

Advances in Managing Alternaria & Botryosphaeria Blights; and Controlling Aflatoxin with AF36



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Pistachio Day, 18 Jan 2012

Alternaria Sp. A Pathogen Of Pistachio?

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Introduction

On June 4, 1985, G. S. Sibbett (Farm Advisor, Tulare County) provided samples of pistachio fruits with small black surface lesions. Isolations from surface sterilized lesions showed 62% *Alternaria alternata* (Table 1). Another sample sent on June 13 by R. Stiefvater (Pacific Producers of California, Madera) also showed small black lesions, not typical of *Botryosphaeria blight*. *A. alternata* was also isolated from this sample. Isolations from various plant parts showed a high incidence of *Botryosphaeria blight* on leaves, buds,

purpose of this study was to determine whether *A. alternata* is pathogenic to pistachio.

Materials and Methods

Cluster inoculations. Kerman tree clusters (8-10 fruit/cluster) were surface sterilized with 400 μ l a.i. chorine (household bleach, 5.25% sodium hypochlorite) for 3 min, allowed to dry, placed over wire screens in plastic containers, and sprayed to runoff with a 1×10^5 conidia/ml suspension. Spore suspension was prepared from a 30-day-old culture grown on acidified potato-dextrose agar. The same experiment was made on abscised fruit from both 'Kerman' and 'Tahiti' pistachios. They were sprayed with a 1×10^5 , 2.5×10^5 , and 5×10^5 conidia/ml suspension of *Alternaria alternata*.

Leaf inoculations. Leaves of 'Kerman' pistachio were surface sterilized with 400 μ l bleach, allowed to dry, placed in plastic containers, and sprayed with a 1×10^5 conidia/ml suspension. The plastic containers were sealed and stored at $25 \pm 1^\circ \text{C}$. Leaves were sprayed with sterile water, shoots were covered with both sides with a brown paper bag for 3 days, and were recorded 3, 10, and 20 days after inoculation.

Results and Discussion

The *Alternaria* sp. from various sample sources collected in November (Table 1) showed characteristics of *A. alternata* (Fries). The spores resembled those of *A. alternata* (Fries) and were described by Auct., which is *A. alternata* (Fries). Two clusters of *A. alternata* were isolated from the leaves of 'Kerman' pistachio.

...ance by crows and scrub

Acceptance

Poor

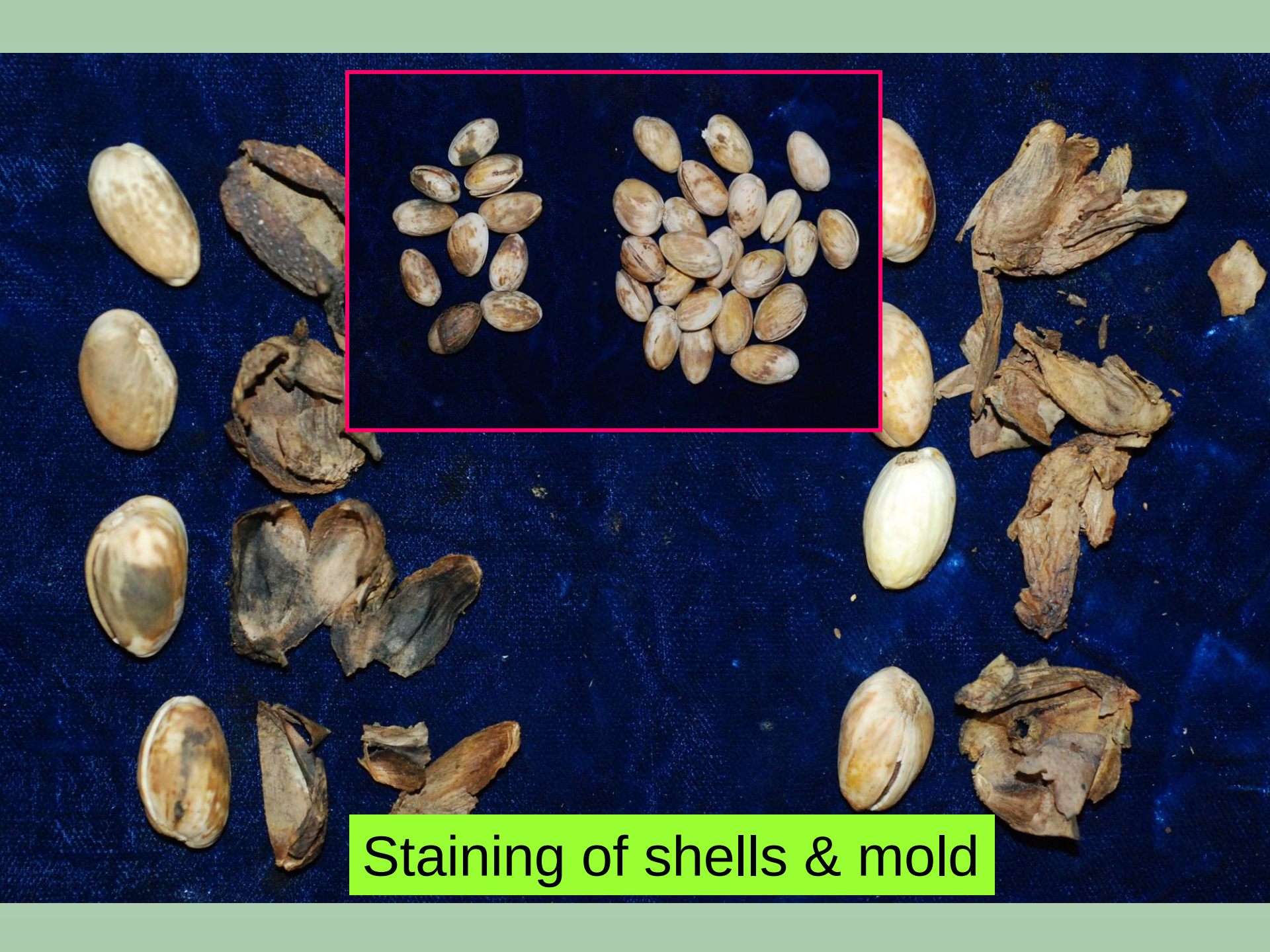
Poor

Alternaria late blight (severe leaf symptoms)



Alternaria late blight (severe leaf symptoms)





Staining of shells & mold



Management of Alternaria late blight:

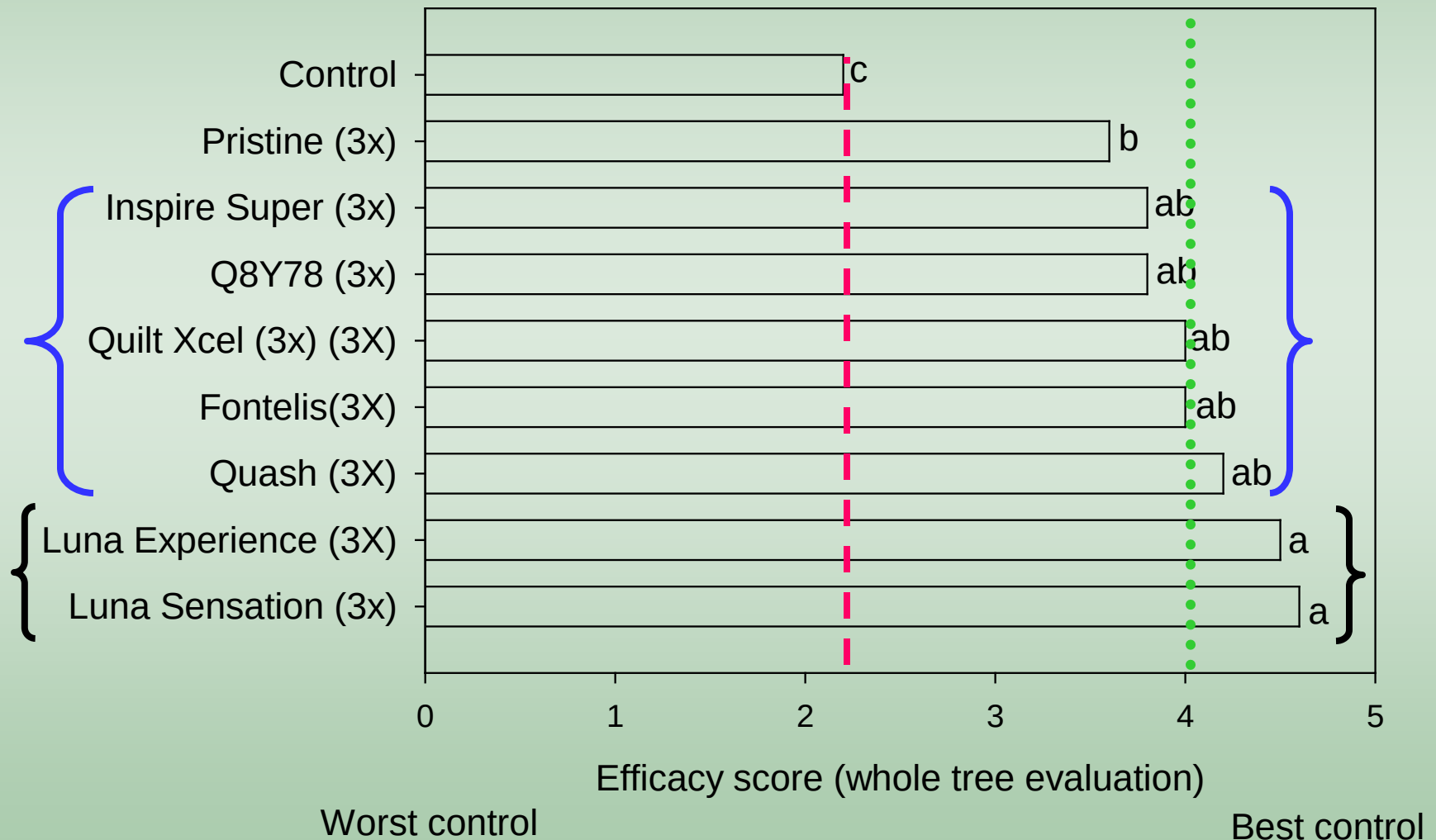
- **Cultural practices** (manage irrigation, improve water infiltration, buried drip, hedge trees to increase orchard ventilation, no cover crops, etc.)
- **Chemical control** (apply fungicides)...**difficult.**
- **Integrated disease control** (use both cultural & chemical control).....**the best effect!**

Alternaria late blight management with buried drip irrigation





Most effective fungicides against Alternaria (Kearney, 2011)



Fungicides registered for Alternaria late blight

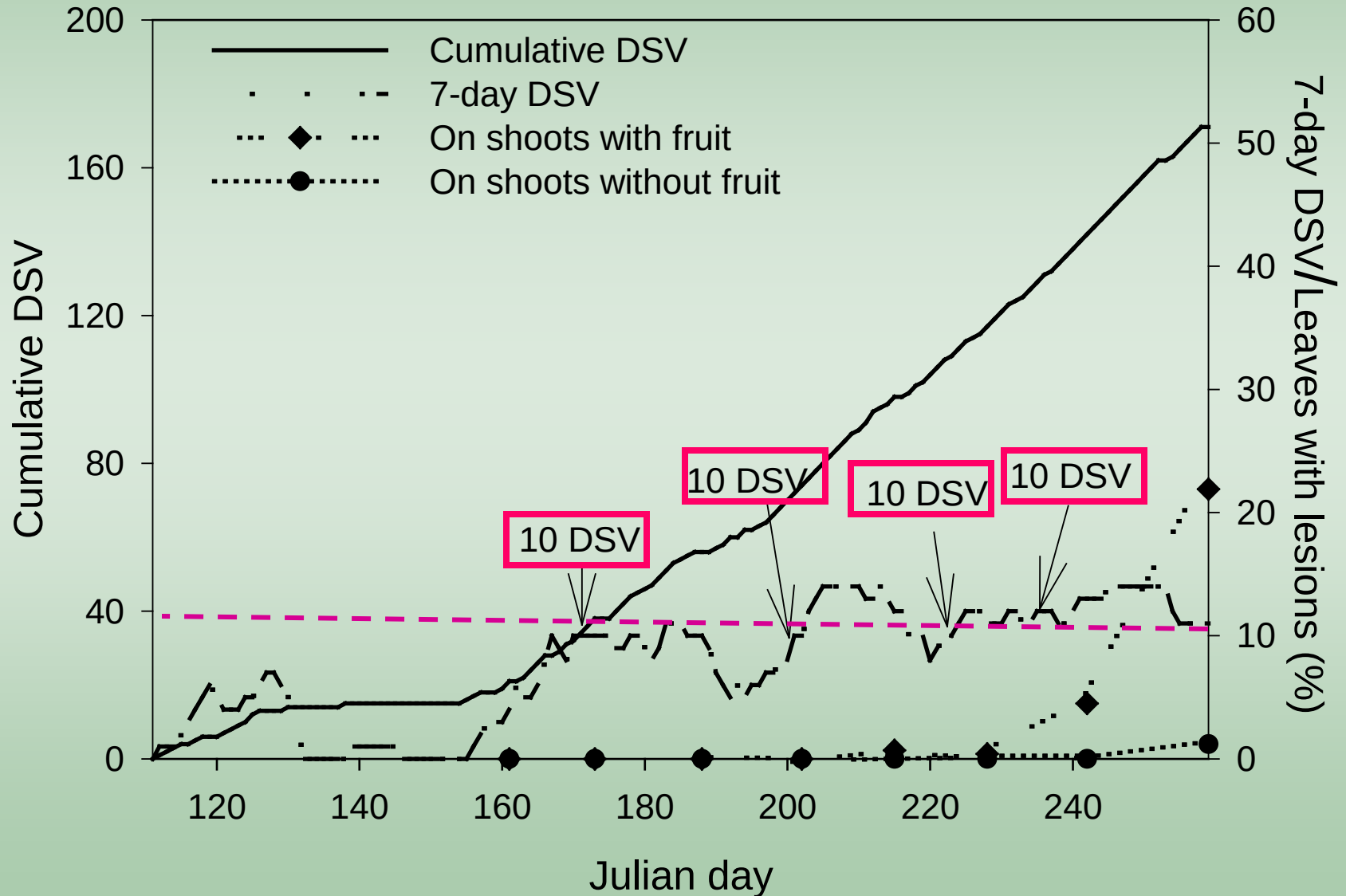
Fungicide	Active ingredient	Efficacy
Abound	Azoxystrobin	++
Adament	Trifloxystrobin+tebuconazole	+++
Bravo	chlorothalonil	++
Bumper/Tilt	propiconazole	+++
Cabrio	pyraclostrobin	+++
Gem	trifloxystrobin	+++
Quash	metconazole	+++(+)
Fontelis	penthiopyrad	++++
Pristine	boscalid+pyraclostrobin	+++(+)
Inspire Super	difenoconazole+cyrodinil	+++(+)
Quilt-Xcel	azoxystrobin+propiconazole	+++(+)
Scala	pyrimethanil	++
Switch	cyprodinil+fludioxonil	+++
Tebuzol	tebuconazole	+++
Copper	Copper	+



