Evaluation of new fungicides for control of olive leaf spot (peacock spot) *and* Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi*

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Current Limits of Copper Usage For Managing Olive Diseases

Max. single application rate
6.0 lbs Cu^{2+}/A

Max. annual application rate
18.0 lbs Cu^{2+}/A

The US EPA is reviewing further reductions in copper usage on many crops.

Symptoms of Peacock Spot

Fusicladium oleagineum (syn. *Spilocea oleaginea, Venturia oleaginea*)

Overview of peacock spot project

1. **Peacock spot is an ongoing fungal disease problem and can be serious in some locations**
   a. Infection occurs in the fall resulting in defoliation
   b. Optimum conditions 58-75F, with 14-48 h of wetness for spore germination and infection to occur depending on temperature
   c. Reduces bloom and crop production

2. **Management**
   a. Fall or Fall and spring application timings if conditions are conducive.
   b. Only copper is currently registered

Objectives of Peacock Spot Project

1. **Evaluate the performance of new and older fungicides in field trials.**
   a. Multisite MOA fungicides - Dithiocarbamates (ziram) and chlorinated hydrocarbons (chlorothalonil), (FRAC Codes – M3, M5)
   b. Single-site MOA fungicides - DMIs (FC 3), polyoxins (FC 19), or mixtures such as FC 3/9, and FG 3 + 19.

2. **Evaluate application timing and adjuvants of selected treatments.**
   a. Fall, spring, or fall and spring.
   b. Adjuvants: NuFilm-17, capric/caprylic acids, oil

3. **Evaluate new fungicides for their in vitro activity.**
   Determine the in vitro activity of selected fungicides that are effective in field trials.

Efficacy of fungicide treatments for management of peacock spot of Arbequina olive, Yuba Co. 2018-19

No.	Treatments*	Product rate/A	Applications		No. leaves diseased**	LSD^
			10-18-18	1-17-19		
1	Control	---	---	---	28.3	a
2	Champ	128 oz	@	@	14.5	b
3	Syllit	48 oz	@	@	11.3	bc
4	Ziram	128 oz	@	@	11.0	bcd
5	Inspire Super	20 fl oz	@	@	8.5	cd
6	Bravo	64 fl oz	@	@	7.3	cd
7	Ph-D***	6.2 oz	@	@	6.8	d

* - Treatments were applied using an air-blast sprayer at 100 gal/A.
Treatments on 1-17-19 were applied with NuFilm-P (8 fl oz).

**- Disease was evaluated on 5-1-2019. for this, the number of leaves with typical disease symptoms was counted on each tree.

*** - Polyoxin-D is a biofungicide and exempt from tolerance. Efficacy data is only needed for registration (no GLP residue studies required). Label for Ph-D is being amended for 2020.

The IR-4 Program

- The purpose of the IR-4 program is to enable the crop protection industry to provide safe, effective, and economical crop protection products for growers and consumers of minor/specialty crops.
- The crop protection industry cannot justify the costs associated with the research and development, registration, production, and marketing of crop protection products for minor/specialty crops due to the smaller market base and limited sales potential.
- The IR-4 program ensures that new and more effective crop protection products are available to minor/specialty crop producers. These efforts require effective collaborations among federal agencies, the crop protection industry, and land-grant colleges and universities.

The IR-4 Program

Steps involved:

1) Nomination of fungicides for managing a fungal disease
    a) Requires chemical industry support (2018)
    b) Requires agricultural commodity support (2018)
2) Acceptance at the Food Use Workshop – Efficacy data (2017 to 2019)
3) Protocol Development and QA assignments (2019)
4) Steps:
    a) Year 1 – GLP Field Trials for Pesticide Residues (Fall 2019)
    b) Year 2 – GLP Lab Analysis – Residues, Decline Studies (Fall 2020?)
    c) Year 3 – Pesticide Petition Report (2021?)
        i. Field and lab data
        ii. QA reports
    d) Year 4 – EPA Review (2022/23?)
    e) Year 5 – California EPA Review (2024?)

Summary of new fungicides accepted into the IR-4 Program at the Food Use Workshop in Sept. 2018/ongoing in 2019

Fungicide	Active Incredient(s)	FRAC	Registrant	IR-4/EPA	Approved for Residue Trials
Ziram	ziram	M3	Supported	Supported	In 2018/19 season
Inspire	difenoconazole	3	Not supported	Supported	
Inspire Super	difenoconazole + cyprodinil	3/9	Supported	Supported	In 2018/19 season
Topsin-M	thiophanate-methyl	1	Supported	Not supported	

- Ongoing IR-4 project for Ziram and Inspire Super based on the after-harvest and winter season usage with expected zero to limit-of-detection residues on the crop in the following harvest season.
- Ziram is a FRAC Code M3; Inspire Super is a FRAC Code 3/9. Integration is an effective anti-resistance strategy for disease control.
- These fungicides are also highly effective against newly described *Neofabraea* and *Phlyctema* diseases of olive in California.
- Topsin-M was not accepted due to a low probability of registration because of the EPA RED concerning its human safety and potential for resistance in the *F. oleaginum*

