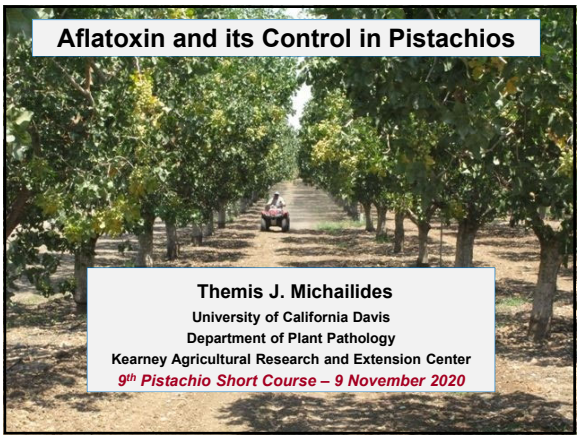
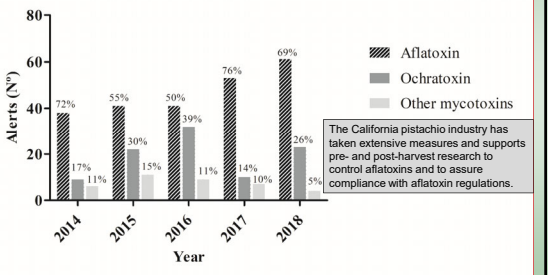


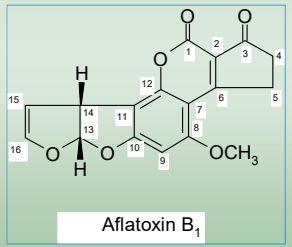
Percent Rapid Alerts on aflatoxins, ochratoxins, & other mycotoxins in various crops



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

Definition of aflatoxin

- **Aflatoxins B_1, B_2, G_1, G_2, M_1**
- *Aspergillus flavus* and *A. parasiticus*



Aflatoxin B_1

Regulatory limits for aflatoxins

- **USA**

Total aflatoxins ➔ 15 ppb
Aflatoxin B1 ➔ 10 ppb

- **European Union**

Total aflatoxins ➔ 10 ppb
Aflatoxin B1 ➔ 8 ppb

(in pistachios for direct consumption)

Commodities contaminated with aflatoxins:

Highest risk of aflatoxin contamination:

Corn
Peanuts
Cottonseed

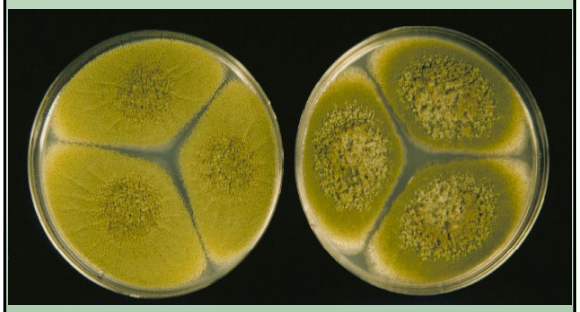
Occasionally contaminated:

Tree nuts (almonds, pistachios, walnuts)
Figs
Sorghum
Spices
Others

Outline of this course:

- Fungi producing aflatoxin (causes)
- Fungal variability
- Risk factors for aflatoxin contamination
- Defects associated with contamination and reduction of these defects
- Biocontrol of aflatoxin

The pathogens: Molds that can produce aflatoxin in pistachio orchards in California



Aspergillus flavus *Aspergillus parasiticus*

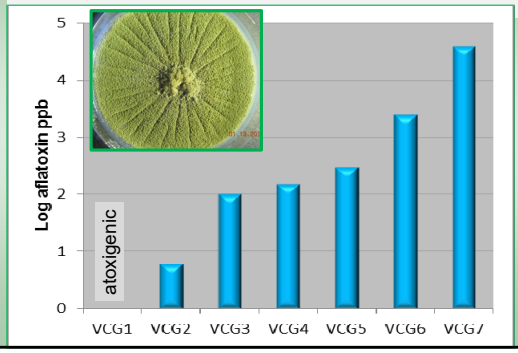
Fungal Variability:
Strains of *Aspergillus flavus*

L - strain → **about 50:50 toxigenic: atoxigenic**

M - strain → **?**

S - strain → **most toxigenic**

***Aspergillus flavus* L strain**
Vegetative Compatibility Groups or VCGs



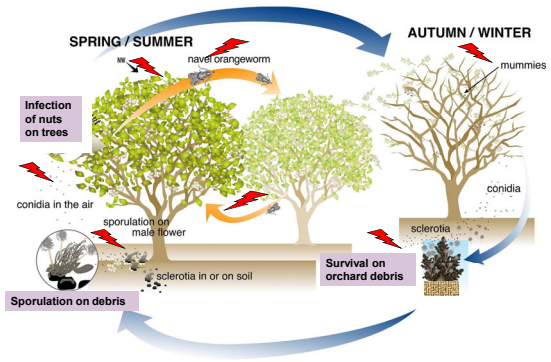
Density of *A. flavus*/*A. parasiticus* in California soil

