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

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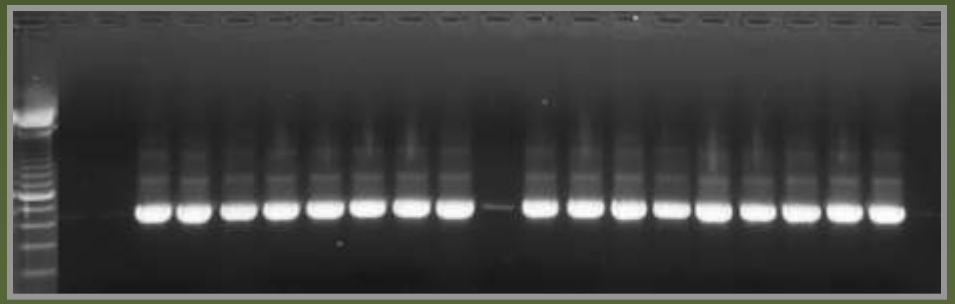
Objective: Collection of strains

- Collection of *P. syringae* pv. *savastanoi* strains from Sutter/Yuba, Glenn, and Colusa Co.
- Evaluation of isolation media (higher recovery rate on KMB than on PVF-1)
- Identification by cultural morphology and by PCR using specific primers (Appl. Environ. Microbiol. 66:267-2677)
- A total of 80 isolates were obtained from 7 orchards (more orchards were sampled and more isolates will be obtained).

Isolation of *P. syringae* pv. *savastanoi* on KMB (left) and PVF-1 medium and visualized under long-wave UV light

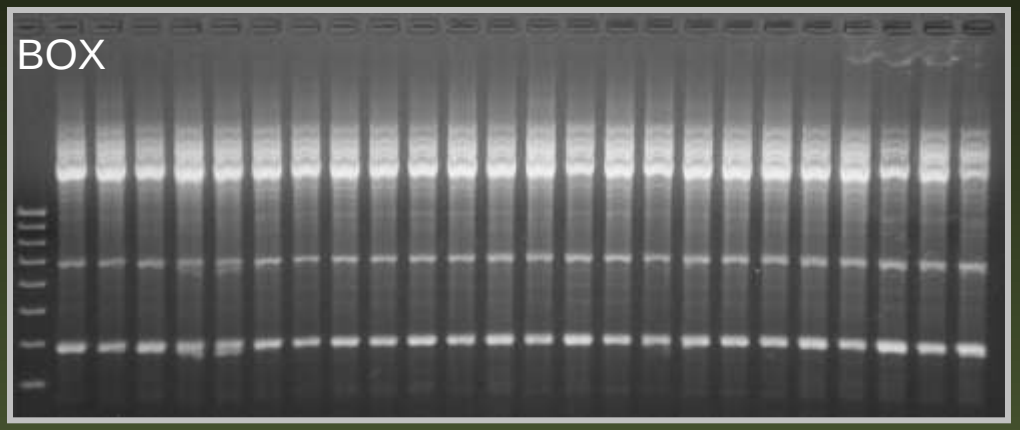


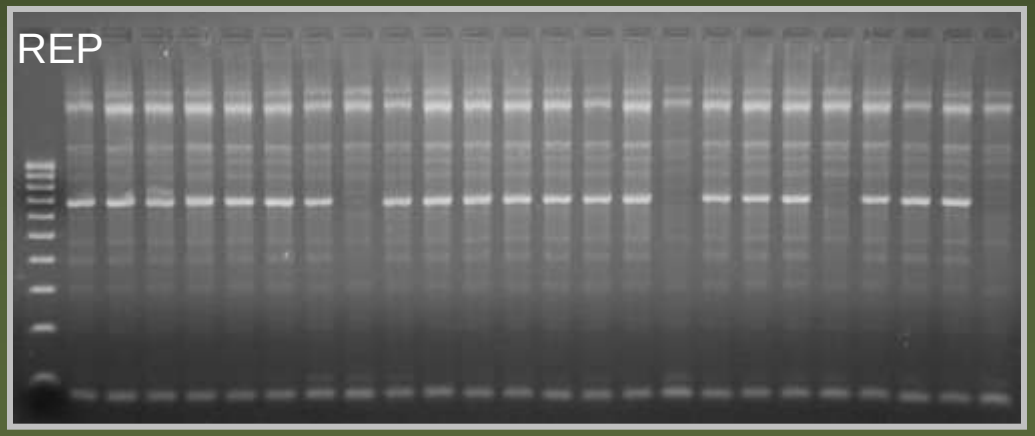
Specific amplification of *P. syringae* pv. *savastanoi* using primers targeting the IAA-lysine synthethase gene

