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Title: Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi (Psv)*

2016 Research Objectives:

1) **Continuation of quaternary ammonium compound (QAC) trials.**
   a. Evaluate the performance of the quaternary ammonium compound Deccosan 321 as an equipment sanitizer under field conditions in comparison to chlorine by itself and in conjunction with additional foliar treatments (copper and kasugamycin).
   b. Test the effect of pH on Deccosan 321 activity against Psv in direct contact assays.
2) **Efficacy of new bactericides.**
   a. Optimize the efficacy of antibiotic treatments (kasugamycin, oxytetracycline, streptomycin) against Psv in greenhouse and field trials using various formulations (technical and commercial grades), application timings, and additives (UV blockers, buffering agents, etc.)
   b. Develop copper activity-enhancing (CAE) materials such as Terrazole, Tanos, and amino-thiadiazole (ATD) when using maximum rates of copper.
   c. Field trials on the persistence of copper-antibiotic mixtures after a rain event using stickers and oils vs. hydrated lime.
   d. Field trials using high rates of copper mixed with antibiotics in tank mixtures as a resistance management strategy using copper-resistant strains of Psv.
3) **Epidemiology and management under different environmental conditions with copper-resistant strains of the pathogen.**
   a. Continue to conduct growth chamber studies to reproduce systemic infections of Psv.
   b. Determine if protective treatments can reduce infection of olives under low-temperature conditions using different rates and application timings
   c. Greenhouse studies on soil drench application of antibiotics (e.g., Kasumin) against olive knot systemic infections using potted olive plants.

Summary of Progress in 2016 including ongoing studies:

1a. We repeated field studies on the efficacy of Deccosan 321 for the sanitation of field equipment in the fall of 2015 and spring of 2016. Results indicated that sanitation of a *Psv*-contaminated hedger with Deccosan 321 before pruning significantly reduced the number of knots developing on pruning wounds on cvs. Arbequina and Manzanillo by 55% to ≥ 87.3%, as compared to sodium hypochlorite with 48%. When combined with an application of copper or copper-kasugamycin mixtures after pruning, disease incidence was reduced by ≥ 86%. Thus, sanitation of *Psv*-contaminated equipment is a highly effective method to reduce disease spread in mechanized olive production and low levels of disease result when these treatments are followed by spray applications with effective bactericides.

1b. The performance of Deccosan 321 against *Psv* was influenced by the pH of the contact solution. It was significantly more effective at pH 6, 7, 8, or 9 (mean log CFU mL^{-1} reduction of ≥ 3.5 = 99.9% reduction) with no significant differences between these pH values. At pH 5, the mean log CFU mL^{-1} reduction was

only 0.5. Therefore Deccosan 321 should be prepared in solution with near neutral or slightly basic pH to obtain maximum biocidal activity.

2a. In an IR-4 efficacy trial, kasugamycin was significantly more effective than oxytetracycline; providing management of olive knot similar to copper. Wound inoculation studies were done over the winter and early spring season. Evaluation of additives to prevent the rapid degradation of oxytetracycline is ongoing.

2b. Field trials were performed in the fall of 2015 and evaluated in the spring of 2016 to test copper treatments (at maximum field rates) mixed with etridiazole (Terrazole), amino-thiadiazole-thiol (ATD), mancozeb (Manzate Prostick), famoxadone + cymoxanil (Tanos), and dodine (Syllit) to possibly improve copper efficacy against a copper-resistant *Psv* strain. On cv. Arbequina, copper at low rates was the least effective in reducing knot formation on lateral wounds (65% reduction), whereas copper at the high rate alone and in mixtures reduced disease by ≥ 75%. The copper-Tanos treatment resulted in no disease. On leaf scar wounds, low rates of copper reduced disease by 16.2%; whereas copper-Tanos was again the best treatment with an 83.8% reduction. On lateral wounds of cv. Manzanillo, low rates of copper resulted in a 29.6% reduction, whereas high rates of copper reduced knot formation by 37%. Copper-Terrazole was the best treatment with a reduction of 96.3%, followed by copper-Manzate (85.2% reduction) and copper-ATD (70.4% reduction). On leaf scar wounds, treatments with copper-Tanos had the best efficacy. These results indicate that copper activity can be improved, but more studies are needed to identify the most effective mixture partners.

