Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi*

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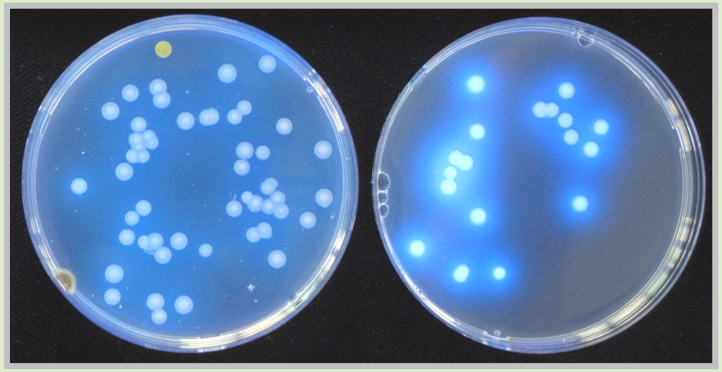
Overview

I. **Epidemiology**
   A. Olive knot - Entry points of the pathogen
   B. Effect of inoculum level on disease development
   C. Wound healing and susceptibility to infection
   D. Disease development: Localized knots vs. systemic infection

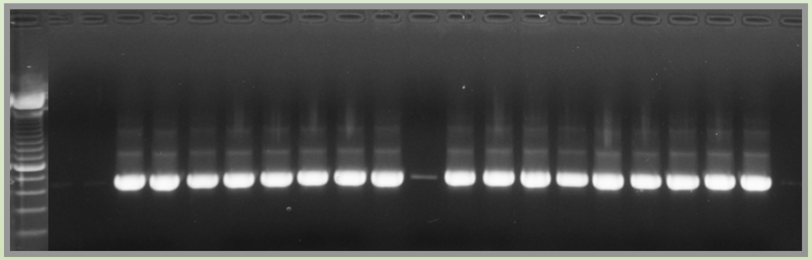
II. **Management of olive knot by sanitation**
   A. A new sanitizer for field equipment

I. **Management of olive knot with field applications of chemicals**
   A. Field surveys on sensitivity of Psv to copper and antibiotics
   B. Efficacy of new bactericides and optimization
      - Copper
      - Oxytetracycline accepted into IR-4 program Sept 2015.
      - Kasugamycin accepted into IR-4 in 2014
      - Field trials on the persistence of copper-antibiotic mixtures after a rain event
   C. Timing of applications relative to injuries

Olive Knot - *Pseudomonas savastanoi* pv. *savastanoi*



Isolation plates of *Psv* on KMB (left) and PVF-1 (right) under long-wave UV.



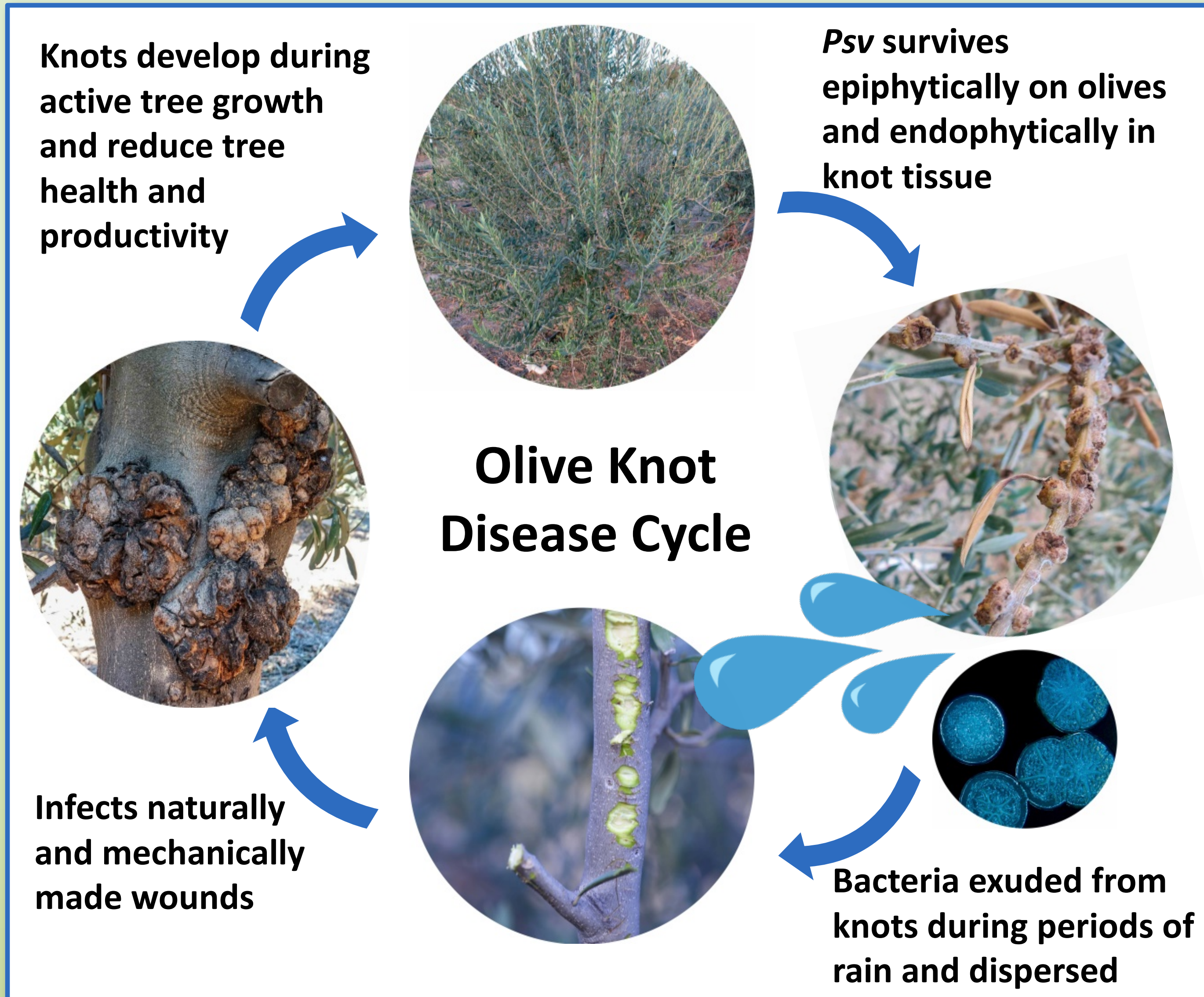
Specific amplification of *Psv*

- Economically important worldwide
- All olive varieties are susceptible to Psv.
- Pathogen gains entry into host through wounds.
- Psv found as an epiphyte on surface and as an endophyte inside knots.
- Produces phytohormones that cause hyperplastic and hypertrophic outgrowths (knots, galls).
- Infections cause tree defoliation, branch dieback, and reduced tree vigor.

Olive Knot – Disease Cycle

Pseudomonas savastanoi **pv.** ***savastanoi (Psv)***

- Gram-negative bacterium
- Epiphytic, opportunistic wound pathogen
- Naturally disseminated by rain and water splash



Epidemiology

- **Entry points of the pathogen and environmental conditions for infection**
- **Inoculum availability**
- **Effect of inoculum level on disease development**

