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

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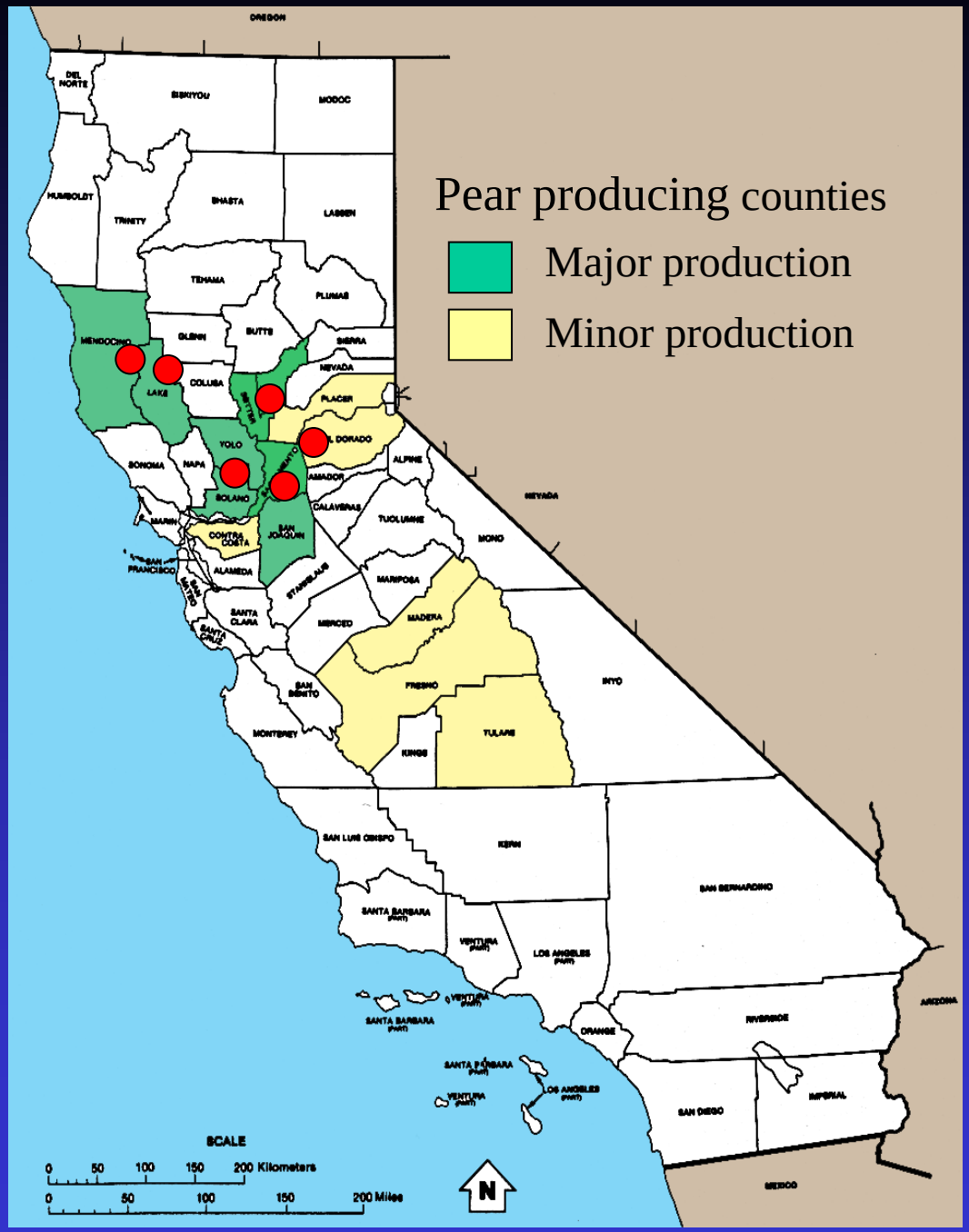
Fire blight - Chemical and biological control - Currently registered treatments -

Class	Compound	Products	Registration	Efficacy	Resistance
Antibiotic	Streptomycin	Agric. Strep.	+	++++	+
		Firewall	+	++++	+
	Kasugamycin	Kasumin	CA Pending	++++	-
	Oxytetracycline	Mycoshield	+	+++	+/-
		Fireline	+	+++	+/-
Biological	*Aureobasidium* sp.	Blossom Protect	+	++/+++	-
	Pantoea sp.	Bloomtime Bio	+	+/+++	-
	Pseudomonas sp.	Blightban	+	+/++	-
	Actinomyces sp.	Actinovate	+	+/++	-
Inorganic	Copper	Various	+	+/+++	-
SAR	Acibenzolar S-methyl	Actigard	-	+/-	-
	Citrus Extract	ProAlexin	+	+/-	-
	PO_3	K-Phite	+	+/++	-
Sanitizer	Peroxyacetic acid	Oxidate/Perasan	+	-	-
	Citrus Extract	Citrox	+	-	-

* - Newer copper products include Badge X2, Kocide 3000, etc. have lower MCE values compared to older products.



