



CALIFORNIA **OLIVE** COMMITTEE
2565 Alluvial Ave. • Suite 182
Clovis, CA 93611
PHONE 559/456-9096 FAX 559/456-9099

AGENDA
Ripe Olive Research Subcommittee Meeting
Double Tree • Sonoma Room
Thursday, November 5, 2015
11:00 a.m.

- I. Call to Order
 - a. Roll call
 - b. Approval of 7-30-15 Research Subcommittee minutes (action item)
 - c. Elect Research Subcommittee Chairperson (action item)
- II. Discussion and Review of 2015 Projects
 - a. Budget Status Update
- III. Presentation of 2016 Proposals (action item)
- IV. Approval of 2016 Budget (action item)
- V. Approval of authority to the Chairman to approve No-Cost extensions (action item)
- VI. Other Business
- VII. Adjournment

COC Subcommittees for 2015-2017

Executive Subcommittee:

12 Michael Silveira, G-1
Mark Hendrixson, G-2
Dennis Burreson, MUS
James Thomas, BCF
Tim T. Carter, BCF
Ed Curiel, G-1
Janet Edwards, MUS
Felix Musco, MUS
Edward Garcia, G-1
Mark Heuer, G-2
Pat Ricchiuti, G-2
Doug Reifsteck, BFC

Marketing Subcommittee:

16 Bill McFarland, MUSCO
James Thomas, BCF
Tim T. Carter, BCF
Ed Curiel, G-1
Wai Wu, MUS
Julia Interoza, G-2
Pat Ricchiuti, G-2
Scott Hamilton, MUS
Mark Hendrixson, G-2
Maria Belshaw, BCF
Edward Garcia, G-1
Michael Silveira, G-1
Rick Benson, G-2
Pablo Nerey, G-1
Felix Musco, MUS
Doug Reifsteck, BFC

Inspection Subcommittee:

16
Julia Tinsley, BCF
Paul Danielson, G-2
Julia Interoza, G-2
Dennis Burreson, MUS
Pablo Nerey, G-1
Rick Benson, G-2
Janet Edwards, MUS
Ben Hall, MUS
Chris Henderson, G-1
Doug Reifsteck, BCF
Cody McCoy, BCF
Larry McCutchen, MUS
Vito Deleonardis, G-2
Phil Quigley, BCF
Art Hutcheson, G-2
Edward Garcia, G-1

Research Subcommittee:

16
Dennis Burreson, MUS
Julia Tinsley, BCF
Pat Ricchiuti, G-2
Scott Patton, G-1
Bert Ouezada, G-2
Michael Silveira, G-1
Vito Deleonardis, G-2
Chris Henderson, G-1
Cody McCoy, BCF
Ben Hall, MUS
Phil Quigley, BCF
Felix Musco, MUS
Pablo Nerey, G-1
Ed Curiel, G-1
Carla Anderson, BCF
Janet Edwards, MUS



CALIFORNIA OLIVE COMMITTEE
Research Subcommittee Meeting Minutes
Thursday, July 30, 2015
10:00 a.m.
Double Tree- Modesto, CA
1150 9th Street

I. CALL TO ORDER

A meeting of the Research Subcommittee was called to order by Dennis BURRESON at 10:13 a.m., and the following members were present:

<u>Members</u>	<u>Affiliation:</u>
Dennis BURRESON	Musco
Bert QUEZADA	Grower
Mark HEUER	Grower
Art HUTCHESON	Grower
Janet EDWARDS	Musco
Mike SILVEIRA	Grower
Pablo NEREY	Grower
Ed CUIEL	Grower
Phil QUIGLEY	Bell-Carter
Chris HENDERSON	Grower
Pat V. RICCHIUTI	Grower
Ben HALL	Musco
Felix MUSCO	Musco
Mark HENDRIXSON	Grower
Jacob Peters	Bell-Carter
Tim T. CARTER	Bell-Carter
Carla ANDERSON	Bell-Carter
Julia TINSLEY	Bell-Carter
Julia INESTROZA	Grower
Edward GARICA	Grower
Rick BENSON	Grower
Paul DANIELSON	Grower

Others Present:

Alexander OTT
Denise JUNQUEIRO
Terry VAWTER
Adin HESTER

COC
COC
USDA
Olive Grower Council

With a majority of the Subcommittee members present, a quorum was established.

MOVED BY Pat V. RICCHIUTI, duly seconded by Julia TINSLEY, and unanimously carried THAT the minutes of the December 9, 2014 Research Subcommittee meeting be approved. (Motion 7-30-15 #1)

II. DISCUSSION AND APPROVAL OF 2016 PRIORITIES

Each year, the Research Subcommittee sets research priorities. Once the priorities are set they are provided to the University of California liaisons for their request for proposals. Proposals will be reviewed for funding in November by the Subcommittee.

2015 List of Priorities:

Olive Knot
Olive Fruit Fly
Olive Leaf Scorch
Black Scale
Resistance to Weed Control (New Product)
Pollination/Shot Bearing
Acrylamide
Fruit Fly Monitoring
Reserve for emergency use
Hedge Rowing
Alternate Bearing (Last year)

MOVED BY Mike SILVEIRA, duly seconded by Chris HENDERSON, and unanimously carried THAT the priorities be approved. (Motion 7-30-15 #2)

IV. ADJOURNMENT

MOVED BY Mark HENDRIXSON, duly seconded by Pat V. RICCHIUTI, and unanimously carried THAT the meeting be adjourned. (Motion 7-30-15 #3)

July 31, 2015
Date July 31, 2015

Liza Ramon
Liza Ramon, California Olive Committee

SUMMARY OF MOTIONS FOR JULY 30, 2015

Motion 7-30-15 #1

APPROVED

MOVED BY Pat V. RICCHIUTI, duly seconded by Julia TINSLEY, and unanimously carried THAT the minutes of the December 9, 2014 Research Subcommittee meeting be approved.

Motion 7-30-15 #2

APPROVED

MOVED BY Mike SILVEIRA, duly seconded by Chris HENDERSON, and unanimously carried THAT the priorities be approved.

Motion 7-30-15 #3

APPROVED

MOVED BY Mark HENDRIXSON, duly seconded by Pat V. RICCHIUTI, and unanimously carried THAT the meeting be adjourned.

***** INFORMATION *****

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: PROGRESS REPORTS for 2015

BACKGROUND: Each year, the Subcommittee funds research projects and request progress reports from researchers. Provided in your packet are the current research project progress reports.

2015 Research Projects

Updated 7/14/2015

Researcher	Project	Amount	Finalized MOU	Paid thus far	% Paid	No Cost Extension
Lovatt & Fichtner	Alternate Bearing in Olives	\$ 33,357.00			0%	6/30/2016
Ferguson	Propagating Dwarfing Rootstocks and Establishing a long Term Orchard	\$ 13,245.00		\$2,649.00	20%	7/31/2016
Wang, Avena, Friedman	Evaluation of Several Promising Processing Variables and Additives for Reducing Acrylamide in Black Ripe Olives	\$ 64,000.00		\$12,800.00	20%	
Pickett	Biological Control of Olive Psyllid Parasitoid	\$ 35,304.00			0%	6/30/2016
Adaskaveg	Olive Knot	\$ 21,000.00		\$4,200.00	20%	
Lightle	Evaluation of Magnet OL Attract-&-Kill Device for Efficacy Against Olive Fruit Fly and Alternative to GF-120					
Fleishman-Hillard	Health Benefits	\$ 11,592.00			0%	
Schramm, Williams & Associates	World Trade Study-Ripe Olives	\$ 12,000.00		\$7,740.00	65%	
	Contingency	\$ 15,000.00		\$8,964.50	60%	
Ernie Simpson	Northern Fly Trapping	\$ 41,000.00			0%	
Jim Stewart	Southern Fly Trapping	\$ 6,400.00			0%	
	Total	\$ 6,333.00		\$2,375.01	38%	
		\$ 259,231.00		\$1,502.00	1%	

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.