Olive knot - Epidemiology

Leaf scar infection

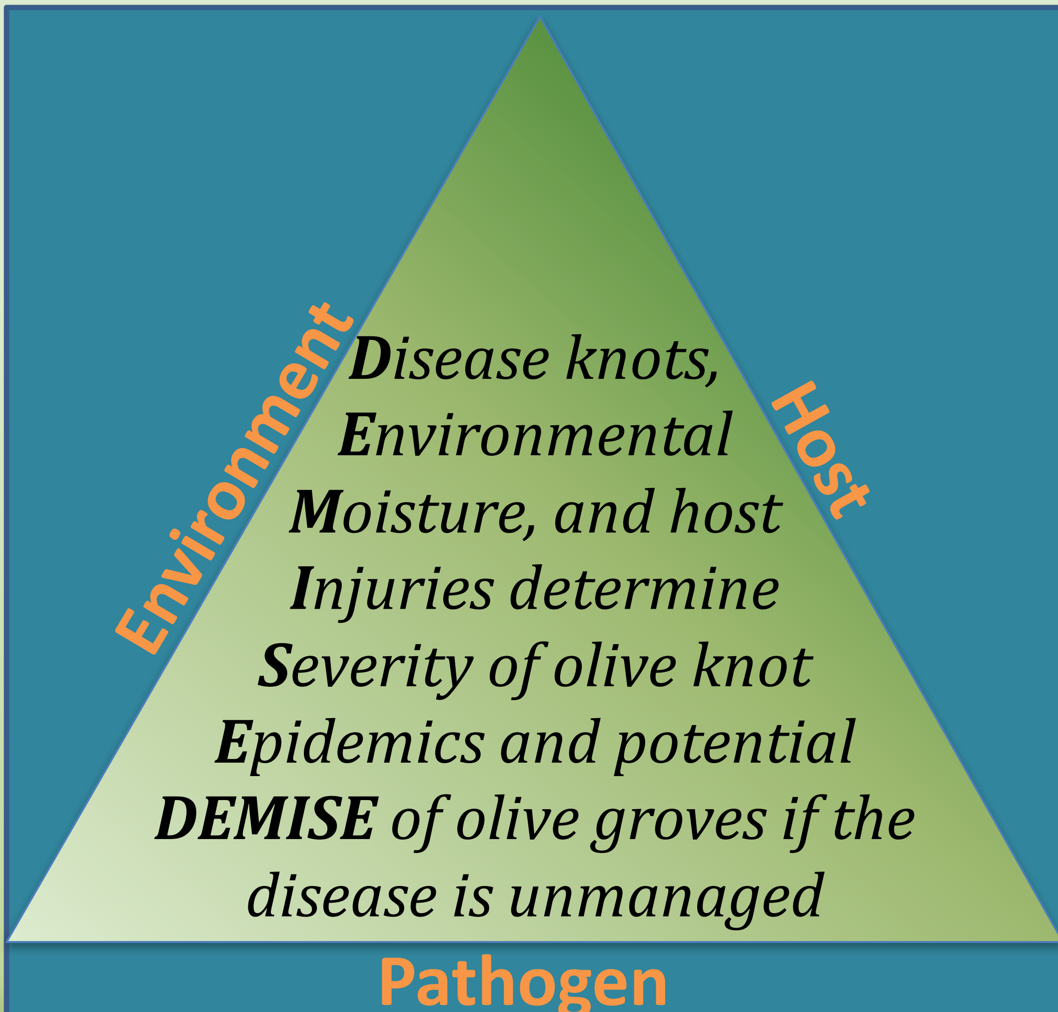
Mechanized pruning

Mechanized harvest

- **Infection through:**
  - **Leaf scars** – spring leaf drop
  - **Cold injury** - frost
  - **Mechanical injury** - pruning, harvesting, hail
- **Increase in olive knot**
  - High-density plantings, mechanical harvesting, and pruning operations to optimize yield and reduce labor costs are causing an increase in bark injuries.
  - Olives (especially oil varieties) growing areas have expanded into areas that are more prone to winter freezes.

Olive knot - Epidemiology

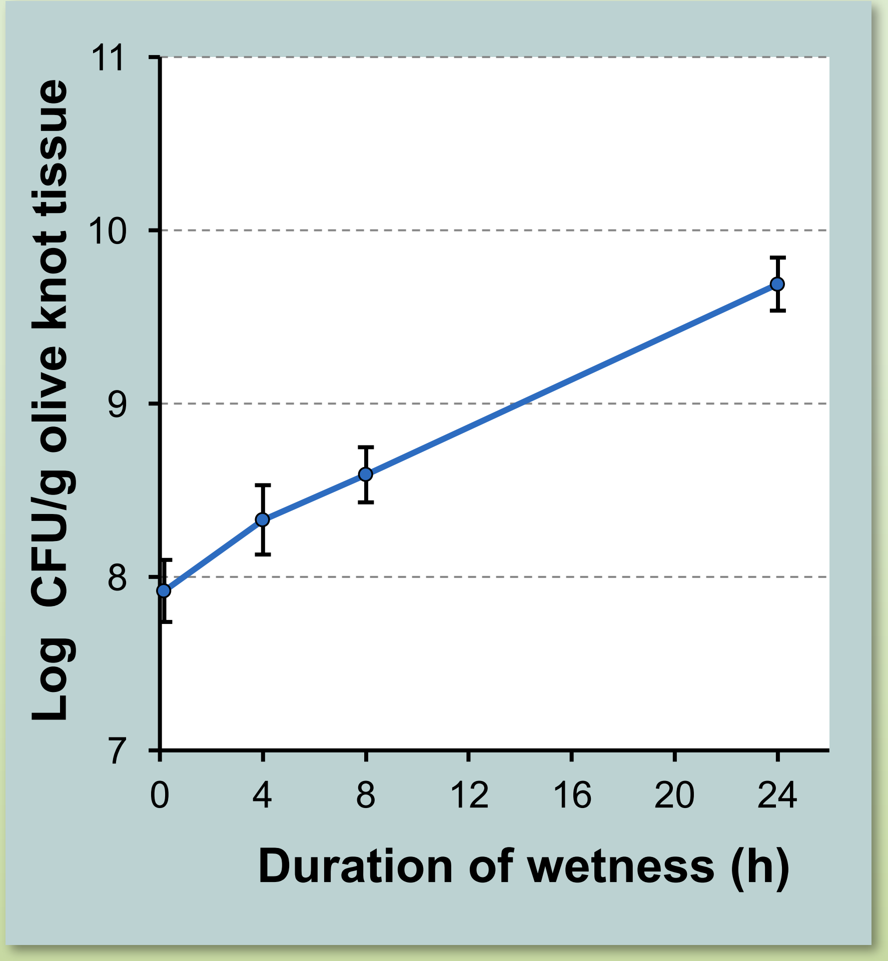
- **Time of infection in CA**
  - Late fall, winter, spring (rainy / cold season)
  - Knot development in the spring and summer



- **Environmental conditions**
  - **Infections** occur over wide range of temperatures
  - **Wetness** is the main environmental factor favoring disease development

Olive knot – Epidemiology
Knots are inoculum sources

- Knots with living host tissue contain viable inoculum
- Re-hydrating olive knots for **<u>one</u>** hour led to bacterial oozing from most of the knots.
- **<u>Nearly all</u>** knots tested continued to ooze the pathogen after 18 to 24 h of hydration.



Wounding and inoculation technique

Leaf scar and lateral wound

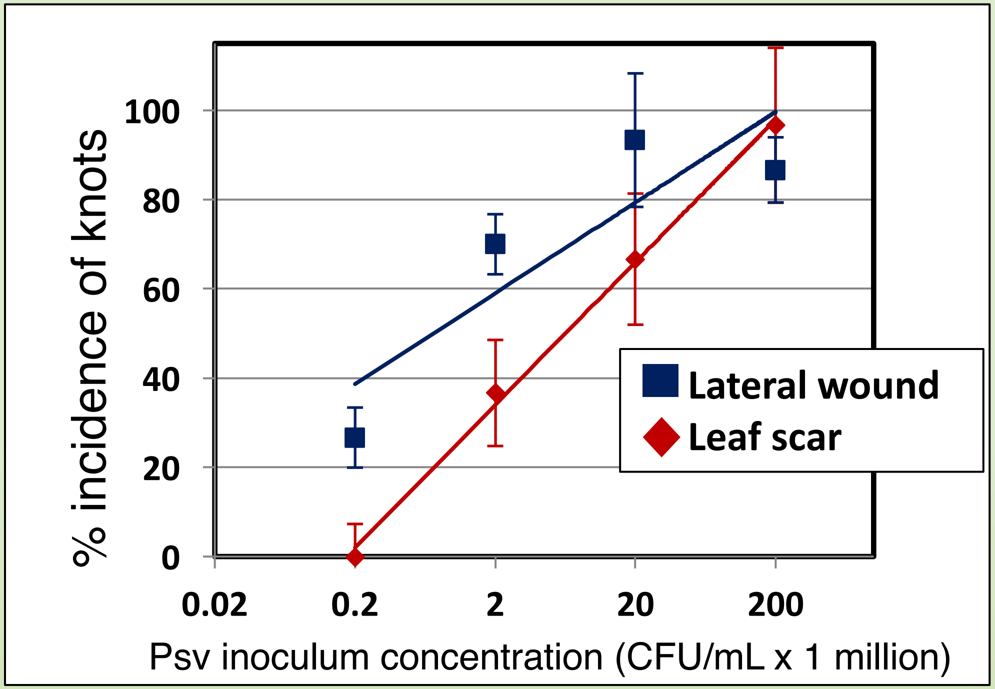
- 1- to 2-yr-old twigs were laterally injured with a sterile scalpel or leaves were pulled off to make leaf scars
- Bacterial inoculum was sprayed onto wounds
- Plants evaluated after 3-6 months

Healed lateral wound

Lateral wounds developing knots

Epidemiology: Effect of inoculum concentration on development of olive knots

- Leaf scars and lateral wounds were inoculated with Psv using selected inoculum concentrations
- Rating for incidence of knot development
- Regression of inoculum concentration on disease incidence
- Symptoms develop after **3-6 months** in the field



Conclusion:

Naturally occurring Psv concentrations can cause a high incidence of disease.

