Evaluation of new bactericides for control of fire blight of pears caused by *Erwinia amylovora*

and

Update on postharvest decay control

J.E. Adaskaveg

Department of Plant Pathology and Microbiology

UC Riverside

Fire blight - Chemical and biological control
- Currently registered and new materials -

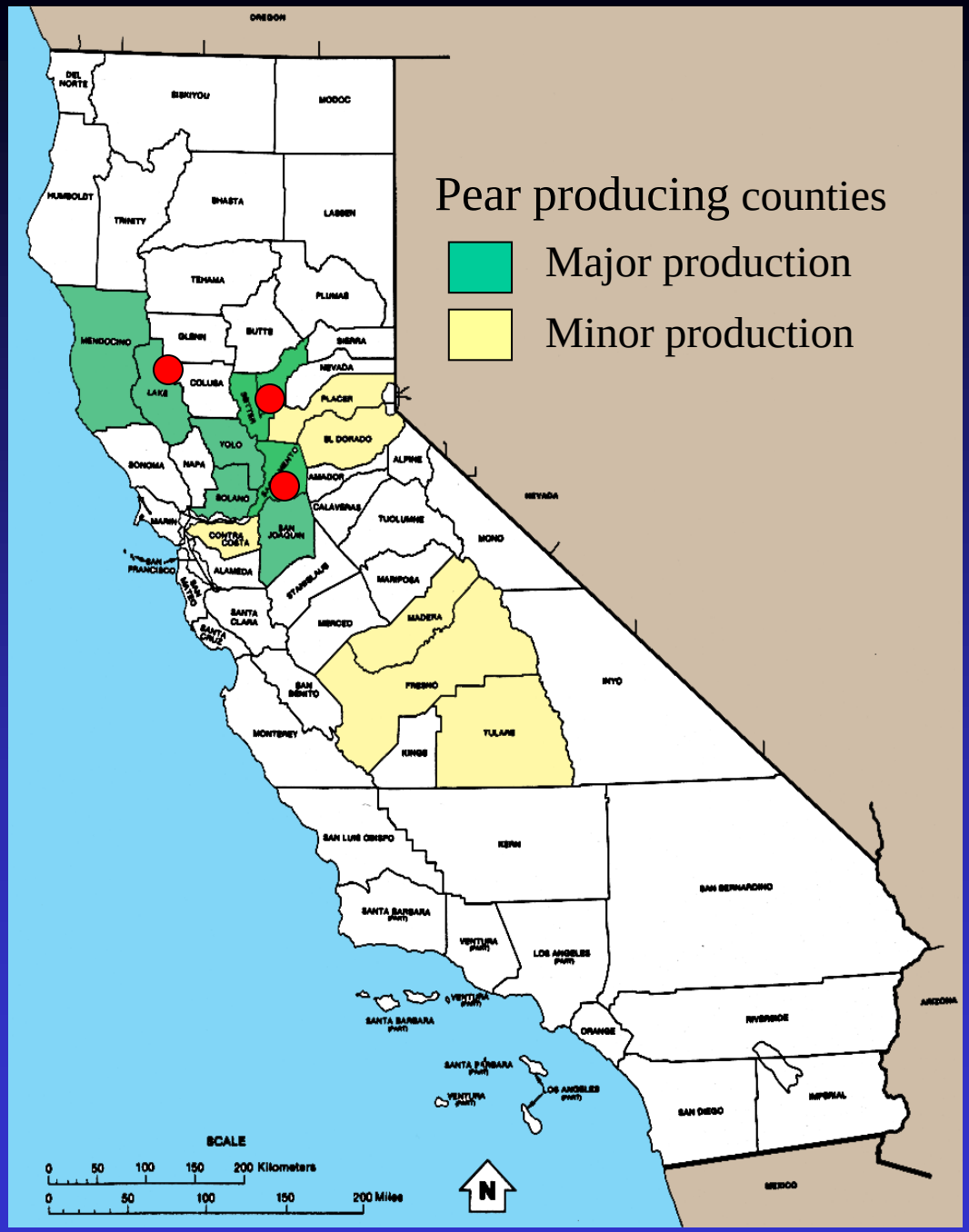
Copper compounds	Antibiotics	Biologicals
Fixed coppers: Historically used only during dormant and bloom periods because of phytotoxic effects on developing fruit (russetting)	Streptomycin (1950s): ***Resistance widespread***	Bloomtime Biological *Pantoea agglomerans* E325 and Blightban *Pseudomonas syringae* A506: Efficacy variable
Efficacy intermediate	Terramycin (oxytetracycline) (1970s): **Effective but less than strep., resistance limited**	Blossom Protect: Efficacy intermediate
Kocide 3000; Badge X2: New copper formulations are less phytotoxic but labels will not change.	Kasugamycin: high efficacy in multiple years, also at other US locations, registration expected 2013	Other materials have been evaluated: Cerebrocide, Actinovate, Blossom Protect, Citrox/ProAlexin

Few consistently effective available for fire blight control.
Goals: provide new conventional and biological treatments, limit spread of existing resistance and development of new resistance

Fire blight - Chemical and biological control
- Currently registered treatments -

Compound	Registered	Efficacy
Copper – Reduced MCE	+	+++
Dithane	+	++
Streptomycin	+	++++/+
Oxytetracycline	+	+++/+
Bloomtime Biological	+	+/+++
BlightBan	+	+/++
Actinovate	+	+/++

Streptomycin is the only bactericide currently registered that is highly effective, but resistance is widespread.