Development of New Fungicides for Managing Peacock Spot of Olive

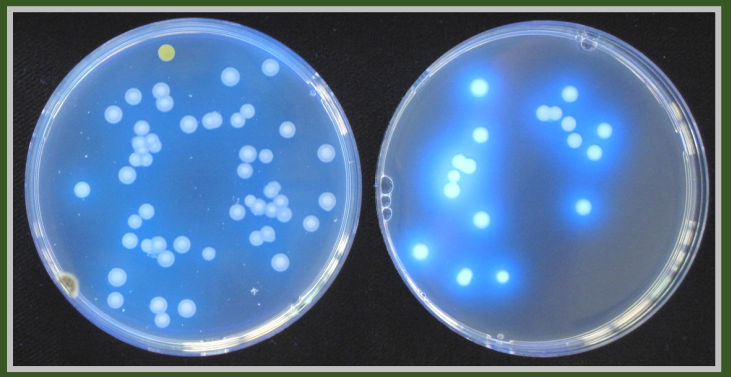
- Ziram – Conventional Registration (IR-4)
  - A DMDC fungicide with long residual activity.
- Inspire Super – Conventional Registration (IR-4)
  - A mixture of difenoconazole and cyprodinil that has strong affinity to plant leaf waxes.

- Ph-D – Biopesticide
  - A polyoxin fungicide with long residual activity.
  - Exempt from tolerance
  - Efficacy data will allow adding olive to the label.

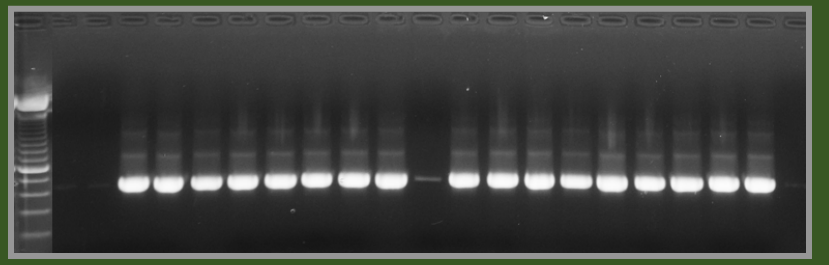
- **Timing will be the same as copper (Fall, Fall and Spring).**
- **Rotations will allow for resistance management.**
- **Less dependency on copper.**

