AES/CE MAR 84

Workgroup: Olive
Department: Plant Pathology/ UCR

University of California
Division of Agricultural Sciences
ANNUAL PROJECT/RESEARCH GRANT REPORT
December 2014

Project Year: 2014 Anticipated Duration of Project: 1st year of 3 years

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

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

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JUSTIFICATION/ BACKGROUND

Olive knot caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi (Psv)*, is a significant pathogen of olives worldwide including California. The bacterium is an opportunistic wound pathogen but also exist as an epiphyte on olives. Disease symptoms include tumors, galls, or knots that may lead to tree defoliation and branch diebacks with subsequent reductions in yield, fruit size, and oil quality. Historically, the most susceptible olive cultivars are: Manzanillo, Sevillano, Ascolano, and Mission, but many of the newer oil varieties are also highly susceptible which include: Arbequina, Arbosana, and Koroneiki.

The pathogen survives on olive leaves and stems but high populations mainly reside within actively growing knots. Bacterial cells are exuded to the knot surface during periods of wetness and are readily water splash/wind-dispersed or may be disseminated by insects or birds. It is not known if inoculum production varies at different times of the year but exudation relies on free water. Leaf and blossom scars as well as injuries to the twig bark caused by any biological (e.g., other diseases), environmental (e.g., freeze cracks), or physical (e.g., mechanical harvesting, pruning) means may serve as sites of infection. Twig injuries occurring at harvest are major potential entry points. The period of susceptibility of injuries is not known and is currently under evaluation. In California, infection occurs mostly during the rainy season (late fall, winter and spring) but the knots do not develop until new vegetative growth begins in the spring. Infections can occur at fairly low temperatures (5-10 C) and thus, wetness and availability of susceptible wounds are the main limiting factors for the disease.

Sanitation and prevention are the most successful strategies for management of olive knot. Any horticultural practice that promotes plant health, minimizes tree stress, and results in less leaf drop (i.e., control of peacock spot) 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 shears, frequent disinfection of equipment is necessary. Painting galls with Gallex® is an effective therapeutic treatment but is very labor intensive. Spray applications of copper-containing bactericides have been very effective in minimizing the disease, but they often may need to be repeated to protect new wounds. A minimum of two applications is usually necessary: one in the fall before the rainy season starts and one in the spring when most leaves have been shed. 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 in all isolates, but higher rates of copper were still effective in preventing bacterial growth. Because olive knot infections occur mostly during the rainy period, knowledge on the persistence of treatments is critical. Thus, we will determine the efficacy of copper alone and in mixtures with materials to increase its persistence by inoculating treated twig wounds at selected times after application and overhead irrigation. Rainfall will be simulated using overhead sprinklers established in orchards at UC Davis.

We have been instrumental in the development of the new agricultural antibiotic kasugamycin (commercial name 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. We found it to be the most promising new treatment for preventing olive knot in our field studies, including in a commercial application to inoculated branches and we are continuing its evaluation. Kasugamycin is currently federally registered on pome fruit crops (e.g., apples and pears), whereas use on olives was approved as an "A" priority by IR-4 for the 2015 season. We will be involved with the IR-4 residue studies in the spring of 2015. Several systemic acquired resistance (SAR) compounds (e.g., Actigard, Regalia, quinoxyfen - Quintec, and USF2018A) were also effective in some studies, but not equivalent to copper or kasugamycin. SAR treatments need to be applied several days in advance of favorable disease conditions (or inoculation) but they potentially have a longer lasting effect on plant health and may provide sustainable treatments that could be developed for an integrated approach with other treatments. Initial studies on the use of biocontrols were not successful, but further evaluation with other biocontrol agents may provide disease control.

We have also been working on sanitation treatments for orchard equipment as part of an integrated olive knot management program. We demonstrated that quaternary ammonia compounds, guanidine, and chlorhexidine 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 quaternary ammonia sanitizers are volatile compounds that do not leave residues and are not corrosive to equipment. Deccosan 321 has a federal label and is used in Florida for disinfesting field equipment to prevent the spread of bacterial diseases on citrus and tomatoes caused by *Xanthomonas* species. Deccosan 321 was submitted to the State of California as a Section 24c, Special Local Need, registration on harvesting and pruning equipment used by the olive industry. Registration is expected in the spring of 2015. We will test additional parameters that may affect the efficacy of the sanitizer including inoculum concentration and post-inoculation treatment time. Field evaluations of the material as an equipment sanitizer will be accomplished once registration is approved.

RESEACH OBJECTIVES

1. **Epidemiology – pathogen variability, inoculum availability and period of susceptibility of selected injuries (leaf scars, pruning injuries, etc.) to infection**
    a. Evaluate genetic pathogen variability using DNA markers
    b. Monitor galls for production of inoculum over time
    c. Duration of susceptibility of injuries under different environmental conditions (wetness and temperature)
2. **Evaluate populations of the pathogen for laboratory sensitivity to chemicals**
3. **Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions once registration has occurred.**
4. **Evaluate the efficacy of protective treatments such as new copper formulations, antibiotics (Kasumin, Mycoshield), captan, dodine, SAR compounds (acibenzolar-S-methyl - Actigard, PM-1, quinoxyfen, ProAlexin, Regalia), and combination treatments**
    a. Field trials with and without adjuvants
    b. Timing studies: Protective (pre-infection) vs. post-infection activity of treatments; proper timing of SAR compounds; treatment at spring leaf drop or after harvest.
    c. Persistence of different copper treatments with and without the addition of lime or other additives under simulated rain conditions.
    d. Develop copper activity-enhancing materials such as mancozeb and other products
5. **Systemic infection of Psv**
    a. Investigate potential factors leading to systemic movement of Psv and twig dieback as was observed in evaluations of field trials done in the fall of 2013.