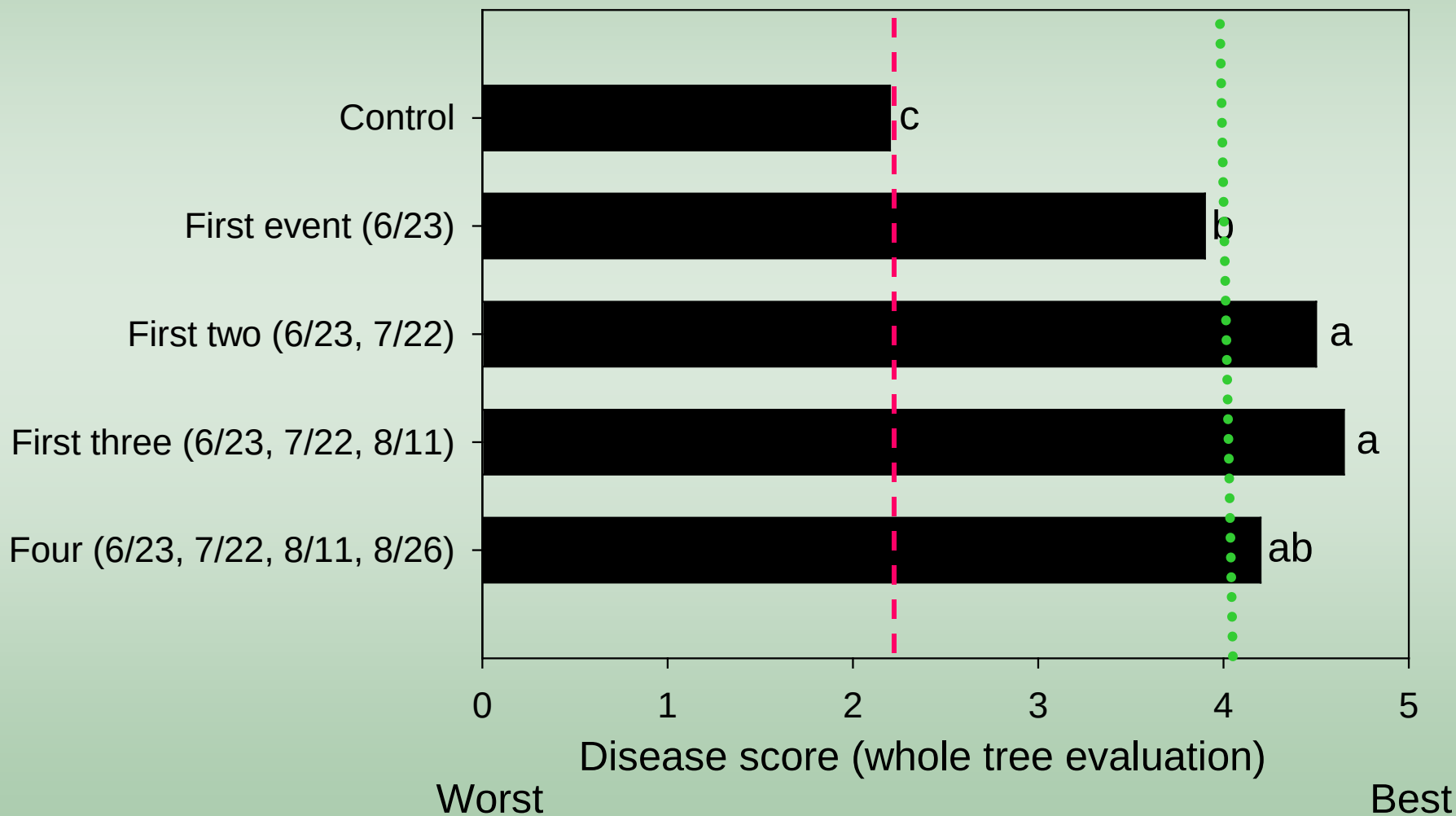
Disease Severity Values (DSV) for Alternaria blight of tomato

	<u>Leaf wetness hours</u> required to produce disease severity values (<u>DSV</u>) of:				
<u>Mean air temp °F</u>	0	1	2	3	4
55-63	0-6	7-15	16-20	21+	
→ 64-68	0-3	4-8	9-15	16-22	23+
69-78	0-2	3-5	6-12	13-20	21+
79-84	0-3	4-8	9-15	16-22	23+

Disease Severity Value Kearney 2011

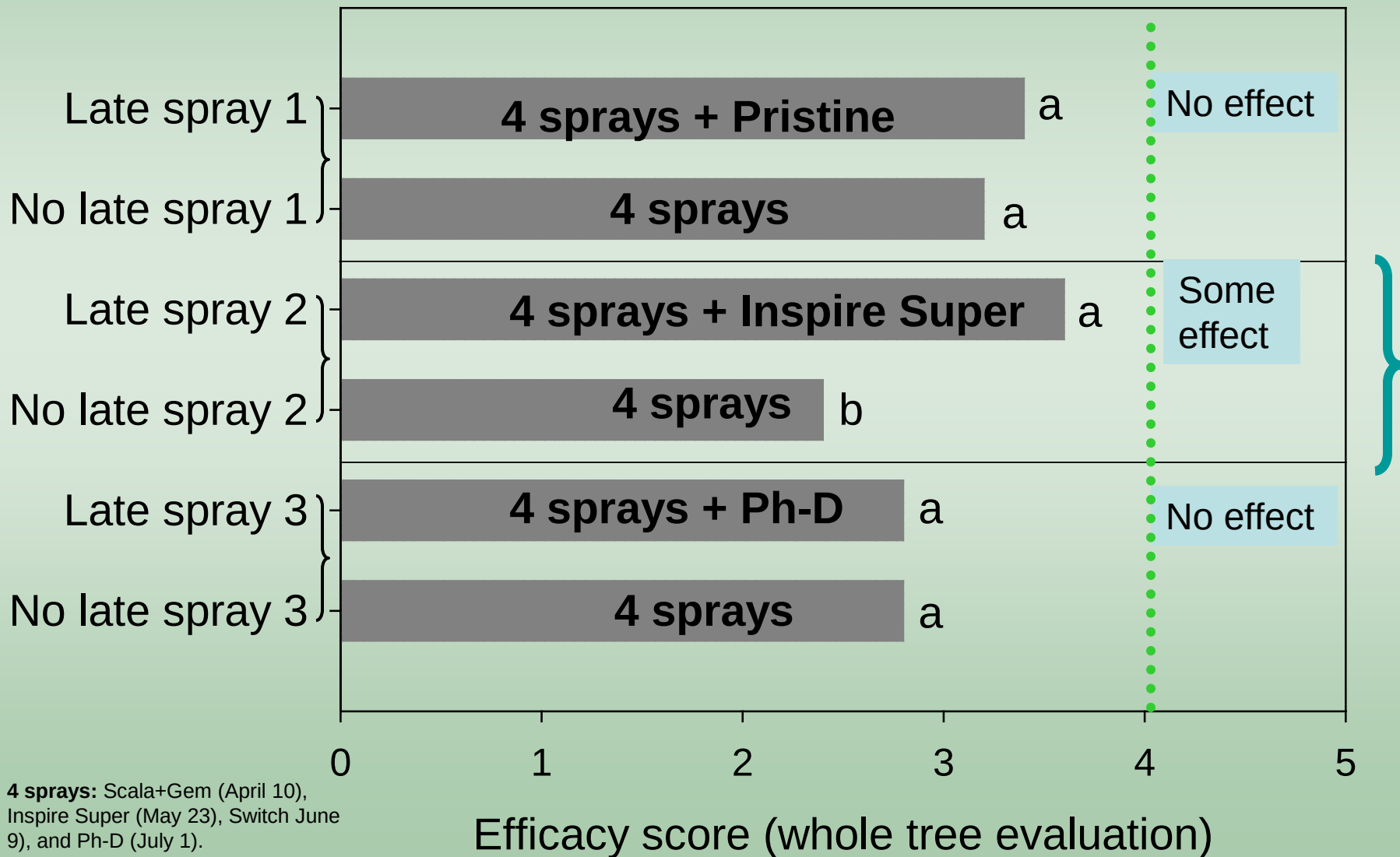


Spraying Luna Sensation after 10 unit DSV events – Kearney 2011



Efficacy of a late spray (3 Aug 2011)

(orchard with severe Alternaria blight)



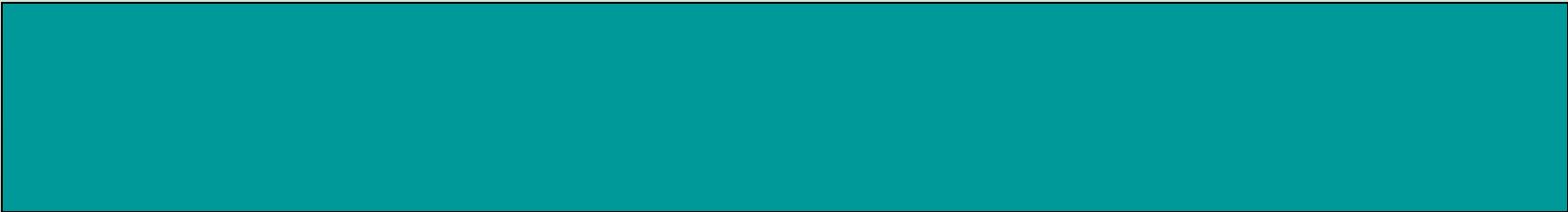


General conclusions:

- ✓ Although there are several fungicides now for the growers to choose, we still need stronger fungicides.
- ✓ Follow label recommendations, do not use reduced rates, rotate fungicides of different classes, and aim for **good coverage**.
- ✓ New fungicide registrations can provide alternatives to overcome the challenge of the *Alternaria* resistance.

PISTACHIO—Fungicide treatment timing

Disease	Dormant	April May	June	July	August
Alternaria	----	----	+++	+++	



Source: <http://www.ipm.ucdavis.edu>



Take-home message

(It is important to diagnose the disease in the orchard correctly)

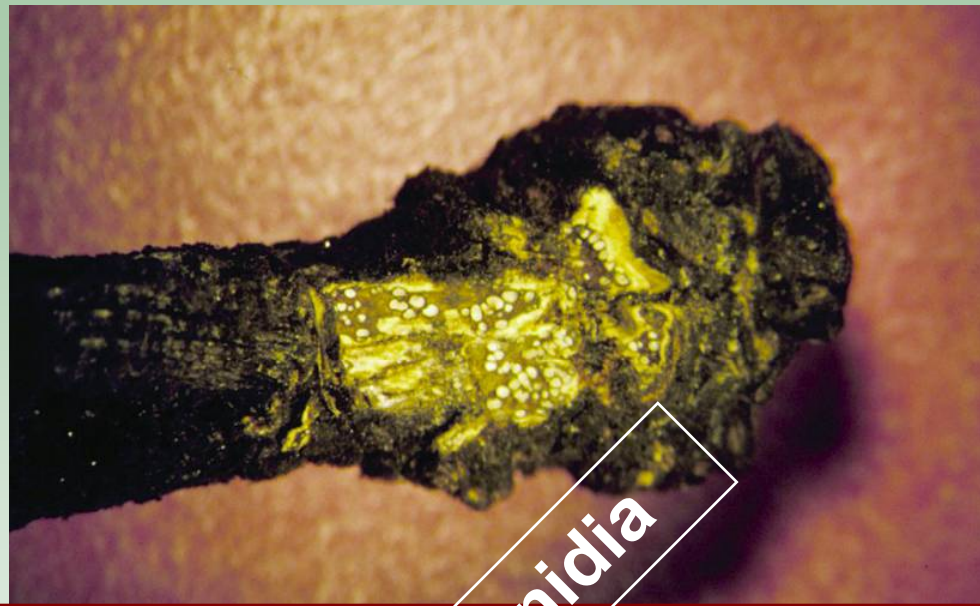
- Start sprays in early June and finish by end of July).
- One critical spray: end June /early July.
- Bloom sprays and August sprays are not effective. (The bloom/early-season sprays cannot be considered when you are trying to control Alternaria late blight.)

