**ANNUAL RESEARCH REPORT**

**California Olive Board and California Olive Oil Commission**

**December 2015**

Project Year:   2015

Principal Investigators: J. E. Adaskaveg

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

Cooperating:   D. Thompson, K. Nguyen, H. Förster (UC Riverside), D.M. Lightle (UCCE - Glenn Co.), and E. Fichtner (UCCE-Tulare Co.)

Keywords: Bactericides, Biological controls, and Systemic Acquired Resistance (SAR) compounds

## BACKGROUND

The bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv) is the causal agent of olive knot and occurs throughout olive (*Olea europaea*) growing regions of the world including California (Young, 2004). The pathogen enters through wounds causing hyperplastic outgrowths (knots, tumors, galls, etc.) on branches and infrequently on leaves and fruit. Olive knot is one of the most economically important diseases of olives as infection may lead to tree defoliation, dieback, and reduced tree vigor, which ultimately lowers fruit yield and quality (Schroth, 1973). Psv can be found as both an endophyte and epiphyte of the olive phyllosphere, but the main source of inoculum are Psv residing in olive knots. Inoculum production of the pathogen is promoted during wet periods when it is exuded from knots and disseminated by rain, wind, insects, birds, as well as human activity. We demonstrated that inoculum is produced very rapidly after wetting olive knots. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission, frost, and hail damage, as well as cultural practices such as pruning and harvesting. These latter orchard practices also lead to direct mechanical damage of the knots and exposure of inoculum. After entering its woody host, the pathogen actively induces knot formation by production of indoleacetic acid (IAA) and cytokinins. In California, infections occur mostly during the rainy season (late fall, winter, and spring) but knots do not develop until active growth initiates in the spring. Infections can occur at fairly low temperatures (5-10 C) and thus, wetness is the main limiting factor for the disease. Historically, the most susceptible table olive cultivars are Manzanillo, Sevillano, Ascolano, and Mission but the oil cultivars are also very susceptible. None of the currently grown olive cultivars is resistant to the pathogen. For our studies, we are using cvs. Manzanillo and Arbequina, and in our experience both can have high incidence and severity of disease. Development of olive knots on wounded, inoculated branches depends on inoculum concentration, environmental conditions, and olive cultivar (Penyalver, 2006).

We determined the minimum threshold inoculum concentration for cvs. Manzanillo and Arbequina for knot development. Management strategies should keep Psv populations below these threshold values. Knot formation is usually localized to the initial entry point of the bacterium. Systemic movement of the pathogen, however, has also been observed in rare cases (Wilson and Magie, 1964). In spring 2014 evaluations of our fall 2013 trials in commercial and experimental olive orchards, we observed systemic movement of Psv which we never observed in any of our previous trials. Infections caused bark blistering and cracking as well as development of knots in proximity to and away from the initial point of inoculation. In most severe cases, inoculated branches and whole trees died. Potential causes of systemic movement have not been well characterized. Thus, one of our objectives is to determine environmental or other factors leading to these symptoms and whether the pathogen is migrating internally or externally on the host. In preliminary investigations, we have been able to reproduce environments in growth chamber studies that lead to systemic movement of the bacterium in olive plants. More detailed studies are currently in progress and some preliminary studies are reported here. This information will contribute to knowledge on the epidemiology of the pathogen and possibly identify new management strategies.

Sanitation and prevention are the most successful strategies for management of olive knot. Any horticultural practice that promotes tree health, minimizes tree stress, and results in less leaf drop will reduce infections. Pruning and removal of knots during dry periods (i.e., summer and early fall) reduces inoculum and avoids re-infection at pruning sites. Because the bacteria may be carried on pruning equipment, frequent disinfection of equipment is necessary. Painting galls with Gallex is an effective therapeutic treatment but is very labor intensive and is considered impractical. Spray applications of copper-containing bactericides have been very effective in minimizing the disease, however repeated applications are generally needed to protect wounds as they occur over the year. A minimum of two applications is usually necessary: one in the fall immediately after harvest (before the rainy season) and a second one in the spring just prior to leaf drop. Additional applications may be needed during winter rains or spring/summer hail-storms. New copper formulations have been developed to reduce the metallic copper equivalent while maintaining the efficacy of the treatment. Our evaluations of copper sensitivity in populations of the olive knot pathogen indicated a reduced sensitivity of all strains with several strains showing an increased level of resistance. These results demonstrate a potential risk of resistance development of Psv to copper with its continued and often exclusive use. Although the combination of copper and mancozeb is highly toxic to strains of Psv less sensitive to copper, the EPA will not allow additional crops to be added to the mancozeb label. Thus, we initiated a search for other compounds that could be mixed with copper to increase its activity. We have identified amino-thiadiazole (ATD), a food-grade additive, as a synergistic compound that increases the activity of copper against copper sensitive and less sensitive strains in the laboratory. Field trials have been completed in fall of 2014 and spring of 2015. Still other compounds need to be developed to reduce exposure of any one mode of action to populations of the pathogen. Additionally, because olive knot infections occur mostly during the rainy period, knowledge on the persistence of treatments is critical. Thus, the efficacy of copper and other compounds like antibiotics alone and in mixtures with adjuvants that may increase the persistence of these treatments has been evaluated.

We have been instrumental in the development of the new agricultural antibiotic kasugamycin (Kasumin) for several bacterial diseases of agronomic crops in the United States. Kasugamycin has high activity against *Erwinia* and *Pseudomonas* species and moderate activity against *Xanthomonas* species and other plant pathogenic bacteria. A second antibiotic oxytetracycline (Mycoshield, Fireline) has also been identified. We found these compounds to be the most promising new treatment for preventing olive knot in our field studies, including in a commercial application to inoculated branches. Additional field trials have been performed to compile data to support the registration of kasugamycin and oxytetracycline on olives. Kasugamycin and oxytetracycline are currently federally registered on pome fruit crops (e.g., apples and pears), whereas their use on olives has been approved as "A" priorities by IR-4 for the 2015 and 2016 seasons. We are involved with these IR-4 residue studies. Other products including antimicrobial peptides from commercial sources were also evaluated in 2015. Several systemic acquired resistance (SAR) compounds (e.g., Actigard, Regalia, quinoxyfen - Quintec, and USF2018A) were effective in our previous studies, but not equivalent to copper or kasugamycin. New trial data from 2015 suggest that it may not be possible to achieve consistent disease control using SAR compounds by themselves, and their development in combination with conventional treatments such as with antibiotics and copper may be more realistic.

We have also been working on sanitation treatments as part of an integrated olive knot management program. We demonstrated that guanidine, chlorhexidine, and quaternary ammonia compounds (QACs) were highly toxic against the olive knot pathogen in laboratory studies. Citrox, a natural product derived from citrus extracts, and the quaternary ammonia sanitizers were also highly effective in disinfecting hard surfaces that were contaminated with Psv. The QACs are volatile, leave near zero residues, and are not corrosive to equipment. Deccosan 321 (MaQuat 615-HD) was registered for use on olives in California in 2015. We initiated field trials in the spring of 2015 to compare QAC performance to chlorine in reducing the spread of Psv on olives from contaminated field equipment. We will test additional parameters that may affect the efficacy of the sanitizers such as inoculum concentration and post-inoculation treatment time. Field evaluations of the material as an equipment sanitizer was accomplished this year with very promising results. Additionally, we evaluated a new non-phenolic QAC, KleenGrow, for use as a protective treatment directly on trees. Unfortunately, KleenGrow was not effective when used as a protective treatment in a foliar spray on olive wounds before inoculation.