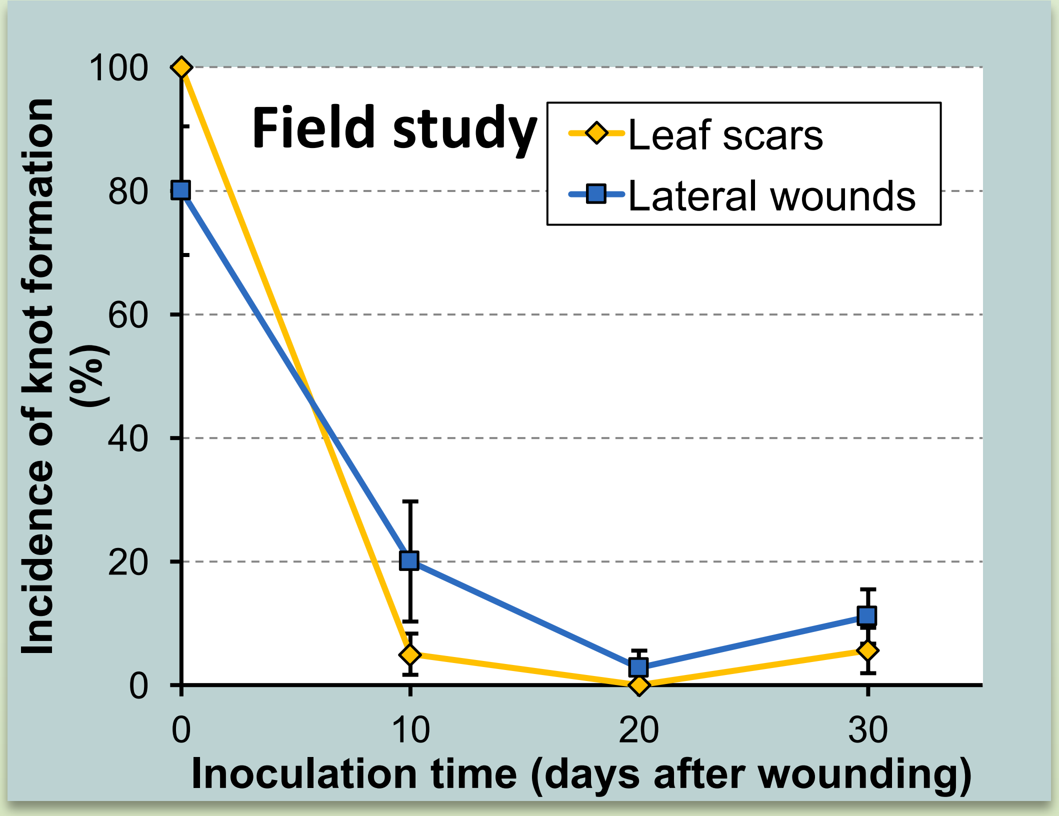
Various developmental stages of knots on wounds

Epidemiology

Wound healing
Leaf scars and lateral branch wounds

- Leaf scar wounds and lateral wounds were inoculated after 0 days, 1 week, 2 weeks, or 3 weeks.

Duration of susceptibility of injuries to infection

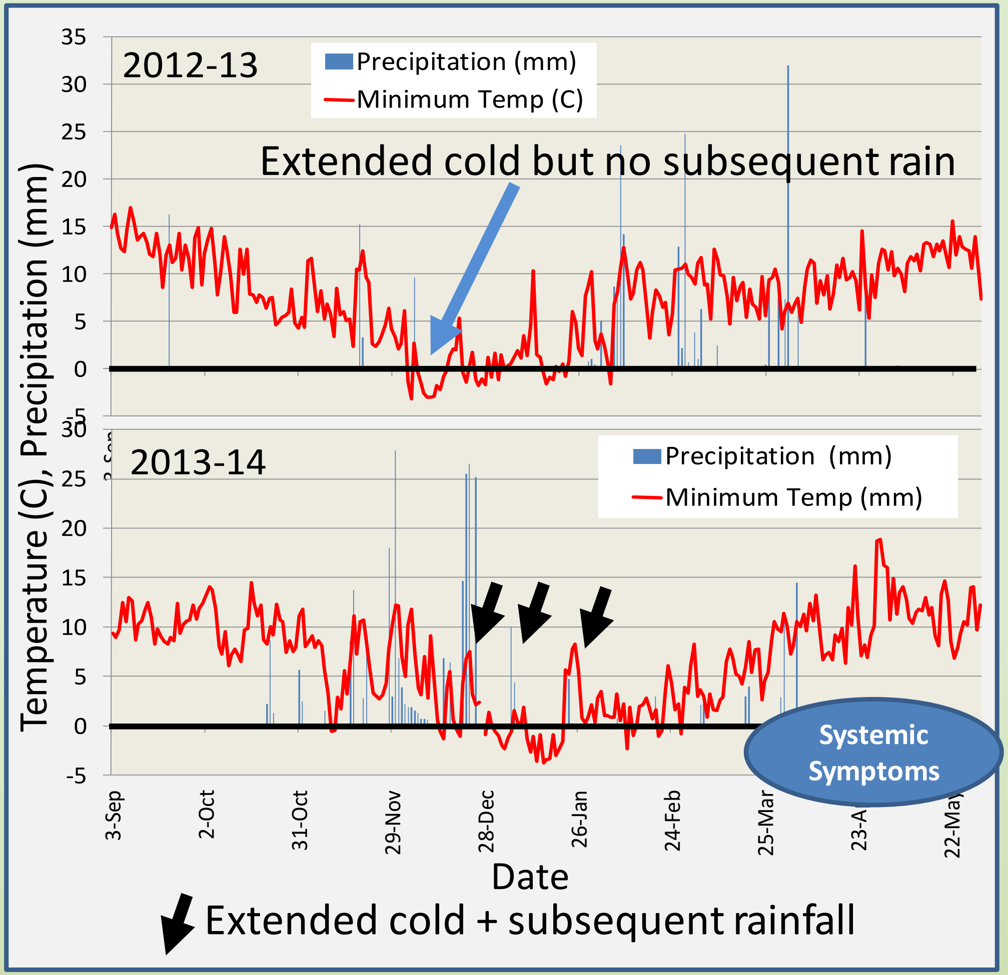


Studies	Leaf Scars	Lateral wounds
Greenhouse	10 days - >90% reduction	14 days - >90% reduction
Field	10 days - >90% reduction	10 days - 80% reduction 20 days - >90% reduction

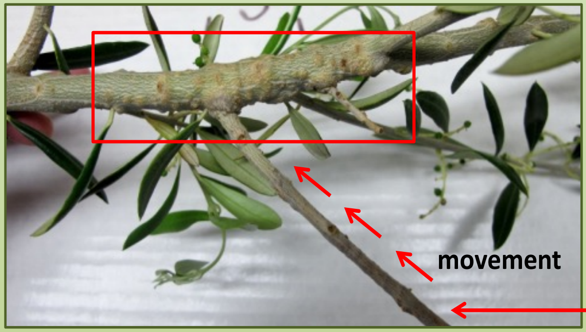
Age of the injury is a critical factor - Wound-healing occurs over time and is not affected by wetness.

Epidemiology - Localized knots vs. systemic infection

- Psv systemic movement rarely observed
- High incidence of systemic infection in field trials in the spring of 2014
- Weather data indicate periods of low temperatures (≤0°C) with intermittent rains
- Frost damage and subsequent wetness may have provided ideal conditions for Psv movement.



Typical *Psv* knots



Systemic infection

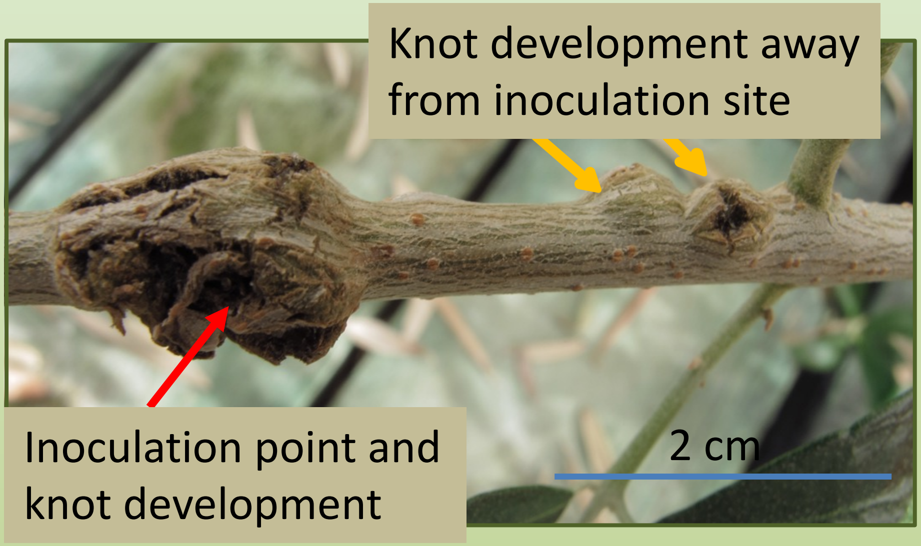
Inoculation point

Potential factors causing Psv systemic movement

Low-temperature growth chamber studies-

- Cv. Manzanillo and Arbequina olives wounded, inoculated with Psv, and exposed to -5°C
- Extensive defoliation and branch dieback
- Systemic movement (nodules) observed away from inoculation sites

