

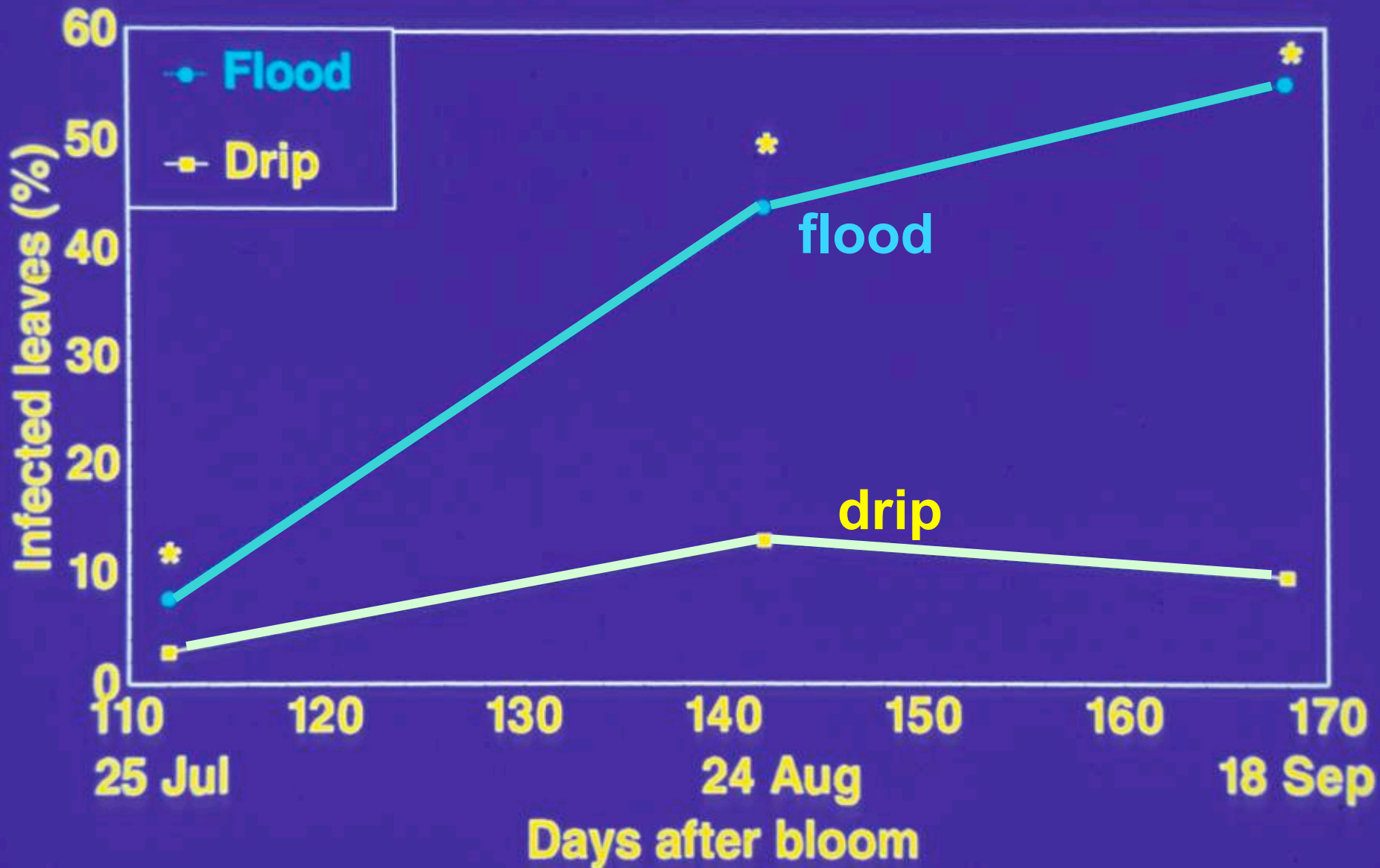
Diagnosis and Management of Alternaria Late Blight: A Resistance Challenge



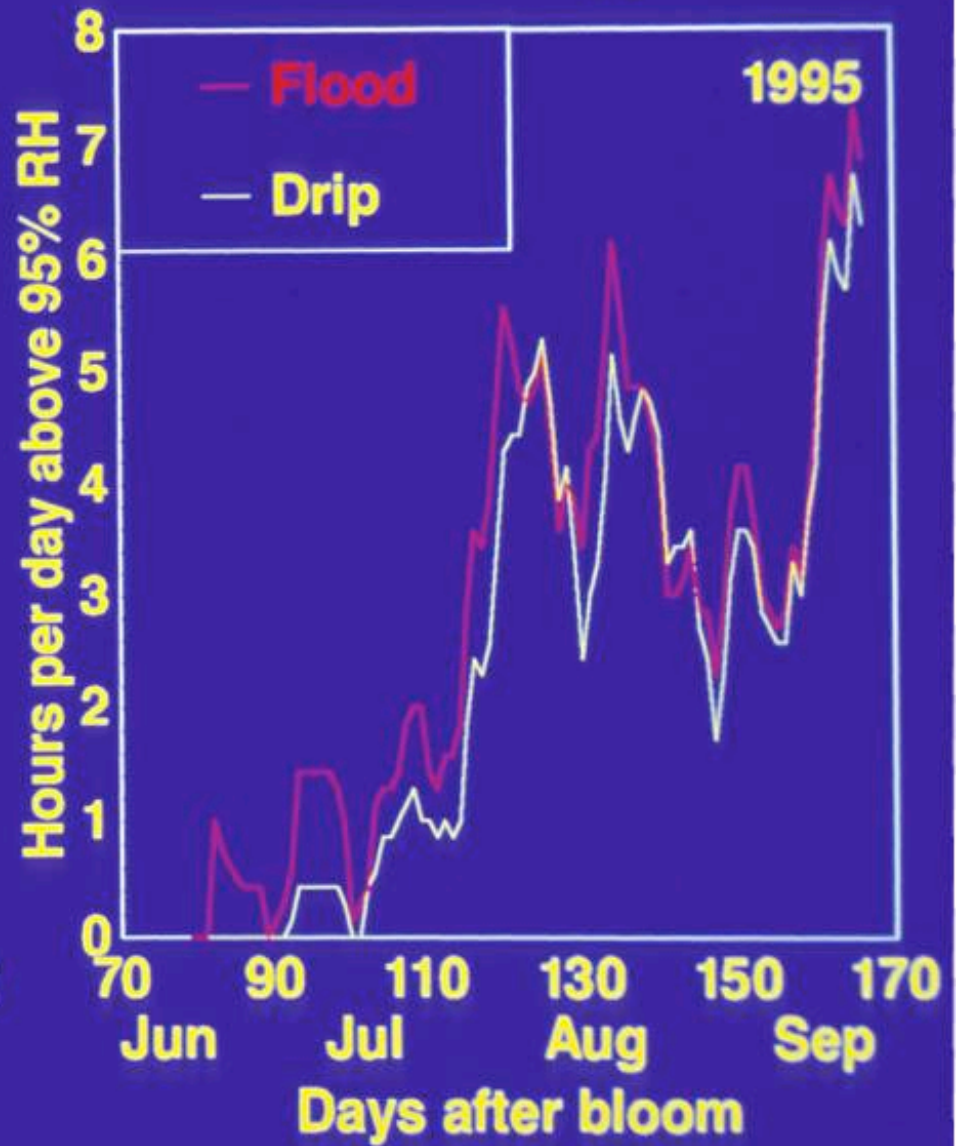
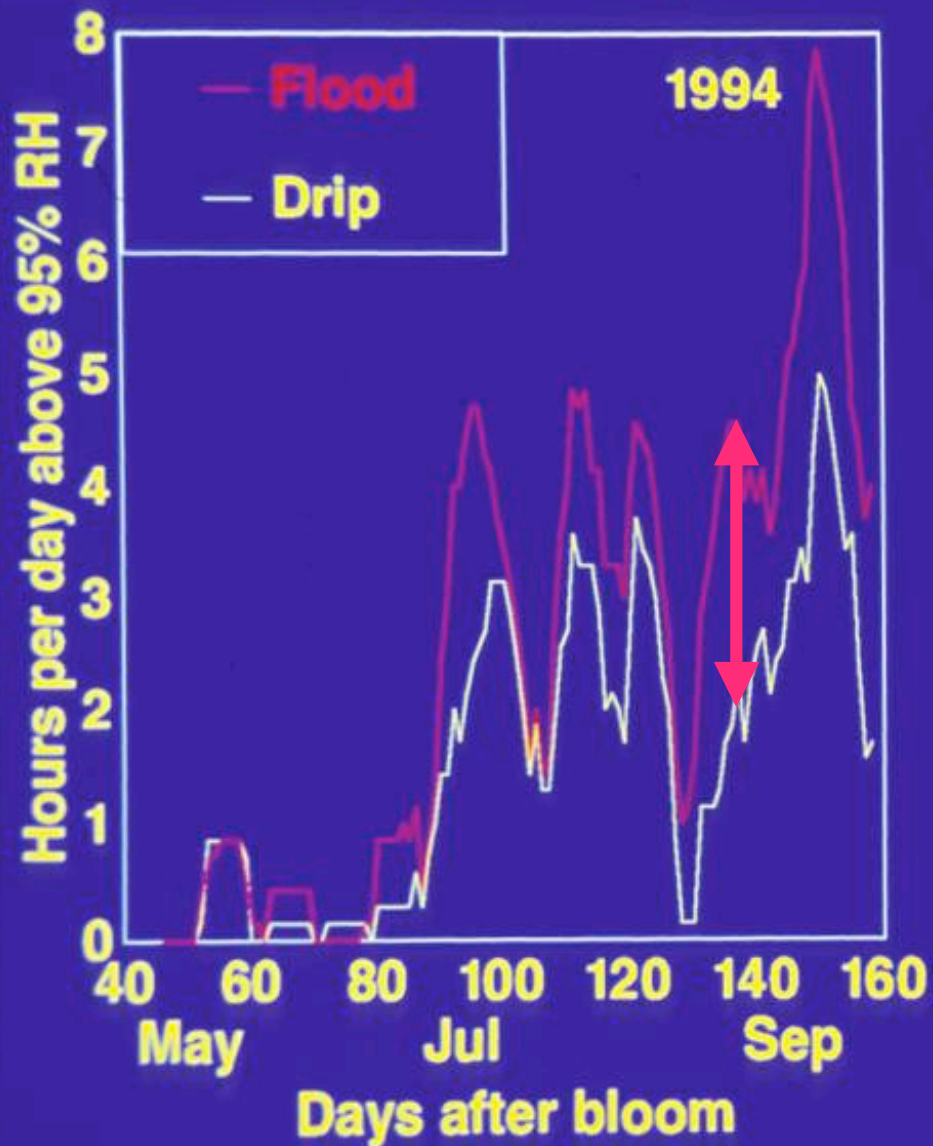


