

Aflatoxin Management in Pistachio

(Successes, Challenges, and More...)



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Kearney Agricultural Research and Extension

Pistachio Day – 19 January 2022

People Involved

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4. Victor Gabri ¹
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6. Ryan Puckett ¹
7. Juan Moral ^{1,3}
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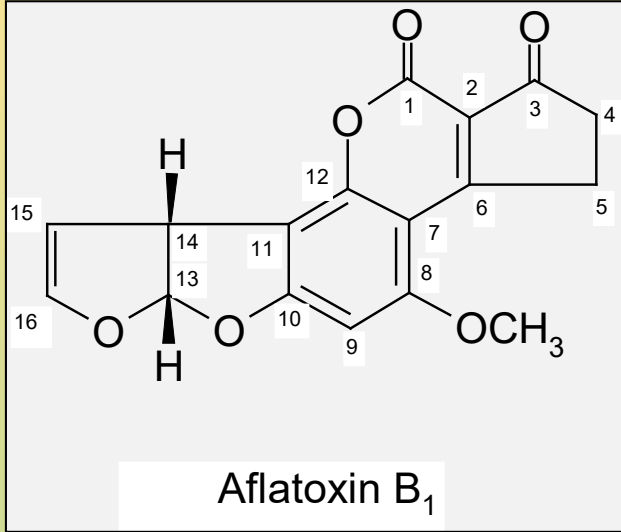
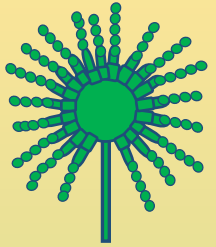
² University of Bari, Italy

³ University of Cordoba, Spain

⁴ Agric. University of Athens, Greece



Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*



B1: The most potent; it can cause liver cancer



Aspergillus flavus (B1,B2)

Aspergillus parasiticus (B1,B2,G1,G2)

Aflatoxins: **B**₁, B₂, G₁, G₂, M₁

Regulatory limits for aflatoxins

- USA
Total aflatoxins → 20 ppb
- European Union
Total aflatoxins → 10 ppb
Aflatoxin B1 → 8 ppb

Pistachios and almonds for direct consumption

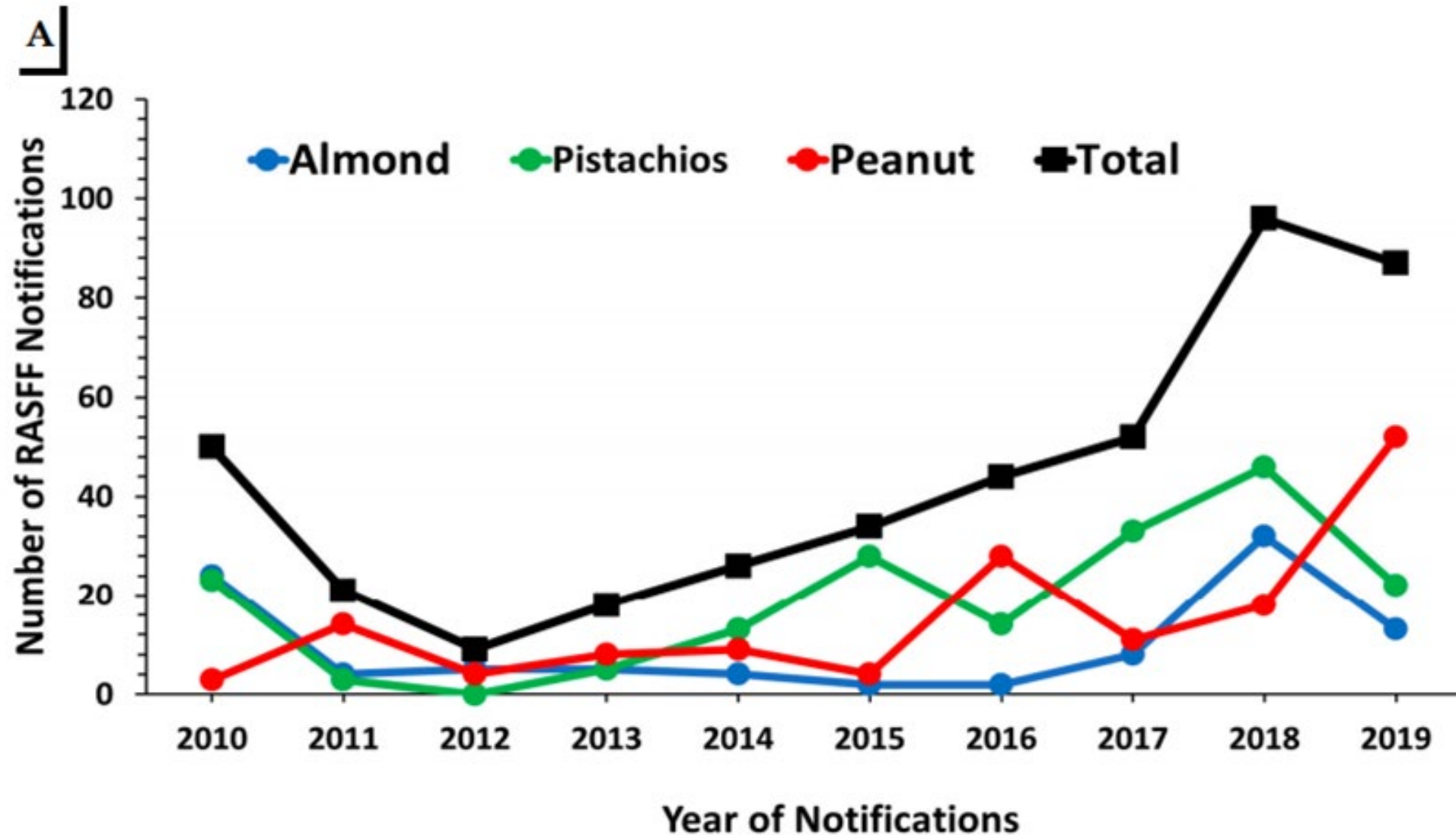
Total aflatoxins → 4 ppb
Aflatoxin B1 → 2 ppb

walnuts and dried fruit

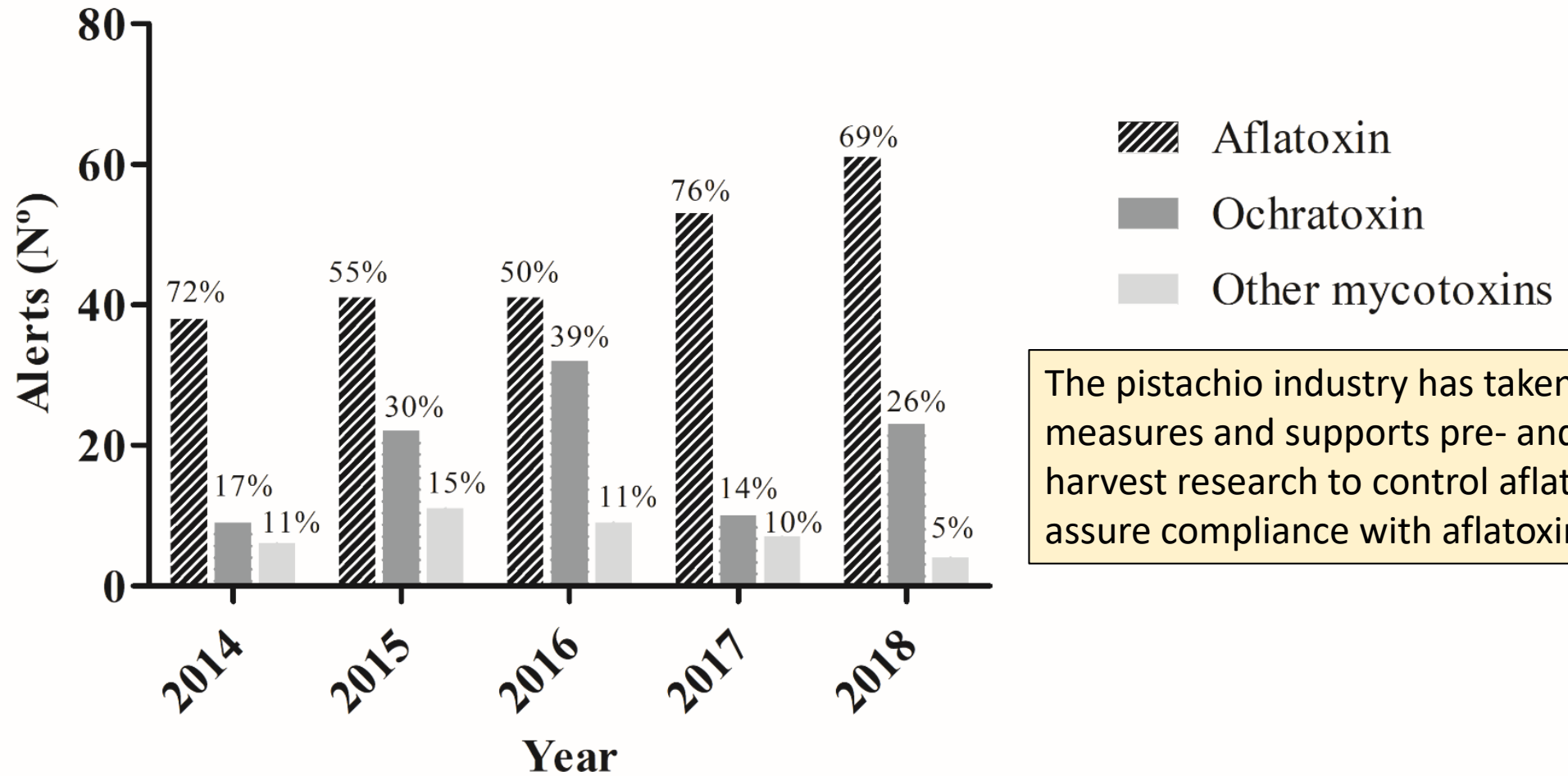
Total aflatoxins → 10 ppb
Aflatoxin B1 → 6 ppb

dried figs

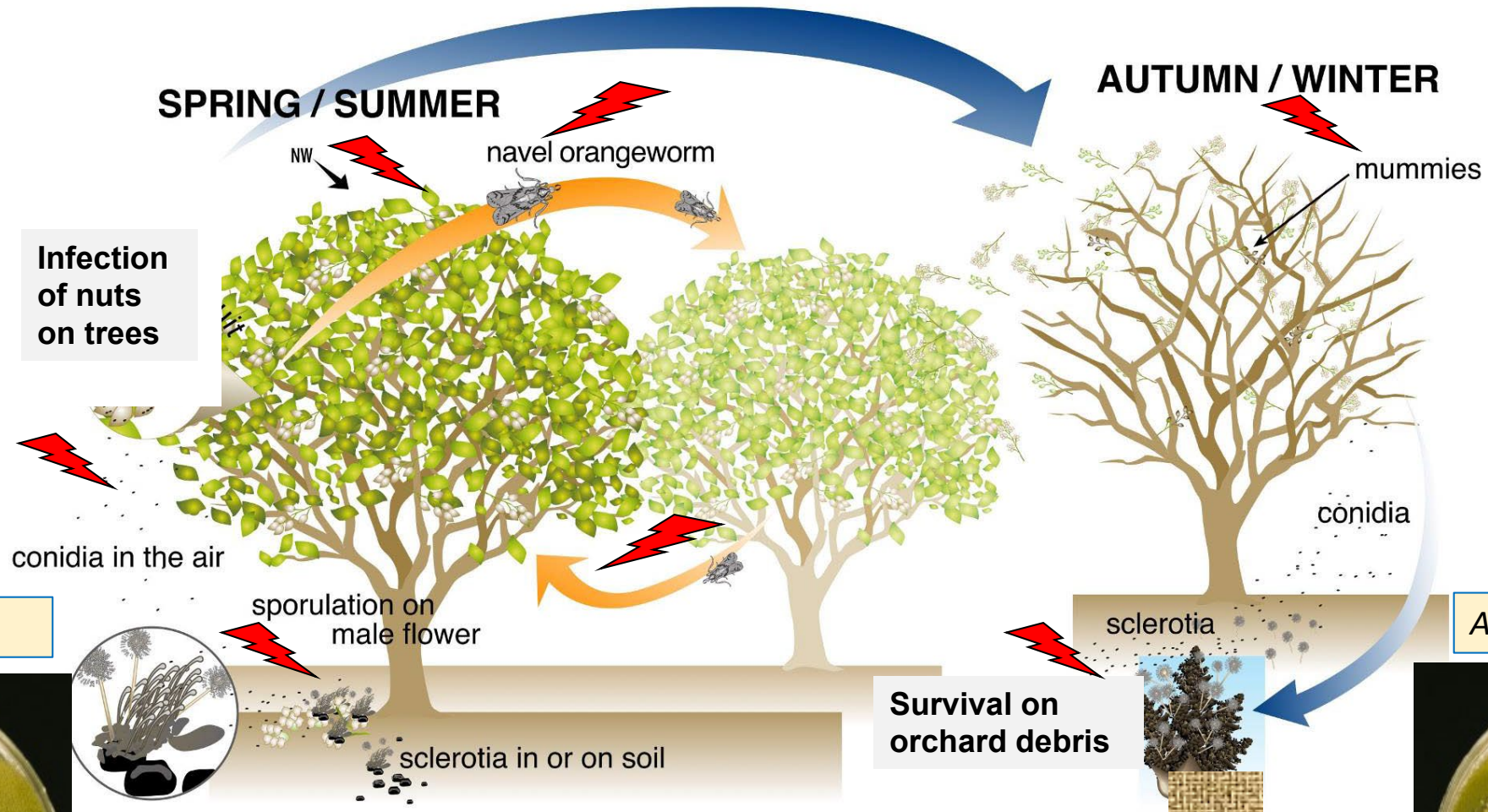
Number of Rapid Alert System for Food and Feed (RASFF) Notifications of mycotoxin reported in almond, pistachios, and peanut crops in the last 10 years (2010-2019)



Percent Rapid Alerts on aflatoxins, ochratoxins, & other mycotoxins in various crops



The pistachio industry has taken extensive measures and supports pre- and post-harvest research to control aflatoxins and to assure compliance with aflatoxin regulations.