Managing Botryosphaeria blight





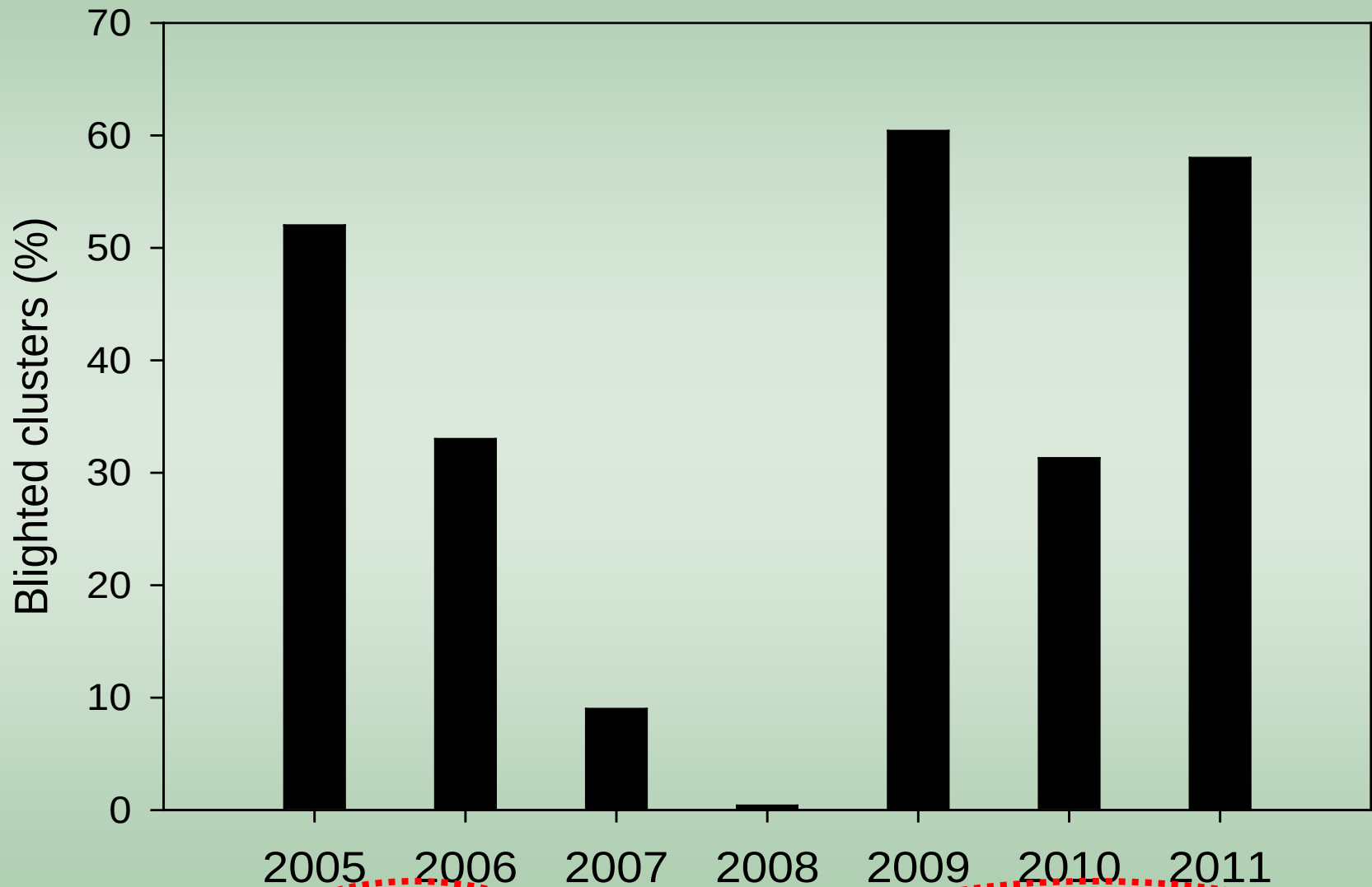
BOT cankers



BOT pycnidia



Botryosphaeria blight in unsprayed control (orchard in Glenn Co.)



Total rain



19"

19"

10"

14"

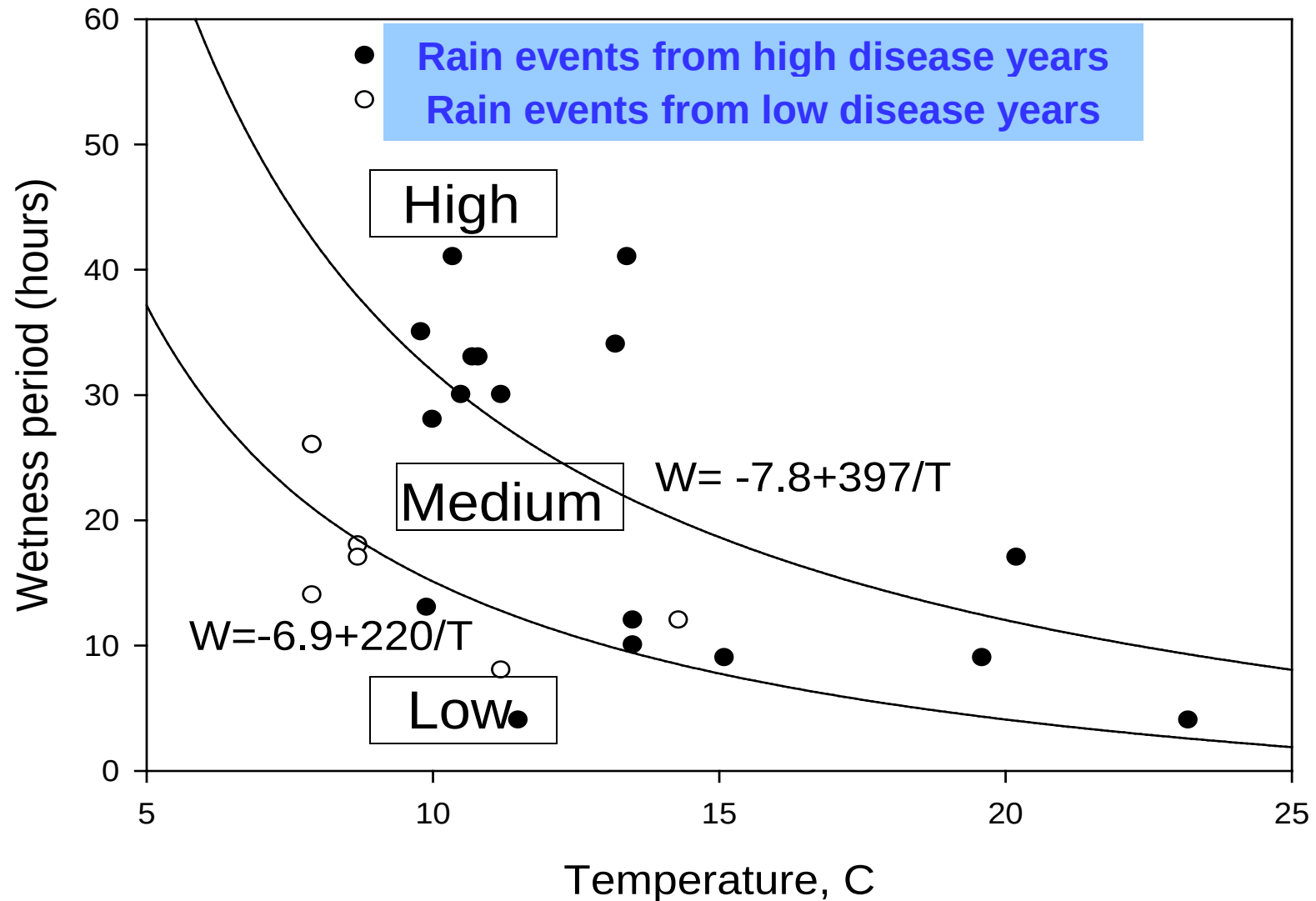
16"

24"

19"

MODEL

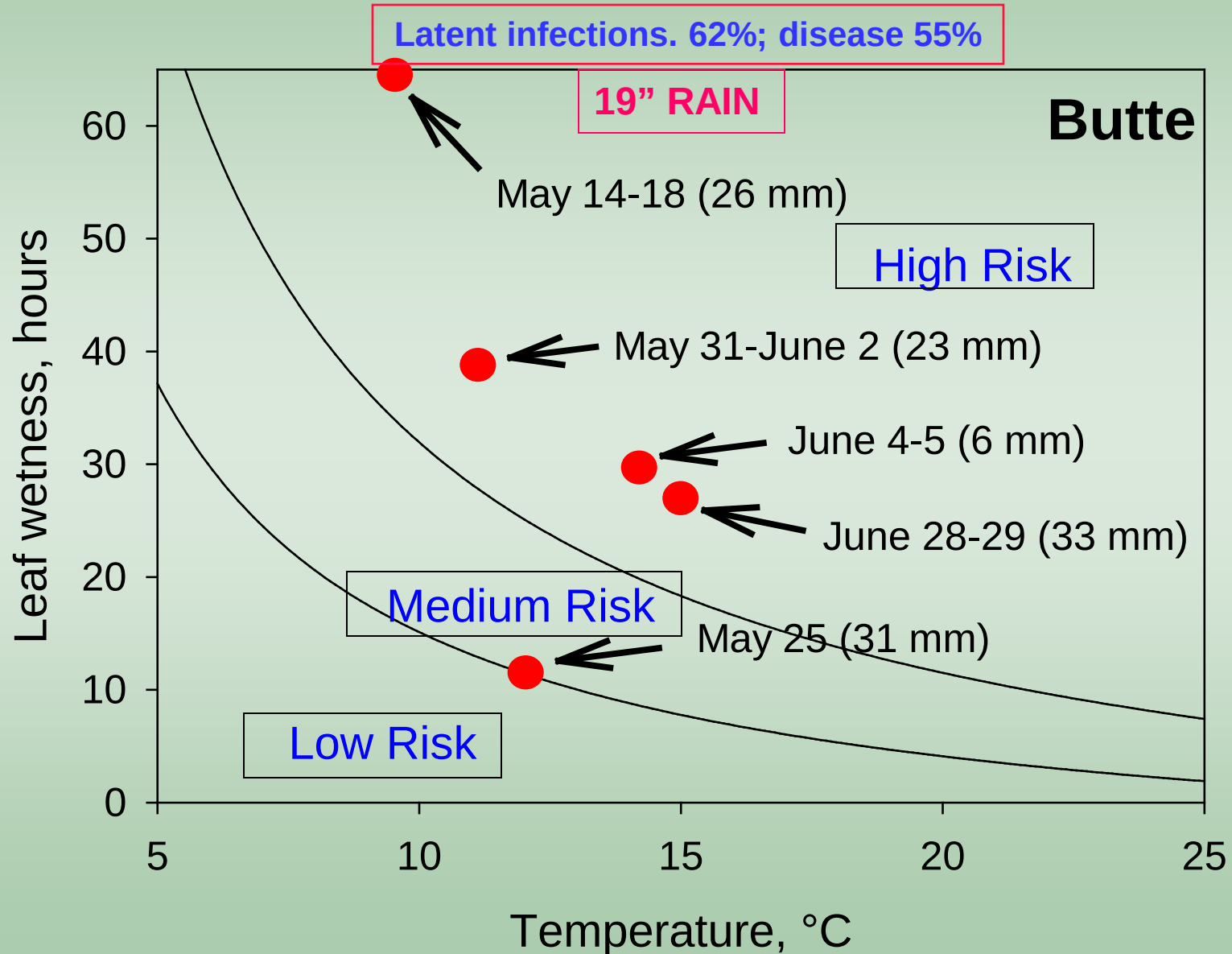
Relative risk of infection of pistachio fruit by *Botryosphaeria*



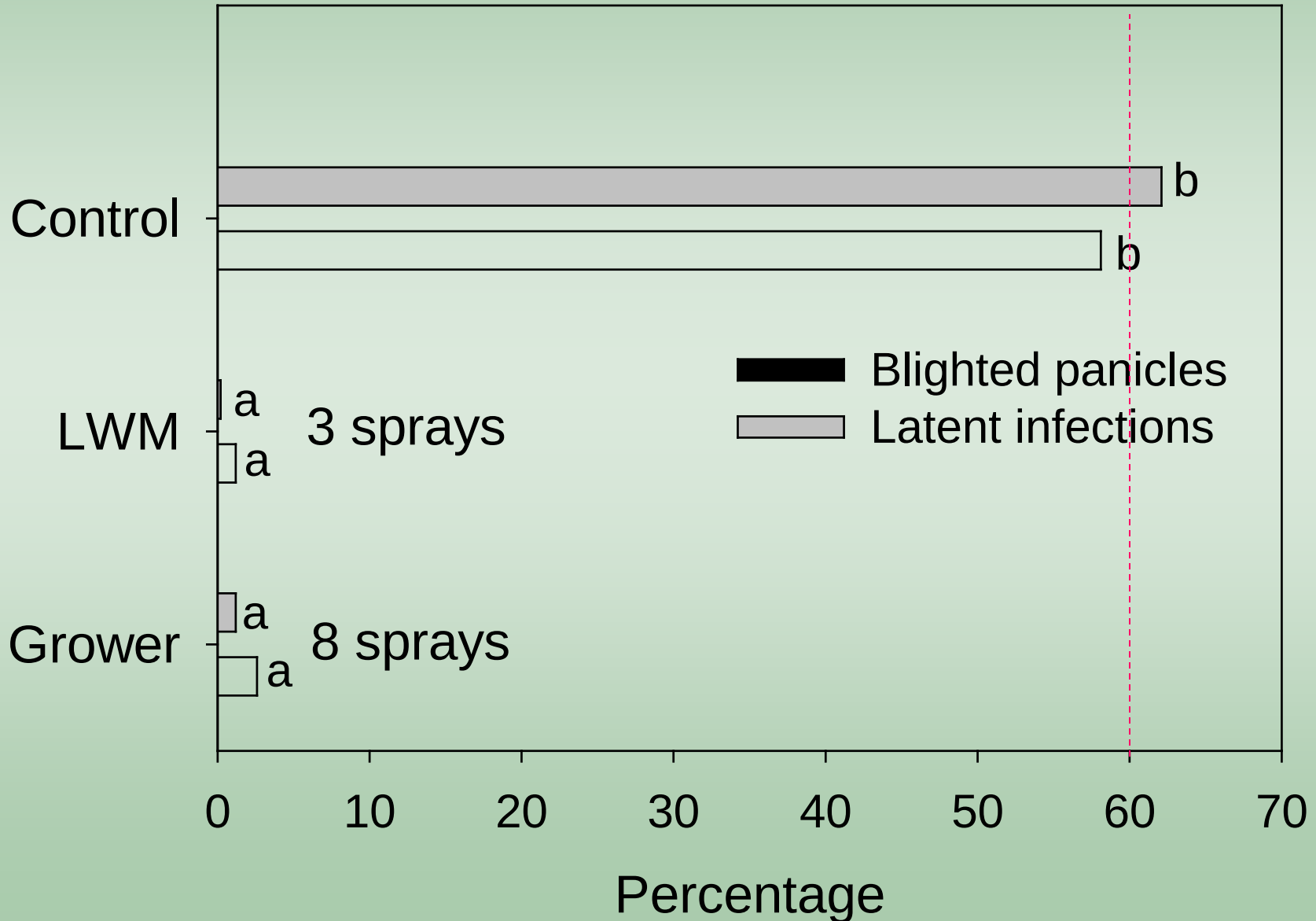


Leaf Wetness Model (LWM) in Butte County

(Medium and high risk infection events in 2011)

