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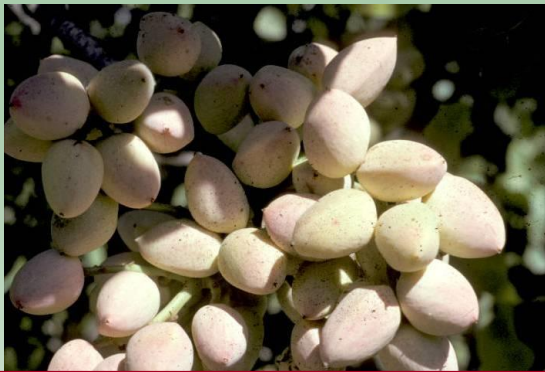
AF36 in Almonds – Another Grower Tool for Aflatoxin Control in the Orchard

Themis Michailides

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& Dr. Peter Cotty

USDA/ARS & University of Arizona, Tucson, AZ



AF36 Registration in Feb 2012

- **Acreage:** 330,000 acres
- **In production:** 250,000 acres
- **Production:** 690 million lbs. (= 313,500 tons of in shell pistachios)



AF36 Registration in Aug 2017

- **Acreage:** 1,110,000 acres
- **In production:** 900,000 acres
- **Production:** 2,050 million lbs. (= 930,000 tons of kernels)



AF36 Registration in Aug 2017

- **Acreage:** 7,500 acres
- **In production:** 7,000 acres
- **Production:** 21,000 lbs (= 9,560 tons of dried figs)

Molds that can produce aflatoxin in almond (also in pistachio and fig) orchards in California



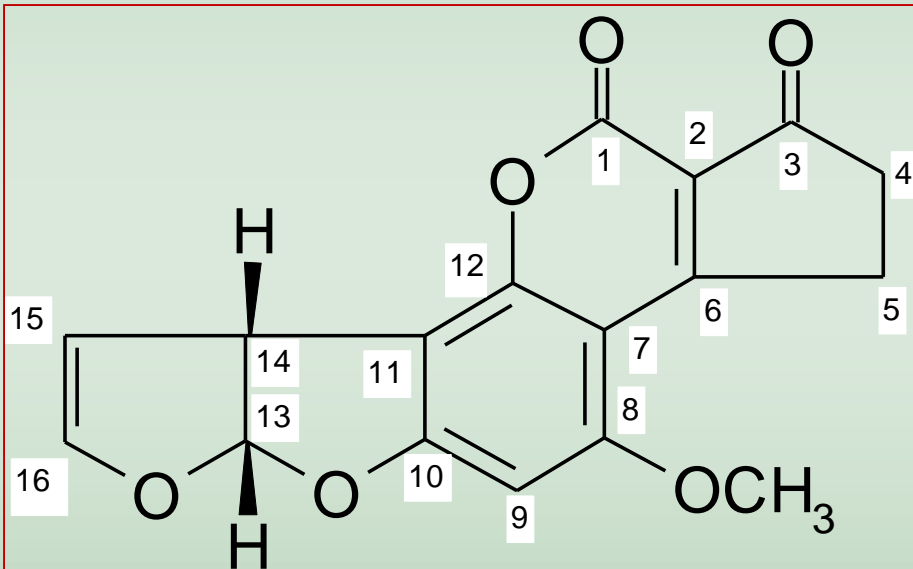
Aspergillus flavus



Aspergillus parasiticus

Aspergillus flavus and *A. parasiticus* produce:

Aflatoxins **B**₁, B₂, G₁, G₂, M₁



Aflatoxin B₁

B₁ is the most potent aflatoxin; it can cause liver cancer

Frequency and tolerance limits of aflatoxin contamination in California almonds

Frequency: 1 nut in 32,000 nuts

Regulatory limits for aflatoxins

- USA
Aflatoxin B1 → 10 ppb
Total aflatoxins → 15 ppb
- European Union
Aflatoxin B1 → 8 ppb
Total aflatoxins → 10 ppb

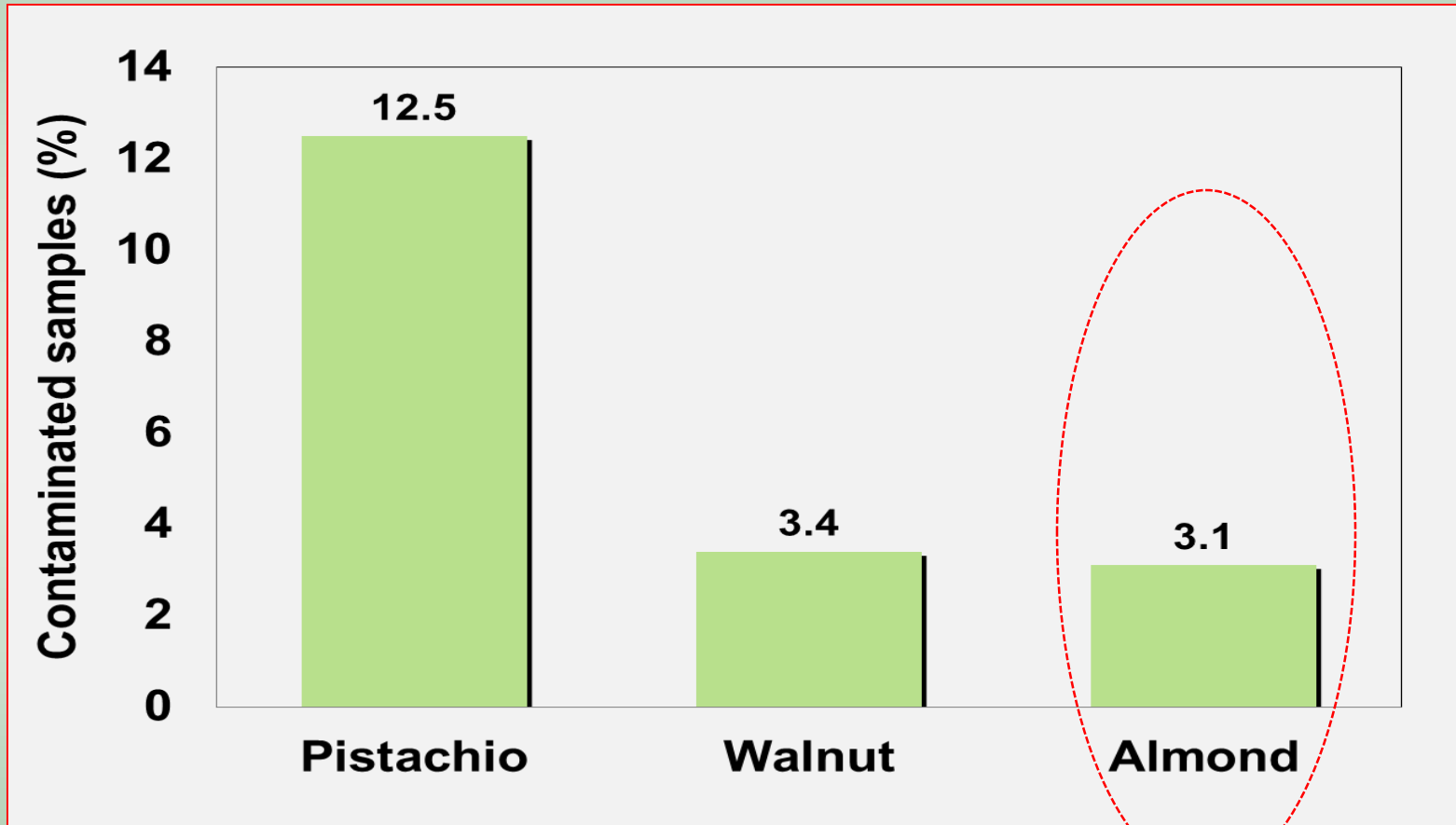
(in almonds for direct consumption)

... in milk: 0.5 ppb

In almonds "further processed": 12 ppb B1; & 15 total aflatoxin

Nut samples contaminated with aflatoxins

(data of Dried Fruit Association in Fresno from 1985-1989 aflatoxin analyses)



Aflatoxin contamination of almonds

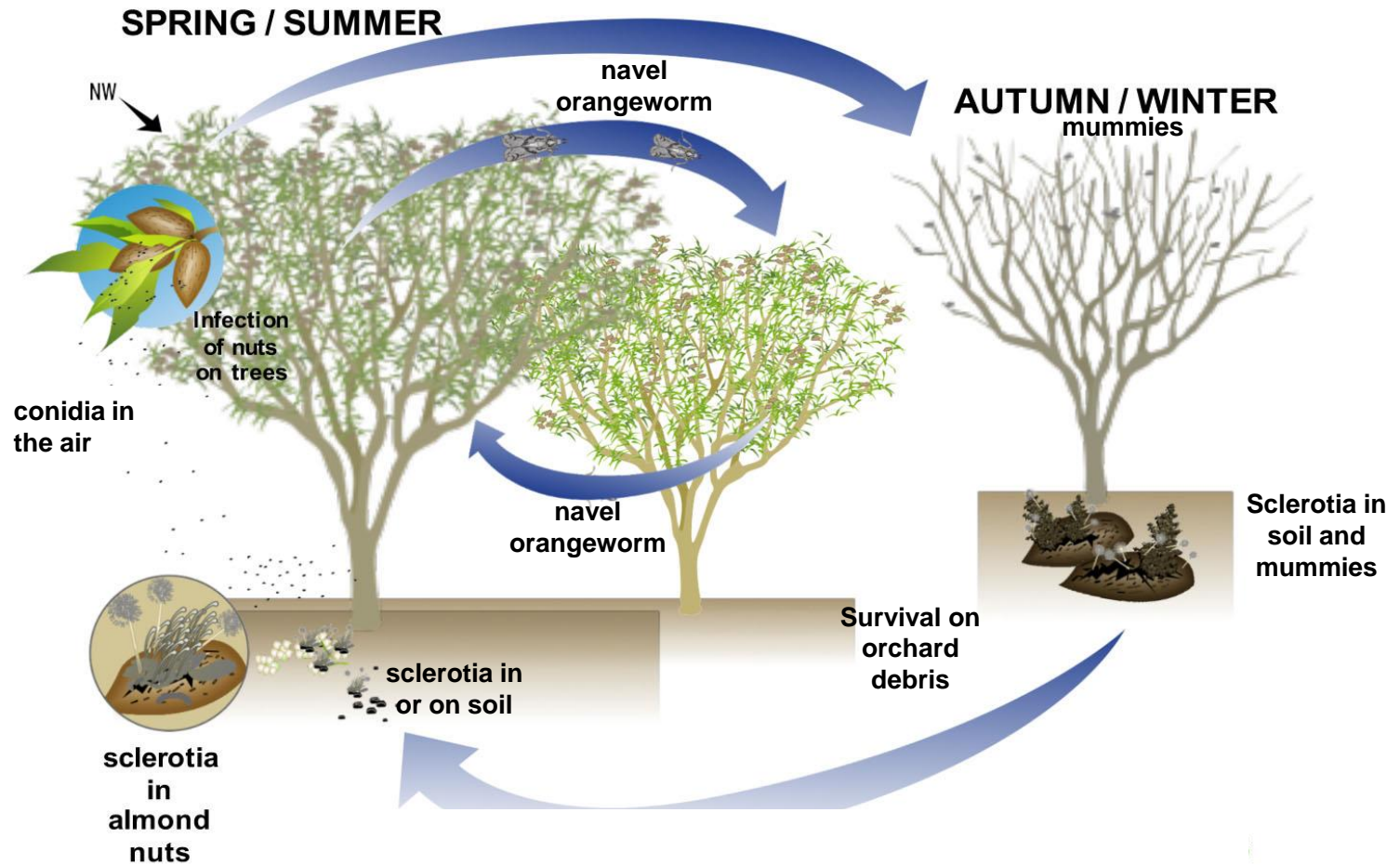
Preharvest vs. Postharvest?

