

Beauveria bassiana application strategies and effectiveness

Stephen Wraight¹, Lisa Keith², Sandra Galaini-Wraight³,
Louela Castrillo⁴, and Tracie Matsumoto²

¹USDA-ARS R. W. Holley Center for Agriculture and Health, Ithaca, NY

²USDA-ARS D. K. Inouye Pacific Basin Agricultural Research Center, Hilo, HI

³University of Hawaii at Manoa, CTAHR, Hilo, HI

⁴Cornell University, Department of Entomology, Ithaca, NY

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

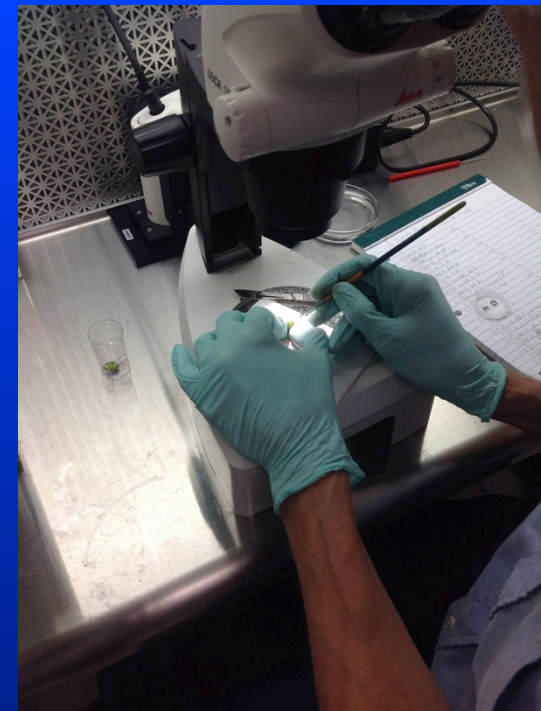
Field:

- 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.

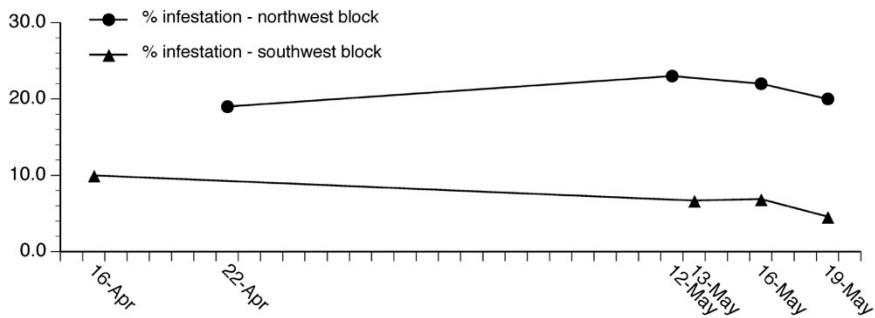
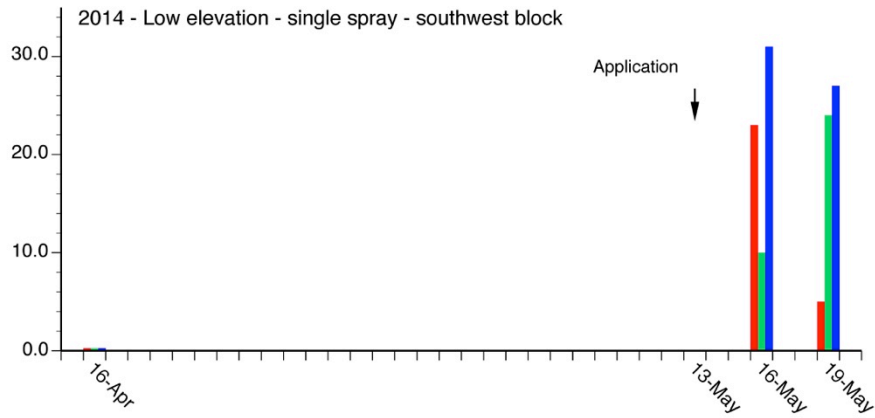
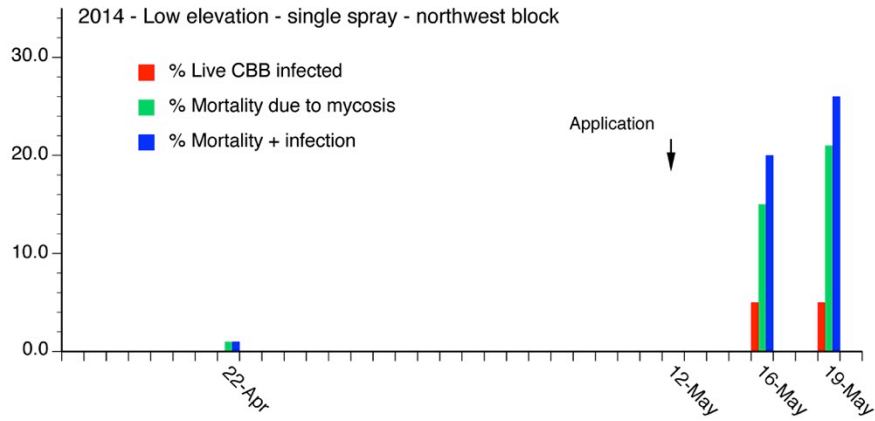
- Percent of pest population actively infected with *Beauveria* but not yet killed by the pathogen (rough measure of weekly disease incidence)