Buried drip

Effect of Buried Drip Irrigation on Alternaria Blight



Relative Humidity under Flood and Subsurface Irrigation



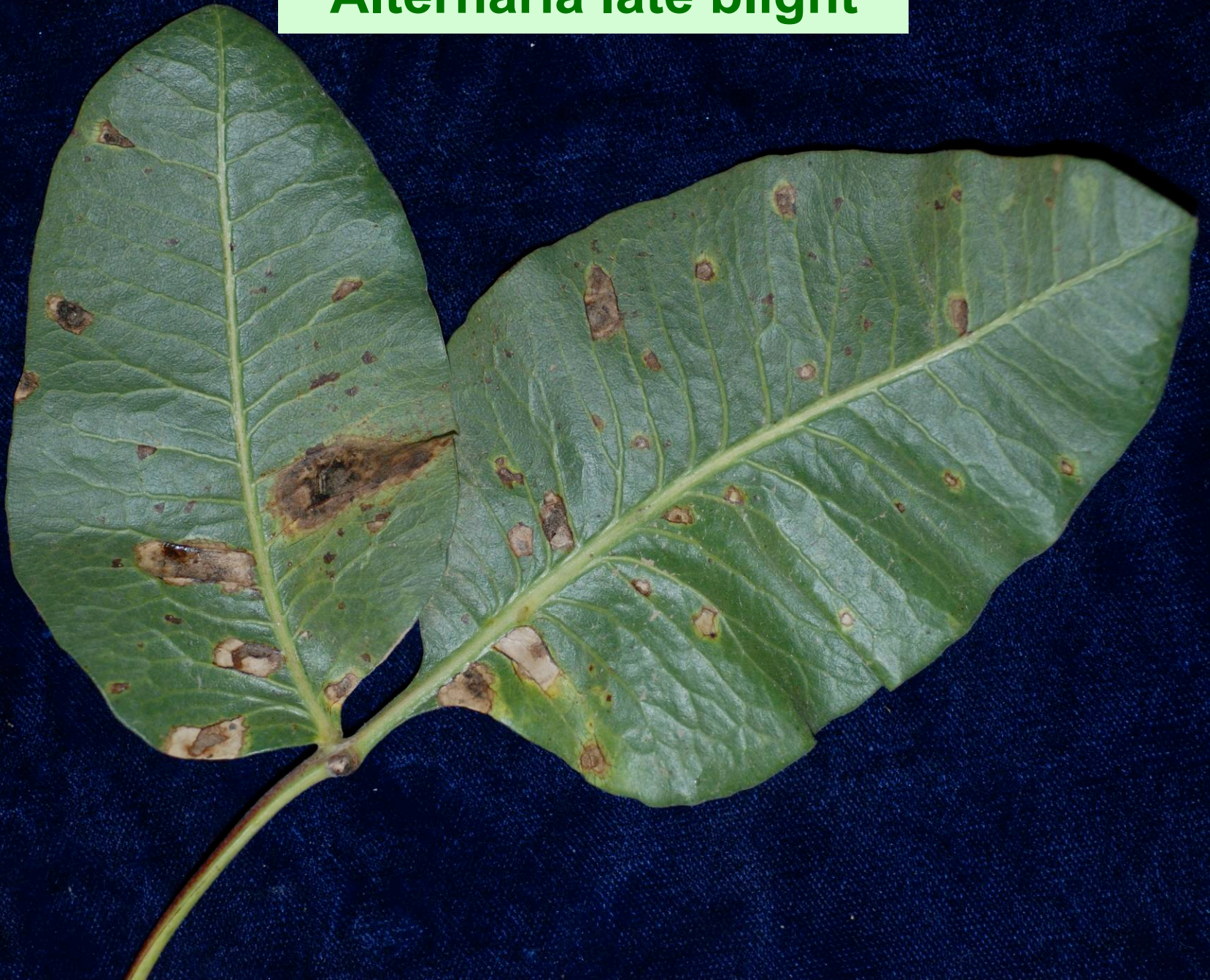
Pathogens of Alternaria late blight

- ✓ *Alternaria alternata*
- ✓ *A. tenuissima*
- ✓ & *A. arborescens*

} “*Alternaria alternata*”