(if almond nuts are dried quickly after harvest, stored properly, and kept dried).

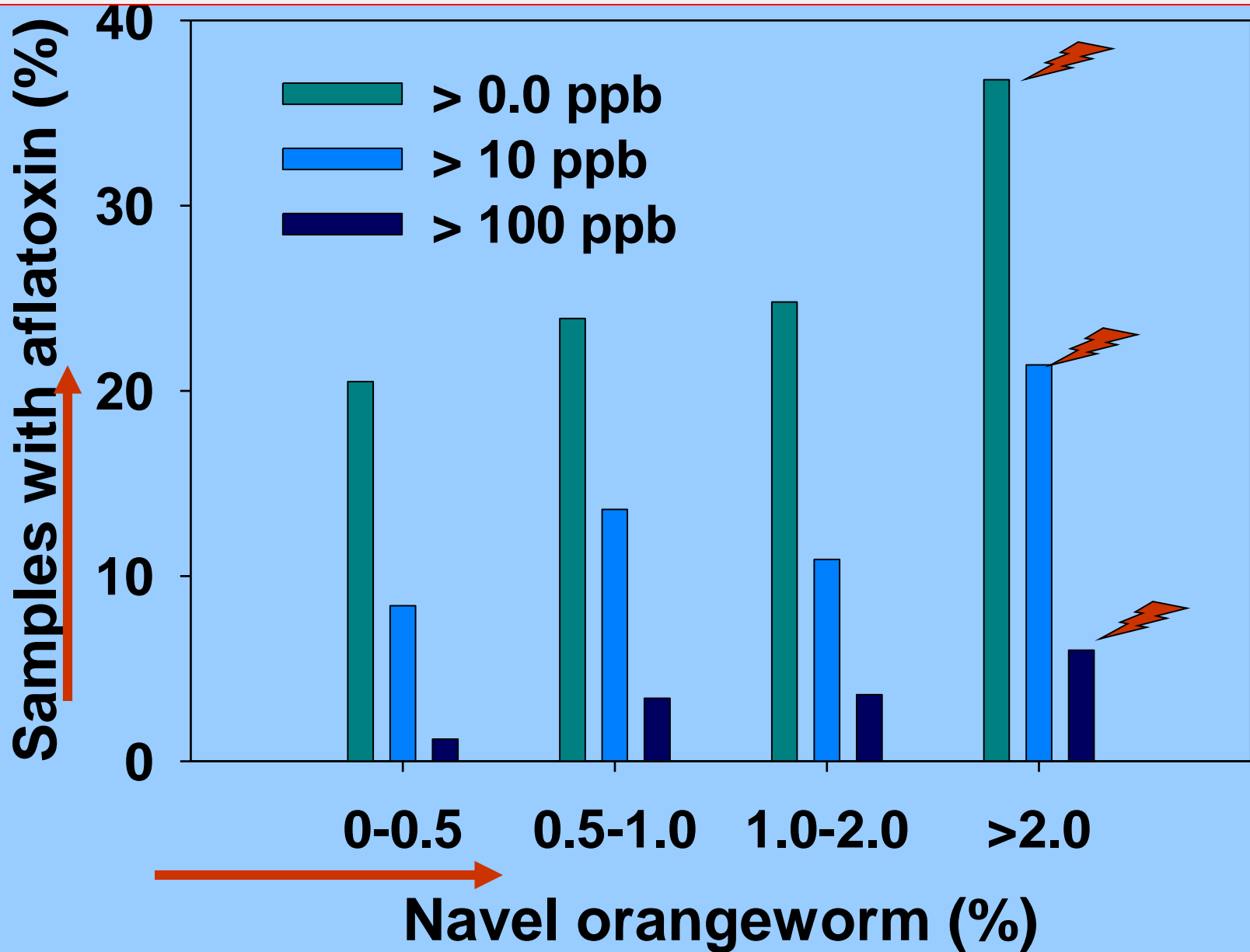
□ Preharvest problem!

□ Leaky stockpiles; no proper storage, etc... → Postharvest

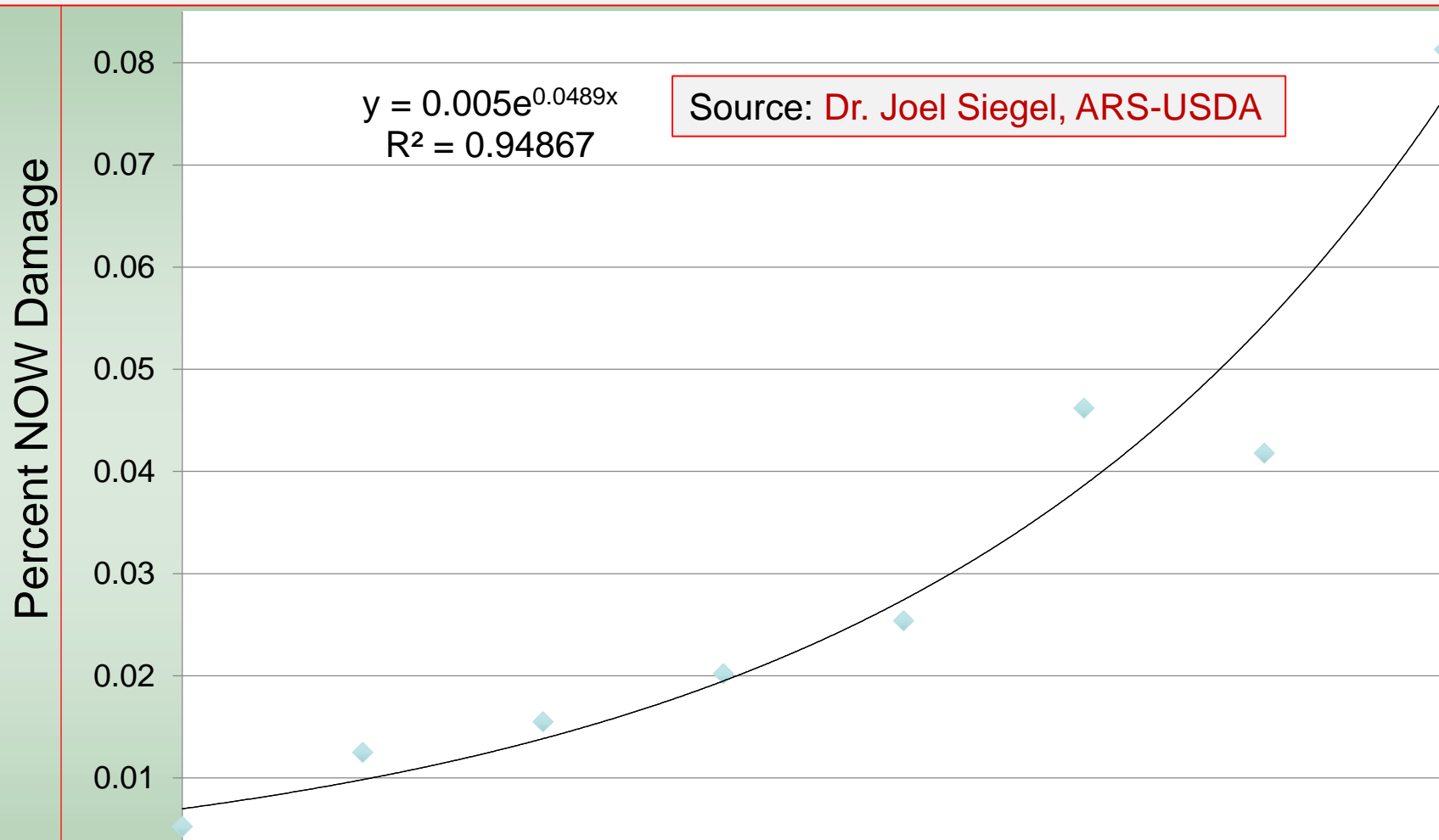
Life cycle of *Aspergillus flavus* in almond orchards



Relationship of navel orangeworm infestation and aflatoxin levels



Kings County 2016: Regression of Average Weekly NOW damage over time (Day 7 = August 28)



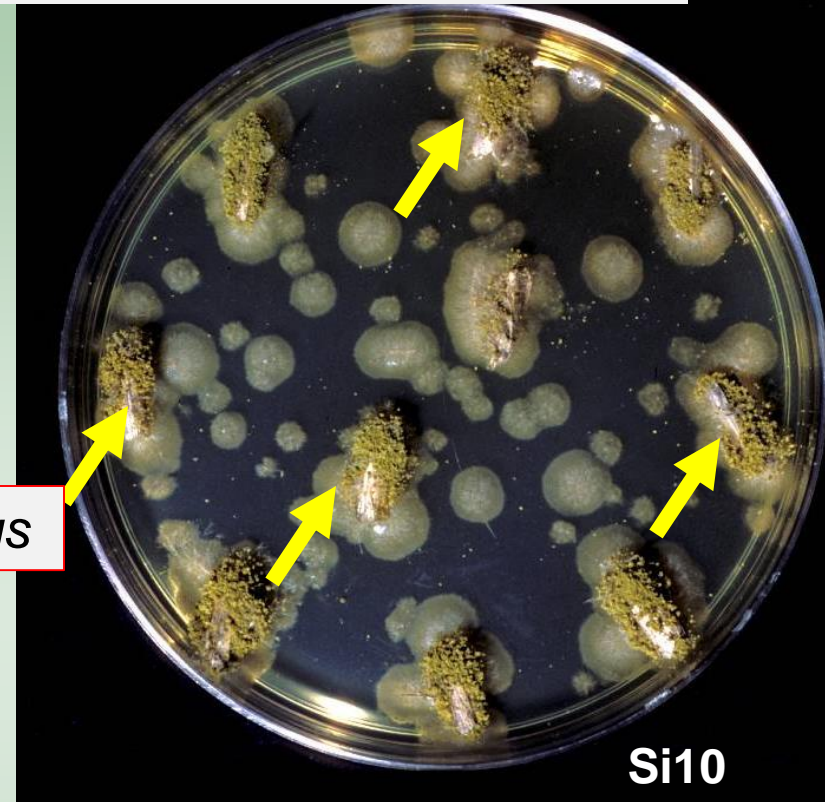
In general, the damage of NOW you have after the first week of harvest will be doubled following the third week of harvest (according to Dr. Joel Siegel)

