

Ramon Jaime, Pummi Singh, John Lake, Giuseppe Fiore, Victor Gabri, Apostolos Papagelis & Themis J. Michailides

> University of California Davis Kearney Agricultural Research and Extension

> > Pistachio Day – Visalia, 18 January 2023

Aflatoxins are produced by Aspergillus flavus and A. parasiticus

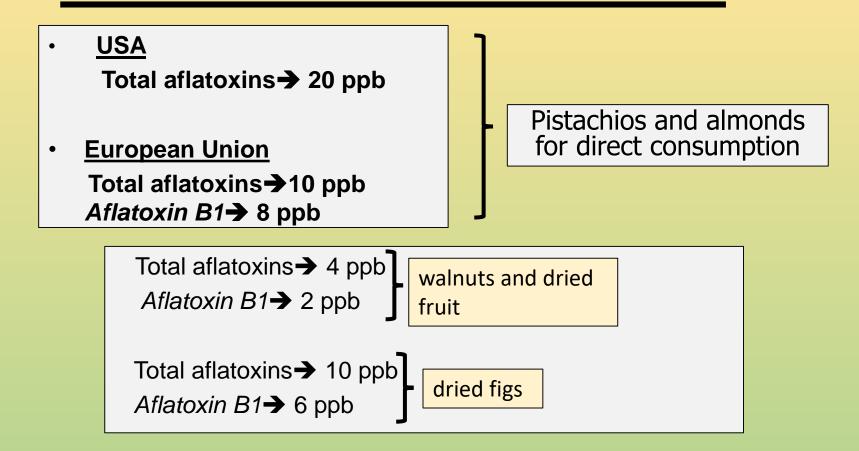


Aspergillus flavus

Aspergillus parasiticus

Aflatoxins: **B**₁, B₂, G₁, G₂,

Regulatory limits for aflatoxins



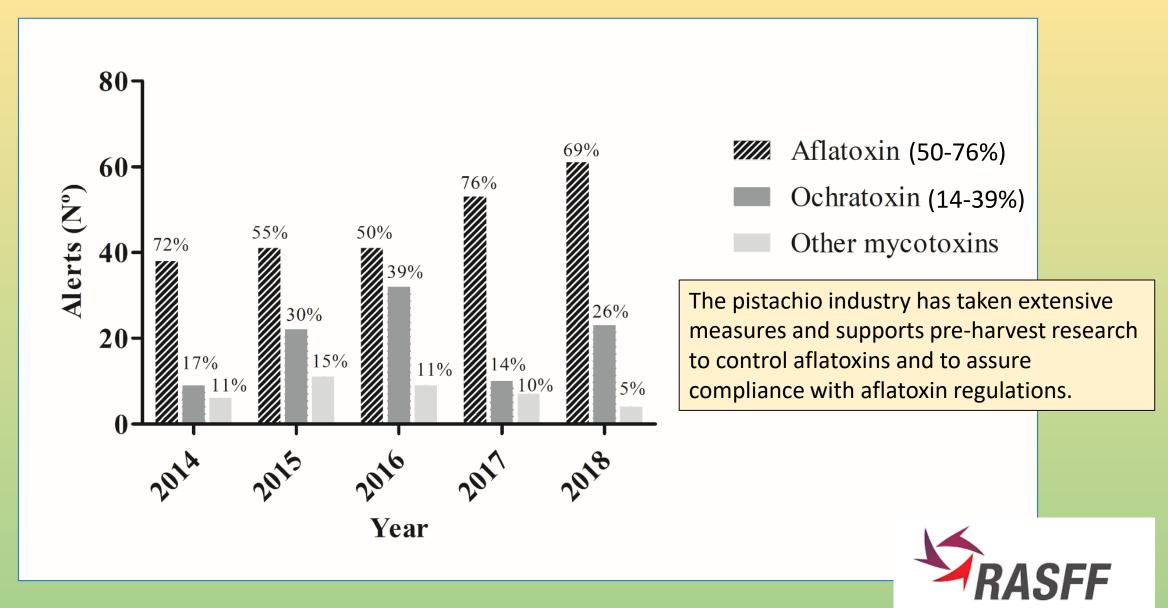
Regulatory limits for Ochratoxins (OTA)

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

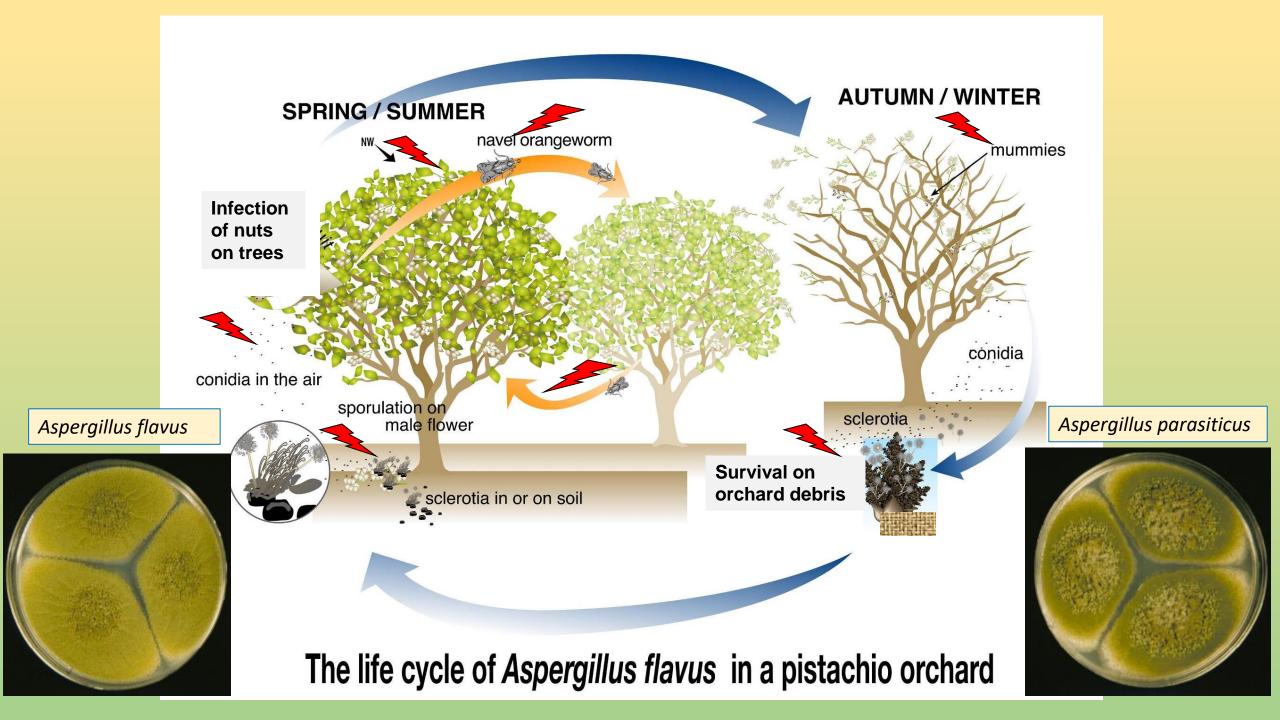
- OTA limit for dried vine fruit (currants, raisins, sultanas): 8 ppb
- OTA limit for other dried fruit: 2 ppb



Percent <u>Rapid Alerts</u> on aflatoxins, ochratoxins, & other mycotoxins in various crops



Rapid Alert System for Food and Feed



Accomplishments of CPRB funded research over the years:

- Determined that Early Splits (ES) contribute a lot to aflatoxin contamination.
- Determined the <u>special features</u> 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