Aspergillus flavus

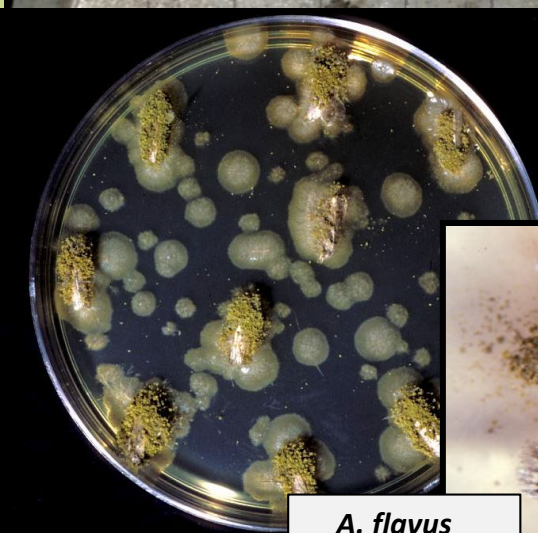
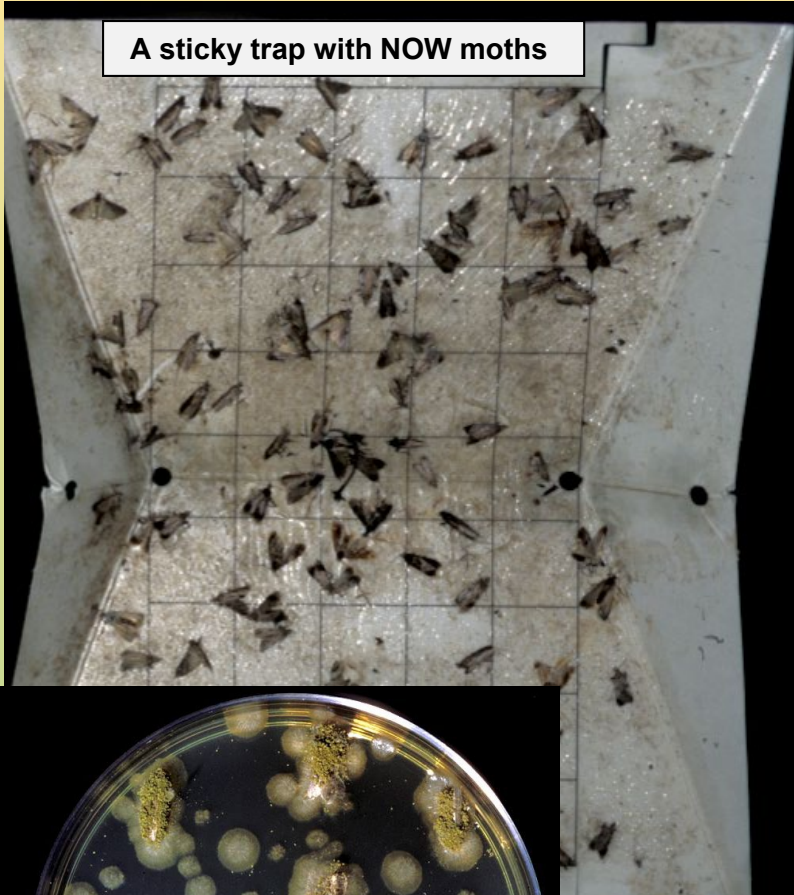
Aspergillus parasiticus



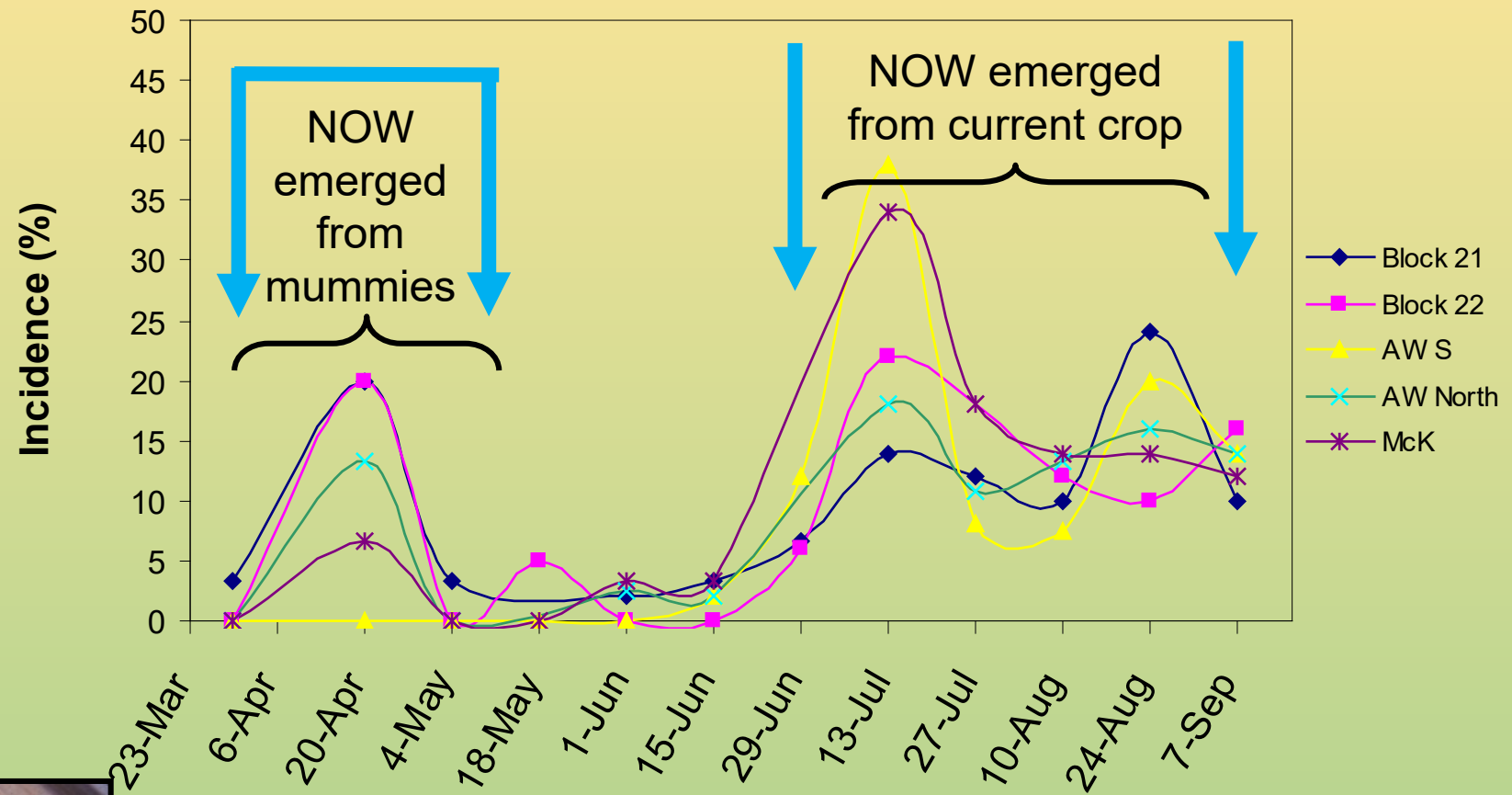
The life cycle of *Aspergillus flavus* in a pistachio orchard

Aspergillus sect. *Flavi* on NOW moths from 5 pistachio orchards (Madera Co.)

A sticky trap with NOW moths



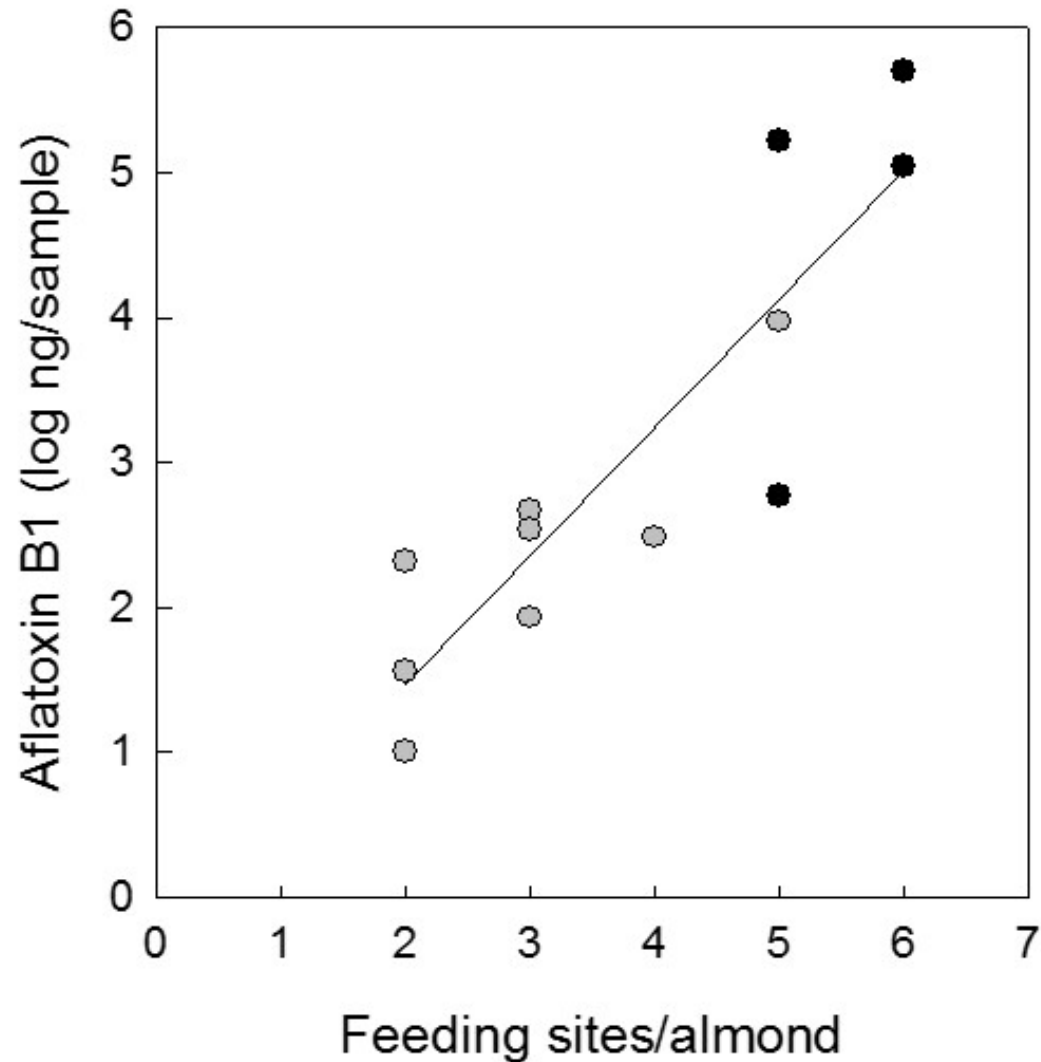
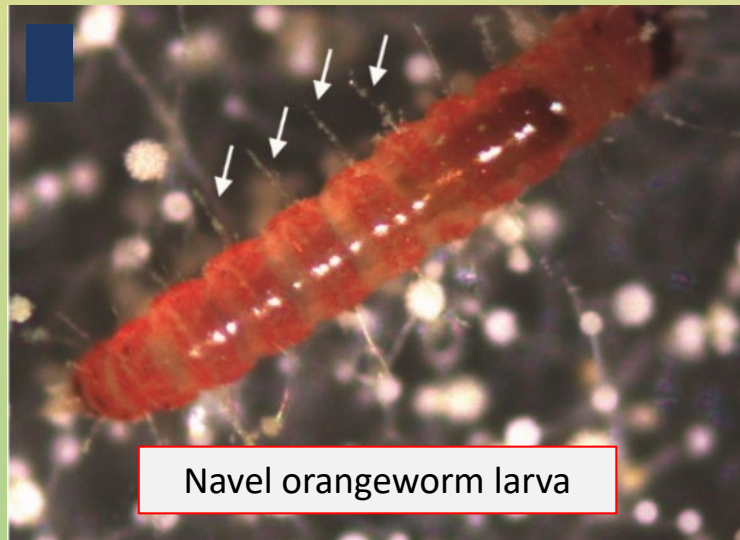
A. flavus



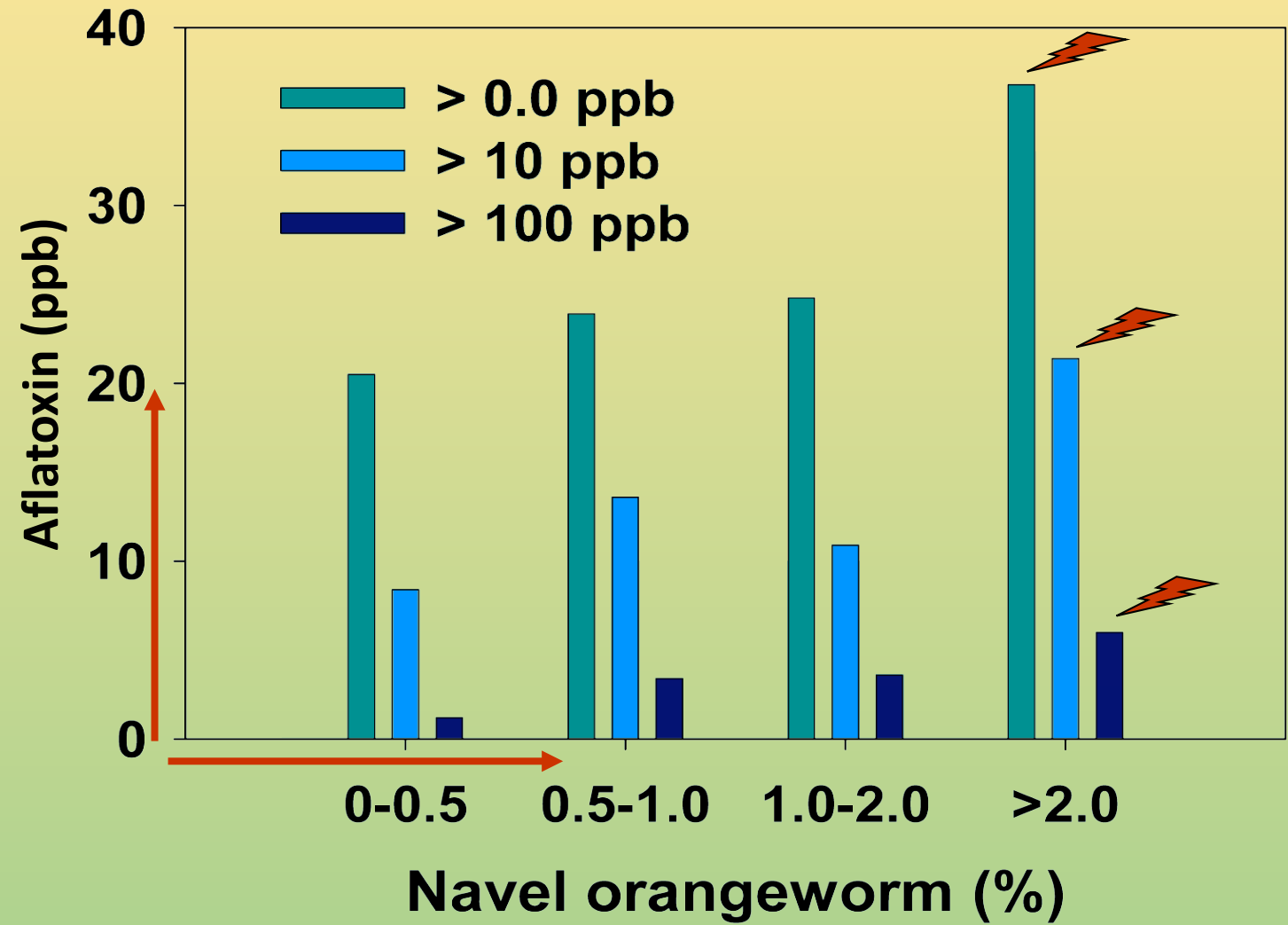
→ Up to 4.2% of the strains on NOW were toxigenic.