Objective: Genotypic diversity among strains of *P. syringae* pv. *savastanoi*

Preliminary data on a sub-set of strains:

- Genetic diversity was evaluated using BOX, ERIC, and REP primers
- Diversity was found to be limited.
- Based on our current California population sample, the pathogen genetically is rather homogeneous.





Objective: Monitor galls for production of inoculum over time in different environments (seasonal availability of inoculum)

Preliminary studies

- All sampled knots contained viable inoculum inside the knot
- Re-hydrating olive knots after field collection for one hour led to bacterial oozing from some of the knots.
- Nearly all knots tested oozed the pathogen after 18 to 24 h of hydration.
- A direct association between the common epiphytic bacterium *Pantoea agglomerans* and *P. syringae* pv. *savastanoi* was found as reported previously by others (Marchi et al., 2006). These authors showed that both organisms produce indole-3- acetic acid, but cytokinins were only produced by *P. s.* pv. *savastanoi*.

Objective: Duration of susceptibility of injuries to infection by *P. syringae* pv. *savastanoi*

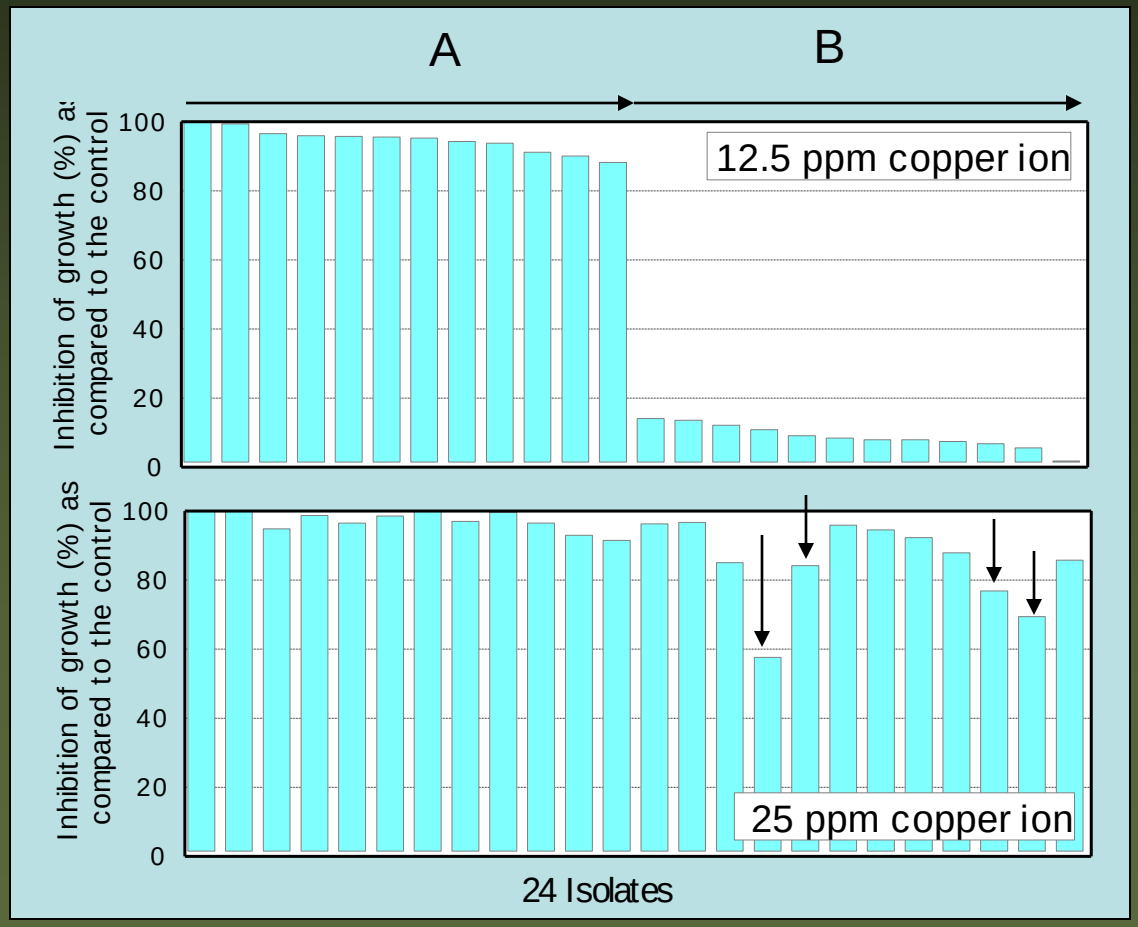
- Olive twigs in a commercial orchard were wounded by terminal cuts and by lateral wounds in late October.
- Wounds were inoculated after 0 days, 1 week, 2 weeks, or 3 weeks.
- Data on disease development are pending.

