Epidemiology and Management of Walnut Blight

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Cooperating:

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L. Milliron - *UC Cooperative Extension, Butte, Glenn, Tehama Co.*

Objectives in 2022

I. **Natural host resistance** against walnut blight. New genotypes to be evaluated as supplied by the Walnut Breeding program (P. Brown/C. Leslie) .

A) Evaluate natural fruit blight incidence and conduct fruit and bud inoculation studies to evaluate genotype susceptibility and survival of *Xaj* in bud tissues.

B) Identify other indicators of host susceptibility. Inoculate wounded and non-wounded green walnut shoots of selected cultivars with *Xaj*.

II. **Epidemiology** - Evaluate *Xaj* populations using molecular methods or culturing.

A) Conduct surveys to determine the sensitivity of strains to copper, copper-mancozeb, kasugamycin, and kasugamycin-mancozeb in collaboration with PCAs and farm advisors.

B) Compare *Xaj* populations in cankers and in near or distant female buds.

III. **New treatments**

A) New liquid and dry formulations of copper

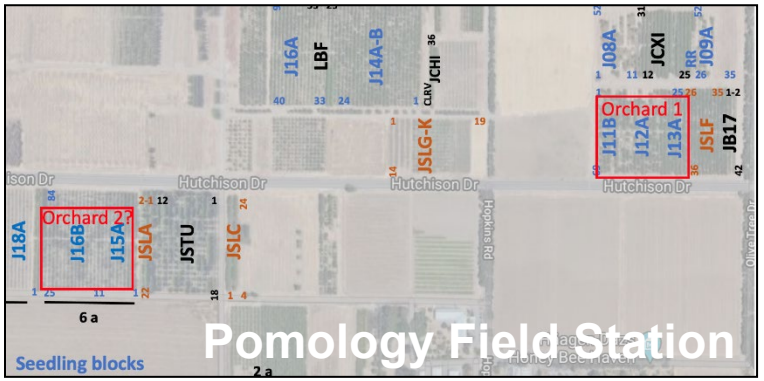
B) Bactericides and enhancers - Nisin, EPL, ningnanmycin, oxytetracycline, and mixtures with dodine and other possible additives as enhancers

C) Biologicals and natural products: biocontrols, essential oils and other plant extracts, and additives

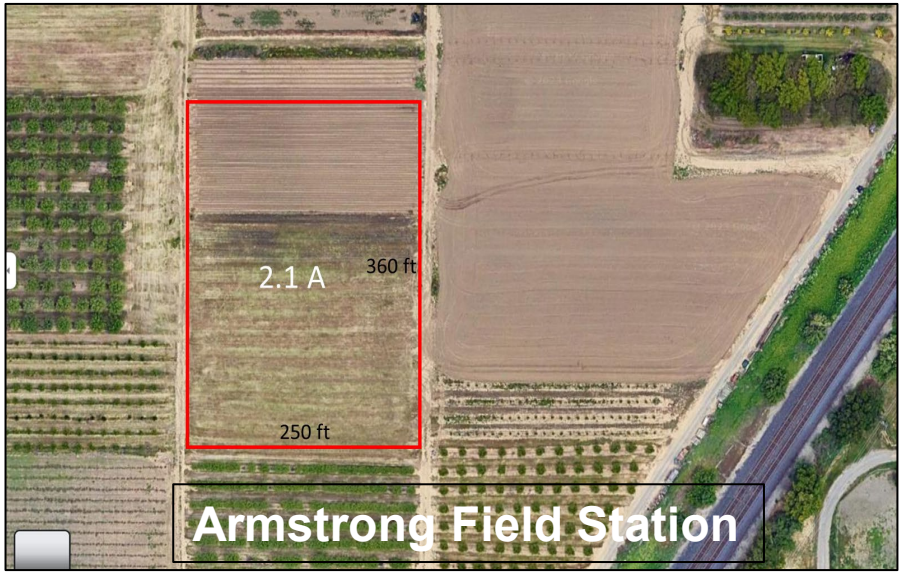
Evaluating Natural Host Resistance in Walnut to Blight

- Continued to cooperate with the UCD breeding program.
- Multiple genotypes that for 3 years consistently did not support the survival of *Xaj* in female buds and had low incidence of fruit infections were identified.

