Project Title

Project No.: **PATH17.Vannette**

Project Leader: Rachel Vannette
Mailing Address: 43 Briggs Hall Davis, CA 95616
Phone: 530-752-3379
Email : rlvannette@ucdavis.edu

Project Cooperators and Personnel:
Florent Trouillas (UC Davis), Elina Nino (UC Davis), Amber Crowley-Gall (UC Davis), Robert Schaeffer (Utah State University)

A. Summary *(In laymen's terms – emphasize key findings and recommendations)*

Sustainable microbial biocontrol of brown rot blossom blight in almond

Flower-inhabiting microbes provide a natural, sustainable form of biocontrol for brown rot blossom blight (BRBB) while potentially minimizing costly non-target effects on almond pollinators and the services they provide, and potentially increasing attractiveness to pollinators. We aim to isolate and identify effective microbial antagonists that can reduce BRBB growth while maintaining pollinator health and attraction. To date, we have isolated and identified more than 300 candidate microbes. We have completed in-vitro screening assays for antagonistic effects towards *M. laxa* and identified 9 strains with excellent potential for BRBB reduction. Current work is examining microbial effects on bee health and attraction to flowers.

B. Objectives *(300 words max.)*

- Obj 1: Identify candidate BCAs for BRBB-Our first step was to isolate potential BCAs naturally present on almond flowers collected in several conventional and organic almond orchards throughout CA, and natural habitats.
    - We have successfully isolated and identified over 300 potential antagonist strains from both orchard and natural flowering materials including filamentous fungi, yeasts and bacteria
- Obj 2: Test isolates for efficacy in suppressing *Monilinia laxa* growth in culture.
    - Our in-vitro screening assays have been completed on 59 strains and revealed at least 10 good candidates for microbial antagonists that suppress *M. laxa* in culture
- Obj 3: Evaluate effects of BCAs on floral attractiveness and HB pollination-
    - Candidate agents are being screened for impacts on floral attractiveness to HBs using a laboratory behavioral assay, as well as their impacts on pollination quality through field testing.

C. Annual Results and Discussion *(This is the core function of this report)*

Identification of candidate BCAs for BRBB: Isolation work from almond flowers resulted in a collection of approximately 250 microorganisms. Thirty-eight isolates, from *Aureobasidium* sp., *Bacillus* sp., *Penicillium* sp. and *Epicoccum nigrium*, were selected from these almond isolates to be tested for antagonistic activity again *M. laxa*. Twenty-

one bacterial and fungal isolates were selected from the microorganisms isolated from natural flower populations (**Fig 4, 5**). These isolates include species associated with bee hosts suggesting they will be safe for use with honey bees as well as species associated with flower stigmas, the location of initial *Monilinia* colonization. Out of the 59 isolates tested, 26 produced inhibition zones. K661, K781 and Ba95 (**Fig 2A, 6, 7**) produced the largest inhibition zones which averaged 1 cm. Several isolates (A33, A17, EC_084, K768, K769 and MAV_P_B1) did not produce inhibition halos; however, they showed some growth inhibition on *M. laxa* which suggests that these isolates may act as substrate competitors of *M. laxa* in the absence of antimicrobial production (**Fig 2B, 5, 8, 9**).

Evaluate effects of BCAs on floral attractiveness and HB pollination: Ten isolates were selected as candidate BCAs to test for their effects on HB attraction. We first validated the CAFÉ assay by providing honey bees with the choice of 45% or 5% sucrose solutions (**Fig 10**) and a preference was observed for the higher sugar concentration. We have begun utilizing this method to test potential natural occurring microbial antagonists for brown rot blossom blight (BRBB), caused by *Monilinia laxa*. To date we have completed trials for *Pseudomonas veronii* (MAV_P_B1, **Fig 11a**) and *Aureobasidium pullulans* (A17, **Fig 11b**) and begun testing another *A. pullulans* (A33, **Fig 12a**) strain and *Bacillus subtilis* (Ba95, **Fig 12b**). Based on currently results we have found that some microbes have exhibit no effect on honey bee feeding (**Fig 11a, 12b**), while others appear to have a negative effect on food consumption. Overall, these preliminary results reveal that CAFÉ assay provides a useful system to examine the effects of potential pest management practices on pollinators.