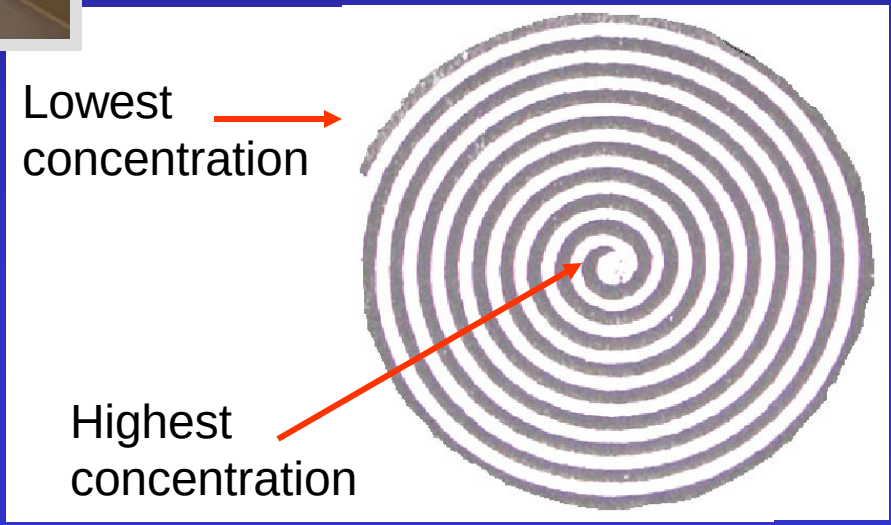
Surveys on antibiotic resistance in populations of *Erwinia amylovora*

- Collection of isolates from major pear growing regions in CA (2006 - 2014)
  - Sacramento Co.
  - Sutter/Yuba Co.
  - Lake/Mendocino Co.
  - El Dorado Co.
  - Solano 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 radially streaked over gradient, incubated, and evaluated.



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 (e.g., MI).

Table 1. Incidence of streptomycin resistance in isolates of *Erwinia amylovora* collected in surveys in 2014

County	Number of orchards	Number of isolates	Incidence of Streptomycin resistance (%)	Incidence of Oxytetracycline resistance (%)	Incidence of Kasugamycin resistance (%)
Sacramento	18	130	2.3 (1 location)	0	0
Lake	6	19	0	0	0
Mendocino	1	7	0	0	0
Total	35	152			

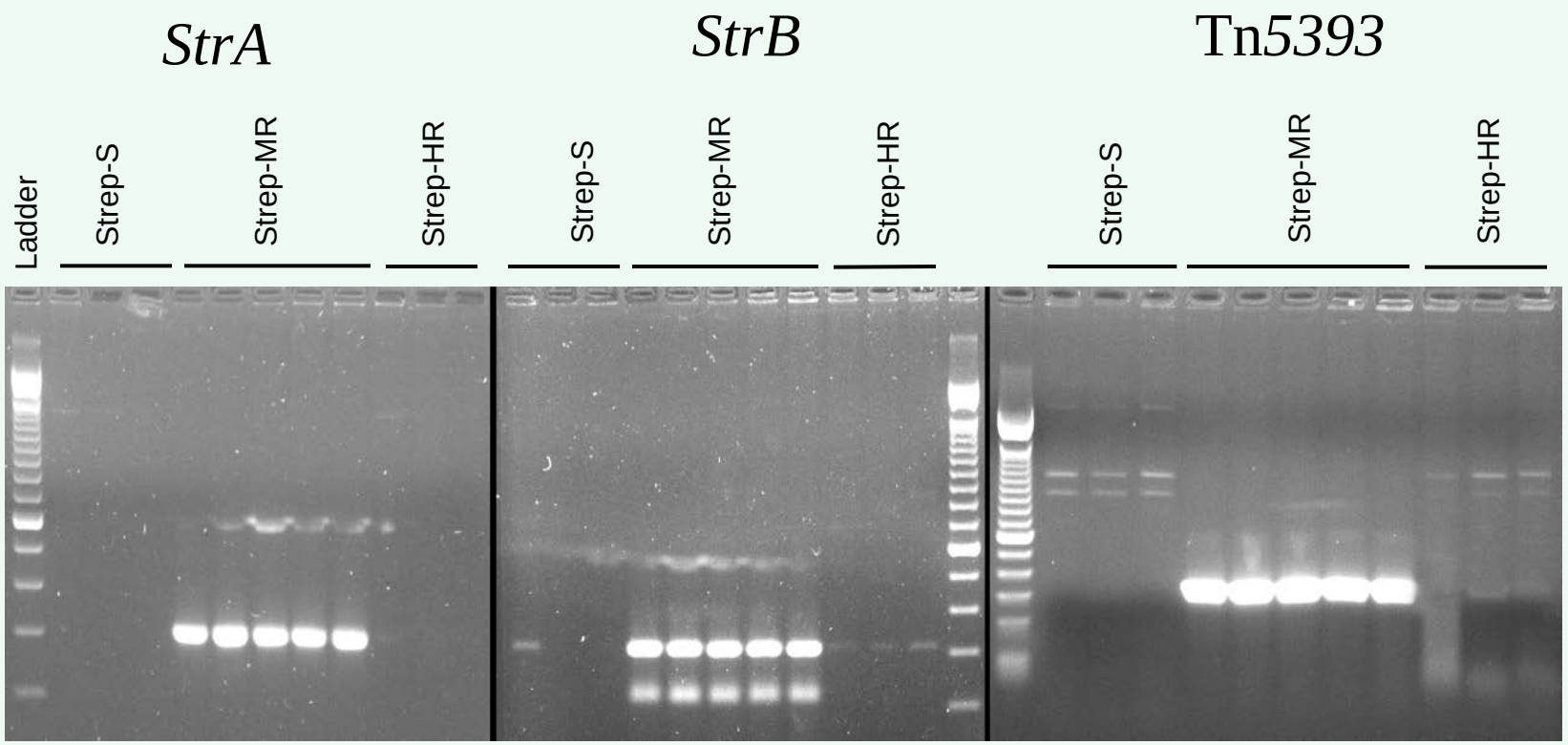
* - Inhibitory concentrations were determined on nutrient agar using the SGD method. Minimum inhibitory concentrations (MIC, >95% inhibition) of isolates sensitive to streptomycin were 0.14-2.5 ppm (>3-25 ppm resistant, >25 highly resistant).

