

2016-2021: CRIS 2030-42000-039-00-D

Biocontrol Interventions for High-Value Ag Commodities

Sub-objective:

Natural Product Research for Mycotoxin & Fungal Control

Jong Heon Kim

Foodborne Toxin Detection & Prevention Research
Western Regional Research Center, USDA-ARS
Albany, CA 94710



STRATEGY

- **REDOX-ACTIVE** small molecules from molecular screening
- **BENZALDEHYDE ANALOGS**
 1. Antioxidant & prooxidant characteristics
 2. Disrupting cellular components susceptible to oxidative stress
 3. Aflatoxin control by targeting mycotoxin machinery
 4. Pathogen control by resistance management

RESULTS

Three benzo analogs for industry application

Producing
BA-1

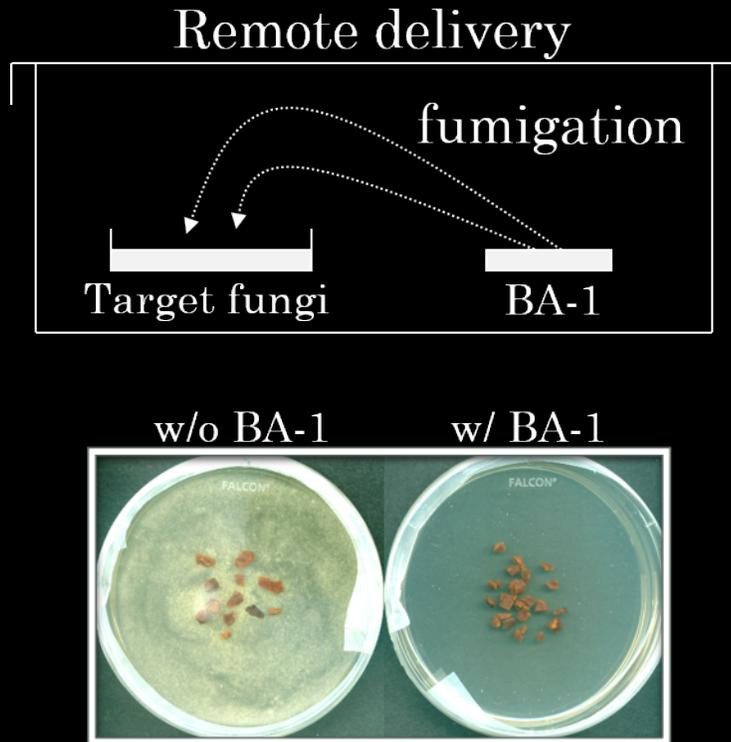
Processing
BA-2

Products
BA-3

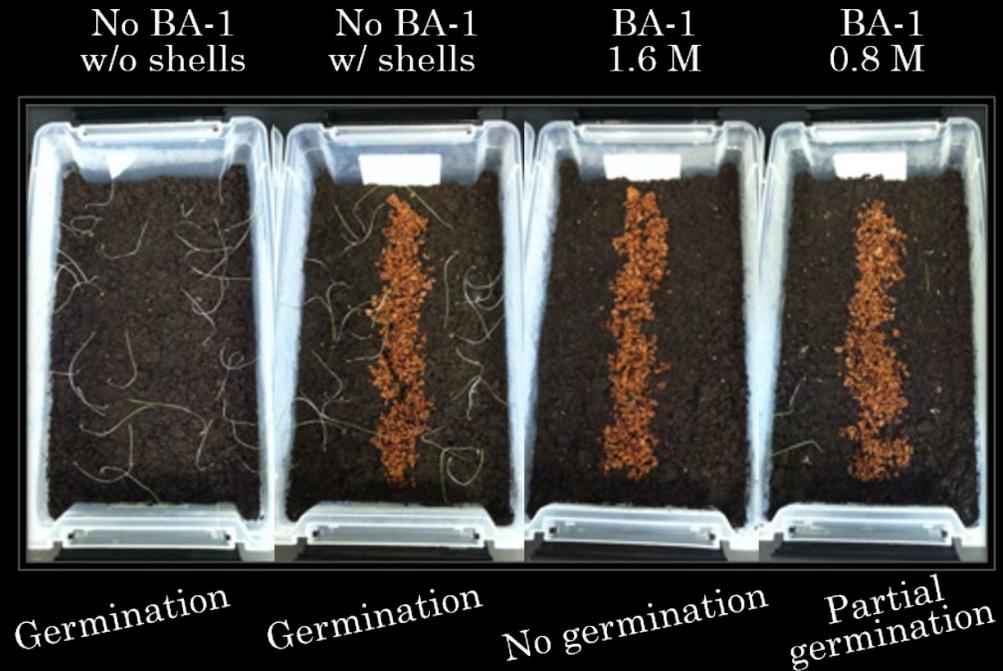
- **BA-1**: Fumigant compound; Possesses both fungicidal & herbicidal activities
- **BA-2**: Heat sensitizing activity; Enhances the efficacy of thermal sanitation processes
- **BA-3**: Rapid elimination (> 99.9% fungal death) of fungal contaminants from food matrices; Heat-independent