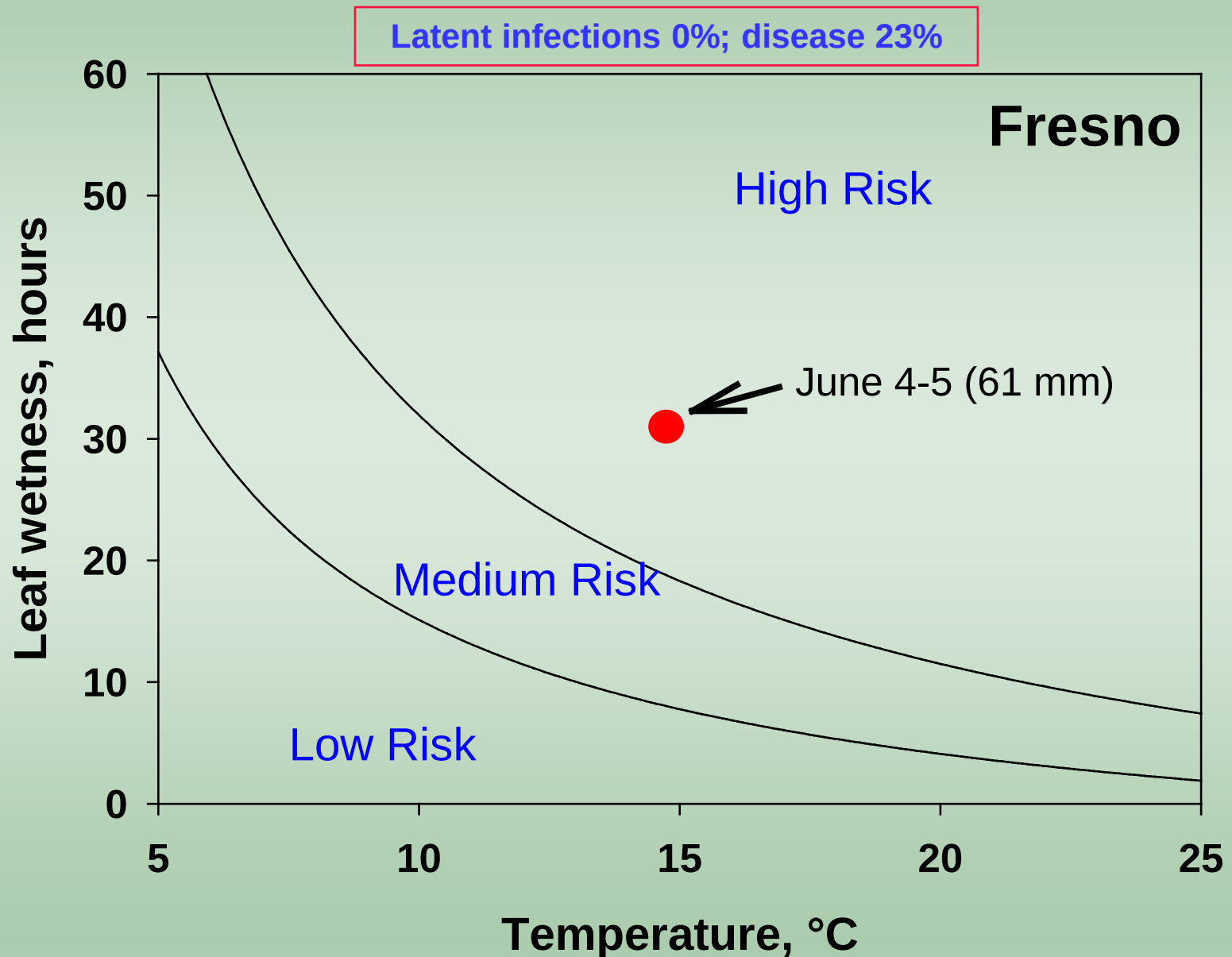


Results of Leaf Wetness Model predicated fungicide sprays in Butte County - 2011

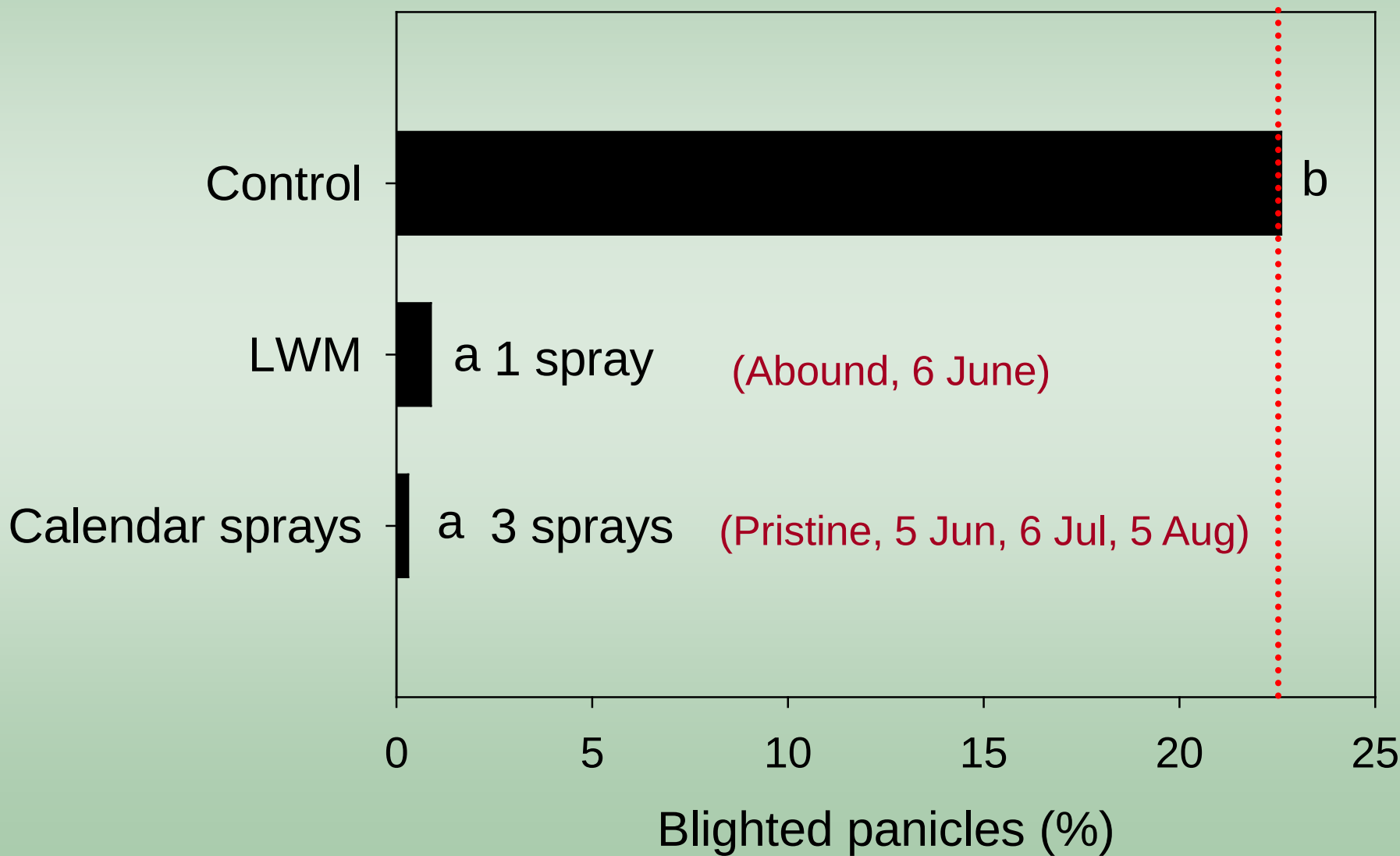




Leaf Wetness Model at Kearney Agric. Center- 2011

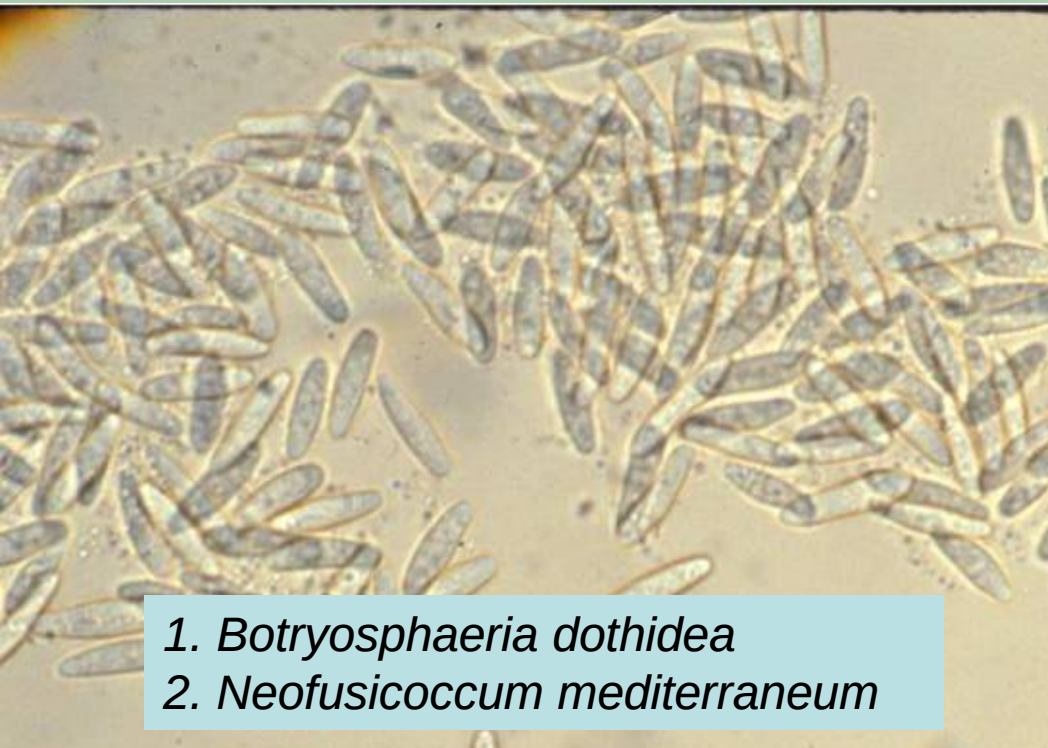


Results of **Leaf Wetness Model** predicated fungicide sprays at Kearney Agric. Center - 2011



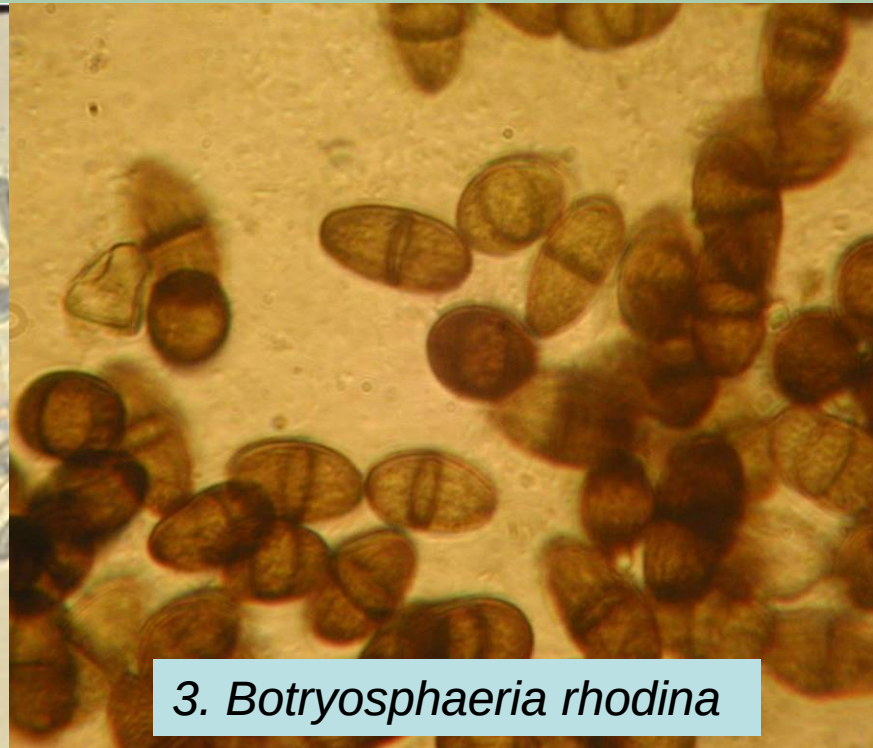
Inoculations of pistachio cultivars with *Botryosphaeria*