Association of NOW with aflatoxigenic fungi



Sticky traps with NOW moths

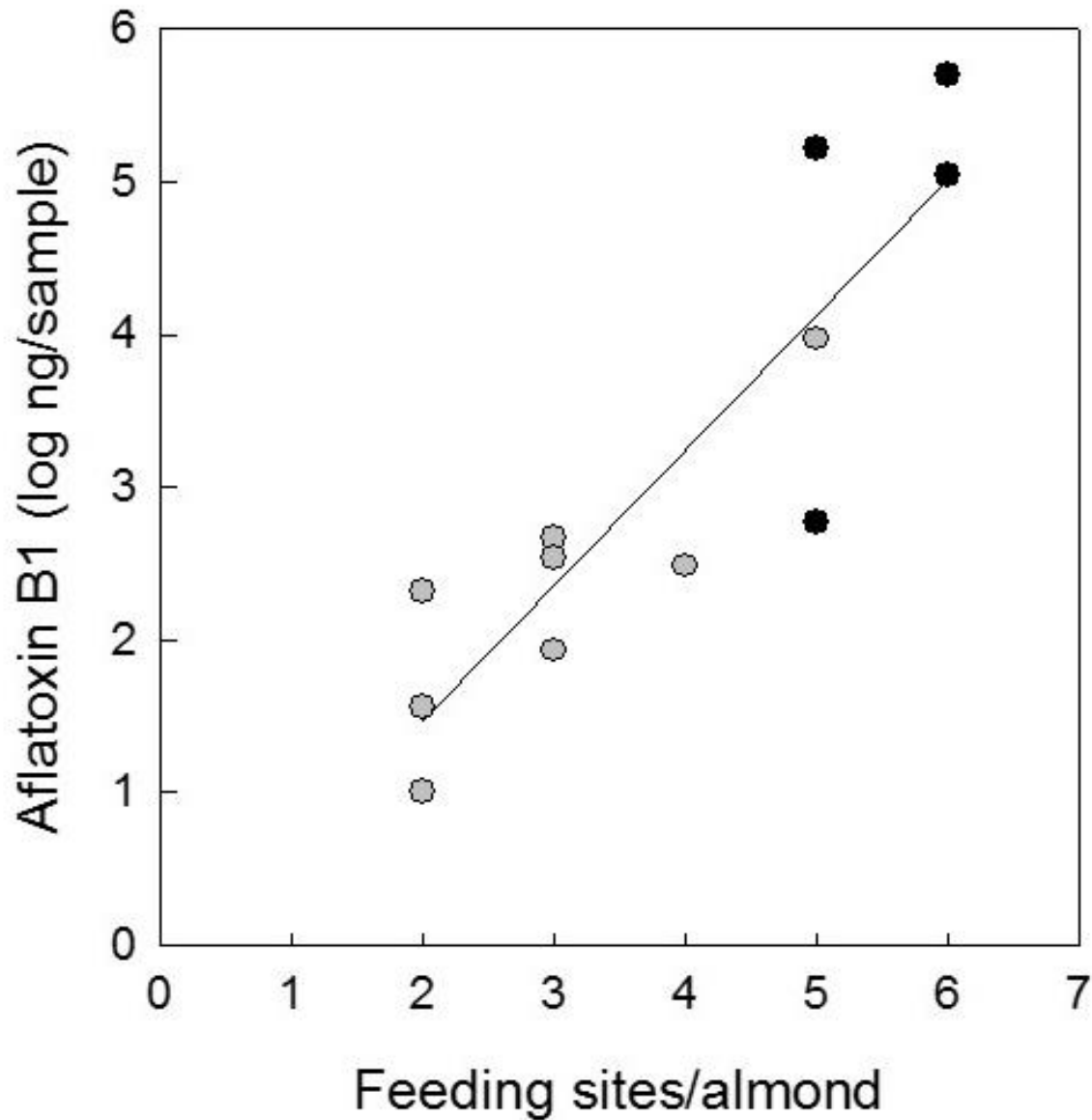
A. flavus



Si10



Effect of feeding sites (wounds) in almond on levels of aflatoxin contamination



Sanitation

Sanitation

Sanitation

Aspergillus flavus in almond orchards




S strain
(small sclerotia): almost all
toxigenic



L strain
(large sclerotia): 50%:50%
toxigenic:atoxigenic

Delivery of AF36 inoculum for treatment of pistachio orchards in 2012



A man wearing a blue hard hat, sunglasses, a light-colored long-sleeved shirt, and blue jeans is riding a red four-wheeled utility vehicle (ATV) through an orchard. The ATV is equipped with a large, light-colored plastic bucket mounted on the back. The bucket is filled with a yellowish-orange liquid. A red arrow points from a text box to the bucket. The orchard has rows of trees with green leaves, and the ground is dry and dusty. The scene is brightly lit, suggesting a sunny day.

**AF36
Inoculum**



Application rate: 10 lbs. per acre



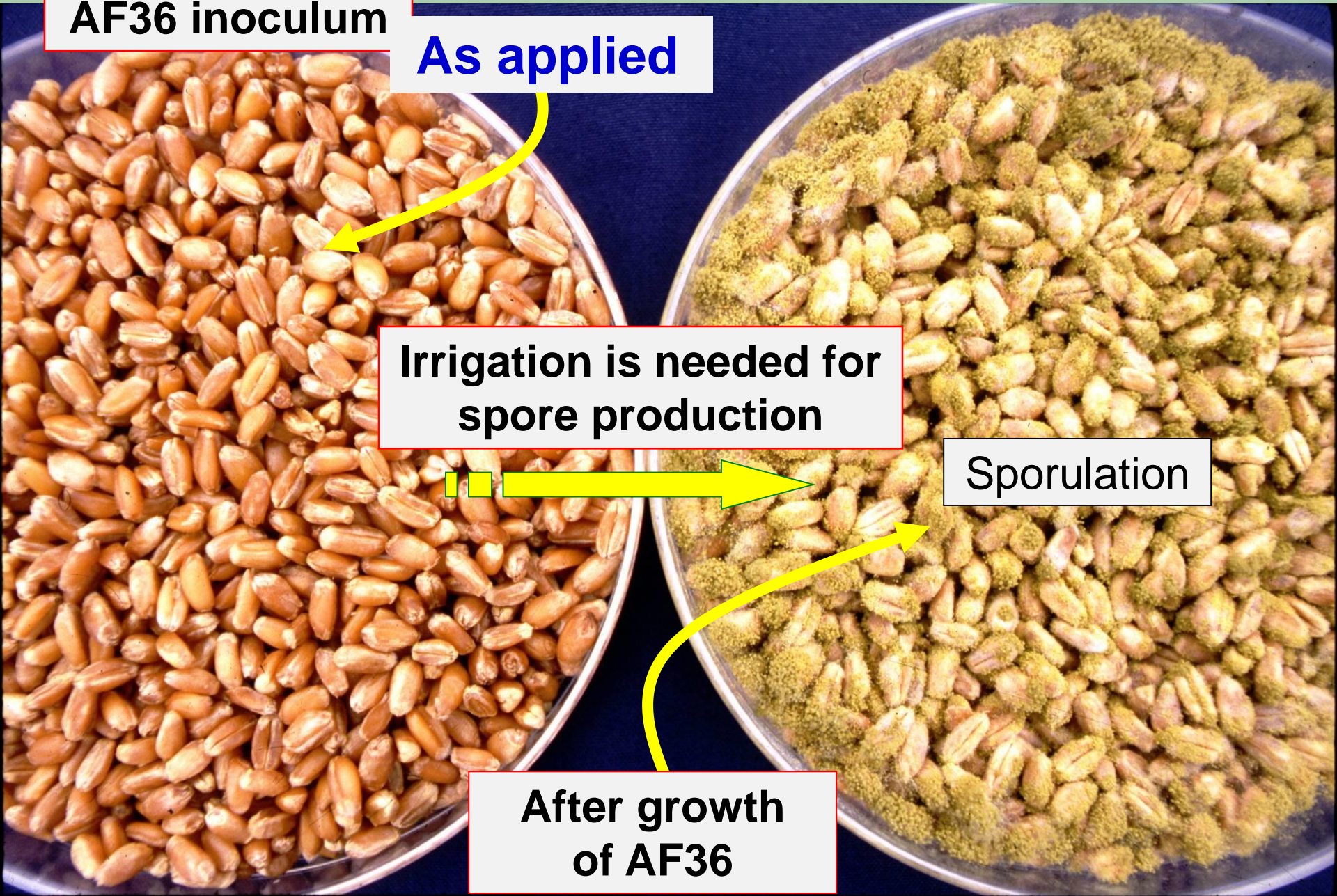
AF36 inoculum

As applied

**Irrigation is needed for
spore production**

Sporulation

**After growth
of AF36**



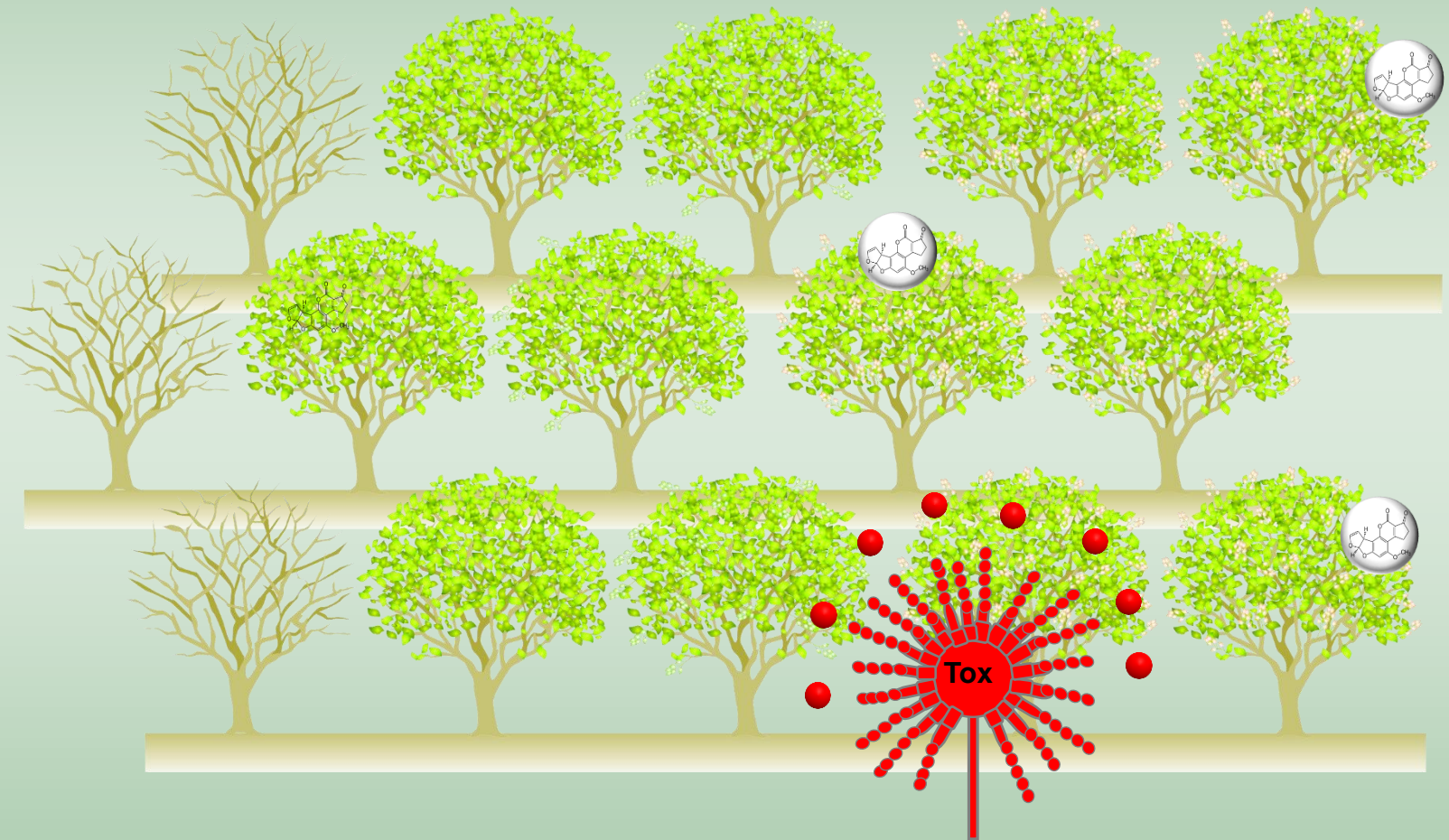
Sorghum is now used as a carrier of AF36 atoxigenic strain