BA-1: New fumigant; Simultaneous elimination of both aflatoxigenic *Aspergillus flavus* & weeds (*Lagurus ovatus*)

(A) Fungicidal activity



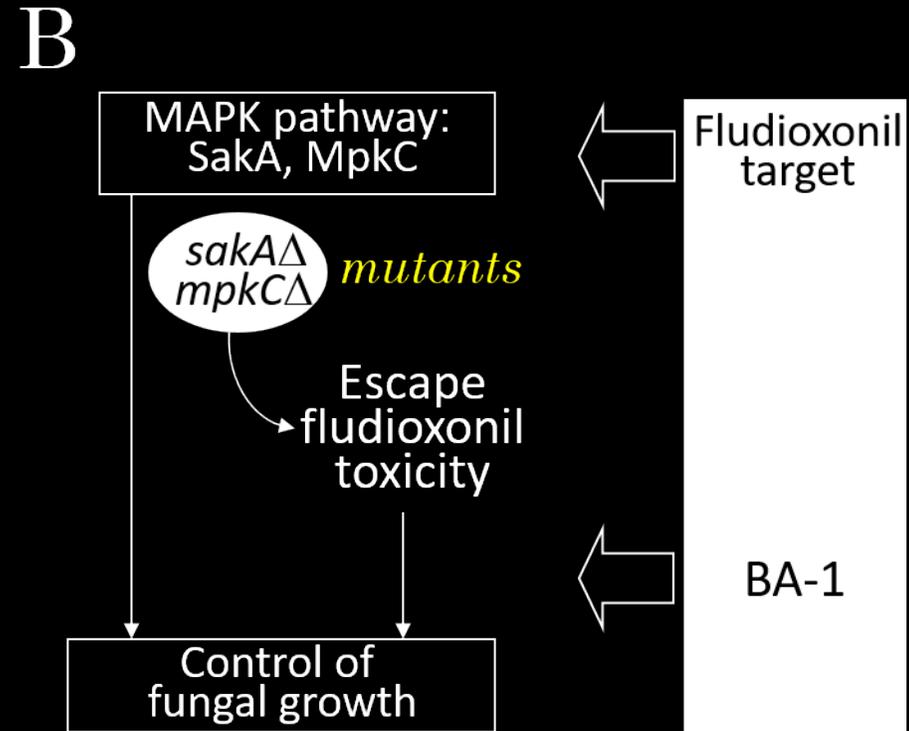
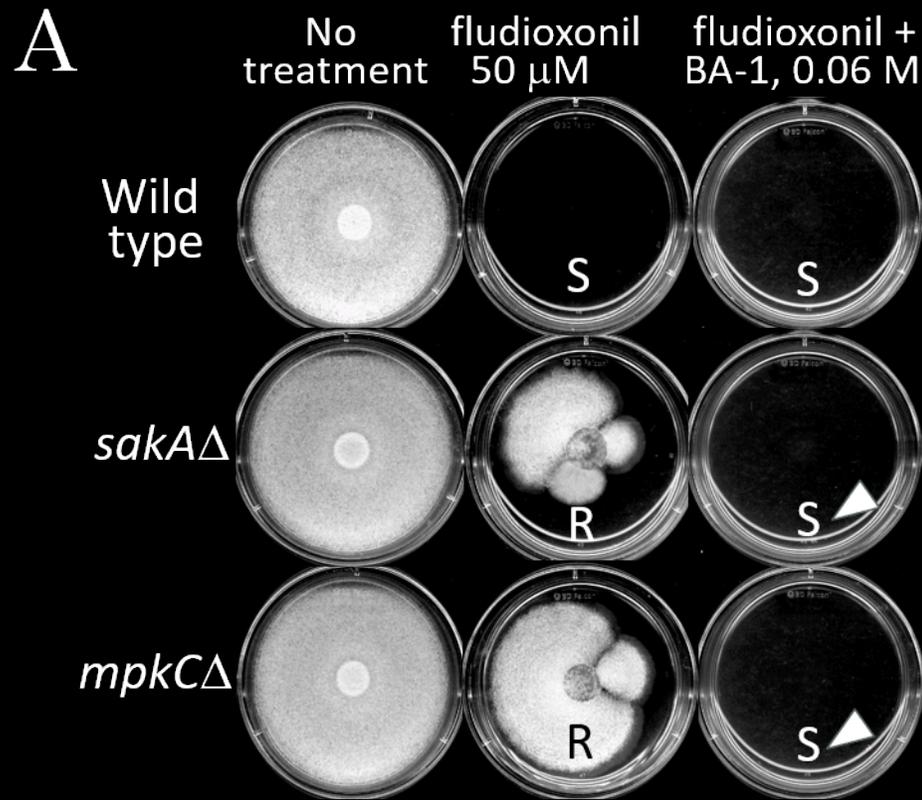
(B) Herbicidal activity



