CALIFORNIA OLIVE COMMITTEE

INTERIM REPORTS

TOPIC	LEADERS
Improving Mechanical Harvesting Efficiency with Engineering and Pruning	Louise Ferguson John Miles Sergio Castro Garcia
Epidemiology and management of olive knot disease: redefining management strategies based on pathogen population dynamics	Elisabeth Fichtner Bruce Kirkpatrick
Evaluation of New Herbicides for Management of Glyphosate-Resistant Weeds in Table Olive Orchards	Brad Hanson
Host Testing for Olive Psylla Parasitoid	C. H. Pickett
Development of attract-and-kill traps for olive fruit fly	Vicki Yokoyama
Use of PGRs to increase bud break and growth of vegetative shoots during the on-crop year and bud break in Spring the following year to increase return bloom and yield – (continuation project)	Carol Lovatt Elizabeth Fichtner
Epidemiology and management of olive knot caused by *Pseudomonas syringae* pv. *savastanoi*	J.E. Adaskaveg
Determination of Styrene in Imported and Domestic Table Olives	Selina Wang

November 16, 2012

CALIFORNIA OLIVE COMMITTEE

INTERIM PROGRESS REPORT: OCTOBER 25, 2012

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2012 Anticipated Duration of Project: One Year

Project Title: **Improving Mechanical Harvesting Efficiency with Engineering and Pruning**

Project Leaders:

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Cooperating Ranches:
Rocky Hill Ranch; Exeter California: Mark Pascoe; Pascoe32@MSN.com
Nickles Soils Laboratory; Arbuckle: Stan Cutter; CutterFarms@Frontier.net

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2012 Current Funding Request**: 65,804.00**

Overall 2012 Season Summary:

Objectives:

Improve the canopy contact head harvester and evaluate it and ENE Inc. improved trunk-shaking harvester* in two hedgerow orchards, a mature 139 trees per acre hedgerow and a young 202 trees per acre hedgerow.

Evaluate the ability of mechanical pruning to create more easily harvestable hedgerows evaluated with the canopy contact harvester.

Evaluate the effects of mechanical pruning on yield and fruit size.

*
The ENE Inc. trunk shaker was improved and evaluated independently during commercial harvest trials in a mature orchard prepared for trunk shaking. Further development and evaluation of trunk shakers has now moved into the commercial sector

Results:

Improving the canopy contact head increased final harvest efficiency to an aver age of 77% on young, 202 trees to the acre, hedgerow orchards.

Mechanical topping and hedging increase canopy contact head efficiency.

Mechanical topping and hedging does not decrease yield and may decrease alternate bearing.

Progress through October 26th, 2012:

Proposed Specific Objectives for 2012:

I. **Improving Canopy Contact Harvesting Technology**

Hypothesis: I:

Incorporating the proposed engineering changes will increase the harvester efficiency.

Dr. Miles has incorporated the proposed improvements into the harvester head; the rings of rods are now decoupled to move independently, the boom has been shortened for better operator visibility and the bearings have been replaced with solid fittings. The mobile Bobcat platform has been fitted with new treads. The reach on the head has been extended from 1 foot off the ground to 18 feet high. The unit was tested September 27th, 2012. The initial test demonstrated we needed shorter rods and a stronger Honeymoon joint. These modifications were done in time for field harvesting at Nickles October 15th, 2012.

Fig. 1. Modified harvester on October 17th, 2012 harvesting prepared trees at Nickles Soils Laboratory near Arbuckle in Colusa County.

Objective II: Improving Canopy Contact Efficiency with Canopy Management:

Objectives 1 and IIA:
Block 17 W Mechanical Pruning Trial with hand Harvest
Rocky Hill Ranch, Exeter, Tulare County: Manager, Mark Pascoe
17-year-old 'Manzanillo' orchard with 'Sevillano' pollinators (irregularly placed)
13, 83 tree, rows spaced at 12' X 26' (139 trees per acre)

Pruning Treatments Completed: June 6 and 7th, 2012
6 control rows were hand pruned
6 mechanically pruned rows were hedged 4 feet from the trunk on the east side
and
3/6 mechanically pruned rows were topped at 12 feet and three left untopped
All 12 rows were skirted up at 42 inches

Fig. 2. Tree mechanically topped and hedged on June 6th, 2012 photographed on September 5th, 2012. Flanking trees were hedged east side the same date but not topped.

<u>Data Collection</u>: Rocky Hill Ranch, 17-19th, September 2012.

Due to a weather related total crop failure in this block the block was only hand harvested for effects of pruning on yield and fruit size. However, the yield was so low that, as in 2008 and 2009, the data is invalid for treatment effects. However, the hedging and topping, and hedging did significantly decrease yield relative to the hand-pruned controls. The hand pruned controls yielded 1.35 tons per acre versus for 0.92 for hedged trees and 0.24 for hedged and topped trees. However, over the 5 years of mechanical pruning the hedged and topped trees yielded 3.1 tons per acre annually versus 3.2 for hedged trees and 3.1 for hand pruned controls. Therefore, over the five years of mechanical harvesting research the mechanical topping hedging has not decreased annual average yields.

Data Collection: Nickles Soils Laboratory, 15-18th, 2012.

This 2 year old pruning trial produced strong effects on yield and value. In the attached preliminary report handed out at our field day on October 25th it can be seen that the mechanically pruned trees yielded 6.65 tons per acre valued at $982 per ton for a final per acre value of $6,503 per acre versus hand pruned trees which yielded 5.09 tons/acre valued at 1004 per ton and $5,110 per acre. Over the two years of this mechanical harvesting experiment the hand pruned trees have yielded a cumulative total of 5.89 tons per acre for an average annual yield of 2.95 tons versus 7.05 tons/acre and an average annual yield of 3.53 tons per acre for the mechanically pruned trees. The hand pruned control trees had larger, more highly valued fruit that ripened earlier.

Fig. 3. This picture demonstrates how the mechanical pruning, a hedging on the east side 2 feet from the trunk and a topping 10 feet from the ground in 2011, and a tipping at 11 feet from the ground in 2012 produced a tree with fruit on the west side in 2012, and growth on the west side and top for production in 2013.

Experimental Procedures: Objectives I and IIB.
Mechanical Pruning Trial with Canopy Contact Harvester:
Nickels soils Laboratory, Arbuckle, Colusa County, California: Stan Cutter, Manager
11 year-old 'Manzanillo' orchard with center Sevillano pollinator row
Olive Hedgerow Block: 13, 31 tree, rows of spaced at 12' X 18' (204 trees per acre)

Pruning Treatments Completed: July 7th, 2012
Trees were chemically thinned with NAA on May 27th, 2012
Four replications of three rows, 7-8 tree sets, were mechanically pruned

- topped 12 feet
- hedged on the east side 2 feet from the trunk
- Four replications of three row, 7-8 tree sets were mechanically pruned

Four replications of three rows of 7-8 tree sets, were hand pruned to simulate mechanical pruning

Fig. 3. Pictured above is the hand and mechanical pruning treatments at Nickles Soils Laboratory on July 20th 2012. The trees on the left were hand pruned to a hedgerow and the trees on the right were mechanically topped and hedged to a hedgerow with pruning and topping in 211 and 2012. When harvested with the canopy contact harvester October 15th 2012 the harvester removed an average of 77% of the olives from the mechanically pruned trees versus 70% from the mechanically pruned trees. The hand-pruned trees badly damaged the harvester by breaking the picking rods, demonstrating there is a design flaw in how the rods are constructed.

Data Collection: October 15th 2012.
The canopy contact harvester was evaluated in this plot the week of October 15th, 2012:
Fruit removal force was tested prior to harvest, a hand harvest control was taken from each replication of both hand and mechanically pruned blocks prior to mechanical harvesting of each replication followed by hand gleaning to for harvester efficiency.

A field day was held in the plot October 23rd to demonstrate the harvester.

Desired Results:
Objective I: Mechanical harvester improvements will increase final harvester efficiency to 80% or more.

Objective IIA and IIB: Demonstrate mechanical pruning and hedgerow training will increase canopy contact mechanical harvesting efficiency versus hand-harvested trees.

Results:
As can be seen in the attached preliminary report handed out at the field day the canopy contact harvester removed an average of 77% of the fruit from the mechanically pruned trees versus an average of 70% for the hand pruned trees. Additionally, the hand pruned trees, with more of their crop close to the trunk badly damaged the harvester by breaking the picking rods. This is a design flaw in the picking head that will need to be solved.