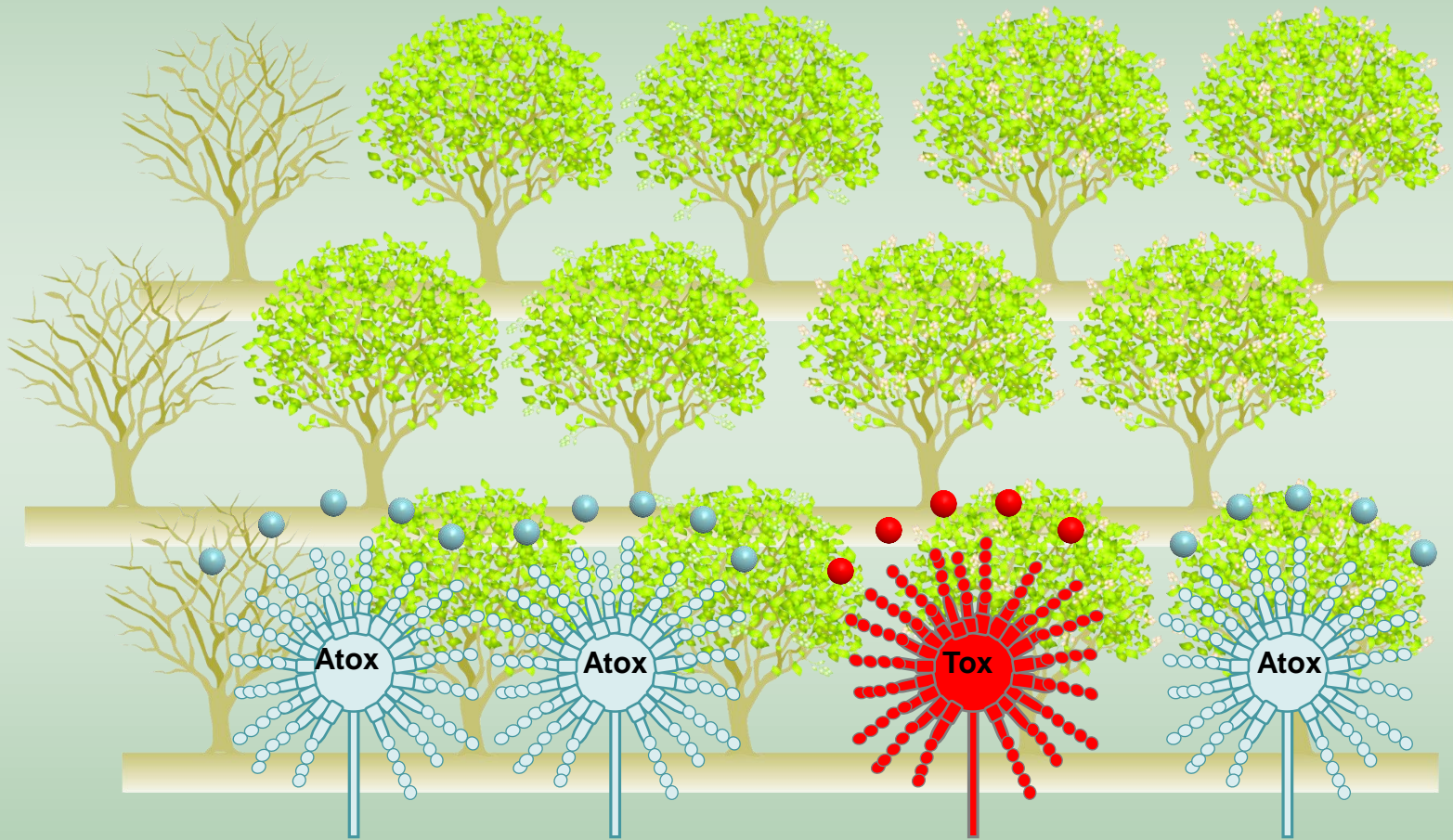
After irrigation, the wet seeds will produce spores of AF36



Non-treated orchard



Treated Orchard

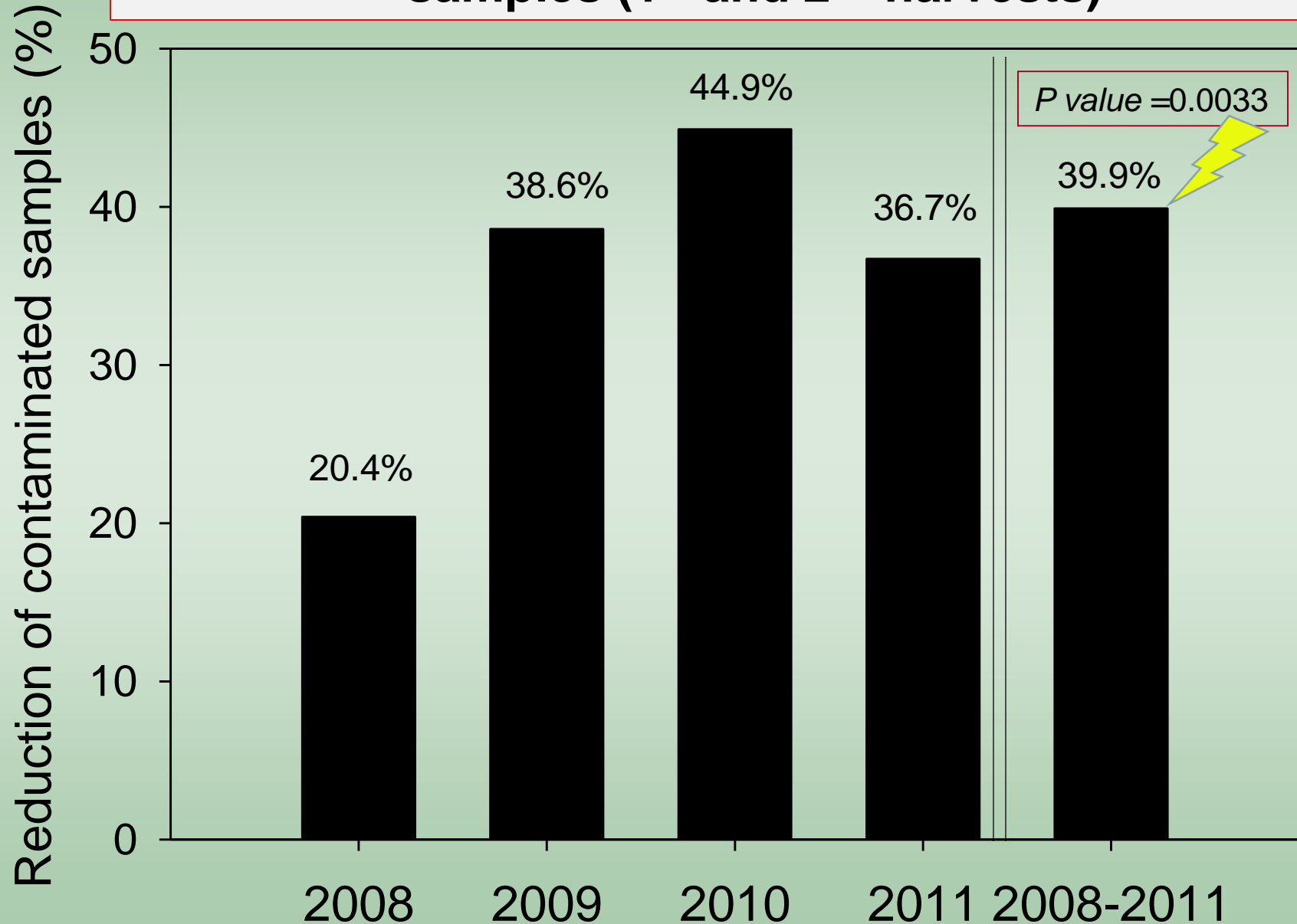


Library samples for aflatoxin analysis



Samples taken at processing plant as nuts are being unloaded.

