Epidemiology and Management of fire blight of apple and pear caused by *Erwinia amylovora*

- North Coast Region -

J.E. Adaskaveg

Department of Microbiology and Plant Pathology

UC Riverside

Cooperating: H. Forster, D. Thompson, D. Cary

Fire blight - a disease of rosaceous plants

Fire blight is a disease of rosaceous plants, including apples and pears.

The disease is caused by the gram-negative bacterium *Erwinia amylovora.*

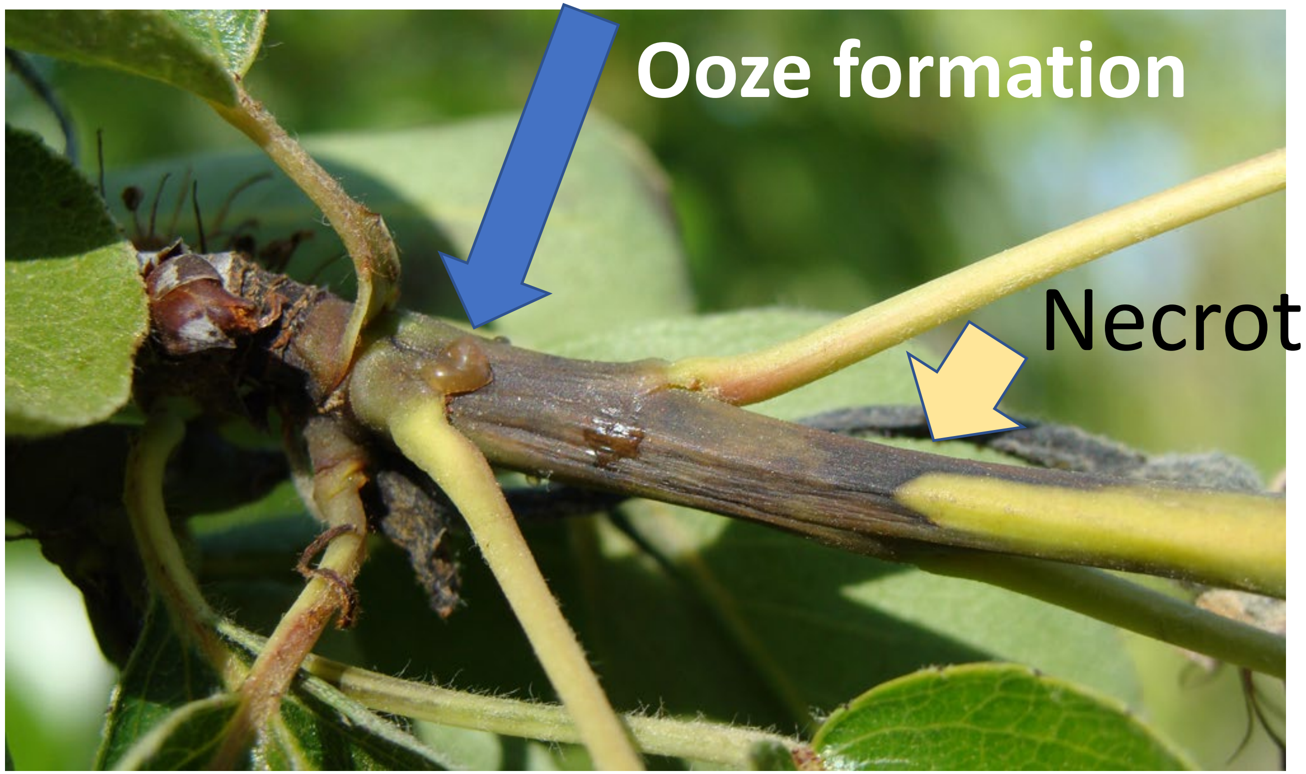
Symptoms: Blossom, twig, leaf, fruit, limb and trunk, collar and root blights

Flower infection

Shoot infection

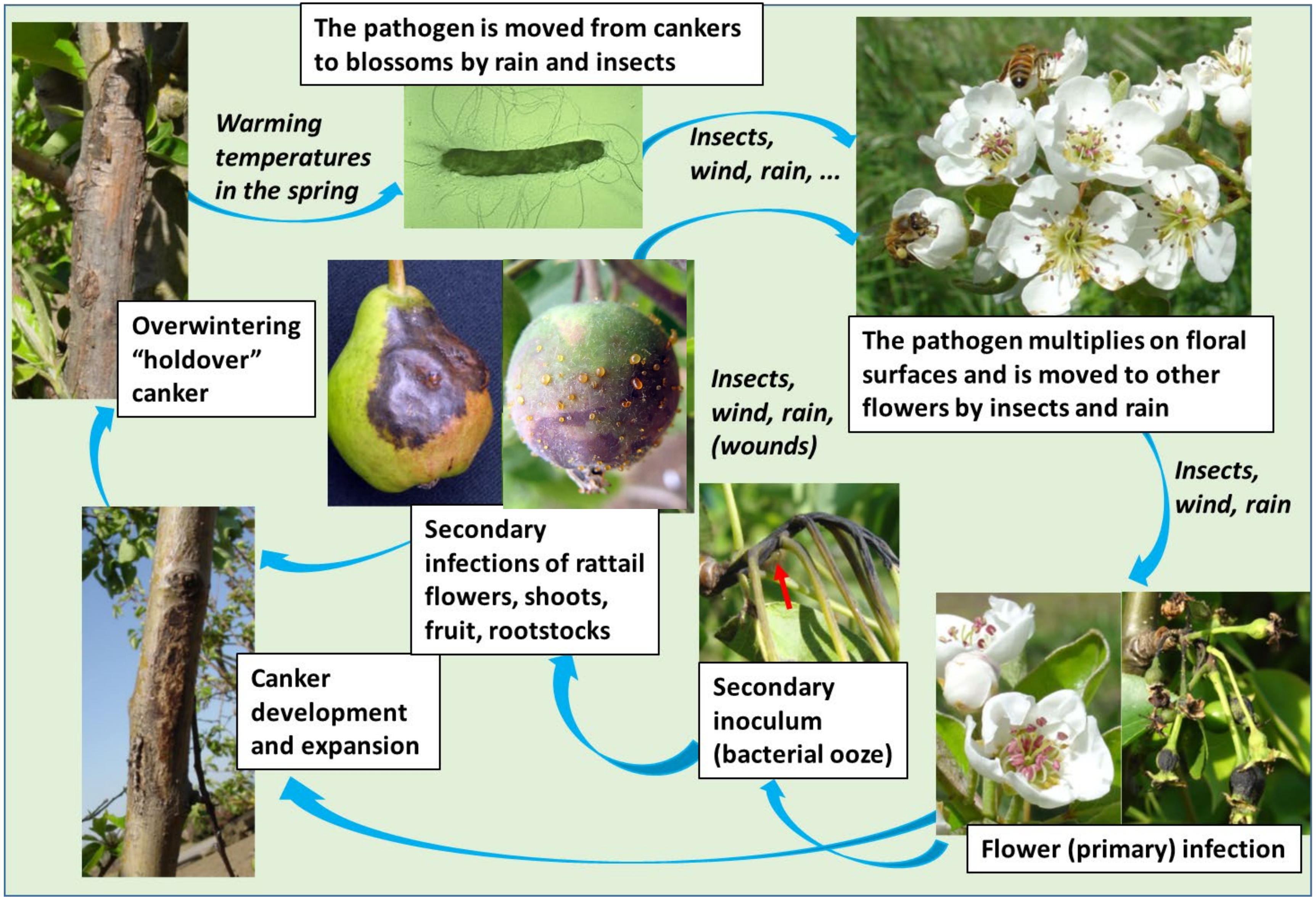
Canker

Bacterial exudation from plant infections



- Bacterial survival time in ooze is approx. 1 year
- Bacterial strands – smooth or beaded, several cm in length, wind disseminated, instantly water dispersible

Disease cycle of fire blight



Critical growth parameters

Optimum Temperature – 21-28°C (70-82.5°F)

Minimum Temperature – 3-12°C (38-54°F)

Maximum Temperature – 35-37°C (95-99°F)

Thermal Death – 45-50°C (113-122°F)

pH range 4 - 8.8, optimum 6 - 7.5

Generation time under optimal conditions = 96 min

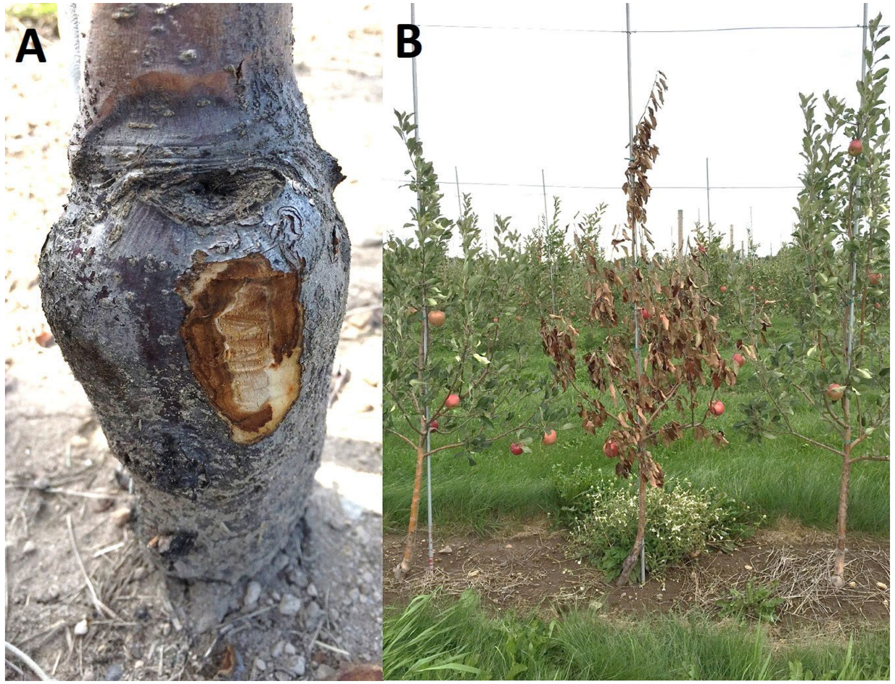
Epidemiology – epiphytic and endophytic life styles