Current Directions

The CAFÉ assay will be continued to complete the laboratory behavior trials assessing the effects of the remaining candidate BCAs on HB food consumption. The selected BCAs will also be tested for their effects on HB attraction and orientation preference when foraging using an artificial flower assay (**Fig 13**). Additionally selected BCAs will be used for our remaining objective: *Determine the safety of BCAs on HB brood and adults*. Safety of prospective BCAs will be tested *in vitro* using contact toxicity trials on adults and by adding potential BCAs to the larval diet during larval rearing trials.

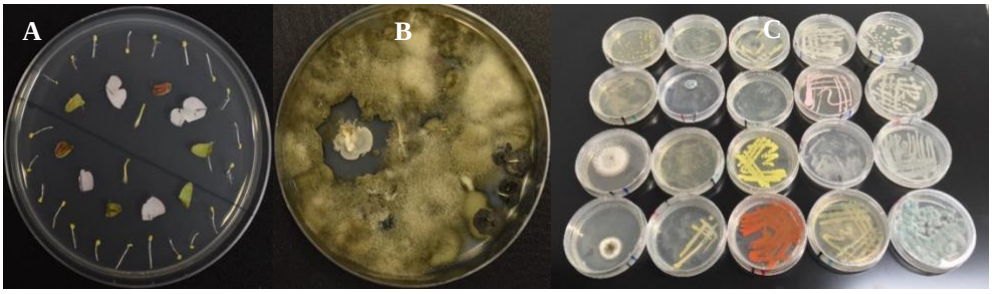

Figure 1. A: Dissected almond flowers on PDA medium. B: Growing and isolation of candidate microorganisms for biological control. C: Bacteria and fungi isolated from flowers and floral nectar.

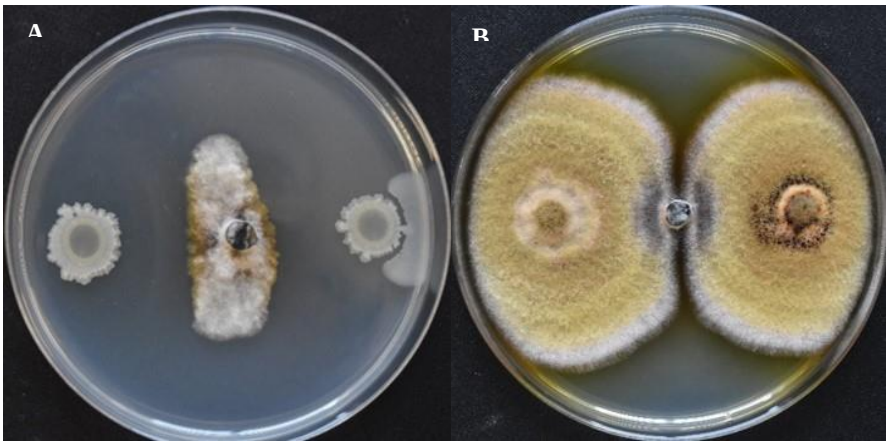

Figure 2: Dual culture technique with growth inhibtion halo (A) and overall growth inhibition (B). *M. laxa* [KARE1135, center] and microbial isolates [sides: *Bacillus subtilis* (Ba95, A) and *Epicoccum nigrum* (KARE768, B)].

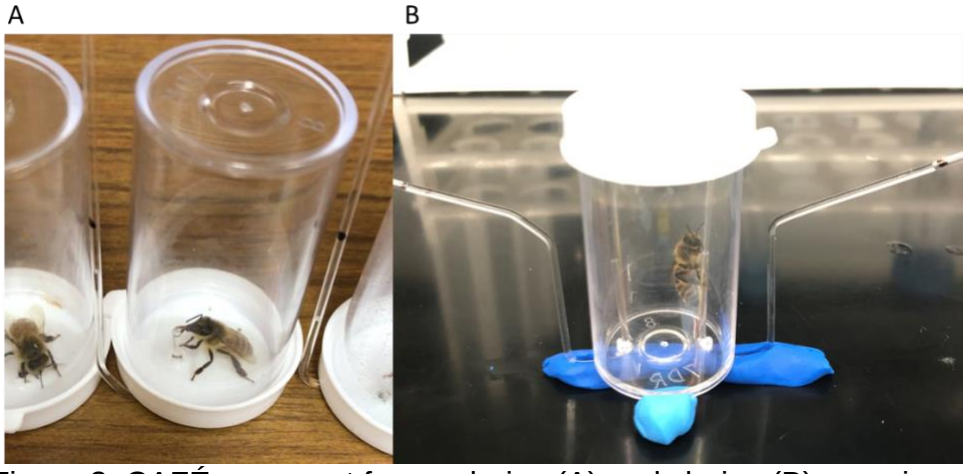

Figure 3. CAFÉ assay set for no choice (A) and choice (B) experiments.