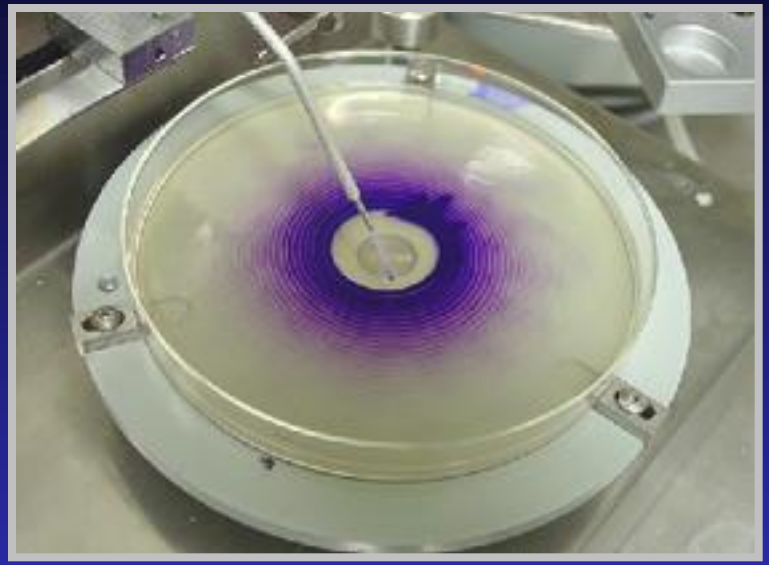


Surveys on antibiotic resistance in populations of *Erwinia amylovora*

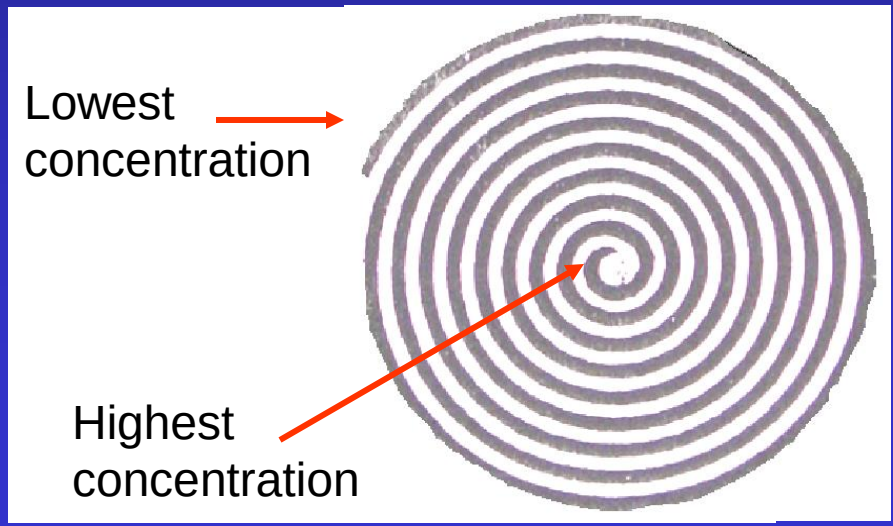
- Collection of isolates from major pear growing regions in CA (2006 - 2012)
  - Sacramento valley – Sacramento, Sutter/ Yuba Co.
  - Lake Co.
- Evaluate sensitivity
  - Streptomycin
  - Oxytetracycline
  - Kasugamycin

Determining inhibitory concentrations using the spiral gradient dilution method

Spiral plater



- A continuous 2.5-log antibiotic gradient is produced on an agar plate using a spiral plater.
- Bacteria are streaked along the gradient and after a 2-day incubation, growth measurements are taken.



In vitro sensitivity of *E. amylovora* isolates to antibiotics

Spiral gradient dilution plate showing isolates with different sensitivity against streptomycin

Concentration for 95% inhibition of growth	Rating for sensitivity to streptomycin
0.6 mg/L	Sensitive
20 mg/L	Moderately resistant
>70 mg/L	Highly resistant

- Molecular basis for high and moderate resistance is different.
- Molecular basis for moderate resistance in CA is different from other locations (MI).

Resistance to streptomycin in *E. amylovora* in California

- **Isolates with high levels of resistance** were common in the Western US in the 1990s.
- This type of resistance is now rare – it was only found at one location in our surveys in CA from 2006 to 2011 and at two locations in 2012.
- **Isolates with moderate levels of resistance** have mostly replaced highly resistant isolates
- Among the major production areas, the incidence of resistance was low in samplings from Lake Co.
- This has been attributed to the widespread use of mixture applications (strep + oxy)

Resistance to oxytetracycline and kasugamycin in *E. amylovora* in California

Oxytetracycline

- Isolates with reduced sensitivity to oxytetracycline were detected at a few locations in 2007-2009 and **in 2012**.
- Minimum inhibitory concentrations: 0.82 – 1.5 ppm as compared to 0.09 – 0.5 ppm for sensitive populations
- This reduced sensitivity to oxytetracycline in CA isolates was *confirmed* to us by other labs.
- At these locations, treatments with Mycoshield lacked efficacy – field resistance.

Kasugamycin

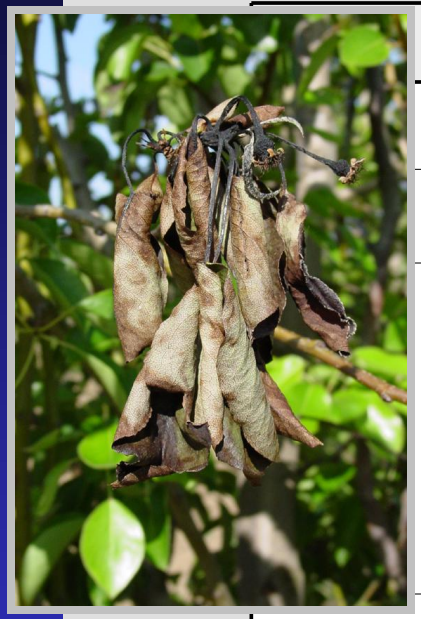
- In our surveys from 2006 to 2012 with 100s of isolates evaluated, no reduced sensitivity to kasugamycin has been detected.