UC ANR
Relevant AES/CE Project No.: Y11-893

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2011 – 2012 **Duration of Project: year 2 of 2**

Project Leaders:
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Title: Epidemiology and management of olive knot disease: redefining management strategies based on pathogen population dynamics.

Cooperators:
Louise Ferguson, PhD, UCCE Specialist, UC Davis
William H. Krueger, MS, UCCE Farm Advisor, Glenn and Tehama Counties
Carolyn DeBuse, MS, UCCE Farm Advisor, Yolo and Solano Counties.
Janet Zalom, Programmer II, UC Davis

2012 Objectives:

I. Investigate survival potential of *P. savastanoi* in gall tissue.
II. Assess epiphytic populations of *P. savastanoi* throughout year and determine whether epiphyte populations reflect disease severity in orchards.
III. Determine potential for two polymer products to mitigate cold damage to olive.
IV. Efficacy of film-forming polymers as physical barriers to infection, as well as for enhancing persistence of copper on leaf surfaces.

Progress to Date (November 2012):

I. In order to generate galls of equal age for commencement of a pathogen survival study, exposed leaf scars on mature 'Manzanillo' branches were inoculated in April 2011. Five branches were flagged on each of seven trees, and 10 pairs of leaf scars were exposed on flagged branches. Leaf scars were inoculated by misting with a 10^6 cfu/ml suspension of *P. savastanoi*. The inoculum cocktail was composed of 9 isolates of the pathogen, all collected from the UC Davis Ag Experiment Station (Solano Co., CA). Symptoms were not observed until October 2011. Quantification of bacterial populations in galls was conducted in Dec 2011, Feb 2012, and May 2012. Psv populations have not changed significantly between December 2011 and the May

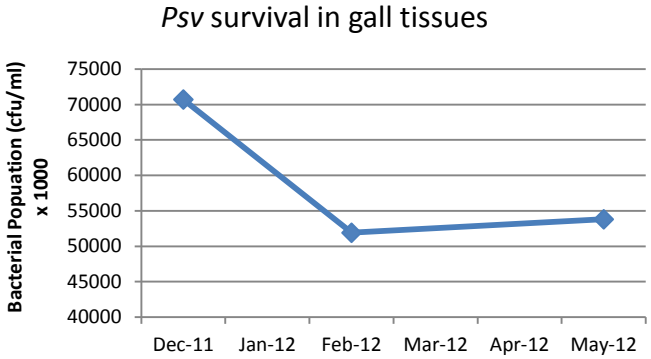

Figure 1. *Psv* populations in galls formed at inoculated leaf scars.

2012 sample timepoint (Fig 1). Galls will be sampled again in November 2012 to determine pathogen survival over a summer survival period.

II. A leaf wash technique was employed to quantify populations of *Psv* on olive leaves. The technique was employed in May and November 2011 to assess epiphyte populations at two sites in Arbuckle, CA. In January 2012, the sampling was expanded to 12 sites ranging from Tulare County to Tehema County, and was conducted again in June 2012 and November 2012 (results pending) at 11 sites. Five trees were flagged in each of 11 orchards with varying levels of disease for repeated sampling over time. The canopy of each flagged tree was divided into 4 quadrants, and 25 leaves were sampled from each quadrant for a total of 100 leaves per tree. To assess bacterial populations over equal leaf surface areas, a single 1.2 cm diameter leaf disk was cut from each leaf, and the 100 leaf disks from a given tree were constantly shaken in phosphate buffer for 2 h at 200 rpm. The resulting bacterial suspension was dilution plated onto both KB and PVF media using a spiral plater. KB medium was utilized to quantify total bacterial populations on leaf surfaces, whereas PVF was utilized to assess fluorescent Pseudomonads, and

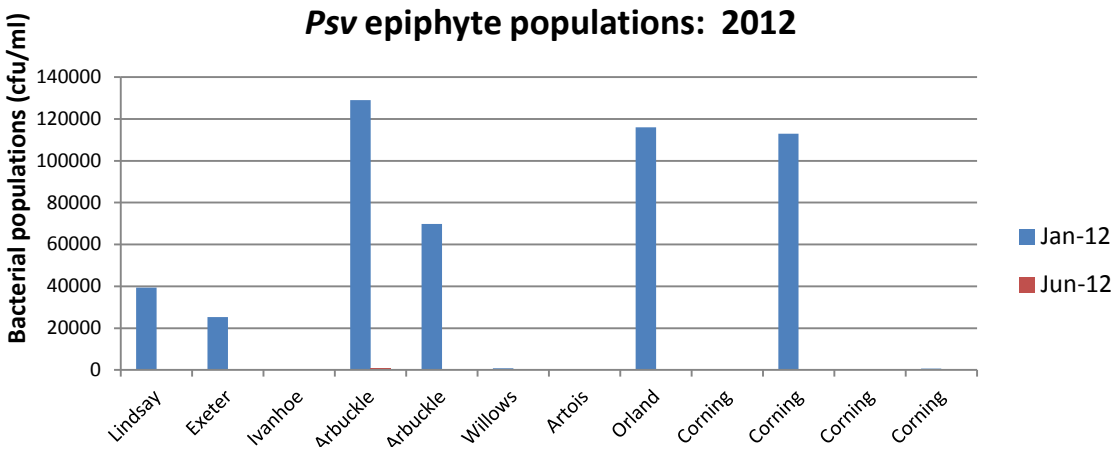

Figure 2. Epiphytic *Psv* populations detected on PVF medium in January and June 2012. Epiphytic pathogen populations were below detectable levels at all but one site during the summer.

specifically *Psv*. The only site in the state with detectable summer populations of the pathogen was in Arbuckle, CA, only 2 miles from the Nickels Site which remains disease-free (Fig 2). Of the 8 sites with high disease severity, only two exhibited high population levels (ie. ≥ 10,000 cfu/ml) of *Psv*.

Preliminary results suggest that epiphytic populations may not serve as a predictor of disease severity or potential risk of disease. Additionally, in most CA orchards, epiphytic populations decline to undetectable levels during the summer months. No influence of geographic area on epiphytic populations has been observed, suggesting that factors other than pathogen population (ie. weather) may be more important predictors of disease risk.

III. A controlled study was conducted to determine the potential for either of two polymer products to offer cold protection to olive. Potted 'Manzanillo' plants were exposed to three treatments, a coating with either Anti-Stress 550 or FreezePruf, or an untreated control sprayed with water. After polymer products were applied, plants were exposed to natural sunlight for approximately 10 hr to allow for product polymerization on the plant surface. Plants were then placed in a cold chamber at -5 °C and 5 replicate plants of each treatment were removed from the cold chamber after the following durations of cold exposure: 0 hr (control), 0.5 hr, 1 hr, 3 hr, 6 hr. Plants were assessed for cold damage upon removal from chambers, and then again 1 week,

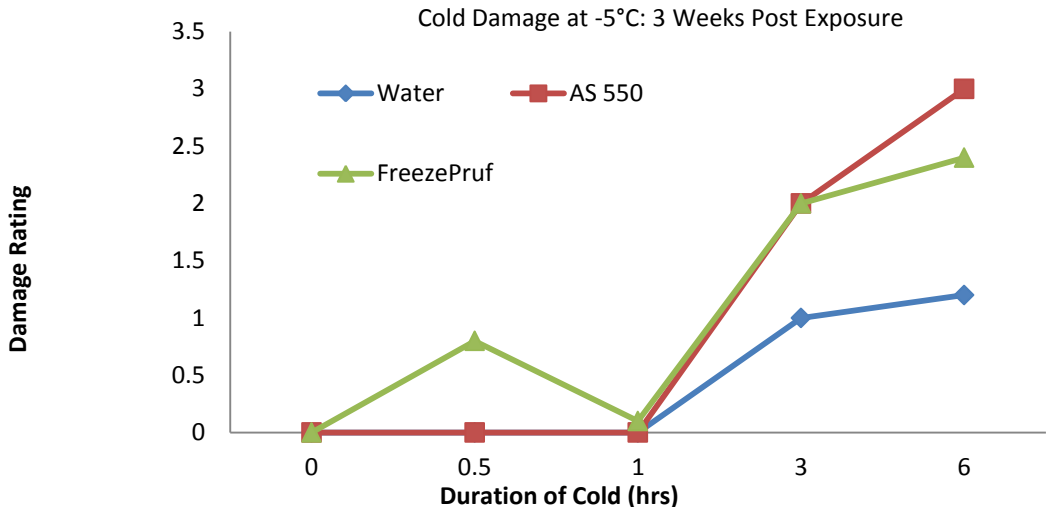

Figure 3. Cold injury on olive as a result of exposure to -5°C. Higher damage ratings represent more cold injury, with "0" indicating no damage, and "4" indicating mortality.

