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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Objectives of Peacock Spot Project

1. **Evaluate the performance of new and older fungicides in field trials.**
   A) **Multisite MOA fungicides** - Dithiocarbamates (ziram) (FRAC Code – M3) and ziram+copper (FC M1/M3) (Second year data needed for this tank mixture)

   B) **Single-site MOA fungicides** – DMIs (e.g., difenoconazole) pre-mixed with other fungicides like cyprodinil (Inspire Super - FC 3/9) and azoxystrobin (Quadris Top - FC 3/11), polyoxins (Ph-D - FC 19), or guanidines (Syllit - FC U12).
2. **Evaluate application timing and adjuvants of selected treatments.**

   A) Timing: Fall, Spring, or Fall and Spring.

   B) Adjuvants: NuFilm-17 or oil (to increase persistence or activity over the fall/winter season)

Efficacy of fungicide treatments for management of peacock spot - 2020-21

				Manzanillo Glenn Co.		Arbequina Sutter Co.	
			Application	Infections		Infections	
No.	Treatments*	Product rate/A	11-6-20	No.	LSD	No.	LSD
1	Control	---	---	35.8	a	35.0	a
2	Ziram	128 oz	@	15.3	b	8.0	b
3	Abound	12.5 fl oz	@	12.5	bc	not done	
4	Champ	96 oz	@	11.8	bc	9.5	b
5	Syllit + Ph-D	32 + 6.2 oz	@	11.5	bc	8.5	b
6	Ziram + Champ	80 oz + 80 oz	@	11.0	bc	12.8	b
7	Syllit	48 oz	@	9.8	bc	7.5	b
8	Quadris Top *	14 fl oz	@	9.8	bc	7.8	b
9	Ph-D	6.2 oz	@	7.0	bc	12.3	b
10	Inspire Super	20 fl oz	@	5.5	c	8.5	b

- Treatments were applied using an air-blast sprayer at 100 gal/A.
- Disease was evaluated on 4-28-21 and 100 random leaves of each tree were assessed for the presence of typical disease symptoms.

* -Polyoxin-D is a biofungicide and exempt from tolerance. Efficacy data is only needed for registration (no GLP residue studies required). UPL advised us that the Section 3 is pending Oct. 2022 and that we should request a concurrent review.

Summary of new fungicides accepted into the IR-4 Program at the Food Use Workshop

Year	Fungicide	Active ingredient(s)	FRAC Code	IR-4/EPA	Status
2018	Ziram	ziram *	M3	Supported	Ongoing
2018	Inspire Super	difenoconazole-cyprodinil	3/9	Supported	Ongoing
2019	Ph-D	polyoxin-D	19	Biopesticide	UPL label change
2020	Quadris Top	azoxystrobin-difenoconazole**	3/11	Supported	Initiated
2020	Syllit	dodine	U12	Supported	Chem-SAC
2018	Topsin-M	thiophanate-methyl	1	Rejected	Not considered
2018	Bravo	chlorothalonil	M5	Rejected	Not considered

- Ongoing IR-4 project (Field studies conducted in 2019/20; lab residue studies in 2021) for ziram and difenoconazole/cyprodinil); Quadris Top initiated in 2020 based on the after-harvest and winter season usage with expected zero residues on the crop in the following harvest season as demonstrated with Ziram and Inspire Super.
- Syllit has international tolerances justifying an IR-4 Chem-SAC proposal (submitted in Oct. 2020) and UPL will add olive to the Ph-D biopesticide label for Section 3 PRIA date Oct. 2022. Additional crop safety / efficacy data requested by EPA.
- These fungicides are also highly effective against newly described **Neofabraea** and **Phlyctema** diseases of olive in California.

* - Ziram cancellation on all crops was proposed in Feb. 2022.

