Aflatoxin Understanding and Control- All the Tools in the Shed

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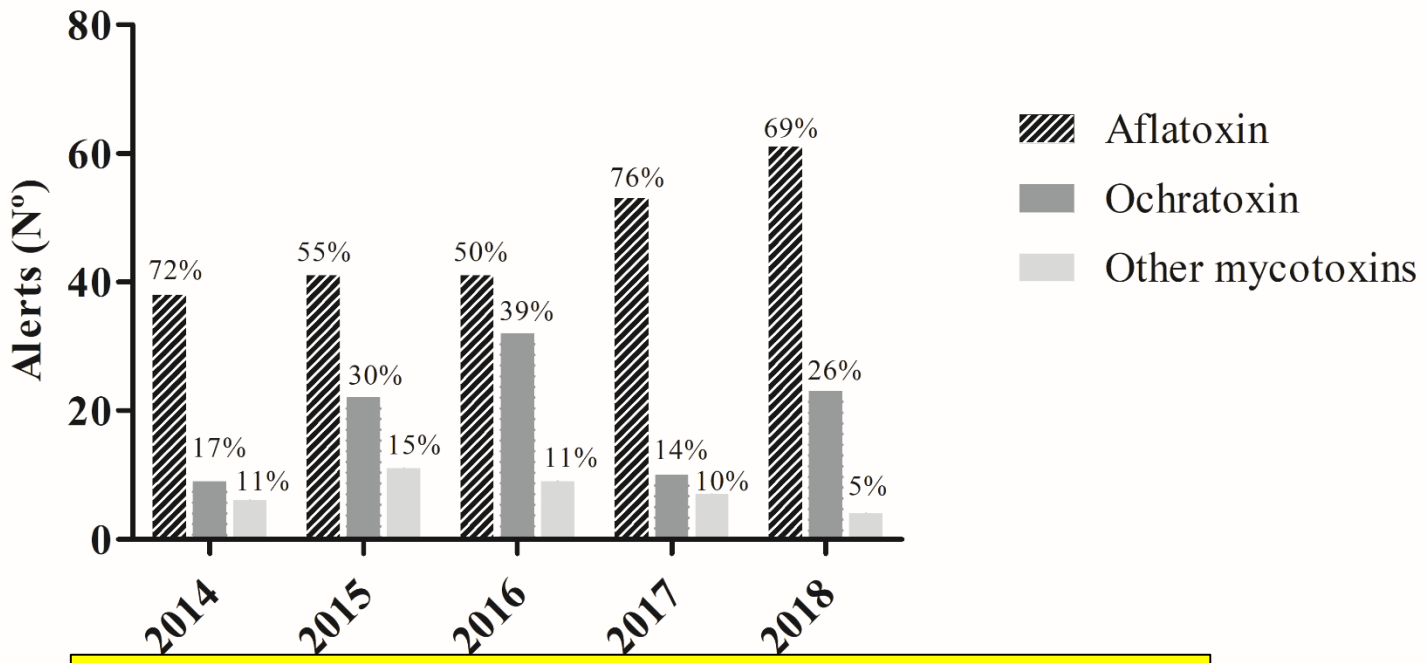
21st Annual Almond Quality & Food Safety Symposium, 13 June 2019, Lodi

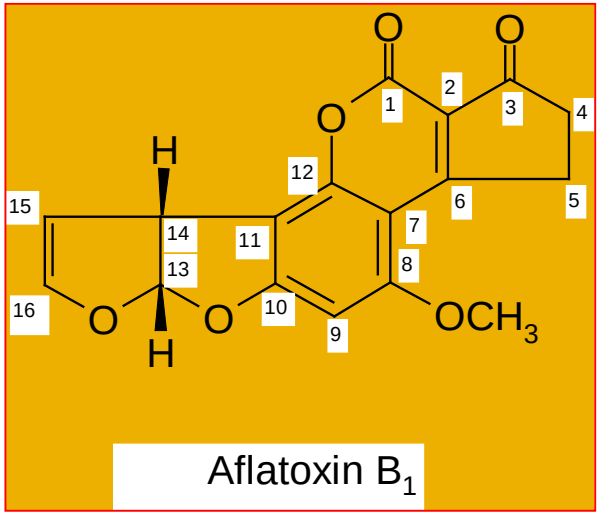
Percent Rapid Alerts on aflatoxins, ochratoxins, & other mycotoxins in various crops



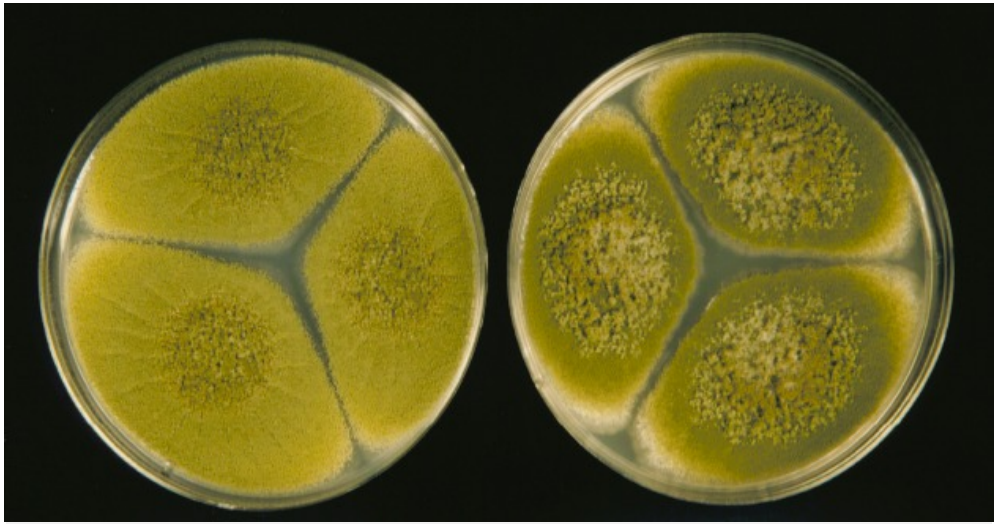
The almond industry has taken extensive measures and supports pre- and post-harvest research to control aflatoxins and to assure compliance with aflatoxin regulations.

Aspergillus flavus and *A. parasiticus* produce: Aflatoxins B_1, B_2, G_1, G_2

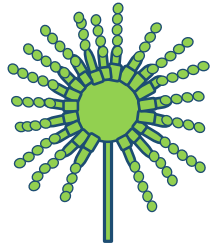
The most potent; it can cause liver cancer



Aflatoxin B_1



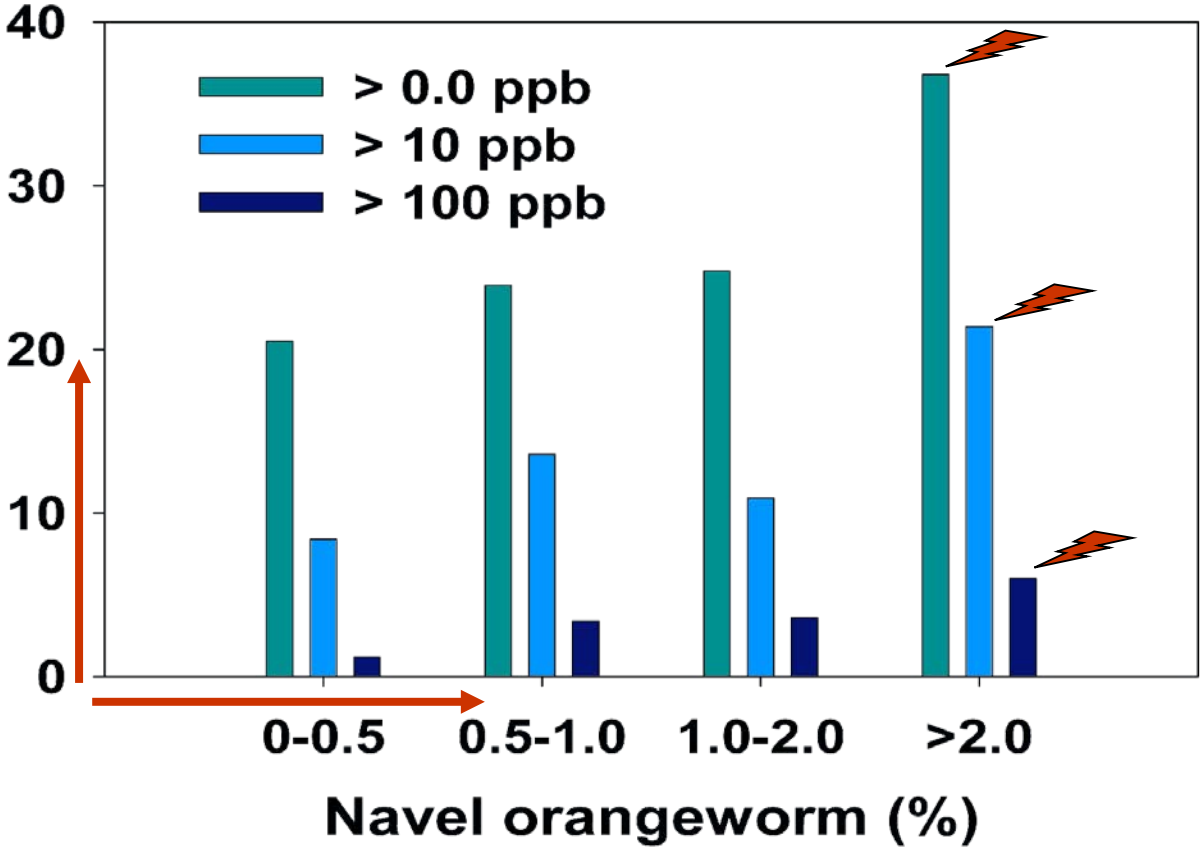
Aspergillus flavus *Aspergillus parasiticus*