- *E. amylovora* overwinters in cankers (dead host tissue) and oozes out in the spring.
- Rain-splash-, wind-, and insect-dissemination to flowers and other plant surfaces. The bacterium colonizes stigmata and nectaries as an <u>epiphyte</u> (10^6 CFU/stigma) and then as a pathogen.
- It can also survive as an epiphyte on other plant surfaces (leaves, fruit, and branches) for limited periods (***several weeks***).
- Can reside as an <u>endophyte</u> within apparently healthy plant tissue, such as branches, limbs, and bud wood.

Epidemiology – Rattail Bloom

- Prolongs the time when highly susceptible host tissue is available
- Increases the number of protectant applications needed
- Requires additional “cutting out” time for workers
- Is the primary factor separating management guidelines in CA and PNW and is the reason why fire blight management in CA is more challenging.

Rootstock and tree susceptibility to fire blight of apple



- Rootstock blight may girdle the rootstock stem or trunk base and kill the tree
- Depending on the level of the rootstock's genetic fire blight susceptibility, infections with *E. amylovora* can express as development of bark cankers
- Infections can remain asymptomatic as latent infections
- There are differences in rootstock susceptibility to fire blight:
  - ➢ Very susceptible: Malling series M.26 and M.9 and its subclones (Nic29, T337, Pajam 2)
  - ➢ Tolerant or moderately resistant: M.7, and Budagovskij series B.9 and B.118
  - ➢ Resistant: Geneva series G.11, G.41, G.202, G.214, G. 890, G.935, G.969

Cultivar susceptibility

Apple

- **Apple cultivars vary in susceptibility and extent of damage.** For example, in Granny Smith, infections are usually limited and do not cause severe structural damage to the tree.
- **More susceptible:** include Golden Delicious, Granny Smith, Gravenstein, Jonathan, Mutsu, Pink Lady, and Yellow Newtown are also.
- **Highly susceptible:** Gala and Fuji trees that may be devastated.

Pear

Most pear tree varieties, including Asian pears (with the exception of Shinko) and red pear varieties, are very susceptible to fire blight.

Most Susceptible:

Bartlett, Bosc, Red Bartlett>Star Crimson

Moderately susceptible:

D'Anjou, Seckel, Comice

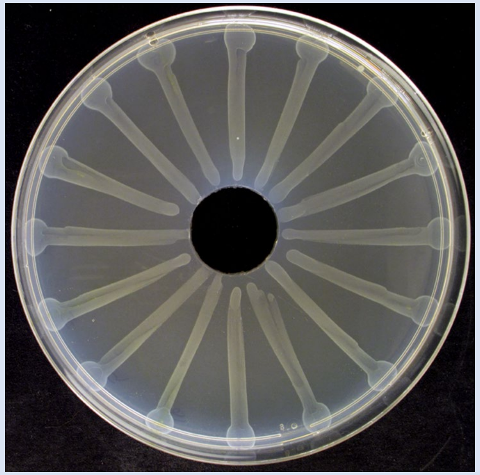
Wherever possible, plant varieties less prone to fire blight damage. Because most infections originate in the flowers, trees that bloom late or throughout the season (i.e., rat-tail bloom) often have severe fire blight damage.

Managing fire blight

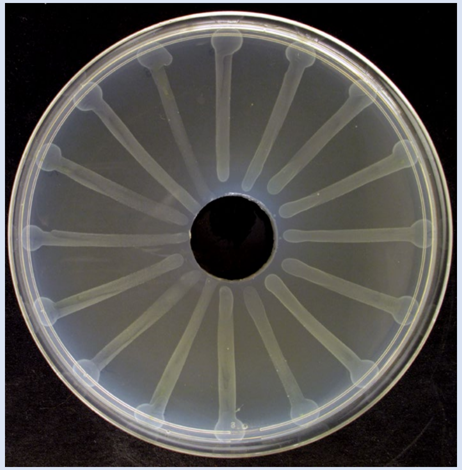
- **Cultural practices –**
  - Rootstock/scion selection
  - Orchard location and design – air movement to reduce humidity
  - Avoid high nitrogen fertilization - Provide balanced nutrition
  - Avoid over-irrigation
- **Sanitation –**
  - Removing infected woody cankers (pruning) and burn
  - In-season removing infected tissue (i.e., strikes)
  - Removal of alternate hosts-*Cotoneaster, Crataegus, Cydonia, Pyracantha, etc.*
  - Cleaning of pruning or hedging tools with HOCl or other sanitizers
- **Chemical –**
  - Antibiotics
  - Early season-copper
  - Biologicals (biological agents and natural products)
  - Plant growth regulators/SARs (e.g., Actigard – 2oz/Apogee 1 oz)
  - Rotation of different modes of action

Sensitivity of *E. amylovora* strains to copper

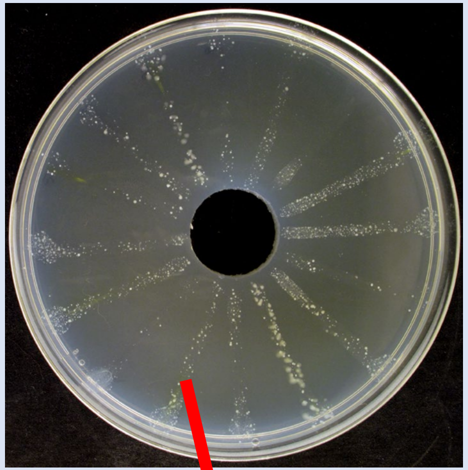
Nutrient agar (rich medium)



Control

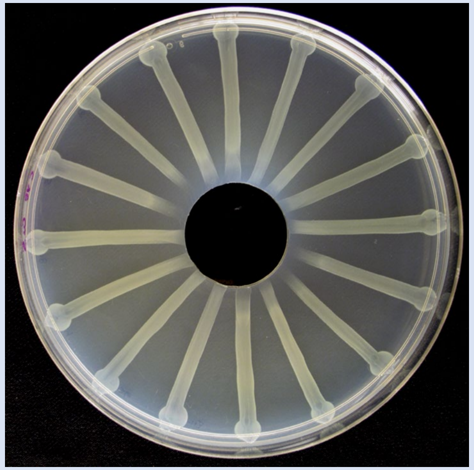


20 ppm MCE

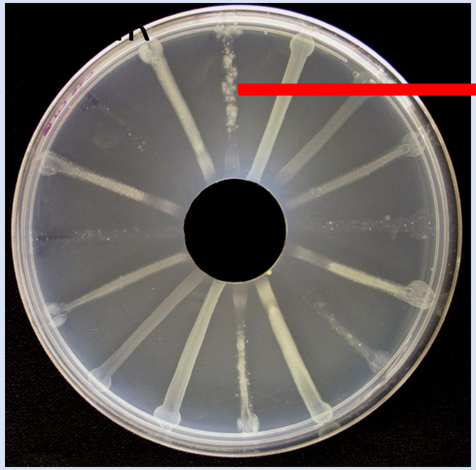


30 ppm MCE

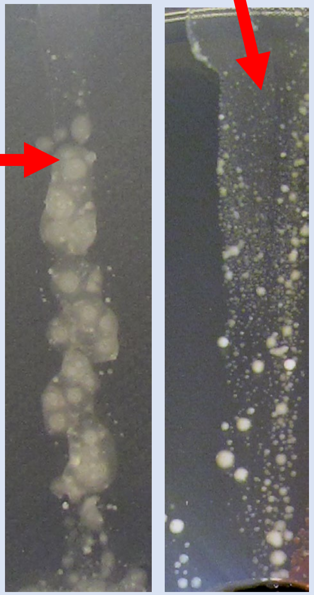
CYE agar (poor medium)



Control



15 ppm MCE



Spontaneous mutants (adaptations) on NA (right) and CYE (left)

Sensitivity of *E. amylovora* strains to copper

Nutrient-rich agar	Nutrient-poor agar
Growth not inhibited at **20** ppm MCE	Growth not inhibited at **10** ppm MCE
Reduced growth of many strains present at **30-40** ppm MCE	Reduced growth of many strains present at **15-20** ppm MCE
Spontaneous mutants often present, growing well at **30-40** ppm MCE	Spontaneous mutants often present, growing well at **15** and sometimes at **20** ppm MCE