Typical knot development at inoculation sites



Management of Olive knot

- **Cultural:**
  - Maintain tree vigor, reduce tree stress, reduce leaf drop
- **Sanitation:**
  - Pruning and removal of knots during dry periods (inoculum reduction)
  - Disinfection of pruning tools (Sodium hypochlorite)
- **Chemical applications to trees:**
  - Painting galls with Gallex
  - Spray applications with copper-containing bactericides to reduce inoculum and protect wounds

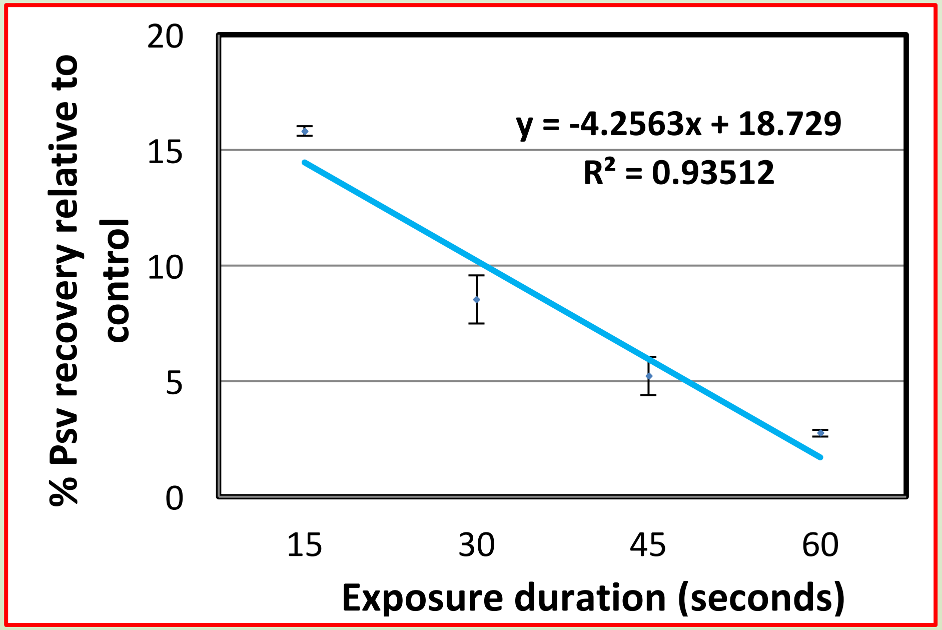
Management of olive knot: Sanitation of equipment

A new sanitizer - quaternary ammonium compound

Direct toxicity of Deccosan 321 against Psv

Direct Contact Assay

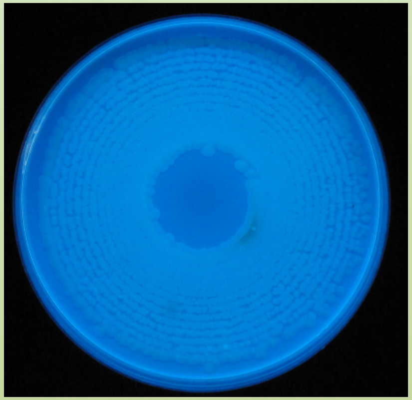
- Psv was exposed to fixed concentrations of QAC for selected time periods.
- Suspensions were diluted and plated.
- Enumeration of viable Psv



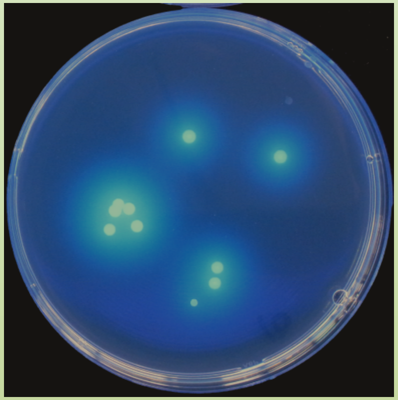
*Psv recovered 100% in the control, mean 1.6×10^5 CFU/mL

Results:

QACs are highly toxic to Psv at low concentrations and very short exposure durations.



Psv recovery in the untreated control

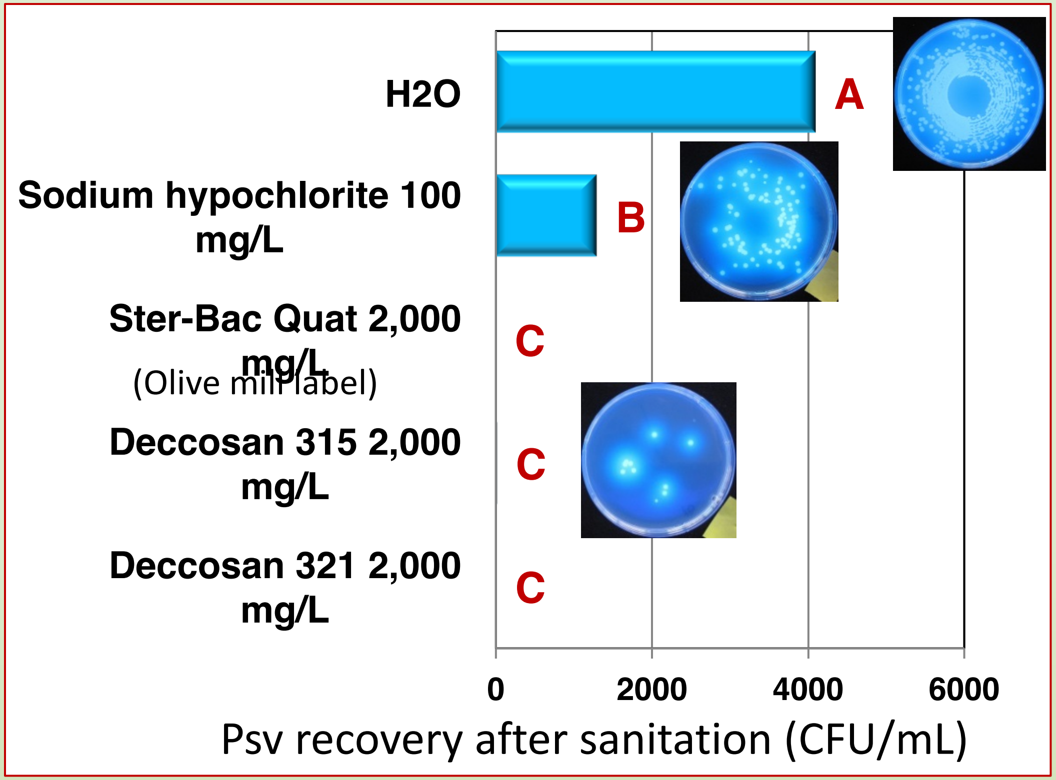


Psv recovery after QAC exposure

Performance of sanitizing agents for olive field equipment

Hard Surface Disinfection Assay

- Simulate olive harvester
- PVC piping contaminated with *macerated olive tissue + Psv*
- Pipes treated with QAC formulations for 90 seconds
- Pipes lightly rinsed with H_2O
- Macerate collected and plated for Psv enumeration

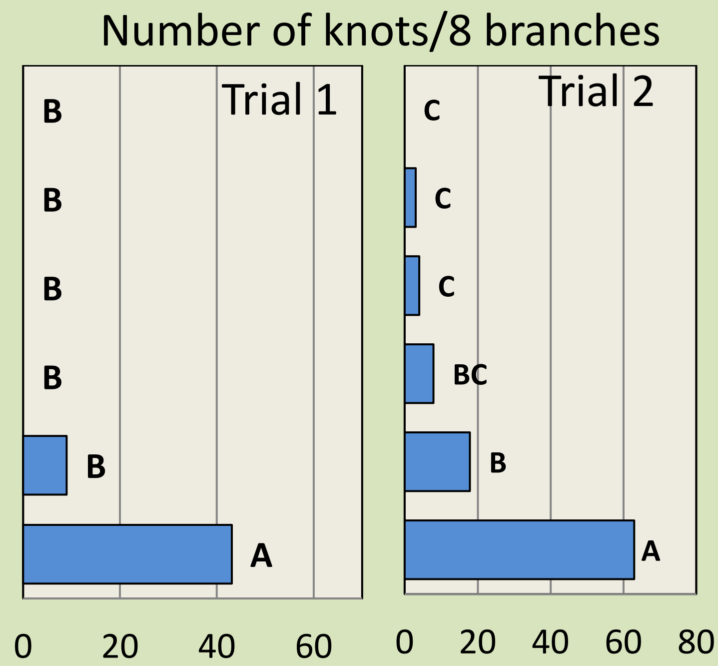


*Same letters are not significantly different based on LSD mean separation procedures ($p < 0.05$)

QACs highly effective - improved performance over sodium hypochlorite in disinfecting hard surfaces in the presence of organic matter.