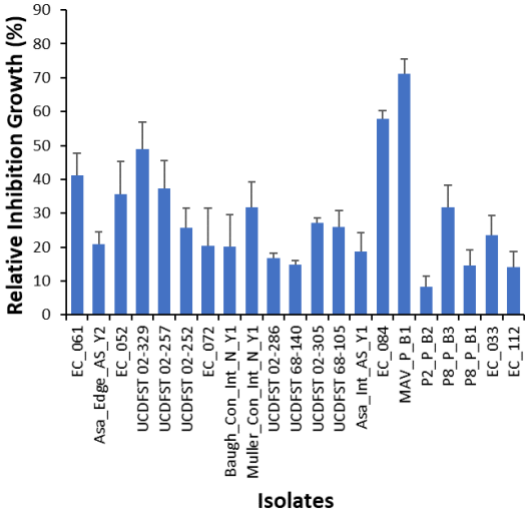

Figure 4. Percentage of relative growth inhibition of natural flower isolates against *M. laxa*.

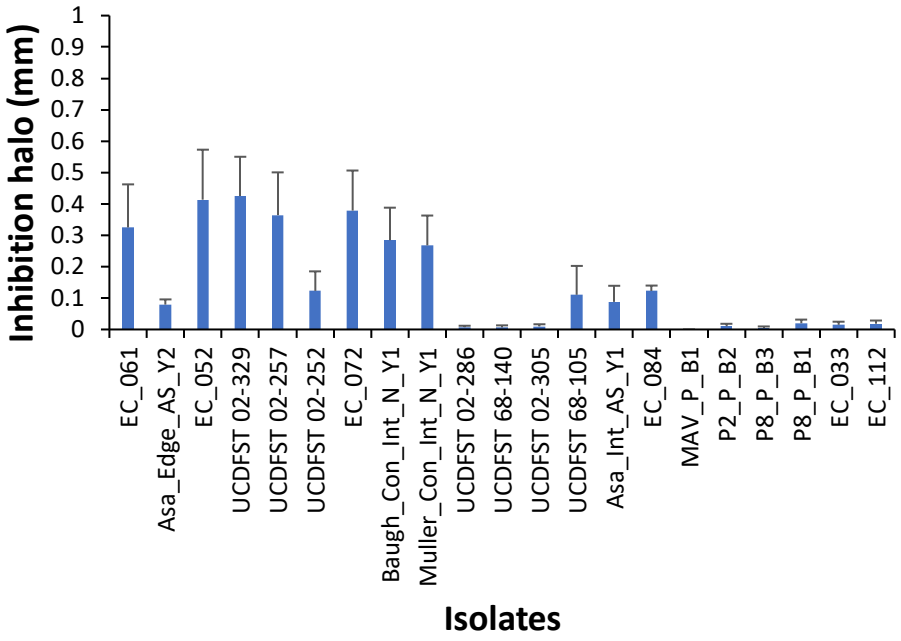

Figure 5. Growth inhibition halo measurements of natural flower isolates against *M. laxa*.

