Aflatoxin Management in Pistachio

(In need of an Area-wide, Long-term Program to Succeed)

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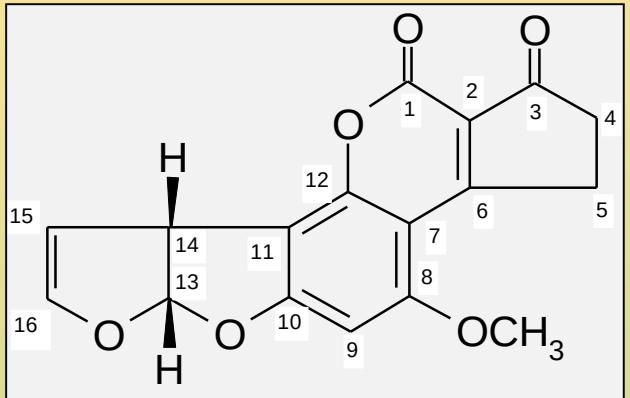
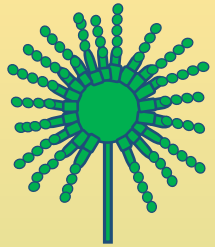
Pistachio Day – Visalia, 18 January 2023

Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*



Aflatoxin B_1

B1: The most potent; it can cause liver cancer



Aspergillus flavus ***Aspergillus parasiticus***

Aflatoxins: **B_1**, B_2, Aflatoxins: **B_1**, B_2, G_1, G_2,

Regulatory limits for aflatoxins

- **USA**

  Total aflatoxins➔ 20 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

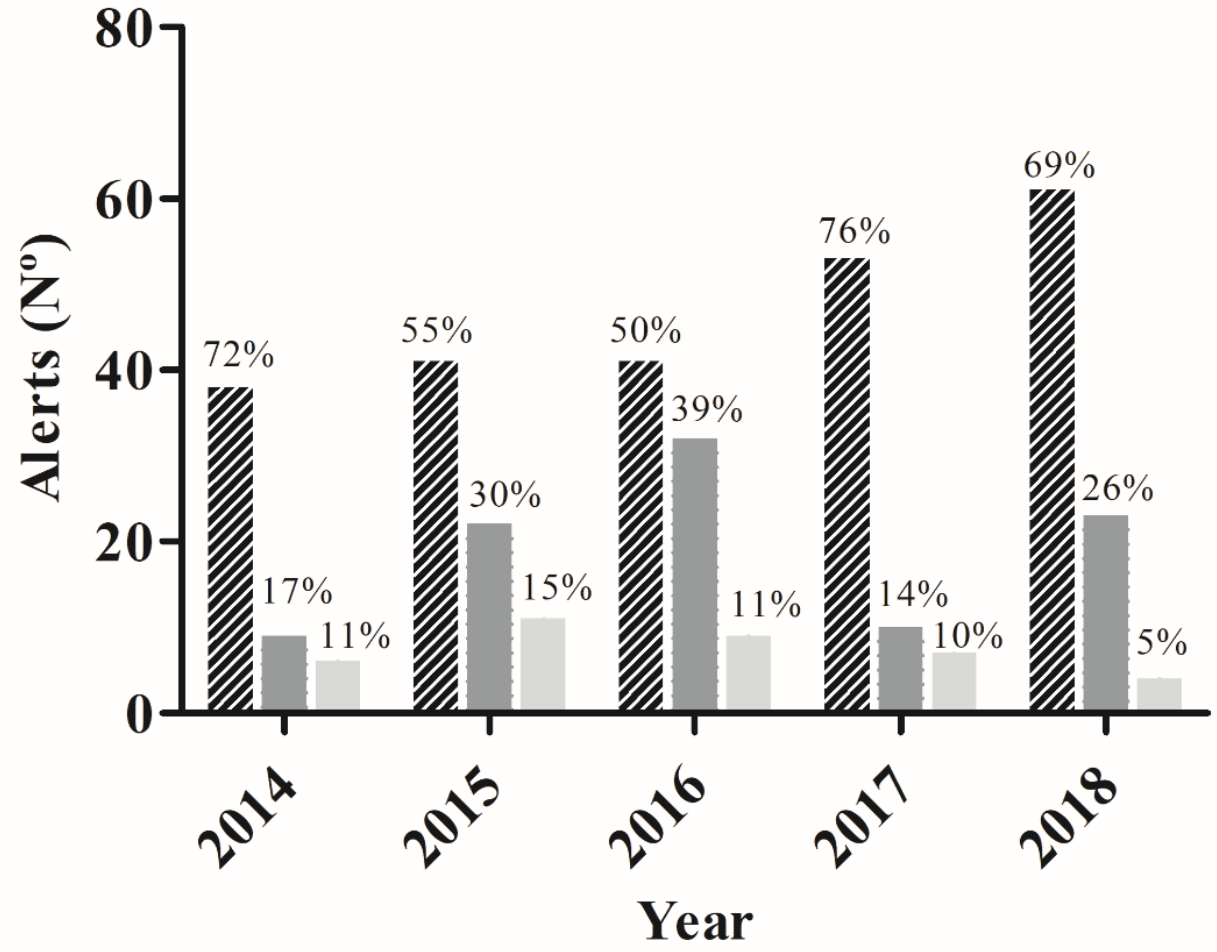
dried figs

Regulatory limits for Ochratoxins (OTA)

- **OTA limit for finished pistachio for consumer use: 5 ppb**
- **OTA limit for pistachios bound for further processing/ physical treatment: 10 ppb**

- OTA limit for dried vine fruit (currants, raisins, sultanas): 8 ppb
- OTA limit for other dried fruit: 2 ppb

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



Aflatoxin (50-76%)

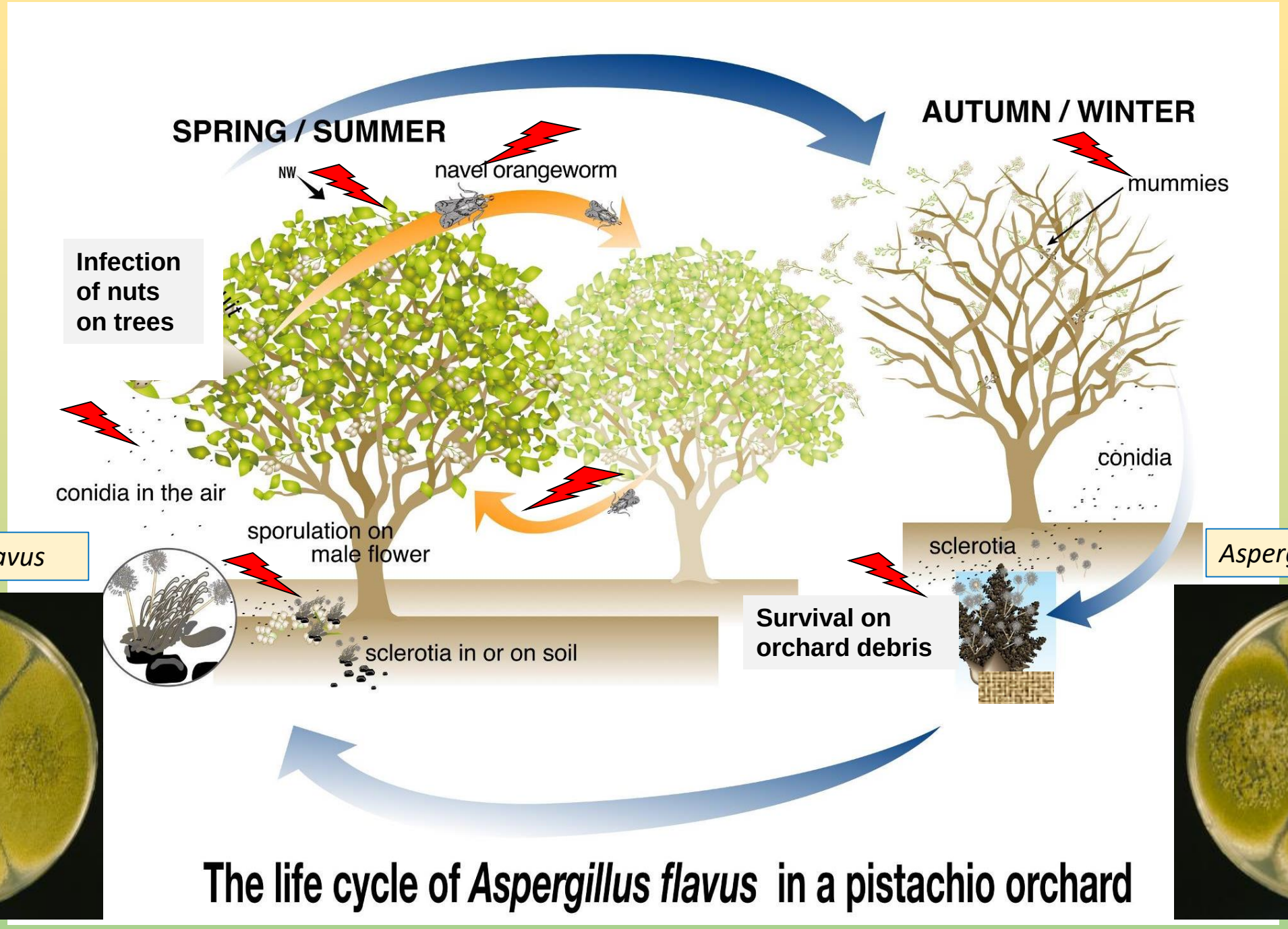
Ochratoxin (14-39%)

Other mycotoxins

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

RASFF

Rapid Alert System for Food and Feed


SPRING / SUMMER
AUTUMN / WINTER
NW
navel orangeworm
mummies
Infection of nuts on trees
conidia in the air
conidia
sporulation on male flower
sclerotia
sclerotia in or on soil
Survival on orchard debris
Aspergillus flavus
Aspergillus parasiticus
The life cycle of Aspergillus flavus in a pistachio orchard

Accomplishments of CPRB funded research over the years:

- Determined that Early Splits (ES) contribute a lot to aflatoxin contamination.
- Determined the special features of ES to help the processors sorting the ES from the marketable product.
- Determined that early irrigation (during May) and rootstock affects ES incidence in Kerman.
- Determined that as the NOW damage increases so does the aflatoxin incidence and the amounts of the toxin.
- Determined that NOW moths in addition to pistachio damage can also carry aflatoxigenic fungi.
- Determined that delaying harvest increases aflatoxin incidence and amounts of toxin.
- Identified “hot spots” for aflatoxin, i.e. Fresno, Madera, and Merced.
- Determined that “Off Years” are expected to have more aflatoxin than “On Years.”
- Determined that defective and stained nuts contribute a lot to aflatoxin contamination
- Determined that the population of *Aspergillus flavus* in pistachio orchards consists roughly from 1:1 ratio of toxigenic vs. atoxigenic

- A major accomplishment: The biological control of aflatoxins

by

using atoxigenic strains of *Aspergillus flavus*

- During the EUP years we showed an average of 40% reduction in aflatoxin contaminated samples and in subsequent years a variable reduction.

Definition: Atoxigenic = a strain that does not produce aflatoxin

Sometimes casually people refer to these as “atoxigenics”

Strains of *Aspergillus flavus*

L – strain: Large sclerotia

about 50:50
toxigenic: atoxigenic

S – strain: Small sclerotia

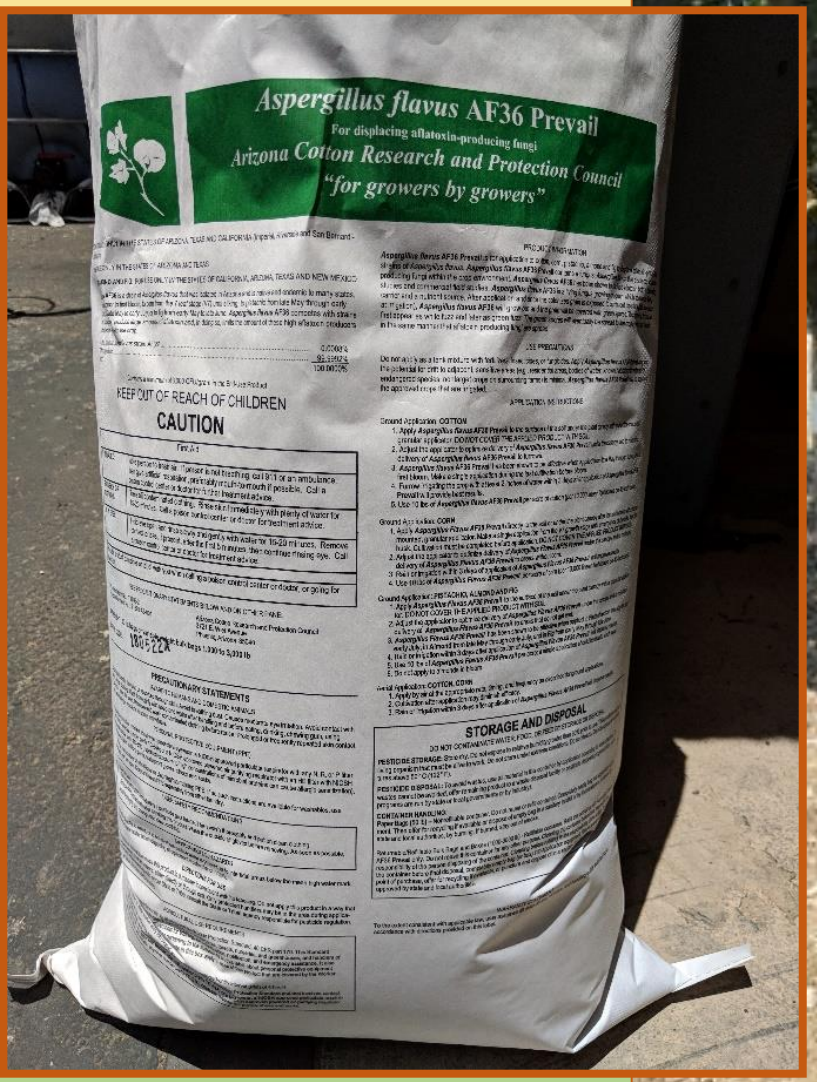
almost all toxigenic

Aspergillus flavus: L-strains

Atoxigenic AF36

0 ppb aflatoxin

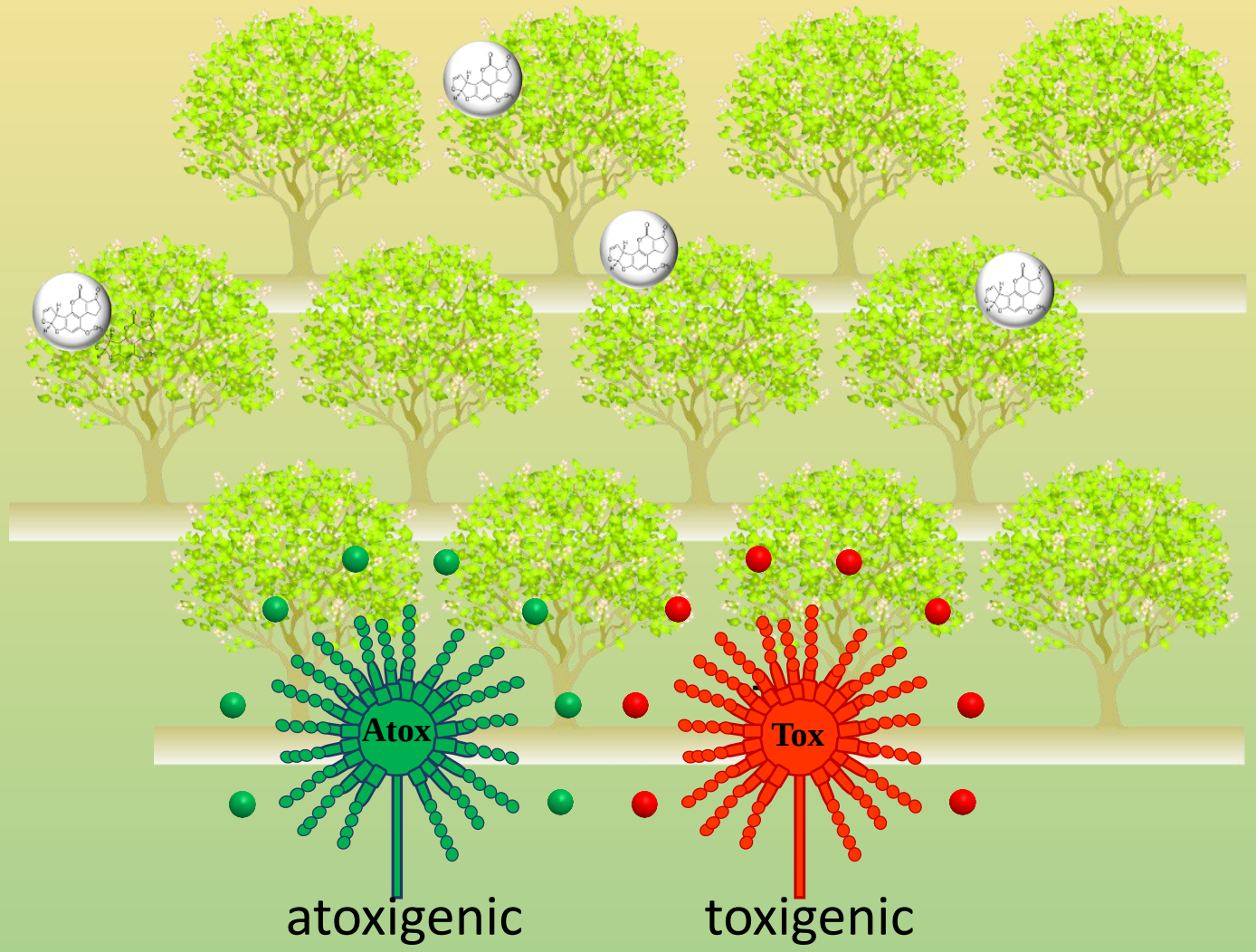
Rationale: Increase the atoxigenic strain population in the orchard to displace the toxigenic strain population.