2 weeks, and three weeks after cold exposure. A rating scale was developed to quantify cold damage: (0) no damage, (1) less than half of leaves browned, (2) ½ to ¾ leaves browned, (3) over ¾ leaves browned, and (4) dead. The experiment was run twice.

Cold damage on olive was first noticed after 3 hr exposure to -5 °C. Less damage was observed on plants that were treated with water as opposed to either polymer product. **Over two runs of the experiment, both the Anti-Stress 550 and the FreezePruf products exacerbated cold injury on olive** (Figure 3). FreezePruf contains five cryoprotective ingredients and has been shown to enhance plant resistance to cold injury and mortality (Francko, et al 2011). The results

of our study indicate that neither product offers cold protection to olive under the parameters utilized in the study. We plan to repeat this study again in 2013.

V. Two experiments (potted plant and tagged branch/whole tree) were conducted in 2011and 2012 to elucidate the efficacy of film-forming polymers as physical barriers to infection, as well as tank additives to enhance persistence of copper on leaf surfaces. These experiments are scheduled to conclude in November 2012.

For the potted plant experiment, olive seedlings were treated with a suite of polymer/copper treatments and exposed to natural inoculum from January through April in 2011 and 2012. A complete factorial treatment design comprising of two copper levels (no copper control; 4.2 g ocide/L) and 4 polymer treatments (no polymer control; 1% VaporGard; 0.11% Nu Film P; 2.5% Anti-Stress 550) was utilized both years; however, in 2012 we included an cryoprotectant polymer-containing product called FreezePruf. Initial spray treatments were conducted on December 24, 2010 and on December 23, 2011. Spray treatments in 2011 were repeated on the following dates: January 16, 2011 and February 28, 2011. Spray treatments in 2012 were repeated on the following dates: February 15, 2012 and April 17, 2012. Twenty pairs of leaf scars were exposed prior to each spray application to test whether polymers created a physical barrier to infection and assess when leaf scars are most susceptible to infection. After each spray application, foliage was sampled to determine the influence of polymers on Cu persistence. Climatologic data (precipitation, temperature, solar radiation) was collected throughout the experiment.

For the potted plant experiment, disease incidence and severity were assessed in November 2011 for the first season of the study, and will similarly be assessed in November 2012 for the second and final season of the study. In 2011, the majority of infections of leaf scars occurred during the spring rain events (>5%), whereas, less than 1% of leaf scars were infected between Dec 24, 2010 and Feb 28, 2011.

During the 2011 season, Anti-Stress 550 enhanced Cu persistence on the leaf surface when applied prior to several consecutive days of incoming solar radiation. This polymer, however, did not enhance copper persistence if applied within 48 h of a rain event**.** Consequently, in 2012, all polymer treatments were conducted in advance of at least two days of low cloud cover. **In 2012, none of the polymer treatments affected Cu persistence on the plant surface. In 2011 only Cu had a significant effect on disease incidence (P≤0.01).**

In the mature-tree trial initiated in April 2011 and April 2012, exposed leaf scars on mature 'Manzanillo' trees were treated with the same compliment of polymer/copper treatments and then artificially inoculated with *P. savastanoi*. In April 2012 an additional polymer treatment 'WiltPruf' was be included in the study. A randomized complete block design was utilized, with each of 12 trees serving as a block. Treatments were therefore "nested" on individual trees. On each tree, treatments were challenged with two inoculum levels of *P. savastanoi*, 10^4 and 10^8 cfu/ml. In 2011 disease incidence was influenced by an interaction of polymer, presence of Cu, and inoculum level (P≤0.045**); however, in 2012, disease severity was only influenced by presence of Cu (P≤0.01)**. **In both seasons, none of the four polymers protected leaf scars**

from infection at either inoculum level, nor did any polymers enhance the efficacy of Cu in reducing disease incidence. In 2011 there was no clear influence of Cu, polymer, or inoculum level on disease severity. Disease severity data for the 2012 field season has not yet been collected.

Project Year: 2012

Anticipated Duration of Project: 1 year (no-cost extension requested 10/9/12)

Project Leader: Brad Hanson
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Location: UC Davis

Project Title: Evaluation of New Herbicides for Management of Glyphosate-Resistant Weeds in Table Olive Orchards

Cooperating personnel: Elizabeth Fichtner (UCCE Farm Advisor)
M. Joy Abit (Post-doctoral Scholar) – left UC Davis in 2012
Bill Krueger (UCCE Farm Advisor) – retired in 2012

Keywords: glyphosate-resistance, weed management, herbicidal weed control, production system

Commodity: olive

Relevant AES/CE Project No.:

COC approved funding: $ 18,129.00

Project Background: Orchard floor management in table olives is a continuing challenge. Common cultural weed management practices such as mowing and disking can provide good control of weeds. However, the shift to moderate- and high-density plantings in table olive production limits the use of mechanical cultivation in tree rows. As mechanical harvesting is incorporated into table olive production, significant changes in tree maintenance are required to accommodate harvesting equipment. These changes also increase light penetration to the ground thereby promoting weed germination and growth. In addition, the shift to low-volume irrigation systems has affected weed emergence and growth, herbicide persistence, and further limited mechanical weed control within the tree row in drip, micro-sprinkler, and sprinkler irrigated olive orchards.

Several new herbicides have been registered in other orchard crops for the control of a broad spectrum of weeds including glyphosate-resistant species. However, these herbicides are not currently labeled for olive. An important first step in broadening herbicide registrations to include olive is to evaluate and demonstrate weed control efficacy and crop safety in California table olive orchards.

Objectives: Overall, this project aims to develop an additional weed management tools for control of glyphosate-resistant weeds in mechanically-harvested table olives. Specifically, the objectives of this proposal are to:

1. Develop crop safety data on currently unregistered herbicides with a potential fit for moderate-density table olive production systems.
2. Evaluate and demonstrate effective herbicide rates and application timings for effective weed control with new herbicides in moderate- and high-density table olive plantings.

Progress to Date: This work was originally planned for the 2011-12 growing season. Because COC funding decisions were not finalized until spring 2012, the project will be started in winter 2012 instead. A one-year, no-cost extension was requested on 10/9/12 to accommodate the change in research schedule.

Although the proposed table olive trials were delayed one year, we were able to conduct two preliminary experiments in young olives (high density oil olive planting) near Yuba City, CA in 2012. These same treatments will form the basis for the proposed table olive trials planned for the coming growing season. Herbicides were applied using a CO_2-pressurized backpack sprayer, with XR8002 nozzles, calibrated to deliver 20 gal/A. Evaluation for crop injury was made at monthly intervals after treatment. The site had been previously treated with registered herbicides so no weed control evaluations were made.

In the first experiment, 1x and 2x use rates of preemergence herbicides were applied to the tree rows in March 2012 (Table 1). These treatments serve as an important first step in evaluating the table olive safety of several herbicide active ingredients registered in other orchard crops. In this trial, no injury was observed with 2x rates of Pindar GT, Tangent, Matrix, Calisto, or Alion herbicides. The plots will be retreated with the same herbicides in the 2013 season. The second experiment (Table 2) was conducted to evaluate the crop safety of Treevix (saflufenacil) an important POST herbicide in nut crops. Because this herbicide is potentially closer to registration and in order to address manufacturer concerns, the treatments were considerably more aggressive in terms of determining crop safety. This trial included Treevix applied at 1, 2, 4, and 8x use rates in March 2012. No crop injury that could be attributed to root uptake of saflufenacil was noted in this experiment which suggests there is not an unusual level of susceptibility to the herbicide in olive. These treatments will also be repeated on the same plots in 2013.

A table olive cooperator for the 2012-13 trials in Tulare Co has been identified. We are still trying to identify a Sacramento Valley table olive cooperator for the proposed project. Because herbicide registration at the Federal and State level do not distinguish between table and oil olive, the ongoing work will be applicable to all market classes of olive.