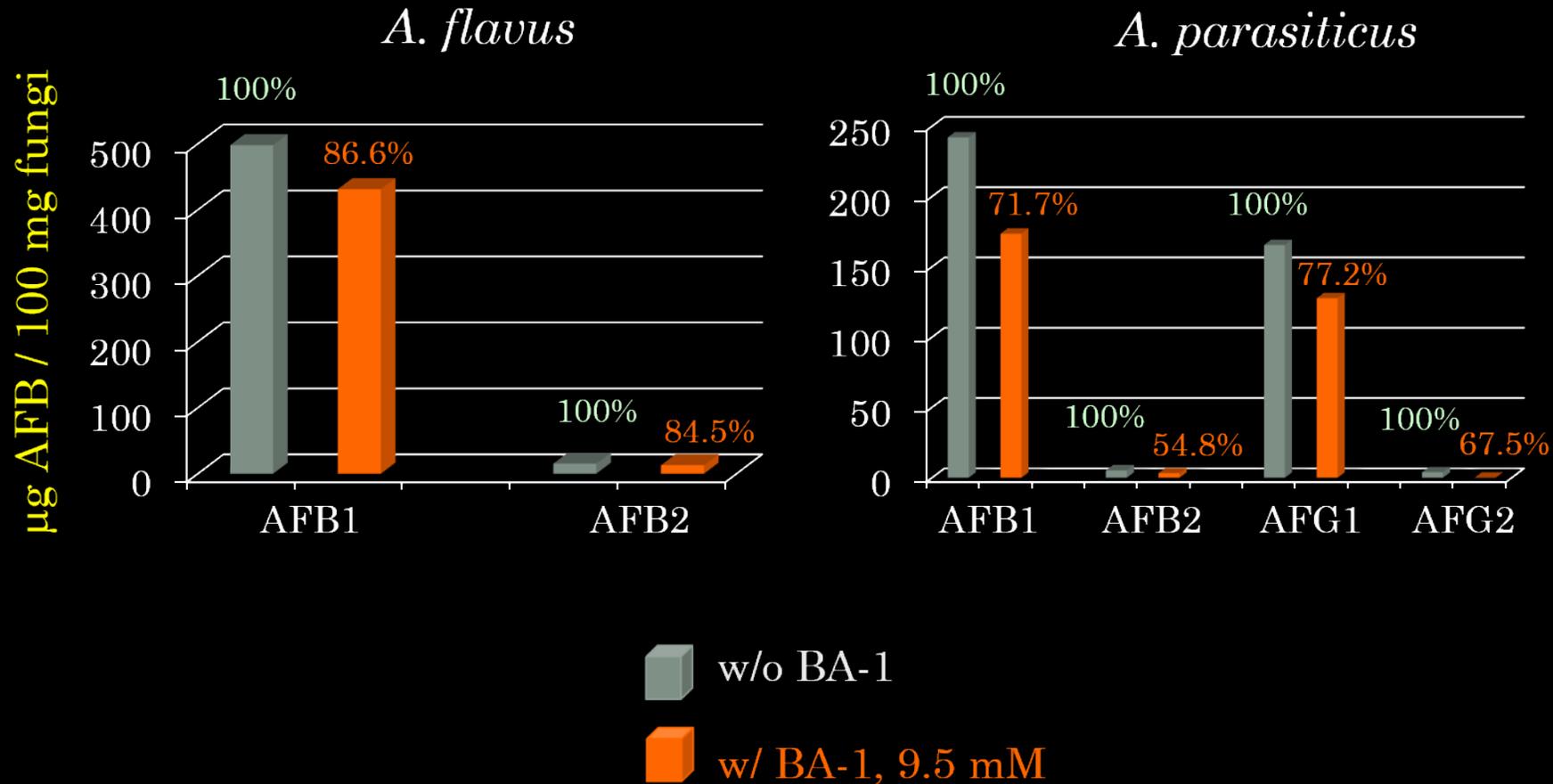
“Lowering the pesticide burden w/ aflatoxin control”