** - Topsin-M was re-classified with potential for registration on olives (IR-4 FUW 2021).

Benefits to the industry

- Chemical management is currently based on the use of copper and lime sulfur, that are increasingly being restricted by regulatory agencies
- Due to the small US acreage of olive production, limited mostly to California, registration of any new material needs to be done through the IR-4 program.
- Ziram is FC M3, Inspire Super is FC 3/9, Quadris Top is FC 3/11, Syllit FC is U12, and Ph-D is FC 19.
- UPI (ziram, polyoxin-D, dodine) and Syngenta (difenoconazole/ cyprodinil, or /azoxystrobin) support their respective products on olive.
- Registration of polyoxin-D and dodine (Chem SAC proposal) have expected registrations in 2022-23 because they are exempt from tolerance or have an established tolerance in other countries, respectively. UPL updated Section 3 registration as fall 2022 but indicated a concurrent review should be requested.
- Five new fungicide registrations will be an expected final outcome that will allow for sustainable management programs for years to come.

Objectives of Olive Knot Project

I. Evaluate new bactericides: GRAS food additives and other experimentals against *Psv*

A. Laboratory in vitro sensitivity studies: bactericides used alone or mixed with capric/caprylic acids (Dart) or other products such as dodine.

B. Field efficacy studies with new bactericides for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv*.

i) Oxytetracycline and kasugamycin formulations in combination with **dodine** or low rates of copper (e.g., Kocide, Champ, Cueva) .

ii) Nisin, Ɛ-poly-L-lysine, and **dodine** alone, in combination with each other, or in mixtures with antimicrobial acids (e.g., capric/caprylic).

II. Continue to support the registration of the antibiotics kasugamycin / oxytetracycline

A. Support letters for both antibiotics from myself and the industry (COC) were provided in the fall of 2020 to IR-4 for inclusion to the IR-4/registrant (UPL for kasugamycin and AgroSource for oxytetracycline) submission petition to EPA. Follow-up requests for registration on olive were made to EPA and UPL in the summer of 2021 for registration in 2022.

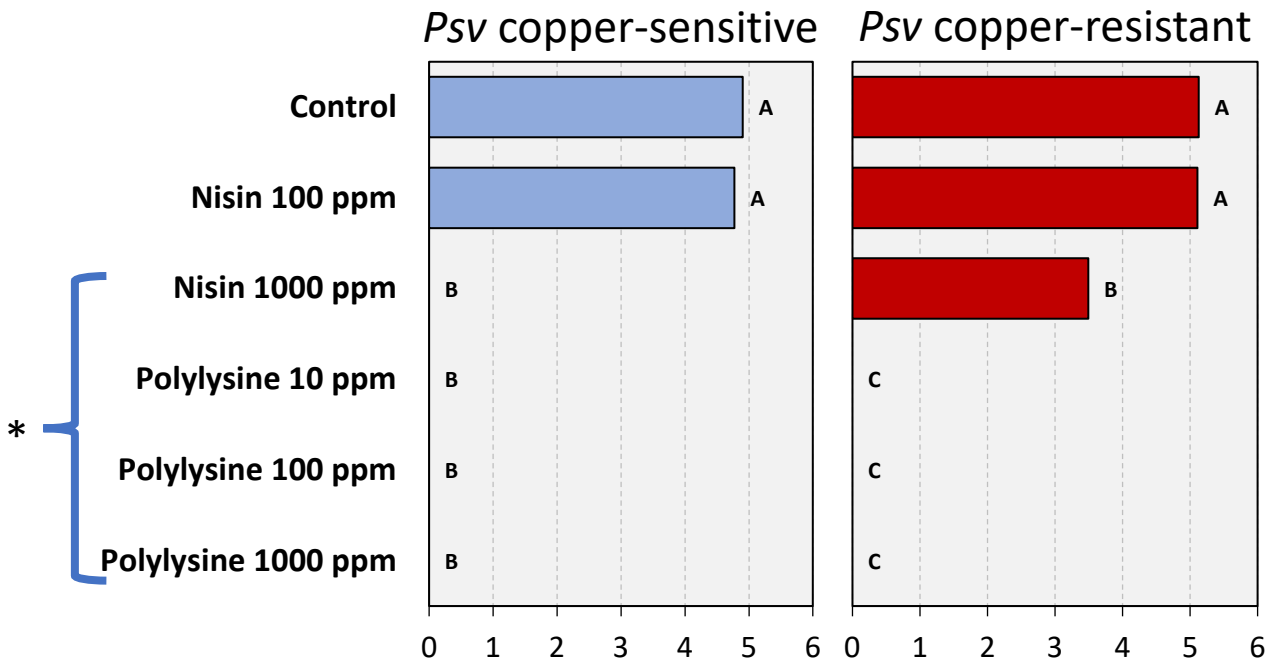
B. Optimize the efficacy of oxytetracycline and kasugamycin, in mixtures with **dodine** under field conditions as they go through the final registration process on olives to ensure guidelines can be provided to the industry for optimal performance.