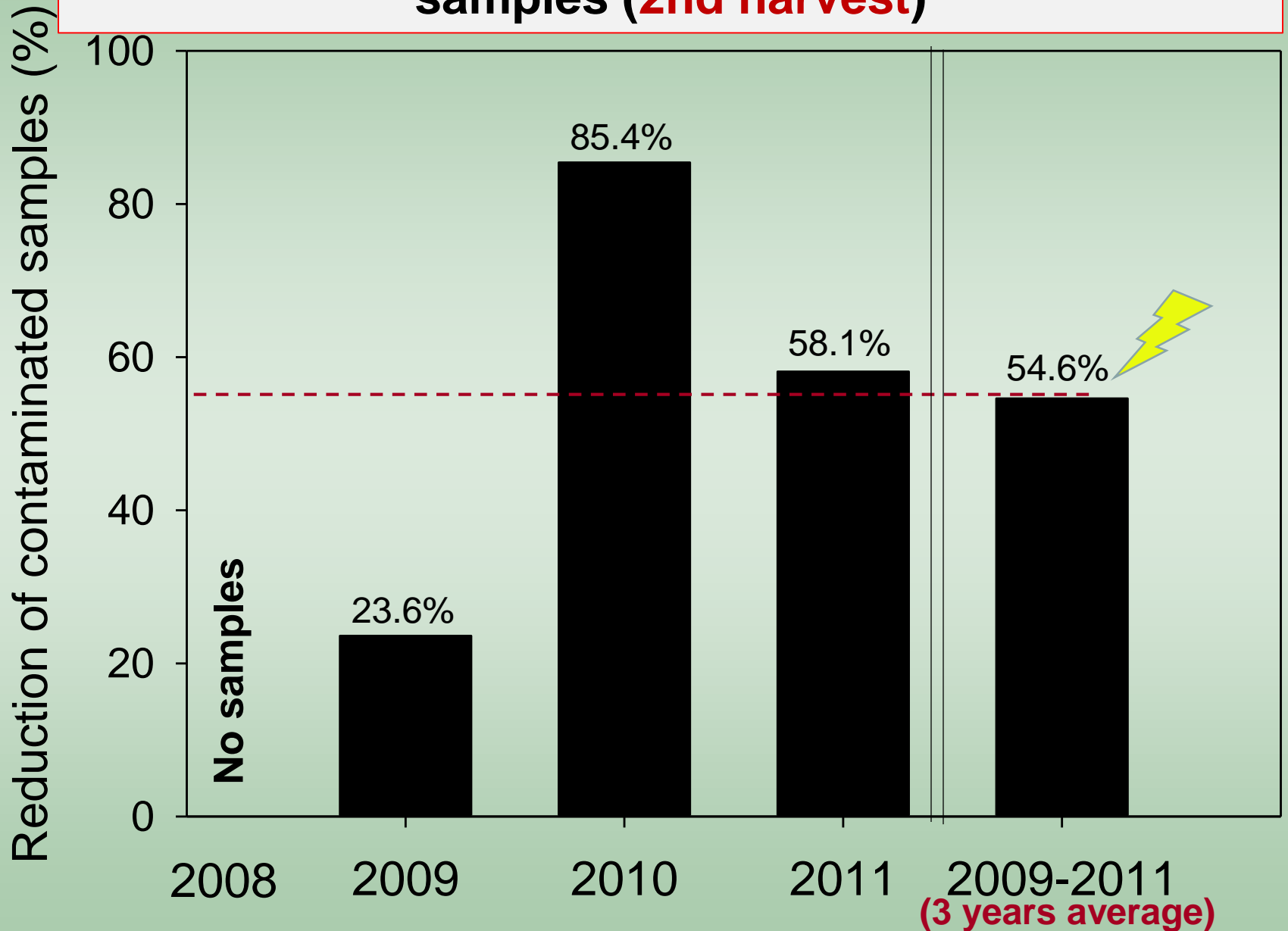
Reduction in aflatoxin-contaminated pistachio samples (1st and 2nd harvests)



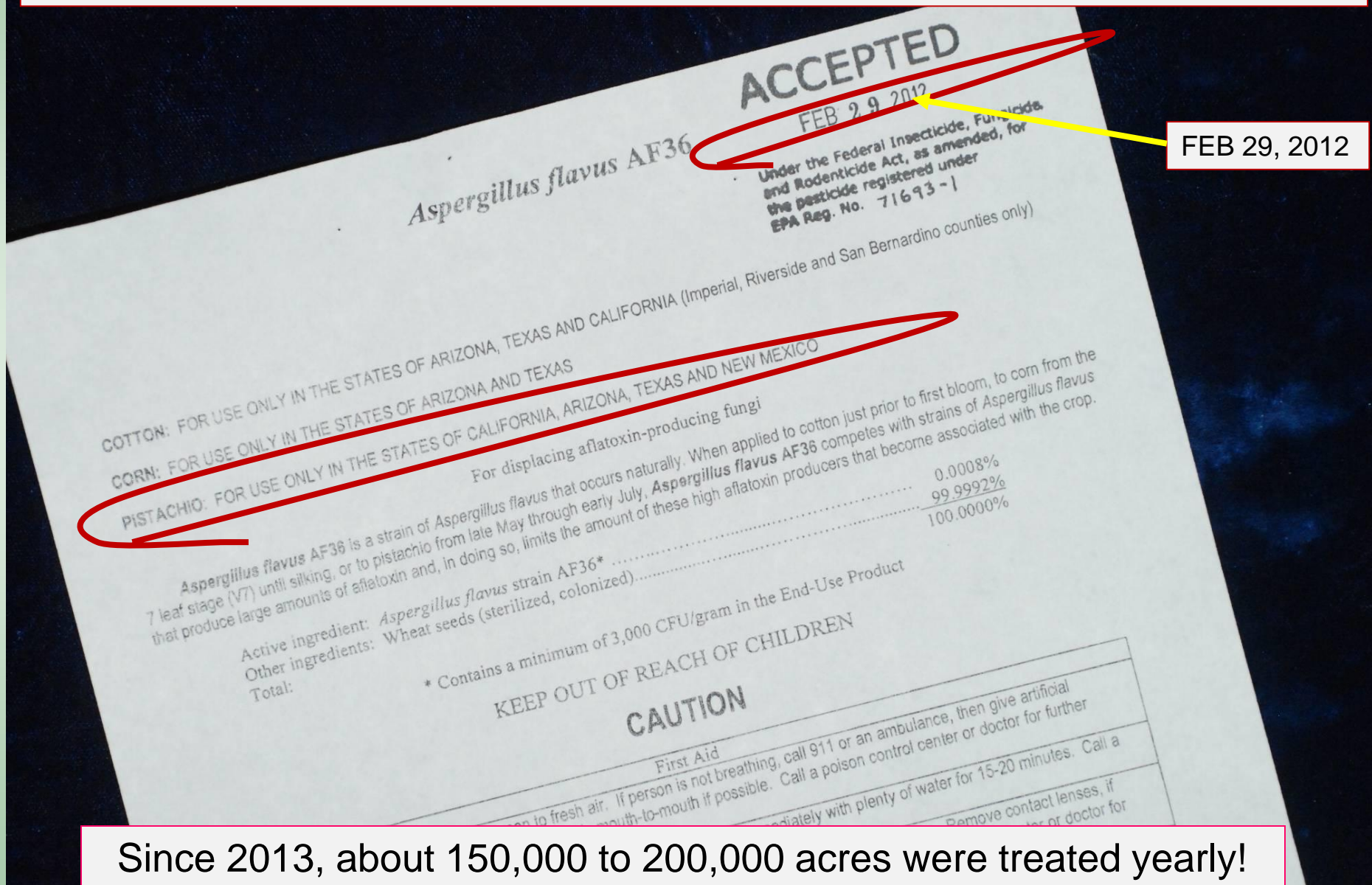
(Doster et al. (2014), Plant Disease 98:948-956)

(4 years average)

Reduction in aflatoxin-contaminated pistachio samples (2nd harvest)



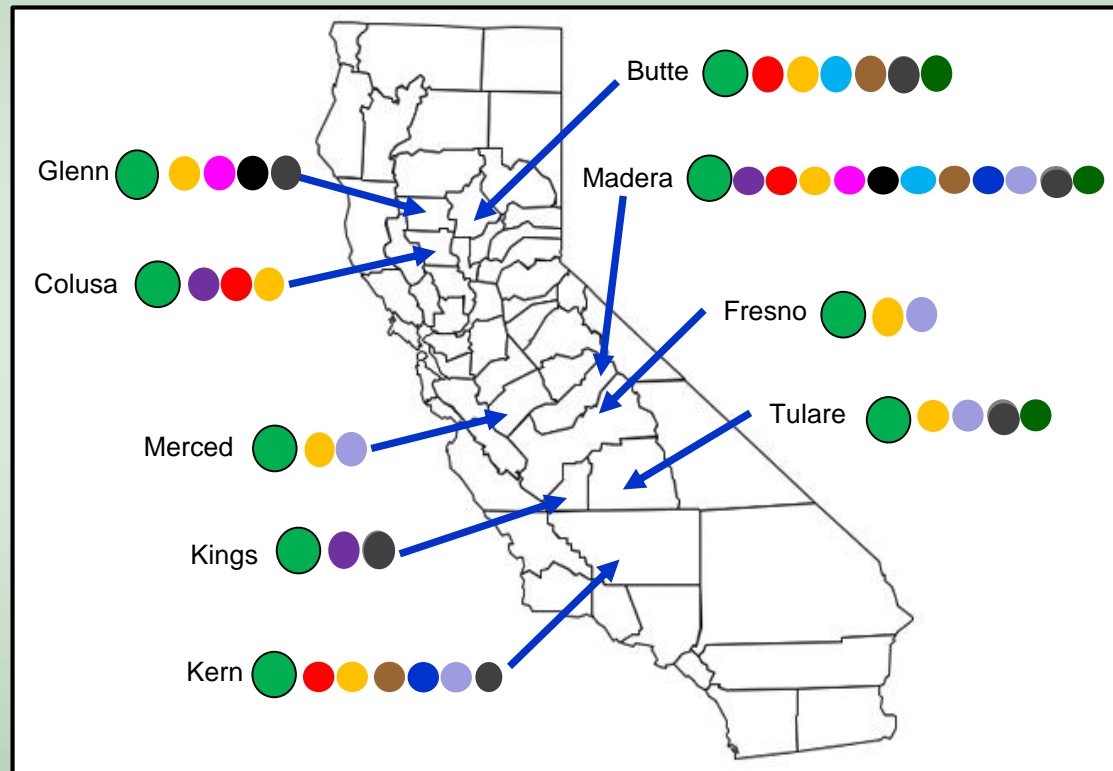
Registration of *Aspergillus flavus* AF36 strain for use in pistachio in 2012



Since 2013, about 150,000 to 200,000 acres were treated yearly!

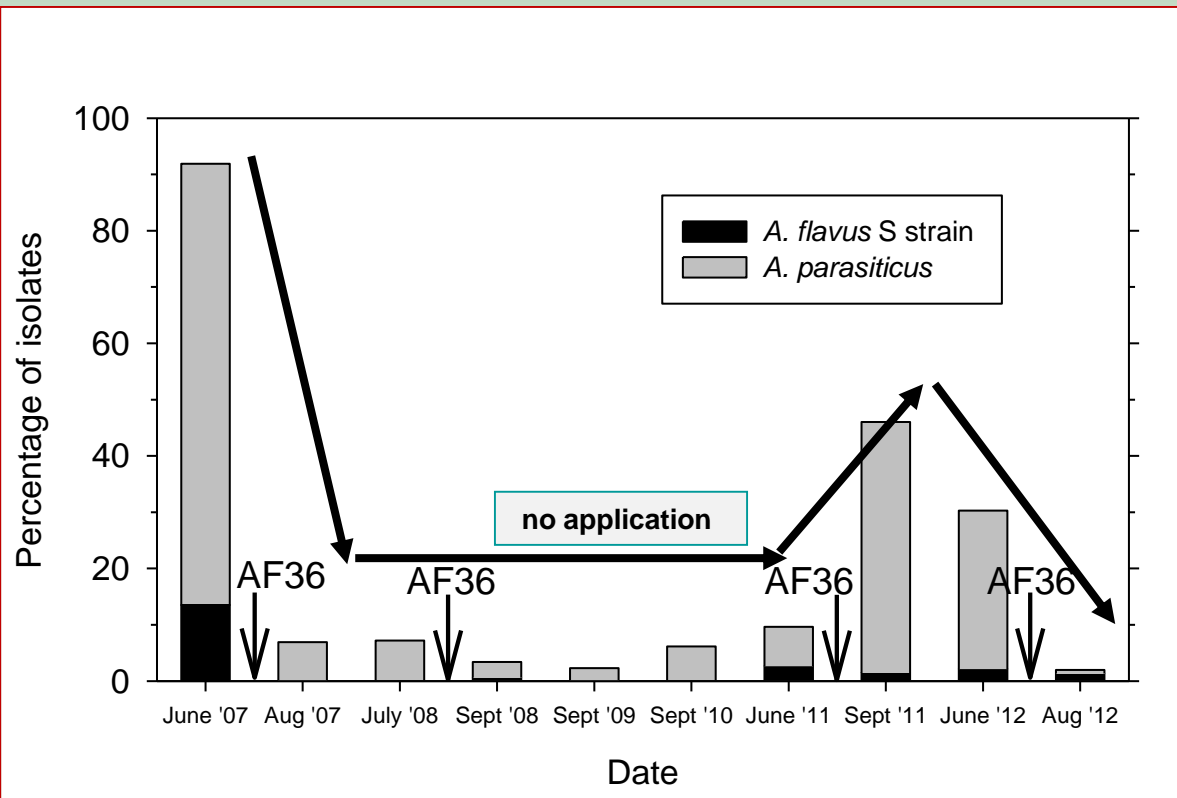
Occurrence of *A. flavus* atoxigenic strains in almond-growing counties of California