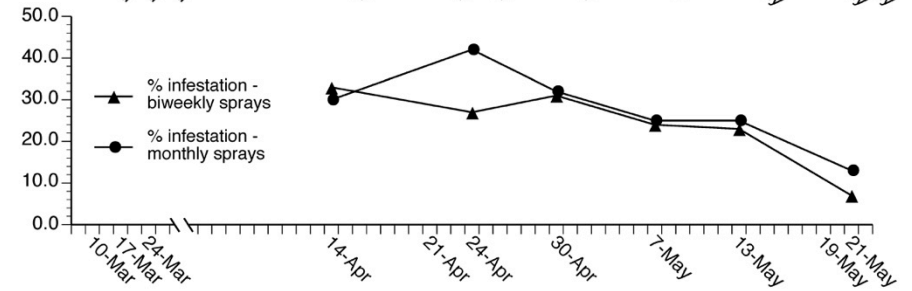
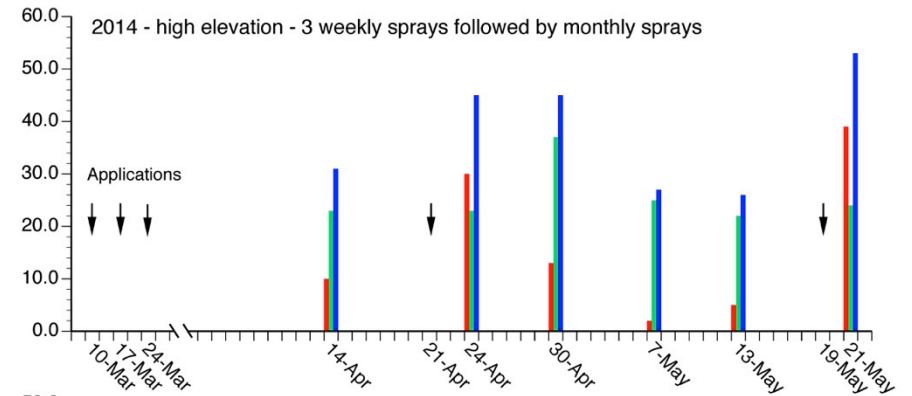
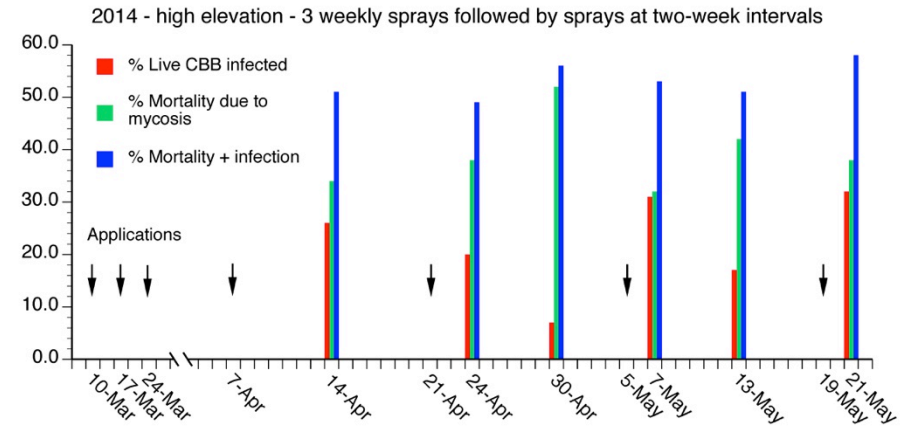
Beauveria mycoses