BA-1 : Overcame fungicide resistance

Aspergillus sp.



BA-1: Inhibits aflatoxin production by *Aspergillus*



BA-1: Application to large-scale soil solarization for control of pathogens & weeds

Film covering:
Microaerobic condition,
Enhancing the activity of fumigant compounds



<http://calag.ucanr.edu>



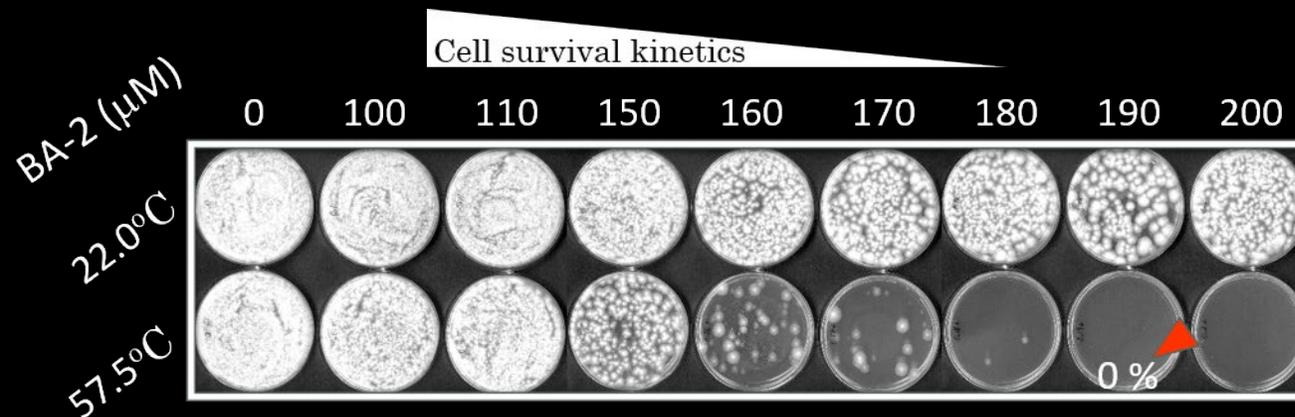
<http://www.apsnet.org>

BA-2: Mild heat sensitizer; Rapid elimination of fungi & bacteria during thermal process

