Aflatoxin and its Control in Pistachios

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Pistachio Short Course – 16 November 2017

Tree nuts

- Low water activity &
- High in oil

} Resistant to spoilage

undesired damage (insects, drought stress, etc.)

← M O L D S →

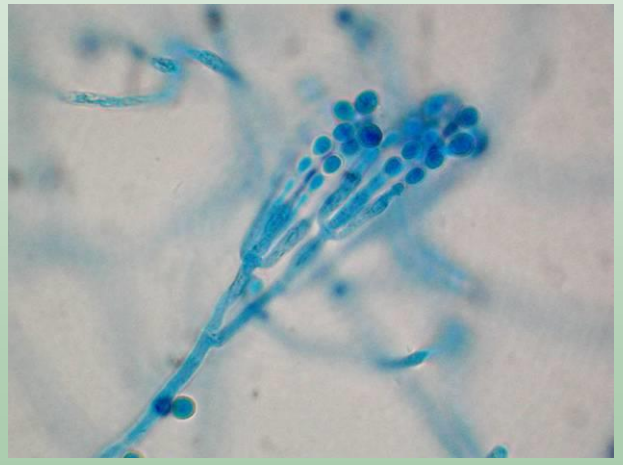
Toxic secondary metabolites

"*Mycotoxins*"

Mycotoxins = **toxic compounds** → **illnesses**, **deaths**

Toxic effects of mycotoxins
- **Carcinogenic**
- **Mutagenic**
- **Teratogenic**

Aspergillus *Penicillium* *Fusarium*

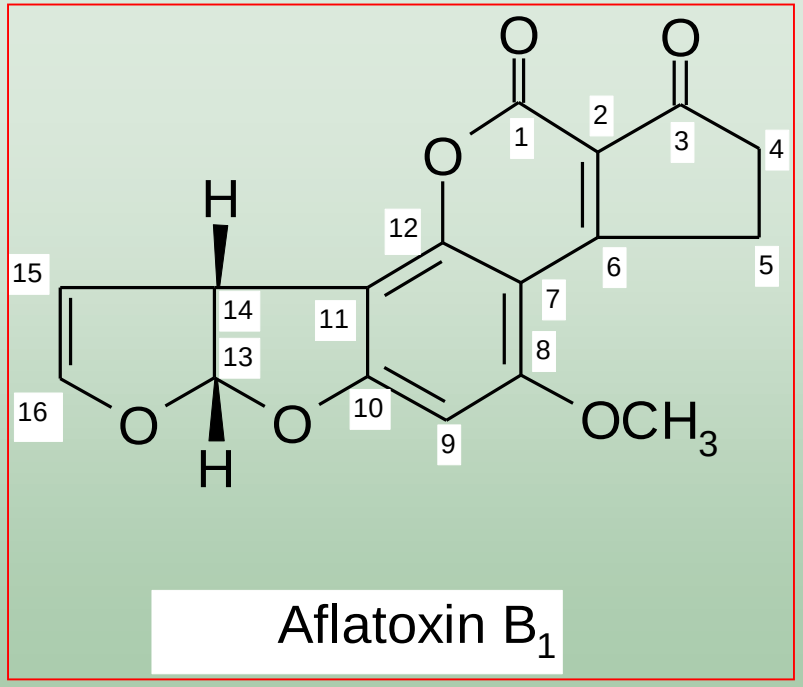


Certain species of *Aspergillus* produce mycotoxins called aflatoxins?

AFs are secondary metabolites produced by:

- *Aspergillus flavus* and *A. parasiticus*

Aflatoxins B_1, B_2, G_1, G_2, M_1



Aflatoxin B_1

} The most potent can cause liver cancer

Commodities contaminated with aflatoxins:

Highest risk of aflatoxin contamination:

Corn
Peanuts
Cottonseed

Occasionally contaminated:

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

Frequency of aflatoxin contamination in California pistachio orchards

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

Regulatory limits for aflatoxins

- **USA**

Aflatoxin B1➔10 ppb

Total aflatoxins➔ 15 ppb

- **European Union**

Aflatoxin B1➔ 8 ppb

Total aflatoxins➔10 ppb

(in pistachios for direct consumption)

OUTLINE:

- Biology and fungal variability of fungi producing aflatoxin
- Risk factors for aflatoxin contamination
- Biocontrol of aflatoxin

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Molds that can produce aflatoxin in pistachio orchards in California

Aspergillus flavus *Aspergillus parasiticus*

Strains of *Aspergillus flavus*

L - strain

about 50:50
toxigenic: atoxigenic

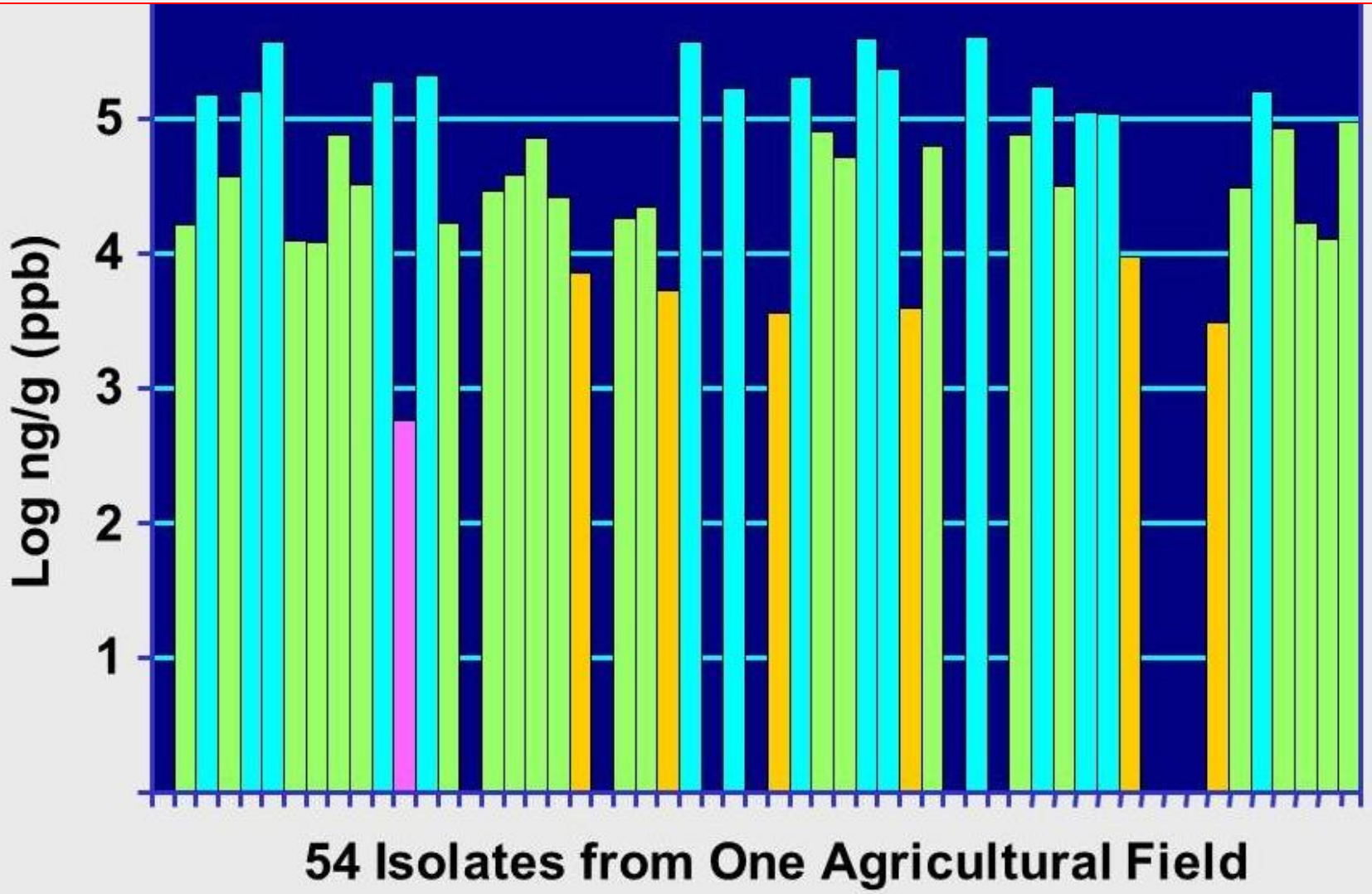
M - strain

?

S - strain

most toxigenic

Aflatoxin production by isolates of *Aspergillus* sect. *Flavi* in liquid culture



Source: Dr. P. Cotty, USDA & Univ. of Arizona

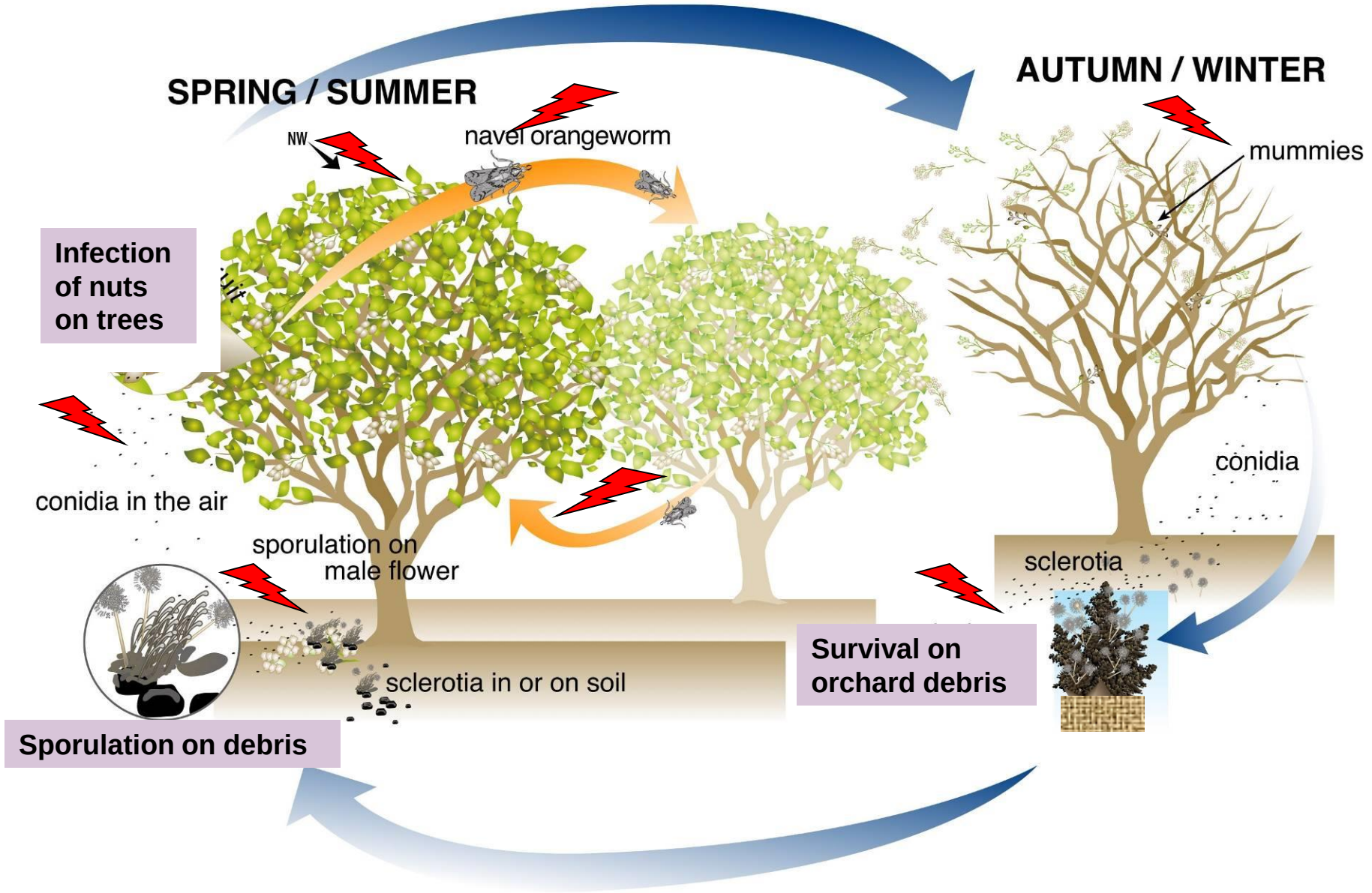
Density of *A. flavus*/*A. parasiticus* in soil