Objective: In vitro sensitivity of *P. syringae* pv. *savastanoi* to copper, kasugamycin, and selected sanitizers

- **Three direct exposure assays**
  - ***Continuous exposure - Microtiter assay.*** Bacteria incubated for 24 to 48 h in liquid growth medium with the addition of **copper** at selected concentrations
  - ***Continuous exposure - Spiral gradient dilution assay.*** Bacteria plated onto agar amended with a continuous gradient of **kasugamycin** concentrations.
  - ***Short-duration exposure.*** Bacteria incubated for 1 min in aqueous dilutions of **Quat 2 Plus, Deccosan 321, Deccosan 315, Vantocil, chlorhexidine, or kasugamycin**. Bacteria then plated out on agar to test viability.
- **Laboratory tests with inoculated olive twig pieces**

Objective: In vitro sensitivity of *P. syringae* pv. *savastanoi* to copper, kasugamycin, and selected sanitizers

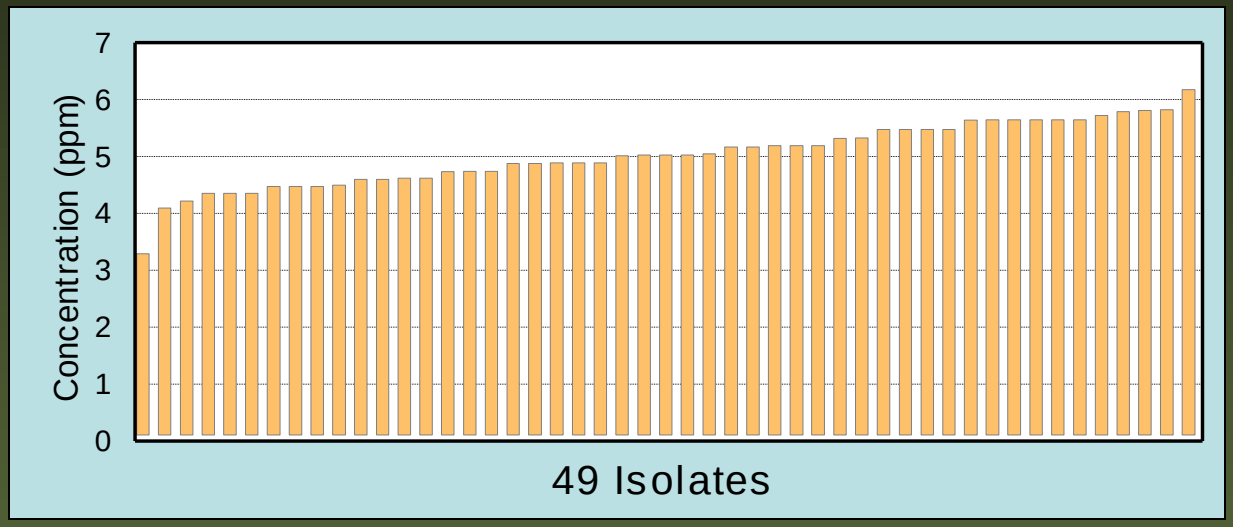
- ***Continuous exposure –*** Microtiter plate assay with copper in liquid medium



