



# **Registration of a Biopesticide to Reduce Aflatoxins in Pistachio**

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***2013 Statewide Pistachio Day***

# Nuts in the field

- High water activity
- Insect damage
- Drought stress, etc.

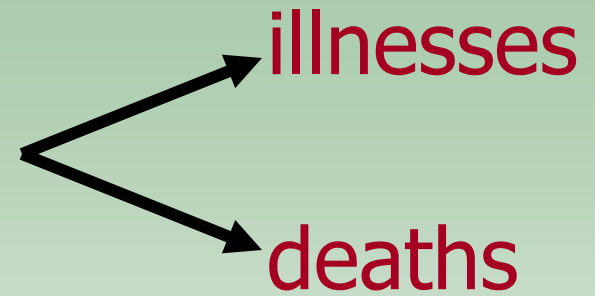
M O L D S

Toxic  
secondary  
metabolites

***"Mycotoxins"***

## *Mycotoxins:*

toxic compounds

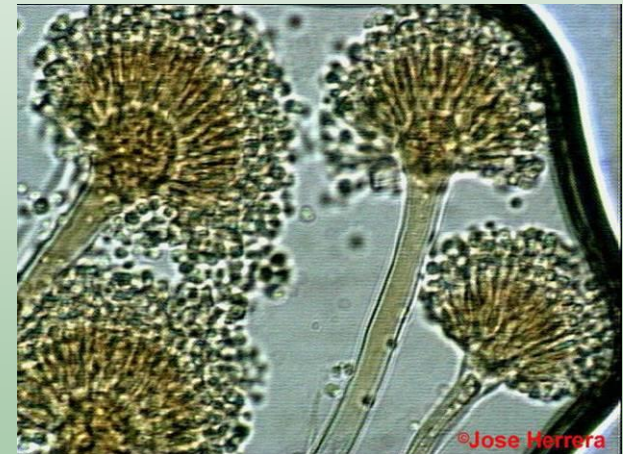


- Carcinogenic
- Mutagenic
- Teratogenic

*Penicillium*

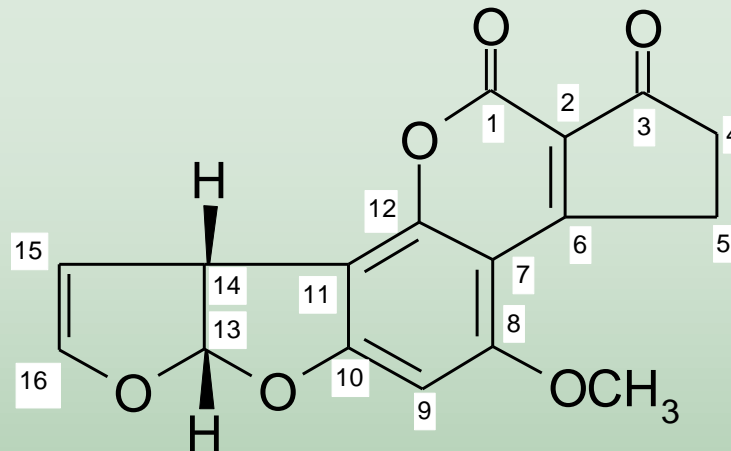
*Fusarium*

*Aspergillus*



- *Aspergillus flavus* and *A. parasiticus*

➡ Aflatoxins **B<sub>1</sub>**, **B<sub>2</sub>**, **G<sub>1</sub>**, **G<sub>2</sub>**, **M<sub>1</sub>**



Aflatoxin B<sub>1</sub>

# Commodities contaminated with aflatoxins:

## Highest risk of aflatoxin contamination:

Corn

Peanuts

Cottonseed

## Occasionally contaminated:

Tree nuts (almonds, pistachios, walnuts)

Figs

Sorghum

Spices

Others

# Incidence 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**

(in pistachios for direct consumption)

- European Union

**Aflatoxin B1 → 8 ppb**

**Total aflatoxins → 10 ppb**

(pistachios for direct consumption)

1 ppb = 0.000 000 001 g per g of pistachio



# Molds that can produce aflatoxin in pistachio orchards in California



*Aspergillus flavus*

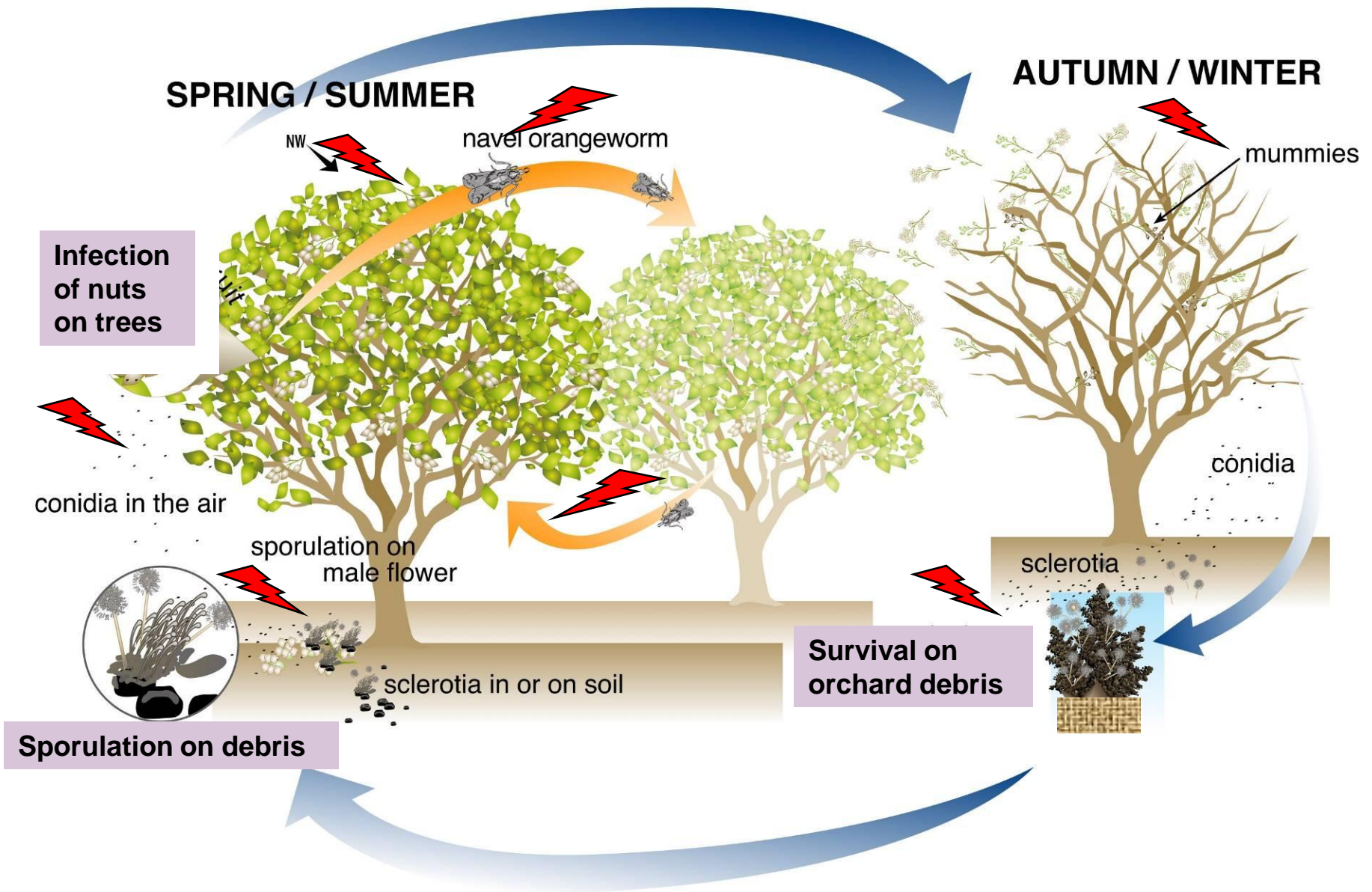


*Aspergillus parasiticus*



**SPRING / SUMMER**

**AUTUMN / WINTER**



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



## Early splits

Infection by  
*Aspergillus* fungi and  
infestation by NOW

Early split nuts: main  
source of aflatoxin  
contamination in  
pistachio nuts

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





Early split nuts  
develop in the  
orchard  
in July and  
continuing until  
harvest;  
(2-5% of the crop)