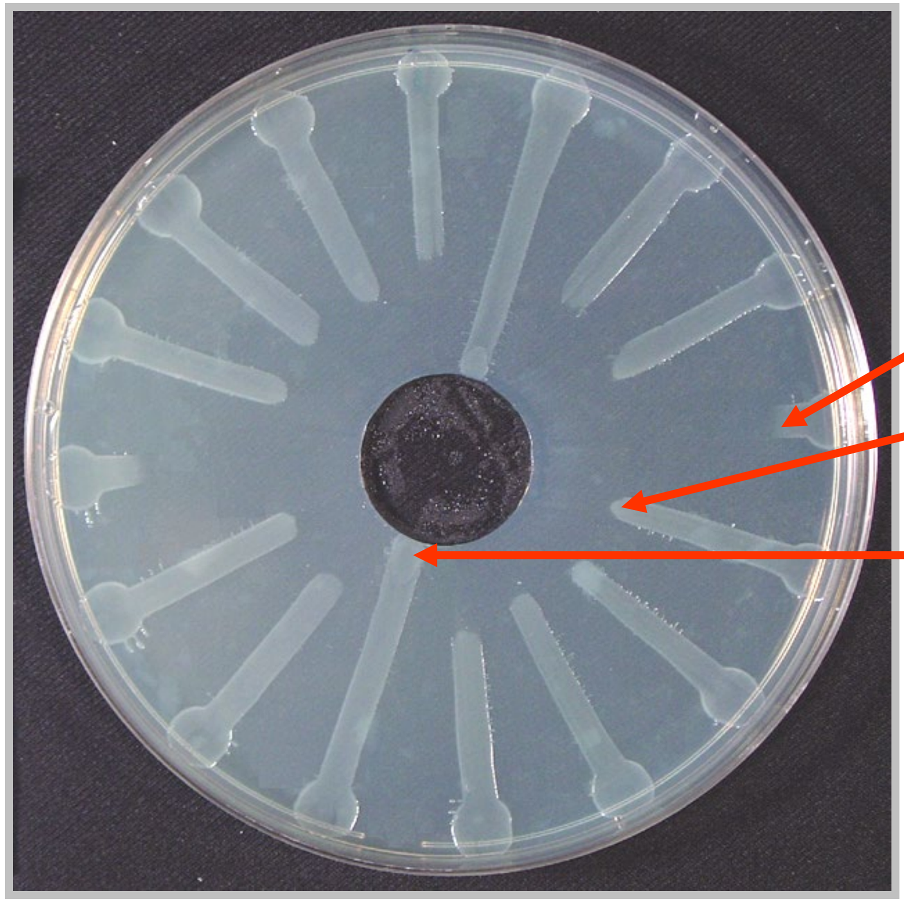
In other bacterial systems:
<10 ppm - Sensitive
10-50 ppm - Moderately resistant
>50 ppm - Resistant

- No high resistance.
- <u>Some</u> strains sensitive, <u>many</u> strains are considered moderately copper-resistant.
- Spontaneous mutants are not stable, but persist in the presence of copper (adaptation)

Copper and fire blight management

1. Copper is rated in UCIPM Efficacy Tables +/++.
2. Copper is a contact material and mostly only suppresses growth of the pathogen – when transferred to copper-free medium, bacteria resume growth.
3. Copper is applied at low rates: e.g., 0.5 lb Kocide 3000 = 180 ppm – this concentration is further diluted on the plant (e.g., 18-25 ppm) and has low solubility.
4. Under highly favorable environmental conditions copper performance will be low:
   a) "Moderate copper resistance" is present in the pathogen.
   b) Spontaneous mutants will persist in the presence of copper and cause disease.
5. **Copper use suggested for early season (bloom)** but not later by itself (can cause russeting of fruit)

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

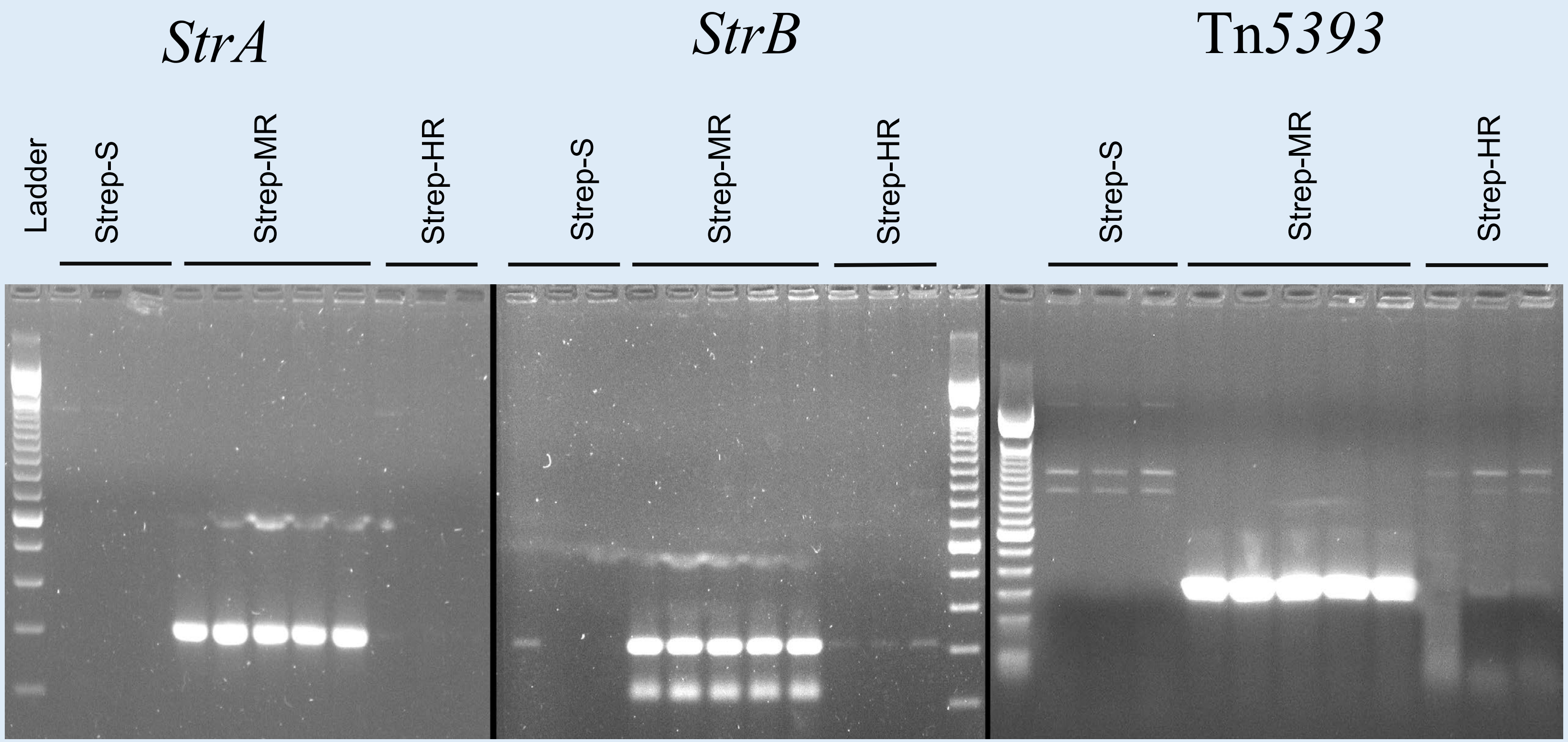


Spiral gradient dilution plate showing isolates with different sensitivity against streptomycin

