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

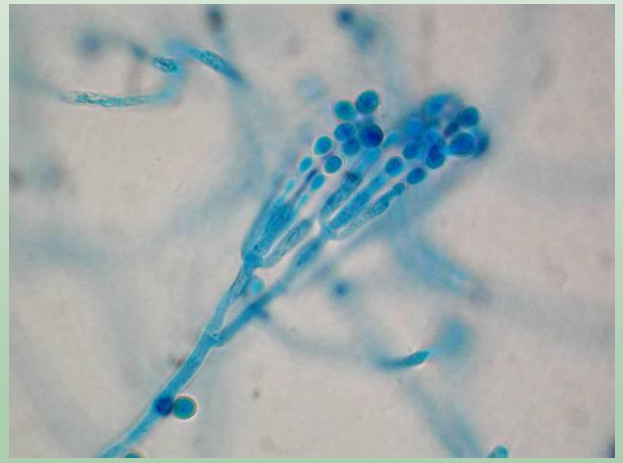
Toxic secondary metabolites

"Mycotoxins"

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

- Carcinogenic
- Mutagenic
- Teratogenic

Penicillium

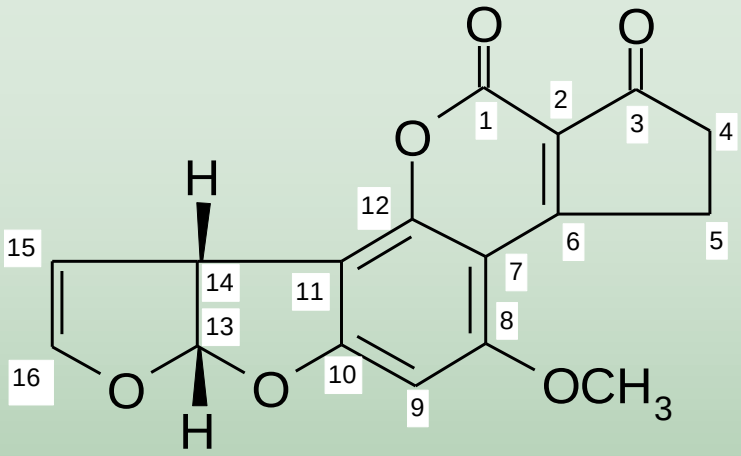


Fusarium

Aspergillus

- ***Aspergillus flavus*** and ***A. parasiticus***

➡ **Aflatoxins B_1, B_2, G_1, G_2, M_1**



Aflatoxin B_1

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 (in pistachios for direct consumption)

Total aflatoxins➔ 15 ppb

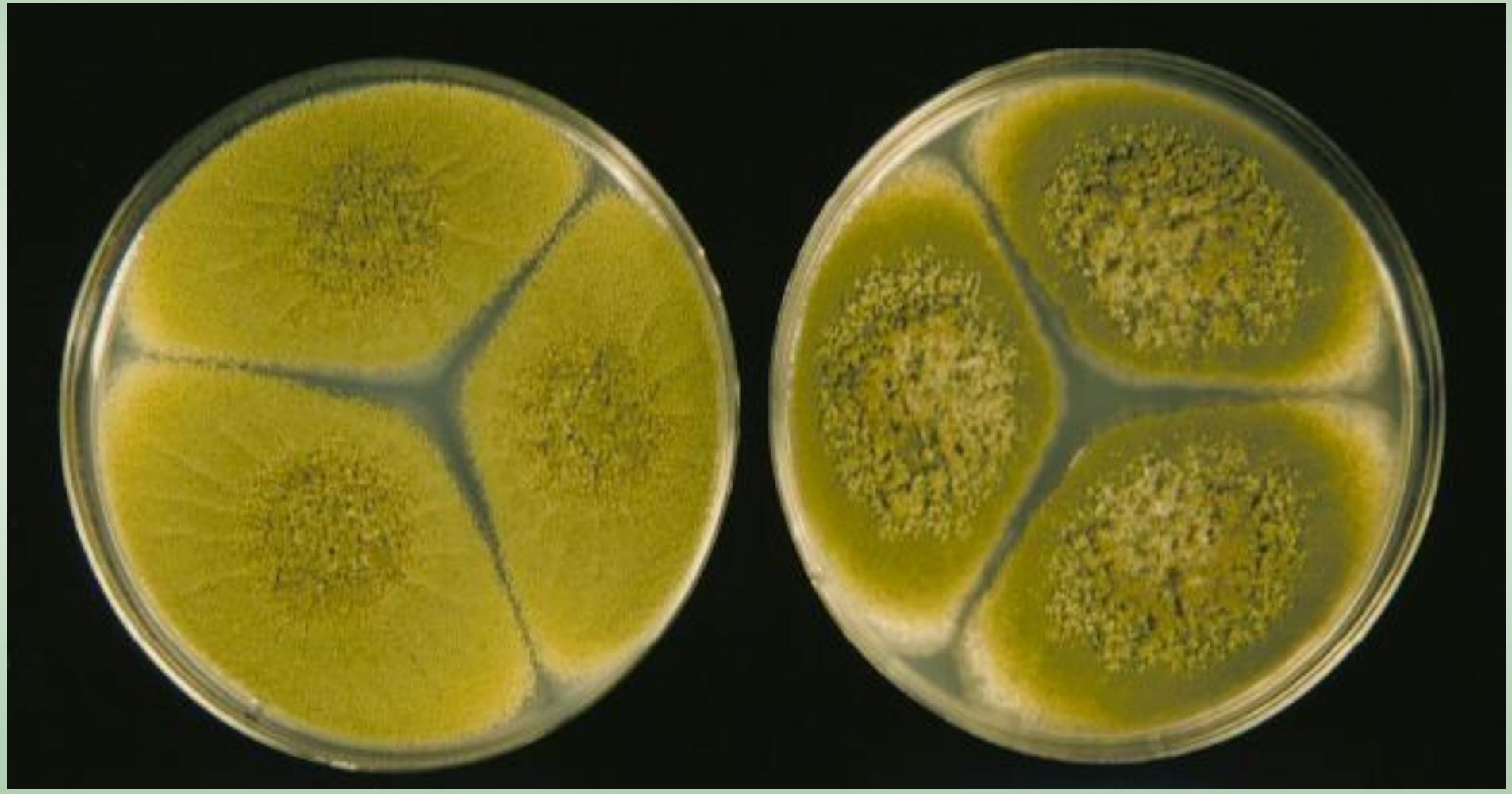
- **European Union**

Aflatoxin B1➔ 8 ppb (pistachios for direct consumption)