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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 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-biuret 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 cytokinin 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:

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

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

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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.

1 **Evaluation of Thermal Processing Variables for Reducing Acrylamide in Canned Black**
2 **Ripe Olives**

3

4 **Shuaikun Tang¹, Roberto J. Avena-Bustillos^{3*}, Molly Lear¹, Ivana Sedej², Dirk M.**
5 **Holstege⁴, Mendel Friedman³, Tara H. McHugh³, and Selina C. Wang^{1,2*}**

6

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24 **Keywords.** acrylamide reduction, black ripe olives, thermal processing, LACF lethality.

25 **ABSTRACT**

26 Acrylamide formed in plant foods at elevated cooking temperatures has been identified as a
27 probable carcinogen. A wide variation and high acrylamide concentration in commercial canned
28 black ripe olives has been reported. The objective of this study was to determine if different safe
29 sterilization conditions during thermal processing can reduce substantially acrylamide levels in
30 black ripe olives. Sterilization time and temperature for six thermal processes were adjusted and
31 by heat penetration tests, process F_0 was measured and correlated to acrylamide formation and
32 changes in quality attributes of black ripe olives.

33 Acrylamide concentration followed a positively correlated second order polynomial regression
34 with process F_0 . Similar process F_0 , obtained by different processing conditions, gave similar
35 acrylamide concentrations. Solids leaching from olives increased while pH decreased in brine at
36 higher thermal processes. Skin color did not change, while increasing thermal processing
37 reduced firmness of whole olives. Optimization of safe thermal processing conditions is a
38 practical and efficient alternative to reduce acrylamide formation and improve quality of black
39 ripe olives.

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47 INTRODUCTION

48 Acrylamide [(CH₂=CH-CO-NH₂)] is a conjugated reactive molecule that acts as a biological
49 alkylating agent that is reported to induce numerous adverse effects in cells, animals, and
50 possibly also humans. These include antifertility, carcinogenicity, neurotoxicity, and
51 teratogenicity. Acrylamide has been identified as a probable carcinogen^{1, 2}. The chemistry of
52 acrylamide formation in food, and its *in vivo* reactions with essential enzymes, DNA, and
53 neurons that cause the mentioned adverse effects are described in detail elsewhere³⁻⁵.

54 Dietary acrylamide is largely derived from the heat-inducing Maillard-type reactions between the
55 amino group of the amino acid asparagine and the carbonyl groups of glucose and fructose in
56 plant-derived foods including cereals, coffees, almonds, olives, and potatoes during baking,
57 frying or canning^{3, 6-8}. Reported methods to reduce acrylamide levels include selecting
58 commercial food with a low acrylamide content, selecting cereal and potato varieties with low
59 levels of asparagine and reducing sugars, selecting processing conditions that minimize
60 acrylamide formation, adding food-compatible compounds and plant extracts to food
61 formulations before processing that inhibit acrylamide formation during processing of cereal
62 products, coffees, olives, potatoes, and reducing multi-organ toxicity with the aid of
63 antioxidative natural compounds, sulfur amino acids, and flavonoids^{4, 9}.

64 Acrylamide is formed in foods at elevated and typical cooking. Studies with laboratory-heated
65 foods revealed a temperature dependence of acrylamide formation, though it is not detected in
66 unheated or boiled foods⁶. Various levels of acrylamide have been found in black ripe olives. In
67 a survey of black ripe olives in US market, a wide range of acrylamide (375-1925 µg/kg) was
68 found¹⁰. Casado and Montaña¹¹ screened 11 black ripe olives from Spain and found levels of
69 acrylamide also ranged broadly from 176 to 1578 µg/kg of olive pulp. Similarly, in our lab, we

70 also found 288-1192 $\mu\text{g}/\text{kg}$ acrylamide in seven black ripe olives samples (unpublished results).
71 Charoenprasert and Mitchell¹² also reported relatively high concentrations of acrylamide in black
72 ripe olives (226-1925 $\mu\text{g}/\text{kg}$).

73 It has been shown that acrylamide in black ripe olives is primarily formed during sterilization,
74 and that sterilization time and temperature can significantly influence the formation of
75 acrylamide^{9, 11, 13}. Black ripe olives are processed through several lye immersion and water
76 rinsing steps^{12, 14} and canned or bottled and sterilized as a low-acid canned food (LACF) with a
77 flesh pH around neutrality^{14, 15}. LACF heat treatment require assurance of at least 12D reduction
78 of *Clostridium botulinum* spores and minimum presence of other more thermal resistant spoilage
79 spores, around 4D¹⁶, where D refers to a one log cycle reduction (or 90% reduction) in the initial
80 number of spores in the LACF¹⁷.

81 California-style black ripe olives, as a LACF, usually require sterilization temperatures above
82 110°C¹². In contrast Spanish- and Greek-style table olives are considered acidified foods (AF)
83 and only require pasteurization temperatures below 65°C and/or additives for preservation and as
84 a consequence acrylamide is negligible in these two olive styles¹². Lowering the sterilization
85 temperature and shortening the sterilization time could reduce the formation of acrylamide, while
86 continuing to achieve appropriate commercial sterilization to assure low hazards of pathogenic
87 *C. botulinum* and spoilage thermophylic bacterial spores survival. It is hypothesized that the
88 wide variation of acrylamide in commercial canned black ripe olives is a consequence of
89 different thermal processes used by industry due to different container sizes and ways to deliver
90 the thermal processing in different retort types, as well as required heat sterilization established
91 by thermal process authorities and regulatory agencies. Even though it has been reported that
92 acrylamide in canned black ripe olives is caused by high temperature processing^{11, 13}, there is a

93 lack of systematic studies to minimize acrylamide occurrences by reducing thermal processing
94 during commercial sterilization. For LACF it is important to assure commercial sterility and then
95 determine if it is still possible to reduce acrylamide concentrations as compared to common
96 commercial canning practices. We examined the proposed simple and logical approach to reduce
97 acrylamide content of olives.

98 The objective of this study was to determine if different safe sterilization conditions during
99 thermal processing could reduce substantially the acrylamide levels in canned black ripe olives.
100 We adjusted the sterilization time and temperature for six different processing conditions. From
101 heat penetration data, process lethality was calculated and correlated to acrylamide formation
102 and changes in physical quality attributes of black ripe olives.

103 **MATERIALS AND METHODS**

104 **Reagents**

105 For the olive preparation, sodium hydroxide solution (50%) was obtained from Loeffler
106 Chemical Corp. (Atlanta, GA, USA) Sodium chloride (canning and pickling salt, food grade)
107 was purchased from Morton Salt Inc. (Chicago, IL, USA). Ferrous gluconate (food grade) were
108 purchased from VWR (Radnor, PA, USA).