Selective 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

Total fungal count = 58,000 cfu/g



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

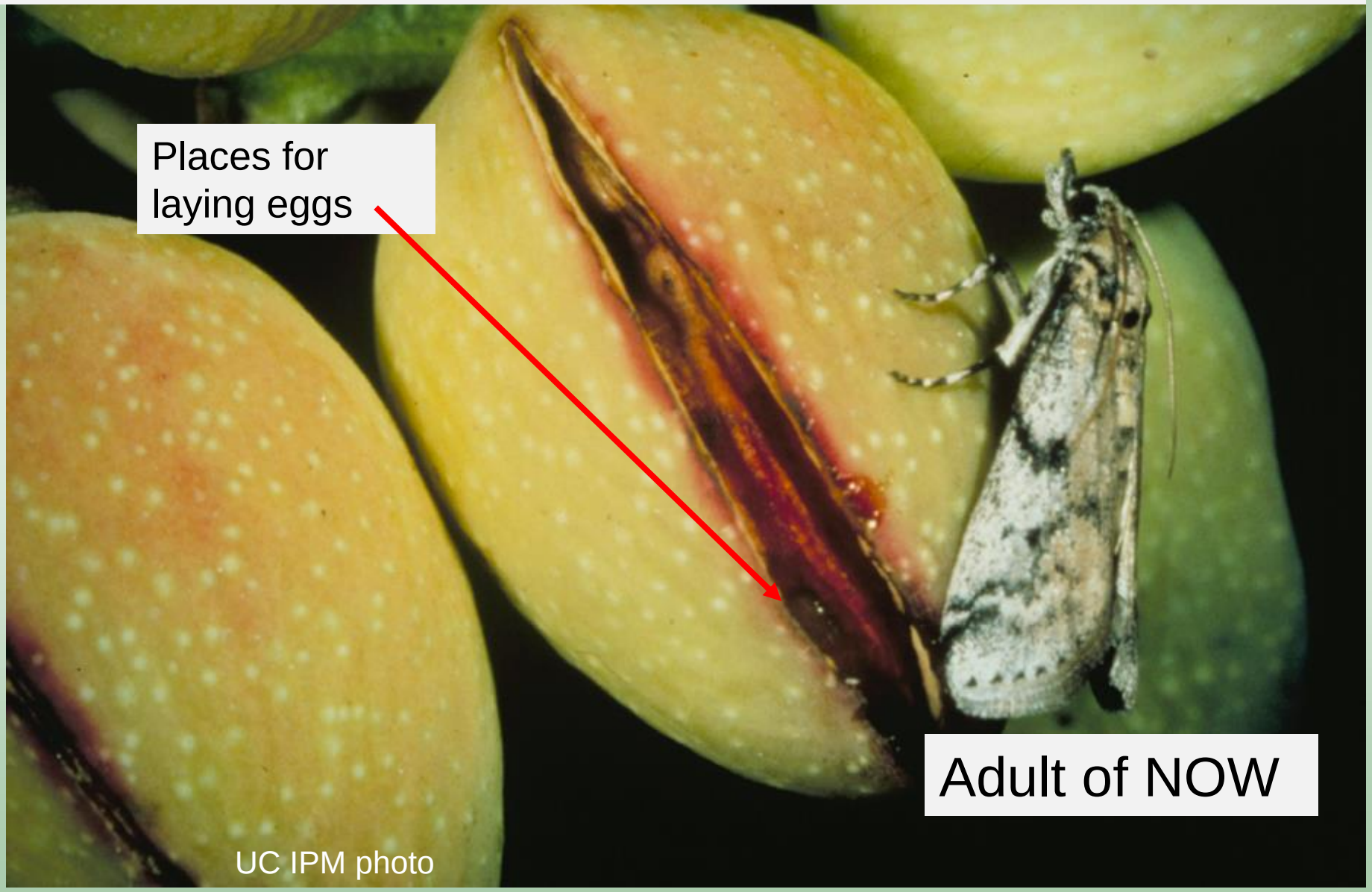
OUTLINE:

- Biology and fungal variability of fungi producing aflatoxin
- Risk factors for aflatoxin contamination
- Biocontrol of aflatoxin

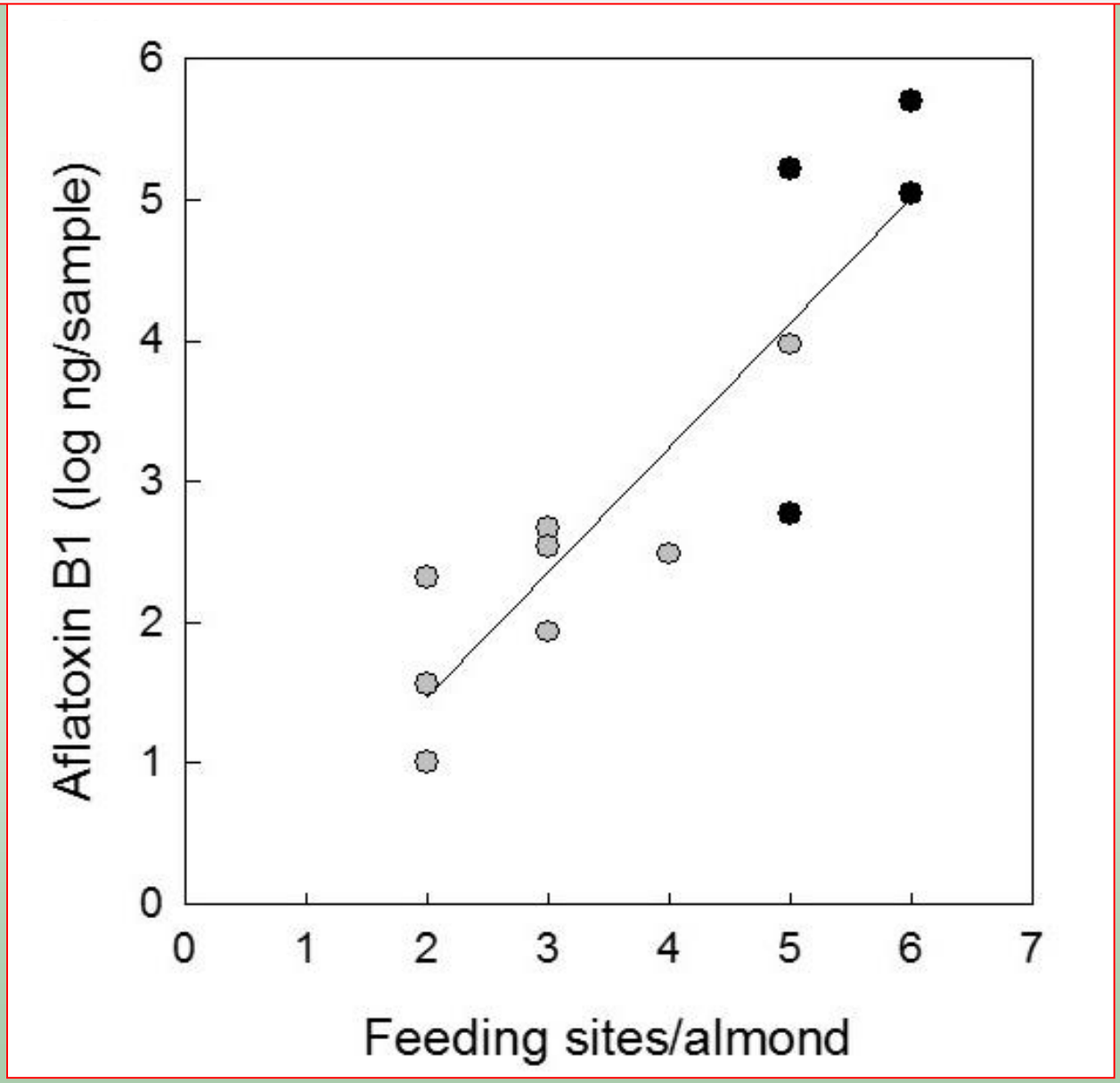
Risk factors:

1) Navel orangeworm (NOW)
2) Harvest date
3) Location
4) Year (on /off)
5) Cultural practices
6) Rootstock
7) Various nut defects

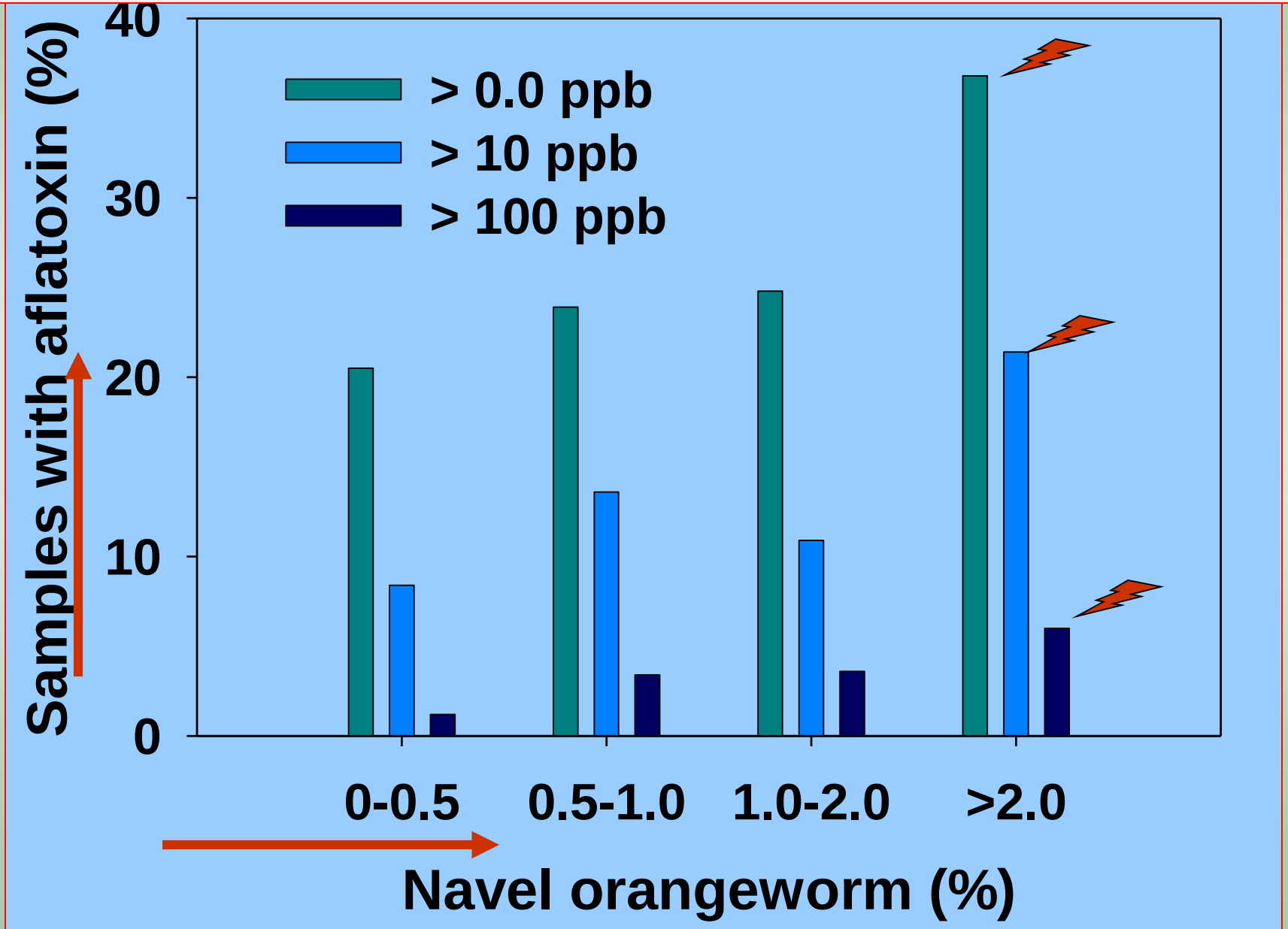
1. Navel orangeworm (NOW):
A moth on an early split pistachio