** - MIC ranges for oxytetracycline and kasugamycin: 0.201 - 1.268 ppm and 3.54 - 25.59 ppm., respectively.

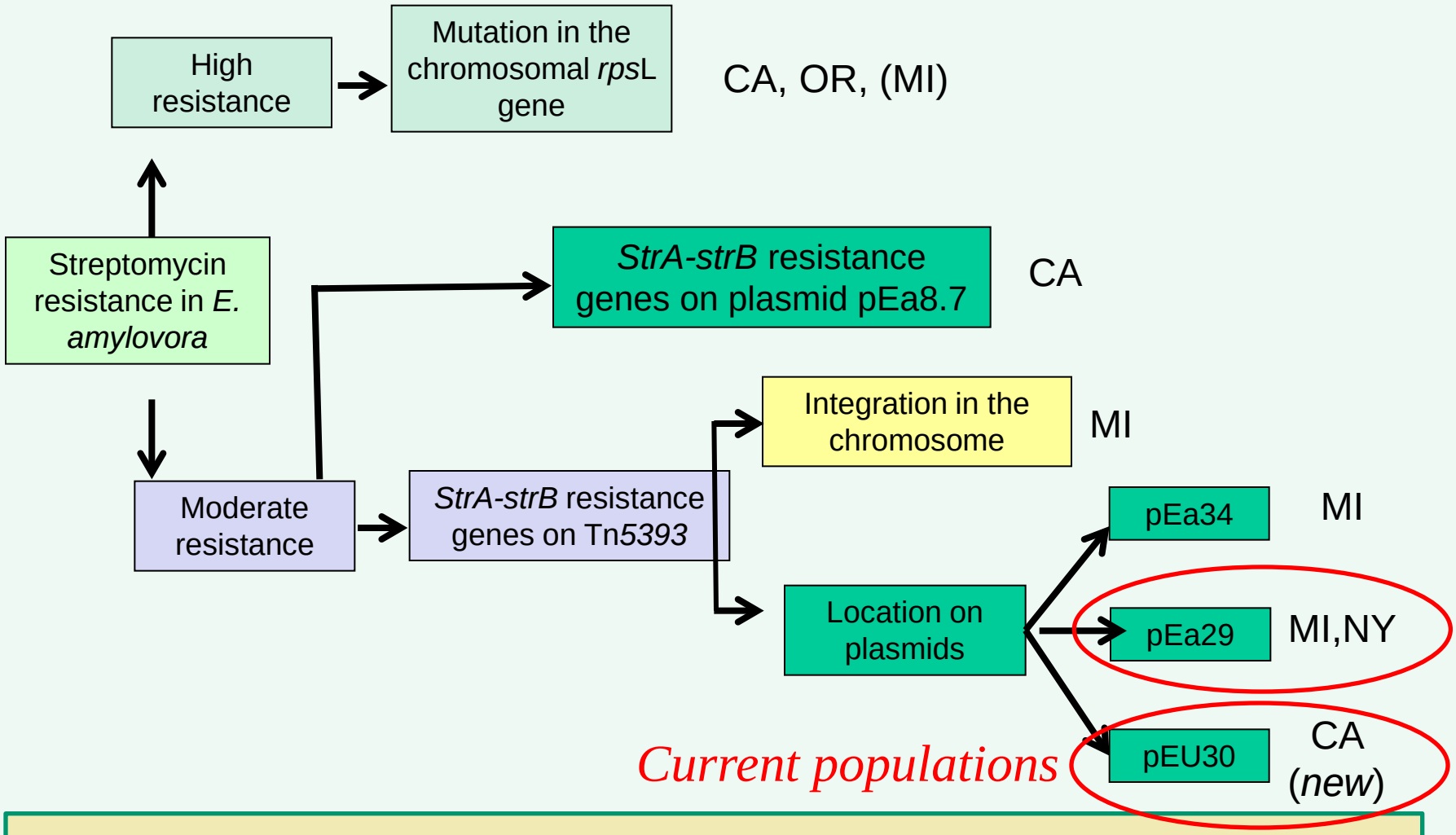
Streptomycin resistance in *E. amylovora* in California