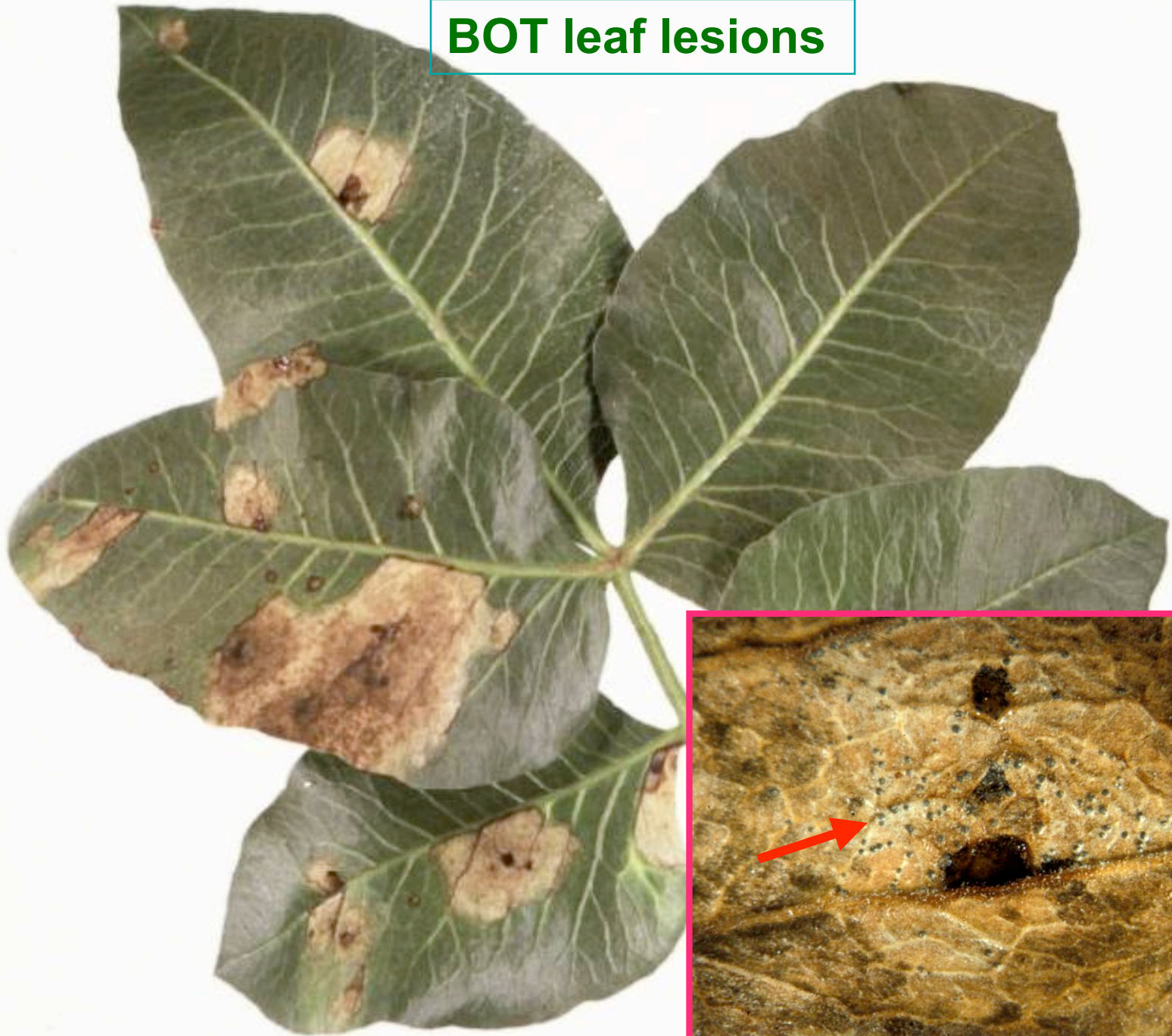
Alternaria late blight



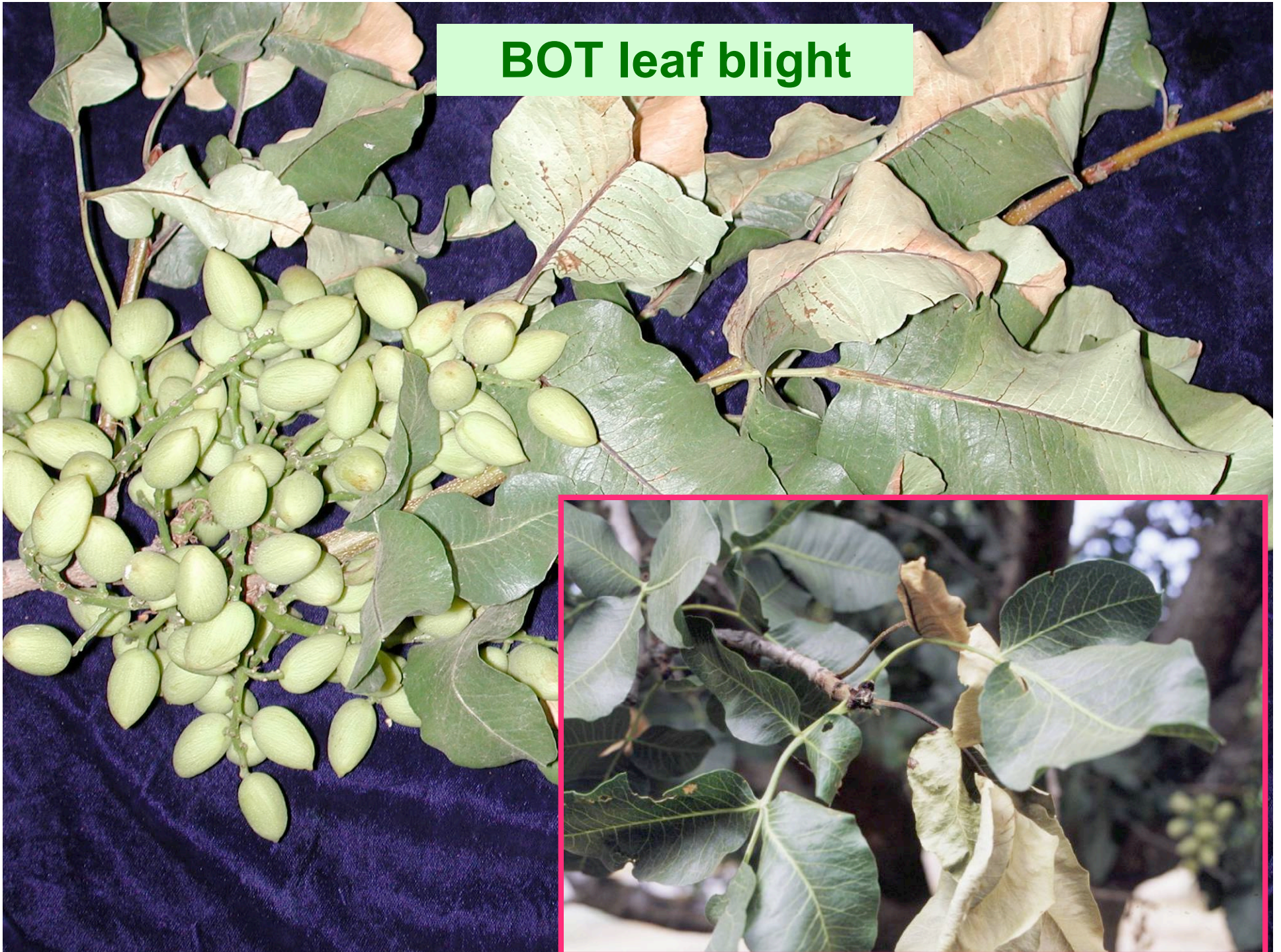
Alternaria lesions



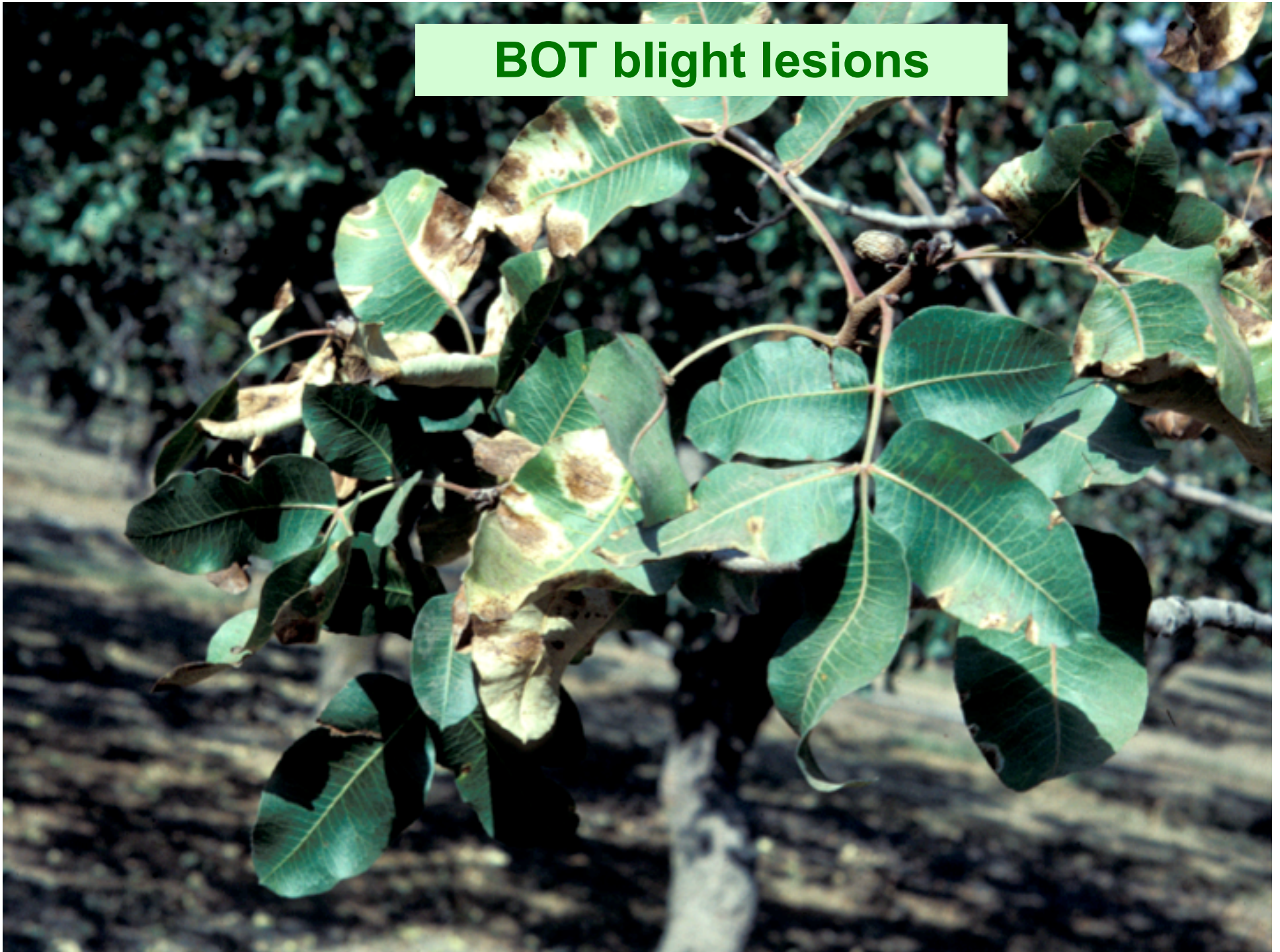
BOT leaf lesions



BOT leaf blight



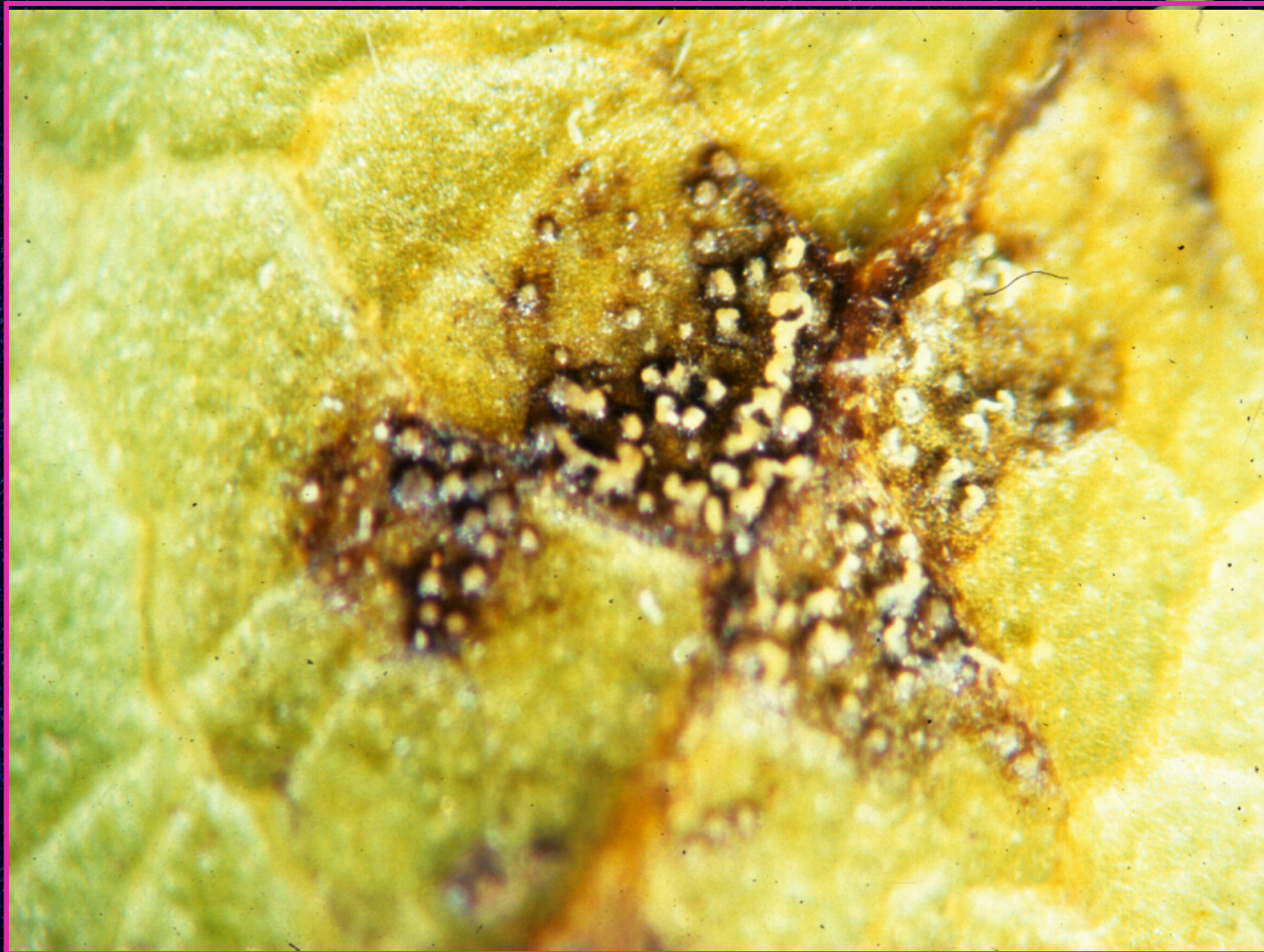
BOT blight lesions



Peter's scorch lesions



Septoria leaf spot



Alternaria late blight



Alternaria late blight



Botryosphaeria panicle blight



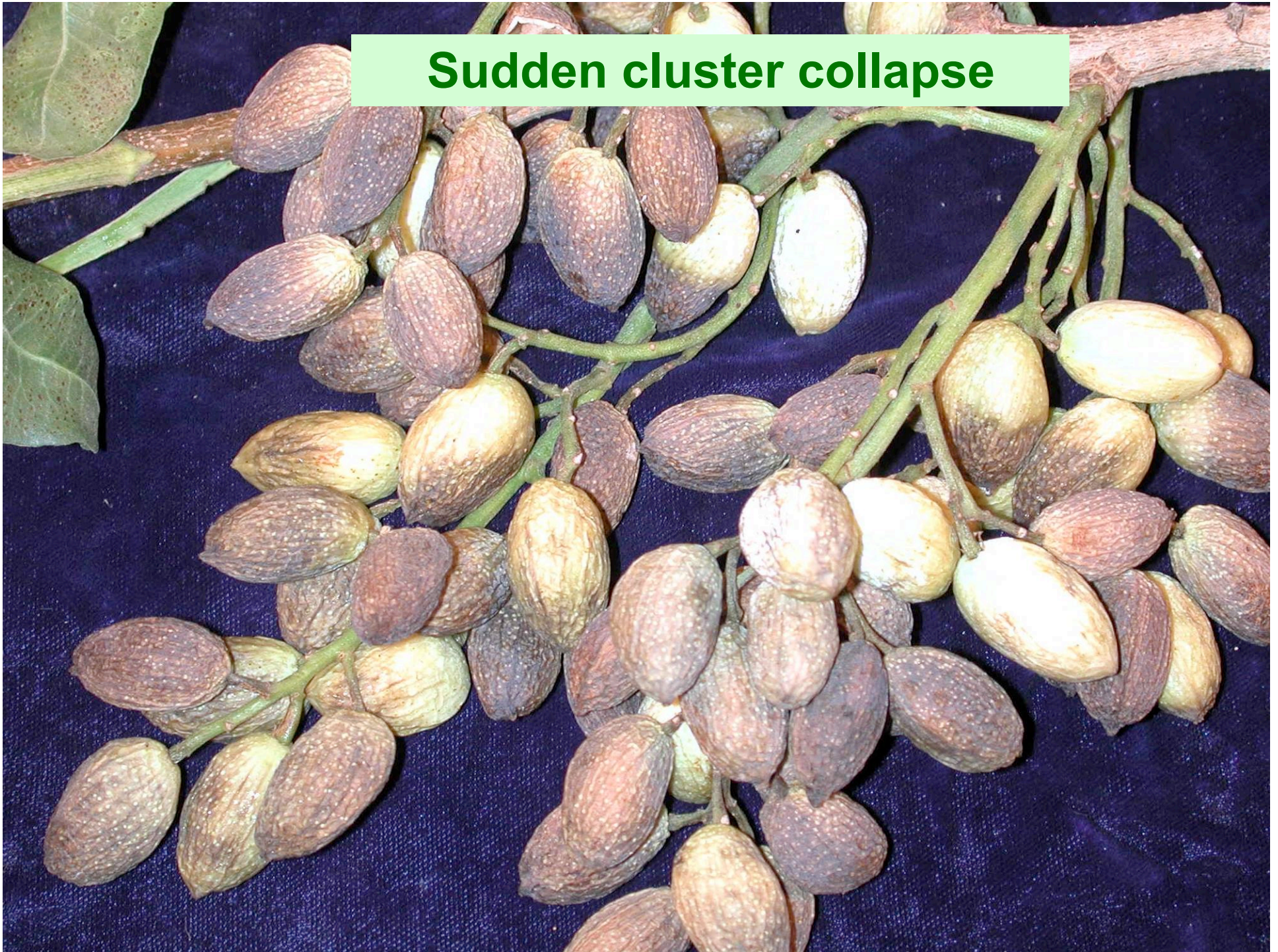
Botryosphaeria panicle blight: total kill