Chemicals to be used for evaluation as potential bactericides against olive knot in 2021

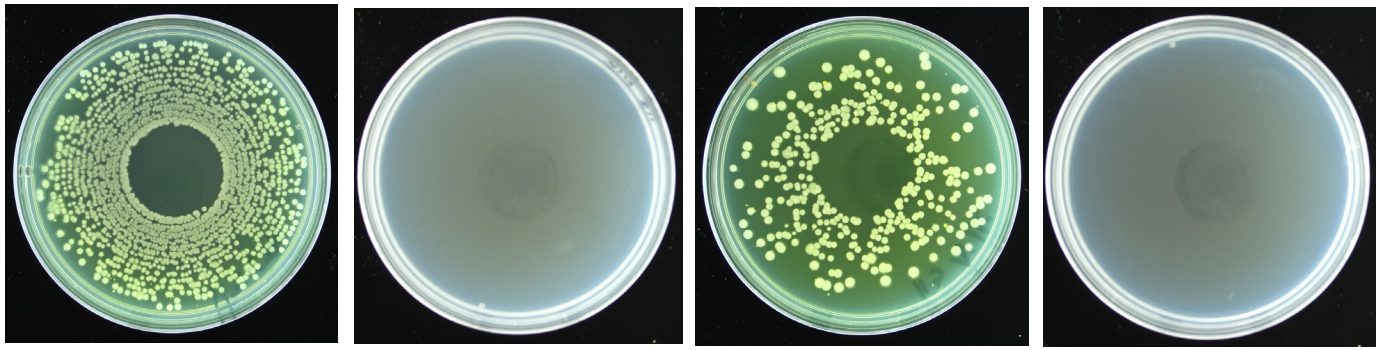
No.	*Treatment*	*Trade name*	*Chemical class/use*
1	Capric/caprilic acids	Dart	Antimicrobial, adjuvant
2	Citric acid	---	Organic acid, antimicrobial
3	Copper hydroxide	ChamplON++	Fungicide, bactericide
4	ε-poly-L-lysine	---	Food preservative
5	Kasugamycin	Kasumin	Antibiotic (aminoglycoside)
6	Essential oils	ET-91, Thymox	Organic acid, antimicrobial
7	Nisin	Niprosin	Food preservative
8	Oxytetracycline	Fireline, Mycoshield	Antibiotic (tetracycline)
9	TDA-NC	TBA	New (food grade)

- UPL will provide commercial agricultural formulations of kasugamycin, nisin, and Ɛ-poly-L-lysine for performance and efficacy trials.
- AgroSource and NuFarm will provide commercial formulations of oxytetracycline.
- Woodbridge will provide commercial formulations of capric/caprylic acid.

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 min and then plated onto agar media. Other materials (e.g., Quam, ET91, and Ninja NSTK-29 less effective).

