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

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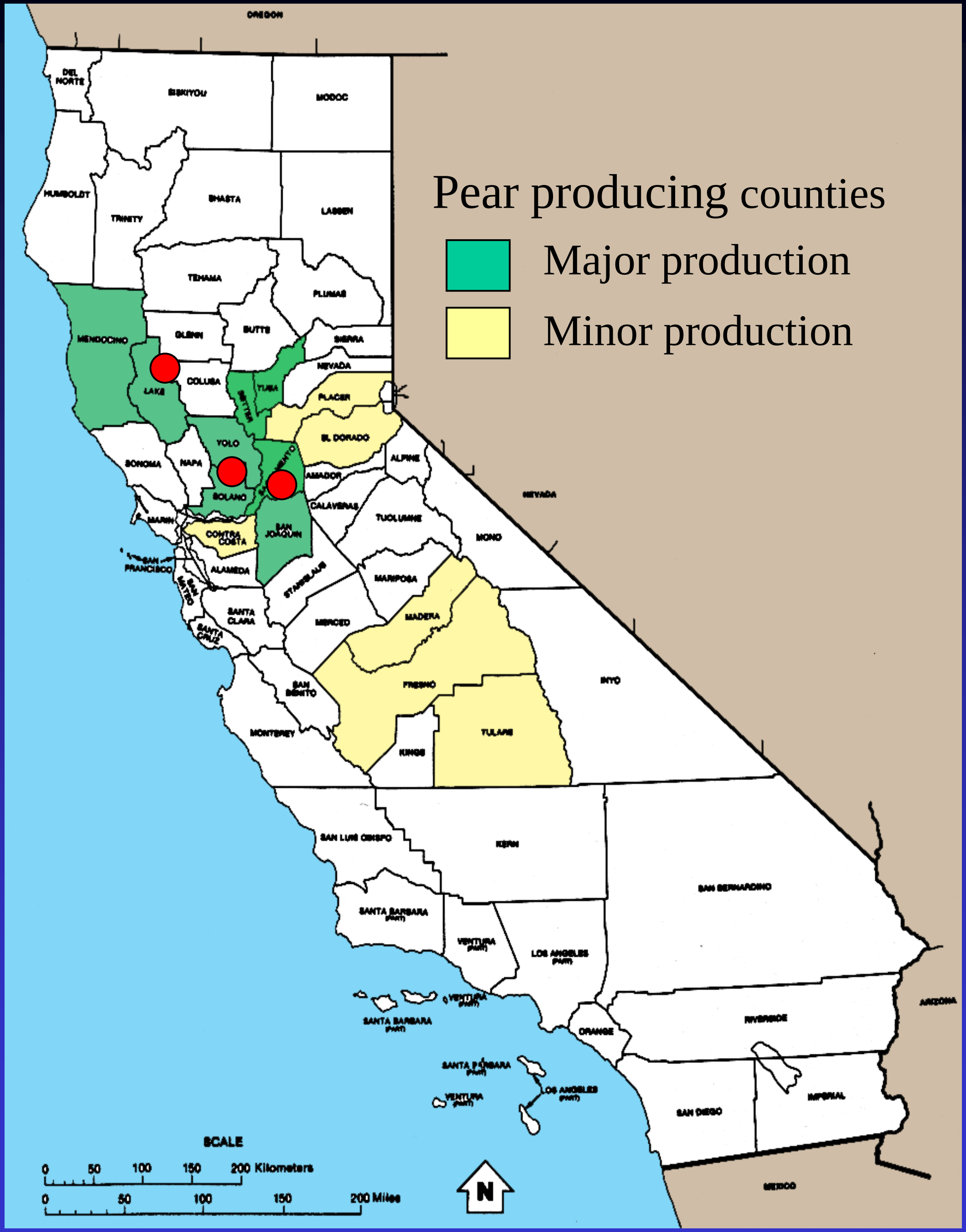
Fire blight - Chemical and biological control
- Currently registered treatments -

Class	Compound	Products	Registration	Efficacy	Resistance
Antibiotic	Streptomycin	Agric. Strep.	+	++++	+
		Firewall	+	++++	+
	Kasugamycin	Kasumin	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.