- **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 a few locations in our surveys in CA from 2006 to 2013.
- These isolates have been displaced by **isolates with moderate levels of resistance**.
- Additionally, a **different genetic mechanism** of resistance among isolates and locations is found between years.

Streptomycin resistance genes in *E. amylovora*



PCR amplification of streptomycin resistance genes A) *StrA* and B) *StrB,* as well as C) transposon Tn*5393* in isolates of *Erwinia amylovora* sensitive (Strep-S), moderately resistant (Strep-MR), or highly resistant (Strep-HR) to streptomycin. Transposon 5393 has different versions based on geography of strains



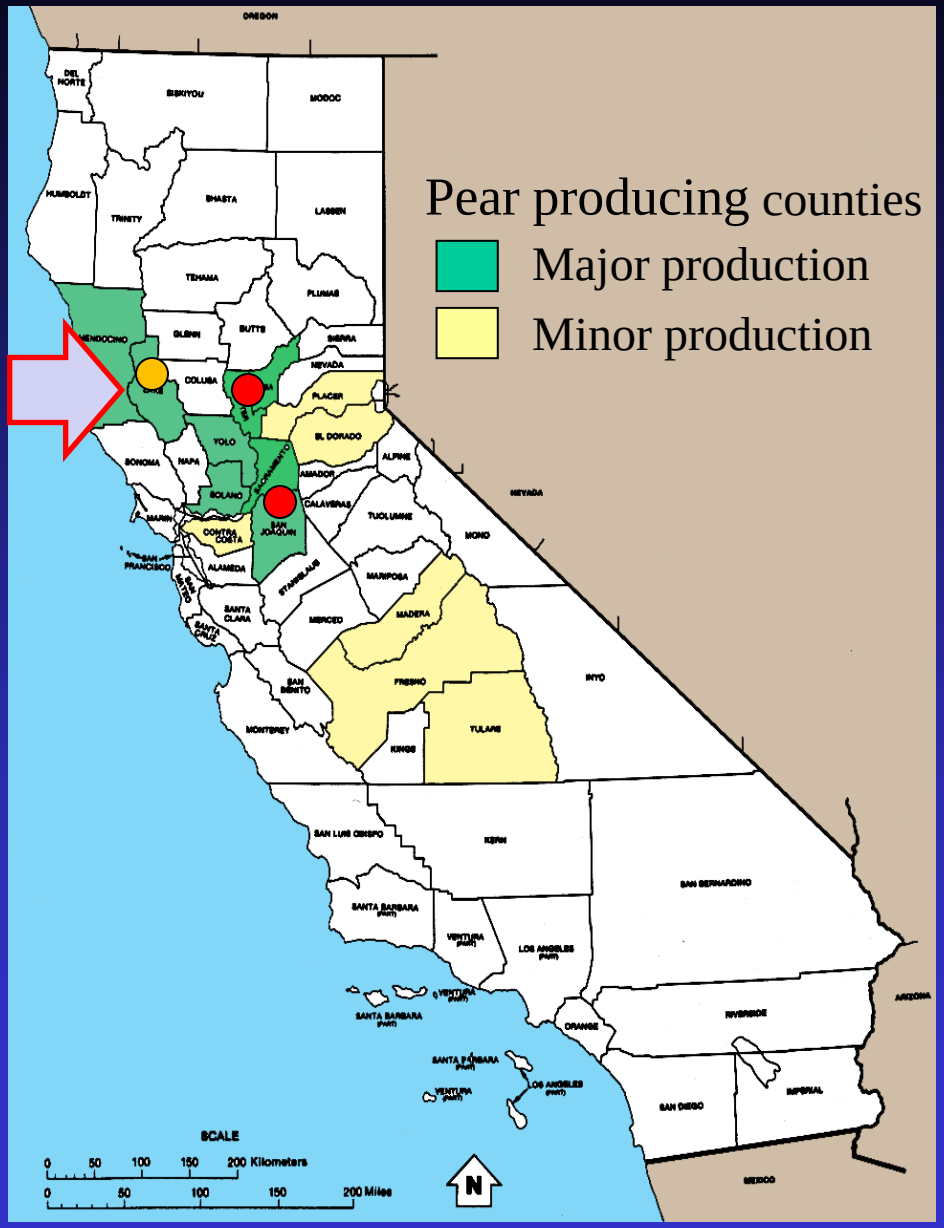
Genetic mechanisms of streptomycin resistance in *Erwinia amylovora.* State abbreviations indicate where each mechanism has been reported. Tn*5393* is a transposon.