Each colored circle represents a different atoxigenic strain; ● = AF36

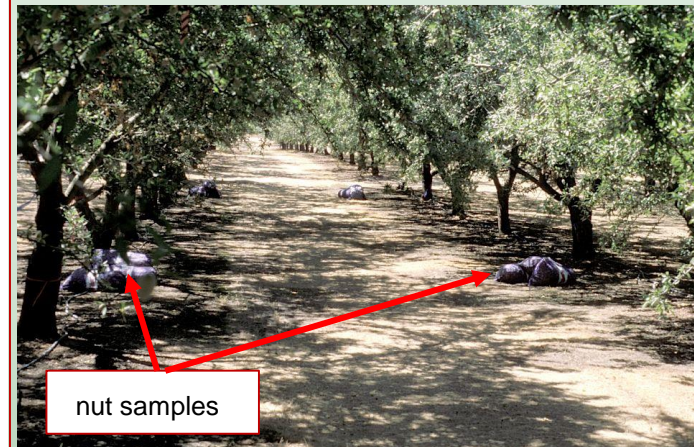


● AF36 incidence: 3.0% to 8.5%

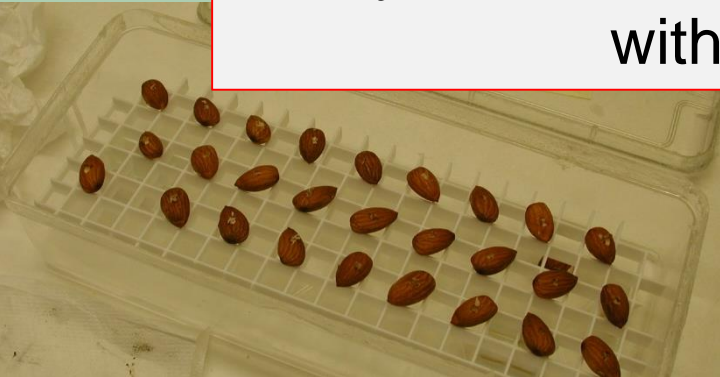
Reduction of aflatoxigenic *Aspergillus flavus*/*A. parasiticus* in areas of an almond orchard treated with the AF36



Nickels Soil Laboratory

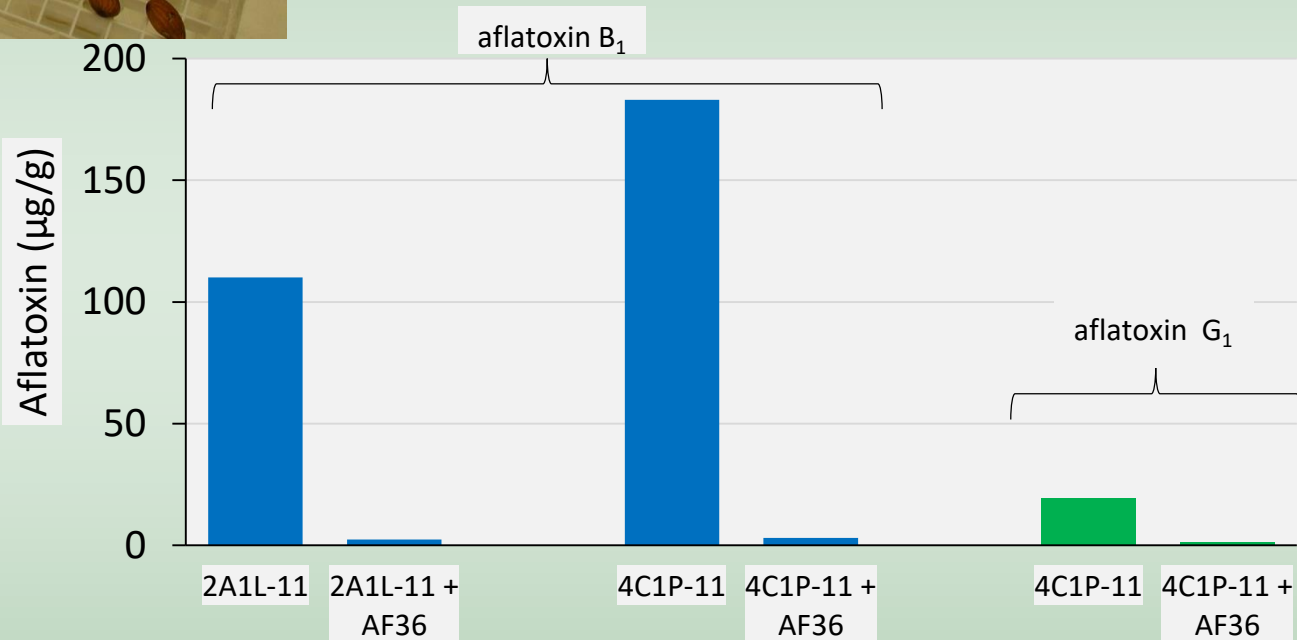


Ability of AF36 to reduce aflatoxins when co-inoculated with highly toxigenic isolates



2A1L-11 :
toxigenic isolate
of *A. flavus*

4C1P-11 :
toxigenic isolate
of *A. parasiticus*

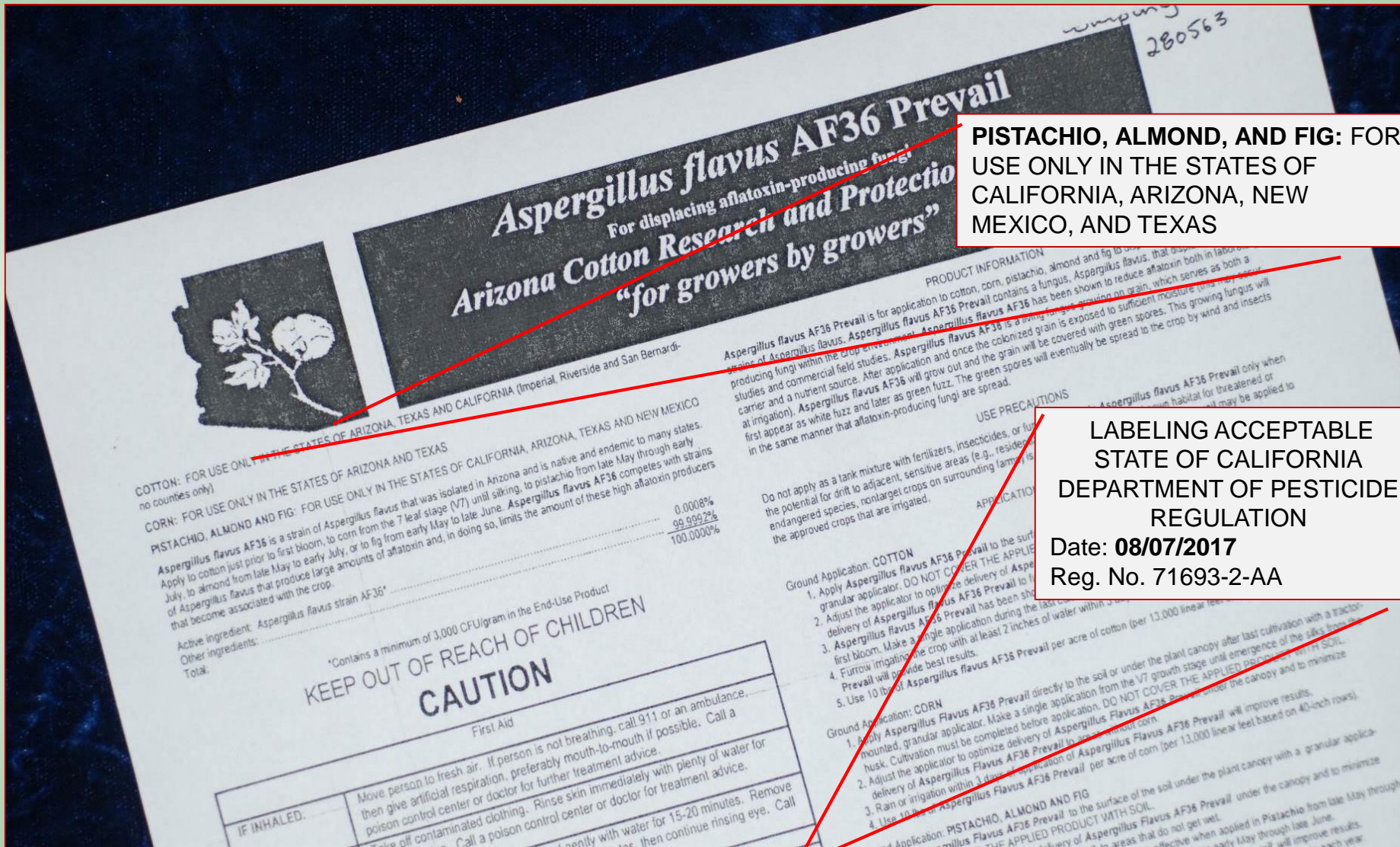


Greater than 94% reduction in aflatoxins in comparison to levels in kernels inoculated with the toxigenic isolate alone

Burkard spore trap in a pistachio orchard

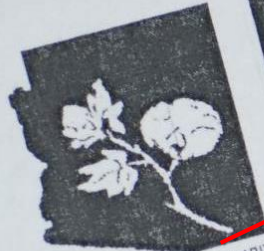


Registration of *A. flavus* AF36 Prevail



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

**LABELING ACCEPTABLE
STATE OF CALIFORNIA
DEPARTMENT OF PESTICIDE
REGULATION**
Date: **08/07/2017**
Reg. No. **71693-2-AA**



Aspergillus flavus AF36 Prevail For displacing aflatoxin-producing fungi Arizona Cotton Research and Protection "for growers by growers"

COTTON: FOR USE ONLY IN THE STATES OF ARIZONA, TEXAS AND CALIFORNIA (Imperial, Riverside and San Bernardino counties only)
CORN: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, TEXAS AND NEW MEXICO
PISTACHIO, ALMOND AND FIG: FOR USE ONLY IN THE STATES OF CALIFORNIA, ARIZONA, TEXAS AND NEW MEXICO
Aspergillus flavus AF36 is a strain of Aspergillus flavus that was isolated in Arizona and is native and endemic to many states. Apply to cotton just prior to first bloom, to corn from the 7 leaf stage (V7) until silking, to pistachio from late May through early July, to almond from late May to early July, or to fig from early May to late June. Aspergillus flavus AF36 competes with strains of Aspergillus flavus that produce large amounts of aflatoxin and, in doing so, limits the amount of these high aflatoxin producers that become associated with the crop.

