Department of Plant Pathology
Relevant AES/CE Project No.: 11103864

University of California
Division of Agricultural Sciences
INTERIM PROJECT/RESEARCH PROGRESS REPORT
July, 2015

Project Year: 2015

Project Leader:

Dr. J. E. Adaskaveg, Professor
Department of Plant Pathology
University of California, Riverside
Riverside, CA 92521
(O) 951-827-3880 FAX: 951-827-7577 (M) 951-288-9312
jim.adaskaveg@ucr.edu

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

2015 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. Timing studies: 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.

Summary of Progress in 2015 including ongoing studies:

1a. Ongoing.

1b. Greenhouse and field trials were performed on two olive cultivars (Manzanillo and Arbequina) to investigate the effects of Psv inoculum concentration on disease incidence for both leaf scar and lateral wounds using a copper-sensitive and a -resistant Psv isolate. In greenhouse trials done during the spring of 2015, leaf scars appeared to be less susceptible to infection as compared to lateral wounds at all except one inoculum concentration used, ranging from 2×10^5 to 2×10^8 CFU/mL Psv, for both cultivars and isolates tested. Higher disease incidence was observed for the higher Psv concentrations (2×10^7 and 2×10^8 CFU/mL) in most cases, but other factors may to contribute to disease incidence (e.g., the growth stage of olive plants - less active growth have fewer knots developing). 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.

In field trials conducted during the fall of 2014 at UC Davis (trees planted in 2011) using the same inoculum concentration range and a copper-sensitive Psv isolate, higher disease incidence was observed on cv. Arbequina olives 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 2×10^5 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 olives, respectively.

In a trial repeat using younger olive trees (planted in 2013), similar observations were noted as in the previous experiment using the lowest inoculum concentration (disease incidence of 0 and 10% for cvs. Manzanillo and Arbequina, respectively). For concentrations ranging from 2×10^6 to 2×10^8 CFU/mL, very high disease incidence was observed of at least 66% on both cultivars and both types of wounds.

Past trials have shown that concentrations of 2×10^5 CFU/mL of Psv can produce some disease depending on factors such as humidity and temperature (inoculated greenhouse plants had higher disease incidence than field grown plants with faster developing knots), plant age, wound type, or Psv strain. Thus, this could be considered a threshold concentration for disease induction. Consistent high levels of disease resulted when plants were inoculated with 2×10^7 CFU/mL Psv, and somewhat lower consistent disease levels were achieved with 2×10^6 CFU/mL. We have found that these higher concentrations can be naturally exuded from living knots. Therefore, chemical treatments must be able to provide control at these inoculum levels in order to be effective. Cultivars Manzanillo and Arbequina had some variability in disease incidence among trials, but both should be considered as very susceptible to Psv.

1c. Ongoing.

1d. Ongoing.

2b. In 2015, we obtained additional olive knot samples from an orchard where we previously detected copper resistance in Psv. Isolation from these samples recovered an additional 20 Psv strains. Copper sensitivity tests indicated that total of 3 and 4 of the isolates were resistant or moderately resistant to copper of 147 strains collected. All isolates, however, were sensitive to the antibiotics kasugamycin, streptomycin, and oxytetracycline.

3b. The quaternary ammonium compound (QAC), MaQuat 615-HD, that we have been evaluating as a sanitizing agent for olive field equipment was registered for use on olives in California in 2015 under the trade name Deccosan 321. We initiated field trials in the spring of 2015 to compare QAC to chlorine, as well as QAC in combination with foliar applied copper treatments. We utilized a handheld gas-powered hedger to replicate larger commercial pruning equipment on an experimentally feasible scale. The hedger was used to trim and injure olive branches, simulating damage that could occur during commercial pruning operations. The hedging blades (metal teeth) were contaminated with a high concentration of Psv, and the hedger was subsequently used to prune healthy olive trees. For treatment, the contaminated blades were sprayed with various disinfectants before use. In some treatments, hedging was followed by additional copper and copper-kasugamycin applications to possibly obtain even better disease control. These trials were performed at UC Davis and Riverside and data are pending.