2c. The persistence of copper and copper-antibiotic treatments on wounds inoculated with a copper-resistant *Psv* strain after a 30-min simulated rain event was studied in the fall of 2015 and again evaluated in the spring of 2016. On cv. Manzanillo, copper hydroxide (Kocide 3000)-oxytetracycline-NuFilm treatments were more effective than copper alone or other mixtures, reducing disease by 37.5% and 45.7% on leaf scar and lateral wounds, respectively. A basic copper (i.e., Cuprofix Disperss) mixed with hydrated lime was not effective. On lateral twig wounds of cv. Arbequina, all mixture treatments were similarly effective as copper hydroxide alone, but again basic copper was not effective.

2d. Among the antibiotics tested, kasugamycin was significantly more effective than oxytetracycline or streptomycin and the efficacy of all antibiotics was generally improved when mixed with copper hydroxide.

3a. Additional low-temperature growth chamber greenhouse studies were completed in 2015. Systemic movement of the pathogen was only observed on trees that were wounded and inoculated before exposure to low temperatures. No movement was noted on wounds inoculated after cold exposure and no knots formed on unwounded plants inoculated before or after cold exposure. Low-temperature studies with olives are difficult to do because many twigs die due to frost injury before disease can be evaluated. Current data indicate that systemic movement of *Psv* mostly occurs, when plants are infected before a freezing event, and plants are not killed by the low-temperature exposure.

3b. Ongoing

3c. Application of kasugamycin as a soil drench treatment 3 or 6 days before wounding and inoculation of olive twigs was not successful in reducing the incidence of knots. This could be due to low uptake of the antibiotic by the root system, or to its rapid degradation in the soil. Additionally, kasugamycin injection treatments were performed on mature olive trees (cv. Arbequina) with an EnTree injection system (Brandt Consolidated, Inc.). Large branches were injected several inches below a large knot to test whether the treatment could reduce or eradicate *Psv* within the knot. Knots were sampled for *Psv* recovery several months after inoculation. Injections did not eradicate Psv inside knots and viable bacteria were recovered. Phytotoxicity (branch dieback and leaf drop) was observed, indicating that kasugamycin moved in the water transpiration stream.

Conclusions

Data presented in this interim-report add information on improving the management of olive knot. QAC sanitizers are effective in reducing inoculum of *Psv*-contaminated field equipment, thus reducing the spread of the pathogen to healthy trees. Application of foliar treatments of copper or of copper-kasugamycin mixtures to pruning wounds can further reduce knot formation. QACs (specifically Deccosan 321) perform well on a broad pH range from 6 to 9, but acidic solutions (pH ≤ 5) have a negative effect on QAC biocidal activity.

The addition of selected activity enhancing materials to copper treatments improved disease control. ATD, mancozeb, and etridiazole are not likely to be registered on olive, but Tanos has potential for registration. We are currently testing other enhancing materials on other bacterial plant diseases and identified SBH as very promising compound. In preliminary in vitro studies, SBH significantly increased the performance of copper against a copper resistant *Psv* strain. We plan to evaluate SBH in field trials.

Effective control of olive knot caused by a highly virulent, copper-resistant *Psv* strain could be achieved using high rates of copper hydroxide (equivalent to 2.1 lbs MCE/A). The addition of kasugamycin or oxytetracycline further improved performance. Using a copper sensitive strain, copper alone and kasugamycin were highly effective in reducing disease incidence. In persistence studies, high rates of copper mixed with oxytetracycline and NuFilm were the best treatments against a copper resistant strain. Several oxytetracycline formulations were not as effective as kasugamycin when used alone, but increased copper performance in mixtures. Studies are ongoing to improve persistence of oxytetracycline.

We demonstrated that systemic movement of *Psv* can occur due to frost damage, but studies to evaluate protective treatments under low temperatures are ongoing.