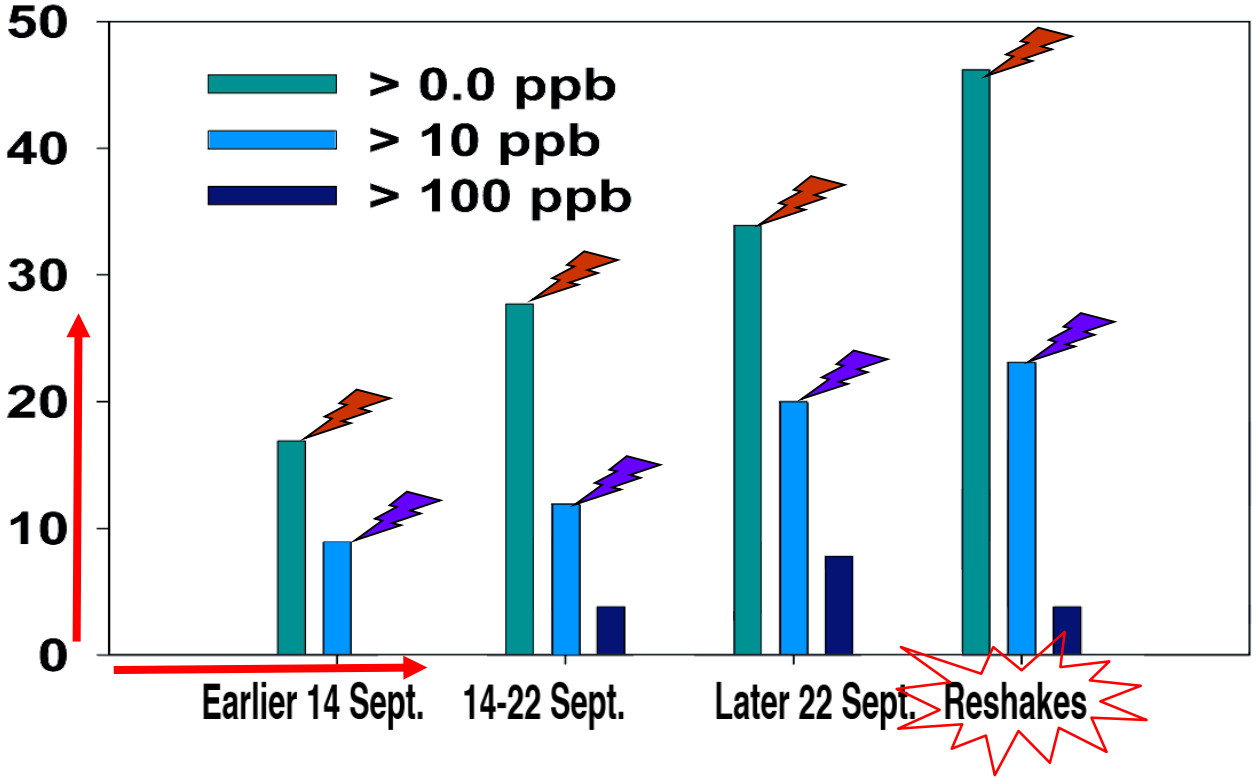
Risk factors for aflatoxin contamination in pistachios and almonds:

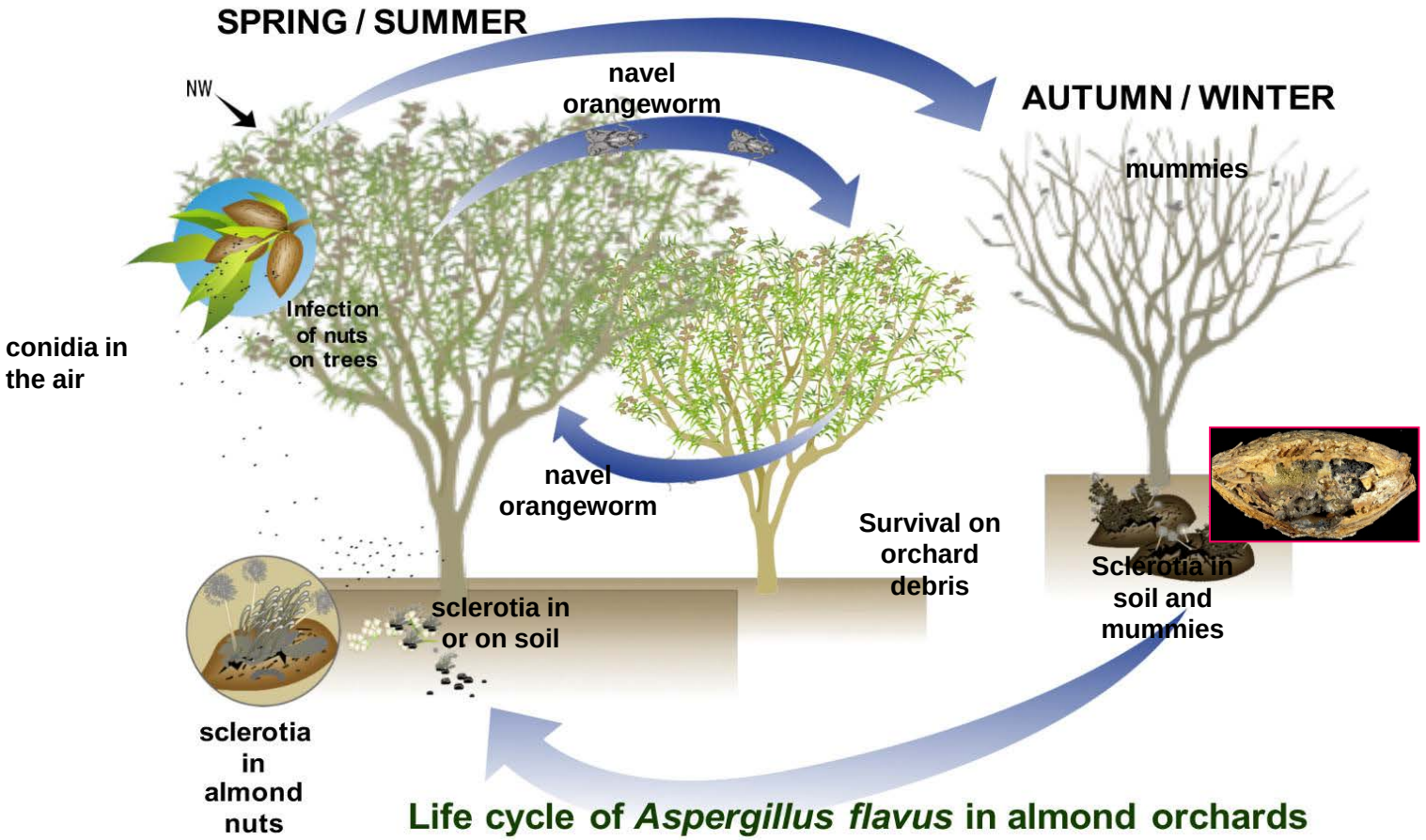
Risk factor	Pistachio	Almond
1) Navel orangeworm (NOW)	✓ Yes	✓ Yes
2) Harvest date	✓ Yes	Most likely
3) Location	✓ Yes	Most likely
4) Year (on /off)	✓ Yes	---
5) Cultural practices	✓ Yes	✓ Yes (Removal of mummies)
6) Rootstock	✓ Yes	???
7) Various nut defects	✓ Yes	✓ Most likely
8) Stockpiling	---	✓ Yes

Relationship of navel orangeworm infestation and aflatoxin levels



Harvest dates, incidence, and amounts of aflatoxin contamination in pistachios




SPRING / SUMMER
NW
navel orangeworm
AUTUMN / WINTER
mummies
Infection of nuts on trees
conidia in the air
navel orangeworm
Survival on orchard debris
Sclerotia in soil and mummies
sclerotia in or on soil
sclerotia in almond nuts
Life cycle of Aspergillus flavus in almond orchards

Almonds touching the soil … when soil wet … major risk…

Area-wide NOW Management Project (2007-2012)

Main Objectives:

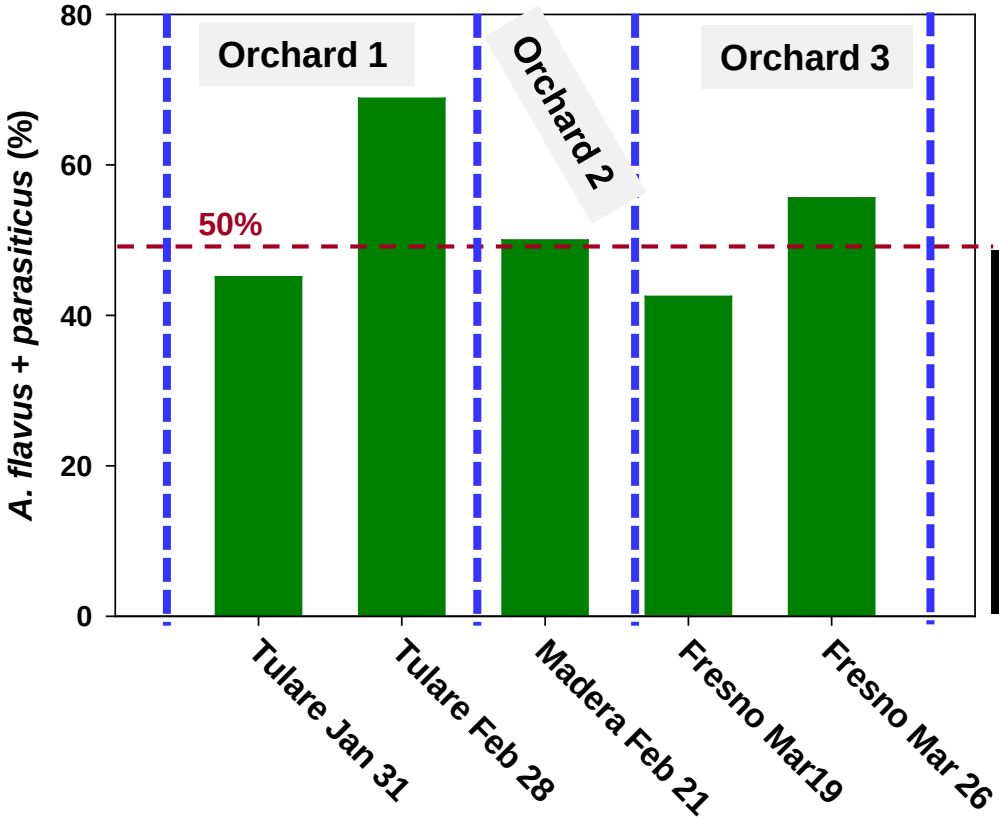
1. Determine the association of navel orangeworm (NOW) with the incidence of aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus.*

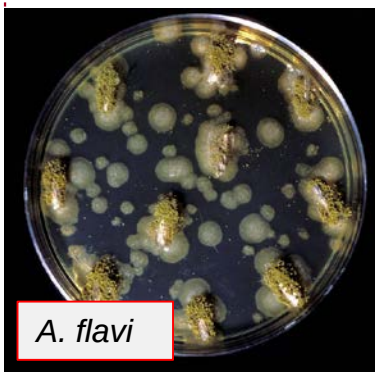
2. Develop management of aflatoxins using biocontrol approaches (i.e. atoxigenic *A. flavus*).

UC IPM photo

Incidence of *Aspergillus flavus* and *A. parasiticus* on NOW moths emerged in spring from almond mummies collected from 3 orchards.