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

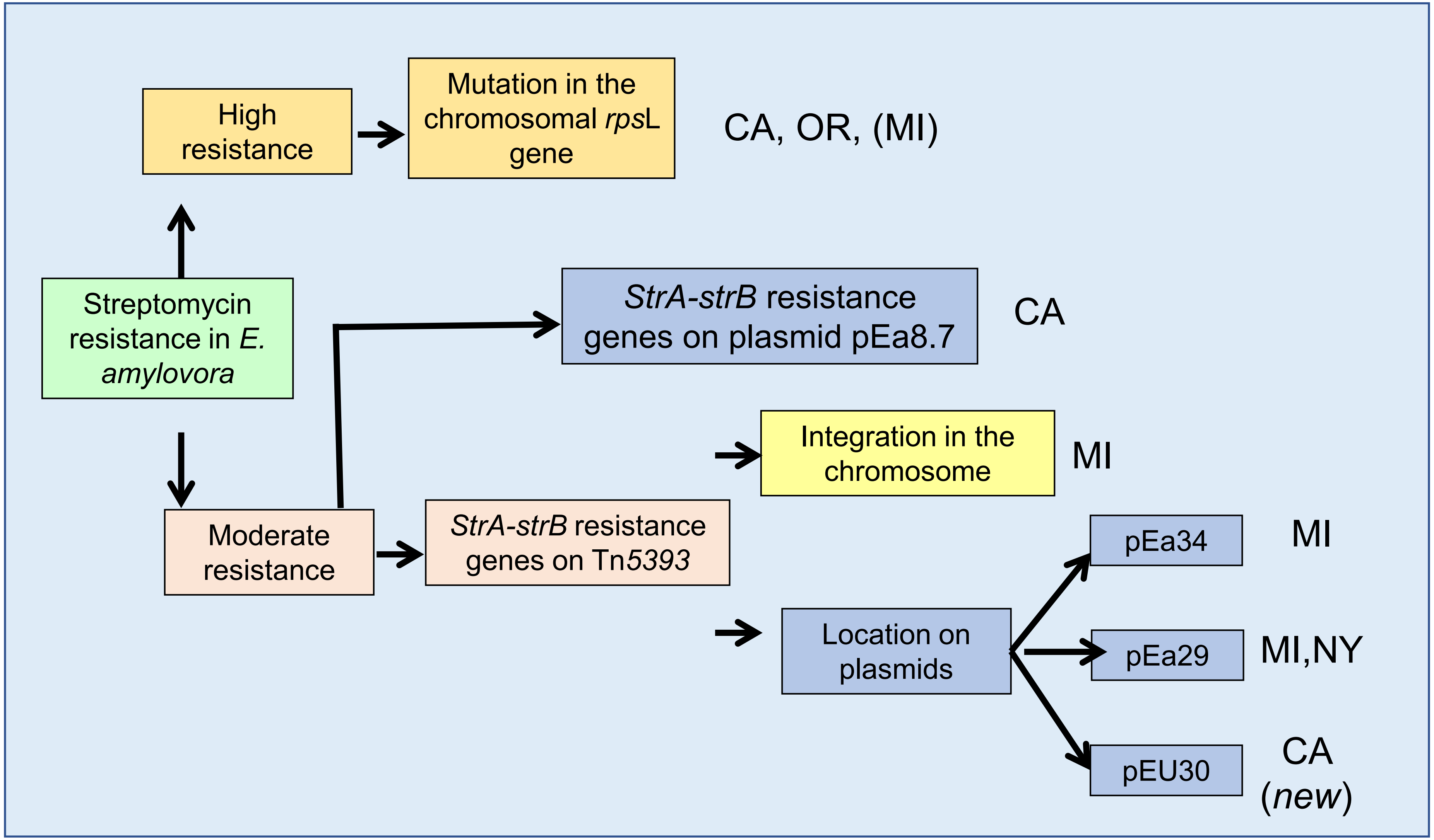
- Molecular basis for high and moderate resistance is different.
- Molecular basis for moderate resistance in CA is different from other locations (MI).

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.

Resistance surveys for streptomycin, oxytetracycline, and kasugamycin in *E. amylovora* populations from pear orchards in California

- Annual surveys have been conducted for over 15 years
- **Moderate (MIC 20-35 ppm) and high (MIC >100 ppm) STR resistance has been detected in pears and apples.**
- **Moderate (MIC <4 ppm) and high (MIC >100 ppm) OXY resistance has been identified in pears.**
- **No resistance to kasugamycin detected**

2023

County	Orchard No.	No. isolates	In vitro sensitivity (MIC)			
			Streptomycin		Oxytetra-cycline	Kasuga-mycin
Sacramento	1	10	8 MR	2 HR	S	S
	2	8	6 S	2 MR	S	S
	3	12	10 S	2 MR	S	S
	4	14	MR		S	S
	5	14	4 S	10 MR	S	S
	6	15	1 S	14 MR	S	S
	7	3	MR		S	S
Lake	1	1	S		S	S
	2	1	S		S	S
	3	1	S		S	S
Yolo	1	3	MR		S	S
total	11	82				

2021

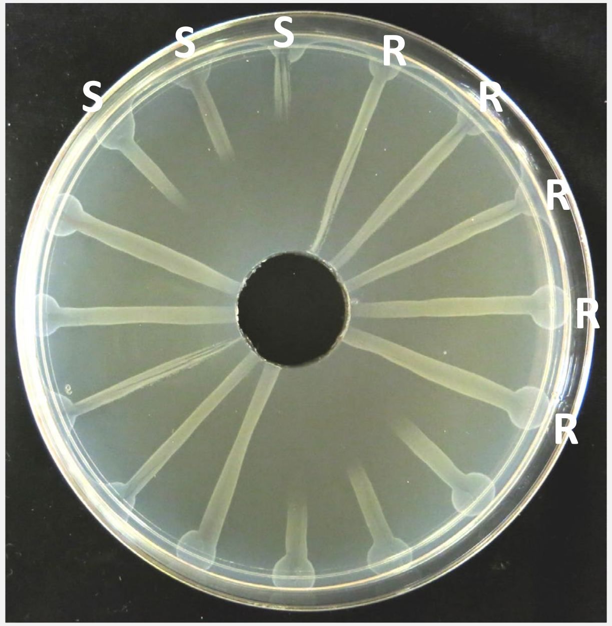
Orchard No.	County	Streptomycin	Oxytetracycline	Kasugamycin
1	Lake	S	S	S
2	Lake	MR	S	S
3	Lake	S	S	S
4	Lake	S	S	S
5	Lake	MR	S	S
6	Lake	S	S	S
7	Lake	S	S	S
8	Lake	S	S	S
9	Lake	S	S	S
10	Mendocino	HR	S	S
11	Sac	MR	S	S
12	Sac	HR	HR	S
13	Sac	S	S	S
14	Sac	MR	S	S
15	Sac	HR	HR	S

2022

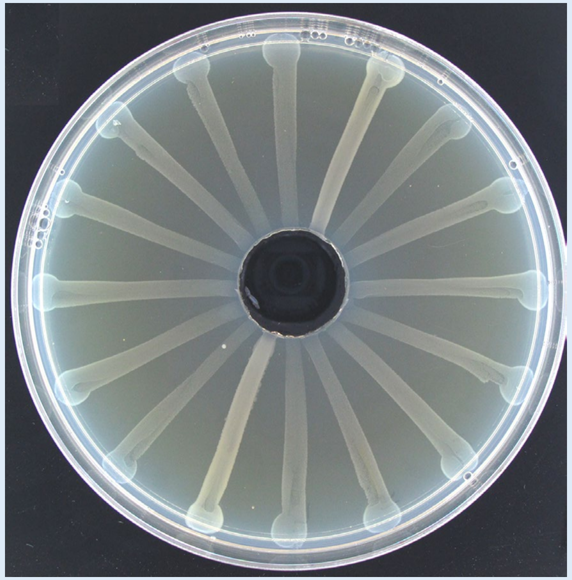
County	Orchard No.	No. isolates	In vitro sensitivity (MIC ppm)		
			Streptomycin	Oxytetracycline	Kasugamycin
Mendocino	1	1	S	S	S
	2	1	S	S	S
	3	1	MR (33.2)	S	S
	4	1	S	S	S
	5	1	S	S	S
	6	1	S	S	S
	7	1	S	S	S
	8	1	S	S	S
	9	3	S	S	S
	10	2	HR (> 40)	S	S
Lake	1	1	S	S	S
	2	1	S	S	S
	3	1	S	S	S
	4	1	S	S	S
	5	1	S	S	S
	6	1	S	S	S
	7	1	S	S	S
	8	1	S	S	S
	9	2	S	S	S
	10	1	S	S	S
	11	5	S	S	S
	12	2	S	S	S
	13	5	S	S	S
Sacramento	1	1	S	S	S
	2	2	S	S	S
	3	1	MR (22.9)	S	S
	4	1	MR (21.5)	S	S
	5	6	S	S	S
	6	2	S	S	S
	7	2	S	S	S
	8	4	S	S	S
	9	3	S	S	S
	10	4	S	S	S
	11	2	S	MR (3.1)	S
	11	4	S	S	S
	12*	9	S	S	S
Total	35	77			

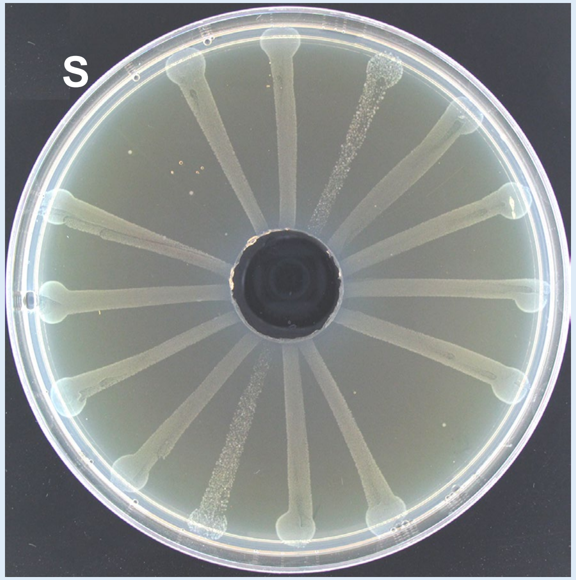
MIC rating:	S = 0.6 - 1.4 ppm	MR = 20 - 35 ppm	MIC >100 ppm

Detection of oxytetracycline-resistance in *E. amylovora* in orchard surveys



Concentration gradient from 0.02 ppm (edge of plate) to 40 ppm (center of plate)