109 For the acrylamide analysis, acrylamide (>99.9%) and methanol (HPLC grade, 99.9%) were
110 purchased from Fisher Scientific (Pittsburgh, PA, USA). Acrylamide-d₃ standard solution (500
111 mg/L in acetonitrile) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Reversed-phase
112 cartridge columns (Sep-Pak™ C18, 1 g and 6 cc) were obtained from Waters Corp. (Miford,
113 MA, USA).

114 **California-style black ripe olive preparation**

115 Pilot-plant olive preparation method was modified from the work done by Charoenprasert and
116 Mitchell¹² to mimic industrial California-style black ripe olive processing. The olives were
117 harvested in October, 2014 and stored in 2% acetic acid solution since late October 2014 by the
118 processor prior to the preparation. Thirteen kg olives were immersed in 1% sodium hydroxide
119 solution (19 L) for 5 h for lye treatment, and then rinsed eight times before placed in fresh water
120 for 19 h to remove the residual sodium hydroxide. During the washing step, air was constantly
121 bubbled into the water to oxidize olives. This lye-wash cycle was performed twice. For the last
122 cycle, the olives were soaked in lye for 2 h and washed for 20 h. The pH was measured after the
123 final lye treatment to ensure the lye penetrated to the pit and pH was between 8 and 9.5. Then the
124 olives were treated with 0.15% ferrous gluconate solution (19 L) for 3 h to fix the color. During
125 the ferrous gluconate treatment, carbon dioxide was bubbled into the solution to neutralize
126 olives. When the pH reached 7, the olives were rinsed to remove residual ferrous gluconate.

127 **Blanching, canning and heat penetration testing**

128 Cruess¹⁸ indicated that tendency to "buckle" was greatly reduced when olives in cans were
129 thoroughly heated in an exhaust box to 195°F before sealing. To prevent can buckling, blanching
130 conditions under saturated steam conditions for whole olives should be around 2-4 min. This
131 additional processing step eliminates tissue gases and bulging of cans without increasing
132 acrylamide levels. Blanching of whole olives on single layers on S.S. trays moving in a
133 continuous belt was done in the University of California, Davis FST food processing pilot plant
134 custom built steam blancher for a residence time of 4 min, thus reaching the whole olives a
135 maximum temperature of $200.6 \pm 3.3^\circ\text{F}$ from an initial temperature of $77.0 \pm 2.0^\circ\text{F}$ (n = 6).

136 Filling was done by hand. Fill weights (790 g) for 401x411 cans were 322.5 g (41%) whole
137 olives and 467.5 g (59%) previously boiled brine (2-2.5% NaCl in water), allowing a ¼ inch

138 headspace in cans to facilitate internal agitation during intermittent rotation of cans inside the
139 rotary retort.

140 Temperature of the can content before sealing was recorded with a digital thermometer. Model
141 7500T DataTrace MPIII data loggers (Mesa Laboratories, Inc., Lakewood, CO, USA) were
142 installed in each of six cans using S.S. bendable retainers to fix the 1” rigid probe tip at the
143 geometric center of cans for measuring temperature during sterilization. Sealing was done with a
144 Dixie Double Seamer model UVGMD-ALCC (Dixie Canner Co., Athens, GA, USA) using the
145 vacuum setting provided for 401x411 cans. After sealing, cans were immediately sterilized.

146 A steam-heated discontinuous rotary retort (FMC Rotary Steritort, Madera, CA, USA) built in
147 1963 and refurbished in 2010 by the Precision Canning Equipment Co. from (Woodland, CA,
148 USA) was used. This rotary retort requires 10-17 min to reach 215°F for venting, and another 2-
149 8 min for coming up time (CUT) depending on processing temperatures. This retort can run up to
150 72 cans spaced in a balanced weight fashion. Retort was adjusted to constant processing
151 temperatures in the range of 230°F-260°F for different processing times. After cooling, cans with
152 data loggers were opened and data collected in a laptop computer through a DT Pro MPIII PC
153 Interface with DT Pro Basic software.

154 **Assessment of commercial sterilization of canned black ripe olives**

155 Heat penetration data at six different thermal processing conditions were obtained during
156 sterilization of canned black ripe olives with wireless data loggers inside 401x411 cans. This
157 information was used to establish lethality values to assure safe (inactivated) *Clostridium*
158 *botulinum* spores and reduction of other more thermo-resistant spoilage bacterial spores. Process
159 lethality was calculated as process F_0 according to the evaluation of the integral alternative using

160 the following equation that defines required or process F_0 lethality, either from microbiological
161 or heat penetration data¹⁷:

$$F_{T_{ref}}^z = D_{T_{ref}} (\log(C_a) - \log(C_b)) = \int_{t_a}^{t_b} 10^{\frac{T(t) - T_{ref}}{z}} dt \quad (1)$$

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163 In this equation, by definition of F_0 , $Z = 18^\circ\text{F}$, $T_{ref} = 250^\circ\text{F}$ in order to calculate F_0 as an integral
164 equation with the DT Pro Basic software and Excel spreadsheet, where $T(t)$ was temperature
165 inside the cans during all the heating and cooling sterilization cycle from the start of the thermal
166 processing (t_a) to the end of cooling (t_b), taken at 15 sec intervals (dt). Safe required F_0 thermal
167 processing times for different retort temperatures and intermittent convection heating mode were
168 applied according to process recommendations defined by Stumbo, Purohit and Ramakrishnan¹⁶
169 and the state of California Department of Health Services¹⁹ in Table 1.

170 For LACF it is required to design the thermal processing in a way to assure very low
171 probabilities of survival of thermophilic spoilage bacterial spores and even lower probabilities of
172 survival of *Clostridium botulinum* spores¹⁶. Sterilizing value F_0 is defined as the time in minutes
173 equivalent at 250°F to destroy bacterial spores with a thermal sensitivity of $Z = 18^\circ\text{F}$ (indicating
174 that there is a ten times increase in the rate of spores destruction when temperature is raised
175 18°F , or 10°C). Process F /required F_0 defines lethality of these spores by two ways (measuring
176 microbial survival or measuring the sum of different lethalties as temperature rises and
177 decreases during the heating and cooling processing) as indicated by Equation 1. Required F_0 is
178 defined not only by microbiological control needs, but industry previous process results, unique
179 processing equipment, or even subjective concerns on process safety factors²⁰. Process F_0 is
180 applied in laboratory tests or by canning industries and could become required F_0 . The goal of

181 commercial sterilization is to achieve Unit Lethality, when process F_0 is equal to required F_0 , as
182 lethality is defined as the ratio of process F_0 /required F_0 .

183 **Acrylamide Analysis Sample preparation**

184 Whole canned black ripe olives (~20 g) were drained and crushed in a mortar with a pestle.

185 Sample pulp (2 g) was placed in a centrifuge tube and spiked with 0.5 μg d_3 -acrylamide as an

186 internal standard. Water (4 mL) was added to the centrifuge tube. After 10 min shaking, hexane

187 (1 mL) was added, followed by further shaking for 10 min. The samples were then centrifuged at

188 8,000 rpm in a Sorvall SS-34 centrifuge (Thermo Scientific, Waltham, MA) for 10 min to

189 separate the aqueous and hexane layers. The aqueous layer was vacuum filtered using a 125 mL

190 Buchner funnel. Nitrogen was then blown on the top of the filtrate to remove residual hexane.

191 A Sep-Pak C18 cartridge was activated with methanol (2 mL) followed by water (2 mL). The

192 filtrate was then loaded on the cartridge and passed through without vacuum (about 1.5 mL/min).