**NOW pupa**

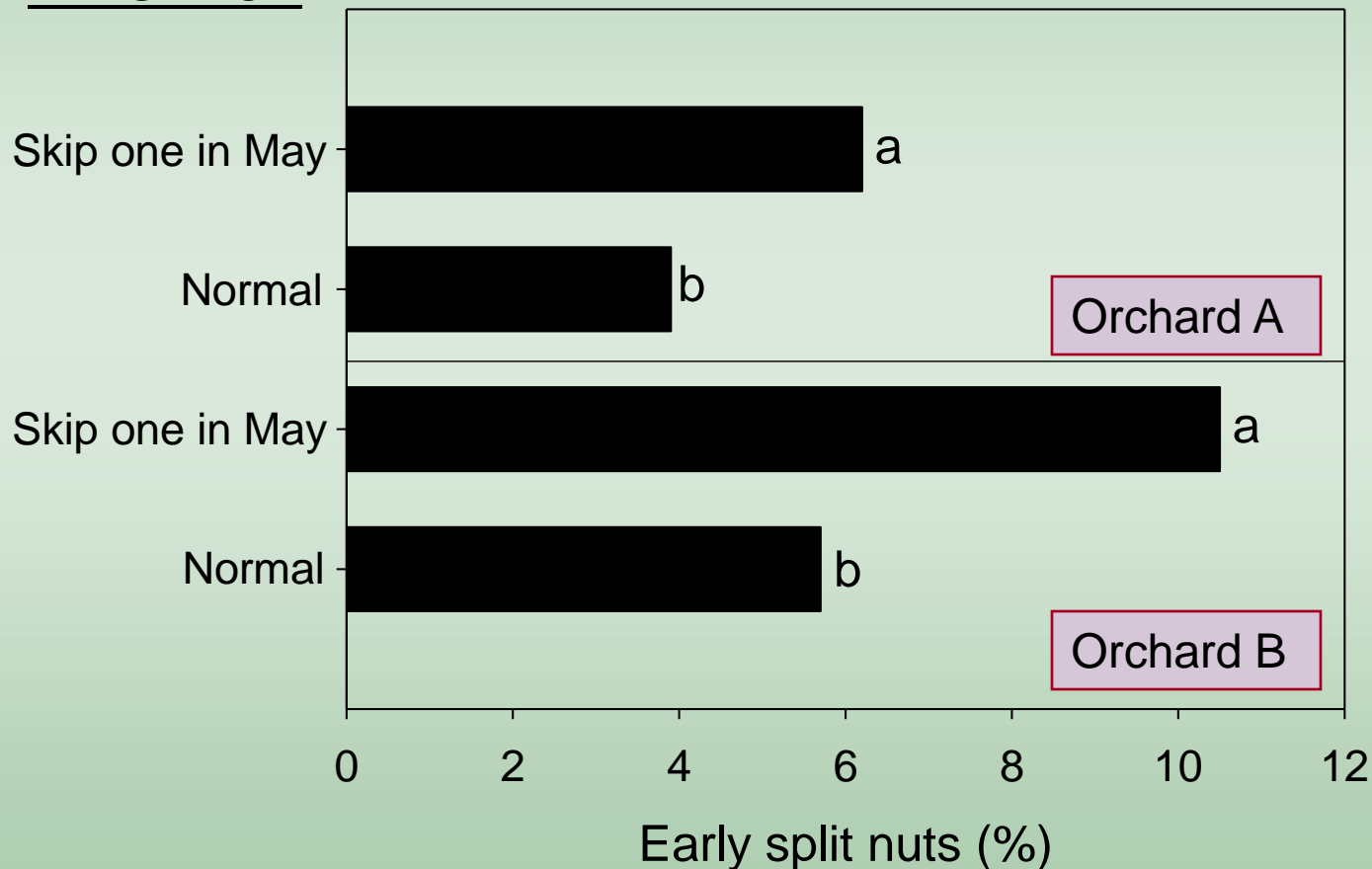


**Suture staining**

Reduce early split nut formation

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

IRRIGATION:



→ Use a rootstock that minimizes early split nuts.

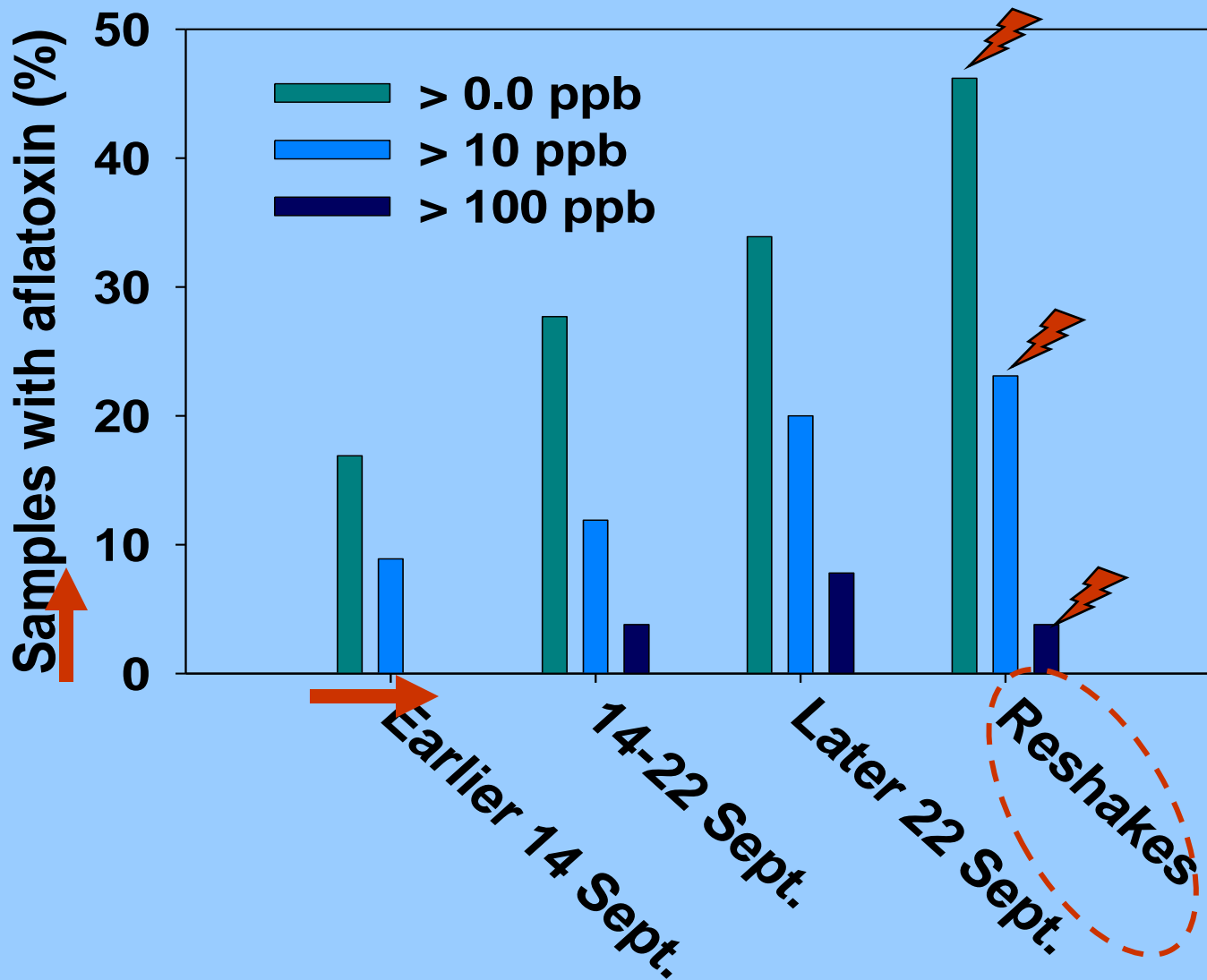
## Percentage of early split nuts in rootstock trials