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

Olive Knot - Pseudomonas savastanoi pv. *savastanoi*



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



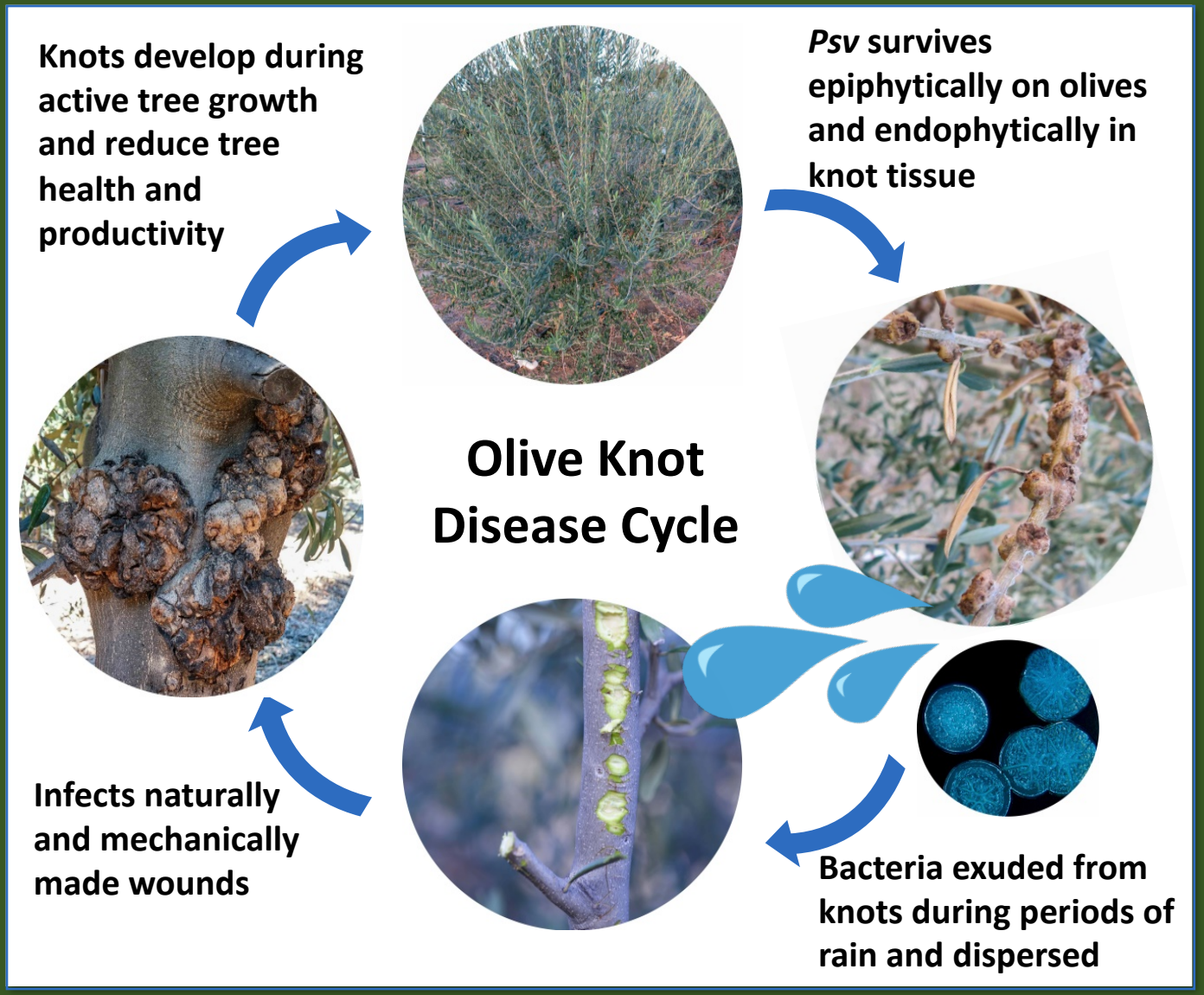
Specific amplification of *Psv*

- ✓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



Objectives of Olive Knot Project

1. **Evaluate new bactericides: Antibiotics, GRAS food additives, sanitizers, and other experimentals against Psv**
   a) In-vitro sensitivity of *Psv* and efficacy against olive knot:
      - Food additives: nisin, Ɛ-poly-L-lysine, and others
      - Sanitizers: lactic and citric acid, capric/caprylic acids (DART)

2. **Support the registration of kasugamycin & oxytetracycline –**
   a) Support registration efforts for kasugamycin and oxytetracycline
      - Kasugamycin - repeated GLP field and lab studies in 2019

   b) Improve performance of oxytetracycline
      - UV blockers and stabilizers and EPA policy.

Registration update for antibiotics

Kasumin (kasugamycin) received California registration on apple/pear, cherry, and walnut in 2018; olive pending.

- GLP residue field and analytical studies completed
- The rate is 64 fl oz/100 gal/A - ground application.
- The maximum number of applications is two-four per season with up to two sequential applications before rotation to other modes of action. Re-entry time is 12-h

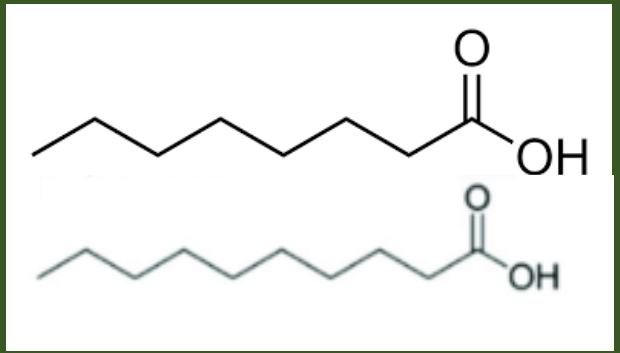
Oxytetracycline registration on apple and pear, olive pending.

- GLP residue field and analytical studies completed
- EPA review is pending.