## Research Objectives

1) Epidemiology – pathogen genetic variability, inoculum availability, threshold inoculum level for disease induction, systemic movement of Psv
   a. Monitor galls for production of inoculum over time
   b. Evaluate the effects of inoculum concentration on disease development
   c. Investigate environmental factors that may lead to systemic movement of Psv
   d. Track the systemic movement (endophytic or epiphytic) of Psv on the olive host using selective re-isolation techniques and microscopy
2) Evaluate populations of the pathogen for laboratory sensitivity to chemicals
   a. Population dynamics of copper-resistant in relation to copper-sensitive strains of Psv
3) Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions in comparison to chlorine.
4) Field trials on efficacy of bactericides and SAR compounds.
   a. Protective (pre-infection) vs. post-infection activity of treatments; proper timing and application of SAR compounds; effects of inoculum concentration on the efficacy of SAR compounds
   b. Develop copper activity-enhancing materials such as mancozeb, amino-thiadiazole (ATD), and dodine
   c. Determine the efficacy of a new, non-phenolic-based quaternary ammonium formulation (i.e., KleenGrow) for use as a protective treatment on olives
   d. Persistence of different copper formulations with and without the addition of lime, pinolene, or carnauba-based additives under simulated rain conditions.

## Materials and Methods

***1a. Monitor galls for production of inoculum over time.*** Olive knots attached to twigs were washed and sampled after selected time periods in order to enumerate Psv population levels and to determine the extent of inoculum produced or exuded by knots during multiple rain events.

***1b. Evaluate the effects of inoculum concentration on disease development.*** Greenhouse and field trials were performed on cvs. Manzanillo and Arbequina to investigate the effects of Psv inoculum concentration on disease incidence for leaf scar and lateral wounds inoculated with either a copper-sensitive or copper-resistant strain. Olive twigs were wounded and inoculated with selected concentrations of these strains ranging from $2x10^5$ to $2x10^8$ CFU/ml and scored for disease incidence after symptoms (knots) developed. In field studies, treatments of copper hydroxide or kasugamycin were applied to wounds that were inoculated with various inoculum concentrations to examine treatment efficacy under different disease pressures. A greenhouse trial was carried out in the spring of 2015 at UCR. Field trials at UC Davis were done in the fall of 2014 and were repeated in the spring of 2015. All field plant inoculation studies that are described in this report have durations of at least four to six months because knots do not develop until plant growth occurs. In growth chamber and greenhouse studies, young and succulent plants that are continuously growing were incubated for 2 to 3 months before symptoms developed.

***1c. Investigate environmental factors that may lead to systemic movement of Psv.*** Young potted olive trees of cvs. Manzanillo were placed into a growth chamber and exposed to -5°C for 8 h. Multiple inoculation and wounding scenarios that were tested included:

i. Wounding and inoculating plants with Psv before placing into the cold chamber. This experiment simulates olive trees that are damaged and inoculated (i.e., hail storm, harvest damage followed by rain event, etc.) before occurrence of a freezing event.
ii. Spray inoculating plants without wounding before placing into the cold chamber. This determines if a freezing event creates Psv-susceptible tissue that can be infected by the bacterium that is already present on the surface during the event (Psv pre-existing as an epiphytic colonizer).

iii. Wounding and placing plants into the cold chamber and inoculating wounds afterwards. This simulates olive trees that are wounded (mechanically or naturally) and are then exposed to a freezing event with subsequent rain and inoculum dispersal.

iv. Placing plants into the chamber followed by spray inoculating the whole plant. This determines if a freezing event creates Psv-susceptible tissue that can be infected when the bacterium is introduced afterwards (i.e., rain dispersal of Psv from knots after a freezing event).

These scenarios allow us to elucidate whether freezing damage can predispose olives to Psv infection by creating new wounds or increasing colonization of existing tissue damage, and if the migration of the bacterium on frost-damaged tissue is external, internal, or possibly both.

*1d. Track the systemic movement (endophytic or epiphytic) of Psv on the olive host using selective re-isolation techniques and microscopy*. Plants used in objective 1c. studies will be further evaluated by tracking movement of the bacterium using selective re-isolation techniques and microscopy. Olive twig tissue samples will be taken at various distances away from the initial inoculation point over several months to monitor movement of the bacterium. Tissue will be examined using scanning electron microscopy as well as re-isolating Psv on selective media. The Psv strain used will have unique characteristics (copper-resistance) that will allow for the discrimination of the inoculated strain from Psv strains that may be residing epiphytically on the olives (although all plants used did not have olive knot symptoms).

*2. Population dynamics of copper-resistant and copper-sensitive strains of Psv*. Additional strains of Psv were collected from an orchard in Glenn Co. where a copper-resistant Psv strain was recovered previously. Strains were tested for sensitivity to copper using a serial dilution method and for sensitivity to the antibiotics kasugamycin, oxytetracycline, and streptomycin using the spiral gradient endpoint (SGE) method.

*3. Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions in comparison to chlorine*. The quaternary ammonium compound (QAC), MaQuat 615-HD was tested in field trials in the spring of 2015 and compared to sodium hypochlorite (bleach solution) in reducing the spread of Psv by contaminated field equipment. We utilized a handheld gas-powered hedger to simulate larger commercial pruning equipment. The hedger was used to trim and injure olive branches, simulating damage that would likely occur during commercial pruning operations. The hedging blades (metal teeth) were contaminated (sprayed with a suspension of Psv) and the hedger was subsequently used to prune healthy (symptomless) trees. For treatments, the contaminated blades were sprayed with selected disinfectants at experimental or labeled rates and exposure durations before pruning trees. In some treatments, hedging was followed by additional copper and copper-kasugamycin foliar applications on newly hedged olives to possibly obtain greater reduction in disease incidence. These trials were performed at UC Davis.

*4a. Protective (pre-infection) vs. post-infection activity of SAR compounds and effects of inoculum concentration on the efficacy of SAR compounds.* SAR compounds were field-tested against olive knot during the fall of 2014 and evaluated in the spring of 2015, focusing on the effects of Psv inoculum concentrations. Foliar sprays of SAR compounds were applied to entire cvs. Manzanillo and Arbequina olive trees until runoff 3 days before wounding and inoculating with a copper-sensitive Psv strain. Psv inoculum concentrations ranged from $2x10^5$ to $2x10^8$ CFU/ml. SAR compounds evaluated included Regalia, Proalexin, Stout, Actigard, and Quintec at experimental or field labeled rates.

*4b. Develop copper activity-enhancing materials such as mancozeb, amino-thiadiazole (ATD), and dodine.* Field trials were performed during the fall of 2014 and spring of 2015 in two olive orchards (UC Davis and Yuba Co.) to test copper treatments mixed with etridiazole (Terrazole), amino-thiadiazole-thiol (ATD), mancozeb (Manzate Prostick), famoxadone + cymoxanil (Tanos), or dodine (Syllit) to determine if any enhancement in disease control could be achieved as compared to copper alone. Treatments using kasugamycin at low (100 ppm/A) and high rates (200 ppm/A) were also evaluated along with high rates of copper hydroxide (7 lb/A).

*4c. Determine the efficacy of a new, non-phenolic-based quaternary ammonium formulation (i.e., KleenGrow) for use as a protective treatment on olives.* The non-phenolic quaternary ammonia compound KleenGrow was tested as a protective treatment in a greenhouse trial in fall of 2014 and in several field trials

in the fall of 2014 and spring of 2015. KleenGrow treatments were applied to olive twig wounds before being inoculated with a Psv suspension.

***4d. Persistence of different copper formulations with and without the addition of lime, pinolene, or carnauba-based additives under simulated rain conditions.*** A copper persistence trial was performed on young cv. Manzanillo trees in the fall of 2014 and repeated in the spring of 2015 at UC Davis. Olive twigs were wounded and treated with several copper and copper-adjuvant treatments (lime – calcium hydroxide, pinolene – NuFilm-P, or a carnauba-based additive – Washgard). After air-drying, trees were overhead irrigated with micro-misters to simulate a 30-min rain event. Treated wounds were then spray-inoculated with a copper-sensitive Psv strain.