Performance of Deccosan 321 as an equipment sanitizer under field conditions on cv. Arbequina

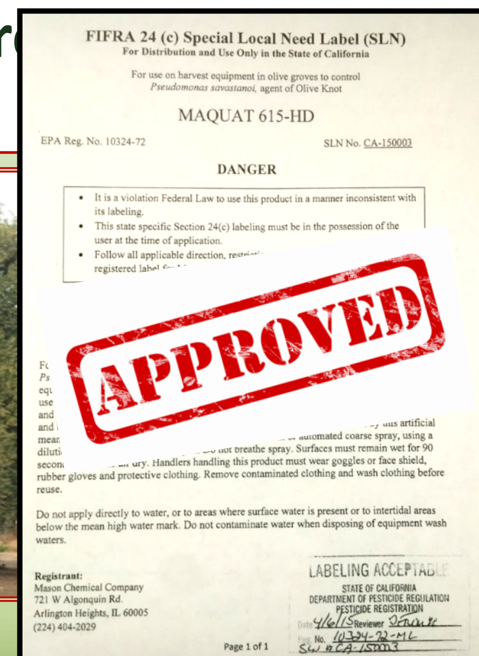
Sanitation treatment	Foliar application
Non-inoculated	-
Deccosan 321 (2000 ppm)	Kasumin + Kocide
Deccosan 321	Kocide
Deccosan 321	-
NaOCl (50 ppm)	-
Non-sanitized	-



Olive branches were pruned with a contaminated hedger (control) or sanitized with Deccosan 321 (2000 mg/L) or sodium hypochlorite (50 mg/L). Some branches were treated with an additional foliar application of Kocide 3000 (3.5 lb/A) or Kocide 3000 + Kasumin (100 mg/L). Disease evaluations were done after 6 months.

Summary: Equipment sanitation in the field

- **Mechanized olive production creates a high risk for olive knot.**
- **Quaternary ammonium compounds (QACs) are highly toxic to *Psv* at low concentrations and short exposure durations.**
- **QACs remain efficacious in the presence of organic load over a wide pH range (6-9).**
- **QACs are non-corrosive.**
- **Deccosan 321 (MaQuat 615-HD) was registere[d] … olives in early 2015.**



FIFRA 24 (c) Special Local Need Label (SLN)
For Distribution and Use Only in the State of California

For use on harvest equipment in olive groves to control
Pseudomonas savastanoi, agent of Olive Knot

MAQUAT 615-HD

EPA Reg. No. 10324-72 SLN No. CA-150003

DANGER

- It is a violation Federal Law to use this product in a manner inconsistent with its labeling.
- This state specific Section 24(c) labeling must be in the possession of the user at the time of application.

rubber gloves and protective clothing. Remove contaminated clothing and wash clothing before reuse.

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment wash waters.

Registrant:
Mason Chemical Company
721 W Algonquin Rd.
Arlington Heights, IL 60005
(224) 404-2029

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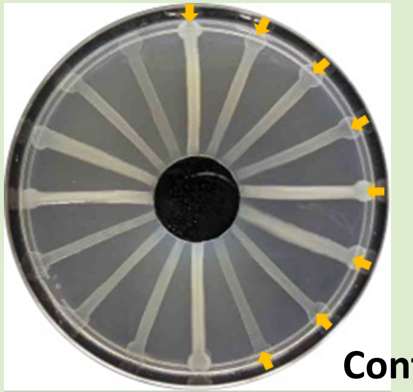
LABELING ACCEPTABLE
STATE OF CALIFORNIA
DEPARTMENT OF PESTICIDE REGULATION
PESTICIDE REGISTRATION
Date 4/6/15 Reviewer
Reg. No. 10324-72-ML
SLN # CA-150003

Management of olive knot:

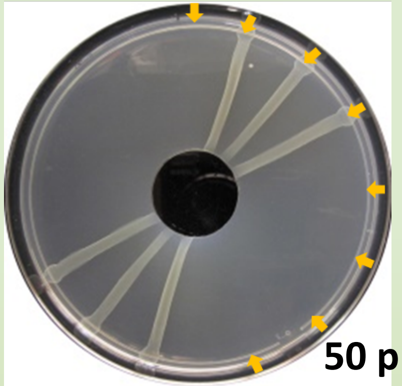
Field surveys on sensitivity of Psv to copper and antibiotics

Copper Sensitivity of *Psv* in California

- ✓ Surveyed orchards in Butte, Colusa, Glenn, Tehama, and Sutter/Yuba Co. from 2012 to 2015.
- ✓ Most strains copper-sensitive (MIC <20 ppm Cu)
- ✓ Copper-resistant strains were recovered in an orchard where copper has been used for >100 years
- ✓ Thus, copper-resistant strains are present at low incidence and are residing in the population



Control



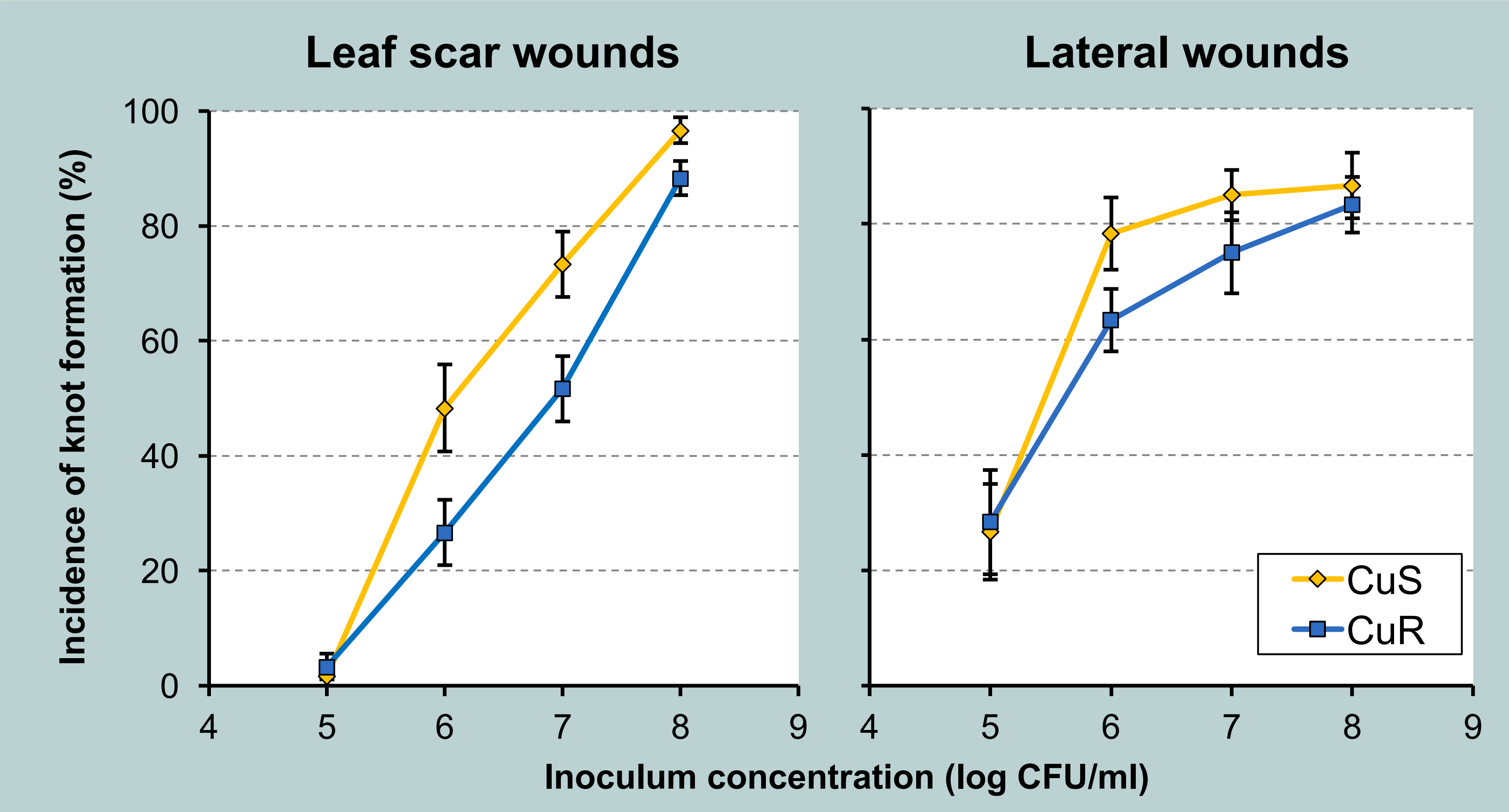
50 ppm MCE

Copper sensitivity*	Number of isolates	% of total
Sensitive	140	95.3
Moderate	4	2.7
Resistant	3	2.0

Total of 147 strains
Sensitive: growth ≤20 mg/L Cu
Moderate: growth 20-50 mg/L Cu
Resistant: growth ≥50 mg/L Cu

* - Relative to labeled rates of registered copper products.