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

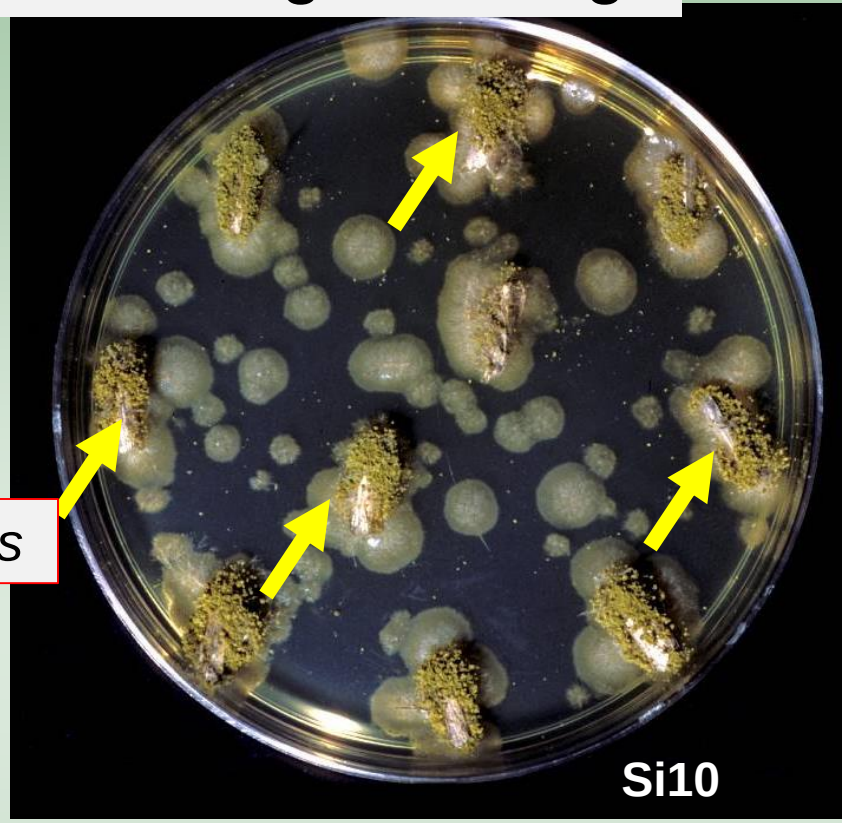


Relationship of navel orangeworm infestation and aflatoxin levels

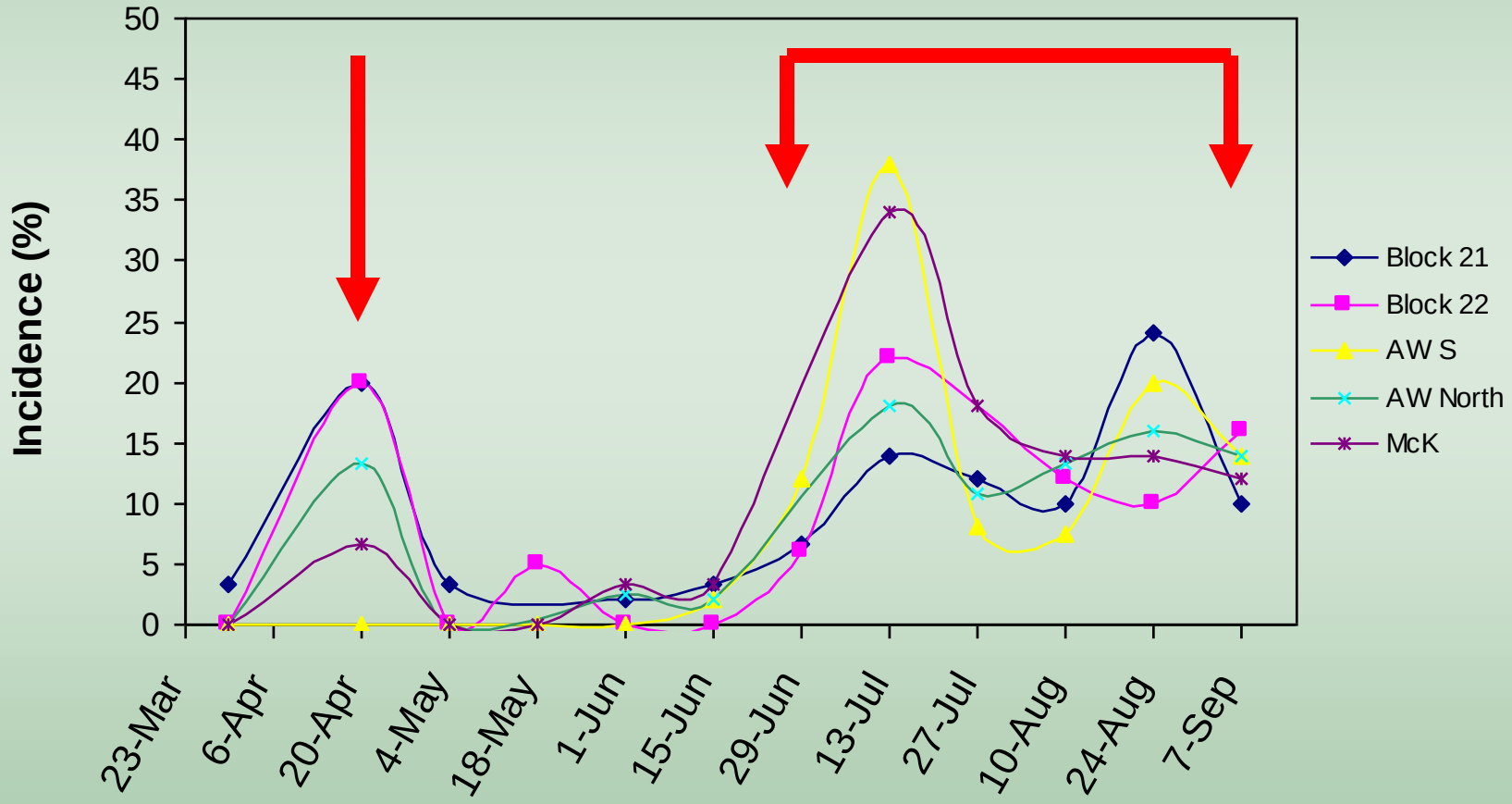


Association of NOW with aflatoxigenic fungi

Sticky traps with NOW moths



Aspergillus sect. *Flavi* on NOW moths from pistachio blocks (Madera Co.; 2012)



Frequency estimate of aflatoxin-producing isolates carried by NOW

Isolates/strains from NOW	Orchard 1- Madera	Orchard 2 - Madera
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. Harvest date: Delaying harvest increases NOW infestation