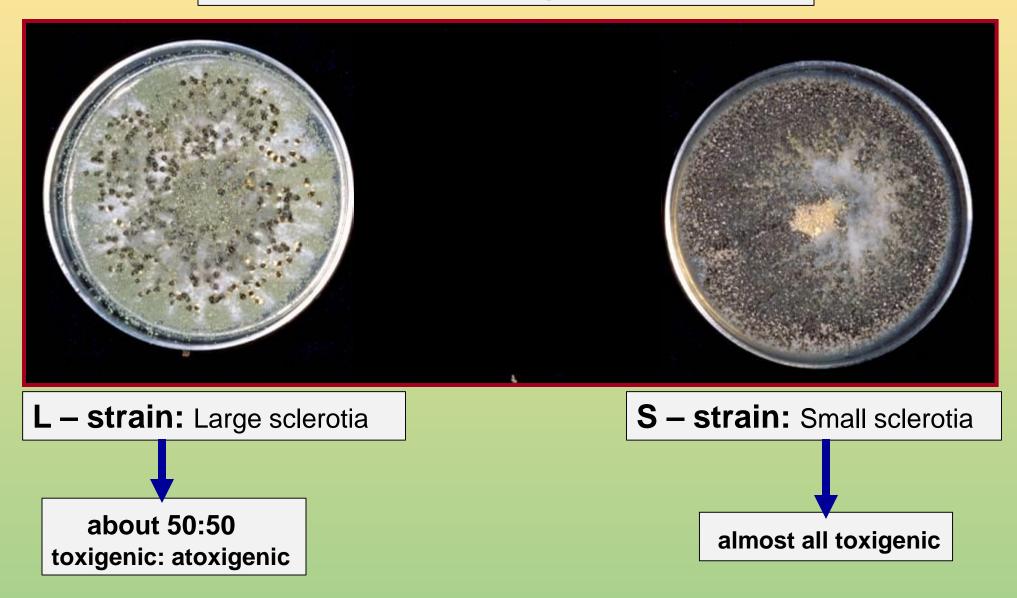
 A major accomplishment: The biological control of aflatoxins by using atoxigenic strains of Aspergillus flavus

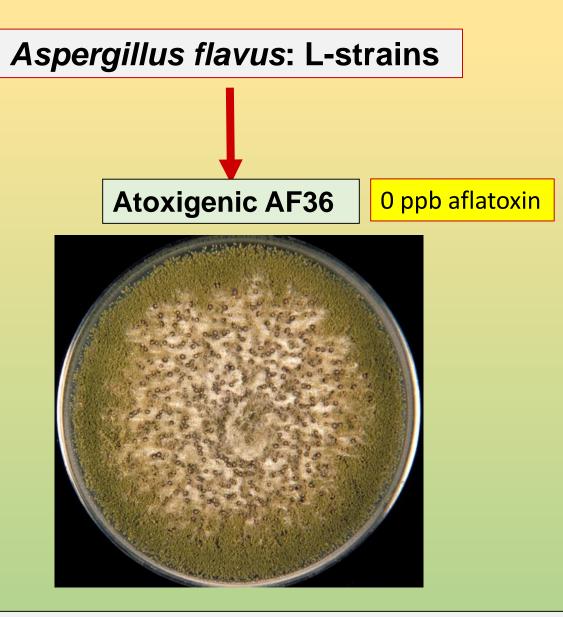
• During the EUP years we showed an average of 40% reduction in aflatoxin contaminated samples and in subsequent years a variable reduction.

<u>Definition:</u> Atoxigenic = a strain that does not produce aflatoxin

Sometimes casually people refer to these as "atoxigenics"

Strains of Aspergillus flavus





Rationale: Increase the <u>atoxigenic strain population</u> in the orchard to <u>displace the toxigenic strain</u> population.



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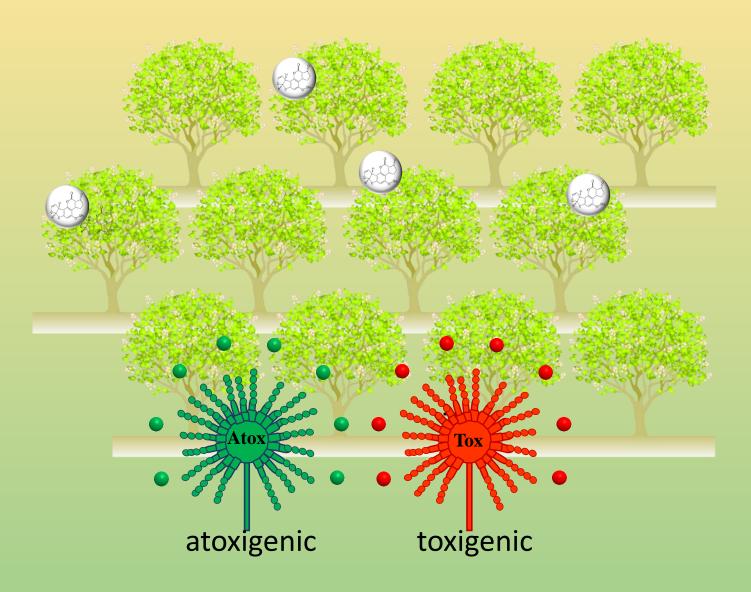
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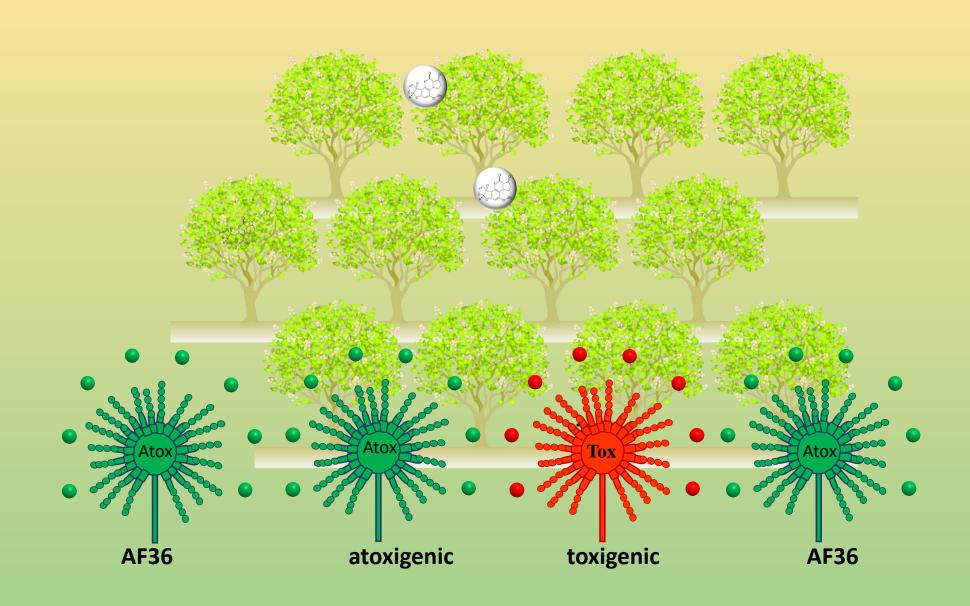
AF36 Inoculum