## Results and Discussion

***1a.*** **Monitor galls for production of inoculum over time.** This trial is currently in progress. Results are pending and will be available in the next report.

***1b. Evaluate the effects of inoculum concentration on disease development.*** In greenhouse trials performed during the spring of 2015, similar results were obtained as reported in 2014. Leaf scars were less susceptible to infection as compared to lateral wounds except for the highest inoculum concentration for both cultivars and strains tested. Higher disease incidence was observed for the higher Psv concentrations ($2x10^7$ and $2x10^8$ CFU/ml) in most cases, but other factors may contribute to disease development (e.g., the growth stage of olive plants – when growth is less active, fewer knots develop). Knots were also substantially larger on some plants while much smaller or absent on others. Inoculated young, succulent, green twigs produced knots more readily than older woody twigs. Both copper-sensitive and -resistant strains were equally virulent under greenhouse conditions.

In field trials conducted during the fall of 2014 at UC Davis using the same inoculum concentration range and a copper-sensitive Psv strain, higher disease incidence was observed on cv. Arbequina than on cv. Manzanillo for both leaf scar and lateral wound inoculations. Again, inoculated leaf scars typically developed fewer knots at the lower inoculum concentrations (incidence of 12.5 and 22.5% for cvs. Manzanillo and Arbequina, respectively, using $2x10^5$ CFU/ml) while lateral wounds had high levels of disease for all concentrations ranging from 45-75% and 80-100% incidence for cvs. Manzanillo and Arbequina, respectively. Copper hydroxide performed well in reducing disease incidence at all inoculum concentrations on both lateral and leaf scar wounds for either cultivar ($\leq$ 22.5% incidence). Kasumin 2L at the 100-ppm rate reduced disease on lateral wounds when lower inoculum concentrations were used (Fig. 1). The different slopes of the regression lines indicate that bacterial concentration affected the performance of kasugamycin more than that of copper.

Past trials have shown that concentrations of $2x10^5$ CFU/ml of Psv can produce some disease depending on plant age, wound type, and Psv strain. Also, inoculated greenhouse plants had higher disease incidence than field grown plants and knots developed faster (2.5 months in the greenhouse vs. 4-6 months in the field). Thus, $2x10^5$ CFU/ml could be considered a threshold concentration for disease induction. Consistent high levels of disease resulted when plants were inoculated with $2x10^7$ CFU/ml Psv, and somewhat less consistent disease levels were achieved with $2x10^6$ CFU/ml. We have found that these higher concentrations may potentially be exuded from living knots but dilution occurs during their dispersal due to precipitation, runoff, or plant canopy size. Thus, reduced inoculum levels are likely present on susceptible tissue. Therefore, treatments that are less effective when artificially inoculated with a substantial amount of bacteria may not be indicative of actual effectiveness under field conditions. Thus, we evaluated treatment efficacy using very high disease pressure. High copper concentrations are needed to maintain some level of effectiveness even under these conditions and higher rates of kasugamycin showed similar results in our chemical field trials (see data for objective 4b). The cultivars Manzanillo and Arbequina had some variability in disease incidence among trials, but both should be considered as highly susceptible to Psv.

***1c. Investigate environmental factors that may lead to systemic movement of Psv.*** In a growth chamber-greenhouse study performed in October 2015, olive plants that were wounded and inoculated developed typical knots localized to the wound after 2.5 months. Only in scenario (ii) where plants were wounded, inoculated, and then exposed to cold did we observe symptoms of systemic movement. Small nodules were

noted several centimeters from the point of inoculation. On some branches, nodules were produced even more than 10 cm away from the inoculation point. Interestingly, for twigs that were wounded and cold exposed before inoculation, no knots developed away from the inoculation point. Symptoms were examined recently (10 weeks after inoculation), and nodules will be sampled and used for re-isolation at a later time to confirm the presence of Psv. Eight hours of cold exposure was too severe for cv. Manzanillo plants because this caused major branch dieback and no data could be obtained. Repeat trials will limit cold exposure to 4 h to reduce dieback while still providing some frost damage. Non-wounded plants that were spray-inoculated before or after cold exposure have presently not developed any symptoms. Thus, existing injury may be necessary for infection, and subsequent frost damage may assist in systemic movement of the bacterium. In these preliminary studies, cold injury alone did not facilitate infection of Psv. Additional studies will be undertaken in 2016 to repeat the current study as well as including the addition of protective treatments on frost-injured plants inoculated with Psv to determine if treatments can reduce disease intensity.

***1d. Track the systemic movement (endophytic or epiphytic) of Psv on the olive host using selective re-isolation techniques and microscopy***. This is pending on development of symptoms (on surviving branches of objective 1c) as well as repeating the trial with shorter cold exposure duration to reduce branch dieback.

***2. Population dynamics of copper-resistant in relation to copper-sensitive strains of Psv.*** An additional 20 Psv strains were recovered from a location where we previously detected copper-resistance. Copper sensitivity tests indicated that 2 and 3 of the 20 strains obtained were resistant (>50 mg/L MCE) or moderately (20 to 30 mg/L MCE) resistant to copper, respectively. All strains, however, were sensitive to the antibiotics kasugamycin, streptomycin, and oxytetracycline (Tables 1 and 2).

***3. Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions in comparison to chlorine***. Deccosan 321 performed exceptionally well when used to sanitize pruning equipment that was contaminated with a high concentration of Psv (Fig. 2). For disease control on Manzanillo olives, Deccosan 321 sanitation alone was similar in efficacy as compared to chlorine or the Deccosan plus subsequent copper or copper-antibiotic foliar treatment. On cv. Arbequina, additional foliar applications of copper hydroxide or of copper hydroxide-kasugamycin mixtures to newly hedged olives significantly decreased the occurrence of olive knots from that of the chlorine treatment and numerically improved the performance from the Deccosan alone treatment. Equipment sanitation with sodium hypochlorite was also effective, but with less disease reduction as compared to Deccosan 321 (Fig. 2).

***4a. Pre-infection (protective) vs. post-infection activity of SAR treatments and effect of inoculum concentration on the efficacy of SAR compounds.*** Most SAR compounds tested did not significantly reduce disease incidence as compared to controls treated with water. At the lowest concentration of Psv, Proalexin resulted in a significant decrease in disease incidence on lateral wounds (3.3% incidence) as compared to the water control with 27% incidence in one study. In a few cases, Quintec resulted in some reduction of knot formation when trees were inoculated with $2 \times 10^6$ CFU/ml Psv, but not to satisfactory levels. Still, none of the SAR treatments provided a consistent reduction in disease. Possible explanations include: rates evaluated may not have been sufficient for activating plant defensive mechanisms, timing of application was not appropriate, or these compounds may not trigger a SAR reaction in olive plants. In comparison, Kocide 3000 at 3.5 lbs/A that was used as a control treatment in these studies provided high and consistent levels of disease control for the entire range of Psv inoculum concentrations used.

***4b. Develop copper activity-enhancing materials such as mancozeb, amino-thiadiazole (ATD), and dodine.*** In the fall 2014 Davis trial on cv. Arbequina where a copper-sensitive Psv strain was used, all copper-containing treatments performed similarly, reducing disease incidence by at least 74% on lateral wounds (Fig. 3). On cv. Manzanillo, similar results were obtained using the copper-sensitive strain, with disease incidence reduced by treatments containing copper by at least 90% (Fig. 4). When a copper-resistant strain was used in the fall 2014 trial, copper alone at the highest labeled rate (7 lb/A) performed better than any of the mixture treatments on lateral wounds of both cultivars (disease reduction by at least 74%; Figs. 5 and 6).