Honaunau, 2014

Low elevation - one spray application

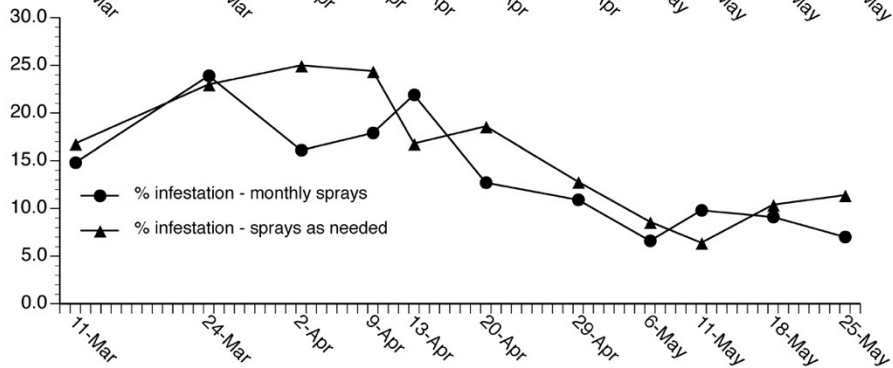
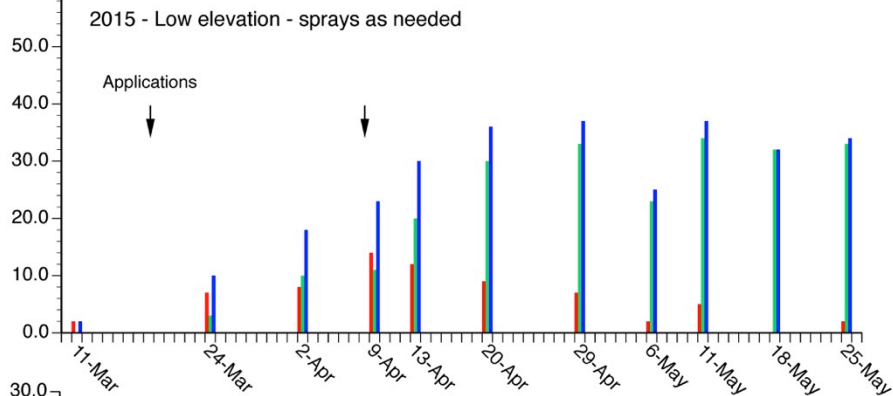
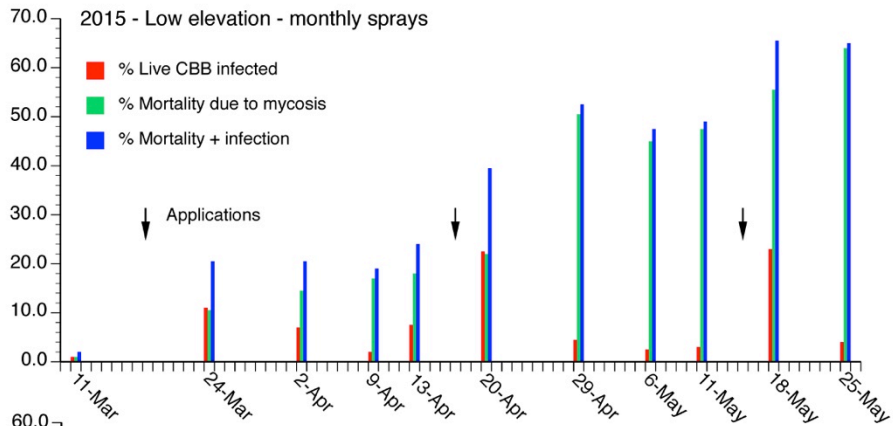