4a. Systemic acquired resistance (SAR) compounds were field-tested against olive knot during the fall of 2014, focusing on the effects of Psv inoculum concentrations on SAR performance. Foliar sprays of SAR compounds were applied to olive cvs. Manzanillo and Arbequina 3 days before wounding and inoculating with a copper-sensitive Psv isolate. Psv inoculum concentrations used ranged from 2×10^5 to 2×10^8 CFU/mL. SAR compounds evaluated included Regalia, Proalexin, Stout, Actigard, and Quintec at experimental or field labeled rates. Most SAR compounds tested did not significantly reduce disease incidence as compared to control trees treated with water. In a few cases Quintec resulted in some reduction of knot formation when trees were inoculated with $2x10^6$ CFU/mL Psv, but not to satisfactory levels. 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. Thus, none of the SAR treatments resulted in a consistent reduction of 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. Field trials were performed during the fall of 2014 in two olive orchards (UC Davis and Yuba county) to test copper treatments mixed with several compounds including etridiazole (Terrazole), amino-thiadiazole-thiol (ATD), mancozeb (Manzate Prostick), famoxadone + cymoxanil (Tanos), and dodine (Syllit), to determine if any enhancement in disease control could be achieved as compared to copper alone. In the Davis trial on cv. Arbequina where a copper-sensitive Psv isolate was used, all copper-containing treatments performed similarly, reducing disease incidence by at least 82.5% and 74% on leaf scars and lateral wounds, respectively. On cultivar Manzanillo, similar results were obtained using the copper-sensitive isolate, with disease incidence reduced by treatments containing copper by at least 87% or 90% for leaf scar and lateral wounds, respectively. Using a copper-resistant isolate, 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%). Poor control was achieved on leaf scar wounds for all treatments, with the best treatment (i.e., copper at the high rate) reducing disease by approximately 30% on both cultivars. 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 isolate, respectively. 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 isolate, while kasugamycin at 200 ppm worked well against a copper-resistant isolate. The incidence of knots developing at leaf scars in the controls was <3% at this trial location.

4c. The non-phenolic quaternary ammonia compound KleenGrow was tested as a protective treatment in several field and greenhouse trials. 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. The antimicrobial compound Ceragenin was also tested for its unique mode of action against bacteria, but also did not provide any reduction in disease incidence in several field trials.

4d. A copper persistence trial was performed on young Manzanillo olives in the fall of 2014 at UC Davis. Olive twigs were wounded and treated with several copper and copper-adjuvant treatments. 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 isolate. All copper treatments significantly reduced disease incidence on inoculated lateral wounds as compared to the control, but only a few treatments were effective in reducing knots on leaf scars. No disease developed on lateral wounds treated with Kocide 3000 (7 lbs/A) or Kocide 3000 (3.5 lbs/A) - Washgard (2.5 gal/A). For inoculated leaf scars, Kocide 3000 (3.5 lb lbs/A) with addition of Quintec (6 fl oz/A), Omni Supreme Oil (2%), or NuFilm (1 pt/A), reduced disease incidence by at least 79%. Copper hydroxide alone at 7 lbs/A performed better than copper hydroxide (3.5 lbs/A) - adjuvant mixtures and thus, persisted well without addition of an adjuvant. In all field trials, a very high level of control was achieved in most cases when copper formulations were used at the high rate, which provided 2,520 ppm metallic copper equivalent, even when a copper-resistant isolate was used. No phytotoxicity was observed. Therefore, high rates of copper still overcome copper resistance mechanisms in Psv.

Contains confidential information for the COC. Please do not post online.

Department of Botany and Plant Sciences
Relevant AES/CE Project No.: 4556

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL PROGRESS REPORT

Project Year: 2015 **Duration of Project: 1 year**

To collect and analyze the yield data for the harvest following the on-crop year (i.e., putative off-crop year) in order to evaluate the efficacy of the PGR treatments applied during the on-crop year.

Project Leaders:

Carol Lovatt, Ph.D.
Department of Botany and Plant Sciences-072
University of California
Riverside, CA 92521-0124
(O) 951-827-4663 FAX: 951-827-4437 (M) 951-660-6730
carol.lovatt@ucr.edu

Elizabeth Fichtner, Ph.D.
University of California Cooperative Extension
4437 S. Laspina St.
Tulare, CA 93274
(O) 559-684-3310 FAX: 559-685-3319 (M) 559-684-2057
ejfichtner@ucdavis.edu

Title: Alternate Bearing in Olive - *Determining when fruit exert their negative effect on return bloom, whether the effect includes inhibition of floral development or only inhibition of bud break, and refining PGR treatments to break AB ON/OFF cycles*

Cooperators:

Lindcove REC — Commercial table olive grove, Lindcove

Objectives for Year 2: Objective (1) to determine whether buds on bearing shoots on ON-crop trees are viable floral buds that only inhibited from undergoing bud break or become nonviable floral buds due to inhibition of floral development by quantifying the expression of key genes in the floral development pathway. Objective (2) to test the ability PGR treatments applied as foliar sprays during the ON-crop year to break the AB cycle by increasing vegetative shoot length (node number), increasing floral bud retention and floral bud break the next spring to increase floral intensity and yield the year following the ON-crop to produce high back to back yields.