Virulence of CuS and CuR strains of *Psv*



The CuS and CuR strains were similarly virulent on both types of injuries.

Management of olive knot:

New bactericides and optimization of efficacy

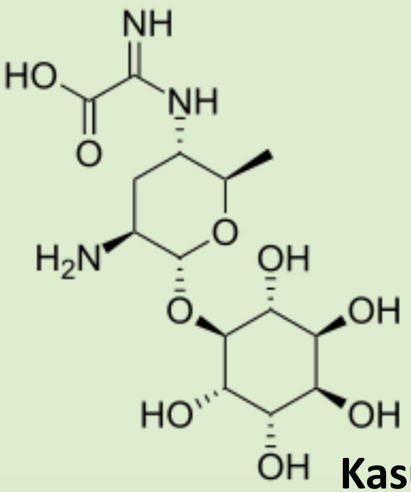
Kasugamycin and Oxytetracycline

Kasumin (kasugamycin)

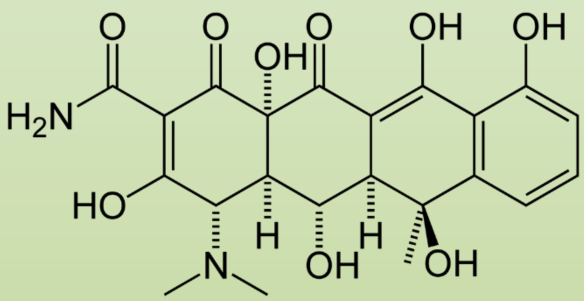
- Produced by *Streptomyces kasugaensis*
- First discovered in the 1960s
- Antifungal and antibacterial activity
- Different mode of action from other antibiotics
- Registered on crops in Asia, Europe, & Central America
- US-EPA import tolerance
- US-EPA registration on pome fruits

Mycoshield (oxytetracycline)

- Registered for the management of fire blight and bacterial spot of pome and stone fruits, respectively



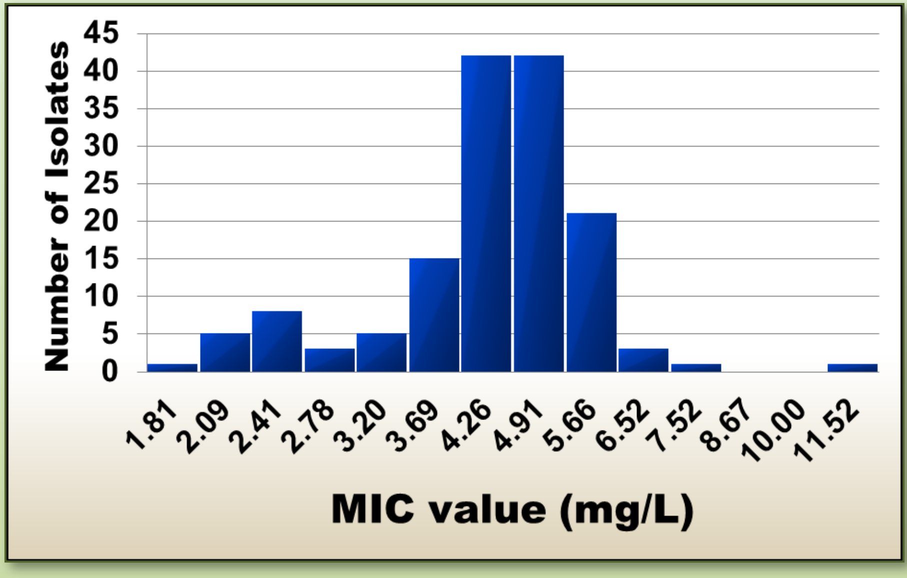
Kasugamycin – an aminoglycoside antibiotic



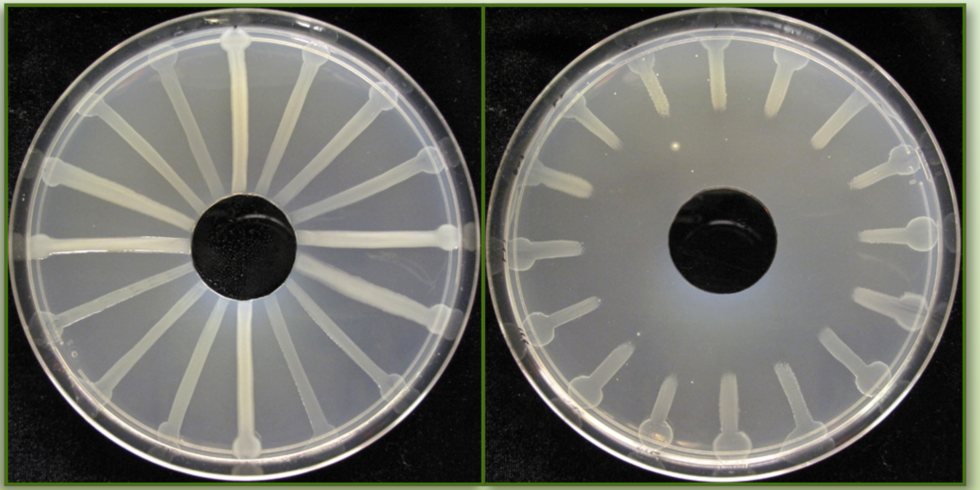
Oxytetracycline – a tetracycline antibiotic

Antibiotic registration

- ✓ Kasumin registered for use on pome fruits in 2014/2018 CA
- ✓ Kasugamycin given "A" priority for olives in IR-4 program
- ✓ Continuing field trials and comparisons with oxytetracycline
- ✓ Oxytetracycline also in the IR-4 program since 2015



Scott's distribution of minimal inhibitory values of 147 Psv isolates to kasugamycin



Control **Kasugamycin**

SGE plates

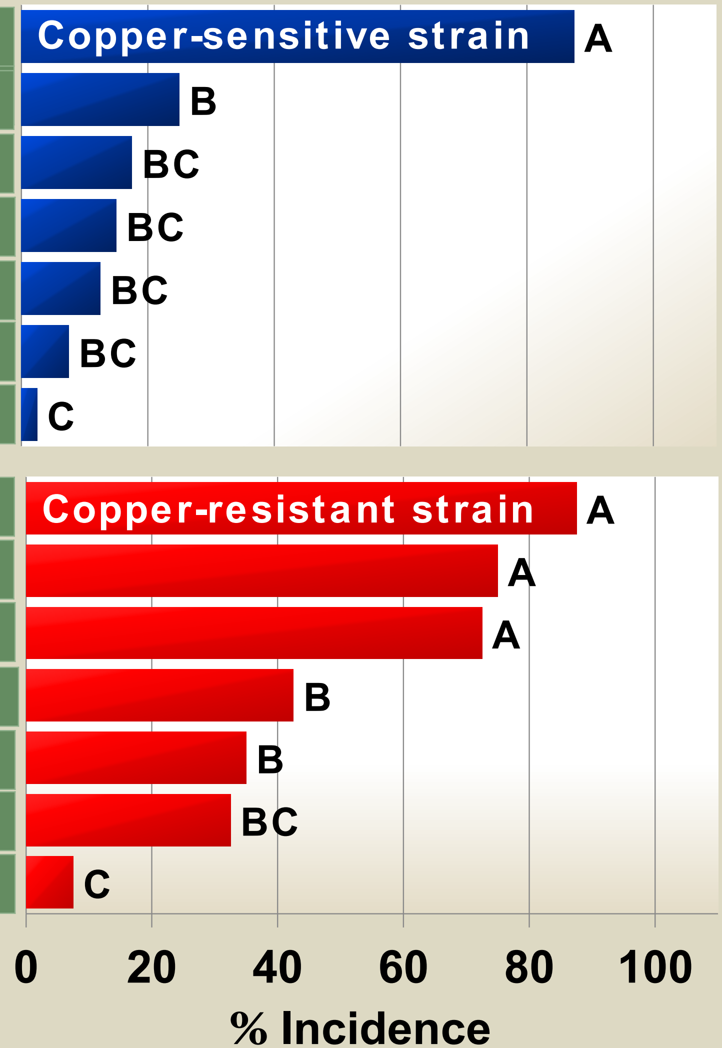
✓All strains sensitive to kasugamycin and oxytetracycline.