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



Avoid late harvests

# Harvest dates, incidence, and amounts of aflatoxin contamination



# Navel orangeworm (NOW) infestation increases with delaying harvest

✓ **Avoid late harvests.**

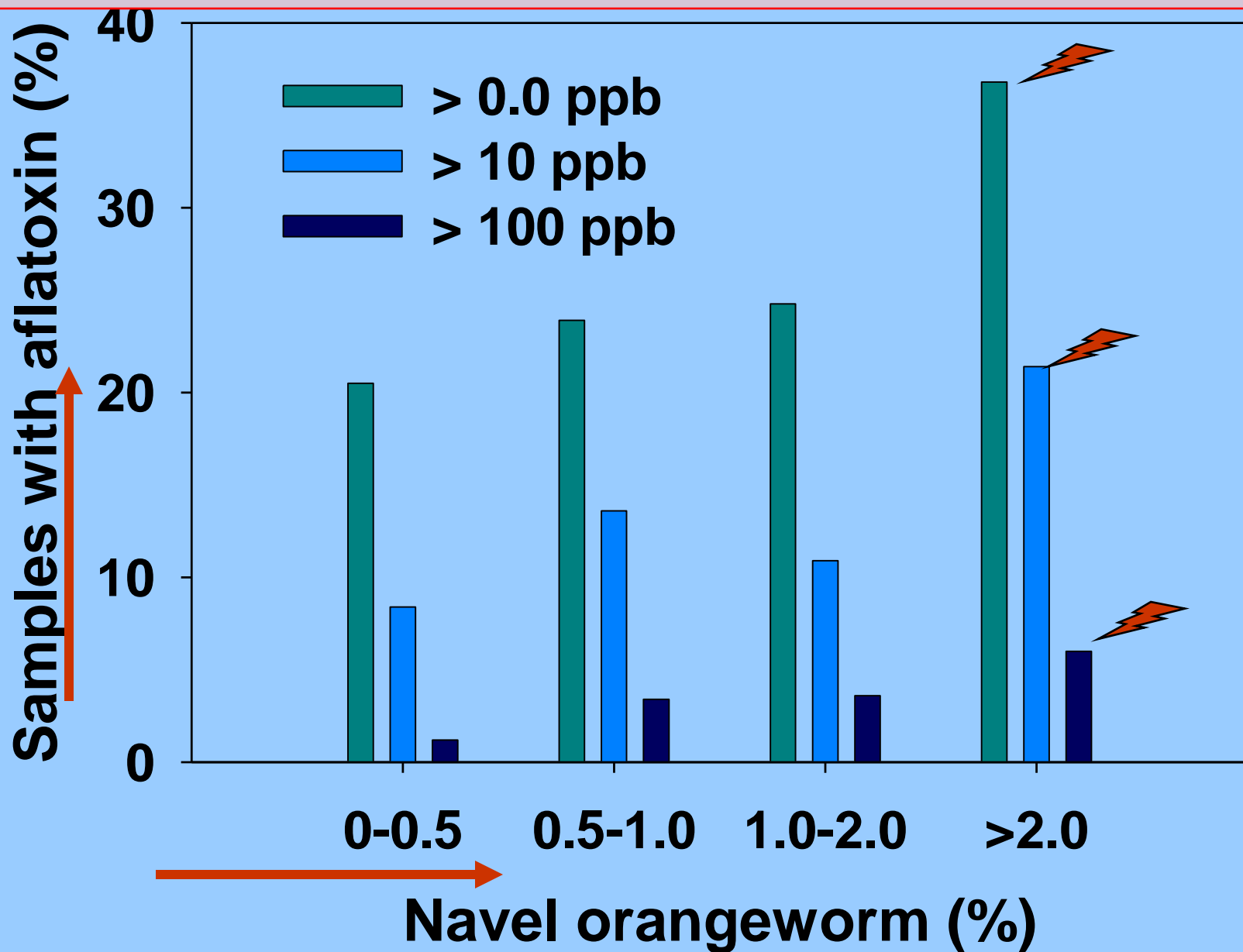
## Year 1

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

# Relationship of navel orangeworm infestation and aflatoxin levels



# Steps to reduce aflatoxins

Field

Processing plant

**NOW**

Lower  
damage

**Early Splits  
(ES)**

**Irrigation  
Management**

**Special  
Characteristics**

Avoid  
water  
stress in  
spring  
(May)

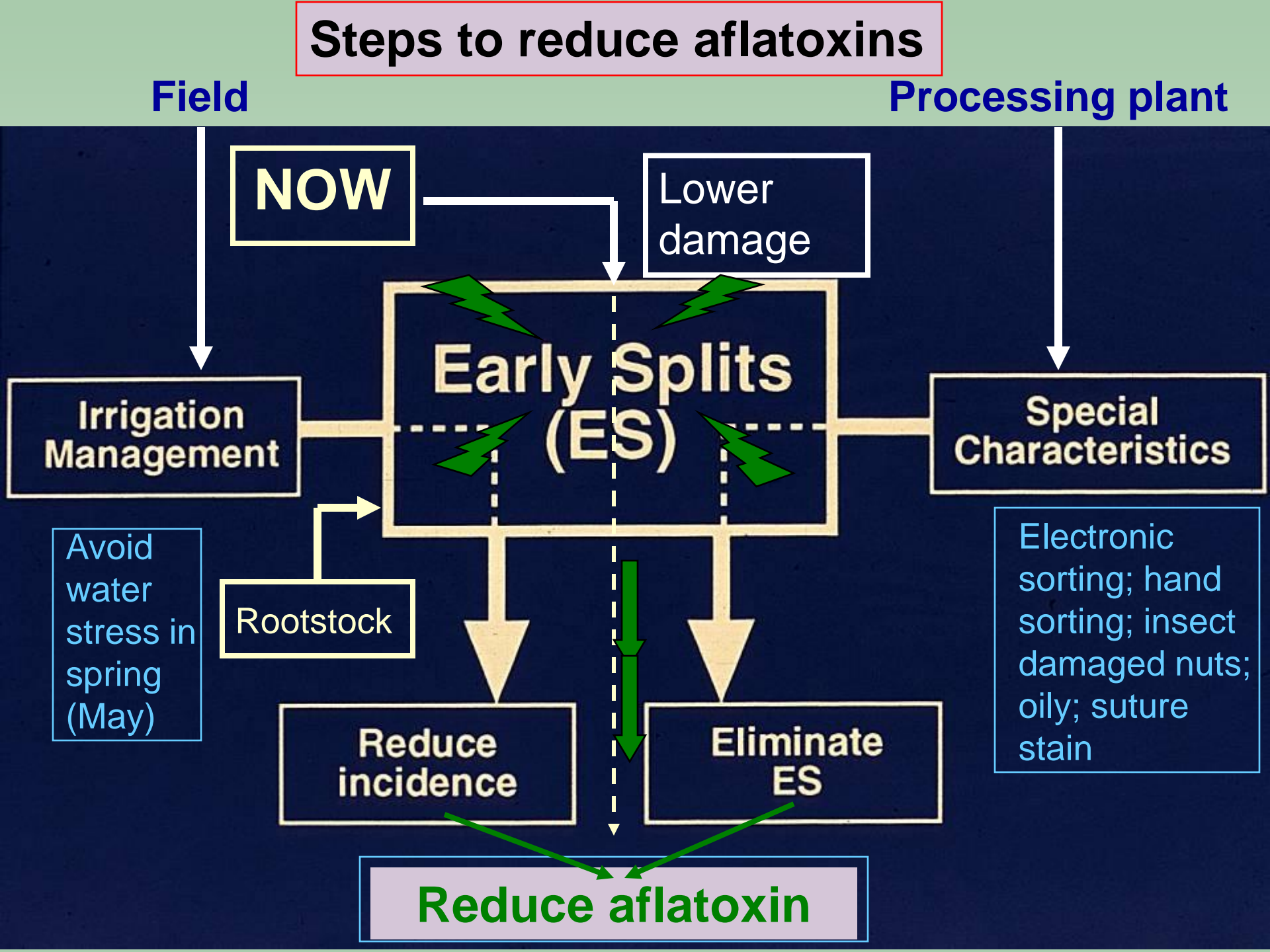
**Rootstock**

Electronic  
sorting; hand  
sorting; insect  
damaged nuts;  
oily; suture  
stain

**Reduce  
incidence**

**Eliminate  
ES**

**Reduce aflatoxin**



# Use of **atoxigenic strains** of *Aspergillus flavus* as biopesticides 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

**M - strain**



?

**S - strain**



most toxigenic

# Occurrence of the atoxigenic strains of *Aspergillus flavus* in pistachio orchards

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*A. flavus* atoxigenic **A564, A815, etc.:** **1-2%**

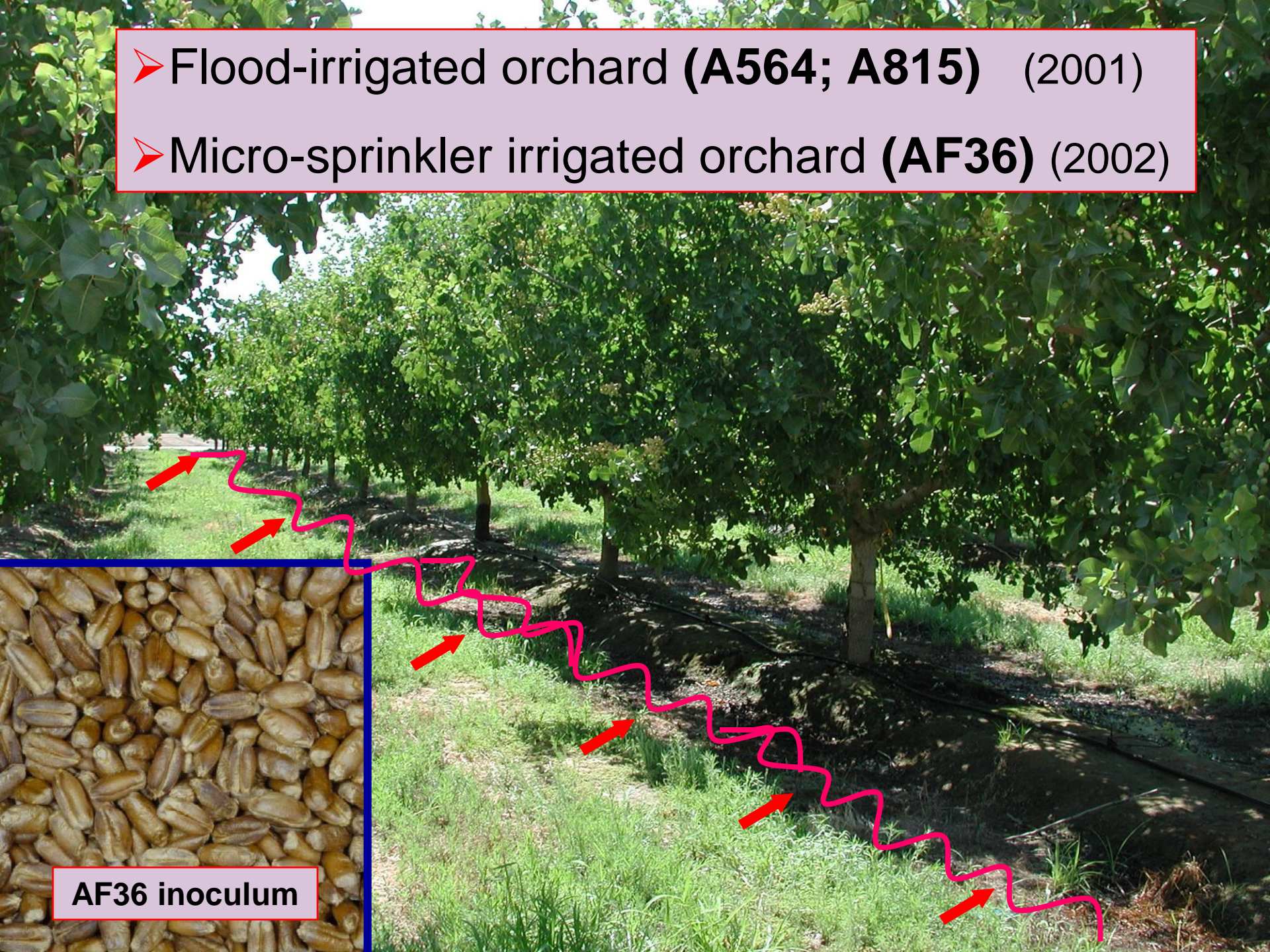
*A. flavus* atoxigenic **AF36** strain .....**4.5 - 7.5%**