Control

100 ppm oxytetracycline

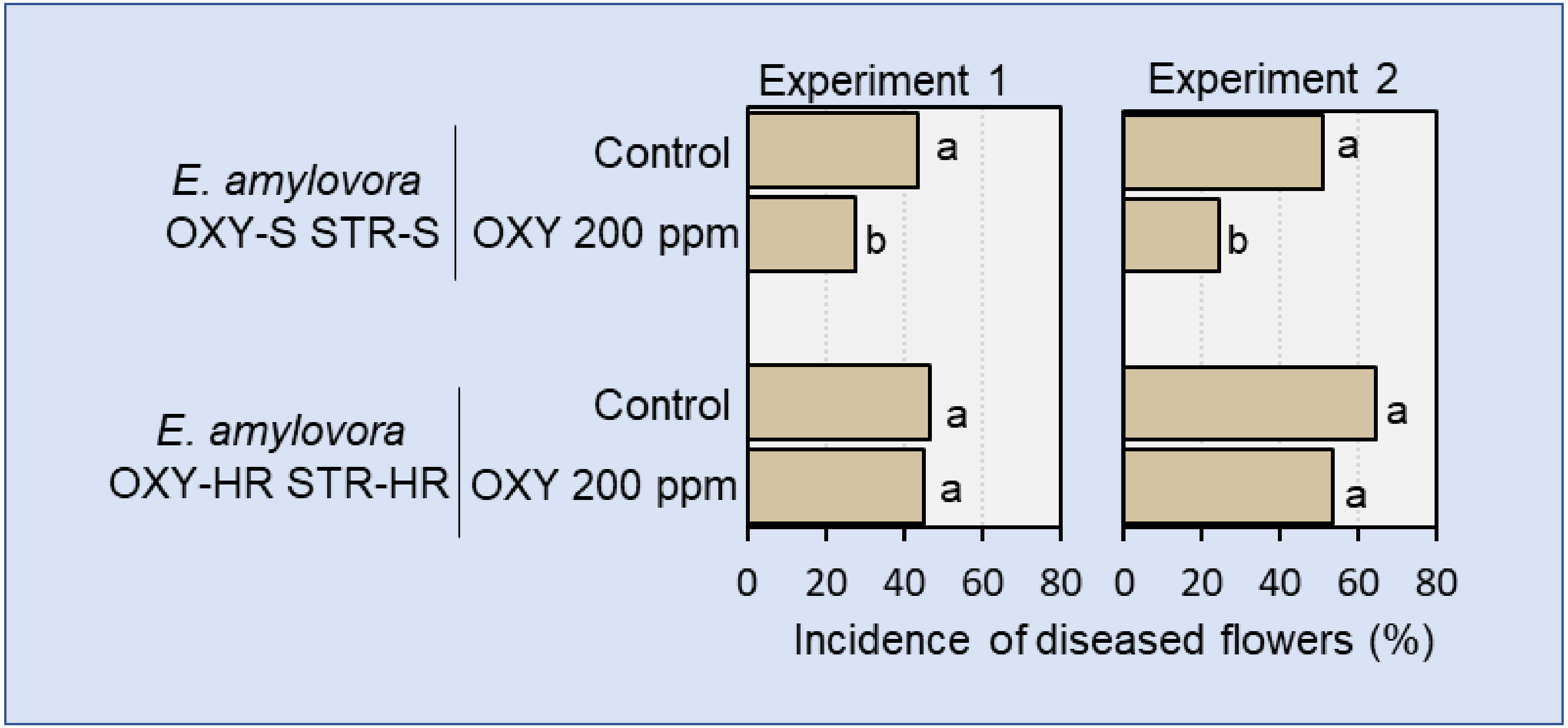
8 strains duplicated in opposite streaks: all except one (S) are **highly resistant** to OXY.

Moderate resistance found before 2018 had sensitivity levels of ca. 1-3 ppm

Spiral gradient endpoint and direct dilution assays:

Resistant strains were not inhibited in growth at ≥100 ppm oxytetracycline.

Efficacy of oxytetracycline treatments against fire blight caused strains of *E. amylovora* OXY-S/STR-S or OXY-HR/STR-HR in laboratory studies



Twigs with pear flowers were placed in beakers with water on a light bench in the laboratory. Flowers were spray-treated and inoculated after 4-5 h with strains of *E. amylovora* either sensitive to oxytetracycline and streptomycin (OXY-S STR-S) or highly resistant to both antibiotics (OXY-HR STR-HR) (10^7 cfu/ml). Disease was evaluated after 7 days.

High resistance to oxytetracycline in *E. amylovora* detected in pear orchards 2018-2020

Resistant strains:

- Not inhibited by ≥100 ppm oxytetracycline.
- Also highly resistant to streptomycin (≥100 ppm)
- Not controlled by labeled rates of oxytetracycline or streptomycin
- Competitive in the presence of sensitive wild type isolates
- Persisted in orchards to the next growing season

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Pathogen Spotlight

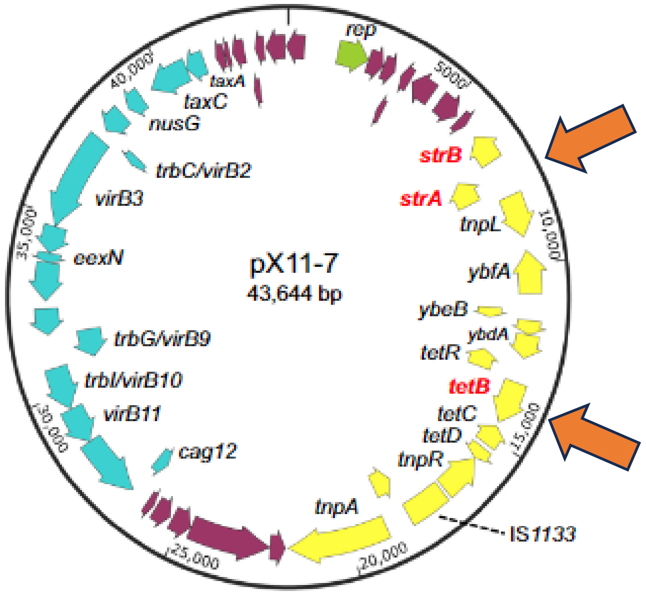
Bacteriology

A Novel IncX Plasmid Mediates High-Level Oxytetracycline and Streptomycin Resistance in *Erwinia amylovora* from Commercial Pear Orchards in California

George W. Sundin,[1,†] Jingyu Peng,[1] Lindsay E. Brown,[1] Quan Zeng,[2] Helga Förster,[3] and James E. Adaskaveg[3,†]

[1] Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824
[2] Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06511
[3] Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521
Accepted for publication 5 August 2023.

Molecular characterization of resistant strains:
Acquisition of resistance on a new plasmid.



Evaluate and optimize the performance of antibiotics, new formulations of copper, new natural products, GRAS food additives, biocontrols, and additives in laboratory and field studies

a) Kasumin in combination with exempt-from-tolerance antimicrobials including copper sulfate

b) Oxytetracycline – new formulations of Mycoshield and FireLine

c) Low-copper concentration products such as copper sulfate pentahydrate (MasterCop) and copper octanoate (Cueva)

d) New formulations of ε-poly-L-lysine and nisin

e) The new FDA GRAS TDA-NC-1, aluminum potassium sulfate (Alum), cinnamaldehyde (Seican), and new essential oils (Cinnerate). The latter two are OMRI-certified.

New bactericides under evaluation in field studies on fire blight

Category	FRAC Code	Active ingredient	Trade name/Code
Antibiotics	24	kasugamycin	Kasumin 2L
	41	oxytetracycline	FireLine 45, Mycoshield NUP-17010
	25	streptomycin	AgriMycin, FireWall
Natural products	BM 01	capric/caprylic acids	Dart
	BM 01	*Acacia* sp. bark extract	QAM
	BM 01	cinnamaldehyde	Seican
	BM 01	cinnamaldehyde + EPL	JAX-1
	BM 01	cinnamon oil	Cinnerate
	BM 01	thyme oil	Thyme Guard
	BM 01	potassium aluminum sulfate	Alum
	BM 01	bacterial metabolite	RAA-A
Food preservatives	BM 01	nisin	food additive
	BM 01	ε-poly-L-lysine	food additive
Biocontrols	BM 02	*Aureobasidium pullulans*	Blossom Protect
	BM 02	*Papiliotrema terrestris*	YSY
	BM 02	*Bacillus subtilis* QST 713	Serenade ASO
Other antimicrobials	U12	dodine	Syllit
	---	water-soluble zinc	Manniplex Zn
	---	peroxyacetic acid	Oxidate