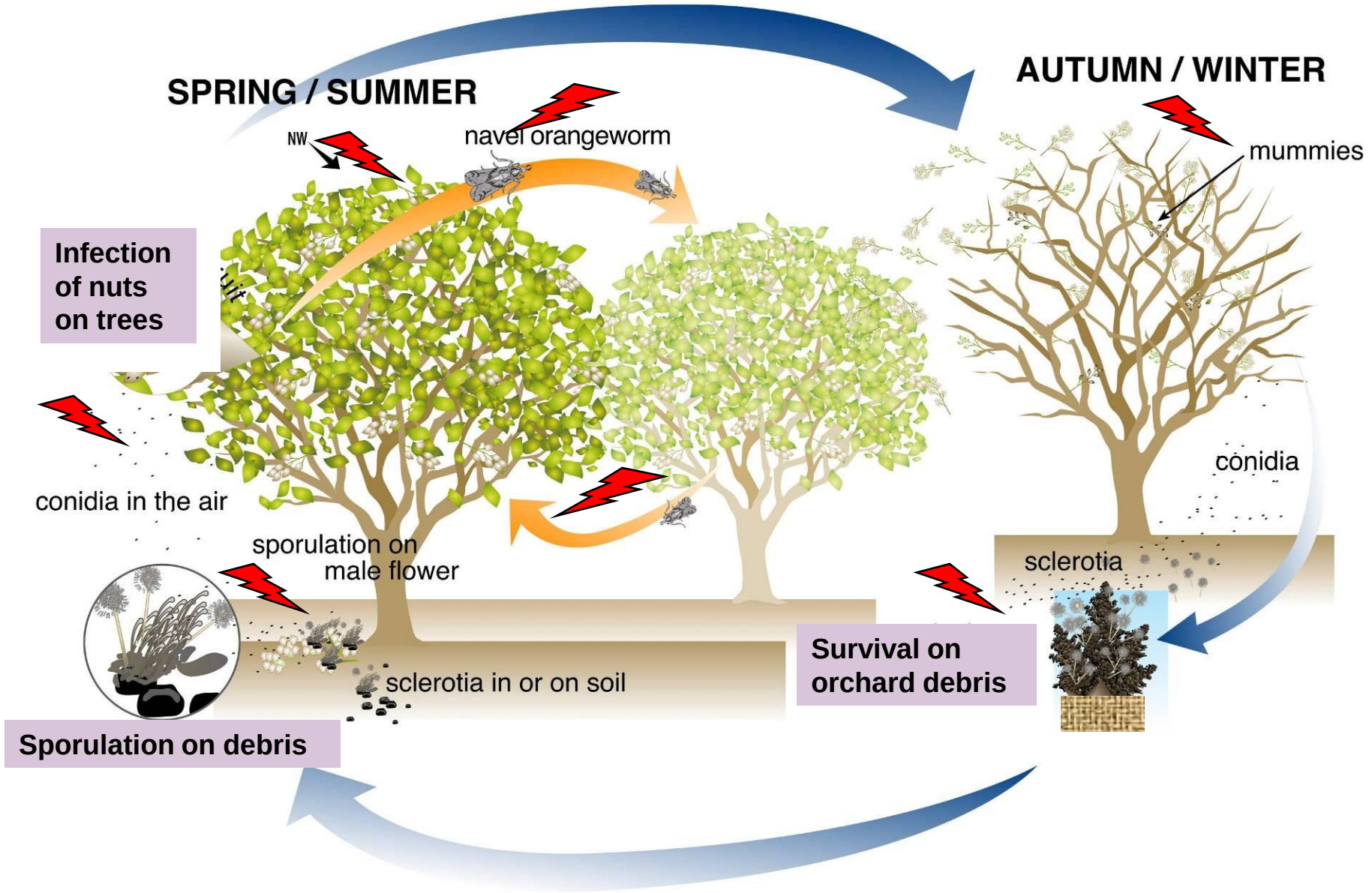
Total aflatoxins➔10 ppb

1 ppb = 0.000 000 001 g per g of pistachio

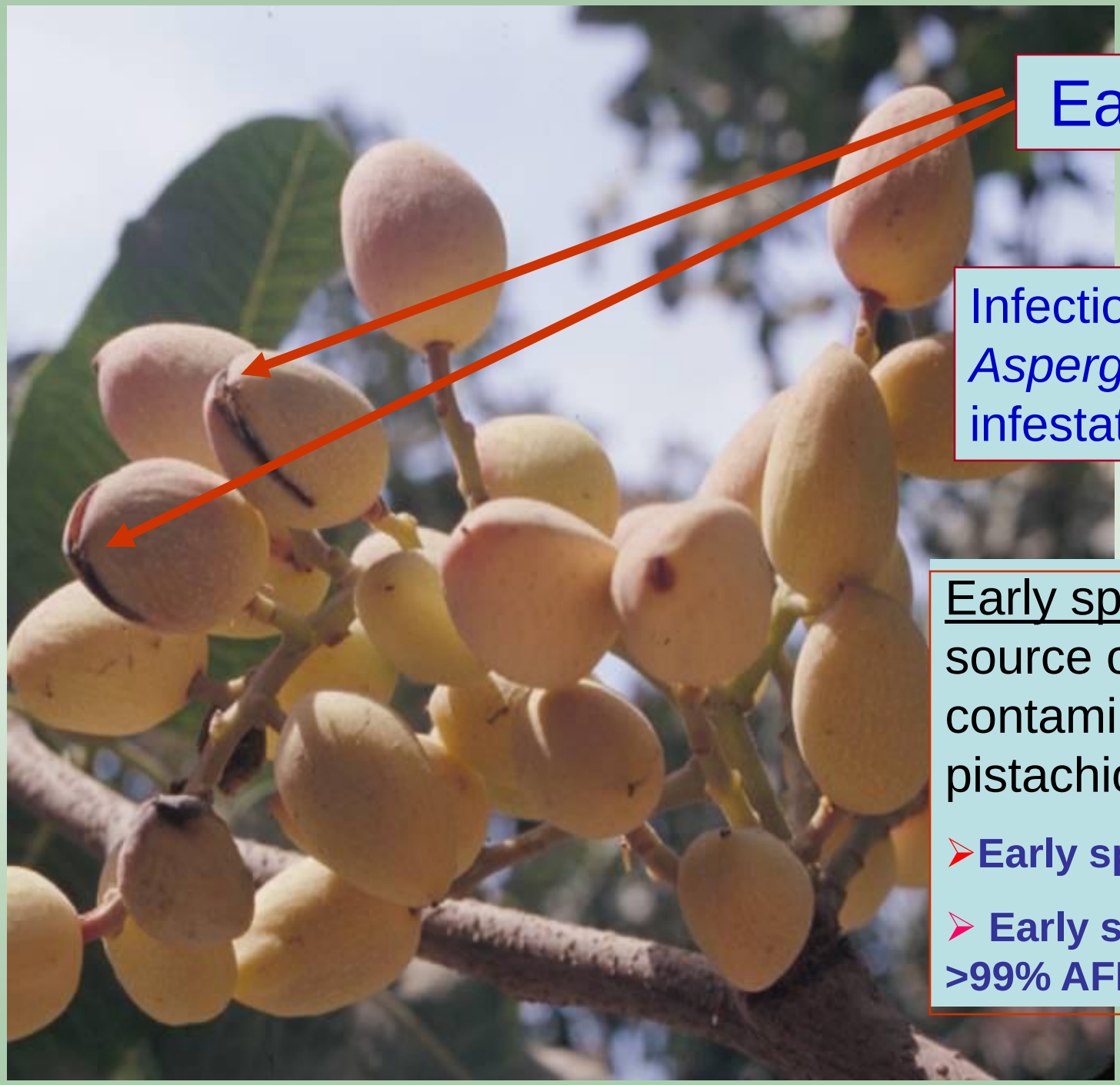
Molds that can produce aflatoxin in pistachio orchards in California



Aspergillus flavus *Aspergillus parasiticus*



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

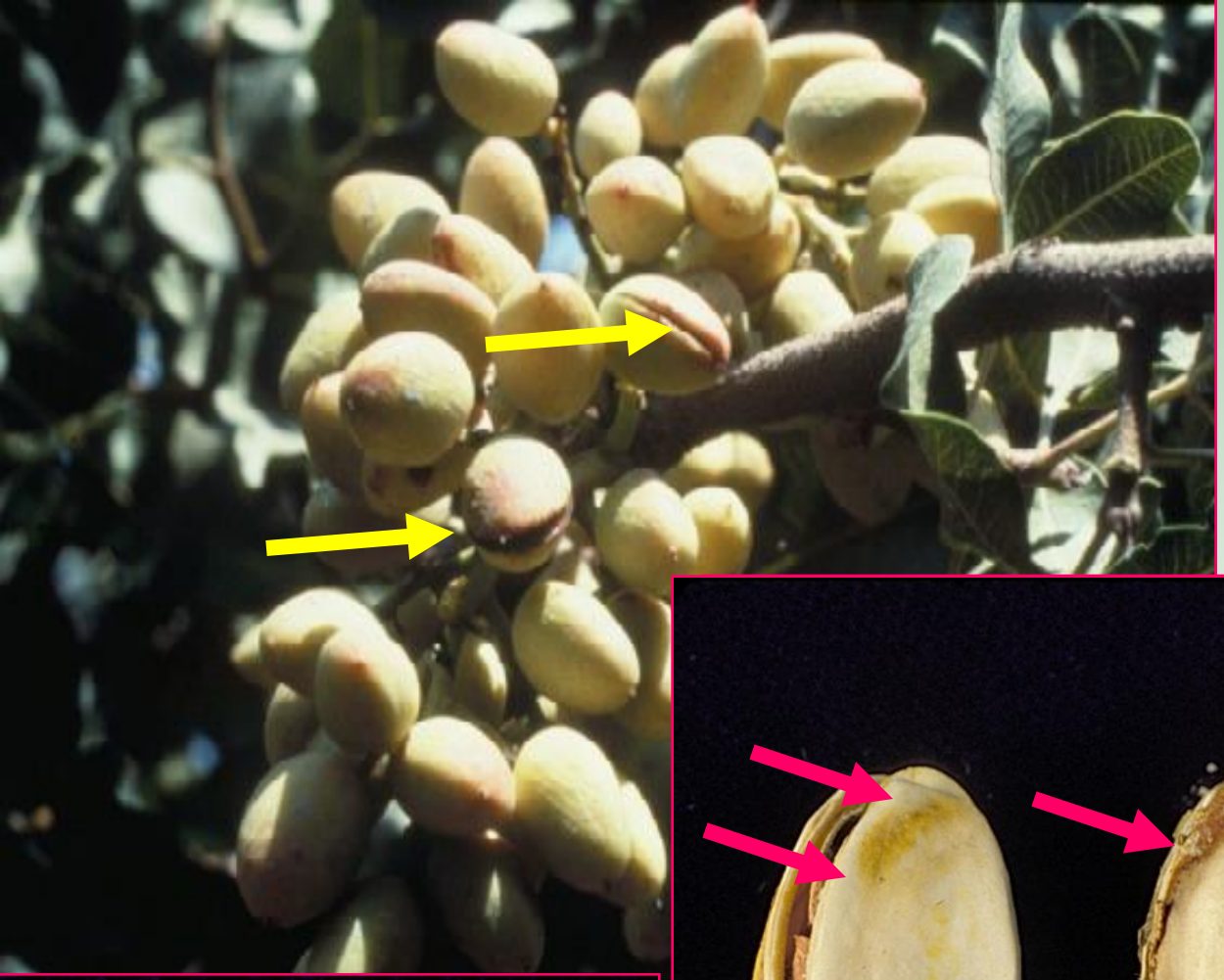


Early splits

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;