BA-2 + 22.0 or 57.5°C, 90 sec

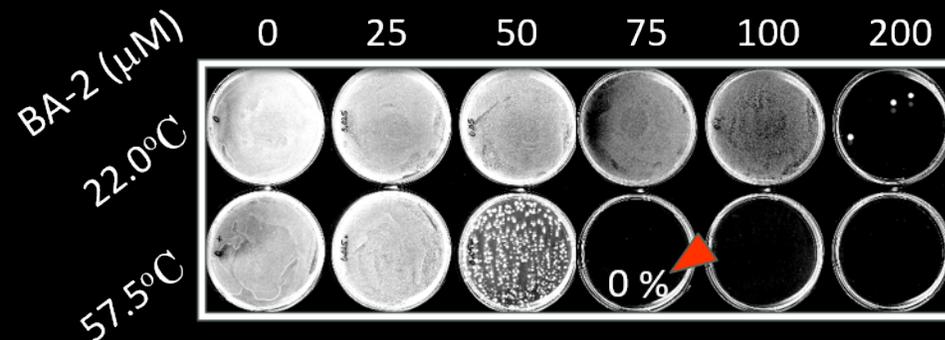
Fungi:

Aspergillus flavus
Aspergillus parasiticus
Aspergillus brasiliensis
Penicillium expansum
Penicillium italicum
Penicillium gresiofulvum



Bacteria:

