



# Aflatoxin Contamination of Nut Crops: Biocontrol by Using Atoxigenic Strains, Successes and Recent Challenges

Themis J. Michailides

Mark Doster, Mattias Donner, Alejandro Ortega-Beltran,  
Juan Moral, Paulo Lichtemberg, Ramon Jaime, M. Teresa  
Garcia, Victor Gabri, John Lake, and Apostolos Papagelis

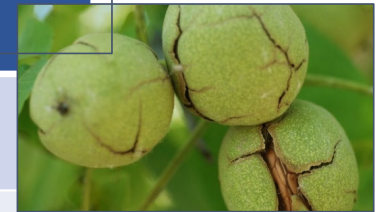
**University of California Davis/Dept of Plant Path**  
**Kearney Agricultural Research and Extension Center**  
**Parlier, CA 93648, USA**



## Total and bearing acreages and Farm Gate Value (FGV) of nut crops in California



Acreage	Almond	Pistachio	Walnut
<b>Total</b>	1,650,000 acres = 668,000 ha	520,000 acres = 210,000 ha	445,000 acres = 180,000 ha
<b>Bearing</b>	1,350,000 acres = 546,000 ha <b>FGV: \$3.88 billion</b>	446,000 acres = 180,500 ha <b>FGV: \$1.6 billion</b>	390,000 acres = 158,000 ha <b>FGV: \$1.2 billion</b>



**FGV = Farm Gate Value**

A large % of the nut production is exported to Europe and other countries

## *Mycotoxins:*

- Carcinogenic
- Mutagenic
- Teratogenic

toxic compounds



*Penicillium*



*Fusarium*

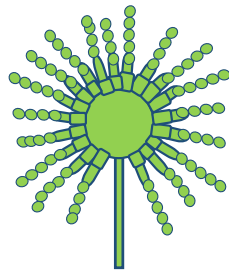
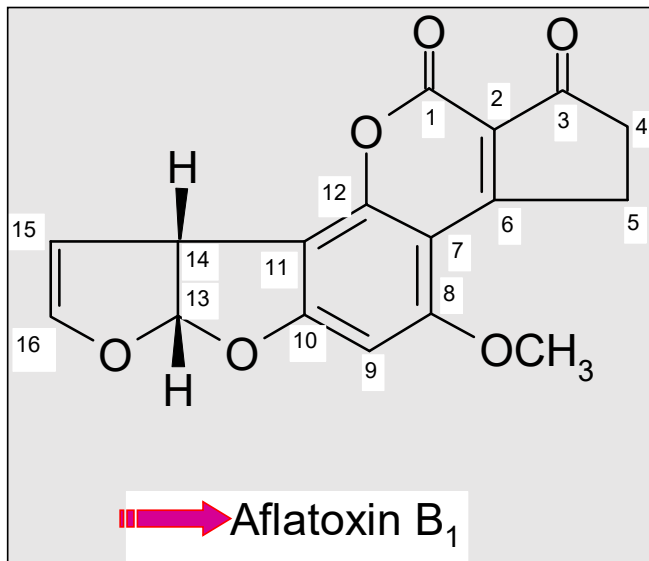


*Aspergillus*



*Aspergillus flavus* and *A. parasiticus* are present in nut crop orchards in California; they produce aflatoxins  $B_1$ ,  $B_2$ ,  $G_1$ , &  $G_2$

B1 is the most potent Class 2A carcinogen; it can cause liver cancer



*Aspergillus flavus*

$B_1$ ,  $B_2$

*Aspergillus parasiticus*

$B_1$  &  $B_2$ ,  $G_1$  &  $G_2$

Incidence of aflatoxin contamination in California  
pistachio, almond, and fig orchards

**1 nut in 20,000** pistachio nuts (“**on**” production  
to  
**1 nut in 5,000** pistachio nuts (“**off**” production y

Pistachio is an  
alternate crop:  
 (“on” & “off”  
production years)

**1 nut in 24,000** almond nuts

**1 fig in 5,000** fig fruits

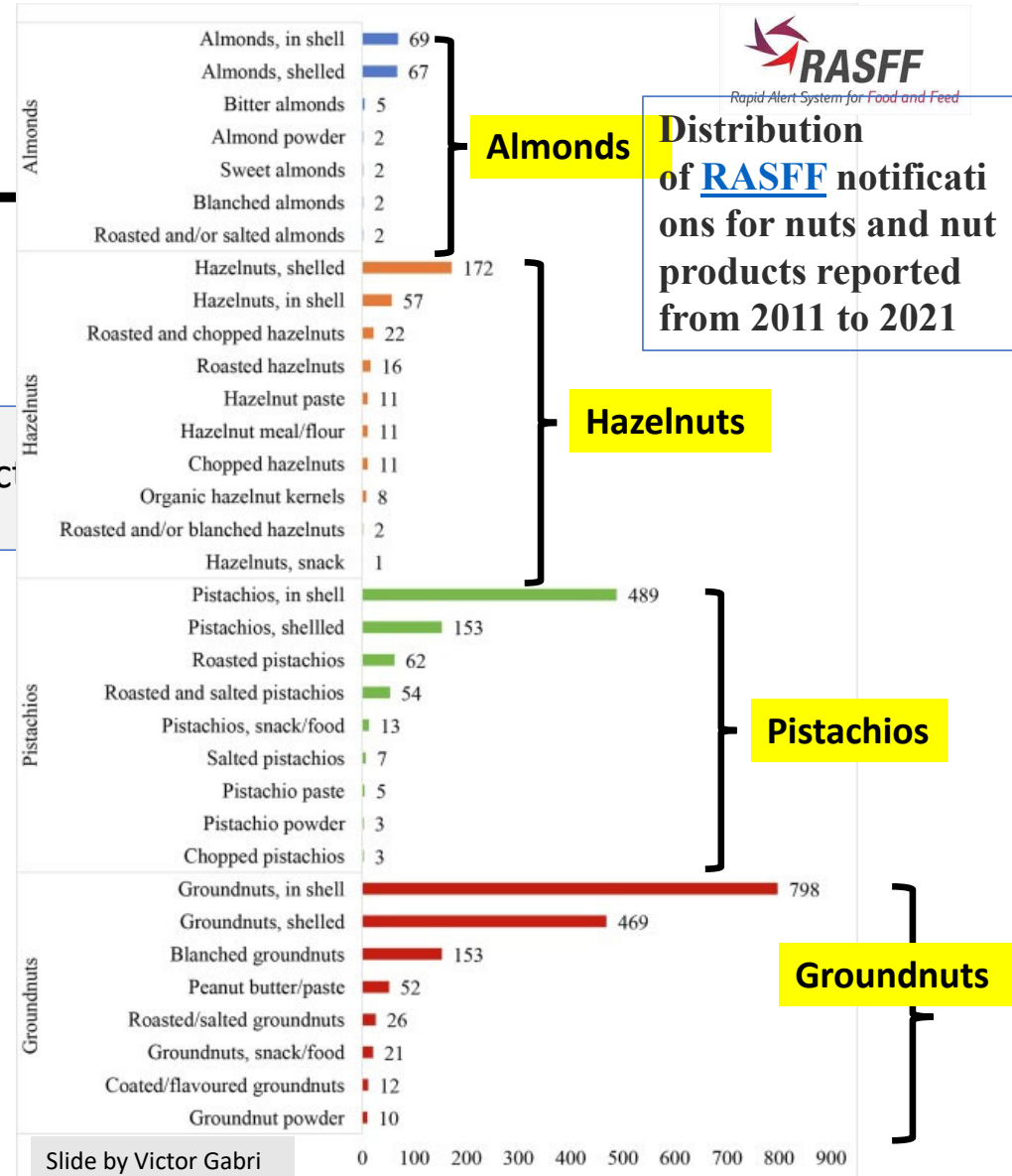
# Regulatory limits for aflatoxins

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

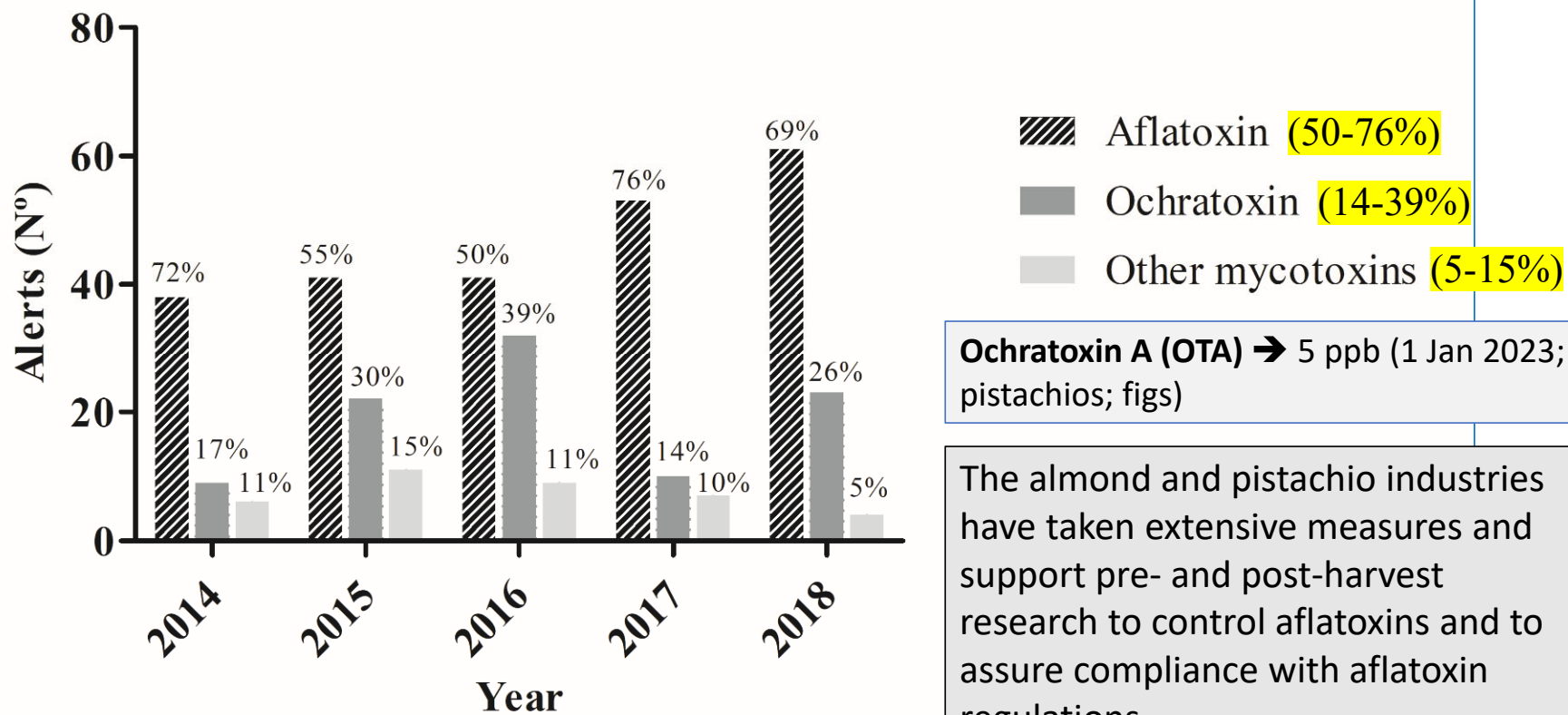
Pistachios and almonds for direct consumption

Total aflatoxins → 4 ppb  
Aflatoxin B1 → 2 ppb } walnuts and dried fruit

Total aflatoxins → 10 ppb  
Aflatoxin B1 → 6 ppb } dried figs



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



**Ochratoxin A (OTA)** → 5 ppb (1 Jan 2023; pistachios; figs)

The almond and pistachio industries have taken extensive measures and support pre- and post-harvest research to control aflatoxins and to assure compliance with aflatoxin regulations.

Comparative Incidence of aflatoxin contamination of nut crops in California (avg. of 5 years analyses by Dried Fruit Association)

pistachios



12%\*\*\*

almonds



3.5%\*

walnuts



3.2%\*

# Aflatoxin contamination of nuts

## Preharvest vs. Postharvest?

(if the nuts were dried quickly after harvest, stored properly, and kept dried at 6% moisture, then it would not be any postharvest contamination)

➤ **Preharvest contamination!**

➤ Leaky silos or stockpiles; no proper storage, etc... ➔ **Postharvest**

Silos for storage of nuts in California



Stockpiling of almonds in California

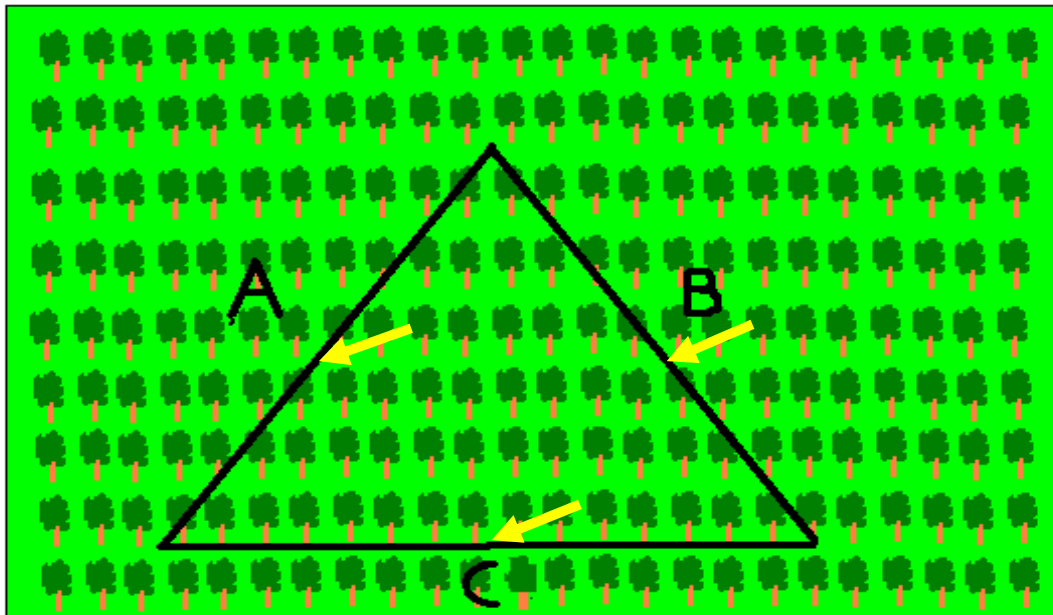


## Outline:

- Brief pathogen variability of the aflatoxigenic fungi in nut crops
- Major risk factors for aflatoxin contamination of nuts
- Pre- and postharvest practices to reduce aflatoxin
- Biocontrol of aflatoxins - successes and challenges