Selective agar medium

propagules

Orchard	cfu/g
A	1.7
F	4.4
D	11.3
C	12.7
G	12.7
H	13.1
J	15.2
I	23.1
E	27.0
B	35.5

Other crops, i.e. peanuts, cotton, corn can have thousands of cfu/g soil

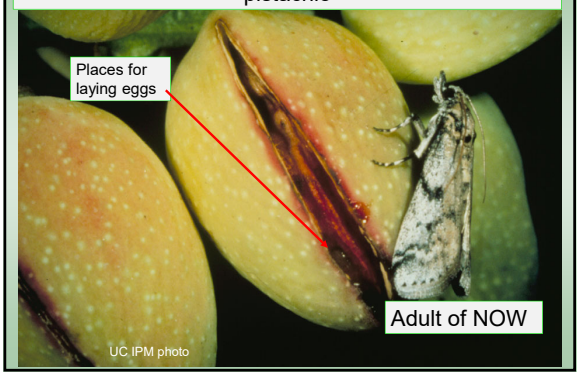


The life cycle of *Aspergillus flavus* in a pistachio orchard

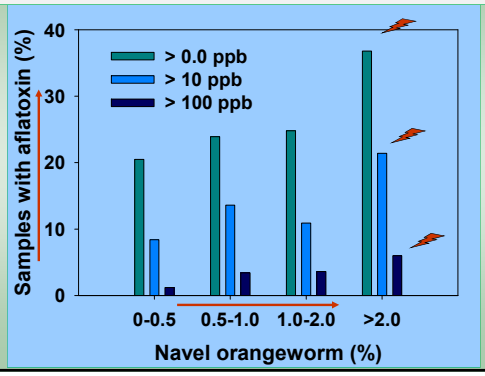
Pre-harvest risk factors affecting aflatoxin contamination in pistachio

1) Navel orangeworm (NOW)
2) Harvest date
3) Early Splits (ES)
   - Cultural practices (affecting ES)
   - Rootstock (affecting ES)
4) Location (“hot spots”)
5) Year (on /off)
6) Various nut defects (DBOM, etc.)

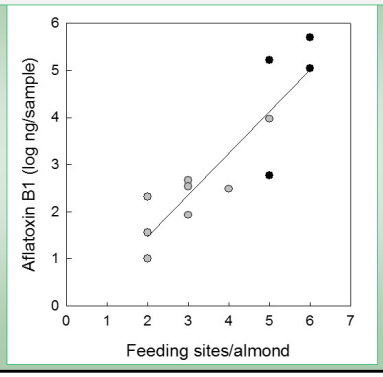
1. Navel orangeworm (NOW) moth on an early split pistachio



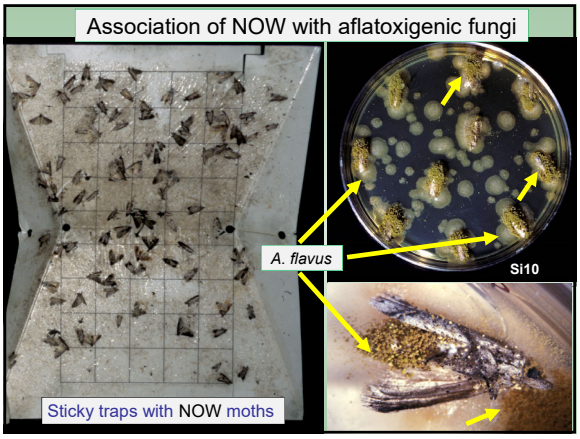
Relationship of navel orangeworm infestation and aflatoxin levels



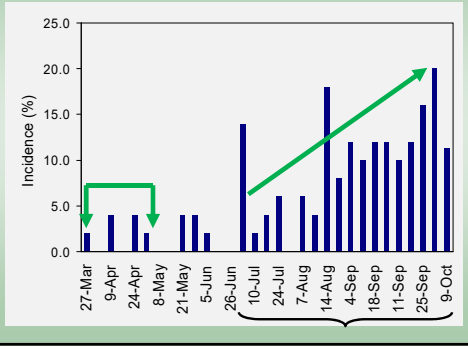
Effect of feeding sites (wounds) on levels of aflatoxin contamination



Association of NOW with aflatoxigenic fungi



Incidence of NOW moths with *Aspergillus flavus* collected with sticky traps from a pistachio orchard



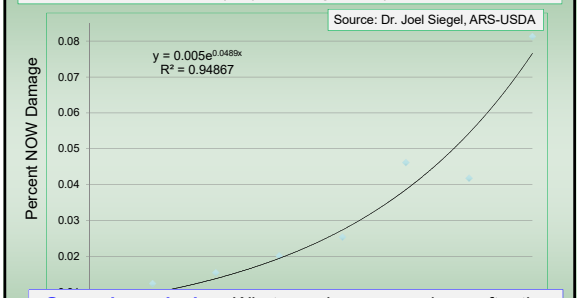
Frequency estimate of aflatoxin-producing isolates carried by NOW in pistachio orchards in Madera Co.