Annual fluctuations in streptomycin resistance in isolates of *Erwinia amylovora* 2006-2014

- Annual fluctuations in streptomycin resistance correlate with disease pressure and subsequent selection pressure from streptomycin applications.
- Isolates of *E. amylovora* with moderate levels of resistance (currently the common type in California) to streptomycin appear to be less fit but more fit than highly-resistant strains.
- This provides an opportunity: When rotated with new bactericides (removal of selection pressure) built-up of streptomycin resistance can be prevented and streptomycin can still be used effectively.

Streptomycin resistance in *E. amylovora* in California

Geographic distribution



- Among the major production areas, the incidence of resistance is consistently zero in samplings from Lake Co.
- This has been attributed to a) the widespread use of mixture applications (strep + oxy) and b) cooler climate, less disease, and fewer applications of strep.

Summary: Sensitivity of *Erwinia amylovora* to antibiotics in surveys from California pear orchards

- Streptomycin resistance is widespread but not in Lake/ Mendocino Co. where strep is applied in mixtures with oxy.
- Isolates with moderate resistance to streptomycin have replaced isolates with high resistance.
- Moderately resistant isolates are less fit but more fit than highly resistant strains and are replaced by sensitive isolates in the absence of selection pressure.
- Populations adapt quickly to changing selection pressure: Resistance management strategies using rotations with new treatments will be very effective in managing the disease.
- Resistance to oxytet. has not be detected since 2009, with the exception of a few strains in 2012 but the potential exists.
- To date, no resistance to kasugamycin has been found in CA populations of *E. amylovora*.

Evaluation of alternative treatments to streptomycin and oxytetracycline

Goals:

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

Evaluation of new bactericides for fire blight management of Bartlett pears in a field trial in Live Oak CA - 2014

Treatment	Rate (/100 gal)	Natural incidence	Inoculated
Control	---	a	a
Ceragenin + Acetic Acid + NF-P	5.1 + 8 fl oz	ab	ab
Kasumin 2L + Magna Bon	100 + 100 ppm	abcd	abc
Magna Bon	100 ppm	abc	abcd
Fireline	200 ppm	cde	abcd
Champion2++	8 oz	bcde	abcd
Kasumin 2L + Actigard	100 ppm + 2 oz	de	abcd
Kasumin 2L	100 ppm	bcde	bcd
Kasumin 2L + Fireline	100 + 150 ppm	e	bcd
Fireline + Firewall	200 + 100 ppm	cde	cd
Champion + Mycoshield	8 oz + 16 oz	cde	d
Kasumin 2L + Manzate ProStik	100 ppm + 5 lb	cde	d
Kasumin 2L + Captan 80WDG	100 ppm + 5 lb	de	bcd
Kasumin 2L + Firewall	100 + 100 ppm		

Rotation

0 10 20 30 40 50

0 20 40 60 80 100

Incidence of disease (%)

4 applications between 3-12 (bloom) and 4-4-14 (rat tail). Inoculation on 3-27-14 after air-drying of treatments. Disease was evaluated on 4-10-14.

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 Sept. 2014 (Jan. 2015, all states approved except CA)
- **New copper materials with lower mce use**
  - Kocide 3000, Badge X2, others
  - Effective
  - No phytotoxicity observed after 3 applications.
  - Can be part of a rotation program.

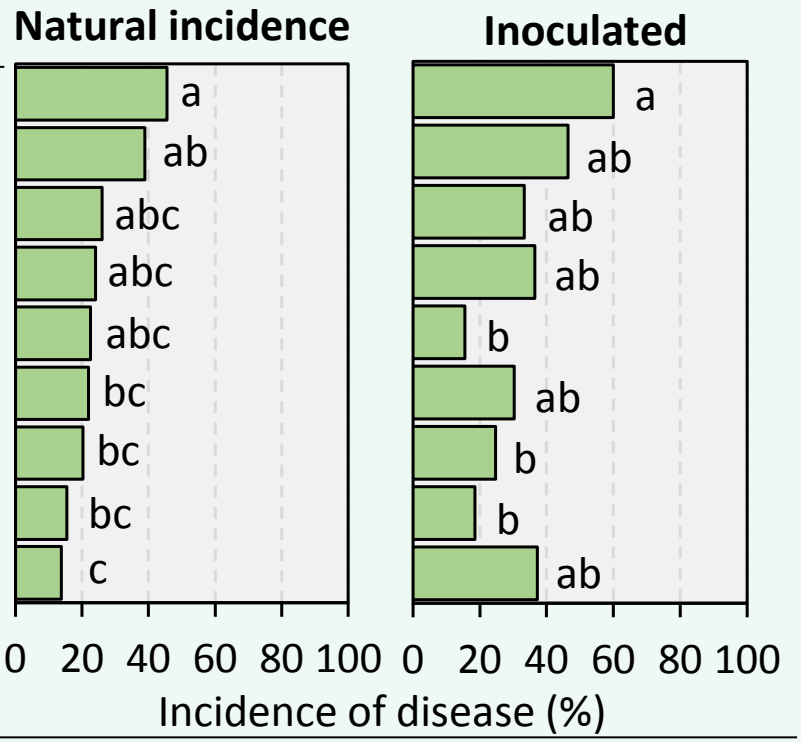
Evaluation of alternative treatments to streptomycin and oxytetracycline