Acquisition and transmission of *Aspergillus flavus* by navel orangeworm

(in coop. with Dr. Palumbo, ARS/USDA, Albany)



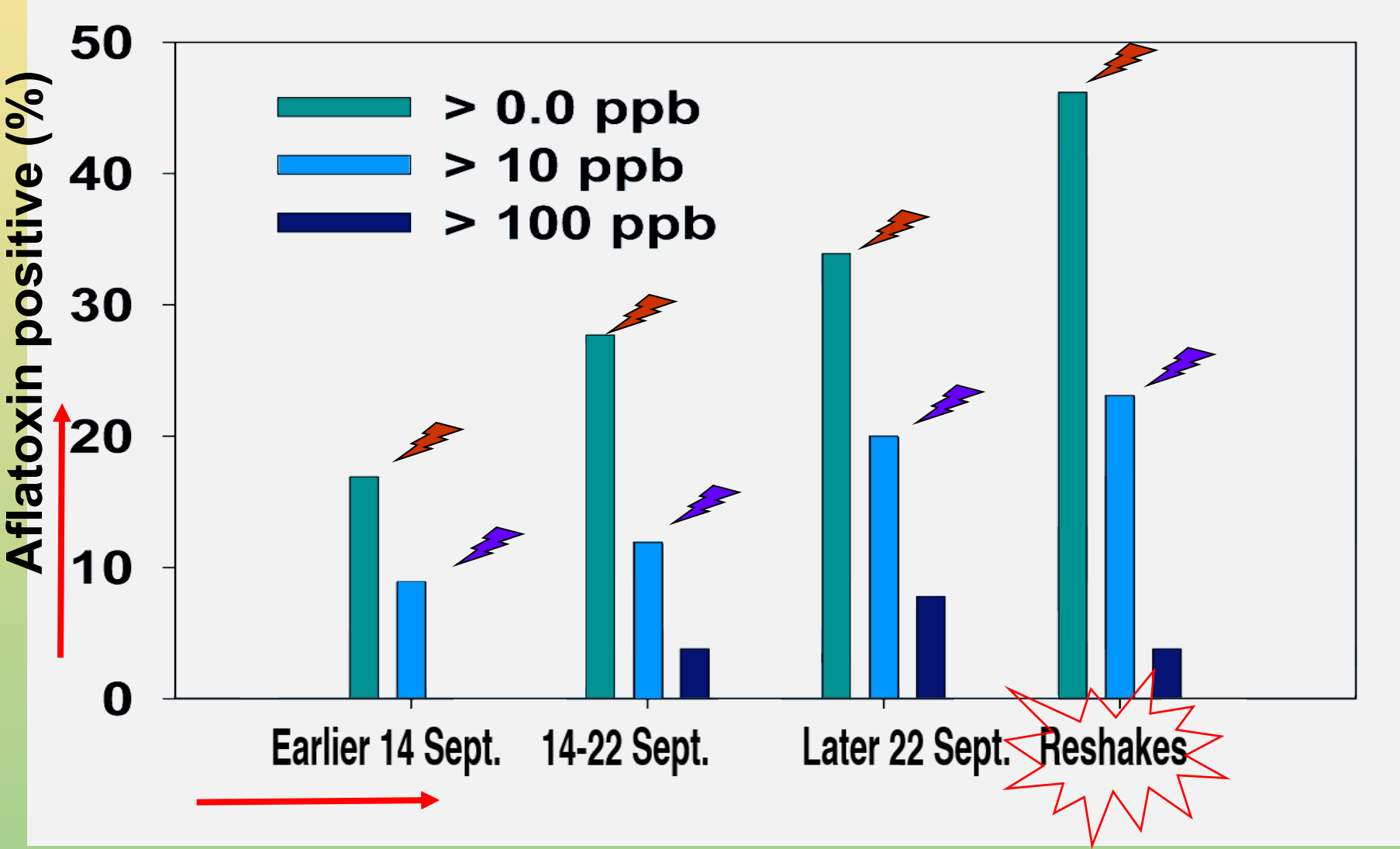
Relationship of navel orangeworm damage and aflatoxin levels



Factors affecting aflatoxin contamination in pistachio

- 1) Navel orangeworm (NOW)
- 2) Harvest date
- 3) Early Splits (ES) and various other defects (DBOM)
- 4) Cultural practices (affecting ES)
- 5) Rootstock (affecting ES)
- 6) Location (“hot spots”)
- 7) Year (on / off)

Effect of harvest date on incidence and amounts of aflatoxin contamination in pistachios



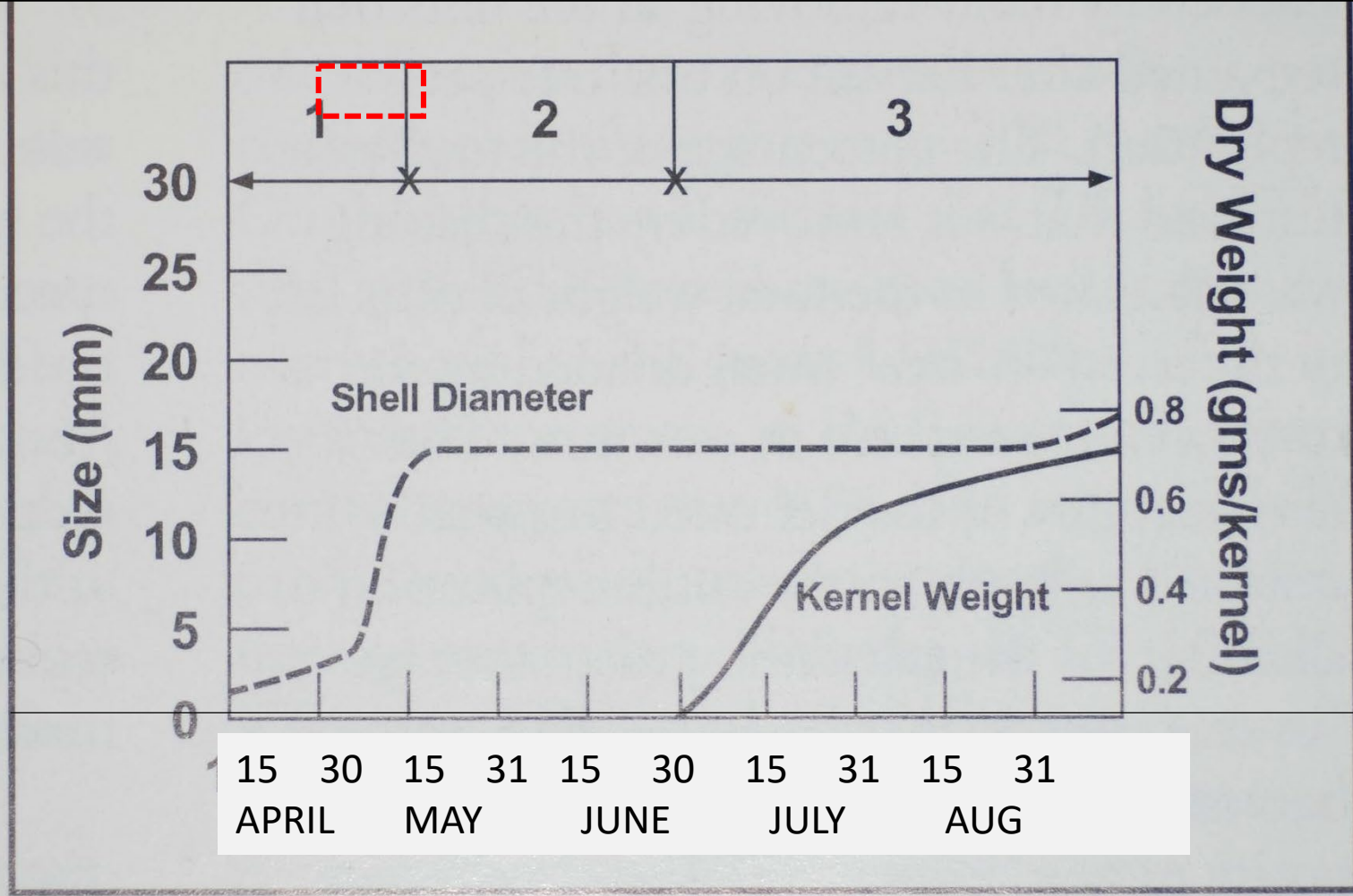


Early splits



Early split nuts: a source of *Aspergillus* spp., NOW, & aflatoxin contamination in pistachio

Phenological stages of shell and kernel development of pistachio (Goldhamer & Beede, 1992)



➤ Apply sufficient irrigation during spring to avoid tree stress.

Rootstocks used for pistachio in California

Greater incidence of ES

Pistacia atlantica

Smaller incidence of ES

UCB1

PG I (Pioneer Gold)

Greater incidence of ES

PGII

} *P. integerrima*



Use a rootstock that minimizes early split nuts.

Available data supporting recommendations to successfully reduce aflatoxins:

- Control navel orangeworm (NOW). *****
- Reduce early splits. ****
 - ❖ Apply sufficient irrigation during spring to avoid tree stress.
 - ❖ Use a rootstock that minimizes early split nuts (i.e. UCB1, PGI).
- Do not delay harvest. *****
- Sort out damaged and defected nuts. ****

Biological control of aflatoxins

Strains of *Aspergillus flavus*



L - strain



**about 50:50
toxigenic: atoxigenic**

S - strain



most toxigenic

***Aspergillus flavus*: L-strains**



about 50:50
toxigenic: atoxigenic

Atoxigenic AF36

0 ppb aflatoxin

Rationale: Increase the atoxigenic strain population in the orchard to displace the toxigenic strain population.