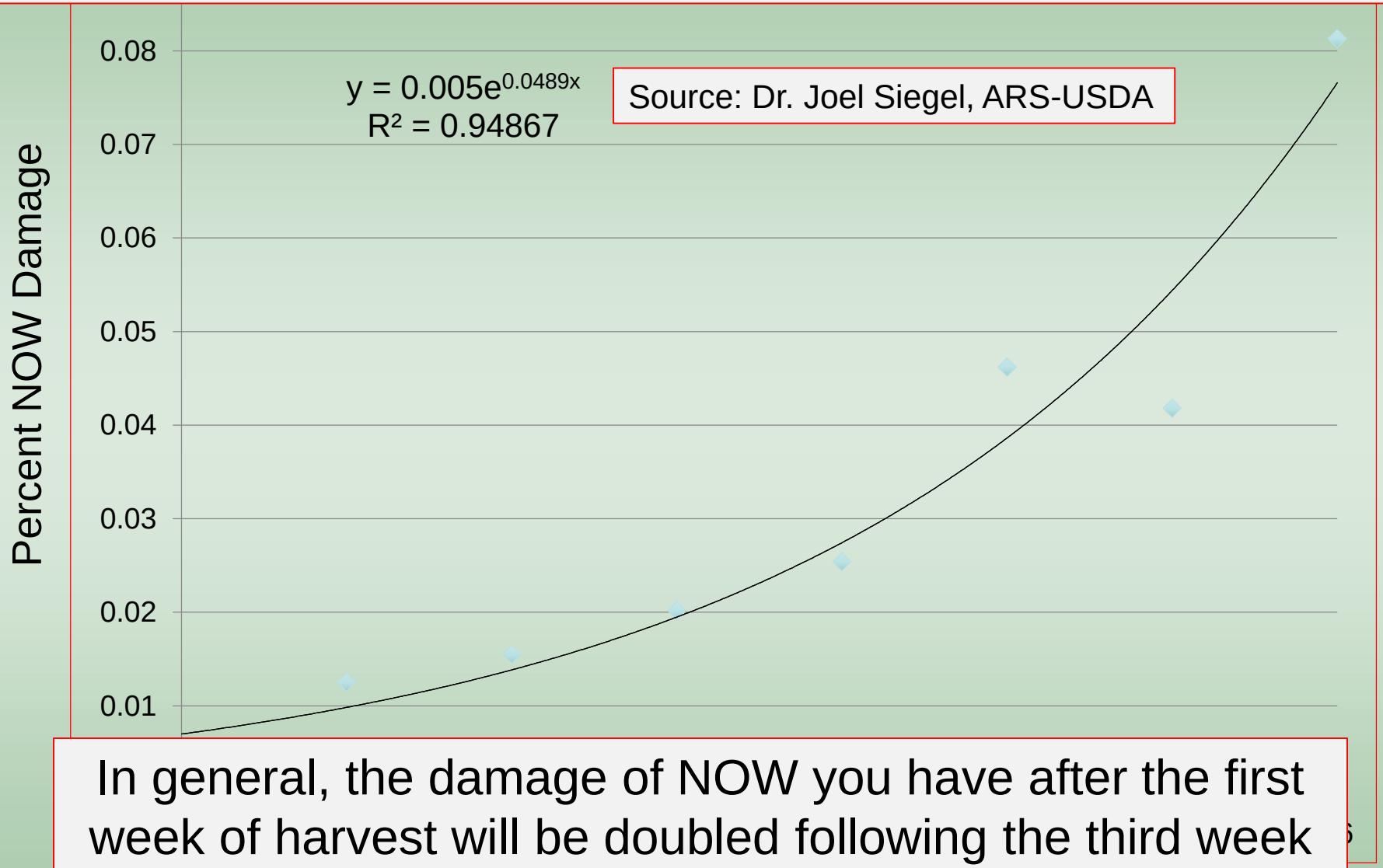
Year 1

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

Year 2

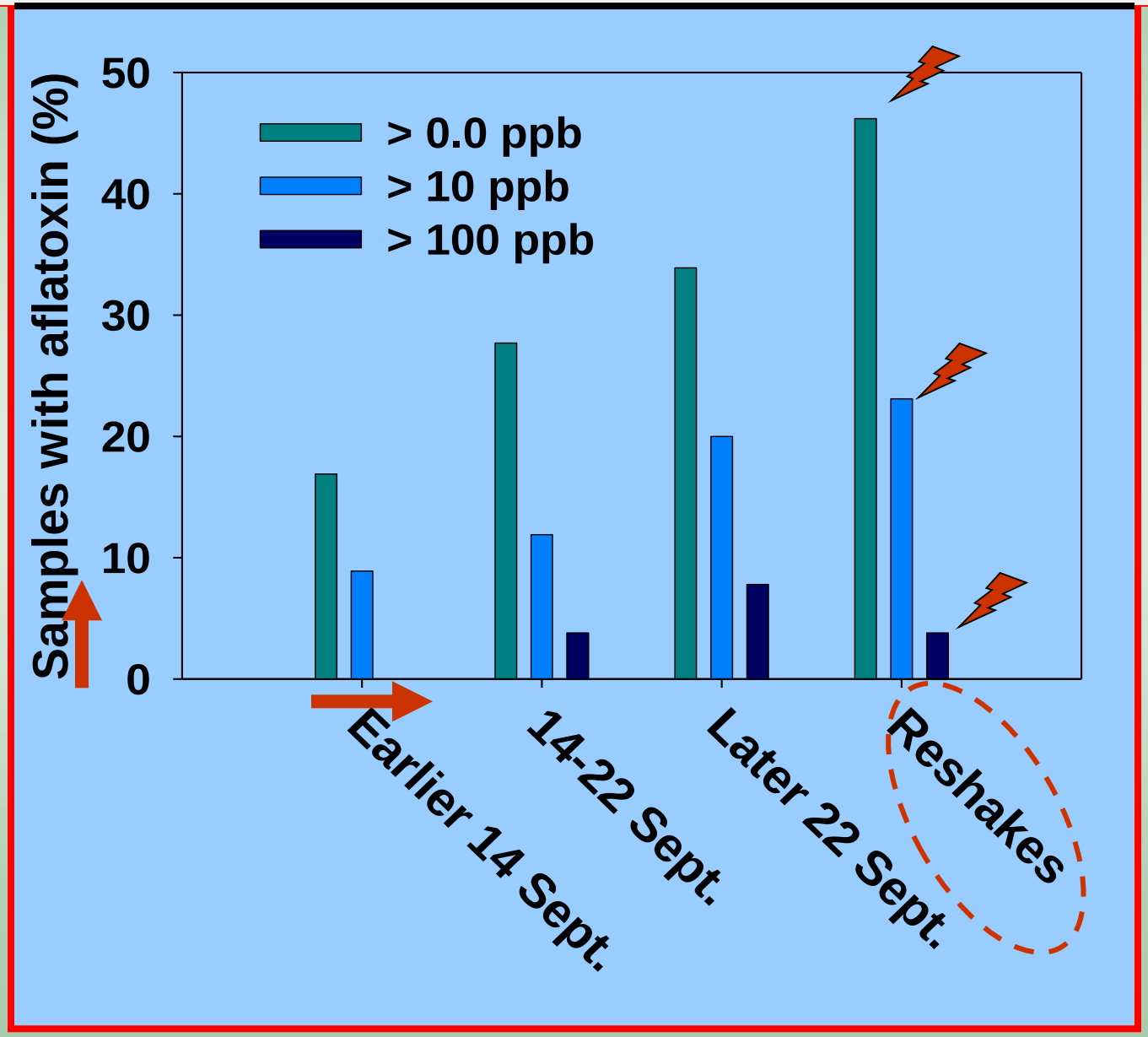
Harvest date	NOW(%)
14 Sept.	1.8
20 Sept.	3.1
28 Sept.	6.1
4 Oct.	9.1
12 Oct.	14.2

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. Siegel)

Harvest dates, incidence, and amounts of aflatoxin contamination



Recommendations:

- ✓ Do not delay harvest.

Reduce damage by NOW

- ✓ Follow an aggressive insecticide program (spray according to recommendations given by UC entomologists).
- ✓ Decrease number of nuts left after harvest.
- ✓ Winter sanitation should be an annual practice.


Sweep the berms and grind nuts in the middles

3. 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

4. Effect of year (on or off): 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

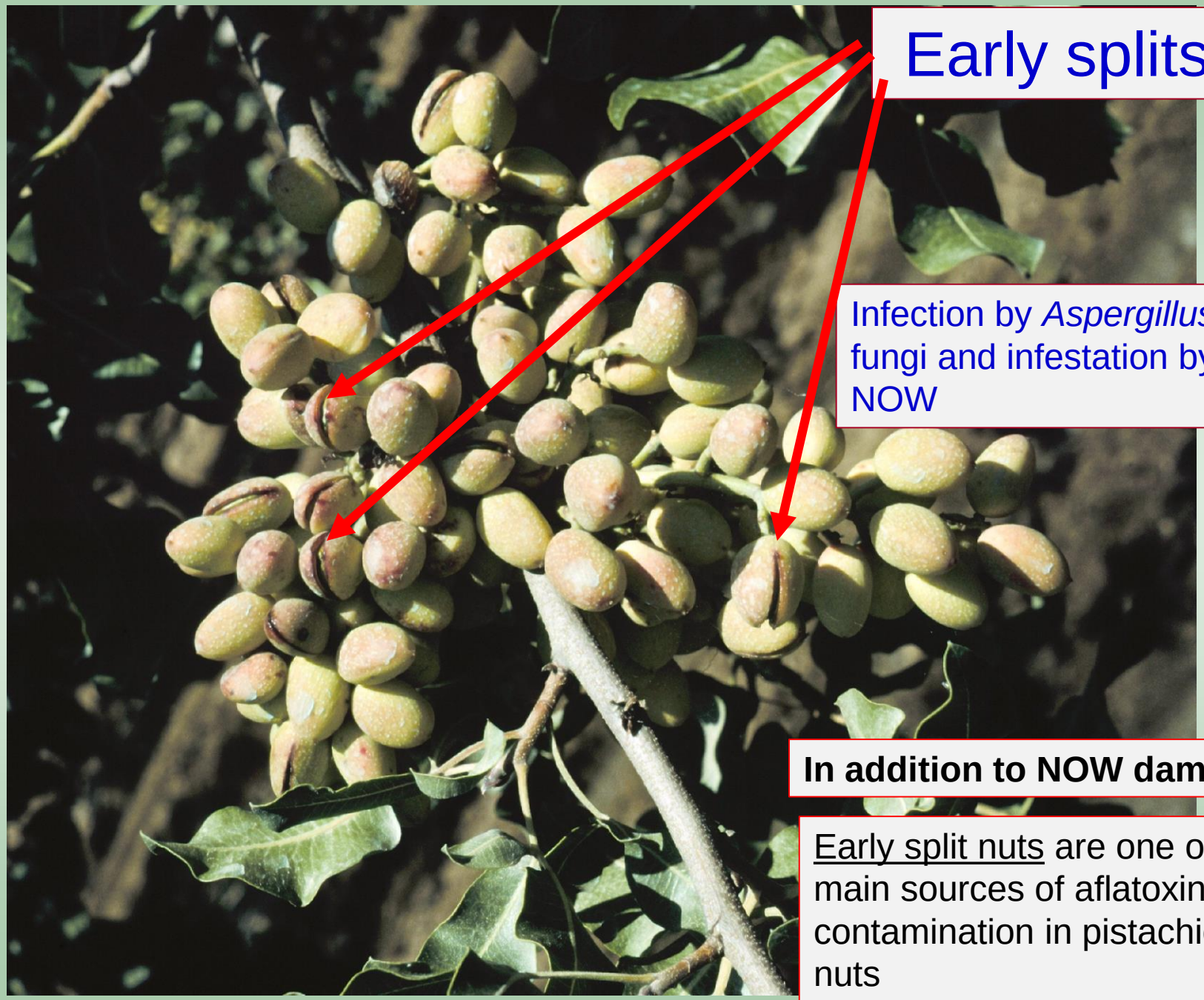
4. Effect of year (on and off): Frequency of aflatoxin contamination in pistachio orchards

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

to

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

5. Cultural practices



Early splits

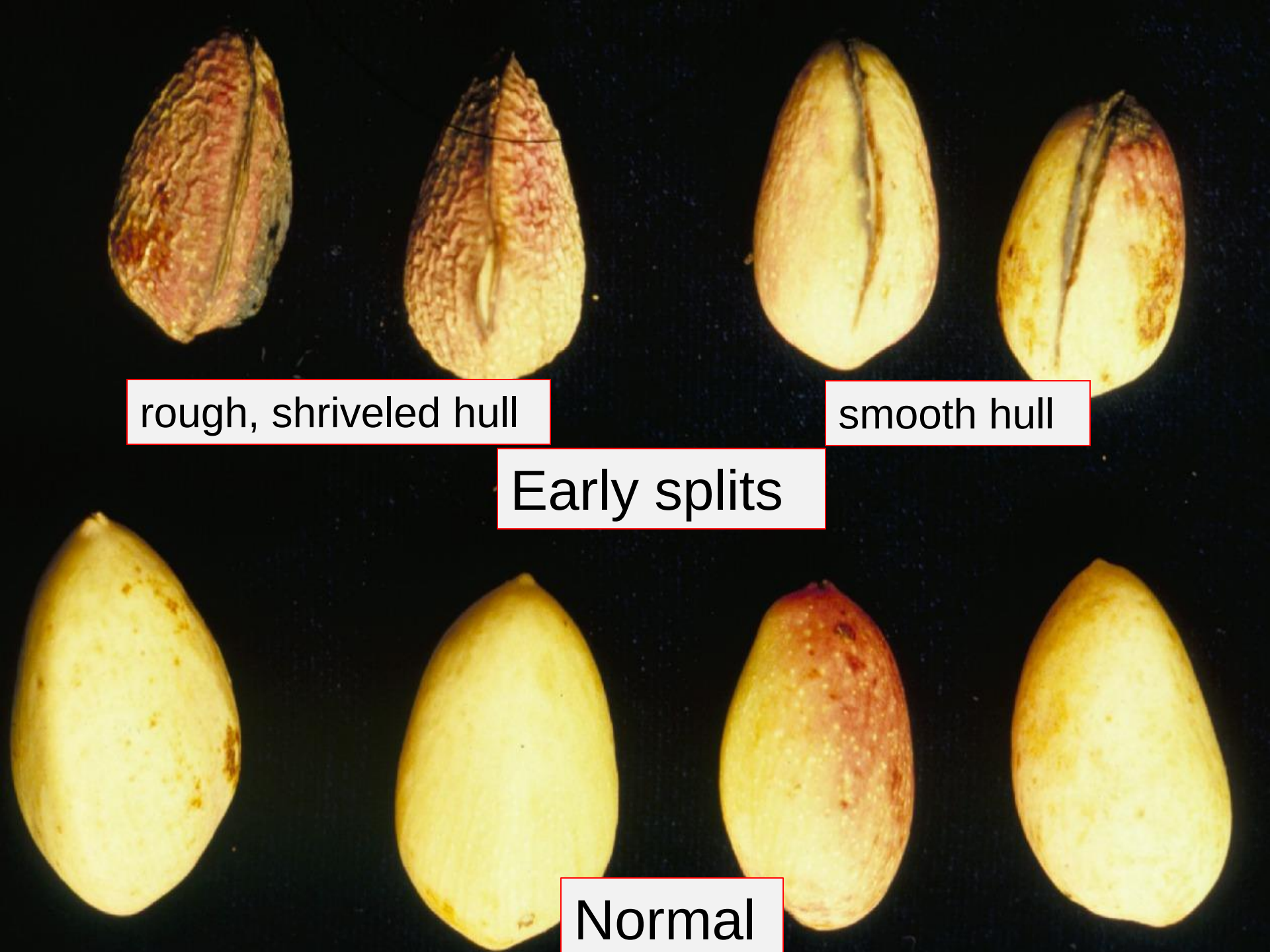
Infection by *Aspergillus* fungi and infestation by NOW