MATERIALS and METHODS

1. Epidemiology.

a. *Evaluation of Psv genetic variability.* Olive knots were collected in collaboration with farm advisors and PCAs. Knots were surface-sterilized with sodium hypochlorite, internal tissue was removed, suspended in sterile water, and the suspension was plated onto KMB medium. Single bacterial colonies were cultured and

species identity was verified using primers that target the IAA-lysine synthase gene (Penyalver et al., 2000). Genetic variability of our current collection of Psv strains was evaluated using REP primers in PCR reactions as described previously.

b. ***Monitor galls for production of inoculum over time.*** This objective was studied in the previous funding season and additional experiments are planned for 2015.

c. ***Duration of susceptibility of injuries and effect of inoculum concentration on development of olive knot.*** In field studies on the effect of inoculum concentration on knot development, leaf scar and lateral twig wounds of cv. Arbequina twigs were inoculated in June 2014 with a copper-sensitive or a -tolerant strain of Psv using selected inoculum concentrations ranging from $2x10^5$ to $2x10^8$. The incidence of knot formation was evaluated in October of 2014.

2. ***Evaluation of pathogen populations for sensitivity to copper, antibiotics, and sanitizers in the laboratory.*** The effect of exposure time to Deccosan 321 or sodium hypochlorite on viability of Psv was tested in a laboratory study. For this, Psv was suspended in 5 ppm Deccosan 321 or 5 ppm sodium hypochlorite. After exposure for 15, 30, 45, or 60 seconds, the suspensions were diluted 1:1000 with sterile distilled water and spiral-plated onto King's B medium. Bacterial colonies were enumerated after 2 to 3 days of incubation at 25C. Psv recovery (cfu/mL) for each exposure time was calculated as percentage of the water control and was the average of three replicates.

3. ***Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions once registration has occurred.*** These studies are planned for 2015.

4. ***Evaluation of protective treatments in greenhouse and field studies.*** In the fall of 2013, greenhouse studies on cv. Manzanillo were done at UC Riverside. Field trials on cvs. Arbequina and Manzanillo were done at UC Riverside, UC Davis, and in a commercial orchard in the fall of 2013 and spring of 2014. Twigs were wounded laterally with a razor blade or leaves were removed to create leaf scar wounds. Wounds were either first inoculated and then treated; or first treated, air-dried, and then inoculated (see Figures of the Results). Inoculations were done by hand-spraying of bacterial suspensions (2×10^7 to 1×10^8 cfu/ml). Treatments of bactericides and SAR compounds were done by spraying the wound sites to run-off. Additionally, a trial was done at UC Davis on cv. Manzanillo on the persistence of selected treatments (Kasumin, Kocide 3000, Kocide with lime and zinc, and Kasumin with Kocide) after simulated rain application. Wounds were first treated, allowed to dry, subjected to simulated rain for 1 h, and then inoculated. Plants were evaluated for the presence of knot formation in the spring of 2014 or fall 2014. All data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

5. ***Investigate potential factors leading to systemic movement of Psv and twig dieback.*** Environmental data (precipitation, temperature) were obtained from a CIMIS station in proximately to our field trials, graphed out, and visually analyzed for differences between the 2013/14 and previous winter seasons. To simulate field conditions, in an initial controlled temperature study, wounds of cvs. Arbequina and Manzanillo olive plants were inoculated and then exposed to freezing temperatures (-5°C) for 4 to 12 h. Plants were then moved to the greenhouse and observed for disease development.

RESULTS

1. Epidemiology

a. ***Evaluation of Psv genetic variability***. Based on rep-PCR, two main genotypes (comprising >95% of the strains evaluated) were identified in our current collection of 120 Psv strains from northern California. Thus, variability was found to be very limited and additional genetic markers will be tested in 2015.

b. ***Monitor galls for production of inoculum over time.*** This objective was studied in the previous funding season and we showed that after external application of water to galls, inoculum was immediately produced at very high concentrations. Galls continued to produce inoculum with continued wetness duration. Thus, short wetness periods in the field can result in a high potential for new infections to occur. Additional experiments are planned for 2015.

c. ***Duration of susceptibility of injuries and effect of inoculum concentration on development of olive knot.*** Studies on susceptibility of injuries of cv. Manzanillo olives under different wetness conditions (overhead irrigation to simulate rain or no irrigation) were conducted in the fall season of 2013 and data were presented

in our July 2014 interim report. Our results indicated that wounds were less susceptible to infection when provided with simulated rain as compared to no rain and this was explained by possible washing off of inoculum from the wounding sites by excess simulated rain. Injuries that received no irrigation remained susceptible to Psv even after 17 days (the maximum wound healing time tested) while irrigated wounds had very low incidence of olive knot after 10 days.

In previous studies on cv. Arbequina, susceptibility of wounds declined significantly after only 10 days and there were no differences between irrigated and non-irrigated trees. To find out if differences between the two studies were due to the olive cultivar used, comparative studies with cvs. Manzanillo and Arbequina are planned for 2015 under controlled conditions in the greenhouse.

The effect of inoculum concentration on knot induction on cv. Arbequina olive was tested in a field study. Fewer knots developed on leaf scar injuries than on lateral wounds when inoculum concentration was reduced for the two Psv strains tested (Fig. 1A). This suggests that leaf scar wounds in this study were less sensitive to infection than lateral wounds. For lateral and leaf scar wounds, a significant difference between the two strains in the incidence of knots was only observed at the lowest inoculum concentration of 2×10^5 cfu/ml and fewer knots developed using the Cu-sensitive strain, indicating that this strain may be less virulent. For the leaf scar wounds, the incidence of knot formation decreased with decreasing inoculum concentration for both strains (Fig. 1B).

Improved control of olive knot may be achieved by understanding threshold inoculum concentrations that are needed for disease development. Different inoculum levels (disease pressure) may require different treatment strategies. Thus, SAR compounds may only provide adequate control when disease pressure is low and may require integrated use with conventional treatments such as Kocide and Kasumin.

2. *Evaluation of pathogen populations for sensitivity to copper, antibiotics, and sanitizers in the laboratory.* Laboratory studies were conducted on the in vitro sensitivity of more than 100 strains of Psv to potential bactericidal treatments (e.g., oxytetracycline, streptomycin, and kasugamycin as well as different copper formulations) and results were presented in our July 2014 interim report. Briefly, there was a range of sensitivities to the three antibiotics, but all strains were all considered sensitive. Most strains showed reduced copper sensitivity in the 10- to 20-ppm range, however, one strain was considered copper-resistant with growth at 50 ppm MCE. We are currently sampling this site. Thus, copper resistance does occur in the pathogen population at some locations and its spread from overuse of copper products has to be minimized. This emphasizes the need for alternative treatments and for new copper-enhancing alternatives.