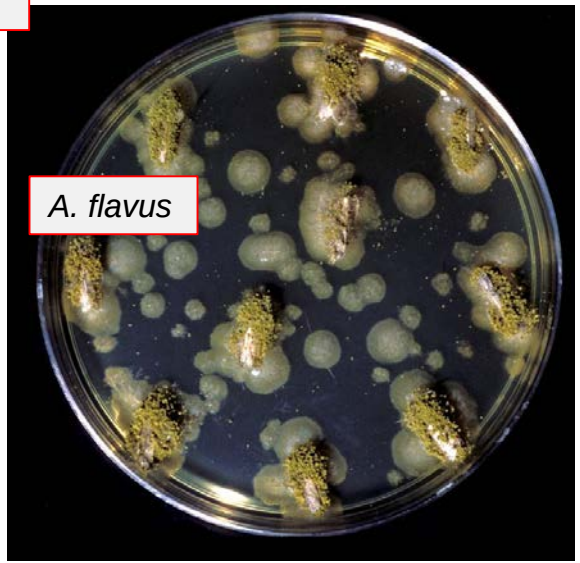
Emergence of moths in buckets

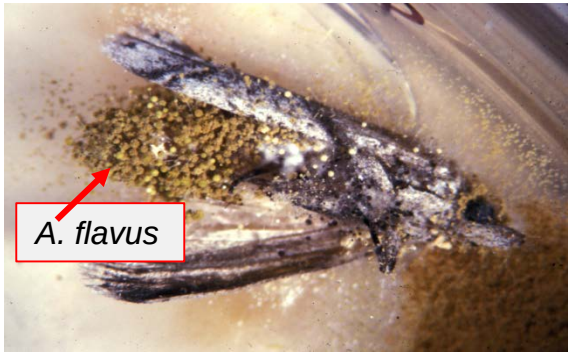




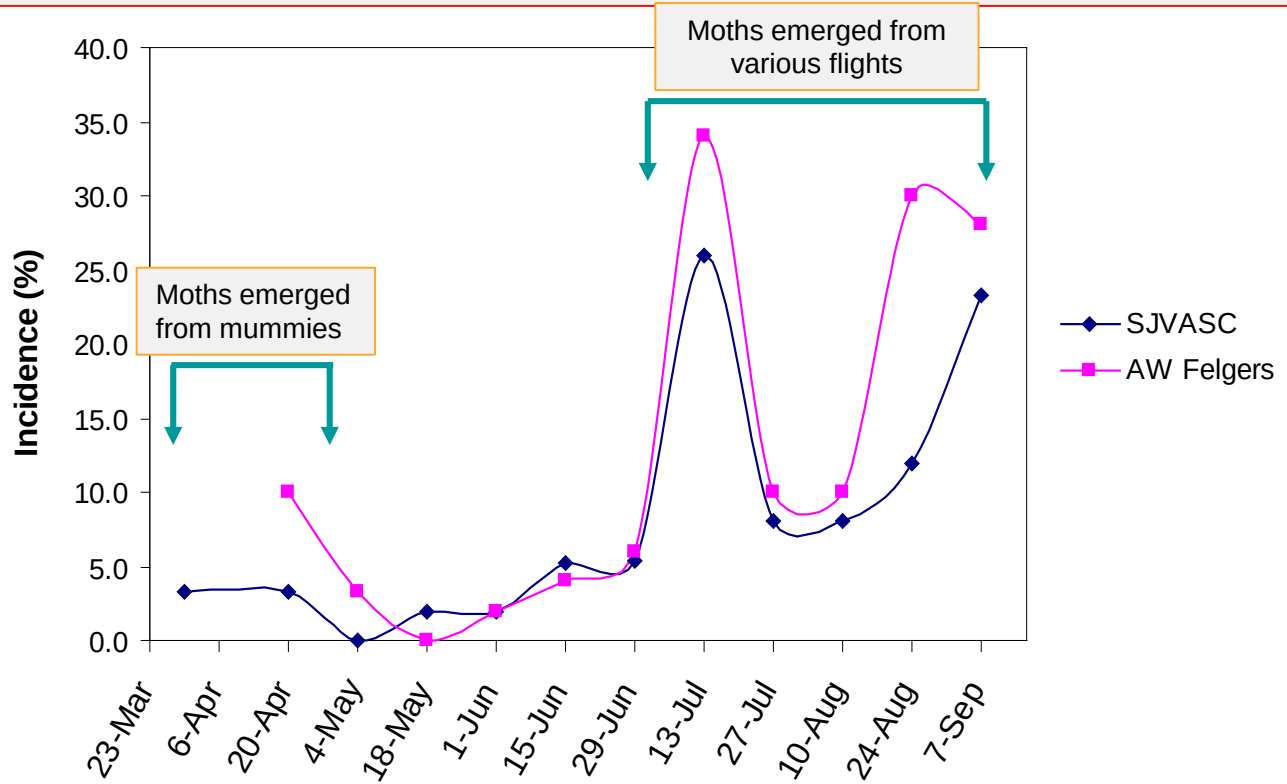
Association of NOW with aflatoxigenic fungi

A sticky trap with NOW moths

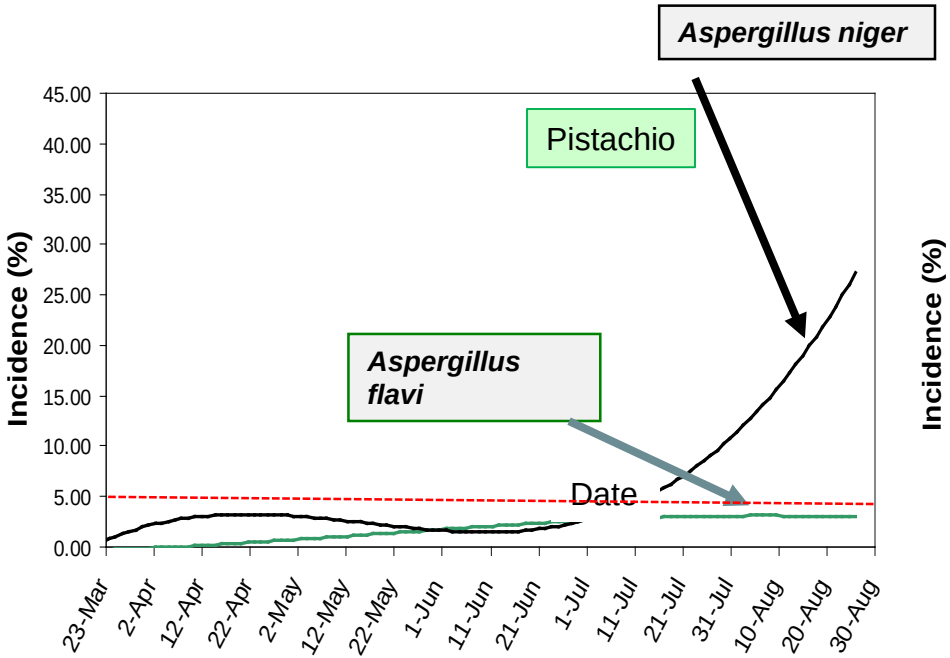


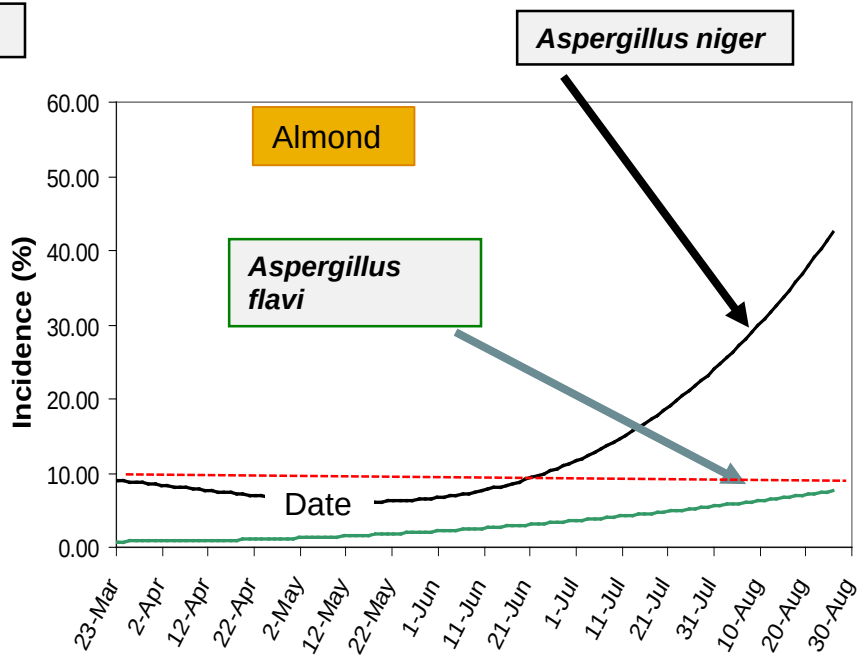


Aspergillus sect. *Flavi* on NOW moths trapped in two almond orchards (in Madera Co. **(in coop. with Dr. Joel Siegel, ARS/USDA, Parlier)**



Incidence of *Aspergillus* Section *Flavi* (green line) and *Aspergillus niger* (black line) from NOW moths collected (2011)

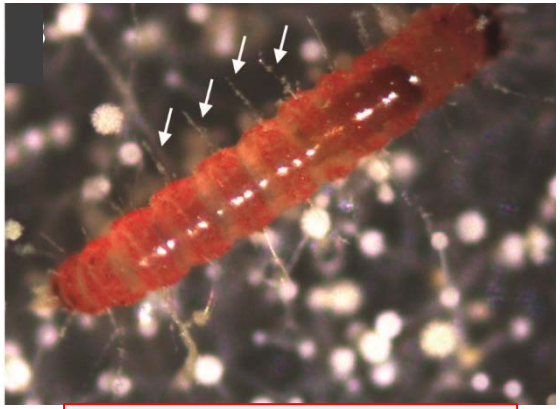




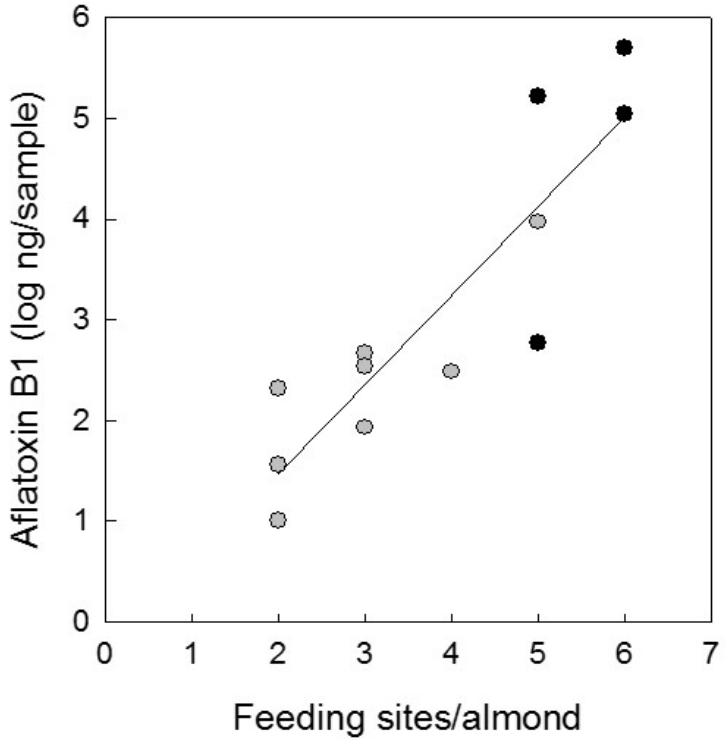
***Aspergillus niger* can cause hull rot**

Acquisition and transmission of *Aspergillus flavus* by navel orangeworm

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



Navel orangeworm larva



Palumbo et al. 2015, Plant Disease 98:1194-1199.