- Two sensitivity ranges found for copper among isolates.
- Testing of additional isolates is ongoing.

Objective: In vitro sensitivity of *P. syringae* pv. *savastanoi* to copper, kasugamycin, and selected sanitizers

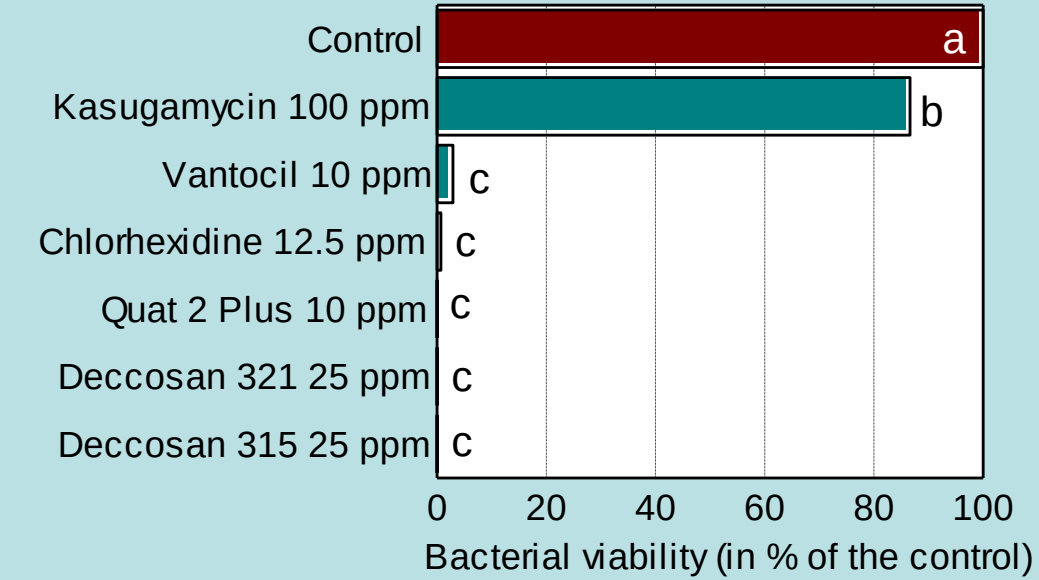
- ***Continuous exposure - Spiral gradient dilution assay for kasugamycin (Bacteriostatic Assay)***