High elevation - treatments following suppression sprays

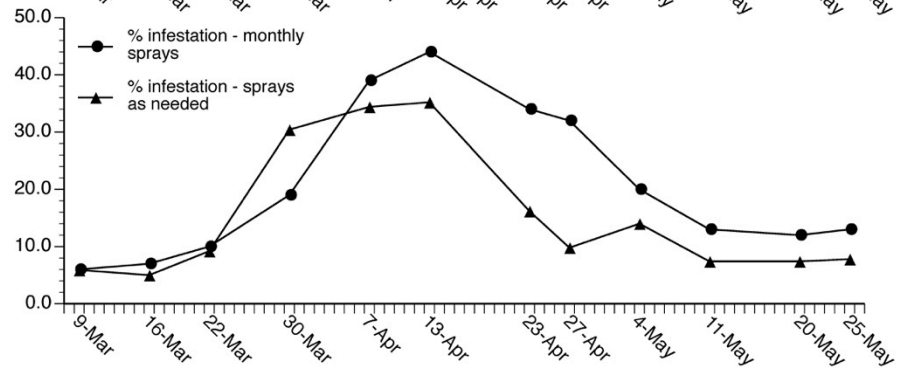
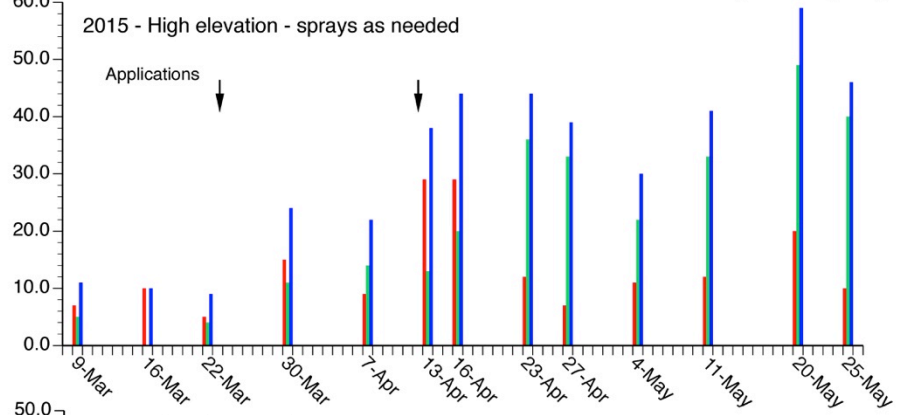
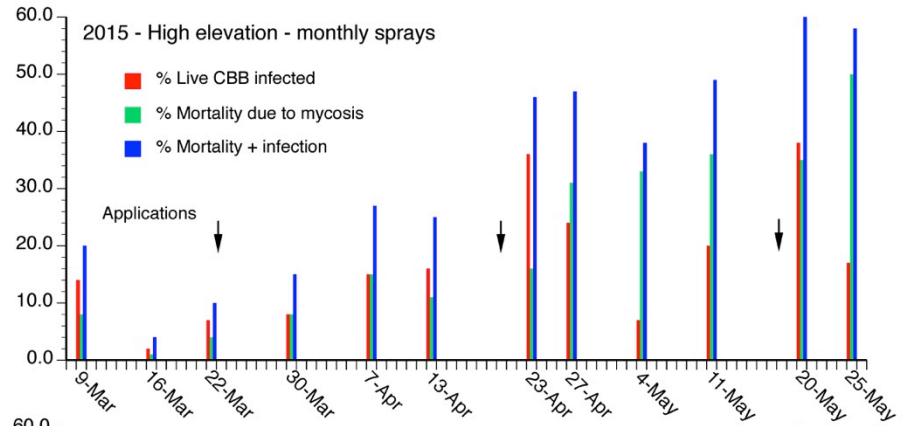