193 The eluate was collected and evaporated to a volume of less than 1 mL followed by addition of

194 water to a volume of exactly 1 mL. Acrylamide determination was performed by LC-MS/MS.

195 **LC-MS/MS analysis**

196 The quantification of acrylamide and d_3 -acrylamide was performed on a Sciex API 2000 triple-

197 quadruple MS system (Perkin-Elmer, Shelton, CT, USA) controlled by Analyst 1.4.2 software

198 (Applied Biosystems, Foster City, CA, USA). HPLC instrumentation consisted of a Perkin-

199 Elmer Series 200LC system with a quaternary pump, autosampler, and in-line mobile phase

200 degasser. The samples were separated using a Hypersil-Keystone Hypercarb column (50 \times

201 2.1mm, i.d., particle size 5 μ ; Thermo, Waltham, MA, USA). The mobile phase was isocratic

202 methanol/water (80:20, v/v) at 200 $\mu\text{L}/\text{min}$ for a total run time of 5 min. The column was

203 operated at room temperature. Injection volume was 35 μ L. The retention time of acrylamide and
204 d_3 -acrylamide was 1.56 min.

205 The mass spectrum data were acquired with positive ion atmospheric pressure ionization (APCI)
206 utilizing the multiple-reaction monitoring (MRM) mode. The ion source settings were as
207 follows: temperature was 350°C; the curtain gas, collision gas, gas 1 and gas 2, 50, 6, 40 and 10
208 psi, respectively; the nebulizer current, 6 kV. The declustering potential, focusing potential,
209 entrance potential, collision energy and collision cell exit potential were 55, 400, 1.6, 17 and 7.2
210 V, respectively. Transitions for acrylamide and d_3 -acrylamide were monitored at m/z (mass-to-
211 charge ratio) $72 \rightarrow m/z 55$ and $m/z 75 \rightarrow m/z 58$, respectively with dwell times of 250 μ sec.

212 **Quantitation**

213 Quantitation was performed by internal calibration using the ratio of integrated area of
214 acrylamide against d_3 -acrylamide. The calibration solutions were injected before, in the middle
215 of, and after the analysis of the samples. The calibration curve used weighted (1/x) second-order
216 regression. The calibration range is 19.70 – 5,000 ng/ml with the correlation coefficient (R^2)
217 0.996.

218 **Method Validation**

219 The method was validated by determining the accuracy and precision of authentic acrylamide
220 added (spiked) at 100 and 500 μ g/kg (n=2). All the results are the averages of duplicates. Method
221 precision was assessed by measuring the repeatability of the results of one sample (n=5).

222 **Physicochemical Properties of Black Ripe Olives and Brine**

223 Three cans from each thermal treatment were used for physicochemical analyses of black ripe
224 olives and brine. All analyses were done in five replicates per can. After opening a can, olives

225 were drained for 5 min and then both olives and brine were weighted. Approximately half of the
226 olives were depitted, blended in a laboratory Waring blender (Waring Commercial, Torrington,
227 CT, USA) and squeezed through a cheesecloth to obtain liquid for soluble solids and pH
228 determination.

229 Soluble solids were determined with a digital refractometer LR-01 (Maselli Misure S.p.A.,
230 Parma, Italy). pH was measured using a Beckman Φ 40 Series pH meter (Beckman Coulter,
231 Fullerton, CA, USA).

232 Tristimulus (L^* , a^* , b^*) color values of whole olive skins were measured using a portable
233 spectrophotometer model CM-508c (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA)
234 through a 3 mm diameter circular opening.

235 Texture analysis was performed on the TA-TX2 texture analyzer (Stable Micro Systems, Ltd,
236 Godalming, Surrey, UK) with a puncture stainless steel probe (3 mm diameter, 25 mm long).
237 Texture analyzer settings were as follows: operation mode – measure force in compression, pre
238 test speed – 10.0 mm/s, test speed – 1.0 mm/s, post test speed – 10.0 mm/s, distance – 2.0 mm,
239 trigger type – auto, trigger force – 0.20 N.

240 Olives were cut with a razor blade alongside without touching the pit to obtain a flat surface. The
241 probe reached olives' skin on the opposite side from the flat surface. The described setup
242 allowed testing the firmness of the olives. The recorded parameters were maximum force area
243 (g.s), maximum force (g), and time (s). Temperature of olives immediately after texture analyses
244 was measured with an Omega HH176 data logger thermometer with a precision fine wire
245 thermocouple (Omega Engineering, Inc., Stamford, CT).

246 **Statistical Analysis**

247 Data were statistically processed by One-Way ANOVA using Minitab® Release 14.12.0
248 statistical software (Minitab Inc., State College, MA, USA) program. Means were compared by
249 HSD Tukey's family error test at the significance level of $p \leq 0.05$. Treatment replicates varied
250 from 2 to 15 depending on analysis accuracy and sample property variability.

251

252 **RESULTS/DISCUSSION**

253 **Thermal processing evaluation of canned black ripe olives**

254 The average temperature of the brine with black ripe olives inside the 401x411 cans, just before
255 vacuum sealing, was $99.6 \pm 10.2^\circ\text{F}$. The initial temperature varied from 70 to 110°F in
256 commercial runs for canned olives. The temperature reported in this study is within the range of
257 commercial runs. The rotary retort heated by saturated steam used for sterilization of canned
258 black ripe olives provided intermittent agitation for induced convection heating every time the
259 cans rotated freely in the bottom of the internal retort body. Table 2 reports the relevant
260 sterilization conditions for the applied six different thermal processes. Initial temperature was
261 obtained from the wireless data loggers at the start of the thermal processes. Venting time and
262 coming-up time varied depending on steam feeding rate and retort initial temperature. Processing
263 time was calculated at different retort processing temperatures to achieve previously defined
264 process F_0 which was determined by using preliminary heat penetration data obtained from
265 wireless thermocouples inside 401x411 cans filled with whole black ripe olives and brine. The
266 defined F_0 is in proportion to that commonly used by industry and in our food pilot plant batch
267 discontinuous rotary retort.

268 **Effect of thermal processing on acrylamide formation in canned black ripe olives**

269 Table 2 reports the acrylamide concentrations measured in six different thermal processes. These
270 acrylamide concentrations are within the range found in the screening study of commercial black
271 ripe olives in the USA and Spain. The acrylamide concentrations in black ripe olives are
272 relatively high compared to of the ones in other foods such as French fries (20-1325 $\mu\text{g}/\text{kg}$),
273 baked foods (<364 $\mu\text{g}/\text{kg}$) and nuts (<457 $\mu\text{g}/\text{kg}$)¹². Acrylamide concentration increased
274 significantly as process F_0 reached up to 2.7 times (or lethality) the required F_0 values to assure a
275 high degree of safety to commercial sterility, exceeding a high degree of assurance according to
276 Stumbo and others¹⁶ for the can size used in this study. Statistical analysis on treatments
277 230°F/175 min and 240°F/45 min showed very similar process F_0 (11.26 ± 0.04 - 11.91 ± 0.19
278 min, respectively). The result shows that there is no significant difference between these two
279 treatments in acrylamide concentrations (154.2 ± 27.9 – 156.2 ± 25.0 $\mu\text{g}/\text{Kg}$, respectively). These
280 observations indicate that we can obtain the same safe F_0 process and similar acrylamide
281 concentrations by different combinations of processing times and temperatures. Related
282 observations by Montañó and others²¹ reported that for similar thermal processes, or analogous
283 cumulative lethality expressed by process F_0 , acrylamide formation was lower for the processes
284 carried out at higher temperature. However, because their study was not published it was not
285 possible to compare our results to the actual process F_0 values or temperature/time processing
286 conditions.

