer for making grids on sticky cards
- Fine paintbrush/tweezers
- White tray/detergent
- Measuring spoons 1/8 tsp and up
- Bucket for mixing
- Microbalance (1/100g best)
- Sieve 30 mesh (600 micron)



Keep beneficials in box and release soon after delivery

## PREDATORY MITES

*Phytoseiulus*, *Amblyseius* and *Neoseiulus* spp.

### LOOSE MATERIAL

- Determine total weight/volume of product using microbalance
- Mix thoroughly in a bucket (use 'tumble' method)
- Immediately take samples for counting and mix between samples

### Dish-ring method (for red persimilis)

- Spread sample (e.g., 0.5g/3ml) on a white tray inside a detergent ring
- Count all live mites running out of material at 5-10X
- Use heat lamp to improve mite movement
- Squash predatory mites to avoid double counting

### Sticky card method (more accurate for most other species)

- Spread smaller sample (e.g., 0.15g/1ml) evenly on a sticky card with 1-cm counting grid
- Count live/dead predatory mites
- Use fine tweezers to examine mites stuck to carrier material
- Using a 30-mesh sieve to separate mites from carrier material can be helpful



Microbalance, sieve method and sticky counting board

### SACHETS

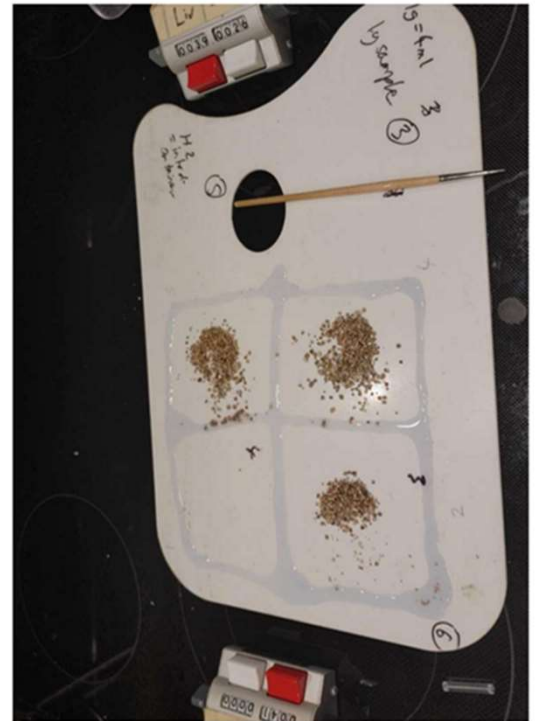
- On arrival: open and count as for loose material
- Release rate: place sachet on sticky card in shaded area of greenhouse. Count emergence over time until no more occurs
- Alternative - place sachet in heavy glass inside plastic container filled with a water 'moat' containing a few drops of dish soap
- Best release obtained under higher humidity (>70% RH)

### NOTES

- Total weight/sample weight x live count = number in product
- Take average of multiple (3 or more) samples for better accuracy
- Do not count 'feeder mites' which move more slowly, with shorter legs, longer hairs and are not 'pear shaped.'



LEFT shows a 'food mite' (*Carpolyphus*), RIGHT Shows a predatory mite (*Amblyseius*)



Detergent ring counting method



Monitoring release of predatory mites from sachet

# BENEICIAL NEMATODES

*Steinernema* and *Heterorhabditis* species

Store nematodes unopened in refrigerator. Note expiration date!

### Bag/container

- Place small amount (0.05g) of material in glass container containing 5ml room temperature water.
- Wait 10-20 minutes for nematodes to rehydrate and become active
- Observe under dissecting microscope.
- Count number of live and dead nematodes (use dark background).
- Live nematodes will move or have an S- or J-shape curvature.
- Dead nematodes are straight, and may contain water droplets. They do not respond to stimulus with a pin.

### Sponge

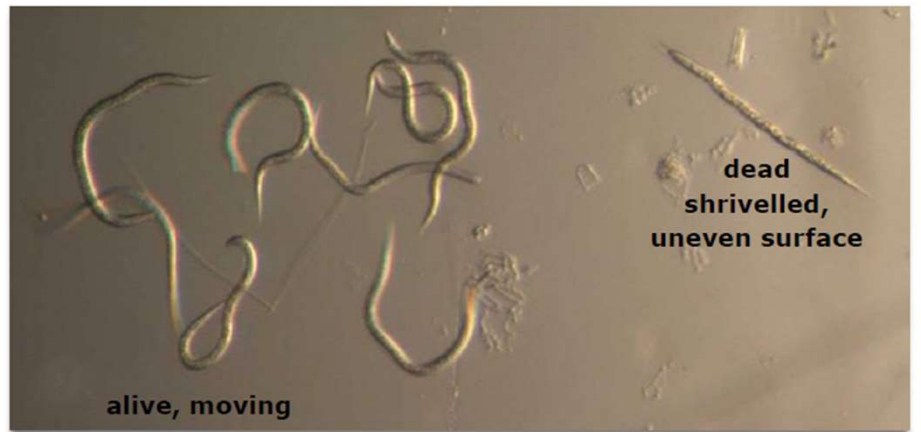
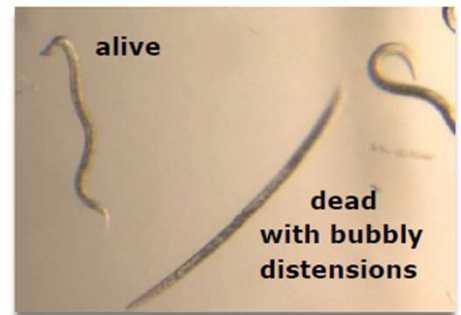
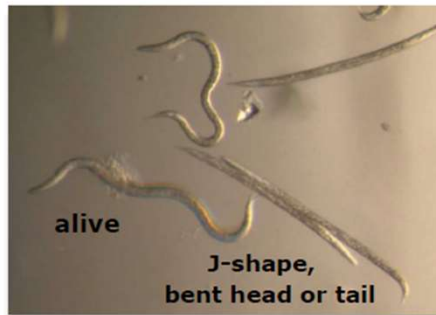
- Wring sponge in clean water before assessment