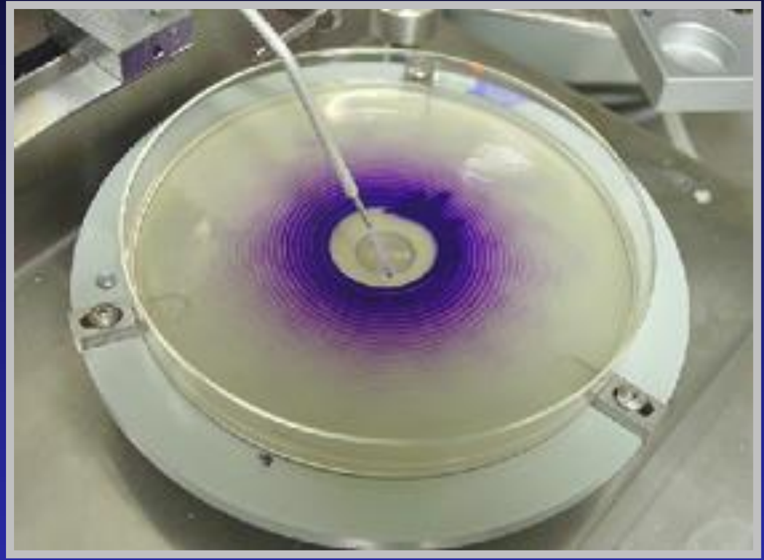


Surveys on antibiotic resistance in populations of *Erwinia amylovora*

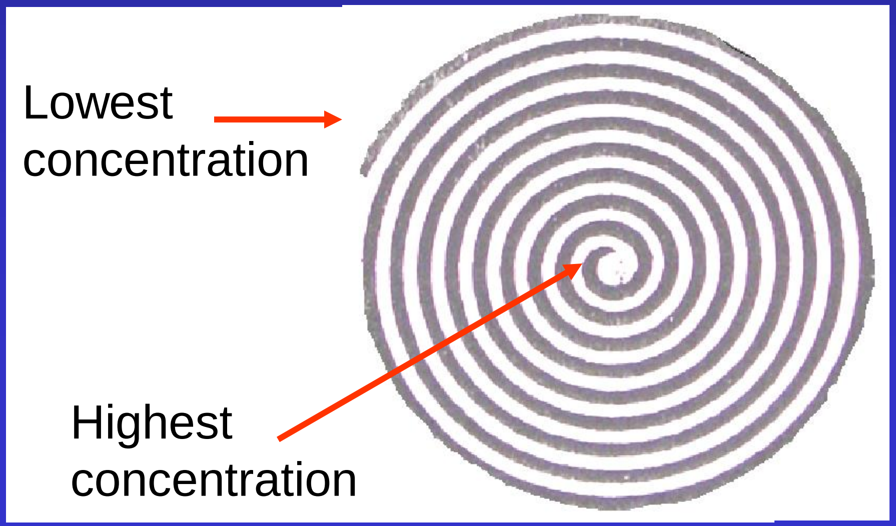
- Collection of isolates from major pear growing regions in CA (2006 - 2013)
  - Sacramento Co.
  - Solano Co.
  - Lake Co.
- Evaluate sensitivity
  - Streptomycin
  - Oxytetracycline
  - Kasugamycin

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).

Table 1. Incidence of streptomycin resistance in isolates of *Erwinia amylovora* collected in surveys in 2013

County	Number of orchards	Number of isolates	Incidence of Streptomycin resistance (%)	Incidence of Oxytetracycline resistance (%)	Incidence of Kasugamycin resistance (%)
Sacramento	13	105	6.7 (2 locations)	0	0
Lake	21	44	0	0	0
Solano	1	3	0	0	0
Total	35	152			