From orchard 1: 17.61, 18.66, 18.65, 14.65, 24.60
Others: 17.64, 23.49, 24.13, 17.63, 24.27, 24.44, 3.2
From orchard 2: 36, 57, 67, 43, 23, 27



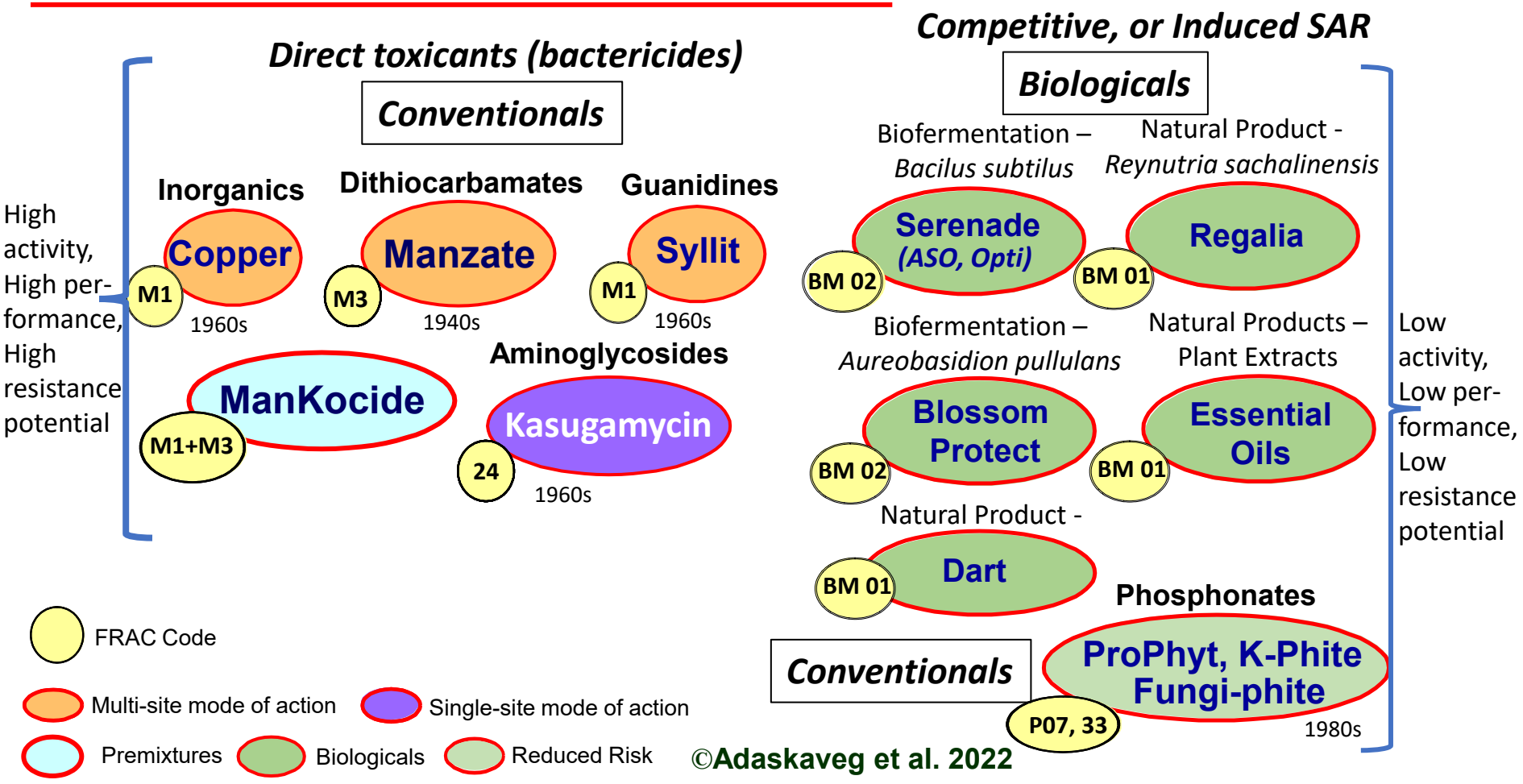
- These have been propagated and will be planted at UC Davis in 2023 and will be evaluated for blight incidence.
- Orchard will be canopy-irrigated, high-density, interplanted with highly susceptible cultivars (e.g., Ashley, Vina)
- Still, other resistance mechanisms to blight are present: some genotypes with high bud populations had a low incidence of disease.



Management of Walnut Blight

Overall, moderate disease levels in 2022

Treatments for Managing Walnut Blight



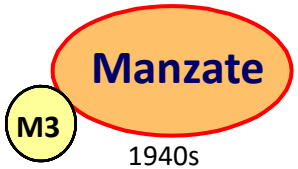
Regulatory Challenges

I . EPA Proposed Cancellation of Ziram in 2022

Dithiocarbamates

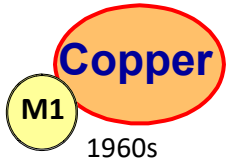
New dermal toxicity assays replace previous mammalian tests and now use human dermal skin cultures to determine toxicity to humans. EPA ignores 70 years of use data with no documented cases of poisoning and the agronomic importance to major crops across the United States. More cancellations planned that will remove most multi-site compounds.