Honaunau, 2015 – treatments following strip sanitation

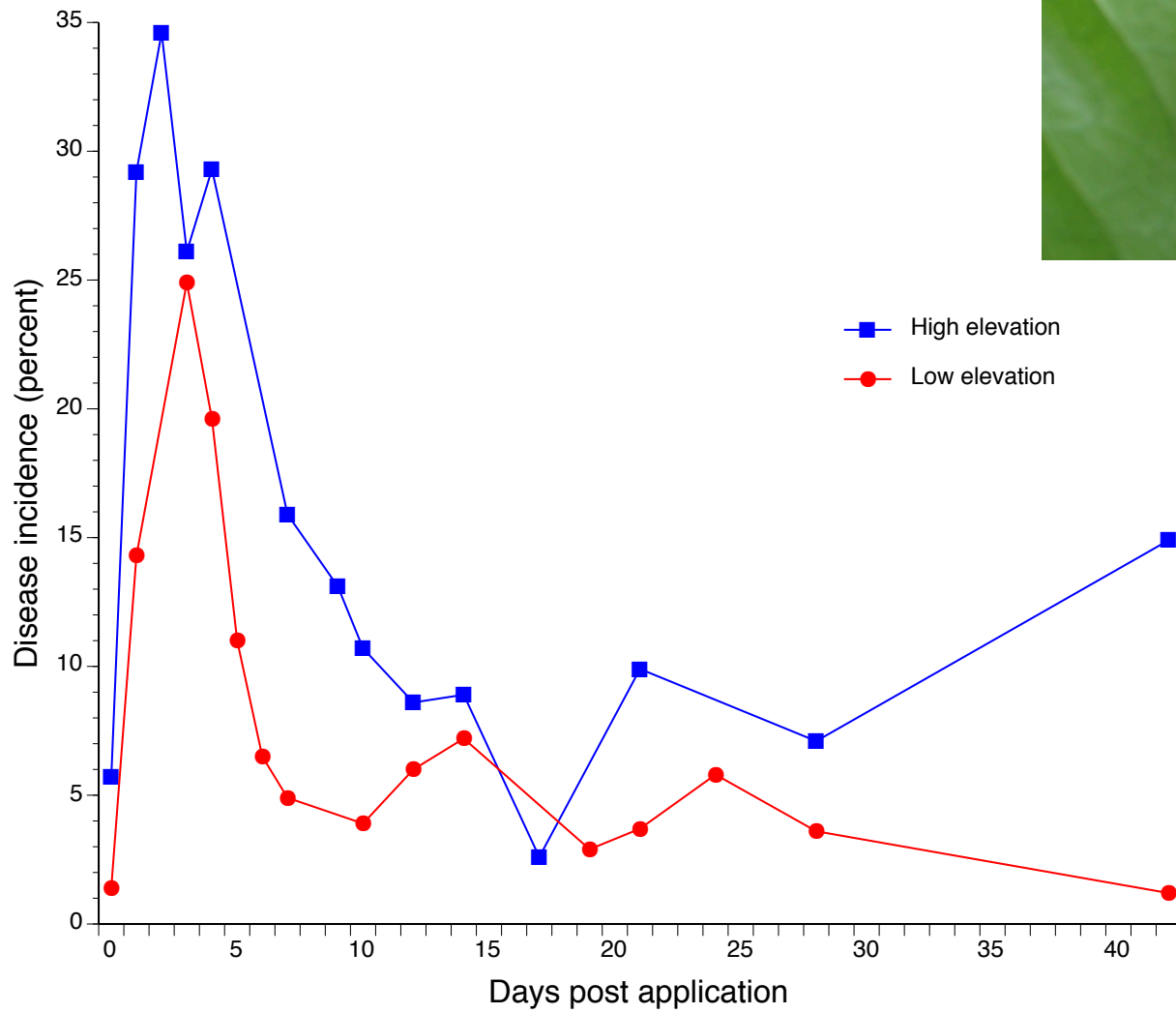
Low elevation



High elevation



Disease incidence (active infection) post application



Caveat with regard to data presented in 2015

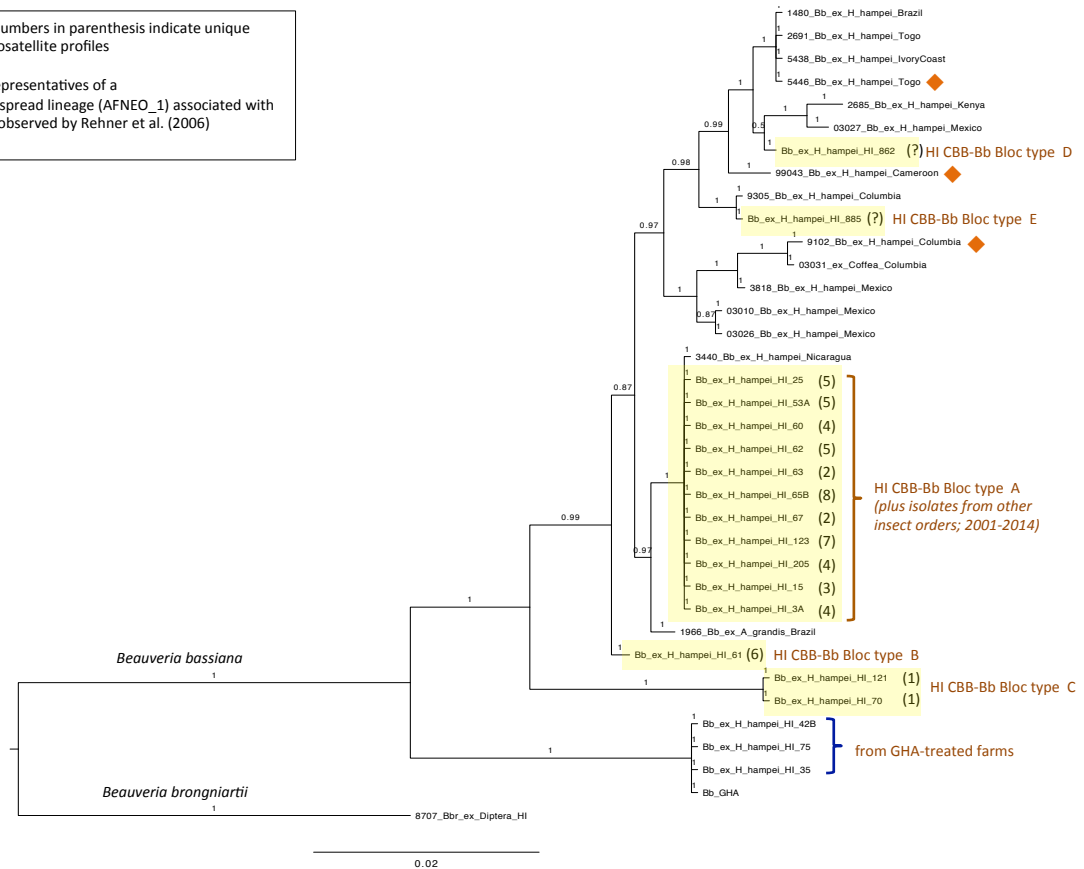
- We did not know what proportion of the observed infections were caused by commercial strain GHA vs. feral strains of *B. bassiana*