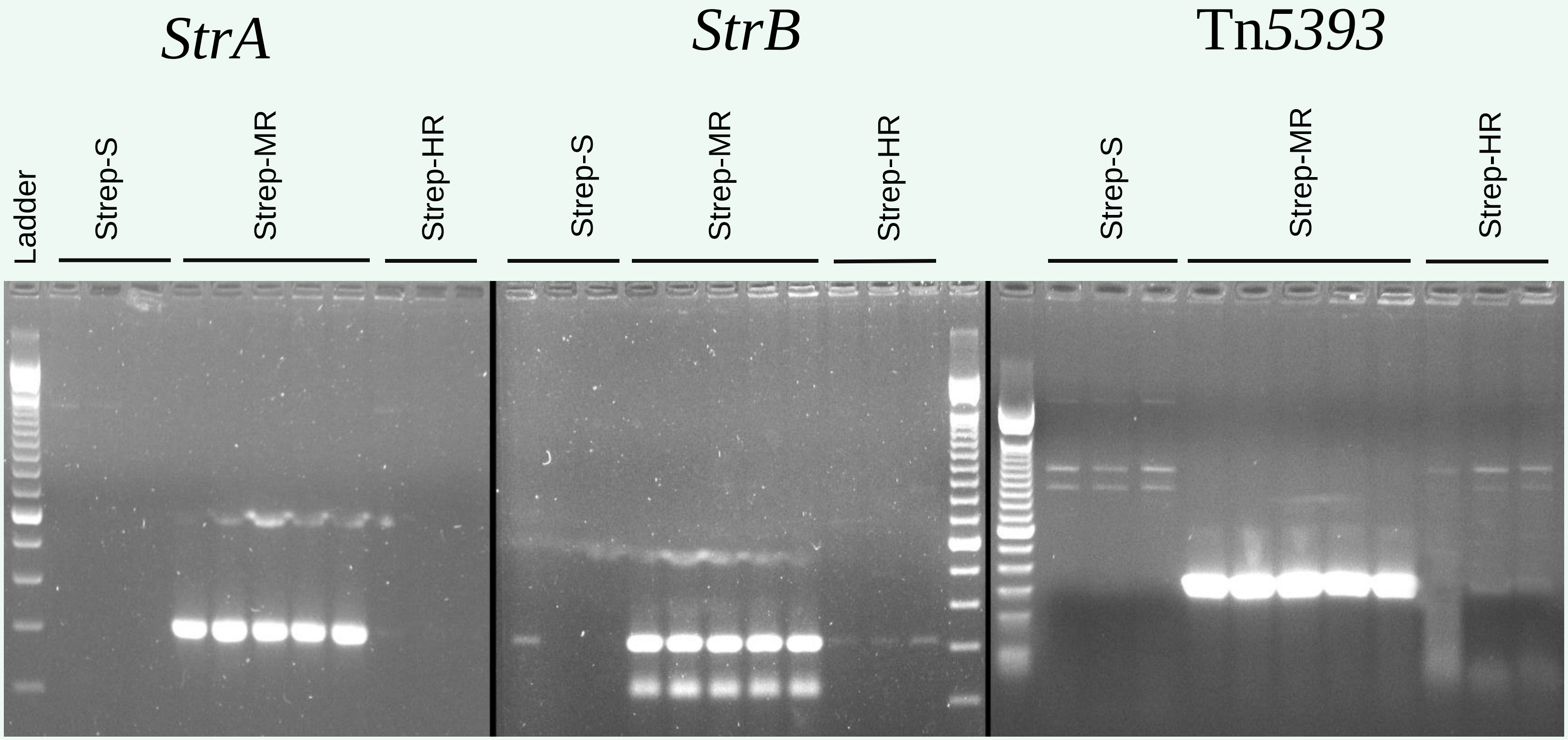
* - Inhibitory concentrations were determined on nutrient agar using the SGD method. Minimum inhibitory concentrations (MIC, >95% inhibition) of isolates sensitive to streptomycin were 9.0-35.5 ppm; Lowest inhibitory concentration (LIC, any inhibition) to streptomycin was 5.8-22.8 ppm.