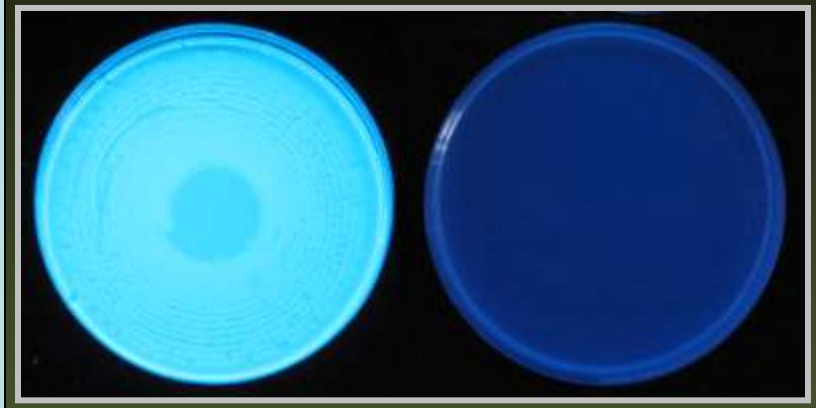
- Range of MICs (concentrations that inhibit growth by >95%) of 49 isolates: **3.3 to 6.2** ppm (mean 5.0 ppm)
- For comparison, range for *Erwinia amylovora* is approx. **3.4 to 25** ppm
- Arysta LifeScience is wiling to support registration on olive and efficacy data will need to be generated.

Objective: In vitro sensitivity of *P. syringae* pv. *savastanoi* to copper, kasugamycin, and selected sanitizers

Short-duration exposure assay (bactericidal assay).



Bacteria were incubated for 1 min in aqueous test solutions and then plated out on agar to test viability.