New formulations

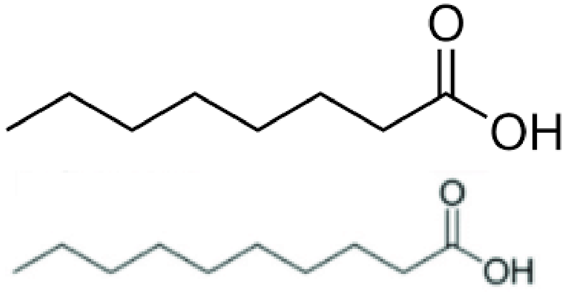
New combination

Biofermentations

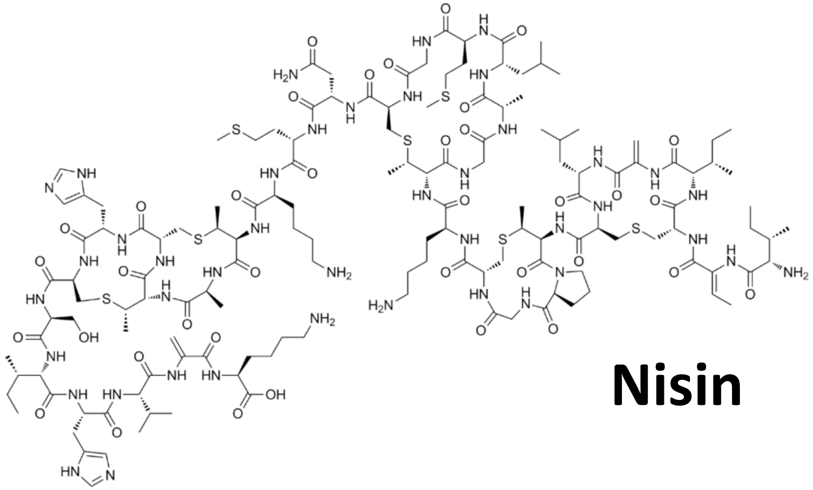
Ongoing evaluations

Mixture partner

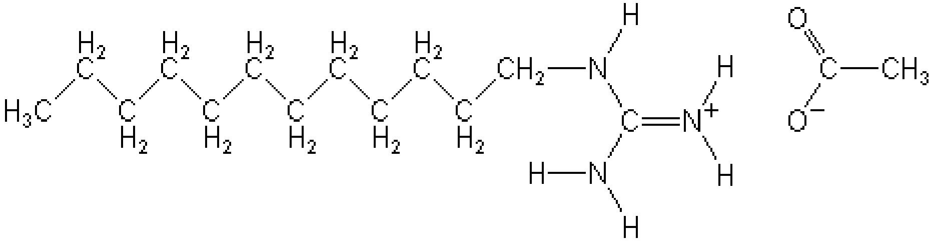
Polymer mixture partners with bactericides for fire blight management



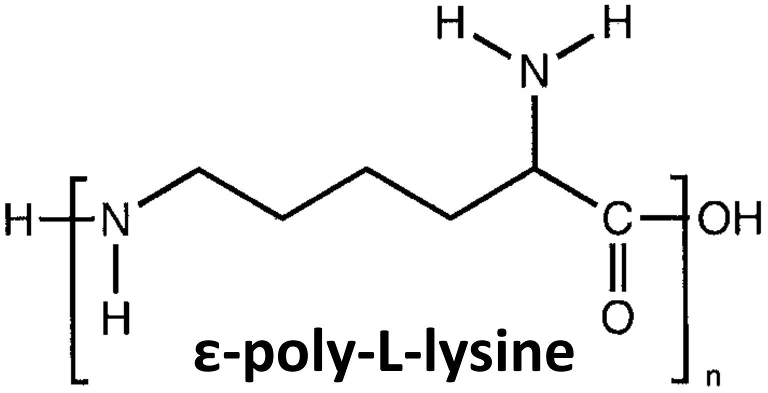
Capric & caprylic acids (Dart)



Nisin



Dodine (Syllit)

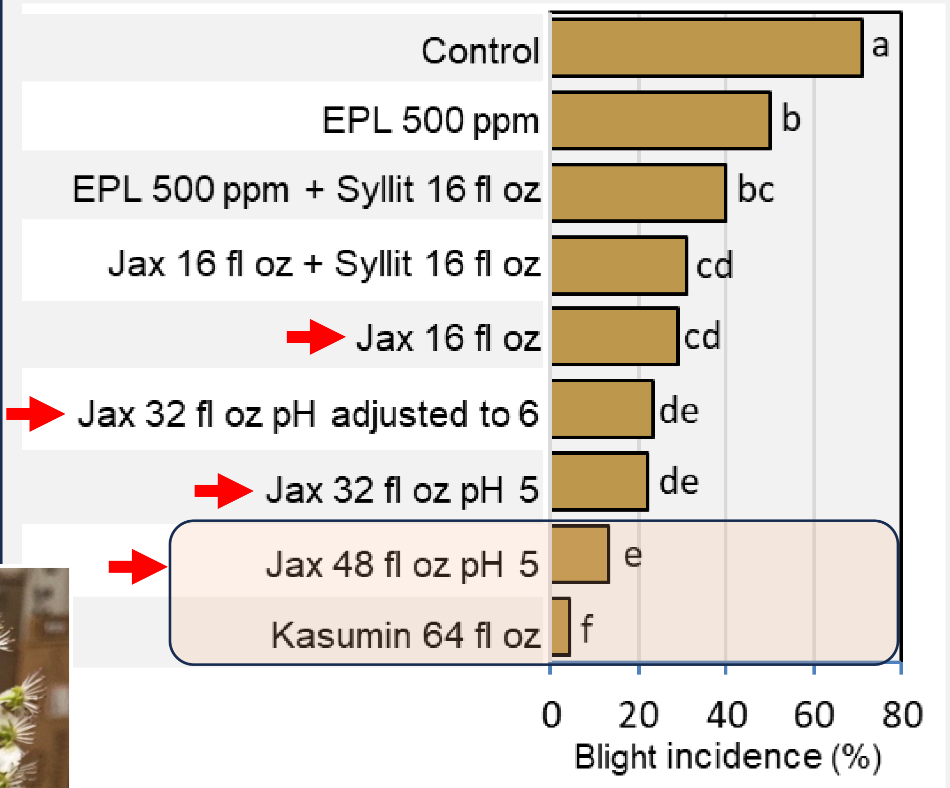


ε-poly-L-lysine

Nisin and ε-poly-L-lysine (EPL): Moderate to very good efficacy in previous trials. An enhancer of activity that we identified in lab studies was evaluated in the field with promising results. **Syllit and Dart** as mixture partners with antibiotics.

Screening of new treatments in small-scale studies with ornamental pear flowers in the laboratory 2023

These experiments supported the high in vitro toxicity of an EPL-cinnamaldehyde (Seican) mixture and helped in integrating formulated JAX-1 treatments in field studies.

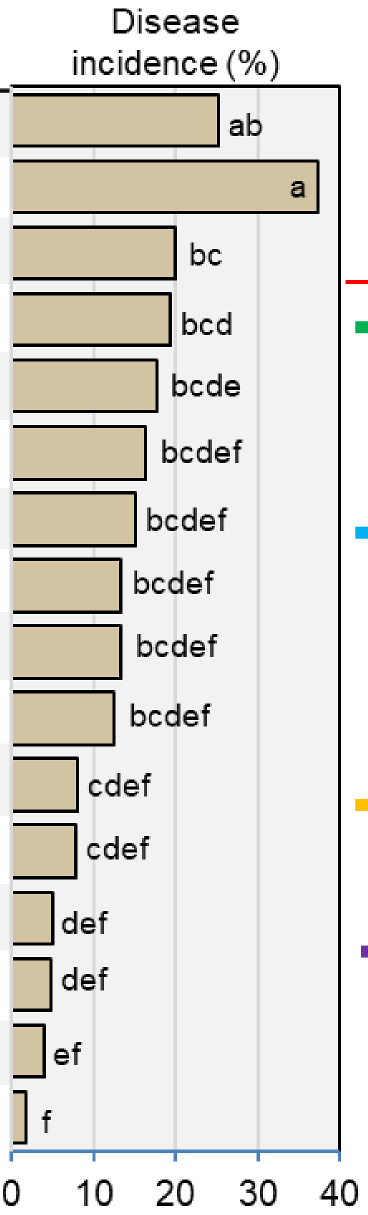


Flowers were spray-treated, allowed to air dry, inoculated with *E. amylovora*, and incubated for 5-7 days.