Isolates/strains from NOW	Orchard 1	Orchard 2
Isolates tested	41	24
S-strain recovered	2	3
L-strain positive for aflatoxin	7	7
Total toxigenic strains	**9**	**10**
Toxigenic strains (%)	**22 %**	**42 %**
Aspergillus sect. *Flavi* carried by NOW at harvest (%)	10 %	10 %
Toxigenic strains carried by NOW at harvest (%)	2.2 %	4.2 %

2. Delaying harvest increases NOW infestation

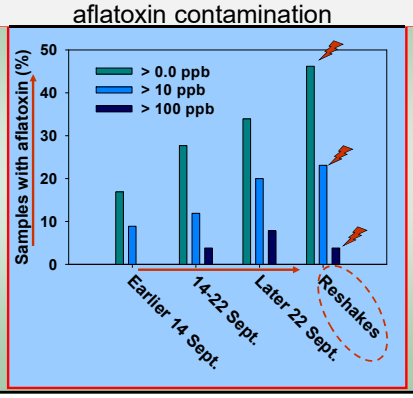
Year 1		Year 2	
Harvest date	NOW(%)	Harvest date	NOW(%)
10 Sept.	1.8	14 Sept.	1.8
20 Sept.	5.2	20 Sept.	3.1
30 Sept.	12.1	28 Sept.	6.1
		4 Oct.	9.1
		12 Oct.	14.2

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



General conclusion: Whatever damage you have after the 1st week of harvest it will be doubled after the 3rd week of harvest (according to Dr. Siegel, USDA, ARS)

Harvest dates, incidence, and amounts of aflatoxin contamination

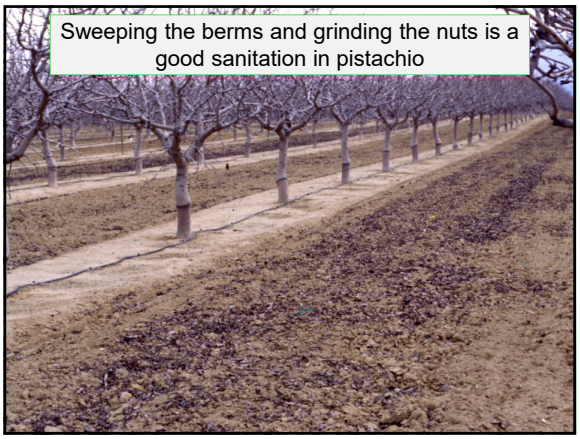


Recommendations:

Reduce damage by NOW

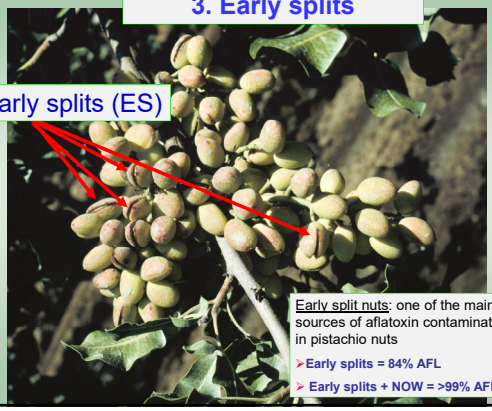
- ✓ Follow an aggressive insecticide program (sprays in summer/ sprays postharvest).
- ✓ Decrease number of nuts left after harvest
- ✓ Winter sanitation should be an annual practice.
- ✓ Do not delay harvest.