In addition to NOW damage:

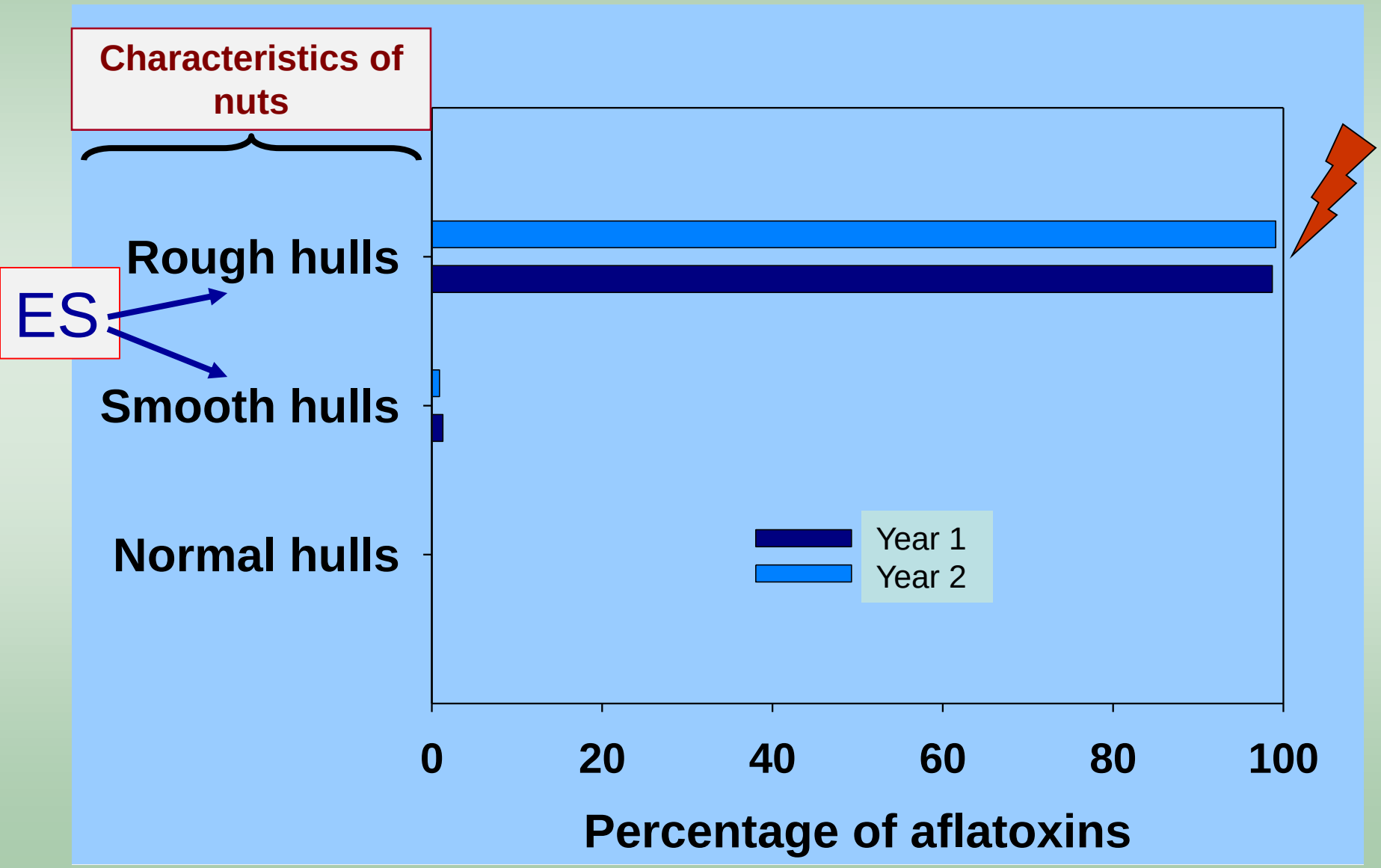
Early split nuts are one of the main sources of aflatoxin contamination in pistachio nuts

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
H	2.7
F	3.4
D	3.4
A	4.3
B	5.7
E	6.5


rough, shriveled hull
smooth hull
Early splits
Normal

Incidence of total aflatoxins detected in ES and normal pistachio nuts



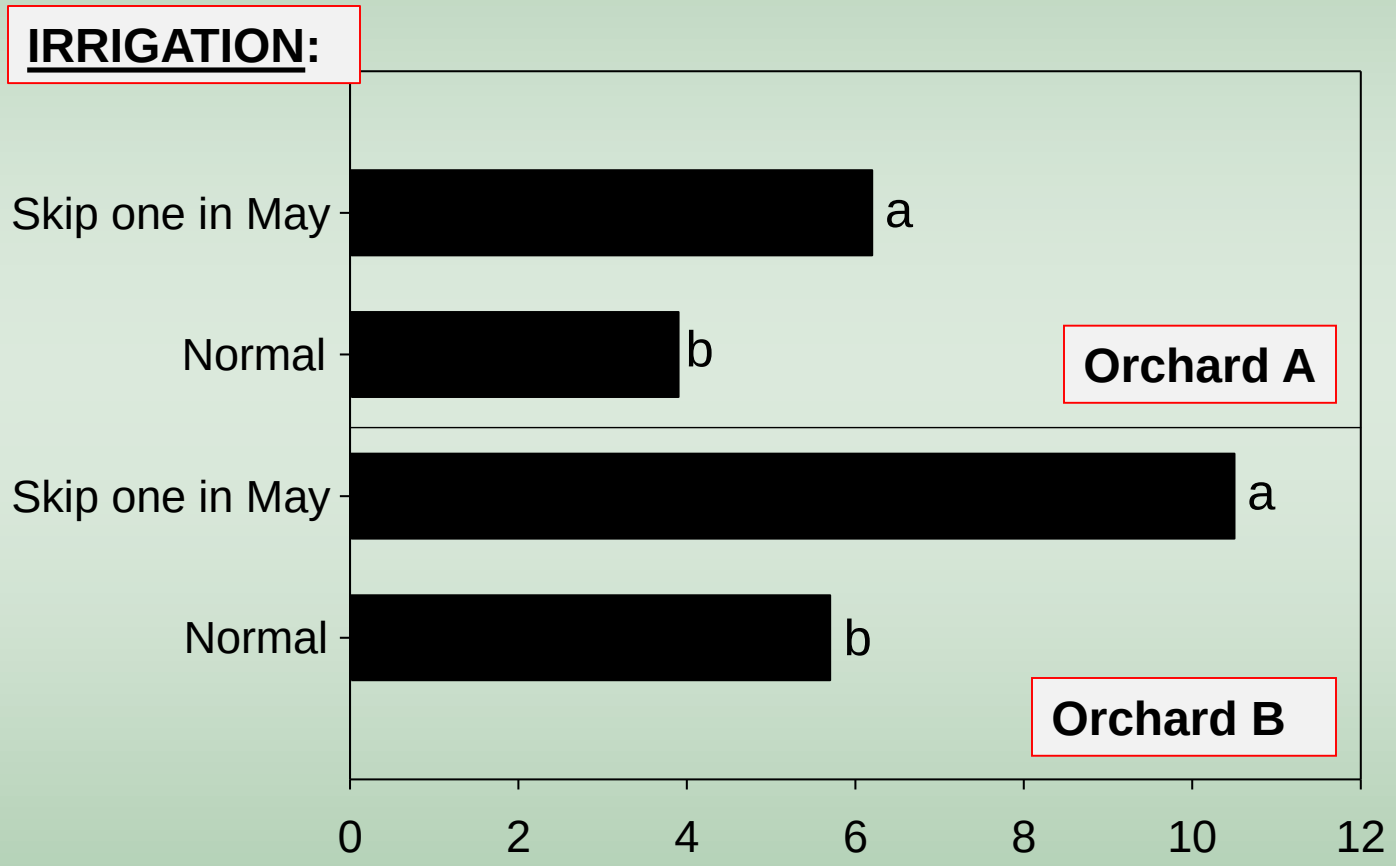
Relationship of the hull appearance and NOW infestation with kernels of early split pistachios

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.

Effect of irrigation on incidence of early splits (ES)



Recommendation: Apply sufficient irrigation during spring to avoid tree stress and reduce ES.

6. Rootstock:

Four major rootstocks are used in pistachio in California

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

} *P. integerrima*

Effect of rootstock on the early split nuts in four rootstock trials

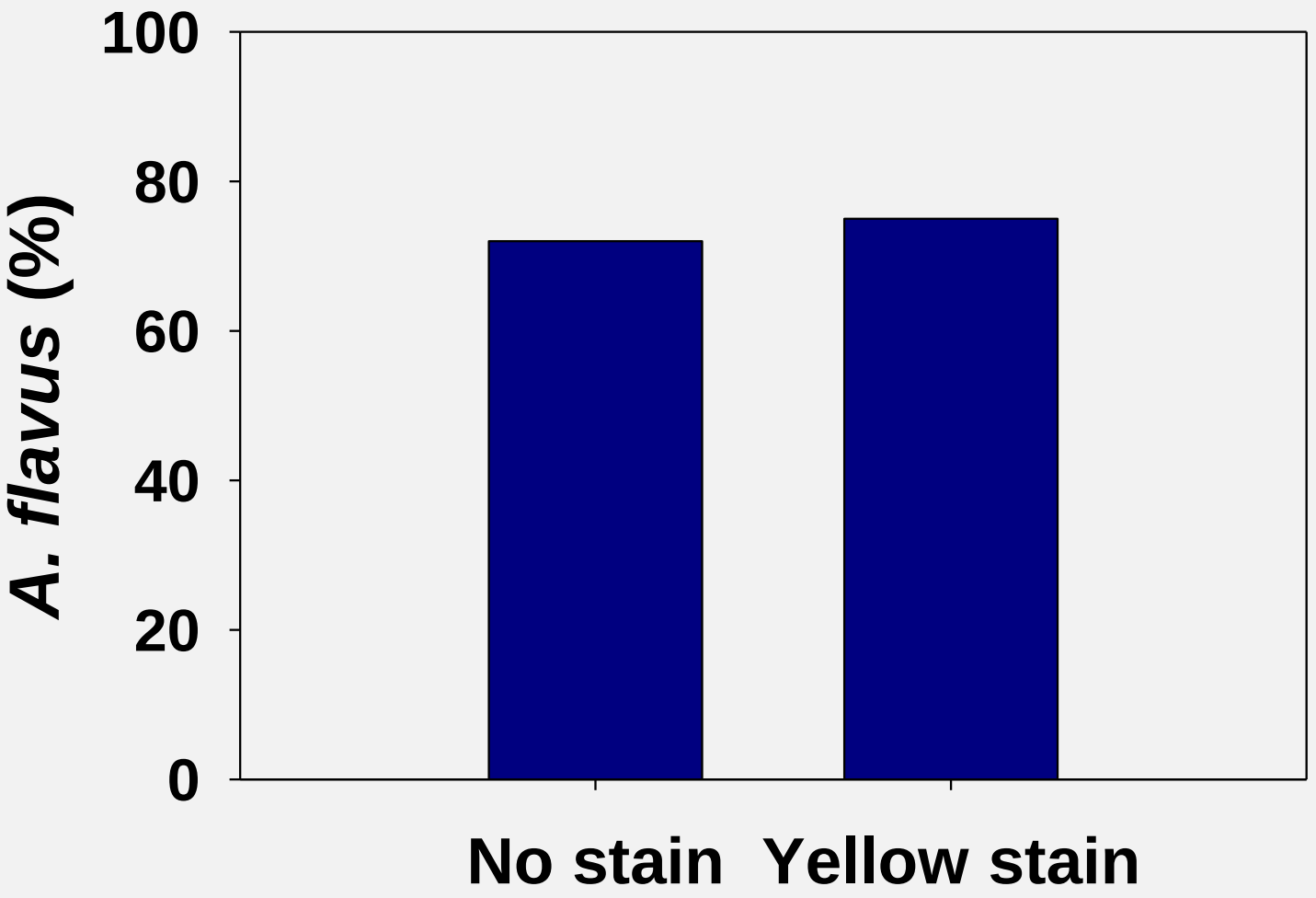
	Early splits (%)			
	Fresno County			
Rootstock	KAC	Westside	Kern Co.	Madera Co.
Atlantica	4.7 a	0.8 a	0.4 b	16.6 a
PGII	4.0 ab	1.8 a	0.9 a	10.3 b
PGI	4.0 ab	0.1 b	0.3 bc	9.3 b
UCB-1	2.1 b	0.1 b	0.1 c	7.2 b