Dithiocarbamates



- Scheduled for EPA Review in 2023

Inorganics



- Ongoing EPA Review in to reduce copper use in agriculture and environment

Monitoring for copper and mancozeb sensitivity in *Xaj* populations in California walnut orchards 2020, No samples in 2021 or 2022

Sample number	Total isolates recovered	No. of isolates/total		Mancozeb MIC (ppm)	Mancozeb MIC (ppm) in presence of 50 ppm MCE
		Resistant to 50 ppm MCE	Resistant to 100 ppm MCE		
1020	8	8/8	1/8	3.9 - 5.6	<0.005 to 0.07
1021	4	3/4	3/4	3.9 - 5.6	<0.005 - 0.07
1037	8	1/8	1/8	4.9 - 6.2	<0.005 - 0.09
1038	3	0/3	0/3	5.6 - 6.2	<0.005
1039	1	0/1	0/1	7.0	<0.005
Total	24	12/24	5/24		

Diseased fruit samples were submitted by farm and pest control advisors.

MIC: minimum inhibitory concentration (growth inhibited by >95%). MCE: metallic copper equivalent (from copper sulfate pentahydrate). Growth at 50 ppm MCE is considered resistant, growth at 100 ppm MCE is considered highly resistant.

Interpretation: *Because copper resistance developed before the introduction of mancozeb, the selection for copper resistance continues with each copper application and thus, higher copper resistance (i.e., 100 ppm) are now found in CA. In China levels of 150 and 200 ppm are common. This emphasizes the need to have more modes of action for rotations.*

Preparing for the Future

The issues:

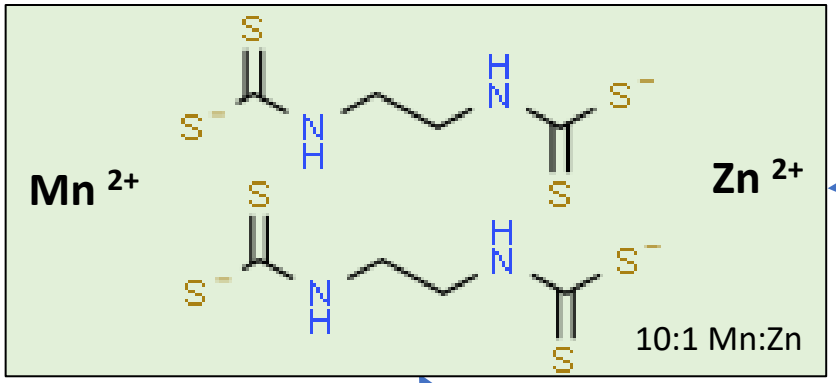
- **Dry spring cycles prevailed in the last two years, but wet cycles will return.**
- **Overuse of copper leads to higher resistance in the pathogen.**
- **Overuse of copper-mancozeb has led to complete resistance in other patho-systems.**
- **Uncertain regulatory future of mancozeb in the EU and other countries including the U.S. (EPA proposed cancellation of dimethyl-dithiocarbamates in spring 2022)**

The goals:

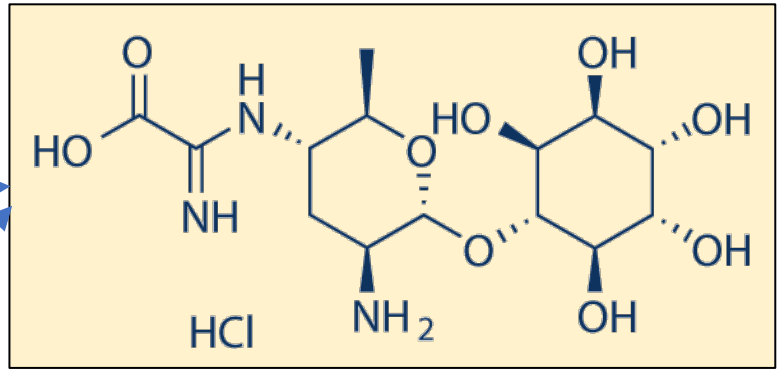
- **Develop effective alternatives that have high performance in favorable environments**
- **Discover new and different modes of action (MOA) against bacterial pathogens**
- **Identify synergistic mixtures with different MOAs that can be used in mixture rotations**
- **Move through the registration and regulatory processes, knowing the issues with EPA and other agencies, to bring new bactericides to the walnut industry**

Current and potential antibacterial treatments for managing walnut blight

Mancozeb (Manzate)

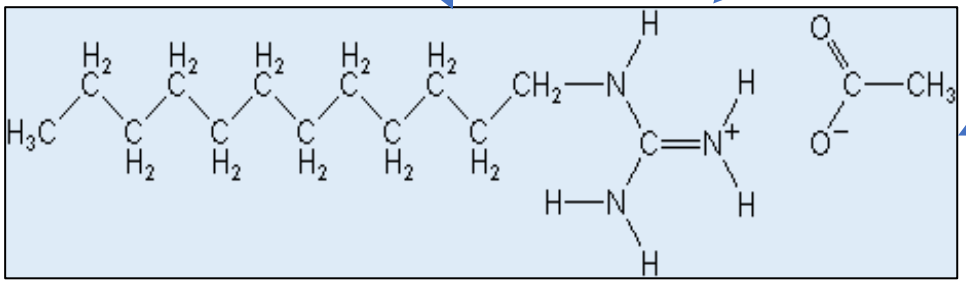


Kasugamycin (Kasumin)