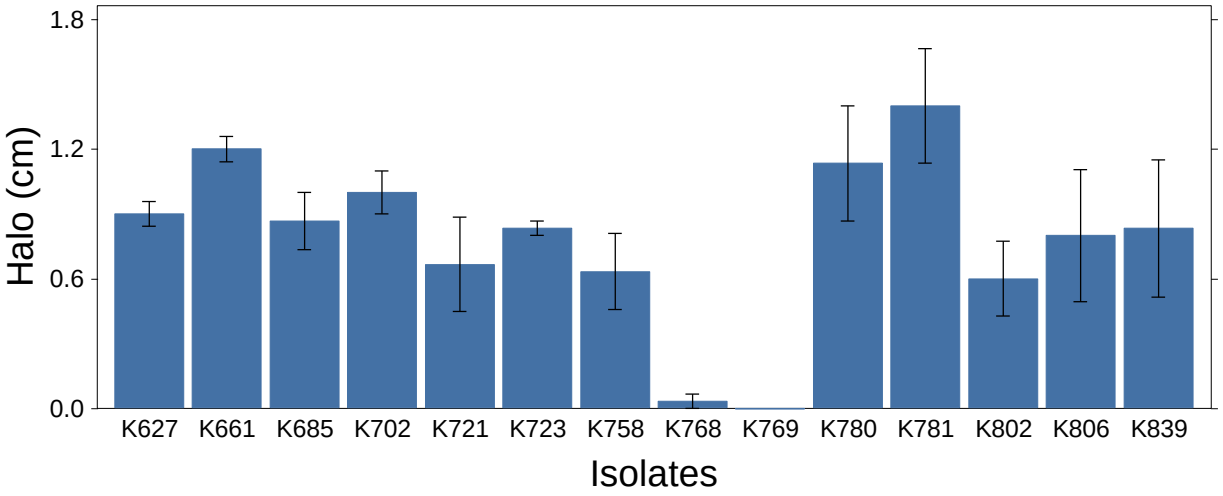

Figure 6: Growth inhibition halo measurements of *Epicoccum nigrum* isolates against *M. laxa*.

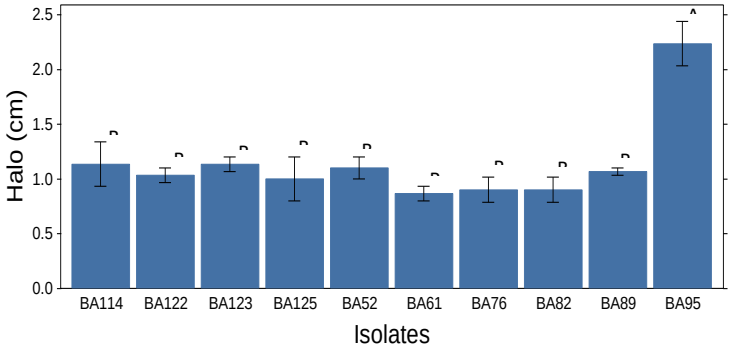

Figure 7. Growth inhibition halo measurements of *Bacillus* sp. isolates against *M. laxa*.

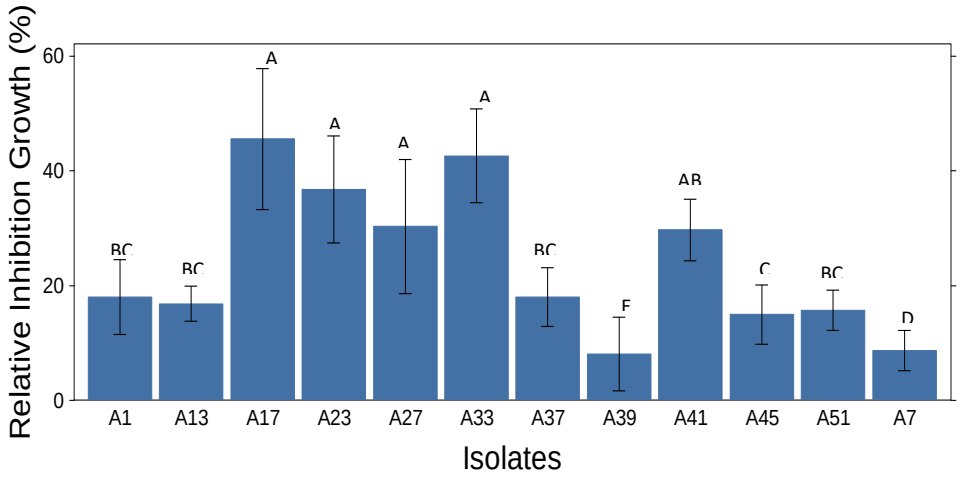

Figure 8. Percentage of relative growth inhibition of *Aureobasidium pullulans* isolates against *M. laxa*.