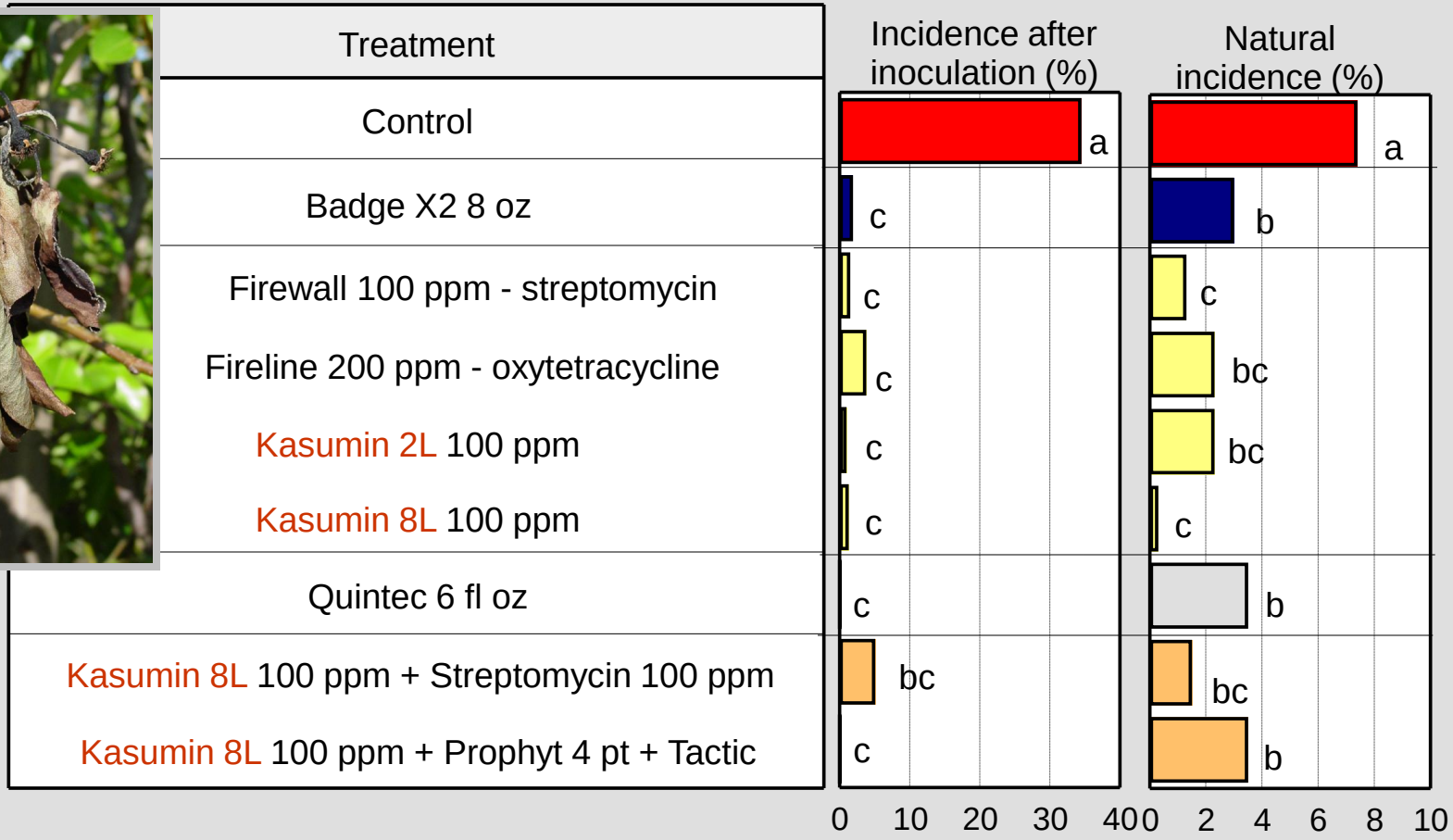
Evaluation of alternative treatments to streptomycin and oxytetracycline

Goals:

- Identify rotation/mixture materials for streptomycin and oxytetracycline
- Limit spread of existing resistance and development of new resistance
- Identify new compounds that can be used for organic production: biocontrols, natural products

Evaluation of new bactericides for fire blight management on Bartlett pears in a field trial in Live Oak 2011

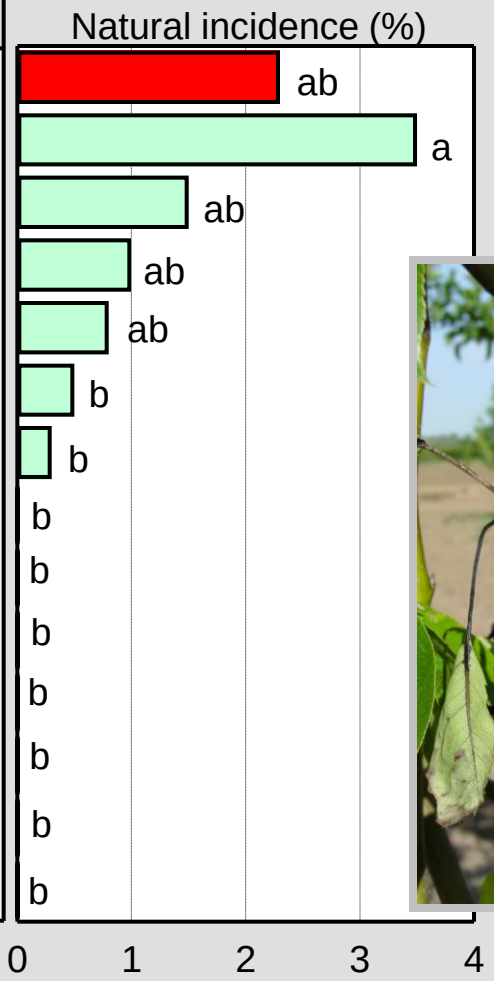




4 treatments (starting at 80% bloom) were applied using an air-blast sprayer at 100 gal/A.