In the fall 2014 trial in the Yuba county cv. Arbequina orchard, the 7-lb rate of copper again was the best treatment on lateral wounds reducing disease by 96 or 83% using a copper-sensitive or -resistant strain, respectively (Fig. 7a and 8a). Kocide 3000 (3.5 lb/A) - mancozeb (2.4 lb/A) mixture treatments performed equally well to copper (7 lb/A) for a copper-sensitive strain (Fig. 7), while kasugamycin at 200 ppm worked

well against a copper-resistant strain (Fig. 8). Data from the repeat trial in spring of 2015 indicated that copper-containing and kasugamycin treatments performed well in reducing knot incidence when a copper-sensitive strain was used (Figs. 7). Using a copper-resistant strain, kasugamycin containing treatments and copper at the highest rate were the best treatments on lateral wounds (Fig. 8).

In summary, the addition of experimental compounds to copper hydroxide did not improve copper performance compared to copper alone in most cases, and copper at the maximum labeled rate gave excellent disease reduction, especially to copper-sensitive Psv strains. Kasugamycin at 200 ppm/A gave comparable results to high rates of copper. Thus, mixtures of copper at maximum rates with high rates of kasugamycin should give exceptional olive knot control and field tests have been performed to evaluate this treatment in the fall of 2015 (results pending). This may be the best strategy for disease prevention and the mixture with two modes of action will minimize the development and spread of copper and potential kasugamycin resistance. All field trials performed in fall of 2014 and spring of 2015 included lateral wound inoculations, and leaf scar wounds will be examined in future studies using different concentrations of Psv inoculum.

*4c. Determine the efficacy of a new, non-phenolic-based quaternary ammonium formulation (i.e., KleenGrow) for use as a protective treatment on olives.* KleenGrow did not reduce knot incidence when sprayed at the maximum labeled rate (0.38 fl oz/gal) to wounds that were subsequently inoculated with Psv in all trials (greenhouse and field). The labeled rate may not be effective for olive knot control or this material may not be effective when used as a protective treatment directly on olives.

*4d. Persistence of different copper formulations with and without the addition of lime, pinolene, or carnauba-based additives under simulated rain conditions.* In the fall 2014 trial, all copper treatments significantly reduced disease incidence on inoculated lateral wounds as compared to the control. No disease developed on wounds treated with Kocide 3000 (7 lbs/A) or Kocide 3000 (3.5 lbs/A) - Washgard (2.5 gal/A) (Fig. 9). Still, there was no significant difference in disease incidence between the high rate of copper and treatments with the half-rate of copper mixed with adjuvants (e.g., Washgard, Quintec, Omni Oil, or NuFilm). In a spring 2015 repeat trial, copper at the high rate had the lowest disease incidence numerically, but statistically was similar to all other Kocide treatments with adjuvants (Fig. 9). The low rate of Kocide (3.5 lb/A) without adjuvants had statistically higher disease incidence than the high rate.

## Acknowledgements

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**Table 1.** In vitro sensitivity to copper of 20 Psv strains collected from one orchard in Glenn Co. in 2015

| Copper sensitivity* | No. strains |
|---------------------|:-----------:|
| Sensitive | 15 |
| Moderate | 3 |
| Resistant | 2 |
| Total | 20 |

\* - Sensitivity to copper was determined by growing strains on media amended with 0, 10, 20, 30, or 50 mg/L metallic copper equivalent (MCE). Copper sensitive: growth at ≤10 mg/L MCE; moderate copper sensitivity: growth at 20 and 30 mg/L MCE; and copper-resistant: growth at ≥50 mg/L MCE.

**Table 2.** In vitro sensitivity to three antibiotics of 20 Psv strains collected from Glenn Co. in 2015

| Oxytetracycline | | Streptomycin | | Kasugamycin | |
|:---:|:---:|:---:|:---:|:---:|:---:|
| LIC* | MIC | LIC | MIC | LIC | MIC |
| 0.15 | 0.23 | 0.15 | 0.4 | 2.46 | 4.92 |

\* - Average lowest inhibitory concentrations (LIC) and minimal inhibitory concentrations (MIC; growth inhibited by ≥95%) for 20 Psv strains from an olive orchard where copper resistance was previously detected. Inhibitory values were determined using the SGE method.

**Fig. 1. Effect of inoculum concentration and pre-infection foliar spray treatments on disease incidence of cvs. Arbequina and Manzanillo olives in field trials in the fall of 2014**
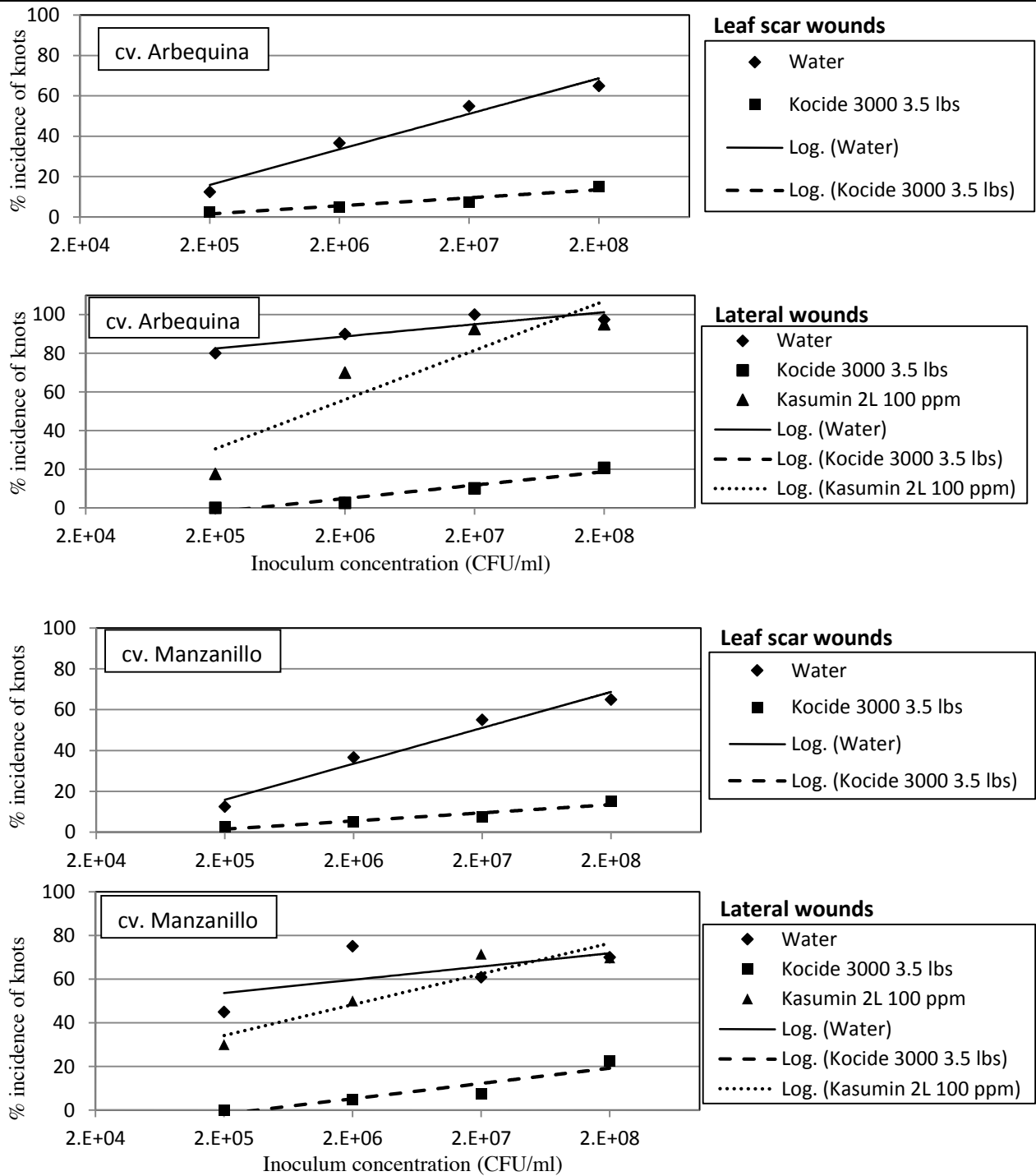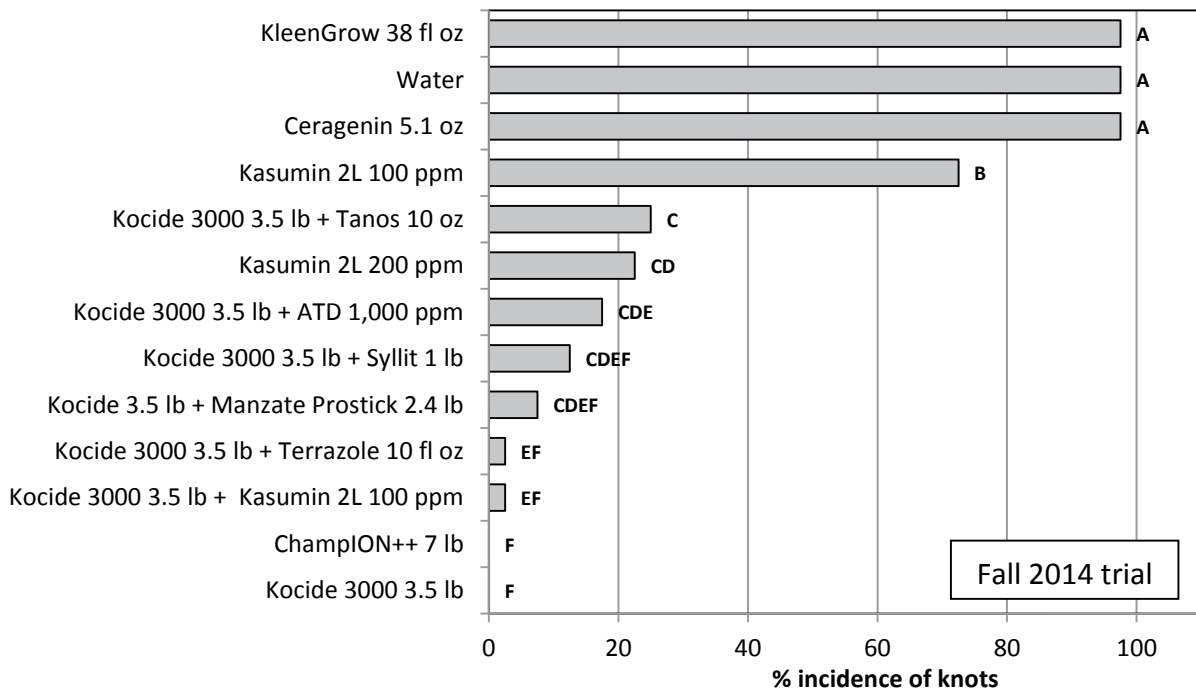