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

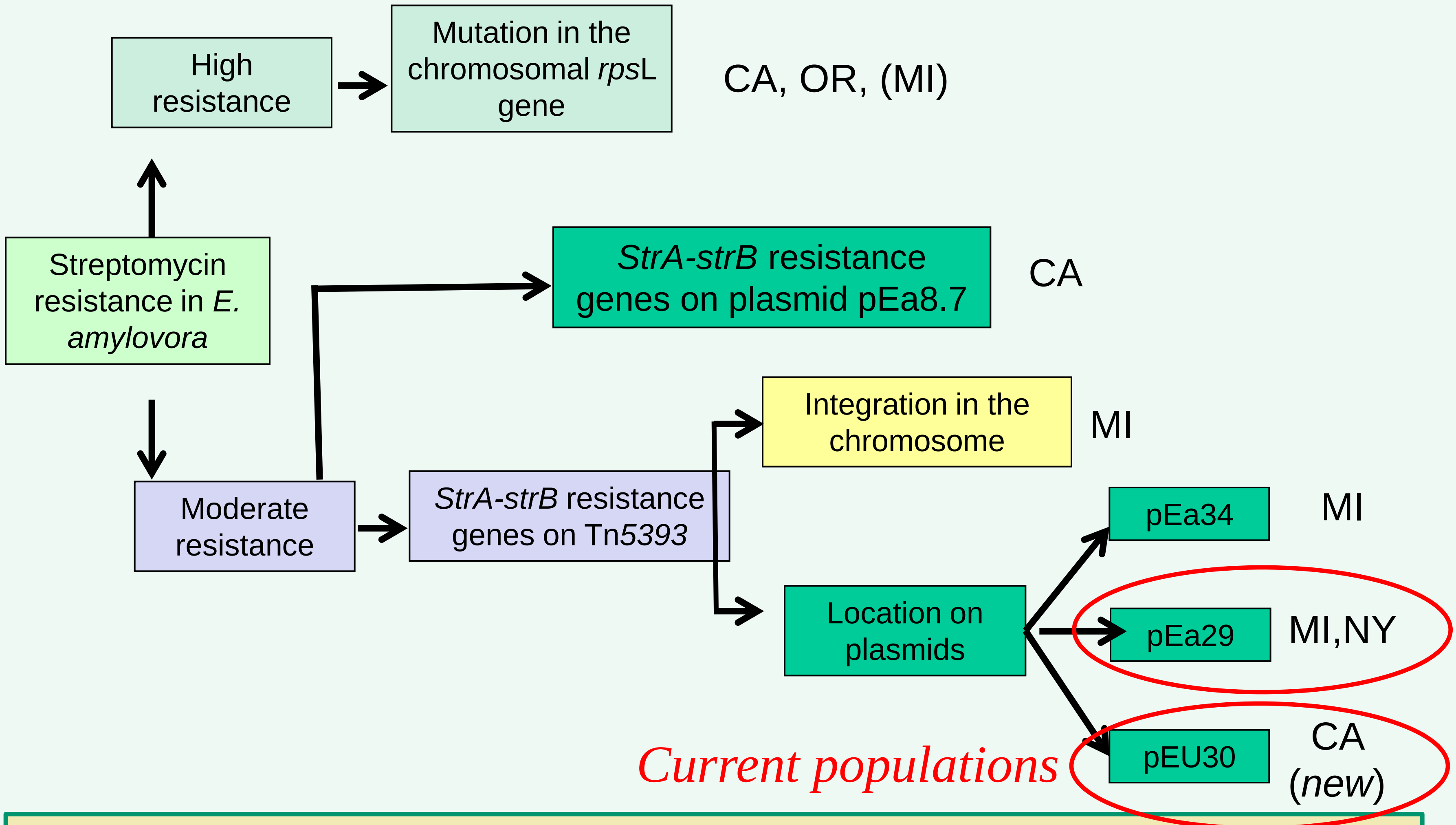
Streptomycin resistance in *E. amylovora* in California