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

Non-treated orchard

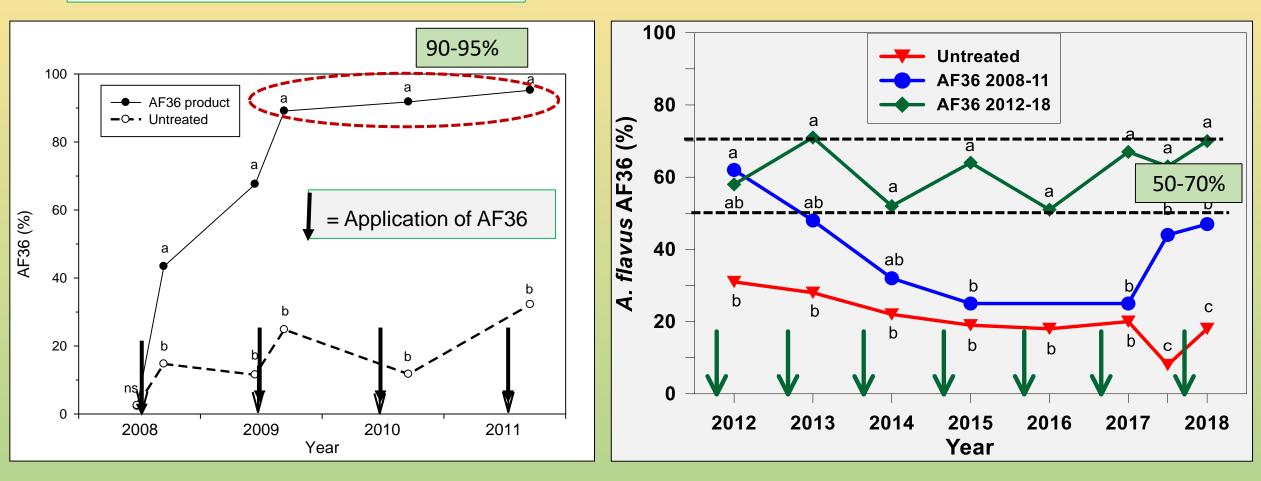


Treated orchard with the AF36 Prevail®



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

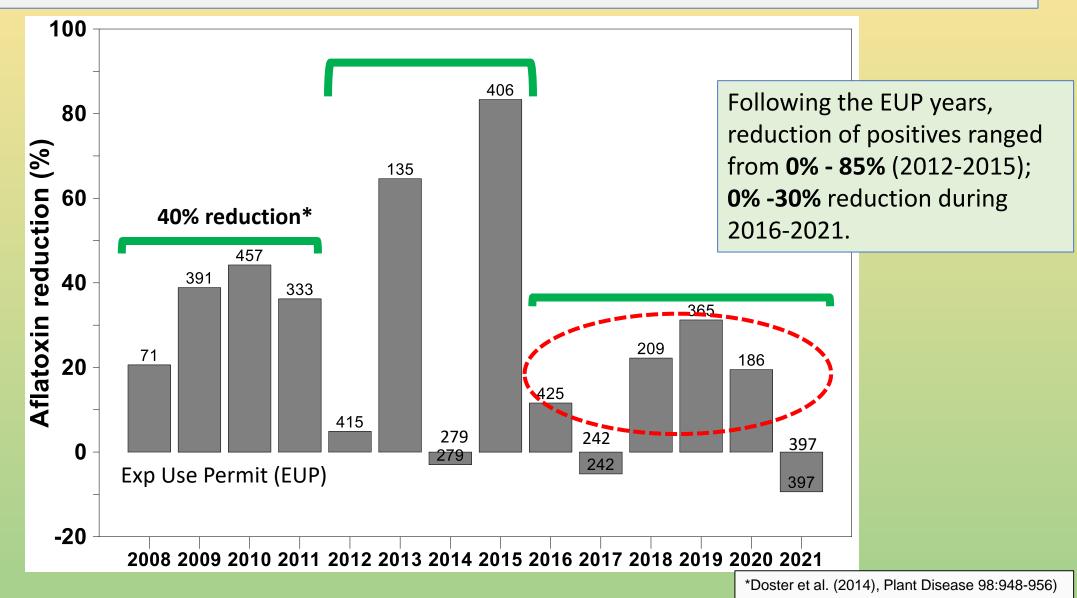
The AF36 displaced the toxigenic strains in soil only by 50 - 70%



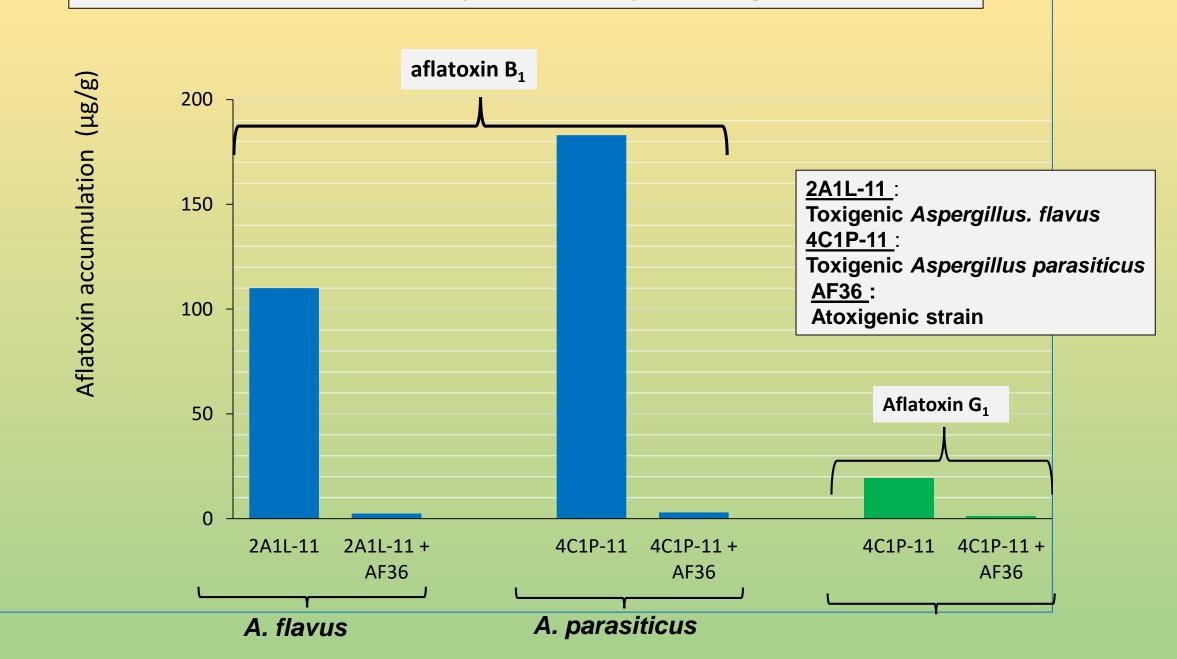
2008 - 2011

2012 - 2018

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



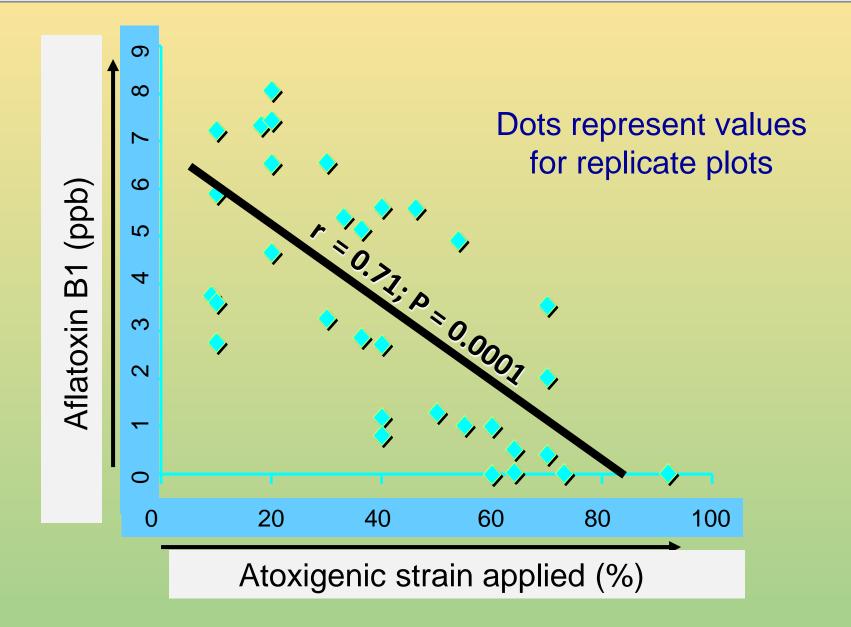
The AF36 reduced aflatoxin production by the toxigenic strains



Application in cotton fields: Humid environment under the plants good sporulation of the product



Aflatoxin in cottonseed versus strain AF36 incidence



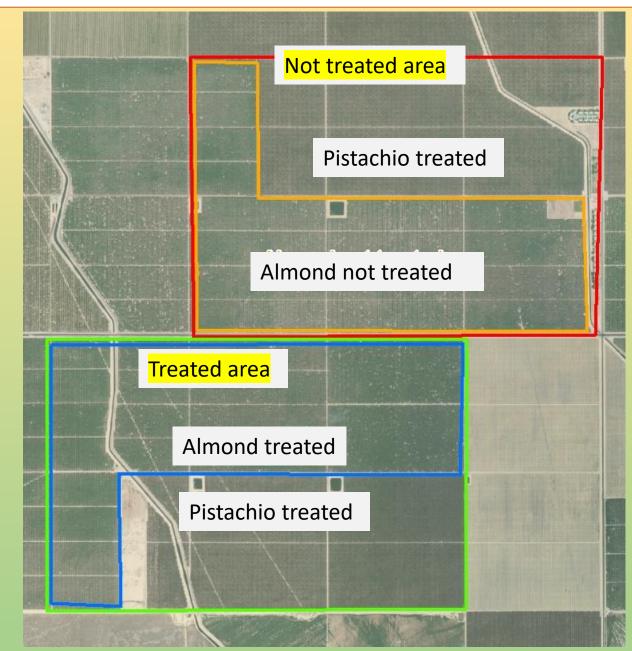
Slide of Peter Cotty



Excellent reduction in aflatoxin contamination by using biocontrol products in Ghana

| | | Total aflatoxin concentration (ppb) | | | |
|-------------|-----------|-------------------------------------|-------------|-------|-------------|
| Region | Treatment | 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:

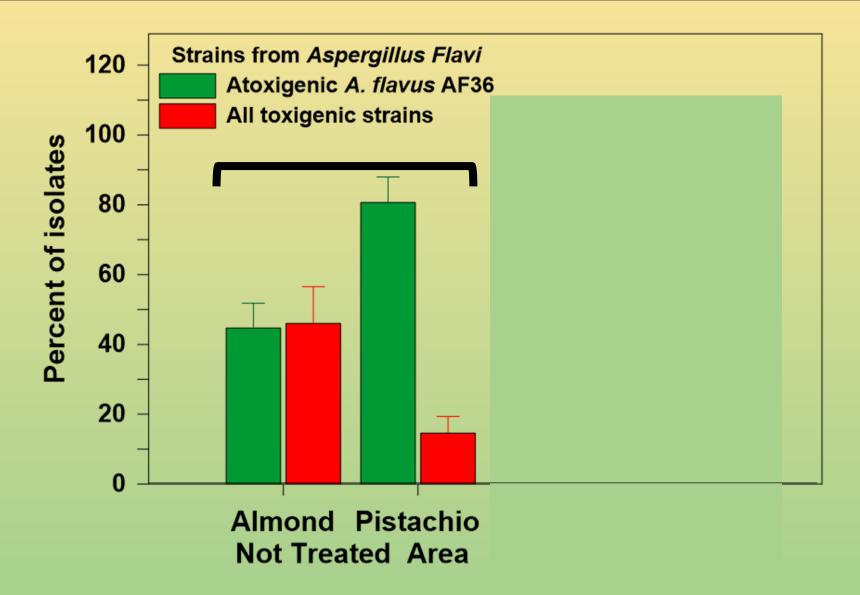
In the not treated area

pistachio orchards were treated; almond orchard were not treated.

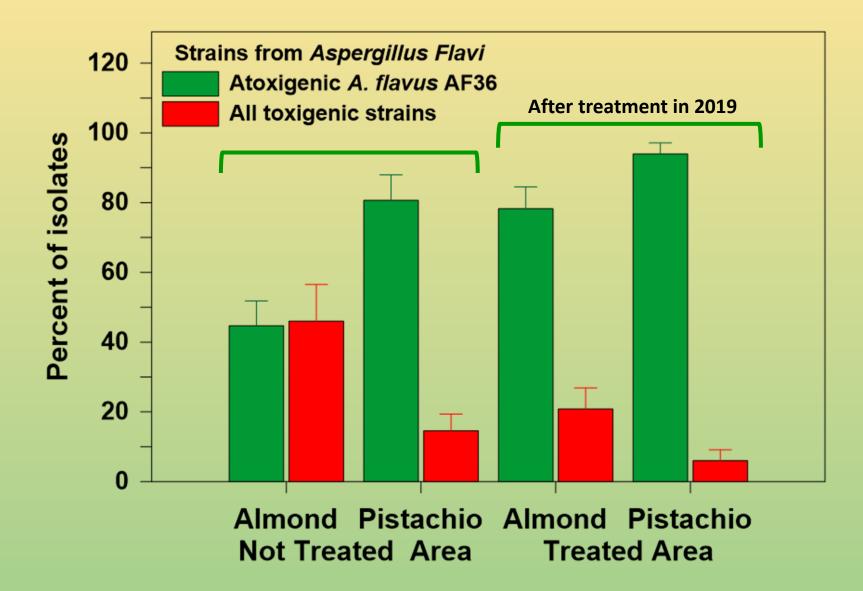
In the treated area

both almond and pistachio orchards were treated.

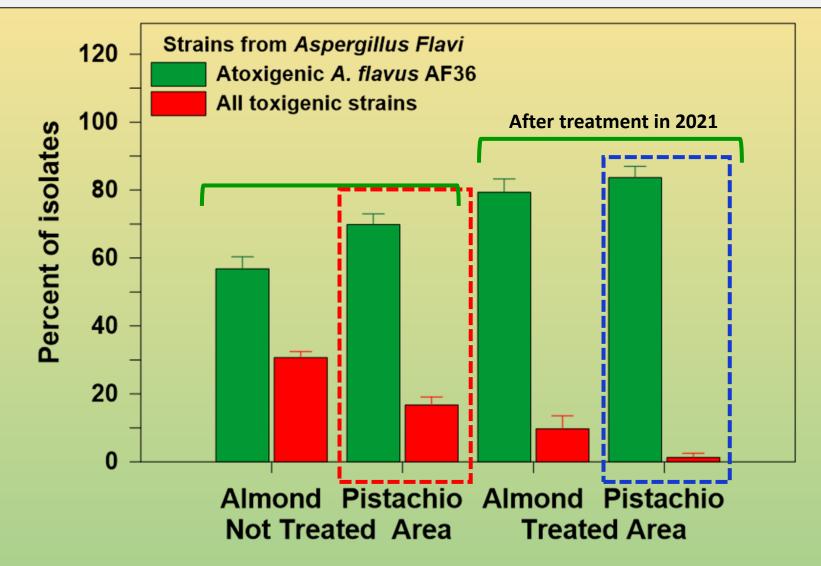
Area-wide, long-term treatment with AF36 Prevail atoxigenic strain in almond and pistachio orchards (2019)



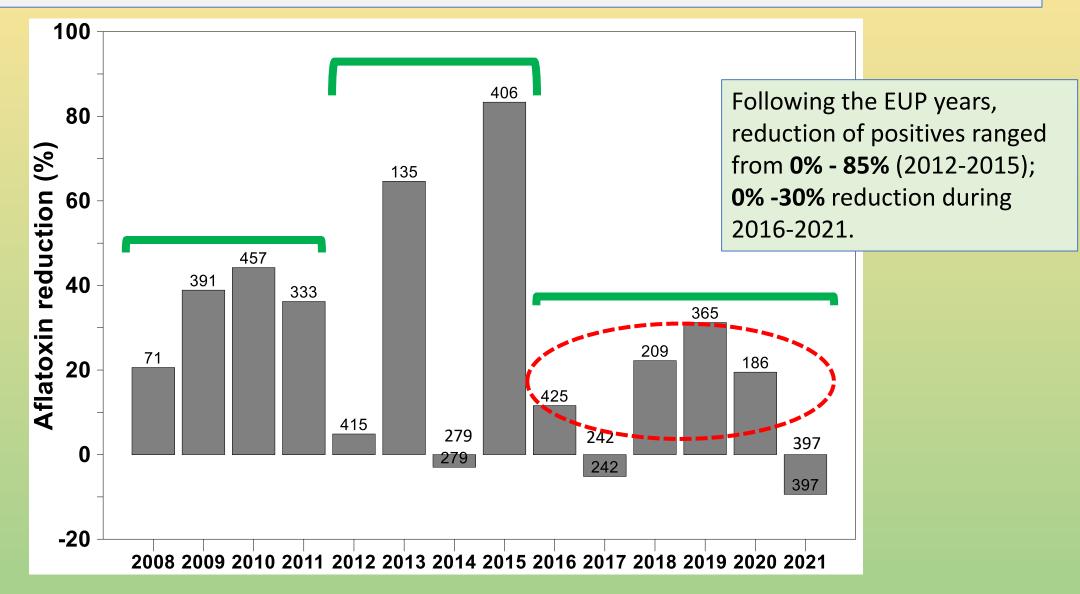
Area-wide, long-term treatment with AF36 Prevail atoxigenic strain in almond and pistachio orchards (2019)