Branches were wounded (leaf scar and lateral wounds) and inoculated with selected concentrations ($2 \times 10^5$ to $2 \times 10^8$ CFU/ml) of a **copper-sensitive** Psv strain. Some wounds were also spray-treated with Kocide 3000 or Kasumin 2L. Knot development on inoculated wounds was evaluated 9-months post-inoculation.

**Fig. 2. Performance of Deccosan 321 (QAC) as an equipment sanitizer under field conditions**
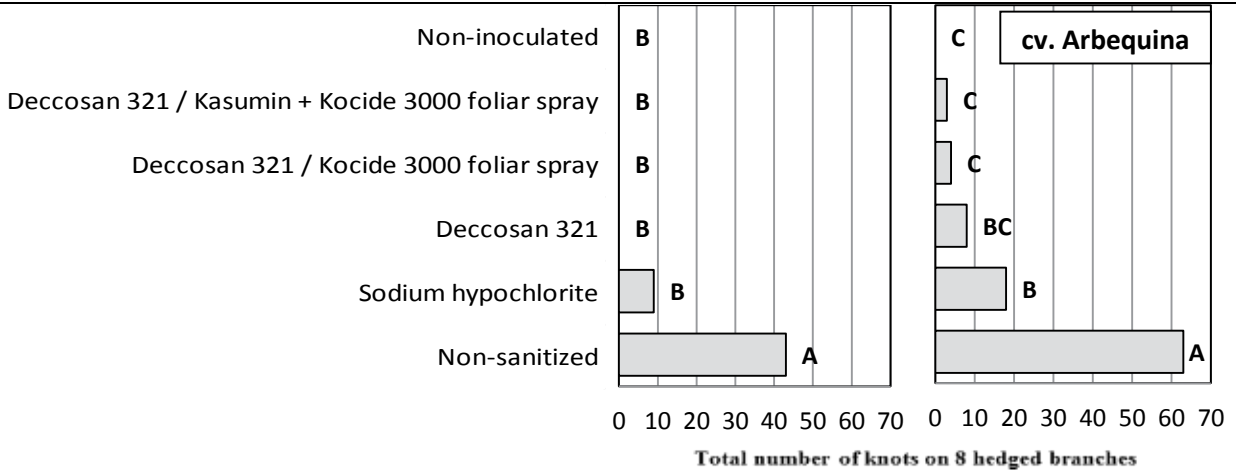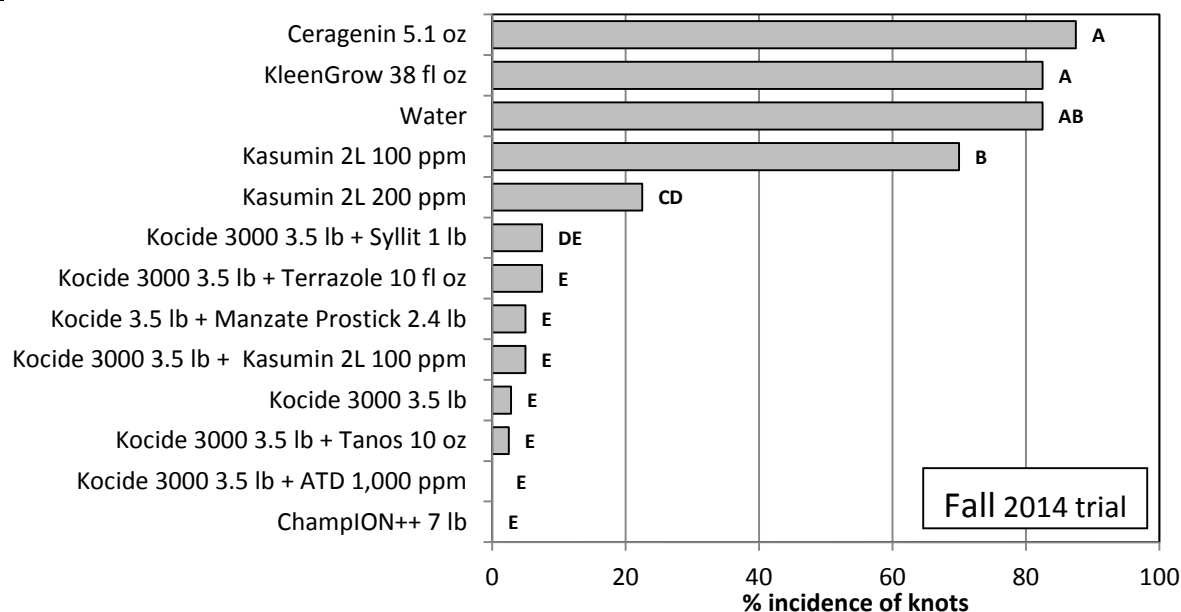


Olive branches were pruned with a hedger that was contaminated with a **copper-sensitive** strain of Psv ($2x10^7$ CFU/ml). The hedger was not sanitized or sanitized with Deccosan 321 (2000 mg/L) or sodium hypochlorite (50 mg/L). Branches were not treated or treated with a foliar application with Kocide 3000 (3.5 lb/A) or Kocide 3000 + Kasumin (100 mg/L). Disease evaluations were performed 6 months after the start of the trial.