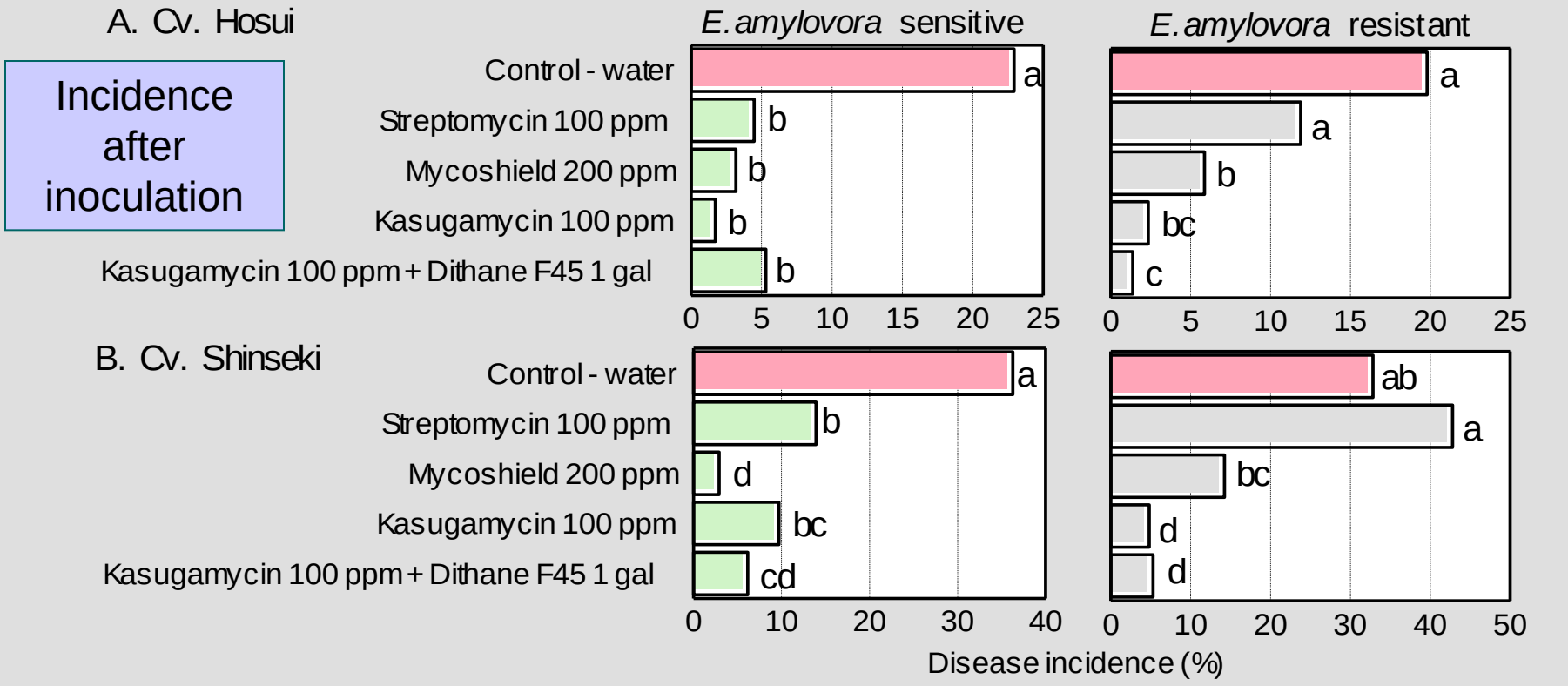
Evaluation of new bactericides for fire blight management on Bartlett pears in a field trial in Live Oak 2012

No.	Treatment*	Rate/A
1	Control	---
2	Ph-D	6.2 oz
3	Quintec	6 fl oz
4	Kasumin 8L + Quintec	100 ppm + 6 fl oz
5	Badge X2	8 oz
6	Fireline (Oxytetracycline)	200 ppm
7	Kasumin 8L + Badge X2	100 ppm + 8 oz
8	Kasumin 8L + Firewall	100 ppm + 100 ppm
9	Kasumin 8L + Fireline	100 ppm + 200 ppm
10	Kasumin 8L	100 ppm
11	Kasumin 2L	100 ppm
12	Kasumin 8L + Ph-D	100 ppm + 6.2 oz
13	Firewall (Streptomycin)	100 ppm
14	Fireline + Firewall	200 ppm + 100 ppm



5 treatments (starting at 70% bloom) were applied using an air-blast sprayer at 100 gal/A. Evaluation in May 2012.

Efficacy against fire blight caused by *E. amylovora* resistant to streptomycin and oxytetracyline
Small-scale field test on Asian pear



Inoculations 1 h after treatment. Resistant = reduced sensitivity to streptomycin and oxytetracycline

- Kasugamycin is effective against isolates of *E. amylovora* streptomycin/oxytetracycline-sensitive or -resistant.
- No cross-resistance

Summary: New bactericides for management of fire blight that can be used in programs with strep and oxy

- **Kasugamycin- Kasumin**
  - Efficacy equivalent or better than terramycin or streptomycin.
  - Effective against strep/oxy-resistant isolates of *E. amylovora*.
  - Mixtures with strep, oxy, Dithane/Manzate, Quintec, Syllit and other compounds are effective and can be part of a resistance management program.
  - No phytotoxicity observed after 3 applications.
  - Federal registration in 2013? Conditional federal registration for March 2013 (EPA Posting for Public Notice – Feb. 7?). CA registration only after federal approval.
- **New copper materials with lower mce use**
  - Kocide 3000, Badge X2, others
  - Moderately effective
  - No phytotoxicity observed after 3 applications.
  - Can be part of a rotation program.

Conclusion: Kasugamycin

- The most promising new bactericide for control of fire blight
- This aminoglycoside antibiotic is not used in medicine
- Antifungal and antibacterial activity
  (Also effective against scab)
- Different mode of action from other antibiotics
- Registered on crops in Asia, Europe, & Central America
- US-EPA approved an import tolerance in 2005
- US-EPA reviewing 2010 submission for 2013 registration