Sweeping the berms and grinding the nuts is a good sanitation in pistachio



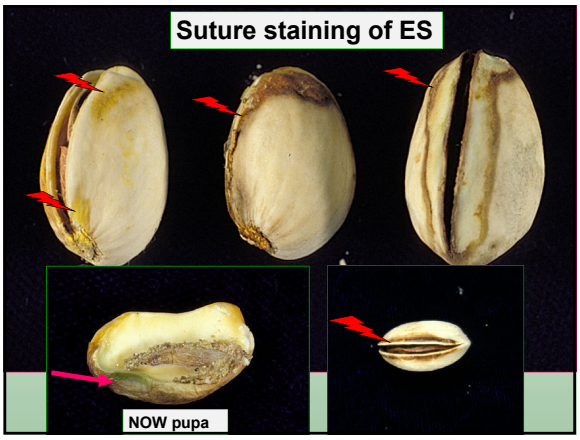
3. Early splits

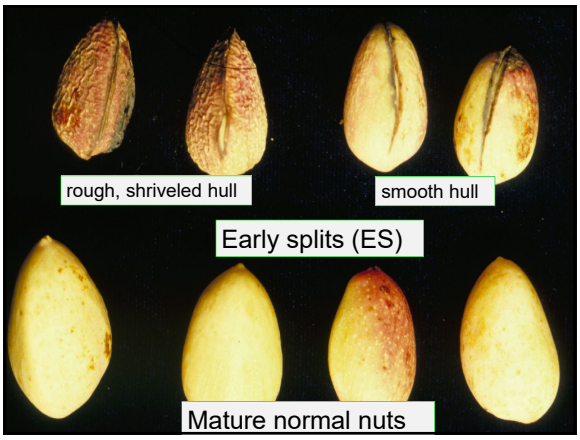
Early splits (ES)

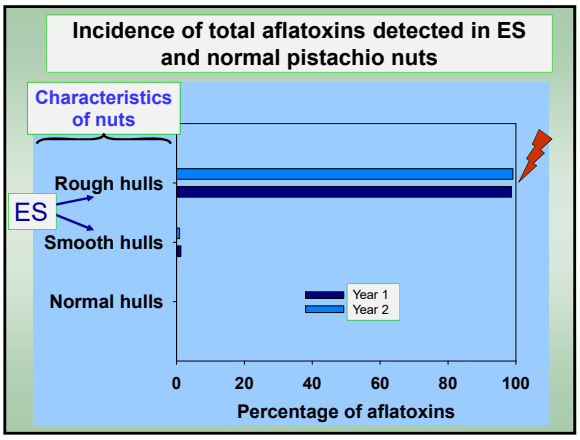


Early split nuts: one of the main sources of aflatoxin contamination in pistachio nuts

- Early splits = 84% AFL
- Early splits + NOW = >99% AFL


Suture staining of ES
NOW pupa


rough, shriveled hull
smooth hull
Early splits (ES)
Mature normal nuts


Incidence of total aflatoxins detected in ES and normal pistachio nuts
Characteristics of nuts
ES
Rough hulls
Smooth hulls
Normal hulls
Year 1
Year 2
0
20
40
60
80
100
Percentage of aflatoxins

Relationship of the hull appearance and NOW infestation with kernels of early split pistachios

Characteristics of early split fruit	positive samples (%)	aflatoxins per nut (ppb)	% of total aflatoxins
Rough hull;NOW	60	2998	83.7
Rough hull; no NOW	20	141	16.2
Smooth hull; NOW	20	2	0.1
Smooth hull; no NOW	0	0	0.0

99.9%

The normal nuts had no aflatoxins.

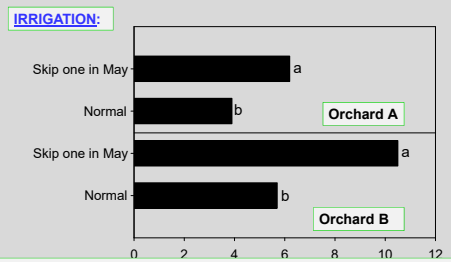
Incidence of early splits (ES) in 10 pistachio orchards

Orchard	Early split nuts (%)
I	0.6
J	0.6
C	0.8
G	2.0

➔ **Recommendation: Reduce the incidence of ES**

F	3.4
D	3.4
A	4.3
B	5.7
E	6.5

✓ **3. - Cultural practices, irrigation:** Effect of irrigation on incidence of early split



➔ Recommendation: **Apply sufficient irrigation during spring to avoid tree stress.**

✓ **3. - Rootstock:** Four major rootstocks were evaluated

- *Pistacia atlantica*
- UCB1 (hybrid)
- PG I (Pioneer Gold)
- PGII (Pioneer Gold)

} *P. integerrima*

Effect of rootstock on ES: Four trials in four different locations were evaluated

	Early splits (%)			
	Fresno County			
Rootstock	KAC	Westside	Kern Co.	Madera Co.
Atlantica	**4.7 a**	**0.8 a**	**0.4 b**	**16.6 a**
PGII	4.0 ab	1.8 a	0.9 a	10.3 b
PGI	4.0 ab	0.1 b	0.3 bc	9.3 b
UCB-1	**2.1 b**	**0.1 b**	**0.1 c**	**7.2 b**

➔ Recommendation: Use a rootstock that minimizes early split nuts.