- Flood-irrigated orchard (**A564; A815**) (2001)
- Micro-sprinkler irrigated orchard (**AF36**) (2002)



**AF36 inoculum**





# AF36 wheat inoculum

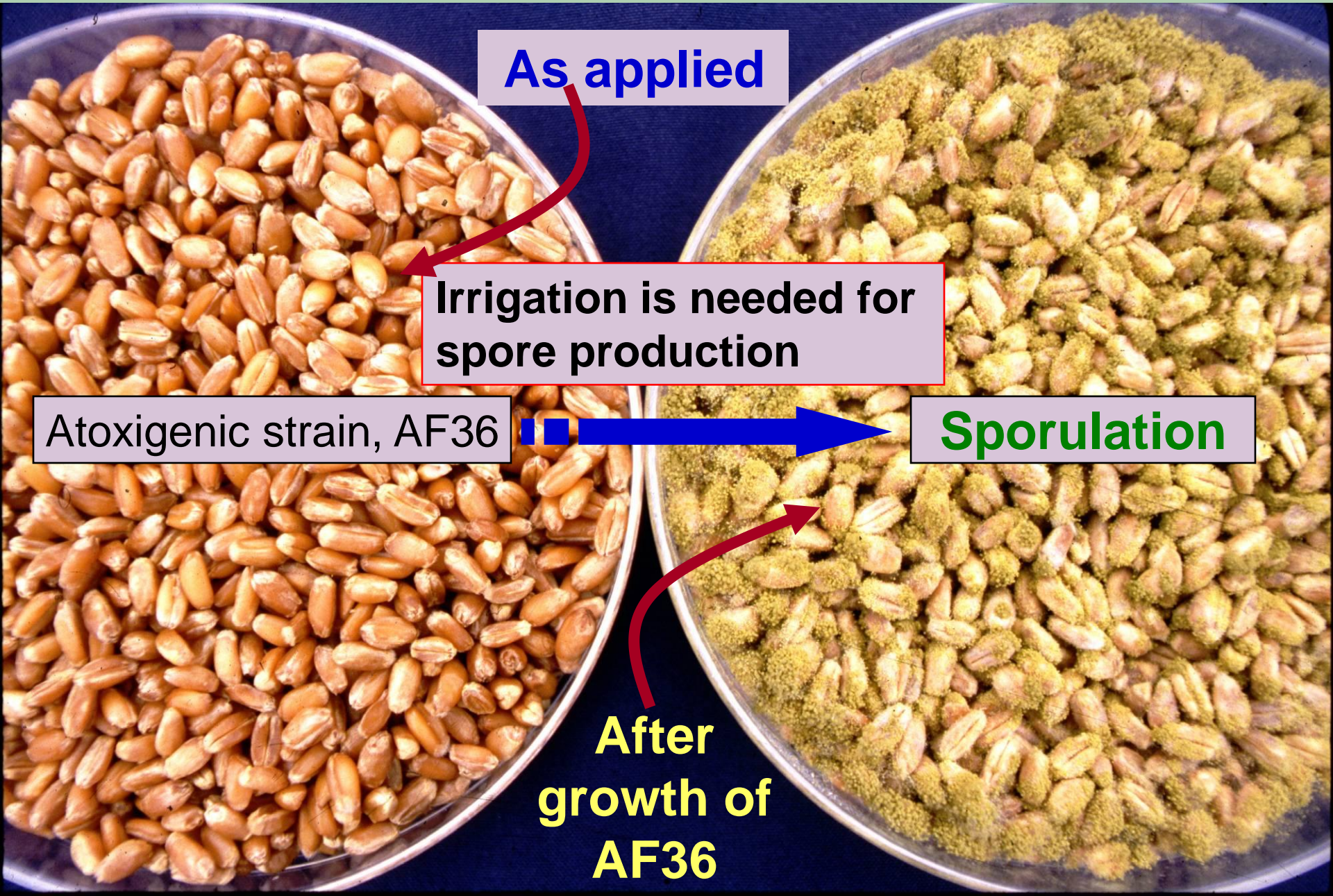
**As applied**

**Irrigation is needed for  
spore production**

**Atoxigenic strain, AF36**

**Sporulation**

**After  
growth of  
AF36**





# Advantages in selecting the atoxigenic *Aspergillus flavus* strain AF36



- ✓ AF36 is a naturally-occurring in high levels.
- ✓ AF36 displaces toxigenic strains (micro-plot studies).
- ✓ Wheat is infected with AF36 shows no signs of fungal growth.

# Process leading to EUP and registration of AF36 in pistachio

- Early 2007 ready; However, requested by EPA & CDPR to do spore trapping in orchards.
- January 2008: Experimental Use Permit (EUP) approved by the EPA and CDPR.
- In early summer of 2008, a single application of the wheat-AF36 product was applied to 3,000 acres (limit of the EUP) of commercial orchards in Kern, Kings, Madera, Merced, and Tulare Counties.
- In 2009, 2010, and 2011, a single application was also applied to the same orchards.