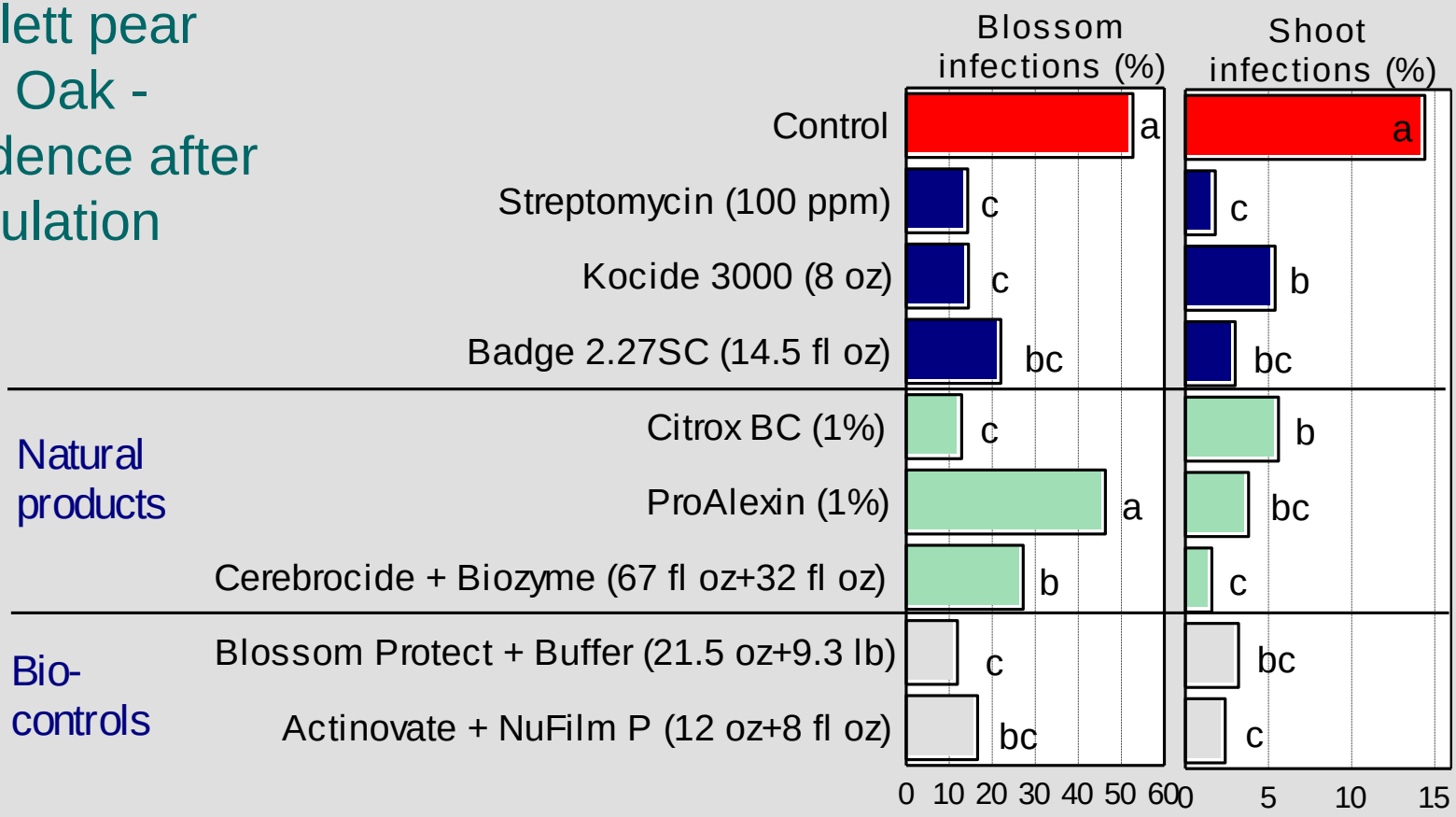
Evaluation of alternative treatments to streptomycin and oxytetracycline

Goals:

- Identify rotation/mixture materials for streptomycin and oxytetracycline
- Identify new compounds that can be used for organic production: biocontrols, natural products

Evaluation of natural products and biocontrols for fire blight management

Bartlett pear
Live Oak -
Incidence after
inoculation



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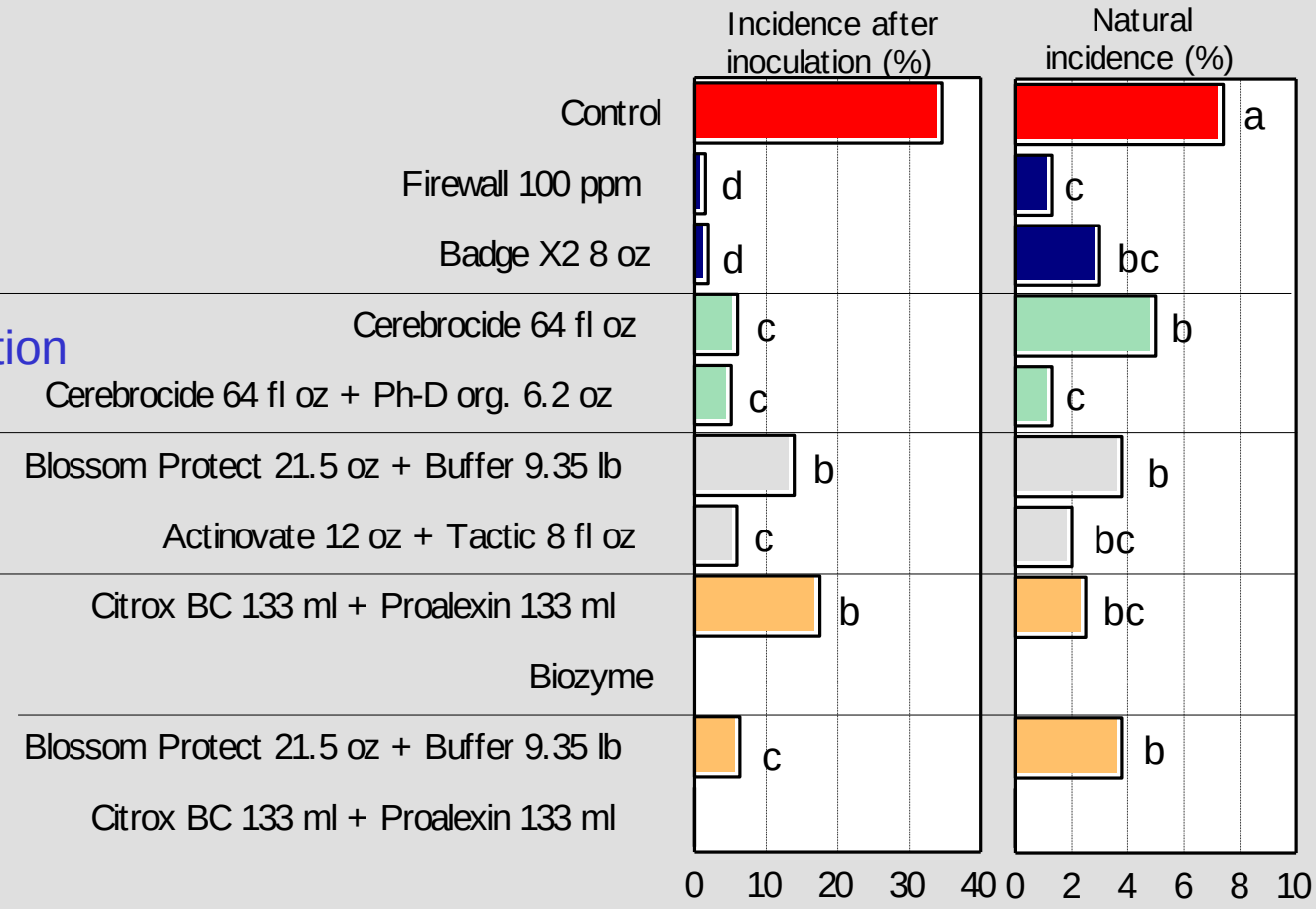
Evaluation of natural products and biocontrols for fire blight management