Mummies on trees contribute both NOW moths and *Aspergillus flavus*

Incidence of *Aspergillus* section Flavi isolates isolated from almond mummies of different cultivars

Almond Mummy Status (+/- NOW)	Nonpareil		Butte		Padre	
	n	mean[a]	n	mean[a]	n	mean[a]
no-damage	48	9.1% a	11	2.3% a	8	2.0% a
damage	9	12.2% a	1	5.6% a	0	0.0% b
Overal Incidence[b]	**57**	**9.5% a**	**12**	**2.4% b**	**8**	**2.0% b**

Perhaps kernels from mummies contribute to aflatoxin contamination of the current season's crop

Dark kernels represent mainly the kernels of mummies from the previous year

Tools in the Shed:

Strains of *Aspergillus flavus*

Strains of *Aspergillus flavus* (S, L)

L - strain

about 50:50
toxigenic: atoxigenic

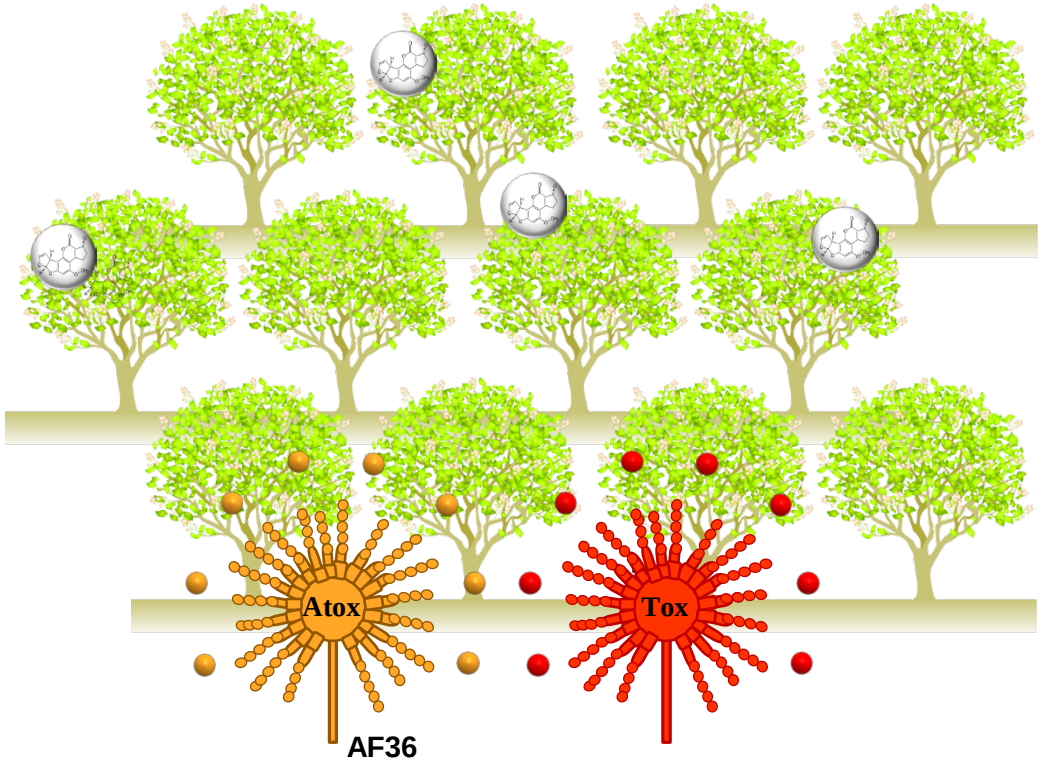
Atoxigenic AF36

Rationale: Increase the atoxigenic strain population in the orchard to reduce (displace) the toxigenic population of *Aspergillus flavi*