287 Table 1 reports four required F_0 that can be used for the design of thermal processing of canned
288 black ripe olives, as a LACF. The optimum value is the lowest F_0 required for safety assurance.
289 The olive canning industry can use these required F_0 values to estimate process F_0 for different
290 can sizes and retort systems. As there are several combinations of processing time and
291 temperature to obtain process F_0 equal to required F_0 for unit lethality, it allows industry to use

292 the processes with highest retort temperature but minimum time that can practically be
293 achievable for their retort systems and can sizes and be certain on the expected acrylamide level.

294 From results of this study it appears that the main reason there are wide-ranging reports of
295 acrylamide in canned black ripe olives is because the olive canning industry may be using a large
296 range of different process F_0 . The State of California Department of Health Services advises to
297 use a required F_0 of 13 min as an official required sterilization control process for olives, ripe,
298 whole, pitted or broken pitted, in brine in 300x407 cans.

299 Mathematical fitting of the relationship between process F_0 and acrylamide concentration
300 follows a second order polynomial equation with high correlation coefficient ($R^2 = 0.9932$) as
301 indicated in Figure 1. This non-linear regression is typical of biological and chemical phenomena
302 such as the progression of disease epidemics or the growth rate of tissues. Extrapolation of this
303 polynomial equation to process F_0 values of 32-34 min, usually obtained in canned olives at
304 highest retort temperatures (255-265 °F), gives acrylamide concentrations values in the range of
305 1750-1950 $\mu\text{g}/\text{Kg}$, as found in samples of commercial canned black ripe olives in USA and
306 Spain.

307 According to the observed relationship between F_0 and acrylamide formation in canned black
308 ripe olives it is expected a concentration of 250 $\mu\text{g}/\text{Kg}$ for this required F_0 . This concentration is
309 within the range of lowest acrylamide concentrations found in commercial canned black ripe
310 olives by Charoenprasert and Mitchell¹² and by our group, 226 and 288 $\mu\text{g}/\text{Kg}$, respectively. It is
311 important to consider that an acrylamide concentration of 250 $\mu\text{g}/\text{Kg}$ formed by adhering to the
312 current required F_0 established by the state of California Department of Health Services is not a
313 serious health hazard considering the low consumption of canned black ripe olives compared

314 with fried potato products and chips; crackers, cookies, cakes, and; bread, the three most
315 important food sources of acrylamide in the USA diet (at 38%, 17%, and 14% of total
316 acrylamide in the USA diet, respectively)⁴.

317 Moreover, based on lower required F_0 reported by Stumbo and others¹⁶, the olive canning
318 industry could petition for the reduction of the current thermal processes to a new required F_0 of
319 10 min, as recommended by Stumbo and others¹⁶ for LACF in 300x407 cans under convection
320 heating for commercial sterility with high degree of assurance, without jeopardizing health
321 safety. This can potentially reduce acrylamide concentration to 150 $\mu\text{g}/\text{Kg}$, that is, 60% of the
322 lowest acrylamide concentration found in Manzanilla canned olives sterilized now at required F_0
323 of 13 min. Casado and Montaña¹¹ stated that by paying strict attention to sterilization conditions
324 it could be possible to reduce the acrylamide concentration to $<100 \mu\text{g}/\text{Kg}$, at least for
325 Hojiblanca variety ripe olives. However, the influence of time and/or temperature of sterilization
326 on acrylamide formation was not covered in their study. We suggest that the canning industry
327 invest in devising new optimized thermal processes suited for specific industrial conditions,
328 either by hiring an external Thermal Processing Authority or using their in-house Thermal
329 Processing Authority teams.

330 Other alternative approaches to reduce acrylamide in black ripe olives include sterilization of
331 fresh olives either without brining or after 2-8 months of brining; not using calcium chloride in
332 the brine solution; and decreasing air oxidation, and; carbon dioxide neutralization¹². These four
333 modifications to the traditional processing steps for black ripe olives can achieve reductions in
334 the acrylamide concentration to 62, 44, 55, and 50%, respectively from control concentrations.
335 However, reports on the effects on sensory properties of the three last options most likely will

336 affect negatively the quality of canned black ripe olives. Casado and others¹³ evaluated different
337 additives (salts, amino acids and antioxidants) to reduce acrylamide in canned black ripe olives,
338 and most of these additives turned out to be unsuccessful. Exceptionally, 25mM sodium
339 bisulphate in this report reduced acrylamide concentration to 13% of control values without
340 negative effect on sensory quality. However, sodium bisulphite is allergenic and requires a
341 labeling warning by food safety regulations. Also in this study olives were sterilized after seven
342 months of storage in the brine solution when it was reported by Charoenprasert and Mitchell¹² to
343 have the lowest acrylamide formation during sterilization, compared to sterilization at one month
344 storage. Irrespective of this fact, acrylamide values increased to the highest possible values,
345 obtained if processed at one month of brining according to Charoenprasert and Mitchell¹², at the
346 highest process F_0 values applied in this study. None of these alternatives can by itself reduce
347 acrylamide, without negative effects on sensory quality and regulatory constraints, at the level
348 that can be achieved by optimized thermal processing.

349 **Effect of thermal processing on physical attributes of brine and whole black ripe olives**

350 Soluble solids increase in brine at the highest thermal processing (process $F_0 = 27.7$ min) can be
351 explained by leaching of pulp from whole olives due to the higher temperature. Otherwise, pH of
352 the brine and whole olives decreased at the highest thermal process as shown in Table 3. Casado
353 and others¹⁵ also reported a pH decrease due to harsh thermal processing conditions of black ripe
354 olives and hypothesized it could be related to organic acids formation from sugars degradation
355 and/or degradation of Maillard reaction products, or saponification of pectin ester groups.

356 Color parameters of whole canned olives were not significantly different for the different thermal
357 processing conditions (Table 4). Casado and others¹⁵ reported loss of green color and increased

358 darkening by increasing thermal processing, presumably due to Maillard reactions and
359 autoxidation of phenolic compounds.

360 Firmness of whole olives was significantly reduced by increasing thermal processing (Table 4).
361 Romero and others¹⁴ also reported an decrease in firmness of canned black ripe olives at
362 increasing process F_0 , indicating that changes in firmness of ripe olives during sterilization
363 follows first order kinetics, with activation energy values within the range reported for fruits and
364 vegetables.

365 **Conclusions**

366 Optimization of thermal processing conditions is a practical and efficient alternative to reduce
367 acrylamide formation and improve quality of canned black ripe olives. Modification of thermal
368 processing conditions will require for the olive canning industry to file new processes with Food
369 and Drug Administration (FDA). The proposed processes should be supported by specific heat
370 penetration studies and thermal processing evaluation conducted by LACF thermal processing
371 authorities.