2015 Progress to Date:

Summary of the results of completed research.

Overview - Once alternate bearing is initiated by a climatic event that reduces yield, the OFF/ON yield cycles are perpetuated by four possible mechanisms known to function in fruit and nut tree crops. Through one or more of these mechanisms the ON crop reduces floral intensity the next spring, whereas the OFF crop increases return bloom the following spring. Interestingly, only a subset of these mechanisms functions in most tree crops, but the results of our research have now documented that all four mechanisms operate to perpetuate alternate bearing in olive (reported here as the effect of the ON crop): (*i*) inhibition of summer vegetative shoot growth, including lateral shoot growth, which reduces the number of nodes that can bear inflorescences the following spring; (*ii*) inhibition of spring bud break; (*iii*) abscission of next year's floral buds; and (*iv*) repression of key genes in the floral development pathway. Prior to our research, only inhibition of summer vegetative shoot extension growth was known to perpetuate alternate bearing in olive (Sibbett 2000). Further, the results of our research have identified when the ON crop of fruit exert their negative effects on summer vegetative shoot growth, floral bud abscission, floral development and spring bud break.

(i) The ON crop of fruit inhibits summer vegetative shoot growth starting in July, resulting in 76% fewer node pairs by September. A reduction in the number of node pairs means a reduction in floral intensity for the spring bloom following the ON-crop year because every node pair has the potential to produce 2 to 4 inflorescences.

(ii) Only nonbearing shoots on OFF- and ON-crop crop trees produce inflorescences the following spring. However, buds on nonbearing shoots of OFF-crop trees producing significantly more inflorescences than buds on nonbearing shoots of ON-crop trees.

(iii) This is because 89%, 67% and 63% of buds at the first 8 node pairs, second 8 node pairs and basal 5 node pairs, respectively, on nonbearing shoots on ON-crop trees remain inactive (dormant) at spring bloom following the ON-crop year.

(iv) For bearing shoots on ON-crop trees, 75%, 76% and 71% of the floral buds abscise from the first 8 node pairs, second 8 node pairs and basal 5 node pairs along the shoot, respectively, at spring bloom. The major period of floral bud abscission occurs between September and October.

(v) This spring we collected individual buds from the first 8 nodes of nonbearing shoots of OFF-crop trees and nonbearing and bearing shoots of ON-crop trees. The results confirmed that key regulatory genes known to promote flowering are expressed in all buds, but to a greater degree in buds collected from nonbearing shoots from either OFF- or ON-crop trees than in buds from bearing shoots of ON-crop trees. Our results also confirmed that the expression of genes essential to the development of reproductive structures in the flower are significantly reduced in buds collected from nonbearing shoots on ON-crop trees and totally repressed in buds from bearing shoots of ON-crop trees.

In conclusion, the answer to the question addressed by Objective 1 is that a significant percentage of the inactive (dormant) buds remaining on bearing shoots of ON-crop 'Manzanilla' olive trees after significant floral bud abscission are not viable.

The following PGR treatments were applied during the first week of June and again during the first week of July (to increase shoot growth as node number, floral bud number and floral bud retention and prevent inhibition of floral gene expression) and were applied again in February (to increase spring bud break): (1) ON-crop control trees; (2) ON-crop trees treated with 6-BA + low-biuret urea; (3) ON-crop trees treated with cytokinin X (a natural product) + low-buiret urea; (4) OFF-crop trees treated with cytokinin X; and (5) OFF-crop control trees. The PGRs were applied at 0.9 g/tree, low-biuret urea at 0.18 kg N per tree. In our previous proof-of-concept research in two different orchards, 6-BA and cytokinin X were as effective when used alone as when they were combined with an auxin-transport inhibitor in significantly increasing the number of inflorescences produced by nonbearing shoots on ON-crop trees the following spring; the increase in flowering by bearing shoots on ON-crop trees was increased but not significantly. We tested the cytokinins with low-biuret urea for three reasons: (1) to supply N to support PGR-stimulated shoot growth; (2) 6-BA plus urea increases floral bud retention 2.5- to 3-fold during the ON-crop year of pistachio and increases yield to the same degree (this use is on the Valent BioSciences 6-BA label; if successful, it should be easy to add olive to the label); and (3) to increase the potential for either cyokinin to be available to olive growers sooner, we used them alone without the complication of a second hormone, the auxin-transport inhibitor, which is owned by a different company and would require determination maximum residue levels.