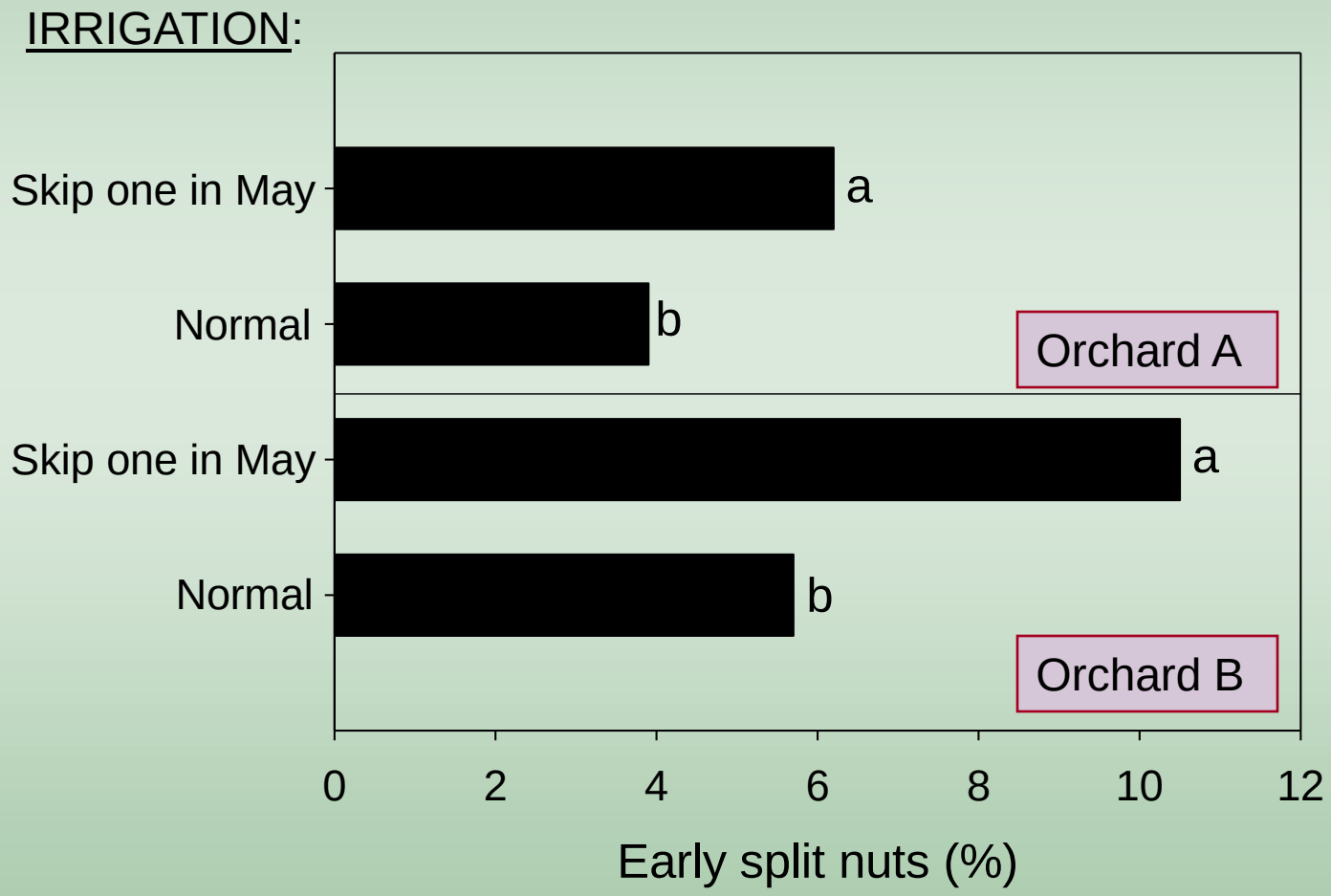
(2-5% of the crop)

NOW pupa

Suture staining

Reduce early split nut formation

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



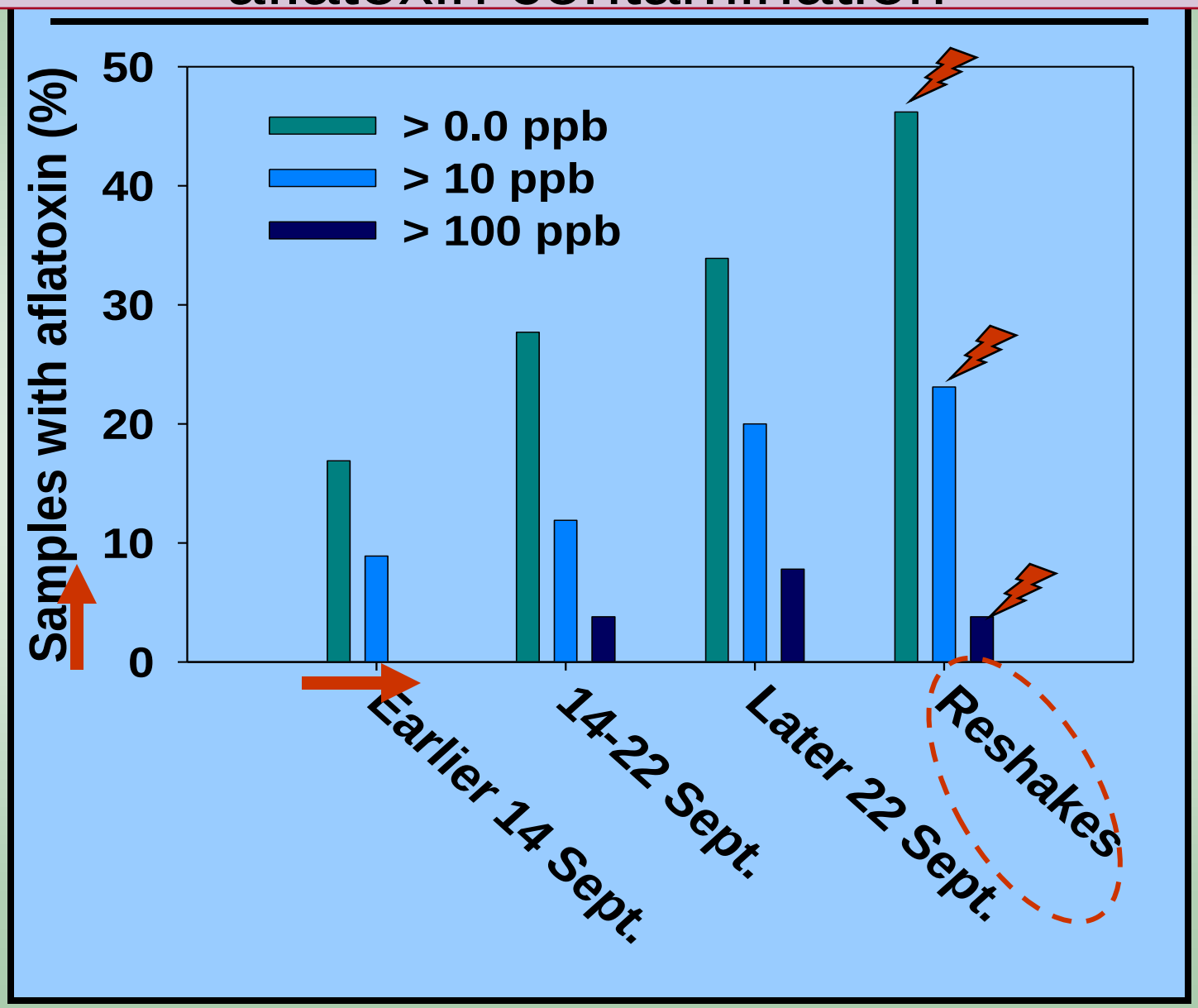
➔ Use a rootstock that minimizes early split nuts.