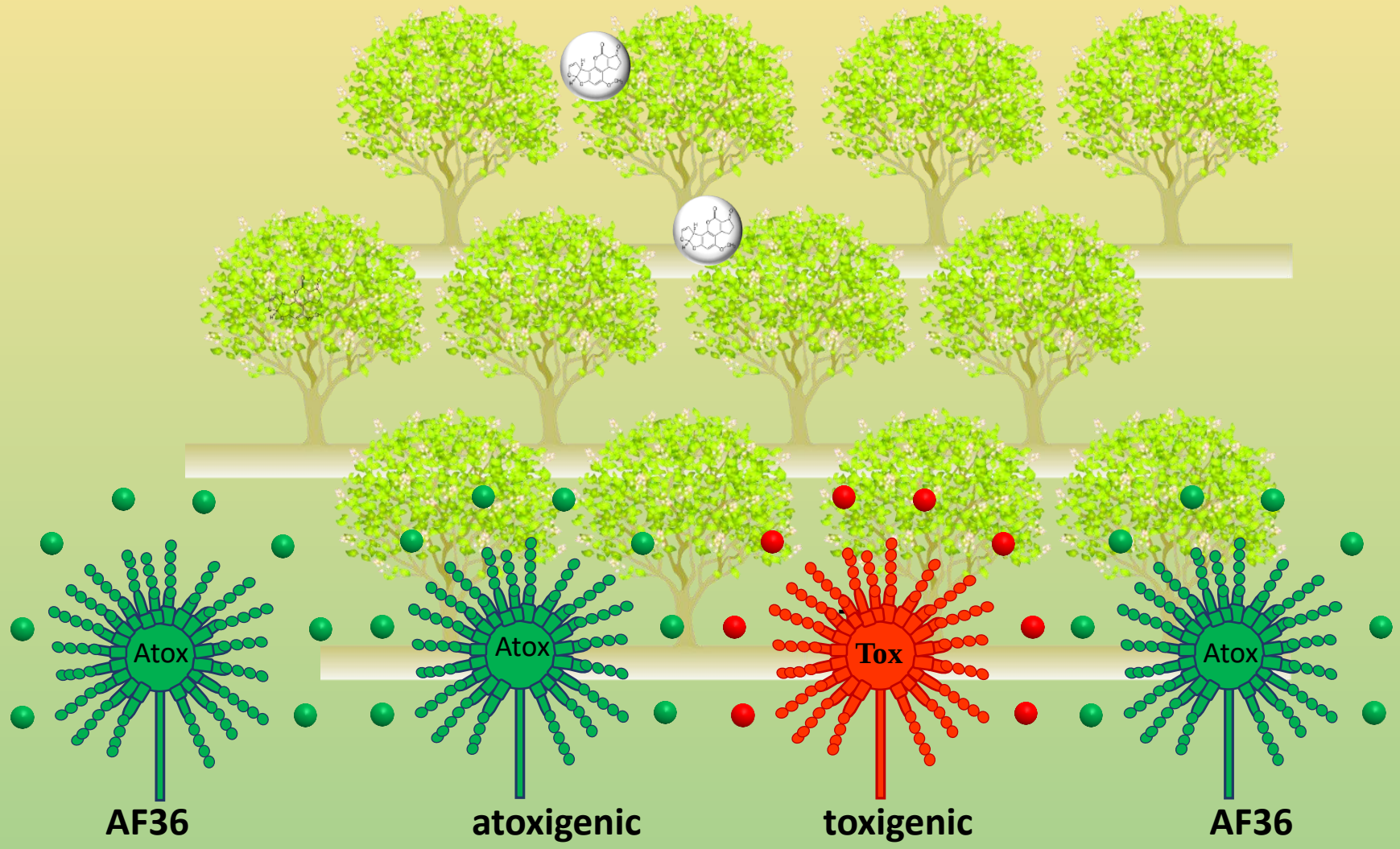
AF36 Inoculum

Rate of application: **10 lbs /Acre** … (~ 11.2 kg /Hectare)