1. *Botryosphaeria dothidea*

2. *Neofusicoccum mediterraneum*



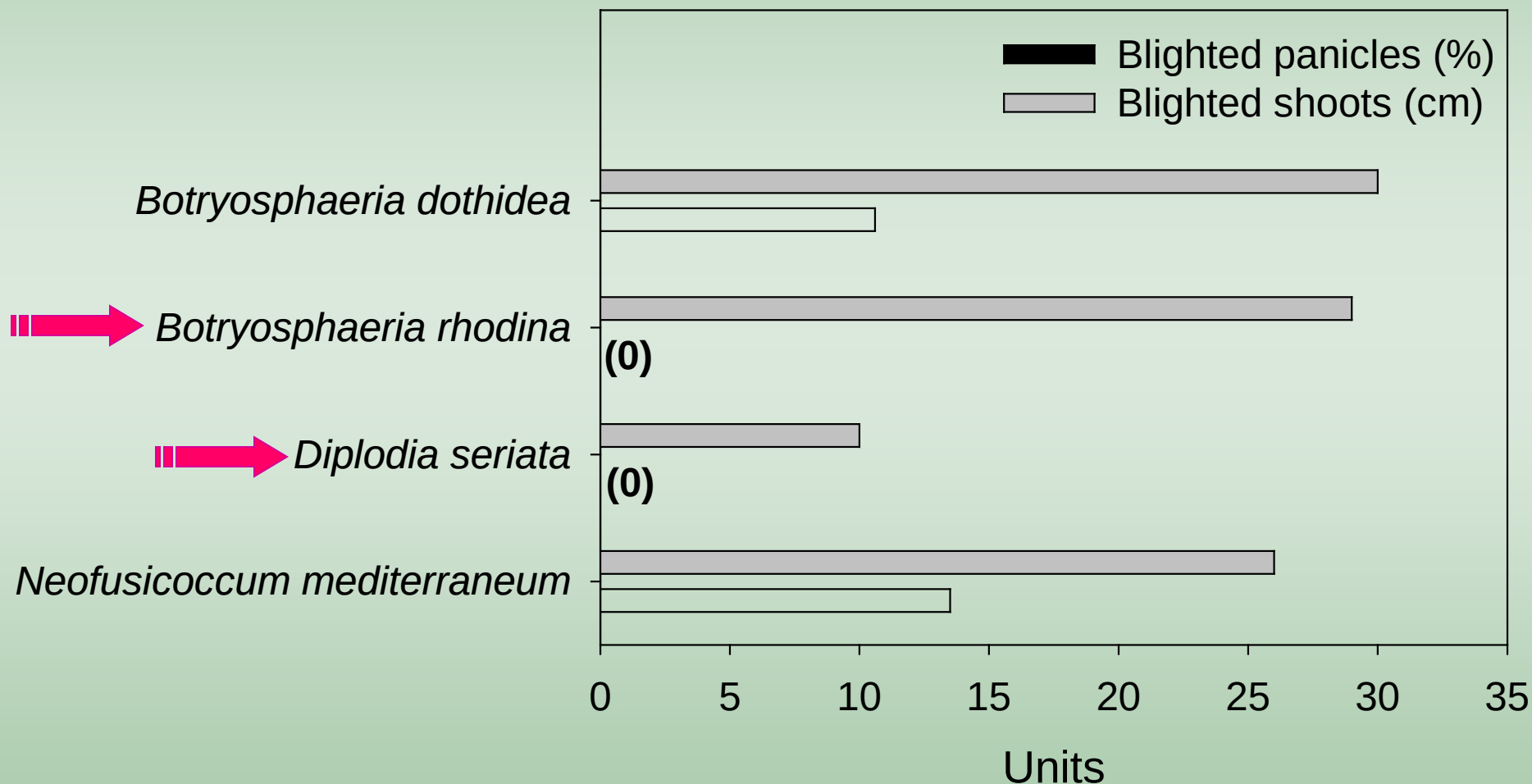
3. *Botryosphaeria rhodina*



4. *Diplodia seriata*



Inoculation results by fungal species



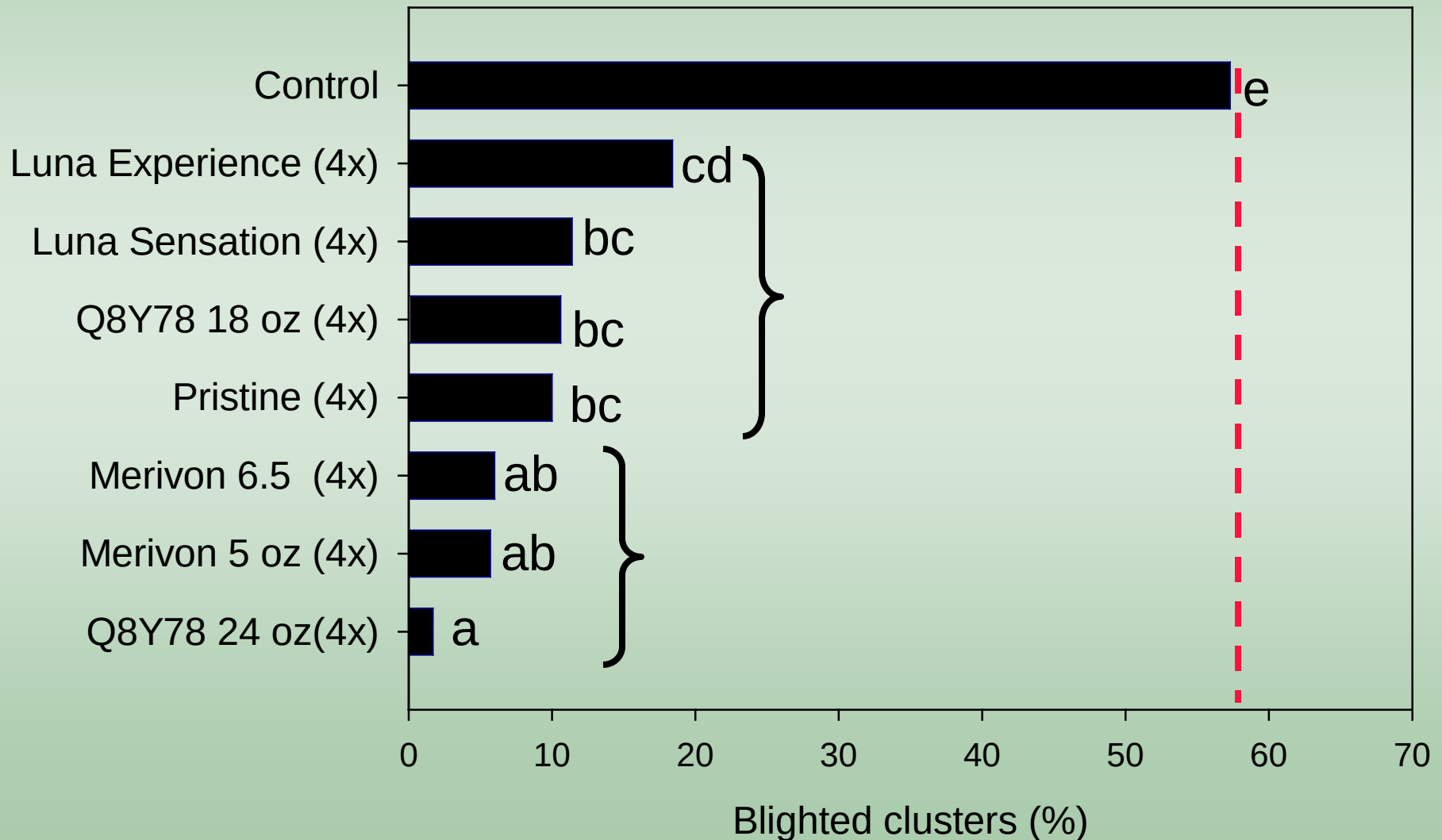
Sanitation:

*** Summer pruning

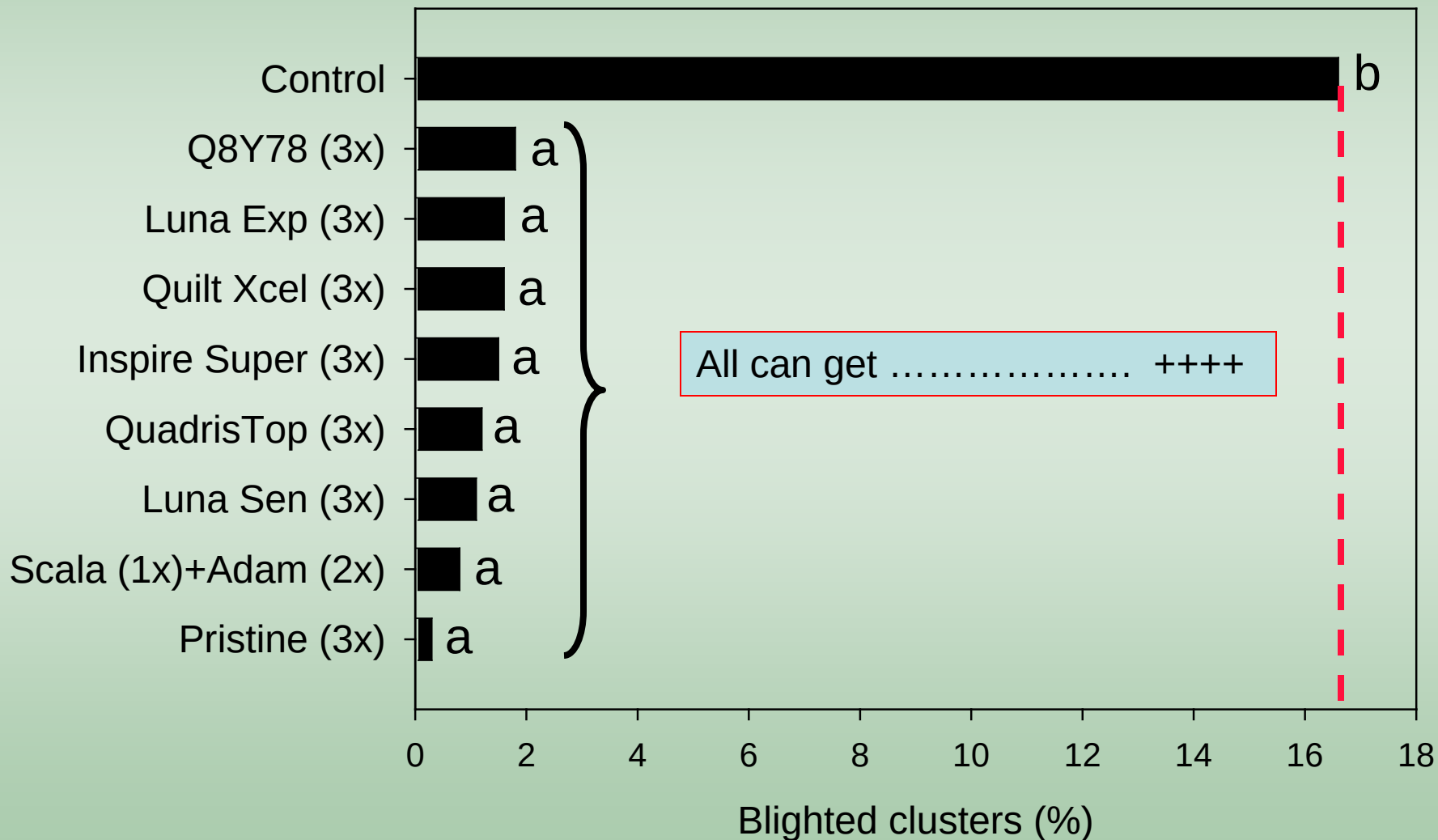
*** Winter pruning to remove the inoculum