Sudden cluster collapse



Sudden cluster collapse

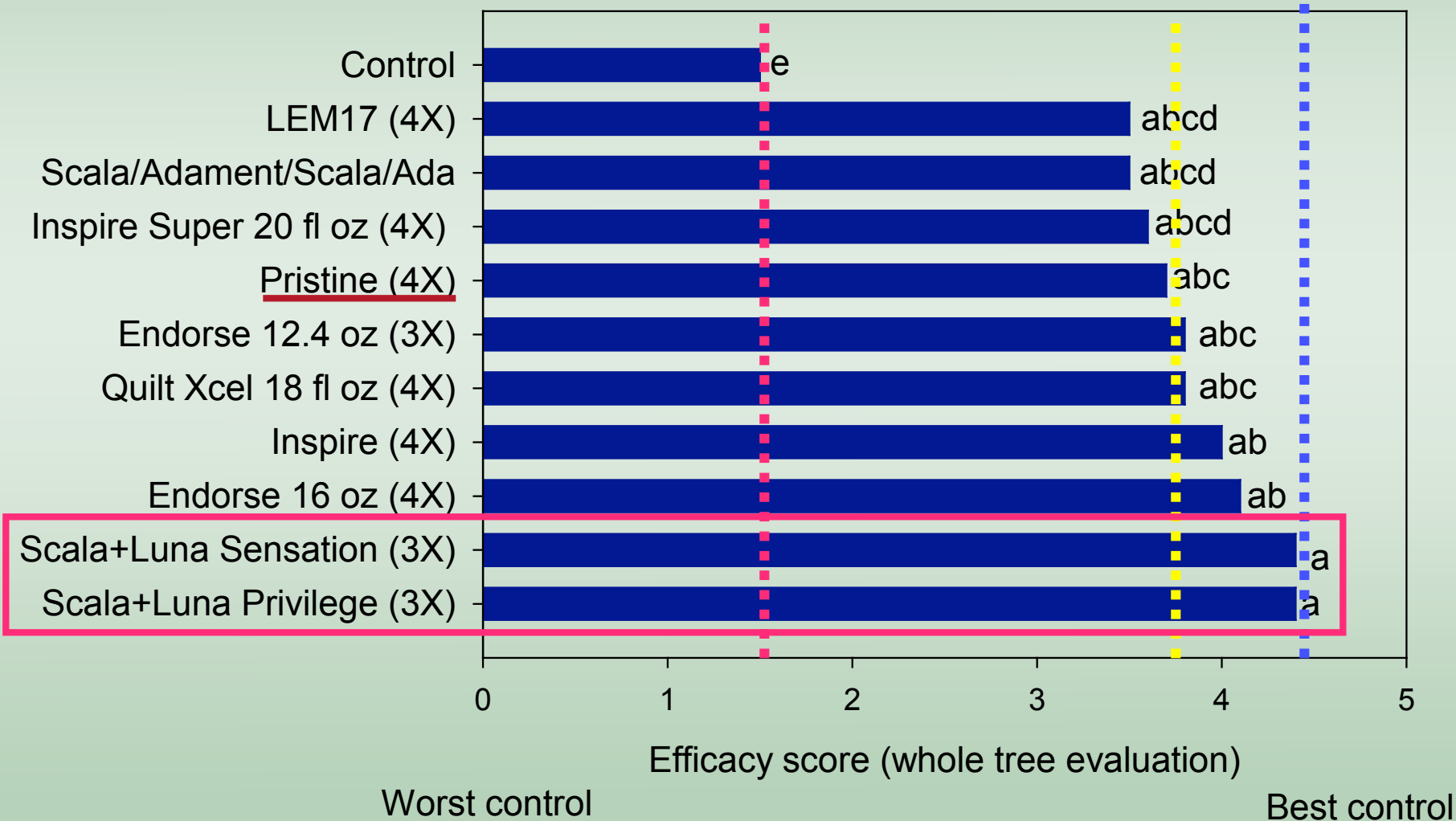




Management of Alternaria late blight:

1. **Cultural practices** (manage irrigation, buried drip, improve water infiltration, hedge trees, no cover crops, etc.)
2. **Chemical control** (apply fungicides)
3. **Integrated disease control** (use both cultural & chemical control)

Efficacy of fungicides against *Alternaria* late blight in 2009 - KAC



Alternaria: A resistance challenge

History of strobilurin resistance in *Alternaria* of pistachio:

2000: Abound (azoxystrobin) and other strobilurins registered.

2002: Suspect existence of resistant isolates?

2003: Yes, resistance; determined the G143A in Cyt b conferring high resistance.

↓ Amino acid position 143

A039(Sen) FLGYVLPYGGQMSLWGATV

A068(Sen) FLGYVLPYGGQMSLWGATV

A067(Sen) FLGYVLPYGGQMSLWGATV

37B12(Res) FLGYVLPYGGQMSLWAATV

37D5(Res) FLGYVLPYGGQMSLWAATV

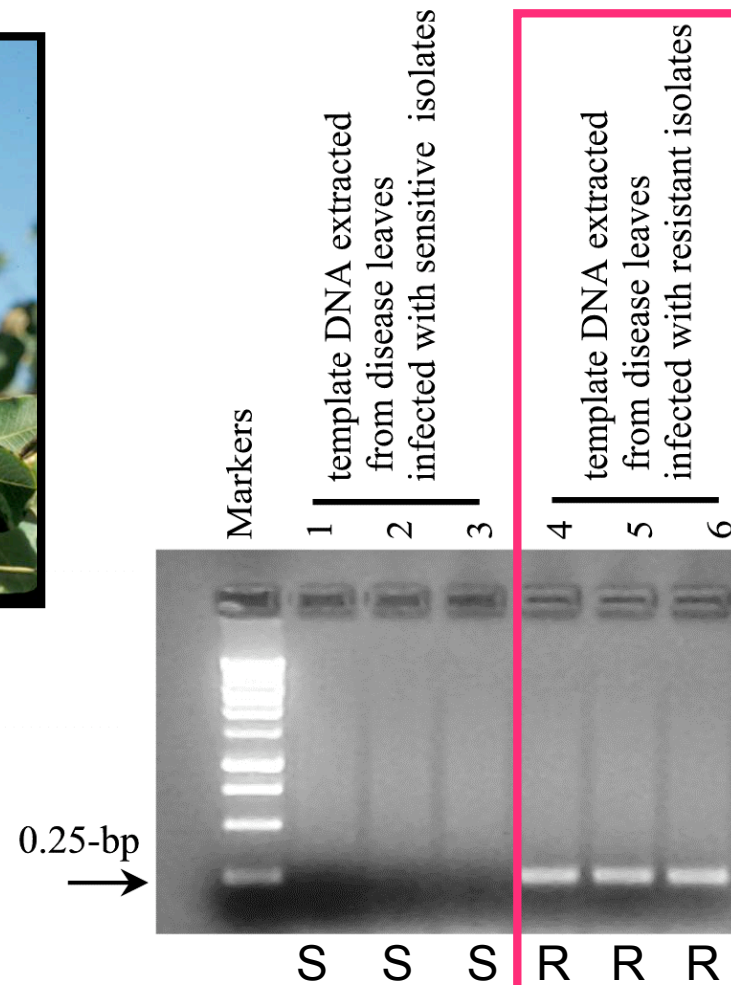
37C3(Res) FLGYVLPYGGQMSLWAATV

37E3(Res) FLGYVLPYGGQMSLWAATV

143

amino acid mutation of the cytochrome b gene from azoxystrobin-sensitive (Sen) and azoxystrobin-resistant (Res) *Alternaria* isolates from pistachio

Allele-specific PCR assay for detecting azoxystr.-resistant *Alternaria* in pistachio leaves infected by azoxystrobin **Res** and azoxystrobin **Sen** isolates



Objective: How widespread is azoxystrobin resistance among pistachio and almond orchards?