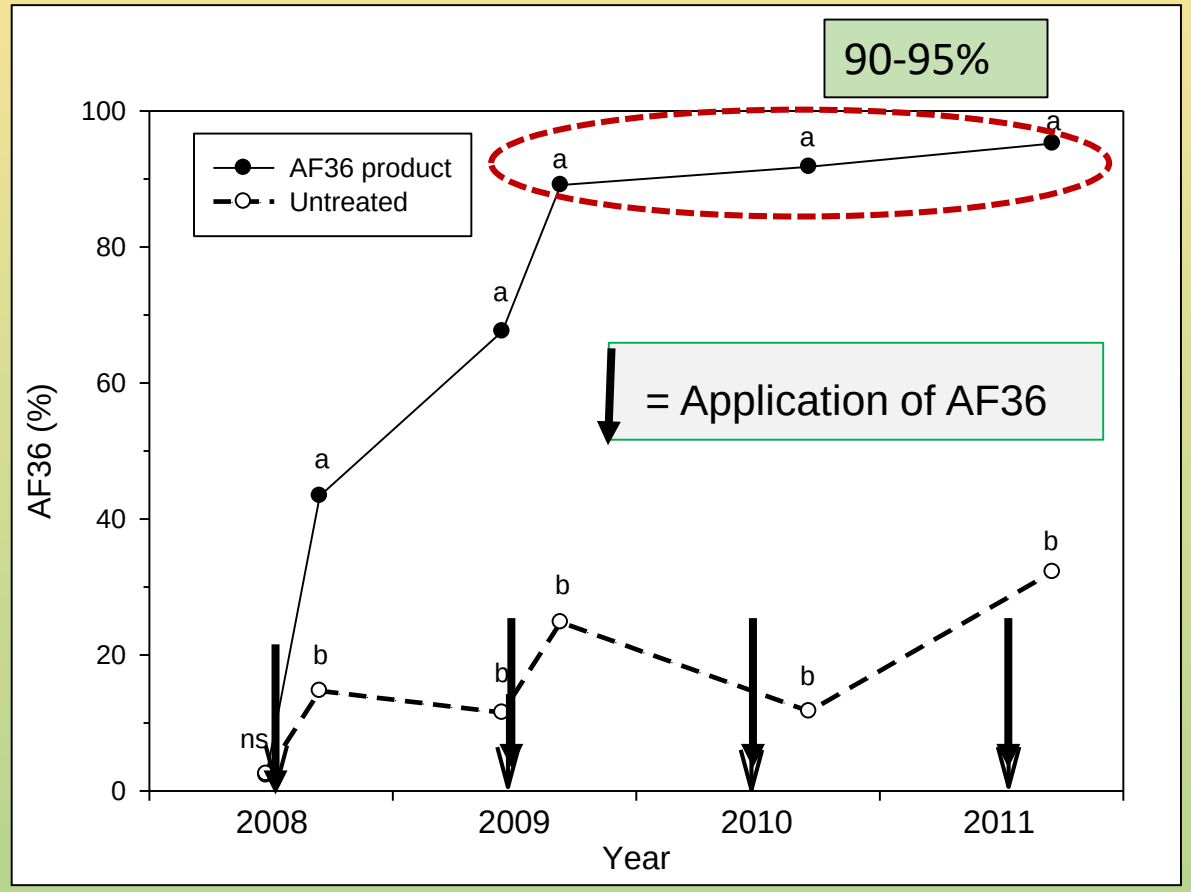
Non-treated orchard



Treated orchard with the AF36 Prevail®

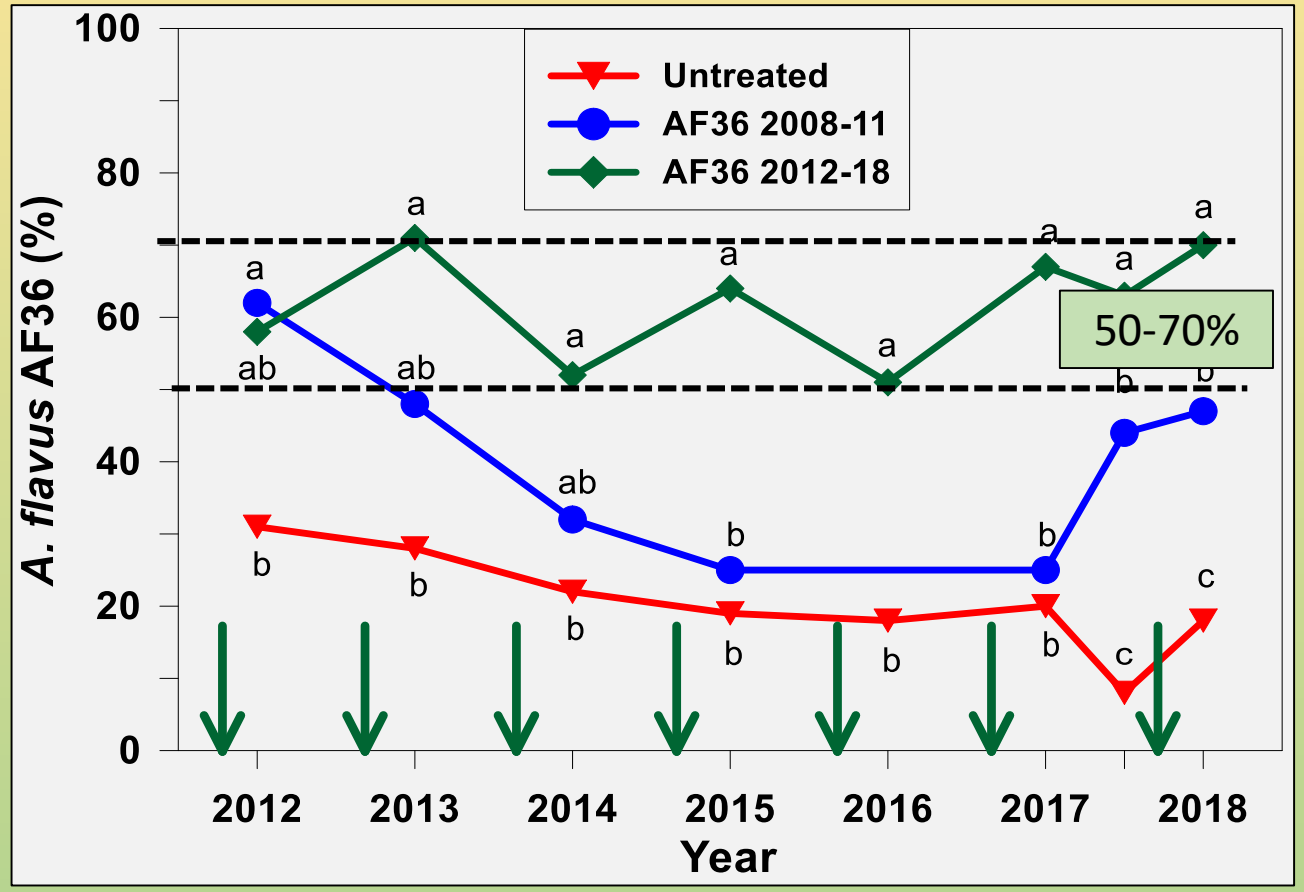


The AF36 displaced the toxigenic strains in soil by 90 - 95%



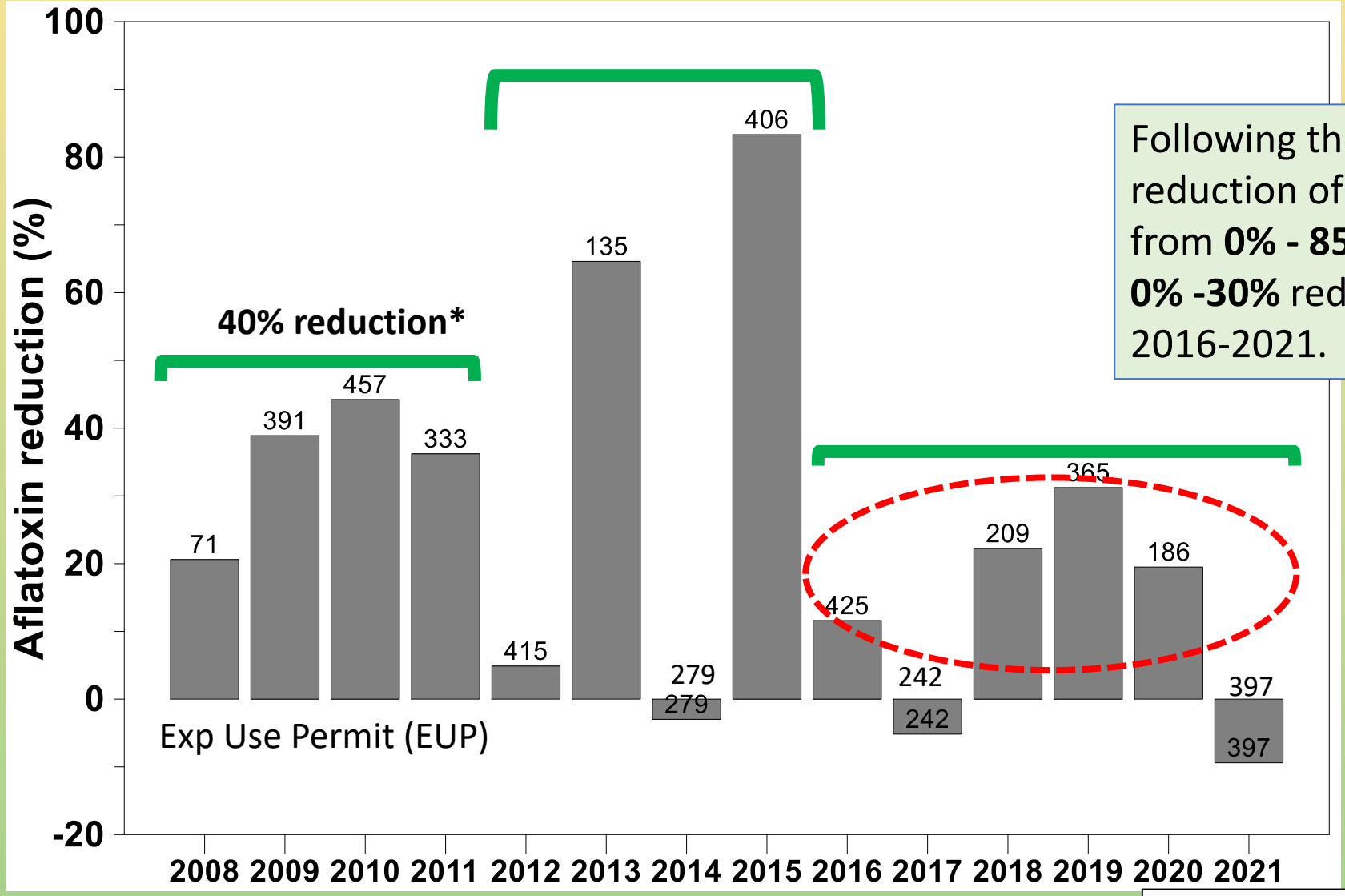
2008 - 2011

The AF36 displaced the toxigenic strains in soil only by 50 - 70%



2012 - 2018

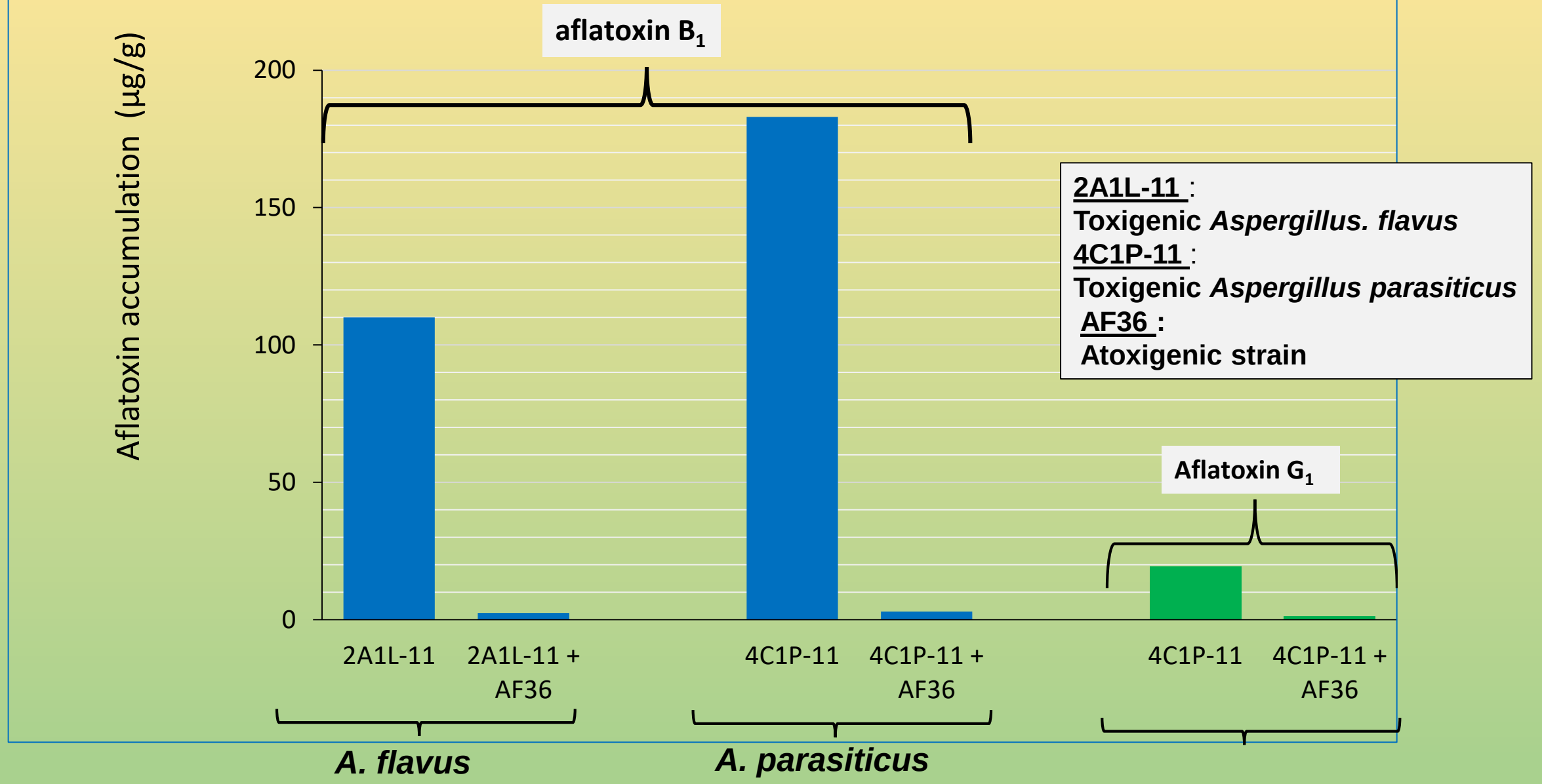
Reduction of aflatoxin contaminated library samples after treatment with AF36 or AF36 Prevail



Following the EUP years, reduction of positives ranged from **0% - 85%** (2012-2015); **0% -30%** reduction during 2016-2021.

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

The AF36 reduced aflatoxin production by the toxigenic strains

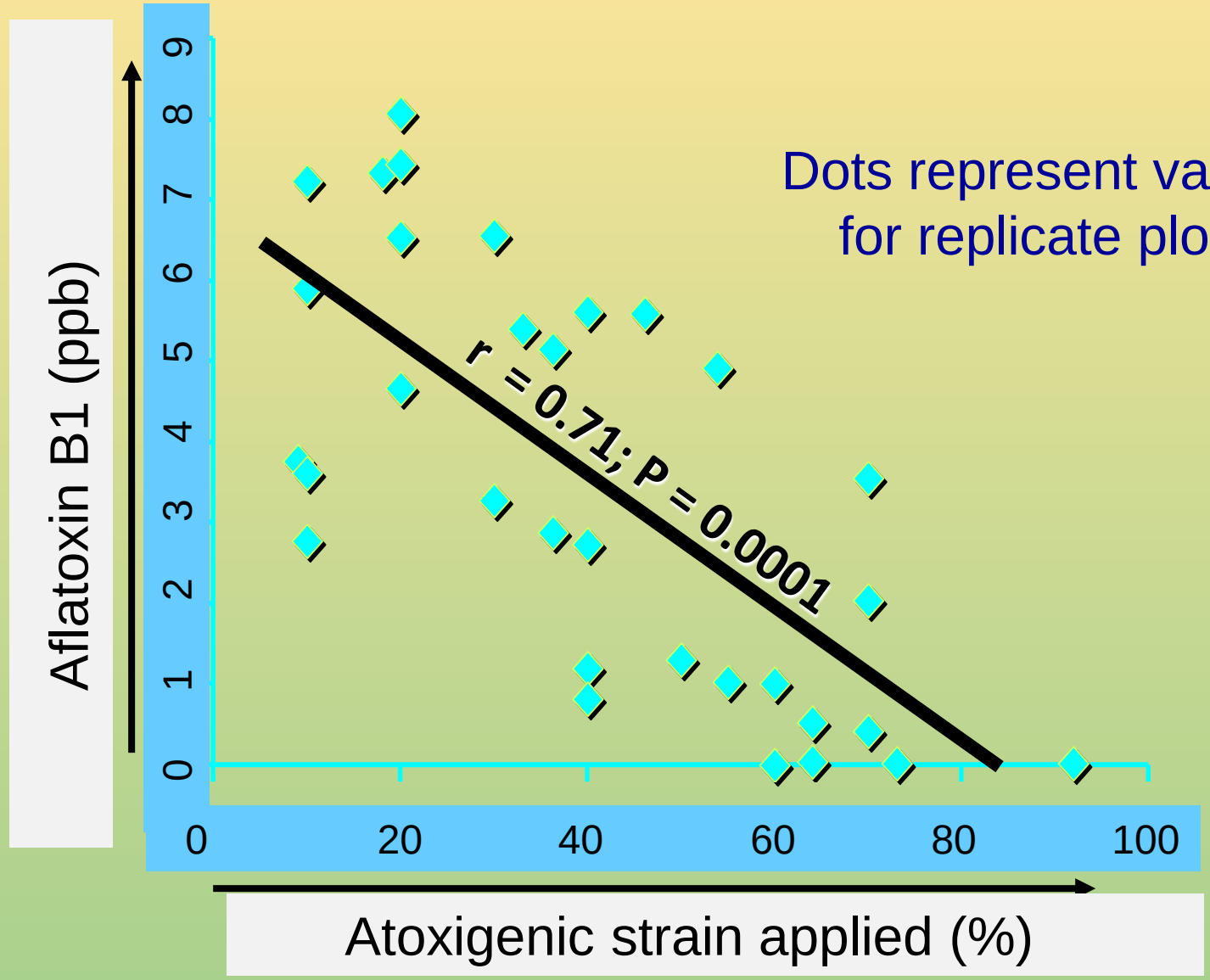


Application in cotton fields: Humid environment under the plants

→ good sporulation of the product

Leading to 80% to 90% displacement of toxigenic strains

Aflatoxin in cottonseed versus strain AF36 incidence



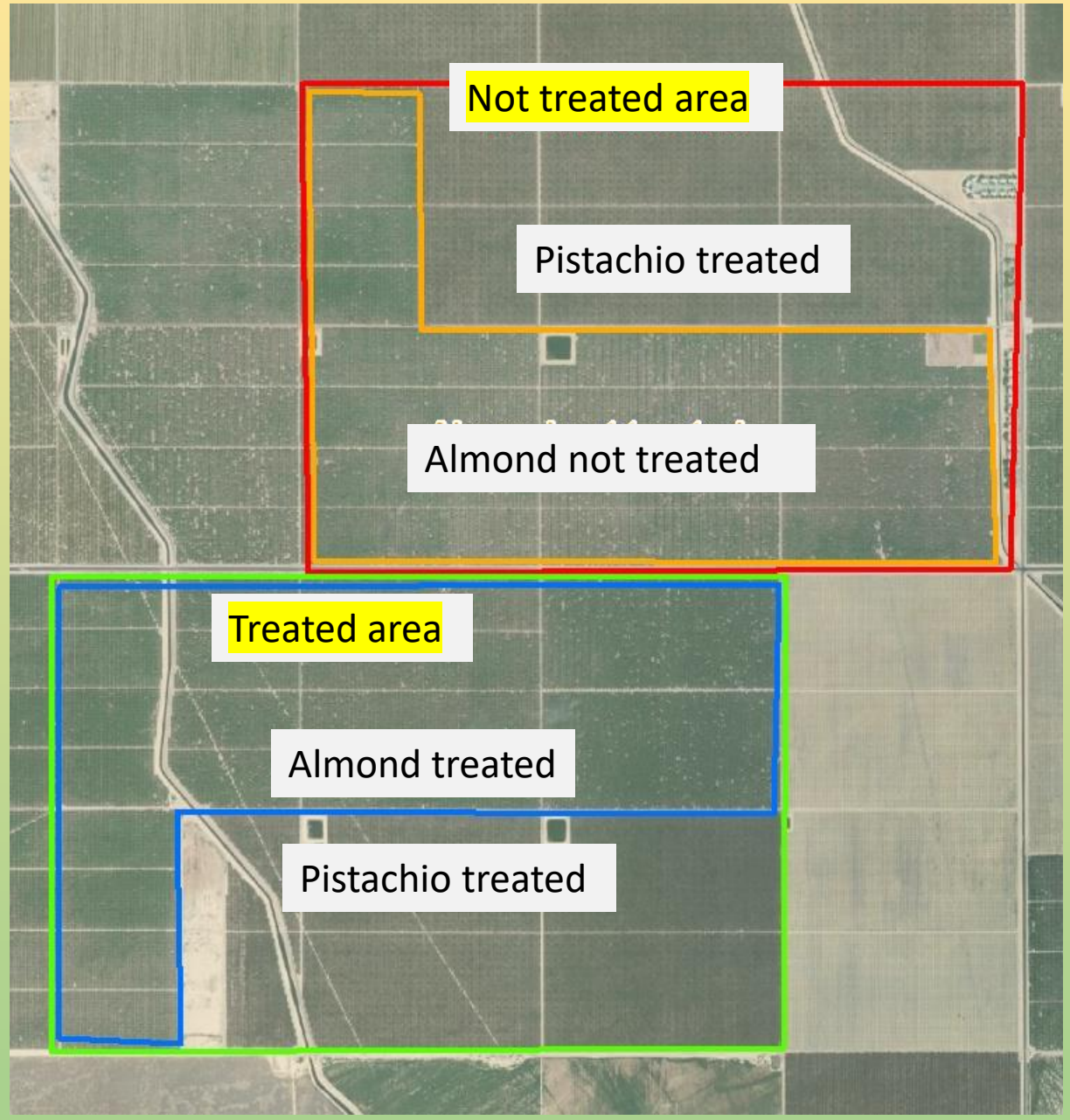
Dots represent values for replicate plots

Slide of Peter Cotty

Excellent reduction in aflatoxin contamination by using biocontrol products in Ghana

Region	Treatment	Total aflatoxin concentration (ppb)			
		Groundnut		Maize	
		Mean	% Reduction	Mean	% Reduction
Ashanti	Control	352	96	8	100
	Treated	15		0	
Brong Ahafo	Control	81	99	12	100
	Treated	1		0	
Northern	Control	199	100	238	100
	Treated	0		0	
Upper East	Control	200	100	122	100
	Treated	0		0	
Upper West	Control	939	100	301	98
	Treated	0		6	

Area-wide, long-term biocontrol with the atoxigenic strain AF36 Prevail

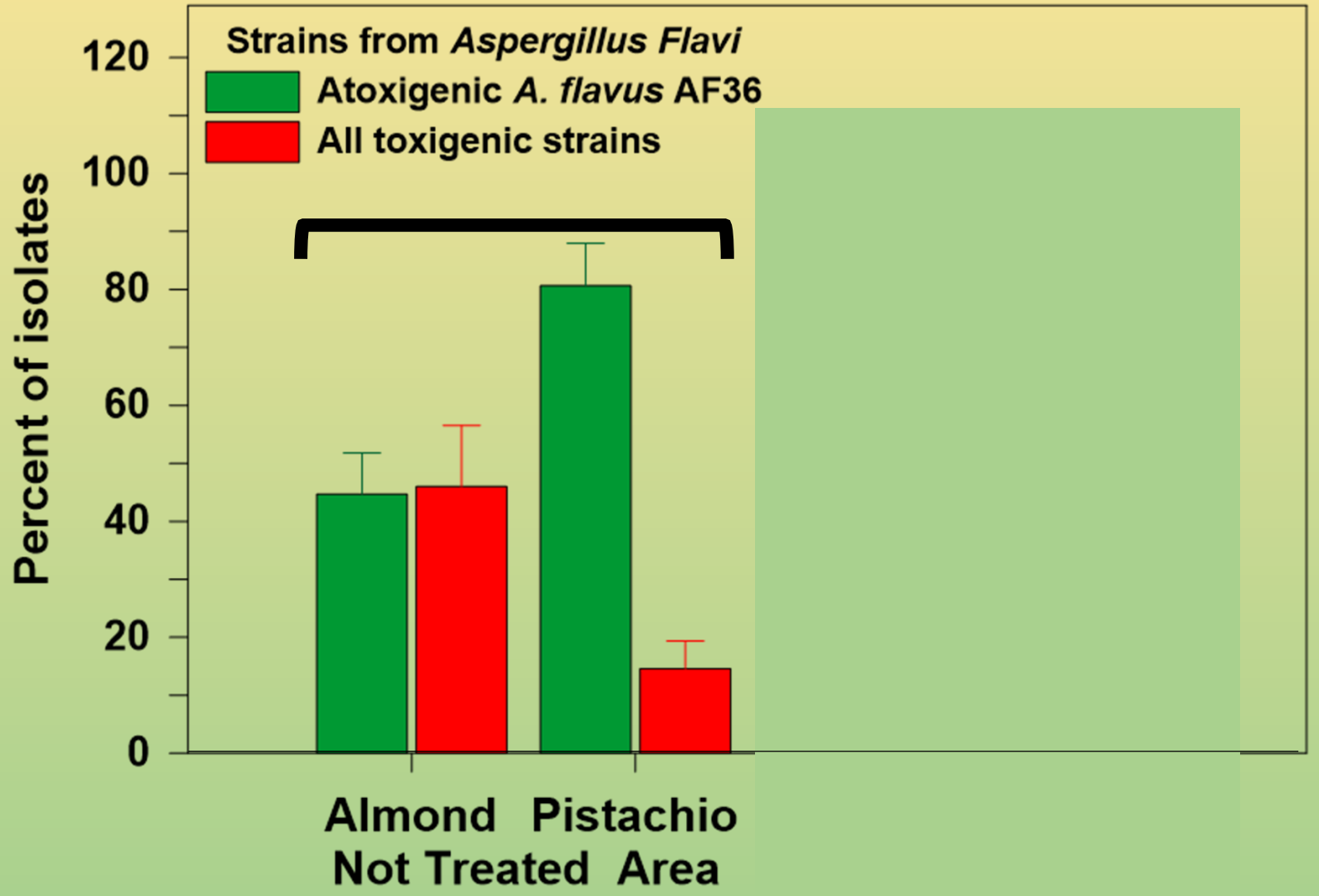


Note:

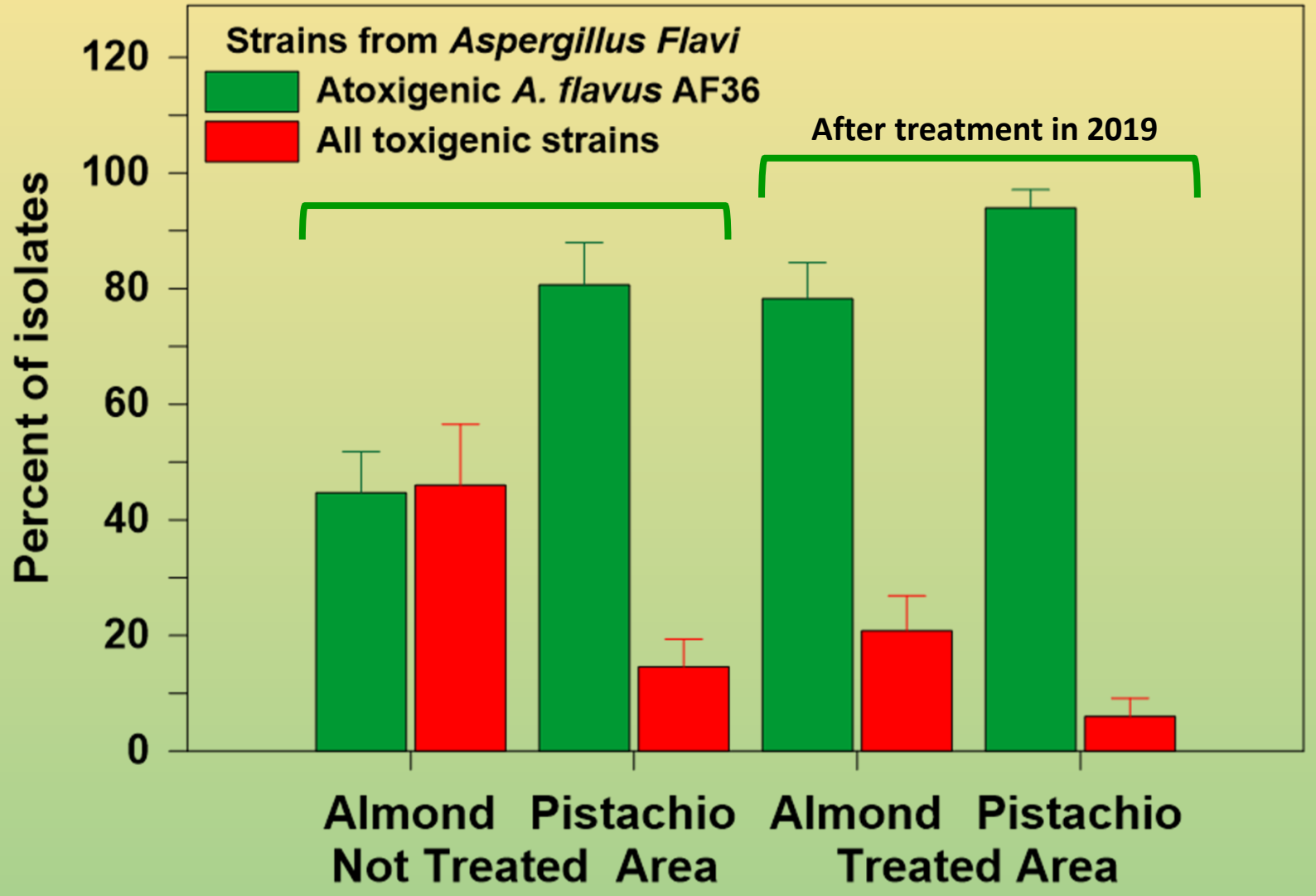
- **<u>In the not treated area</u>**
  pistachio orchards were treated;
  almond orchard were not treated.

- **<u>In the treated area</u>**
  both almond and pistachio orchards were treated.

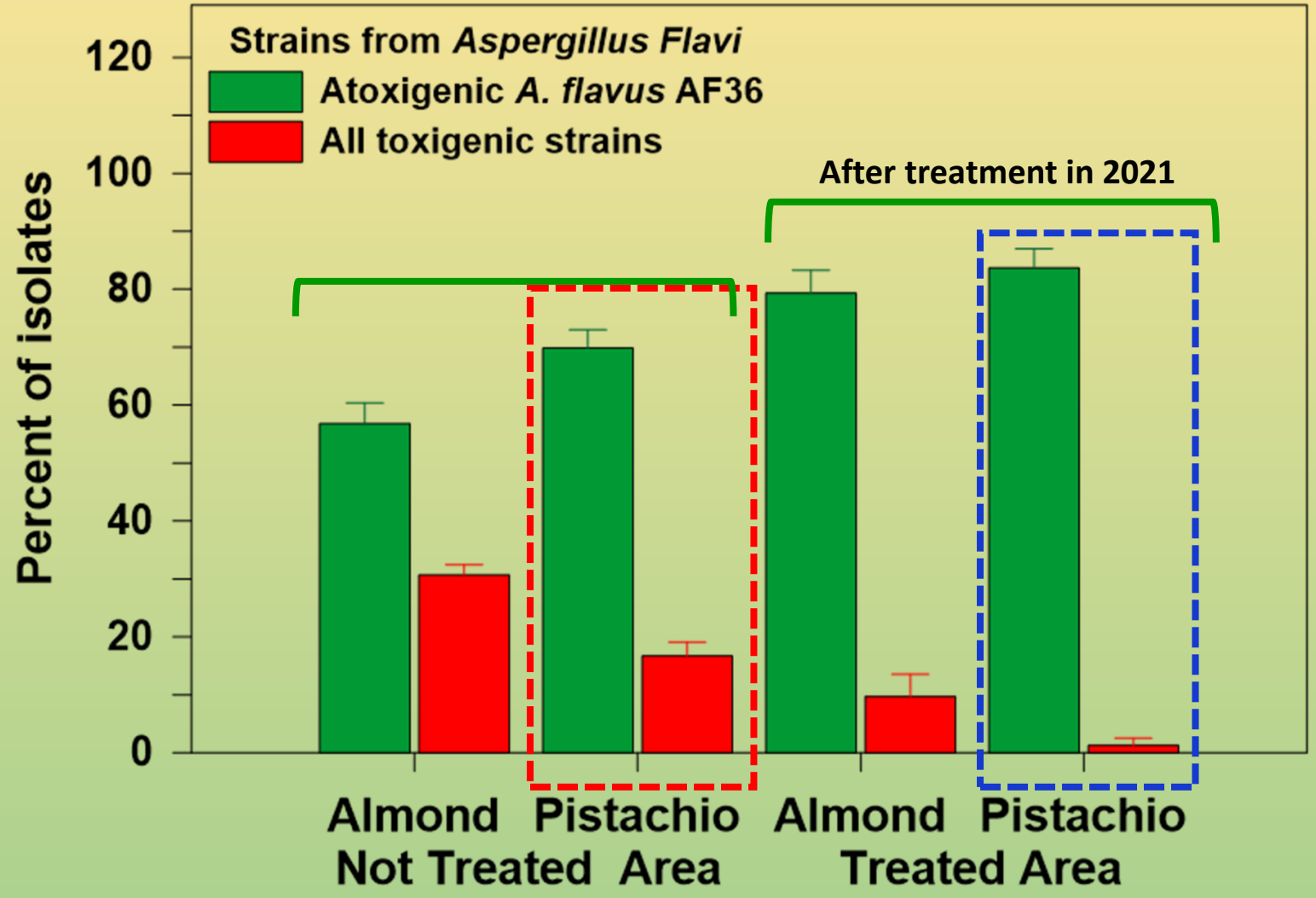
Area-wide, long-term treatment with AF36 Prevail atoxigenic strain in almond and pistachio orchards (2019)



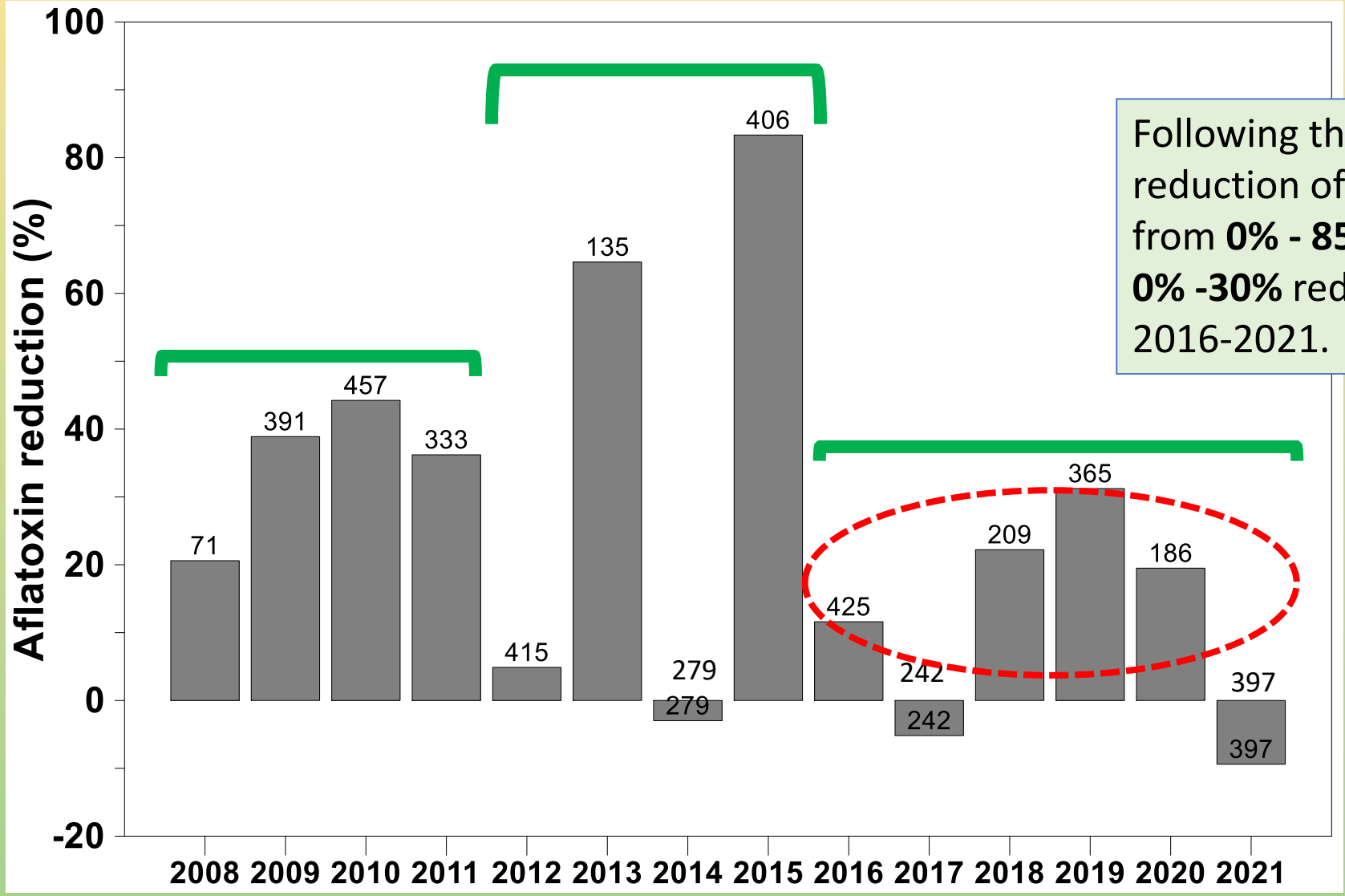
Area-wide, long-term treatment with AF36 Prevail atoxigenic strain in almond and pistachio orchards (2019)



Area-wide, long-term treatment with AF36 Prevail® atoxigenic strain in almond and pistachio orchards (2021)



Reduction of aflatoxin contaminated library samples after treatment with AF36 or AF36 Prevail