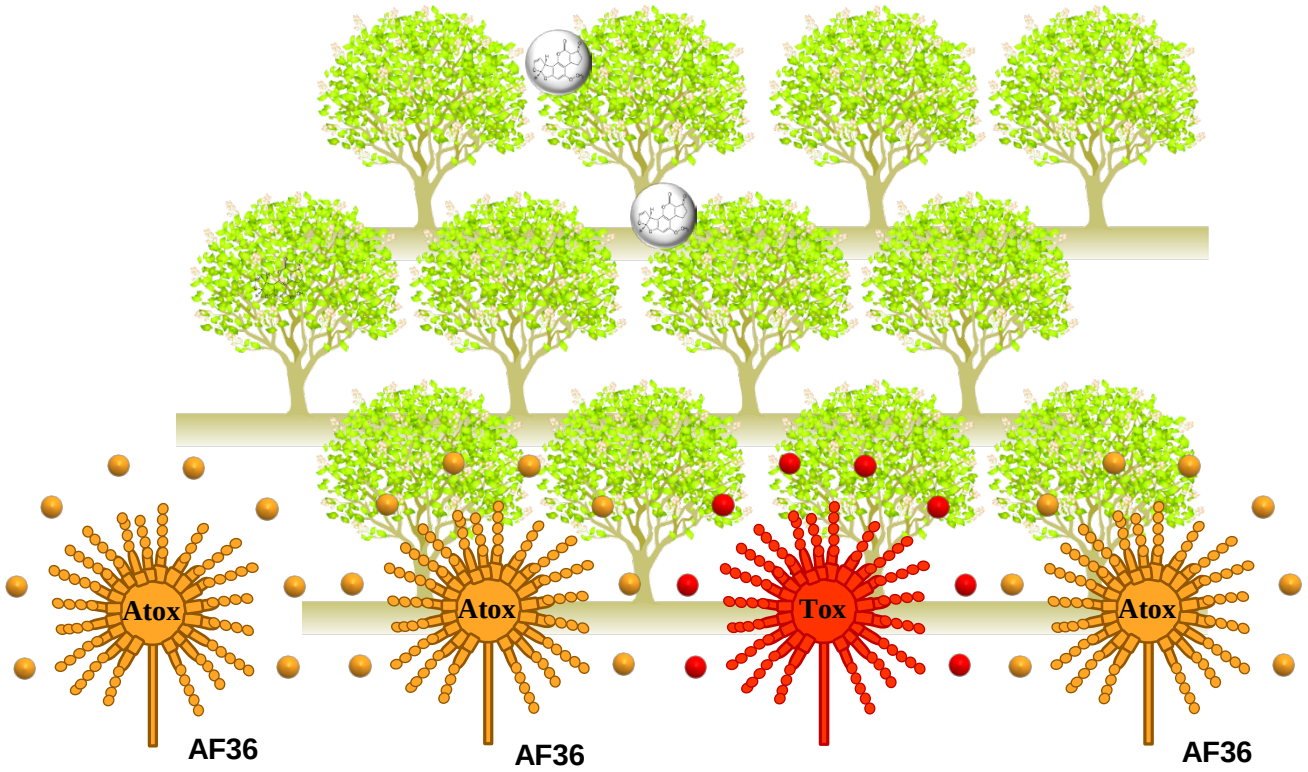

AF36
Inoculum

Application rate: 10 lbs. per acre

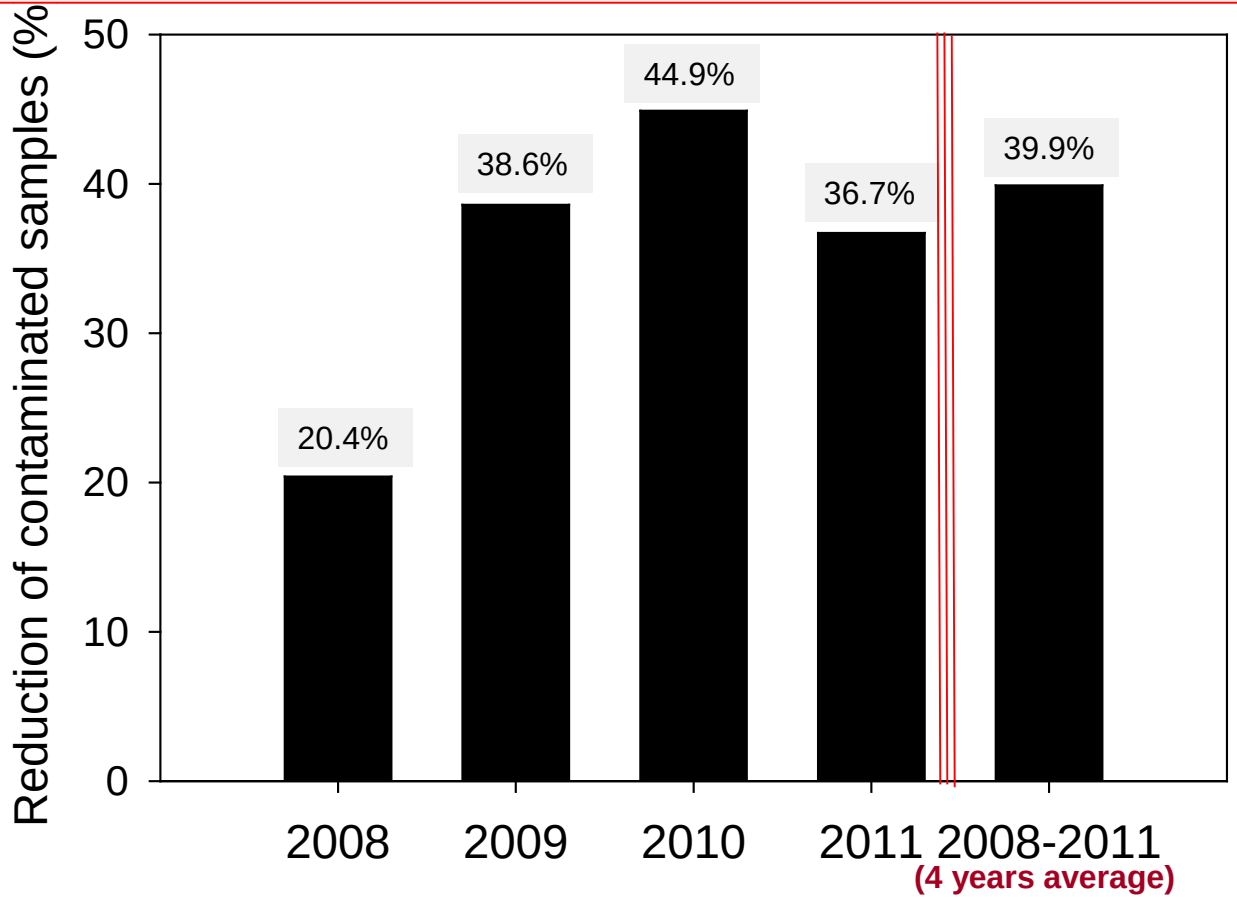
Non-treated orchard



Treated orchard



Reductions in aflatoxin-contaminated pistachios in the 1st and 2nd harvests

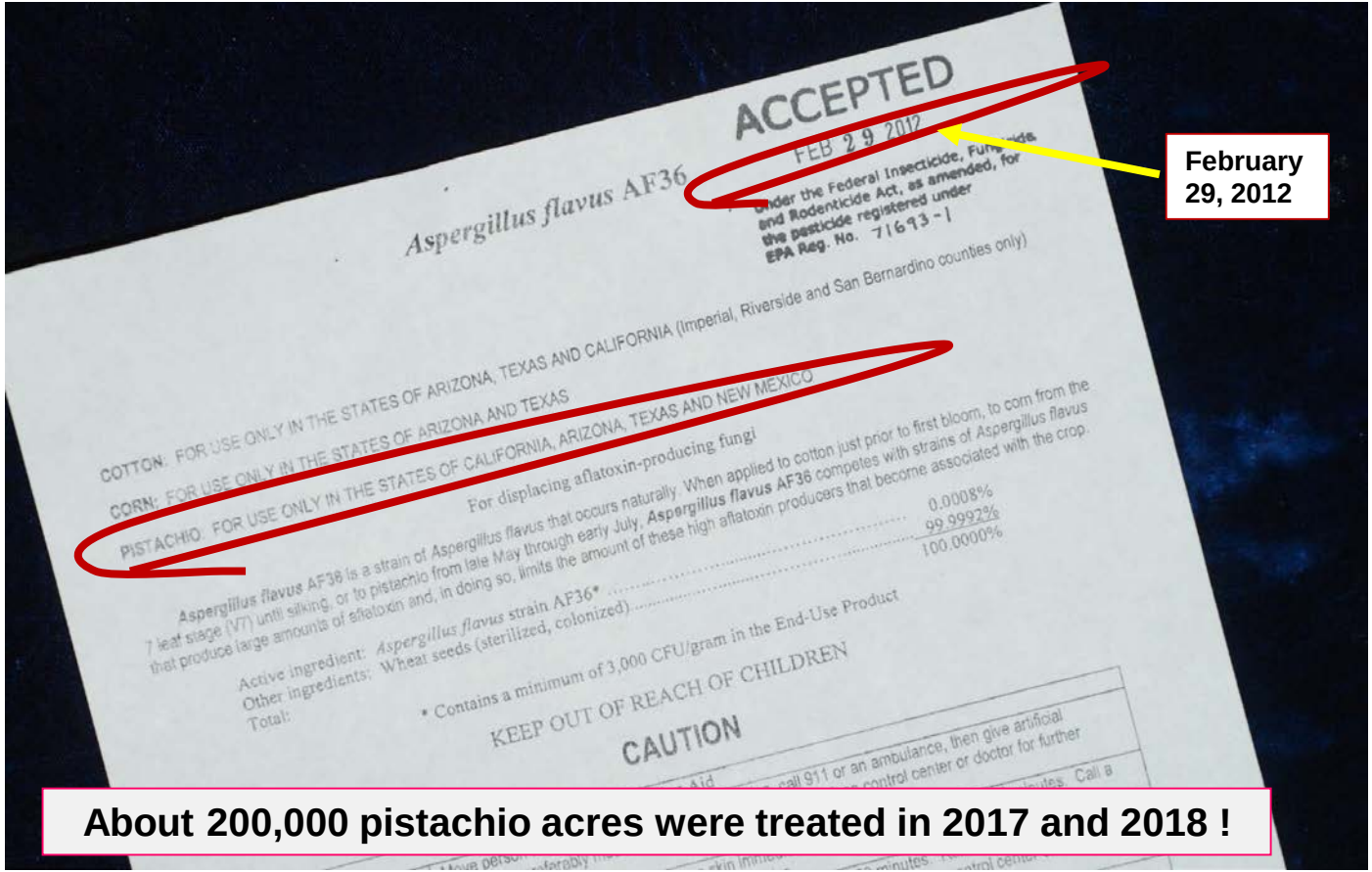


40% reduction

Significant:
***P value* =0.0033**

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

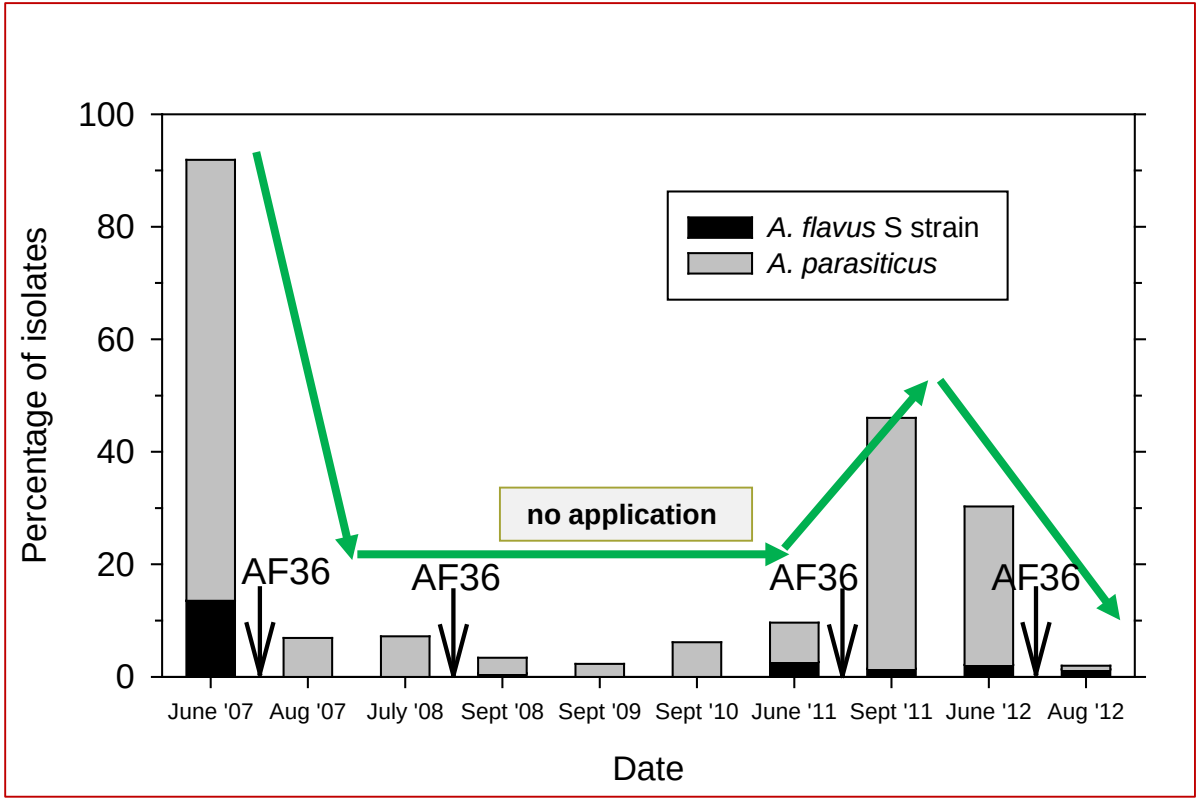
Registration of *Aspergillus flavus* AF36 strain for use in pistachio in 2012



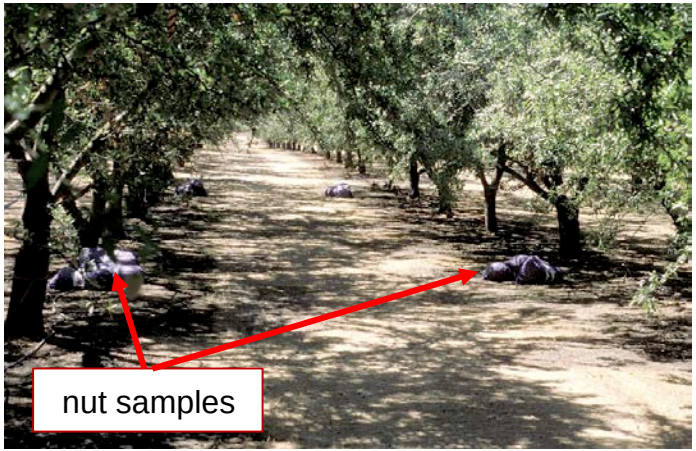
February 29, 2012

About 200,000 pistachio acres were treated in 2017 and 2018 !

Reduction of aflatoxin-producing *Aspergillus flavus/A. parasiticus* isolates in areas of the almond orchard treated with the AF36 product



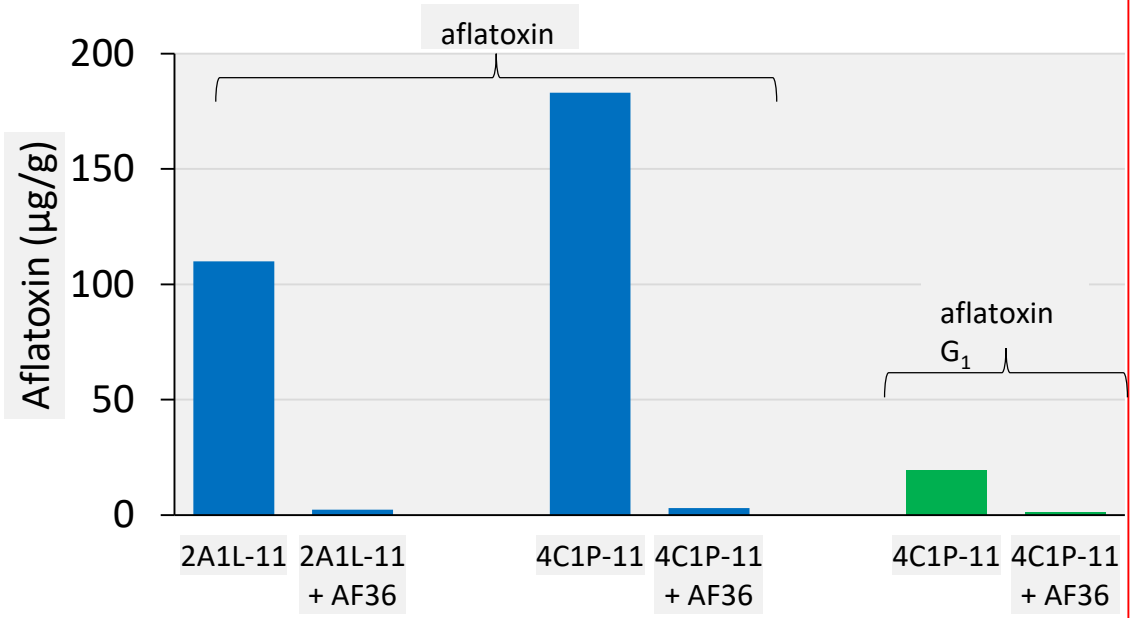
Nickels Soil Laboratory



Ability of AF36 to reduce aflatoxins in almond kernels 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