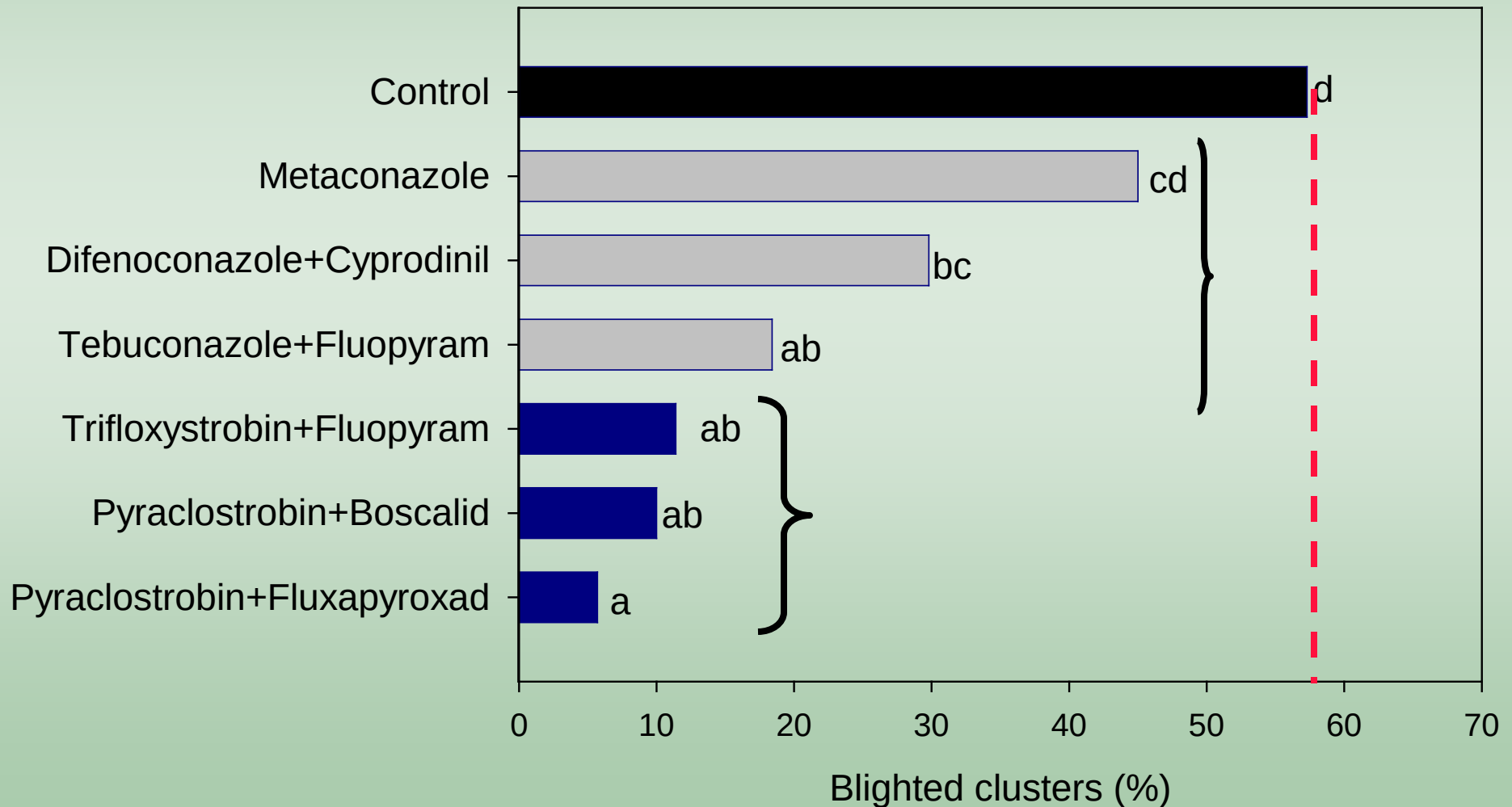
Most effective fungicides against Botryosphaeria - Butte County in 2011



Most effective fungicides against Botryosphaeria - Kearney in 2011



Efficacy of strobilurin mixtures against *Botryosphaeria* vs. those that feature triazole (Butte Co., 2011)

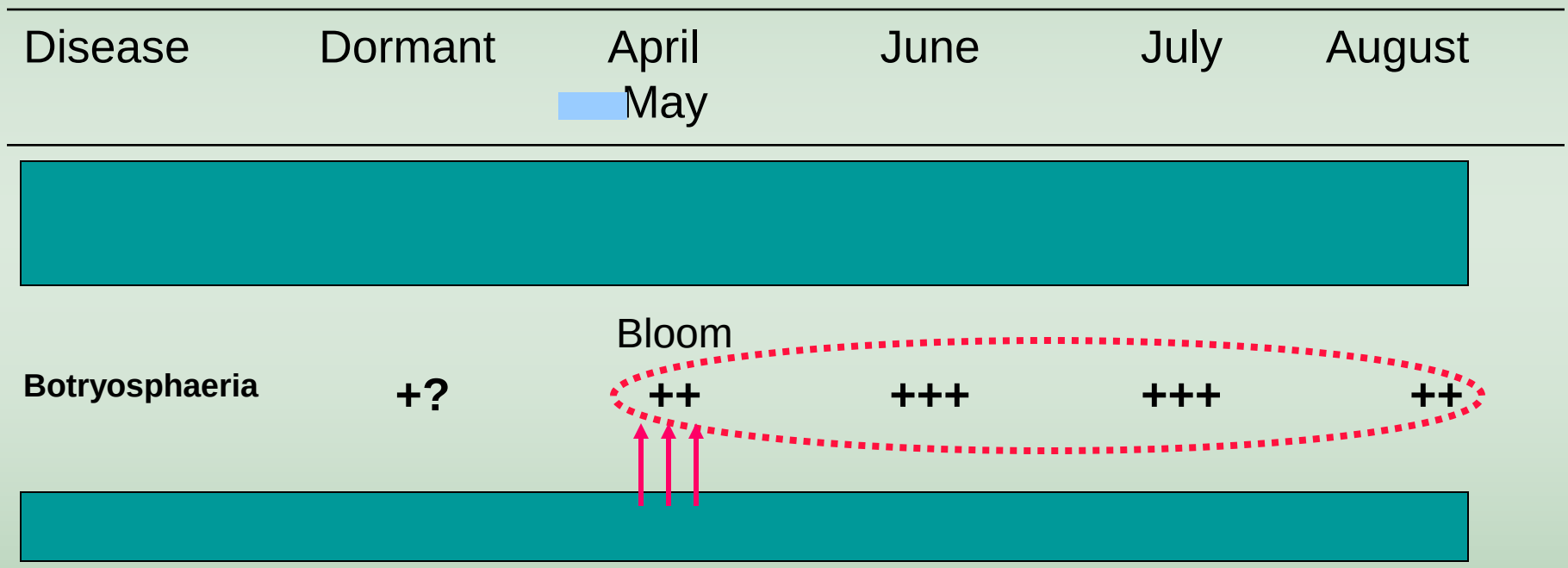


Fungicides registered for Botryosphaeria blight

Fungicide	Active ingredient	Efficacy
Adament.....	Trifloxystrobin+tebuconazole	+++
Abound	azoxystrobin	++++
Bravo.....	chlorothalonil	++
Bumper/Tilt.....	propiconazole	++
Cabrio.....	pyraclostrobin	++++
Gem	trifloxystrobin	++++
Quash.....	metconazole	+++
Inspire Super...	Difenoconazole + cyprodinil	++++
Pristine	boscalid+pyraclostrobin	++++
Quilt-Xcel.....	azoxystrobin+propiconazole	++++
Scala.....	pyrimethanil	+++
Switch.....	cyprodinil+fludioxonil	++
Tebuzol.....	tebuconazole	+++
Topsin-M.....	thiophanate-methyl	++
Copper.....	Copper	+/-

➡ Source: <http://www.ipm.ucdavis.edu>

PISTACHIO—Fungicide treatment timing



Source: <http://www.ipm.ucdavis.edu>







Take-home message

(It is important to diagnose the disease in the orchard correctly)

- Monitor orchard frequently to determine any arrival of inoculum.
- If you do not find *Botryosphaeria* inoculum (**BUDMON assay**) in your orchard, disease won't develop.
- Prune blighted shoots/clusters (*Botryosphaeria* behaves like “**The Sleeping Dragon**”).
- Spray before or after rain events in April/ May, June, & July (**rains cause infection events**).
- Learn and use the leaf wetness (LWM) model to make decisions when and how many times to spray and reduce unnecessary spray costs.
- Good news again!..... No resistance & lots of effective fungicides!

Controlling Aflatoxin with AF36



Molds that can produce aflatoxin in nut crops in California



Aspergillus flavus



Aspergillus parasiticus

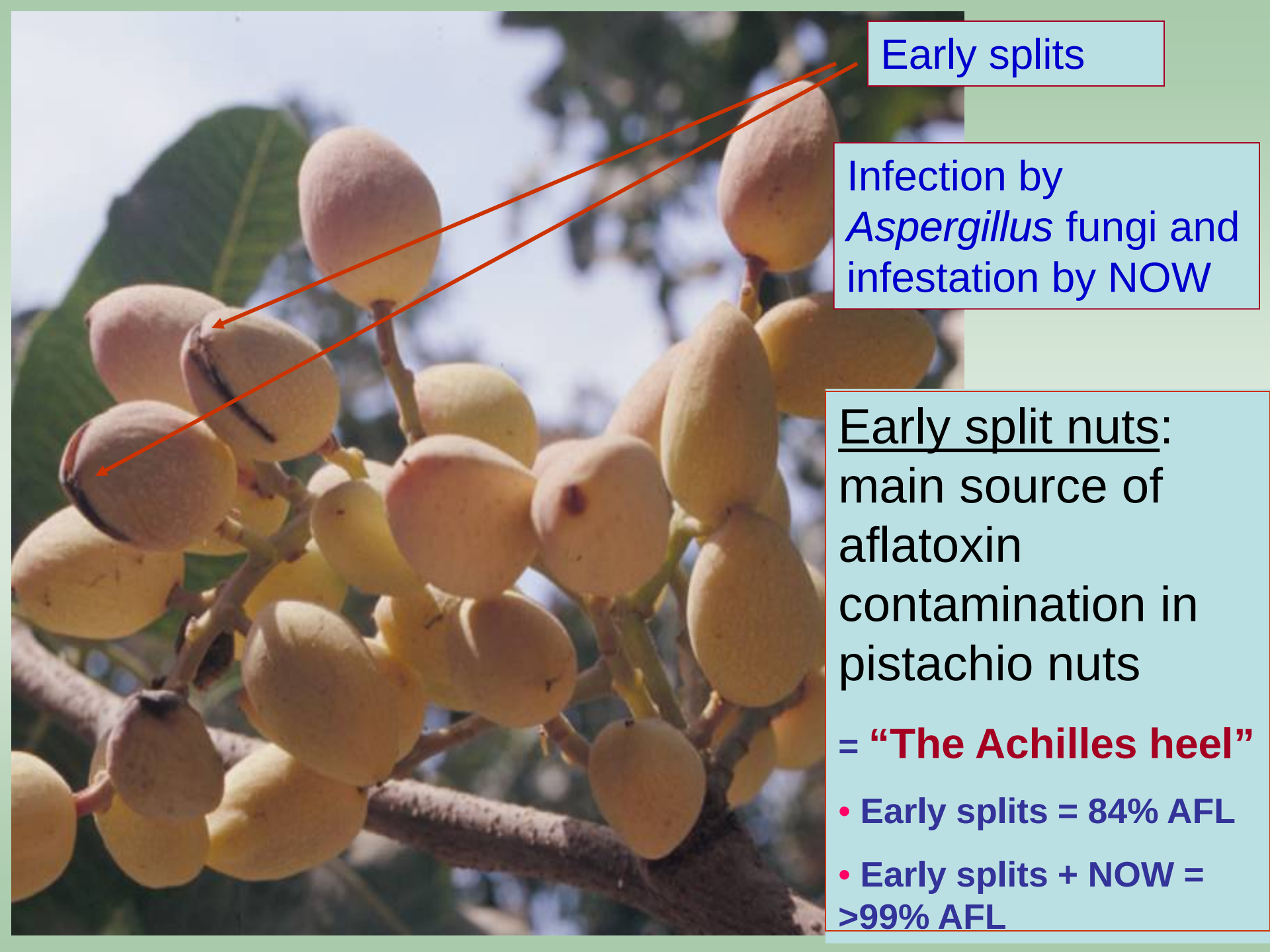
Aflatoxin contamination of nuts

Preharvest vs. Postharvest?

(if nuts are dried quickly after harvest, stored properly, and kept dried)...

→ Preharvest problem!

→ Postharvest (leaky silos; no proper storage)



Early splits

Infection by
Aspergillus fungi and
infestation by NOW

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

= “**The Achilles heel**”

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



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

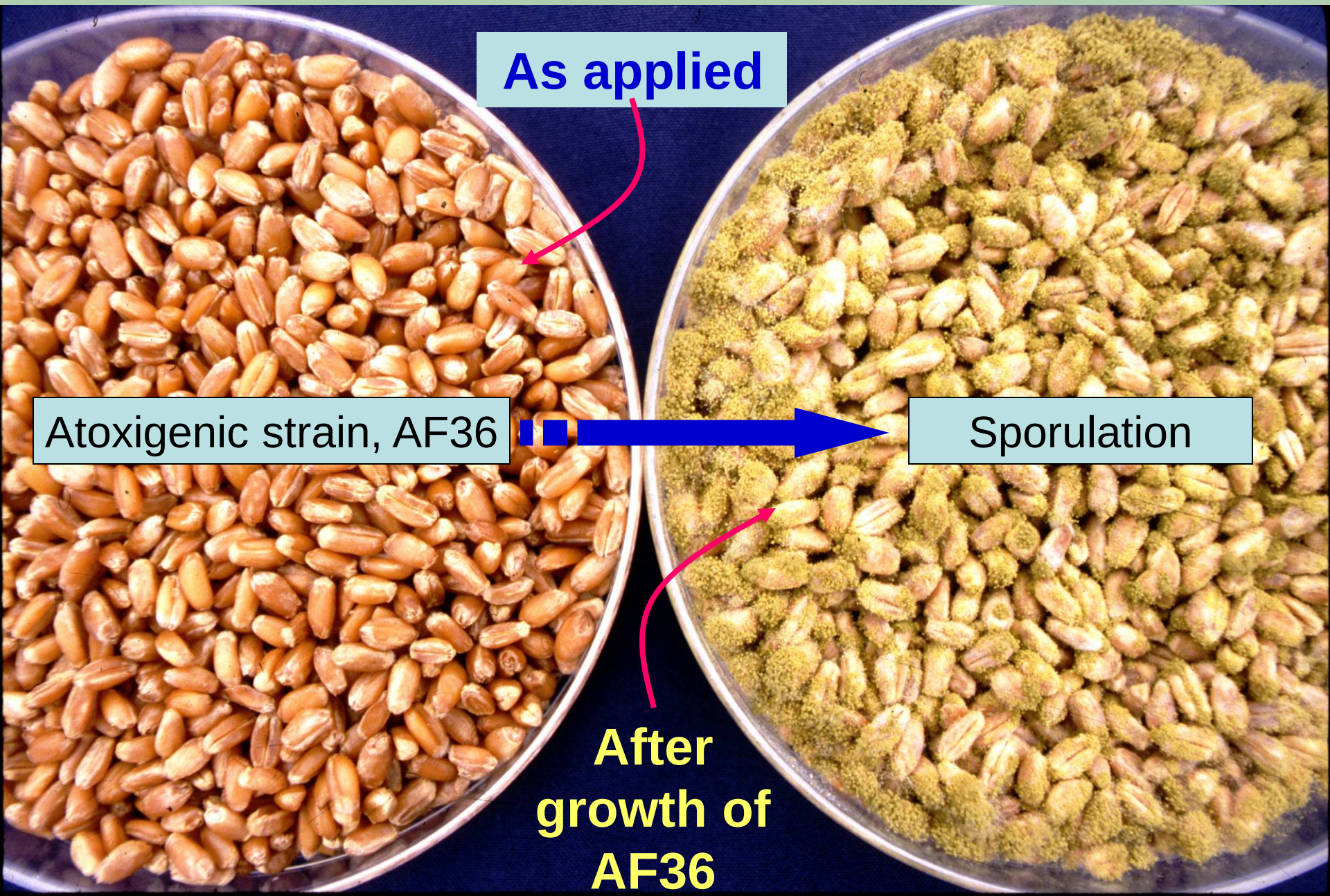


Use of atoxigenic strains of *Aspergillus flavus* as biopesticides to reduce aflatoxins

(atoxigenics = strains not producing aflatoxins)

Rationale: The atoxigenics when applied in the field increase in numbers and displace the toxigenic strains.