**Fig. 3. Evaluation of new foliar treatments for management of olive knot of cv. Arbequina caused by a copper-sensitive Psv strain - Field trial at UC Davis**



Olive branches of cv. **Arbequina** were injured with lateral wounds and foliar treated. After air-drying, wounds were spray-inoculated with a **copper-sensitive** Psv strain at $1x10^8$ CFU/ml. Treatments with same letters are not significantly different based on a least significant difference mean separation test.

**Fig. 4. Evaluation of new foliar treatments for management of olive knot of cv. Manzanillo caused by a copper-sensitive Psv strain - Field trial at UC Davis**



Olive branches of cv. **Manzanillo** were injured with lateral wounds and foliar treated. After air-drying, wounds were spray-inoculated with a **copper-sensitive** Psv strain at $1x10^8$ CFU/ml. Treatments with same letters are not significantly different based on a least significant difference mean separation test.

**Fig. 5. Evaluation of new foliar treatments for management of olive knot of cv. Arbequina caused by a copper-resistant Psv strain - Field trial at UC Davis**
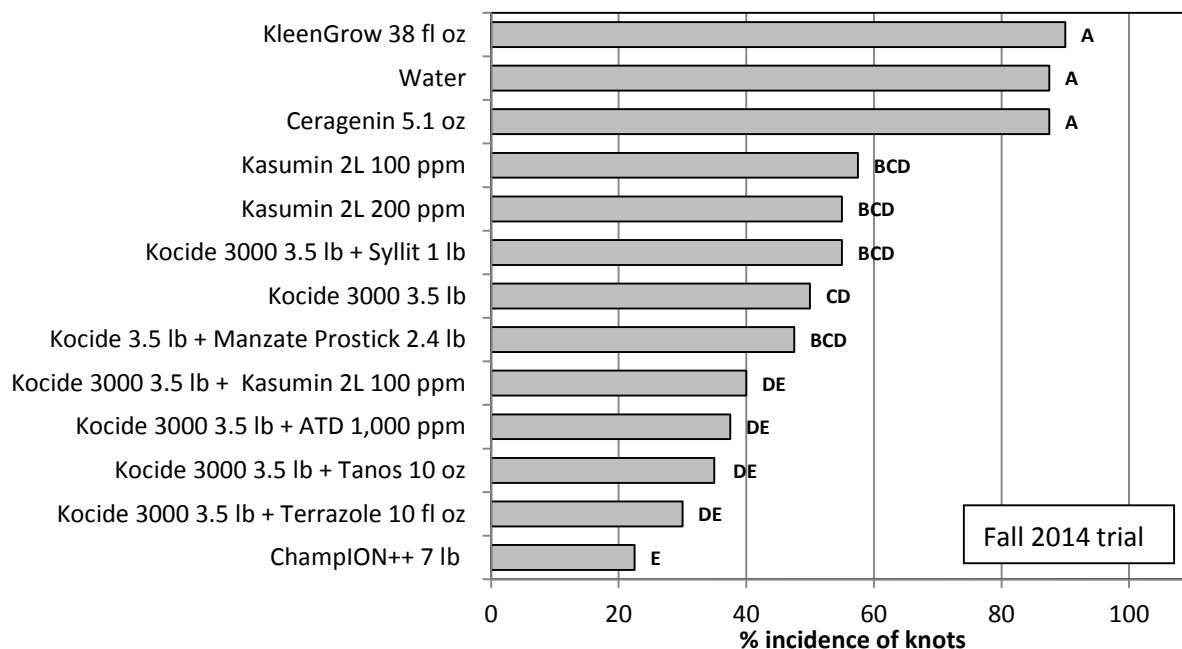


Olive branches of cv. **Arbequina** were injured with lateral wounds and foliar treated. After air-drying, wounds were spray-inoculated with a **copper-resistant** Psv strain at $1x10^8$ CFU/ml. Treatments with same letters are not significantly different based on a least significant difference mean separation test.

**Fig. 6. Evaluation of new foliar treatments for management of olive knot of cv. Manzanillo caused by a copper-resistant Psv strain - Field trial at UC Davis.**
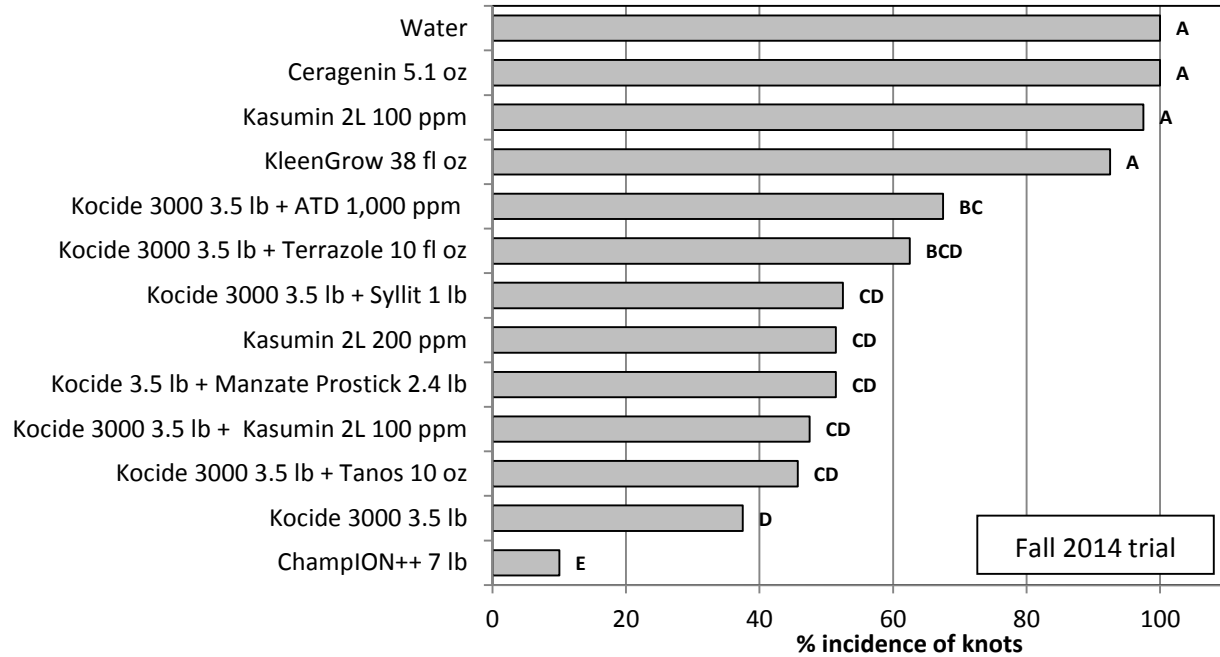


Olive branches of cv. **Manzanillo** were injured with lateral wounds and foliar treated before being spray inoculated with a **copper-resistant** Psv strain at $1\times10^8$ CFU/ml. Treatments with same letters are not significantly different based on a least significant difference mean separation test.