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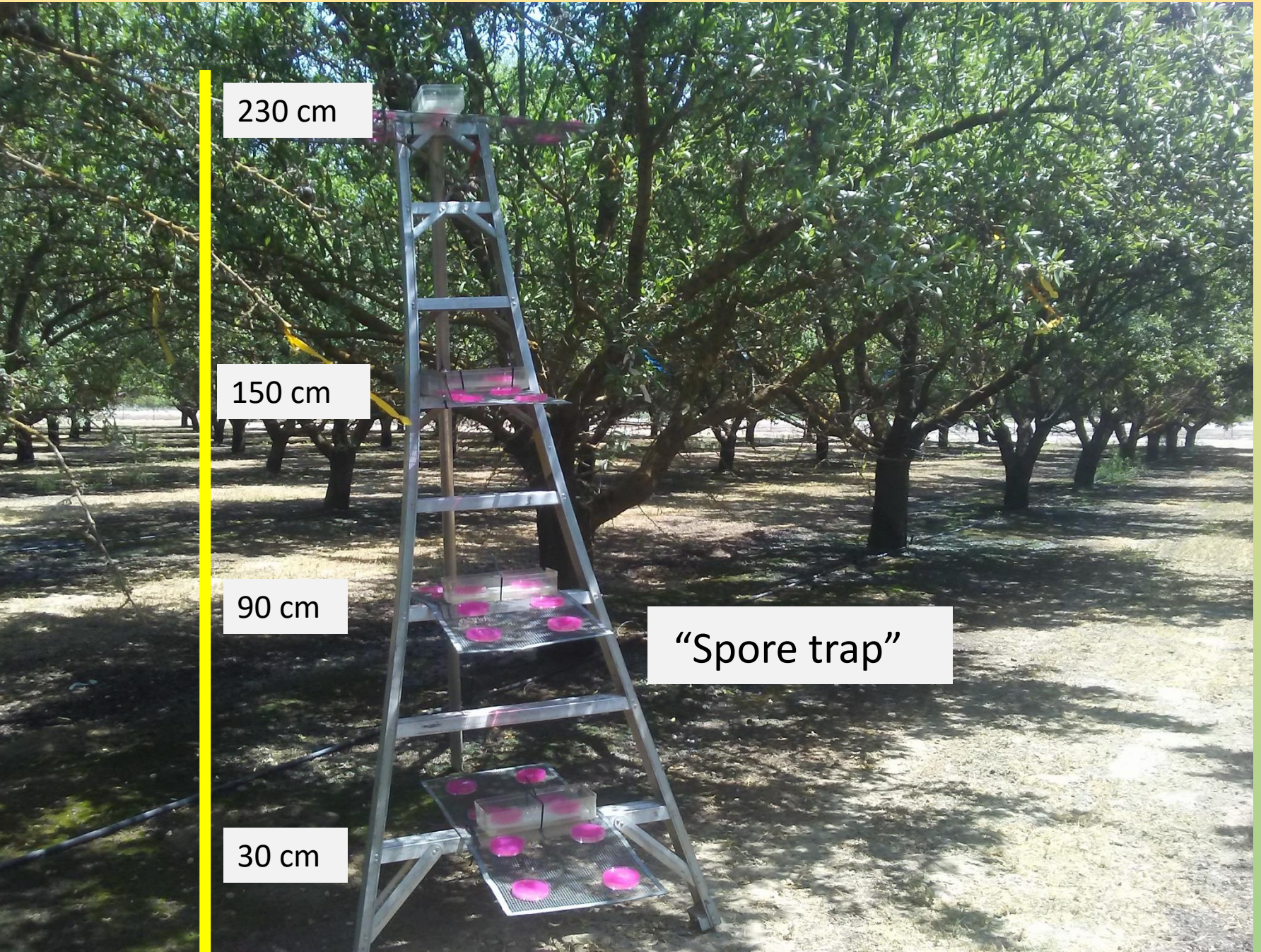
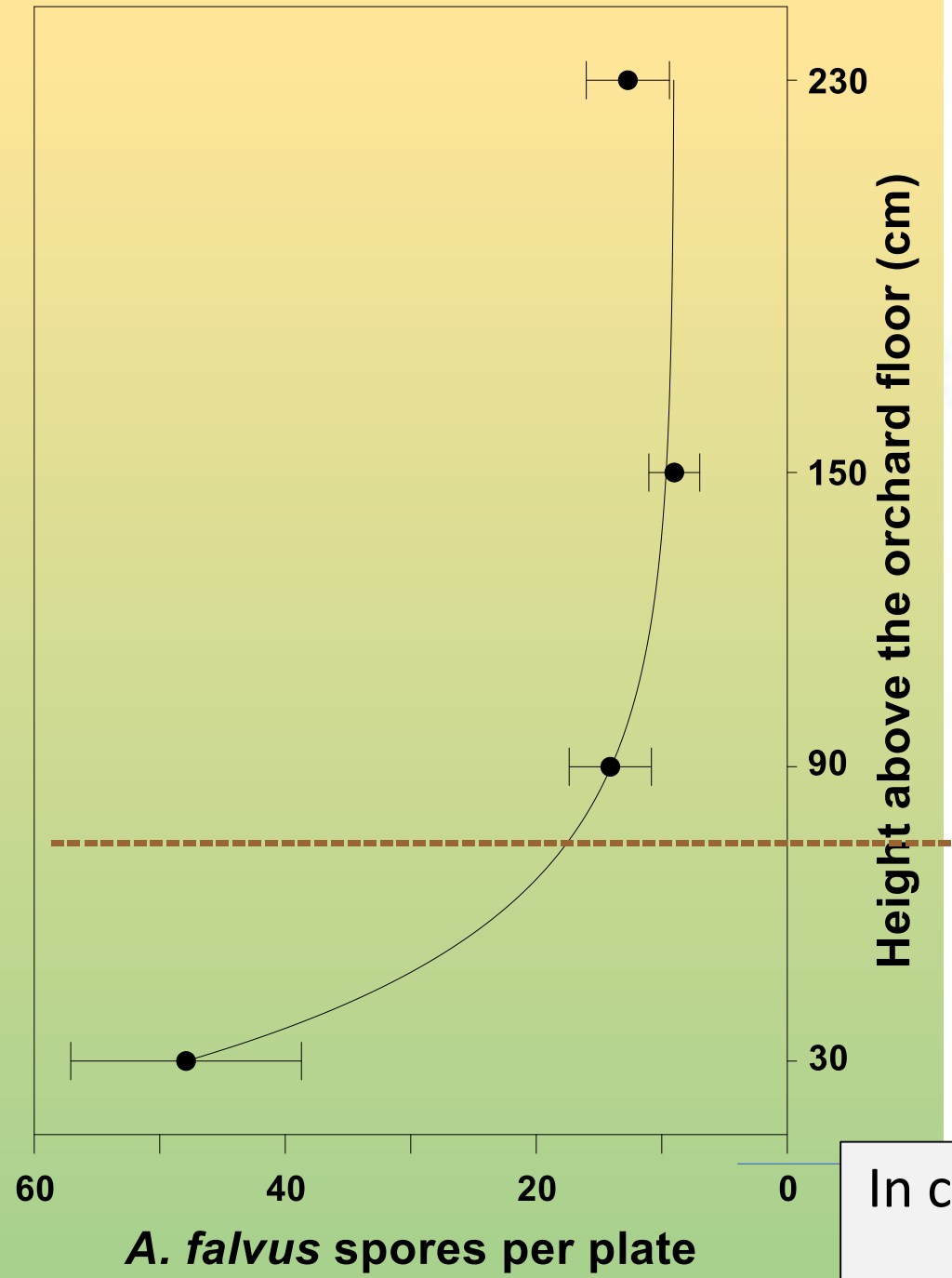
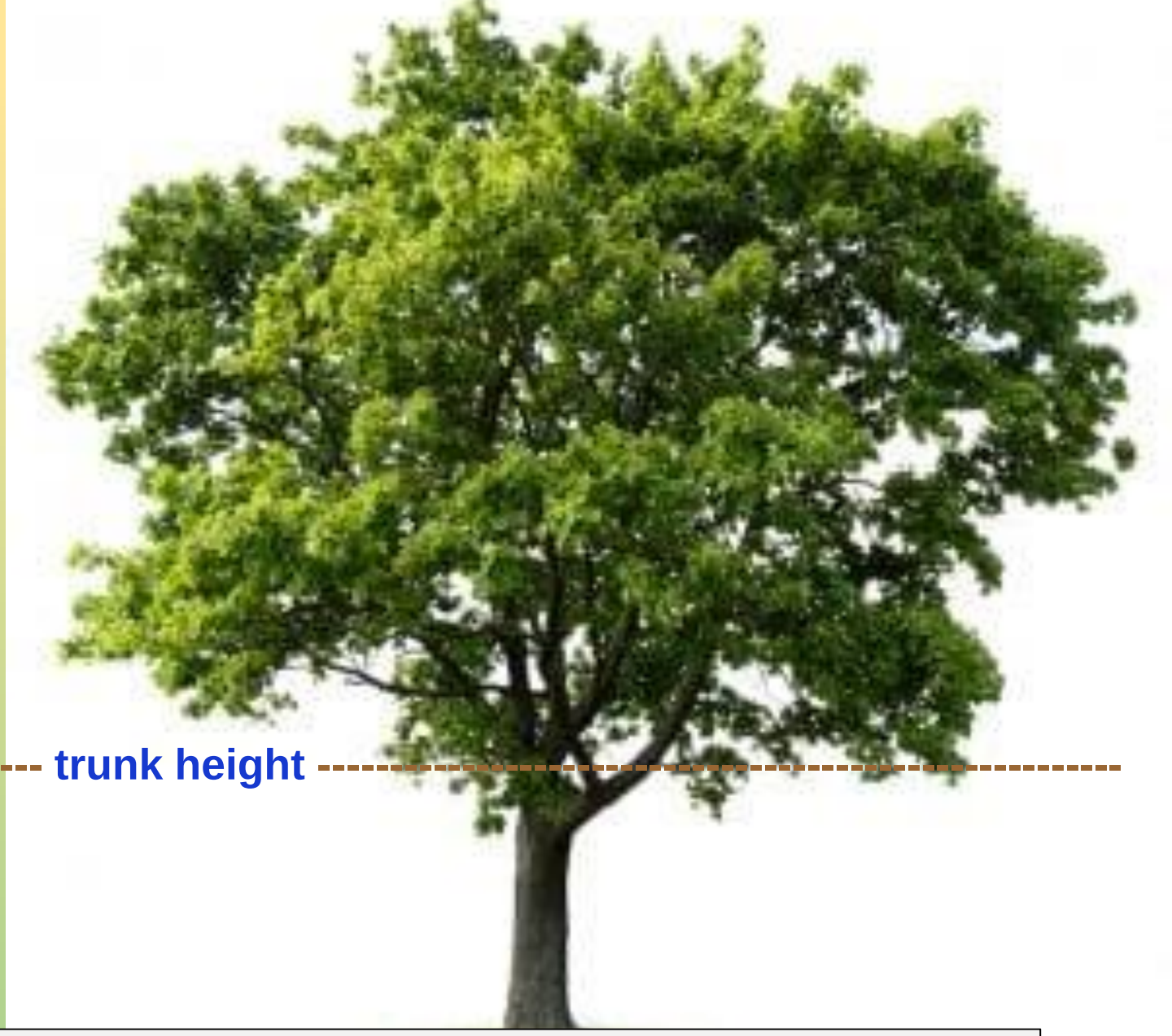

230 cm
150 cm
90 cm
30 cm
“Spore trap”

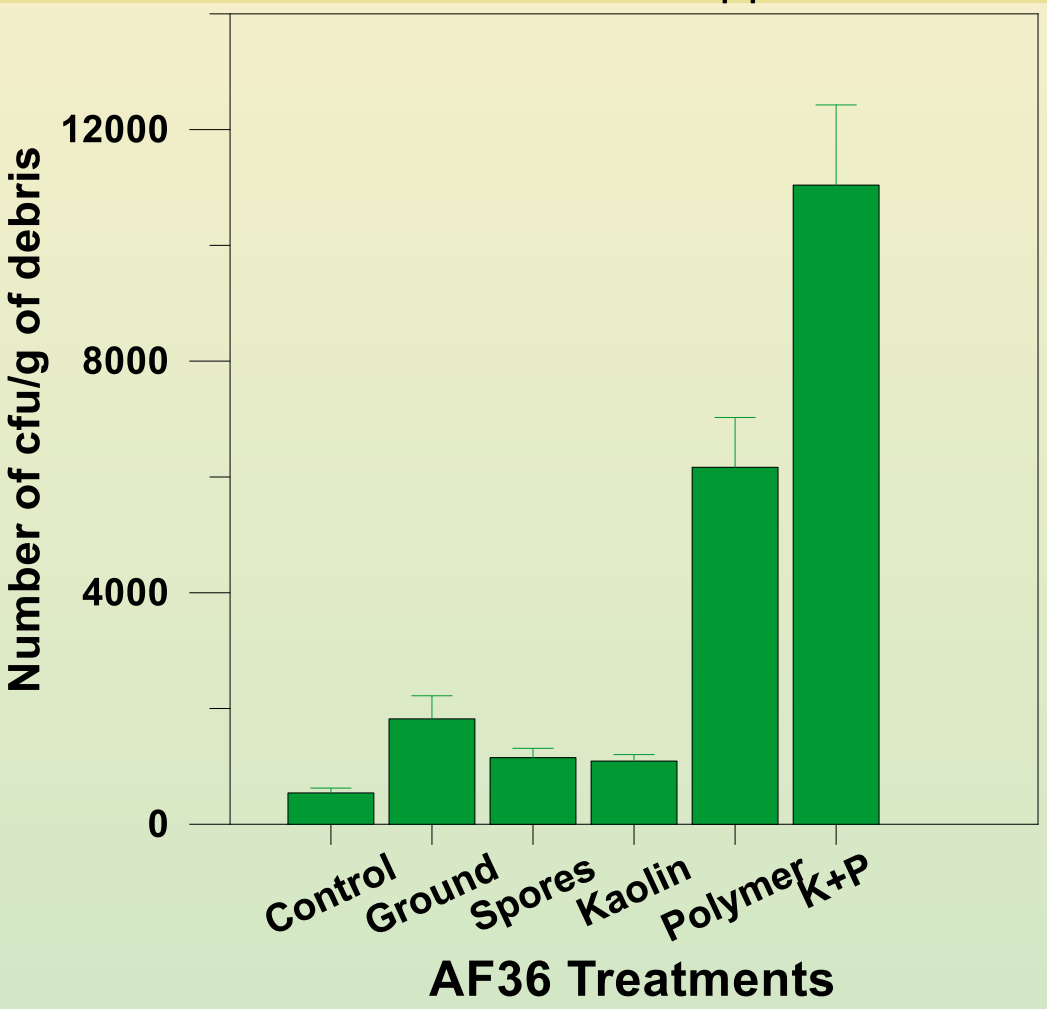




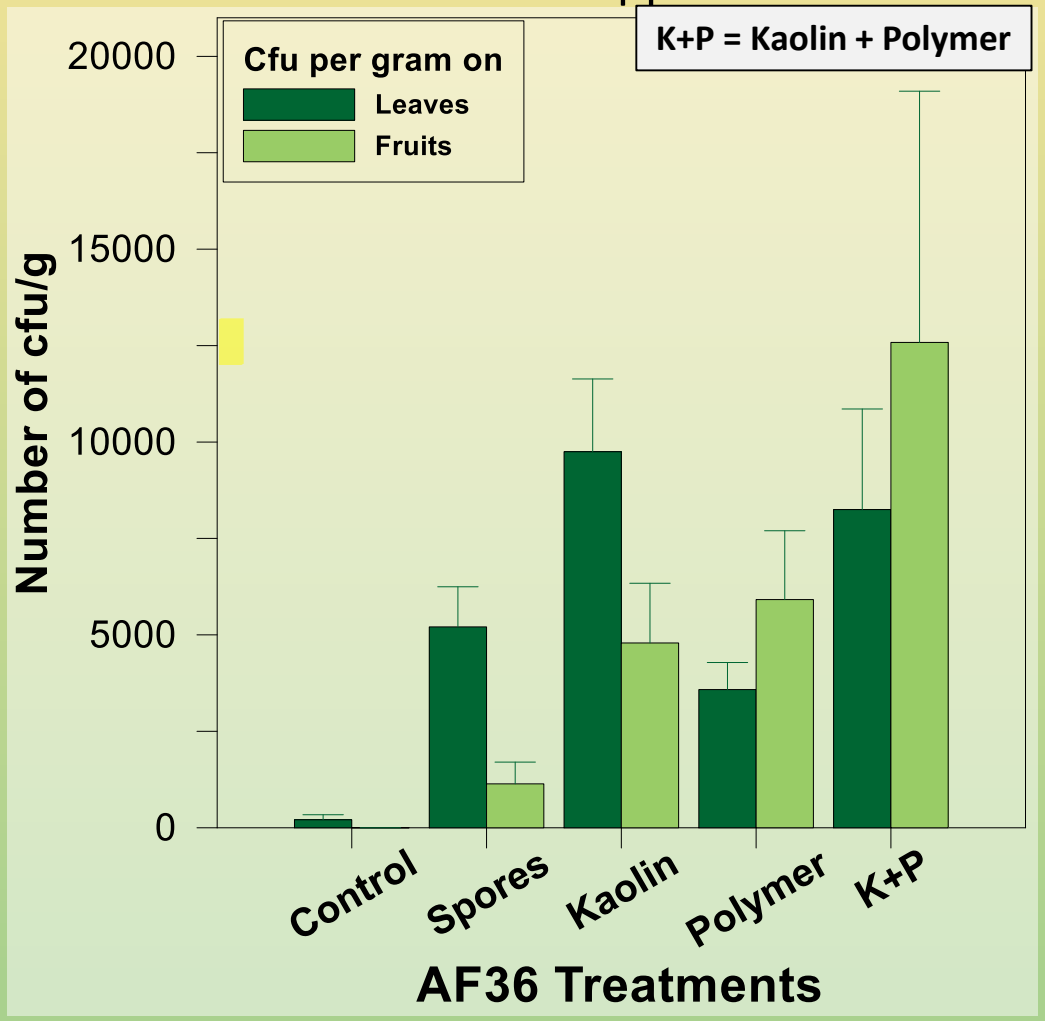
In calm days, limited spores of the biocontrol agent reach the canopy (challenge)

Evaluate the microflora /colonization of NOW-damaged, ES, normal nuts as affected by the method of application of AF36 (ground or canopy)

