Sustainable microbial biocontrol of brown rot blossom blight in almond

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Abstract

Almonds face pressure from an array of damaging bloom diseases such as brown rot blossom blight (BRBB; caused by *Monilinia laxa*), and currently growers primarily rely on the use of fungicides for their control. Flower inhabiting microbes may represent a sustainable alternative for biocontrol of BRBB, with the potential to limit *M. laxa* establishment during bloom. Over 250 microbial isolates were identified from almond and natural flower populations. 59 isolates were tested for antagonist activity against *M. laxa* in culture. Ten microbial isolates were selected as potential biocontrol agents (BCA) to be tested for their affects on honey bee health and pollination services.

Background

BRBB is one of the most economically important fungal diseases of almond in California. Infection by the pathogen occurs at bloom and current management depends strongly on chemical fungicides. Fungicide treatments can lead to rapid resistance by *M. laxa*, as well as negative effects on almond fruit set and non-target organisms, such as honey bees, which are vital for almond pollination. Honey bees have the potential to serve as vectors for the delivery of BCAs potentially reducing the number of conventional fungicide applications. Effective screening of microbial agents and assurance that they do not impose additional costs on honey bee health or the pollination services they provide are necessary for creating sustainable biocontrol systems.

Figure 1. Symptoms of BRBB in inoculated almond flowers

Objective

<u>Identify candidate biocontrol agents for brown rot blossom blight:</u>

1. Isolate potential biocontrol agents naturally present in almond, conventional and organic orchards, and natural flower populations collected throughout California.
2. Test for their efficacy in suppressing *M. laxa* growth in culture.

Methods

Microbial Identification

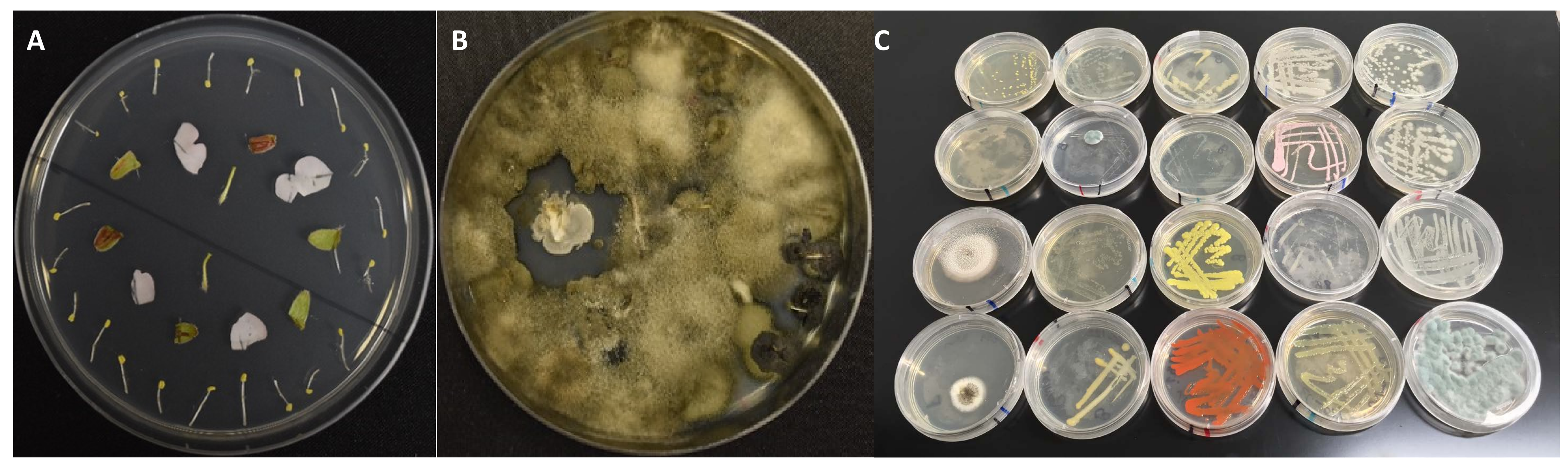

Figure 2. A: Dissected almond flowers on PDA medium. B: Growing and isolation of candidate microorganisms for biological control. C: Bacteria and fungi isolated from flowers and floral nectar.