Table 1. Preemergence herbicide treatments for crop safety and efficacy testing in olive orchards. Rates include a 1x use rate and a 2x rate needed for minimum safety for registration consideration. No injury was observed in olive in this trial

Treatment	Active ingredient	Rate
1	untreated	--
2	penoxsulam (Tangent)	2 oz/A
3	penoxsulam (Tangent)	4 oz/A
4	penoxsulam + oxyfluorfen (Pindar GT)	3 pt/A
5	penoxsulam + oxyfluorfen (Pindar GT)	6 pt/A
6	rimsulfuron (Matrix)	4 oz/A
7	rimsulfuron (Matrix)	8 oz/A
8	mesotrione (Callisto)	3 fl oz/A
9	mesotrione (Callisto)	6 fl oz/A
10	indaziflam (Alion)	5 fl oz/A
11	indaziflam (Alion)	10 fl oz/A

Table 2. Treevix (saflufenacil) treatments in an olive crop safety trial near Yuba City in 2012. Treatments were applied in March 2012 to young oil olive trees. The use rate of Treevix in nut orchards is 1 oz/A. No olive injury was observed from up to 8x rates of Treevix herbicide in this preliminary trial.

Treatment	Active ingredient	Rate (product)
1	untreated	--
2	saflufenacil (Treevix) + MSO + AMS*	1 oz/A
3	saflufenacil (Treevix) + MSO + AMS	2 oz/A
4	saflufenacil (Treevix) + MSO + AMS	4 oz/A
5	saflufenacil (Treevix) + MSO + AMS	8 oz/A

* all treatments included 1% v/v methylated seed oil (MSO) and 2 qt/A ammonium sulfate (AMS)

Progress Report: Host Testing for the Olive Pyslla Parasitoid

Preprared by C. H. Pickett

November 1, 2012

Project: Host Testing for Olive Pyslla
Project leaders: Thomas Miller, Charles Pickett
Length of Grant: One year
Grant Amount: $32,250

Summary
Work on this grant was initially delayed due to invoicing problems. This was resolved through a non-salary, guest appointment given to C. H. Pickett at UC Riverside. Billing can now proceed smoothly, without delays or problems associated with a third entity (CDFA). Progress has been made on establishing psyllid cultures in the UC Riverside quarantine, propagation of plants for culturing insects, and obtaining additional parasitoids from Spain through help from the USDA ARS European Biological Control Laboratory. Lastly, a specialty crops block grant was awarded to Charles Pickett that will allow us to expand the scope and duration of the project when funds become available.

UCR Riverside Progress for COC Project *Host Testing for Olive Psylla Parasitoid*

Permits and establishing cultures:

We applied for a permit in the name of the UCR Co-PI, Dr. Thomas A. Miller required to house the olive psyllid in the UCR quarantine. Permit # 2886 was granted in April and cultures were transferred into Quarantine in May. USDA Permit # P526P-12-02123 was also granted for Dr. Miller to receive and maintain the *Psyllaephagus* parasitoid in the UCR Quarantine facility.

Cultures in UCR I & Q Facility:

Postdoctoral Researcher, Genet Tulgetske, underwent training and obtained permission to maintain cultures and perform research in the UCR Quarantine Facility. Olive plants infested with olive psyllid were transported from Sacramento to Riverside in May 2012 and moved into Quarantine according to permit conditions and quarantine regulations. The culture was maintained and amplified in the third floor quarantine room 370. However, this room was not ideal for testing since quarantine regulations in the wing housing room 370 do not allow the parasitoid or psyllid to be transferred freely between quarantine rooms. In order to solve this problem, we applied for space in the testing wing of the quarantine facility and continued maintaining the culture while waiting for space to become available.

We have recently acquired three rooms in the UCR ENTOMOLOGY Insectary and Quarantine Facility that will allow efficient rearing and testing of all insects related to the project. In the third floor quarantine area, we have acquired rooms 331 and 350 for housing the olive psyllid and parasitoid, respectively. In

the second floor insectary area, we have recently been assigned room 235 for rearing native psyllids not requiring quarantine conditions. The third floor rooms are currently set up with proper rearing conditions (caging, lighting, humidity, and maintenance supplies) and the olive psyllid is currently in active culture and colony expansion for testing (Figures 1 & 2). We are in the process of preparing the rearing room for native psyllid cultures and expect to acquire native psyllids to begin culturing at the beginning of the year.

Plant propagation and maintenance:

We have acquired and set up greenhouse #1654F at AgOps, UC Riverside for maintaining plants that will host native psyllids, such as tomato, honey mesquite, and sweet acacia, and olive. The larger plants are held in the greenhouse until they are transferred either to the Quarantine's 3rd floor restricted rooms where host testing will be conducted, or to the 2nd floor rearing room used for psyllid culture maintenance. Several olive trees and tomato plants are currently being maintained. We have collected and prepared seeds for growing sweet acacia and honey mesquite. We have not been very successful in germinating seedlings of these two plants at the UCR Greenhouse. However, seedlings were provided by colleagues at the CDFA Mt. Rubidoux Field Station in Riverside. They have agreed to help in acquiring additional seedlings for rearing next year.

Figure 1. Olive psyllid culture.

In addition to providing us with seedlings for growing larger plants to be used for culture maintenance, colleagues at Mt. Rubidoux Field Station have also agreed to provide us with olive and other seedlings needed for testing. We have recently received access to the funding from the COC grant and are currently acquiring supplies for seedling propagation and testing.

Figure 2. Closeup of olive psyllid culture.

Parastioid culture

Like the olive psyllid, the parasitoid *Psyllaephagus euphyllurae* estivates during ~~the~~ summer months. They don't reproduce, and remain as pre-adults in a 'mummy-like' condition. We have been able to maintain some olive psyllid reproduction in containment at CDFA in Sacramento by creating 'spring-like' conditions during the summer. This has been far more effective with the same insects at the UCR quarantine where staff have complete control over lighting, temperature, and humidity in their rooms. As a result, the first set of tests may not be possible until early winter, or next spring, because adult parasitoids are not available. We are making contingency plans to support these studies with field collections from northern Spain next spring, and hope to 'trick' the parasitoid population into reproducing all year after cultures are transferred to UC Riverside. The same parasitoid is being maintained at the UC Berkeley quarantine, and they have experienced the same problem.

USDA, Agricultural Research Service
San Joaquin Valley Agricultural Sciences Center

INTERIM PROGRESS REPORT TO THE CALIFORNIA OLIVE COMMITTEE

Project Year: 2012 Anticipated Duration of Project: 2 Years

Project Leader: Victoria Y. Yokoyama, Ph.D. Location: Parlier, CA
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Project: **DEVELOPMENT OF ATTRACT AND KILL TRAPS FOR OLIVE FRUIT FLY**

Cooperating Personnel: Dr. Jaime Pinero, Lincoln University, Jefferson City, MO
Dr. Roger Vargas, USDA, ARS, PBARC, Hilo, HI

Keywords: Olive fruit fly, cultural control, attract and kill, fruit fly traps
Commodity: Olive

SUMMARY OF WORK

1. Olive fruit fly was collected from infested olive trees in Lodi and San Jose, CA. The infestations have been light this year due to the hot weather in August and September. The larvae and pupae from infested fruit were held at two temperatures to stagger emergence for maintaining a laboratory colony and conducting greenhouse tests.

2. Olive fruit fly adults of known age are released in one cubic meter cages in the greenhouse. The attractiveness of olive fruit fly adults to the prototype yellow bait pans with and without male lures, GF-120 and ammonium carbonate baits is determined based on time and number of visits by females and males.

3. Trap construction has been evaluated with Mr. Richard Alvarado, Better World Manufacturing, Fresno, CA to determine procurement of inexpensive materials and the possibility of creating an economical, durable, long lasting, yellow plastic extruded cover.

4. Trap size has been lengthened to eliminate waste of construction materials and to facilitate bait spray applications by ground.

5. A survey continues for suitable sites for preliminary field tests to determine efficacy of reducing olive fruit fly adult and larval infestations. Local sites have been eliminated by premature fruit drop.

   Note: The olive fruit fly laboratory sustained major damage from a hood fire and is under repair.

RECENT PUBLICATIONS OF RESEARCH SUPPORTED BY COC

Yokoyama, V. Y., X. G. Wang, A. Aldana, C. E. Cáceres, H. A. Yokoyama-Hatch, P. A. Rendón, M. W. Johnson, and K. M. Daane. 2012. Performance of Psyttalia humilis (Hymenoptera: Braconidae) reared from irradiated host on olive fruit fly (Diptera: Tephritidae) in California. Environ. Entomol. 41: 497-507.

Yokoyama, V. Y. 2012. Mobility of olive fruit fly (Diptera: Tephritidae) late third instars and teneral adults in test arenas. Environ. Entomol. 41: 1177-1183.