2019 – Debris 9 months after application



2021 – One month after application



AF36 Prevail®

Aspergillus flavus AF36 Prevail

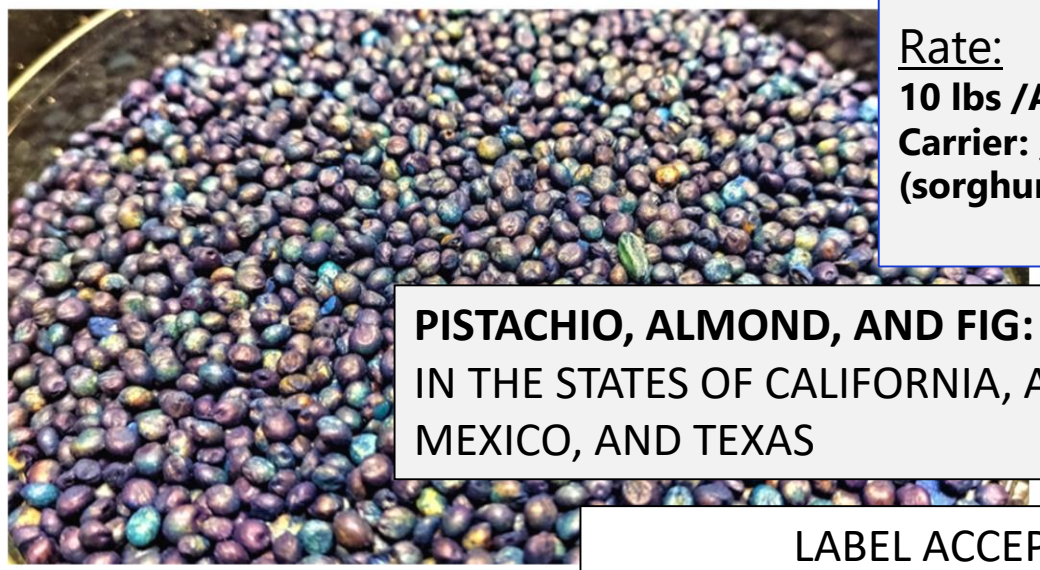
For displacing aflatoxin-producing fungi

Arizona Cotton Research and Protection Cou

"for growers by growers"

KEEP OUT OF REACH OF CHILDREN

CAUTION



Manufactured by:
Arizona Cotton Research and Protection Council

Rate:
10 lbs /Acre
Carrier: coated milo (sorghum) seeds

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

LABEL ACCEPTABLE
STATE OF CALIFORNIA
DEPARTMENT OF PESTICIDE REGULATION
Date: **08/07/2017**
Reg. No. 71693-2-AA

Afla-Guard® GR

syngenta

For agricultural use to displace aflatoxin-producing fungi in almonds, peanuts, pistachios, and corn

OMRI LISTED® For Organic Use

Active Ingredient:
Aspergillus flavus
NRRL 21882* 0.0094%
Other Ingredients: 99.9906%

2000 pounds
Net Weight

PRODUCT ID
43768

List of aflatoxin biocontrol products registered for commercial use

Product	Atoxigenic *Aspergillus flavus* isolate(s)	Responsible organization or entity	Target country	Crops for use	Reference
Aspergillus flavus 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[3]	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]

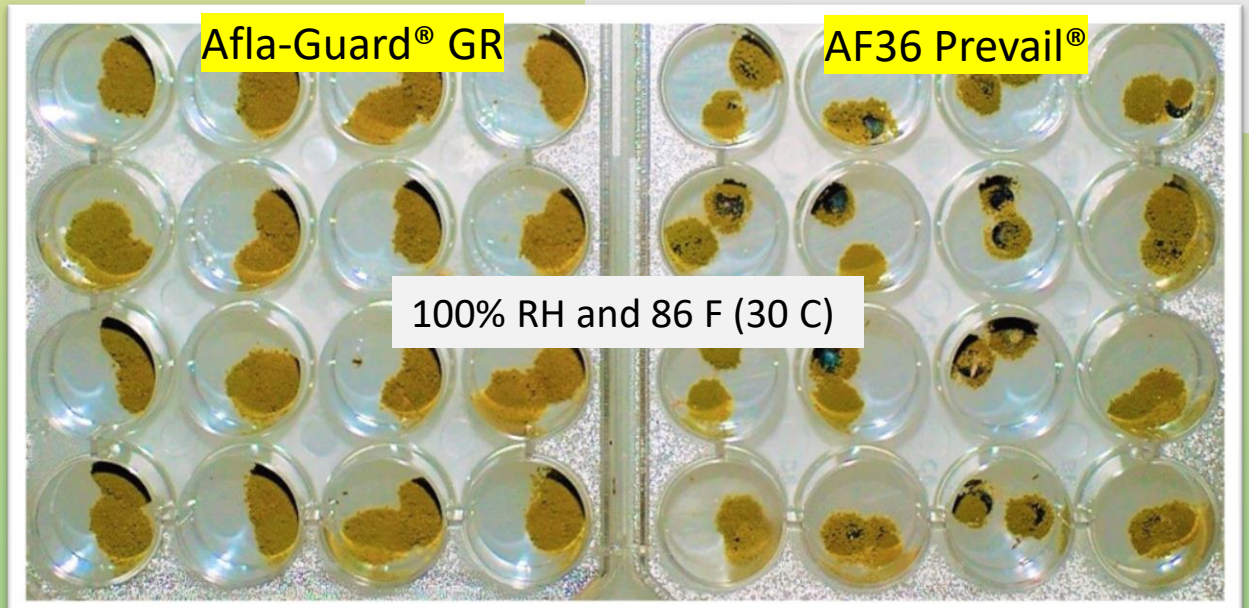
List of biocontrol products registered for commercial use (table continued)

Aflasafe TZ01	TMS199-3, TMH104-9, TGS364-2, TMH 30-8	IITA	**Tanzania**	**Maize and groundnut**	Unpublished registration document
Aflasafe TZ02	TMS64-1, TGS55-6, TMS205-5, TMS137-3	IITA	**Tanzania**	**Maize and groundnut**	Unpublished registration document
Aflasafe MWMZ01[2]	GP5G-8, GP1H-12, MZM594-1, MZM029-7	IITA	**Mozambique**	**Maize and groundnut**	Unpublished registration document
Aflasafe MWMZ01[2]	MW199-1, MW097-8, MW246-2, MW238-2	IITA	**Malawi**	**Maize and groundnut**	Unpublished registration document
Aflasafe MZ02	GP5G-8, MZG071-6, MZM028-5, MZM250-8	IITA	**Mozambique**	**Maize and groundnut**	Unpublished registration document
Aflasafe MW02	MW258-6, MW332-10, MW248-11, MW204-7	IITA	**Malawi**	**Maize and groundnut**	Unpublished registration document
Aflasafe ZM01	110MS-05, 38MS-03, 46MS-02, 03MS-10	IITA	**Zambia**	**Maize and groundnut**	Unpublished registration document

Theoretical and measured RH over saturated salt solutions

Chemicals	Theoretical RH (%)	Measured RH (%)
NaCl	75.5	74.0
$(NH_4)_2SO_4$	80.0	78.8
KCl	84.5	82.6
KNO_3	91.0	90.2
K_2SO_4	96.5	95.8
H_2O	100	99.6

Comparison of sporulation under different relative humidity



Sporulation of the two aflatoxin biological agents after a week incubation at 96.5% RH and 86 °F (30 °C)

Afla-Guard® GR

AF36 Prevail®

Sporulation after a week incubation at 91% RH and 86 °F (30°C)

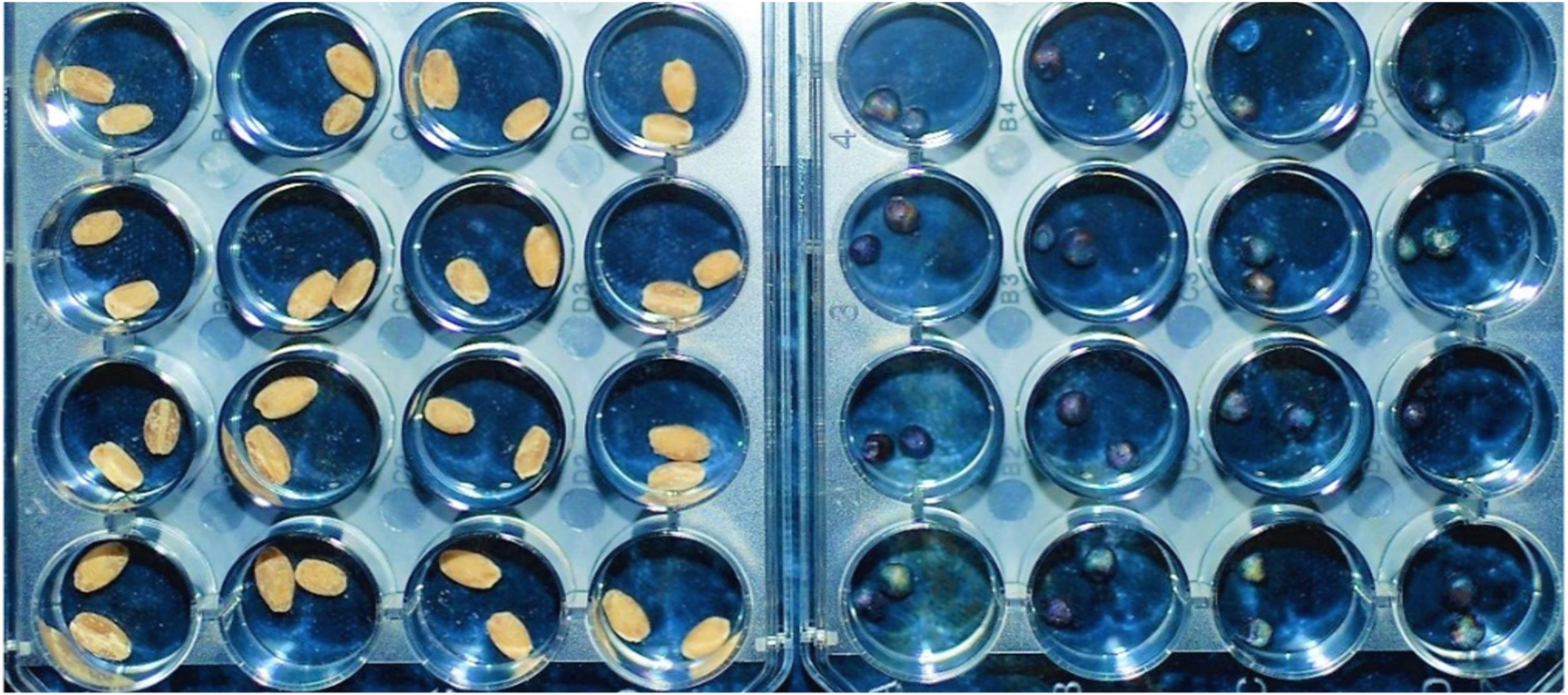
Afla-Guard® GR

AF36 Prevail®

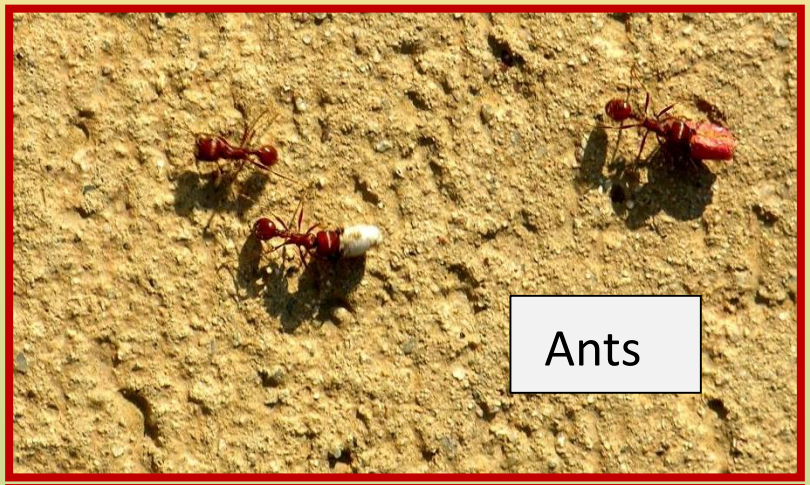
Sporulation after a week incubation at 80% RH and 86 °F (30 °C)