0.0008%
99.9992%
100.0000%

Active ingredient: Aspergillus flavus strain AF36*
Other ingredients:
Total:

*Contains a minimum of 3,000 CFU/gram in the End-Use Product
**KEEP OUT OF REACH OF CHILDREN
CAUTION**

First Aid	
IF INHALED.	Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. Call a poison control center or doctor for further treatment advice.
	Remove person from exposure. Remove contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Remove clothing if contaminated. Call a poison control center or doctor for treatment advice.
	Flush eyes thoroughly with water for 15-20 minutes. Then continue rinsing eye. Call a poison control center or doctor for treatment advice.

PRODUCT INFORMATION
Aspergillus flavus AF36 Prevail is for application to cotton, corn, pistachio, almond and fig to displace aflatoxin-producing fungi within the crop environment. Aspergillus flavus AF36 is a non-toxic, non-pathogenic fungus that produces aflatoxin, a potent carcinogen. Aspergillus flavus AF36 has been shown to reduce aflatoxin both in laboratory studies and commercial field studies. Aspergillus flavus AF36 is a natural carrier of aflatoxin, which serves as both a carrier and a nutrient source. After application and once the colonized grain is exposed to sufficient moisture (this may occur at irrigation), Aspergillus flavus AF36 will grow out and the grain will be covered with green spores. This growing fungus will first appear as white fuzz and later as green fuzz. The green spores will eventually be spread to the crop by wind and insects in the same manner that aflatoxin-producing fungi are spread.

USE PRECAUTIONS
Do not apply as a tank mixture with fertilizers, insecticides, or fungicides in the same manner that aflatoxin-producing fungi are spread. Do not apply to areas that are adjacent to or near residential areas, sensitive areas (e.g., residential areas, endangered species, non-target crops on surrounding farms, etc.) or other areas that may be damaged by the application of Aspergillus flavus AF36 Prevail only when the approved crops that are irrigated.

- APPLICATION**
- Ground Application: COTTON**
1. Apply Aspergillus flavus AF36 Prevail to the soil with a granular applicator. DO NOT COVER THE APPLIED PRODUCT WITH SOIL.
 2. Adjust the applicator to optimize delivery of Aspergillus flavus AF36 Prevail to the soil.
 3. Aspergillus flavus AF36 Prevail has been shown to be most effective when applied during the last irrigation of the crop.
 4. Furrrow irrigating the crop with at least 2 inches of water within 3 days of application of Aspergillus flavus AF36 Prevail will provide best results.
 5. Use 10 lbs of Aspergillus flavus AF36 Prevail per acre of cotton (per 13,000 linear feet).
- Ground Application: CORN**
1. Apply Aspergillus flavus AF36 Prevail directly to the soil or under the plant canopy after last cultivation with a tractor-mounted, granular applicator. Make a single application from the V7 growth stage until emergence of the silks from the husk. Cultivation must be completed before application. DO NOT COVER THE APPLIED PRODUCT WITH SOIL.
 2. Adjust the applicator to optimize delivery of Aspergillus flavus AF36 Prevail to the soil.
 3. Rain or irrigation within 3 days of application of Aspergillus flavus AF36 Prevail will improve results.
 4. Use 10 lbs of Aspergillus flavus AF36 Prevail per acre of corn (per 13,000 linear feet based on 40-inch rows).
- Application: PISTACHIO, ALMOND AND FIG**
- Apply Aspergillus flavus AF36 Prevail to the surface of the soil under the plant canopy with a granular applicator. DO NOT COVER THE APPLIED PRODUCT WITH SOIL.
- Aspergillus flavus AF36 Prevail will improve results when applied in Pistachio from late May through early July, to almond from late May through early July, or to fig from late May through early July. This will improve results.

Conclusions of studies from the AF36 application in almonds

- ✓ The AF36 persists well in the soil.
- ✓ It does not cause any increase in nut decay.
- ✓ Results on almond were similar to pistachio results.
- ✓ The atoxigenic strain AF36 Prevail® is registered now and can be applied in 2018.

Rate: 10 lbs per acre

Application timing: Late May to early/mid-July



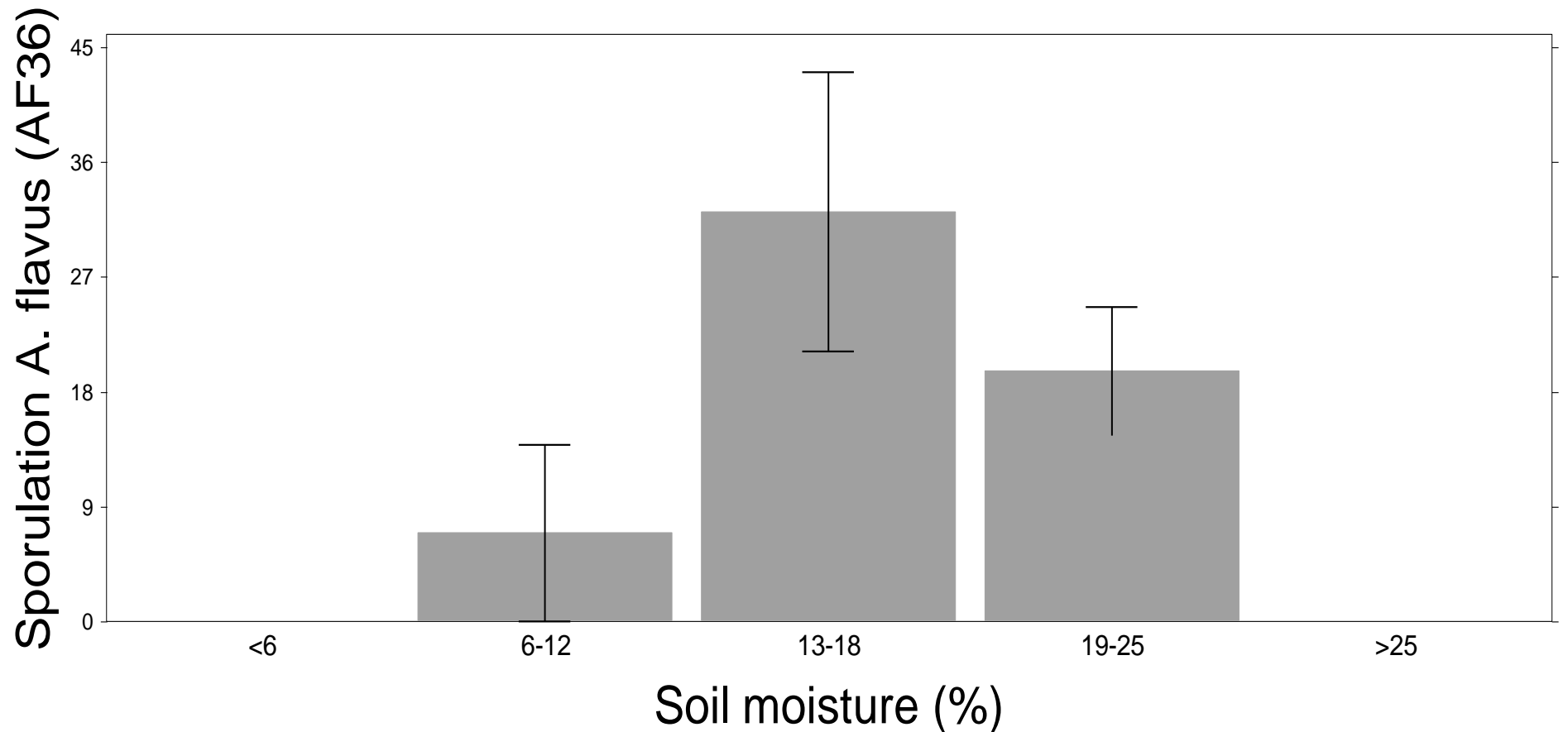
40 lbs capacity



Some Challenges...

- ✓ Soil moisture (and temperature)
 - ✓ Timing of application
 - ✓ Harvest time
 - ✓ Insect seed pests
 - ✓ Predators

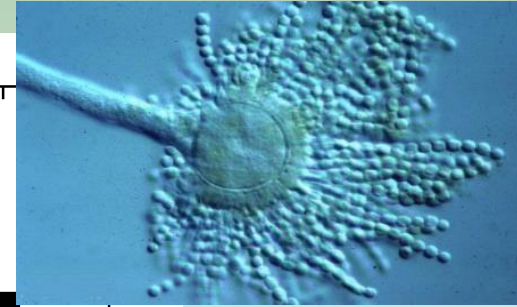
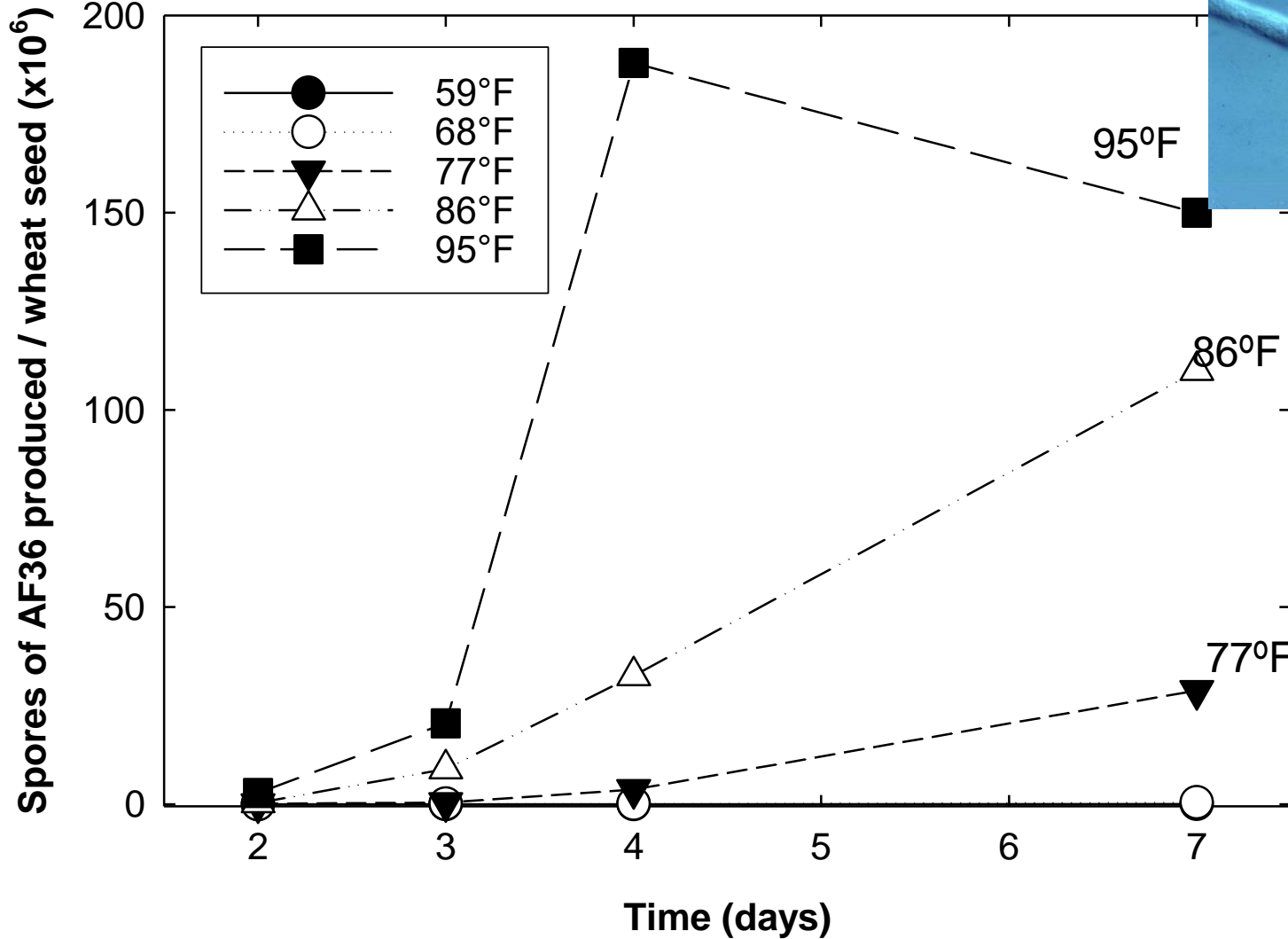
Effect of the soil moisture on sporulation of *Aspergillus flavus* (AF36 Prevail[®]) on grains of product