Yokoyama, V. Y. 2012. Olive fruit fly (Diptera: Tephritidae) in California: Longevity, oviposition, and development in canning olives in the laboratory and greenhouse. J. Econ. Entomol. 105: 186-195.

BUDGET

1. August 1, 2012, Mr. Alexander Ott, COC, was successful in obtaining a waiver of overhead charges for this project, Trust Fund Cooperative Agreement 58-5302-2-266.

2. No funds have been received from the COC at this time. The PL will investigate the status of the agency agreement and invoice activity.

ACKNOWLEDGMENT

I am very grateful for the continuing support from the California Olive Committee for development of methods to control olive fruit fly in canned olives.

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

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL PROGRESS REPORT

Project Year: 2011 – 2012 **Duration of Project: year 2 of 2**

Project Leaders:

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Title: Use of PGRs to increase bud break and growth of vegetative shoots during the ON-crop year and bud break in Spring the following year to increase return bloom and yield – *proof of concept for olive*

Cooperator:

Byron Fox — Commercial table olive grower, Ivanhoe
Clarence Hill — Commercial table olive grower, Exeter
Lindcove REC — Commercial table olive grove, Lindcove

2012 Objectives:

I. To conduct a proof-of-concept experiment testing Summer (Year 1) and Spring (Year 2) applications of an auxin transport inhibitor, tri-iodobenzoic acid or naringenin, combined with a commercial cytokinin, benzyladenine (BA), or the proprietary cytokinin used in our mandarin research, (i) to increase vegetative shoot growth and, hence, the number of nodes that can produce inflorescences at return bloom, (ii) to increase Spring bud break and (iii) to increase return bloom in olive.

2012 Progress to Date:

I. The goal of our research was to determine whether the mechanisms perpetuating the repeating ON- and OFF-crop cycles in 'Pixie' mandarin apply to olive. This included testing the efficacy of an auxin-transport inhibitor combined with a cytokinin (*i*) to increase summer vegetative shoot growth during the ON-crop year to provide more sites at which to bear inflorescences the following spring and (*ii*) to overcome the inhibition of spring bud break to increase inflorescence number following the ON-crop year. Here we summarize the five hypotheses we tested on olive to determine the mechanism by which crop load influences return bloom and yield the following year and the results we obtained, including the results of the PGR treatments listed in objective I.

HYPOTHESIS 1: The ON-crop of olive fruit reduces return bloom in olive by inhibiting bud break for summer vegetative shoot extension growth in Year 1 (2011), thereby reducing the number of nodes that can produce inflorescences the next spring. **This hypothesis is true for olive.** The results in Table 1 (below) provide clear evidence that fruit inhibit summer vegetative shoot extension growth. The results provide evidence that the greatest growth was attained by mid-July by shoots that did not set fruit on OFF-crop trees (OFF/-fruit). These shoots grew more than shoots that did not set fruit on ON-crop trees (ON/-fruit), revealing the strong whole tree inhibitory effect of the ON-crop compared to the OFF-crop. Shoots that set fruit on ON-crop trees (ON/+fruit) produced the least amount of new growth, demonstrating the localized effect of the individual fruit set on the shoot combined with the whole tree effect of the ON-crop. Additional preliminary results of our study removing fruit from shoots of ON- and OFF-crop trees indicated that removing fruit in June increased summer vegetative shoot extension growth from June to September to a greater degree than removing fruit at a later date (even July), suggesting that the effect of fruit on summer shoot extension growth occurs *before* July. It is necessary to now determine how early in the season the setting fruit (possibly the inflorescences) inhibit vegetative shoot extension growth.

HYPOTHESIS 2: The ON-crop of olive fruit reduces return bloom by inhibiting bud break for summer vegetative shoot extension growth in Year 1 by increasing the auxin to cytokinin ratio in the apical bud that produces the new growth. This phenomenon is known as correlative inhibition. If true for olive, treating trees with an auxin-transport inhibitor and/or a cytokinin will overcome the inhibition of bud break and increase shoot growth. In Table 2 (Parts A and B), it is clear that injecting the auxin-transport inhibitors tri-iodobenzoic acid (TIBA) and naringenin (NAR, a natural product) with or without the cytokinin 6-benzyladenine (BA) or the proprietary cytokinin (CPK, a natural product) increased summer vegetative shoot extension growth on ON-crop trees (*i*) for shoots without fruit (Table 2 Part A) and (*ii*) shoots with fruit (Table 2 Part B) relative to the equivalent shoots of untreated ON-crop control trees (ON/-fruit and ON/+fruit) and equal to untreated OFF-crop control trees (OFF/-fruit). See the treatments marked with an asterisk. **This hypothesis is true for olive.** In spring of project year 2013, half the trees treated with PGRs in summer 2012 will receive a second PGR treatment to increase spring bud break. The results will tell us if combining the summer and spring treatments increased floral intensity following the ON-crop year to a greater degree than just treating the trees in summer or spring only.

HYPOTHESIS 3: The ON-crop in Year 1 (2011) reduces the floral intensity of the return bloom in olive in Year 2 (2012). The results in Table 3 provide clear evidence that **this hypothesis is true.** Shoots that did not set fruit on OFF-crop trees in 2011 (OFF/-fruit) produced the most inflorescences in 2012. ***Note:*** In Table 3, shoots that produced many inflorescences concomitantly produced less vegetative shoot extension growth (new nodes) from bud break (BB) through May, resulting in few nodes to bear inflorescences in Year 3. Shoots that did not set fruit on ON-crop trees in 2011 (ON/-fruit) also produced many floral shoots in 2012, but shoots of ON-crop trees that set fruit in 2011 (ON/+fruit) produced few inflorescences. ***Note:*** In Table 3, independent of the number of inflorescences produced per shoot of ON-crop trees the following spring, vegetative shoot extension growth from bud break to May was significantly greater than that of the 2011 OFF-crop trees that produced an intense bloom in spring 2012. For the 2011 ON-crop trees, the low number of inflorescences will result in an OFF-crop in 2012, but the high number of new nodes produced in spring 2012 will result in an ON-crop again in 2013

(Year 3). Thus, the number of new nodes produced during the spring of the ON-crop year is a key determinant of floral intensity the following spring. It is important to determine when the setting crop (or even inflorescences) exerts a negative effect on vegetative shoot extension growth.

There is another important piece of information derived from these results. At bloom following the ON-crop year, it is clear that the shoots that *did not set fruit* on ON-crop trees are the ones producing the majority of inflorescences the following year. The problem is there are too few shoots without fruit on ON-crop trees to achieve a good bloom following the ON-crop. Thus, we must develop a strategy to increase floral intensity on shoots that set fruit on the ON-crop trees. However, at this time, we do not know whether the reduced number of inflorescences on shoots that set fruit on ON-crop trees (ON/+fruit) is due to inhibition of bud break only or inhibition of floral development and, if the case, when the triggering event occurs. In project year 2013, we will answer these questions. We have a partial answer already (see below).

HYPOTHESIS 4: For shoots without fruit on ON-crop trees, bud break, not floral development, is inhibited. If this hypothesis is true, plant growth regulators known to increase bud break in spring will increase floral intensity at return bloom. **This hypothesis is true for olive.** The results in Table 4 (Part A) demonstrate that both cytokinins (BA and PCK) increased the number of inflorescences produced by shoots that did not set fruit on ON-crop trees (ON/-fruit) to a value greater than shoots without fruit on OFF-crop trees (OFF/-fruit). See the treatments marked with an asterisk in Table 4 Part A. In addition, we are also very interested in the PGR treatments (marked with daggers) that increased inflorescence number to a value equal to that of shoots without fruit on OFF-crop control trees (OFF/-fruit) and also increased the number of new nodes from bud break to May to a value similar or equal to the ON-crop control trees, because these are the nodes that will bear inflorescences in Year 3. In our proposed project, we will determine whether treatments that increase bloom in spring and also increase the number of new nodes on vegetative shoots by May go on to produce a large number of inflorescences again the following year, i.e., two ON blooms in a row.

HYPOTHESIS 5: For shoots with fruit on ON-crop trees, failure of PGRs known to stimulate spring bud break to increase inflorescences number following the ON-crop year leaves open two possibilities: (i) the buds are more deeply inhibited but remain viable floral buds or (ii) floral development is inhibited and the buds are no longer viable floral buds. Treating trees with PCK or TIBA plus PCK in February or TIBA plus BA in March increased inflorescence number more than 6-fold over the untreated ON/+fruit control, but the increase was not significant at the 5% level. Moreover, inflorescence number remained 3-fold lower than the OFF/-fruit control. See treatments marked with an asterisk in Table 4 Part B. These results leave open both possibilities posed above. In project year 2014, we will be able determine whether bud break of buds of shoots +fruit on ON-crop trees are simply inhibited (viable) or whether floral development was inhibited (not viable floral buds). The answer to this question is critical for evaluating and improving the efficacy of PGR or flower and fruit thinning treatments to mitigate AB.