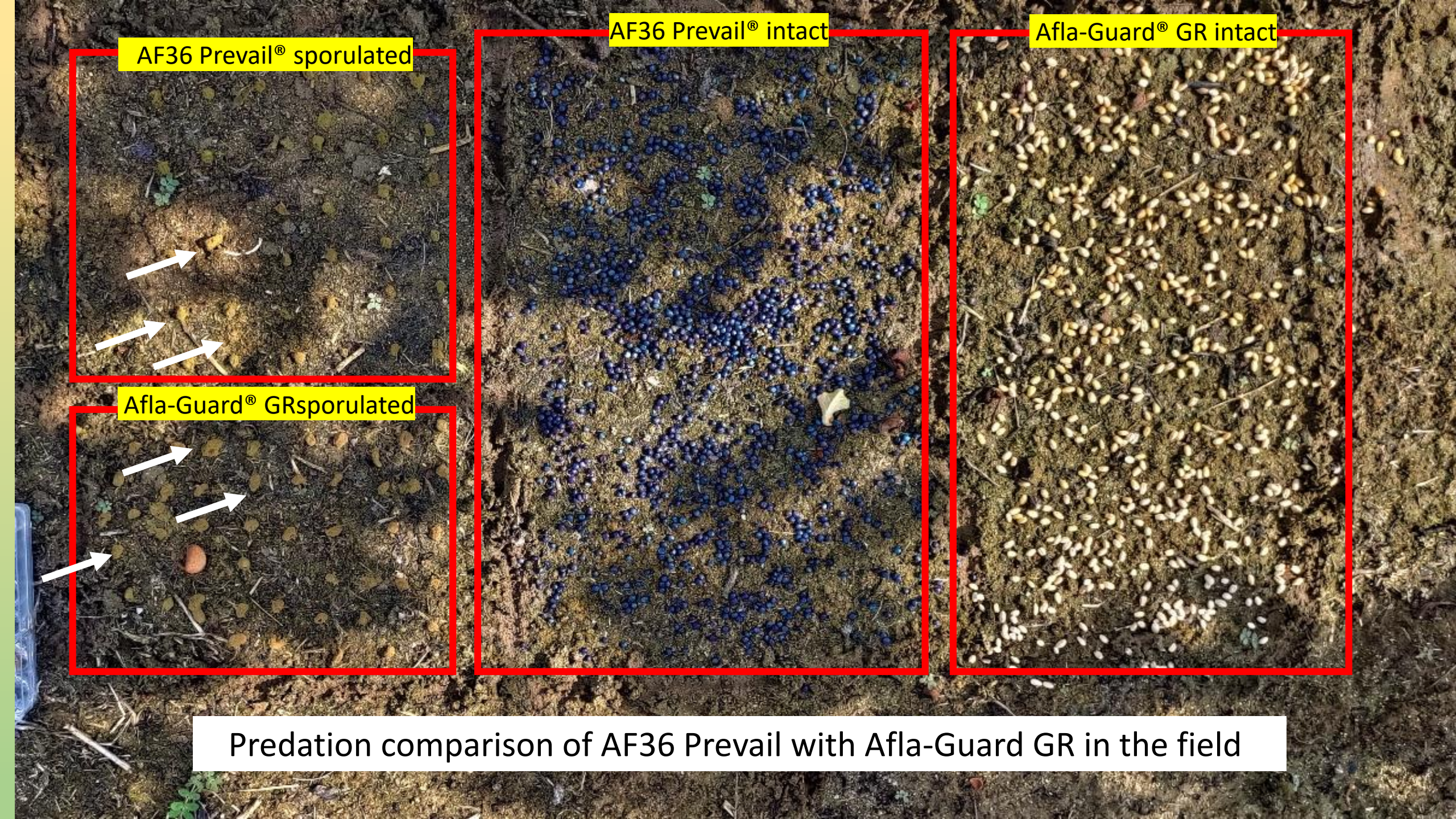
Afla-Guard® GR

AF36 Prevail®



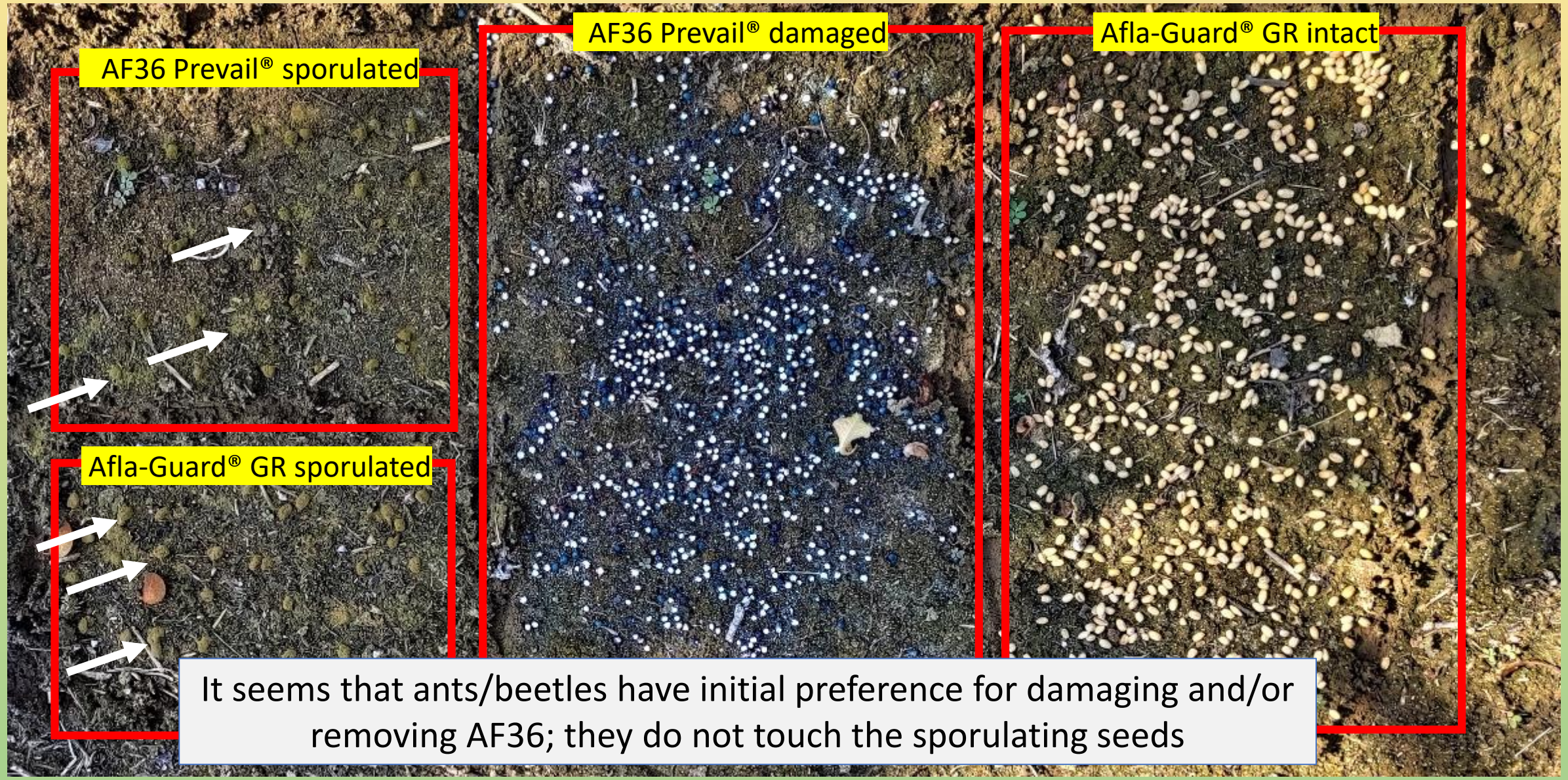
A challenge is the predation of seeds by insects, birds, and decay by other fungi; reduction of inoculum will reduce spore density





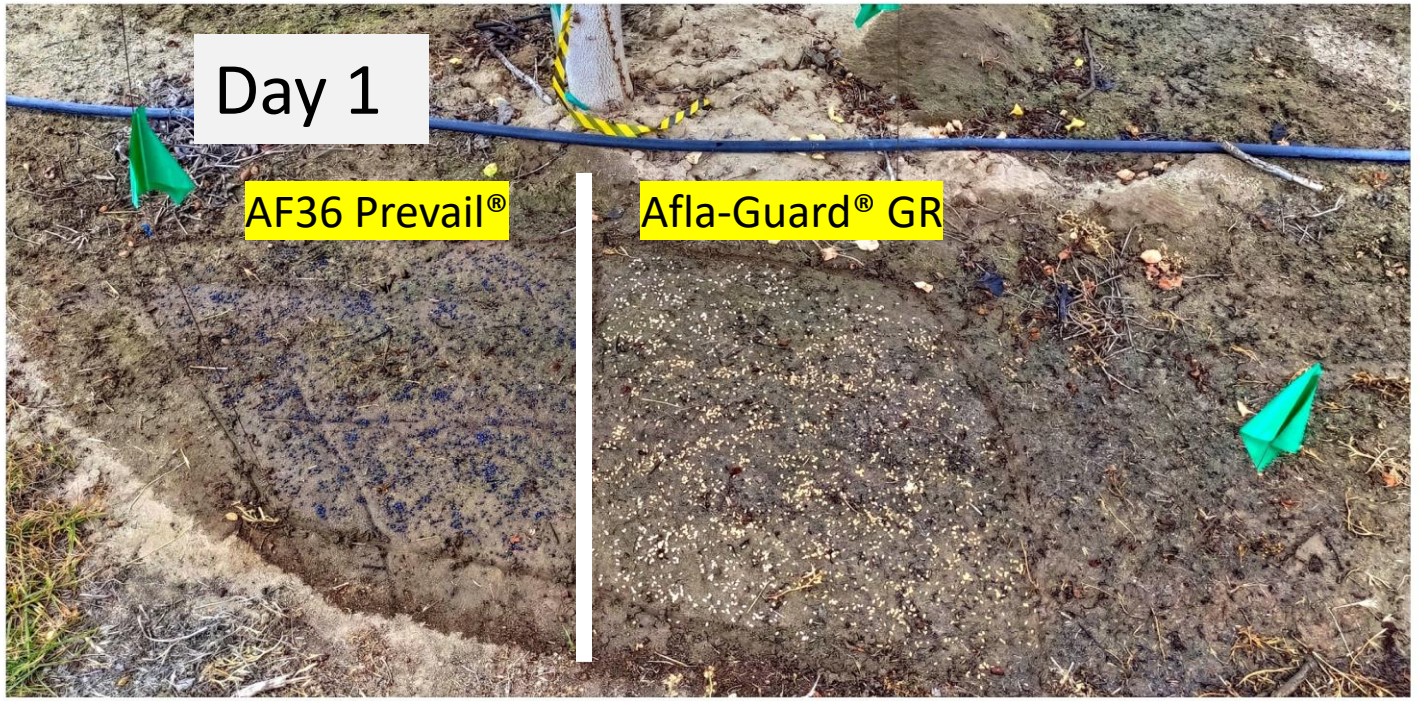
Predation comparison of AF36 Prevail with Afla-Guard GR in the field

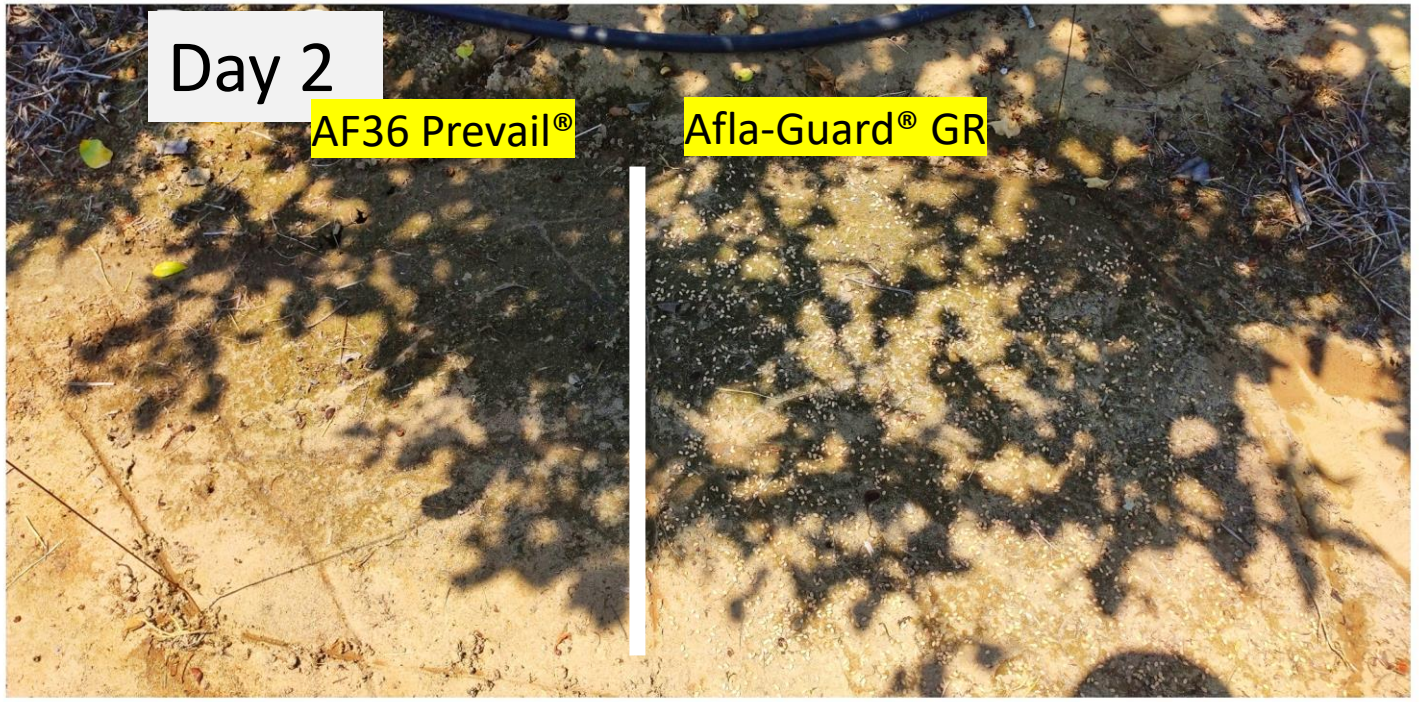
Predation of the two products 24 hours after application in the field



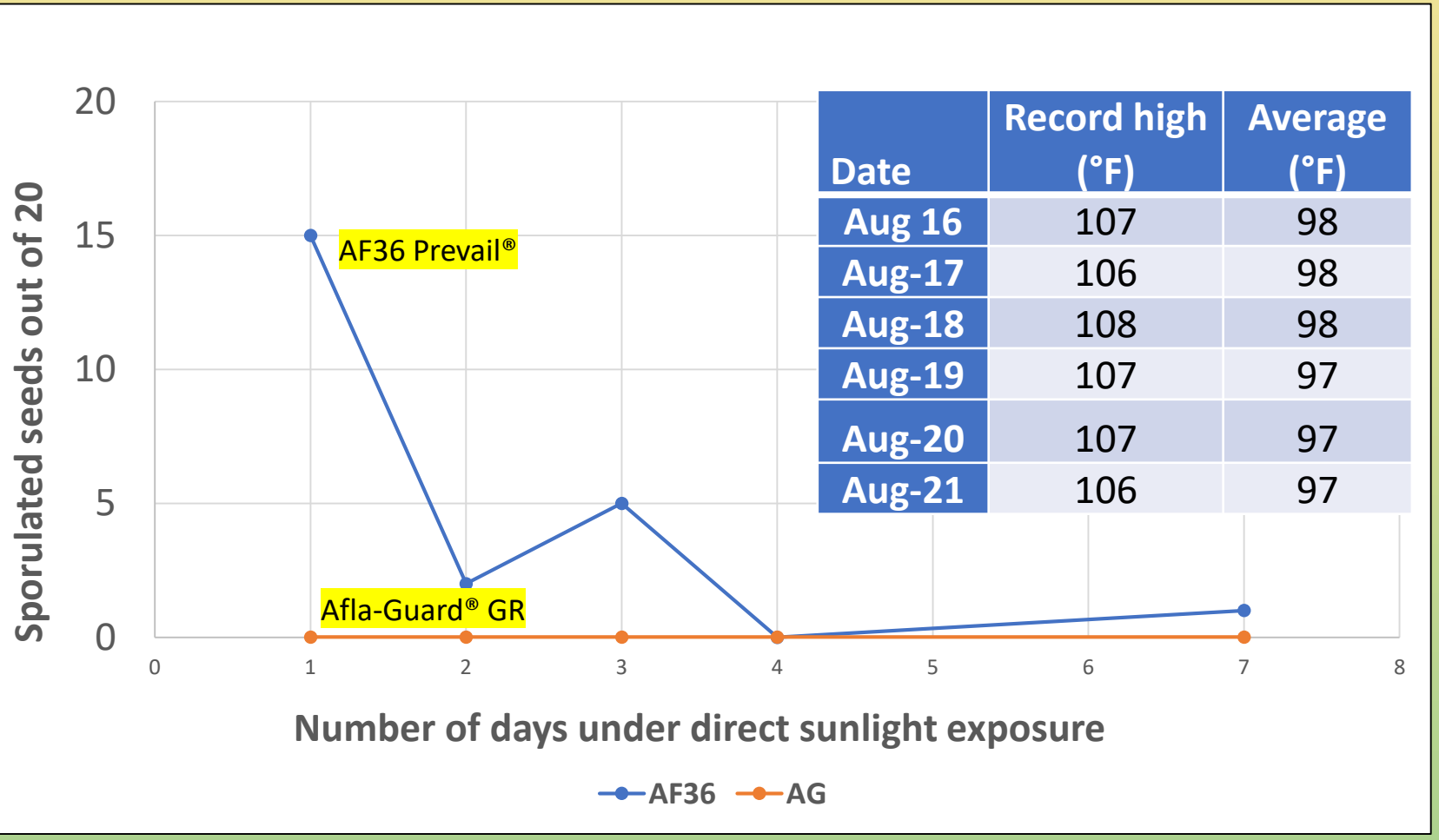
It seems that ants/beetles have initial preference for damaging and/or removing AF36; they do not touch the sporulating seeds