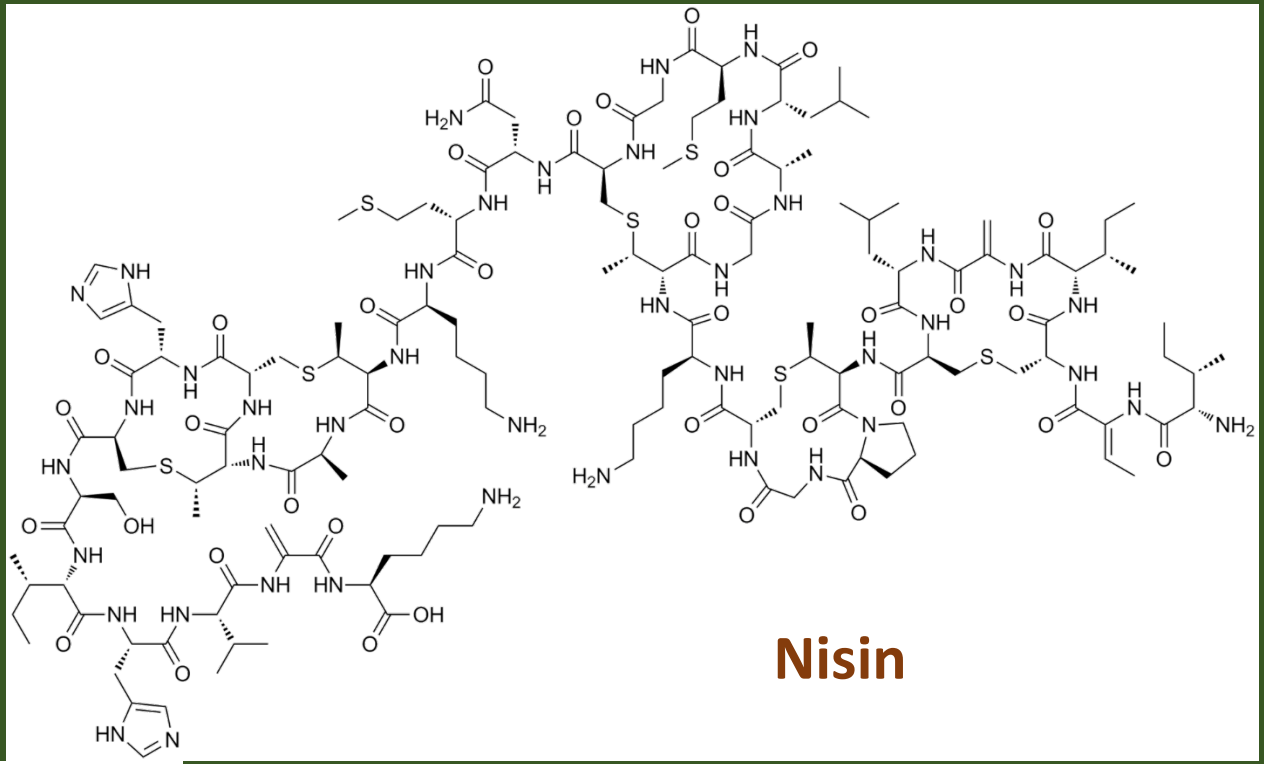
Kasugamycin – a new bactericide

- Different **mode of action** compared to other antibiotics (FRAC 24)
- **No human or animal uses**
- **Breaks down** to near zero levels within 30 days
- **No worker safety issues** - virtually non-toxic to mammals ('Caution' rating)
- **No concern for promoting resistance** in human/animal bacterial pathogens with plant use (field monitoring studies were done)

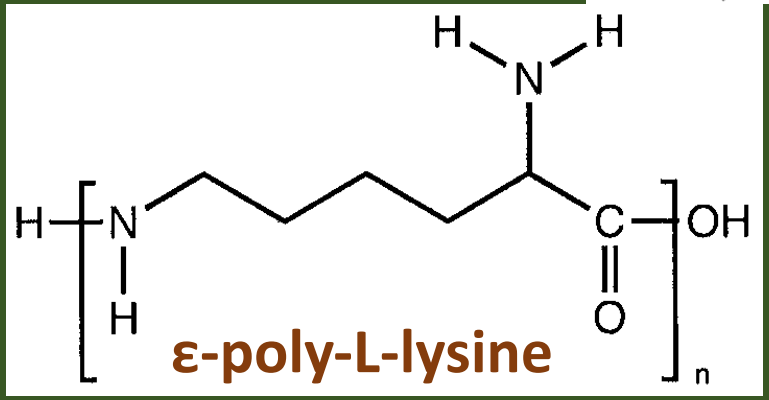
Food preservatives as new bactericides for fire blight blight management



Capric & Caprylic acid (Commercial registration as Dart)