**Fig. 7. Evaluation of new foliar treatments for management of olive knot of cv. Arbequina caused by a copper-sensitive Psv strain - Field trial in Yuba Co.**
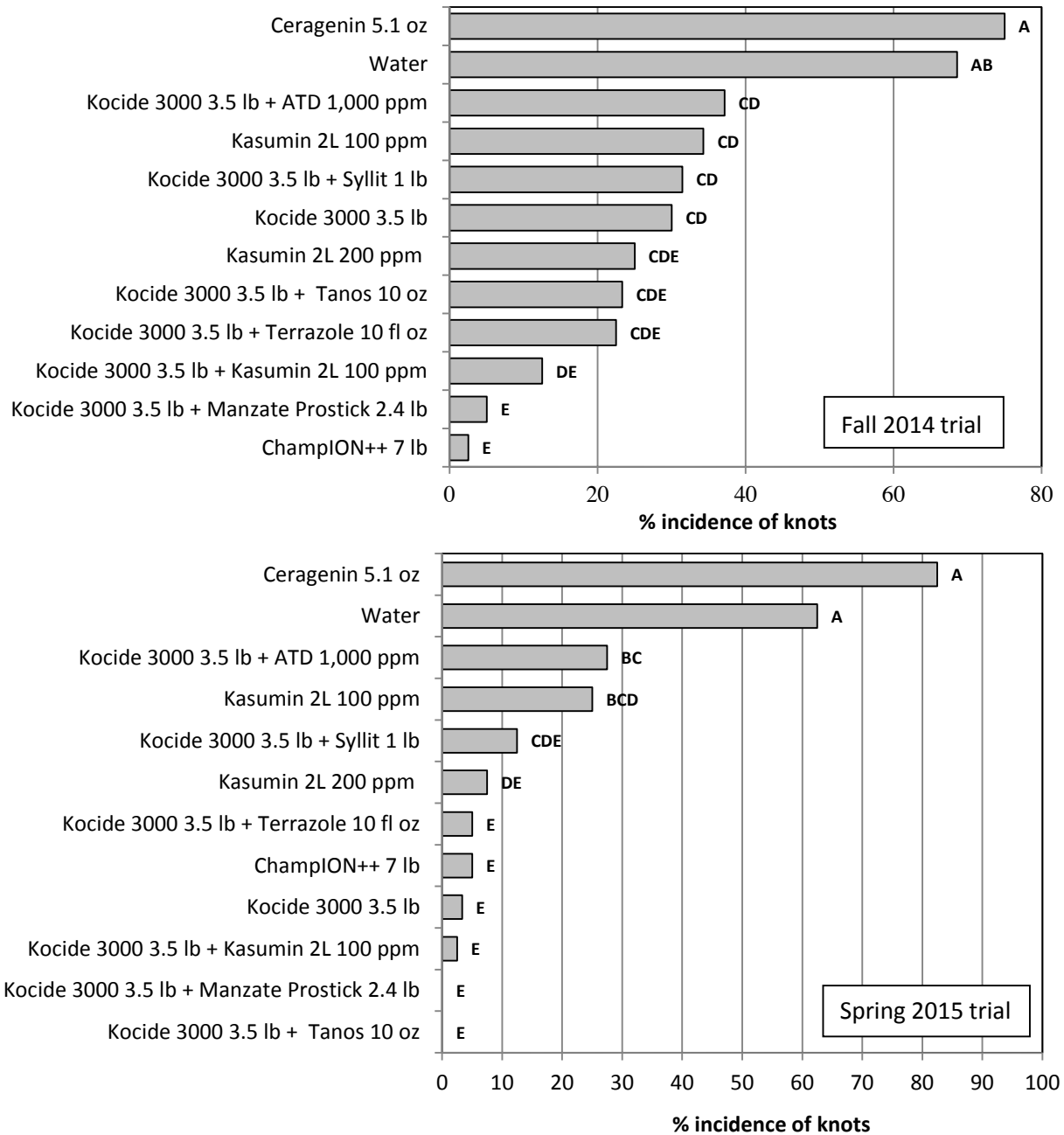
Fall 2014 trial

| Treatment | Significance group |
|---|---|
| Ceragenin 5.1 oz | A |
| Water | AB |
| Kocide 3000 3.5 lb + ATD 1,000 ppm | CD |
| Kasumin 2L 100 ppm | CD |
| Kocide 3000 3.5 lb + Syllit 1 lb | CD |
| Kocide 3000 3.5 lb | CD |
| Kasumin 2L 200 ppm | CDE |
| Kocide 3000 3.5 lb + Tanos 10 oz | CDE |
| Kocide 3000 3.5 lb + Terrazole 10 fl oz | CDE |
| Kocide 3000 3.5 lb + Kasumin 2L 100 ppm | DE |
| Kocide 3000 3.5 lb + Manzate Prostick 2.4 lb | E |
| ChampION++ 7 lb | E |

% incidence of knots (0 – 80)

Spring 2015 trial

| Treatment | Significance group |
|---|---|
| Ceragenin 5.1 oz | A |
| Water | A |
| Kocide 3000 3.5 lb + ATD 1,000 ppm | BC |
| Kasumin 2L 100 ppm | BCD |
| Kocide 3000 3.5 lb + Syllit 1 lb | CDE |
| Kasumin 2L 200 ppm | DE |
| Kocide 3000 3.5 lb + Terrazole 10 fl oz | E |
| ChampION++ 7 lb | E |
| Kocide 3000 3.5 lb | E |
| Kocide 3000 3.5 lb + Kasumin 2L 100 ppm | E |
| Kocide 3000 3.5 lb + Manzate Prostick 2.4 lb | E |
| Kocide 3000 3.5 lb + Tanos 10 oz | E |

% incidence of knots (0 – 100)

Branches were injured with lateral wounds in the fall of 2014 or spring of 2015 and spray treated. After air-drying, branches were inoculated with a **copper-sensitive** Psv strain at $1\times10^8$ CFU/ml (fall 2014) or $2\times10^7$ CFU/ml (spring 2015). Treatments with same letters are not significantly different based on a least significant difference mean separation test.