## Pattern of soil sampling

Almond Field



Colonies of *Aspergillus* Sect. *Flavi* in soil



## Fungal Variability: Strains of *Aspergillus flavus*



Large sclerotia

Medium size sclerotia

Small sclerotia

L - strain



~ 50%:50%  
toxigenic: atoxigenic  
strains

M - strain



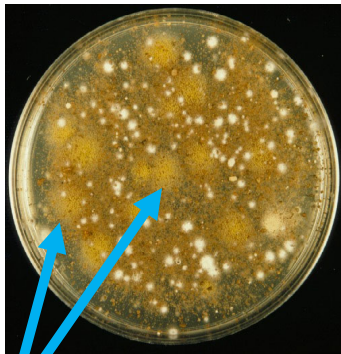
?  
*Aspergillus*  
*minisclerotigenes*

S - strain

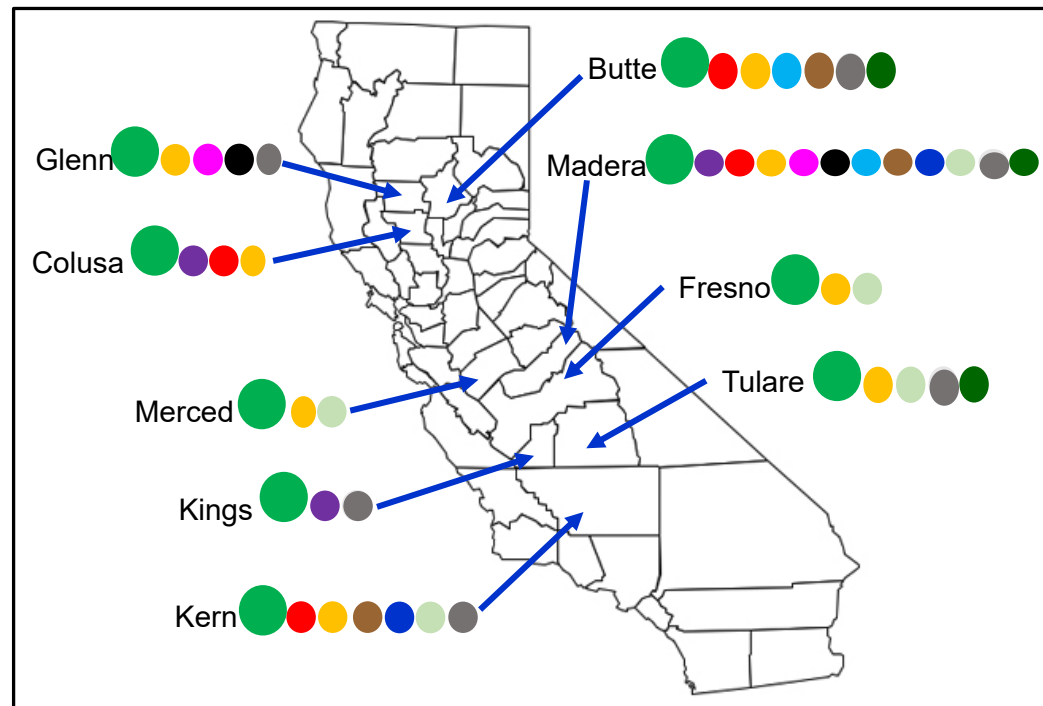


mostly toxigenic  
strains

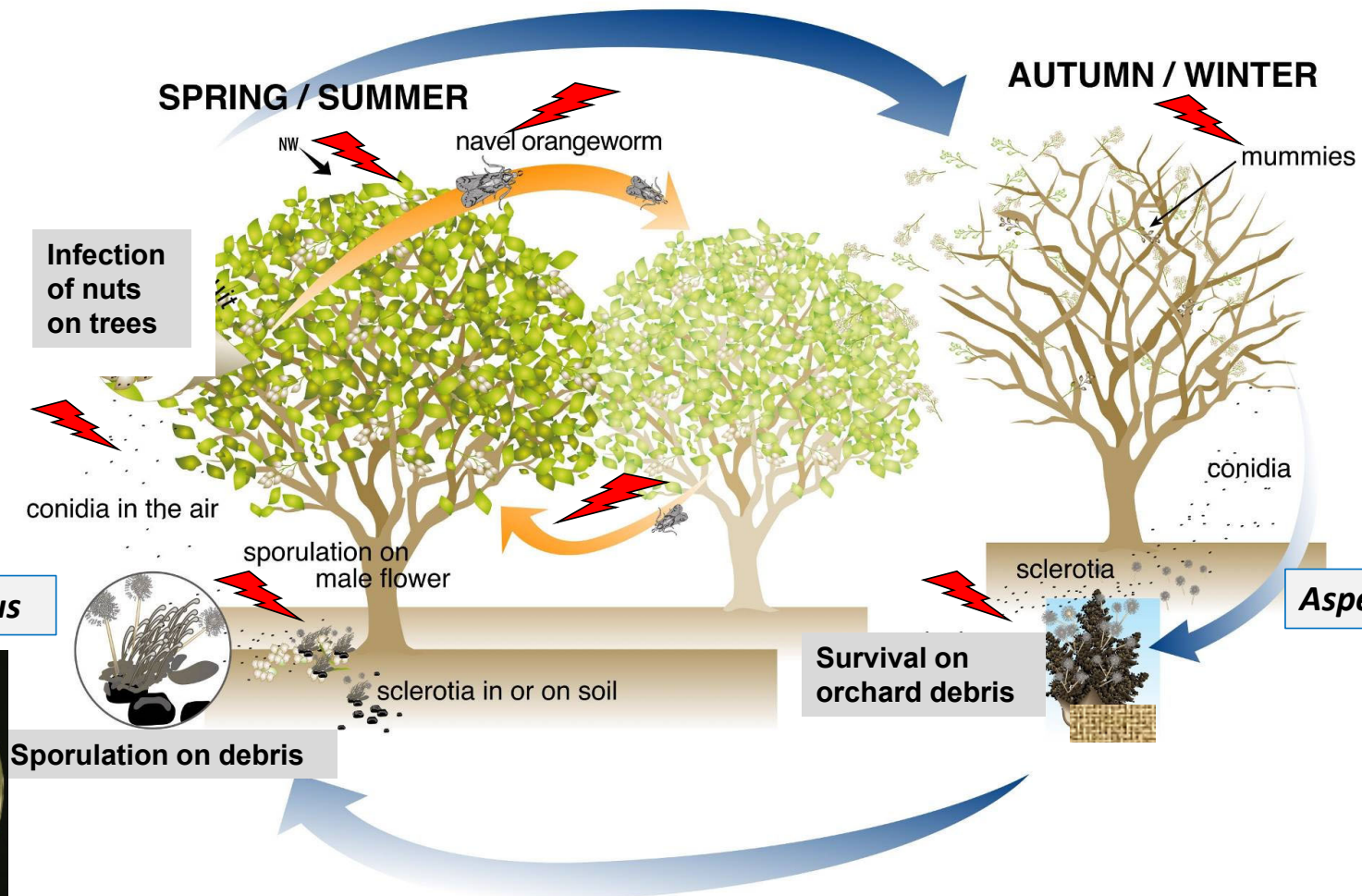
# Occurrence of *Aspergillus flavus* atoxigenic vegetative compatibility groups (VCGs = strains) in almond-growing counties of California



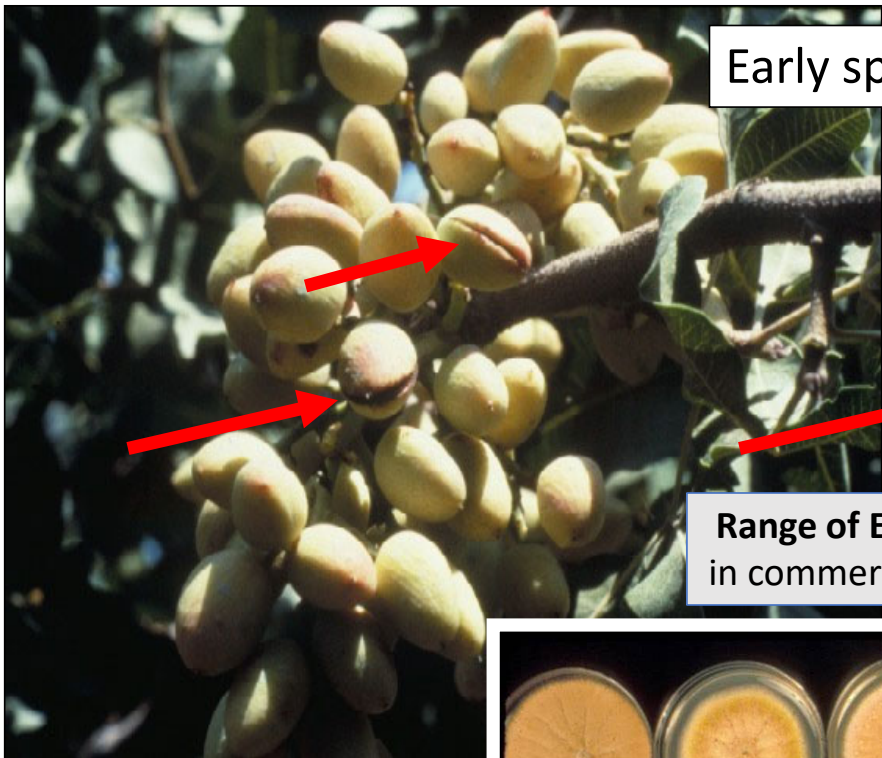
Colonies of *Aspergillus* Sect. *Flavi* in soil



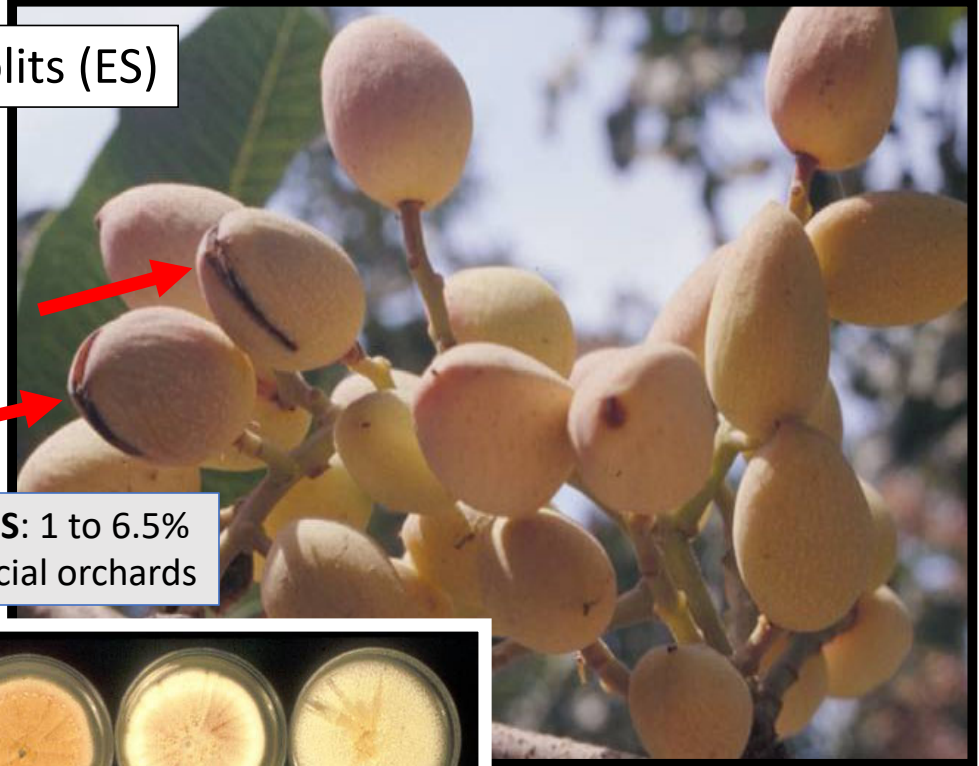
Each circle represents a VCG. The most common VCG, **AF36 in green**, was detected in all examined counties.