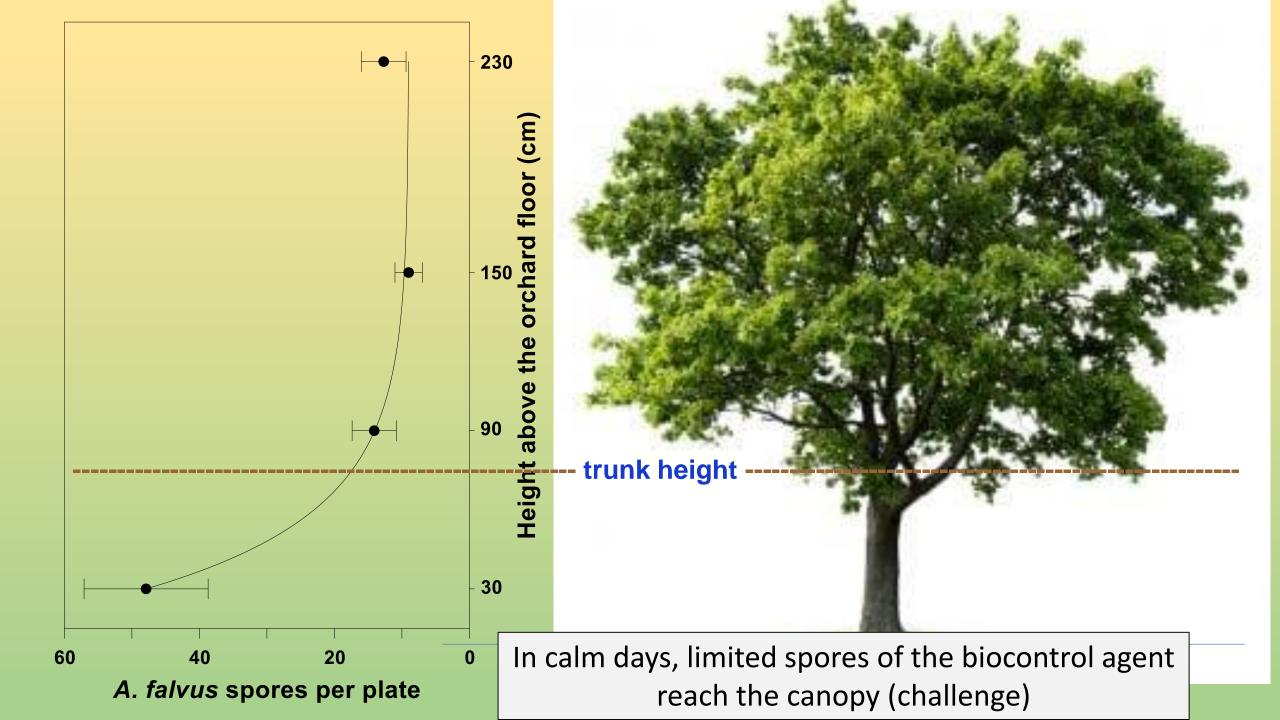
Area-wide, long-term treatment with AF36 Prevail[®] atoxigenic strain in almond and pistachio orchards (2021)



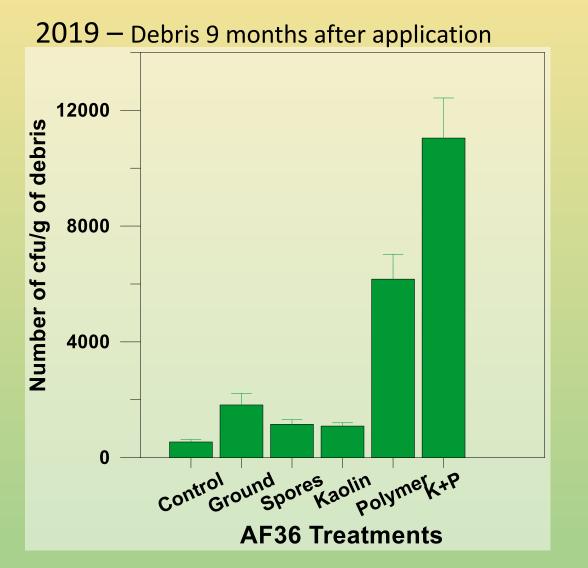
Reduction of aflatoxin contaminated library samples after treatment with AF36 or AF36 Prevail

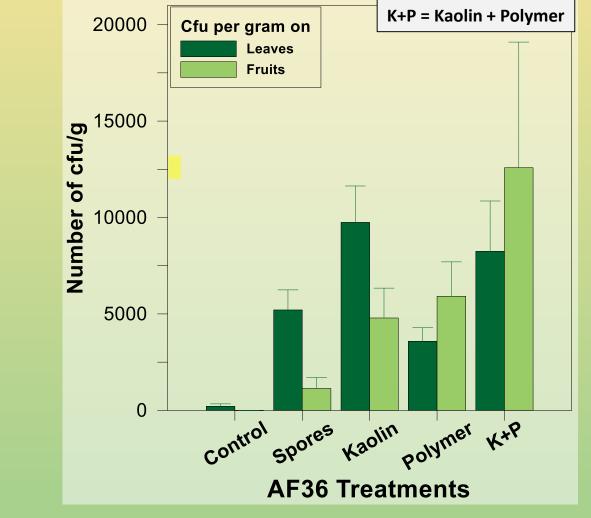






Evaluate the microflora /colonization of NOW-damaged, ES, normal nuts as affected by the method of application of AF36 (ground or canopy)





2021 – One month after application



Aspergillus flavus AF36 Prevail Arizona Cotton Research and Protection Con AF36 Prevail® "for growers by growers"

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PRODUCT INFORMATION

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In the role acceder of **Arizona Cotton Research** and Protection Council

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

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

| Afla | a-Gua | ard® GR | |
|--|----------|------------|--|
| | syngent | :a. | |
| For agricultural use to displa aflatoxin-producing fungi in a peanuts, pistachios, and con | almonds, | | |
| Other Instantients | | | |

2000 pounds **Net Weight**



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 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] |

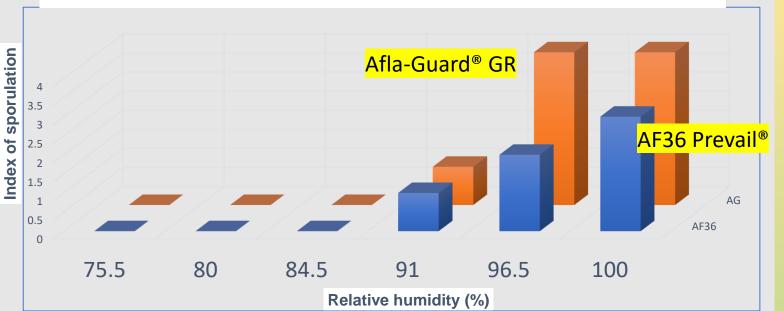
List of biocontrol products registered for commercial use (table continued)

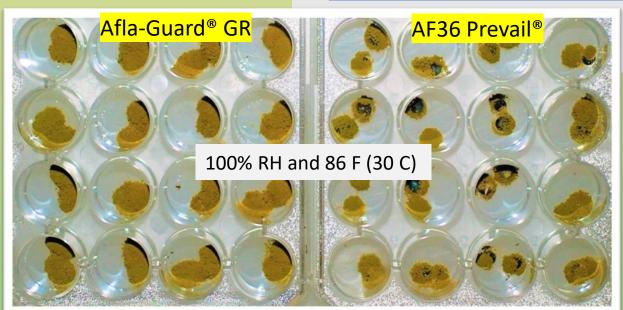
| Aflasafe TZ01 | TMS199-3, TMH104- 9, TGS364-2, TMH 30-8 | IITA | Tanzania | Maize and groundnut | Unpublished registration document |
|------------------------------|---|------|------------|------------------------|---|
| Aflasafe TZ02 | TMS64-1, TGS55-6, TMS205-5, TMS137-3 | IITA | Tanzania | Maize 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) ₂ SO ₄ | 80.0 | 78.8 |
| KCI | 84.5 | 82.6 |
| KNO ₃ | 91.0 | 90.2 |
| K ₂ SO ₄ | 96.5 | 95.8 |
| H ₂ O | 100 | 99.6 |