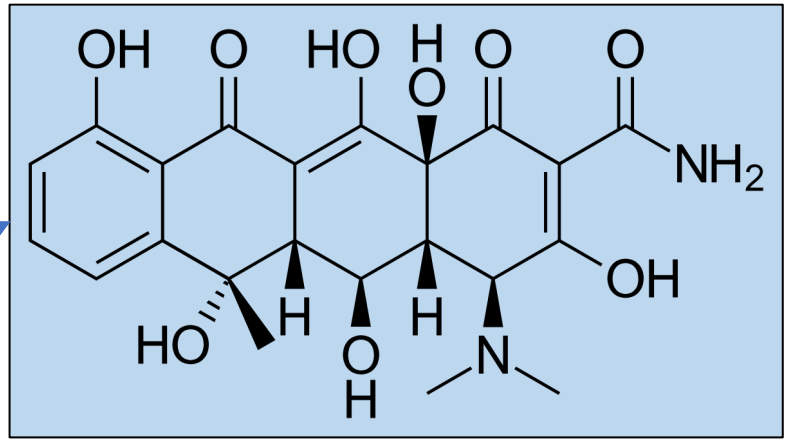


+ Cu^{2+}

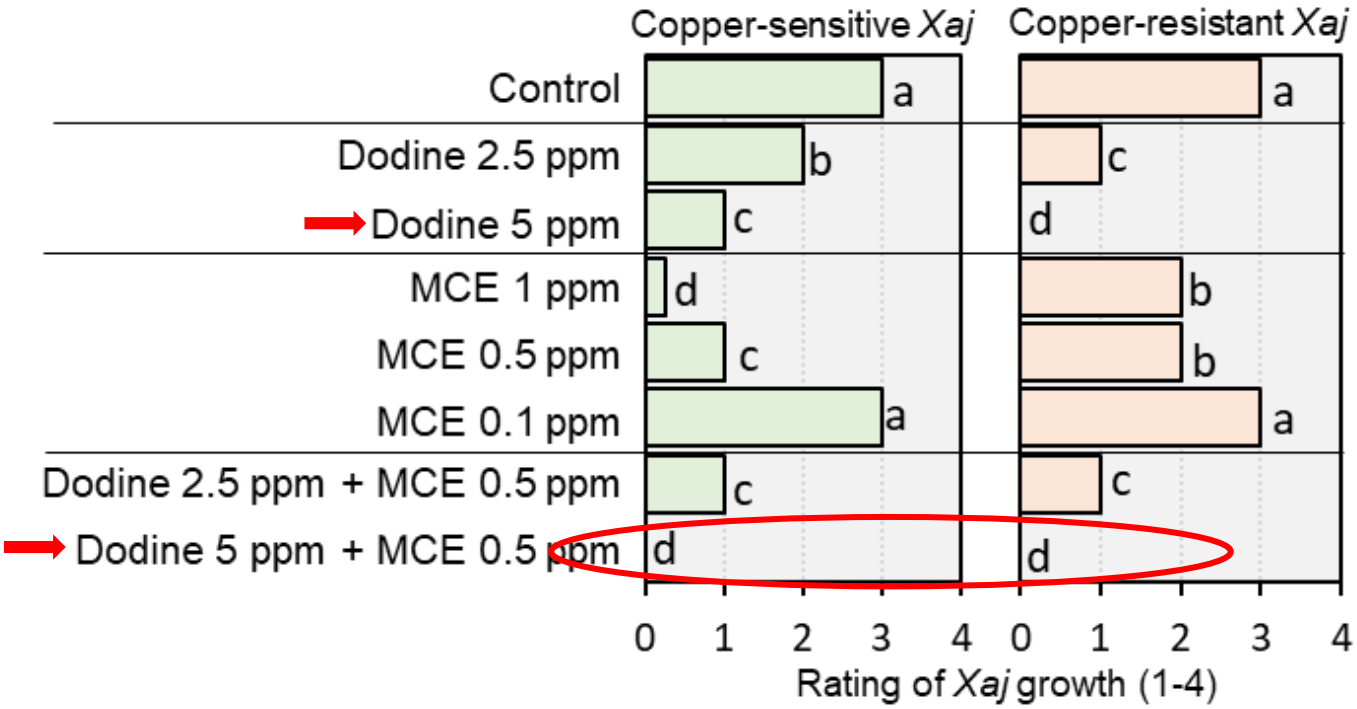
Dodine (Syllit)



Oxytetracycline hydrochloride (FireLine)



In vitro toxicity of dodine and copper against *Xaj* in direct exposure assays



Bacterial suspensions were exposed to the antimicrobials for 30 min. Suspensions were then diluted with water 1:100, plated onto agar media, and growth was evaluated after 2 days.