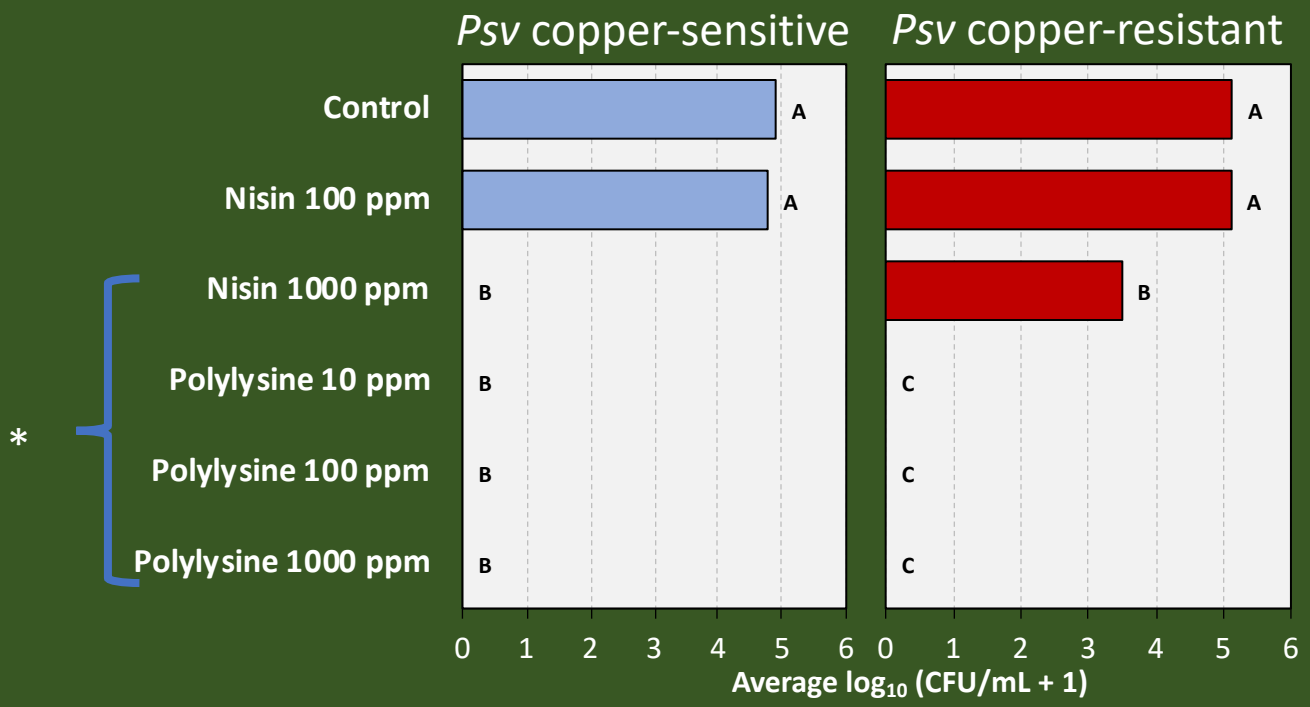
Nisin



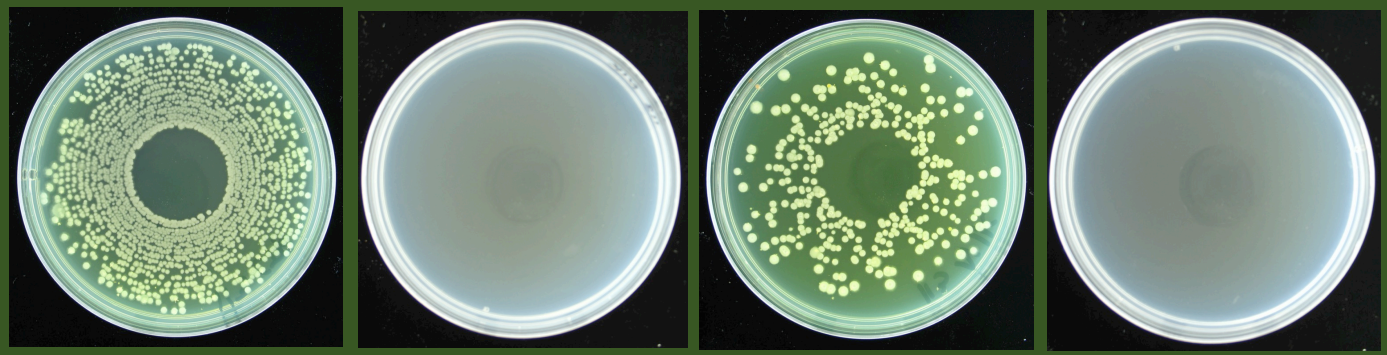
ε-poly-L-lysine

Nisin and ε-poly-L-lysine: Very good efficacy in 2018/19, an enhancer of activity was evaluated in the field in 2019 with promising results.

In-vitro toxicity of selected antimicrobials against *Pseudomonas savastanoi* pv. *savastanoi*



Toxicity was tested using a direct exposure assay. Bacterial suspensions were exposed to the antimicrobials for 60 s and then plated onto agar media.

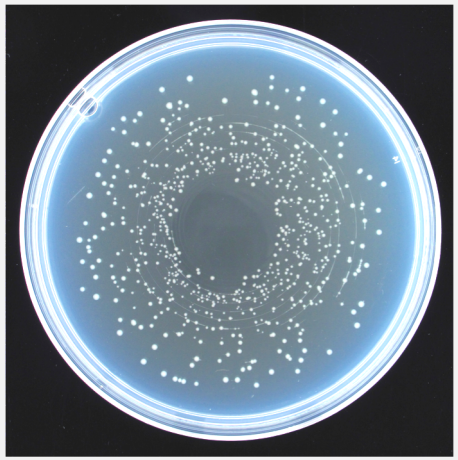


Control
Cu-R

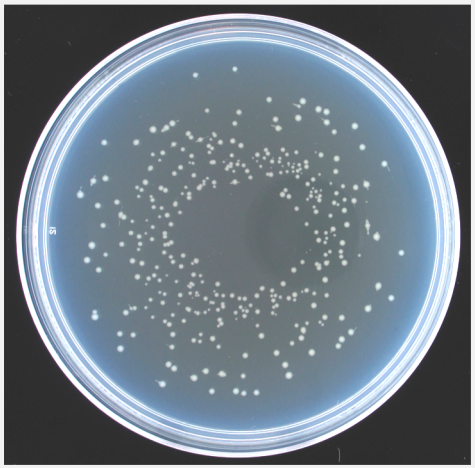
Nisin 1000 ppm
* Cu-S
Cu-R

* Ɛ-poly-L-lysine
10 ppm
Cu-S and -R

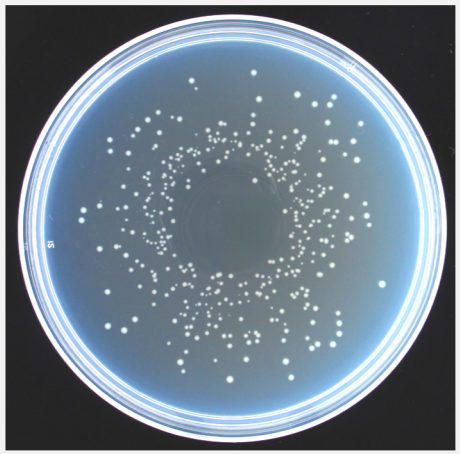
Optimizing the activity of nisin and poly-L-lysine in direct exposure assays in the laboratory