- **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*



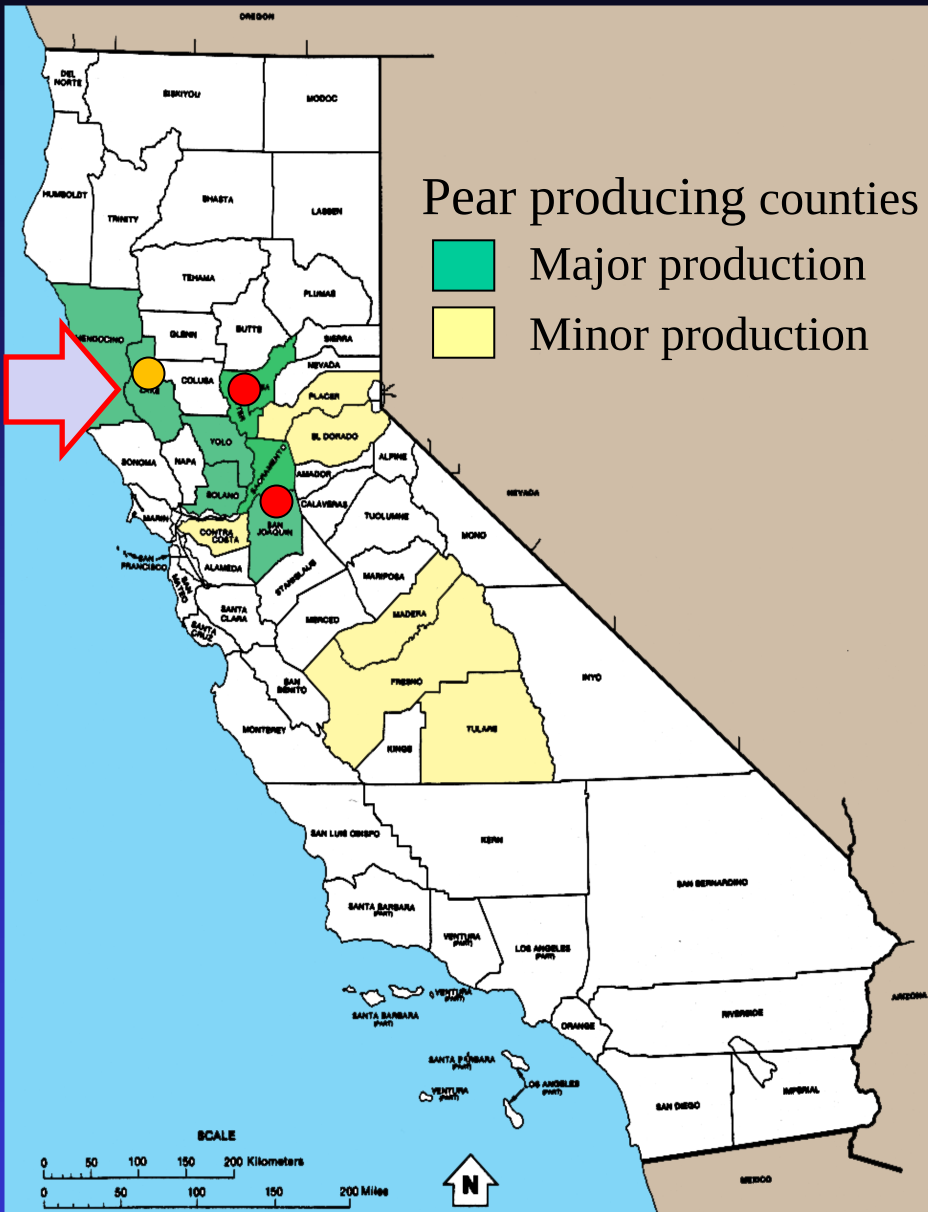
PCR amplification of streptomycin resistance genes A) *StrA* and B) *StrB,* as well as C) transposon Tn*5393* in isolates of *Erwinia amylovora* sensitive (Strep-S), moderately resistant (Strep-MR), or highly resistant (Strep-HR) to streptomycin.



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* 2006-2013

- 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.
- 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 was low in samplings from Lake Co.
- This has been attributed to the widespread use of mixture applications (strep + oxy)

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 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*.