ON-crop trees treated with 6-BA plus low-biuret urea produced significantly more fruit per tree than trees in any other treatment, resulting in a net increase in yield of 15 kg/tree compared to the ON-crop control trees and 126 kg/tree compared to the OFF-crop control trees ($P < 0.0001$). In addition, increasing yield with 6-BA and urea in the ON-crop year did not make yield "more OFF" the following year. All Year 1 ON-crop trees had the same OFF-crop yields. Treatments applied to OFF-crop trees did not increase yield (kg/tree) in either the OFF-crop year or the following ON-crop year. Thus, we failed to reduce the severity of alternate and increase yield in the year following the ON-crop year. This is likely due to the fact that the treatments were applied before we discovered that the major period of floral bud abscission on bearing shoots of ON-crop 'Manzanilla' olive trees is from September to October. Our treatments designed to reduce floral bud abscission were made too early to prevent abscission of the floral buds in olive during the ON-crop year.

Progress Report

Project Title:

Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard

Project Leaders:

Louise Ferguson: Extension Specialist, Department of Plant Sciences, 2037 Wickson Hall, Mail Stop II, UC Davis, 1 Shields Ave., Davis CA 95616, (530) 752-0507 [Office], (559) 737-3061 [Cell], LFerguson@ucdavis.edu

John Preece: Curator, USDA National Clonal Germplasm Repository, Davis CA.

John.Preece@ars.usda.gov, (530)-752-7009

Dan Flynn: University of California Olive Center, Davis CA

JDFlynn@UCDavs.edu; (530)-752-5170

James M. Jackson: Principal Superintendent, Plant Sciences Field Facility, UC Davis CA

JMJackson@ucdavis.edu; (530)-753-2173 and (530)-681-2279

Progress through 9/24/2015

The trees planted in 2014 were maintained and staked and grown through the summer of 2015 to allow the trees to reach sufficient size for grafting. The 'Oblonga' trees were falling over more and in more need of staking (which was done) than the others. In spring of 2015, the border rows of 'Sevillano' pollinizers were completed by planting the last 41 trees. There were insufficient trees available in 2014 to complete the border rows.

Some of the rows of dwarf olives were incomplete, therefore additional cuttings were rooted and trees produced at the National Clonal Germplasm Repository nursery. The exception is that 'Dwarf D' has proven to be extremely difficult to root to produce plants for the wider spacing portion of the study. Therefore, in addition, cuttings of 'Little Ollie' were rooted and this cultivar proved to be easy to propagate. On September 29, the nursery produced plants will fill in the missing plants in the planting and 'Little Ollie' will replace the originally planned 'Dwarf D' at the wider spacing. This also gives a fifth genetically different rootstock to test for dwarfing of olive.

One of the 'Sevillano' trees died during the summer of 2015, but there were a few extra trees from the spring 2015 planting, and that tree will be replaced when the planting is completed on Sept. 29.

Sierra Gold Nursery has been contracted to graft the trees on September 28. Therefore, the planting will be completed and the trees grafted by September 30, 2015. This will give a cooler time of the year for the grafts to heal and take.

8 October 2015

To: California Olive Commission.
From: Charles H. Pickett
Re: Summary of project

Title: Biological Control of the Olive Psyllid
Grant period: 01/01/2015 to 11/30/2015.
Amount: $35,304.00
California Olive Commission.

Introduction

This project was initiated to cover costs during the latter half of 2015. It extends work that was initiated in 2012 with funding from the COC, and one year later funded for a three year period by a Specialty Crops Block Grant that ended June 2015. The goal of this project is to release a highly specific parasitoid into California to control the olive psyllid, an exotic, invasive pest of olive trees. The current funding from COC was to finish up studies needed to obtain a field release permit for the olive psyllid parasitoid, *Psyllaephagus euphyllurae* from the USDA APHIS and maintain continuity in this testing of non-target psyllids until the Specialty Crops Block Grant was renewed. The lead technician on the project is John M. Jones who broke his leg in a motorcycle accident. Therefore work was suspended for much of the year. It just continued in October 2015. We've asked for a no-cost extension through December 2016.

Summary of Work Completed in 2015

The project (and Mr. Jones) moved from the UC Riverside Quarantine facility to the UC Berkeley facility in September. Mr. Jones moved insects, plants, and materials up to UC Berkeley. Prior to his accident, John Jones started surveying the state to determine the current distribution of olive psyllid, the second objective to this project. In addition, some survey work that was completed prior to his accident. John inspected 13 locations in southern California during September, the off season for this pest, and found 2 with olive psyllid infestations; ususally one can't find any stage of psyllids during the summer months. This survey will resume next spring when populations are at their peak for the year. Work on testing additional non-target (native) psyllids at the UC Berkeley quarantine has resumed. Host plants for non-target insects are being cultured and the native psyllids will be collected this fall.