Bartlett pear
Live Oak

Natural/fermentation products

Biocontrols

Rotations



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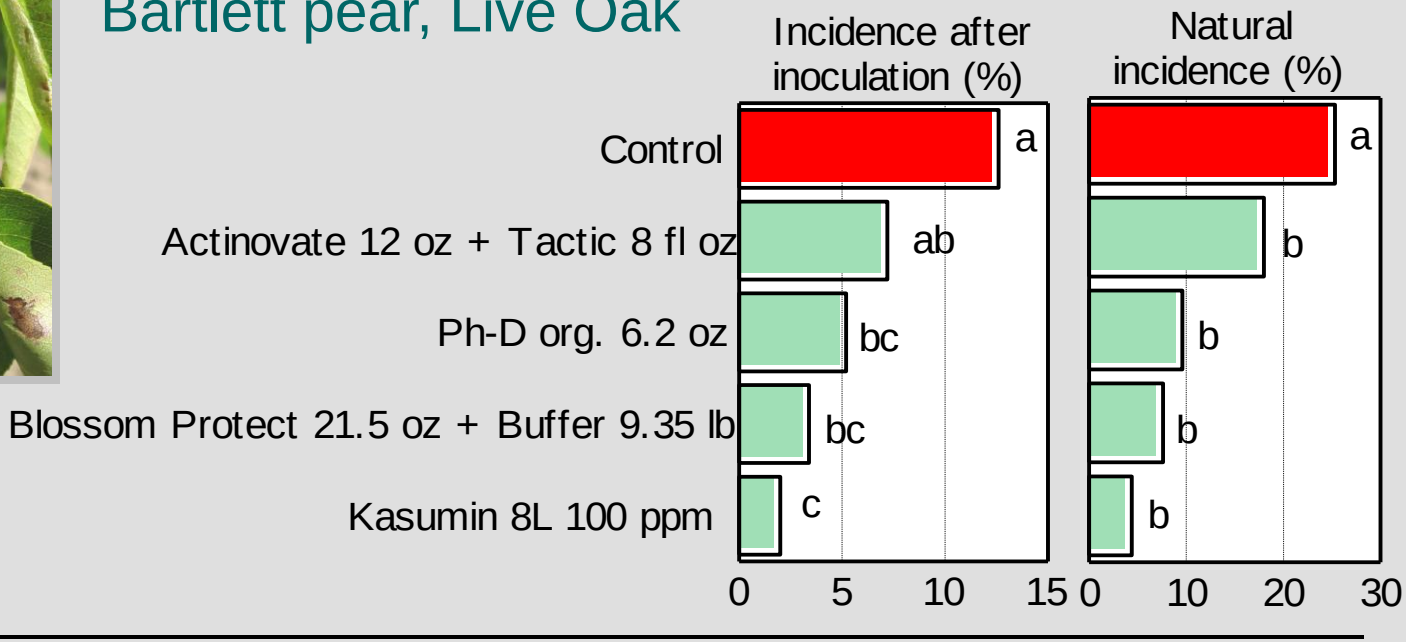
Evaluation of natural products and biocontrols for fire blight management

Bartlett pear, Live Oak

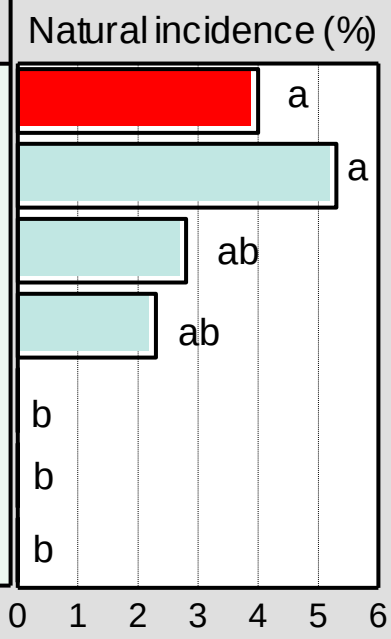


4 treatments (starting at 80% bloom) were applied using an air-blast sprayer at 100 gal/A.

Evaluation of natural products and biocontrols for fire blight management

Bartlett pear, Live Oak 2012

No.	Treatment	Rate/A
1	Control	---
2	AgriTitan	1:25
3	BlossomProtect + Buffer + Actinovate	1.34 lb + 9.35 lb + 12 oz
4	Actinovate	12 oz
5	Kasumin 8L	100 ppm
6	Kasumin 8L + Actinovate	100 ppm + 12 oz
7	BlossomProtect + Buffer	1.34 lb + 9.35 lb



5 treatments (starting at 70% bloom) were applied using an air-blast sprayer at 100 gal/A.

Summary: New natural products and biocontrols for management of fire blight

- Several new products showed promising efficacy and deserve continued evaluation.
  - ***Blossom Protect*** shows the most promise
- Efficacy of biologicals/natural products ranged from low to high and often was inconsistent between years.
- Due to their inconsistent efficacy, natural products and biocontrols will be best used in rotations or when disease pressure is lower.

Summary: Efficacy of new and registered compounds for management of fire blight

Compound	Registered	Efficacy
Copper	+	+++
Dithane	+	++
Streptomycin	+	++++/+
Kasugamycin	2013?	++++
Oxytetracycline	+	+++
Blossom Protect	+	+++/+
Bloomtime Biological	+	+++/+
BlightBan	+	++/+
Actinovate	+	++/+
Cerebrocide	-	++/+
Citrox/ProAlexin	-	++/+

FUNGICIDES, BACTERICIDES, AND BIOLOGICALS FOR DECIDUOUS TREE FRUIT, NUT, STRAWBERRY, AND VINE CROPS - 2013

Statewide IPM Program
www.ipm.ucdavis.edu

Disease	Fall	Delayed dormant	Green tip /White bud	Pink bud	Petal Fall/ Cover Sprays
Scab[1]	++[2]	++[2]	+++	+++	+++
Powdery mildew[3]	----	----	----	+++	+++
Fire blight	----	----	+	+++	+++[4]