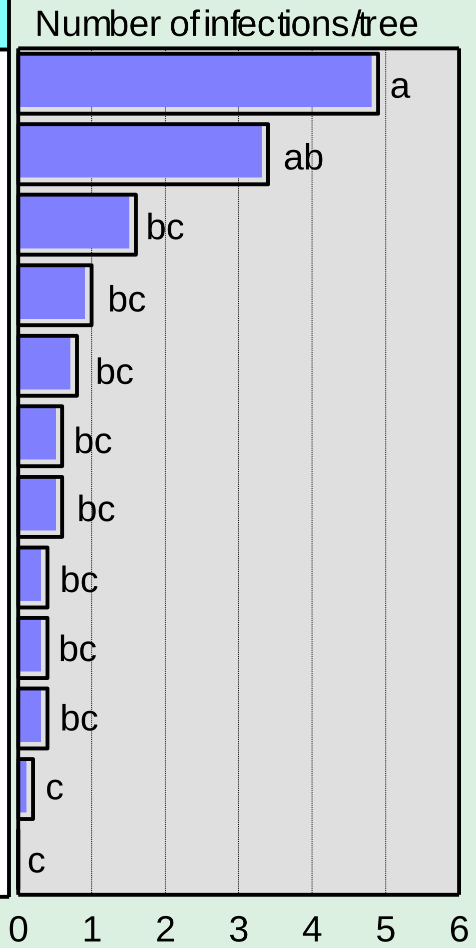
Evaluation of alternative treatments to streptomycin and oxytetracycline

Goals:

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

Fig. 1. Evaluation of new bactericides for fireblight management on Bartlett pears in a field trial in Live Oak CA - 2013

Treatment	Rate
Control	---
K-Phite	96 fl oz
Kasumin 2L	64 fl oz
Kasumin 2L + Manzate ProStik	64 fl oz + 32 oz
Badge X2	8 oz
Kasumin 2L + Fireline	64 fl oz + 16 oz
Kasumin 2L + Badge X2	64 fl oz + 8 oz
Fireline 200 ppm	16 oz
Fireline + Actigard 2 oz	16 oz + 2 oz
Fireline + Firewall	16 oz + 8 oz
Kasumin 2L + Actigard 2 oz	64 fl oz + 2 oz
Kasumin 2L + Firewall	64 fl oz + 8 oz



Treatments were applied on 3-21 (20% bloom), 3-28 (full bloom), 4-2 (petal fall), and 4-11-2013 (begin rattail) using an airblast sprayer at 100 gal/A. Disease was evaluated on April 17, 2013.

Table 2. Evaluation of the additive activity of kasugamycin and captan, mancozeb, or dodine in inhibiting growth of *Erwinia amylovora*

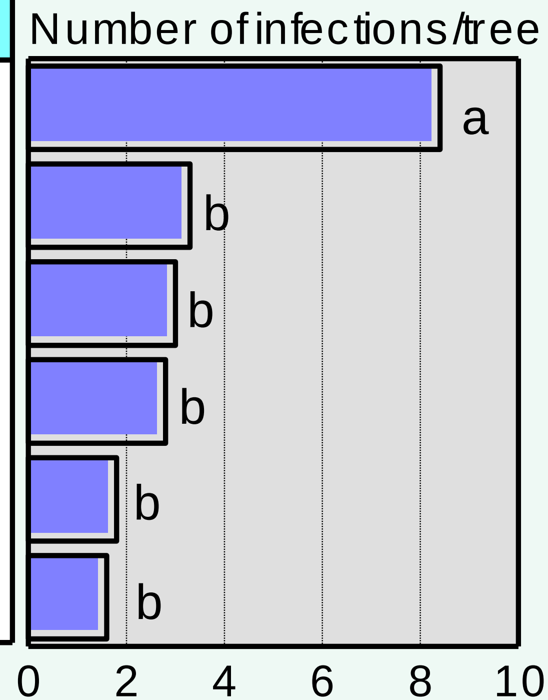
Treatment*	% inhibition of growth**
Kasugamycin 1 ppm	43.8
Captan 5 ppm	53.3
Kasugamycin 1 ppm + Captan 5 ppm	89.7
Mancozeb 10 ppm	37.3
Kasugamycin 1 ppm + Mancozeb 10 ppm	78.9
Dodine 0.5 ppm	99.2
Kasugamycin 1 ppm + dodine 0.5 ppm	100

*- *E. amylovora* was grown in microtiter plates in nutrient broth without or with the addition of test substances. Growth was measured by optical density readings at 600 nm.