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

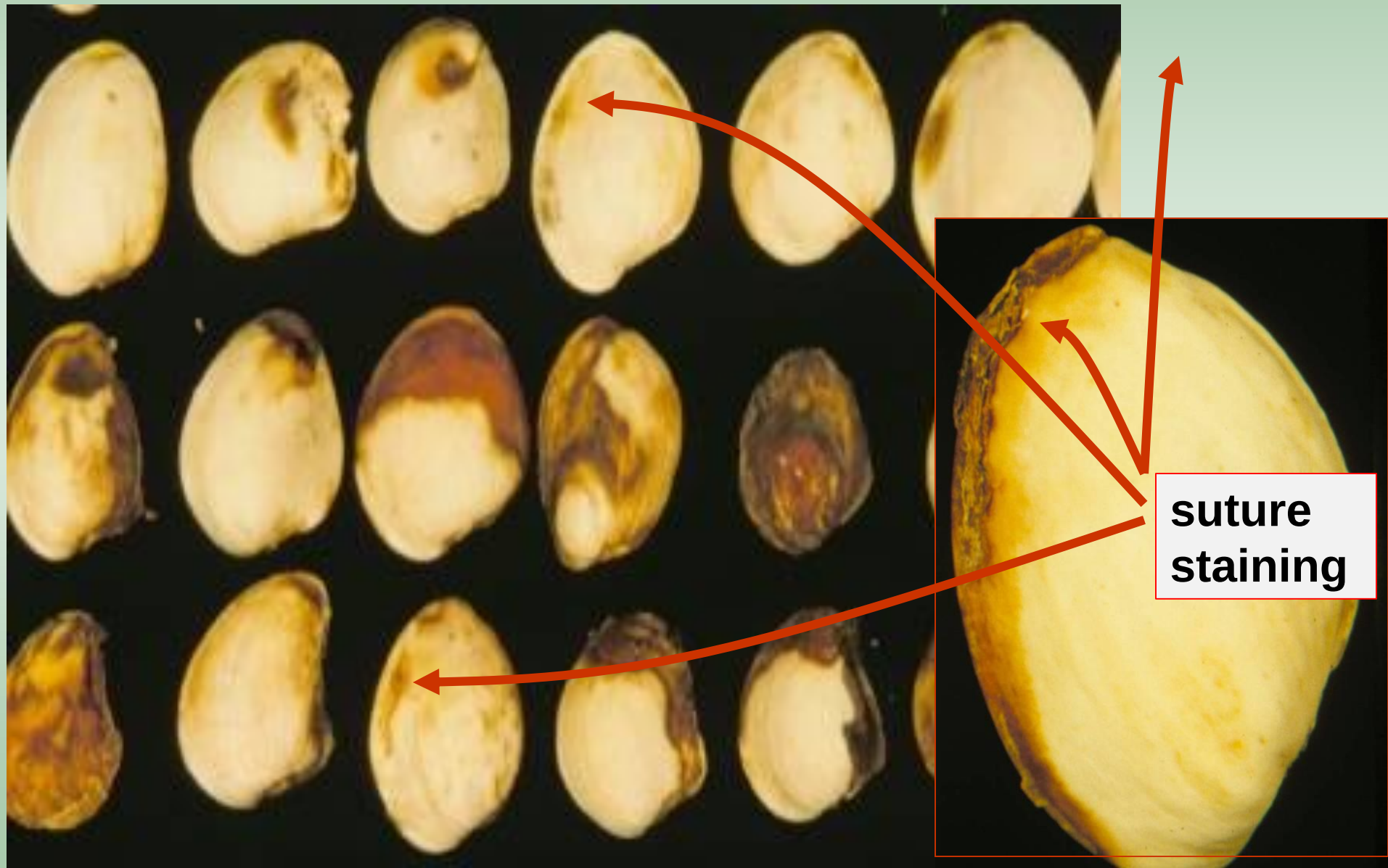
7. Various nut defects: Damaged by other means (DBOM)

High in aflatoxins!

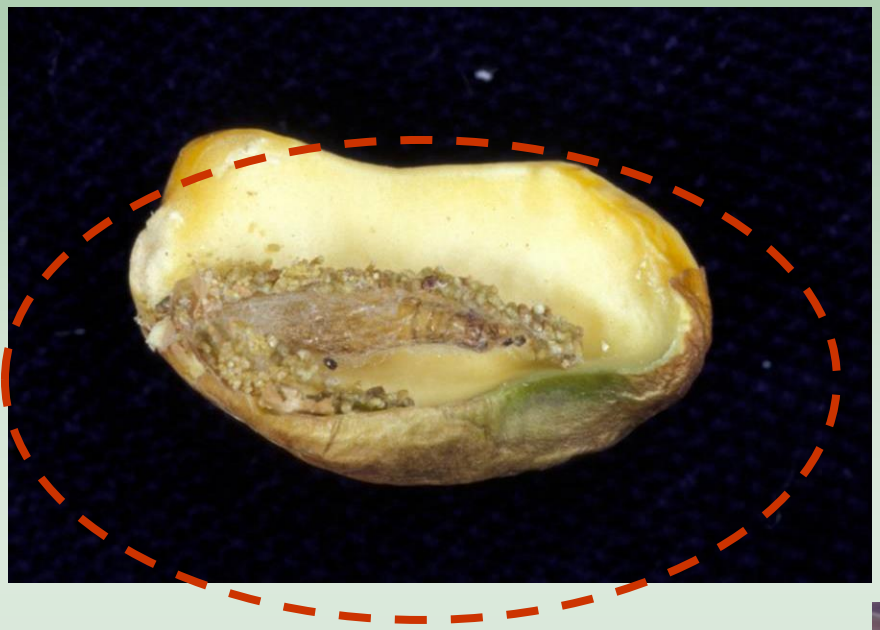
DBOM (or C-nuts) from a processing plant
(after surface sterilization)



7. Various defects: Early splits and stained nuts



7. Nuts with various defects



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 (i.e. UCB1, PGI).
- Do not delay harvest. ***
- Sort out damaged and defected nuts.**

Control of Aflatoxins (biocontrol): Use of **atoxigenic strains** of *Aspergillus flavus* as biopesticides to reduce aflatoxins

(**Atoxigenic strains** = those strains that do not produce aflatoxins)

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

Strains of *Aspergillus flavus*

L - strain

↓

about 50:50
toxigenic: atoxigenic

↓

AF36

M - strain

↓

undescribed

S - strain

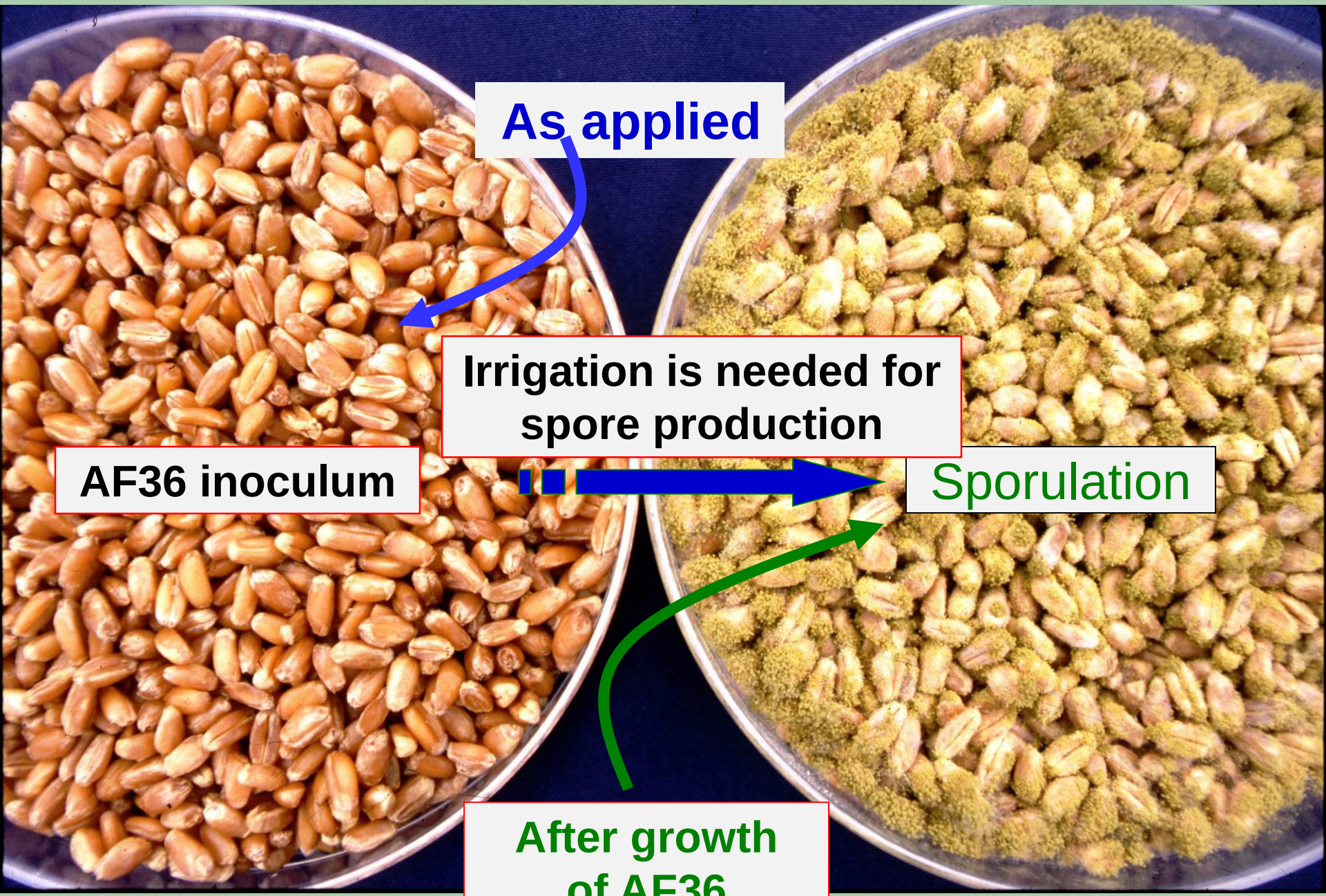
↓

most toxigenic

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

County	AF36 (%)		
	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**	…	…

- Flood-irrigated orchard initially (2001); and since (2002):
- Micro-sprinkler irrigated orchard **(AF36)** (2003)


As applied
Irrigation is needed for spore production
AF36 inoculum
Sporulation
After growth of AF36