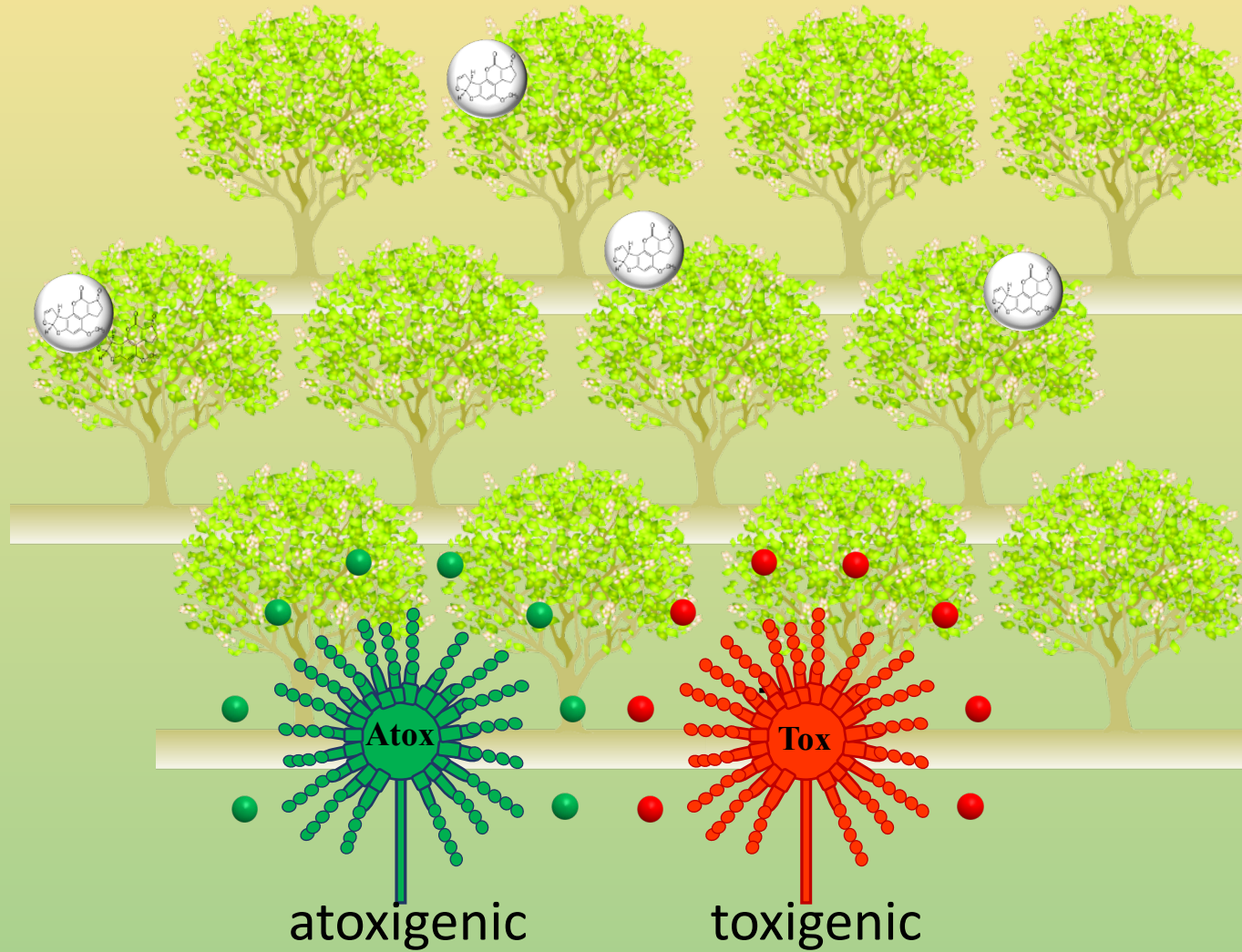


AF36
Inoculum

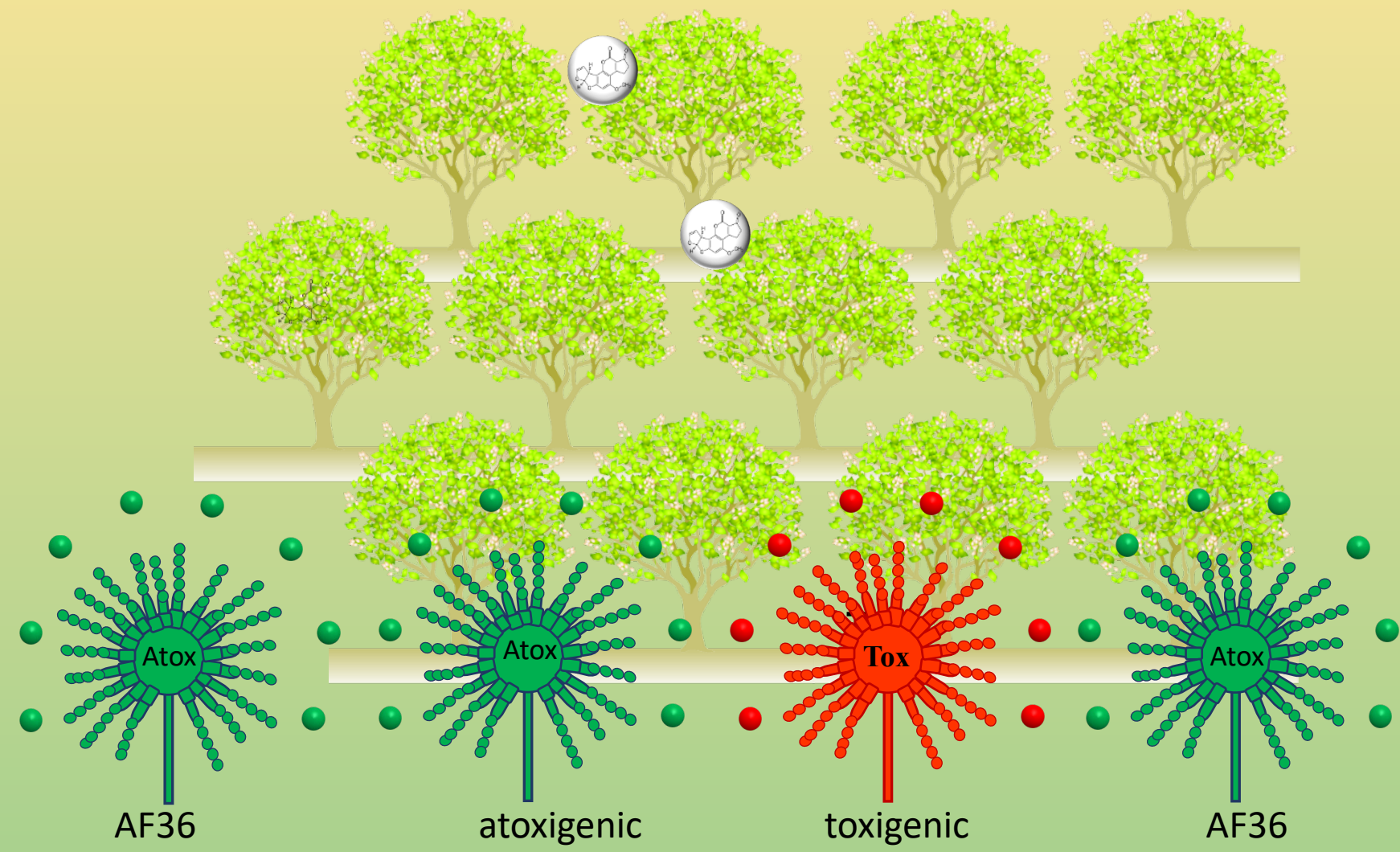
Rate 10 lbs per acre



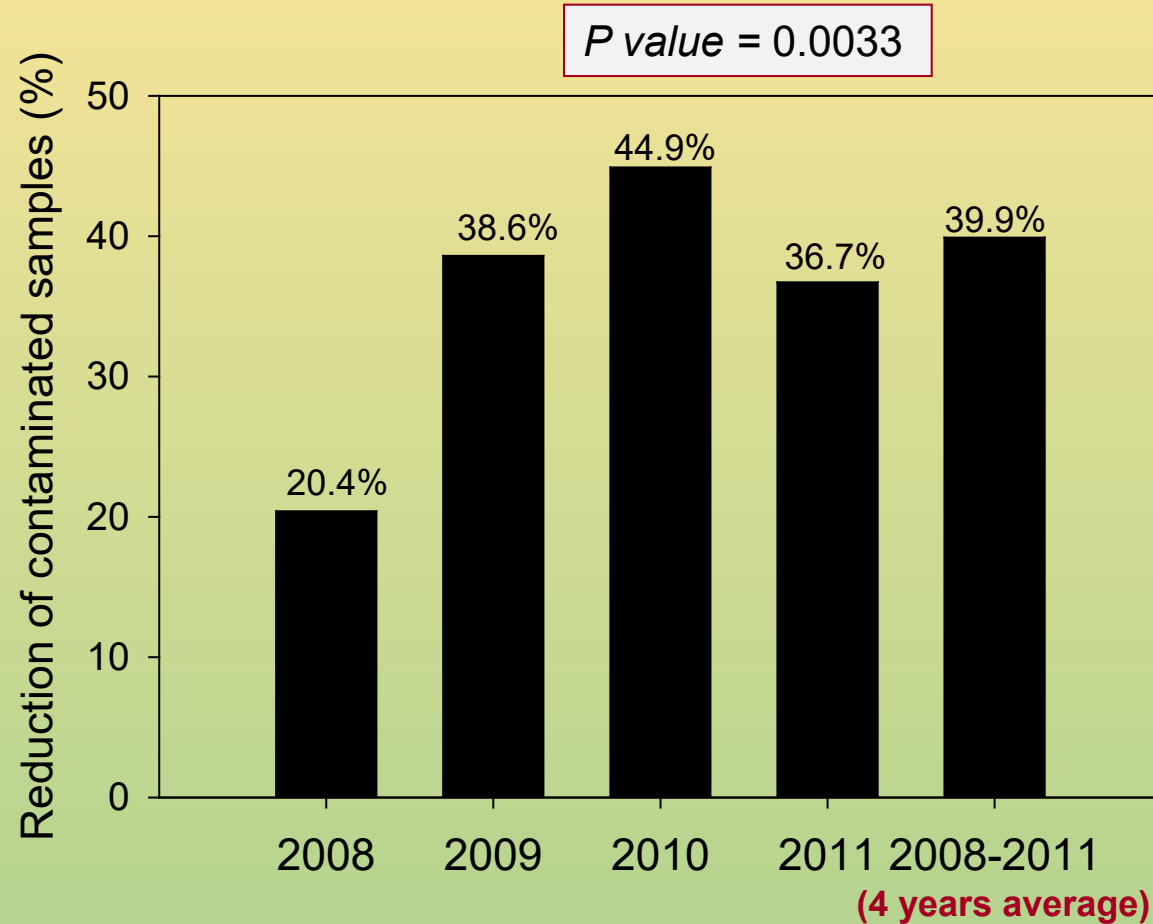
Non-treated orchard



Treated orchard with the AF36 Prevail[®]



Success in reducing aflatoxin-contaminated pistachio library samples (1st harvest & reshakes)

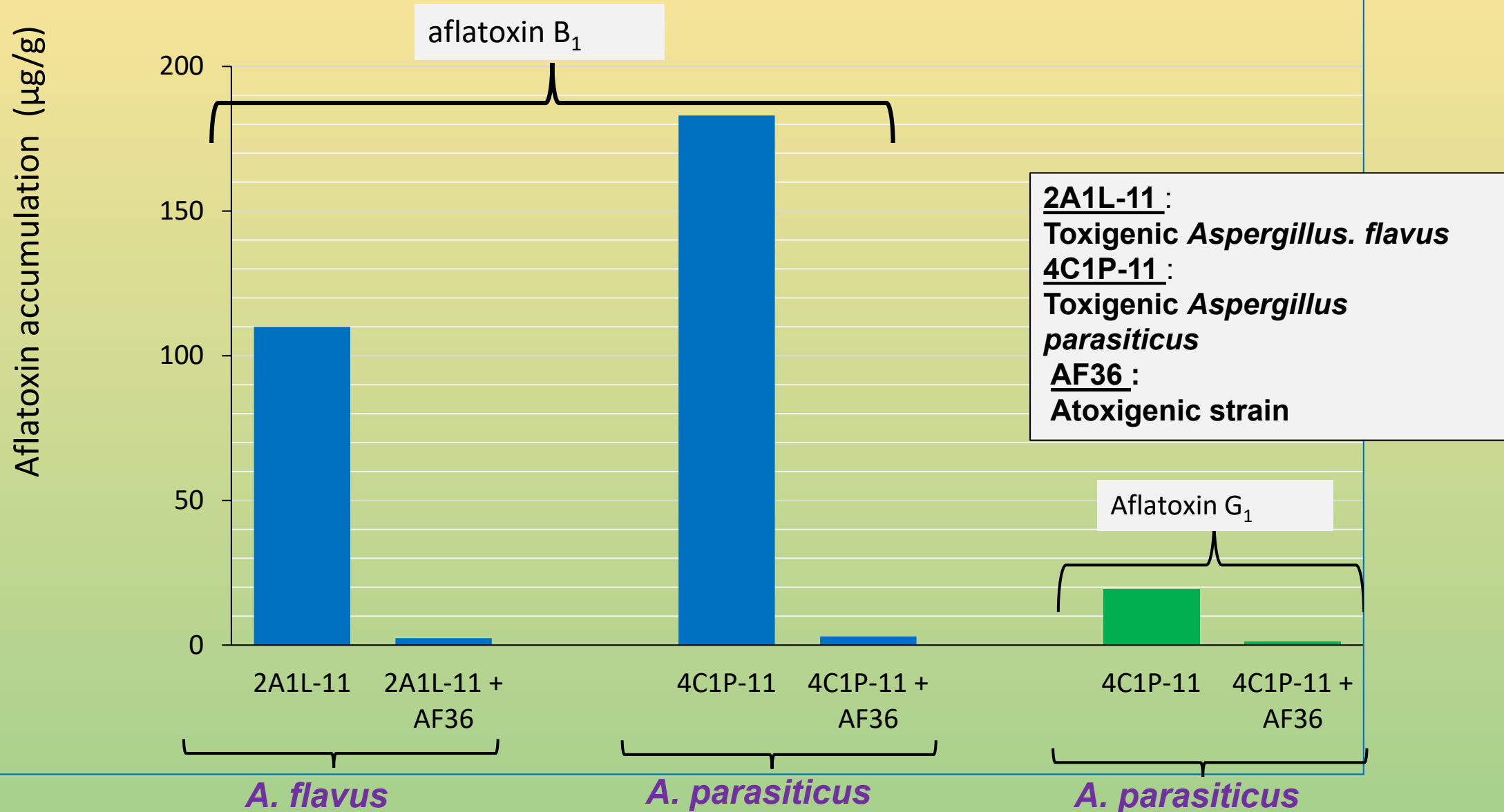


(Doster et al. (2014), Plant Disease 98:948-956)

First harvest:
An average of
40% reduction

In reshakes:
An average of
55% reduction

The AF36 reduced aflatoxin production by the toxigenic strains



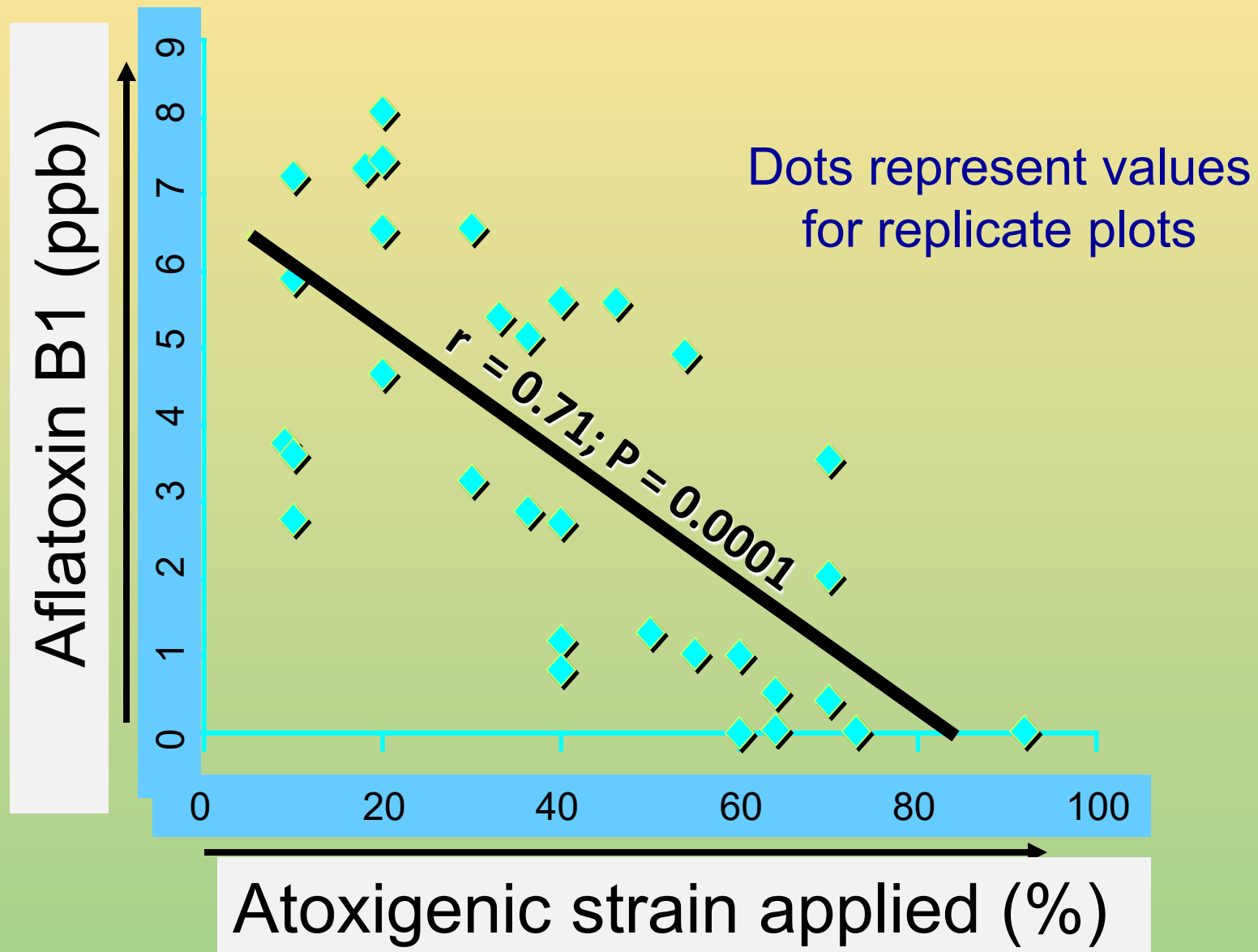
Application in cotton fields: Humid environment under the plants