Goals:

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

Evaluation of biological treatments for fire blight management of Bartlett pear – Live Oak 2014

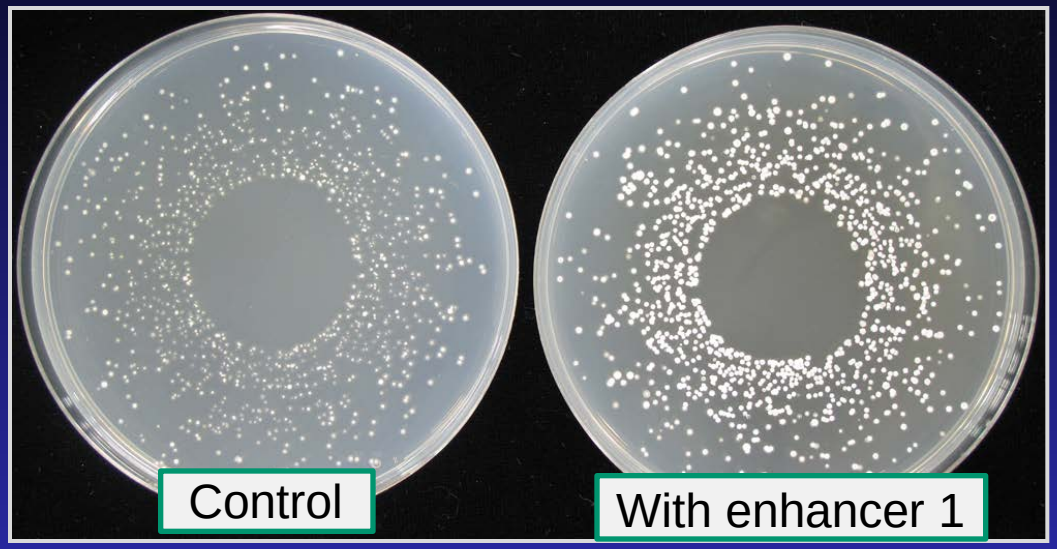
Treatment	Rate (/100 gal)
Control	---
K-Phite + WideSpread	96 + 8 fl oz
Actinovate + NF-P	12 oz
Blossom P. + Buffer + WS	1.25 + 8 + 15 lb
Serenade Optiva + NF-P	20 oz + 8 fl oz
Actinovate + NF-P + Buffer	12 oz + 8 fl oz + 36 oz
Blossom P. + Buffer + BioLink	1.25 + 8 lb + 4.5 fl oz
Blossom P. + Buffer	1.25 + 8 lb
Kasumin 2L	100 ppm

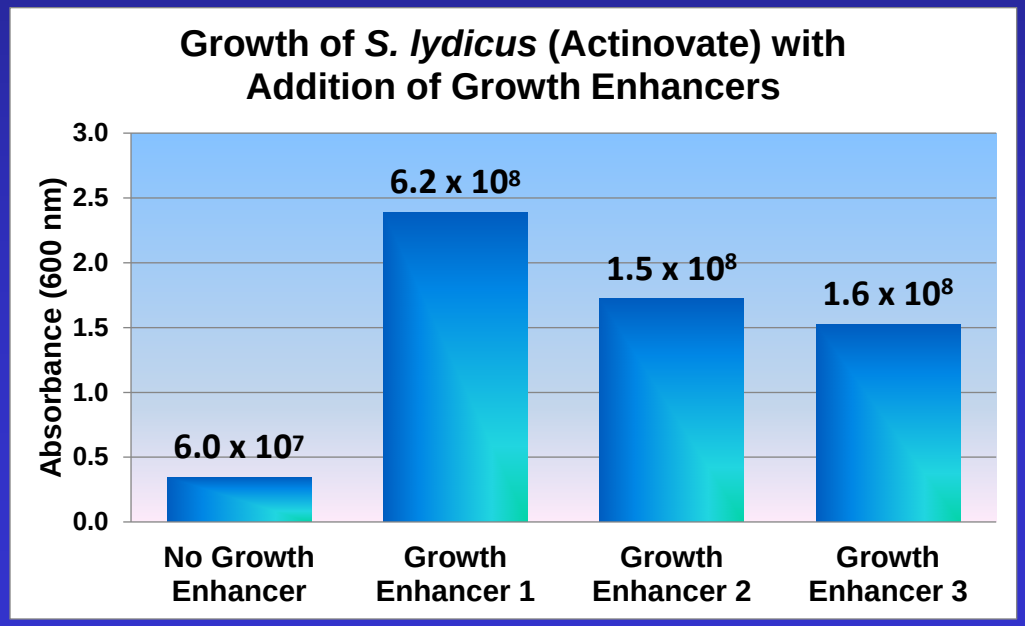


4 applications between 3-12 (bloom) and 4-4-14 (rat tail). Inoculation on 3-27-14 after air-drying of treatments. Disease was evaluated on 4-10-14.