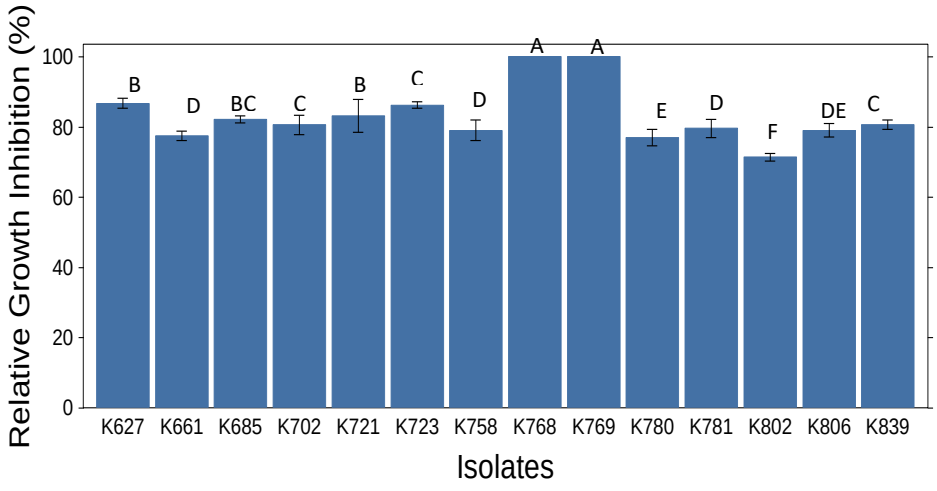

Figure 9. Percentage of relative growth inhibtion of *Epicoccum nigrum* isolates against *M. laxa*.

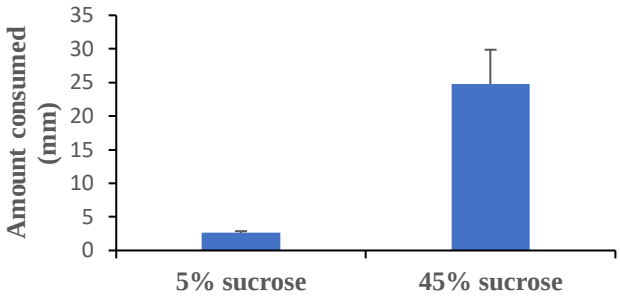

Figure 10. Validation of CAFÉ assay using a choice test between 5% and 45% sucrose solutions (n=8).

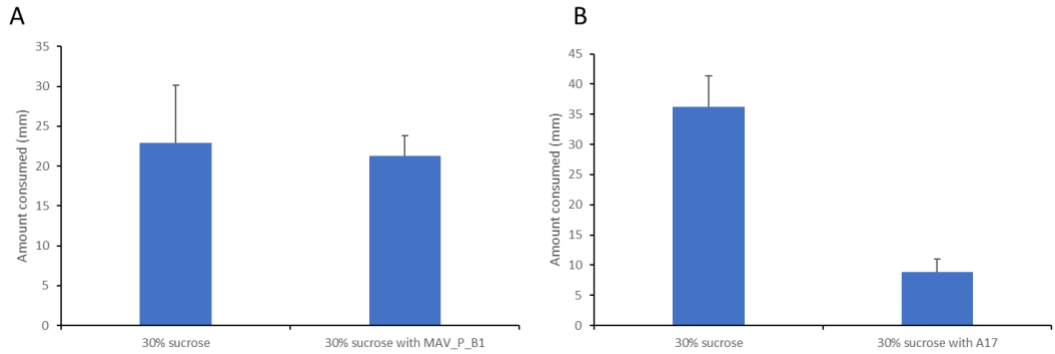

Figure 11. Complete CAFÉ assay trials. Choice test between 30% sucrose control and 30% sucrose inoculated with microbial antagonist: A- *Psedomonas veronii* (MAV_P_B1, n=10); B- *Aureobasidium pullulans* (A17, n=12).

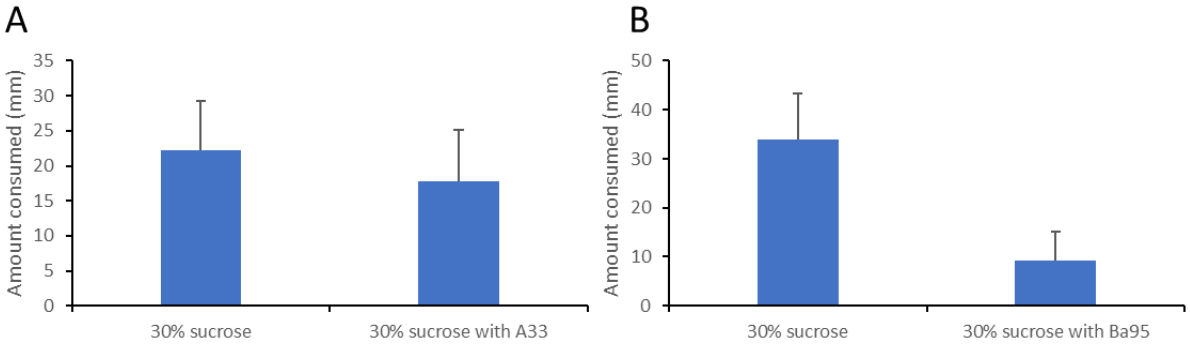

Figure 12. Partial CAFÉ assay trials. Choice test between 30% sucrose control and 30% aucrose inoculated with microbial antagonist: A- *Aureobasidium pullulans* (A33, n =5), B- *Bacillus subtilis* (Ba95, n =3)