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



Early splits (ES)

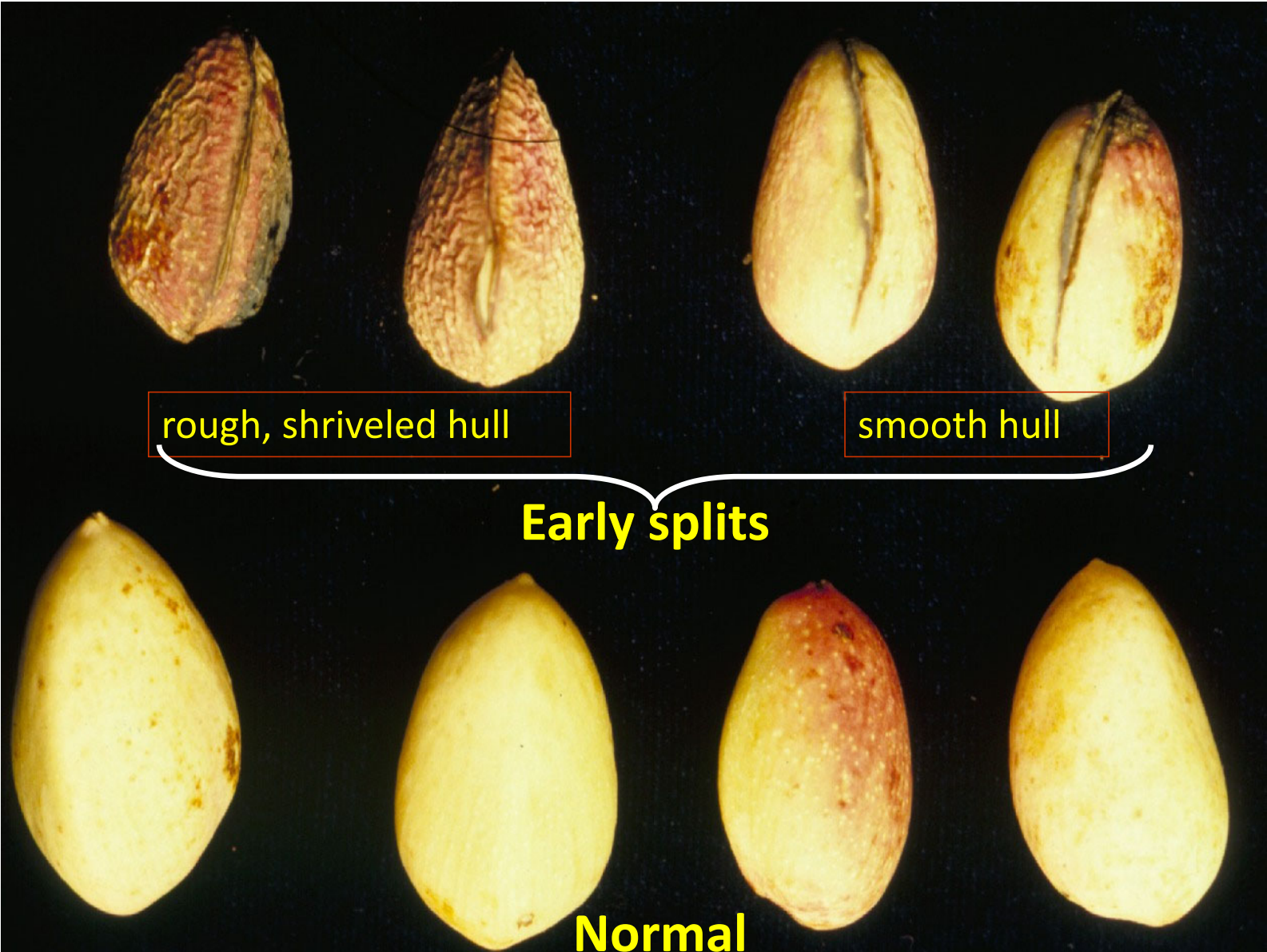


Range of ES: 1 to 6.5%  
in commercial orchards

	Nuts (%)
<i>Aspergillus niger</i>	30.3
<i>A. ochraceus</i>	0.9
<i>A. flavus</i>	0.7
<i>A. melleus</i>	0.5
<i>A. sydowii</i>	0.2
<i>A. tamarii</i>	0.2
<i>A. wentii</i>	0.2
<i>A. parasiticus</i>	0.1



Early split nuts: an excellent source of *Aspergillus* spp., NOW infestation, & good candidates for aflatoxin contamination



rough, shriveled hull

smooth hull

Early splits

Normal

## Risk Factor: Early-splits

Incidence of total aflatoxins detected in ES and normal pistachio nuts

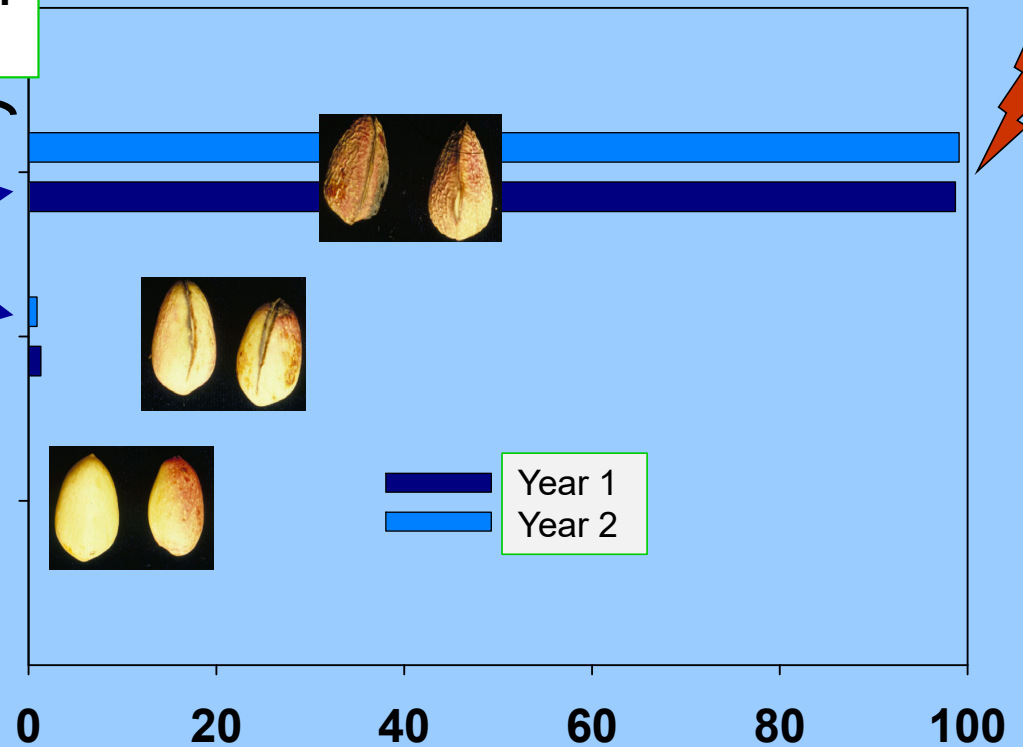
Characteristics of nuts

Rough hulls

ES

Smooth hulls

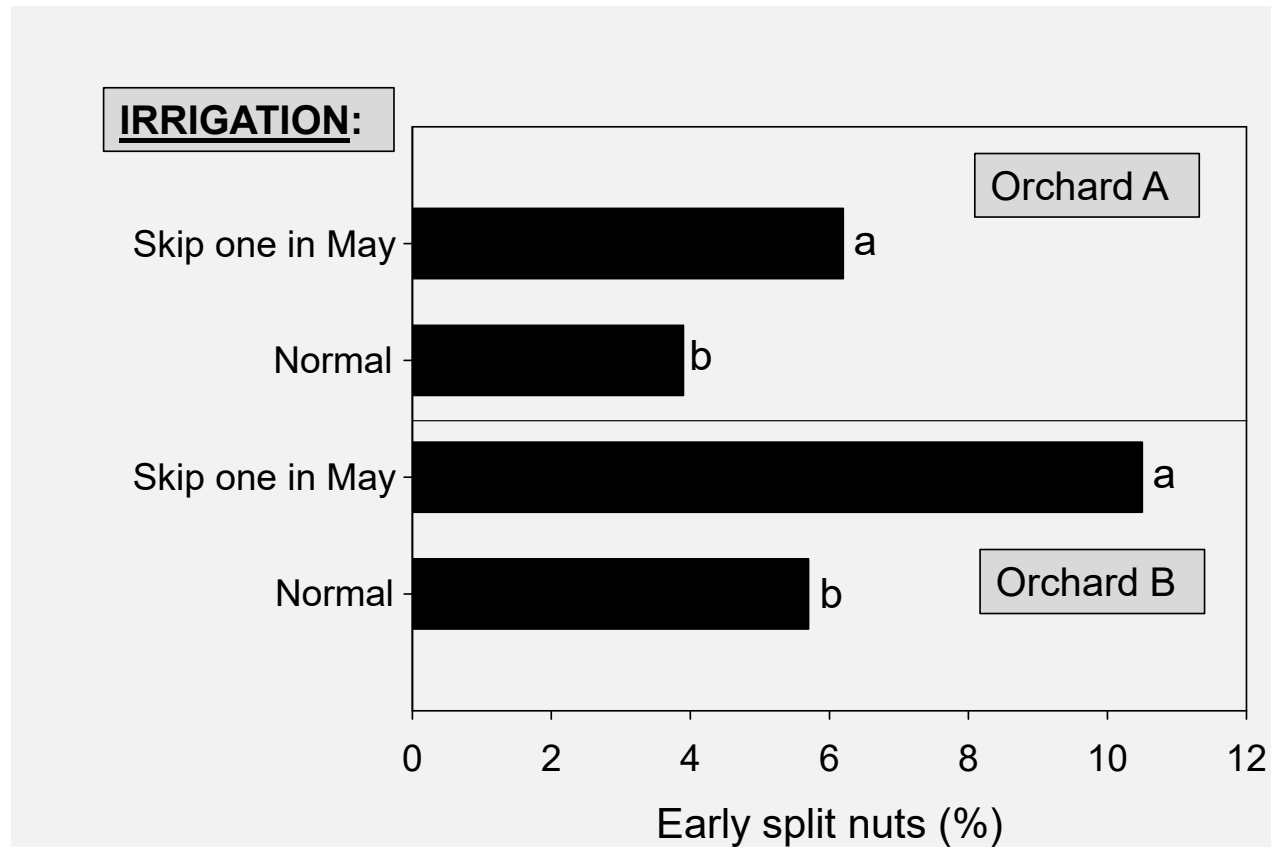
Normal hulls



➤ Reduce early splits (ES)

Percentage of aflatoxins

## Effect of irrigation on early split (ES) development in pistachios



➤ **Apply sufficient irrigation during spring to avoid tree stress & reduce ES**

## Rootstocks used for pistachio in California

*Pistacia atlantica*

UCB1 (hybrid)

PG I (Pioneer Gold I)

PGII (Pioneer Gold II)

*P. atlantica* × *P. integerrima*

*P. integerrima*

Lower % of  
Early splits

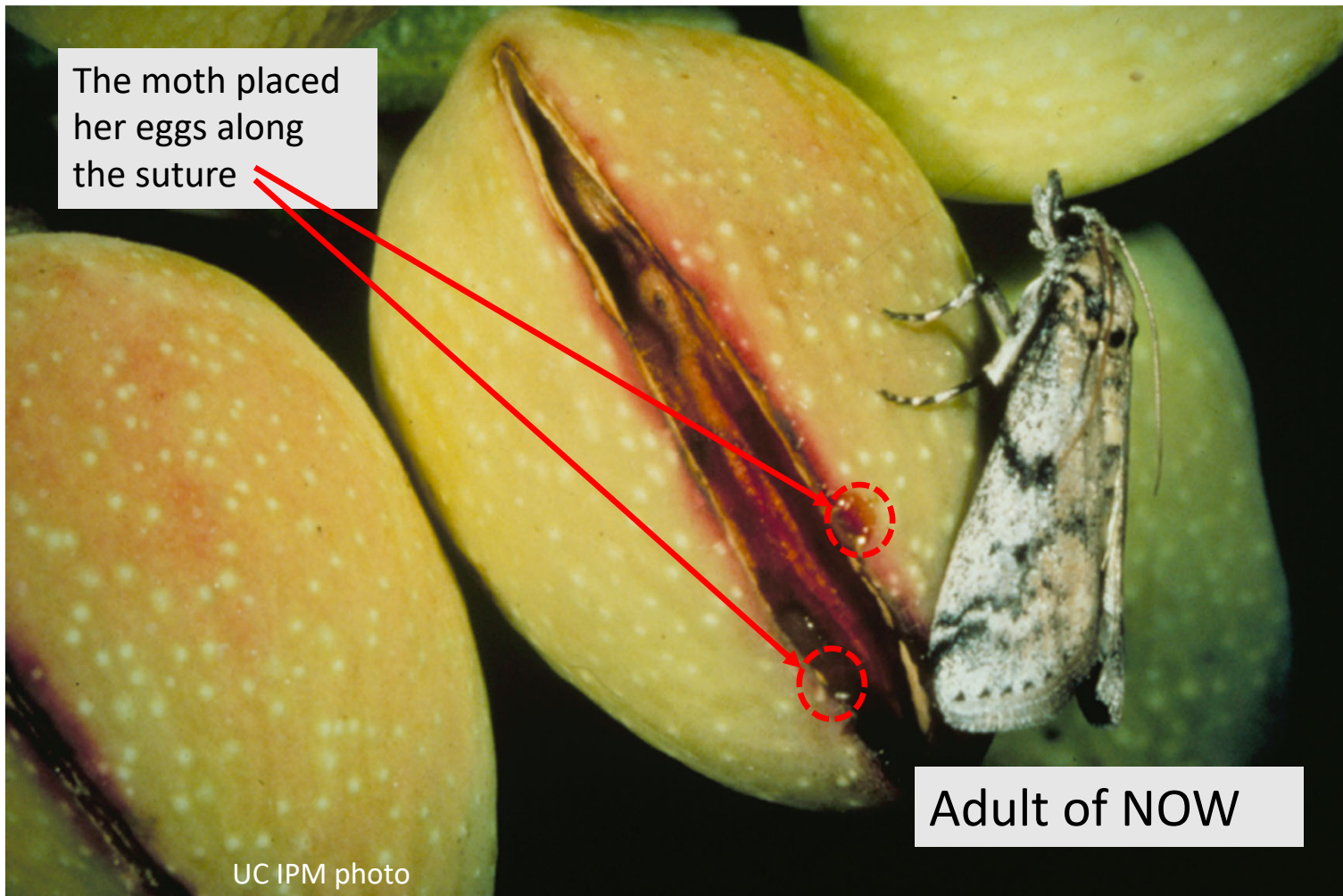
## Effect of rootstock on early split (ES) development in Kerman pistachios

Percentage of early split (ES) nuts in rootstock trials

Rootstock	Fresno County			
	KAC	Westside	Kern Co.	Madera Co.
<i>P. atlantica</i>	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