Percentage of early split nuts in rootstock trials

	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

Avoid late harvests

Harvest dates, incidence, and amounts of aflatoxin contamination



Navel orangeworm (NOW) infestation increases with delaying harvest

✓ **Avoid late harvests.**

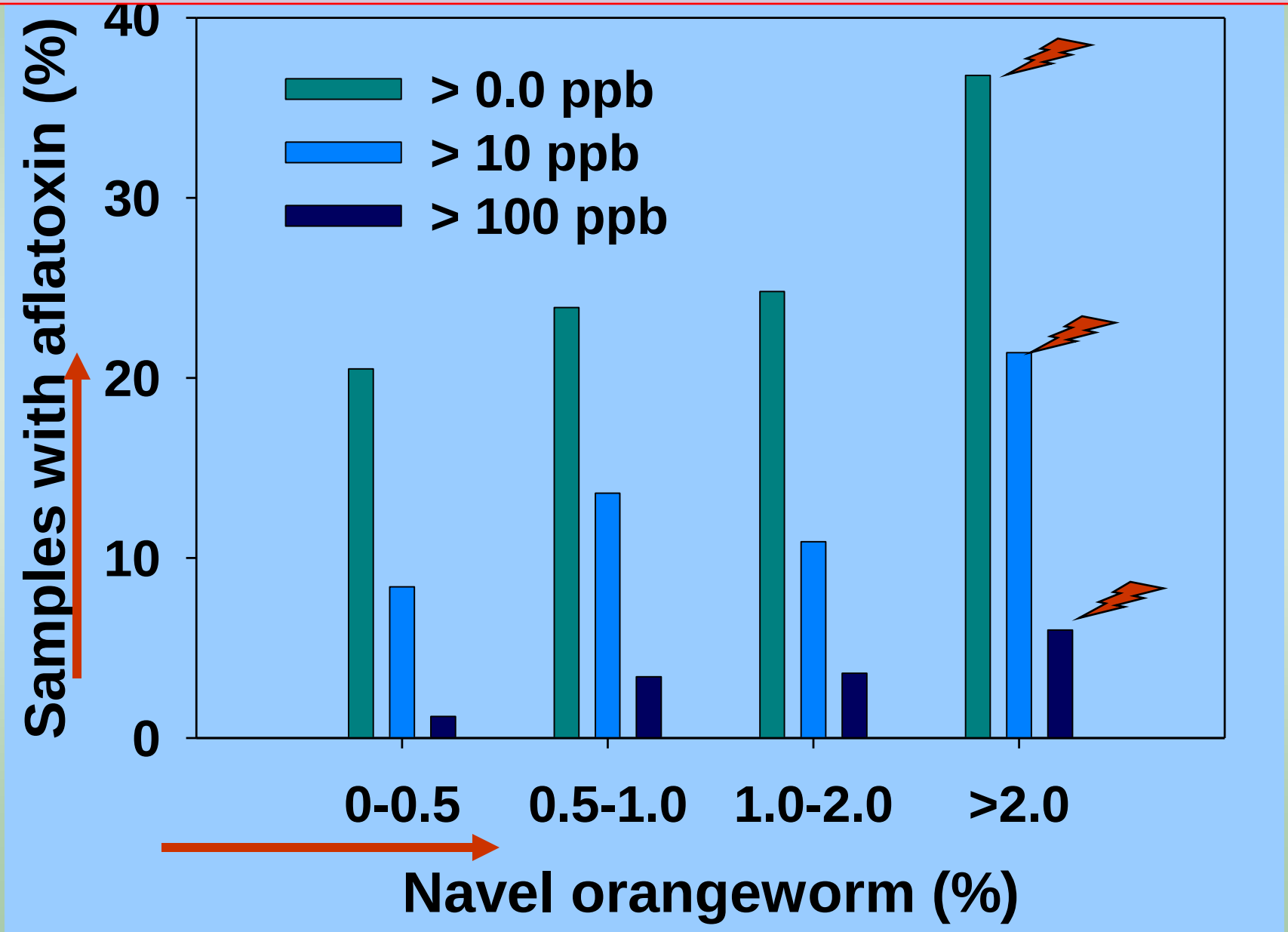
Year 1

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

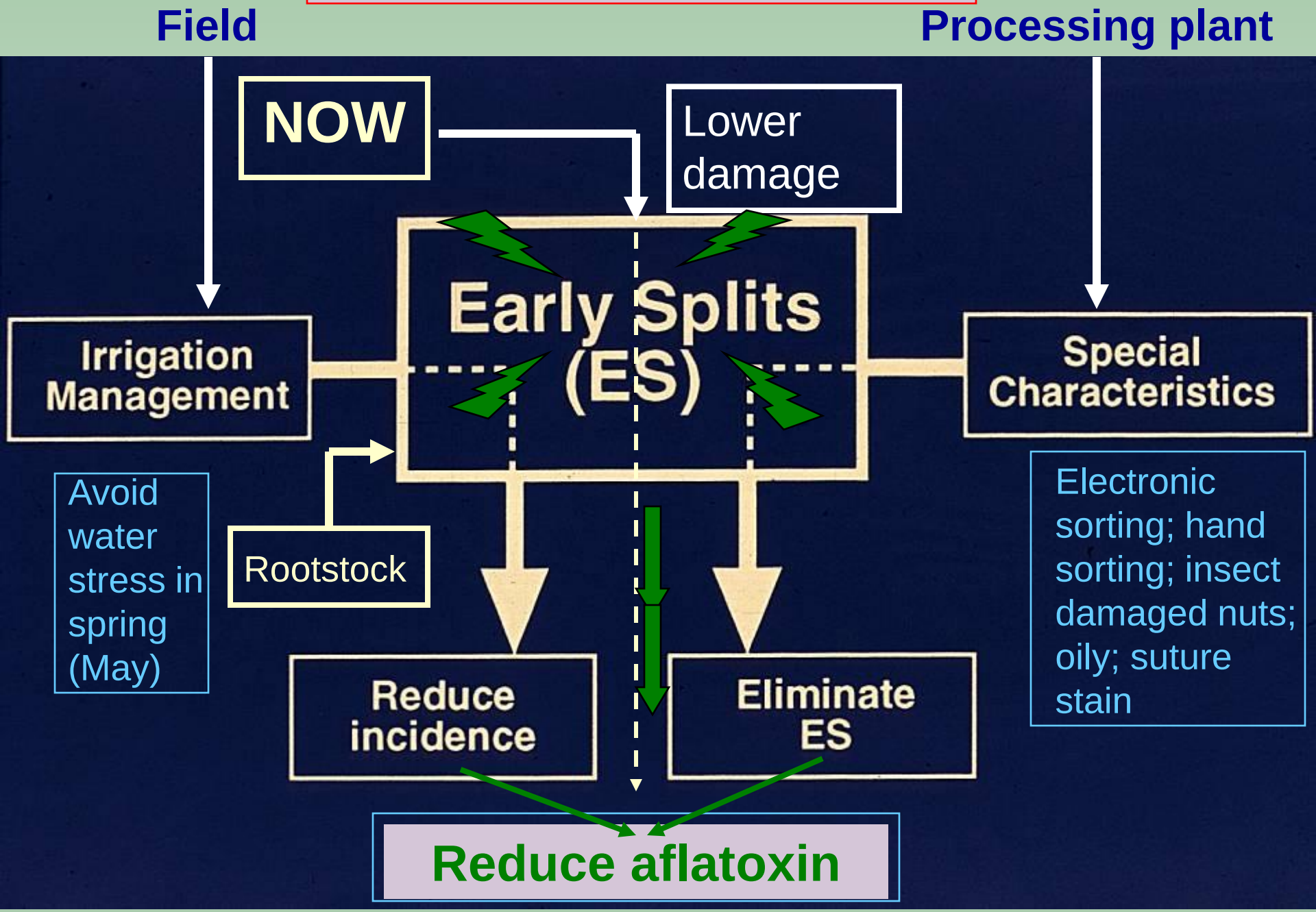
Year 2

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



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

?

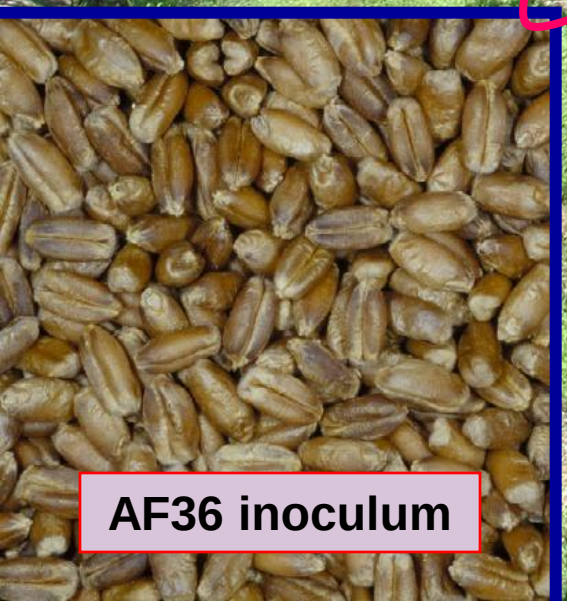
S - strain

most toxigenic

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

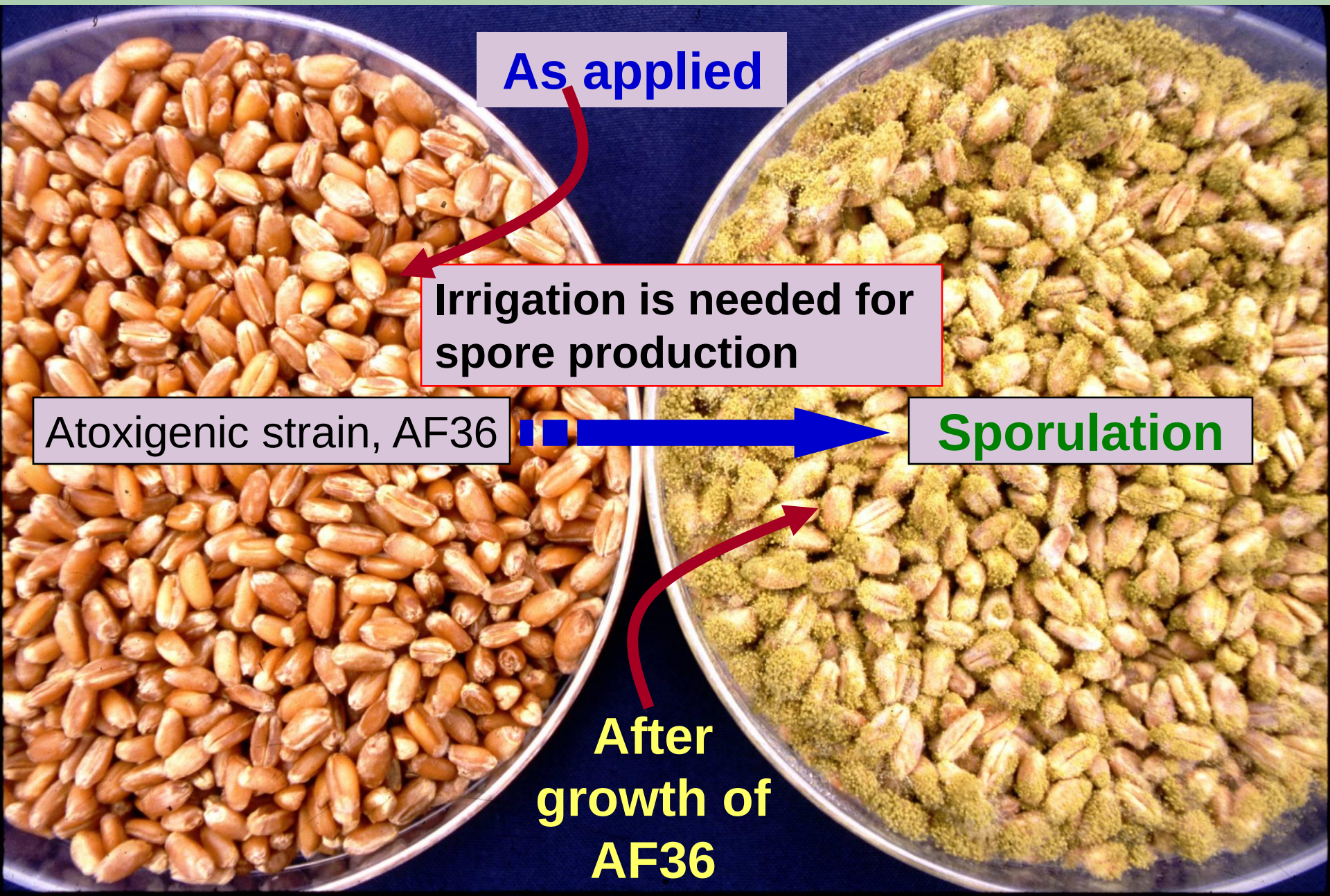
A. flavus atoxigenic **A564, A815, etc.: 1-2%**
A. flavus atoxigenic **AF36** strain ……**4.5 - 7.5%**