In other in vitro assays we tested several strains against new copper products (e.g., Magna Bon) and copper additives (thiadiazoles, dodine, etc.) with very promising results. Copper additives (enhancers) were evaluated due to EPA mandated registration restrictions of mancozeb. At the same metallic copper equivalent (MCE), MagnaBon was more effective in inhibiting bacterial growth in laboratory assays than copper sulfate.

Addition of a thiadiazole (ATD) to copper improved copper activity against copper-sensitive strains (when using copper at a concentration where sensitive strains were still viable), but was not effective against a copper-tolerant strain. In work with copper-tolerant strains of the walnut blight pathogen *Xanthomonas arboricola* pv. *juglandis*, this mixture improved copper activity. Dodine inhibited growth of copper-tolerant and -sensitive Psv strains. Mixtures of copper and dodine are currently being tested.

In vitro direct contact assays indicated an exponential decrease in *Psv* viability with increased exposure duration to Deccosan 321 at very low rates (5 ppm) (Fig. 2). As compared to the water control, there was an 84% reduction after a 15-sec exposure and a 97% reduction after a 60-sec exposure. Deccosan 321 at 25 ppm completely inactivated *Psv* after 15 sec. In comparison, 5 ppm sodium hypochlorite completely inhibited growth at any of the exposure times tested. Thus, Deccosan 321 is a very effective sanitizer and is not corrosive to equipment as chlorine is and performed better than chlorine in the presence of an organic load (i.e., plant debris) as we previously showed in hard-surface disinfestation assays.

3. *Test the performance of an equipment sanitizer (e.g., quaternary ammonium) under field conditions once registration has occurred.* In collaboration with the registrant, we submitted the quaternary ammonium compound Deccosan 321 for a special local need registration for use in California, and approval is expected for March 2015. Once a Section 24C is in place, we plan to test the material on harvesting and pruning equipment in the field.

4. *Evaluation of protective treatments in greenhouse and field studies.*

a. Copper and copper alternatives with adjuvants. In greenhouse trials conducted in the fall of 2013, the effect of two adjuvants, NuFilm-P and Washgard (a carnauba-based adjuvant), on the performance of Kasumin and copper treatments was evaluated. Overall, there was some inconsistency in results among treatments and between lateral wounds and leaf scar wounds. Washgard numerically increased the activity of Kasumin and of Kasumin-Kocide, but not of Kocide in lateral wound inoculations (Fig. 3). For NuFilm-P, there was a trend for reduced activity for the Kasumin and Kasumin-Kocide treatments. For leaf scar wounds, there was a trend of improved activity with Washgard for the Kasumin and Kasumin-Kocide treatments, and a trend for reduced activity with NuFilm-P for the Kasumin and Kocide treatments. The activity of Kasumin-Kocide, however, was significantly improved with the addition of NuFilm-P (Fig. 3B). Thus, these results warrant further studies.

In the fall of 2013, field trials were done at UC Davis and in several commercial cv. Manzanillo and Arbequina groves. In evaluating these trials in the spring of 2014, we noted disease symptoms that we did not observe previously, even at study sites that were used in previous years. On cv. Manzanillo, and to a much lesser extent also on cv. Arbequina, many of the inoculations resulted in major shoot dieback and blistering on the inoculated as well as neighboring branches. Symptoms on non-inoculated neighboring branches are an indication of bacterial movement inside the host, and this was substantiated by bacterial isolation from these distal points. With this severe disease development, performance in our bactericide efficacy studies was often compromised and treatment efficacy was generally very poor. Thus, results are only presented for some of these trials, and mostly for trials on cv. Arbequina because this cultivar appeared to be much less susceptible to Psv infection than cv. Manzanillo.

In a commercial cv. Manzanillo orchard, severe dieback was observed on untreated, inoculated controls and on copper- and copper-mixture-treated trees. Still, treatments with kasugamycin, streptomycin, and oxytetracycline significantly reduced dieback and systemic movement was reduced indicating that these treatments may be more effective than copper.

On cv. Arbequina, Kocide performed mostly well in reducing the incidence of knots on lateral and leaf scar wounds when using a copper-sensitive strain for the inoculations (Fig. 4A, 5, 6A) and was moderately effective when using a copper-tolerant strain (Fig. 4B, 7B). Kasumin treatments resulted generally in very good (Fig. 4A, 6A) to moderate (Fig. 4B, 5A, 7A) control when using a copper-sensitive strain for inoculation; and very good (Fig. 6B, 7B) control when using a copper-tolerant strain. Addition of Syllit or Captan to Kasumin did not improve Kasumin efficacy in one trial where this was studied (Fig. 4).

Efficacy of streptomycin ranged from excellent (Fig. 4A, 5A, 6, 7A), to moderate (Fig. 7B), to ineffective (Fig., 4B, 5B); whereas oxytetracycline had excellent (Fig. 6, 7), moderate (Fig. 4A), or no (Fig. 4B, 5) activity. Trials where these antibiotic treatments had low efficacy were at sites with cold injury. In comparison of copper treatments, Kocide 3000 generally performed similar to Badge X2, and these treatments performed numerically or statistically better than MagnaBon or Previsto at the rates used (Figs. 6, 7). MagnaBon was used at a very low rate (100 MCE) based on the manufacturer's recommendation and based on the risk of phytotoxicity that was observed in our trials with MagnaBon on other crops. The bacterial membrane disruptor Ceragenin was not or only slightly effective in reducing olive knot (Figs. 6, 7). Additionally, ATD that we found to enhance copper activity for some bacterial diseases of other plants did not improve copper activity in our studies on olive. Mancozeb is registered for management of bacterial diseases of some tree crops in California such as walnut where it increases efficacy of copper when disease is caused by copper-resistant strains of the pathogen. Mancozeb, however, cannot be registered on olives. Therefore, we are evaluating alternatives. Quinoxyfen has been reported to improve copper performance against diseases caused by *Xanthomonas* species in Florida and we are planning to apply this compound in mixture with other bactericides for *Psv* control (we previously tested quinoxyfen by itself).