Irrigation is needed for spore production



As applied

Atoxigenic strain, AF36

Sporulation

**After
growth of
AF36**

Experimental Use Permit (EUP) to apply the **AF36** in 3,000 acres of pistachios

Experimental Use Pesticide

EXPERIMENTAL USE ONLY - NOT FOR SALE TO ANY PERSON OTHER THAN PARTICIPANT OR COOPERATOR OF THE EPA - APPROVED EXPERIMENTAL USE PROGRAM THIS LABEL MUST BE IN THE POSSESSION OF THE USER AT THE TIME OF APPLICATION

FOR USE ONLY AT AN APPLICATION SITE OF A COOPERATOR AND IN ACCORDANCE WITH THE TERMS AND CONDITIONS OF THE EXPERIMENTAL PROGRAM - READ SAFETY DIRECTIONS BEFORE OPENING.

FOR USE ONLY ON PISTACHIO IN MERCED, MADERA, KERN,
TULARE and KINGS COUNTY in the state of CALIFORNIA.

ACCEPTED

for shipment and use of product
for experimental purposes under
the provisions of the Federal
Insecticide, Fungicide, and
Rodenticide Act.

Permit No. 71693-EuP-1

Issued on 05/14/07

Aspergillus flavus AF36

For displacing aflatoxin producing fungi

Aspergillus flavus AF36 is a strain of *Aspergillus flavus* that occurs naturally. When applied to crops after last cultivation, *Aspergillus flavus* AF36 competes with strains of *Aspergillus flavus* that produce large amounts of aflatoxin and in so doing 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).....

0.0008%
99.9992%
100%

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

KEEP OUT OF REACH OF CHILDREN

CAUTION

First Aid Statement

IF SWALLOWED:	Call a Poison Control Center or doctor immediately for treatment advice. Have a person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control center or doctor. Do not give anything by mouth to an unconscious person.
IF ON SKIN OR CLOTHING:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Get medical attention if irritation persists. Prolonged or frequently repeated skin contact may cause allergic reactions in some individuals.
IF INHALED:	Move person to fresh air. If not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth to mouth if possible. Call a poison control center or doctor for further treatment advice.
IF IN EYES:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.

SEE ADDITIONAL PRECAUTIONARY STATEMENTS BELOW AND ON OTHER PANEL

Move the product container or label with you when calling a poison control center or doctor, or going for treatment.

1000-3000 lbs

Granted on May 3, 2007



Inoculum





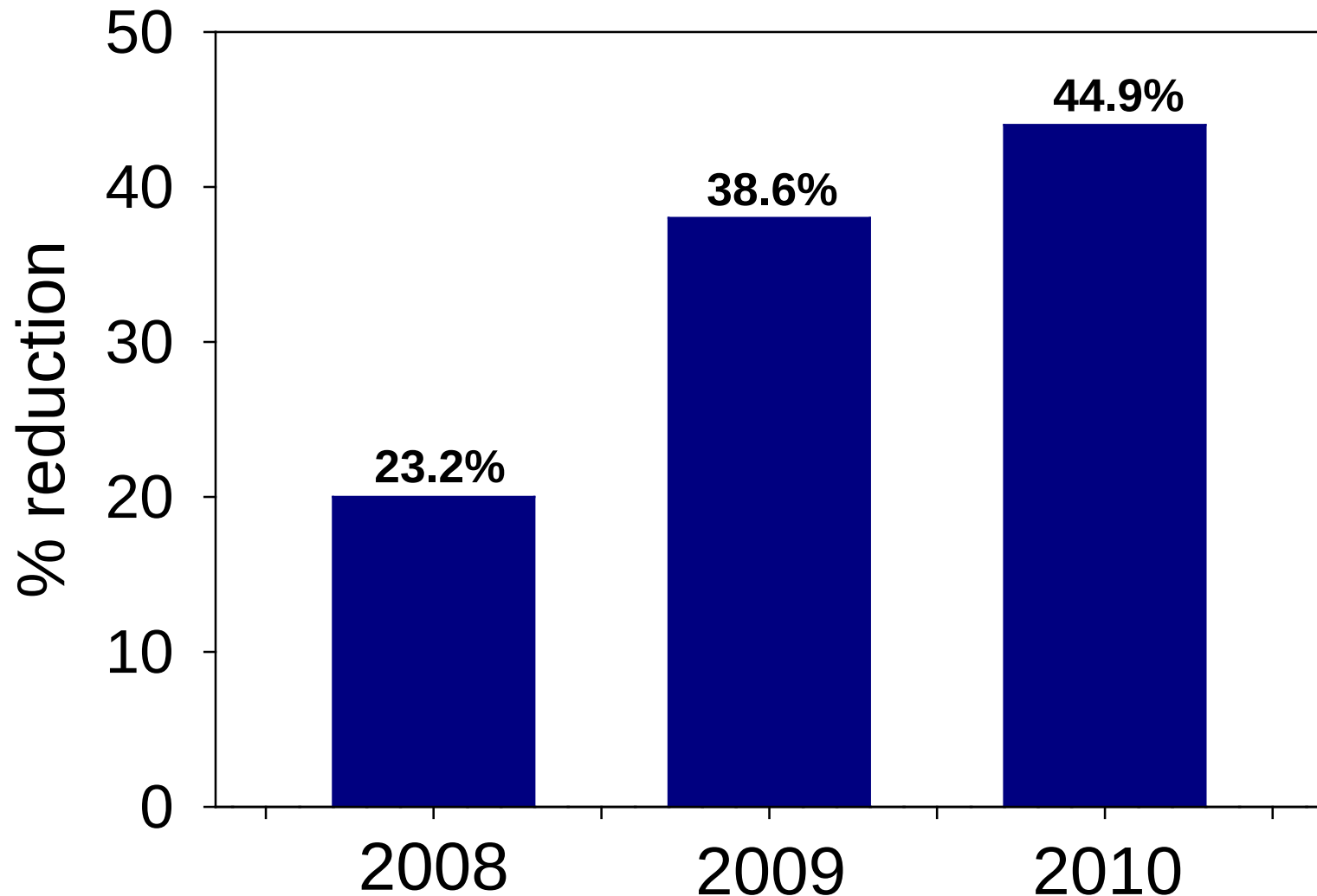
Burkard spore trap in pistachio at canopy height



Library samples

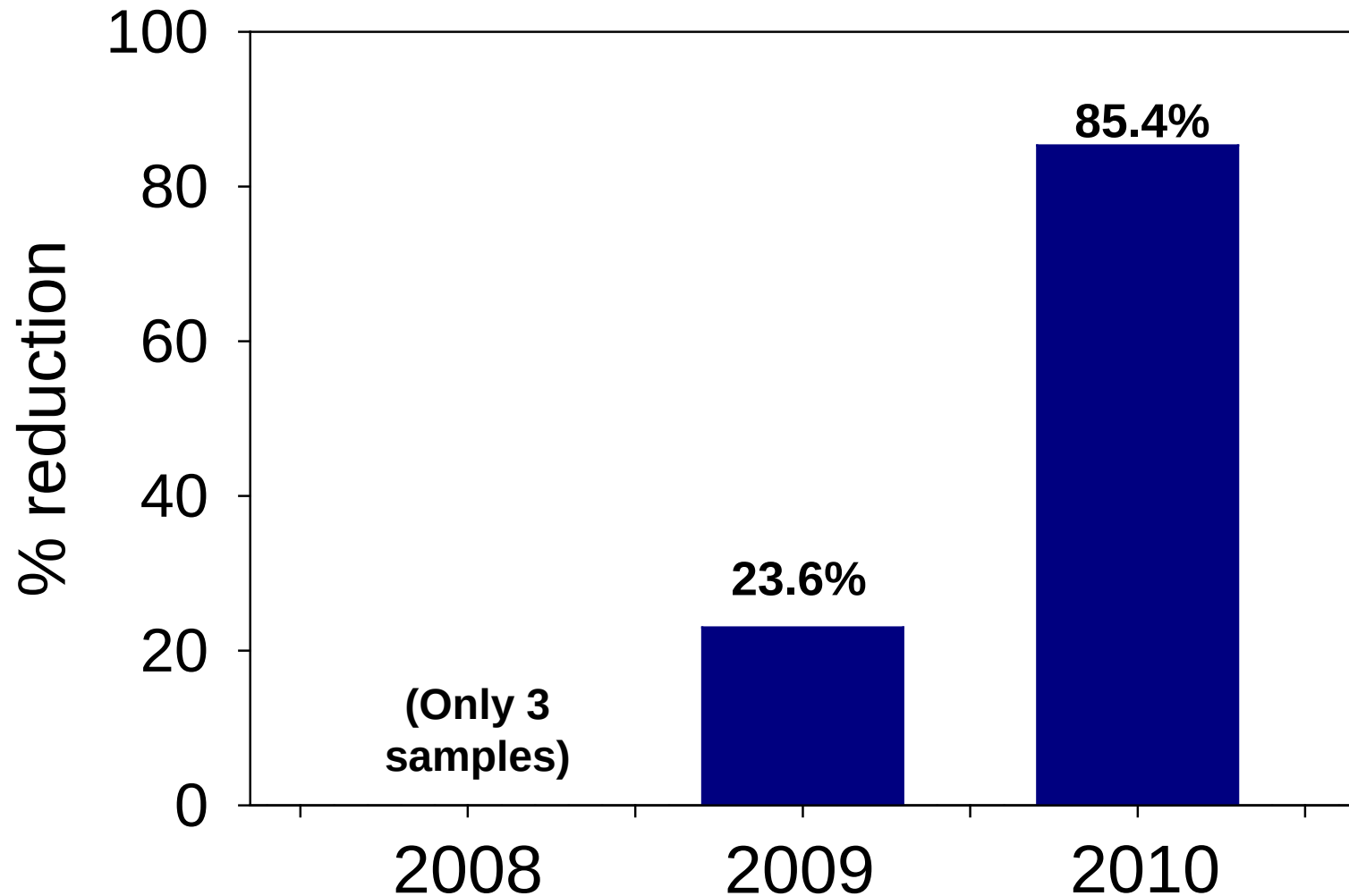


Percent reduction of aflatoxin contaminated pistachio samples after treating orchards with AF36 (all harvests: 1st & 2nd harvests)





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



(Host)

pistachio
almond
walnut

We cannot change

AFATOXIN
CONTAMINATION
RISK IN TREE NUTS

We cannot change

(Agent)

AF36

Aspergillus

(atoxigenic)
(aflatoxins)

We can change

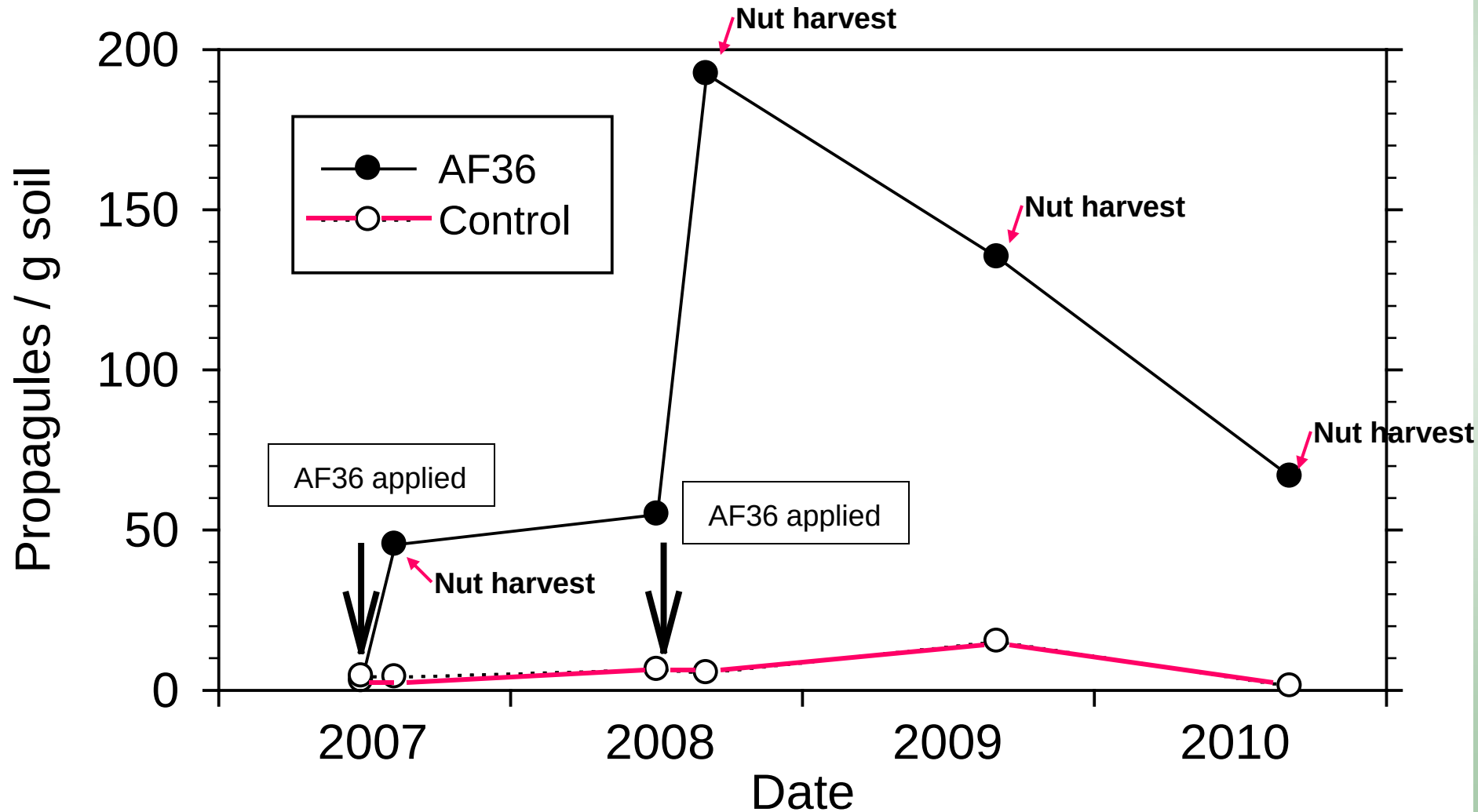
(Environment)