A model illustrating the cycling of ON- and OFF-crops in 'Manzanillo' olive trees. The reciprocity between floral shoot and vegetative shoot development (Tables 3 and 4) results in the annual cycling of ON- and OFF-crops in olive during alternate bearing (Fig. 1).

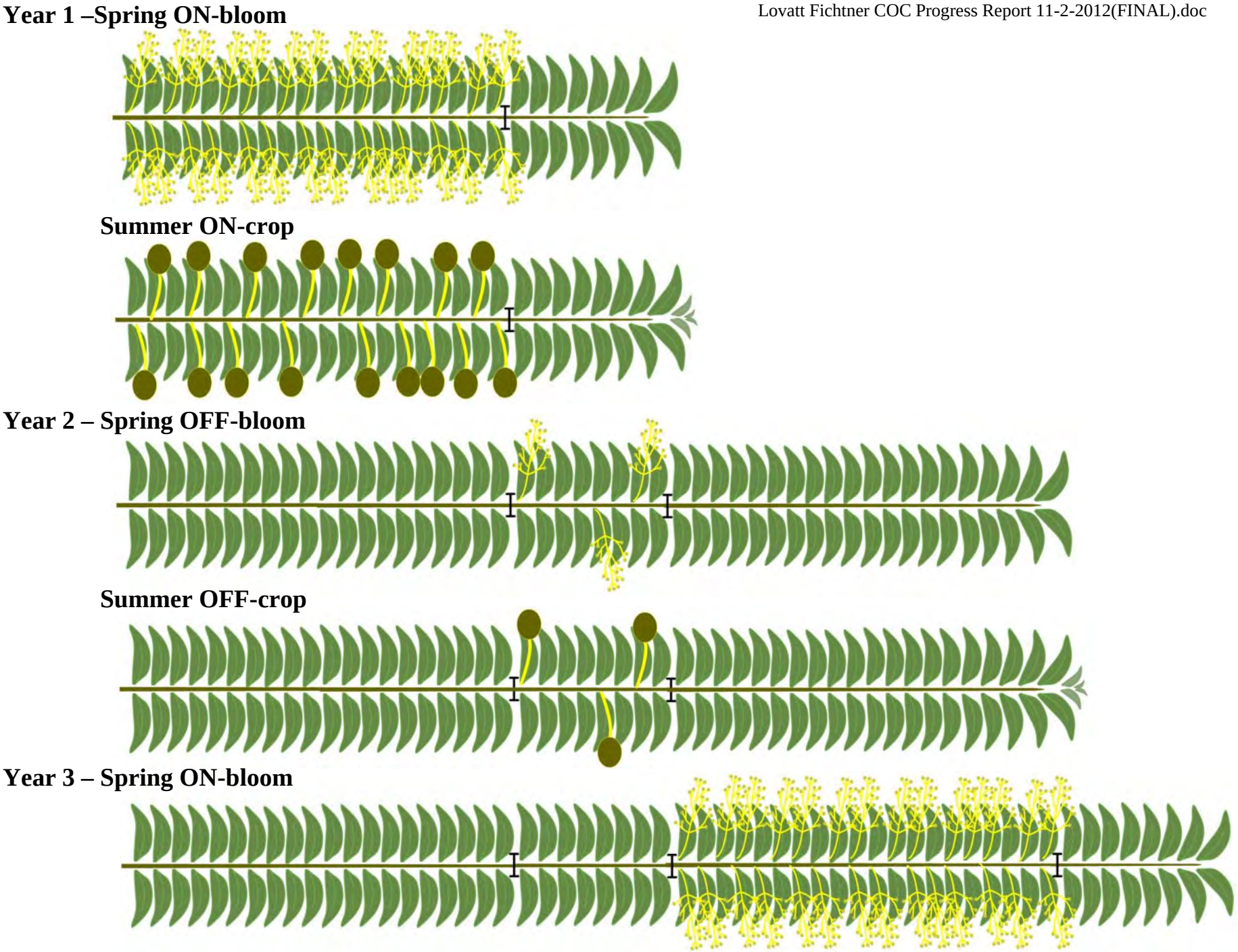

Fig. 1. Alternate bearing in 'Manzanillo' olive. Year 1 ON-crop trees produces little vegetative shoot growth and an OFF-bloom in spring of Year 2 but also produces significant vegetative shoot extension growth in Year 2 that in turn produces an ON-bloom and little vegetative shoot growth in spring of Year 3, which will result in an OFF-bloom in Year 4.

Table 1. **Hypothesis 1: The ON-crop reduces return bloom in olive by inhibiting bud break for summer vegetative shoot extension growth in Year 1 (2011).**

Tree/shoot status (2011)	Fruit per shoot	Net shoot growth and nodes per shoot			
		15 July – 17 Aug		18 Aug – 4 Oct	
		--mm--	-- no.--	--mm--	--no.--
OFF/-fruit	0.0 b	24.0 a	1.3 a	1.0a	0.1 a
ON/-fruit	0.0 b	9.0 b	0.6 b	1.0 a	0.1 a
ON/+fruit	22.8 a[z]	0.0 c	0.1 c	0.0 a	0.1 a
P-value	< 0.0001	< 0.0001	< 0.0001	0.4004	0.6024

[z] Values in a vertical column followed by different letters are significantly different at the *P*-values specified by Fisher's protected LSD.

Table 2 (Part A). **Hypothesis 2: The ON-crop inhibits summer vegetative shoot growth by causing auxin > cytokinin in buds; injecting an auxin-transport inhibitor an/or cytokinin in summer increased vegetative shoot growth (ON/-fruit) in Year 1.**

Tree/shoot status (2012)	New nodes per shoot (Jun-Sept)	Number of fruit per shoot	
		Aug	Sept
OFF/-fruit	3.4 a[z]	0.0. d	0.0 f
ON/-fruit	0.7 e	0.0 d	0.0 f
TIBA+BA	3.3 ab	0.0 d	0.0 f
*TIBA+PCK	3.8 a	0.0 d	0.0 f
*NAR+BA	3.5 a	0.0 d	0.0 f
NAR+PCK	3.1 abc	0.0 d	0.0 f
*TIBA	3.8 a	0.0 d	0.0 f
*NAR	3.5 a	0.0 d	0.0 f
BA	3.4 ab	0.0 d	0.0 f
PCK	3.2 abc	0.0 d	0.0 f
P-value	< 0.0001	< 0.0001	< 0.0001

[z] Values in a vertical column followed by different letters are significantly different at the *P*- values specified by Fisher's protected LSD.

Table 2 (Part B). **Hypothesis 2: The ON-crop inhibits summer vegetative shoot growth by causing auxin > cytokinin in buds; injecting an auxin-transport inhibitor an/or cytokinin in summer increased vegetative shoot growth (ON/+fruit) (Year 1).**

Tree/shoot status (2012)	New nodes per shoot (Jun-Sept)	Number of fruit per shoot	
		Aug	Sept
OFF/-fruit	3.4 a[z]	0.0. d	0.0 f
ON/-fruit	0.7 e	0.0 d	0.0 f
ON/+fruit	0.6 e	8.6 c	8.4 e
TIBA+BA	3.3 ab	10.9 a	11.1 a
*TIBA+PCK	3.8 a	9.2 bc	9.1 cde
*NAR+BA	3.5 a	10.7 a	10.7 ab
NAR+PCK	3.1 abc	8.8 bc	8.5 de
*TIBA	3.8 a	9.6 abc	9.3 bcde
*NAR	3.5 a	9.9 ab	10.5 abc
BA	3.4 ab	9.6 abc	10.0 abcd
PCK	3.2 abc	9.0 bc	9.0 de
P-value	< 0.0001	< 0.0001	< 0.0001

[z]Values in a vertical column followed by different letters are significantly different at the *P*- values specified by Fisher's protected LSD.