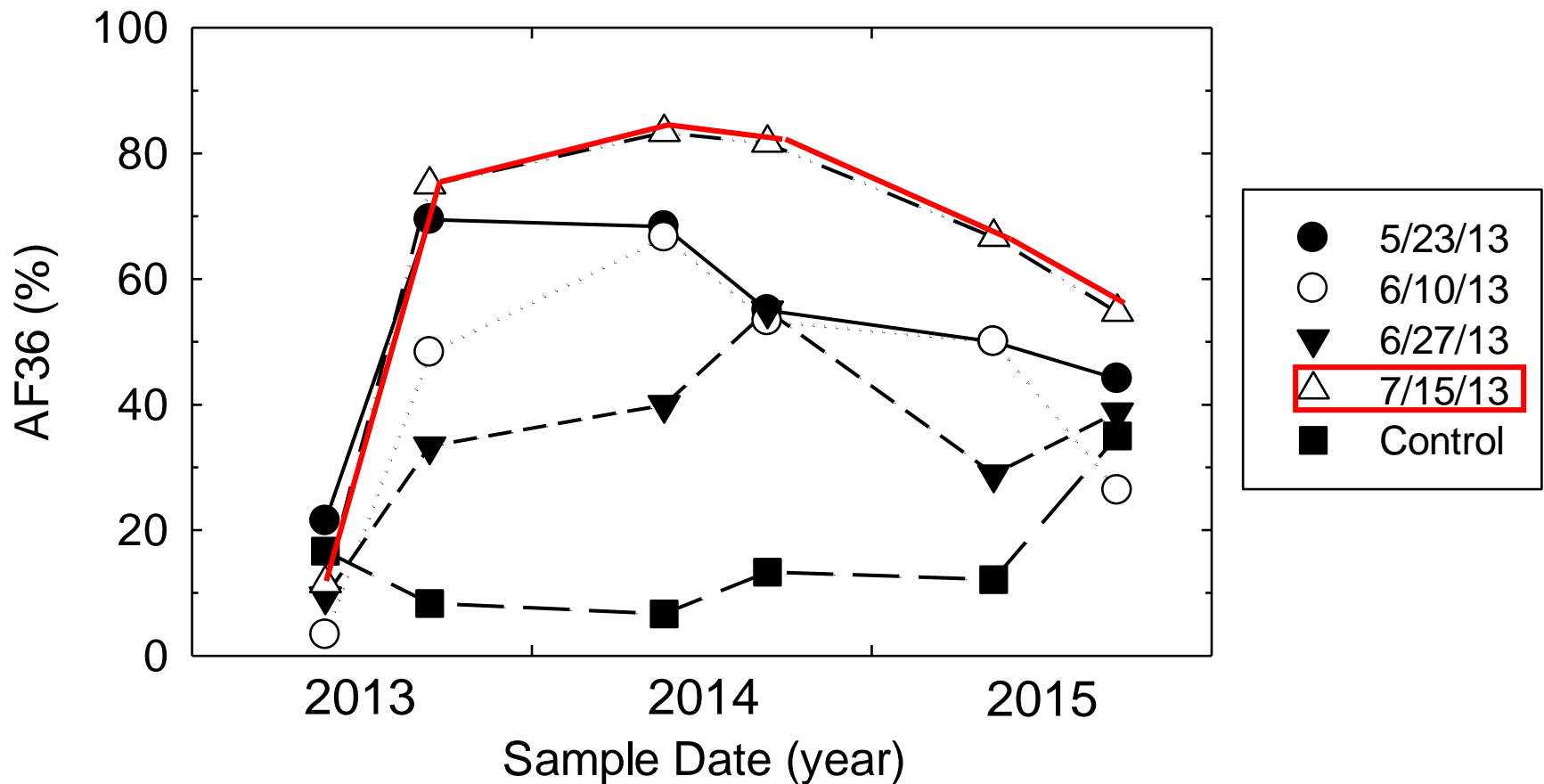
Inoculum dropped into a very wet soil will not produce any spores of AF36; it will decay



Warmer temperatures favor sporulation of the AF36 fungal strain on the AF36 product

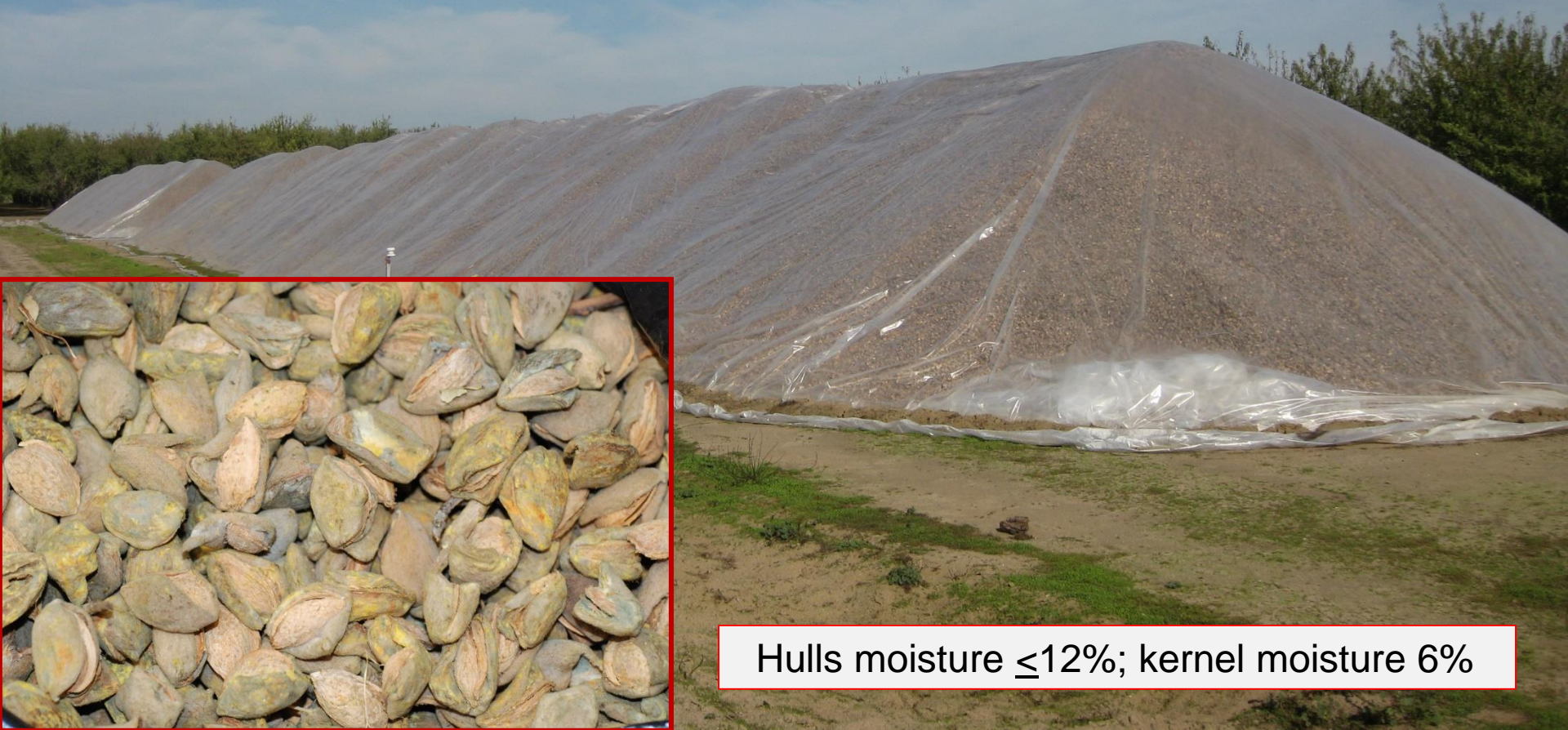


Effect of date of application on sporulation of *Aspergillus flavus* strain AF36



Best results with the 15 July application

Harvest and stockpiling



Hulls moisture $\leq 12\%$; kernel moisture 6%

Predation of grain by insects



Ants



Predation of grain and decay by other fungi

Roly polies

Fusarium spp.



Suggestions for best AF36 application

- ✓ The application method and product rate (10 lbs per acre) are the same as in other crops.
- ✓ Apply product in late May to early / mid-July.
- ✓ Irrigate within 3 days after application.
- ✓ Make sure that most of the inoculum will be spread in the areas wetted by irrigation.
- ✓ Avoid covering the inoculum by plowing or with too much water.
- ✓ Do not spray herbicides 1 to 2 weeks after application.
- ✓ Control the ants, other arthropods, and birds in the orchard.

<< This is a novel approach to reduce aflatoxin contamination >>

Link of video on application of AF36:

www.calpistachioresearch.org/training-videos/

Acknowledgments:

- **Peter Cotty, USDA**
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- **Juan Moral**
- **Teresa M. Garcia-Lopez**
- **John Lake**
- **Peter Cotty, USDA**

USDA/ARS, IR-4 Project/Biopesticide Branch, Almond Board of California, Calif. Pistachio Research Board, Calif. Fig Institute, ...

... and a multitude of growers