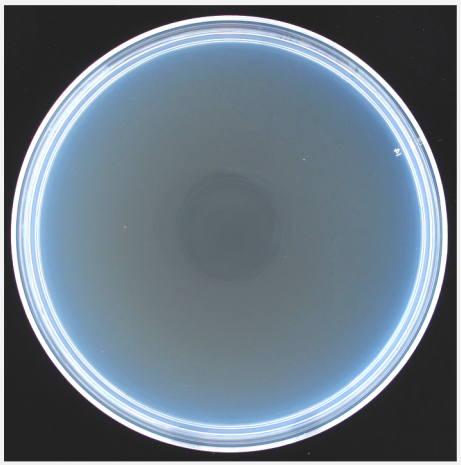
Control



EDTA 500 ppm



Nisin 500 ppm



Nisin 500 ppm + EDTA 500 ppm

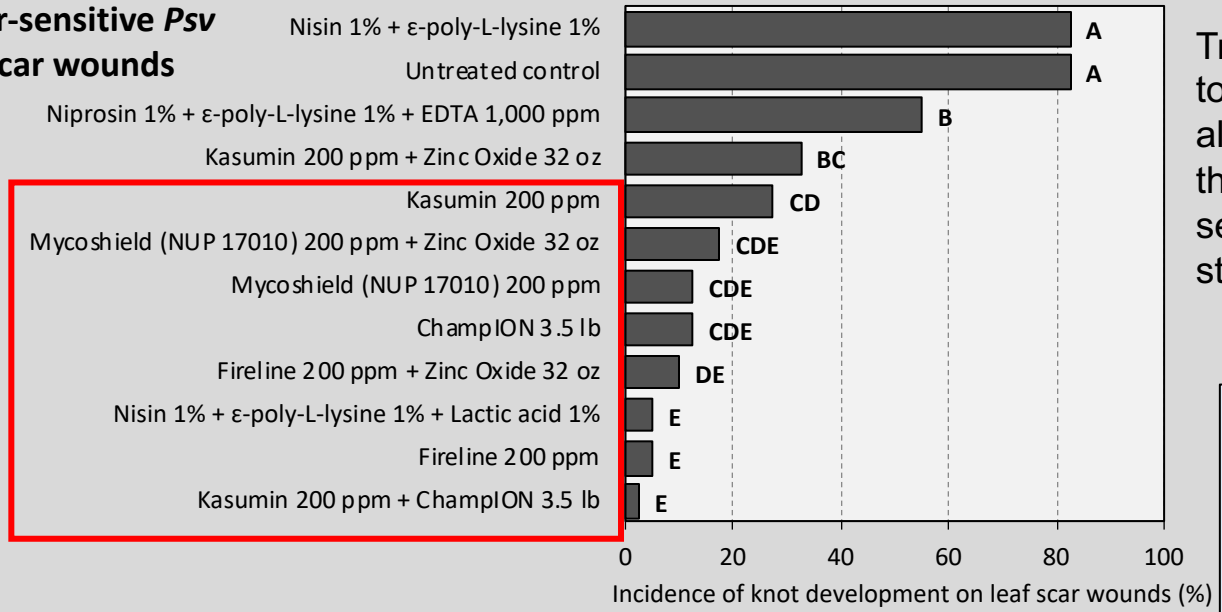
Apparently a synergistic interaction of nisin and EDTA with high activity against Cu-Sensitive and -Resistant Strains.

Field tests in 2019:

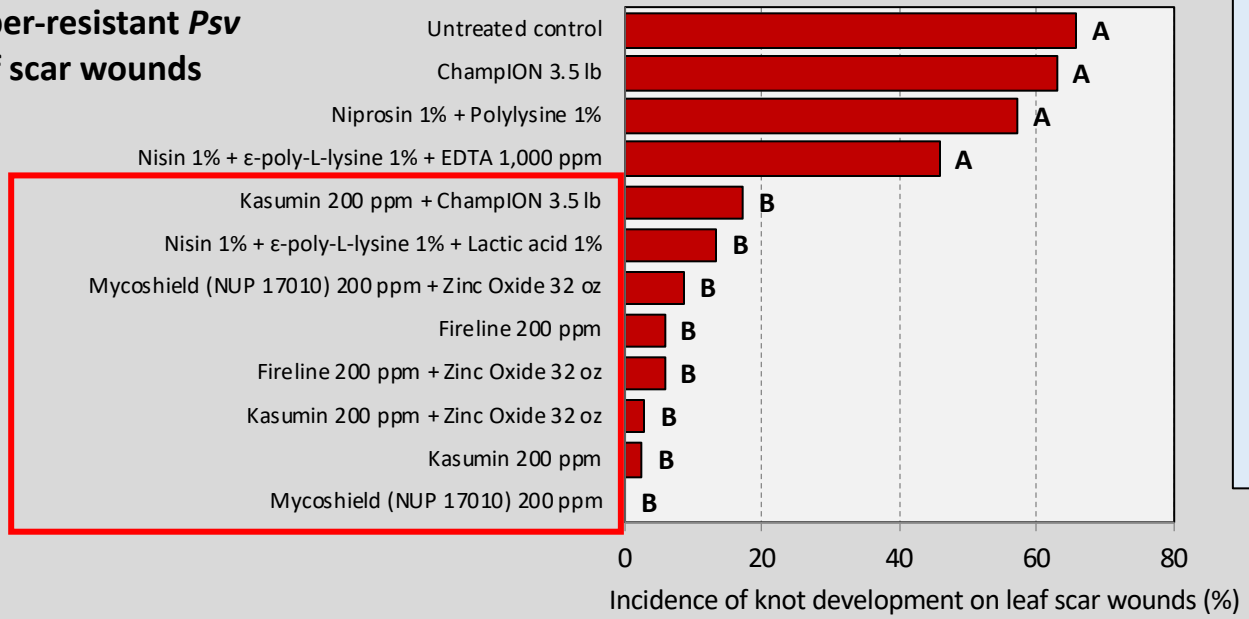
- Nisin
- Nisin+EDTA (synergy)
- Nisin+EDTA+ZnO (prevent UV degrade)
- Nisin+EDTA+ZnNO3 (improve penetration)
- Nisin+EDTA+ZnO+ZnNO3 (all of the above)