-Moisture
-Temperature
-Soil

Acknowledgments

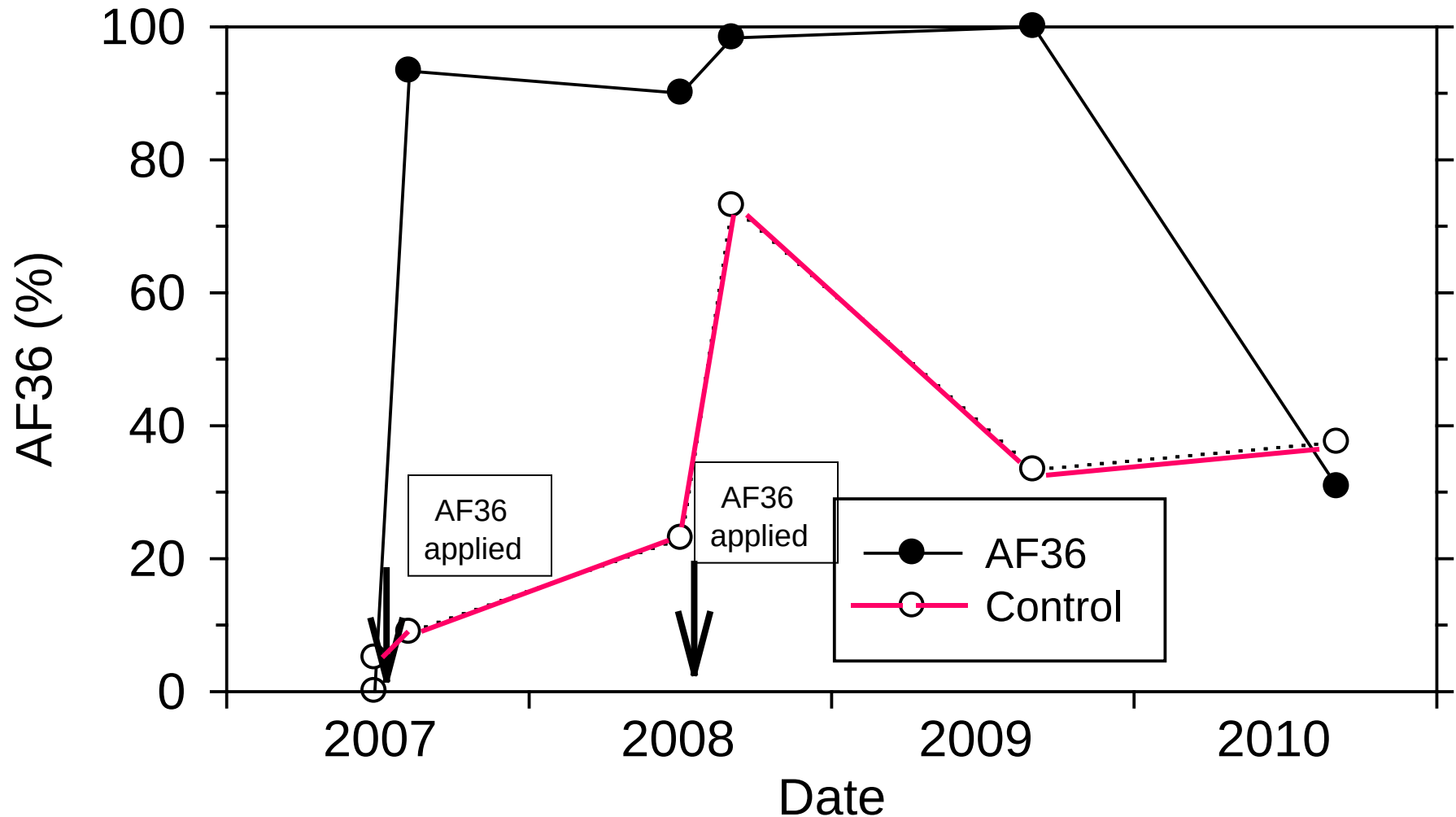
- David Morgan
- Mark Doster
- Matthias Donner
- Ryan Puckett
- Heraclio Reyes

Density of *Aspergillus flavus*/*A. parasiticus* in soil (treated with the wheat-AF36 product vs. untreated)



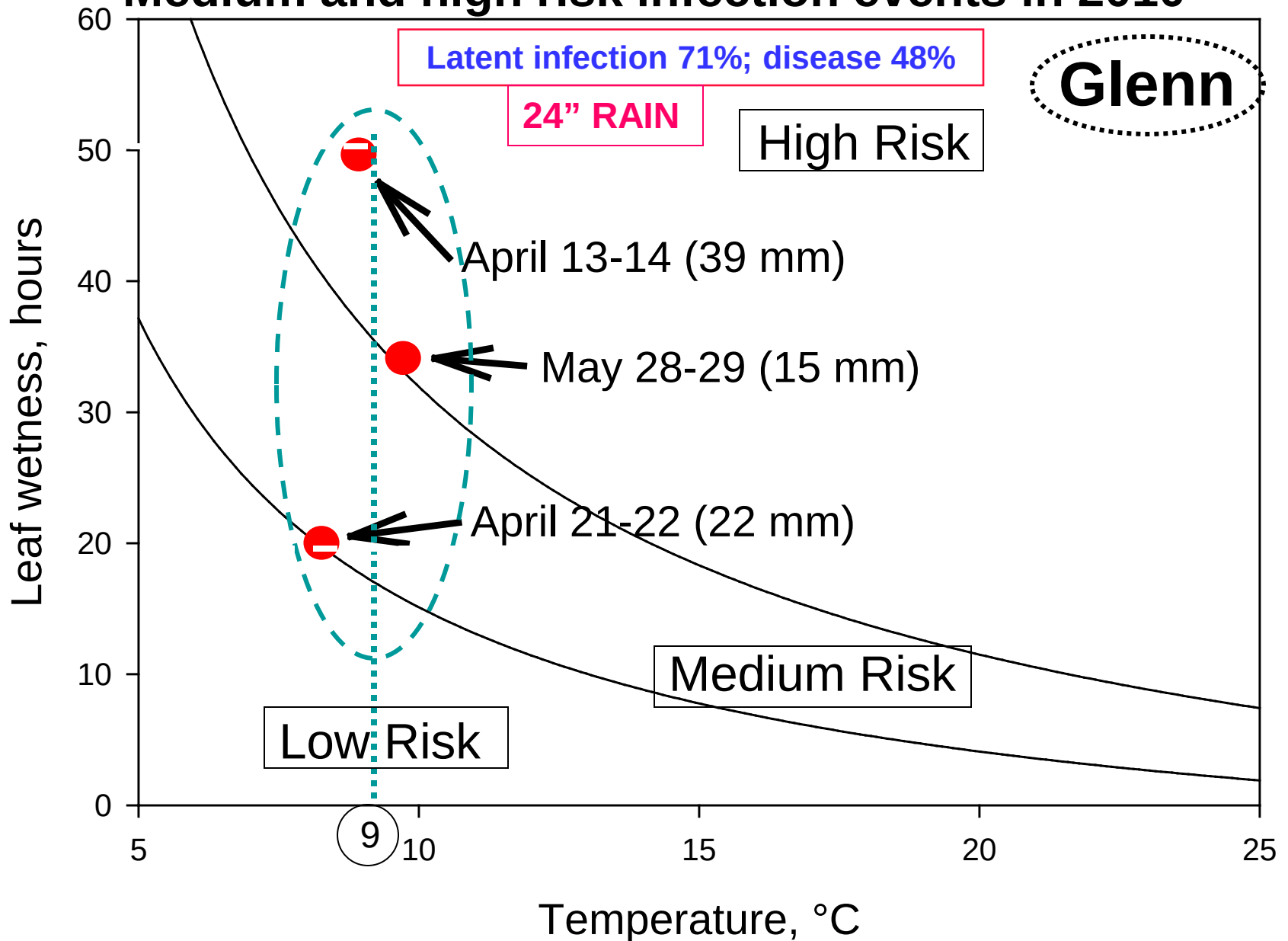


Percentage of *Aspergillus flavus*/*A. parasiticus* in soil (treated with the wheat-AF36 product vs. untreated)

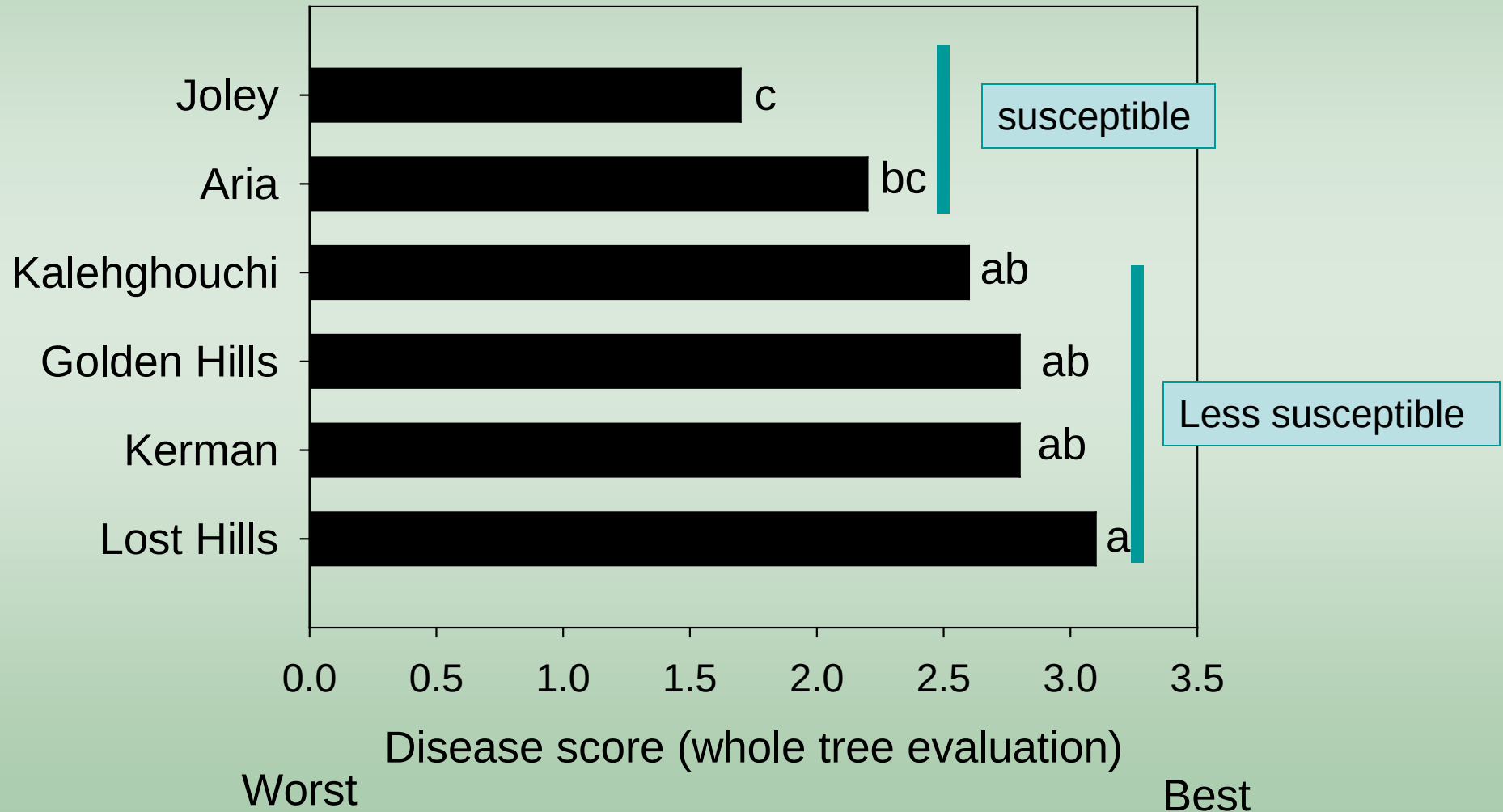


Leaf Wetness Model (LWM) in Glenn County -2010

Medium and high risk infection events in 2010



Alternaria inoculation of cultivars at Kearney 2011



Inoculation of pistachio cultivars with *Botryosphaeria* at Kearney - 2011