Real-time PCR machine (JB Research Unit)



Late blight



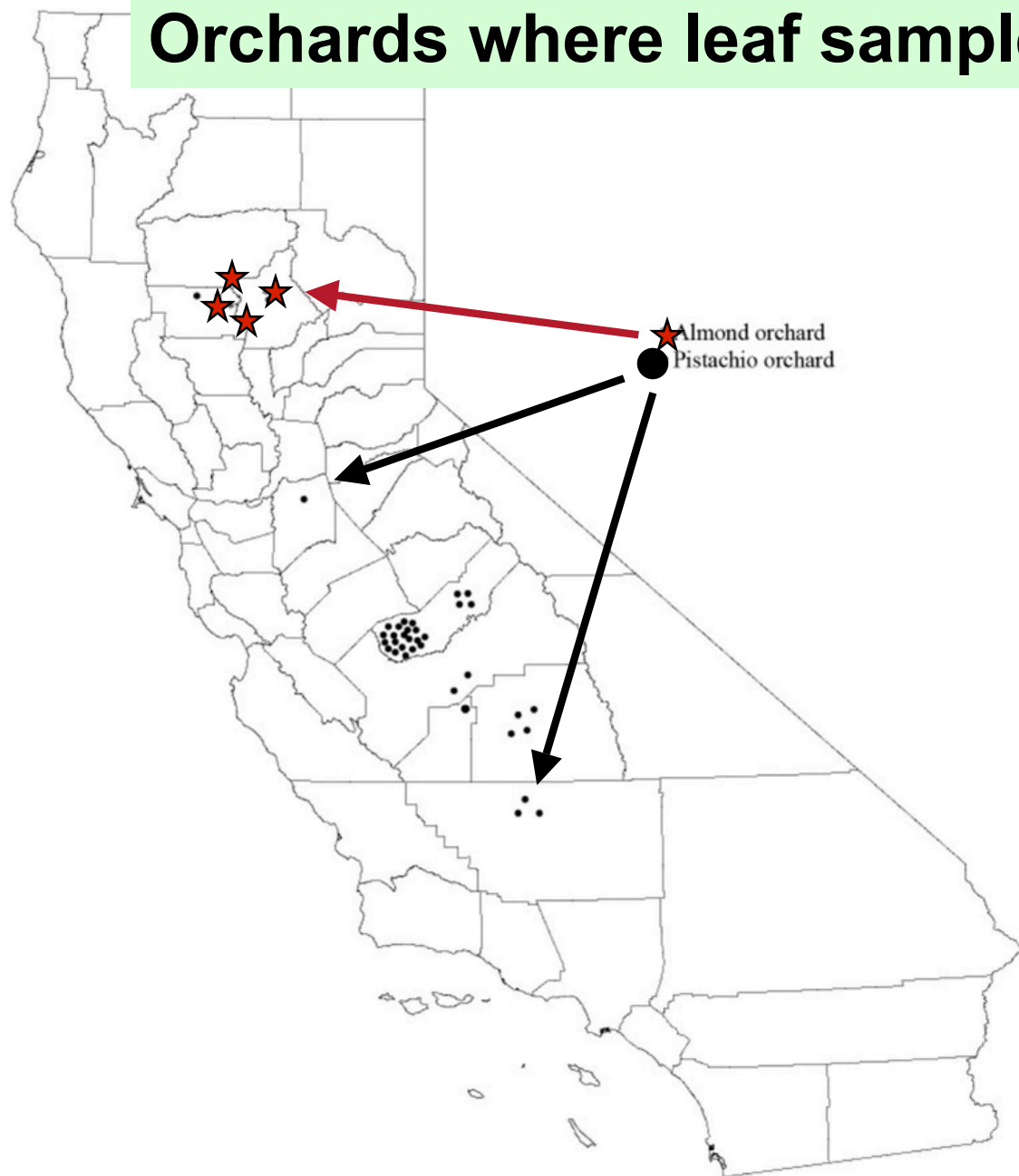
Lesions of Alternaria from leaves

Leaf spot



Orchards where leaf samples were collected

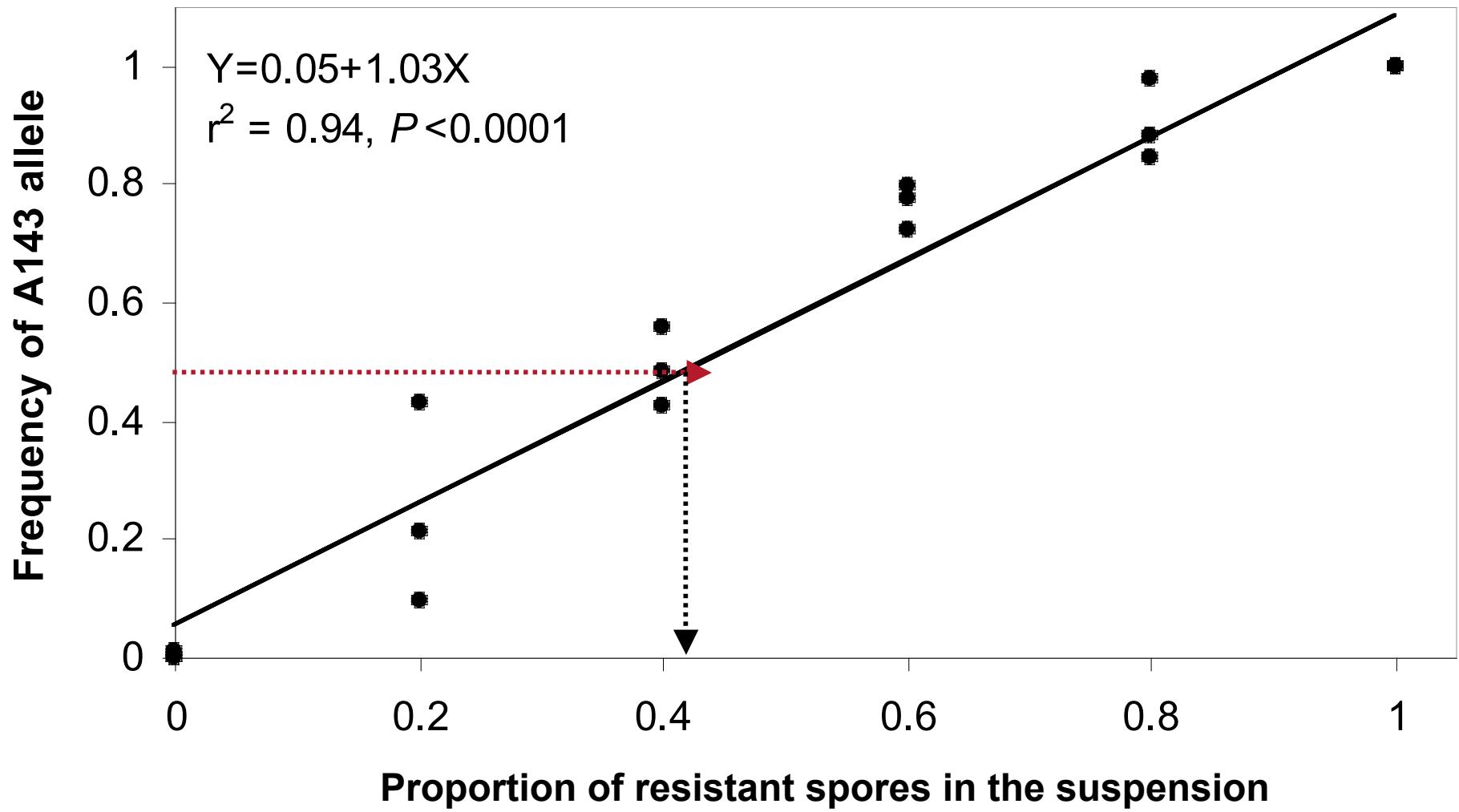
37 pistachio & 4 almond orchards:
(from 7 counties)

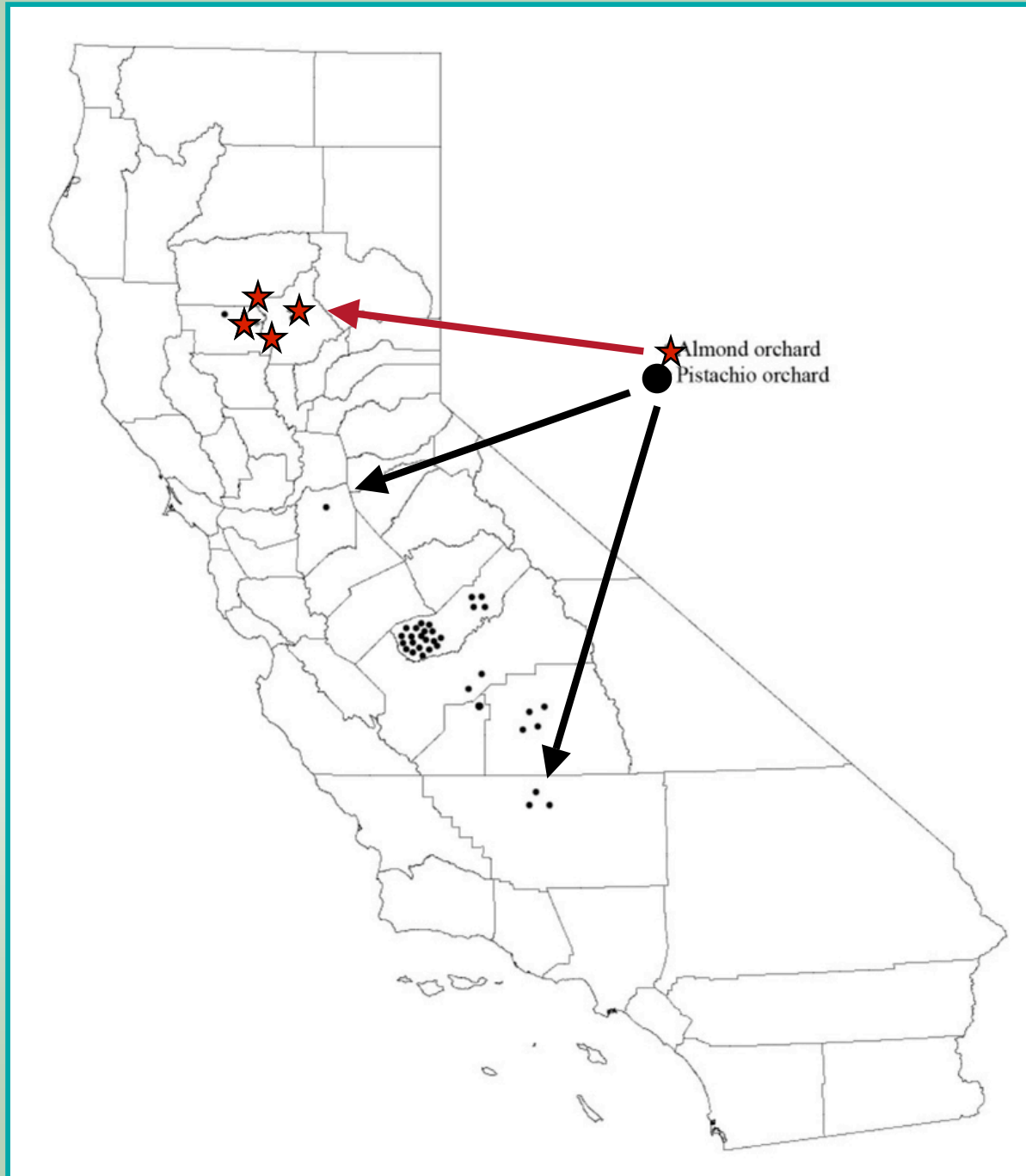


Quantitative determination of frequency of azoxystrobin resistant allele A143 (FA) using a real-time PCR assay

Orchard	Date of collection	County	Host	Mean FA	SD
A1	8/11/2005	Hamilton City, (Glenn)	Almond	0.9910	0.0010
A2	8/5/2005	Hamilton City, (Glenn)	Almond	0.9960	0.0005
A3	8/1/2005	Hamilton City, (Glenn)	Almond	0.9990	0.0011
A4	8/5/2005	Chico, (Glenn)	Almond	0.9998	0.0008
P1	7/28/2005	Orland (Glenn)	Pistachio	0.9926	0.0028
P2	8/19/2005	Fresno	Pistachio	0.0603	0.0489
....
P32	9/6/2005	Madera	Pistachio	0.9899	0.0150
P33	9/6/2005	Madera	Pistachio	0.7885	0.0544
P34	9/6/2005	Madera	Pistachio	0.9926	0.0104
P35	9/6/2005	Madera	Pistachio	0.9874	0.0179
P36	9/8/2005	Butte	Pistachio	0.9915	0.0098
P37	9/8/2005	San Joaquin	Pistachio	1.0000	0.0000

Linear relationship between the frequency of azoxystrobin-resistant allele A143 in *Alternaria* spp. (FA) and the corresponding proportions of azox. **Res** & azox. **Sen** isolates





- 84% of pistachio had >90% resistant isolates;

16% of pistachio had <90% resistant isolates.