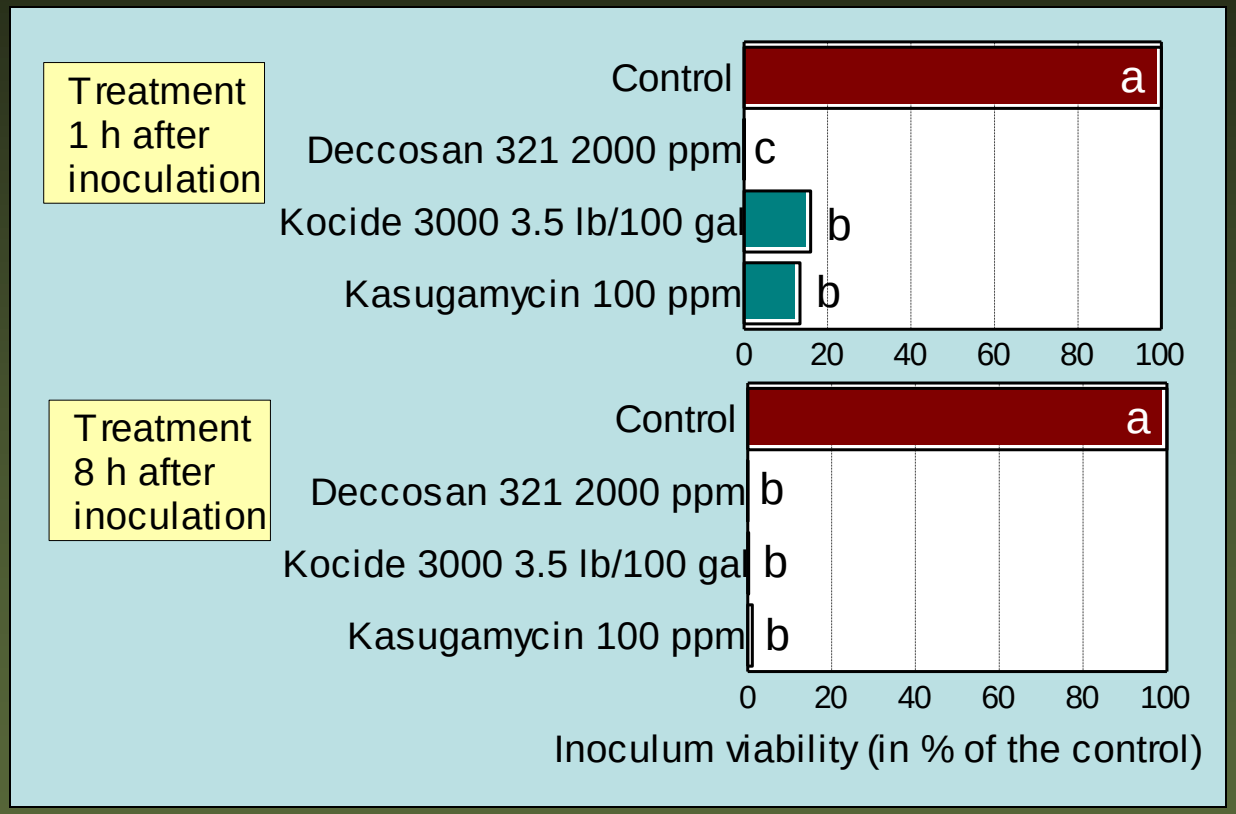


Viability test for bacteria of the control (left) and after exposure to Deccosan 321 (right). Plates are viewed under long-wave UV light.

- The five sanitizing solutions were all highly effective in inactivating *P. syringae* pv. *savastanoi* in 1-min exposures.
- Quaternary ammonia materials are federally registered for use on harvesting and pruning equipment on selected agricultural crops (tomato and citrus).
- Kasugamycin was not effective as a bactericidal compound.

Objective: In vitro sensitivity of *P. syringae* pv. *savastanoi* to copper, kasugamycin, and selected sanitizers

Laboratory tests with inoculated olive twig pieces



Olive twig sections (5 x 5 x 5 mm) were inoculated with *P. syringae* pv. *savastanoi* (5 x 10^6 cfu/ml) by dipping, treated after 1 or 8 h by dipping for 1 h, and washed in water for 20 min. Sections were then cut up, submersed in water and the aqueous suspension was plated out for viability testing.

Objective: Evaluation of copper, kasugamycin, and selected sanitizer treatments for the management olive knot