Table 3. **Hypothesis 3: The ON-crop reduces the floral intensity of the return bloom in olive in Year 2 (2012).**

Tree/shoot status (2011)	Inflor. per 5 shoots (2012)	% Bud break of floral buds (spring 2012)			New nodes BB-May
		Nodes 1-5	Nodes 6-10	Nodes 11-15	
	--no.--	---------- % of Total inflor. ----------			--no.--
OFF/-fruit	76.8 a[z]	142.7 a	108.8 a	48.6 a	8.7 b
ON/-fruit	66.6 a	129.1 a	90.7 a	57.3 a	16.7 a
ON/+fruit	3.8 b	14.7 b	0.0 b	2.0 b	16.7 a
P-value	0.0018	0.0002	0.0060	0.0275	0.0006

[z]Values in a vertical column followed by different letters are significantly different at the *P*-values specified by Fisher's protected LSD.

Table 4 (Part A). **Hypothesis 4: Bud break, not floral development, is inhibited on shoots without fruit on ON-crop olive trees; PGRs known to increase spring bud break increased inflorescence number in Year 2 (2012).**

Tree/shoot status (2011)	Inflor. per 5 shoots (2012)	% Bud break of floral buds (spring 2012) Nodes 1-5	Nodes 6-10	Nodes 11-15	New nodes BB-May
	--no.--	*---------- % of Total inflor. ----------*			*--no.--*
OFF/-fruit	76.8 b[z]	142.7 ab	108.8 bcd	48.6 de	8.7 cdefg
ON/-fruit	66.6 bc	129.1 abc	90.7 cd	57.3 cd	16.7 ab
Jan TIBA+BA	52.6 bcd	88.6 cdefg	89.6 cd	45.6 de	9.0 cdef
†Feb TIBA+BA	81.5 ab	121.3 abcd	123.4 abc	81.1 bcd	13.5 abc
†Feb NAR+BA	84.3 ab	112.0 abcedf	127.7 abc	109.1 ab	14.0 abc
†Feb TIBA+PCK	79.0 ab	121.1 abcd	111.5 bcd	76.5 bcd	12.1 bcd
Feb NAR+PCK	43.3 cde	66.0 fgh	73.1 d	45.8 de	15.5 ab
*Feb BA	110.0 a	155.9 a	158.1 a	103.6 ab	9.4 cde
*Feb PCK	111.0 a	138.3 ab	150.3 ab	127.4 a	8.0 defg
†Mar TIBA+BA	76.6 b	102.1 bcdefg	128.5 abc	78.7 bcd	16.0 ab
†Apr TIBA+BA	79.8 ab	116.0 abcde	125.6 abc	93.2 abc	16.0 ab
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4 (Part B). **Hypothesis 5: Bud break or floral development may be inhibited on shoots with fruit on ON-crop trees since PGRs did not significantly increase return bloom Year 2 (2012).**

Tree/shoot status (2011)	Inflor. per 5 shoots (2012)	% Bud break of floral buds (spring 2012) Nodes 1-5	Nodes 6-10	Nodes 11-15	New nodes BB-May
	--no.--	*---------- % of Total inflor. ----------*			*--no.--*
OFF/-fruit	76.8 b[z]	142.7 ab	108.8 bcd	48.6 de	8.7 cdefg
ON/-fruit	66.6 bc	129.1 abc	90.7 cd	57.3 cd	16.7 ab
ON/+fruit	3.8 f	14.7 l	0.0 e	2.0 b	18.3 a
†Jan TIBA+BA	16.3 ef	59.2 ghi	5.8 e	0.0 f	7.1 defgh
Feb TIBA+BA	17.8 ef	62.7 ghi	9.0 e	0.0 f	5.8 efgh
†Feb NAR+BA	19.3 ef	68.6 efgh	9.1 e	0.7 f	7.4 defgh
*Feb TIBA+PCK	24.3 def	77.1 defgh	16.6 e	9.1 ef	5.0 efgh
Feb NAR+PCK	10.3 f	34.5 hi	8.5 e	0.0 f	2.4 h
Feb BA	20.4 ef	66.3 fgh	14.6 e	5.7 f	3.4 gh
*Feb PCK	25.7 def	70.3 efgh	23.2 e	10.4 ef	5.5 efgh
*Mar TIBA+BA	24.2 def	81.0 defgh	12.0 e	3.6 f	5.4 efgh
Apr TIBA+BA	21.0 def	73.3 defgh	17.8 e	0.7 f	3.7 fgh
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

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

University of California
Division of Agricultural Sciences

PROJECT/RESEARCH PROGRESS REPORT No. 2
October 22, 2012

Project Year: 2012 **Duration of Project: Year 2 of 3**

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

Cooperators:
Dr. Helga Forster — Project Scientist: UCR, Plant Pathology
D. Thompson, K. Nguyen — SRA, graduate student: UCR, Plant Pathology
J. Connell (Butte Co.), B. Krueger (Glenn Co.): and E. Fichtner (Tulare Co.) — UCCE, Farm Advisors

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2012 Research Objectives:

1) Collection of strains, occurrence of inoculum availability, and period of susceptibility of selected injuries (leaf scars, pruning injuries, etc.) to infection (previous reports indicated 7 to 14 days in one season and shorter periods in other seasons).
    a. Collection of strains to determine genetic variability of populations using molecular approaches.
    b. Monitor galls for production of inoculum over time in relation to conducive environments (e.g., rainfall)
    c. Duration of susceptibility of injuries in selected seasons (e.g., fall, spring, summer)
2) Evaluate populations of the pathogen for laboratory sensitivity to:
    a. Copper compounds - fixed and non-fixed formulations
    b. Develop baseline sensitivities of the pathogen to appropriate selected agricultural chemicals such as kasugamycin (Kasumin).
    c. Sanitizing agents – quaternary ammonium (Deccosan) and titanium dioxide (AgriTitan)
    d. Biologicals - Blossom Protect, Actinovate, and others.
3) Evaluation of protective treatments such as new copper formulations, antibiotics such as Kasumin, Mycoshield, combinations of fungicides and copper or antibiotics, and biologicals to determine optimal usage strategies to obtain high performance and efficacy.
    a. Field trials with and without adjuvants to determine optimal performance (i.e., rates)
    b. Protective vs. post-infection activity of treatments
    c. Timing studies: Treatment at spring leaf drop or after harvest. Note: copper or antibiotics will not be used as preharvest treatments, only titanium dioxide will be used for preharvest treatments due to the extremely low concentrations being used.

2012 Progress to Date:

I. ***Collection of strains, occurrence of inoculum availability, and period of susceptibility of selected injuries to infection.*** One hundred and twenty-three strains of *P. savastanoi* were obtained from olive knots in an initial survey from 26 olive groves in Butte, Colusa, Glenn, Tehama, and Sutter/Yuba Co.

and were cultured on nutrient agar and King's medium B. Molecular identification using IAA-lysine synthethase-based primers (Penyalver et al. 2000) confirmed the species identity of the pathogen. Thus, we accomplished our goal to collect over 100 strains of the study to develop baseline sensitivity data for selected antimicrobials.

We are also evaluating the seasonal availability of inoculum from olive knots during the year. This research is ongoing but all knots collected over the year contain viable inoculum. We also showed that re-hydrating the olive knots after field collection for as little as one hour led to bacterial oozing in some of the knots and most knots oozed the pathogen after 18 to 24 h of hydration. Similar to last year's results, the common epiphytic bacterium *Pantoea agglomerans* was also isolated from the bacterial ooze from all hydrated knots evaluated. Both organisms were shown to produce indole-3-acetic acid but cytokinins were only produced by *P. savastanoi* (Marchi et al., 2006).

To determine the susceptibility of olive wounds to *P. savastanoi*, leaf scars and lateral branch wounds of potted olive plants grown in a greenhouse with high humidity and optimal temperature for bacterial growth were inoculated at selected times after injury. Leaf scars were no longer susceptible to knot formation after 10 days; whereas lateral branch injuries were no longer susceptible after 14 days. Incidence of knot formation decreased linearly with duration of post-injury inoculation. Field studies on wound healing were conducted in the fall of 2011 and knots were present at high incidence (60-100%) in the controls in the fall of 2012. There were differences in results in wound healing for wounds that were wrapped with parafilm after inoculations and for wounds that were not wrapped. There were also some differences for twig lateral wounds and terminal cuts. Susceptibility of non-wrapped lateral wounds decreased from 90% at 0 days to 24.2% after one week and to 10% after three weeks. Susceptibility of parafilm-wrapped lateral wounds decreased from 100% at 0 days to 93.3% after one week and to 30% after three weeks. Wound healing apparently is greatly affected by moisture levels in the wound and this likely also varies during the growing season. Thus, these experiments are being repeated.