Comparison of sporulation under different relative humidity





AF36 AG

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

Afla-Guard[®] GR

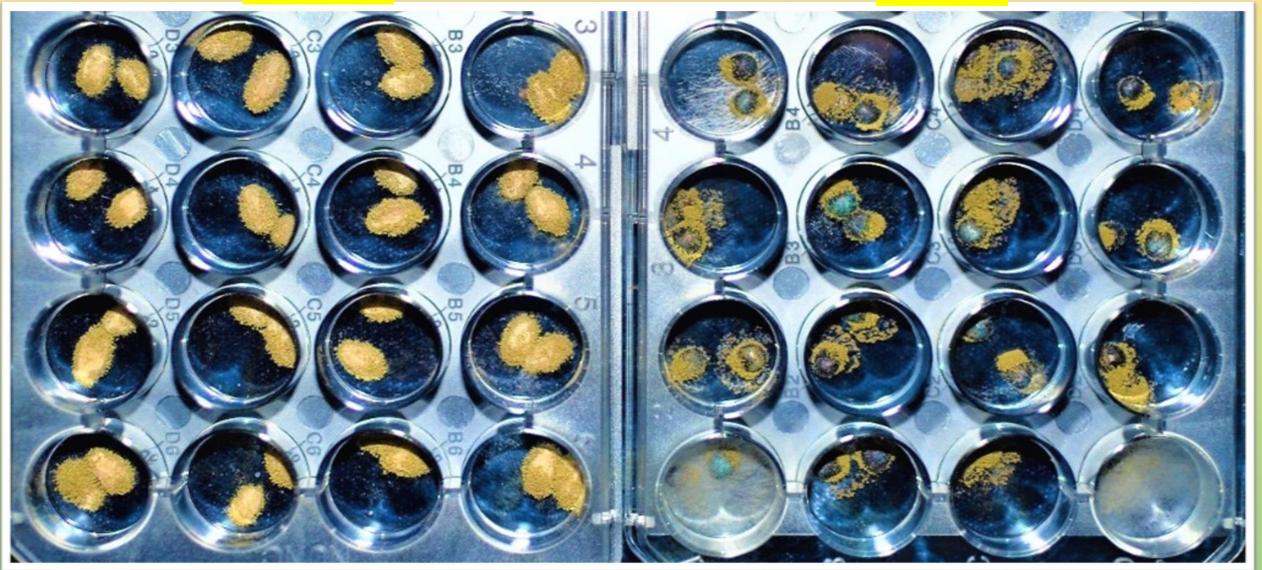
AF36 Prevail[®]



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

Afla-Guard[®] GR

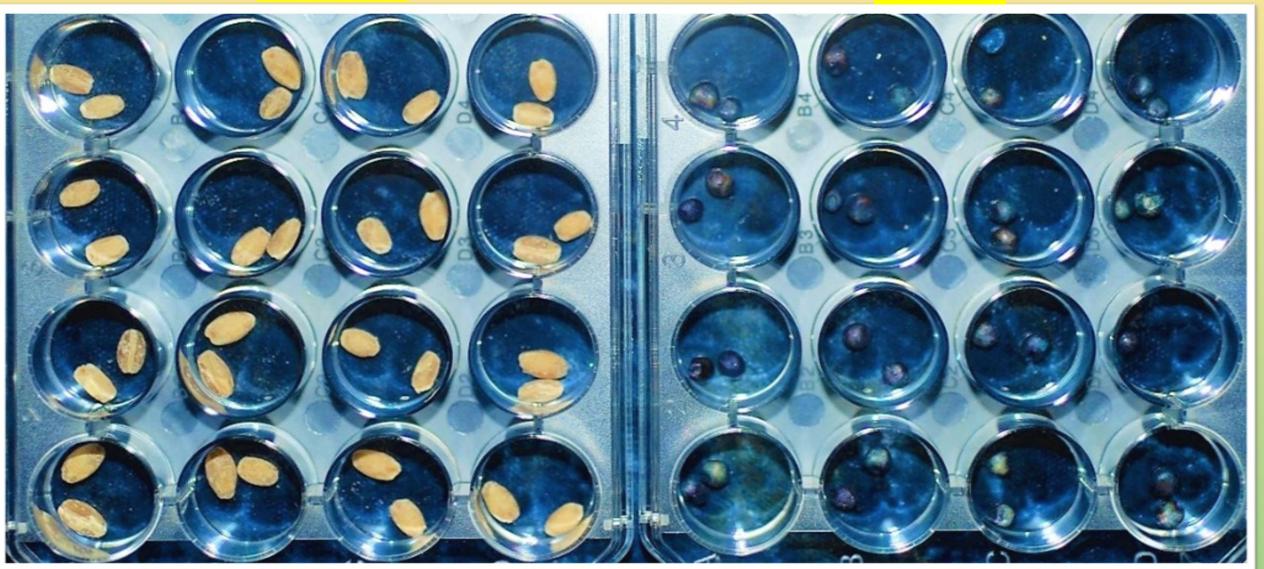
AF36 Prevail[®]



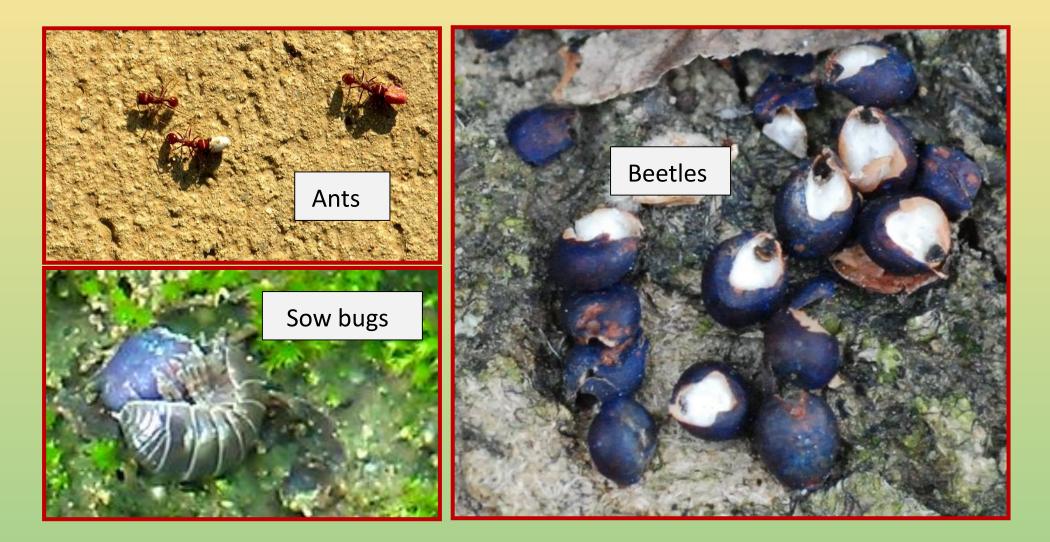
Sporulation after a week incubation at 80% RH and 86 °F (30 °C)

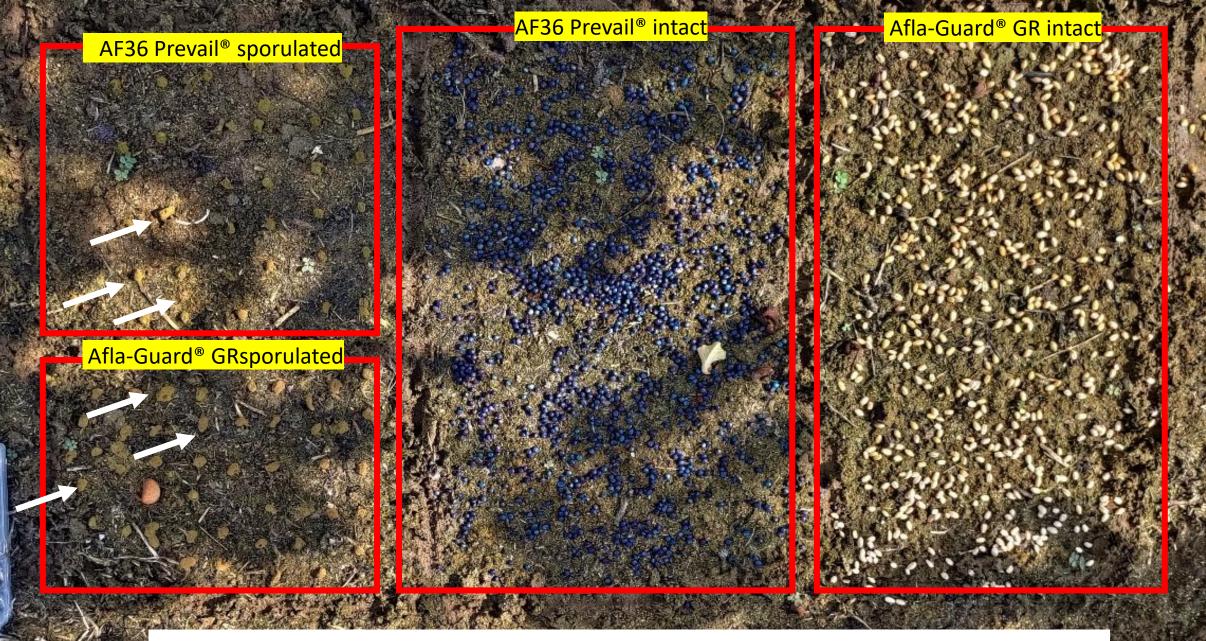
Afla-Guard[®] GR

AF36 Prevail[®]