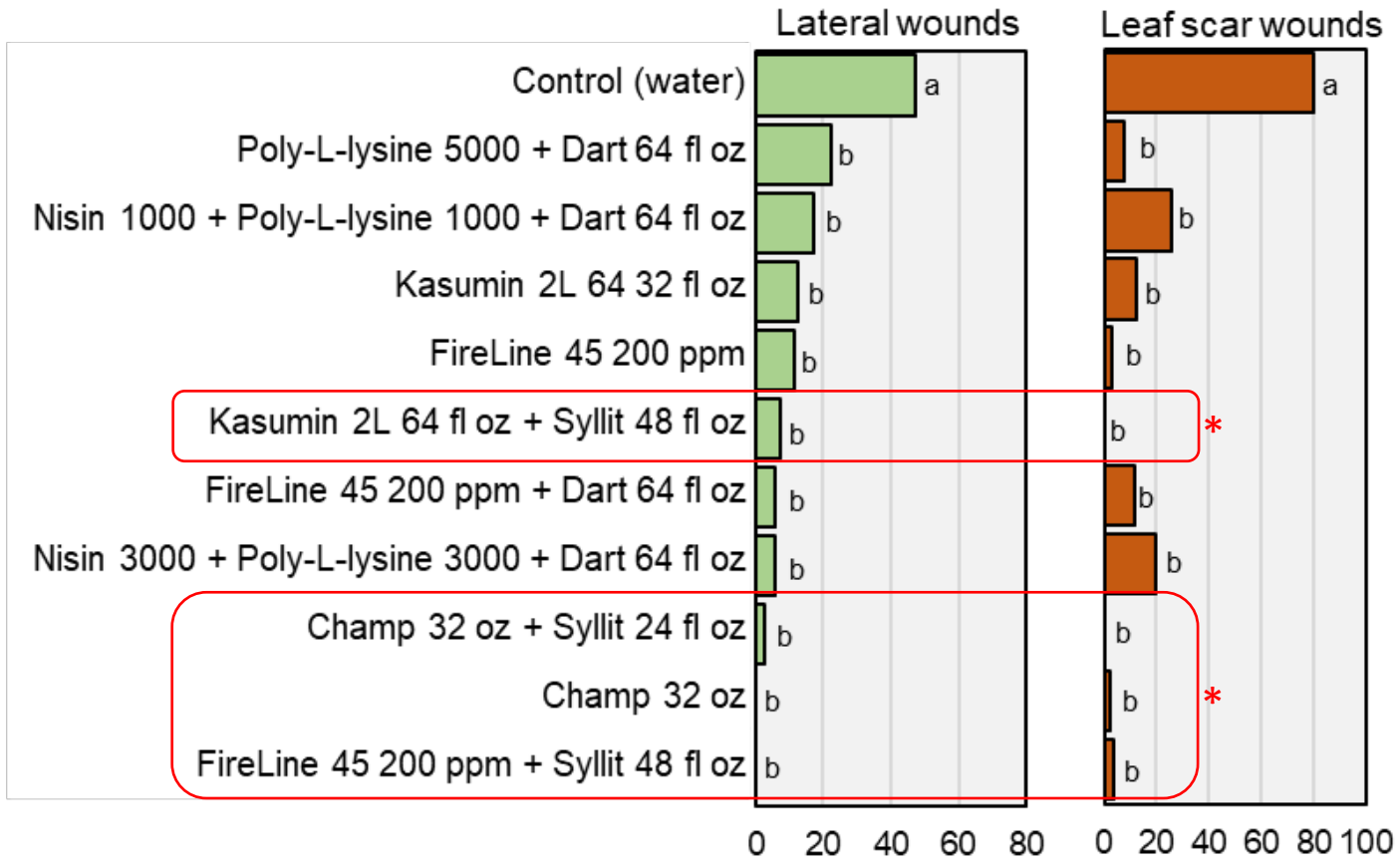


Control
Cu-R

Nisin 1000 ppm
∗Cu-S
Cu-R

∗Ɛ-poly-L-lysine
10 ppm
Cu-S and -R

Efficacy of experimental bactericides against olive knot



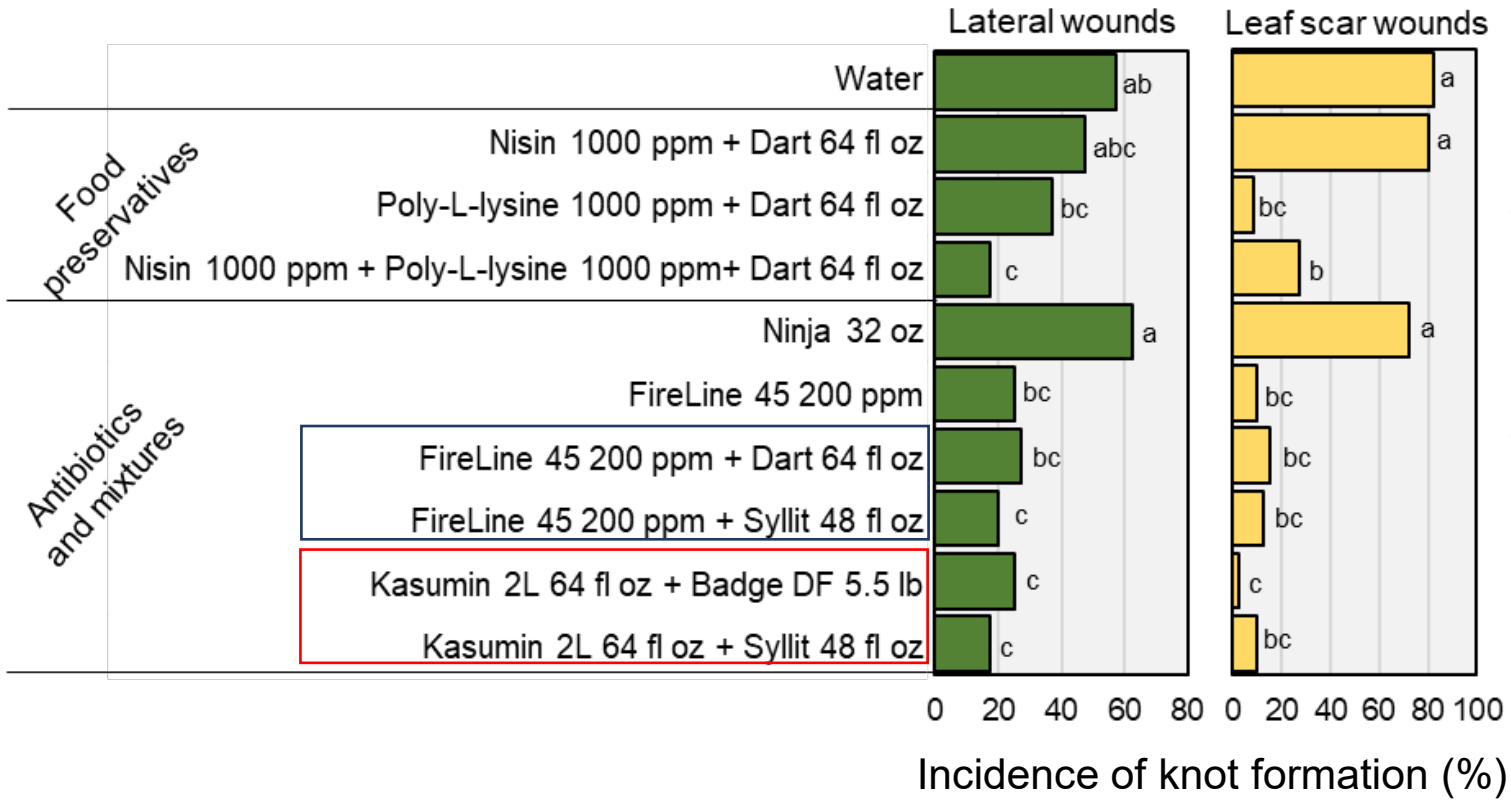
Greenhouse trials on cv. Arbequina - Treatments were spray-applied to wounds until runoff and allowed to dry. Wounds were then inoculated with a **Cu-sensitive *Psv* strain.**

Summary

- **ε-poly-L-lysine (EPL) mixtures with Dart** performed well.
- **Syllit mixed with copper, Fireline, or Kasumin** performed well in protecting lateral wounds and leaf scars against Cu-S strains
- **Nisin, EPL, and Dart mixtures** performed well against Cu-S strains