4. Location/Region: Incidence of aflatoxin contamination by county

County	Samples with (%) >100.0 ppb
Merced	**12.5 (2nd)**
Tulare	**3.6**
Madera	**5.8 (3rd)**
Fresno	**15.4 (1st)**
Kern	**1.4**
Kings	**0.0**
Northern Calif.	**0.0**

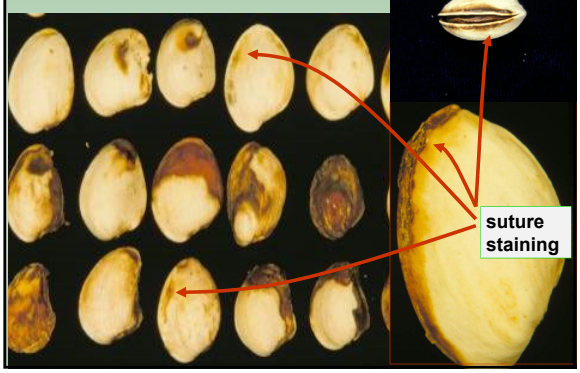
5. Effect of year, on vs. off: Example; results from aflatoxin evaluations of library samples for 2001-2005

	Percentage of samples		
Year	>0.0 ppb	>10.0 ppb	>100.0 ppb
2001 (off)	27.4	13.9	3.8★
2002 (on)	9.8	5.6	0.7
2003 (off)	34.7	19.7	2.7★
2004 (on)	12.2	3.5	0.0
2005 (off)	16.0	4.8	0.9★

5. Effect of year: Frequency of aflatoxin contamination in pistachios collected from orchards

1 nut in **5,000** nuts (**off years**)
to
1 nut in **20,000** nuts (**on years**)

6. Various defects: Early splits and stained nuts

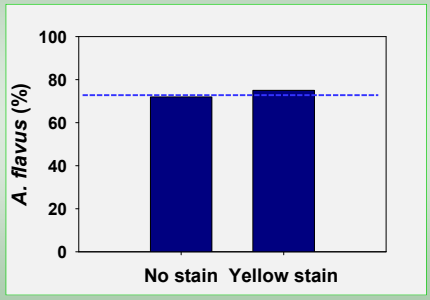


6. Defective nuts: Damaged by other means (DBOM)

High levels of *Aspergillus flavus*

High contamination with aflatoxins

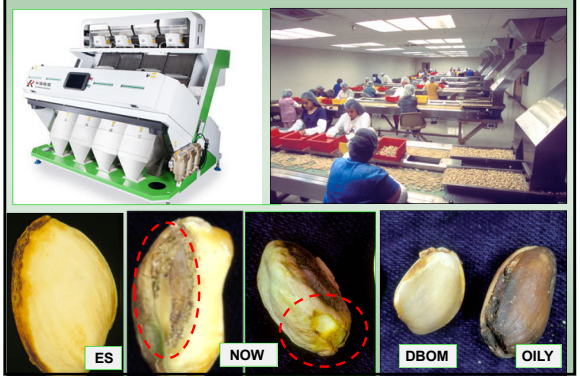
DBOM (or C-nuts) from a processing plant
(after surface sterilization)



Recommendations to reduce aflatoxin:

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

Automated-color and hand sorting for defective nuts



Aflatoxin management in pistachio

Biocontrol: Use of **atoxigenic strains** of *Aspergillus flavus* as bio-pesticides to reduce aflatoxins

(**atoxigenic strains** = strains that do not produce aflatoxins)

Rationale: The atoxigenic strains when applied in the field, increase in numbers, and displace the toxigenic strains.

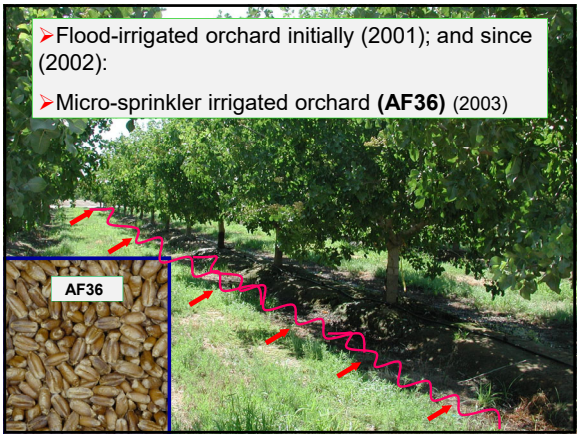
Strains of *Aspergillus flavus*

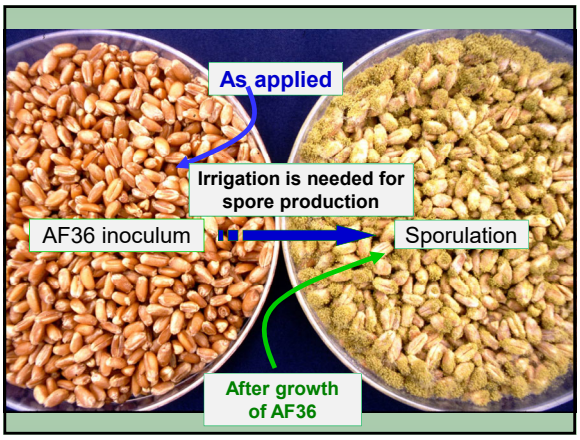
L - strain → about 50:50 toxigenic: atoxigenic → Atoxigenic AF36 strain