Escherichia coli
Agrobacterium tumefaciens

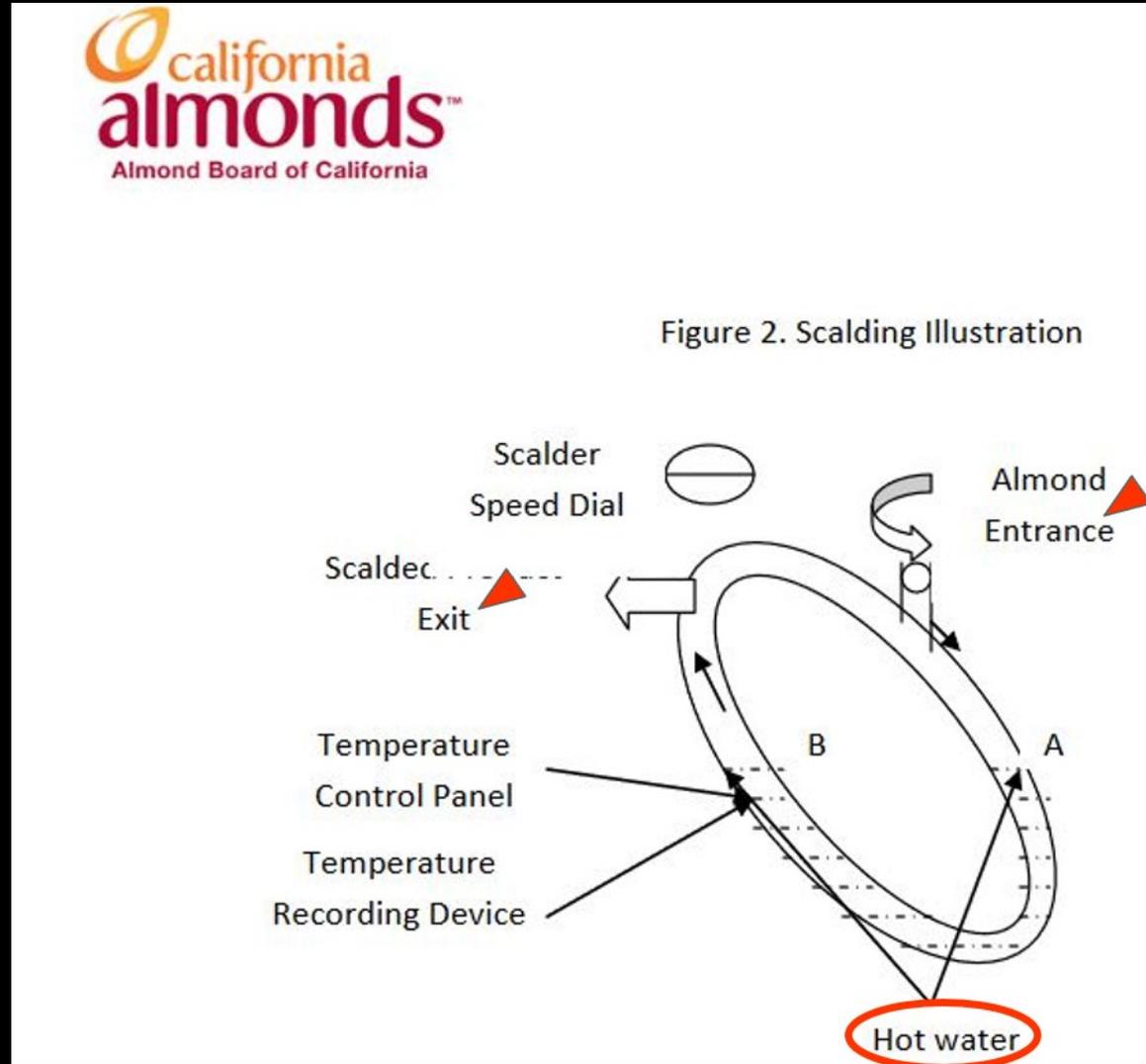


BA-2: Application to tree nut blanching

(57.5°C, 90 sec)

Blanching:

- A thermal process to remove almond skins & to reduce *Salmonella* on almonds in hot water.
- Required > 2.0 minutes, > 190°F (~88°C), > 4-log reduction of *Salmonella* on almonds.
- Ongoing research: Control of aflatoxigenic aspergilli & heat resistant *Salmonella* (*Salmonella* Enteritidis PT 30) on almonds.

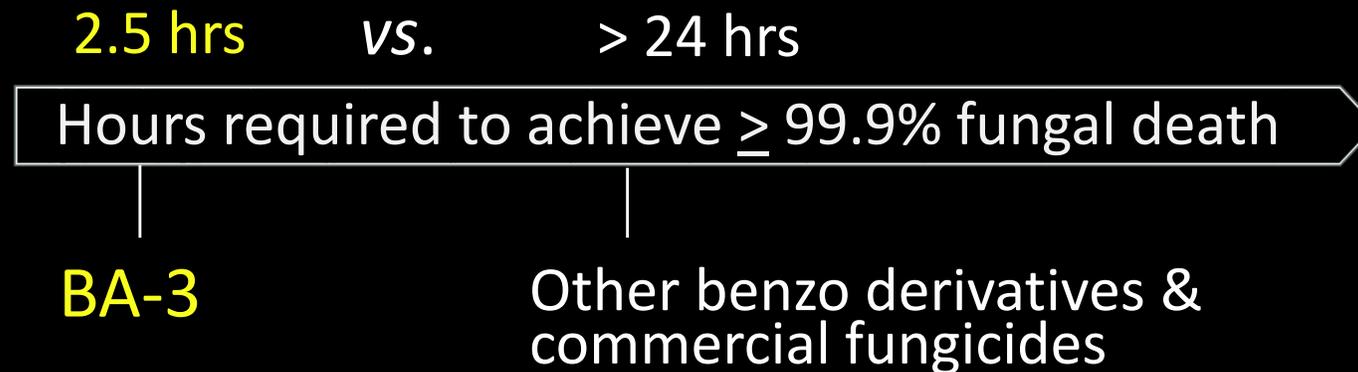


BA-3: Rapid elimination of fungi in food products, RT

TESTS:

- Commercial food matrices: Organic fruit juices (pH 3.0 – 3.5)
- Nine benzoics including BA-3; Six commercial fungicides
- Fungi (*Aspergillus*, *Penicillium*, *Neosartorya*)

RESULTS:

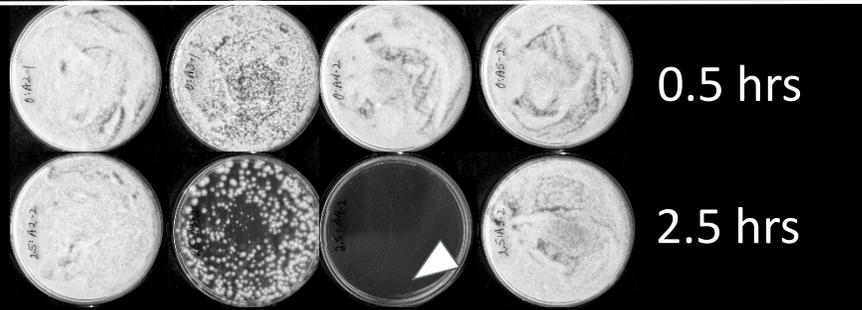


BA-3: Application to fungal control in food products; Heat-independent

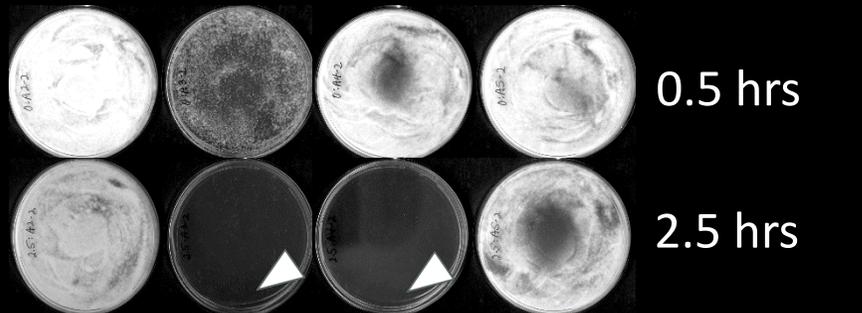
Compounds (0.1%)

None BA-2 BA-3 BA-4

A. flavus
3357



P. expansum
W1



pH 3.5 higher activity > **pH 5.6** lower activity;
As a food additive (antioxidant)

2016-2021: Summary

- Molecular screening identified the redox-active small molecules BA-1, BA-2 and BA-3, which can be applicable to fungal control during crop production, food processing or in food products.
- Identification of more industry application is currently underway.

2021-2026: Proposed plans (tentative)

1. *Testing fungicide* resistance vs. mycotoxin production in *A. flavus* & *A. parasiticus* isolated from California orchards
2. Anti-mycotoxigenic/Antifungal biocontrol agents from tree nut plants (e.g., almond flowers)
3. Compound repositioning for control of food-contaminating microbes

Fungicide potentiation of mycotoxin production

Examples

FUNGI	FUNGICIDES
<i>Aspergillus parasiticus</i>	Anilinopyrimidine
<i>A. parasiticus</i>	Flusilazole
<i>A. parasiticus</i>	Phenylpyrrole
<i>Fusarium graminearum</i>	Carbendazim
<i>Fusarium sp.</i>	Strobilurins
<i>F. sporotrichioides</i>	Carbendazim
<i>Penicillium expansum</i>	Tebuconazole, Fludioxonil
<i>P. expansum</i>	Benzimidazole
<i>P. verrucosum</i>	Iprodione

2021-2026: Proposed plan-1

Testing fungicide resistance vs. mycotoxin production in *A. flavus* & *A. parasiticus* isolated from California orchards

- To examine the level of fungicide resistance and mycotoxin production in *A. flavus* and *A. parasiticus* from California orchards (In collaboration with UC-Davis).
- To identify “control methods” targeting resistant *Aspergillus*.

Proposed plan-2

Anti-mycotoxigenic/Antifungal biocontrol agents from tree nut plants

- Mycotoxin contamination could be inhibited by selected bacteria as biocontrol agents: “Aflatoxins, Ochratoxins, Zearalenone, Deoxynivalenol, Fumonisin.”
- Identify bacterial biocontrol agents from host plants, such as almond flowers.
- Ensure the maintenance of healthy microbiome of pollinators; Might start with the identification of *Lactobacillus* sp., which are routinely found in bees; Isolation methods for *Lactobacillus* sp. from host plants are under development.



Proposed plan-3

Compound repositioning for control of food-contaminating microbes

- **COMPOUND REPURPOSING:** An alternative intervention strategy, whereby new utility of various marketed, non-antifungal compounds could be repositioned as novel antifungal agents.
- **MERIT:** The mechanisms of action, cellular targets or safety of commercial compounds, e.g., drugs, are already identified or well characterized.
- **ANTIFUNGAL CHEMOSENSITIZATION:** A method developed to improve the efficiency of antifungal drug repurposing (Combined application of a second compound as a chemosensitizer with a conventional, non-antifungal drug could greatly enhance antifungal activity of the drug co-applied.)

Summary: Proposed plans

1. Testing fungicide resistance vs. mycotoxin production in *A. flavus* & *A. parasiticus* isolated from California orchards.
2. Anti-mycotoxigenic/Antifungal biocontrol agents from tree nut plants.
3. Compound repositioning for control of food-contaminating microbes.

THANK YOU!