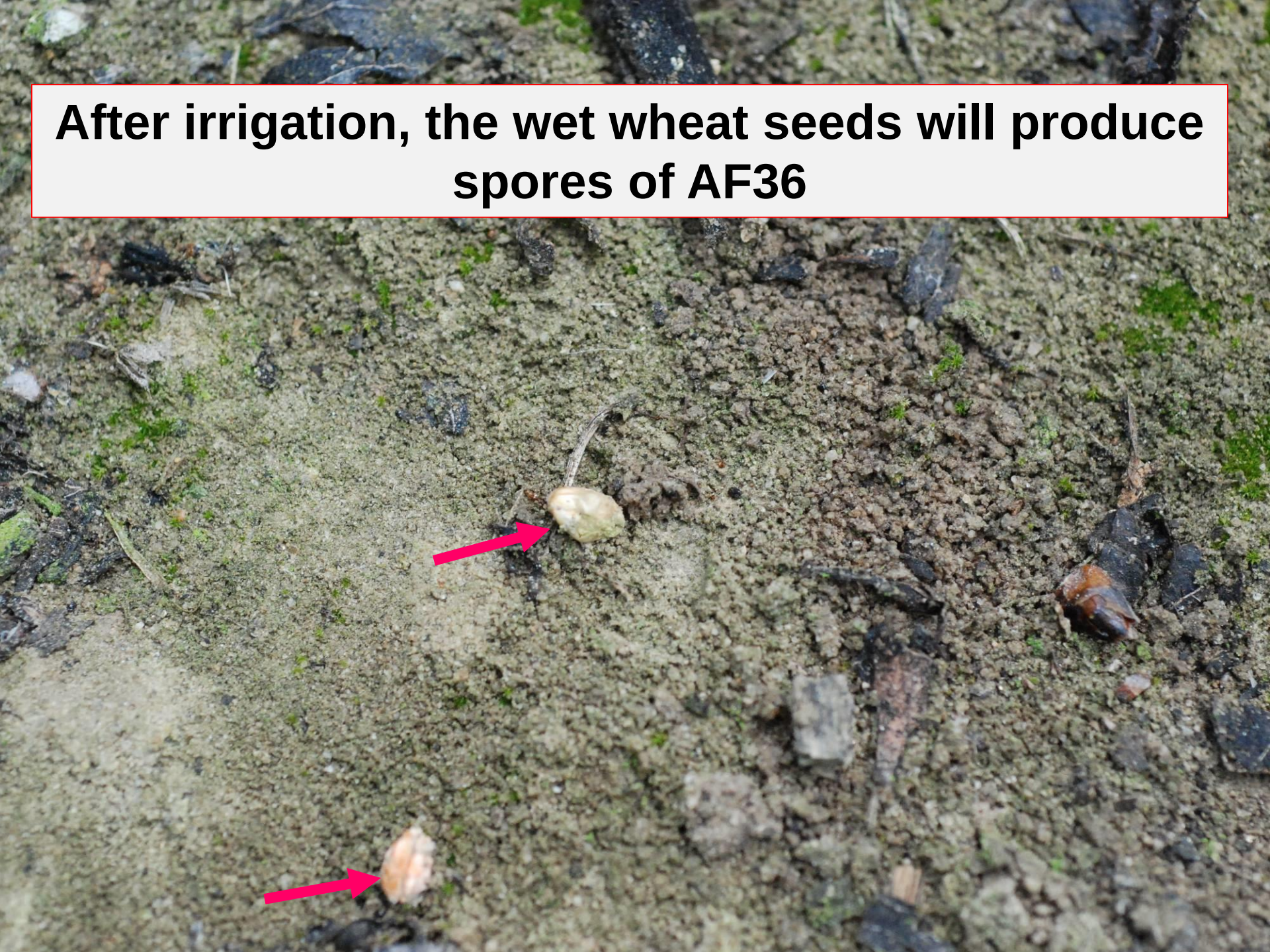
Inoculum of **atoxigenic strain AF36** stored and shipped to the growers

Delivery of AF36 inoculum for pistachio treatment

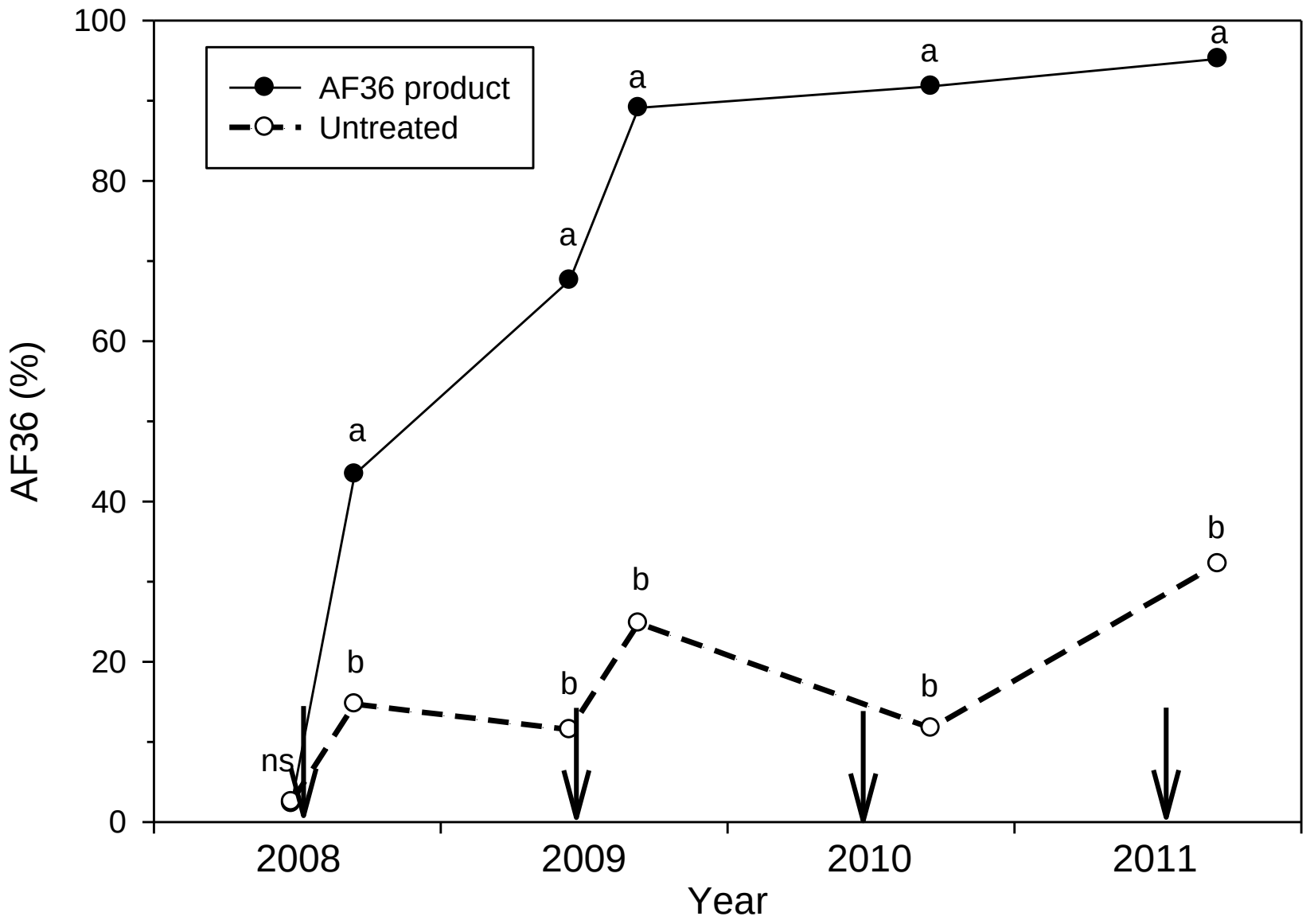

AF36
Inoculum

Application rate: 10 lbs. per acre

(=11.2 kg per ha)