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374 olives for this study and Jane Yegge for technical information about industry practices for
375 processing black ripe olives. We also appreciate the support of Mr. Mark Deniston, Associate
376 Director, Process Engineering at IEH Incorporated for providing information on thermal
377 processing recommendations for whole black ripe olive canning. ST and SCW thank Dajun Yu,
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434

435

436 **TABLES AND FIGURES**

437 Table 1. Required F_0 (in minutes) for black ripe table olives as a low-acid canned food in
 438 401x411 can size.

Sterilization condition/Retort temperature	230°F	240°F	250°F	260°F
Assure high degree of safety ¹	6.38	4.68	4.17	3.58
Commercial sterility with moderate degree of assurance ¹	6.93	6.93	6.93	6.93
Commercial sterility with high degree of assurance ¹	10.40	10.40	10.40	10.40
Dept. of Health Services, State of California ²	—	13.0	13.0	13.0

439 ¹Stumbo and others, 1975¹⁶.

440 ²Dept. of Health Services, 1995.

441

442 Table 2. Thermal processing conditions and acrylamide concentration in black ripe olives
 443 processed in 401x411 cans in a discontinuous rotary retort.

Initial temperature (°F)	Venting time to 115°F (min)	Coming-up time (CUT) (min)	Processing temperature (°F)	Processing time (min)	F ₀ process (min)	Acrylamide (µg/Kg)
91.9±1.1 ^c	10	12	230	90	6.32±0.02 ^a	63.7±15.9 ^a
92.0±0.4 ^c	11	13	240	45	11.26±0.04 ^b	154.2±27.9 ^b
106.6±3.1 ^d	11	13	230	175	11.91±0.19 ^c	156.2±25.0 ^b
74.6±0.3 ^a	17	24	250	10	13.15±0.24 ^d	284.9±49.8 ^c
80.9±1.6 ^b	14	21	250	15	17.97±0.19 ^e	539.7±38.5 ^d
111.4±2.5 ^e	14	22	260	5	27.66±0.43 ^f	1184.5±82.6 ^e

444 Thermal processing values are means of five determinations ± standard deviation. Acrylamide
 445 concentrations are means of six determinations ± standard deviation Different subscript letters
 446 indicate significant difference at p<0.05 by Tukey's difference tests.

447

448 Table 3. Soluble solids and pH of black ripe olives and brines with different thermal treatments.

Process F ₀ (min)	Soluble solids (°Brix)		pH	
	Brine	Olives	Brine	Olives
6.32±0.02 ^a	2.72±0.11 ^a	3.99±0.09 ^{NS}	6.67±0.05 ^c	7.47±0.08 ^b
11.26±0.04 ^b	2.64±0.17 ^a	3.68±0.69	6.70±0.03 ^c	7.39±0.06 ^a
11.91±0.19 ^c	2.79±0.10 ^{ab}	3.70±0.09	6.55±0.06 ^{ab}	7.36±0.07 ^a
13.15±0.24 ^d	2.83±0.14 ^{ab}	3.48±0.11	6.53±0.04 ^a	7.40±0.02 ^a
17.97±0.19 ^e	2.62±0.06 ^a	3.33±0.08	6.59±0.03 ^b	7.38±0.06 ^a
27.66±0.43 ^f	2.97±0.21 ^b	3.51±0.36	6.67±0.06 ^c	7.64±0.13 ^c

449

450 Values are means of fifteen determinations ± standard deviation. Different subscript letters
 451 indicate significant difference at p<0.05 by Tukey's difference tests.

452 ^{NS} No significant difference in olive pulp °Brix by different thermal treatments.

453

454 Table 4. Color and firmness properties of whole black ripe olives with different thermal
 455 processes.

Process Fo (min)	Color			Firmness	
	L*	a*	b*	Maximum force area (g.s)	Maximum force (g)
6.32±0.02 ^a	26.6±0.27 ^{NS}	0.19±0.12 ^{NS}	0.29±0.07 ^{NS}	170.1±11.1b	141.8±11.6 ^{ab}
11.26±0.04 ^b	26.7 ±0.01	0.27±0.09	0.50±0.10	175.5±11.4 ^b	146.6±3.8 ^b
11.91±0.19 ^c	26.8±0.41	0.16±0.10	0.25± 0.18	160.6±4.9 ^{ab}	135.2±9.0 ^{ab}
13.15±0.24 ^d	26.9±0.28	0.30±0.06	0.33± 0.05	151.6±6.2 ^a	131.6±3.4 ^{ab}
17.97±0.19 ^e	26.7±0.43	0.14±0.06	0.30±0.08	149.8±8.2 ^a	135.9±8.0 ^{ab}
27.66±0.43 ^f	26.4±0.32	0.22±0.08	0.25±0.12	140.6±17.0 ^a	121.5±16.9 ^a

456

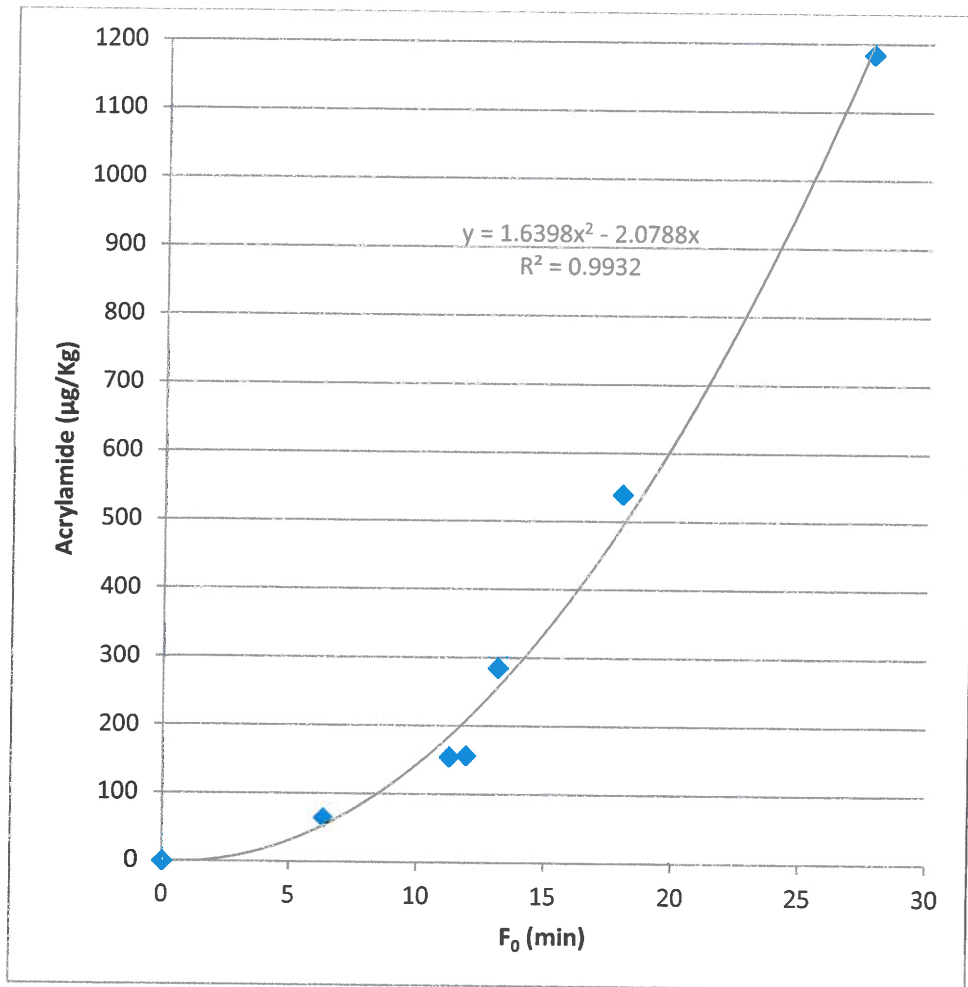
457 Values of color and firmness are means of fifteen determinations ± standard deviation. Different
 458 subscript letters in a column indicate significant difference at p<0.05 by Tukey's difference tests.