* **- Near zero levels**

Efficacy of experimental bactericides against olive knot

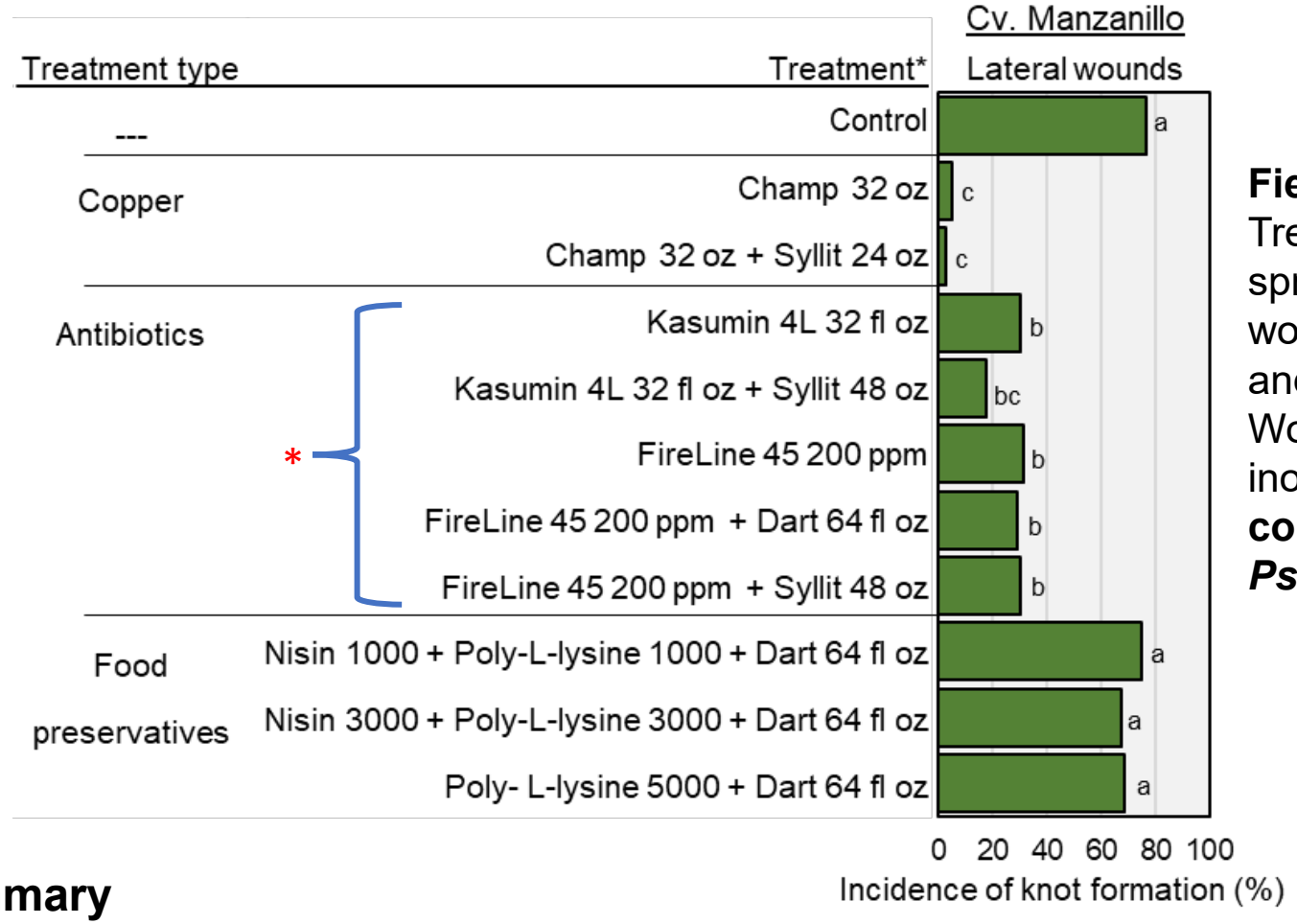


Field trials - Treatments were spray-applied to wounds until runoff and allowed to dry. Wounds were then inoculated with a copper-sensitive *Psv* strain.