### For quantification

- Weigh bag, mix thoroughly and place a 0.5g sample in ½ liter of water
- Stir sample quickly and decant 5ml from the middle.
- Use counting grid and multiple live nematode number in sample by  $100 \times \text{bag weight}/0.5$ .



Package of beneficial nematodes



Alive	Dead
<ul style="list-style-type: none"> <li>• Movement</li> <li>• Resting forms:               <ul style="list-style-type: none"> <li><i>Steinernema</i>: J-Form (head or tail tilted)</li> <li><i>S. feltiae</i>: rolled up (they are more stress resistant in this position)</li> </ul> </li> </ul> <p>⇒ generally, nematodes start moving again after some time in water.</p>	<ul style="list-style-type: none"> <li>• Generally straightened, with shrivelled surface</li> <li>• Old dead EPN: sheer with bubbly distensions in their body               <ul style="list-style-type: none"> <li>⇒ freshly dead EPN are difficult to distinguish from resting ones</li> <li>⇒ Dead by quickly drying out: EPN can show different forms, generally they also show a shrivelled surface</li> </ul> </li> </ul>

## QC FOR PARASITOIDS

*Aphidius*, *Encarsia*, *Eretmocerus*, *Trichogramma* species

### Aphid parasitoids (*Aphidius/Aphelinus*)

- Place in a small fine mesh cage in shaded area of greenhouse.
- Aspirate adults daily on sticky cards until emergence stops.
- Sex ratio can be determined by holding samples until death and examining at 20X
- Sub-samples can be taken to reduce counting effort by mixing material first

### Whitefly parasitoids (*Encarsia/Eretmocerus*)

- In situ - count number of emerged/unemerged mummies at 20X on at least 3 cards/balls/blister packs on arrival.
- Repeat again after 7 and 14 days after placement in the crop.
- Samples can be placed in a Ziploc bag or vial containing yellow sticky card to assess wasp emergence over time.

### Moth parasitoids (*Trichogramma* spp.)

- Cards, count the number of emerged/unemerged pupae on arrival at 20X and after 5 and 10 days in the crop.
- Samples can also be placed in a Ziploc bag or vial containing yellow sticky card to assess wasp emergence over time.

### Leafminer parasitoids (*Diglyphus* spp.)

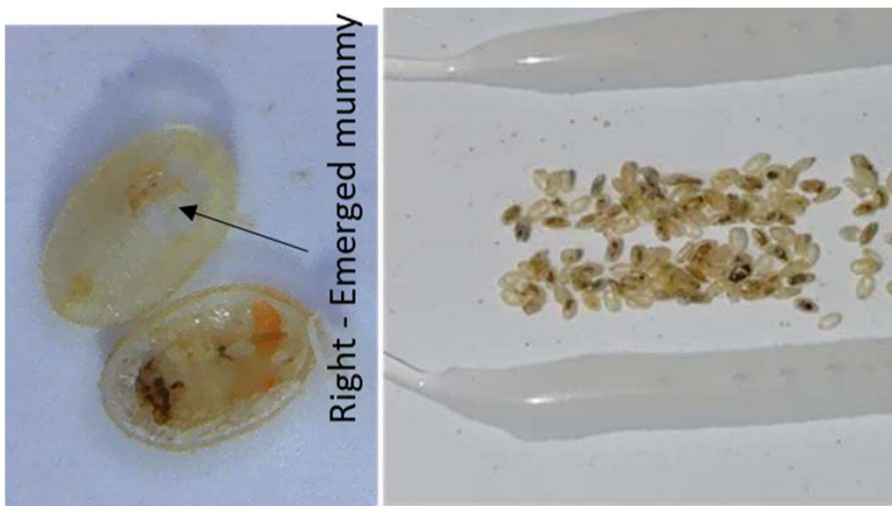
- Most come as adults. Release in fine mesh cage and aspirate to count # live wasps. Also count any dead ones remaining in the bottle.



*Aphidius colmani* with first emerged adults



*Trichogramma* emerged on sticky card



Right - Emerged mummy

*Eretmocerus* - pupae on cards



*Encarsia* balls- emerged in Ziploc bag