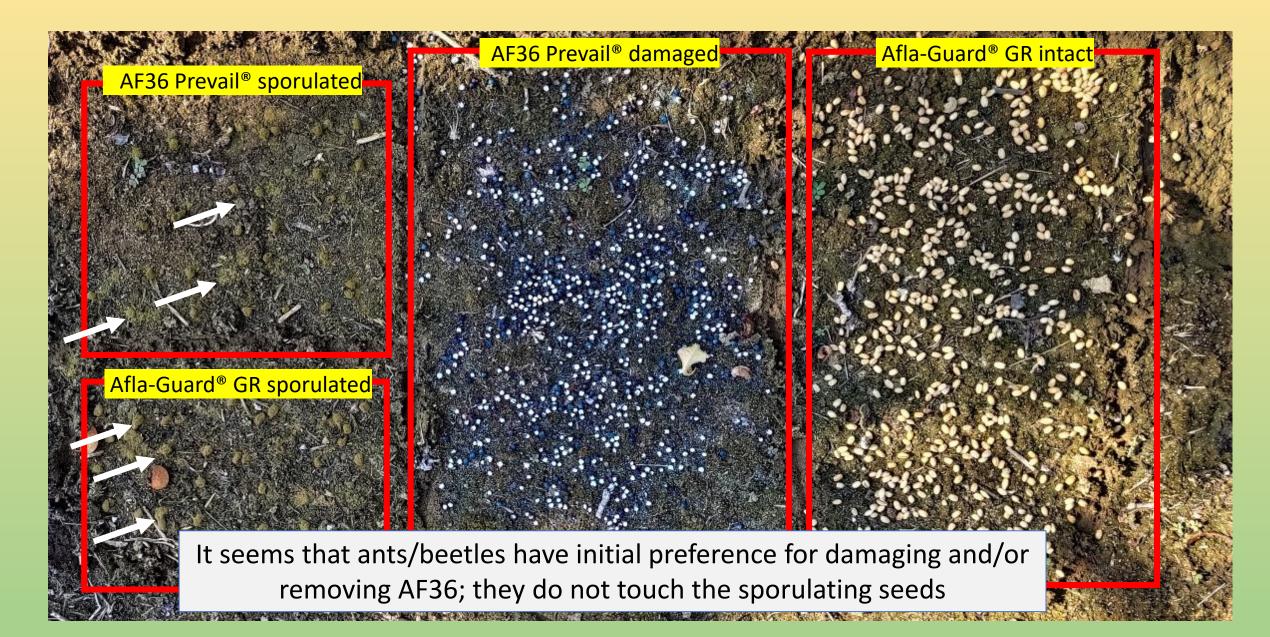
A challenge is the predation of seeds by insects, birds, and decay by other fungi; reduction of inoculum will reduce spore density



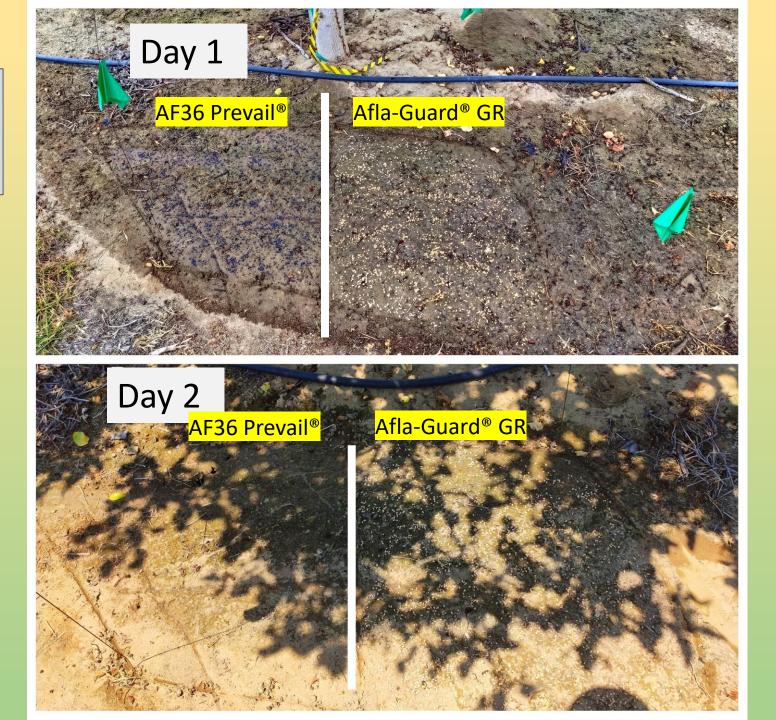


Predation comparison of AF36 Prevail with Afla-Guard GR in the field

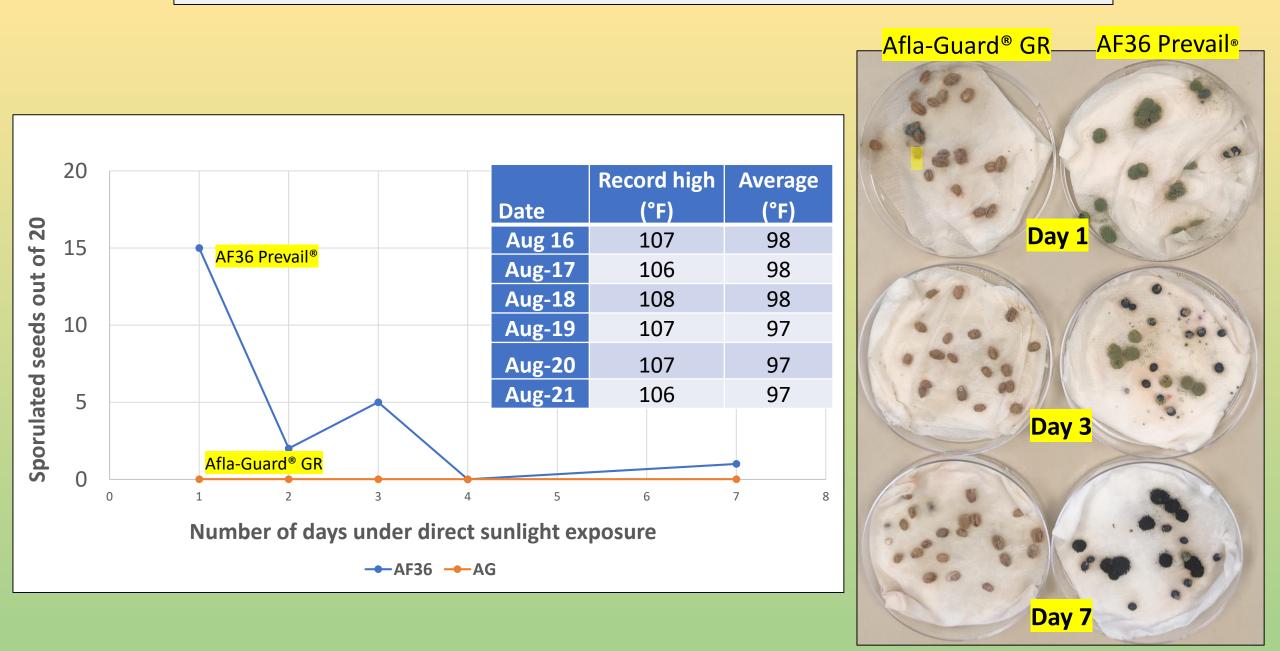
Predation of the two products 24 hours after application in the field



Ants removed all the AF36 Prevail[®] seeds within 24 hours



Comparison of sporulation under direct sunlight exposure



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 <u>no negative effects</u> 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

- 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).