Ants removed all the AF36 Prevail® seeds within 24 hours

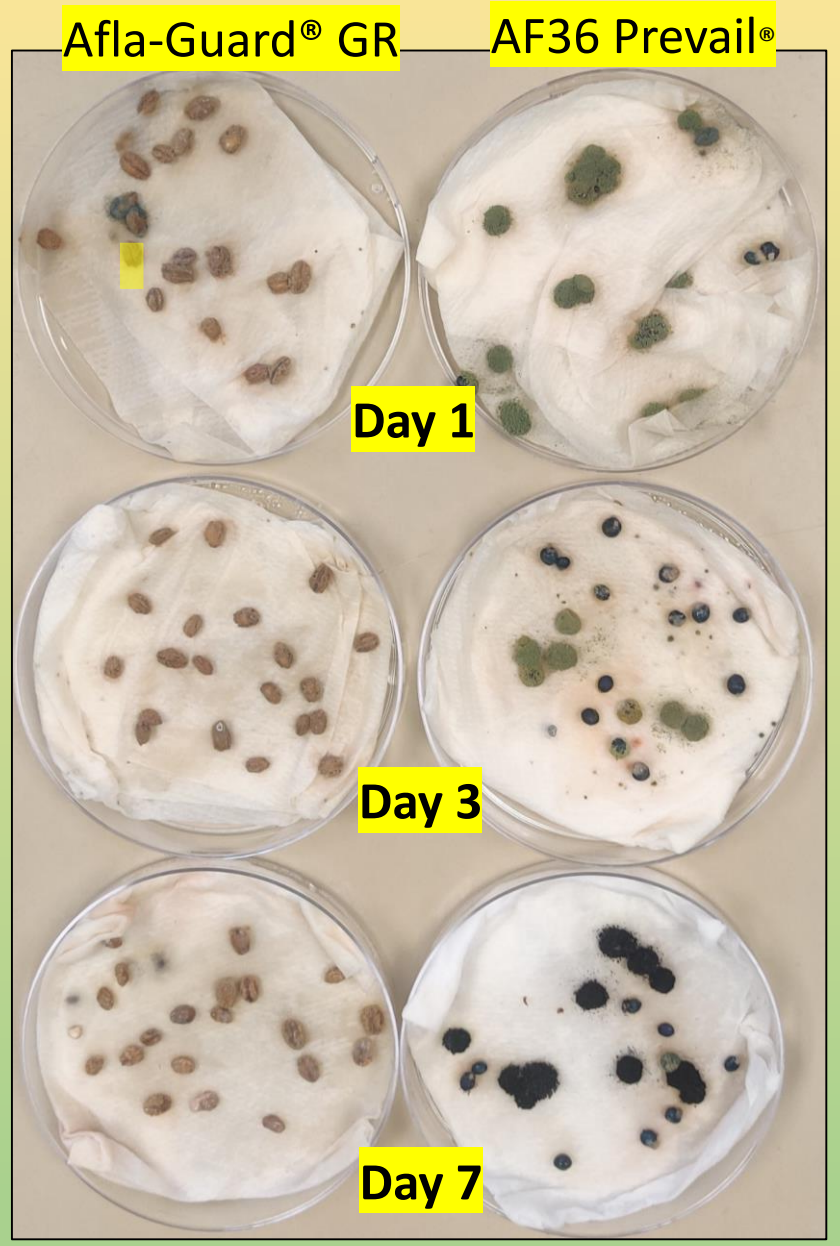




Comparison of sporulation under direct sunlight exposure



Date	Record high (°F)	Average (°F)
Aug 16	107	98
Aug-17	106	98
Aug-18	108	98
Aug-19	107	97
Aug-20	107	97
Aug-21	106	97



Tools to manage aflatoxins in pistachios:

1. Do not stress the trees for water in stage 1 (during May/early June)
2. Remove mummies – orchard sanitation – “mummy shake”…
3. Reduce NOW damage of the crop in season
4. Apply on the soil AF36 Prevail® or Afla-Guard ® GR on late May to mid July at 10 lbs./acre
5. There are no negative effects at all if you have applied AF36 Prevail for several years and you want now to apply Afla-Guard GR
6. Irrigate before or immediately after application of the biological agent
5. Do not spray herbicides 1 to 2 weeks after application
6. Control the ants, other arthropods, and birds in the orchard
7. Sort out stained, suture-stained, and DBOM nuts

There must be a major influence of the treated pistachio acreage from the toxigenic strains of the huge untreated acreage

Total acreage 1,500,000 acres

Total acreage 450,000 acres

Challenges

1. A critical challenge that we have in California is that a major portion of the tree nut industry (mainly almonds) are not treated, which enriching the treated areas with toxigenic strains. (thus, area-wide and long-term programs (mimicking the NOW management program) are essential.

2. Another challenge is the climatic conditions in California do not favor sporulation of biocontrol products in tree nut orchards under our presently used irrigation practices. (emphasis to achieve the best sporulation).

3. A third challenge is the way nuts are produced; the tree canopy is at least 3 feet above the soil, so limited number of the atoxigenic strain products reach the nuts. (developing a spray program may increase the efficacy of the biocontrol products; it looks promising and can be possible).

Special thanks to my lab crew

- California Pistachio Research Board
- CDFA
- Wonderful orchards
- Pistachio growers
- Arizona Cotton Council Assoc.
- Syngenta Co.

Thank you

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Ramon Jaime
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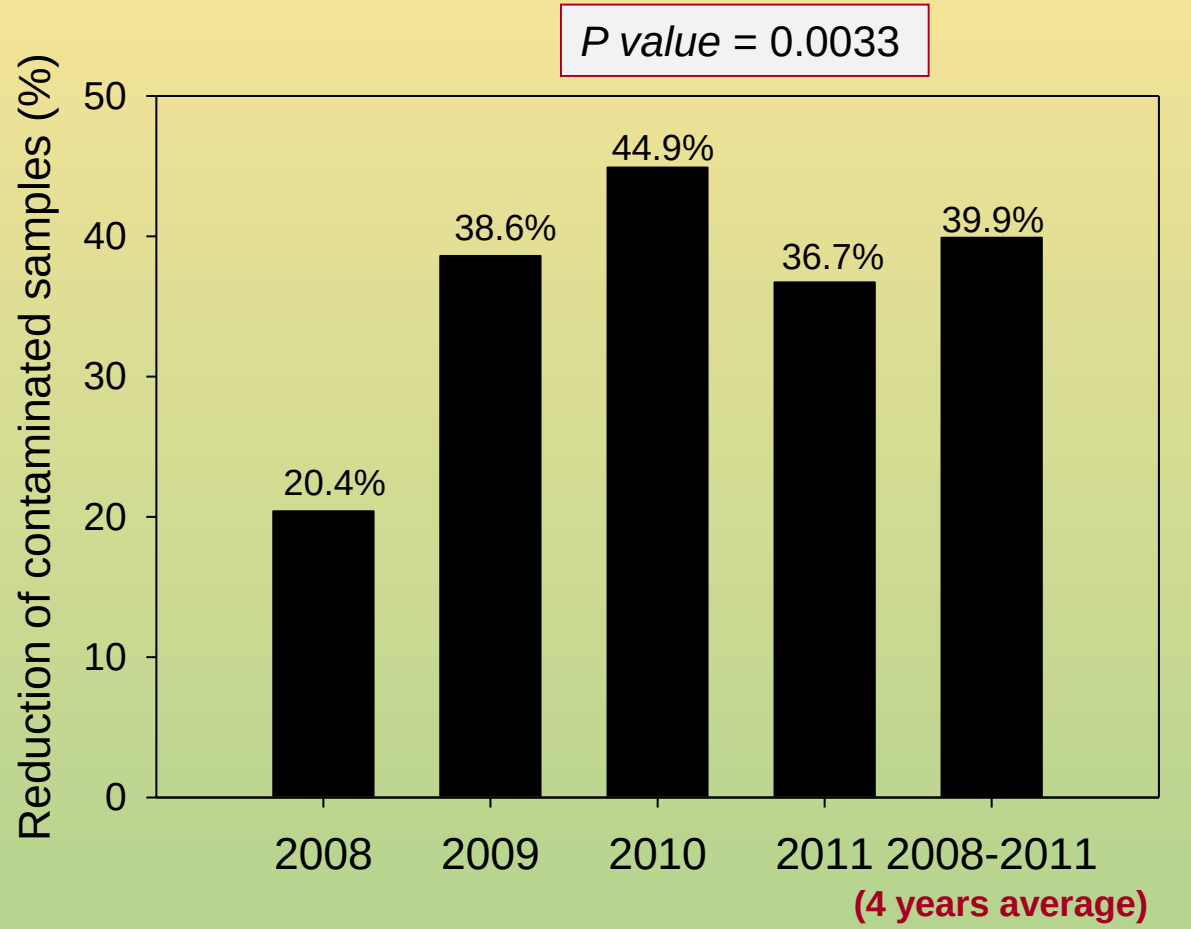
Success in reducing aflatoxin-contaminated pistachio library samples (1st harvest & reshakes)

90-95% displacement of toxigenic strains



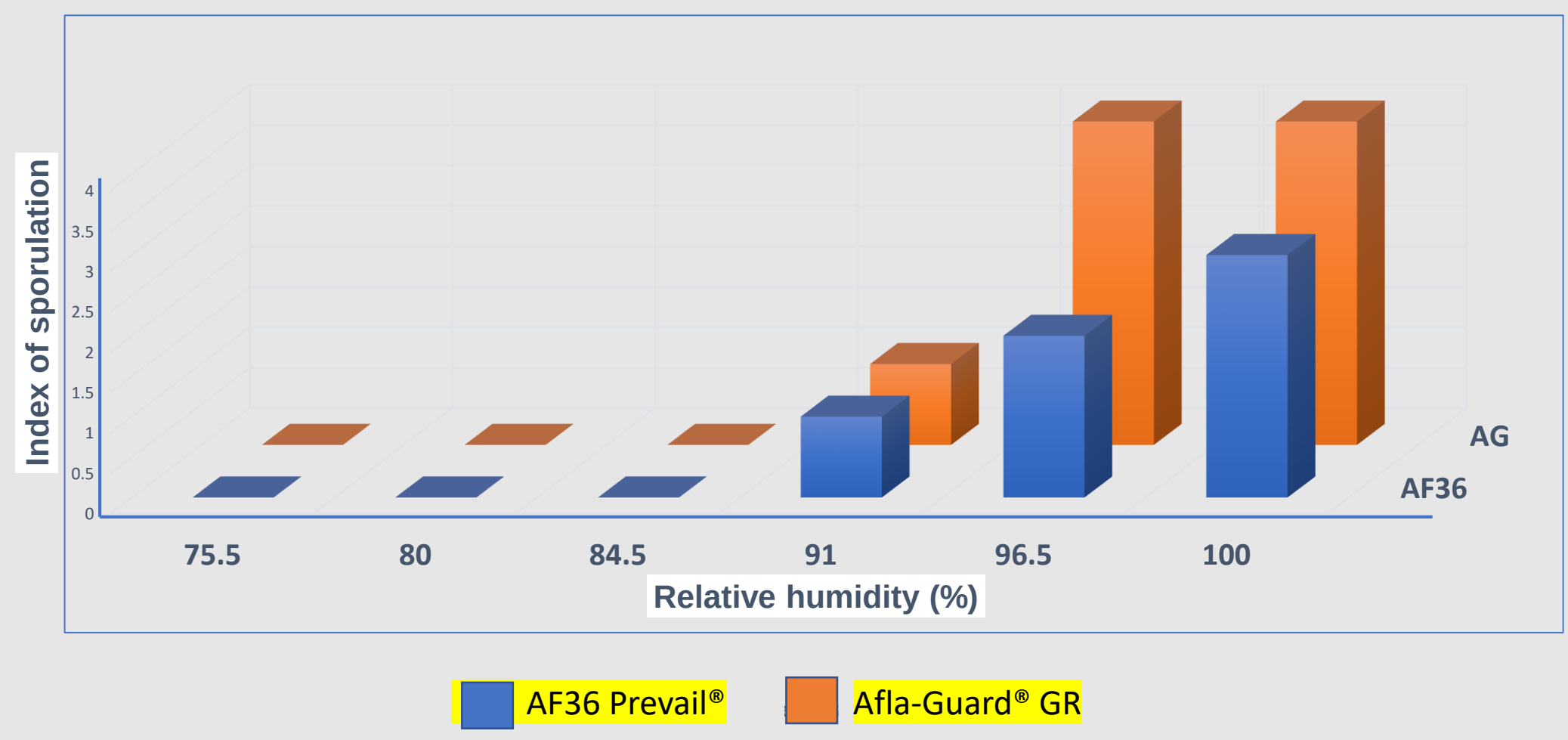
First harvest:
An average of 40% reduction

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

Excellent reduction in aflatoxin contamination by using biocontrol products in Ghana

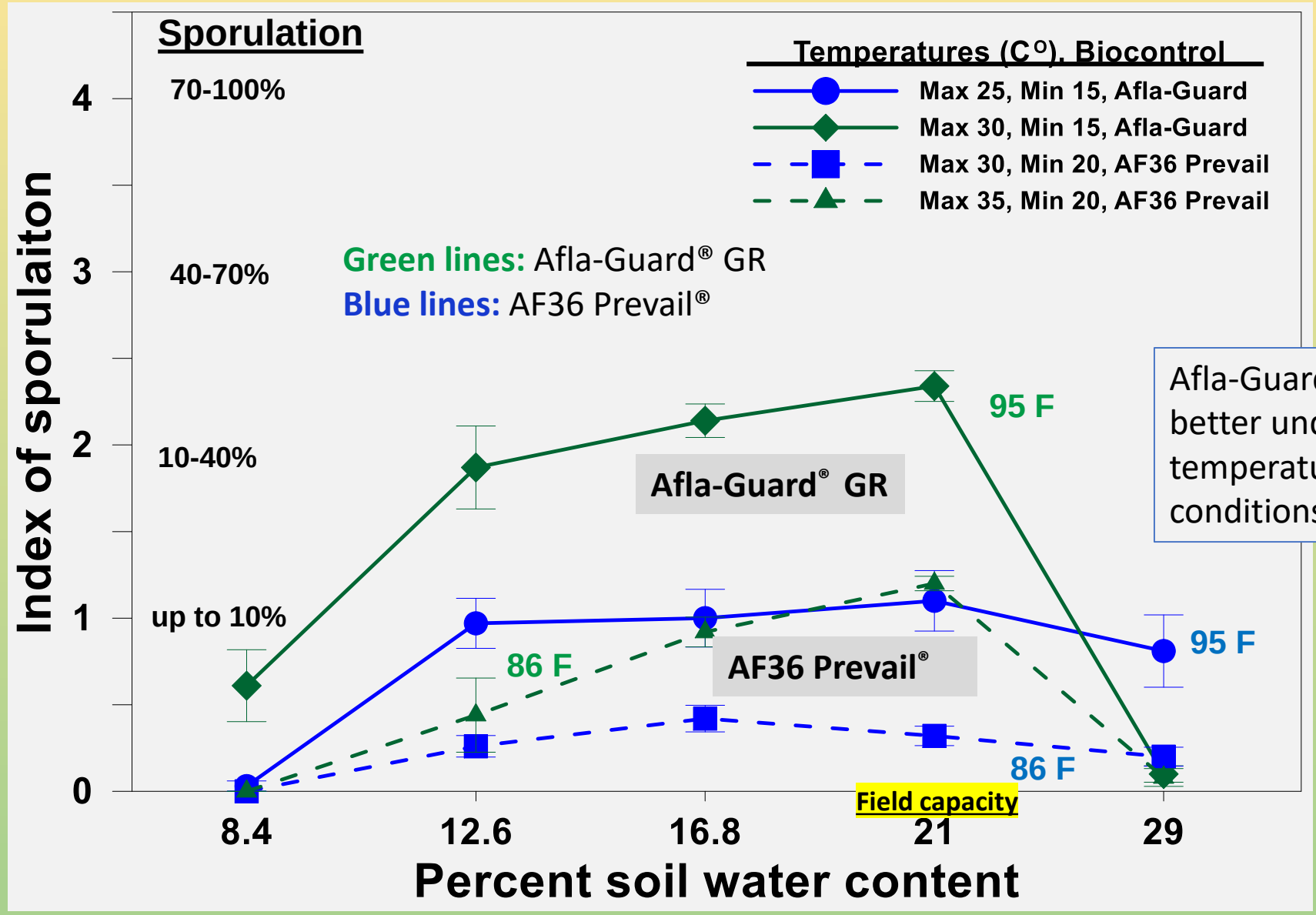
Region	Treatment	Total aflatoxin concentration (ppb)			
		Groundnut		Maize	
		Mean	% Reduction	Mean	% Reduction
Ashanti	Control	352	96	8	100
	Treated	15		0	
Brong Ahafo	Control	81	99	12	100
	Treated	1		0	
Northern	Control	199	100	238	100
	Treated	0		0	
Upper East	Control	200	100	122	100
	Treated	0		0	
Upper West	Control	939	100	301	98
	Treated	0		6	

Sporulation of the two biological agents after 1 week incubation at different RHs and 30°C (86 °F)



0=no sporulation; 1 =25%; 2=50%; 3=75%; & 4=100% sporulation

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



Afla-Guard® GR sporulated better under lower temperatures and drier soil conditions.

In calm days, the spores limited spores of the biocontrol application reach the canopy (challenge)