Bactericide/ Biological	Resistance risk	Fire blight[11] Contact	Fire blight[11] Systemic	Phytotoxicity
Ag Streptomycin/Agri-Mycin /Firewall	high	++++	+++	+/-
MycoShield/FireLine[10] (FlameOut**)	high	+++	+++	+/-
Copper[7]	low (M1)	+++	----	+
Captan[6]	low (M4)	++	----	----
Dithane/Manzate/ Penncozeb[6]	low (M3)	++	----	----
Kasumin*	high	++++	++++	+/-
Blight Ban	low	++/+	----	+/-
Bloomtime Bio	low	+++/++	----	+/-
Blossom Protect	low	+++/++	----	+/-

Update on Postharvest Management of Pear Decays

Experimental packingline at KARE

TBZ resistance rampant among major pathogens – Blue and Gray mold

Multiple products need to be registered – Only single-site mode of action materials available

Registration process takes years to navigate with limited available supporting registrants

Registered and new postharvest fungicides for management of decays of pome fruit

Phthalimides

Captan

M4

Methyl Benzimidazole Carbamates

TBZ (Mertect, Alumni)

1

Phenylpyrroles

Fludioxonil (Scholar)

12

Anilinopyrimidines

Pyrimethanil (Penbotec)

9

Hydroxyanilides

Fenhexamid (Judge)

17

DMI-triazoles

Difenoconazole

3

Pending

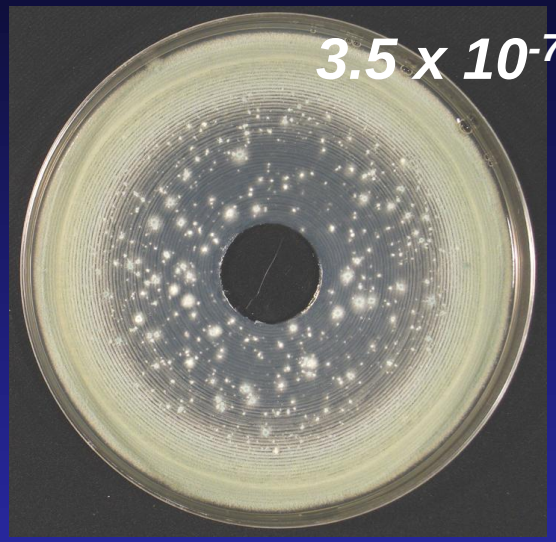
Fungicide	Gray mold	Blue mold	Bull's eye rot	Rhizopus rot	Mucor decay
Captan	+	+	+	-	-
TBZ	++**	++**	++	-	-
Pyrimethanil	+++ **	+++**	+++	-	-
Fenhexamid	+++*	-	-	-	-
Fludioxonil	+++	+++	+	+++	+/-
Difenoconazole	+	+++	+++	+	-
Fludioxonil-Dif'zole	+++	+++	+++	+++	+/-

**Resistance widespread

*Resistance localized

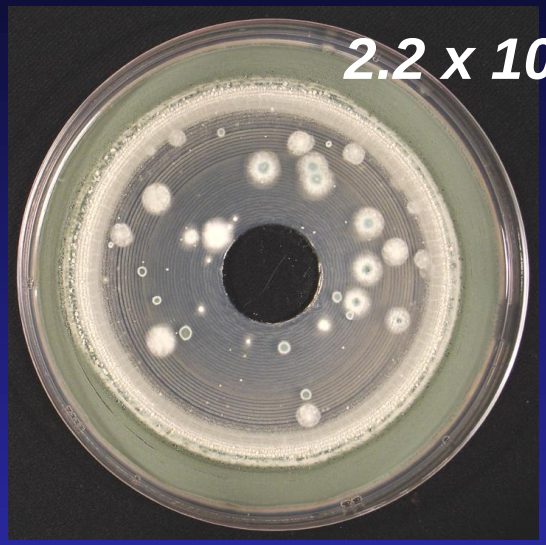
Natural resistance frequencies in P. expansum

Examples of representative selection plates after 5 days of incubation



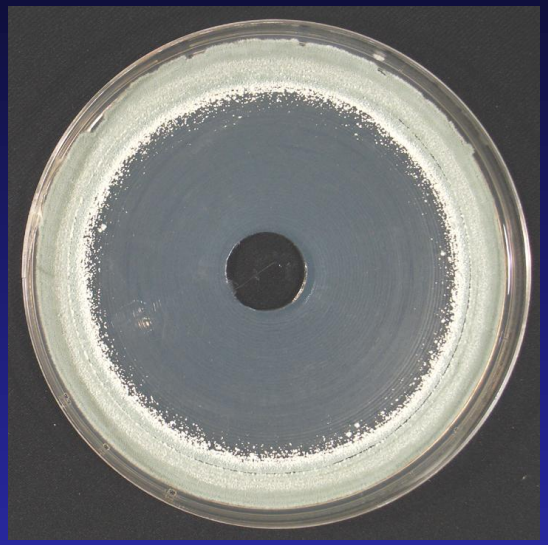
Pyrimethanil (AP agar)

All resistant isolates looked like the sensitive wild-type.



Fludioxonil (PDA)