- ***Sulfur*** reduced activity of Blossom Protect.
- **In vitro studies**: complete inhibition of *A. pullulans* by 12,000 ppm ***sulfur*** (=10 lb/A field rate). Some inhibition at 4000 ppm, no inhibition at 3000 ppm.
- ***Copper*** (up to 400 ppm MCE tested) did not inhibit the biocontrol organism.

Improving growth of biocontrol agents with growth enhancers - Actinovate – *Streptomyces lydicus* -



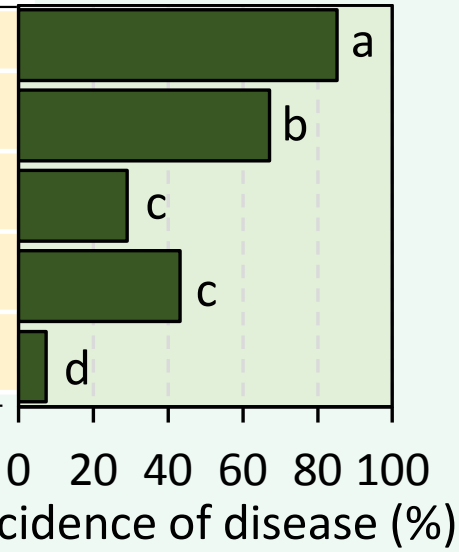


- *S. lydicus* was grown on agar media without or with growth enhancers (selected nutrients).
- Growth was evaluated after 5 days by enumerating spore production.
- More field studies are planned.

Evaluation of biological treatments for fire blight management of Bartlett pear – Live Oak 2014

Small-scale tests

Treatment	Rate (/100 gal)
Control	---
Actinovate + Buffer	12 + 128 oz
Magna Bon	27 fl oz
Blossom Protect + Buffer + Wettable sulfur	20 + 128 + 225 oz
Blossom Protect + Buffer + Cuprofix	20 + 128 + 12 oz



Applications done on 3-21-14 to flowers using a hand sprayer. After air-drying inoculation with *E. amylovora*. Disease was evaluated on 4-4-14.

- **High efficacy of Blossom Protect + copper.**
- **In vitro studies: Copper (up to 400 ppm MCE tested) did not inhibit *A. pullulans.* Sulfur is inhibitory.**

Table 3. Activity of chemicals used for fire blight control against three biocontrol agents

Biocontrol product and agent	Streptomycin	Oxytetracycline	Kasugamycin	Captan	Mancozeb
Actinovate (*Streptomyces lydicus*)	+*	+	+	+	+
Blossom Protect (*Aureobasidium pullulans*)	-	-	-	+	+
Double Nickel 55 (*Bacillus amyloliquifaciens)*	+	+	+	+	+

* - Activity was determined using the spiral gradient dilution assay. + = chemical is active against the biocontrol agent, - = chemical is not effective at maximum concentration of 40 ppm tested.

The antibiotics were ineffective against Blossom Protect because it is a fungal yeast; whereas the fungicides were inhibitory. The antibiotics were inhibitory to bacterial biologicals.

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

- Several new products are promising.
- Efficacy ranged from low to high and often was inconsistent between years, but Blossom Protect most consistent.
- Studies on optimizing efficacy of biocontrols are ongoing.
- Compatibility/Incompatibility:
  - Blossom Protect – Incompatible with S and conventional fungicides; Compatible with label rates of Cu and antibiotics.
  - Actinovate – Incompatible with antibiotics
- For best efficacy, natural products and biocontrols should be used with copper under low disease pressure.