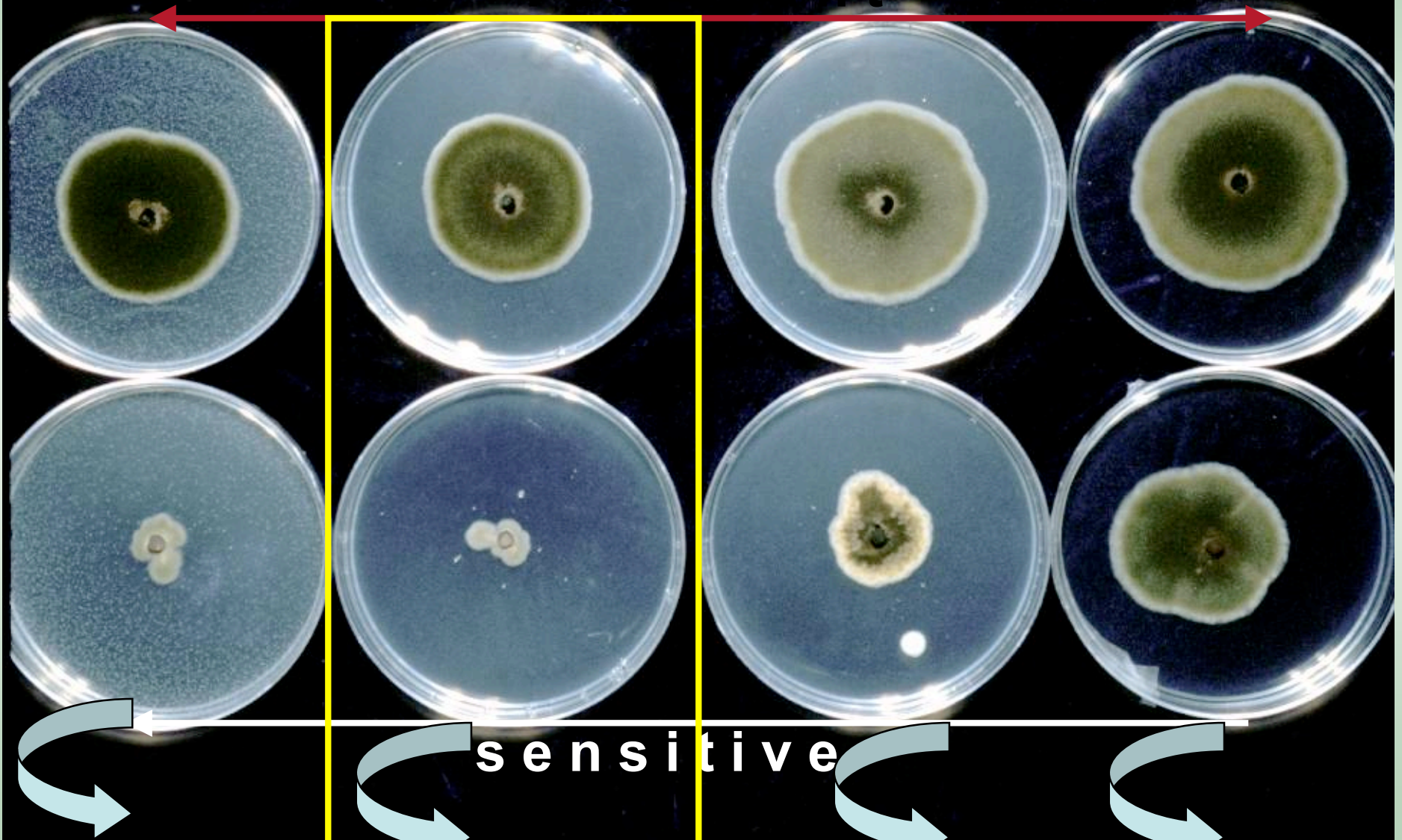
(★ All 4 almond orchards had >90% resistant *Alternaria* isolates.)

Resistance of *Alternaria alternata* from pistachio

- Azoxystrobin resistance widespread and at high levels (pistachios & some almonds orchards).
- Cross resistance to trifloxystrobin and pyraclostrobin.
- In 2003 registration of:
Pristine [a.i. **boscalid** (a carboxamide) + **pyraclostrobin** (strobilurin)];
- Resistance to boscalid in 2 years after its registration.

Boscalid resistant *Alternaria alternata*

r e s i s t a n t



s e n s i t i v e

PDA+100 ppm

PDA+10 ppm

PDA+1 ppm

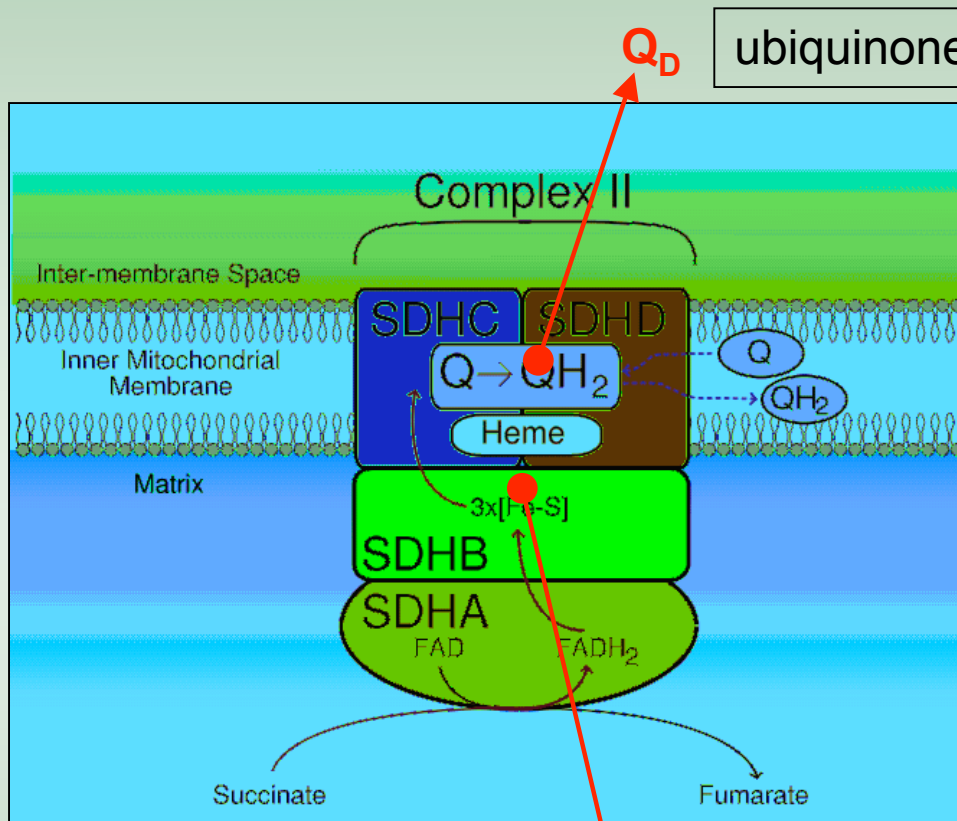
PDA

Cross-resistance between boscalid and carboxin in *A. alternata* isolates (2005)

Phenotype	Number of isolate	Mean EC ₅₀ boscalid (µg/ml)	Mean EC ₅₀ carboxin (µg/ml)
Boscalid-sensitive	7	<1	<20
Boscalid-resistant	38	>100	>50

→ cross resistance between boscalid and carboxin in *A. alternata* → similar mutations could confer resistance to both fungicides

Site of action of carboximides: at the ubiquinone-binding sites succinate dehydrogenase (Sdh)



ubiquinone-binding sites

Four subunits of Sdh complex:

- 1) a flavoprotein (Fp) subunit SdhA,
- 2) an iron-sulfur protein (Ip) subunit SdhB,
- 3) one membrane-anchored protein subunits SdhC,
- 4) And a second membrane-anchored protein subunit SdhD.

Structure and function of Sdh

ubiquinone-binding sites

Conclusions

<i>Alternaria</i> boscalid-resistant mutant	Sensitivity to oxidative stress (preliminary results)	Amino acid modification mutations	Subunit
Type I	+	H 277 → Y H 277 → R	AaSdhB
Type II	+	H134 → R	AaSdhC
Type III	+	D123 → E	AaSdhD
	+	H133 → R	

First report

Orchards with isolates resistant to boscalid (2005 - 2007)

Orchard	History of Pristine	Year	Isolates tested	Boscalid resistant (%)
KAC, Fresno	No	2005	49	2.04
1, Kern	Yes	2005	59	12.00
1, Kern	Yes	2006	42	81.00
2, Kern	Yes	2007	74	88.00
3, Kern	Yes	2007	26	96.15
1, Tulare	Yes	2007	27	89.00
2, Tulare	Yes	2007	53	94.34
3, Tulare	Yes	2007	88	94.32