→ good sporulation of the product

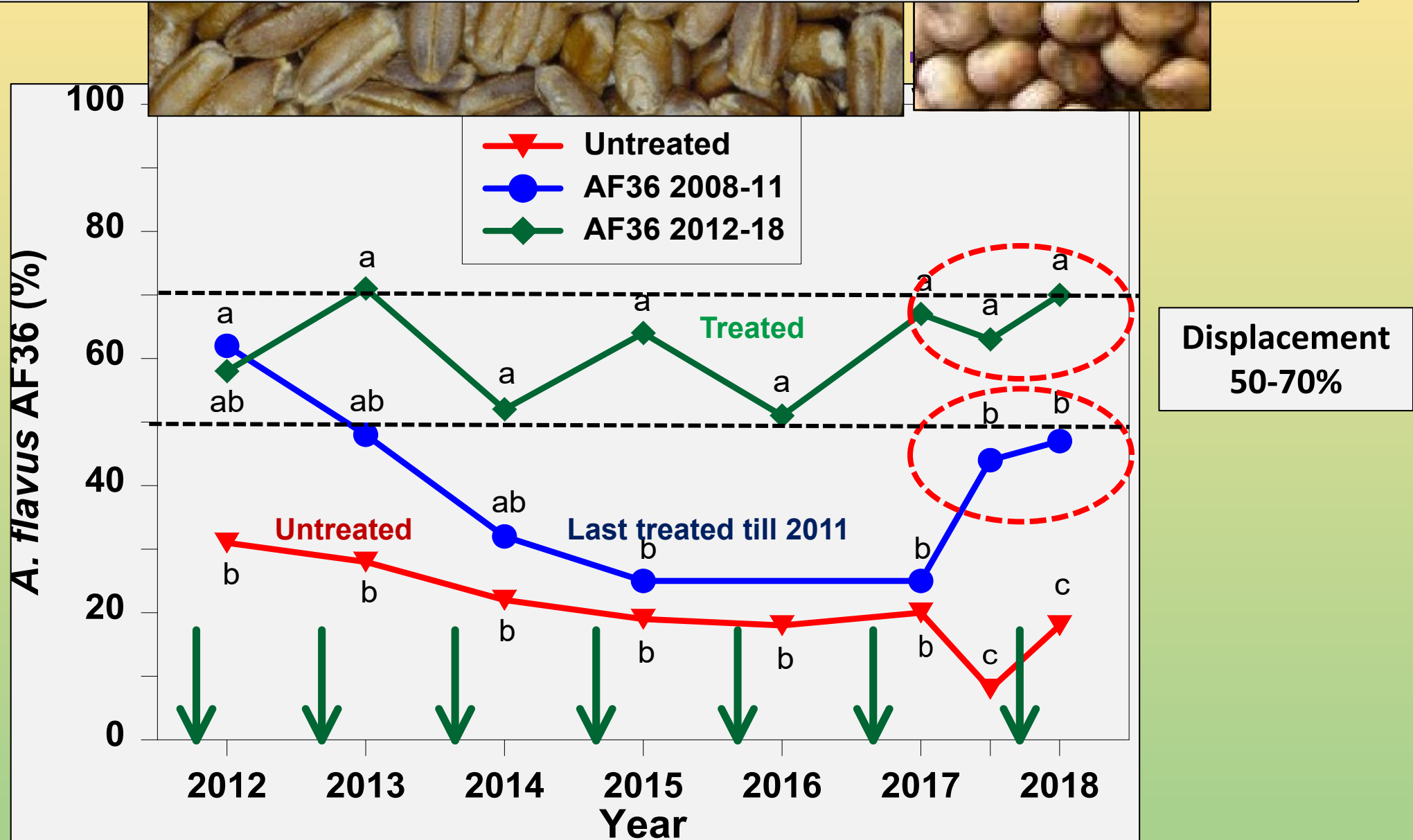


Leading to 80% to 90% displacement

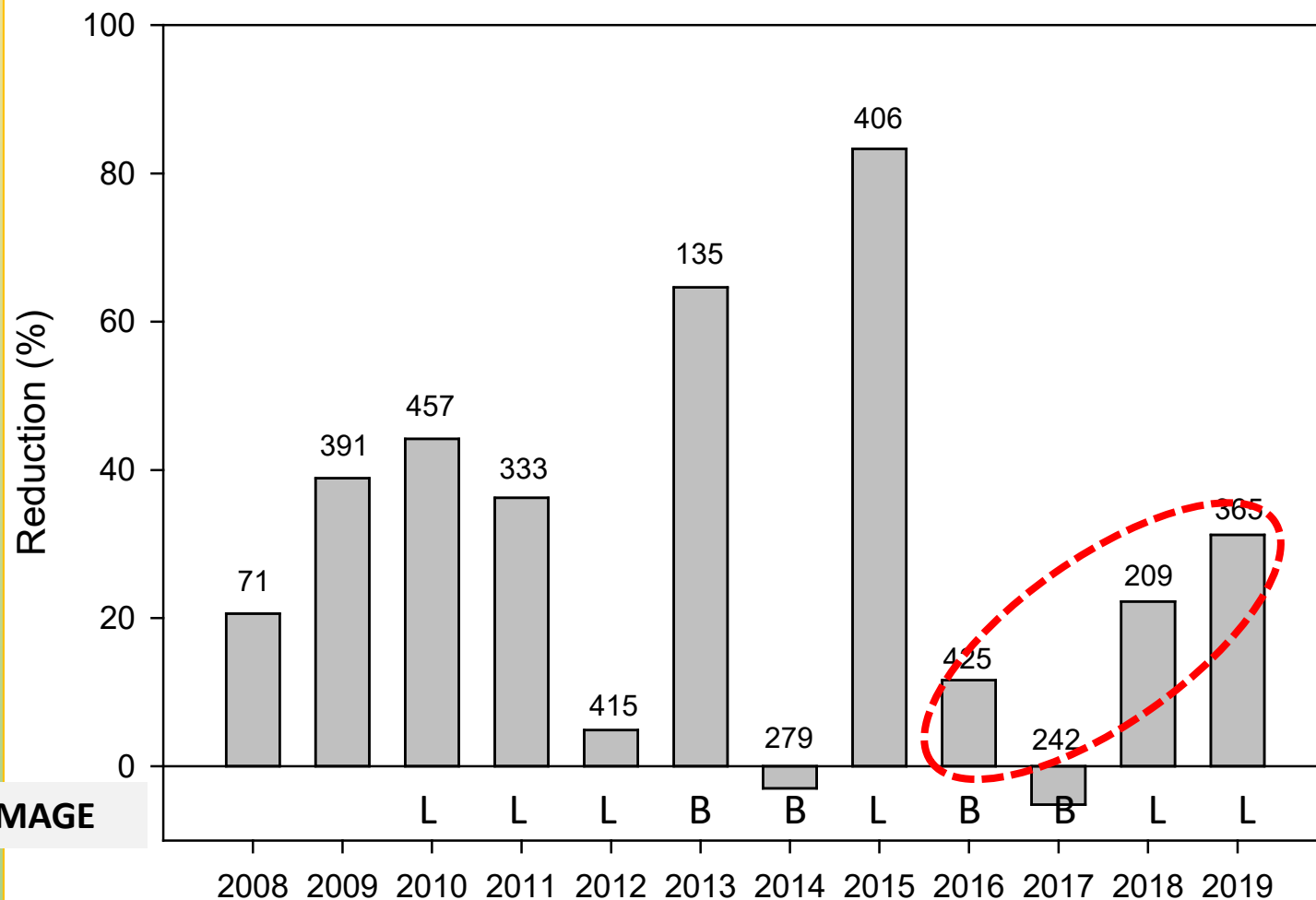
Aflatoxin in cottonseed versus strain AF36 incidence



A challenge in California Orchards: Displacement of toxigenic strains was reduced from 90 - 95% to 50 - 70%



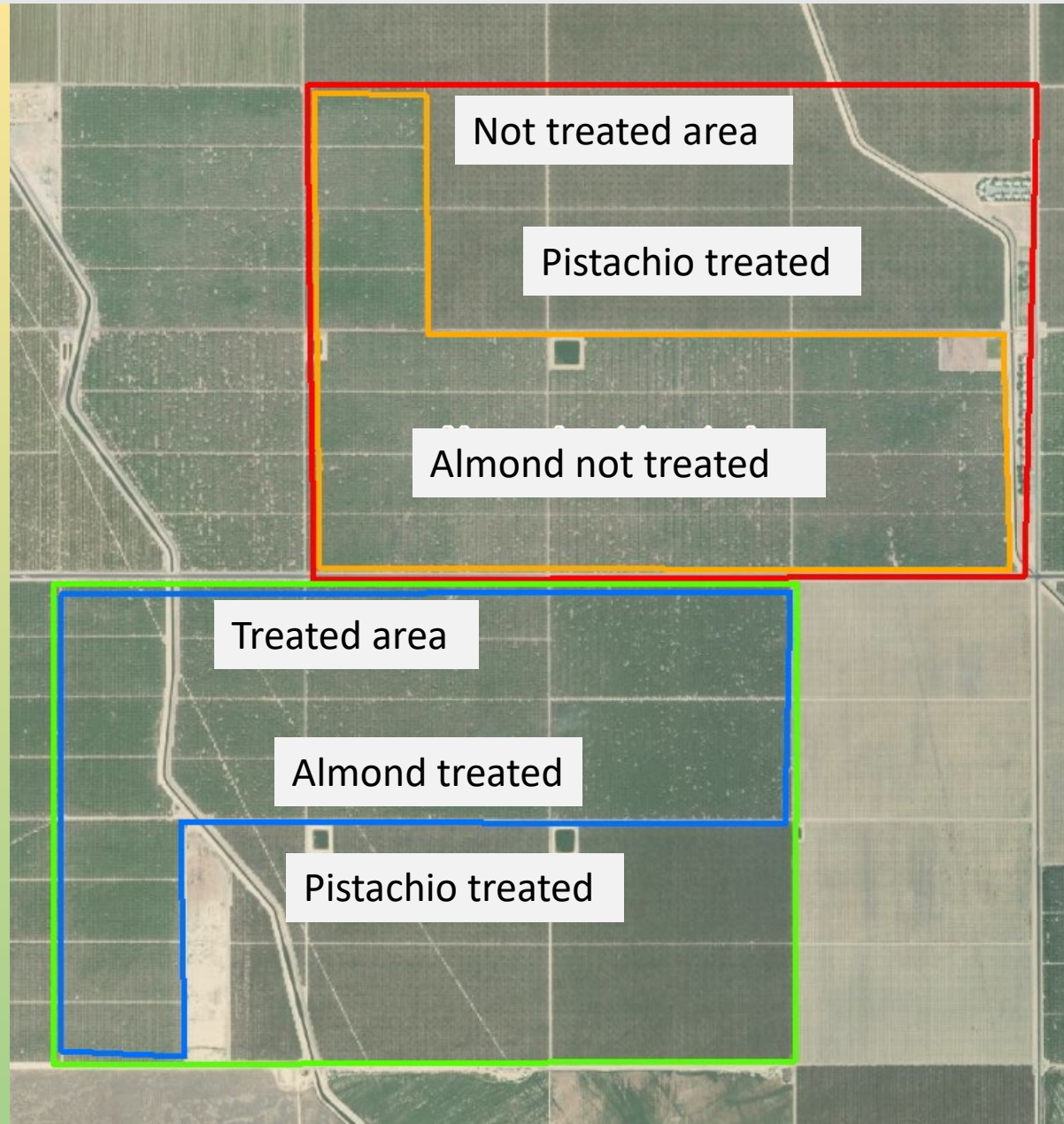
Reduction of aflatoxin contaminated library samples for blocks treated with AF36 with corresponding number of samples analyzed



NOW DAMAGE

In following the EUP years reduction of positives ranged from 5% to 85%; 2016-2019: 0 -30% reduction

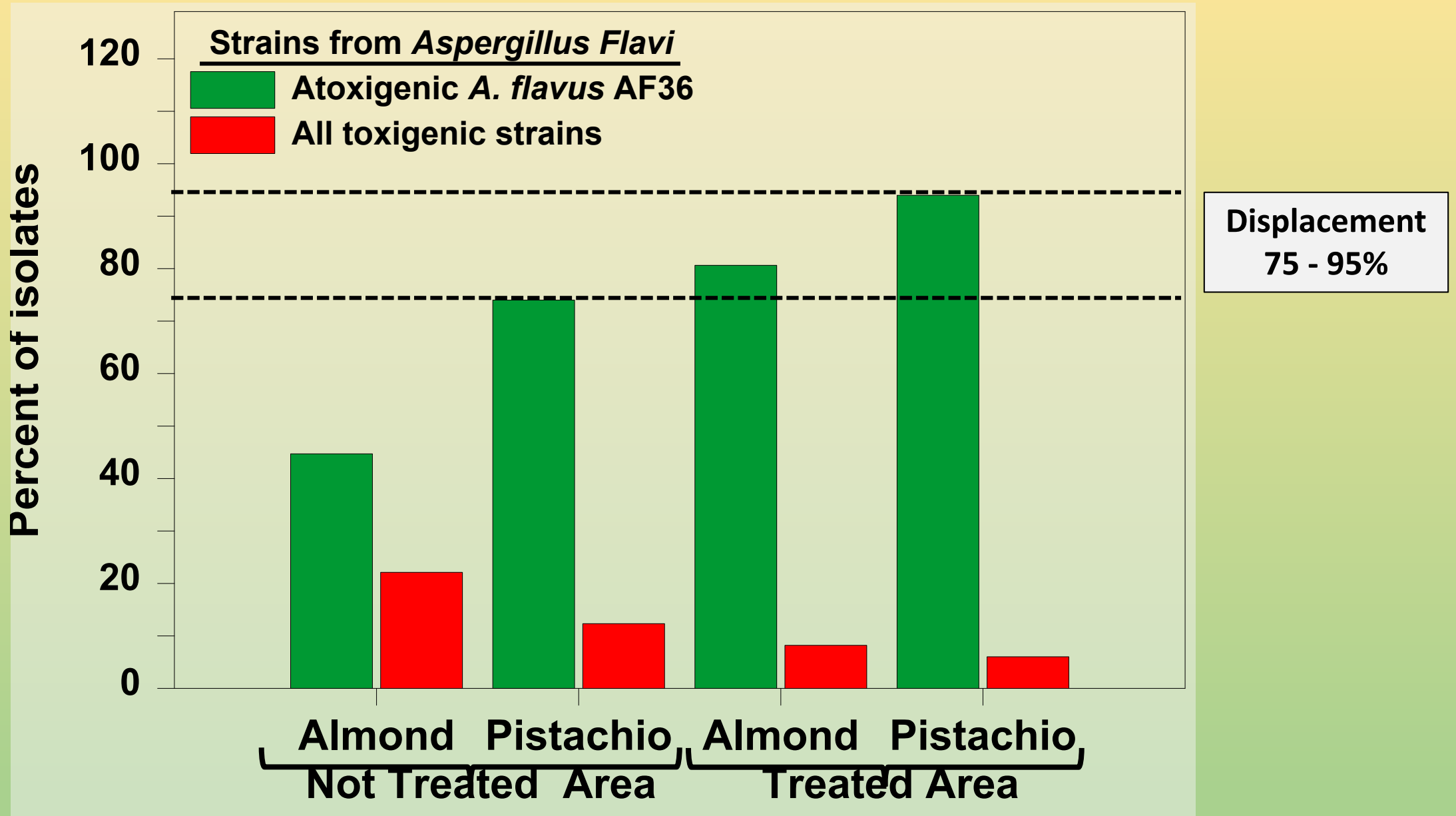
Area-wide, long-term treatment with atoxigenic strain biocontrol



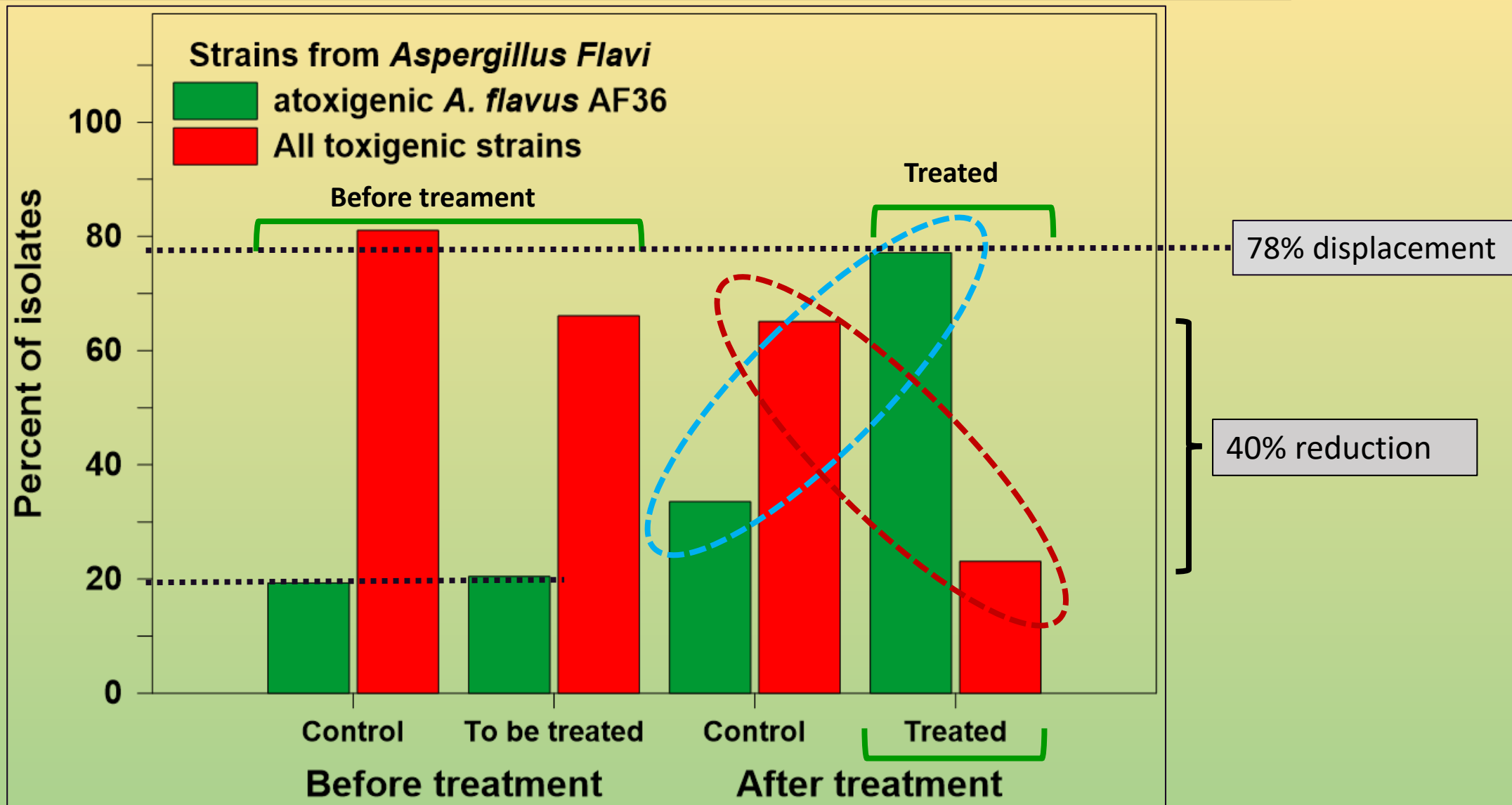
Note:

- In the not treated area
pistachio orchards were treated;
almond orchards were not treated.
- In the treated area
both almond and pistachio orchards
were treated.