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



AF36 inoculum

AF36 wheat inoculum



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

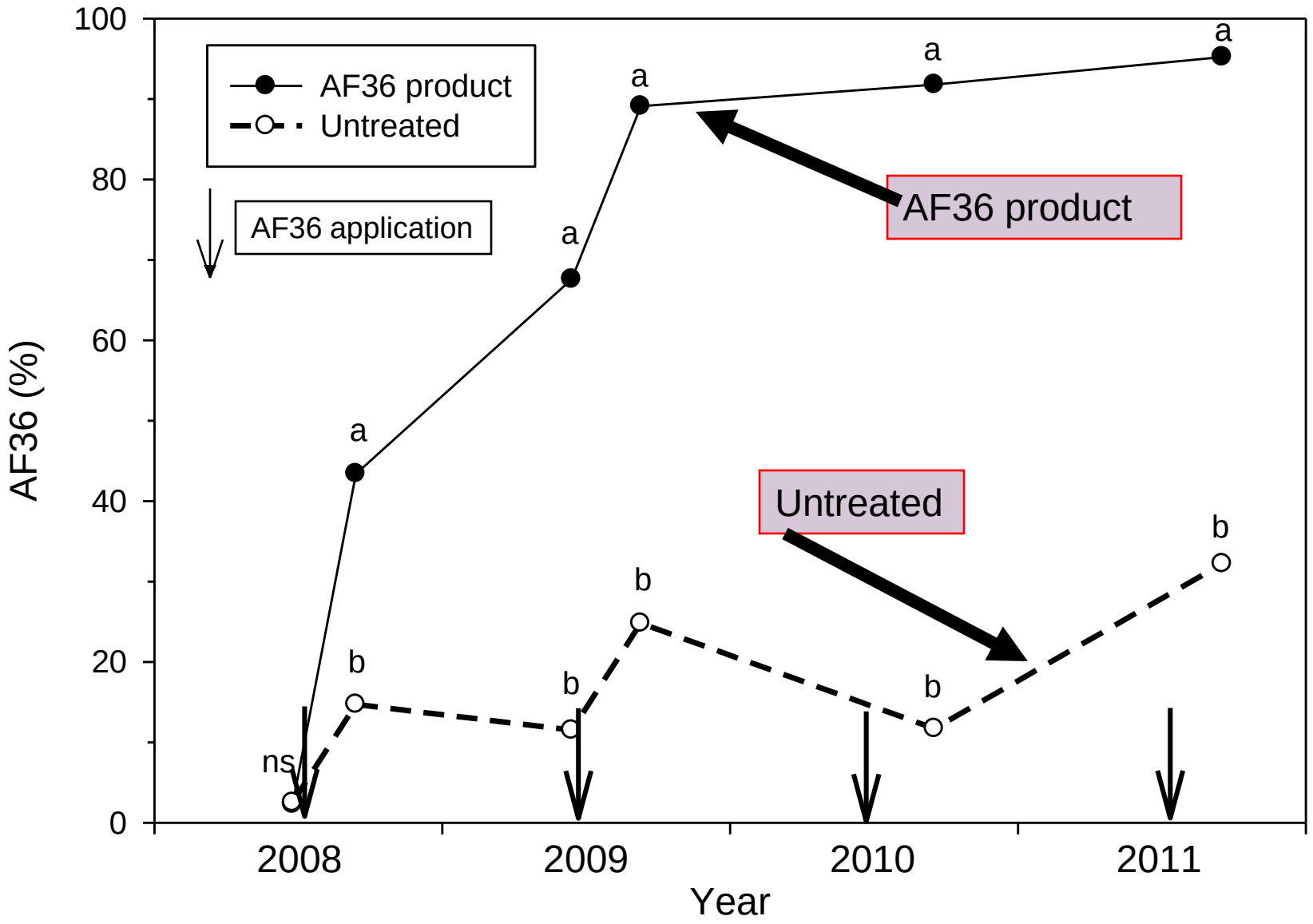
- Early 2007 ready; However, requested by EPA & CDPR to do spore trapping in orchards.
- January 2008: Experimental Use Permit (EUP) approved by the EPA and CDPR.
- In early summer of 2008, a single application of the wheat-AF36 product was applied to 3,000 acres (limit of the EUP) of commercial orchards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

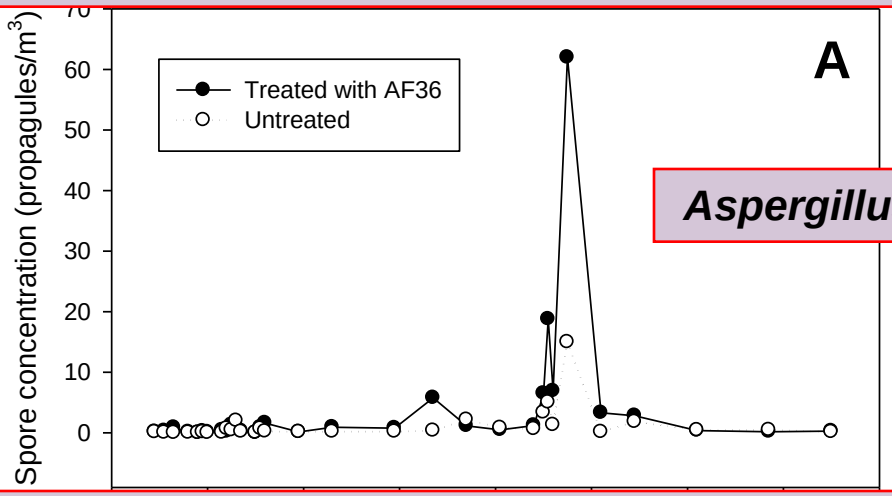

After irrigation, the wet wheat seeds will produce spores of AF36

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



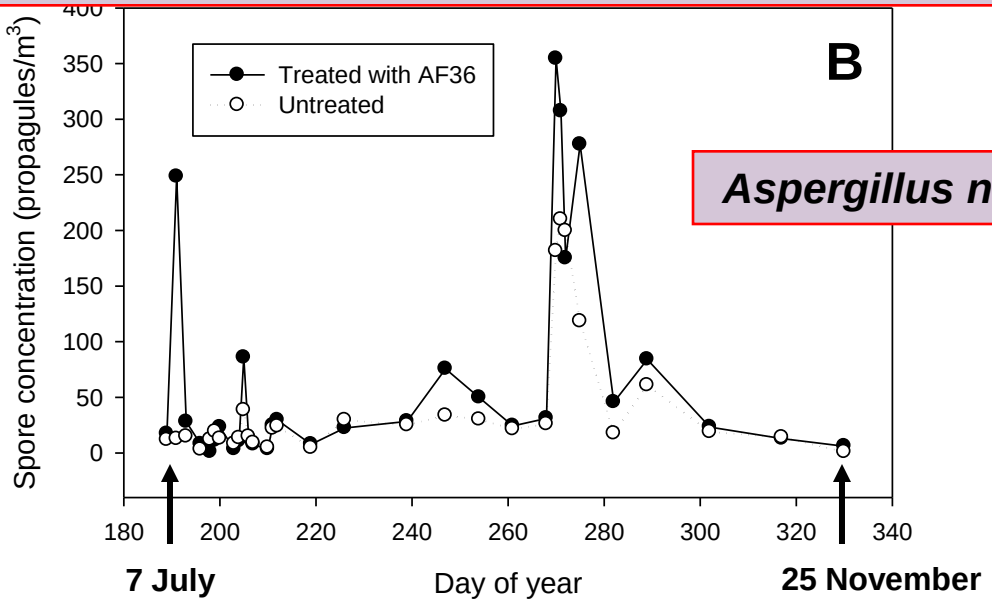
Burkard spore trap in a pistachio orchard