In summary, although reduction in disease by bactericidal treatments was not as high as in previous trials, several treatments still significantly reduced the disease as compared to the control. Higher inoculum levels were used in these studies as compared to previous years and environmental conditions were highly favorable for olive knot, and this may have impacted symptom development and efficacy of treatments. Under these severe conditions, antibiotic treatments were able to significantly reduce dieback providing some measure of control against *Psv* infection.

Overall, we made significant progress in understanding the management of olive knot with chemicals. Copper sensitivity surveys in Psv populations provide information on potential of copper treatments, but also indicate the risk of copper resistance and the need for alternatives. Antibiotics were identified as the most effective alternatives. Our post-infection timing studies indicated that when olives are treated immediately after injury occurs, olive knot development can be reduced significantly to low levels. Thus, under commercial conditions, treatments should be applied as soon as possible after harvest or after injuries from frost or hail occur. Using an equipment sanitizer will reduce inoculum spread during orchard maintenance and harvest.

b. SAR Compounds. Field trials in the fall of 2013 using SAR compounds (i.e., Regalia, ProAlexin, Stout, Actigard, and Quintec) resulted in little or no control of *Psv* when a foliar application was done three days before wound-inoculation. As in other trials conducted over the winter of 2013/14, branch dieback and systemic movement of the bacterium was observed on cv. Arbequina. In previous studies, significant reductions in disease were found using Regalia, quinoxyfen, Actigard, and Stout. Thus, SARs may be effective under specific conditions including timing and application method used. We conducted a new trial using these SAR compounds in October of 2014 on cvs. Manzanillo and Arbequina. We used different inoculum concentrations to find out if SARs possibly are more effective under lower disease pressure. Our goal is to incorporate SAR compounds into an effective spray program along with conventional treatments.

c. Persistence of treatments. Field trials to test the persistence of treatments under simulated rain conditions at UC Davis on cv. Manzanillo olives were performed in the fall of 2013. There was severe systemic movement of the pathogen in all treatments (Kasumin, Kocide 3000, Kocide-lime-zinc, and Kasumin-Kocide) with symptoms including knots, bumps, and blistering at and distant from the initial inoculation point. Disease conditions were highly favorable and we suspect cold injury effected the results of this trial preventing any interpretation of persistence. Trials are ongoing with adjuvants that have been reported to increase the persistence of copper in other crops (e.g., carnauba-based adjuvants, pinolene-based adjuvants).

5. *Investigate potential factors leading to systemic movement of Psv and twig dieback.* The occurrence of symptoms of apparent systemic infections in the fall/winter 2013/14 field trials created new challenges for research on olive knot control and prompted investigations to elucidate the cause. Weather data for the winter 2013/14 season obtained from CIMIS stations in proximately to our field trial locations were compared to those of previous years. It was noted that low temperatures and rainfall were recorded earlier in the winter of 2013/14 as compared to 2012/13. The resulting frost injuries of the olive trees and subsequent rainfall may have led to the dissemination of residual inoculum and infection.

Thus, an initial low temperature study was performed in growth chambers in an attempt to reproduce symptoms of apparent systemic infection. Observations in this study indicated that plants exposed to low temperatures (-5°C) for 4 to 12 hours showed severe responses which included heavy leaf drop and branch dieback. Cvs. Arbequina and Manzanillo plants that were wounded and inoculated before low-temperature exposure showed similar symptoms as observed in the field trials. It is unknown at this time whether Psv was migrating internally within olive tissue (i.e., systemic movement) leading to new infection sites (nodules) or if frost damage created microscopic wounds that were entry points for an external source of Psv inoculum.

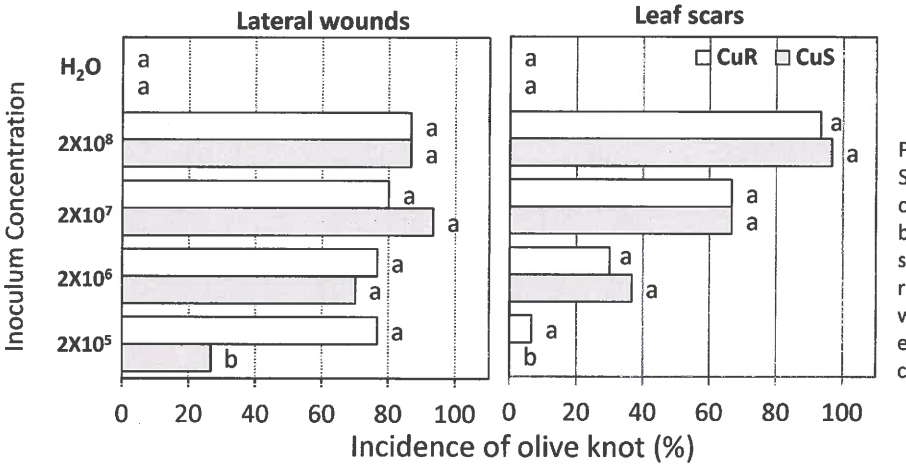
Treatments that were applied in the fall may have been compromised by the cold environments. Possibly some inoculum was inadvertently applied to areas that were not covered by chemical treatments and was able to persist and then infect wounds created by the later occurring frost. All chemical treatments tested are contact materials and good coverage of susceptible tissue is critical for control. Frost may have created new susceptible wounds that were not well protected leading to poor control of olive knot. These hypotheses need further investigation, and thus, chemical treatments before and after occurrence of frost damage will be evaluated in future growth chamber studies. Previously, our wounding studies indicated that the disease could be controlled if copper was applied within 24 h of when the injury occurred. Additional testing with antibiotics will be done under low-temperature conditions because they provided some measure of control against the apparent systemic infection. The goal is to provide an acceptable measure of disease control under these severe conditions.