Efficacy of antibiotics and copper for managing olive knot

Protection of lateral wounds

Control	---
Kasugamycin	200
$Cu(OH)_2 \cdot CuClH_2O$*	1,176
$Cu(OH)_2$	1,260
Oxytetracycline	100
Streptomycin	200
Kasugam. + $Cu(OH)_2$	200 + 1,260

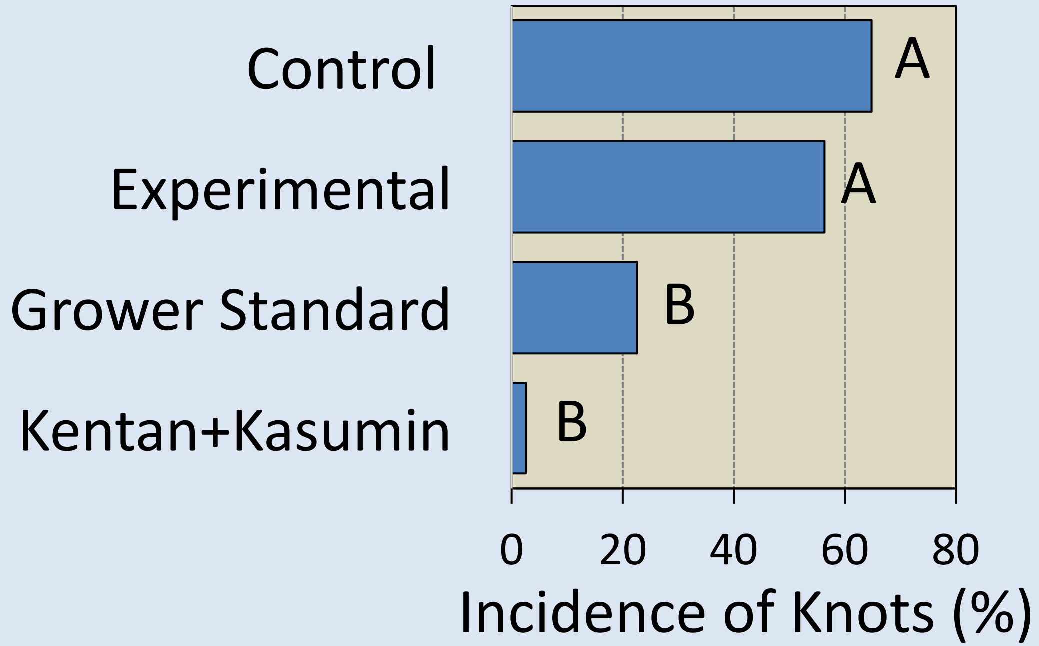
Control	---
$Cu(OH)_2 \cdot CuClH_2O$	1,176
$Cu(OH)_2$	1,260
Kasugamycin + $Cu(OH)_2$	200 + 1,260
Streptomycin	200
Kasugamycin	200
Oxytetracycline	100



*- Copper treatments: Badge X2 or Kocide 3000 were used. Rates based on metallic copper equivalent (MCE).

Evaluation of commercial treatments for the management of olive knot in field studies

Post-inoculation activity – lateral wounds



<u>Grower standard:</u>
4 lb Kocide 3000
2.5 lb zinc sulfate
4 lb lime/50 gal

Time-table:

- Mid-Nov. 2012: wounded, inoculated, treated
- June 2013: Evaluated

All treatments applied using a commercial air-blast sprayer calculated to 70 gal/A. Copper/Kasumin was highly effective in reducing the incidence of knot development after inoculation.

Management of olive knot on natural leaf scars using new bactericides

Location	Treatment	Product Rate/A	% Incidence of knots on natural leaf scar wounds*	LSD
UC Davis	Untreated	---	39.4	a
	ChampION	3.5 lbs	0.0	b
	Kasumin	200 ppm	3.8	b
	ChampION + Kasumin	3.5 lbs + 200 ppm	0.0	b
Commercial	Untreated	---	31.1	a
orchard	ChampION	3.5 lbs	3.0	b
	Kasumin	200 ppm	0.0	b
	ChampION + Kasumin	3.5 lbs + 200 ppm	0.0	b

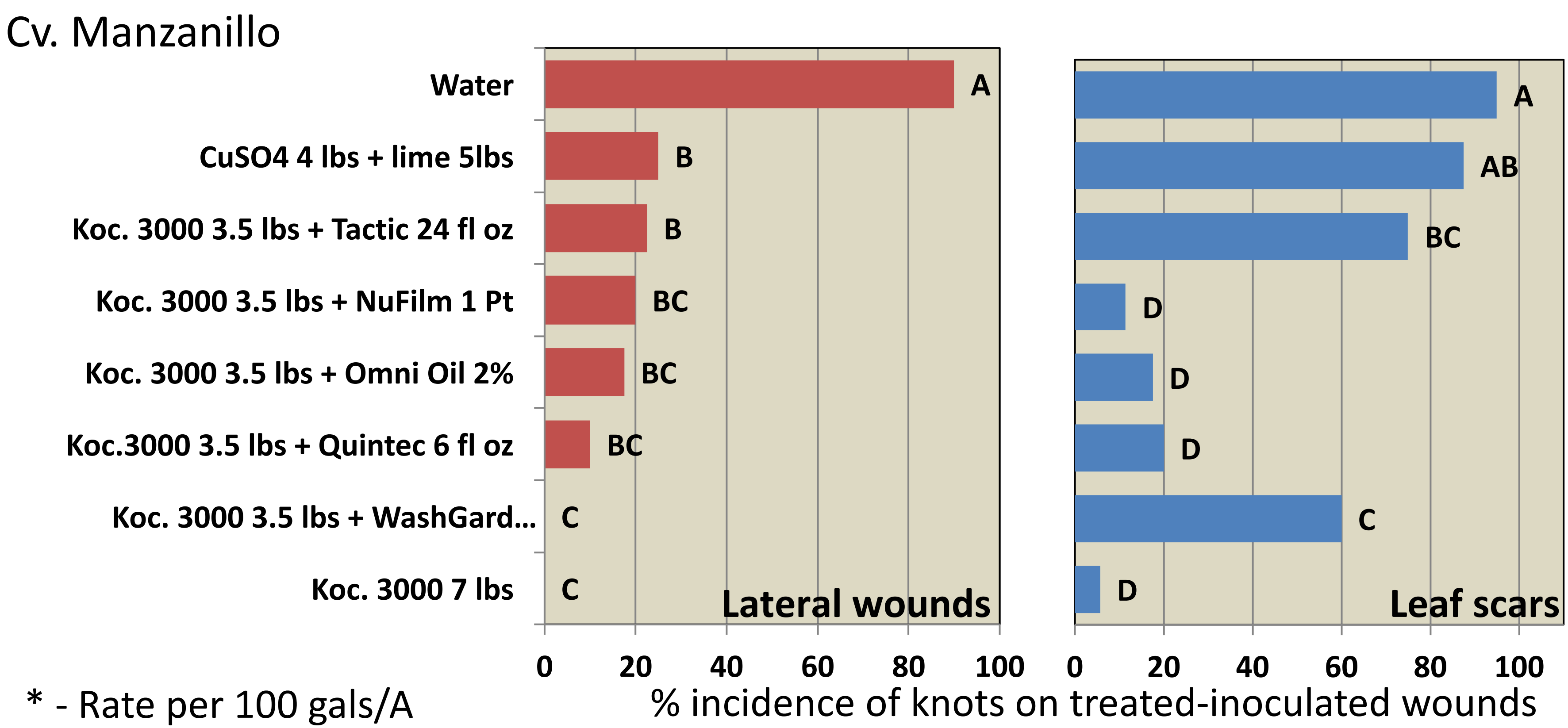
*- Incidence of knots occurring on natural leaf scar wounds made by removing yellow-dying leaves and inoculating the leaf scar after treatment. Experiments done during natural leaf drop in the spring.

Management of olive knot

Persistence of copper-antibiotic mixtures after a rain event using stickers and oils vs. hydrated lime.

