Biological Control Primer: Microbe Entomopathogens and Commercial Formulation Studies

Spotted Wing Drosophila Winter Regional Workshops
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CCE, 24 Martin Road,
Voorheesville, NY 12186

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Hummingbirds feed on small spiders and insects, including mosquitoes, spiders, gnats, fruit flies (genus Drosophila) and small bees.

Important sources of protein particularly needed during the breeding season for proper development of their young. Syrup feeders may increase HB population.

Insects are often caught in flight (hawking); snatched off leaves or branches, or are taken from spider webs. A nesting female can capture up to 2,000 insects a day.
**Beauveria bassiana** Basl.-Criv.
(Hypocreales; Clavicipitaceae)

- Fungal parasite that causes White Muscardine Disease in insects.

- Microscopic spores of fungi come into contact with insect host, which germinate and penetrate the cuticle.

- Fungus then grows inside of insect, killing it within a matter of days.

- White fruiting bodies emerge from insect, releasing spores.

- Anamorph of *Cordyceps bassiana*. 
The in vivo development cycle of the entomopathogenic fungi Beauveria bassiana

- Conidia (spores) adhere to the host cuticle, then the conidia germinate and the germ tube and appressorium (penetration structure) are produced.

- The cuticle is penetrated by a combination of mechanical pressure and the action of cuticle-degrading enzymes.

- The fungus grows by vegetative growth in the host haemocoel and external conidia are produced upon the death of the host.

Can fungal biopesticides control malaria?
Matthew B. Thomas & Andrew F. Read
Nature Reviews Microbiology 5, 377-383 (May 2007)
Plates of *B. bassiana*

A. Dorsal surface
B. Ventral surface
C. Fruiting structures
D. Fruiting structures
E. Conidia
Why use Mycological Warfare?

- **B. bassiana** has been used as biocontrol for insect species.

- Propensity towards humid and cool environments (23° C) (Hallsworth & Magan, 1999), coincides with SWD preferred temperature range (20-25° C) (Walsh et al., 2011)

- Based on field work, SWD prefer shaded and humid areas, where fungal pathogens are more likely to thrive.

- **Research Focus:** Organic growers need new tools to deal with this invasive pest.
Objectives:

• Determine the lethality of *B. bassiana* on SWD adults, larvae, and pupae.

• Determine the effect of *B. bassiana* on oviposition.

• Determine if transfer between gravid females and offspring occurs, and if so, does it affect the next generation’s size?

• Determine if generalized strain (GHA) performs as well as Diptera-specific strain (HF23 - BalEnce).
Methods and Materials

- Two strains of *B. bassiana* tested, each one in the form of a commercially available, EPA registered insecticide. Both are approved for organic uses.

- Strain GHA (Mycotrol O- Bioworks), intended for broader range, advertised as effective against Whiteflies, Thrips, Aphids, Leafhoppers, Lepidoptera, and Coleoptera (among others).

- Strain HF23 (BalEnce- JABB of the Carolinas), intended for use against Diptera, particularly Muscidae.

- BalEnce: (EPA Reg. No. 70787-U) contains 1.18 percent by weight of *B. bassiana* HF23 TGAI. The viability of the active ingredient in the EP is $5.6 \times 10^9$ cfu/gram.

- Mycotrol O: (EPA Reg. No. 82074-3) contains 10.9 percent by weight of *B. bassiana* GHA. The volume of the active ingredient in the EP is approximately $2 \times 10^{10}$. 
• Insects were reared in test tubes with 1 Tbs *Drosophila* media, 1.5 Tbs Distilled Water, and 0.5 tsp Active Dry Yeast at 23° C for up to 14 days.

• F2 Adults were removed for experimentation at or before 24 hours of pupal eclosion. Healthy adults were selected at random for experimentation after removal from colonies.

• Washed and dried blueberries were sprayed at highest labeled rates (Mycotrol O=2 qt/A, BalEnce=1 qt/A). Using airbrush at 20 psi air pressure, sprayed until point of drip on surface of fruit.

• Berries allowed 2 hours to dry under fume hood (reentry interval of both materials).

• 25 random male SWD and 25 random females SWD removed from colony and introduced to berries.
Methods and Materials (con’t)

• At 48 hours, insects removed and eggs were counted. Live insects were counted and placed in isolation. All dead insects separated into isolation to observe potential fungal growth.

• Isolation chambers kept at 23° C and 100% humidity to encourage visible fungal growth.

• After 10 days, insects examined for *B. bassiana*, positive ID’s attained through spore microscopy.

Photo: Svetlana Y. Gouli, University of Vermont, Bugwood.org
Mycotrol-O mycopesticide. (*Beauveria bassiana*) to control *D. suzukii* adults

Advanced stage of fungal growth (~10 days of exposure). Most of the spores have already detached.
Recently deceased SWD adult (~5 days of exposure). Signs of fungal growth present on head (around antennae) and abdomen.

Mycotrol-O mycopesticide. (*Beauveria bassiana*) to control *D. suzukii* adults
(Beauveria bassiana) Infesting D. suzukii larvae

Advanced stage of fungal growth (~10 days of exposure) completely engulfing a cluster of 3rd instar Larvae.
Results: Significant increase in mortality of adults on treated berries compared to untreated check. BalEnce provided higher mortality when compared to Mycotrol O.

P Value <0.001
Beauveria bassiana to manage D. suzukii larvae

Results: Numeric decrease in ovipositional activity (NS).

Eggs per gram after 48 Hours of SWD Adult Exposure

P Value 0.1687
**Beauveria bassiana to manage D. suzukii larvae**

**Results:** SWD larva exposed to *B. bassiana* developed disease significantly more than untreated check. Mycotrol O appeared more virulent.
Results: Eggs laid in berries exposed to *B. bassiana* were somewhat less likely to mature to adulthood than those laid in untreated berries.

Data transformed prior to ANOVA from percentages. P Value: 0.07
Breakdown of Untreated (UTC) Fruit at 14 d. relative to *B. bassiana* treated fruit
Conclusions

• Both *B. bassiana* formulations cause mortality within 48hrs.

• Neither formulation works quickly enough to decrease egg-laying significantly.

• Insects exposed to Mycotrol O appear to express mature *B. bassiana* more readily than those exposed to BalEnce.

• Eggs that are laid on *B. bassiana* exposed berries are less likely to mature.
Questions?
Thank You

Technical staff and assistants