Area-wide, long-term treatment with AF36 Prevail atoxigenic strain



Aspergillus strains of *Aspergillus Flavi* (*Aspergillus flavus* & *A. parasiticus*) recovered from soil samples of almond orchards before and after treatment with AF36 Prevail®





Aspergillus flavus AF36 Prevail

For displacing aflatoxin-producing fungi

Arizona Cotton Research and Protection Council

"for growers by growers"

PISTACHIO, ALMOND, AND FIG: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, NEW MEXICO, AND TEXAS

COTTON: FOR USE ONLY IN THE STATES OF ARIZONA, CALIFORNIA, NEW MEXICO, AND TEXAS

CORN: FOR USE ONLY IN THE STATES OF ARIZONA AND TEXAS

PESTICIDE: ALMOND AND FIG: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, NEW MEXICO, AND TEXAS

PROXIMATE INFORMATION

Aspergillus flavus AF36 Prevail is a highly effective aflatoxin-producing fungus that displaces aflatoxin-producing fungi. It is highly effective in displacing aflatoxin-producing fungi from crops and is safe for humans and animals. It is safe for use on crops and is safe for humans and animals. It is safe for use on crops and is safe for humans and animals.

USE PRECAUTIONS

Do not apply to crops that are under stress. Do not apply to crops that are under stress. Do not apply to crops that are under stress. Do not apply to crops that are under stress.

APPLICATOR PRECAUTIONS

Wear protective clothing and avoid contact with the treated crops. Avoid contact with the treated crops. Avoid contact with the treated crops.

KEEP OUT OF REACH OF CHILDREN

CAUTION

For oral use only

IF INHALED:	Move person to fresh air. If person is not breathing, call 911 for ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. Call a poison control center or doctor for further treatment advice.
IF ON SKIN OR CLOTHING:	Wash skin immediately with plenty of water for 10-15 minutes. Call a poison control center or doctor for treatment advice.
IF IN EYES:	Hold eyes open and flush with clean water for 15-20 minutes. Flush contact lenses, if present, under the flow of water. Then continue flushing eyes. Call a poison control center or doctor for treatment advice.

KEEP OUT OF REACH OF CHILDREN

SEE PRECAUTIONARY STATEMENTS BELOW AND ON OTHER PANEL

EPA Registration No. 758-35
EPA Est. No. 7582-0001

Arizona Cotton Research and Protection Council
1201 E. McQueen Avenue
Tempe, Arizona 85281

NET WEIGHT: 11 lb. bags or variable weight bulk bags 1,000 to 2,000 lb

BATCH CODE:

PRECAUTIONARY STATEMENTS

HAZARD TO HUMANS AND DOMESTIC ANIMALS

CAUTION: Irritant. If swallowed, it may cause severe irritation and may be harmful. If it reaches the eyes, it may cause severe irritation. If it reaches the skin, it may cause severe irritation. If it reaches the lungs, it may cause severe irritation.

PERSONAL PROTECTIVE EQUIPMENT (PPE):

Appropriate clothing and gloves. Wear appropriate clothing and gloves. Wear appropriate clothing and gloves.

HAZARD TO THE ENVIRONMENT

CAUTION: Harmful to fish and aquatic life. Avoid discharge to streams, rivers, and other bodies of water. Avoid discharge to streams, rivers, and other bodies of water.

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CAUTION: Harmful to fish and aquatic life. Avoid discharge to streams, rivers, and other bodies of water. Avoid discharge to streams, rivers, and other bodies of water.

DIRECTIONS FOR USE

Apply to crops that are under stress. Apply to crops that are under stress. Apply to crops that are under stress. Apply to crops that are under stress.

HAZARD TO THE ENVIRONMENT

CAUTION: Harmful to fish and aquatic life. Avoid discharge to streams, rivers, and other bodies of water. Avoid discharge to streams, rivers, and other bodies of water.

FOR CULTURAL USE REQUIREMENTS

Use the product in accordance with the label and the manufacturer's instructions. Use the product in accordance with the label and the manufacturer's instructions. Use the product in accordance with the label and the manufacturer's instructions.

NOTE

The application charges on the Agency's labeling file. The application charges on the Agency's labeling file.

STORAGE AND DISPOSAL

DO NOT CONTAMINATE WATER, FOOD, OR FEEDS

HAZARD TO THE ENVIRONMENT

CAUTION: Harmful to fish and aquatic life. Avoid discharge to streams, rivers, and other bodies of water. Avoid discharge to streams, rivers, and other bodies of water.

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CAUTION: Harmful to fish and aquatic life. Avoid discharge to streams, rivers, and other bodies of water. Avoid discharge to streams, rivers, and other bodies of water.

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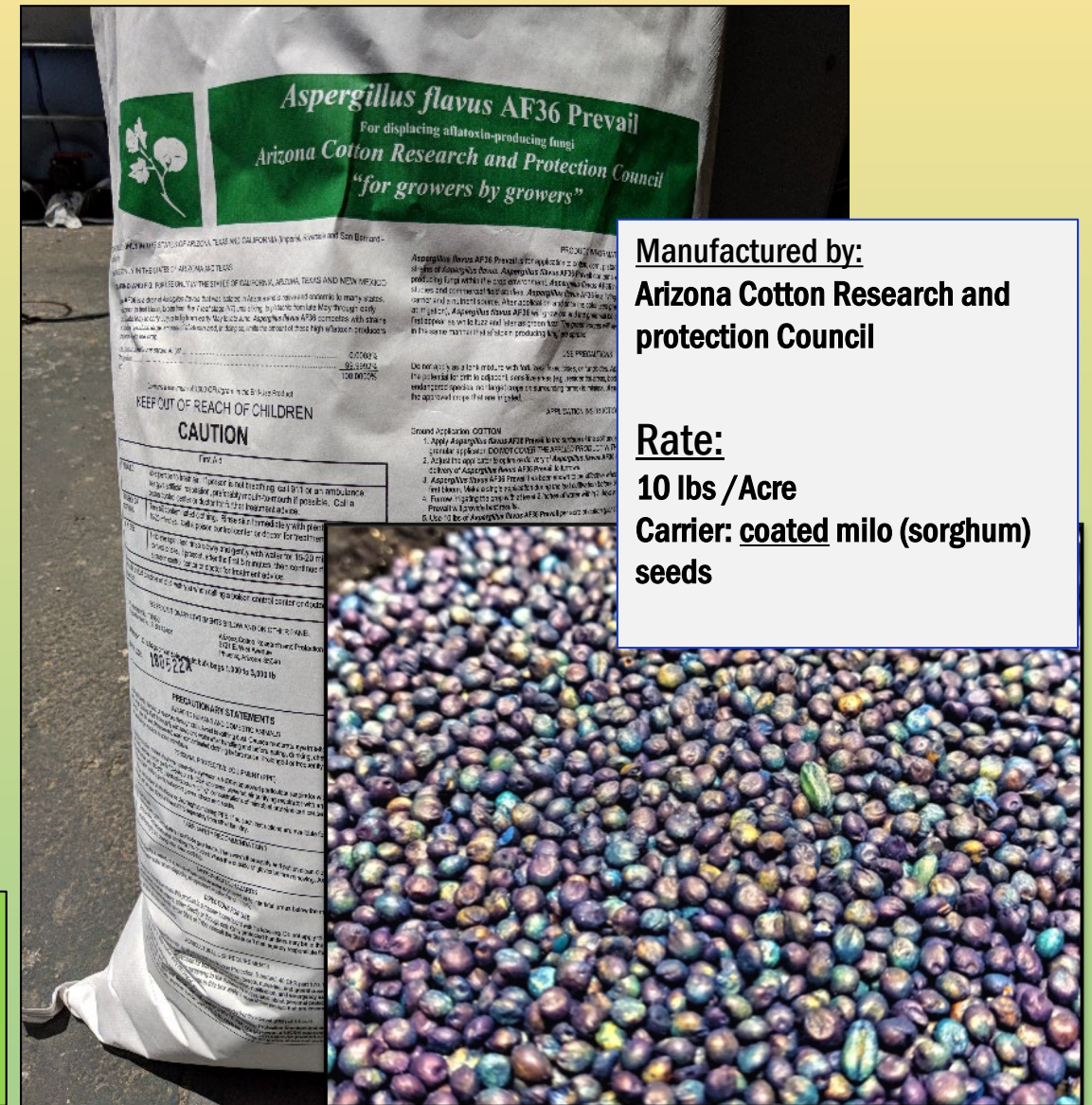
LABELING ACCEPTABLE

STATE OF CALIFORNIA

DEPARTMENT OF PESTICIDE REGULATION

Date: **08/07/2017**

Reg. No. 71693-2-AA



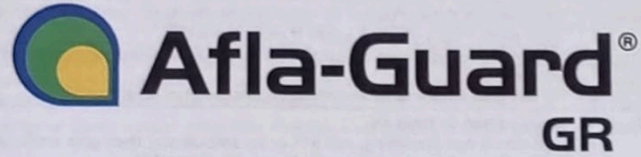
Manufactured by:

Arizona Cotton Research and Protection Council

Rate:

10 lbs / Acre

Carrier: coated milo (sorghum) seeds



For agricultural use to displace aflatoxin-producing fungi in almonds, peanuts, pistachios, and corn



Active Ingredient:
Aspergillus flavus
 NRRL 21882* 0.0094%

Other Ingredients: 99.9906%

Total: 100.0000%

*Contains a minimum of 1.2 x 10⁸ CFU/lb of product

**KEEP OUT OF REACH OF CHILDREN
CAUTION**

See additional precautionary statements and directions for use on the container label to the right.

EPA Reg. No. 100-1469
EPA Est. No. 5905-GA-001
Manufactured for:
Syngenta Crop Protection, LLC
P. O. Box 18300
Greensboro, North Carolina 27419-8300
SCP 1469A-L2B 0321

Not for sale or use after _____

Lot No.: _____

2000 pounds
Net Weight

PRODUCT ID
43768

Active Ingredients:

***Aspergillus flavus* NRRL 21882**

Contains 0.0094% active ingredient with a minimum of 1.2 x 10⁸ CFU/lb

Registered for agricultural use to displace aflatoxin-producing fungi in almonds, pistachios, peanuts, and corn

Manufactured for:

Syngenta Crop Protection, LLC

P.O. Box 18300

Greensboro, North Carolina 27419-8300

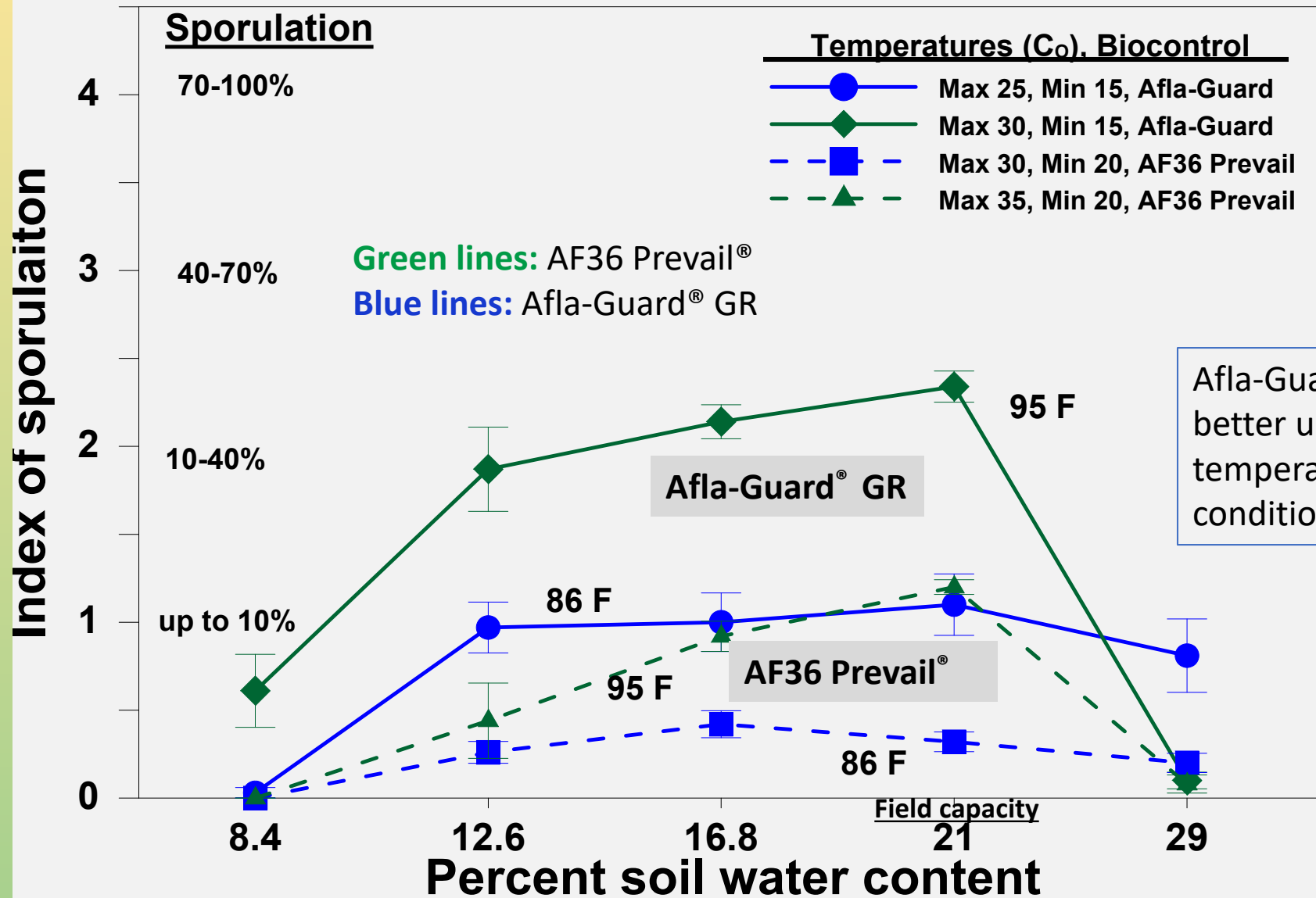
Rate:

10 lbs / Acre

Carrier: inoculated barley seeds



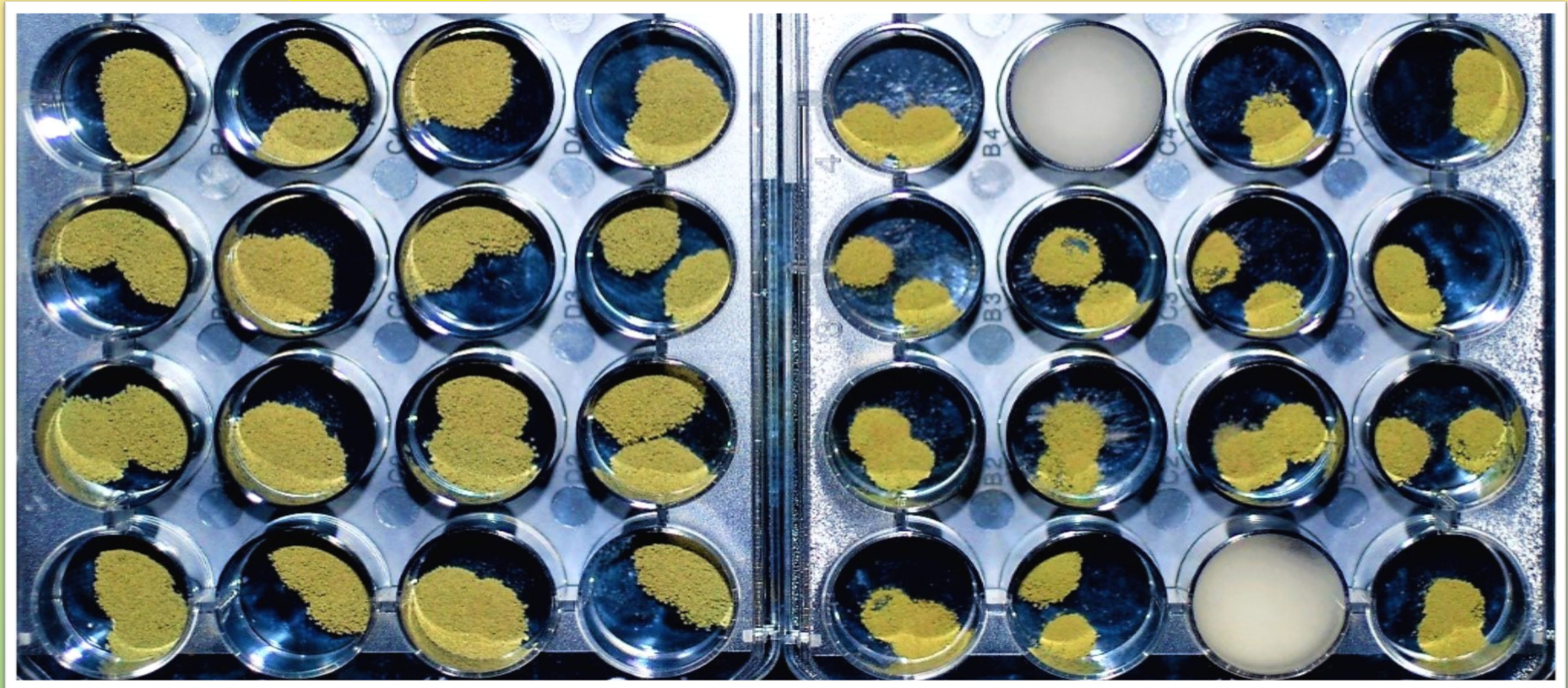
Sporulation of Afla-Guard® GR and AF36 Prevail® different soil moistures and temperatures



Sporulation of the two biological agents after a week incubation at 96.5% RH and 30°C (86 °F)

Afla-Guard® GR

AF36 Prevail®



Sporulation of the two biological agents after a week incubation at 91% RH and 30°C (86 °F)

Afla-Guard® GR

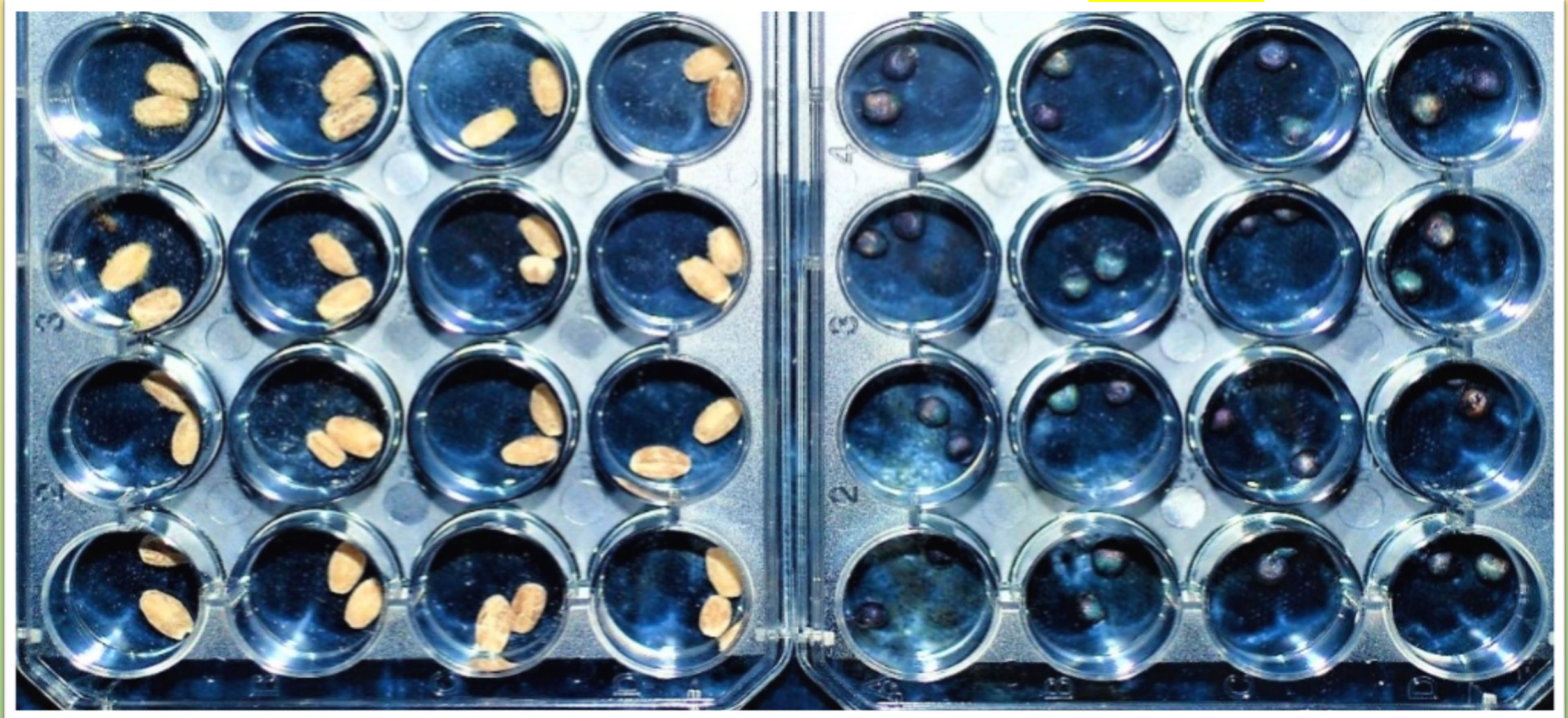
AF36 Prevail®



Sporulation of the two biological agents after a week incubation at 84.5% RH and 30°C (86 °F)

Afla-Guard® GR

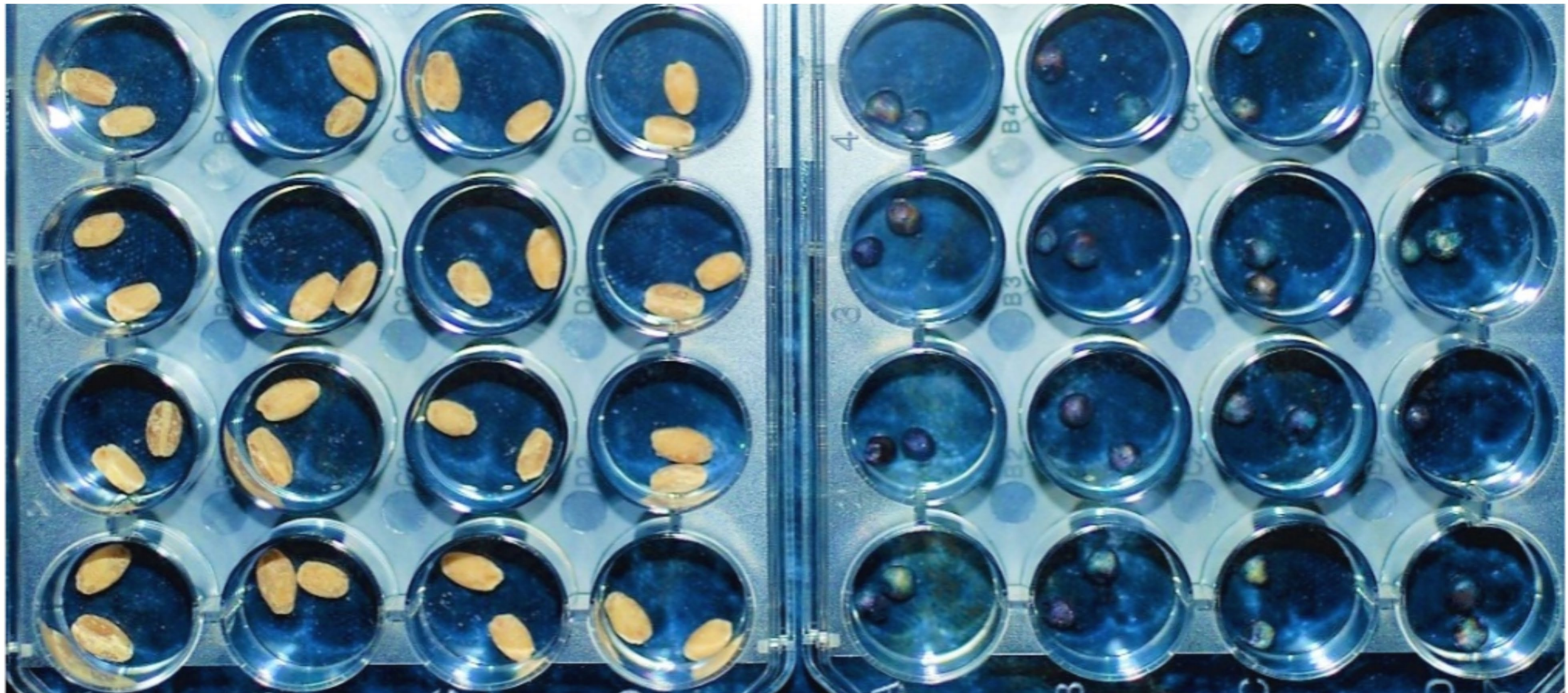
AF36 Prevail®



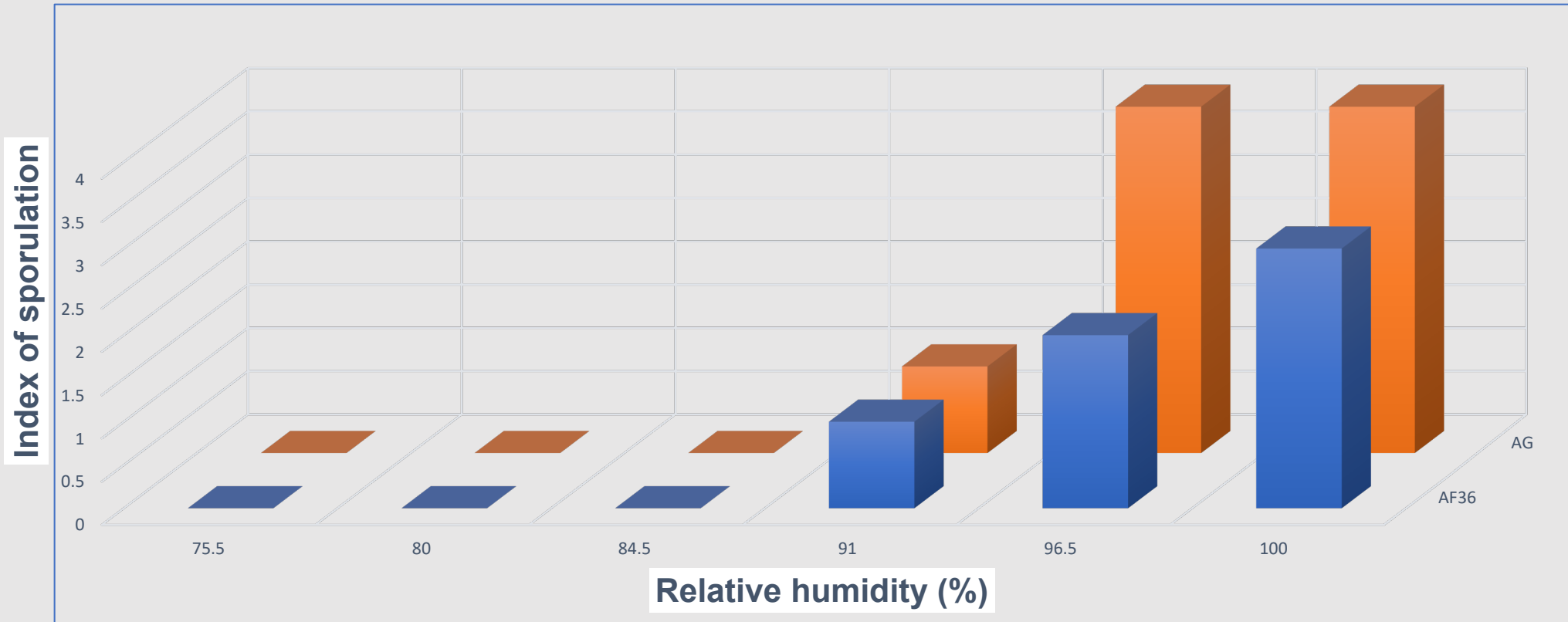
Sporulation of the two biological agents after a week incubation at 80% RH and 30°C (86 °F)

Afla-Guard® GR

AF36 Prevail®



Sporulation of the two biological agents after a week incubation at different RHs and at 30°C (86 °F)



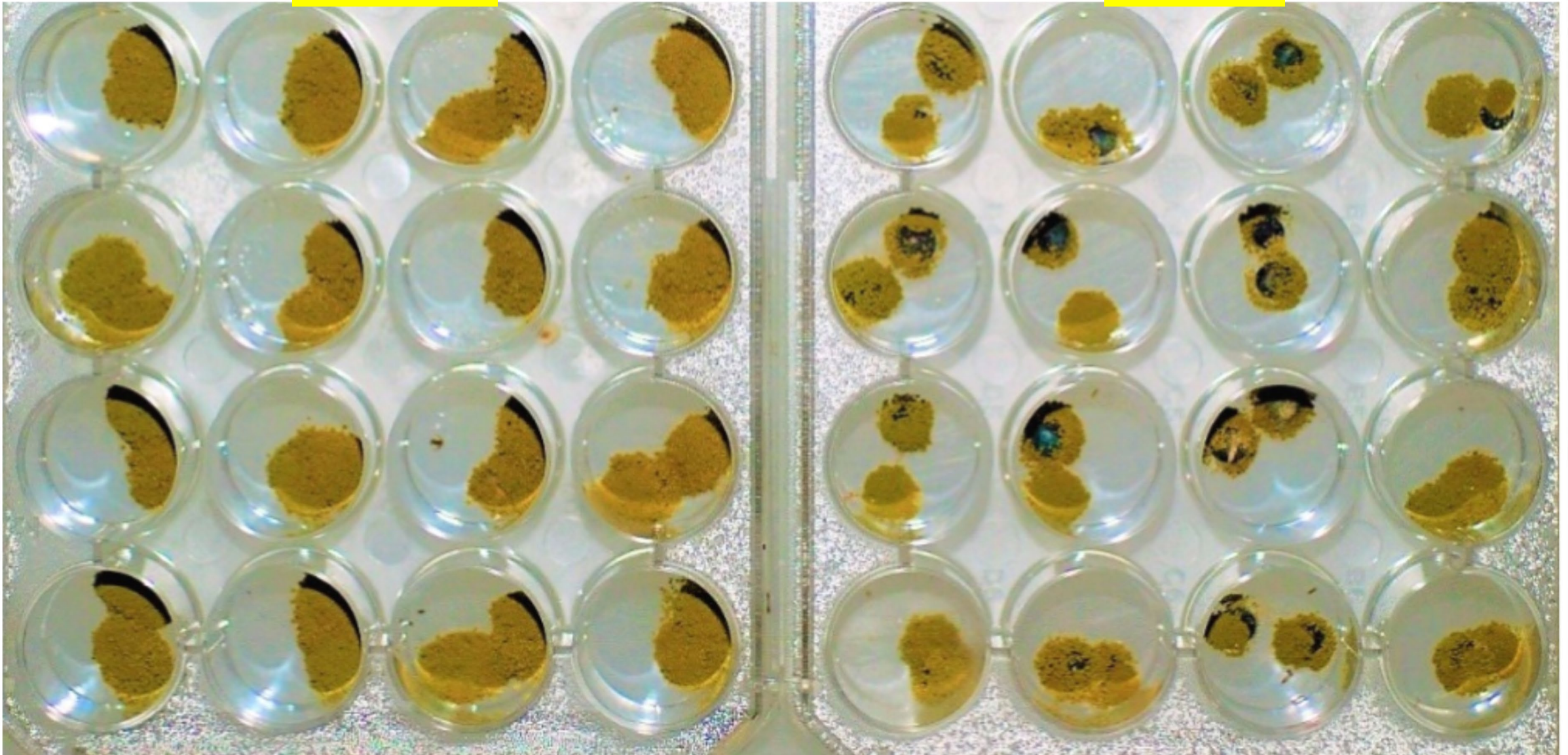
AF36 Prevail® Afla-Guard® GR

0=no sporulation; 1 =25%; 2=50%; 3=75%; & 4=100% sporulation

Sporulation of the two biological agents after 1 week incubation at 100% RH, at 30 C (86 F) following a transfer from 84.5% RH