REFERENCES

1. Adaskaveg, J.E., Forster, H., and Wade, M.L. 2011. Effectiveness of kasugamycin against *Erwinia amylovora* and its potential use for managing fire blight of pear. Plant Dis. 95:448-454.
2. Comai, L., and Kosuge, T. 1980. Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis in *Pseudomonas savastanoi*. J. Bacteriol. 143:950-957.
3. Hewitt, W.B. 1939. Leaf scar infection in relation to the olive knot disease. Hilgardia 12:41-66.
4. Ogawa, J.M., and English, E. 1991. Diseases of Temperate Zone Tree Fruit and Nut Crops. University of California, DANR, Oakland, CA. Publication 3345. 461 pp.
5. Penyalver, R., Garcia, A., Ferrer, A., Bertonlini, E., and Lopez, M.M. 2000. Detection of *Pseudomonas savastanoi* pv. *savastanoi* in olive plants by enrichment and PCR. Appl. Environ. Microbiol. 66:2673-2677.
6. Wilson, E.E. 1935. The olive knot disease: Its inception, development, and control. Hilgardia 9:233-264.

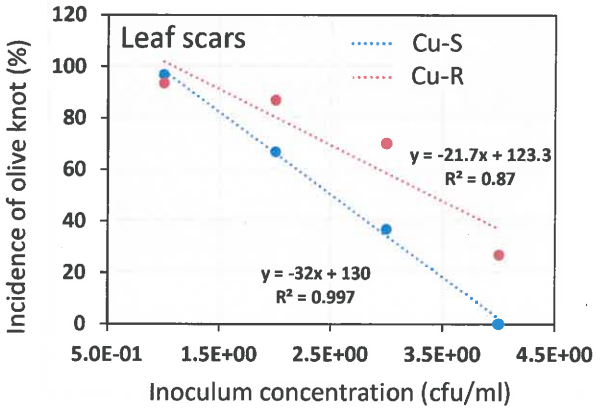
Fig. 1. Effect of inoculum concentration on development of olive knot in field studies at UC Riverside

A. Incidence of olive knot caused by Cu-sensitive and Cu-resistant isolates of Psv using selected inoculum concentrations.



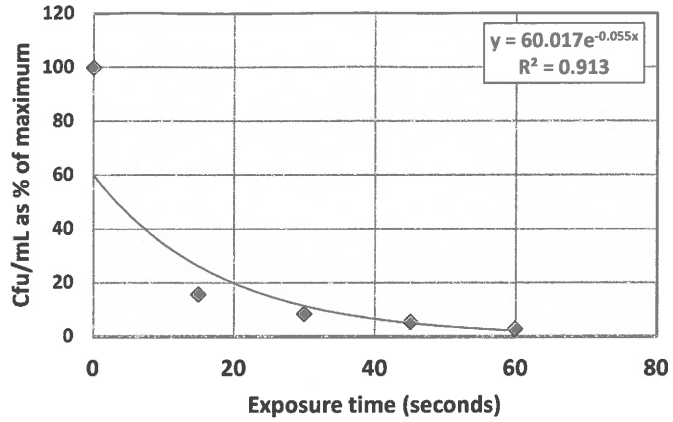
Pairwise Statistical comparisons between Cu-sensitive and Cu-resistant isolates were made for each inoculum concentration.

B. Regression of inoculum concentration on incidence of olive knot caused by Cu-sensitive and Cu-resistant isolates of Psv



In June of 2014, leaf scar and lateral twig wounds of cv. Arbequina twigs were inoculated with either of 2 strains of Psv at selected inoculum concentrations ranging from $2x10^5$ to $2x10^8$ at UC Riverside. Knot incidence recorded in October of 2014.

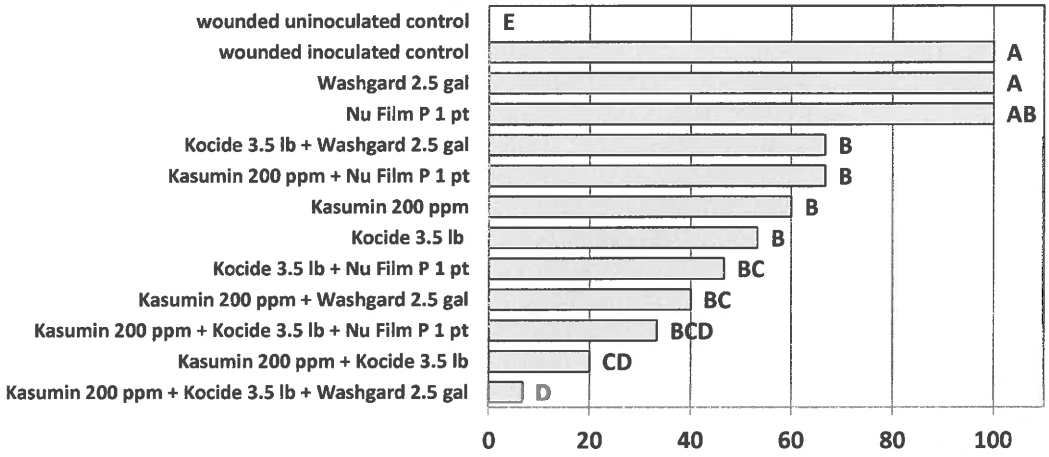
Fig. 2. Viability of Psv after selected exposure times to Deccosan 321 in a direct contact assay



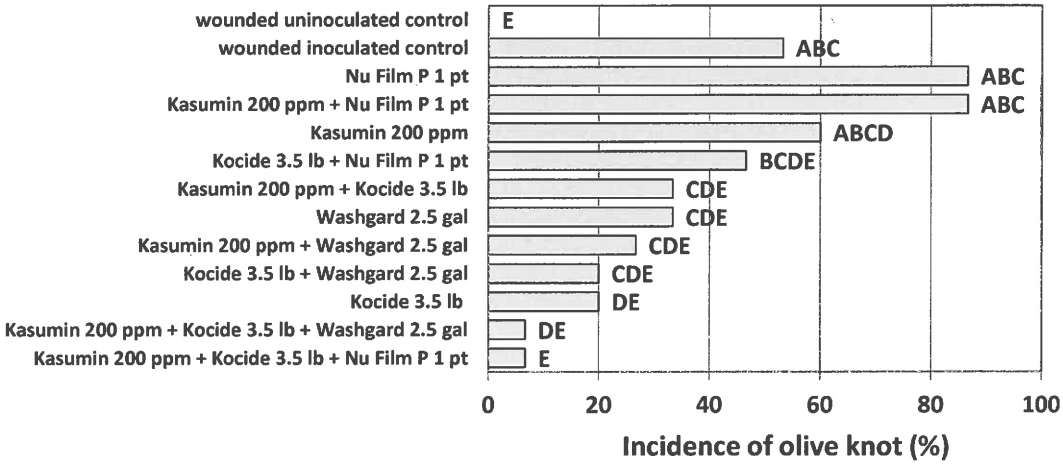
Psv was suspended in 5 ppm Deccosan 321. After 15, 30, 45, or 60 seconds, the suspension was diluted 1:1000 with sterile water and plated onto King's B medium for bacterial enumeration. *Psv* recovery (cfu/mL) for each exposure time is calculated as percentage of the water control and is the average of three replicates.