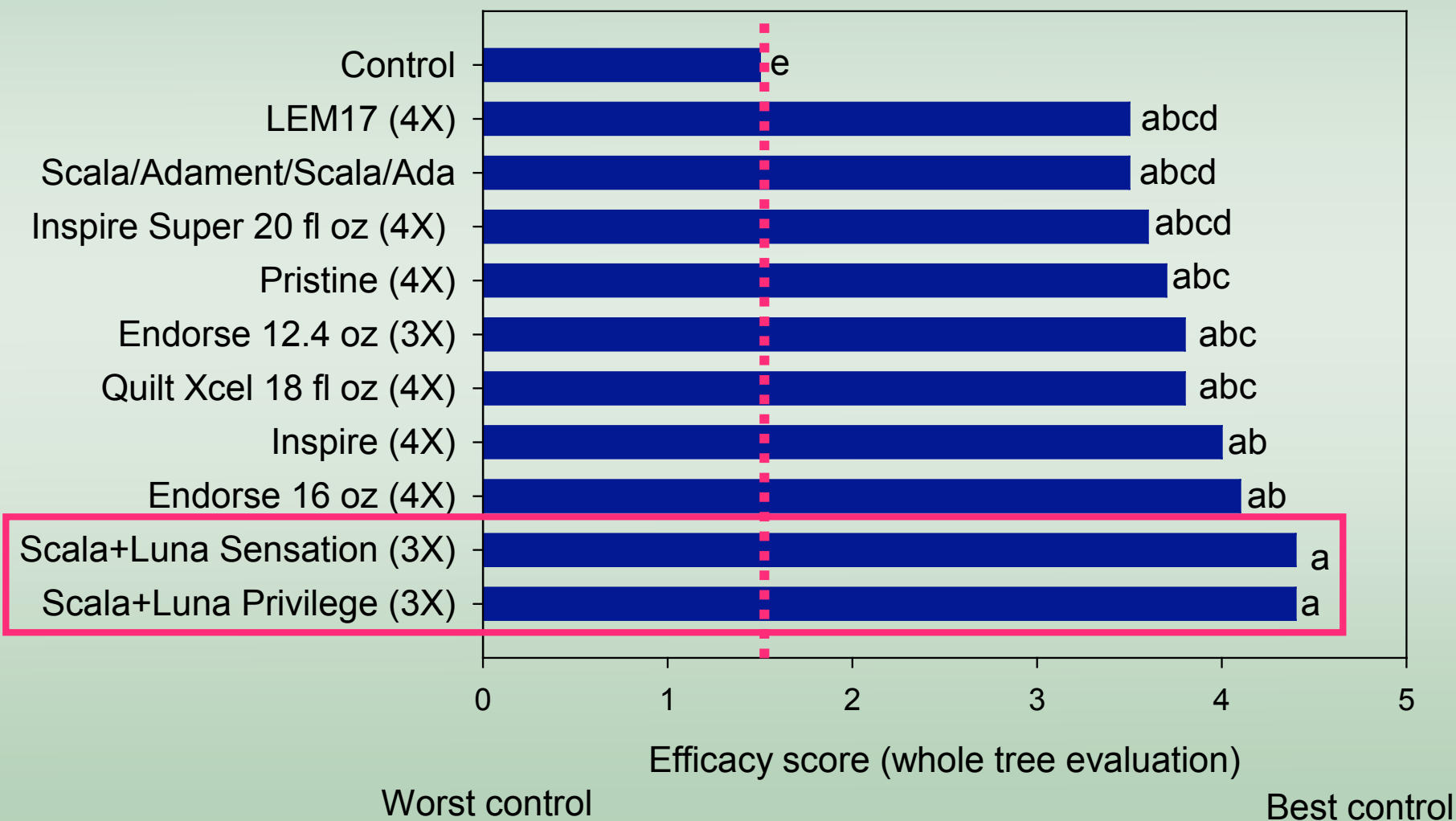
Summary of boscalid resistance in *Alternaria* isolates

Year of isolation	Number			Boscalid resistant isolates (%)
	Counties	Orchards	Isolates	
2005	2	2	59	12%
2006	1	1	42	81%
2007	3	5	180	90%
2008	5	39	300	Variable: up to 100% in some orchards

Proportion of *Alternaria* isolates showing mutations in different SDH subunits

	Mutation in subunit:	Percent of isolates of <i>Alternaria</i>
I	AaSdhB	48%
II	AaSdhC	33%
III	AaSdhD	7.5%

Efficacy of fungicides against *Alternaria* late blight in 2009 - KAC



Sensitivity and cross resistance or lack of cross resistance of *Alternaria alternata* isolates from pistachio

Isolate of <i>Alternaria</i>	Phenotype	Cross resistance (Res) or sensitivity (Sen)	
	Boscalid (10 ppm)	Penthiopyrad (10 ppm)	Fluopyram (10 ppm)
Aa23	Sen	Sen	Sen
Aa4	Sen	Sen	Sen
Aa6	Sen	Sen	Sen
Aa61	Sen	Sen	Sen
Aa35	Sen	Sen	Sen
Aa10	Sen	Sen	Sen
Aa48or	Res	Res	Sen
Aa50	Res	Res	Sen
Aa22rc	Res	Res	Sen
Aa32	Res	Res	Sen
Aa122	Res	Res	Sen
Aa40	Res	Res	Sen
Aa126	Res	Res	Sen
Aa37	Res	Res	Sen
Aa29	Res	Res	Sen

cross resi

negative cross resistance



General conclusions:

Although *Alternaria* poses a challenge in management of resistance, new registrations of fungicides will provide alternatives to overcome this challenge.

For instance, new fungicides to which *Alternaria* does not show resistance will be successful in managing the disease (at least for a couple of years or so ...)

PISTACHIO—Fungicide treatment timing

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

Source: <http://www.uckac.edu/plantpath/>

For managing *Alternaria* late blight:

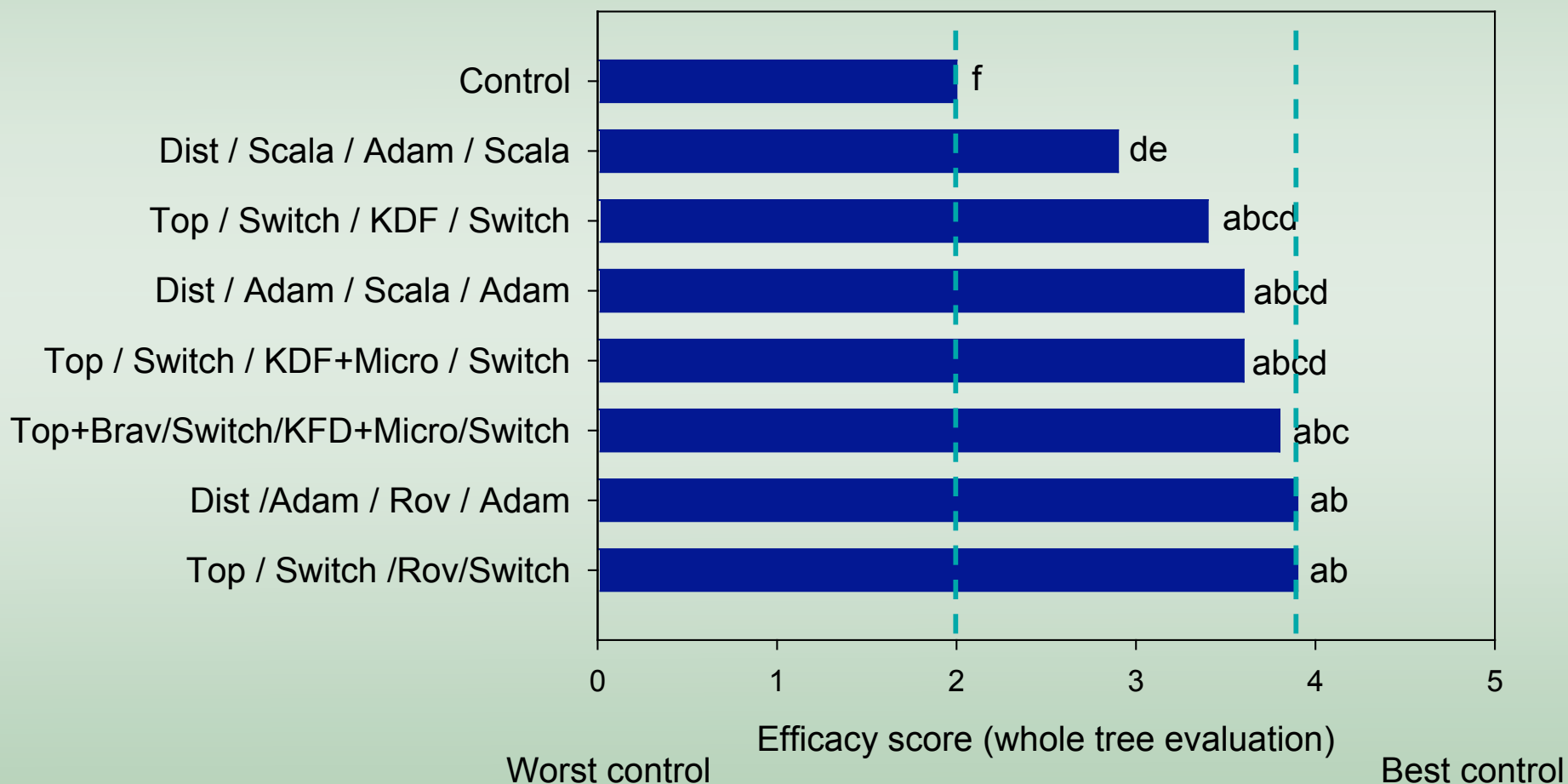
- ✓ Mid season sprays very important and effective (start in June & end late July).
- ✓ When one spray: **end June /early July.**
- ✓ Bloom and late season sprays not effective.

Acknowledgments:

UC Kearney Agricultural Center

- Herve Avenot, Ph.D.
- David Morgan, M.Sc.
- Ryan Puckett
- Heraclio Reyes
- Jessica Windt

Efficacy of rotations against *Alternaria* late blight in 2008- KAC



Resistance management

1. Tank mix or alternate compounds with different mode of action.
2. Limit the number of sprays. **DMI or strobilurins sprays up to 3 or better fewer.**
3. The timing of application is **very important (systemic early in the season to keep down the epidemic).**
4. Blocking (**when strobilurins are used, avoid using more than 2 subsequent strobilurin sprays.**

Examples to extend the use of Pristine in pistachio

Timing	April (bloom)	June	July	August
Program options	Topsin-M (for Botrytis)	None	Pristine + chlorothalonil	Switch+ chlorothalonil
Program options	Topsin-M (for Botrytis)	Switch	Pristine + chlorothalonil	Switch+ chlorothalonil
Program options	Topsin-M (for Botrytis)	Pristine	Switch + chlorothalonil	Pristine + chlorothalonil
Alternaria pressure	---	++	++++	++

Cross resistance

Isolate	Origin (Orchard location)	Year of isolation	EC ₅₀ boscalid (g / ml) ^a	EC ₅₀ carboxin (g / ml)
AaY16	Kern County, CA	2005	0.28	13.21
Aa4	Kern County, CA	2005	0.26	11.27
Aa127	Kern County, CA	2005	0.56	18.49
Aa20	Kern County, CA	2005	>100	77.37
Aa29	Kern County, CA	2005	>100	54.41
Aa65	Kern County, CA	2005	>100	51.23
Aa111	Kern County, CA	2005	>100	43.58
Aa122	Kern County, CA	2005	>100	65.79
AaY1	Kern County, CA	2005	>100	38.06
Aa2	Kern County, CA	2006	>100	55.67
Aa26	Kern County, CA	2006	>100	46.64
Aa13	Kern County, CA	2006	>100	46.41
Aa40	Kern County, CA	2006	>100	64.80
Aa10	Kern County, CA	2006	>100	98.53
Aa54	Kern County, CA	2006	>100	68.71
Aa19	Kern County, CA	2006	>100	80.92
Aa94	Kern County, CA	2007	>100	62.17
Aa105	Kern County, CA	2007	>100	63.67
Aa37	Kern County, CA	2006	>100	45.38
Aa45	Kern County, CA	2006	>100	53.51
Aa33	Kern County, CA	2006	>100	71.31
Aa58	Kern County, CA	2006	>100	80.29
Aa 48o	Tulare County, CA	2007	>100	40.44
Aa 26o	Tulare County, CA	2007	>100	41.17
Aa 19o	Tulare County, CA	2007	>100	52.02