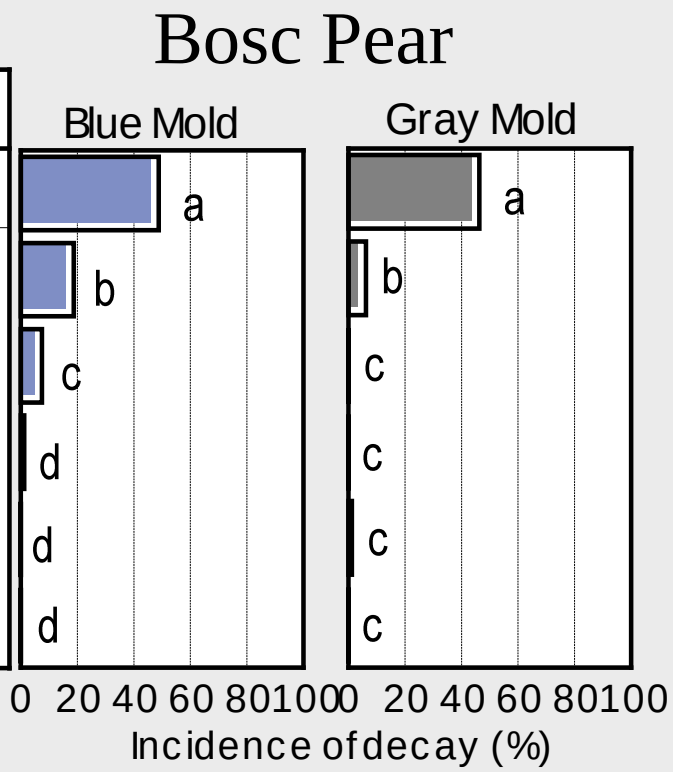
Thank you

Questions?

New treatments for postharvest decay control

EXP-13

Drench application	CDA application
Control (water)	Wax only
N-1 50% form. 1000 ppm	No Wax
N-1 50% form. 1000 ppm	Wax only
N-1 50% form. 1000 ppm	N-1 5% form. 250 ppm in wax
N-1 50% form. 2000 ppm	Wax only
Scholar SC 276 ppm	Wax only

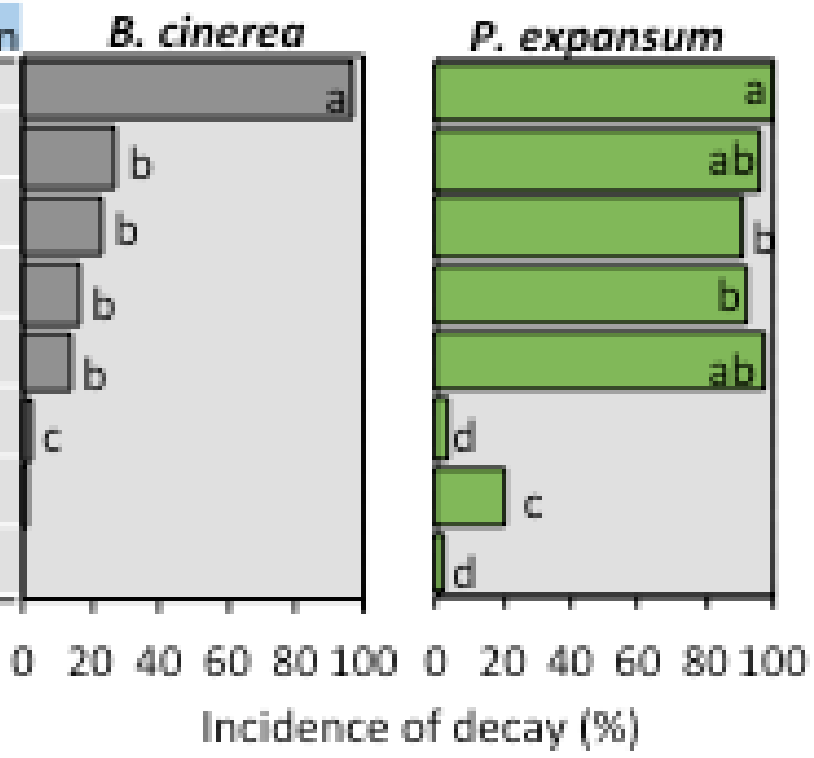


Experimental packingline study. Treatments 17-19 h by in-line drenches.

- EXP-13 was highly effective against blue mold and gray mold and other decays.

Evaluation of new postharvest treatments for management of gray mold and blue mold of Comice pear

Treatment	Rate	Application
Control (water)	---	Drench
EXP-13 WP	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	T-Jet
EXP-13 SC	1000 ppm	Drench
EXP-13 WP	1000 ppm	Drench
EXP-13 WP + Scholar	1000+150 ppm	Drench
Academy	16 fl oz	T-Jet
Academy	16 fl oz	Drench



Fruit were wound-inoculated with conidia of *B. cinerea* (5 x 10^4 conidia/ml) or *P. expansum* (5 x 10^5 conidia/ml) and were incubated for 16-18 h at 20C. Fruit were then incubated at 20C for 5 to 7 days. 16 fl oz Academy = 180 ppm fludioxonil + 300 ppm difenoconazole).

Summary: New Postharvest Strategies

- Several new products will becoming available in 2015.
  - Academy – Difenoconazole + fludioxonil
  - Post-Doc – Polyoxin-D (Exempt from tolerance)
- New Experimentals are being developed:
  - EXP-13 – broad spectrum material with potentially exempt from tolerance status. Research ongoing to determine differential activity on pear varieties.
- Exempt from residue tolerance - New standards of safety
  - Fermentation products of microbiological organisms
  - EPA classification under “Biological Pesticides”
  - Exempt in the US but may be fast-tracked in other countries.