Fig. 3. Greenhouse trial on the effect of selected adjuvants on the efficacy of copper and kasugamycin against olive knot

A. Treatment and inoculation of lateral wounds

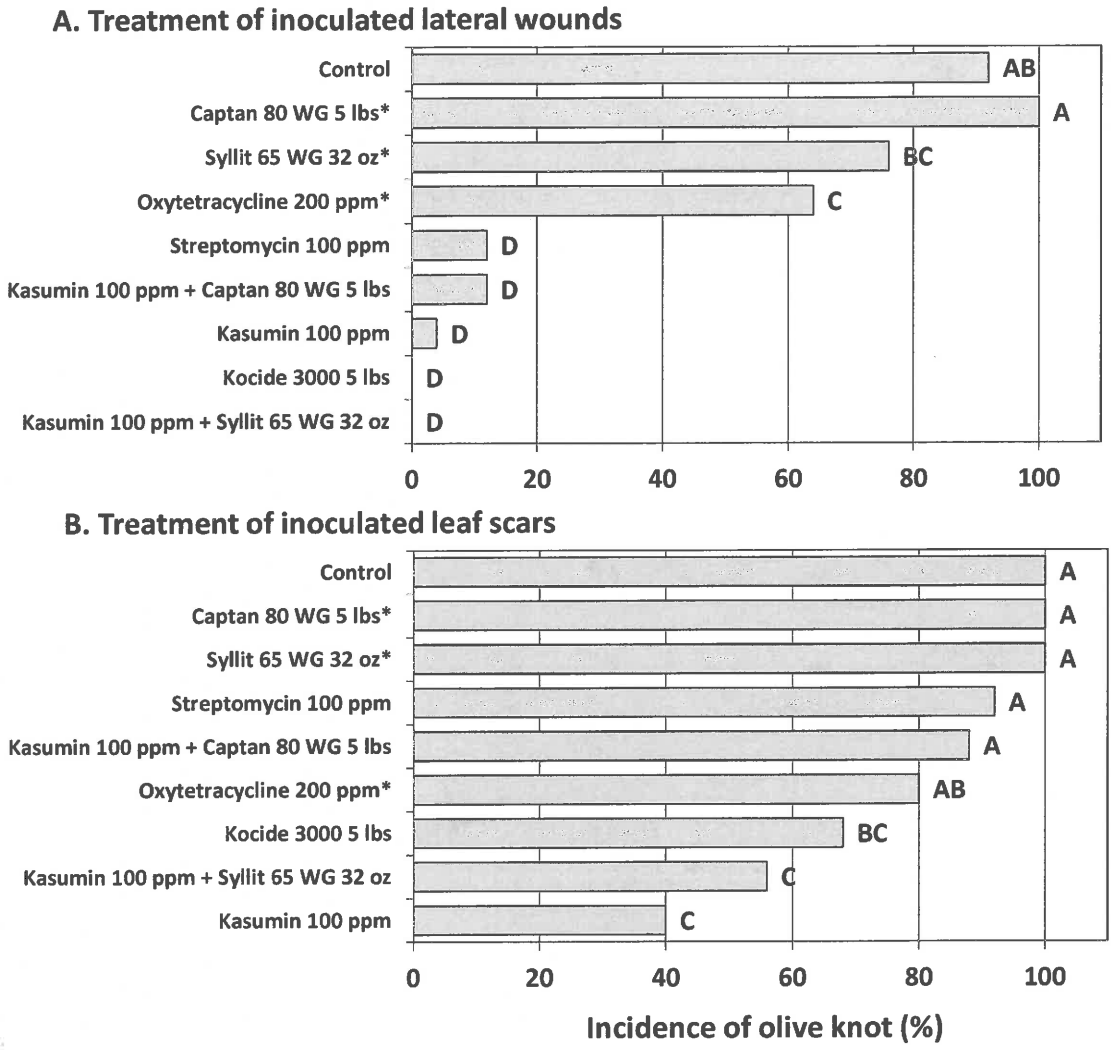


B. Treatment and inoculation of leaf scars



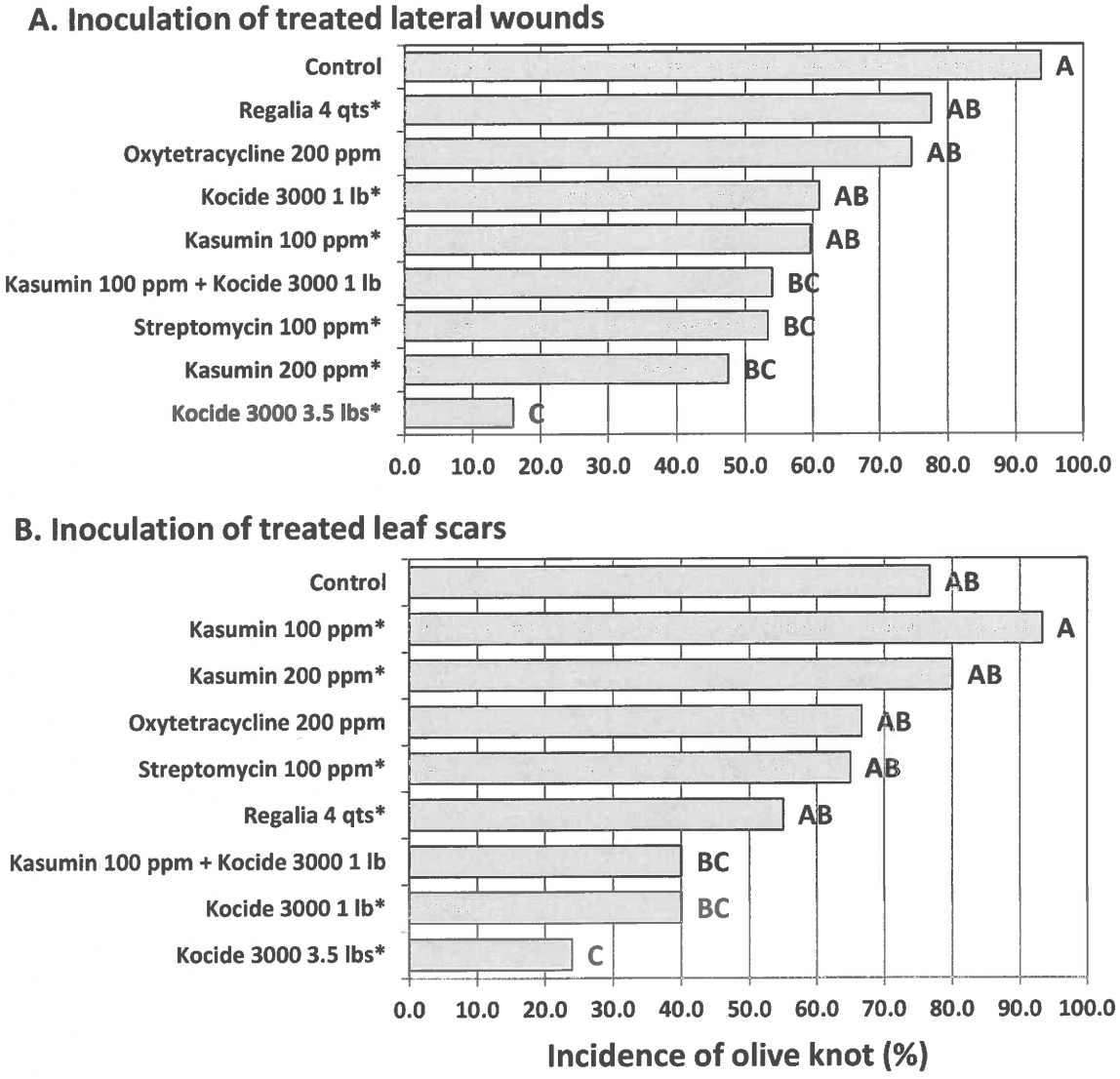
Cv. Manzanillo twigs were wounded (lateral or leaf scar wounds), treated to run-off using a hand sprayer, allowed to air-dry, and then inoculated with a suspension of a copper sensitive isolate of *Psv* ($2x10^7$). Rates specified are per acre/100 gal. Trial performed in late August and incidence of knots recorded in early October.