} sensitive

} resistant

Levels of boscalid resistant *Alternaria alternata* - pistachio

Location		Number of isolates	Year of isolation	History of Pristine sprays	Frequency of boscalid Resist. isolates (%)
Orchard	County				
KAC	Fresno	49	2005	No	2.04
SVO1	Kern	59	2005	Yes	12.00
SVO1	Kern	42	2006	Yes	81.00
SVO2	Kern	74	2007	Yes	88.00
SVO3	Kern	26	2007	Yes	96.15
NCL1	Tulare	27	2007	Yes	89.00
NCL2	Tulare	53	2007	Yes	94.34
ORD3	Tulare	88	2007	Yes	94.32
JV	Madera	19	2008	No	15.50
T8	Tulare	106	2008	Yes	75.50
A1	Fresno	9	2008	Yes	56.00
F1	Kings	15	2008	Yes	0.00
Y1	Yolo	30	2008	-	0.00
SQ1	Kern	15	2008	No	6.67
M1	Madera	38	2008	Yes	18.42
C1	Madera	121	2008	Yes	56.20

Orchards with isolates resistant to boscalid (collected in 2008)

Orchard	History of Pristine	Year	Isolates tested	Boscalid resistant (%)
1, <u>Madera</u>	No	2008	19	<u>15.8</u>
4, <u>Tulare</u>	Yes	2008	106	75.5
2, <u>Fresno</u>	Yes	2008	9	<u>56.0</u>
1, <u>Kings</u>	Yes	2008	15	<u>0.0</u>
1, <u>Yolo</u>	Yes	2008	30	<u>0.0</u>
4, <u>Kern</u>	No	2008	15	<u>6.7</u>
1, Madera	Yes	2008	38	18.4
2, <u>Madera</u>	Yes	2008	121	<u>56.2</u>

Alternaria: A resistance challenge

Strobilurin resistance in *Alternaria* of pistachio: history

2000: Abound and other strobilurins registered.

2002: Suspect existence of resistant isolates?

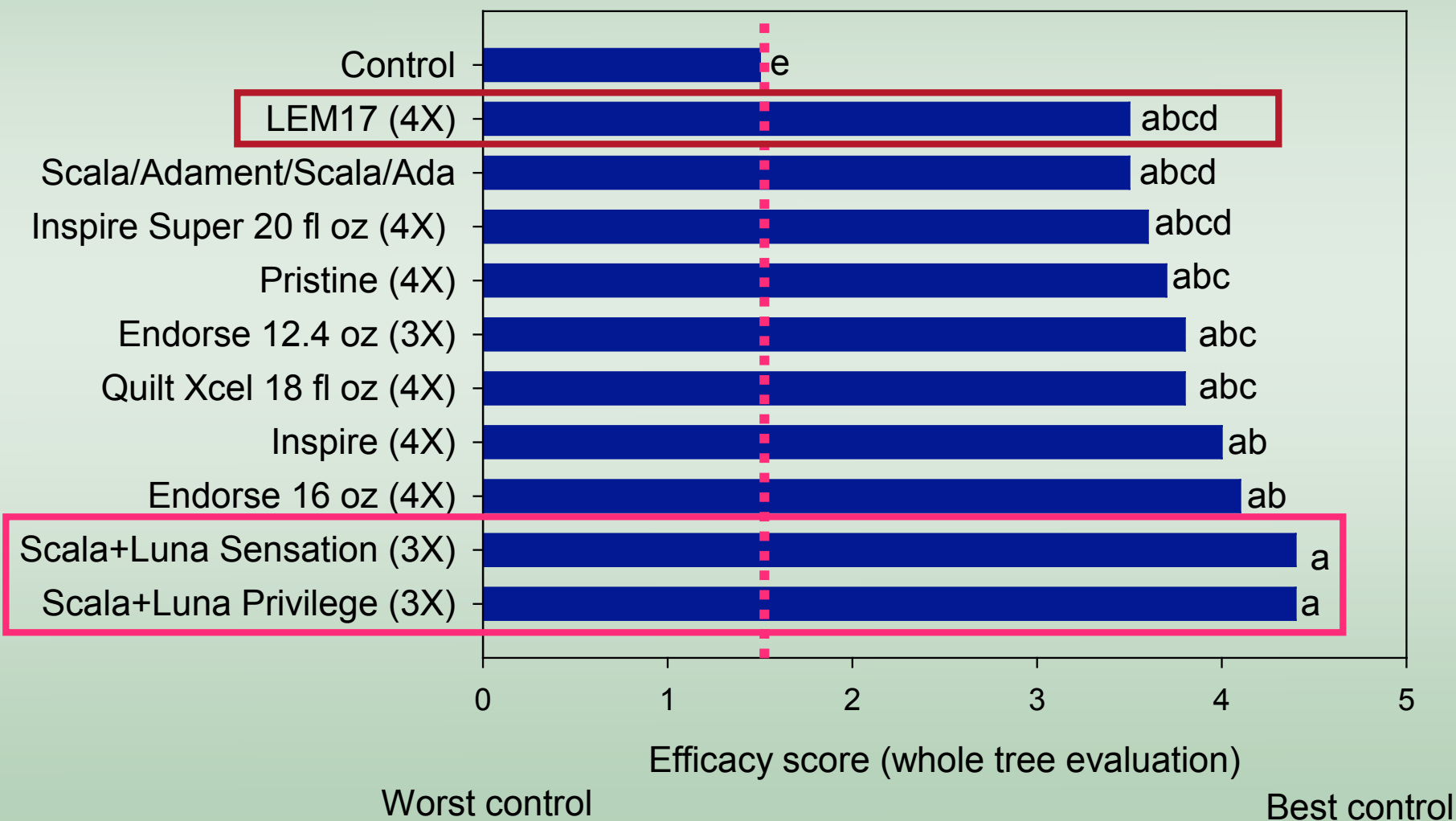
2003: Yes, resistance; determined the G143A in Cyt b conferring high resistance.

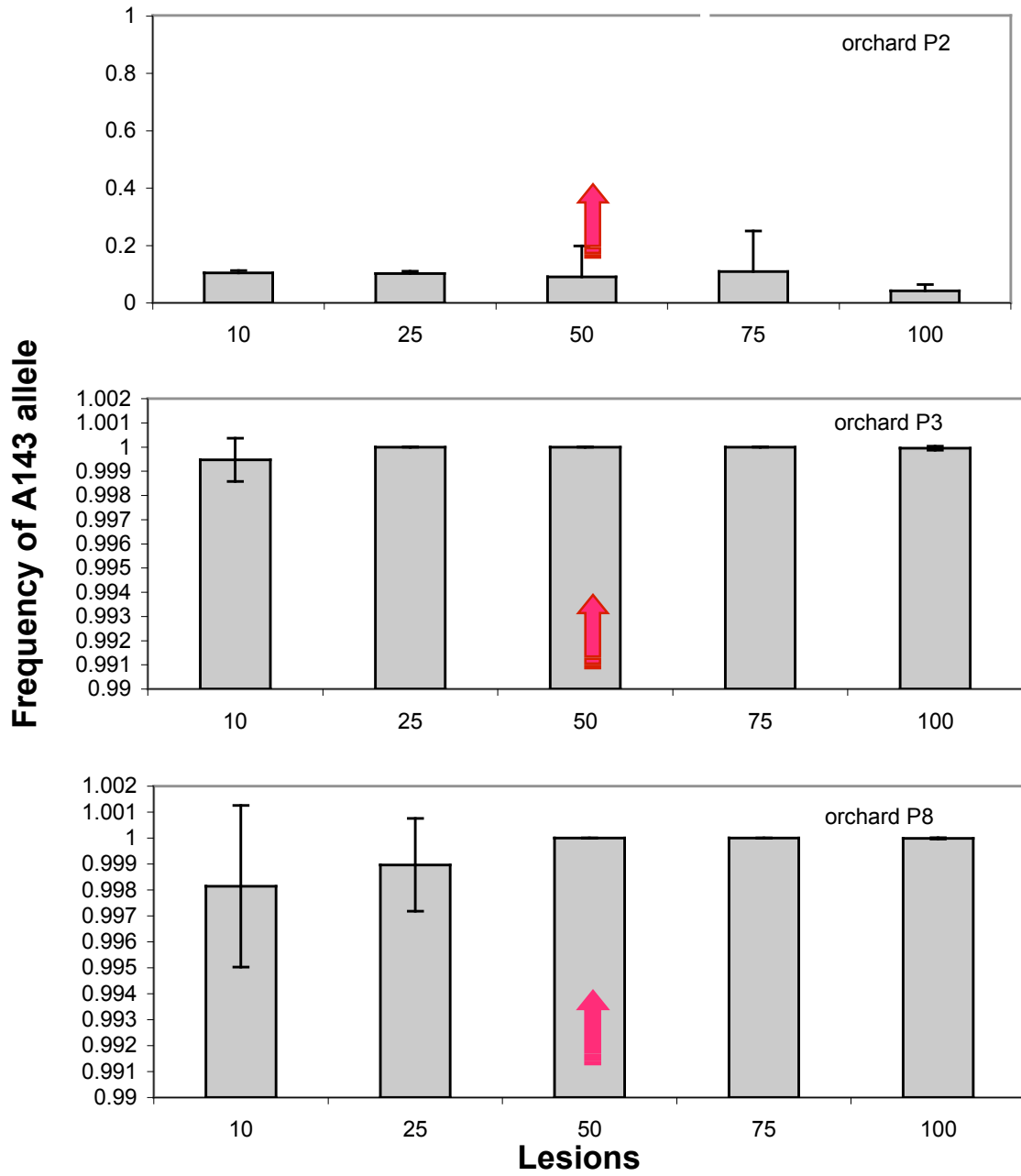
2004: An allele-specific PCR to detect azoxystrobin resistance from cultures.

2004: A real-time PCR assay for detecting azoxystrobin resistant isolates (in cultures).

2006: A real-time PCR assay for detecting azoxystrobin resistance (in the field; leaf lesions from orchards).

Efficacy of fungicides against *Alternaria* late blight in 2009 - KAC





Optimum sample size



Tests with samples:
10, 25, 50, 75, and
100 lesions