AF36  
Inoculum







**Application rate: 10 lbs. per acre**

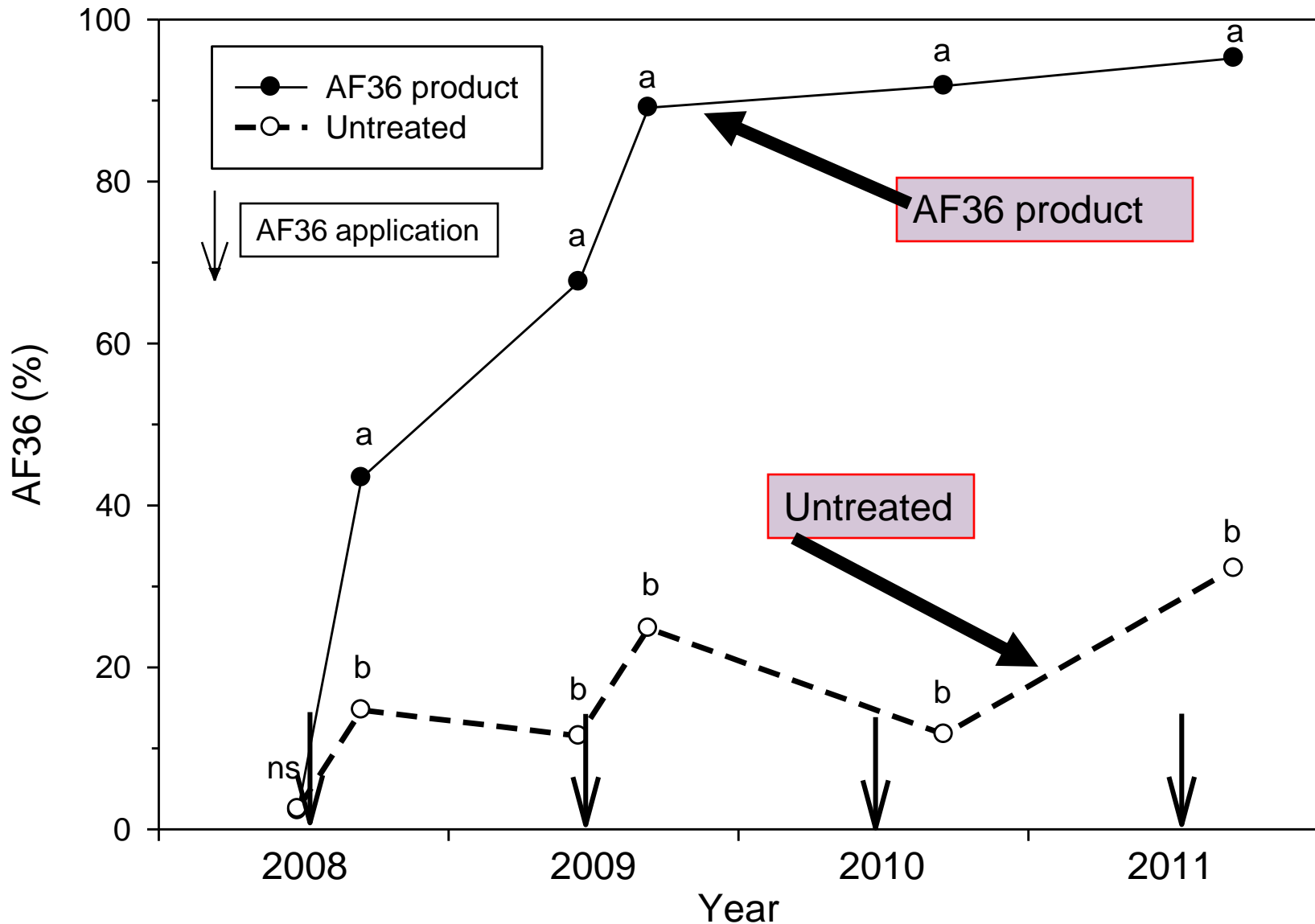


After irrigation, the wet wheat seeds will produce spores of AF36





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



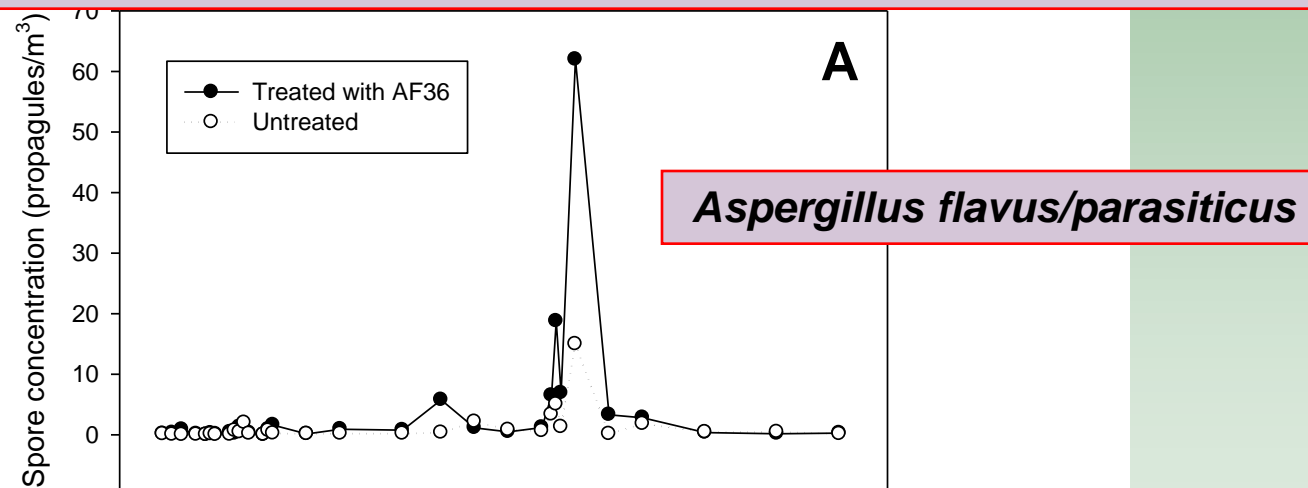


# Burkard spore trap in a pistachio orchard

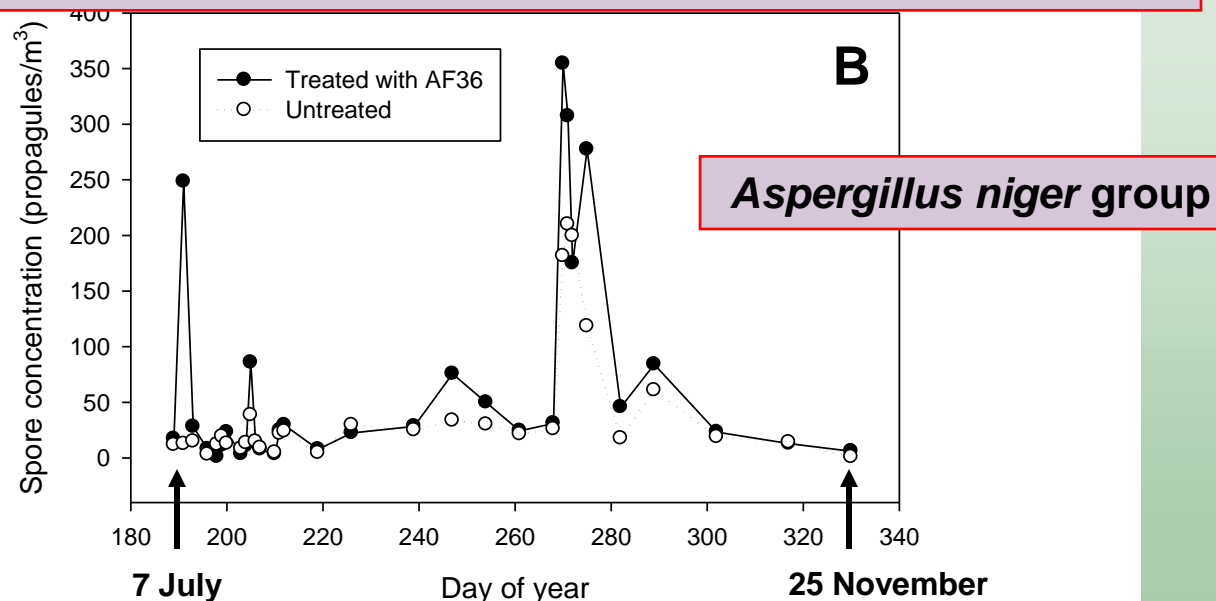




# Concentration of fungal spores in the air in two commercial pistachio orchard (2008)



Similar results in 2009





## Early split nuts decayed

Year	Treatment	%	
		<i>A. flavus</i>	<i>A. niger</i>
2008	Wheat-AF36	0.09 ns	6.39 ns
	Untreated control	0.16	9.85
2009	Wheat-AF36	0.10 ns	5.80 ns
	Untreated control	0.03	5.95
2010	Wheat-AF36	0.06 ns	3.01 ns
	Untreated control	0.01	3.17
2011	Wheat-AF36	0.09 ns	2.00 ns
	Untreated control	0.05	2.21



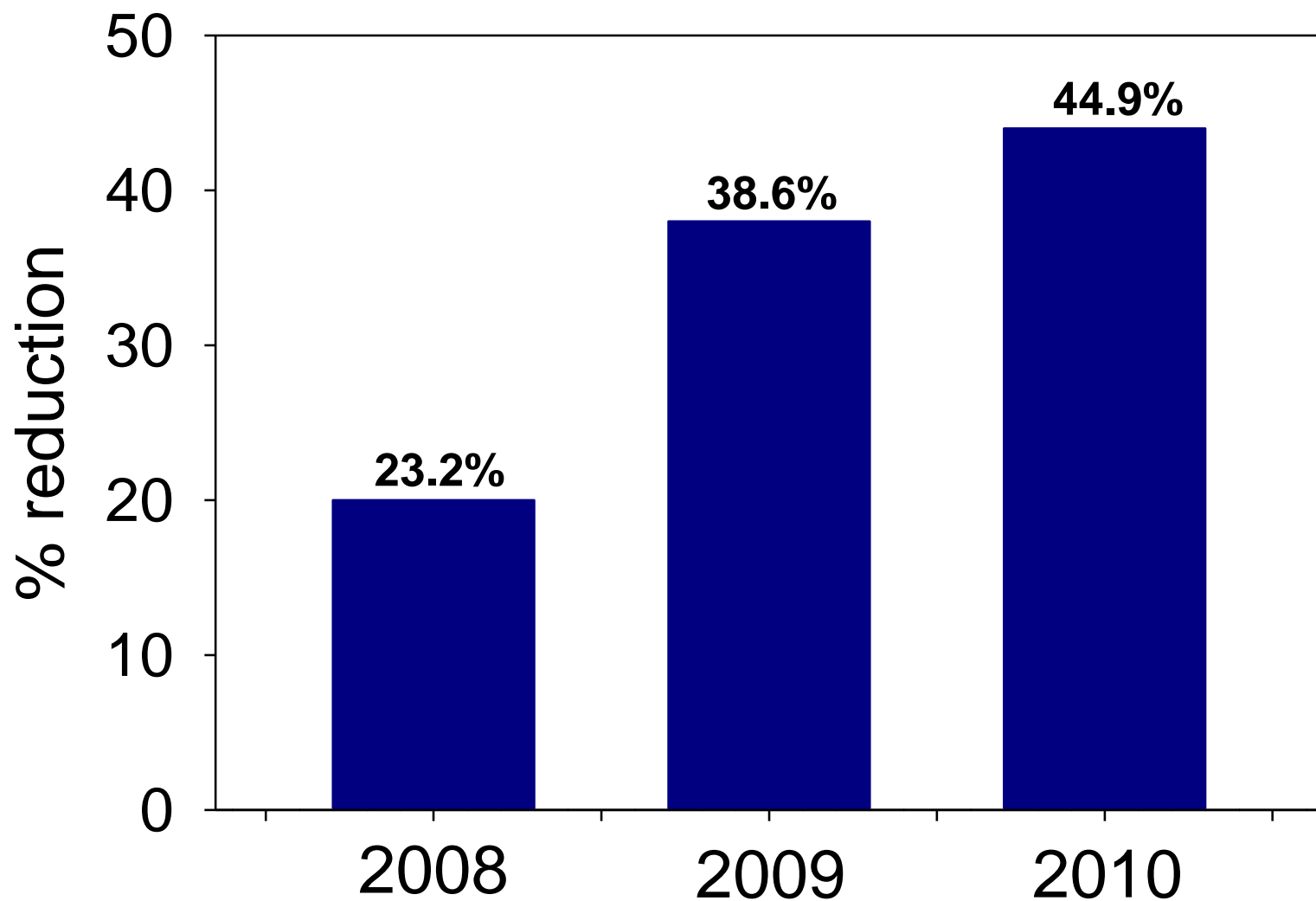
No increase in kernel decay.