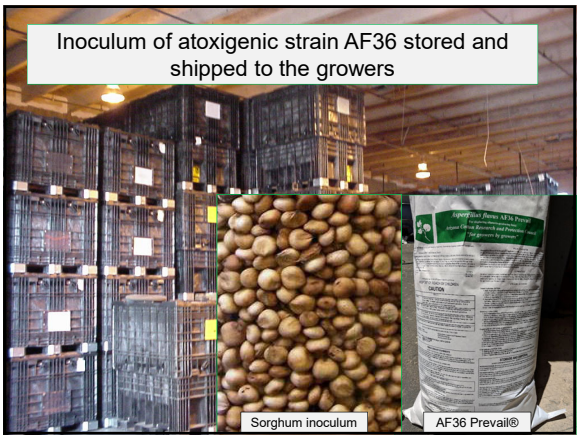
S - strain → most toxigenic

Natural occurrence of the atoxigenic strain AF36 among *A. flavus* isolates from orchards in California

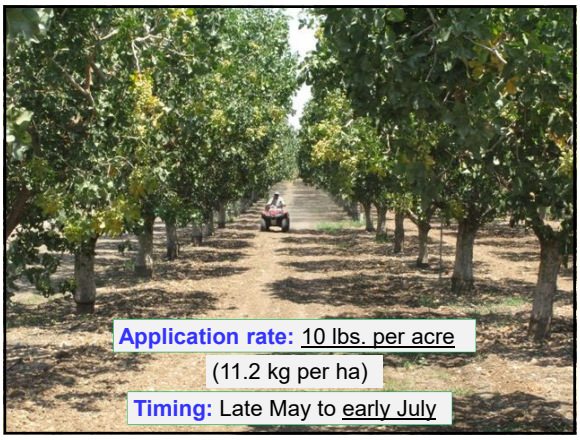
	AF36 (%)		
County	Pistachio	Almond	Fig
Butte	…	6.5	…
Colusa	…	3.0	…
Fresno	3.1	…	6.1
Glenn	…	4.4	…
Kern	12.7	8.5	…
Madera	7.2	5.0	7.2
Merced	15.0	…	5.8
Tulare	2.9	…	…



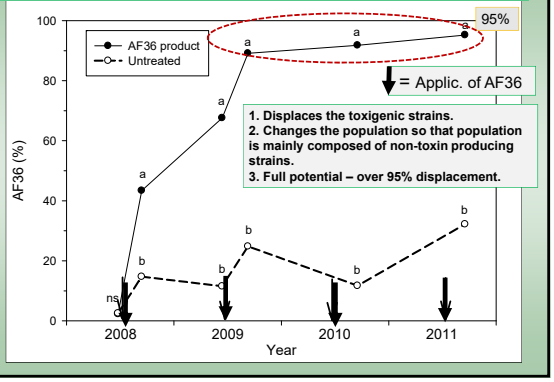


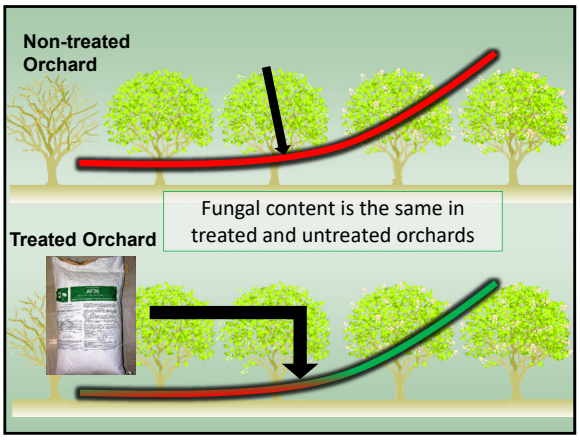

Inoculum of atoxigenic strain AF36 stored and shipped to the growers
Sorghum inoculum
AF36 Prevail®