New bactericides in field studies 2022

Bartlett pear, Live Oak, CA

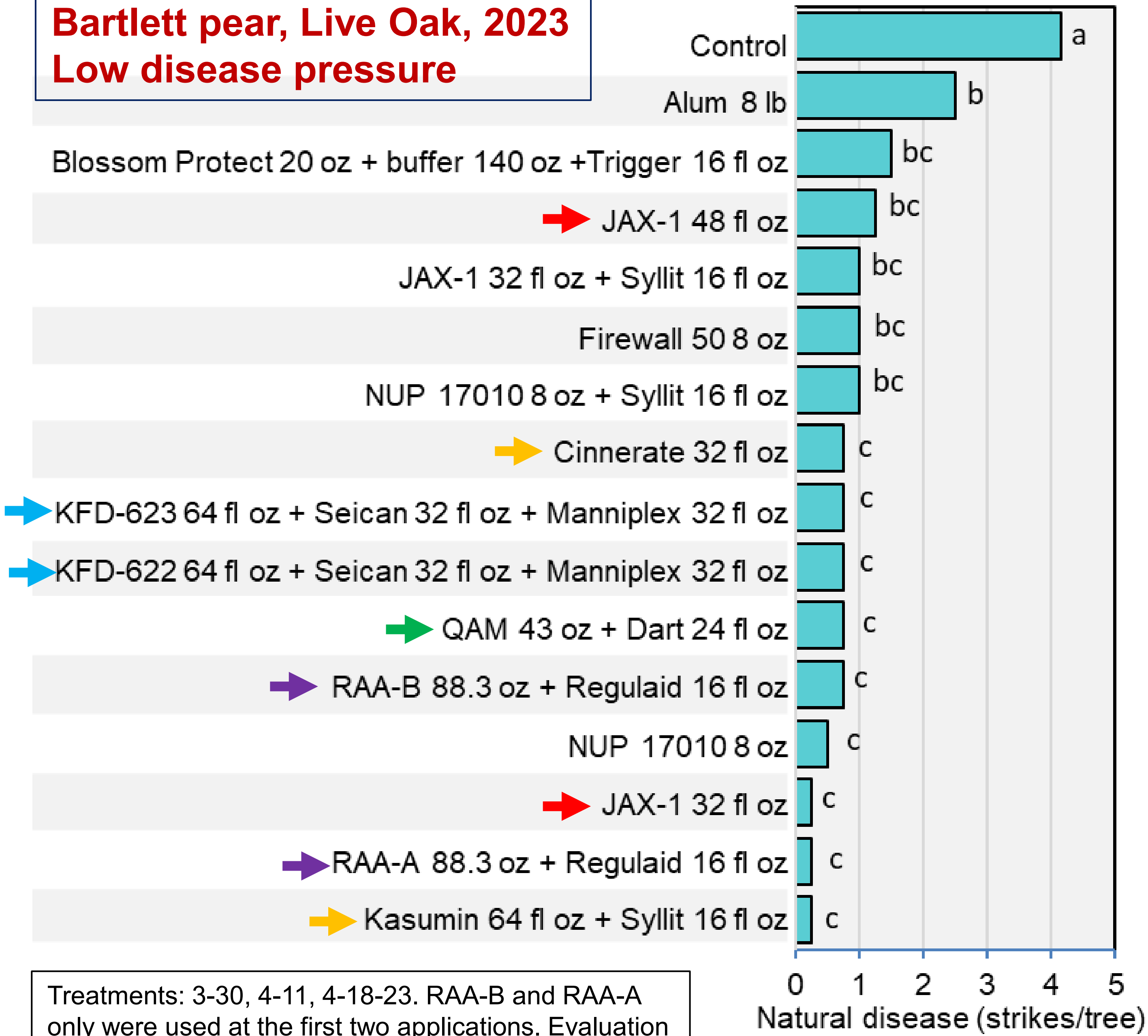
Treatment	3-16	3-22	3-29	4-7
Control	---	---	---	---
Thyme Gard 64 fl oz	@	@	@	@
Cueva 64 fl oz + Double Nickel 55 128 fl oz	@	@	@	@
QAM 43 fl oz + Dart 24 fl oz	@	@	@	@
Alum 8 lb	@	@	@	@
Ninja 64 oz + Dart 24 fl oz	@	@	@	@
Blossom Protect 20 oz + buffer 140 oz	@	@	@	@
TDA -NC-1 20 oz + NuFilm 17 8 fl oz	@	@	@	@
Kasumin 64 fl oz	@	@	@	@
KFD 622-01 64 fl oz + Kas. 32 fl oz + Manniplex 32 fl oz	@	@	@	@
FireLine 45 8 oz + Syllit 16 fl oz	@	@	@	@
Cinnerate 32 fl oz	@	@	@	@
KFD 623-01 64 fl oz + Kas. 32 fl oz + Manniplex 32 fl oz	@	@	@	@
NUP-17010 8 oz + Dart 24 fl oz	@	@	@	@
Kasumin 64 fl oz + Syllit 16 fl oz	@	@	@	@
Kasumin 64 fl oz + Firewall 50 8 oz	@	@	@	@



- A previous formulation of an *Acacia* sp. extract (QAM): not effective
- Field formulations of EPL and nisin in combination with Seican and ManniPlex Zn: very good or poor efficacy, respectively
- Cinnerate similarly effective in 2021-2023 years
- Kasumin-Syllit similarly highly effective in both years

New bactericides in field studies 2023

Bartlett pear, Live Oak, 2023
Low disease pressure

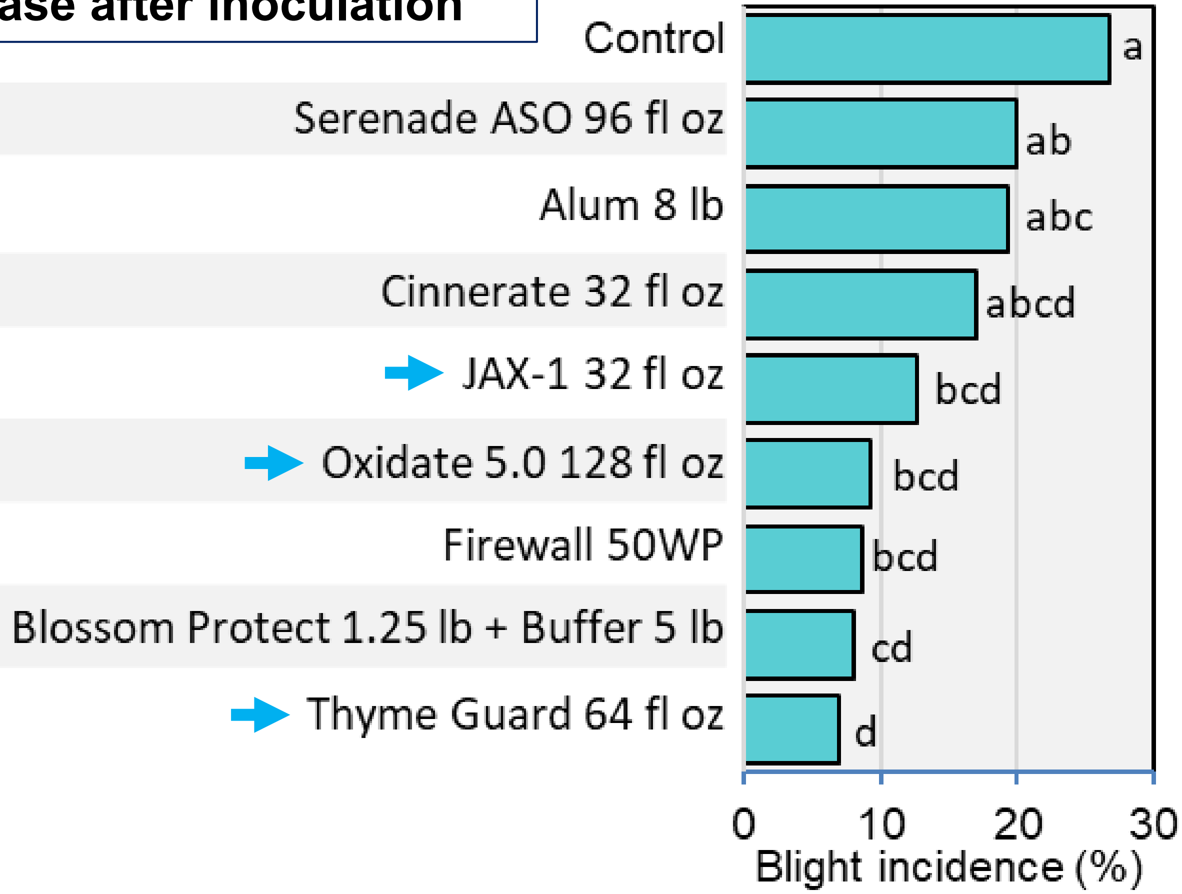


Treatments: 3-30, 4-11, 4-18-23. RAA-B and RAA-A only were used at the first two applications. Evaluation on 5-5-23.

- **JAX-1:** good to very good efficacy
- Field formulations of EPL and nisin in combination with Seican and ManniPlex Zn: very good efficacy
- A new formulation of an *Acacia* sp. extract (QAM): very effective
- Two bacterial (RAA-A,-B) metabolites: very effective
- Other very effective treatments: Cinnerate, Kasumin mixed with Syllit