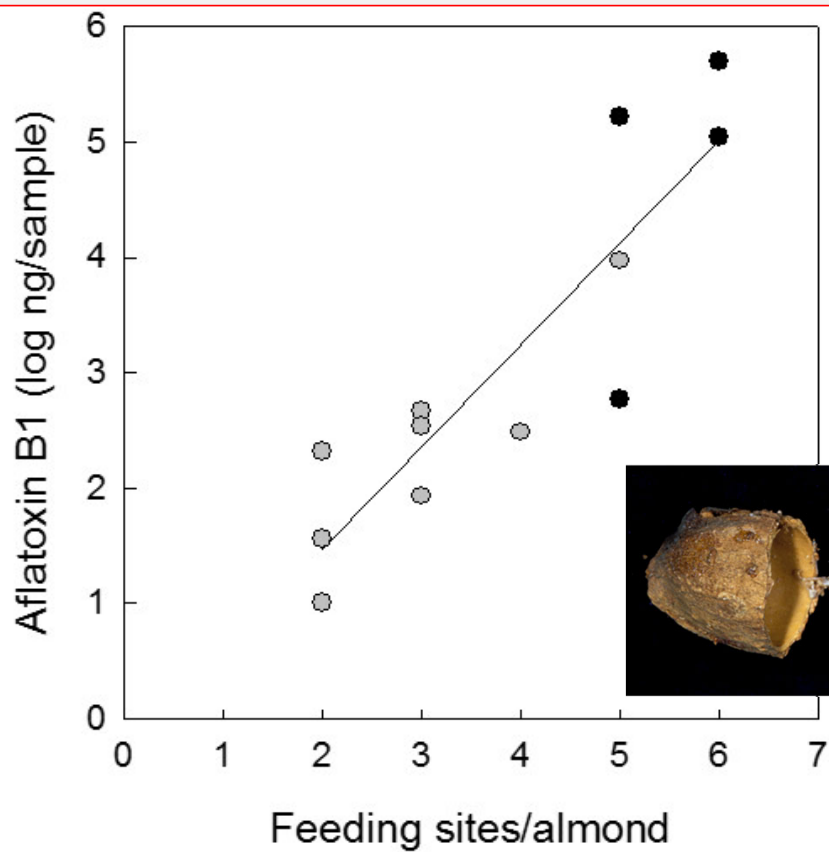
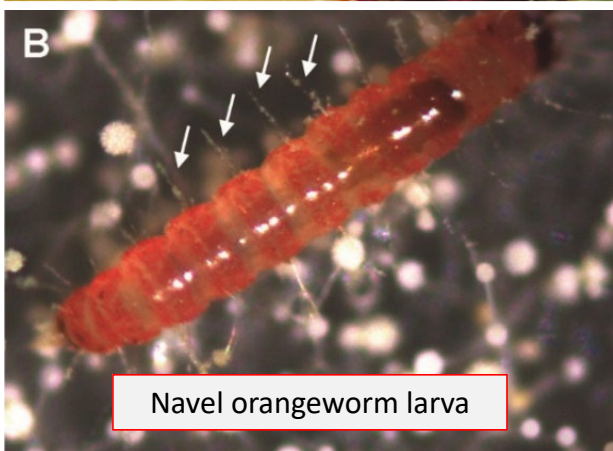
➤ Use a rootstock that minimizes early split nuts (the best is the hybrid UCB1)

## Navel orangeworm (NOW) moth on an early split pistachio



# Acquisition and transmission of *Aspergillus flavus* by navel orangeworm

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

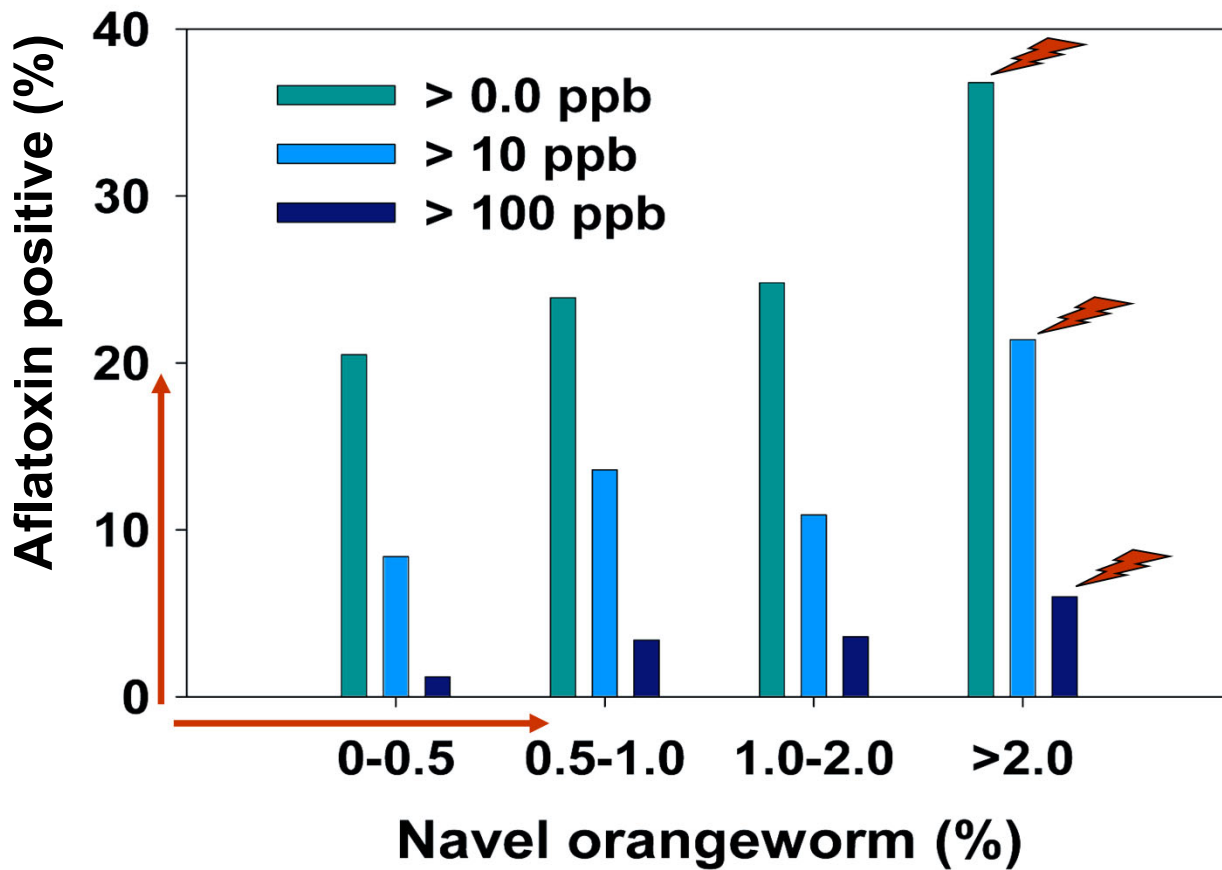


NOW damaged almond



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

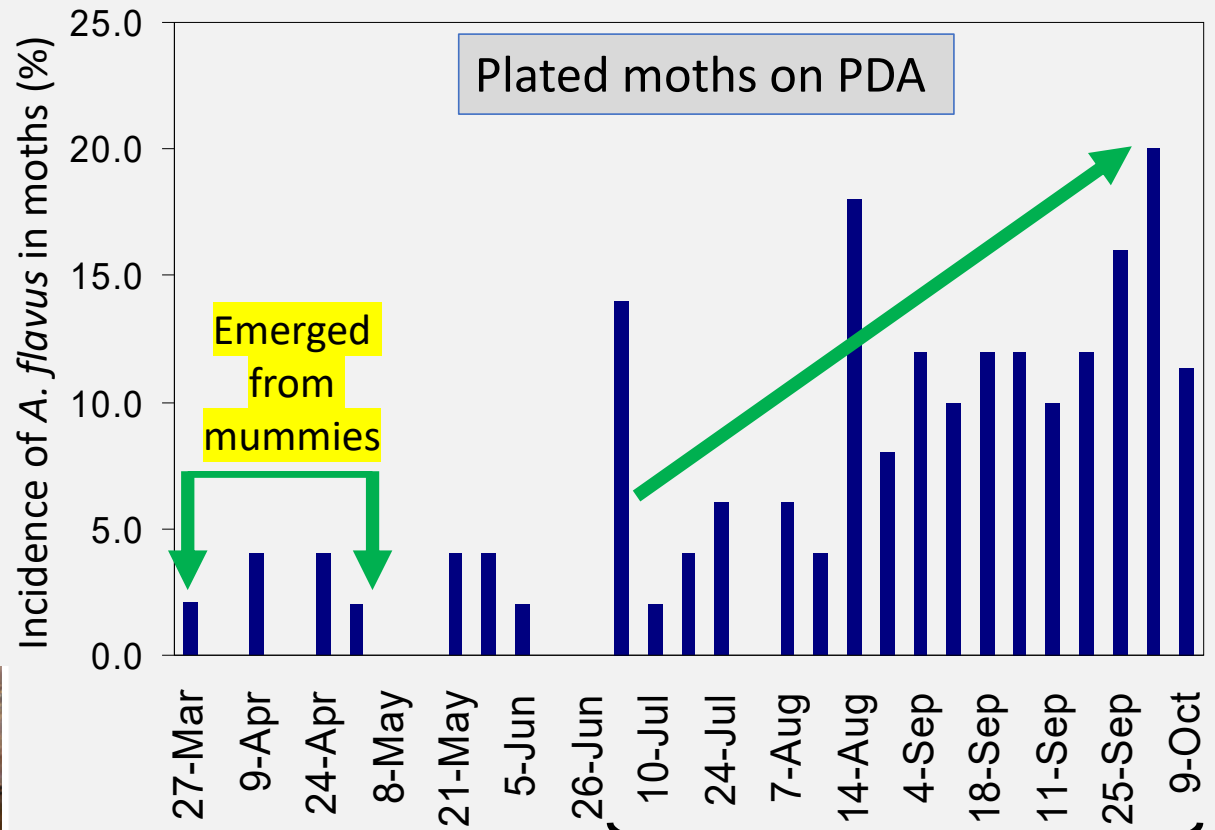
Relationship of navel orangeworm infestation and aflatoxin levels



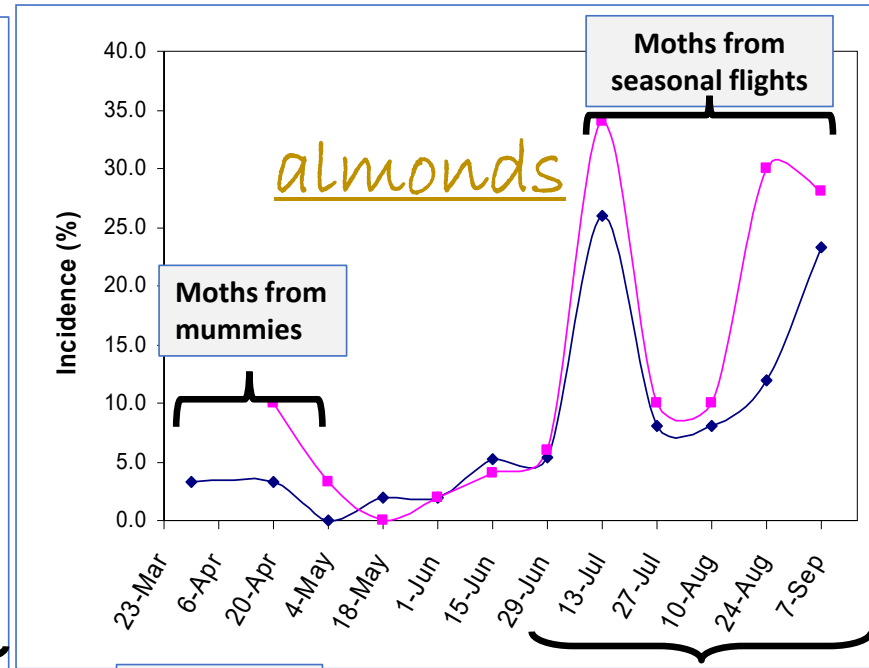
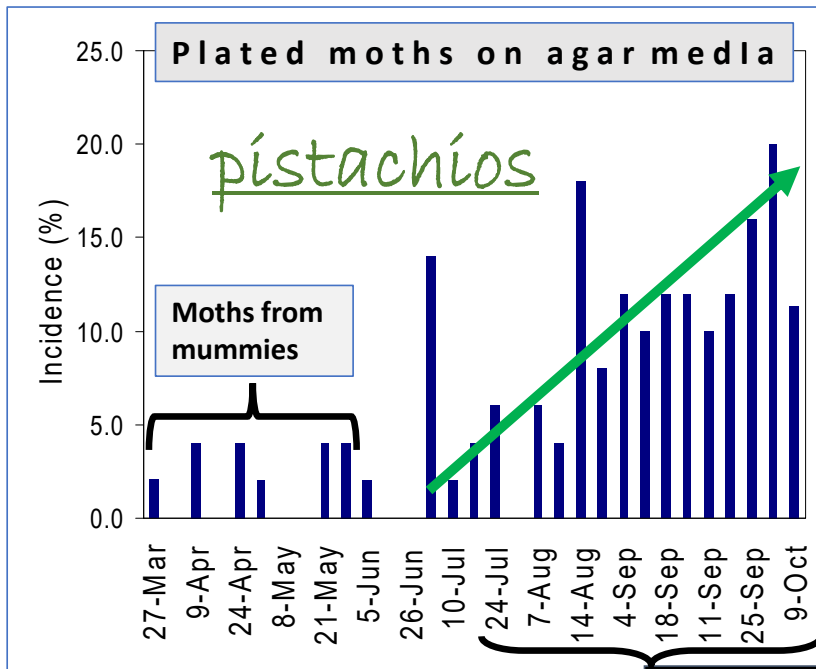
# Incidence of NOW moths with *Aspergillus flavus* collected with sticky traps in a pistachio orchard