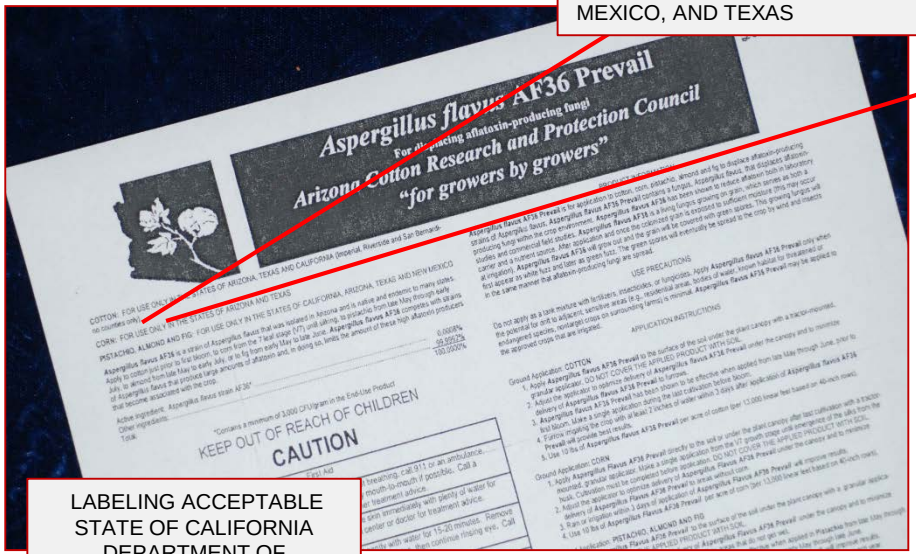
Bridging document along with additional data on efficacy in the laboratory (the pistachio studies helped in the registration of AF36 Prevail)

- Avoided doing an Experimental Use Permit (EUP) trial **(i.e. in pistachio: 3,000 acres treated & 3,000 untreated control; 4 years study)**
- Avoided repeating a spore trapping trial (i.e. in pistachio: **(8 Burkard traps, 2 years spore trapping in orchards)**

Aspergillus flavus AF36 strain

The AF36 atoxigenic was registered for use in almonds in August 2017.

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

Questions still to answer about using AF36 in almond orchards

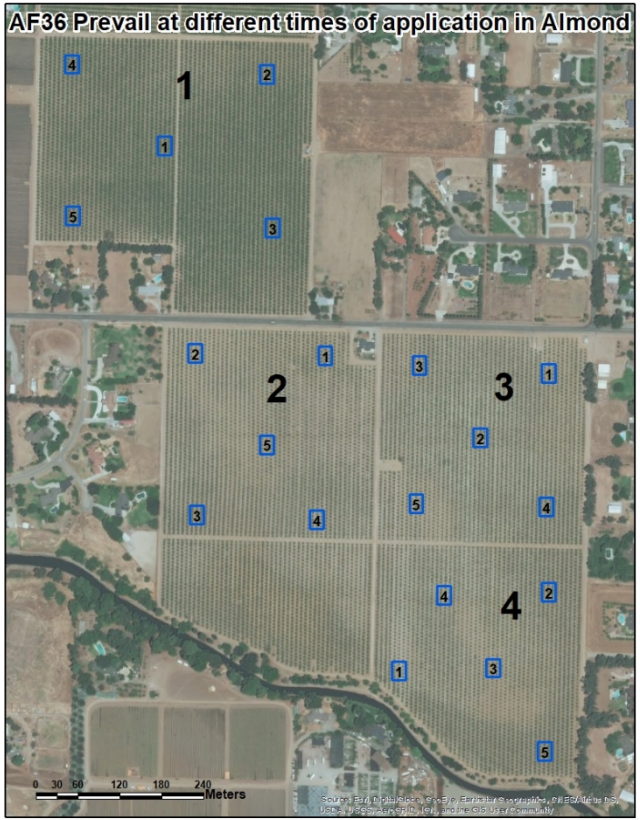
1. What is the optimal time for applying the AF36 Prevail® biopesticide in the almond orchards?

2. What is the risk of infection of almond fruit while they are drying on the ground?

3. What is the efficacy of AF36 Prevail® in reducing AF in commercial almond orchards?

4. Can we develop and use molecular tools to monitor the AF36 strain in orchards?.

Timing of application in a commercial orchard (2018):

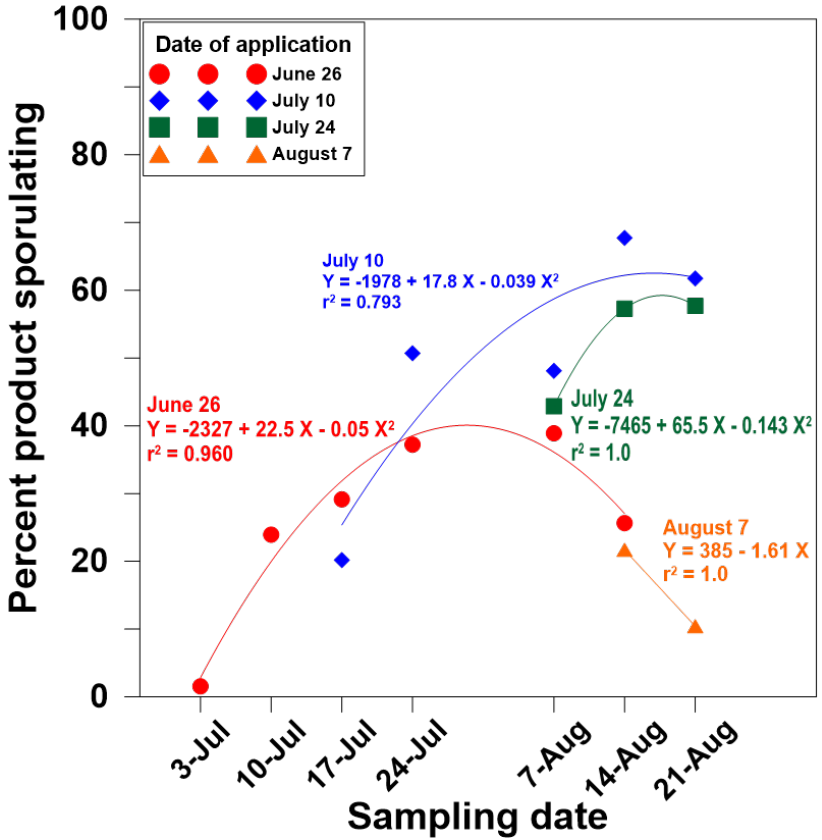


Dates of application of AF36 Prevail in Almond

- **1: 26 June**
- **2: 10 July**
- **3: 24 July**
- **4: 7 August**
- **5: --- (None)**

Collected samples were analyzed: No aflatoxins

Results of experiment for the best application time:

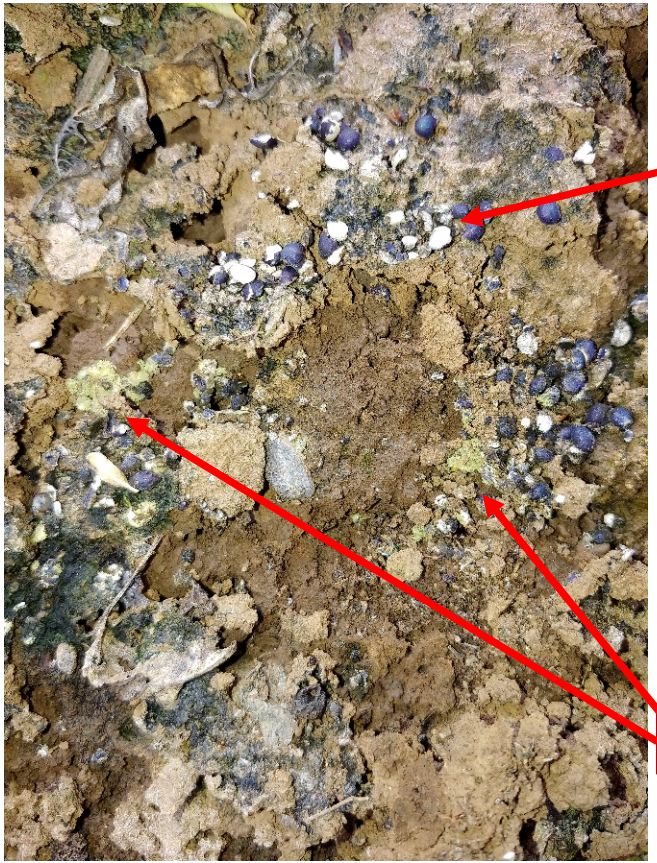


- **Conclusion:** The most sporulation occurred with the **application on 10 July (blue line)**

Efficacy of AF36 Prevail® in a commercial almond orchard (Merced Co., 2018)

Rate of biopesticide:
10 lbs/acre

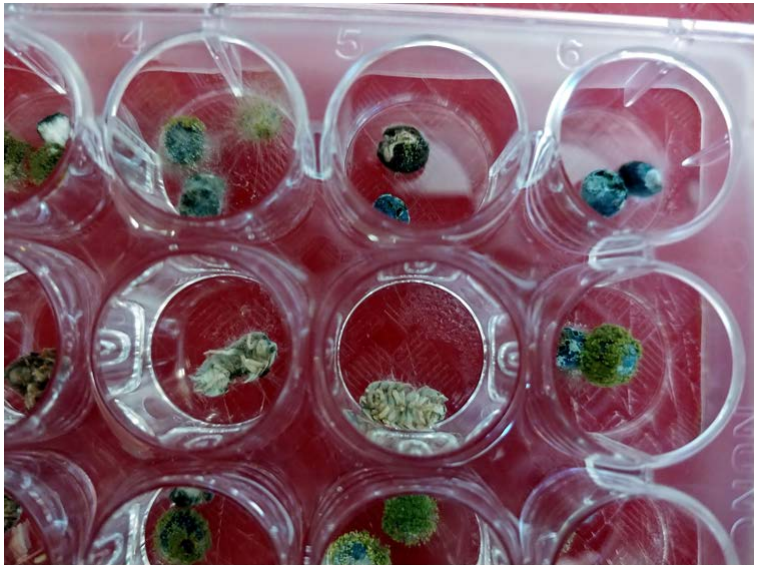
Sporulation of AF36 Prevail and challenges in a commercial orchard



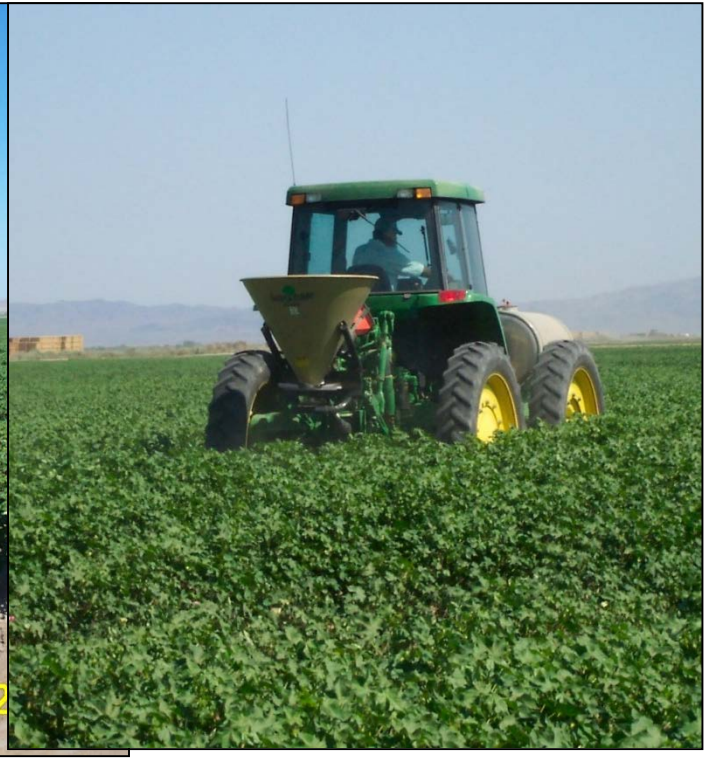
Partially eaten

Sporulating product

Partially eaten product after 4 days of incubation



Application in cotton fields: Favorable environmental conditions there for the product to sporulate (good sporulation!)