Genetic characterization of Hawaiian strains of *B. bassiana* isolated from CBB (collaboration with L. Castrillo and T. Matsumoto)

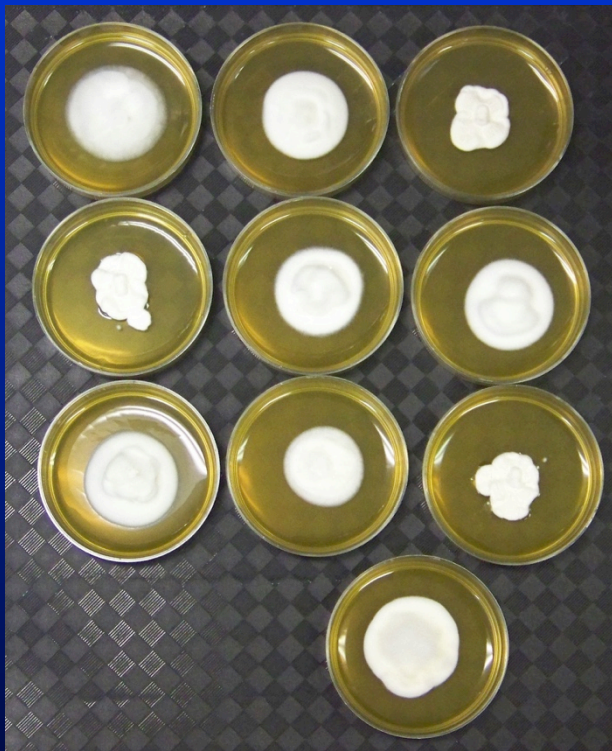


Characterization based on sequencing of intergenic region Bloc and microsatellite profiling (data of Louela Castrillo, Cornell Univ./ USDA-ARS, Ithaca, NY).

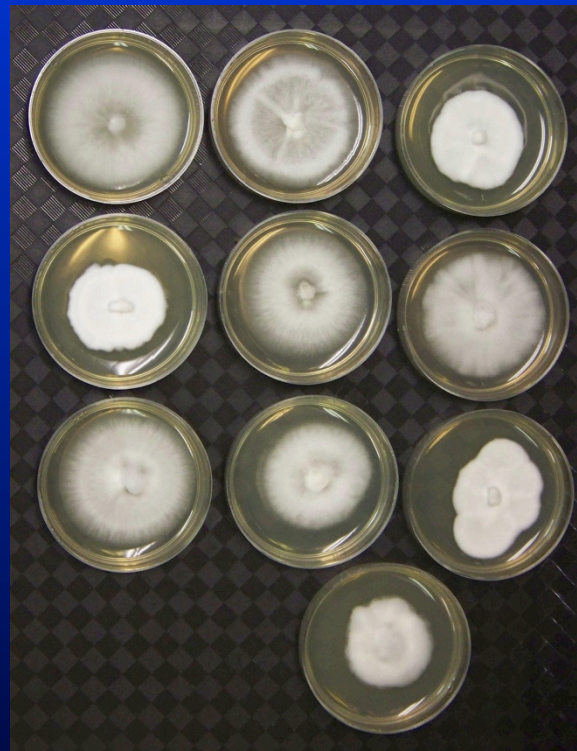
(x) numbers in parenthesis indicate unique microsatellite profiles
 ◆ representatives of a widespread lineage (AFNEO_1) associated with CBB observed by Rehner et al. (2006)