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



Aspergillus flavus/parasiticus

Similar results in 2009



***Aspergillus niger* group**

Early split nuts decayed

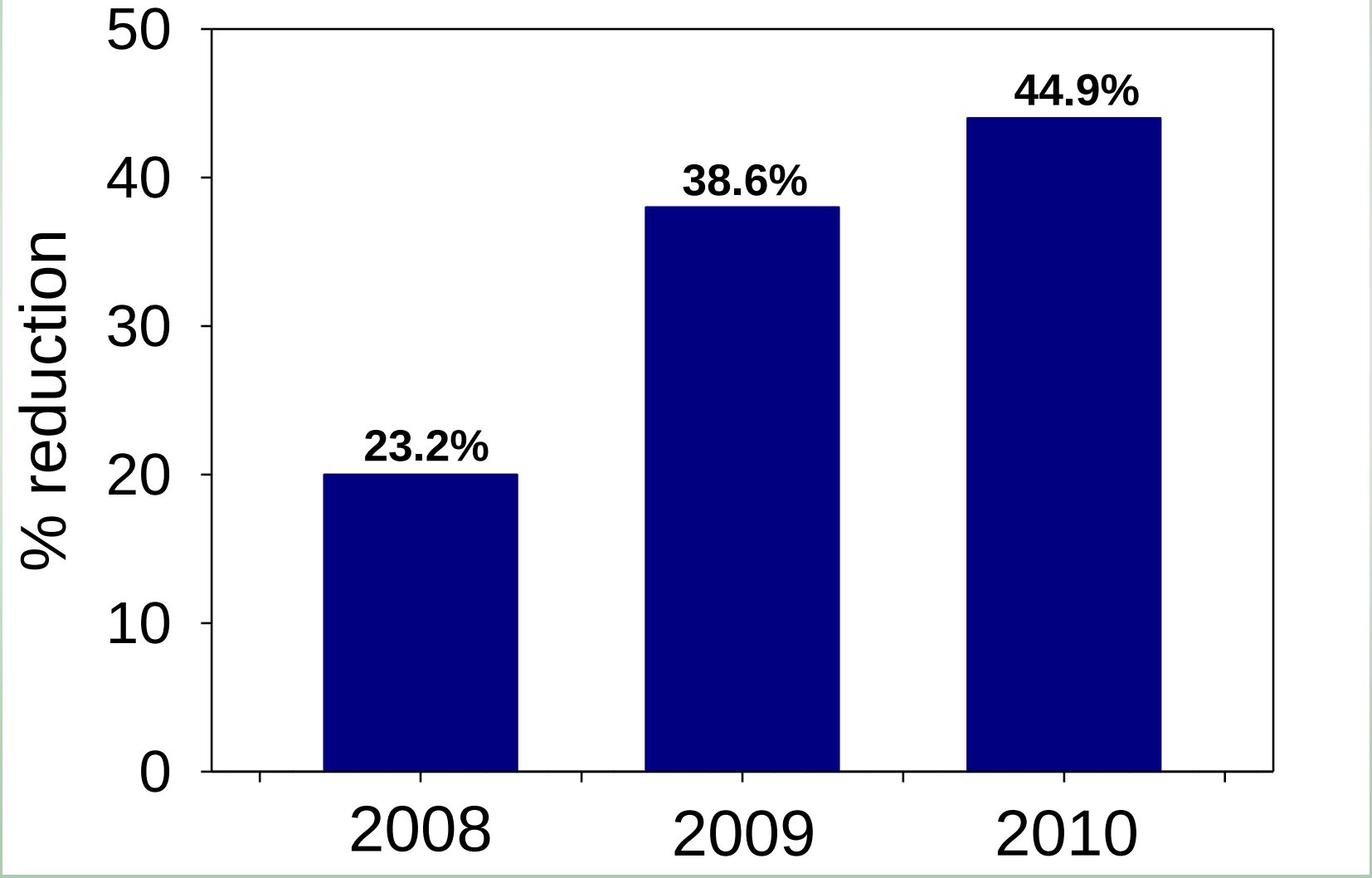
Year	Treatment	% *A. flavus*	% *A. niger*
2008	Wheat-AF36	0.09 ns	6.39 ns
	Untreated control	0.16	9.85
2009	Wheat-AF36	0.10 ns	5.80 ns
	Untreated control	0.03	5.95
2010	Wheat-AF36	0.06 ns	3.01 ns
	Untreated control	0.01	3.17
2011	Wheat-AF36	0.09 ns	2.00 ns
	Untreated control	0.05	2.21

No increase in kernel decay.

Library samples for aflatoxin analysis

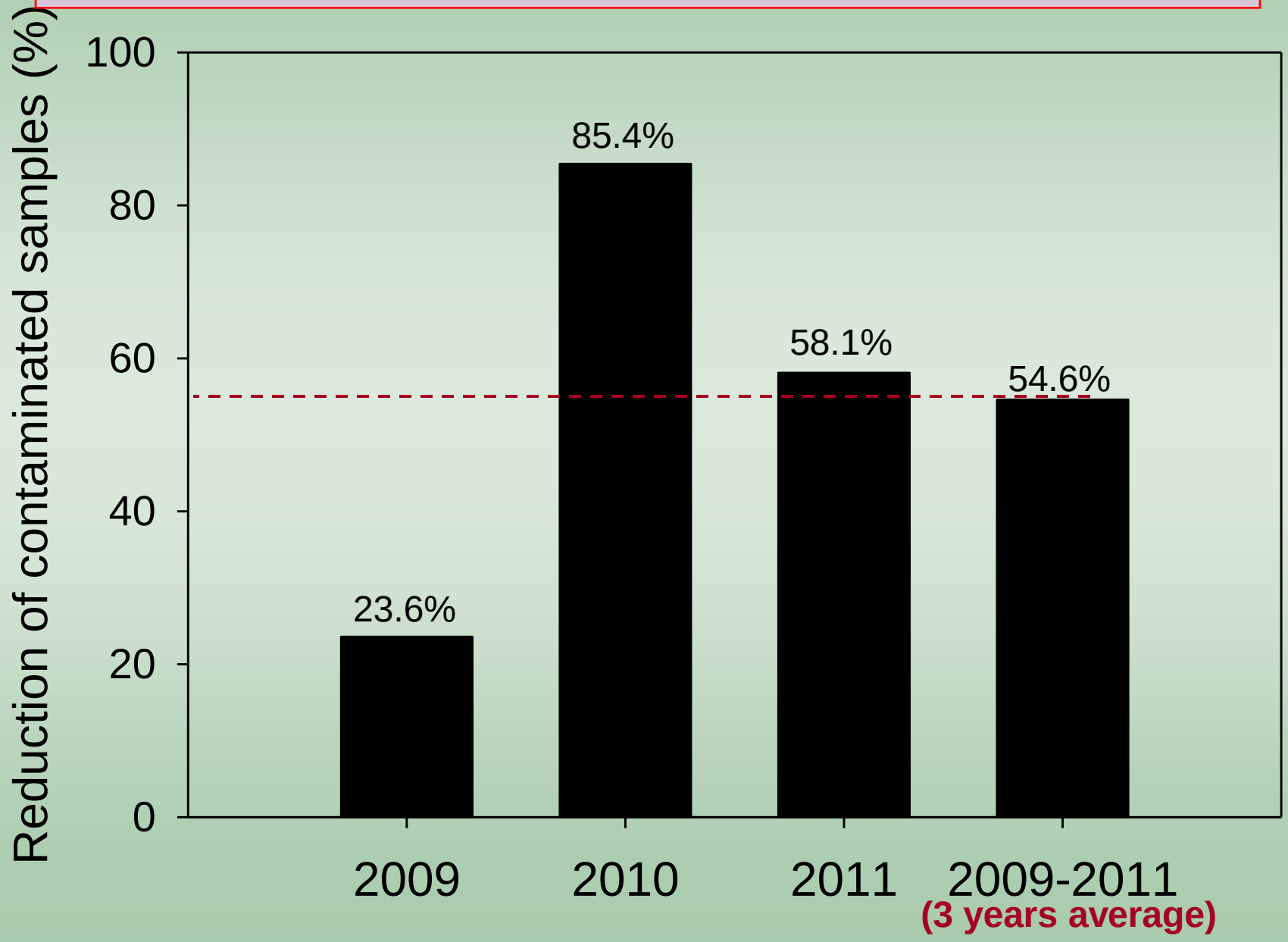
Samples taken at processing plant as nuts are being unloaded.

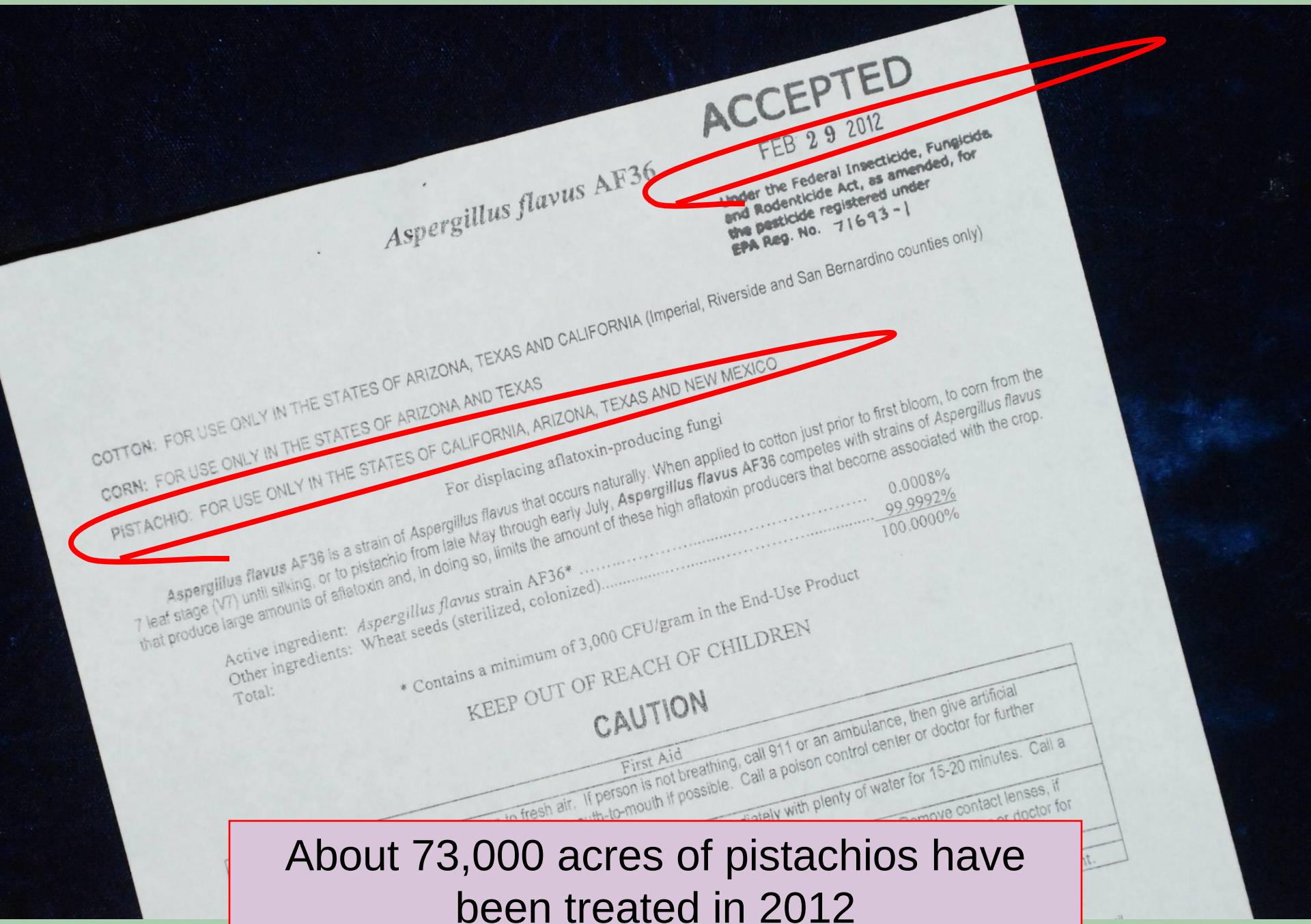
<u>Reduction in aflatoxin-contaminated</u> pistachio samples after treating orchards with AF36 **(all harvests: 1st & 2nd harvests)**