Antagonistic Activity

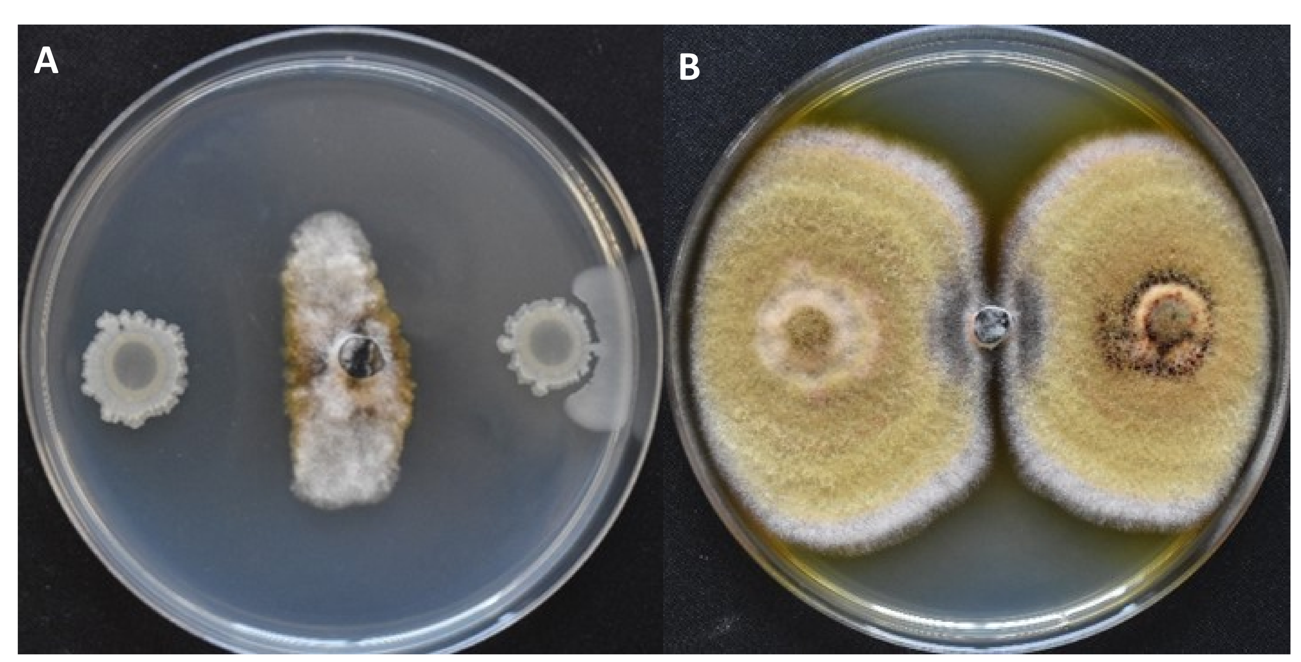

Figure 3. Dual culture technique with growth inhibition halo (A) and overall growth inhibition (B). *M. laxa* [KARE1135, center] and microbial isolates [sides:*Bacillus subtilis* (**Ba95**, A) and *Epicoccum nigrum* (**KARE768**, B)].

Results

Microbial Identification:

- Over 250 microorganisms identified
- 59 microbial isolates were selected to test for antagonistic activity:
  - 38 almond isolate: 14 *Epicoccum nigrum*, 12 *Aureobasidium* sp., 10 *Bacillus* sp. and 2 *Penicillium* sp.
  - 21 natural flower isolates:: 6 bacterial isolates (genera: *Acinetobacter*, *Neokomagataea* and *Pseudomonas*) and 15 fungal isolates (genera: *Metschnikowia*, *Cryptococcus*, *Hanseniaspora*, *Candida*, *Lachancea*, *Meyerozyma*, *Starmerella*, *Sygosaccharomyces*, and *Penicillium*.

Results continued

Antagonistic Activity:

- 26 of the 59 isolates tested produced inhibition zones: Largest produced by *Epicoccum nigrum* (K661, K781) & *Bacillus subtilis* (**Ba95)**
- Several isolates not producing inhibition halos exhibited some growth inhibition on *M. laxa*
  - Almond isolates: *Aureobasidium pullans* (A33, A17) & *Epicoccum nigrum* (**K768**, K769)
  - Natural flower isolates: *Penicillium chloroleucon* (EC_084) & *Pseudomonas veronii* (MAV_P_B1)