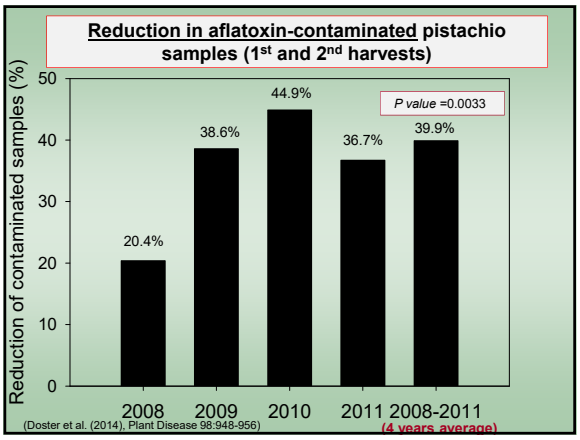

AF36 Inoculum

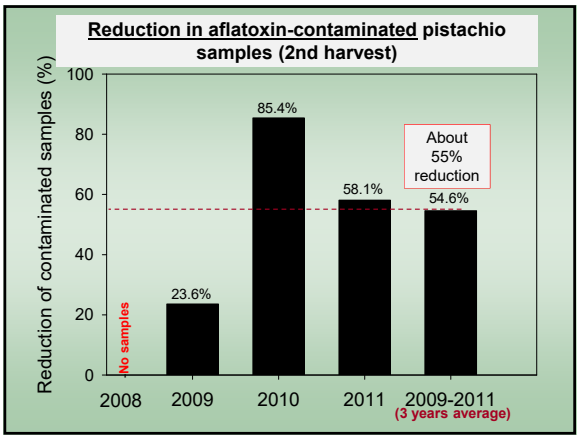

Application rate: 10 lbs. per acre
(11.2 kg per ha)
Timing: Late May to early July

The AF36 displaced the toxigenic ***A. flavus*** **isolates in soil**





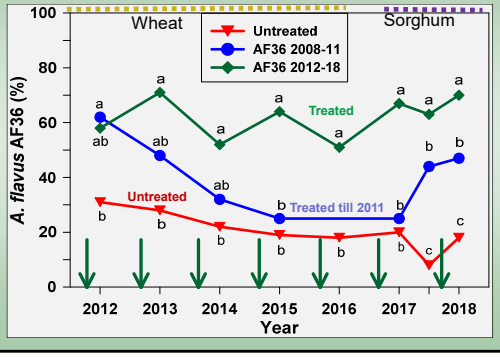




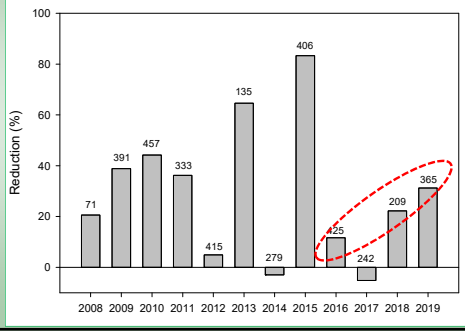
Treated pistachio acreage with AF36

Product	Year	Acreage treated (ha)
AF36-wheat	2012	73,000 acres (29,500 ha)
	2013	150,000 acres (60,700 ha)
	2014	200,000 acres (80,900 ha)
	2015	200,000 acres (80,900 ha)
	2016	200,000 acres (80,900 ha)
AF36 Prevail	2017	200,000 acres (81,000 ha)
	2018	more than 200,000 acres
	2019	
	2020	

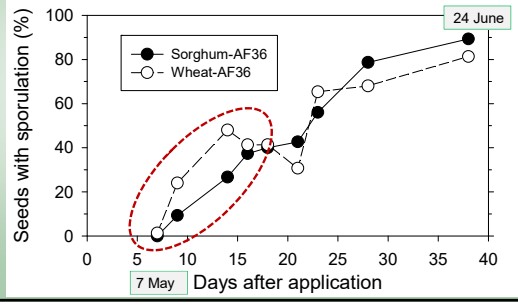
Challenge: Displacement of toxigenic strains was reduced from about 85% - 95% down to 50% - 70%



Reduction of aflatoxin contamination in library samples from blocks treated with AF36 (2008 to 2019)



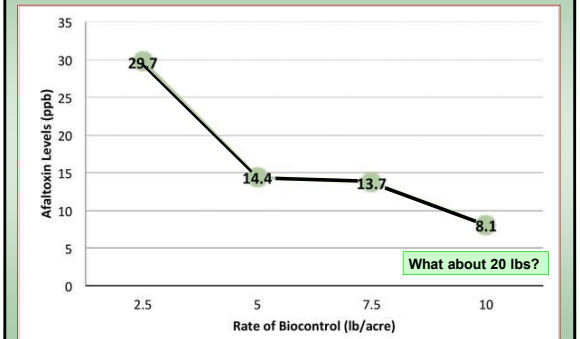
Challenge: Delay of production of spores and lower quantities by *Aspergillus flavus* AF36 in the sorghum product



Problems:

- ✓ Low, or delayed sporulation makes seed available to predation (i.e. insects, birds, and rodents).
- ✓ Predation results in product loss before sporulation.
- ✓ Product loss reduces the displacement of toxigenic *Aspergillus flavus*, so the effect in reducing aflatoxin contamination is minimized.