Copper persistence in field studies

- ✓ Copper persistence after a 30-min simulated rain event
- ✓ Copper at highest rate (7 lb/A) was the best treatment for both leaf scar and lateral wounds – highest persistence
- ✓ Addition of selected adjuvants (NuFilm, Omni oil, Quintec) improved control of olive knot on leaf scars



* - Rate per 100 gals/A

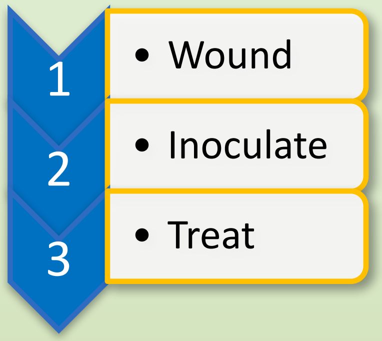
Management of olive knot:

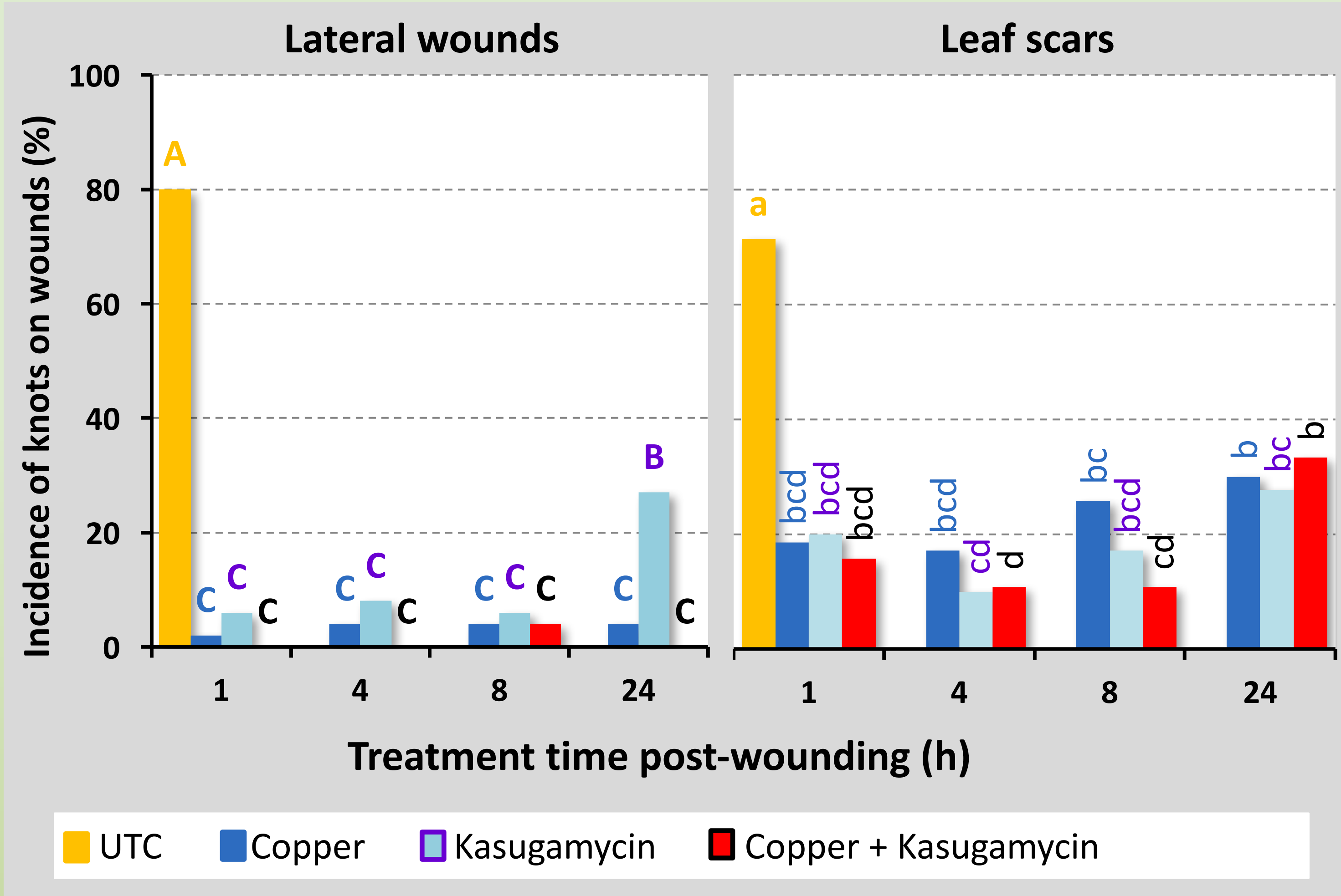
Timing of bactericide applications relative to occurrence of injuries (Post-infection activity)

Branches were inoculated and treated after 0, 1, 2, 3, or 7 days with Kasumin or Kocide 3000.

Timing of copper and kasugamycin treatments after wounding

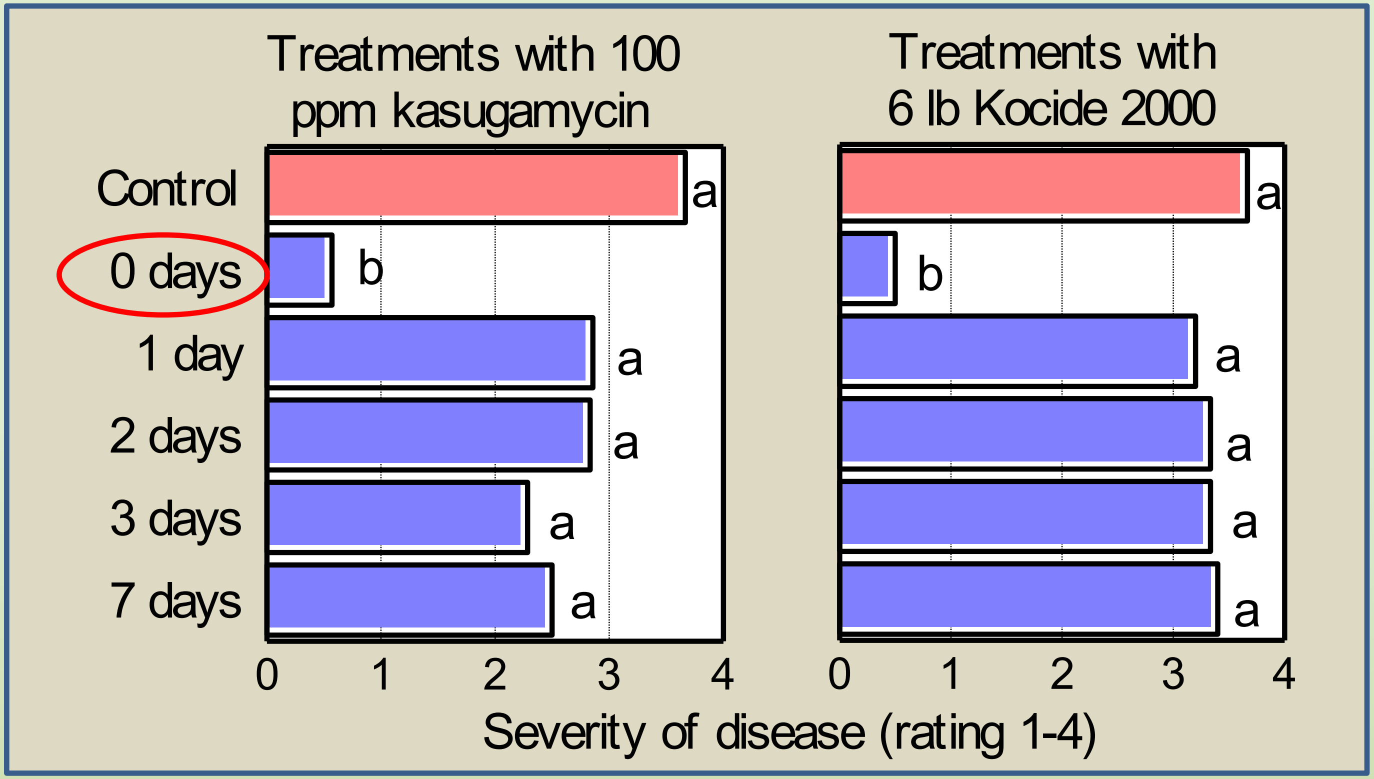
- Cu-S strain
- Arbequina olives





Reduced performance for treatments applied 24 h after wound inoculation

Timing of bactericide applications relative to occurrence of injuries



Treatments are only effective when applied within one day of injury (e.g., pruning, harvest)!

Highlights - 1

Epidemiology

- Injuries needed for entry of Psv – harvesting, pruning, leaf drop, cold.
- Inoculum levels determine incidence and of knot development.
- Wound healing –Wounds heal within 10 to 20 days after injury under field conditions.
- Systemic infection of the tree by Psv may occur after cold injury.

Highlights - 2

Management of olive knot by equipment sanitation in the field

- Quaternary ammonium compounds (QACs) are highly toxic to *Psv* at low concentrations and short exposure durations.
- QACs remain efficacious in the presence of organic load.
- QACs are non-corrosive.
- Effective QAC sanitation of equipment was demonstrated.
- Deccosan 321 (MaQuat 615-HD) was registered for field use on CA olives in early 2015.

Highlights - 3

Management of olive knot with field applications of chemicals

- Psv populations in California are mostly copper-sensitive.
- Copper applications (high rates) are highly effective when properly timed.
- Selected adjuvants improve copper efficacy and persistence while using lower rates of copper.
- New alternatives to copper are being developed to minimize the spread of copper-resistance.
  - Rotation of different modes of action (MOA)
  - Mixtures of different MOA to improve performance

Highlights - 4

Management of olive knot with field applications of chemicals -

Copper alternatives:

- **Oxytetracycline** - accepted into IR-4 program Sept 2015.
- **Kasugamycin** accepted in 2014 – residue field studies done in 2015.

Application of any chemical (copper or antibiotics) has to be done within 24 h of occurrence of injuries (pruning, harvest, cold) or within a week prior to cold injury and rain.

Questions?

Thank you for your support!