Growth of *Asp. flavus* fungi on NOW moths

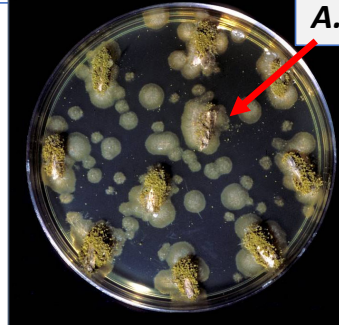
*A. flavus*



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



A sticky trap with NOW moths



*A. flavus*



## Delaying harvest increases NOW infestation

### Year 1

Harvest date	NOW(%)
10 Sept.	1.8
20 Sept.	5.2
30 Sept.	12.1

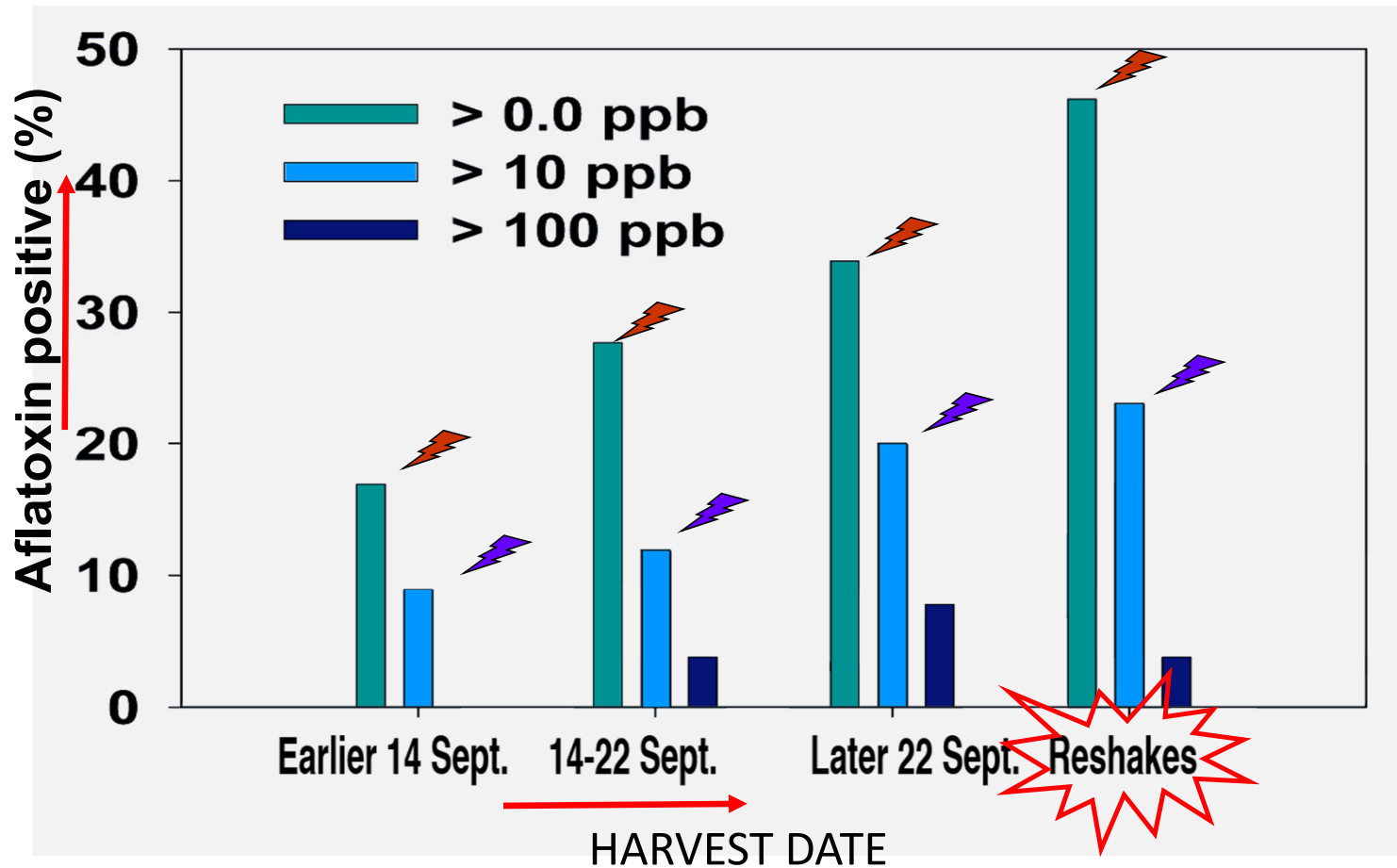
A 3 weeks harvest delay → 6X NOW% damage

### Year 2

Harvest date	NOW(%)
14 Sept.	1.8
20 Sept.	3.1
28 Sept.	6.1
4 Oct.	9.1
12 Oct.	14.2

A 4 weeks harvest delay → 7X NOW% damage

Relationship of harvest date and aflatoxin contamination levels in pistachios





➤ For pistachio: Blowing the nuts off the berms and shredding them is a good sanitation

➤ For almond: Nocking the mummies (“mummy shake”), blow them off the berms, and then shredding them is an excellent sanitation practice

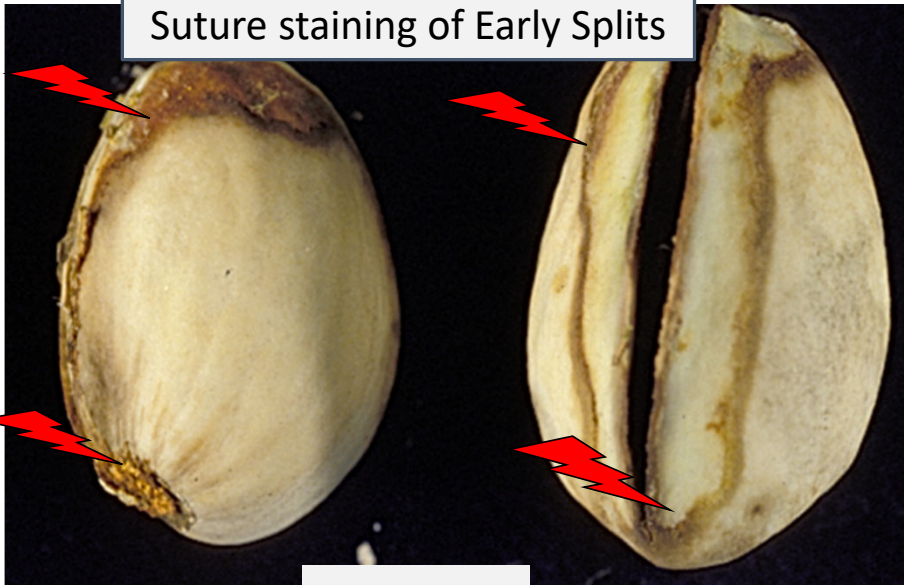
## Available data supporting recommendations to successfully reduce aflatoxins:

- Control navel orangeworm (NOW). \*\*\*\*\*
- Reduce early splits in the field. \*\*\*\*
  - ❖ 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 ES, damaged, and defected nuts. \*\*\*\*

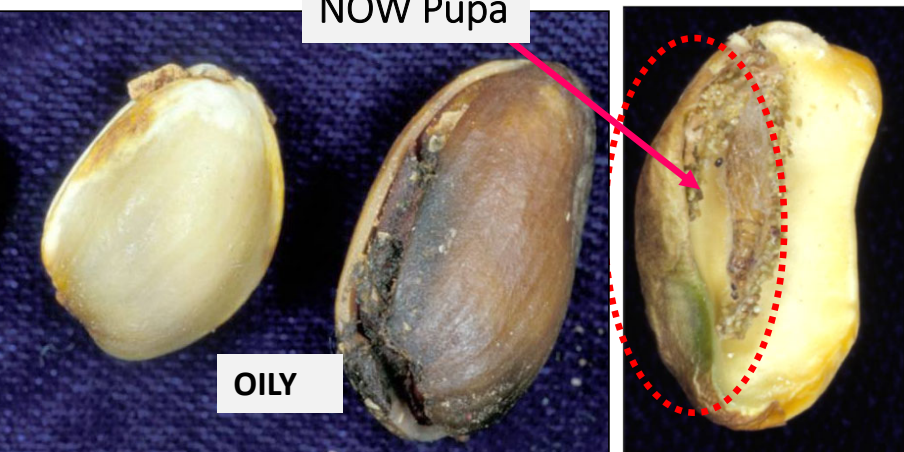


# Automated-color and hand sorting for defective nuts

Suture staining of Early Splits



NOW Pupa



Automated electronic color sorter



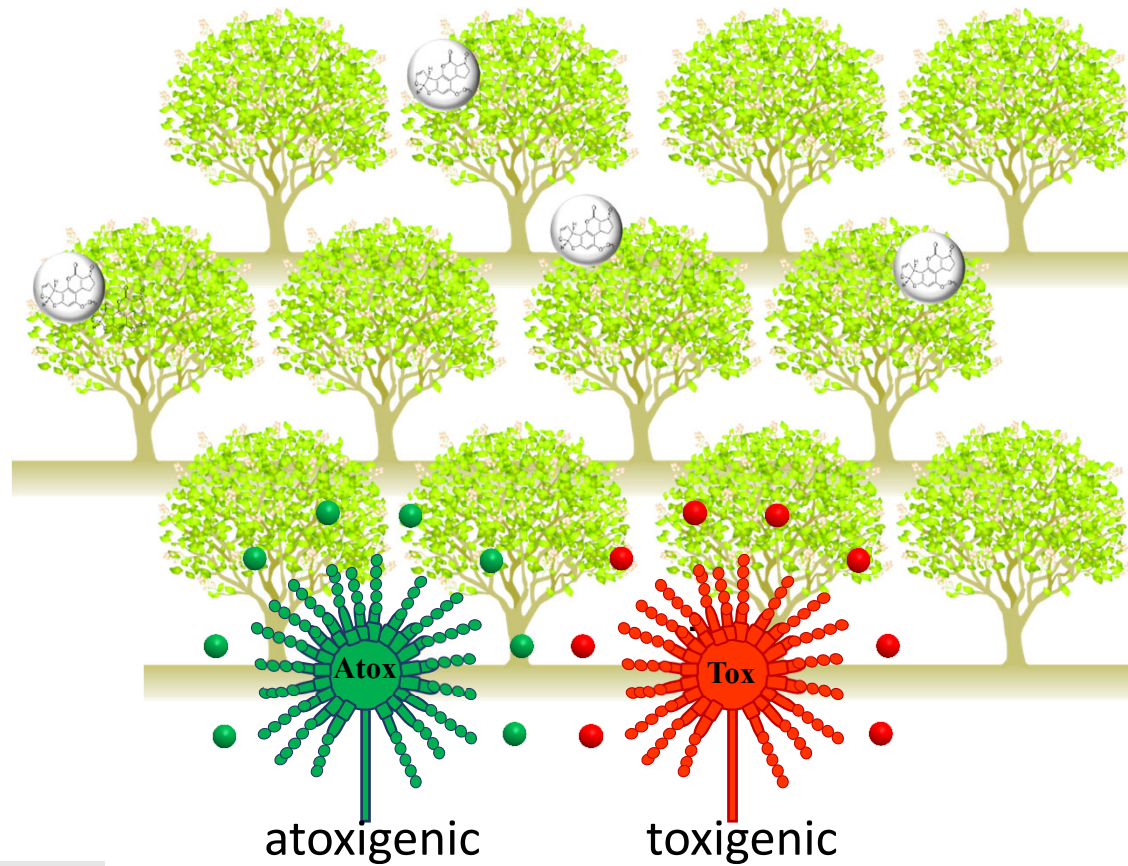
Aflatoxin management in nut crops using the  
**atoxicogenic *Aspergillus flavus* technology**

2002

**Atoxicogenic strains** = strains that do not produce aflatoxins

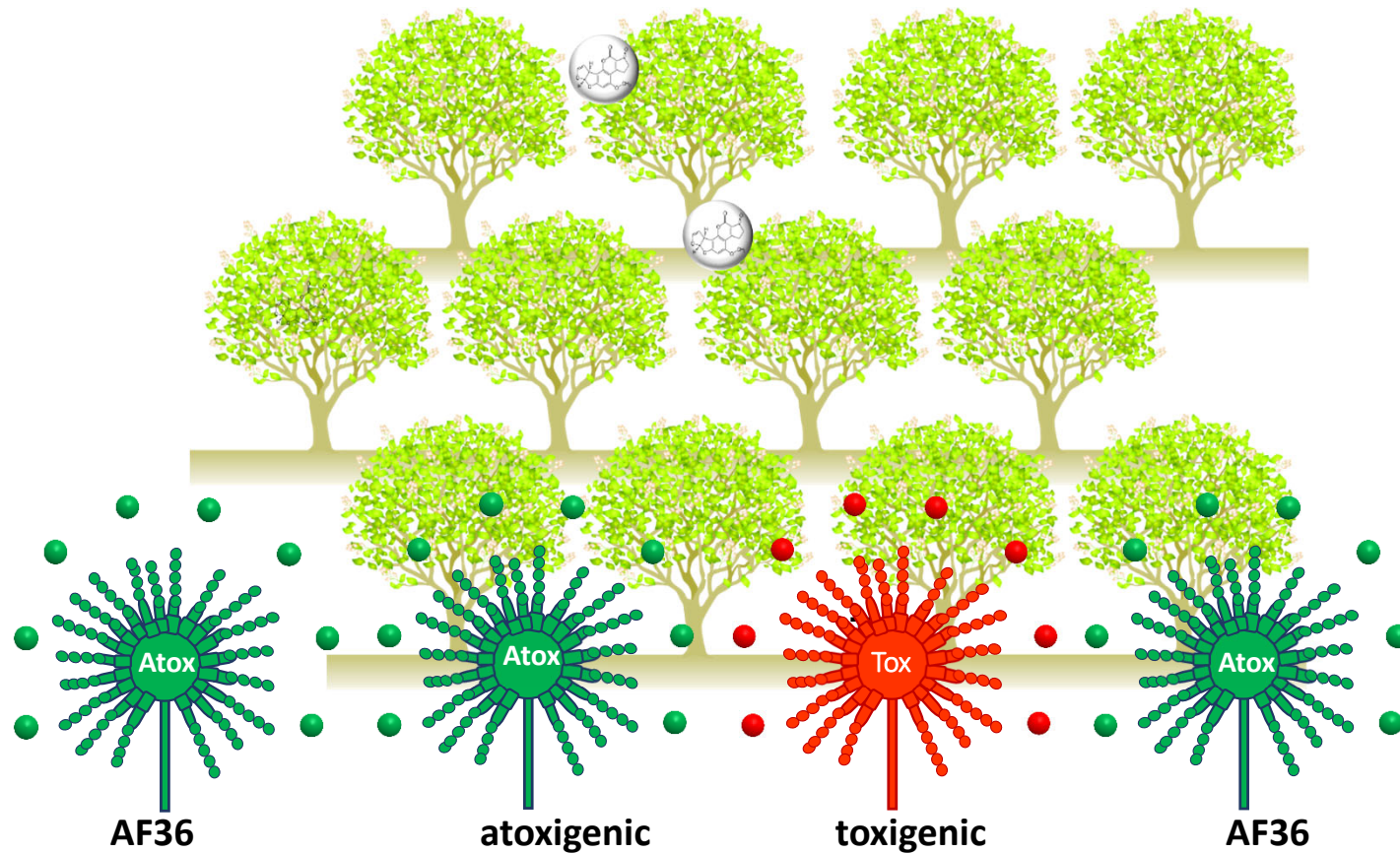
**Rationale:** The atoxicogenic strains when applied in the field, sporulate, increase in numbers, and displace the toxigenic strains