** - % inhibition as compared to the non-amended control

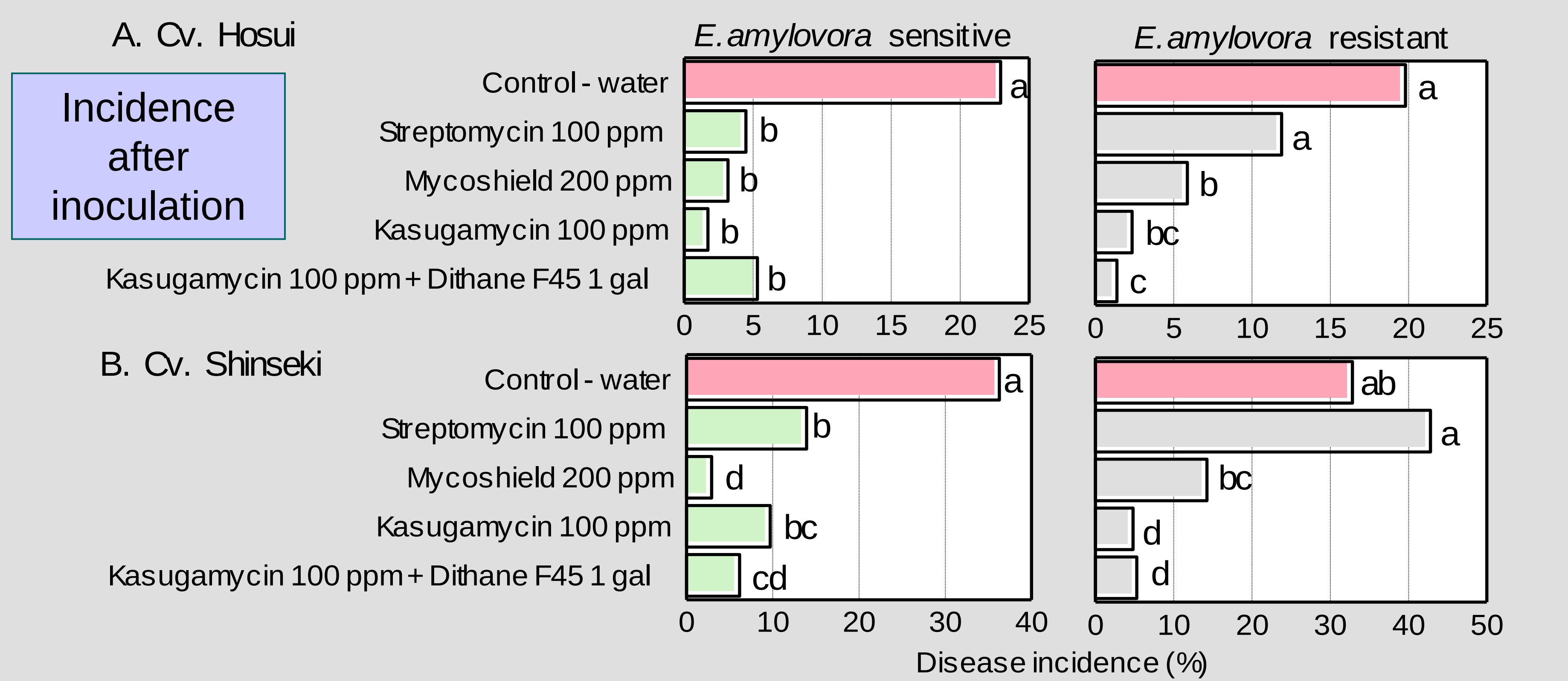
Fig. 2. Evaluation of new bactericides for fireblight management on Comice pears in a field trial at UC Davis, CA - 2013