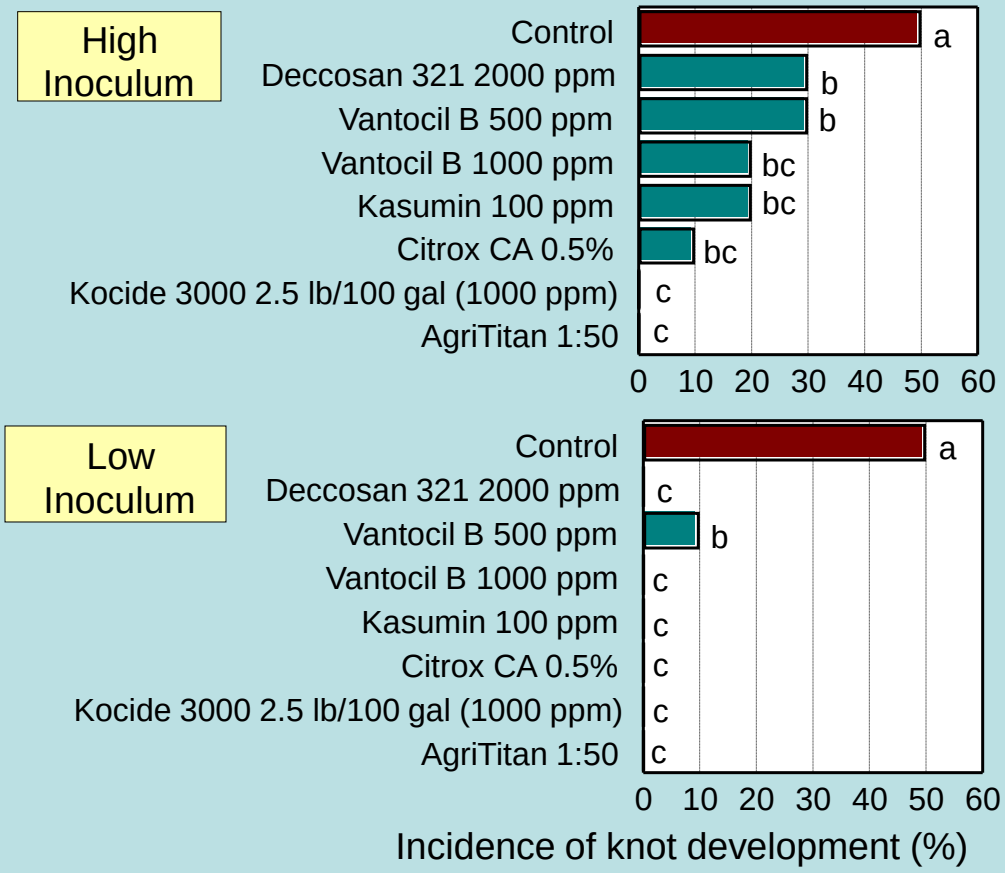
Greenhouse studies

Plants with leaf scar wounds were inoculated and then treated with selected bactericides.

First symptoms of olive knot started to develop after 1 month and final results are pending.

Objective: Evaluation of copper, kasugamycin, and selected sanitizer treatments for the management olive knot

Greenhouse studies – Preliminary results



Treatments applied 4 h after inoculation of leaf scars with a copper-sensitive strain of *P. syringae* pv. *savastanoi*

High inoculum = 10^8 cfu/ml
Low inoculum = 10^5 cfu/ml

Data recorded after 7 weeks

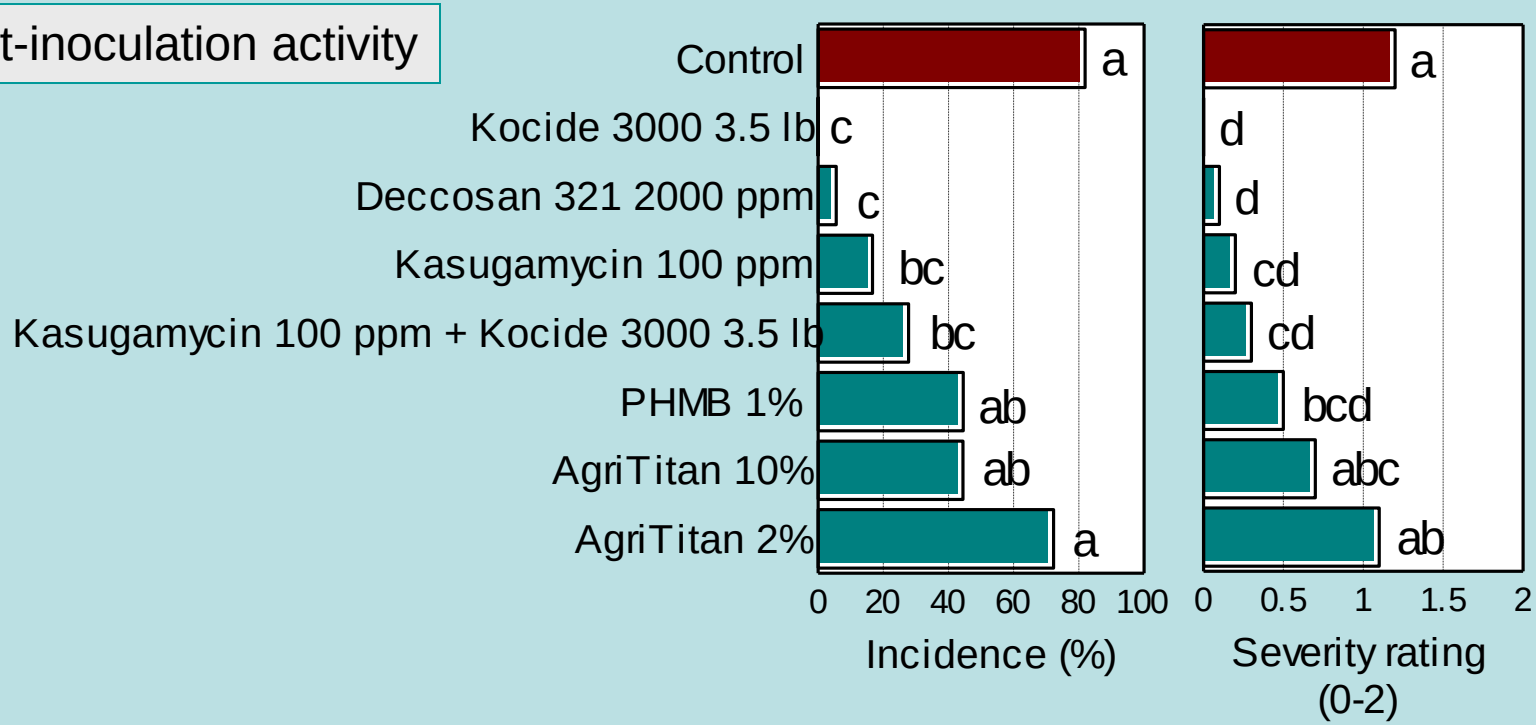
Objective: Evaluation of copper, kasugamycin, and selected sanitizer treatments for the management olive knot