MCE = metallic copper equivalent

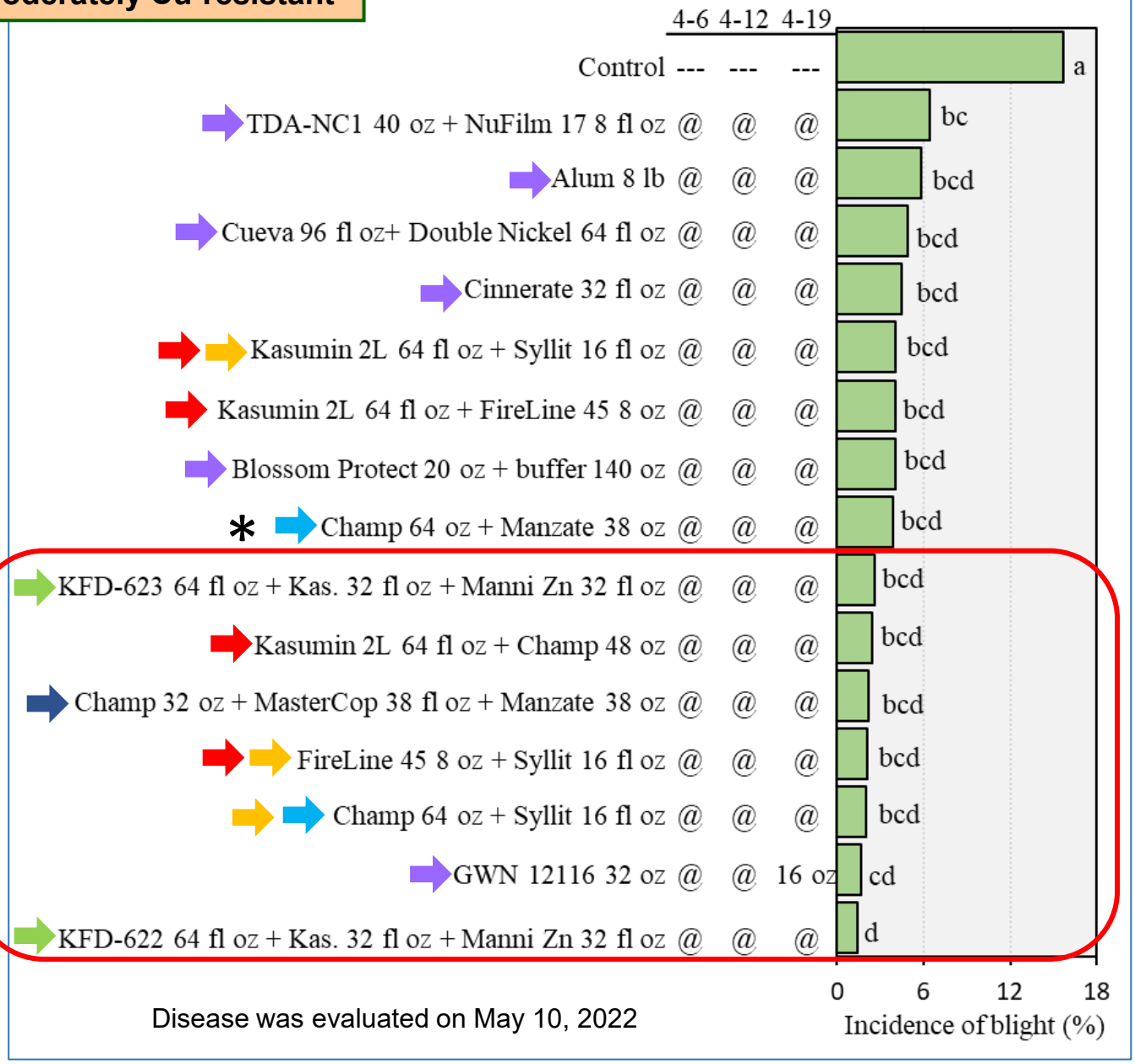
New bactericide treatments for managing blight - Solano Co. 2022

cv. Tulare, Solano Co., *Xaj* moderately Cu-resistant

Natural rainfall conditions

Low/moderate disease pressure:

- **Mastercop+Champ+Manz.**
- **Champ+Manzate, or Champ+Syllit had high efficacy.**
- **Syllit in mixtures excellent performance**
- **Oxytetracycline (FireLine, Mycoshield) and Kasumin performed very well.**
- **Nisin or EPL + ManniPlex + Kasumin showed high to intermediate efficacy.**
- **Experimentals/organics promising:**
  - **Blossom Protect**
  - **KFD (nisin and EPL)**
  - **GWN 12116 (but minor phytotoxic)**

