Beauveria bassiana application strategies and effectiveness

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Field site – low elevation (137m)

Charles T. Onaka Farm, Honaunau, HI
Field site – high elevation (570 m)

Smithfarms, Honaunau, HI
Primary research objective:

Determine effects of *B. bassiana* applications on early-season CBB populations.

Applications by back-pack motorized mist blower:

- 1 oz. BotaniGard ES + 0.25 oz. Silwet per gallon

- Spray volume of ca. 30 gallon per acre.
Sampling protocol

Field:

- 25 trees per research block

- Record CBB infestation in clusters of 10 berries on opposite sides (N vs. S) of each tree (non-destructive)

- Collect 2 infested berries from each side of each tree
Laboratory:

- Dissect berries to identify *Beauveria*-killed CBB, collect fungal isolates, and determine extent of damage.

- Collect a subsample of live CBB (n = 30 to 50):
  - Surface sterilize
  - Transfer individually to small vials containing sterilized coffee berry
  - Hold beetles 8 – 10 days at room temperature
Sampling program designed to generate two primary statistics:

- Percent of pest population killed by *Beauveria* (one measure of disease prevalence)
  
  In early-season samples this measure of prevalence indicates the percent of CBB population that did not successfully reproduce.

- Percent of pest population actively infected with *Beauveria* but not yet killed by the pathogen (rough measure of weekly disease incidence)
Beauveria mycoses
Honaunau, 2014

Low elevation - one spray application

High elevation - treatments following suppression sprays

2014 - Low elevation - single spray - northwest block
- % Live CBB infected
- % Mortality due to mycosis
- % Mortality + infection

2014 - Low elevation - single spray - southwest block
- % infestation - northwest block
- % infestation - southwest block

2014 - high elevation - 3 weekly sprays followed by sprays at two-week intervals
- % Live CBB infected
- % Mortality due to mycosis
- % Mortality + infection

2014 - high elevation - 3 weekly sprays followed by monthly sprays
- % infestation - biweekly sprays
- % infestation - monthly sprays
Honaunau, 2015 – treatments following strip sanitation

Low elevation

High elevation

2015 - Low elevation - monthly sprays
- % Live CBB infected
- % Mortality due to mycosis
- % Mortality + infection

Applications

2015 - Low elevation - sprays as needed

Applications

2015 - High elevation - monthly sprays
- % Live CBB infected
- % Mortality due to mycosis
- % Mortality + infection

Applications

2015 - High elevation - sprays as needed

% infestation - monthly sprays

% infestation - sprays as needed
Disease incidence (active infection) post application

![Graph showing disease incidence over days post application for high and low elevation.](image)

- **High elevation**
- **Low elevation**
Caveat with regard to data presented in 2015

- We did not know what proportion of the observed infections were caused by commercial strain GHA vs. feral strains of B. bassiana
Genetic characterization of Hawaiian strains of *B. bassiana* isolated from CBB (collaboration with L. Castrillo and T. Matsumoto)

Characterization based on sequencing of intergenic region Bloc and microsatellite profiling (data of Louela Castrillo, Cornell Univ./USDA-ARS, Ithaca, NY).

(x) numbers in parenthesis indicate unique microsatellite profiles

● representatives of a widespread lineage (AFNEO_1) associated with CBB observed by Rehner et al. (2006)
B. Bassiana strain GHA is readily distinguished from the feral Hawaiian strains based on colony morphology on common mycological media.
Incidence of GHA vs. feral isolates pre and post spray

2015 - High elevation
Three monthly sprays monitored: mean cumulative mycosis = 56%

2015 - Low elevation
Three monthly sprays monitored: mean cumulative mycosis = 67%
Laboratory virulence bioassays

<table>
<thead>
<tr>
<th>Bb strain</th>
<th>Genetic type</th>
<th>Viable conidia/µg technical powder</th>
<th>No. of assays</th>
<th>Probit-regression slope ± SE</th>
<th>Log LC$_{50}$ ± SE (µg/ml)</th>
<th>LC$_{50}$ as viable conidia/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHA</td>
<td>Bloc F</td>
<td>1.103 x 10$^6$</td>
<td>7</td>
<td>1.39 ± 0.10 a</td>
<td>1.006 ± 0.092 a (10.1)</td>
<td>1.253 x 10$^6$ a</td>
</tr>
<tr>
<td>HI-15</td>
<td>Bloc A, MP 3</td>
<td>2.549 x 10$^6$</td>
<td>2</td>
<td>1.57 ± 0.37 a</td>
<td>0.835 ± 0.099 a (6.8)</td>
<td>1.768 x 10$^6$ a</td>
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<tr>
<td>HI-25</td>
<td>Bloc A, MP 5</td>
<td>2.380 x 10$^6$</td>
<td>2</td>
<td>1.82 ± 0.21 a</td>
<td>0.664 ± 0.224 a (4.6)</td>
<td>1.840 x 10$^6$ a</td>
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<tr>
<td>HI-63</td>
<td>Bloc A, MP 2</td>
<td>2.859 x 10$^6$</td>
<td>2</td>
<td>1.59 ± 0.22 a</td>
<td>0.803 ± 0.136 a (6.4)</td>
<td>1.943 x 10$^6$ a</td>
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<tr>
<td>HI-70</td>
<td>Bloc C, MP 1</td>
<td>3.121 x 10$^6$</td>
<td>2</td>
<td>1.23 ± 0.06 a</td>
<td>0.956 ± 0.163 a (9.0)</td>
<td>2.924 x 10$^6$ a</td>
</tr>
</tbody>
</table>
Conclusions

Findings indicate that observed control is primarily attributable to direct spray contact

- Immediate increase in disease incidence after application, followed by rapid decline

- Observed rates of incidence are significantly lower during periods of heavy CBB attack

- Increasing rates of incidence at points between monthly sprays have been observed only at high elevation under wet conditions

- Difficulty in protecting older coffee berries from CBB attack

- No significant impact on Xylosandrus compactus (black twig borer) populations

- Research on use of B. bassiana vs. other insect pests has shown that direct spray is the most efficient mode of inoculation
Recommendations

- Sanitation is critical to effective CBB management.

- *Beauveria* applications must begin at the beginning of the season (against CBB that have attacked the first significant flush of small, green berries)

- CBB are most vulnerable to *Beauveria* sprays when embedded in small berries (in the AB position). Application should therefore be held off until just after the primary wave of attack has occurred (information from prediction models?)

- Applications should be made late in the day, after the attacking beetles have settled.

- Ideally, *Beauveria* would be applied in this manner against CBB attacking each major flush of coffee berries (although monthly calendar sprays and sprays based on an action threshold of 20% infestation have proven reasonably effective at protecting the early harvests).

- *Beauveria* cannot be relied upon to control CBB attacking berries that have begun to mature. CBB rapidly penetrate the pericarp and enter the endosperm of these berries where they are protected from *Beauveria* sprays.
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