# Non-treated orchard



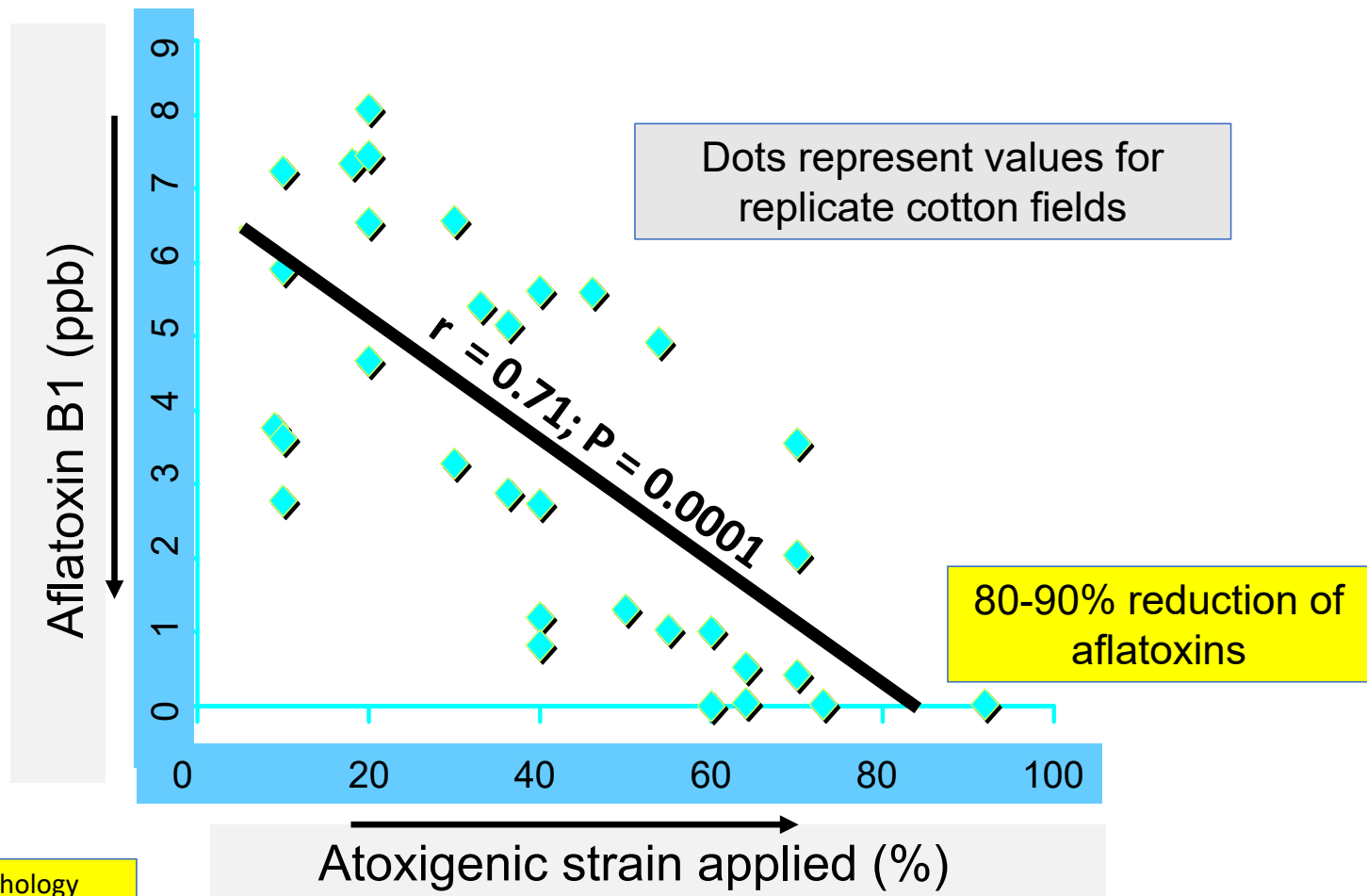
Slide by M. T. Garcia & Moral (modified)

## Treated orchard with the AF36



Slide by M. T. Garcia & Moral (modified)

# Aflatoxin in cottonseed versus strain AF36 incidence

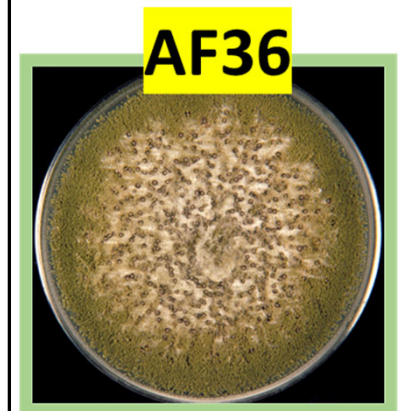
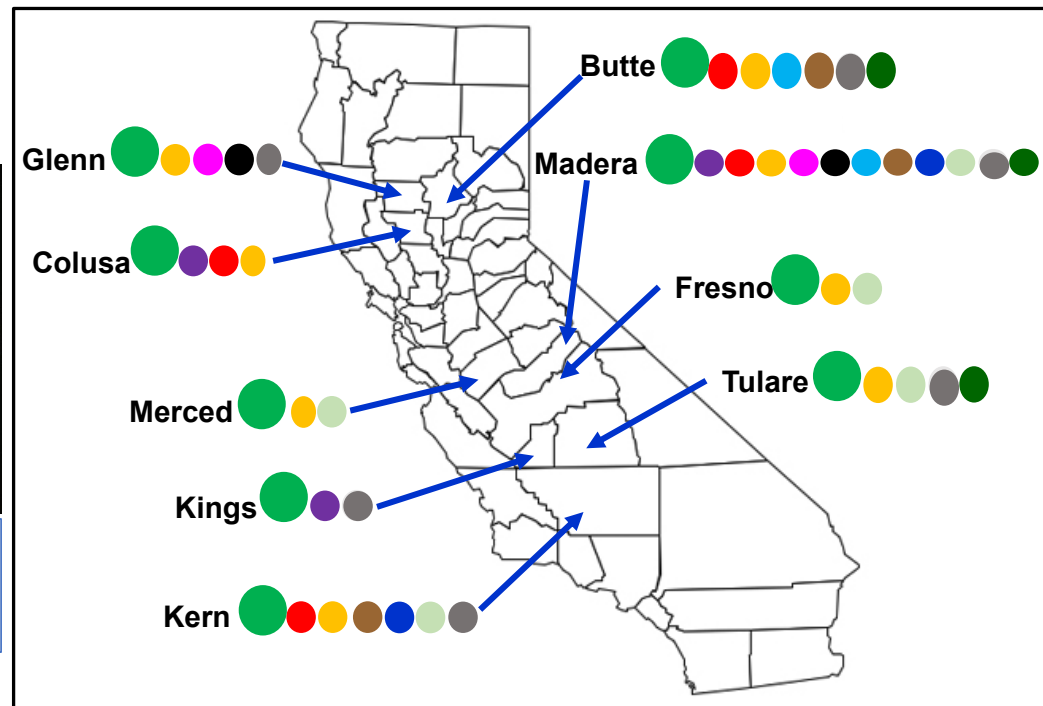


Source: P. Cotty, 1994. Phytopathology  
84: 1270-1277: (USDA & Univ. of Arizona)

# Occurrence of *Aspergillus flavus* atoxigenic vegetative compatibility groups (VCGs) in almond-growing counties of California

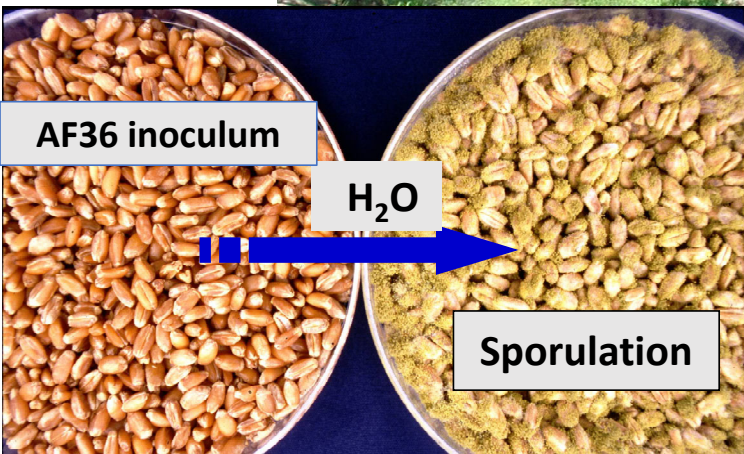
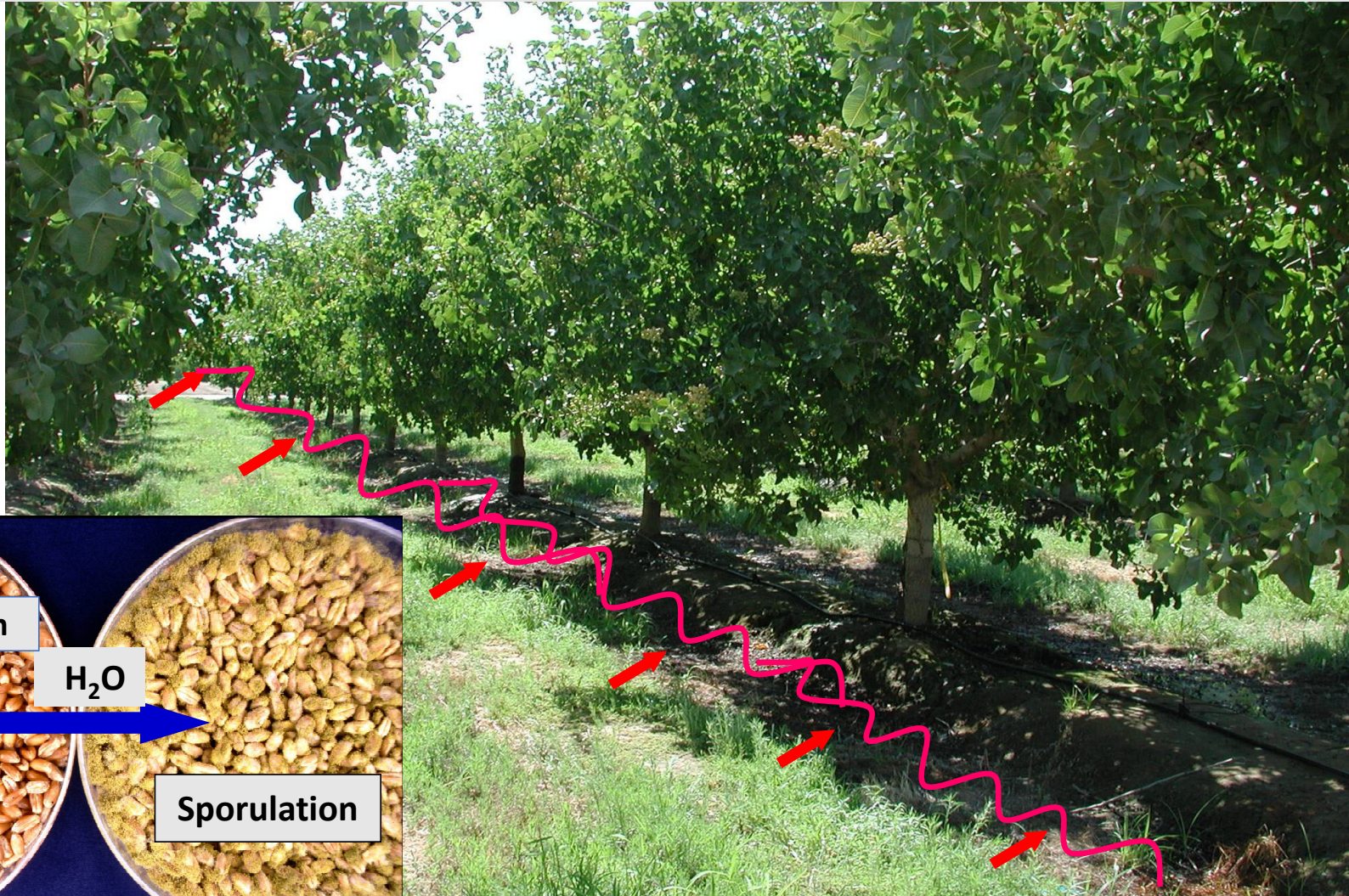


*Aspergillus* from soil



The most common VCG, **AF36 in green**, was detected in all examined counties.