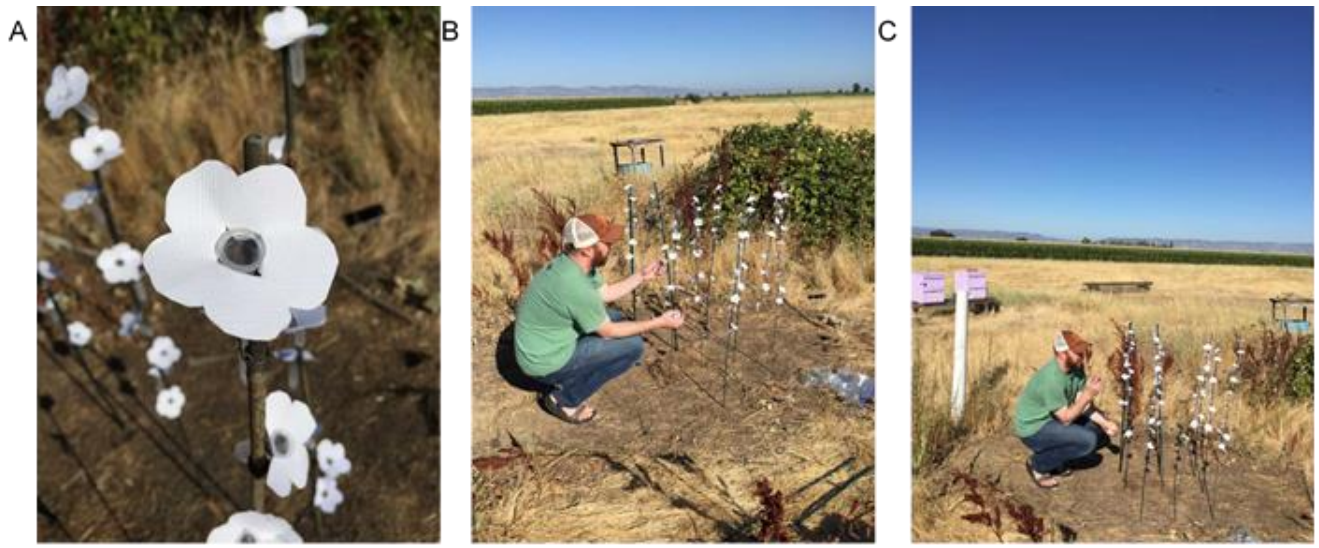

Figure 13. HB orientation preference assay utilizing artificial flowers. A: Artificial flower containing synthetic nectar. B: “Branches” of artificial flowers. C: Set up of branches across from hives (purple, left side of picture).

D. Outreach Activities

1. None to date

E. Materials and Methods *(500 word max.)***:**

Identification of candidate BCAs for BRBB: Almond flowers were collected from both conventional and organic almond orchards in different counties in California. Additional candidate microorganisms were collected from natural flower populations in Yolo and Solano counties in California (**Fig 1**). Purified isolates were identified by the amplification of a conserved DNA or RNA region by polymerase chain reaction. Amplicons were sequenced and compared to sequence already available in Gen Bank. Antagonistic activity of microorganisms was tested using the dual culture technique in Petri dishes filled with potato dextrose agar (PDA) medium (**Fig 2**). Mycelial plugs (5mm diameter) of the pathogen and putative fungal antagonists were placed on the same Petri dish 3 cm from each other. Paired cultures were incubated at 25$_{o}$C for up to 14 days. Plates inoculated with only the pathogen served as controls. The inhibition halo was measures and the percent of relative growth inhibition was calculated.

Evaluate effects of BCAs on floral attractiveness and HB pollination: The capillary feeder (CAFÉ) assay provides a means to examine the effect of potential pathogen antagonist on HB. The no choice CAFÉ assay (**Fig 3a**) measures the amount of food an individual bee will consume and a choice assay (**Fig 3b**) can be utilized to determine if potential blossom treatments will impact bee feeding, and provide an indicator of flower visitation and potential pollination success. HB foragers are collected and starved overnight before being placed in a vial containing two capillary tubes containing either a control 30% sucrose solution or a 30% sucrose solution that has been inoculated with a candidate BCA. HB are allowed to feed during a 3 hr time period with amount of food consumed measured and solutions refilled at half hour intervals.

F. Publications that emerged from this work

1. No publications to date on this work.