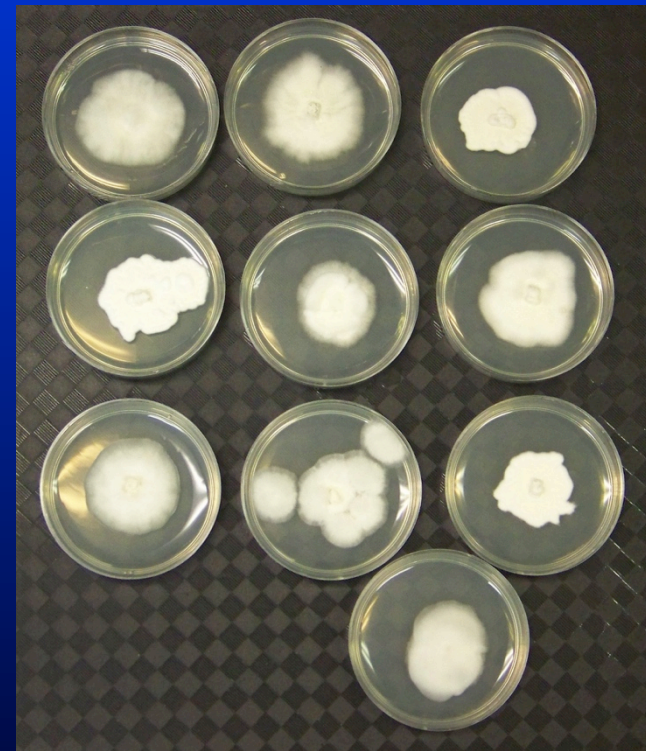
B. Bassiana strain GHA is readily distinguished from the feral Hawaiian strains based on colony morphology on common mycological media



SMA



SDA

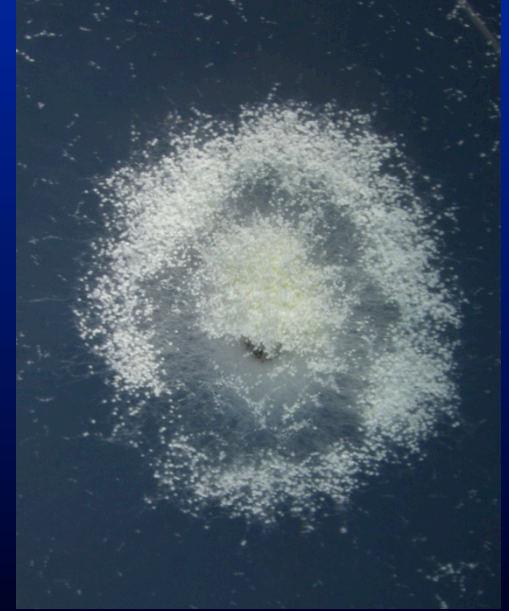


PDA

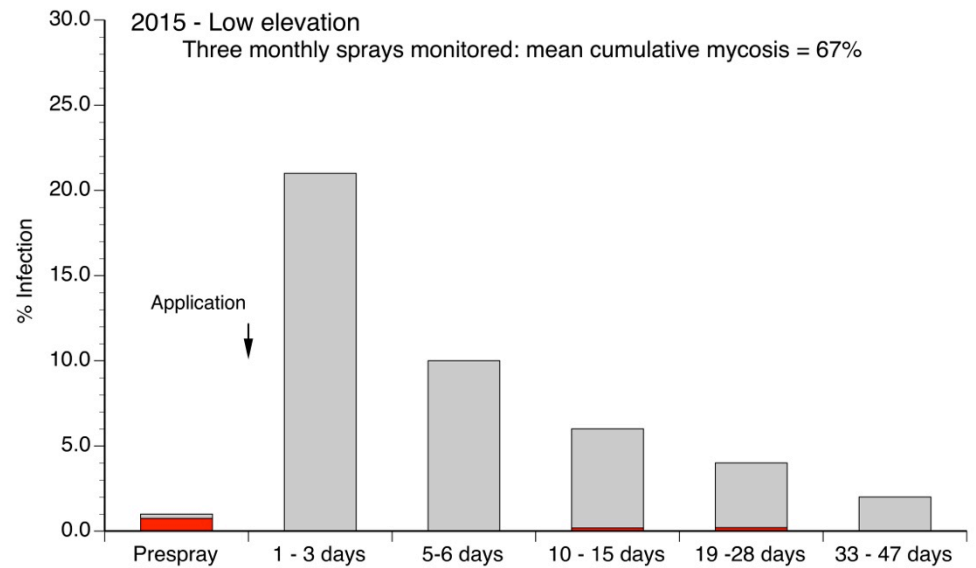
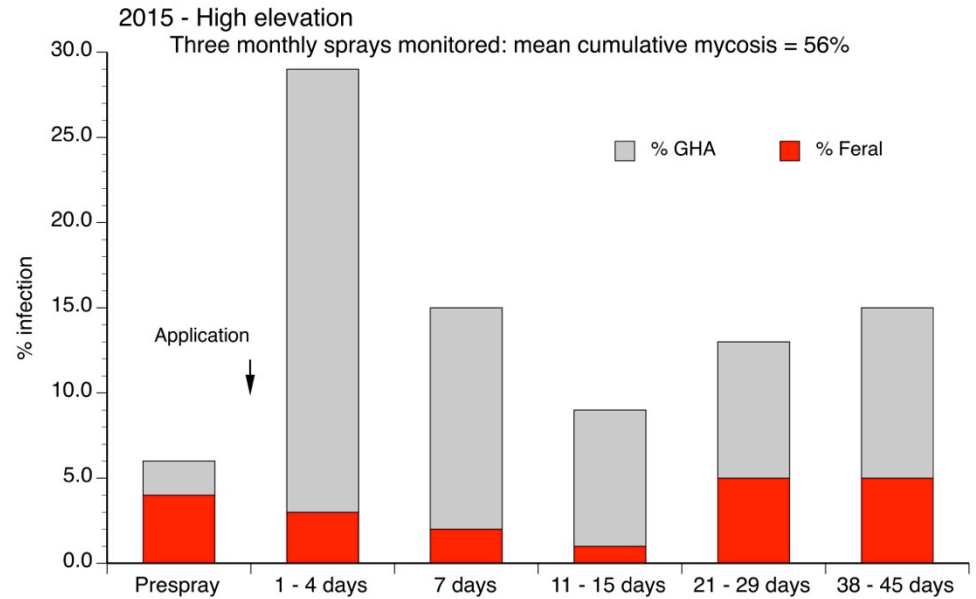
GHA