# Micro-plot experiments at the Kearney Ag Center orchard (2002)



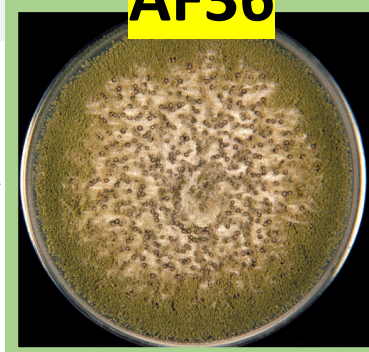
## Strains of *Aspergillus flavus*



**L – strain:** Large sclerotia

about 50%:50%  
toxigenic: atoxigenic

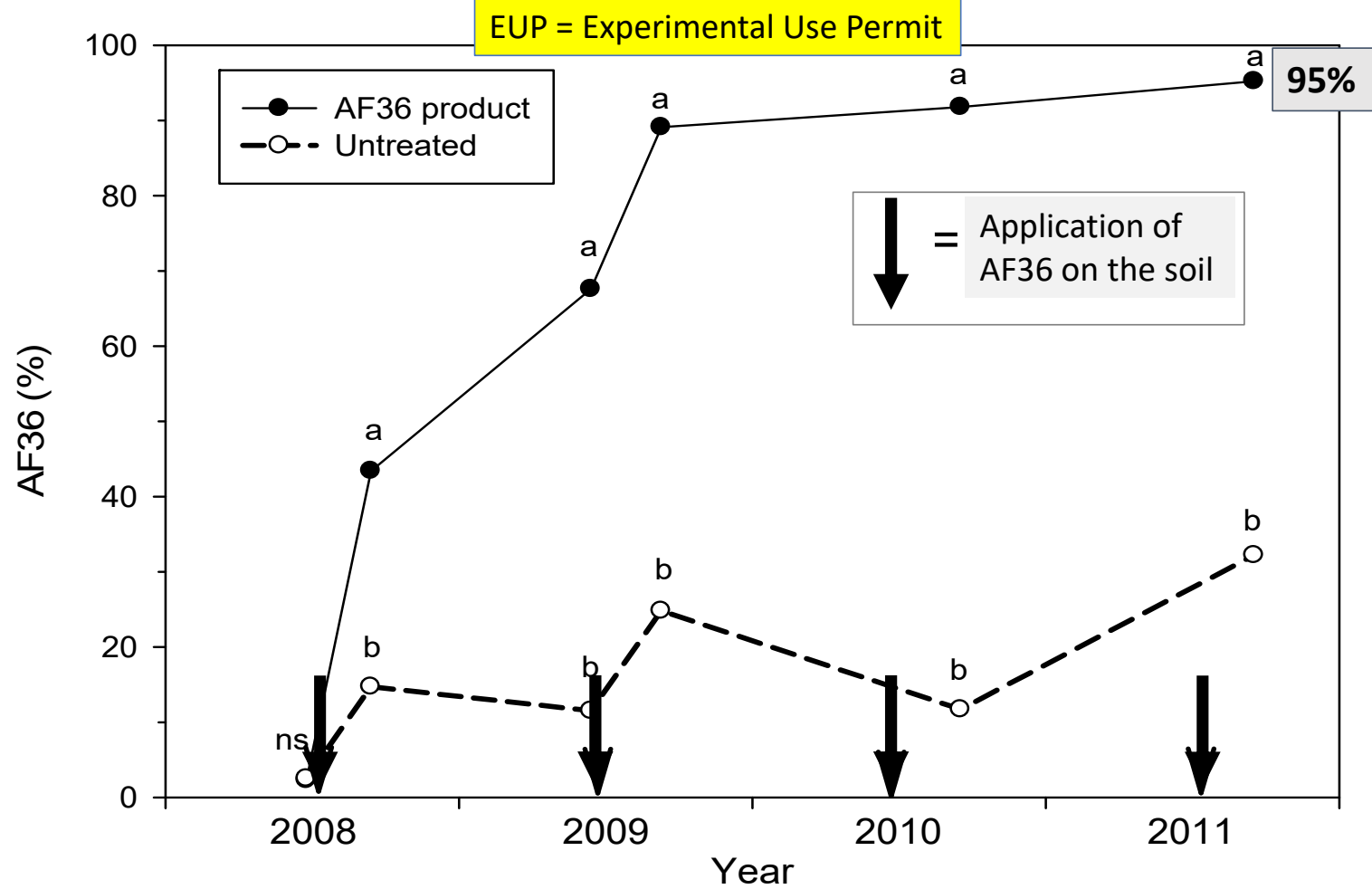
**AF36**



**S – strain:** Small sclerotia

almost all toxigenic

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



## Application of the **AF36 atoxigenic strain** in a pistachio orchard



Rate of application: **10 lbs /acre** ( $\approx$  11.2 kg /Hectare)



40 lbs capacity

Tractors modified to spread the biocontrol inoculum  
in the orchards

## Library samples for aflatoxin analyses

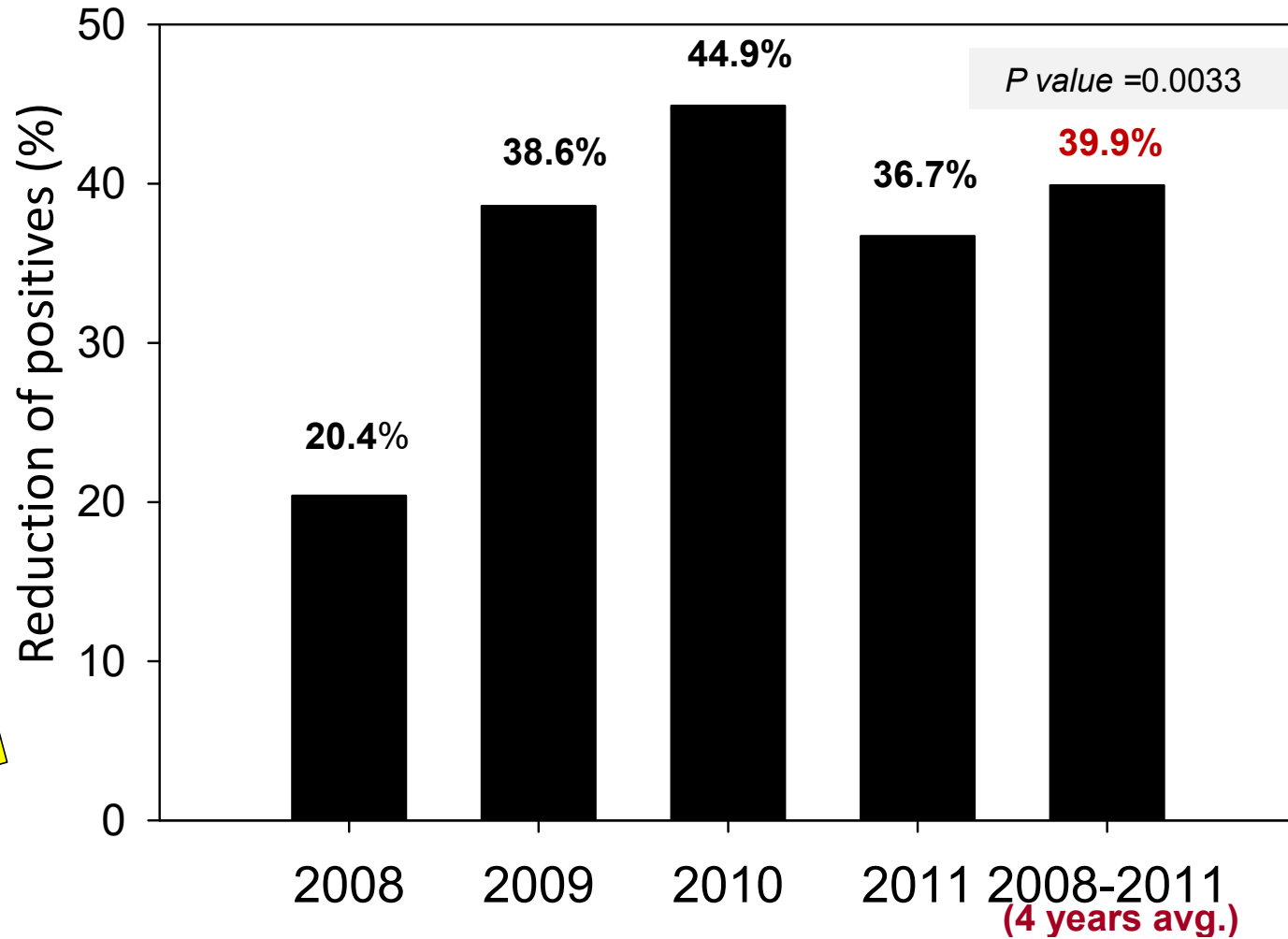


(Doster et al. (2014), Plant Disease 98:948-956)

# Reduction in aflatoxin-contaminated pistachio samples (1<sup>st</sup> and 2<sup>nd</sup> harvests)



**Registration: 17 Feb 2012**  
**Rate: 10 lbs/acre**  
**Application timing: Late May to mid-July**



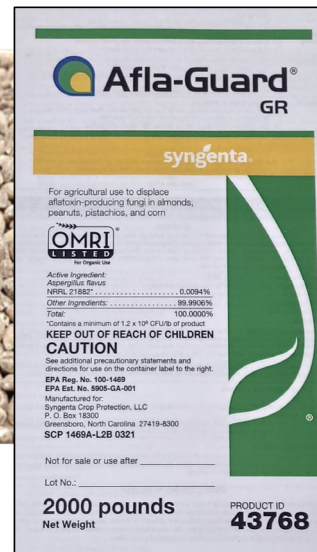
# Pistachio acres treated with biocontrol AF36, AF36 Prevail®, or Afla-Guard® GR



02 17 2012

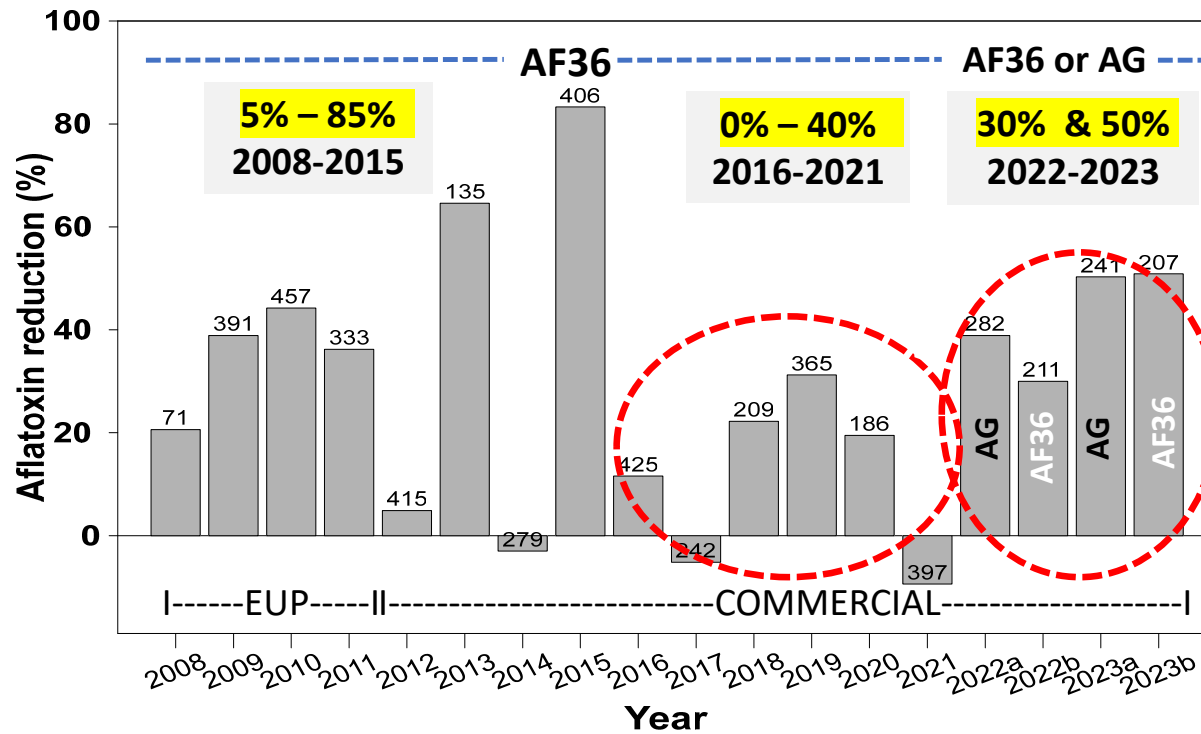


Production year	Acres treated
<b>AF36 (wheat)</b> 2012*	73,000 acres (29,500 ha) 150,000 acres (60,700 ha) 200,000 acres (81,000 ha)
2013	
2014	
2015	
2016	
<b>AF36 Prevail® (sorghum)</b> 2017*	> 200,000 acres (>81,000 ha)
2018	
2019	
2020	
2021	
<b>Afla-Guard® GR (barley)...</b> 2022* } 2023, 2024 }	About <b>300,000+ acres</b> (121,000 ha) <b>(AF36 Prevail® + Afla-Guard® GR)</b>




\* Year of registration

Reduction of aflatoxin contaminated samples treated with AF36 (2008-2023) or Afla-Guard GR (2022 & 2023)



The biocontrol initially developed for row crops...