3 types of colonies



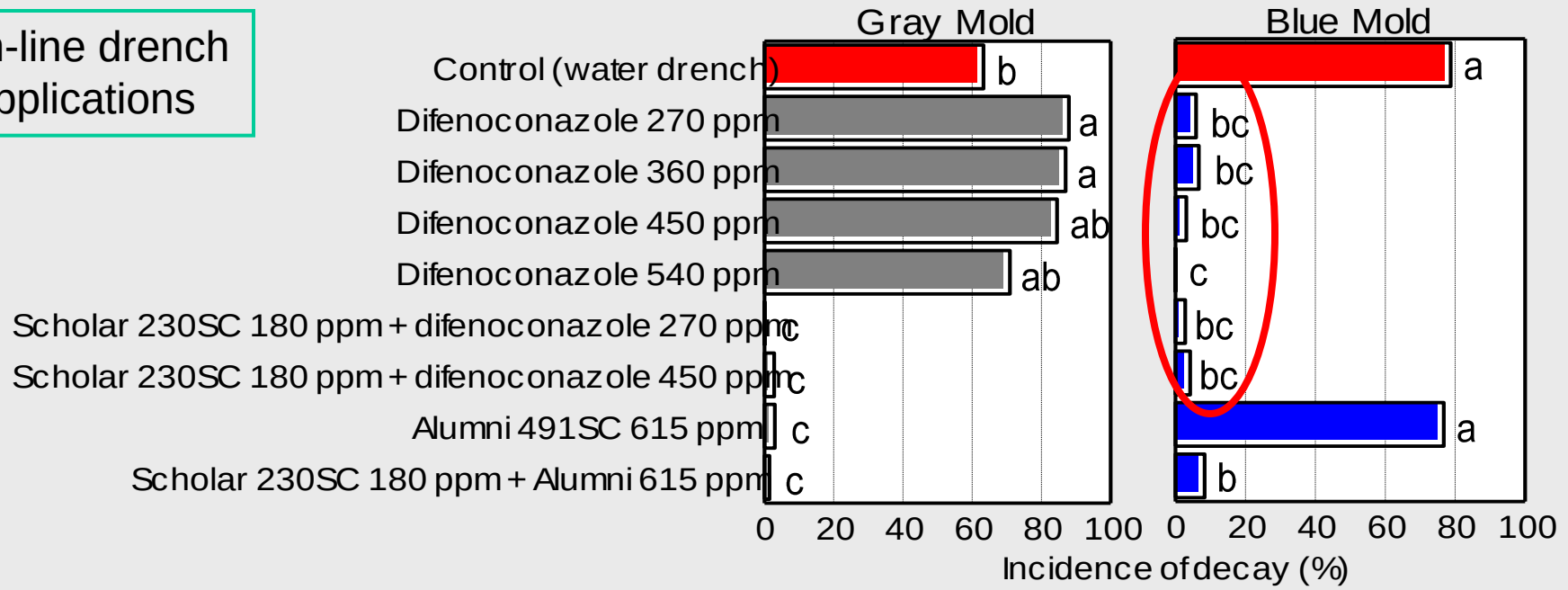
Difenoconazole (PDA)

No resistant isolates recovered

Representative colonies were cultured on fungicide-free agar.

Postharvest treatments of inoculated Bosc pears: *Difenoconazole and Scholar – Gray mold and blue mold*

In-line drench applications



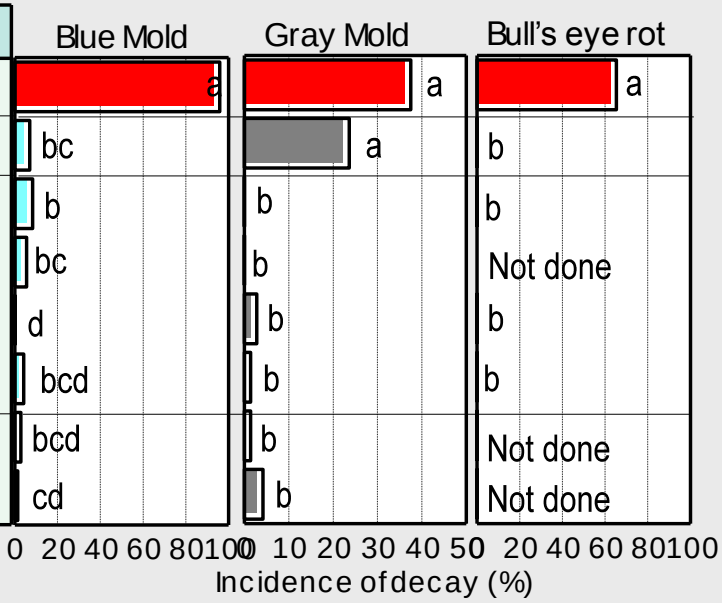
Treatments were applied 16-17 h after inoculation (BC-TBZ^S, Pen TBZ^R) and incubation at 20C.

- **Difenoconazole**: Efficacy high against blue mold. Not effective against gray mold.
- **Scholar - Difenoconazole**: Efficacy high for both decays.
- **Scholar - Alumni (TBZ):** Efficacy high against TBZ-resistant pathogens.

Postharvest treatments of inoculated Bartlett pears:
Difenoconazole - Scholar – Blue mold, Gray mold, Bull's eye rot

In-line drench applications

Fungicide	Active ingredients	Rate	Blue Mold	Gray Mold	Bull's eye rot
Control (water)	---	---	a	a	a
A8574D 360SC	difenoconazole	300 PPM (10.7 fl oz /100 gal/)	bc	a	b
Scholar 230SC + A8574D	fludiox. + difenoc.	180 PPM (10 fl oz /100 gal.) + (10.7 oz /100 gal/)	b	b	b
A20171A 384SC	fludiox. + difenoc.	360 PPM (12 fl oz /100 gal.)	bc	b	Not done
A20171A 384SC	fludiox. + difenoc.	420 PPM (14 fl oz /100 gal.)	d	b	b
A20171A 384SC	fludiox. + difenoc.	480 PPM (16 fl oz /100 gal.)	bcd	b	b
Scholar 230SC	fludioxonil	180 PPM (10 fl oz /100 gal.)	bcd	b	Not done
Penbotec	pyrimethanil	383 PPM (12.8 fl oz /100 gal.)	cd	b	Not done



Treatments were applied 16-17 h after inoculation (Pen TBZ^{R}) and incubation at 20C.

- Effective rates of Scholar and difenoconazole were identified for the design of a pre-mixture.
- The new pre-mixture was highly effective and showed a broad spectrum of activity: ***blue mold, gray mold, bull's eye rot, Rhizopus rot.***
- A postharvest pre-mixture is an important step to prevent resistance from developing. A similar approach is being followed in postharvest decay control of citrus and stone fruits.

Postharvest fungicide treatments for control of gray mold, blue mold, and Bull's eye rot of pears

- Only two of the currently registered postharvest fungicides are highly effective against TBZ-resistant blue mold: Scholar, Penbotec.
- Potential for resistance development:
  - A risk for resistance development against Scholar and Penbotec was demonstrated in our studies previously
  - Penbotec resistance currently at many locations in the PNW
- Anti-resistance strategy - New mode of action/strategies
  - **New AI: Difenoconazole (with *lower* resistance potential)**
  - **New Strategy - Pre-mixtures of Difenoconazole and Scholar or Scholar and TBZ**

Questions