Feral



Incidence of GHA vs. feral isolates pre and post spray

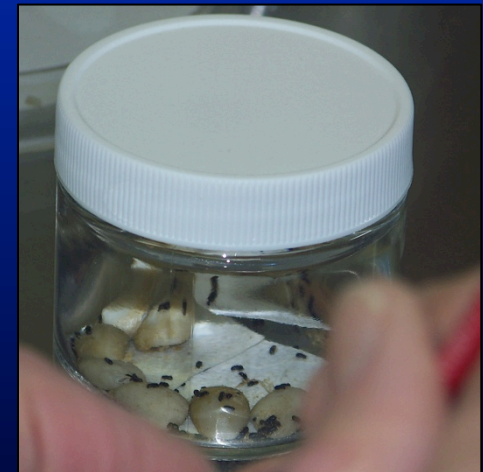


Laboratory virulence bioassays



Virulence of four Hawaiian CBB strains of *B. bassiana* compared to commercial strain GHA

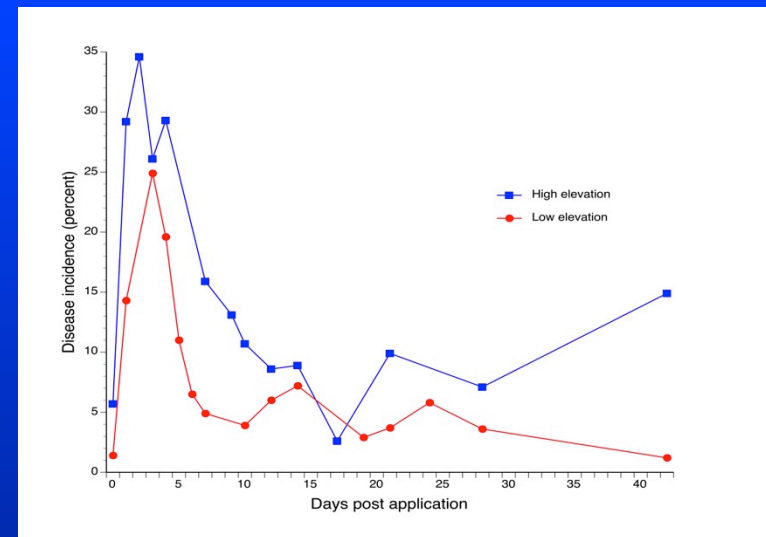
Bb strain	Genetic type	Viable conidia/ μg technical powder	No. of assays	Probit-regression slope \pm SE	Log $\text{LC}_{50} \pm$ SE ($\mu\text{g}/\text{ml}$)	LC_{50} as viable conidia/ml
GHA	Bloc F	1.103×10^6	7	1.39 ± 0.10 a	1.006 ± 0.092 a (10.1)	1.253×10^6 a
HI-15	Bloc A, MP 3	2.549×10^6	2	1.57 ± 0.37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



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.

Acknowledgments

Collaborators:

Kelvin Sewake - University of Hawaii at Manoa

Ray Carruthers - University of Hawaii at Manoa

Bob and Cea Smith - Smithfarms, Honaunau, HI

Charlie and Gwen Onaka – Charles T. Onaka Farm, Honaunau, HI

Technical Support:

Becky Howes

Nicholle Konanui

Dan Sullivan

Lionel Sugiyama

This presentation reports results of research only. Mention of a proprietary product does not constitute a recommendation or endorsement for its use by the U.S. Department of Agriculture.