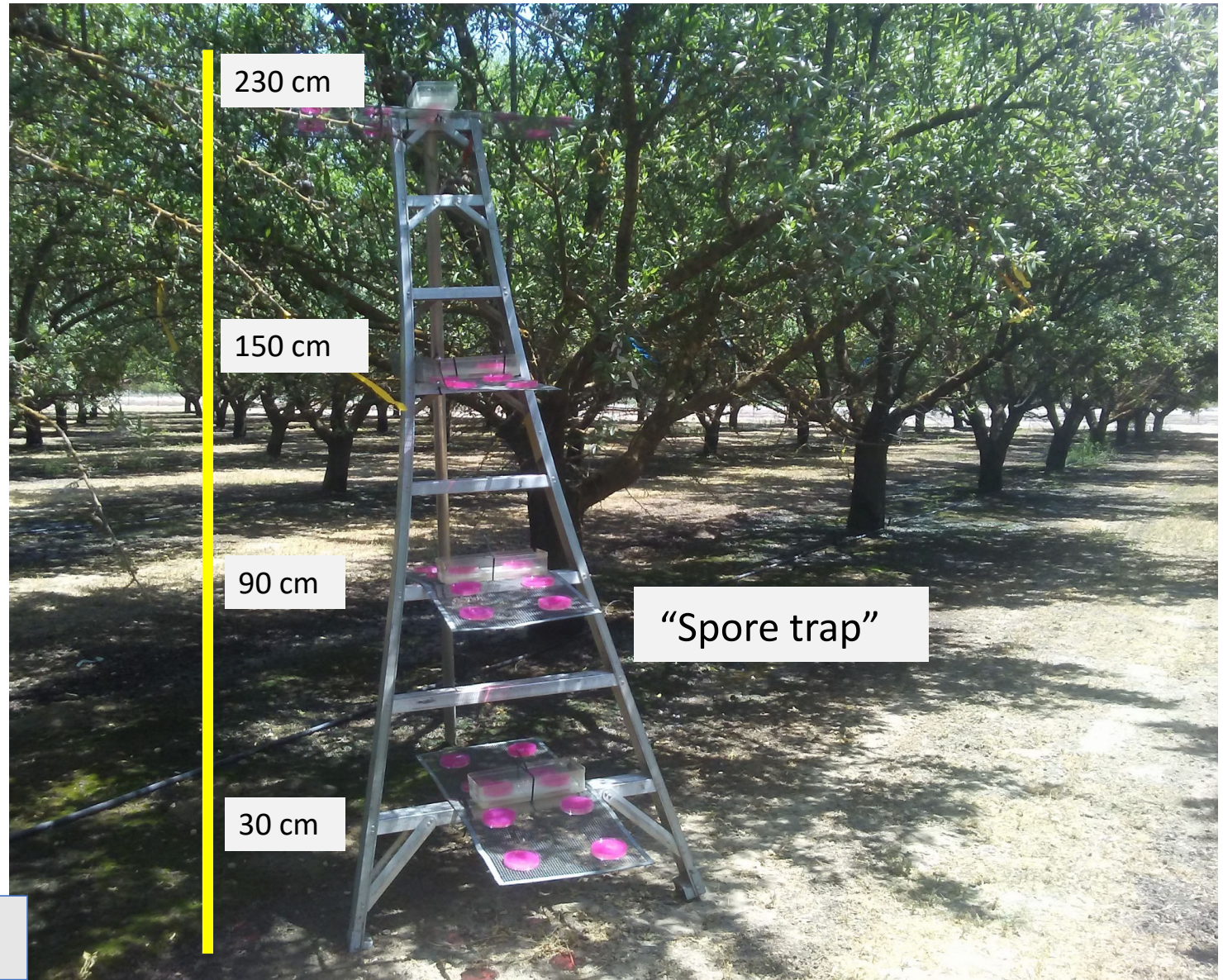


**Challenge #1:** Nut crop orchards have a dry environment;  
not favorable for sporulation of the biocontrol products

...then adapted for tree crops

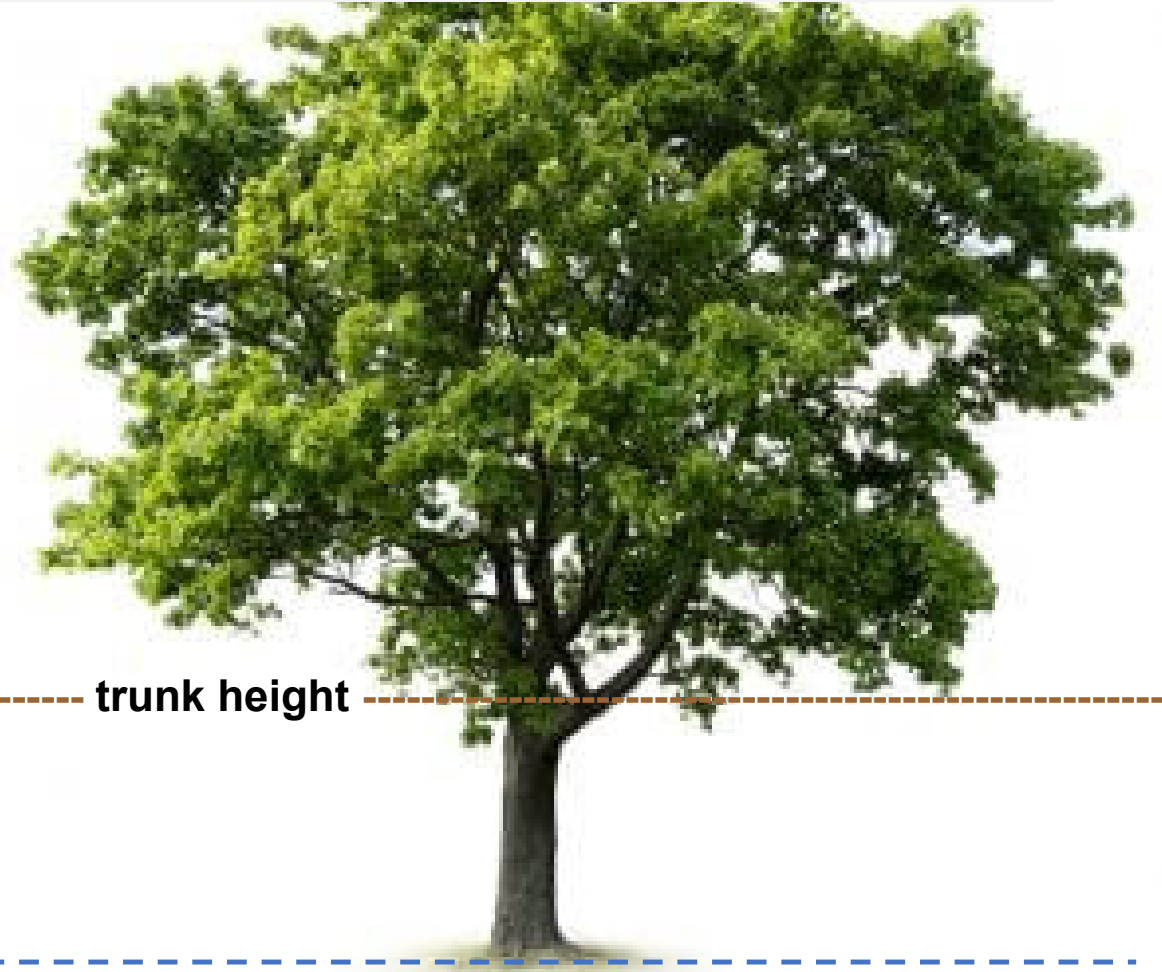
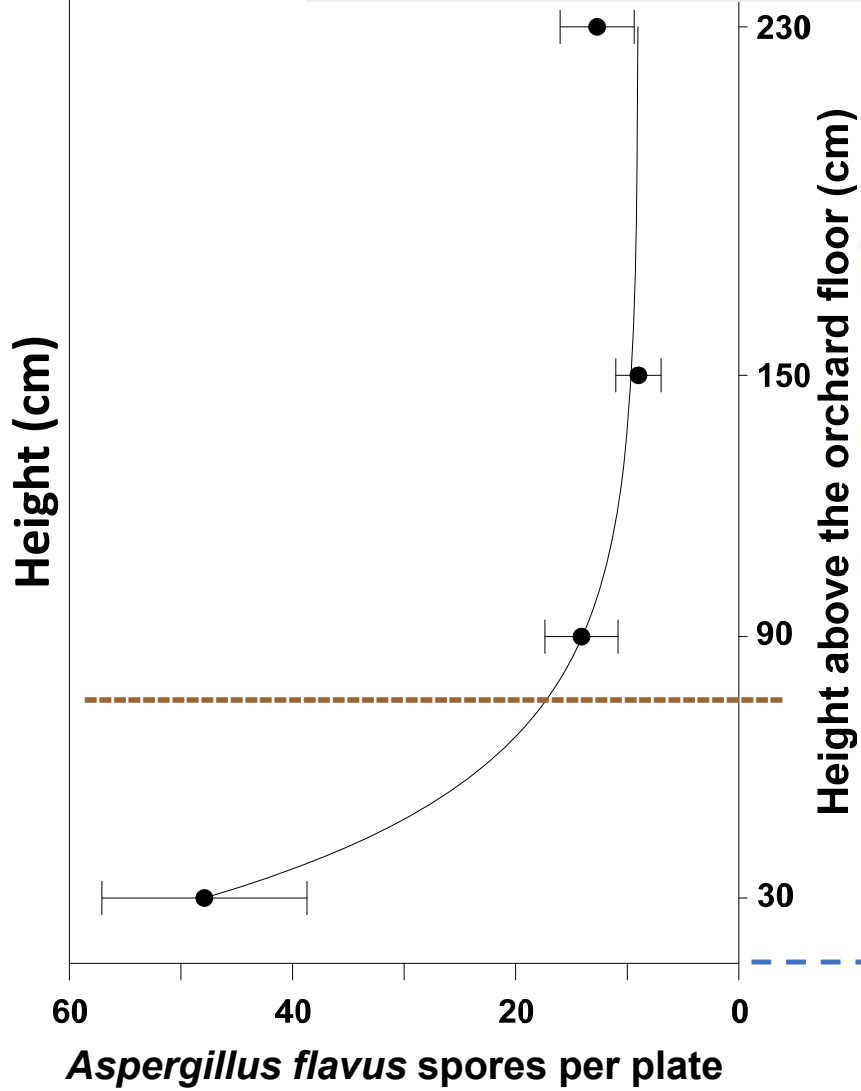


Slide: Victor Gabri



Garcia-Lopez, et al. 2024,  
Phytopathology 114:1118-1125

**Challenge #2:** Limited amounts of atoxigenic spores can reach the canopy



**Challenge #3:** Predation of seeds by insects, birds, and decay by other fungi; reduction of inoculum product will reduce the density of atoxigenic spores



Ants





Sow bugs



Beetles

## List of aflatoxin biocontrol products registered for commercial use

Product	Atoxigenic <i>Aspergillus flavus</i> isolate(s)	Responsible organization or entity	Target country	Crops for use	Reference
 <i>Aspergillus flavus</i> AF36 Prevail®	AF36	Arizona Cotton Research and Protection Council	US	Cotton, maize, pistachio, almond, and figs	[5,48,49,51]
 Afla-guard®	NRRL21882	Syngenta®	US	Maize and groundnut	[91]
Aflasafe™	Ka16127, La3279, La3304, Og0222	IITA <sup>3</sup>	Nigeria	Maize and groundnut	[87]
Aflasafe KE01™	C6-E, C8-F, E63-I, R7-H	IITA	Kenya	Maize	[45]
Aflasafe SN01	M2-7, M21-11, Ms14-19, Ss19-14	IITA	Senegal and The Gambia	Maize and groundnut	[45,88]
Aflasafe BF01	M011-8, G018-2, M109-2, M110-7	IITA	Burkina Faso	Maize and groundnut	[45]
Aflasafe GH01	GHG079-4, GHG083-4, GHG321-2, GHM174-1	IITA	Ghana	Maize, groundnut, and sorghum	[47]
Aflasafe GH02	GHM511-3, GHM109-4, GHM001-5, GHM287-10	IITA	Ghana	Maize, groundnut, and sorghum	[47]

And 7 more Aflasafe products

Slide by Dr. A. Ortega-Beltran

## Tools to manage aflatoxins in orchards of nut crops using the atoxigenic-strain biocontrol technology:



1. We achieved a 40% to 85% (once) reduction in incidence of aflatoxin-positives, but to increase the efficacy consistently, we need to overcome major challenges:
  - a) The poor sporulation due to **dry orchard environment**;
  - b) The **limited spore amount** reaching the susceptible nuts in the canopy;
  - c) The influence of treated pistachios by the **large non-treated almonds**;
  - d) The **high predation level of seeds** (insects, birds, rodents, etc.).
2. The **biological control technology** needs to be combined with the various **cultural practices**, i.e., avoiding tree stress, **removing mummies (sanitation)**, **controlling NOW**, **sorting out ES**, **stained**, **insect/bird, and defected nuts**.

**Prospects**: New application practices are being now tested (i.e., experiments with application of atoxigenic spore suspension directly onto the tree canopy) and also area-wide, long-term studies (almond+pistachio).

# Acknowledgments



Thank you

Special thanks to my former and present  
lab members

**Contacts:** Themis J. Michailides  
[tjmichailides@ucanr.edu](mailto:tjmichailides@ucanr.edu)  
559-646-6546; twitter @PistachioDoctor

- California Pistachio Research Board
- Almond Board of California
- California Fig Institute
- Calif Dept. of Food & Agriculture
- Arizona Cotton Res. & Protection Council
- Syngenta Co.

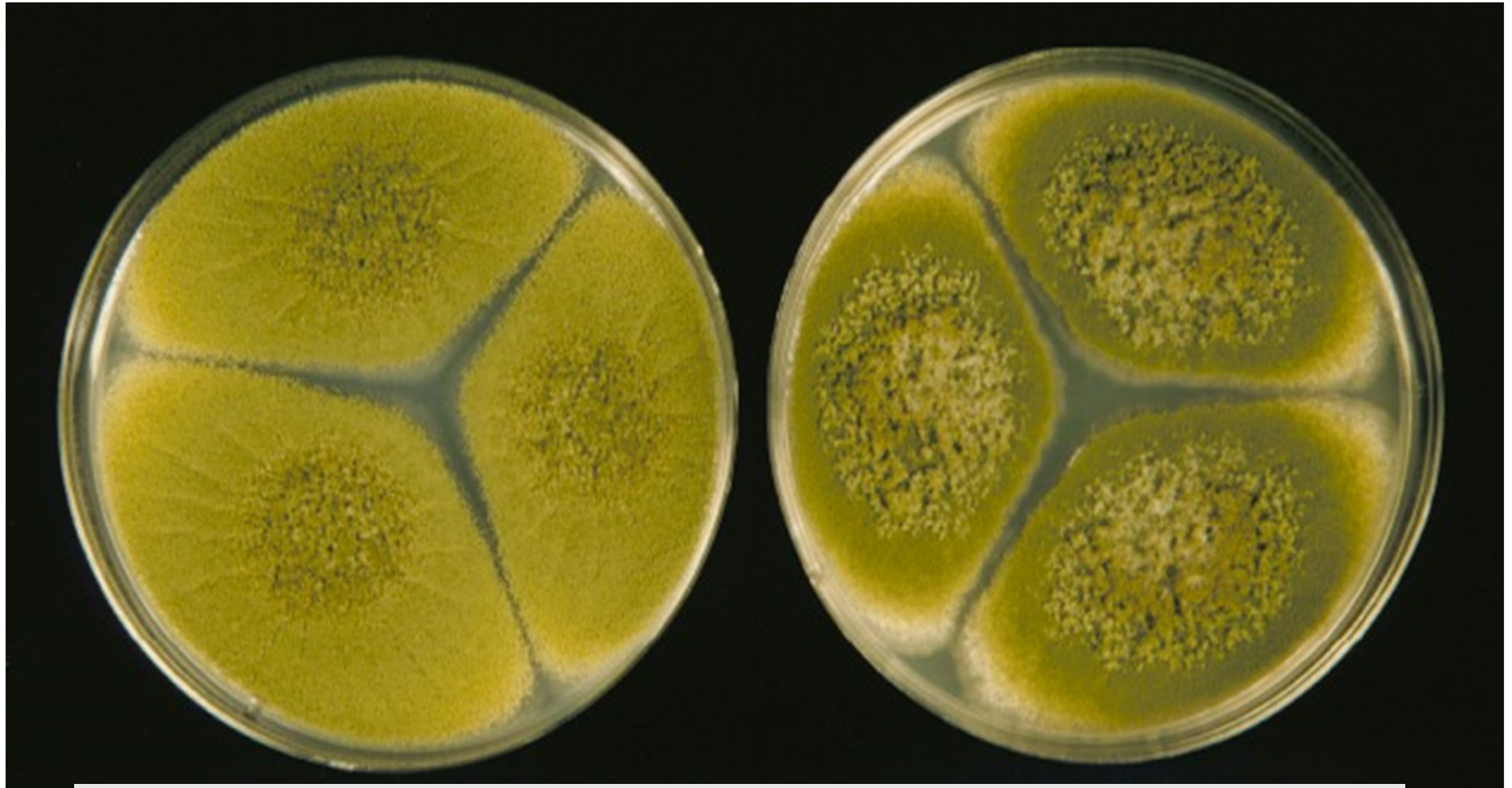
## Major cooperator:

- Dr. Peter Cotty, USDA & Univ. of Arizona

- Wonderful Orchards Co.\*\*\*
- Setton Farms
- Nichols Farms
- Keenan Farms
- & pistachio and almond growers

**Ramon Jaime**  
[rjaime@ucanr.edu](mailto:rjaime@ucanr.edu)  
559-646-6582

Fungi that can produce aflatoxins in nut crop orchards in California



*Aspergillus flavus*

*Aspergillus parasiticus*