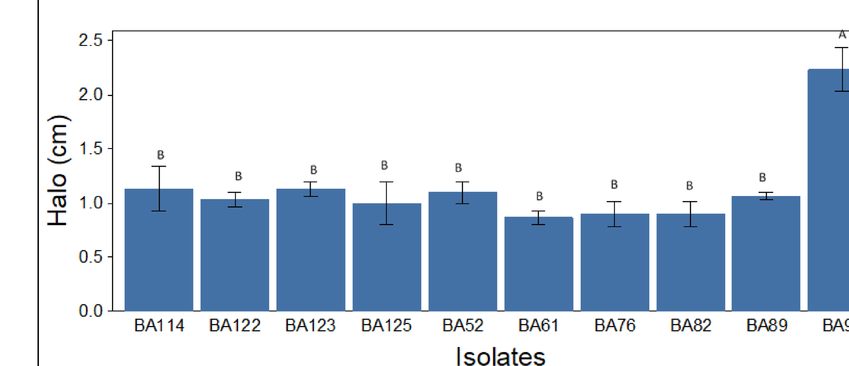

Figure 4. Growth inhibition halo measurements of *Bacillus* sp. isolates against *M. laxa*.

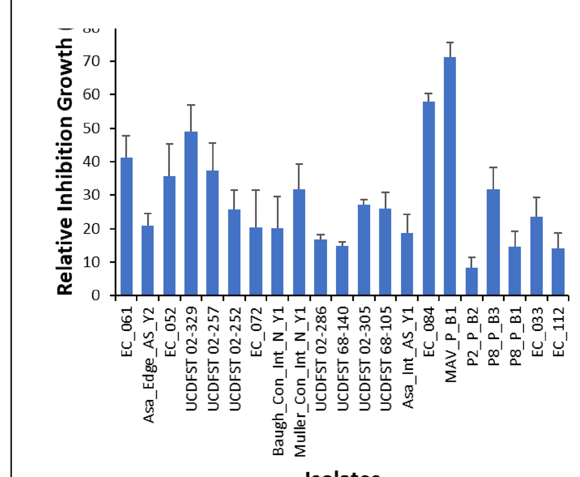

Figure 5. Relative inhibition growth percentage of natural flower isolates against *M. laxa*.

Conclusions

- Microbial isolates producing inhibition zones may be the result of antimicrobial production.
- Isolates that reduced *M. laxa* growth without inhibition zones may act as substrate competitors in the absence of antimicrobial production.
- Ten isolates were selected as candidate biocontrol agents and will be used for our remaining objectives:
  - <u>Determine the safety of biocontrol agents on honey bee brood and adults</u>
  - <u>Evaluate effects of biocontrol agents on floral attractiveness and honey bee pollination</u>

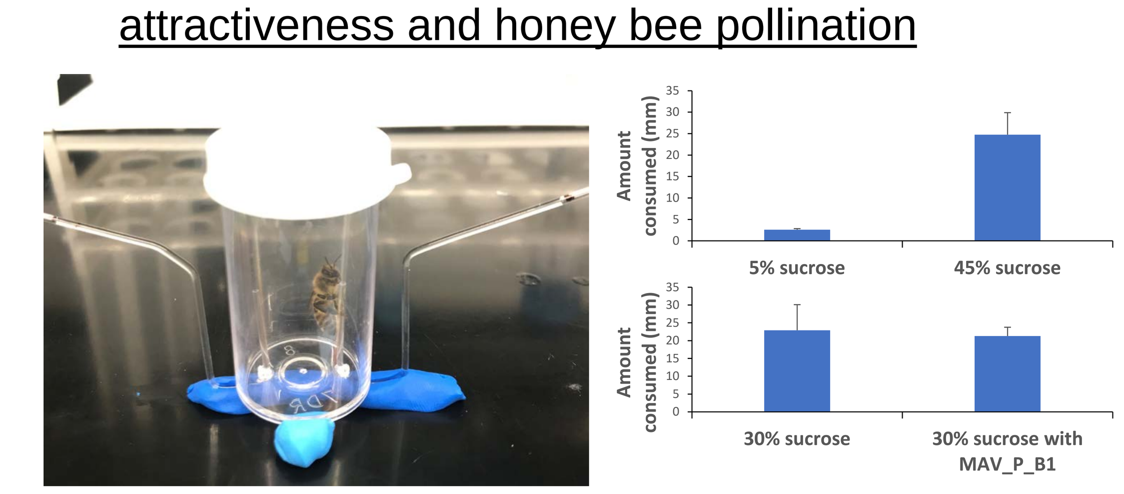

Figure 6. Preliminary results from CAFÉ assay examining microbial effects on honey bee food consumption

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