Afla-Guard® GR

AF36 Prevail®



**A challenge is the predation of seeds by insects, birds, and decay by other fungi;
reduction of inoculum will reduce spore density**



AF36 Prevail® sporulated



AF36 Prevail® intact



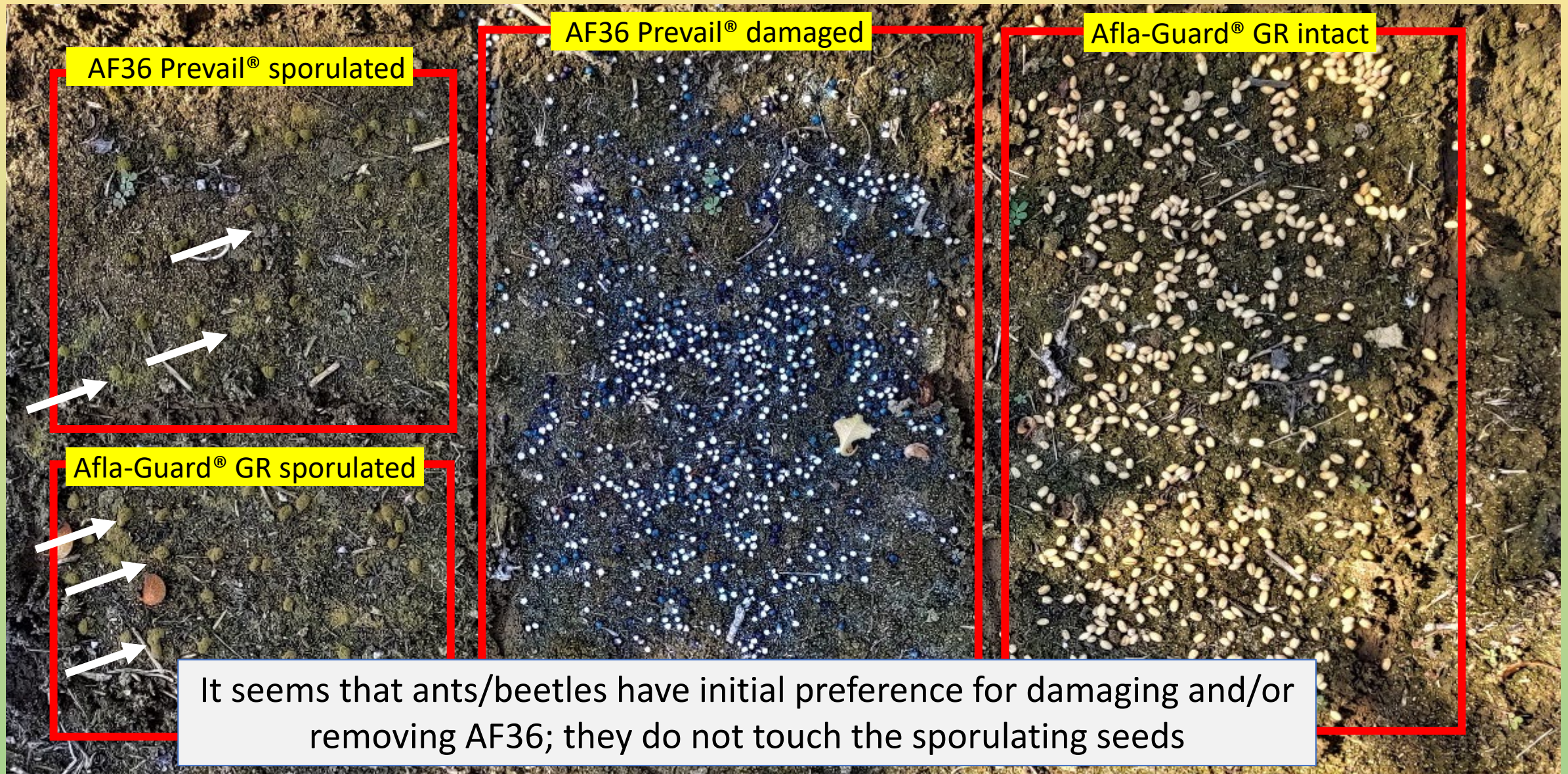
Afla-Guard® GR intact



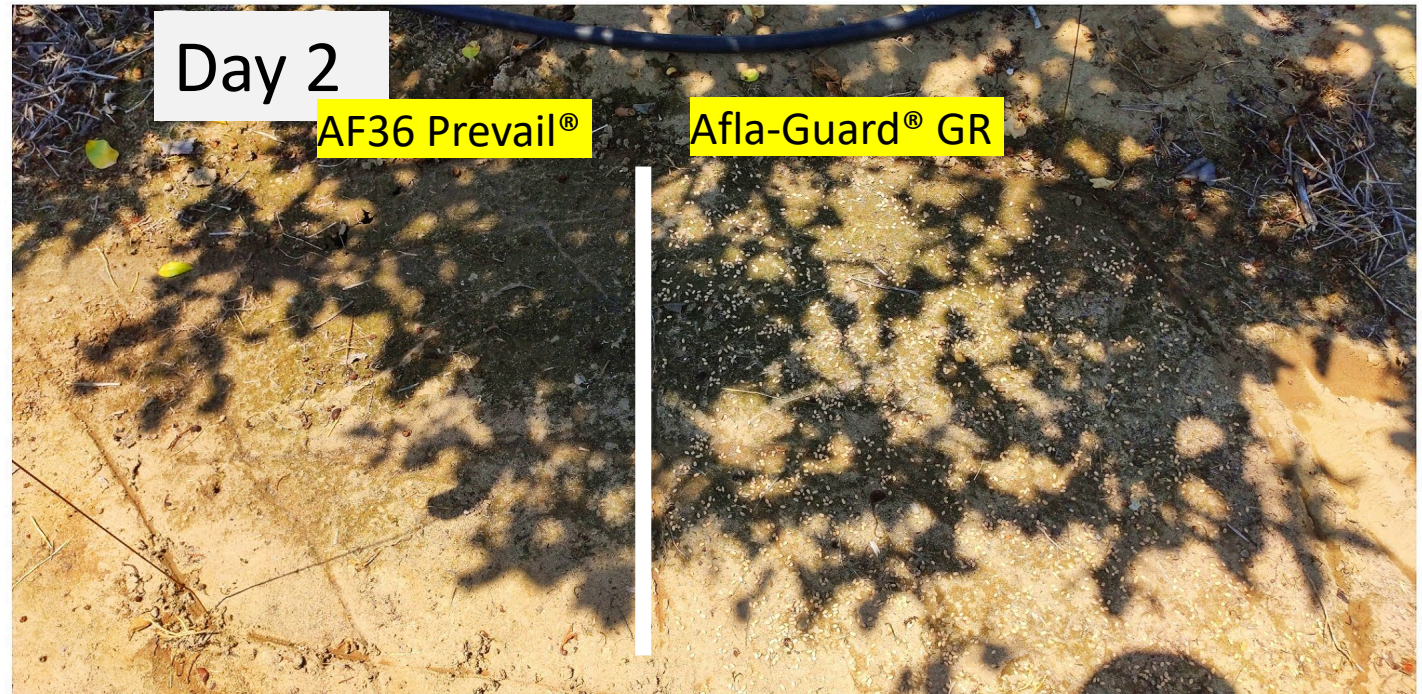
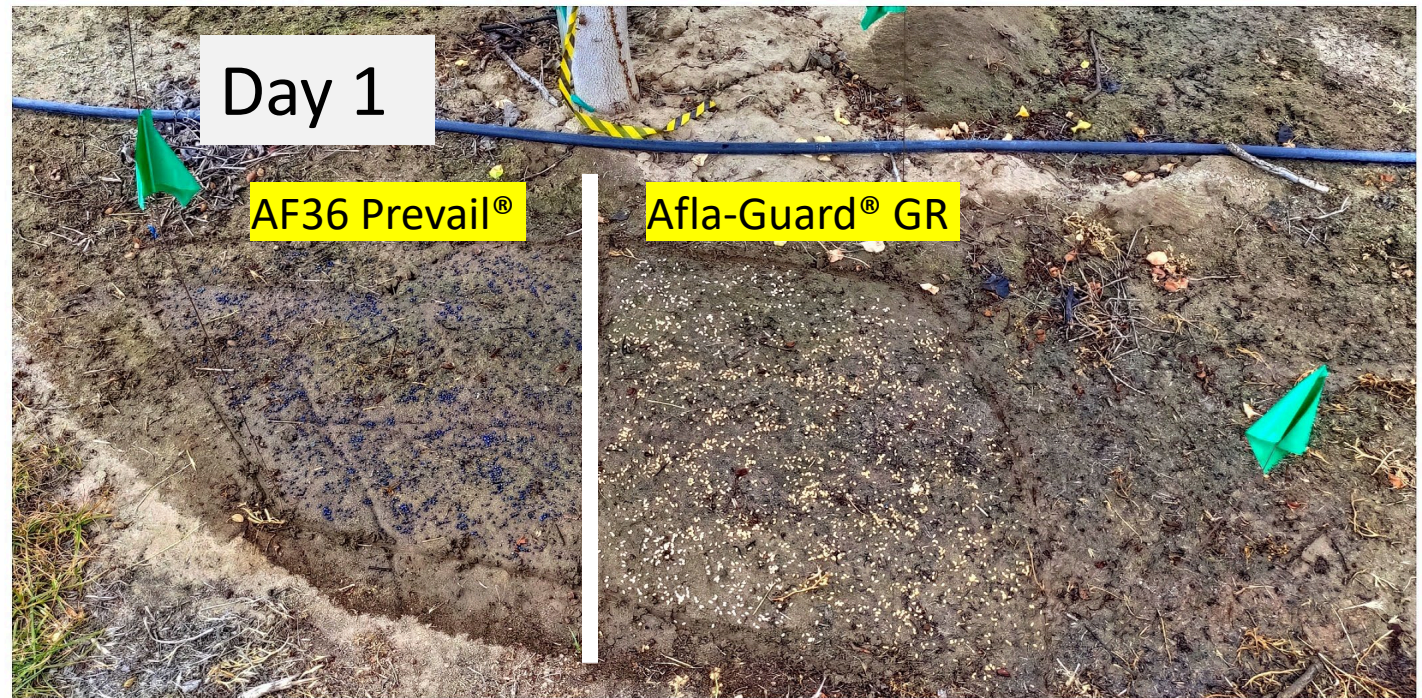
Afla-Guard® GR sporulated



Predation of the two products 24 hours after application in the field



**Ants removed all the AF36
Prevail seeds within 24 hours**



Tools to manage aflatoxins in pistachios:



1. Choose the right rootstock to reduce ES
2. Do not stress the trees for water in stage 1 (May) to reduce ES
3. Remove mummies – orchard sanitation – “mummy shake”...
4. Reduce NOW damage of the crop in season
5. Apply AF36 Prevail® or Afla-Guard® GR on late May to mid July at 10 lbs./acre
6. Irrigate before or immediately after application of the biological agent
5. Do not spray herbicides 1 to 2 weeks after application
6. Control the ants, other arthropods, and birds in the orchard
7. Sort out stained, suture-stained, and DBOM nuts



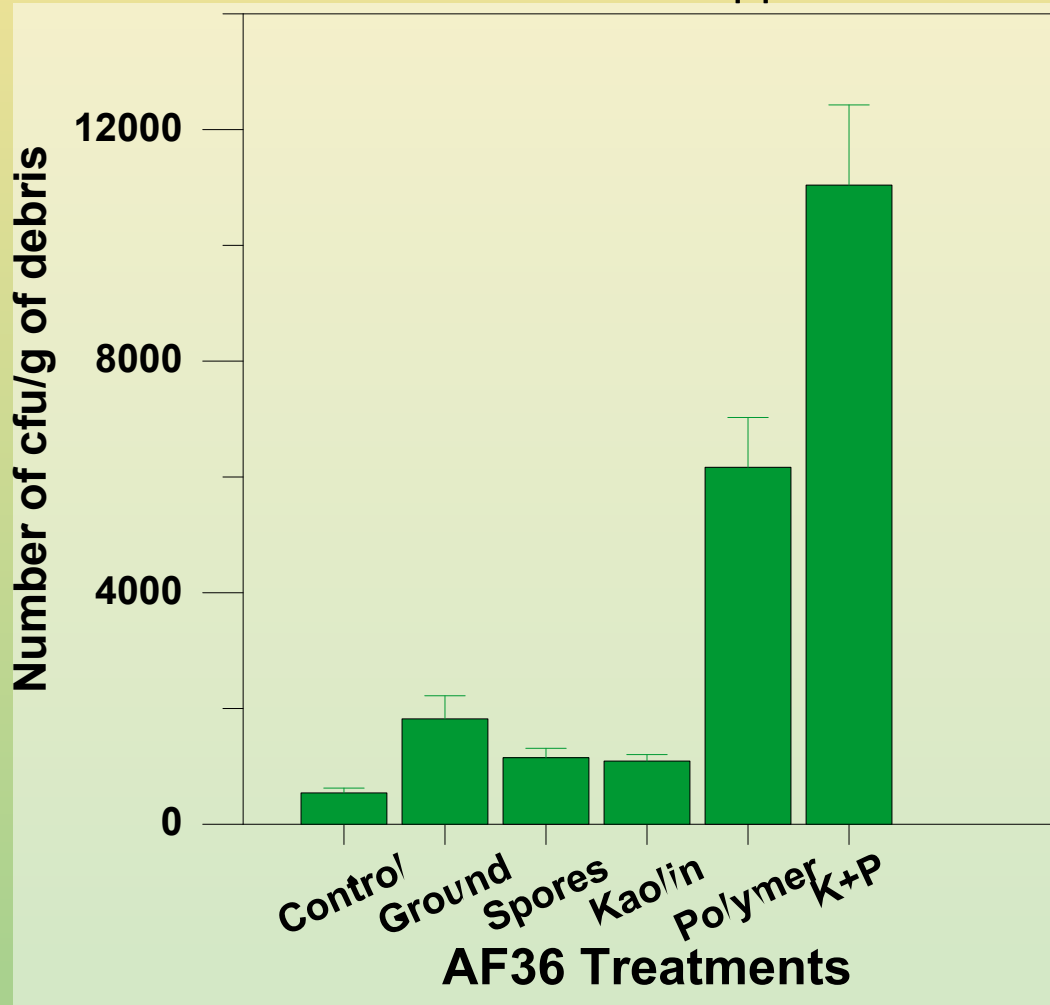
Acknowledgments:
California Pistachio
Research Board, CDFA,
Wonderful orchards,
pistachio growers, and
Syngenta Co.

Special thanks to my
lab crew.

Thank you

Microflora /colonization of NOW-damaged, ES, and normal nuts as affected by the method of AF36 application (ground or canopy)

2019 – Debris 9 months after application



2021 – One month after application