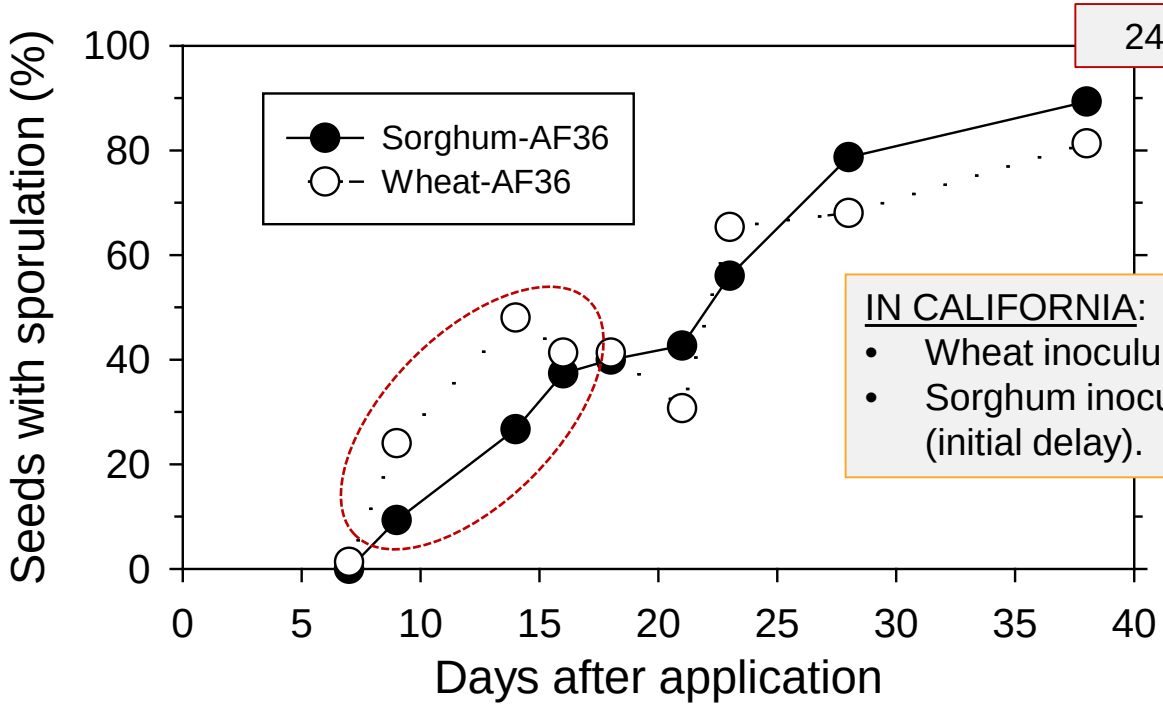
First Challenge: Change of the carrier of AF36

Wheat inoculum
(tested for 15 yrs)

Sorghum inoculum
(tested for only 5 yrs)

And change of the seed inoculation method

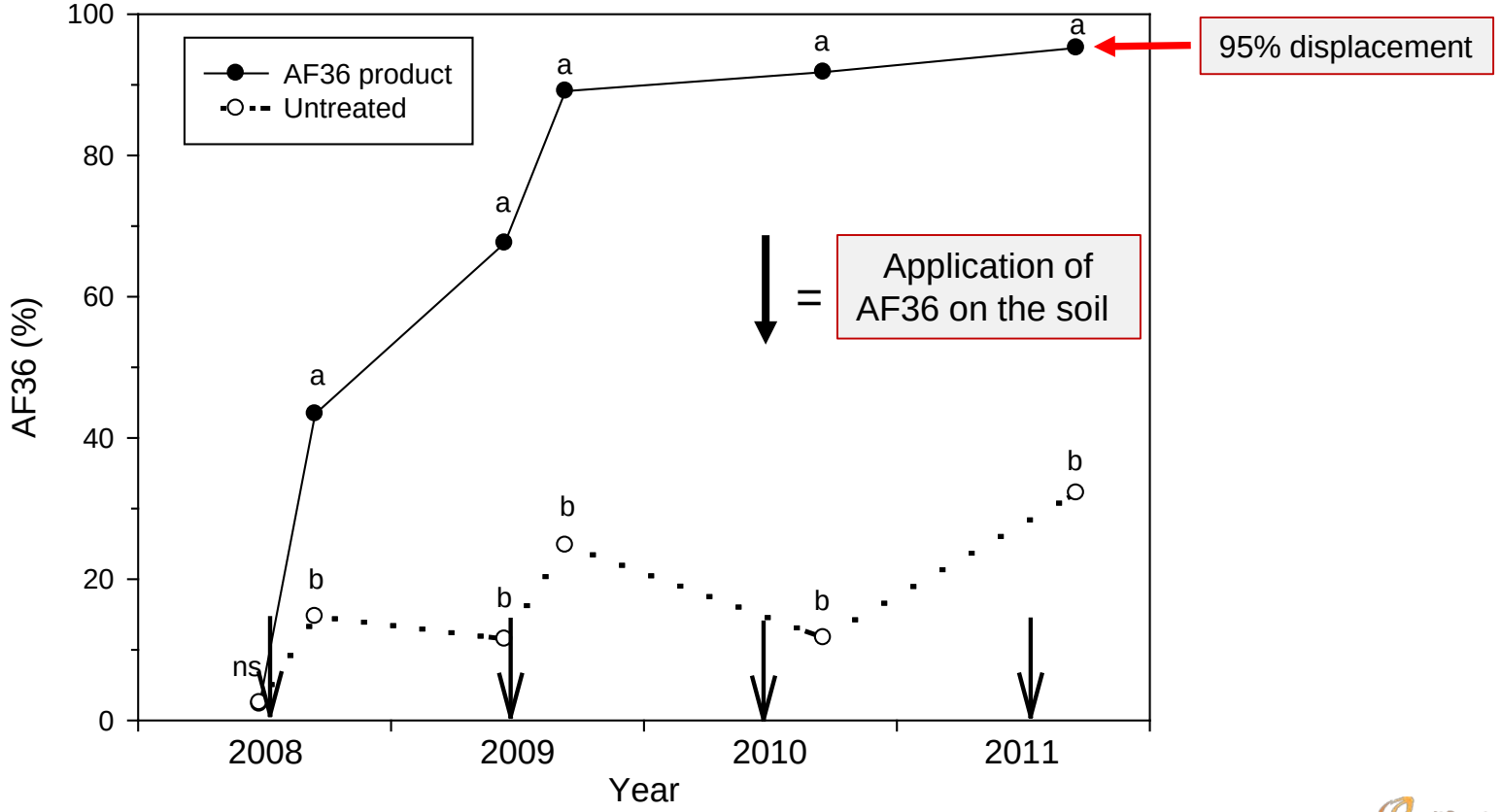
Second Challenge: Production of *Aspergillus flavus* spores on products applied to soil in a research orchard in California



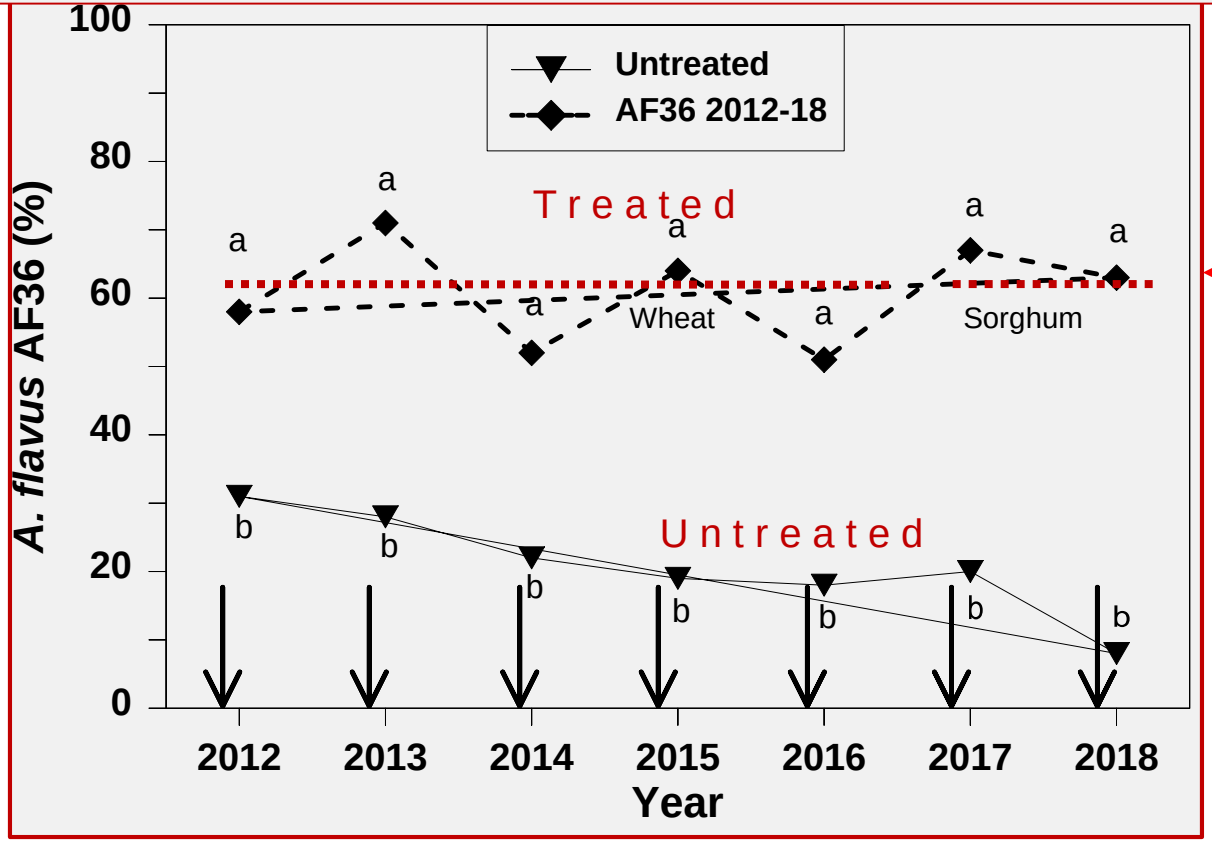
IN CALIFORNIA:

- Wheat inoculum: 0% in 7 days & 50% in 14 days.
- Sorghum inoculum: 0% in 7 days & 25% in 14 days (initial delay).