Summary

- **Oxytetracycline** was effective on both lateral injuries and leaf scars.
- **Copper- or Dodine-kasugamycin** and **Dodine- or Dart-oxytetracycline** mixtures performed well using copper-sensitive *Psv* strains.
- **Nisin-ε-poly-L-lysine-Dart** also performed well.
- **Ninja is an experimental antibiotic for agriculture** that did not perform well at the rate used.

Efficacy of experimental bactericides against olive knot



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

Summary

- **Oxytetracycline** (**FireLine**) performance was similar to that of kasugamycin on lateral wounds.
- **Kasumin-dodine (Syllit) was similar to copper and copper-dodine treatments**.
- **Oxytetracycline-dodine or –Dart mixtures** performed well using copper-sensitive *Psv* strains.
- Nisin, EPL, and Dart mixtures were not effective in this study. Optimization and consistency are needed

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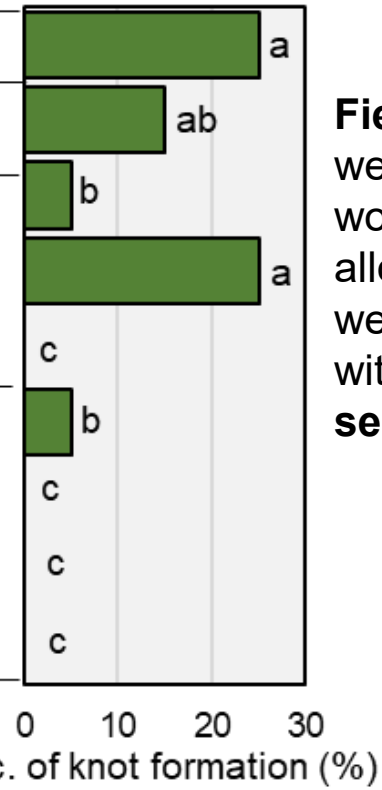
Efficacy of experimental bactericides against olive knot

cv. Arbequina - Inoculation with a Cu-sensitive strain

		Lateral wounds
Water	---	a
TD EXP-NC-1 + BreakThru	40 oz + 1.6 fl oz	ab
Nisin + ManniPlex Zn	13.5 oz + 32 fl oz	b
Poly-L-lysine + ManniPlex Zn	13.5 oz + 32 fl oz	a
Poly-L-lysine + Dart	13.5 oz + 48 fl oz	c
Champ + Syllit	64 + 24 oz	b
Champ + Syllit	64 + 48 oz	c
Champ + Kasumin 2L	64 oz + 32 fl oz	c
Champ + Super Tin 4L	64 oz + 12 fl oz	c



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

Summary

- Nisin-ManiPlex-Zn effective in some trials on lateral wounds but inconsistent.
- **Ε-poly-L-lysine - Dart** very effective on lateral wounds, less effective in mixtures with ManniPlex-Zn on lateral wounds.
- **Copper-Kasumin, copper-Super Tin, and copper-Syllit** continued to perform well.

Summary

- Numerous treatments were evaluated in lab, greenhouse, and field studies.
- **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.
  - UPL are developing ag-formulations as potential registrants for **Nisin and Ɛ-poly-L-lysine**.
- **Kasugamycin** and **oxytetracycline** - High performance on leaf scars and lateral wounds especially in mixtures with copper or **dodine (Syllit)**.
- Registration of **Kasumin** (UPL and IR-4) – redo-residue studies completed and EPA PRIA date for full registration Dec 2022;
- Registration of **FireLine** (AgroSource and IR-4) submitted in 2020 and is pending at EPA Mar. 2022 (However, EPA may delay until Mar 2023).
- **AMR policies** for pesticides in agriculture (WHO/FAO) being developed as guidance documents.