Aflatoxin concentrations (ppb) in response to biocontrol application rate (lb/acre).

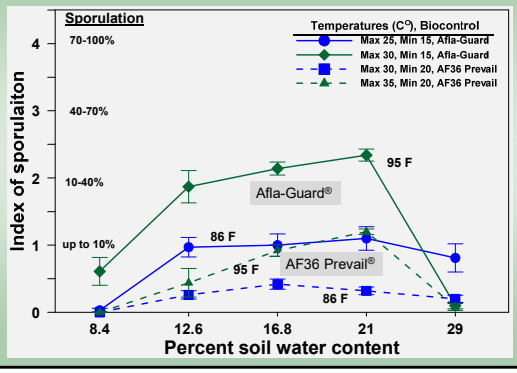


Afla-Guard® GR from Syngenta

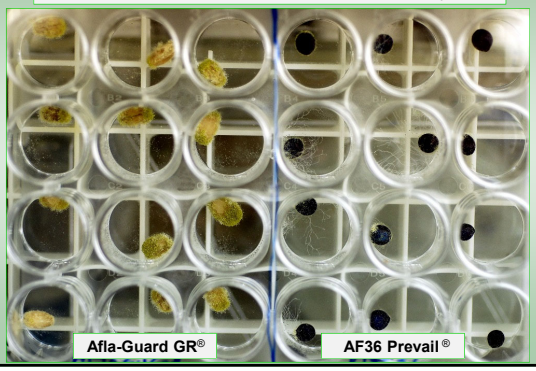
US Environmental Protection Agency Office of Pesticide Programs
BIOPESTICIDE REGISTRATION ACTION DOCUMENT
***Aspergillus flavus* (NRRL 21882)**
(PC Code 006500) - **March 24, 2004**

It has not been registered in California

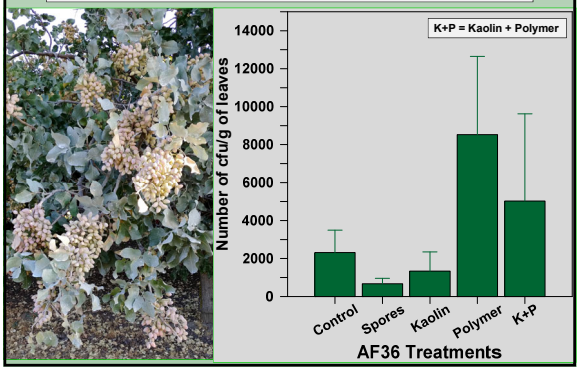
Sporulation of Afla-Guard® and AF36 Prevail® under different soil moistures



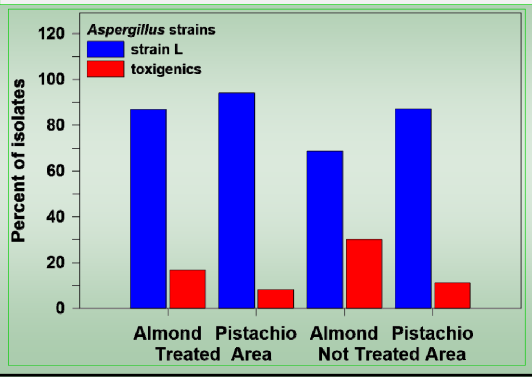
Sporulation: Afla-Guard® vs. AF36 Prevail® 4 days after incubation at 59ºF & optimal humidity

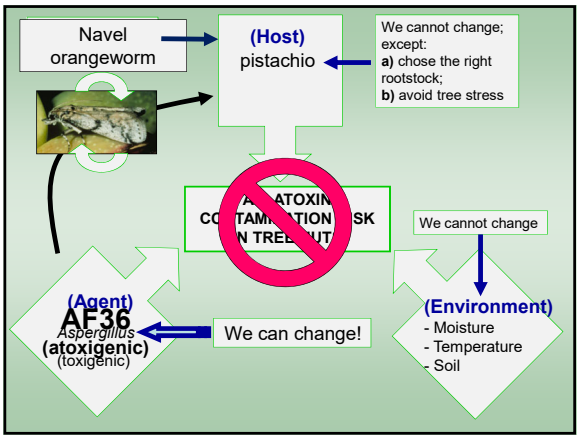


Application of AF36 directly on the canopy alone or with additives



Aflatoxin management in pistachio – Area-wide programs (compare two areas containing pistachio and almonds)





Additional information on AF36 application

Link of video on application of AF36
(Lead actors: Bob Beede & Bob Klein)

www.calpistachioresearch.org/training-videos/

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Thank you !