Third Challenge: Percentage of ***A. flavus* isolates from soil** belonging to AF36



Third Challenge: *Aspergillus flavus* AF36 isolates (%) from soil collected from treated with AF36 and untreated orchards



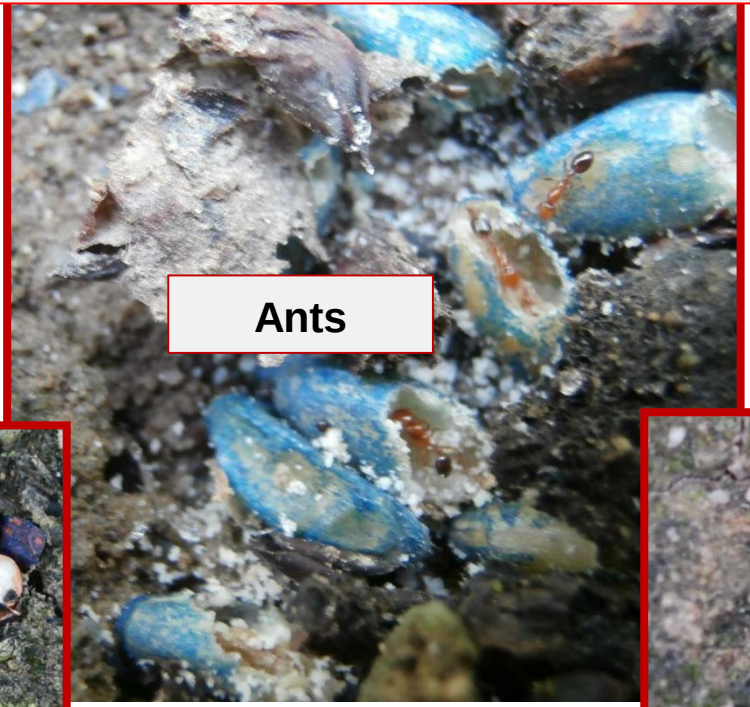
65-70% displacement

Fourth Challenge: Predation of seeds by insects, birds, and decay by other fungi

Ants

Sow bugs

Nitidulid beetles



Ants

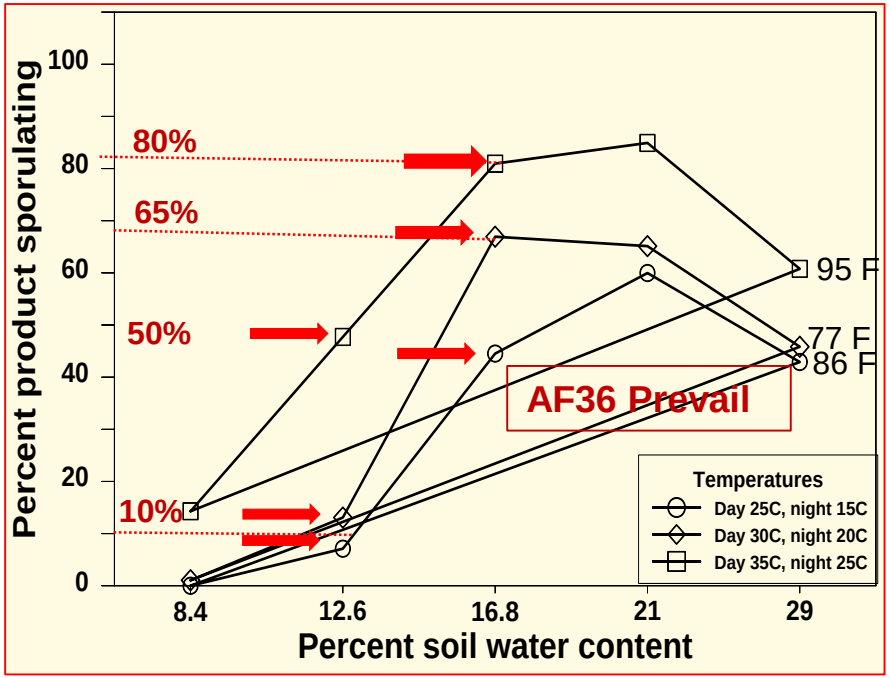
Decay by other fungi (i.e. *Fusarium*, etc.)

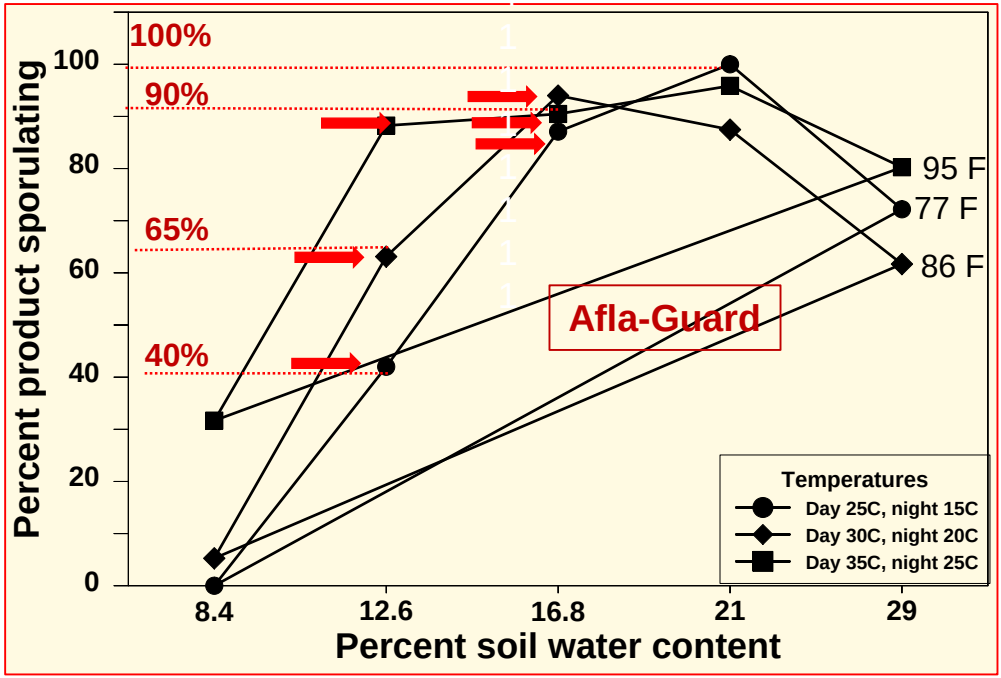
New product: Afla-Guard GR by Syngenta

US Environmental Protection Agency Office of Pesticide Programs:

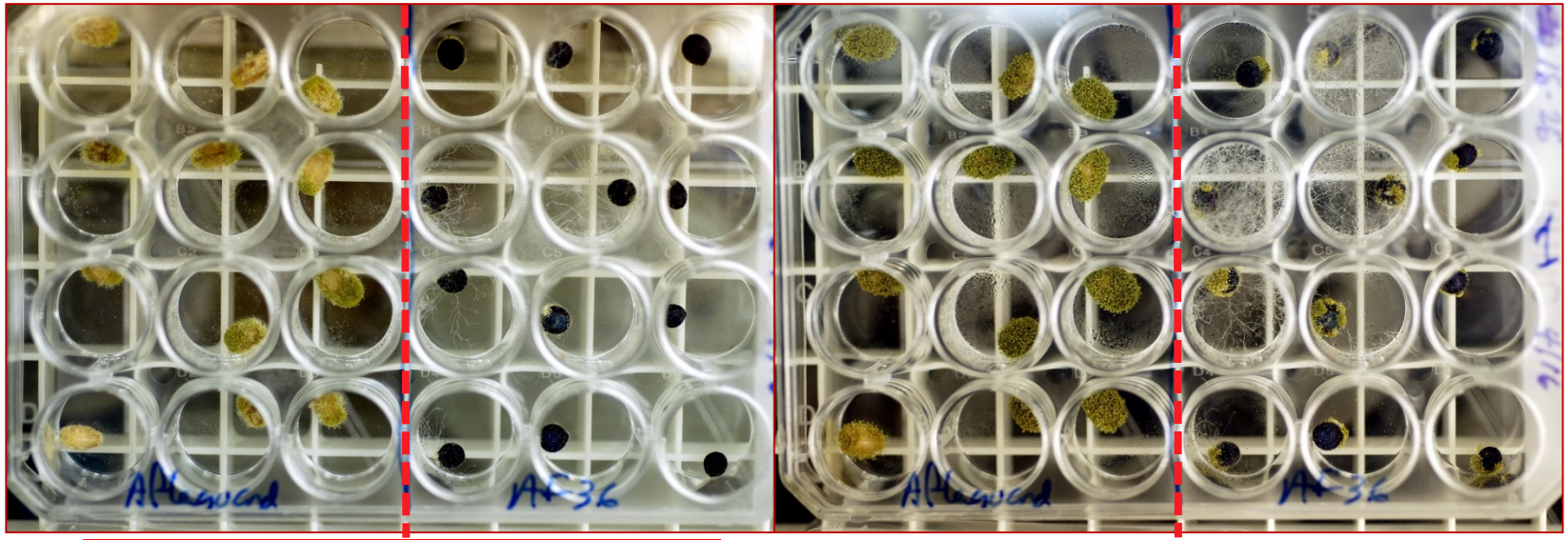
Biopesticide Registration Action Document
Aspergillus flavus (NRRL 21882) (PC Code 006500) **March 24, 2004**

Sporulation of biopesticides under various soil water content and temperature conditions (21% = saturated soil)





Visual Example: Sporulation of Afla-Guard and AF36 Prevail under low night temperature (59 F) and optimal humidity



Afla-Guard	AF36 Prevail

4 days of incubation

Afla-Guard	AF36 Prevail

1 week of incubation

Conclusions and Future Studies

- *First Tool*: Sanitation to reduce mummies and thus NOW damage, *Aspergillus* inoculum, and aflatoxins.
- *Second Tool*: The AF36 Prevail® product is registered and ready to use in almonds.
- *Third Tool*: The best timing for application (sporulation) is in early July.
- *Fourth Tool*: The proper method for stockpiling to avoid moisture under the plastic cover.
- *Fifth Tool on its way*: A new product, Afla-Guard® (Syngenta Chemical Co) showing better sporulation soon to be registered.

Future studies:

- Efficacy of AF36 Prevail® in 5 commercial orchards.
- Exps. with additives to increase sporulation.

Acknowledgments

We thank the **Almond Board of California,** the ARS/USDA, & the CDFA for funding parts of this research

california almonds
Almond Board of California