Field studies on efficacy and timing

- **Pre- and post-infection activity:** Twig end cuts and side wounds were inoculated before or after hand- or air-blast sprayer applications (up to 7 treatments per trial).
- **Timing studies**: branches were inoculated and treated after 0, 1, 2, 3, or 7 days with Kasumin or Kocide 3000.
- **Large-scale field studies to control the natural incidence of olive knot:** Trees were harvested before a rain and then treated with Kasumin, Vantocil, Deccosan 321, or AgriTitan.
- Due to the long incubation time for olive knot, most results for these trials are pending.

Objective: Evaluation of treatments for the management olive knot in field studies

Post-inoculation activity



In early October, lateral wounds on olive twigs were inoculated with *P. syr*. pv. *savastanoi* (10^7 cfu/ml) sensitive to copper and treated by hand-spraying. Wounds were then wrapped with Parafilm. Disease was evaluated in mid-January 2012.

Summary

- **Collection, identification, and characterization of the pathogen using conventional and molecular approaches**
  - Molecular and conventional methods accomplished for positive identification
  - Diversity studies initiated to determine the heterogeneity of the pathogen
- **Pathology of olive knot**
  - Knots contain viable inoculum that can ooze the pathogen within 18 hr of wetness
  - Other organisms also associated with the pathogen in the diseased tissue
  - Duration of wound susceptibility ongoing
- **In vitro toxicity studies**
  - Copper toxicity studies indicate reduced sensitivity in some isolates of the pathogen.
  - Baseline studies being developed for Kasumin, bactericidal effects of other compounds identified

Summary

- **Studies on efficacy and timing are ongoing in commercial orchards and greenhouse trials -**
  - Studies to identify novel treatments including Kasumin, Vantocil, Deccosan 321, and Titan against olive knot.
  - Treated-inoculated vs. Inoculated-treated will help indicate the protective or post-infection activity of treatments, respectively.
    - **Preliminary results from field and greenhouse studies: Deccosan 321 and kasugamycin effectively inhibited olive knot development after inoculation**.
    - **AgriTitan was effective in the greenhouse, but not in the field, possibly because wounds were wrapped with Parafilm after treatment.**
  - Timing studies will help to determine the post-infection activity of antimicrobial treatments and duration of host susceptibility.

Future objectives in addition to current goals

- Collection, identification, and characterization of the pathogen using conventional and molecular approaches
  - Molecular characterization of copper resistance - plasmid screening
- Pathology of olive knot
  - Biological sources of resistance genes
- *In vitro* toxicity studies
  - Screen new synthetic bactericides – PP, Ti, SR-PPA
- Field studies on efficacy and timing are ongoing in commercial orchards and greenhouse trials - inoculation and natural incidence
  - Test persistence, pre- and post-infection activity, evaluate registration potential with IR-4
- Establish olive orchards at UC field stations