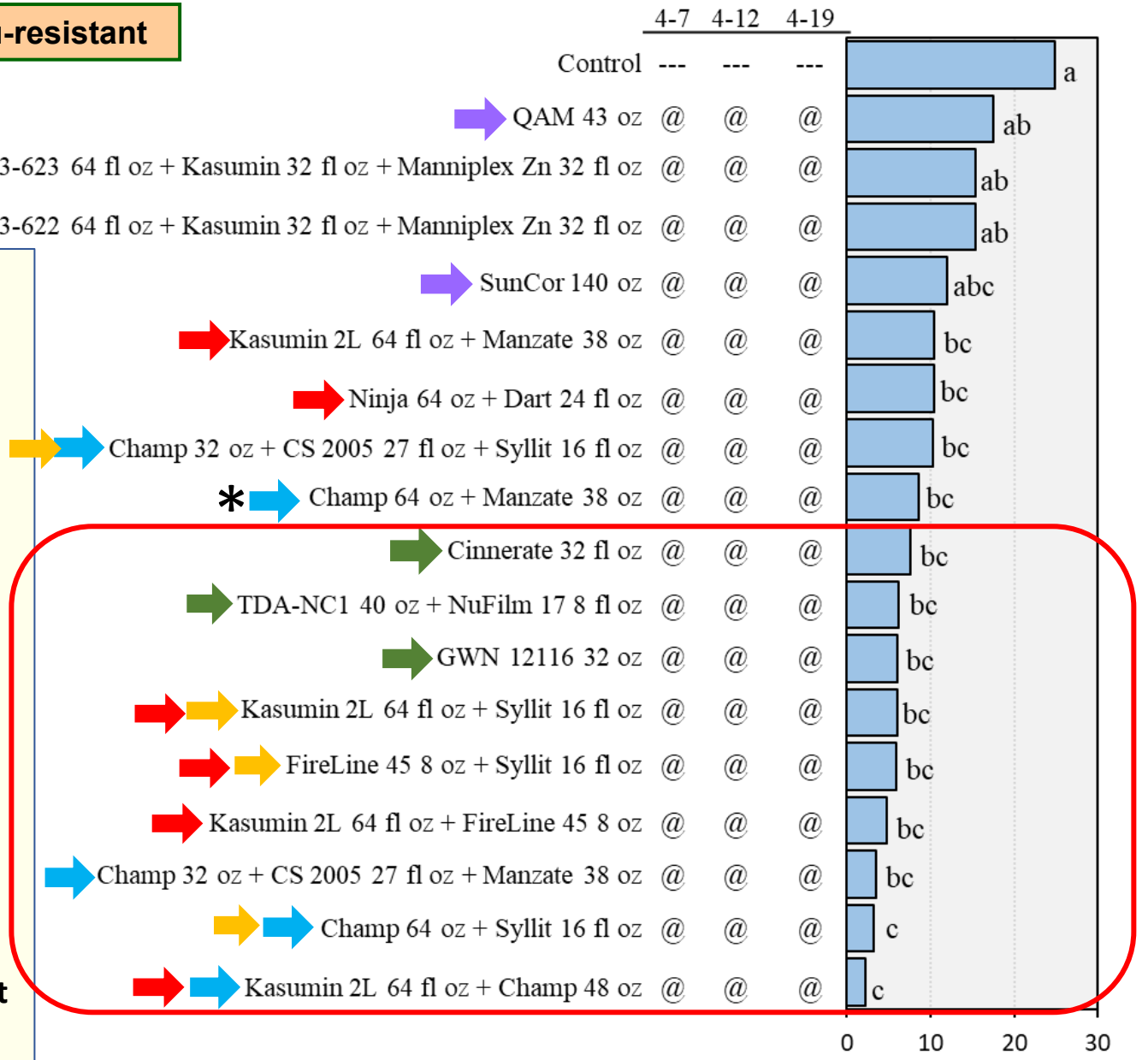


Disease was evaluated on May 10, 2022

New bactericide treatments for managing blight - Sutter Co. 2022

cv. Tulare, Sutter Co., *Xaj* Cu-resistant

Natural rainfall conditions



Moderate disease pressure:

<u>Copper mixtures:</u> CS2005/ MasterCop+Champ+Manz, Cuprofix+Manzate, Cuprofix+ Syllit provided high efficacy.

<u>Syllit</u> in mixtures excellent performance.

<u>Antibiotics:</u> Oxytetracycline (FireLine), Kasumin, Kasumin+FireLine, and Ninja performed very well.

<u>Experimentals:</u> QAM, SunCor, Nisin, poly-lysine, and others showed inconsistency.

<u>Natural products</u> (Cinnerate) /<u>experimentals</u> (GWN 12116, TDA- NC1) performed well but GWN 12116 and SunCor caused phytoxicity.