Reduction in aflatoxin-contaminated pistachio samples (all harvests)

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





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

Acknowledgments:

- Peter Cotty
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- Ryan Puckett
- Heraclio Reyes

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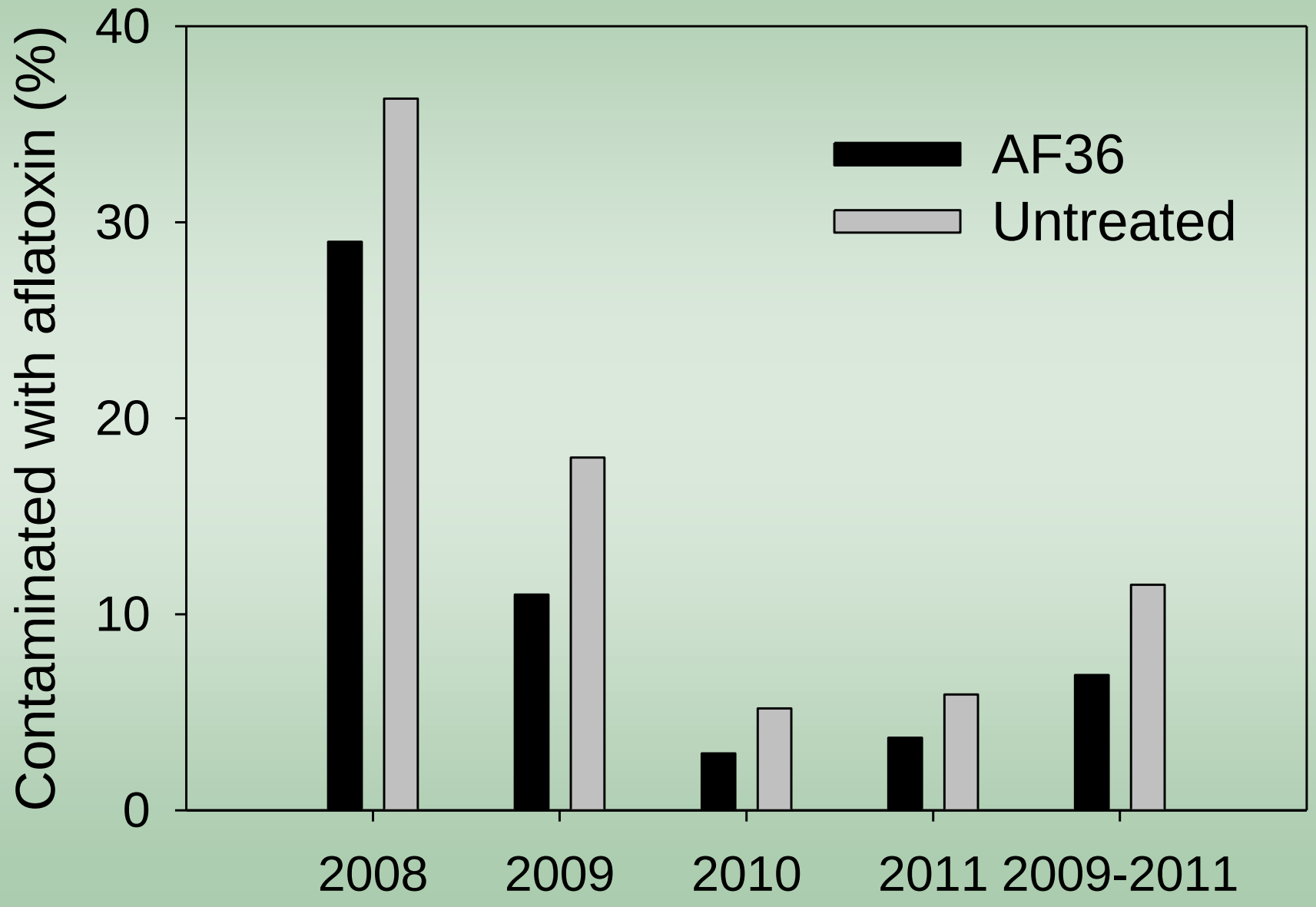
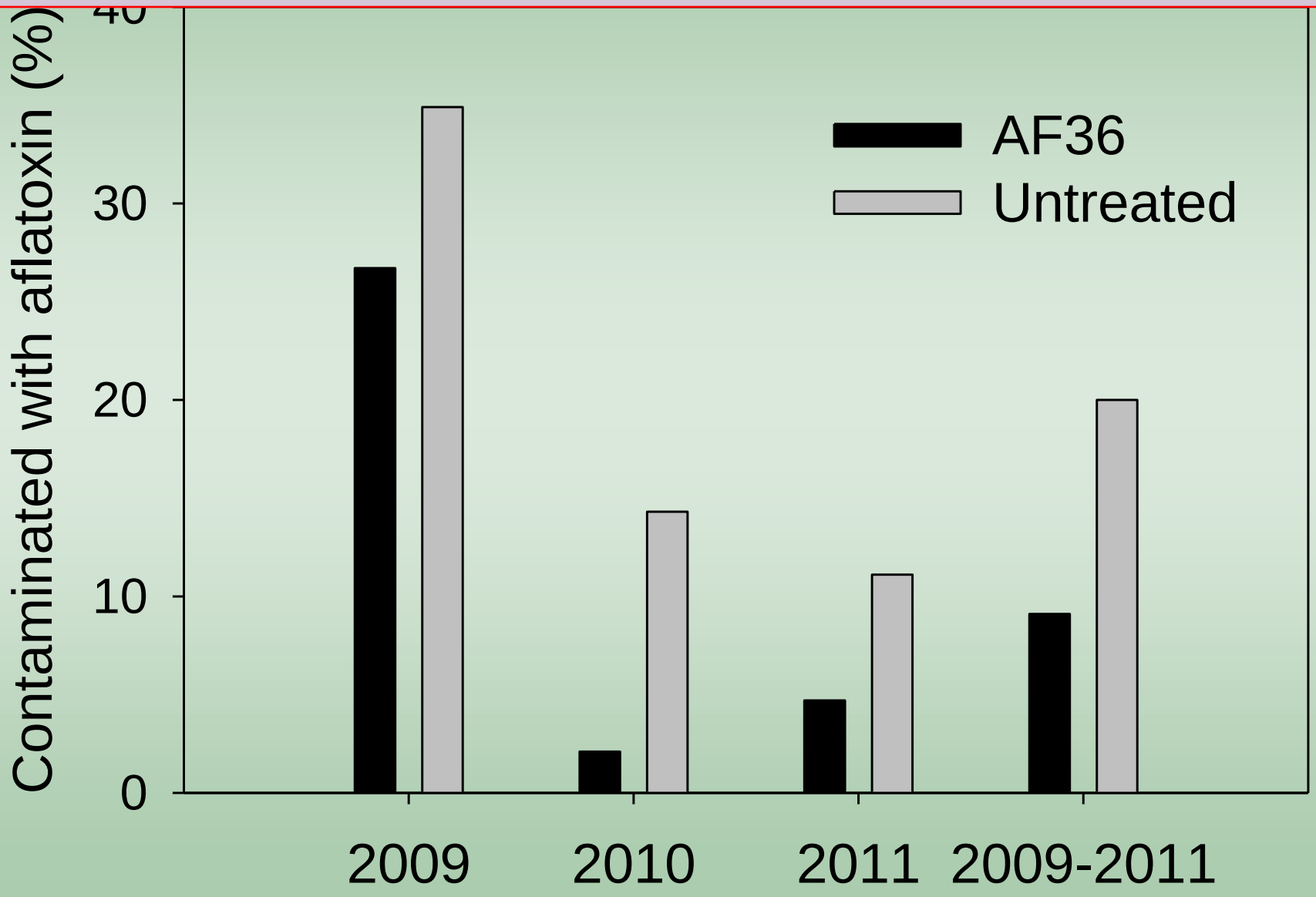
- Mike Smith & Jeff Gibbons, Setton Farms
- April Huber, Nichols Farms Steven Smit, Keenan Farms