Treatment	Rate/A/100 gal
Control	-----
Firewall	8 oz
K-Phite	96 fl oz
Actigard + Blossom Protect	2 oz + 1.34 lb + 9.35 lb
Kasumin 2L + Actigard	100 ppm + 2 oz
Kasumin 2L	100 ppm



Treatments were applied on 3-21, 3-28, and 4-3-13 (rattail) using an airblast sprayer at 100 gal/A. On 3-31-13, trees were at 30% bloom. Trees were inoculate with *E. amylovora* using an air-blast sprayer on 3-29-13. Disease was evaluated mid April 2013.

Efficacy against fire blight caused by *E. amylovora* resistant to streptomycin and oxytetracycline
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.
  - Registration in 2014?
- **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.

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 2014 registration

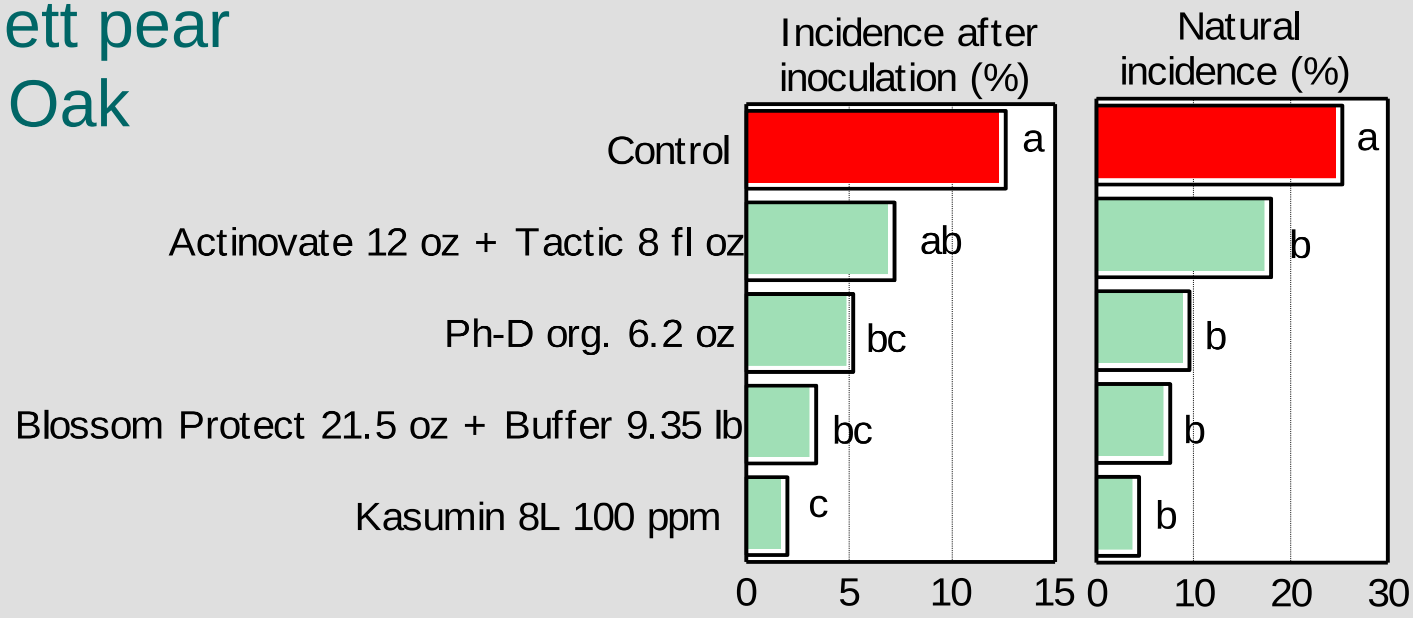
Evaluation of alternative treatments to streptomycin and oxytetracycline

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



4 treatments (starting at 80% 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.
- Efficacy 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.

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 effective against bacterial biologicals.

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		Firewall	+	++++	+
	Kasugamycin	Kasumin	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.

Thank you

Questions?