After irrigation, the wet wheat seeds will produce spores of AF36

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



Non-treated Orchard

Fungal content is the same in treated and untreated orchards

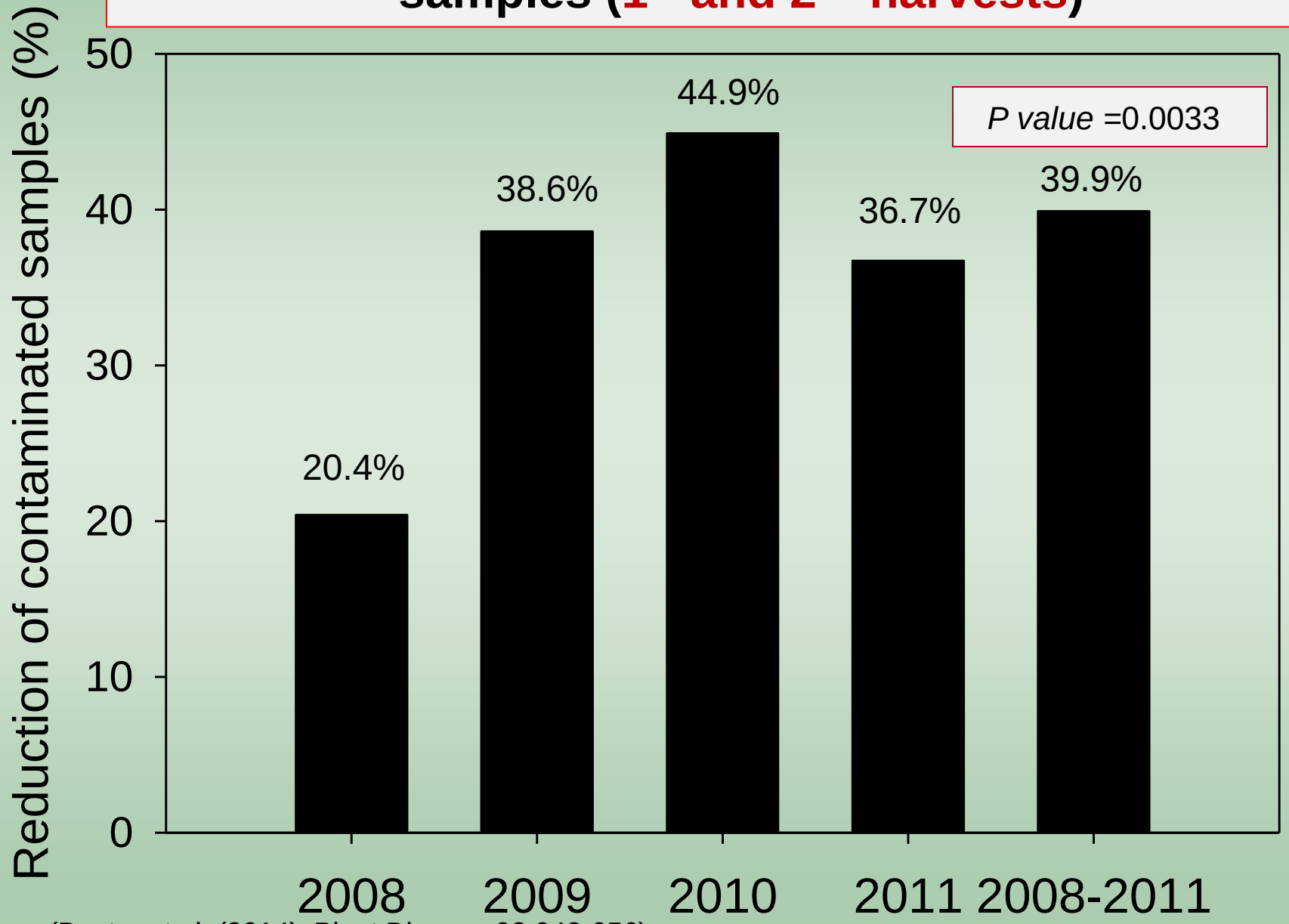
Treated Orchard

Library samples for aflatoxin analyses

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

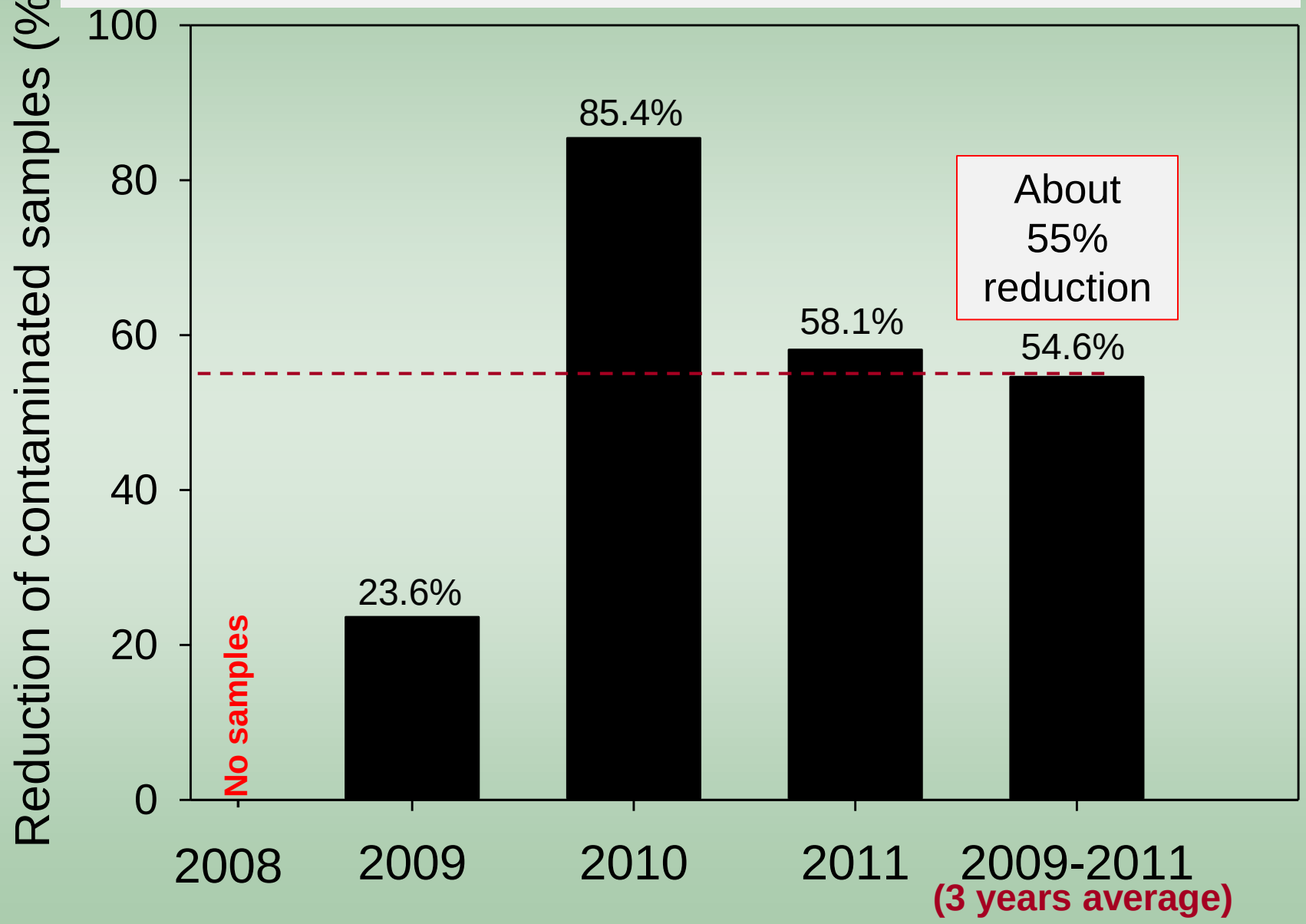
Label has orchard location, date, and grower.

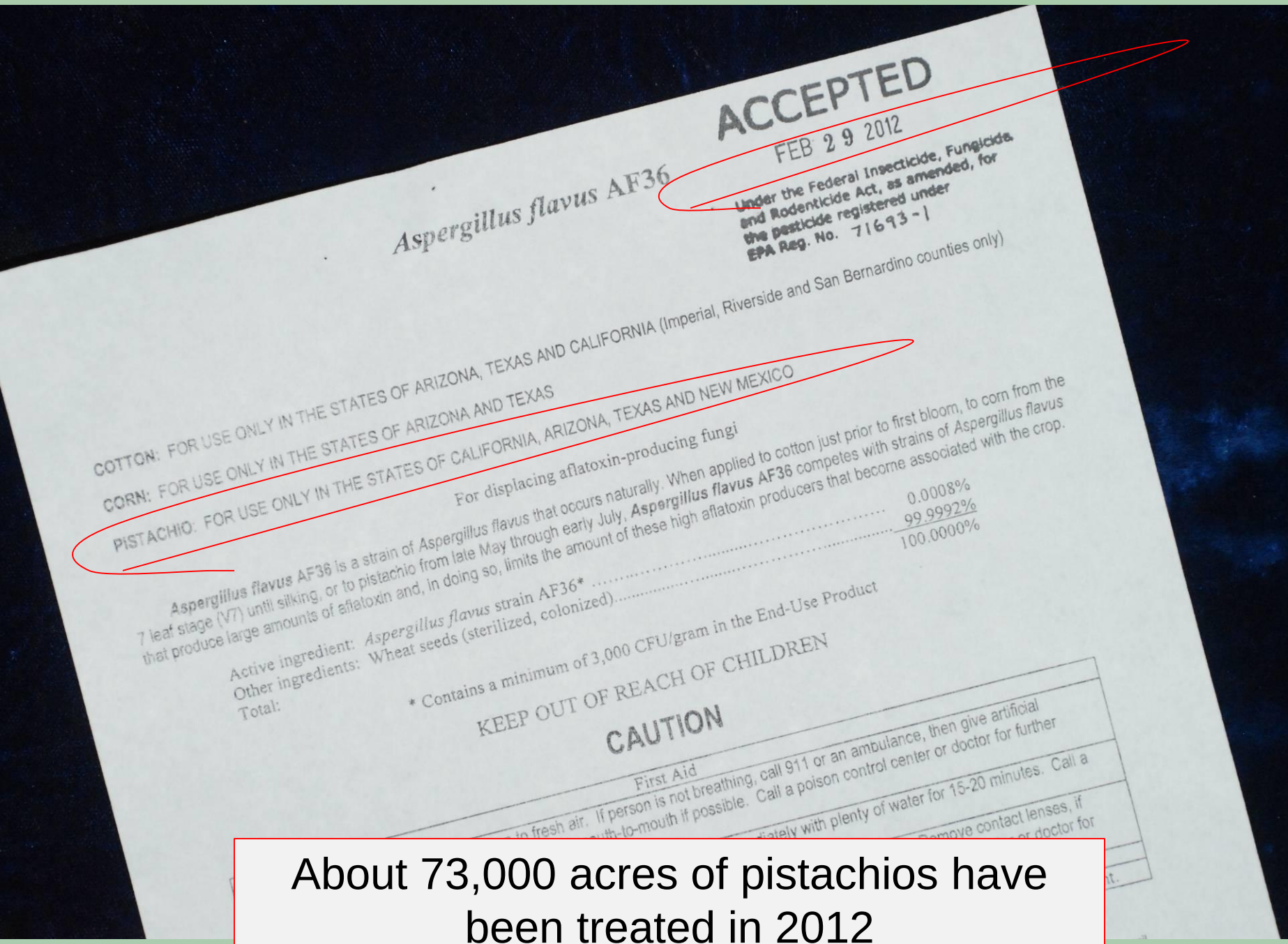
Reduction in aflatoxin-contaminated pistachio samples (1st and 2nd harvests)



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

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



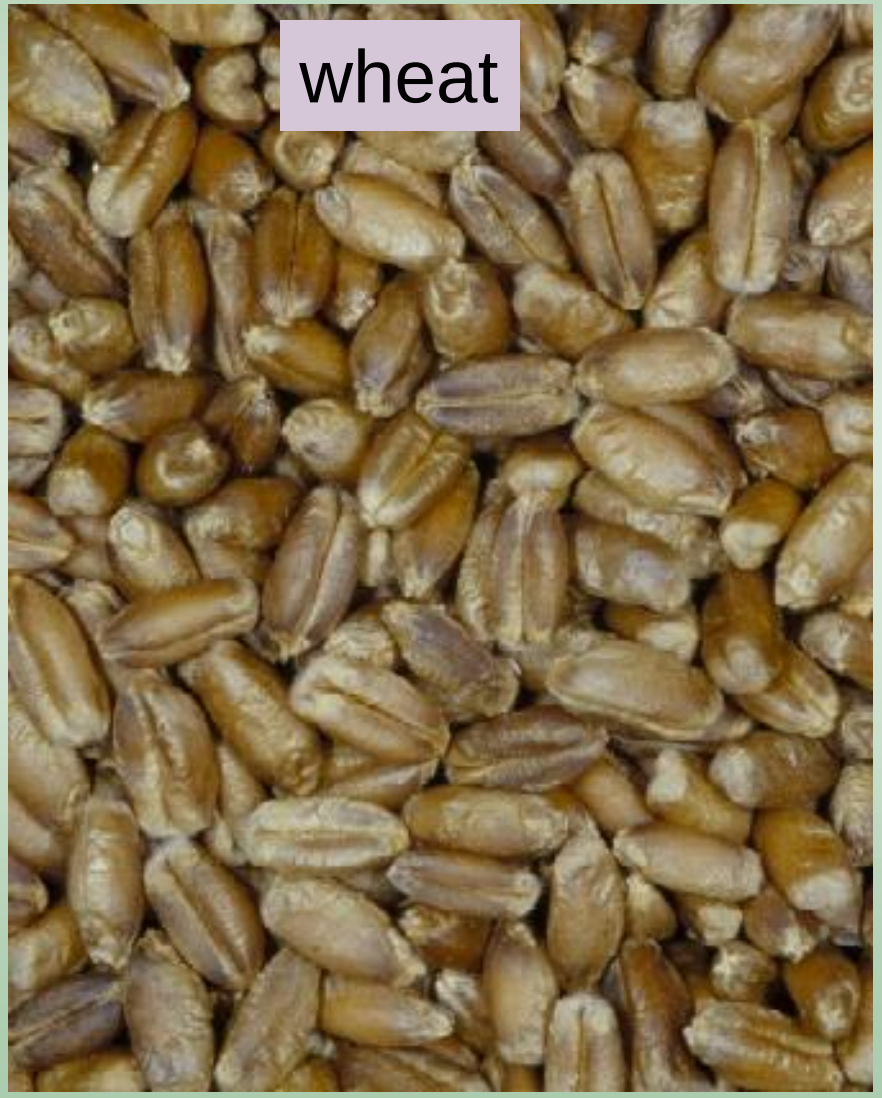


About 73,000 acres of pistachios have been treated in 2012

Treated pistachio acreage with AF36

Year	Acreage treated (ha)
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)
2017	200,000 acres (81,000 ha)

Substrates (carriers) of biocontrol strain AF36



Navel orangeworm

(Host)
pistachio

We cannot change; except:
a) chose the right rootstock;
b) avoid tree stress
c) we can reduce NOW damage

A ATOXIN
CO TAMI ATION SK
N TREE UT

We cannot change

(Agent)
AF36
Aspergillus
(atoxigenic)
(toxigenic)

We can change!

(Environment)
- Moisture
- Temperature
- Soil

Acknowledgments:

- P. Cotty
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- D. Felts
- H. Reyes

California Pistachio Research Board, USDA, and IR-4

- Paramount Farming. Co. (now Wonderful Orchards Co.)
- Setton Farms
- Nichols Farms & Keenan Farms
- & many pistachio growers

Thank you

Nut length, dark stain, and navel orangeworm (NOW) infestation