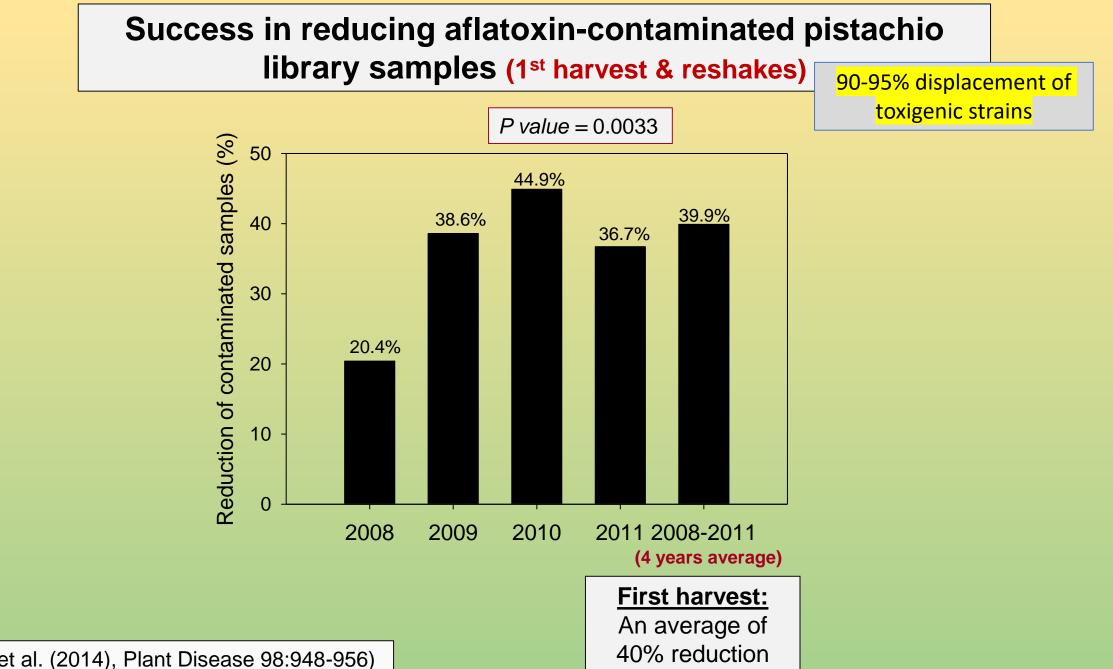
Special thanks to my lab crew

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

Thank you

Contacts: <u>Themis J. Michailides</u> <u>tjmichailides@ucanr.edu</u> 559-646-6546; twitter @PistachioDoctor

Ramon Jaime rjaime@ucanr.edu 559-646-6582

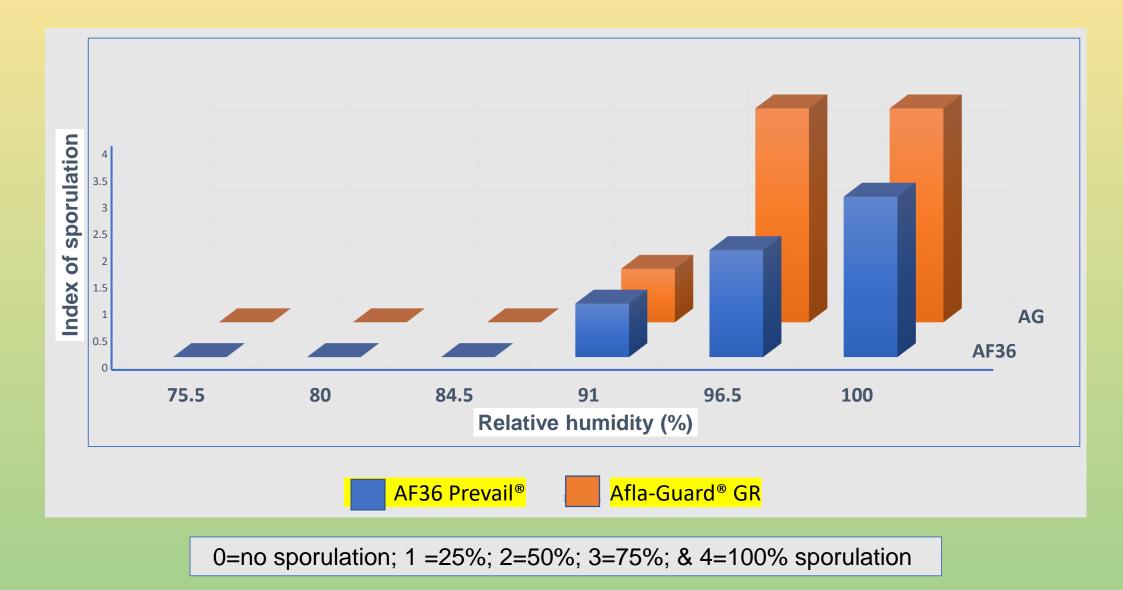


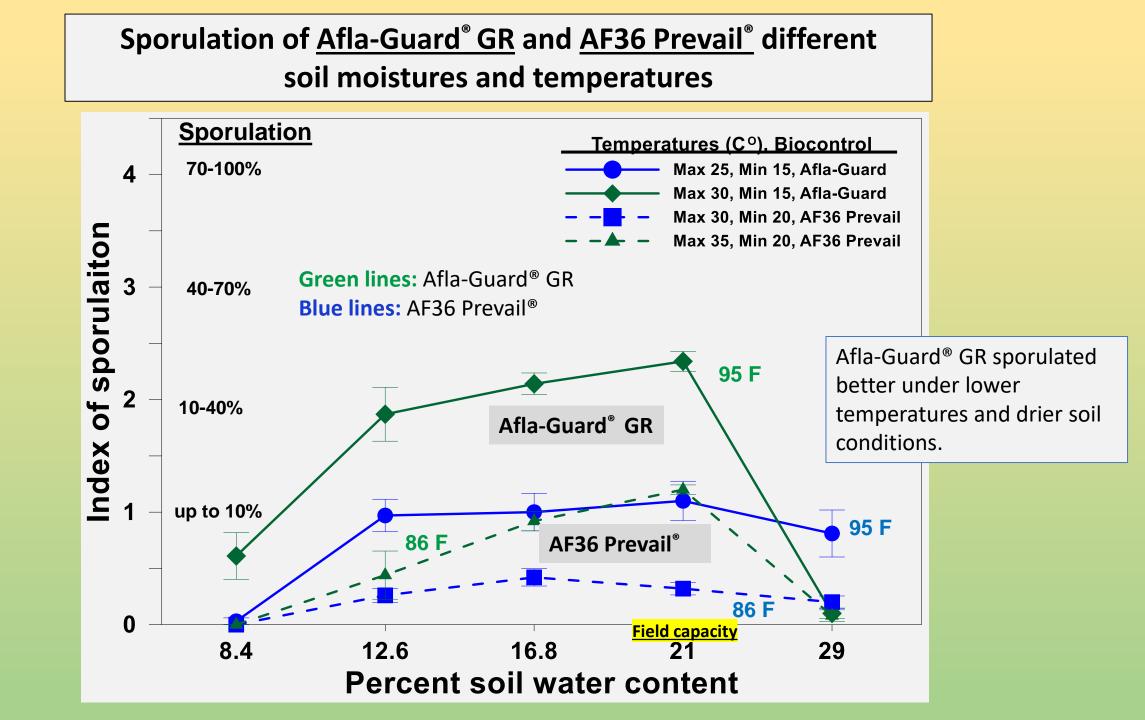
(Doster et al. (2014), Plant Disease 98:948-956)

Excellent reduction in aflatoxin contamination by using biocontrol products in Ghana

| | | Total aflatoxin concentration (ppb) | | | |
|-------------|-----------|-------------------------------------|-------------|-------|-------------|
| Region | Treatment | 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)





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