# Library samples for aflatoxin analysis

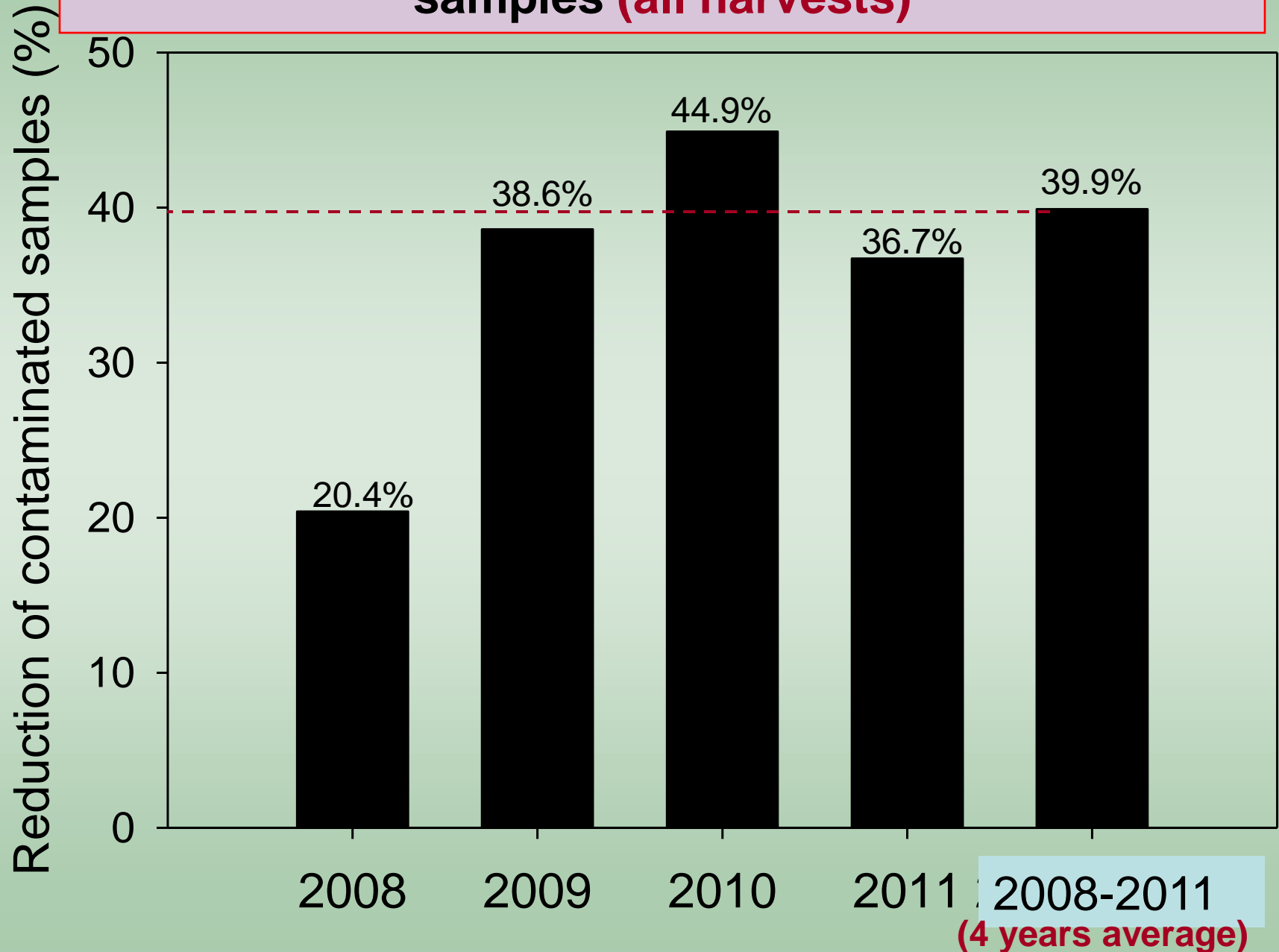


Samples taken at processing plant as nuts are being unloaded.

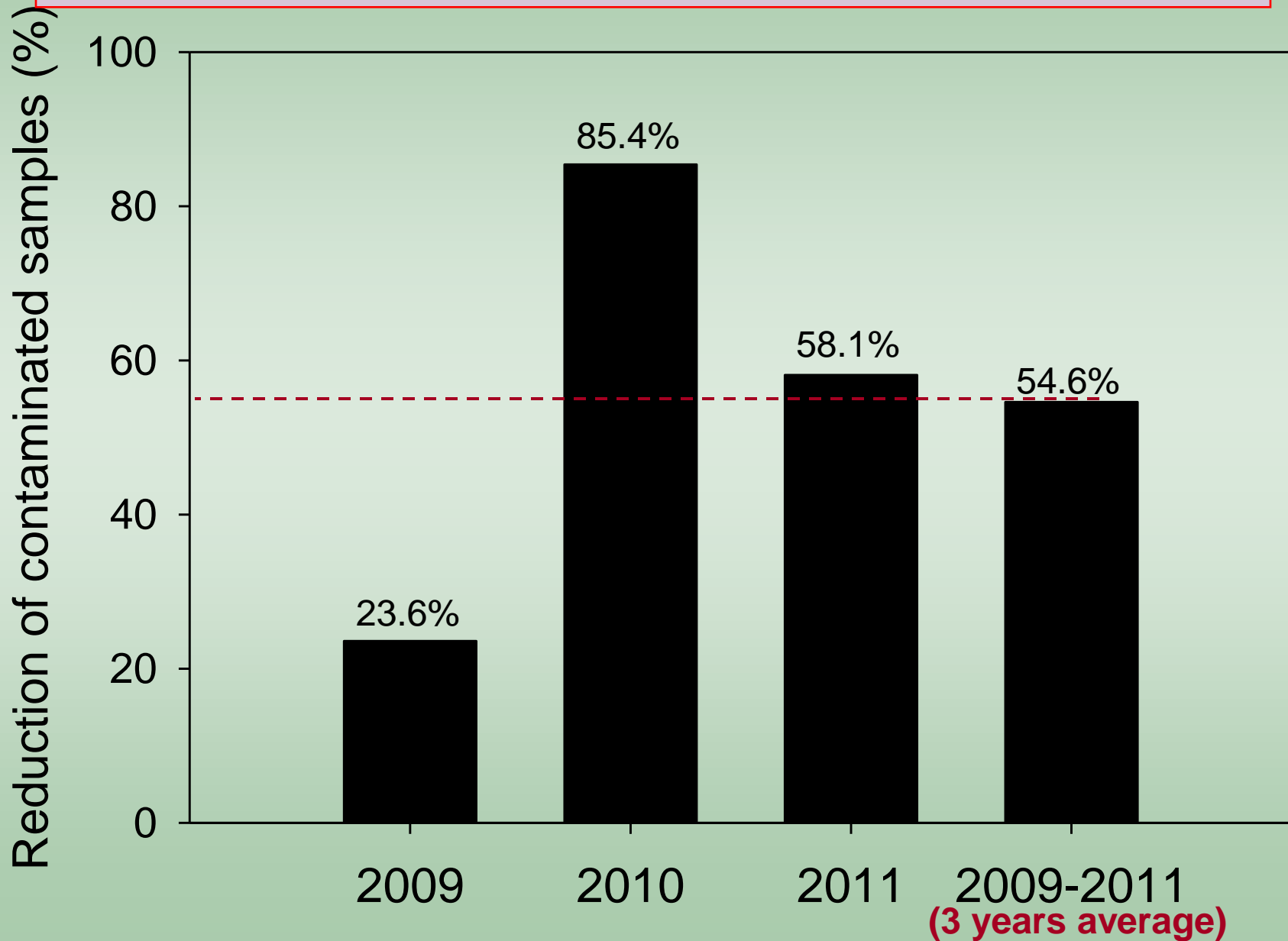
**Reduction in aflatoxin-contaminated pistachio samples after treating orchards with AF36 (all harvests: 1<sup>st</sup> & 2<sup>nd</sup> harvests)**



## Reduction in aflatoxin-contaminated pistachio samples (all harvests)



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





*Aspergillus flavus* AF36

ACCEPTED  
FEB 29 2012

Under the Federal Insecticide, Fungicide  
and Rodenticide Act, as amended, for  
the pesticide registered under  
EPA Reg. No. 71693-1

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 ARIZONA AND TEXAS

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

For displacing aflatoxin-producing fungi  
*Aspergillus flavus* AF36 is a strain of *Aspergillus flavus* that occurs naturally. When applied to cotton just prior to first bloom, to corn from the  
7 leaf stage (V7) until silking, or to pistachio from late May through early July, *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.