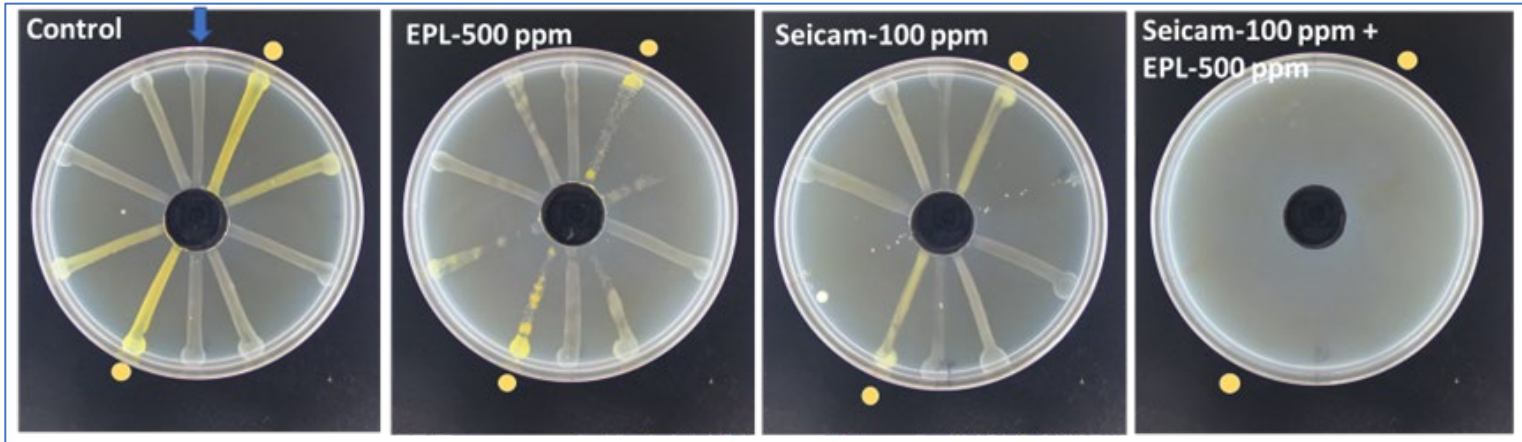
Disease was evaluated on May 10, 2022

Phytotoxicity evaluations

GWN 12116 – 2 lb/A

SunCor – 8 lb/A

Identification of potentially OMRI-approved bactericides active against *X. arboricola* pv. *juglandis* and other bacterial plant pathogens in laboratory amended agar tests



Clockwise from arrow: *Erwinia amylovora*, ***Xanthomonas arboricola pv. juglandis*** (●), *Xanthomonas arboricola* pv. *pruni*, *Pseudomonas savastanoi*, and *P. syringae*. Note: only one concentration on each plate.

Nutrient agar was amended with bactericides and bacteria were streaked out. Growth was evaluated after 2 days at 25C. '+++' indicates that growth was similar as on non-amended agar, '+' indicates that growth was inhibited by >80%, and '-' indicates that growth was completely inhibited.

Treatment	Concentration (ppm)	Growth rating
Control	---	+++
Timorex ACT - tea tree oil	1000	+++
CWP - yeast + yeast extract	1000	+++
Cinnerate - cinnamon oil	100	+++
	500	-
Seican - cinnamaldehyde	100	++
	250	-
EPL	500	++
	1000	-
Nisin	1000	+++
EPL + cinneraldehyde	500 + 100	-
Nisin + cinneraldehyde	1000 + 100	+

Concept: Mixtures of OMRI-approved natural products similar to mixtures of conventional bactericides may be a new direction in developing agricultural bactericides.

Summary of bactericide efficacy trials 2022

- Copper-mancozeb continued to perform well, however, mancozeb is facing potential MRL cancellations in some countries.
- Dodine (Syllit) at **low rates** (16 fl oz) was successfully evaluated as a mancozeb alternative for copper, Kasumin, and FireLine.
- Low copper-containing products (Adama - MasterCop 5.4% MCE and MagnaBon – CS-2005 5.0% MCE) mixed with 8 lb/A Mankocide (30% MCE) or 2 lb of Champ (50% MCE) were very effective, and similar in performance to 5 lb/A Champ mixed with 38 fl oz of Manzate.
- These new mixture treatments use less copper per acre and provide fast-acting free copper ions and a residual reservoir of copper hydroxide (fixed copper) that will solubilize under wet conditions.
- A FireLine-Kasumin or -Syllit mixtures were highly effective.