459 ^{NS} No significant difference in color parameters on olive skins by different thermal processes.

460

461

462 Figure 1. Effect of thermal process lethality F_0 on acrylamide concentration in black ripe olives



463

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.

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

Project Year: 2015

Project Leader:

Dr. J. E. Adaskaveg, Professor

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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 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 2×10^6 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.

16 October 2015

From: Dani Lightle and Emily Symmes
Summary of 2015 COC project

Title: Evaluation of efficacy of attract and kill devices for olive fly control

Objective:

This project aimed to evaluate whether the attract and kill device (Magnet OLI, marketed by Suterra LLC) provided adequate control of olive fly when compared to grower standard control programs (GF-120 and/or Danitol). The study, as proposed, was 1) to evaluate field efficacy of the Magnet OLI using manufacturer's rates and recommendations; and 2) to evaluate, in the lab, how traps were affected after exposure to summer growing conditions. On September 11, 2015, Suterra LLC informed us that they were discontinuing registration of the Magnet OLI devices. Therefore, Objective 1 was mostly completed; however, Objective 2 was not begun.

Introduction:

Currently, many olive growers in the Sacramento Valley growing region are applying insecticides (primarily GF-120) as frequently as every other week during peak olive fly activity periods. Benefits to an alternative control measure such as Magnet OLI are delayed resistance build-up to GF-120 and low impacts on other natural enemies that control scale. Though attract-and-kill devices initially have high costs related to purchasing the devices and the labor to deploy them early in the season, the devices are advertised as effective for six months. If the devices are shown to be effective all season long, the initial investment will be offset by eliminating or reducing the number of sprays required for olive fly control, which lowers labor, fuel, and material costs.

Summary of Work Completed:

Three sites were identified in Glenn and Tehama counties:

Site 1 – Manzanillo orchard, Bayliss (Glenn county)

Site 2 – Manzanillo orchard, Capay (Tehama county)

Site 3 – Seviallano orchard, Orland (Glenn county)

Each site consisted of a 10-acre block treated with the Magnet OLI devices at the label rate (42-45 traps / acre, depending on the tree spacing at each site). A 10-acre block acted as a grower standard control, and was managed by the individual grower at each site. Site 1 was established on May 11th; Site 2 was established on May 14th; and Site 3 was established on May 18th.

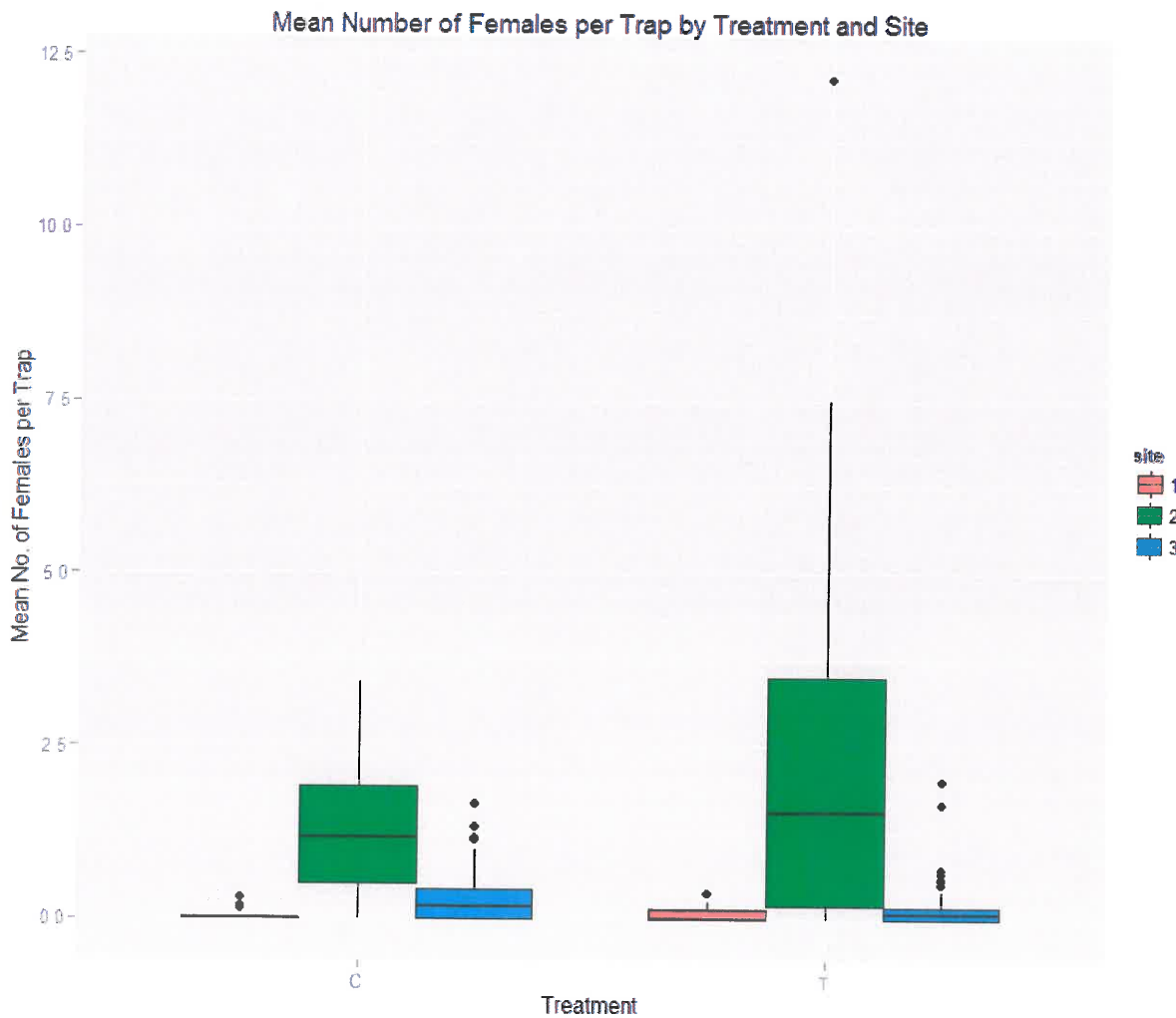
The control and treatment blocks at each location were monitored for olive fly populations using McPhail traps baited with Torula yeast, with one McPhail trap placed per acre. Traps were