II. ***Evaluate populations of the pathogen for laboratory sensitivity to selected antimicrobials*****.** All strains were tested for their sensitivity to the antibiotics kasugamycin, oxytetracycline, and streptomycin using the spiral gradient dilution method. For kasugamycin, minimum inhibitory concentrations (MIC) that prevent growth by 95% and lowest inhibitory concentrations (LIC) where a reduction in growth occurs ranged from 1.87 to 6.29 ppm (mean 4.23 ppm) and from 1.32 to 4.35 ppm (mean 2.98 ppm), respectively. For oxytetracycline, these ranges were 0.123 to 0.537 ppm (mean 0.199 ppm) and 0.075 to 0.201 ppm (mean 0.143 ppm) and for streptomycin these ranges were 0.138 to 1.774 (mean 0.294 ppm) and 0.108 to 0.274 ppm (mean 0.150 ppm), respectively. These LIC and MIC ranges for the antibiotic are similar to those obtained against other bacterial plant pathogens (Adaskaveg, *unpublished*).

Arysta LifeScience is willing to support a second tier registration effort for kasugamycin poised for 2012 once the first tier registration efforts are achieved. These registrations on pome fruit, walnut, and tomato/pepper crops were postponed in 2011 and are pending in the fall of 2012. Thus, efficacy data is being generated as described below to support registration plans.

Of 113 strains of the pathogen from our surveys tested to date for copper sensitivity on CYE dilution plates, five isolates still grew at 20 ppm, one grew at 30 ppm, whereas the remaining strains did not grew at concentrations higher than 10 ppm. Thus, all isolates showed reduced sensitivity to copper, but higher rates of copper were still effective in preventing bacterial growth. Baseline data that describes the activity of copper to *P. savastanoi* is not available because isolates were not available from orchards before copper was introduced to olive culture in California. One ppm metallic copper equivalent, however, is considered toxic to most bacteria that are sensitive to copper. Thus, possibly the pathogen shows a 10X to 30X shift towards reduced sensitivity.

We evaluated the sensitivity of *P. savastanoi* against five different commercial sanitizers – Deccosan 315, Deccosan 321, 2-Quat Plus, Ster-Bac Quat, Vantocil IB, and Nolvasan. The active ingredients are quaternary ammonia compounds, guanidine, and chlorhexidine, for the first four, fifth, and last materials, respectively. All of the tested sanitizers were highly toxic in vitro to the olive knot pathogen with 98% to 100% toxicity obtained at low concentrations.

Because sanitizers can be used to disinfest harvesting equipment and non-corrosive treatments such as quaternary ammonia compounds are needed, we tested the toxicity of chlorine, three quaternary ammonia products (i.e., Deccosan 321, Deccosan 315, Ster-Bac Quat), Perasan (acidified hydrogen dioxide), Novalsan, PHMB (Vantocil), and Citrox to a pathogen-plant extract mixture that was air-dried on PVC pipe sections. Concentrations of the test substances were chosen that were recommended by the manufacturers or that would allow usage on equipment without a water rinse. Pipe sections were sprayed with 100 ppm chlorine or 2000 ppm of the other sanitizers, rinsed with sterile water after 90-120 sec, and then vortexed in a centrifuge tube with distilled water. The supernatant was plated out on KMB agar. The viability of the pathogen by the treatments was reduced by 68.4% to100% in comparison to the water control. At the concentrations tested, chlorine and Perasan were the least effective. Citrox, a natural product derived from citrus extracts, and the quaternary ammonia sanitizers were highly effective at the 1% rate evaluated. The quaternary ammonia sanitizers are volatile compounds that may not leave any residues and they are not corrosive to equipment. The corrosive nature of Citrox is not known at this time, but we plan to evaluate the corrosive effects of the tested compounds in future experiments.

Federal registration of quaternary ammonia materials for use on harvesting and pruning equipment on selected agricultural crops (e.g., tomato and citrus) has been obtained by Decco North America and is currently available on the Deccosan 321 federal label but not in California. We prepared a Section 24C for Deccosan 315 for use on olive harvesting equipment similar to the directions on the Deccosan 321 label. Deccosan 315 is registered in California for postharvest equipment for handling fruit and for food processing equipment. Quaternary ammonia compounds are currently being used for sanitizing mill equipment in the production of olive oil but not for field equipment. We will test additional parameters that may affect the efficacy of the sanitizers such as inoculum concentration and post-inoculation treatment time.

III. ***Evaluation of protective treatments in greenhouse and field trials*****.** Results of a first greenhouse study with potted olive plants using Kasumin, Deccosan 321, AgriTitan, PHMB, and copper were reported on previously. Treatments of inoculated twigs with Deccosan, Kasumin, Kasumin-copper mixtures, or copper were highly effective in preventing the development of olive knots, whereas AgriTitan and PHMB were not effective. Additional studies were initiated in the fall of 2012. Treatments in one study included copper, Kasumin, AgriTitan, mixtures of Kasumin-copper, Kasumin-Manzate, or copper-manzate, as well as the biocontrol Actinovate and the natural product Citrox. In another study we are evaluating the effect of compounds that possibly can enhance the plant defense system and these included foliar applications of quinoxyfen, ProAlexin, and Actigard, as well as soil applications with Actigard. Additional studies are planned. Using a similar spectrum of treatments, to date we also conducted five field studies in the fall of 2012 where inoculated olive twigs were treated before or after inoculation.

In evaluation of the post-infection activity of Kasumin and copper, inoculated twig wounds were treated after 4, 24, 48, 72, or 168 h (7 days) in the fall of 2011. Only the 4 h treatment proved to be effective, indicating that treatments have to be applied within hours of infection. The persistence of copper treatments under simulated rainfall is currently being evaluated. For this, twig wounds were treated and irrigated weekly by overhead sprinklers and inoculated. Additional field trials are planned in commercial, as well as three experimental orchards (i.e., UC Davis, UC Riverside, Kearney Ag Center).

Literature Cited

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Determination of Styrene in Imported and Domestic Table Olives

Midterm Progress Report: 10/22/2012

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Background

Styrene, colorless oily liquid, is an organic volatile compound with a chemical formula $C_6H_5CH=CH_2$. It is described to be a suspected toxin by U.S. EPA and an anticipated human carcinogen. Previous lab work has indicated that styrene existed in table olive produced from some cultivars in Europe. In this project, the styrene concentration in table olives produced from Europe and California was analytically quantified by using solid phase microextraction/gas chromatography-ion trap-mass spectrometry. Styrene concentrations were measured and compared between imported and California table olives samples. Effects of origin, pH and style were also taken into consideration.

Materials and Methods

Table Olive Samples

62 samples were received in total. 51 samples were sent by Musco Family Olive Company and Bell-Carter Company, and 11 samples were directly sampled from port by USDA (origins were unknown). Among these samples, 3 cans were green table olives and 59 cans were black table olives, which included 34 cans of black pitted samples and 25 cans of sliced ripe samples. 38 samples were imported products and 24 samples were California products.

Chemicals

Deuterated styrene and styrene monomer standard were purchased from Sigma-Aldrich.

Methods

30 g table olive sample was weighed and transferred to blender with 50 mL milli-Q water. 1 mL of 0.4 ppm deuterated styrene was spike into blender. Blend the mixture with high speed for 30 seconds. Transfer the olive slush with 100 mL milli-Q water to a bottle containing 54g baked sodium chloride. Stir for 5 mins. Transfer 60 mL olive slush to an amber bottle. Insert the SPME fiber and extract 30 mins. Manually inject the fiber to GC injector.

Result and Analysis

Origin Effect: Average styrene concentrations of table olive samples was 0.267 ppm for

imported products, which was significantly higher compared to 0.0941 ppm for US products. EPA's drinking water regulations for styrene is established by maximum contaminant level goals equal to 0.1 ppm. Comparing with this value, only three US samples were above this value while 24 imported samples were above this level.

pH Effect: pH values were measured after opening the cans. pH values of three green olive products were from 3.4 to 3.7. pH values of black olives varied from 6 to 8. No significant effect of pH on styrene concentrations was found according to current data.

Style Effect: 14 out of 25 sliced ripe olives were detected containing styrene concentrations above 0.1 ppm. 12 out of 34 black pitted olives were detected containing styrene concentrations above 0.1 ppm. Sliced ripe olives may be suspected to contain higher amount of styrene than that of with black pitted style but more samples will be necessary to verify this hypothesis. One California style green olive was detected with fairly high styrene concentration (0.224 ppm). We suspect it may result from spoilage.

This project is on track to finish by December 2012. We look forward to submitting a full report with detailed analysis.