Fig. 4. Efficacy of bactericide treatment against olive knot in a field trial at UC Davis



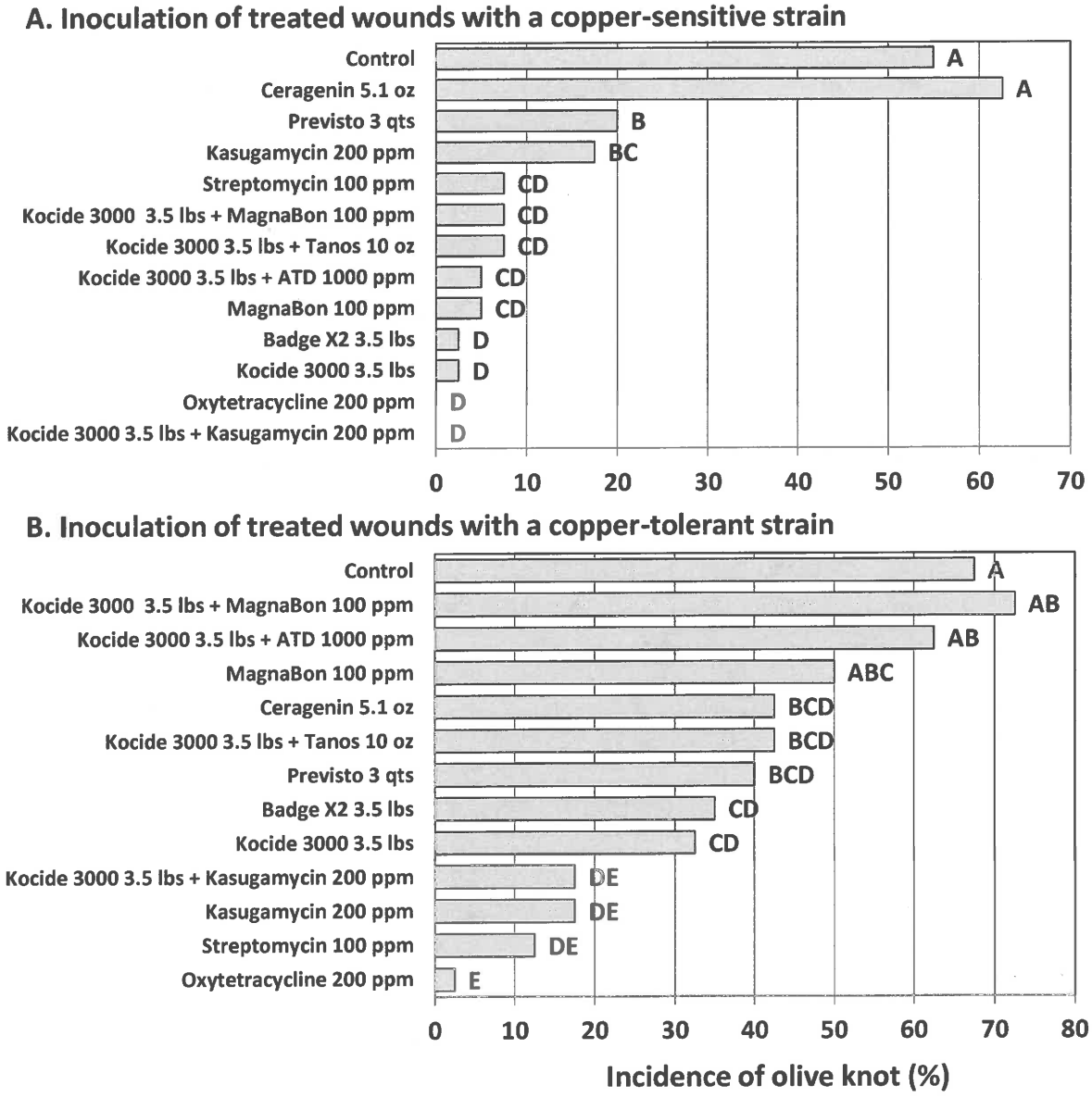
In November 2013, cv. Arbequina olive twigs were wounded, inoculated with a copper-sensitive strain of *Psv* ($1x10^8$), allowed to air-dry, and treated by hand-spraying to run-off. Disease was evaluated in April 2014. * Indicates treatments where some replicates showed symptoms of apparent systemic infection. Rates specified are per acre/100 gal. Knot incidence recorded in May of 2014.