New bactericides in field studies

Apple pear, UC Davis, 2023
Disease after inoculation



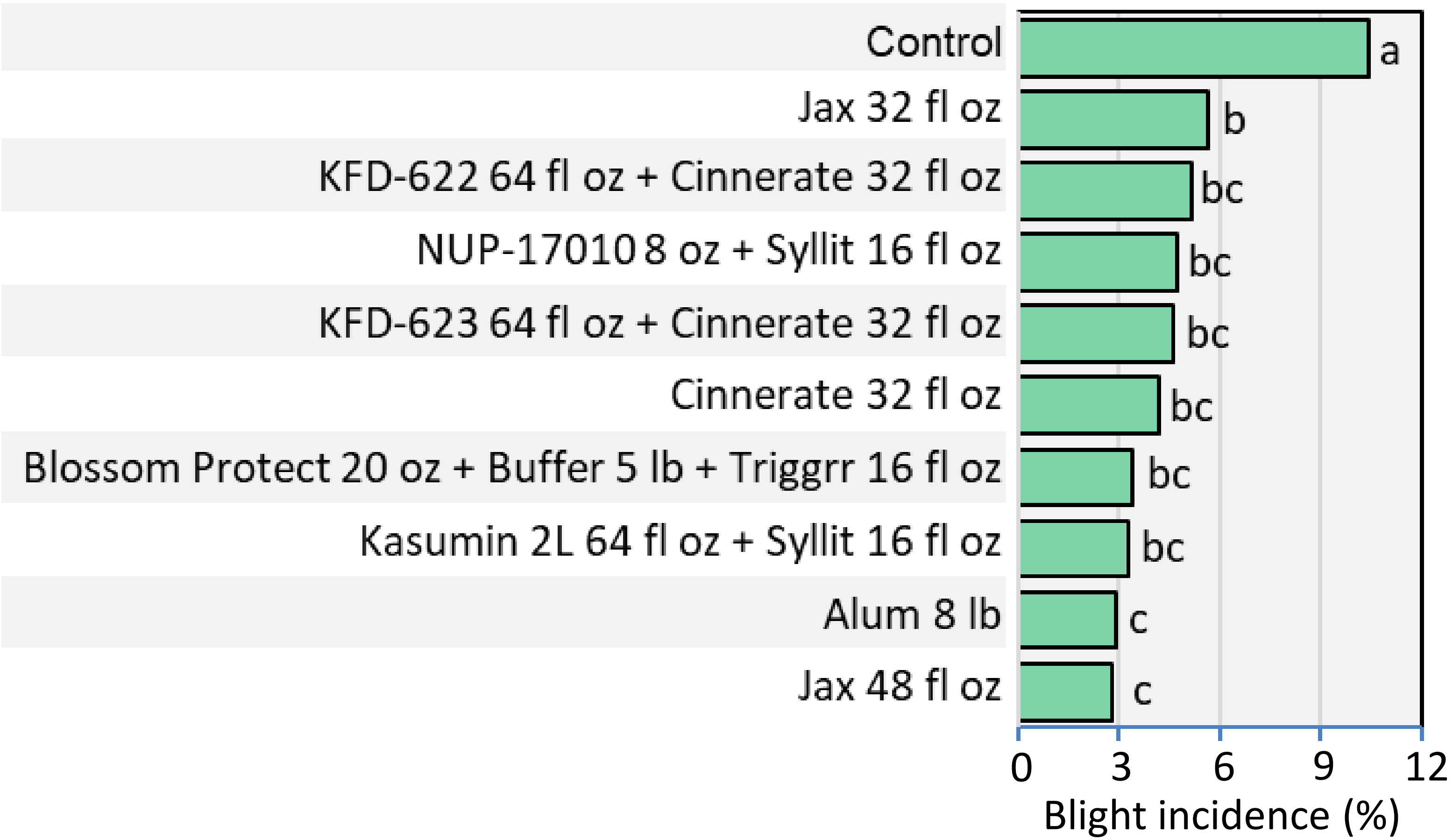
Treatments on 3-23 and 3-28-23. Inoculation with *E. amylovora* on 3-25-23. Evaluation on 4-18-23.

JAX-1, Oxidate, Blossom Protect, and Thyme Guard statistically similar in efficacy to FireWall or Blossom Protect.

Oxidate only has short residual activity.

Part of USDA-SCRI multi-state trial

Efficacy of bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2023



Treatments were applied on 4-3 (king bloom), 4-8 (40% bloom, 4-13 (full bloom), and 4-20-23 (petal fall) using an air-blast sprayer. There were four replications of three trees each per treatment. Disease was evaluated in early June on 100 flower clusters per tree.

Alum and Jax-1 had the highest performance.

NEW treatments for fire blight with promising results that are strongly supported by their registrants for registration

Category	FRAC Code	Active ingredient	Trade name/Code
Antibiotics	24	kasugamycin	Kasumin 2L
	41	oxytetracycline	FireLine 45, Mycoshield NUP-17010
	25	streptomycin	AgriMycin, FireWall
Natural products	BM 01	capric/caprylic acids	Dart
	BM 01	*Acacia* sp. bark extract	QAM
	BM 01	cinnamaldehyde	Seican
	BM 01	cinnamaldehyde + EPL	JAX-1
	BM 01	cinnamon oil	Cinnerate
	BM 01	thyme oil	Thyme Guard
	BM 01	potassium aluminum sulfate	Alum
	BM 01	bacterial metabolite	RAA-A
Food preservatives	BM 01	nisin	food additive
	BM 01	ε-poly-L-lysine	food additive
Biocontrols	BM 02	*Aureobasidium pullulans*	Blossom Protect
	BM 02	*Papiliotrema terrestris*	YSY
	BM 02	*Bacillus subtilis* QST 713	Serenade ASO
Other antimicrobials	U12	dodine	Syllit
	---	water-soluble zinc	Manniplex Zn
	---	peroxyacetic acid	Oxidate

New formulations (FireLine 45, Mycoshield NUP-17010; AgriMycin, FireWall)

New combination (JAX-1)

For evaluation on apple (Alum)

Biofermentation (RAA-A)

Treatments for Managing Fire Blight

Conventionals

Direct toxicants (bactericides)

High activity, High performance, High resistance potential

Inorganics
- **Copper** — M1 — 1960s

Dithiocarbamates
- **Manzate** — M3 — 1940s

Guanidines
- **Syllit** — M1 — 1960s

Aminoglycosides
- **ArgriMycin, FireWall** — 23 — 1950s

Aminoglycosides
- **Kasumin** — 24 — 1960s

Tetracyclines
- **FireLine, Mycoshield** — 41 — 1970s

Phosphonates
- **ProPhyt, K-Phite Fungi-phite** — P07, 33 — 1980s

SARs -
- **Actigard, LifeGard** — SAR

Growth regulator -
- **Apogee, others** — GR

Biologicals

Direct Suppression, Competition, or Induced SAR

Lower activity and performance, Low resistance potential

Biofermentation – *Bacilus subtilus*
- **Serenade** *(ASO, Opti)* — BM 02

Natural Product - *Reynutria sachalinensis*
- **Regalia** — BM 01

Biofermentation – *Aureobasidion pullulans*
- **Blossom Protect** — BM 02

Natural Products – Plant Extracts
- **Cinnerate, Seican, Dart, Thymox** — BM 01

Experimentals -
- **YSY, JAX-1, QAM, RAA-A** — XYZ

FRAC Code | Multi-site mode of action | Single-site mode of action | Biologicals

Fire blight management - Part 1

- **Differences in sensitivity among cultivars and rootstock/scion combinations -**
  - Less susceptible than Bartlett – Comice, Winter Nelis, BPM
  - Rootstock/scions that produce abundant succulent growth, high tree vigor and dwarfing rootstocks are highly susceptible
- **Cultural practices –**
  - Avoid high nitrogen fertilization - Provide balanced nutrition
  - Avoid over-irrigation
  - Orchard location and design – air movement to reduce humidity
- **Sanitation –**
  - Prune out fire blight cankers and burn
  - In-season remove infected tissue (i.e., strikes)
  - Remove alternate hosts - *Cotoneaster, Crataegus, Cydonia, Pyracantha, etc.*

Fire blight management – Part 2

Chemical/biological control -

- **Toxicants** against the pathogen – Copper, antibiotics, natural products
- **New non-antibiotic bactericides** with higher activity are becoming available that need to be tested under different environments and applications need to be optimized (rates, mixtures, adjuvants).
- **Biocontrols** – Competition, antibiosis, site-exclusion, parasitism – a new biocontrol (YSY) will be evaluated in 2024
- **Systemic acquired resistance (SAR)** – Actigard (strong), Apogee (strong), LifeGard (weak), Regalia (weak)
- **Shoot growth inhibition** - Apogee
- **Insect control** – Aphids, ants, flies, etc.

Monitoring for bacterial populations – pruning and timing of chemical control

- Resistance levels to antibiotics and copper

FUNGICIDES, BACTERICIDES, BIOCONTROLS, AND NATURAL PRODUCTS FOR DECIDUOUS TREE FRUIT AND NUT, CITRUS, STRAWBERRY, AND VINE CROPS IN CALIFORNIA 2022

ALMOND APPLE APRICOT CHERRY CITRUS	GRAPE KIWIFRUIT PEACH NECTARINE PEAR PISTACHIO	PLUM POMEGRANATE PRUNE (DRIED PLUM) STRAWBERRY WALNUT

Adaskaveg, Michailides, and Eskalen

Will be updated in 2024

APPLE AND PEAR: BACTERICIDE EFFICACY – CONVENTIONAL

Bactericide	Resistance risk	Fire blight[11]		Phytotoxicity[15]	Plant Growth Regulator/SAR
		Contact	Systemic		
Ag Streptomycin, Agri-Mycin, Firewall	very high (25)	5	4	1	0
Kasumin	high (24)	5	5	1	0
MycoShield, FireLine	high (41)	4	4	1	0
Captan[6]	low (M4)	3	0	0	0
Copper[7]	low (M1)	3	0	2	0
Dithane, Manzate, Penncozeb[6]	low (M3)	3	0	0	0
Actigard[12]	low (P 01)	0	2	0	2
Apogee[11]	low (PGR)	0	2/3	0	3

APPLE AND PEAR: BACTERICIDE EFFICACY – BIOCONTROLS AND NATURAL PRODUCTS

Bactericide	Resistance risk (FRAC Code)[1,13]	Fire blight[11]		Phytotoxicity[15]	Plant Growth Regulator/SAR
		Contact	Systemic		
AgriPhage	low (BM 02)	2/3	0	0	0
Blossom Protect	low (BM 02)	4	0	1	0
Copper[7]	low (M1)	3	0	4	0
Actinovate	low (BM 02)	2/3	0	1	0
BacStop	low (BM 01)	2	0	1	0
Blight Ban	low (BM 02)	2/3	0	1	0
Dart	low (BM 01)	2/3	0	0	0
Double Nickel 55	low (BM 02)	2/3	0	1	0
Regalia	low (P 05, BM 01)	2/3	0	1	0
Sanitizers[14]	low	2/3	0	0	0
Serenade	low (BM 02)	2/3	0	1	0
LifeGard	low (P 06, BM 02)	2	2	0	2
Lime sulfur/sulfur[8]	low (M2)	2	0	4	0

(Rating of 5 is most effective, 0 is not effective)

Thank you