Active ingredient: *Aspergillus flavus* strain AF36\*  
Other ingredients: Wheat seeds (sterilized, colonized)  
Total:

0.0008%  
99.9992%  
100.0000%

\* Contains a minimum of 3,000 CFU/gram in the End-Use Product

KEEP OUT OF REACH OF CHILDREN  
CAUTION

First Aid

to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial  
breath-to-mouth if possible. Call a poison control center or doctor for further  
treatment. Remove contact lenses, if  
wearing them, and continue to breathe oxygen or doctor for

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



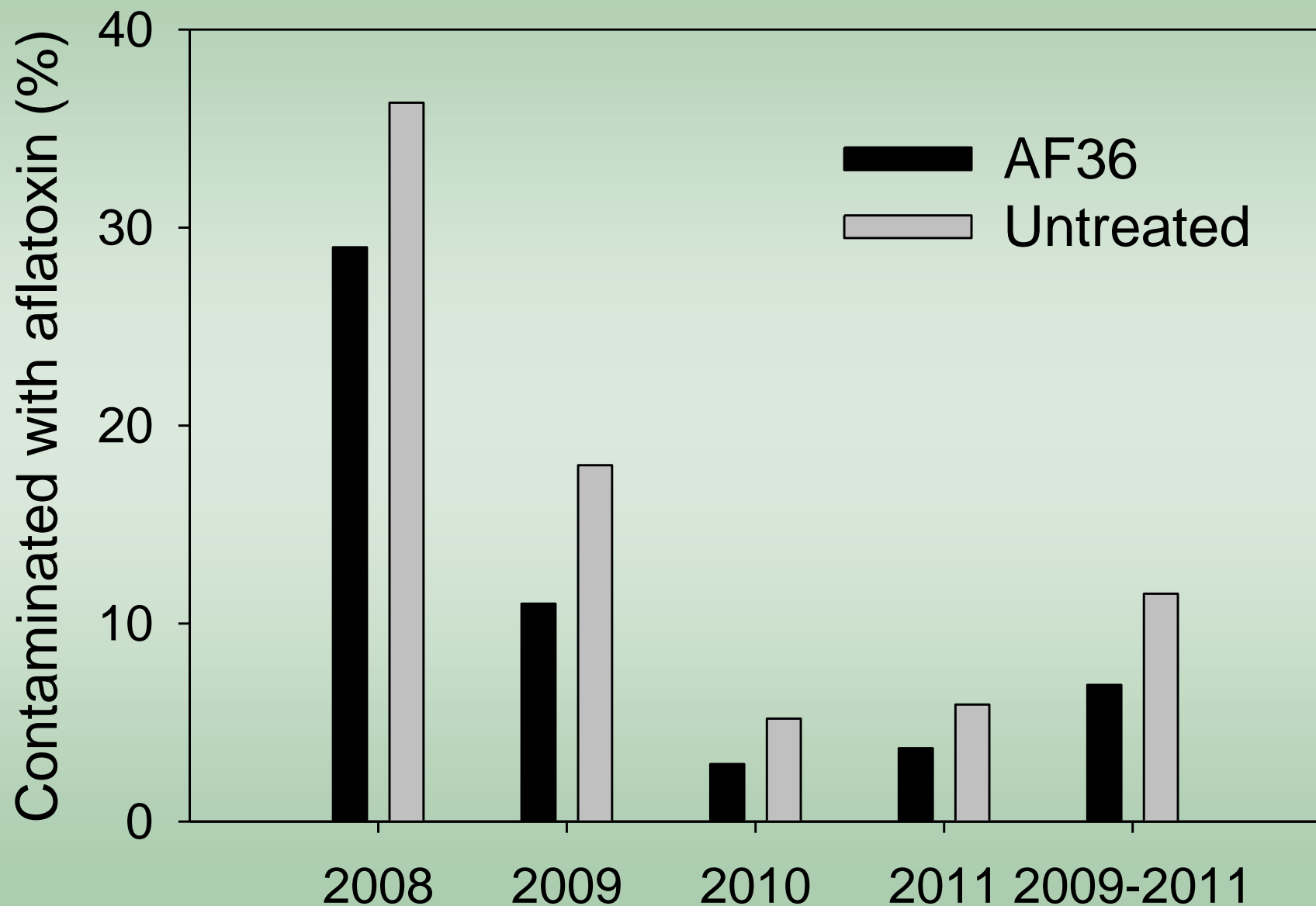
# Acknowledgments:

- **Peter Cotty**
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- **Mark Doster**
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- **Matthias Donner**
- **Ryan Puckett**
- **Heraclio Reyes**

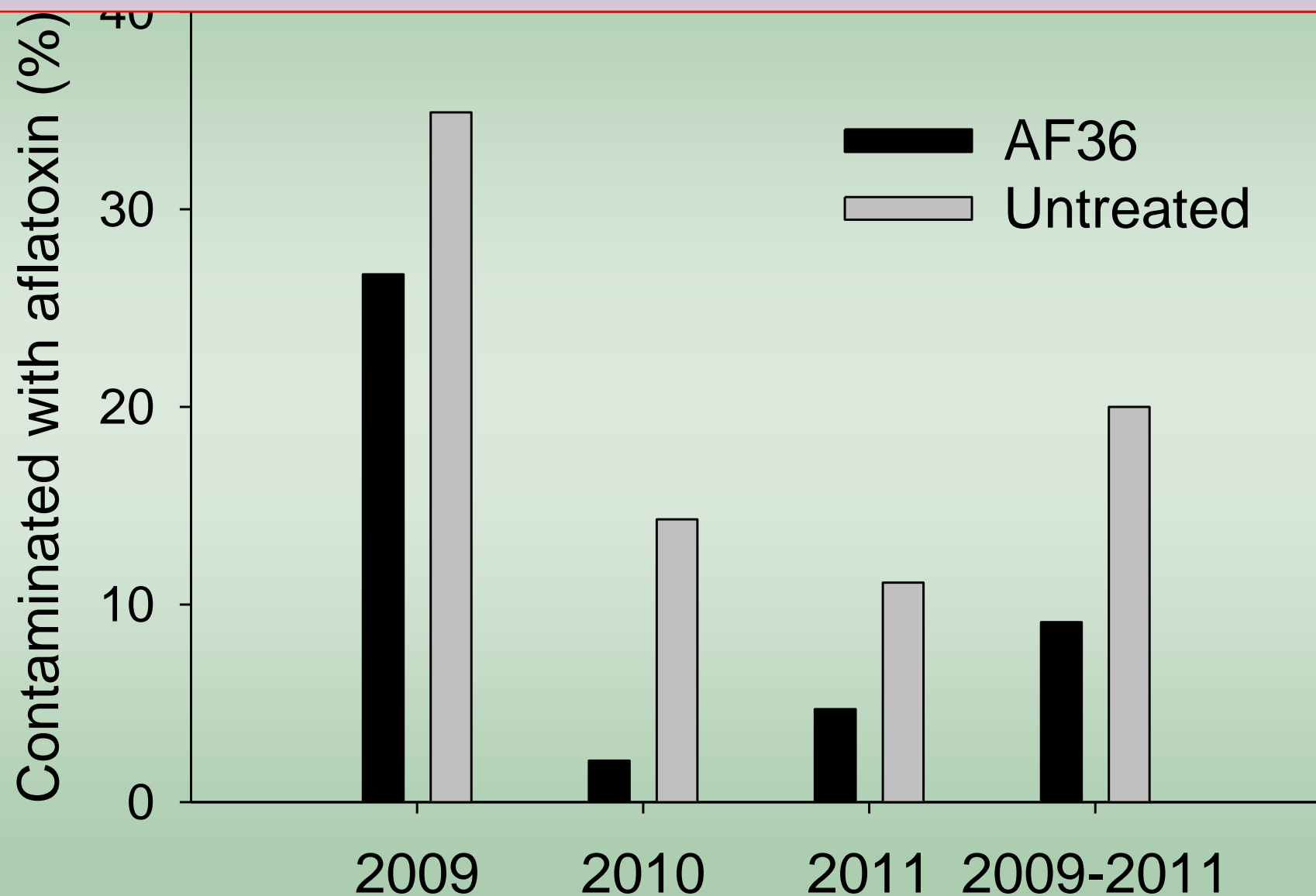
- **Candice Rogers, Paramount Farming. Co.**
- **Mike Smith & Jeff Gibbons, Setton Farms**
- **April Huber, Nichols Farms**  
**Steven Smit, Keenan Farms**