Fig. 5. Treatment efficacy against olive knot in a field trial in a commercial olive orchard after inoculation with a copper-sensitive strain of *Psv*



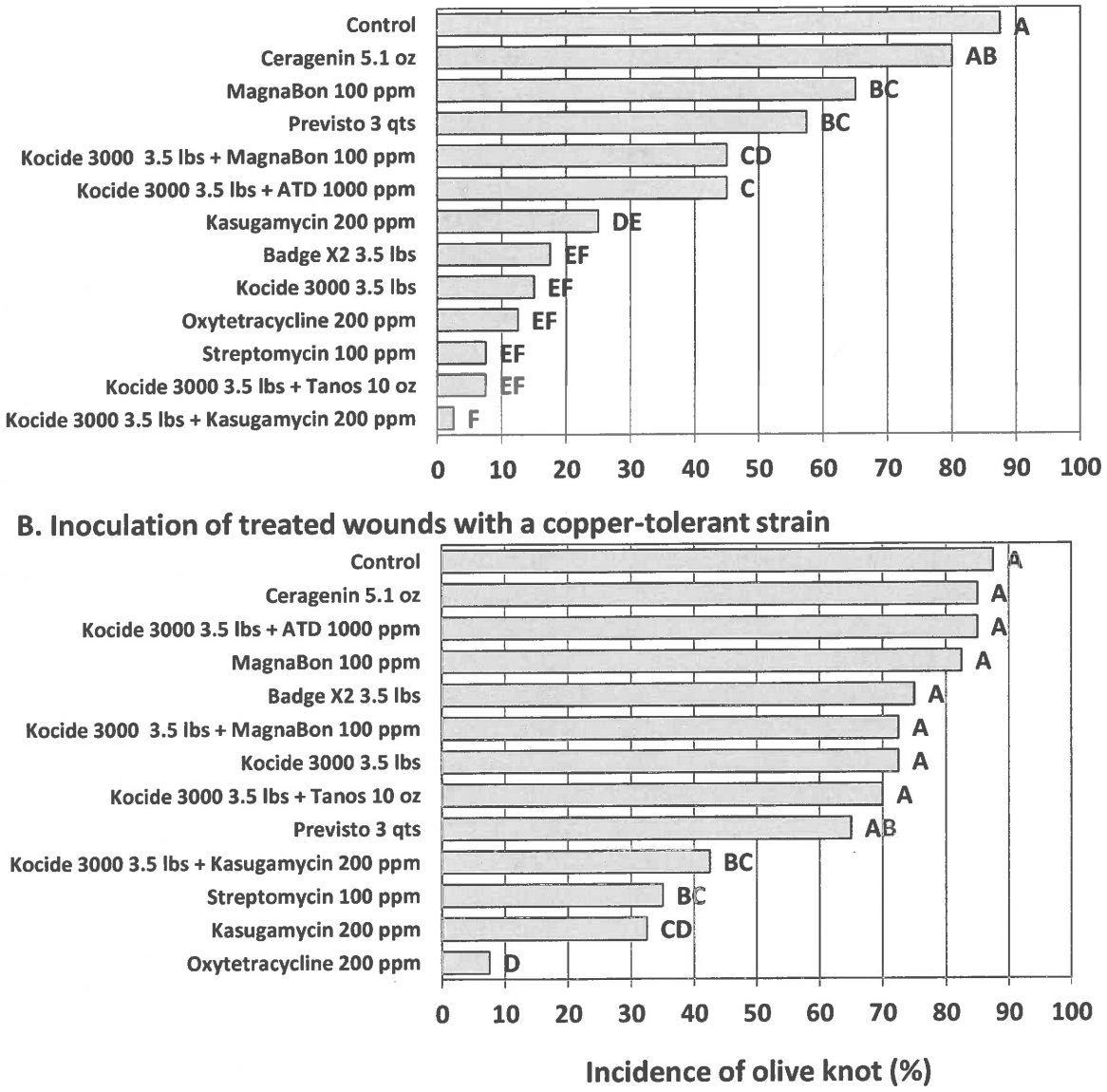
In October 2013, cv. Arbequina olive twigs were wounded, treated, allowed to air-dry, and inoculated with a copper-sensitive strain of *Psv* ($1x10^8$). * Indicates treatments where some replicates showed symptoms of an apparent systemic infection. Rates specified are per acre/100 gal. Knot incidence recorded in early June of 2014.

Fig. 6. Treatment efficacy against olive knot in a field trial at UC Riverside using a copper-sensitive or -tolerant *Psv* strain for inoculation of leaf scar wounds



In late May of 2014, leaf scars on cv. Arbequina were treated, allowed to air-dry, and inoculated with a copper-sensitive or -tolerant strain of *Psv* ($2x10^7$). Rates specified are per acre/100 gal. Knot incidence recorded in early October of 2014.

Fig. 7. Treatment efficacy against olive knot in a field trial at UC Riverside using a copper-sensitive or -tolerant *Psv* strain for inoculation of lateral twig wounds



In May of 2014, lateral twig wounds on cv. Arbequina olive were treated, allowed to air-dry, and inoculated with a copper-sensitive or -tolerant strain of *Psv* ($2x10^{7}$). Rates specified are per acre/100 gal. Knot incidence recorded in early October of 2014.