Efficacy of experimental bactericides against olive knot

Copper-sensitive *Psv* - leaf scar wounds



Copper-resistant *Psv* - leaf scar wounds

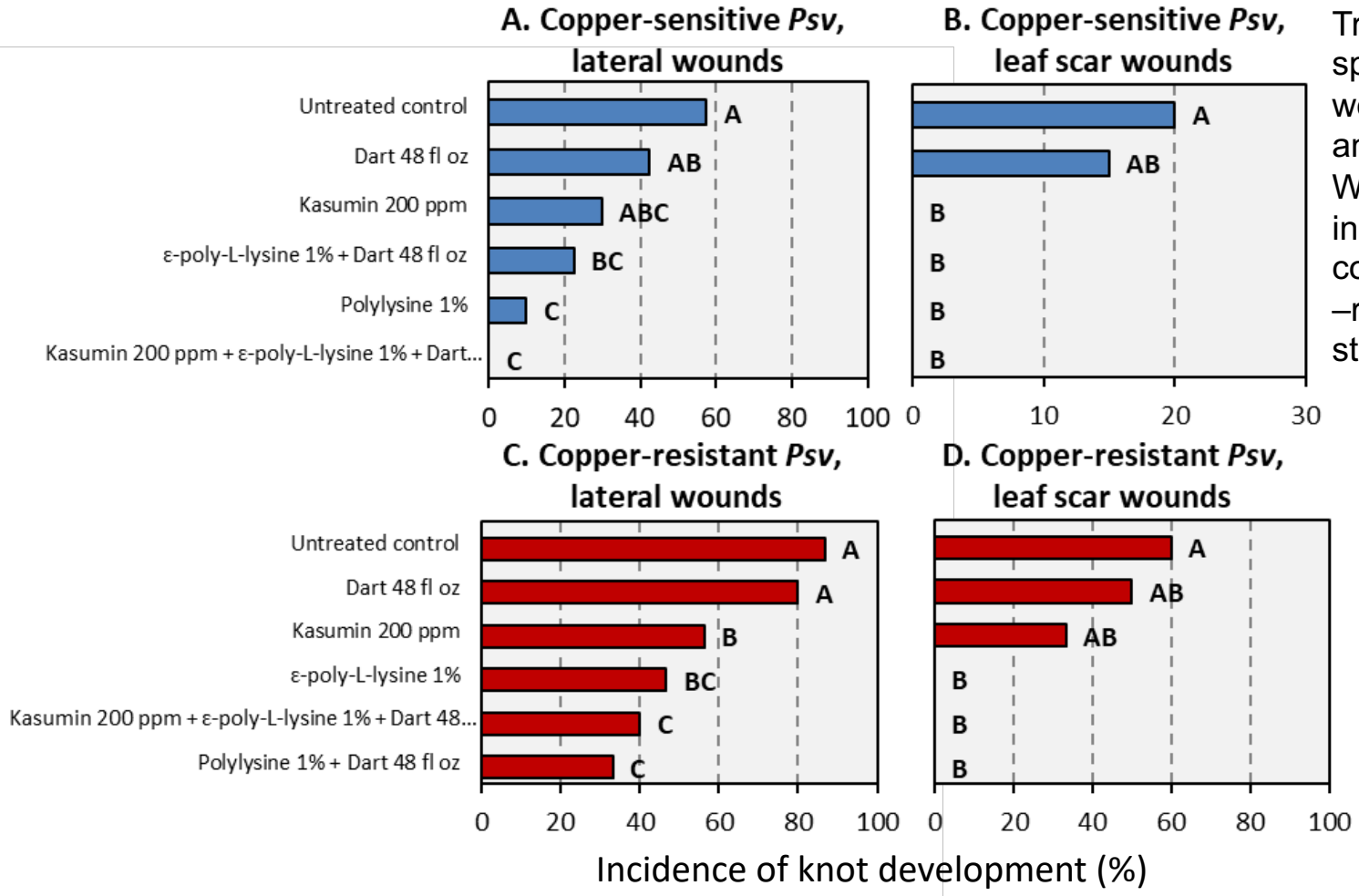


Treatments were spray-applied to wounds until runoff and allowed to dry. Wounds were then inoculated with a copper-sensitive or –resistant *Psv* strain.

Summary

- Oxytetracycline performance was similar to Kasumin on artificial leaf scar wounds.
- Nisin-ε-poly-L-lysine mixtures performed well.
- Copper - kasugamycin mixtures performed well using both strains.