& Thank you

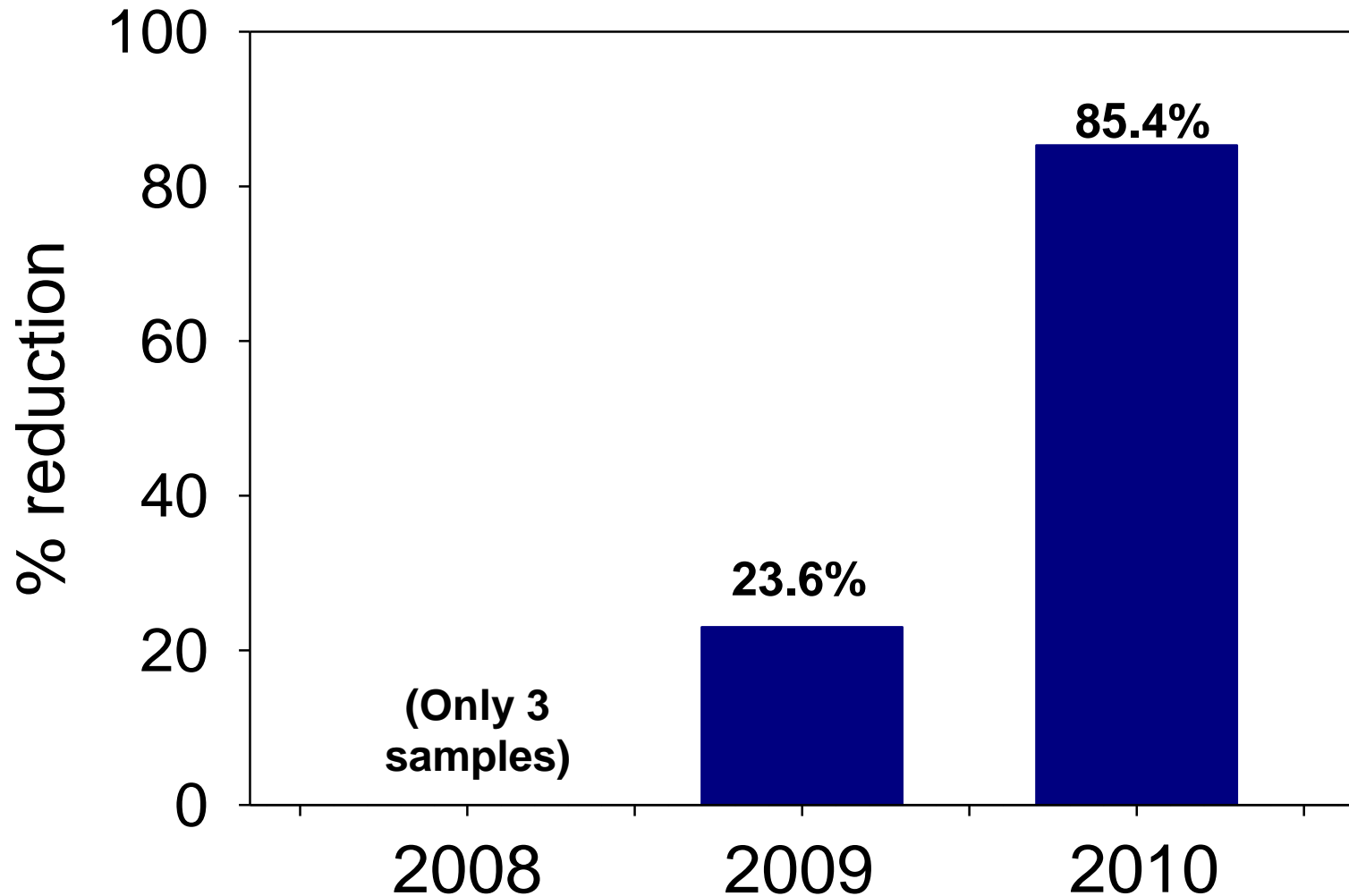
## Samples contaminated with aflatoxin – all harvests



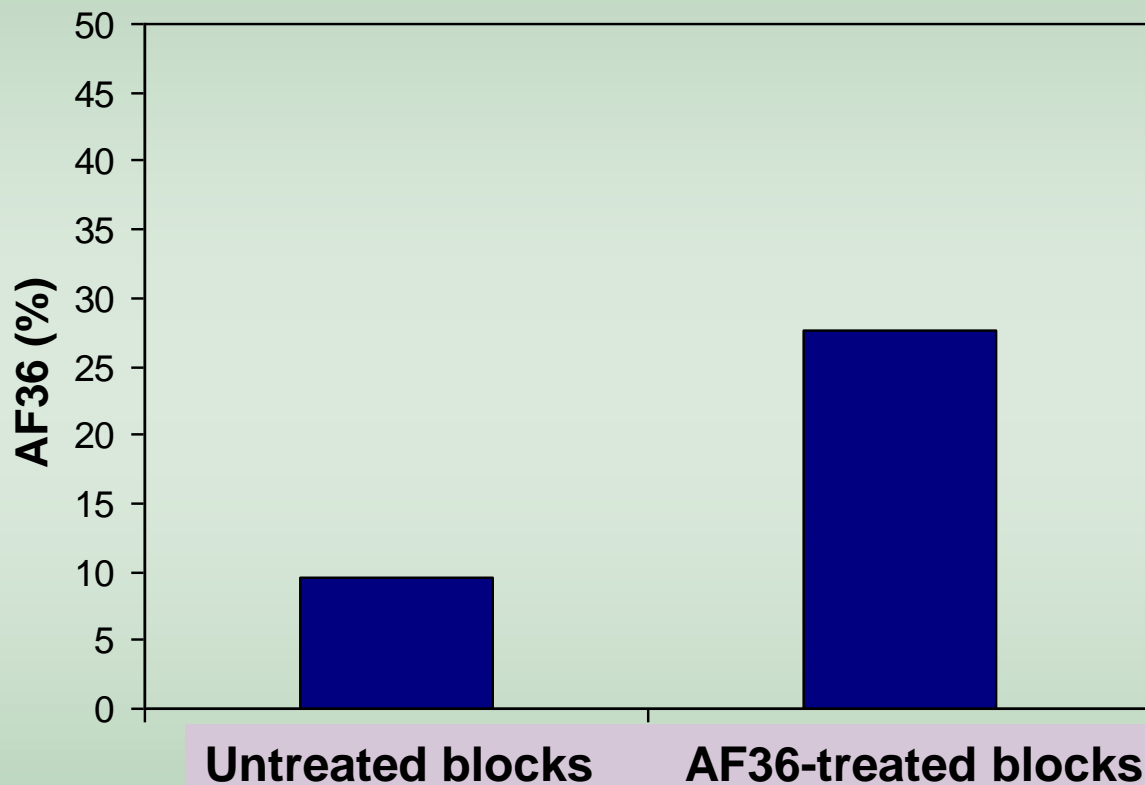
## Samples contaminated with aflatoxin – second harvest



**Percent reduction of aflatoxin contaminated pistachio samples after treating orchards with AF36 (only 2<sup>nd</sup> harvest = “Reshakes”)**



# Incidence of AF36 on NOW moths collected from sticky traps in 2010 (Kern Co).

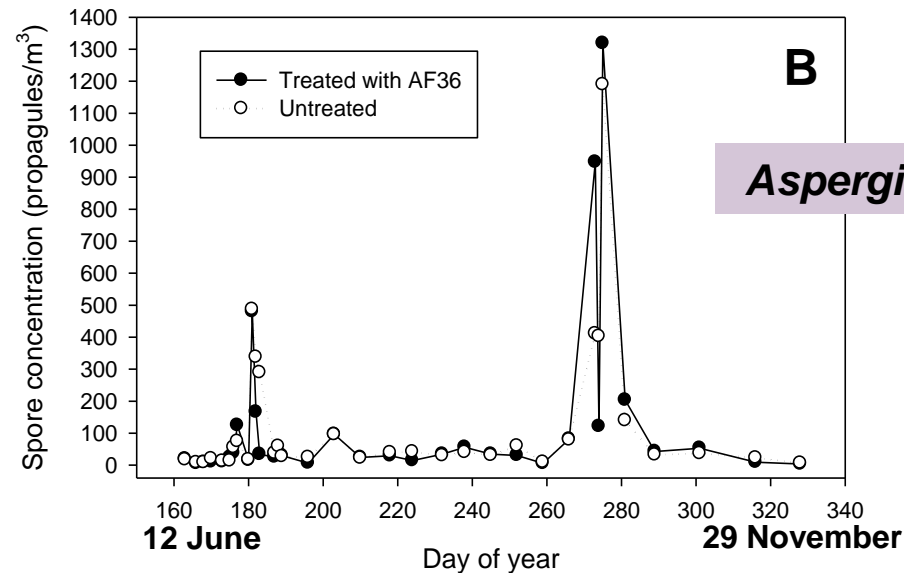
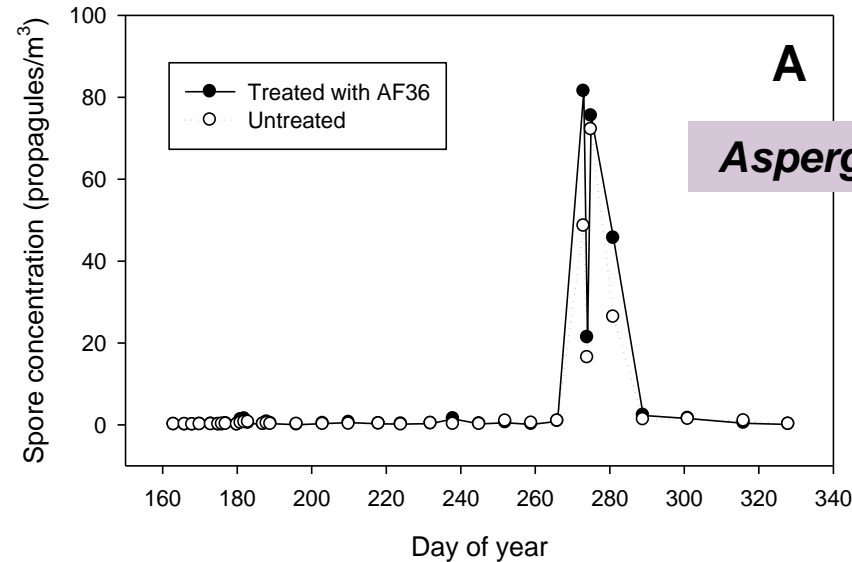


## Isolates obtained from NOW moths (2010)

<b>Pistachios</b>			
	<b>Treatment</b>	<b>Isolates tested</b>	<b>AF36 (%)</b>
Orchard A	AF36	<b>78</b>	<b>24.4</b>
Orchard B	None	<b>20</b>	<b>0</b>
<b>Almonds</b>			
Orchard A	None	<b>26</b>	<b>11.5</b>
Orchard B	None	<b>14</b>	<b>0</b>
Orchard C	None	<b>28</b>	<b>3.6</b>



# Concentration of fungal spores in the air in two commercial pistachio orchard (2009)



**Note:** Other products of AF36 (sorghum /milo) are being now evaluated for sporulation and suitability in pistachios



**sorghum**