Summary of bactericide efficacy trials 2022

- In a second year of testing, the new antibiotic ningnanmycin (Ninja) and the riboflavin product TDA-NC1 significantly reduced blight incidence, but Ninja caused phytotoxicity.
- Cinnerate (an essential oil) showed promising activity, but the alga (SunCor) and the agave (QAM) extracts were not very effective.
- Alum (aluminum potassium sulfate) was inconsistent in its effectiveness.
- The biocontrols Blossom Protect and Double Nickel 55, the experimental GWN 12116 (Gowan) and the formulated food preservatives nisin and ε-poly-L-lysine (KFD-603-622 and -623) provided intermediate and variable efficacy.
- Cinnamaldehyde-EPL/nisin mixtures are promising and will be evaluated in the field in 2023 in cooperation with Summit Agro and UPL (who will provide commercial formulations).

Registration updates

Kasumin (kasugamycin) received California registration on walnut in 2018.

- The rate is 64 fl oz/100 gal/A - ground application.
- The maximum number of applications is **4 per season** (changed from 2/season in 2020) with up to two sequential applications before rotation to other modes of action.

Oxytetracycline submitted to EPA by IR-4

- EPA full registration is pending for **Mar 2023** following EPA timelines (PRIA date changed again) but CA DPR registration is still needed.
- Working with the Walnut Commission to support full registration.

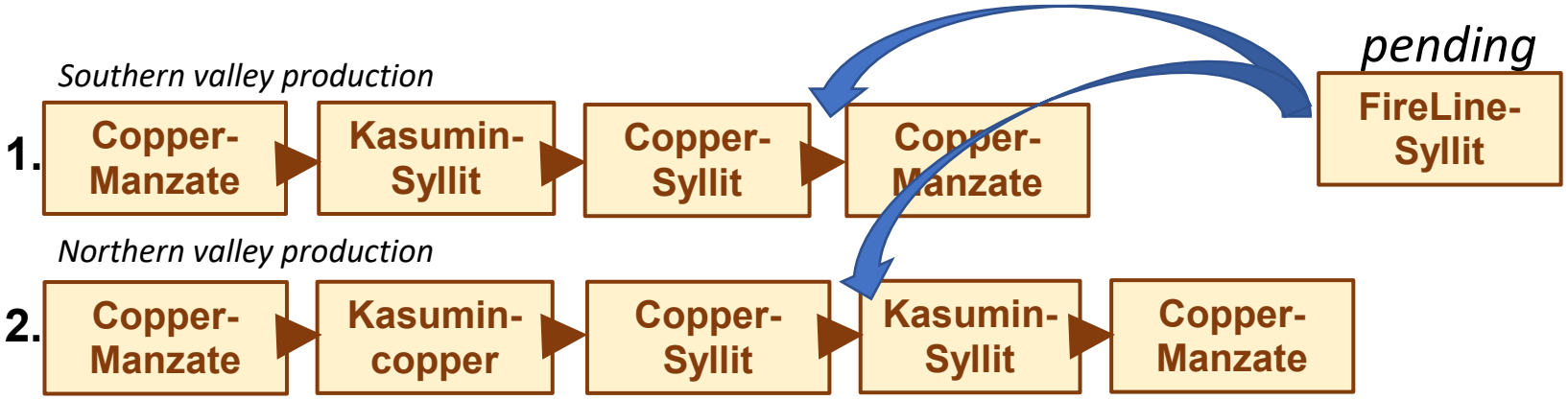
Dodine full registration - Feb. 2021, available in 2022, 2023 – used in mixture rotations. Use at 16 fl oz per acre in combination with copper or kasugamycin.

Kasumin – a new bactericide and Dodine – a new "mancozeb" for tank mixtures

Kasugamycin (Kasumin) and **dodine (Syllit)** were identified, developed, and registered for the purpose of resistance management, reducing over-usage of any one mode of action, and sustaining the CA walnut industry

Future goal is to obtain **oxytetracycline (FireLine)** registration for mixtures

Suggested four- or five-spray **mixture-rotation** programs under ***highly*** favorable conditions for disease:



*** - Seven- to ten-day intervals between treatments**

Objectives for 2023

I. **Natural host resistance**

A. New genotypes will be supplied by the Walnut Breeding (P. Brown/C. Leslie) and planted at UCD. The orchard will be designed to be favorable for walnut blight (high density, canopy irrigation, interplanted with susceptible cultivars such as Vina and Ashley.

B. Identify other indicators of host susceptibility. Inoculate wounded and non-wounded green walnut shoots of selected cultivars with *Xaj*.

II. **Epidemiology** –

A. Monitor *Xaj* populations using molecular methods or culturing for changes in sensitivity to copper, mancozeb, and kasugamycin.

III. **New treatments**

A. New copper formulations (Cueva, MasterCop, CS-2005) in mixtures with other products.

B. Support the registration of oxytetracycline (e.g., FireLine)

C. Support the use of dodine (Syllit) in bactericidal mixtures with coppers or other bactericides (e.g., kasugamycin, oxytetracycline).

D. Evaluate new biologicals and natural products (i.e., nisin, EPL, cinnamon oil - Cinnerate, cinnamaldehyde-Seican) in conjunction with registrants UPL & Summit Agro.