Efficacy of experimental bactericides against olive knot



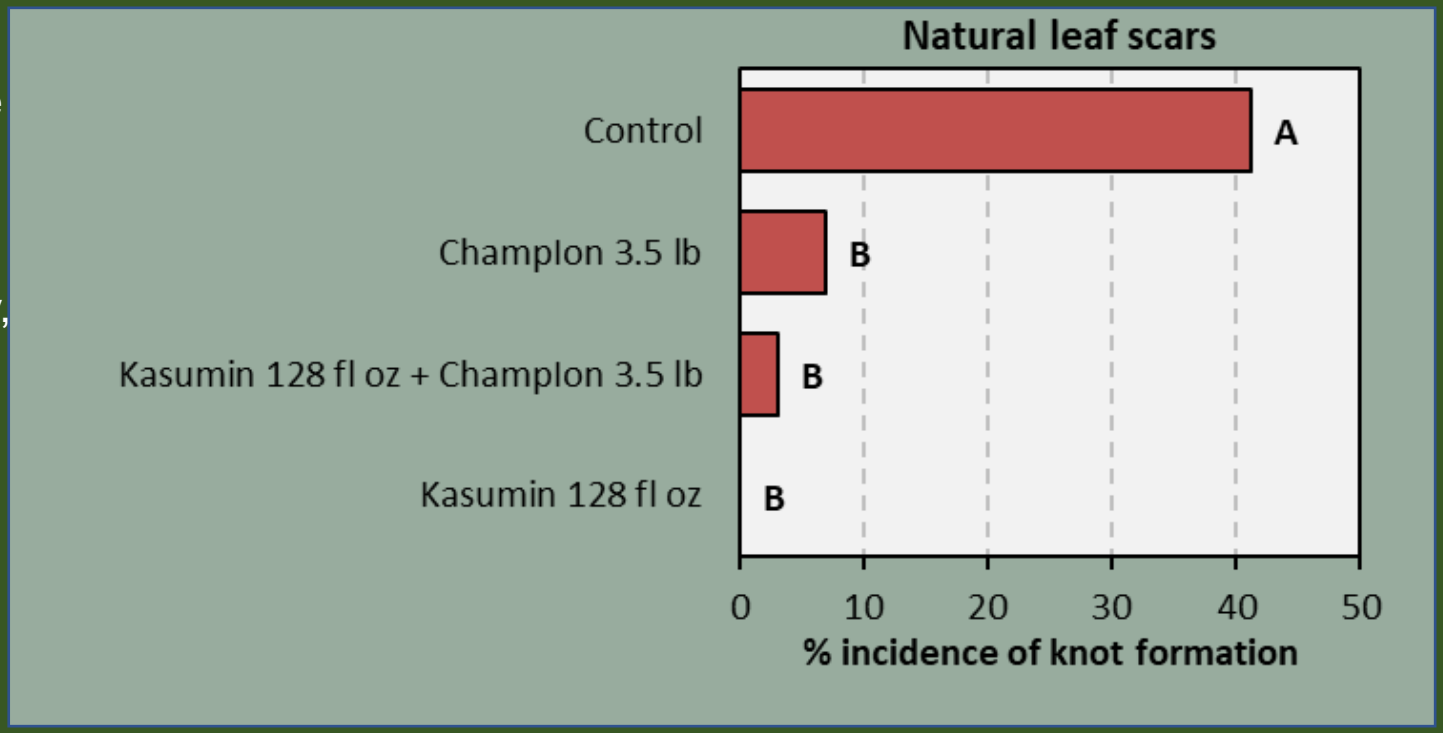
Treatments were spray-applied to wounds until runoff and allowed to dry. Wounds were then inoculated with a copper-sensitive or –resistant *Psv* strain.

Summary

- ε-poly-L-lysine continues to perform well by itself or in mixtures with kasugamycin or Dart.

Efficacy of copper and kasugamycin against olive knot

Field trial on the management of olive on ‘Arbequina’ olive. Treatments were spray-applied until runoff, allowed to dry, and spray-inoculated with a copper-resistant *Psv* strain.



- Copper (Champion++) and kasugamycin (Kasumin) continue to show high efficacy against copper-resistant strains of *Psv* on natural leaf scar wounds.

Timing of bactericide treatments

- *Same Day of Injuries* -

Fall Harvesting

Spring
Leaf
Drop
/Pruning

Winter
Frost

Summer
Hail+Rain
Storms

Summary

- New bactericides are desperately needed in the olive industry. All materials, including copper, are preventative treatments:
- **Kasugamycin and oxytetracycline** - High performance on leaf scars and/or natural leaf scars, as well as lateral wounds. Mixtures with copper should provide optimum performance and duration of activity.
- Registration of **Kasumin** (UPL and IR-4) – redo-residue studies completed in 2019. **Mycoshield/FireLine** registration pending at EPA.
- **Nisin and Ɛ-poly-L-lysine,** bactericidal food additives (biopesticides), showed in vitro toxicity to copper-sensitive and –resistant *Psv* strains, In field trials, mixtures performed well but field formulations need to be developed.
    - NuFarm and UPL are developing ag-formulations as potential registrants for **Nisin and Ɛ-poly-L-lysine**.
- AMR policies for pesticides in agriculture (WHO/FAO) being developed as guidance documents.

What did we learn?

- **The Olive industry is in need for new fungicide and bactericides for disease management to decrease dependency on copper.**
- **Ziram, Inspire Super, and Ph-D are planned for registration to control peacock spot as preventative treatments similar to copper.**
- **Kasumin and Mycoshield/Fireline are planned for registration to manage olive knot in the near future.**
- **Nisin, Ɛ-poly-L-lysine, and other new bactericides are being identified, developed, and potentially registered on olive.**
- **USAGE of fungicides and bactericides:**
  - **Preventative treatments for protecting olive leaves/woody tissue.**
  - **No effective, economical eradication of existing infections.**
  - **For olive knot, treatments should be applied same day that injuries occur.**