**Fig. 8. Evaluation of new foliar treatments for management of olive knot of cv. Arbequina caused by a copper-resistant Psv strain - Field trial in Yuba Co.**
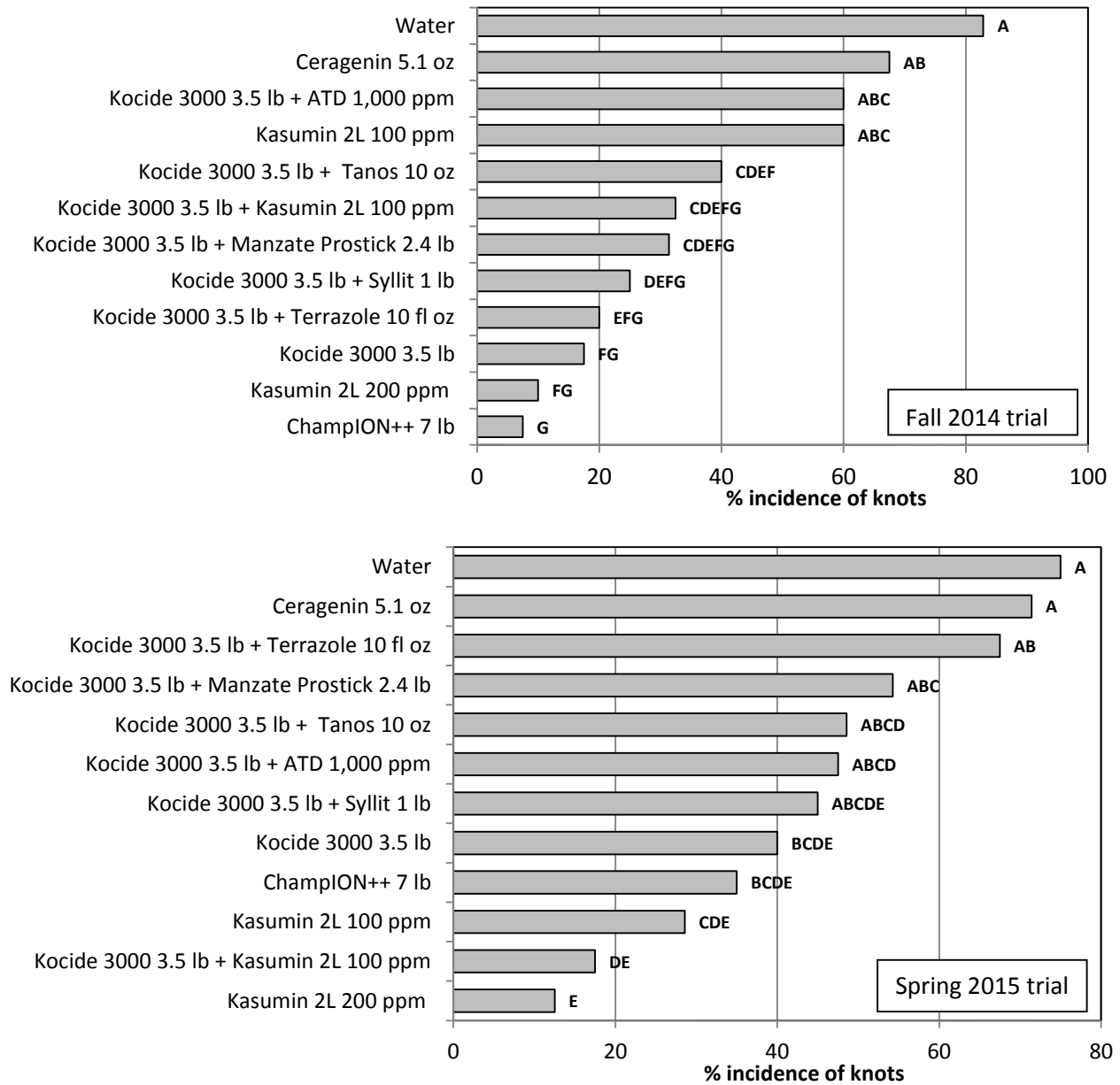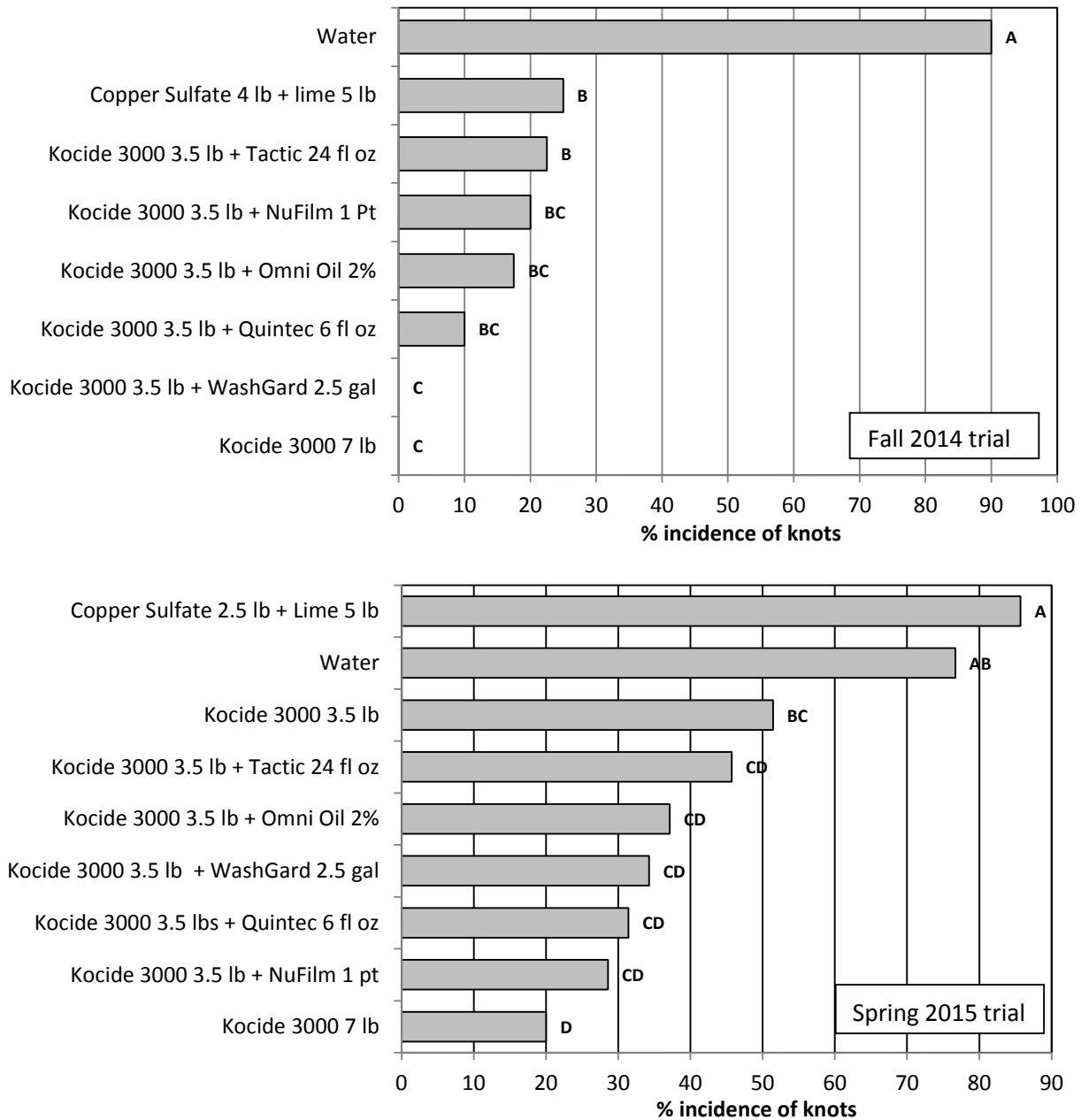


Branches were injured with lateral wounds in the fall of 2014 or spring of 2015 and spray treated. After air-drying, branches were inoculated with a **copper-resistant** Psv strain at $1 \times 10^8$ CFU/ml (fall 2014) or $2 \times 10^7$ CFU/ml (spring 2015). Treatments with same letters are not significantly different based on a least significant difference mean separation test.

# Fig. 9. Persistence of copper treatments in inoculation studies with a copper-sensitive Psv strain on cv. Manzanillo olives in a field trial at UC Davis

**Fall 2014 trial**

| Treatment | % incidence of knots | Group |
|---|---|---|
| Water | 90 | A |
| Copper Sulfate 4 lb + lime 5 lb | 25 | B |
| Kocide 3000 3.5 lb + Tactic 24 fl oz | 22 | B |
| Kocide 3000 3.5 lb + NuFilm 1 Pt | 19 | BC |
| Kocide 3000 3.5 lb + Omni Oil 2% | 17 | BC |
| Kocide 3000 3.5 lb + Quintec 6 fl oz | 9 | BC |
| Kocide 3000 3.5 lb + WashGard 2.5 gal | | C |
| Kocide 3000 7 lb | | C |

**Spring 2015 trial**

| Treatment | % incidence of knots | Group |
|---|---|---|
| Copper Sulfate 2.5 lb + Lime 5 lb | 85 | A |
| Water | 76 | AB |
| Kocide 3000 3.5 lb | 51 | BC |
| Kocide 3000 3.5 lb + Tactic 24 fl oz | 46 | CD |
| Kocide 3000 3.5 lb + Omni Oil 2% | 37 | CD |
| Kocide 3000 3.5 lb  + WashGard 2.5 gal | 34 | CD |
| Kocide 3000 3.5 lbs + Quintec 6 fl oz | 31 | CD |
| Kocide 3000 3.5 lb + NuFilm 1 pt | 29 | CD |
| Kocide 3000 7 lb | 20 | D |

Branches were injured with lateral wounds in the fall of 2014 or spring of 2015 and spray treated. This was followed by overhead irrigation for 30 minutes. After air-drying, branches were inoculated with a **copper-sensitive** Psv strain at $1 \times 10^8$ CFU/ml (fall 2014) or $2 \times 10^7$ CFU/ml (spring 2015). Treatments with same letters are not significantly different based on a least significant difference mean separation test.