& Thank you

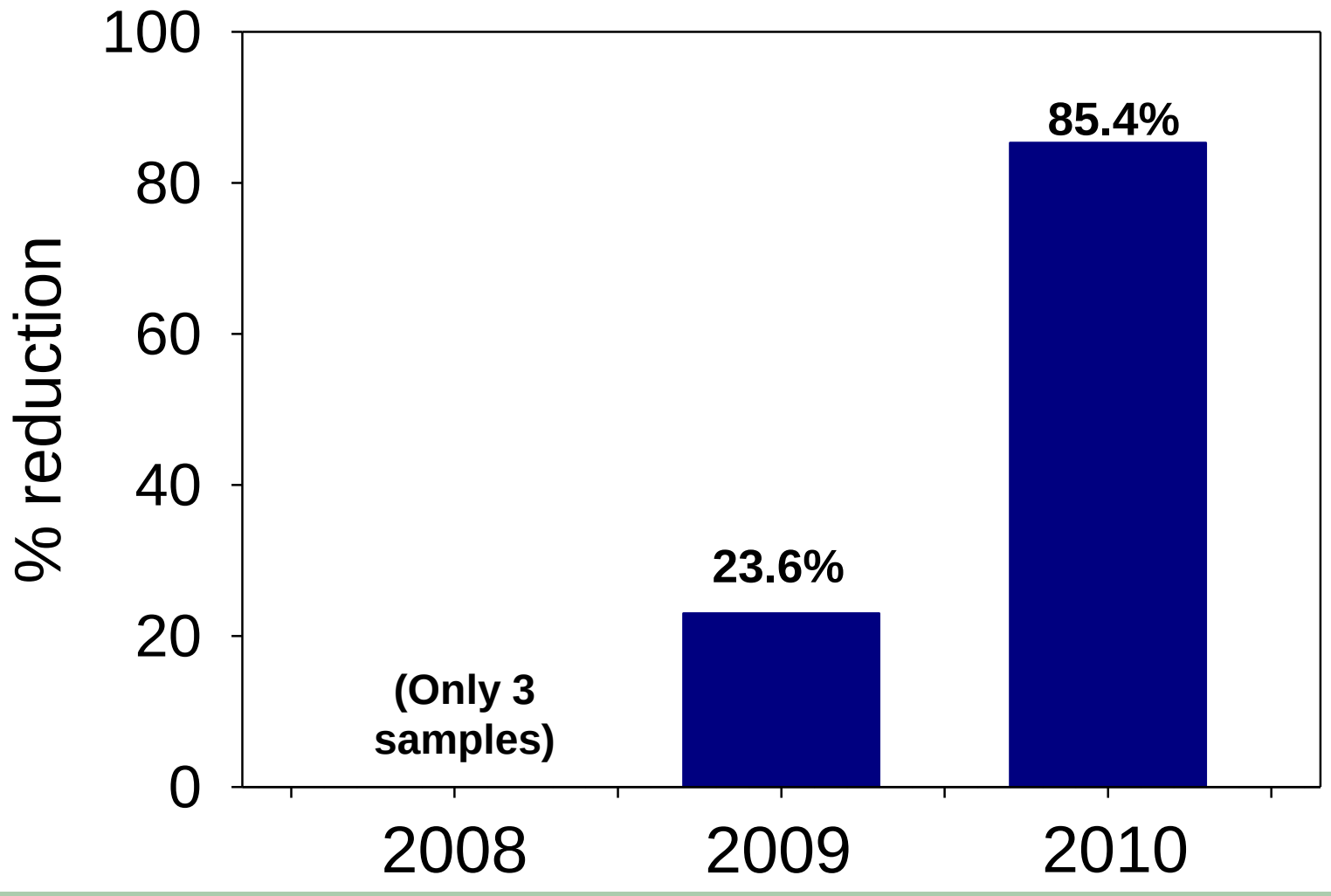
Samples contaminated with aflatoxin – all harvests



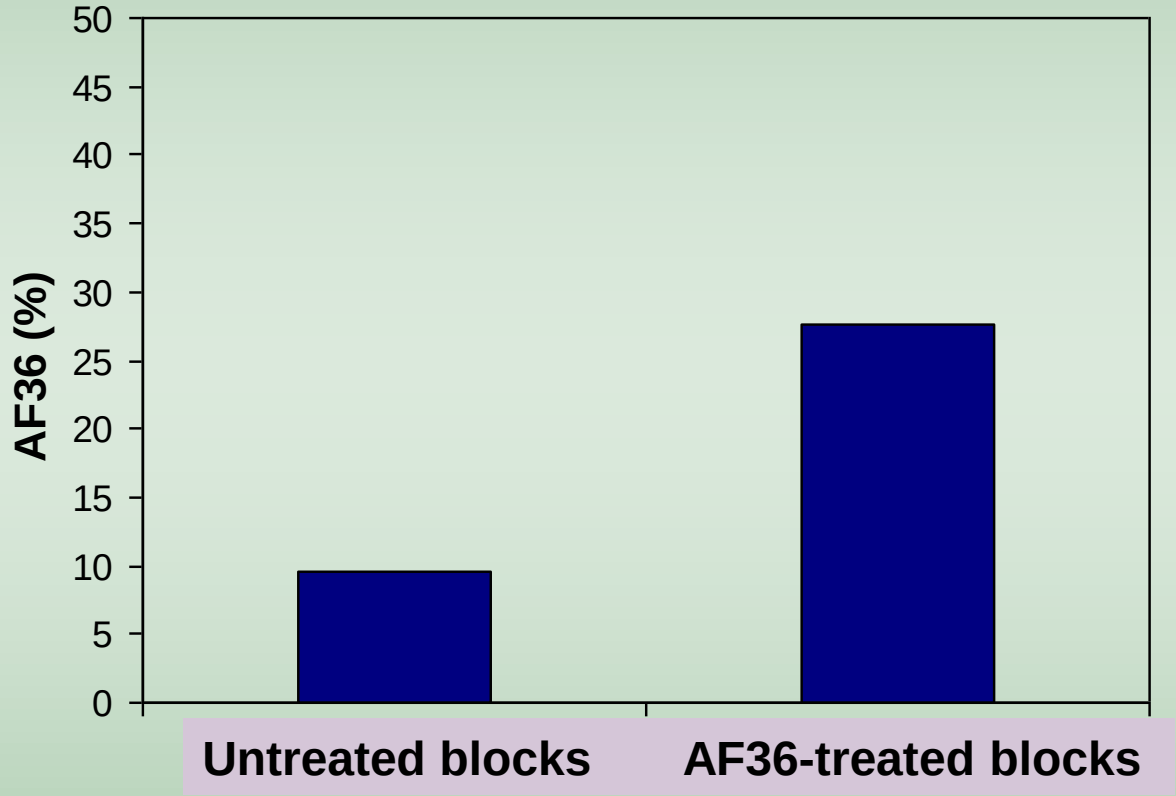
Samples contaminated with aflatoxin – second harvest



Percent **reduction of aflatoxin contaminated pistachio samples** after treating orchards with AF36 (**only 2nd harvest = "Reshakes"**)



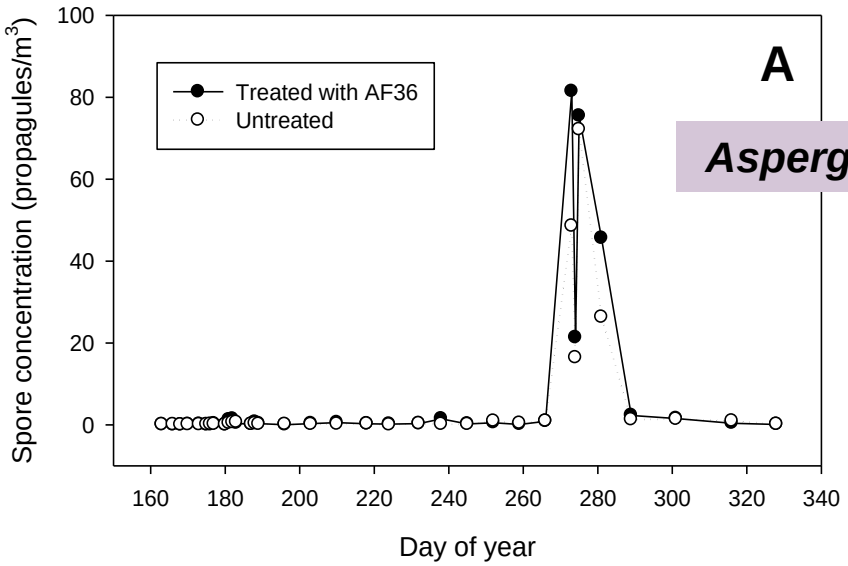
Incidence of AF36 on NOW moths collected from sticky traps in 2010 (Kern Co).



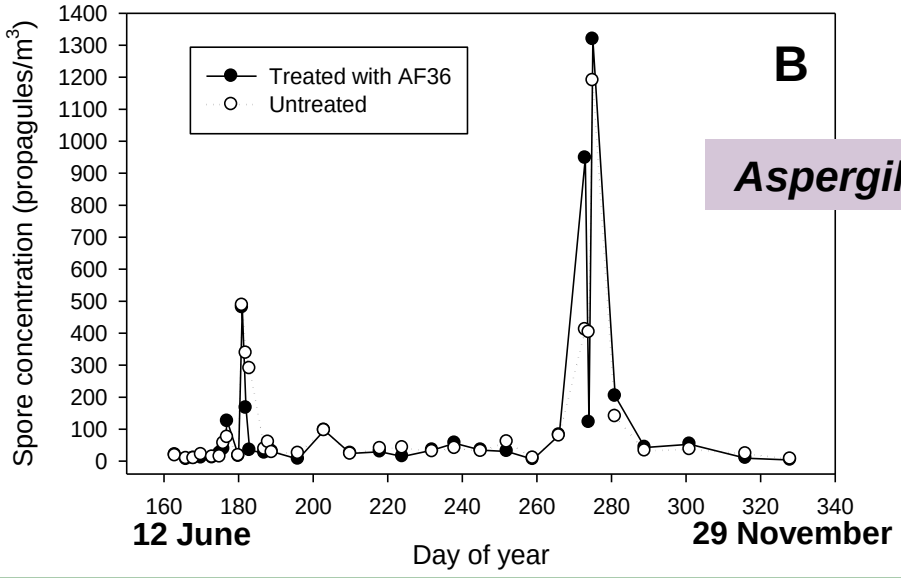
Isolates obtained from NOW moths (2010)

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

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



Aspergillus flavus/parasiticus



***Aspergillus niger* group**

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