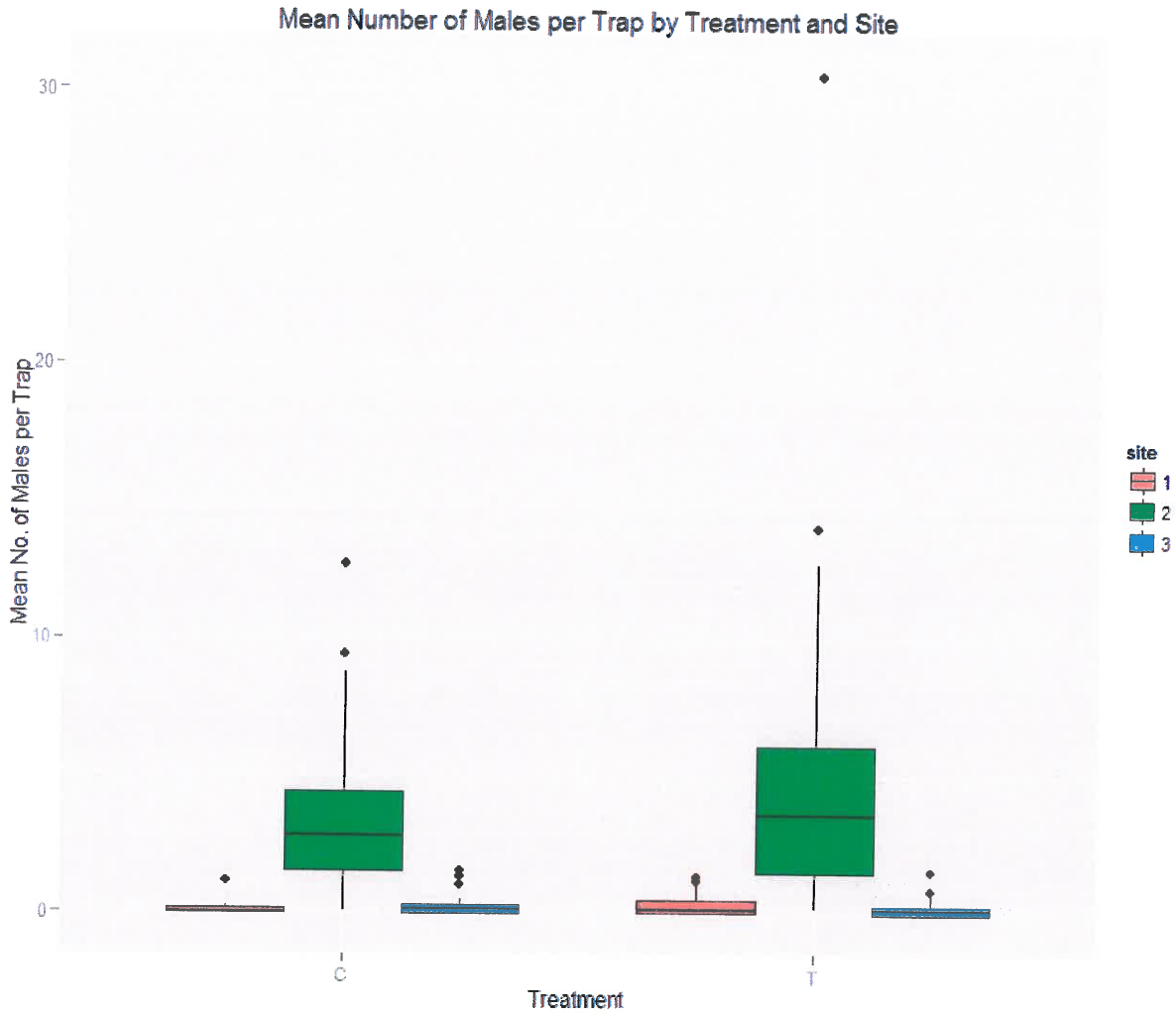
checked on a weekly basis and male and female olive flies were counted. McPhail traps were monitored until olive harvest. Olives were harvested on September 10th. From each treatment block, 1000 fruits were examined for olive fly stings, and an additional 500 fruits were held in rearing cages to monitor for emerging flies.

Results:

McPhail trapping:

Overall, there were more male flies captured than female flies. Additionally, there was a major influence of site on trapping numbers, with Site 2 hosting a very high olive fly population relative to Sites 1 and 3. However, there were no differences in the numbers of male flies or female flies trapped in the attract and kill treated plots (T) vs the grower standard plots (C), after accounting for differences between site.





Harvest damage:

There were no olive fly infested fruit found among the 1000 fruits examined from each treatment. Additionally, no flies have emerged from the olives held in rearing cages.

******* ACTION REQUIRED *******

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: 2016 RESEARCH PROJECT

RECOMMENDATION: THAT the Subcommittee approve research project for 2016.

BACKGROUND: Each year the Research Subcommittee approves various research projects funded by the Committee. The Subcommittee must which proposed projects to recommend to the Committee for funding. A budget of \$ 152,655.00 is purposed based on the submitted projects.

2016 RESEARCH PROPOSALS FOR THE CALIFORNIA OLIVE COMMITTEE

TOPIC	LEADERS	AMOUNT
Northern Valley Olive Fruit Fly Monitoring Project	Erin Simpson	\$6,500.00
Southern San Joaquin Valley Olive Fruit Fly Monitoring Project	Jim Stewart	\$6,334.00
PGRs and pruning treatments to manage alternate bearing	Carol Lovatt Elizabeth Fichtner	\$16,045.00
Real-time removal of insect damaged olives from the processing team	Ron Haff	\$35,000.00
Epidemiology and management of olive knot	Jim Adaskaveg	\$42,000.00
Biological control of olive psyllid (renewal)	Charlie Pickett	\$31,680.00
Prapagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard	John Preece, Louise Ferguson	\$15,096.00
TOTAL		\$152,655

FISCAL IMPACT: \$ 152,655

COC Proposals – Project Year 2016

Project Summaries

Stewart – South SJV olive fly monitoring project - \$6,334

Olive fly monitoring would be continued at the same nine sites as previous years, with an additional site added for 10 sites total. Fly counts in each of the orchards would be available to aid growers in the treatment decision making processes.

Simpson – Sacramento Valley olive fly monitoring project - \$6,500

Olive fly monitoring would be continued at the same 12 sites as previous years. Fly counts in each of the orchards would be available to aid growers in the treatment decision making process.

Haff – Real-time removal of insect damaged olives from the processing stream - \$35,000

Olive fly damaged fruit can be identified mechanically because they give off a different waveband [light] pattern under near-infrared. Similar sorting technology from this lab has used these wavebands to identify damaged pistachios in real-time. This project would build and test a bench-top prototype sorter to remove olive fly damaged fruits from a processing stream. If results are positive, a proposal for a full-size model will be submitted next year.

Adaskaveg – Epidemiology and management of olive knot - \$42,000

This olive knot project builds on previous years of research. First, Deccosan will be evaluated as an equipment sterilizer for preventing infection of pruning wounds. Second, three new antibiotic treatments will be optimized for treatment timings and additives, and additives to enhance copper efficacy will be tested. Third, growth chamber studies will try to reproduce situations that can cause systemic infection of olive knot.

Pickett – Biological control of olive psyllid - \$31,680

The most important parasitoid of olive psyllid in the Mediterranean is currently in colony in California. This project will finish testing to ensure the parasitoid does not affect non-target psyllid species and will begin releases of the parasitoid onto psyllid-infested olives in southern California.

Lovatt/Fichtner – PGRs and pruning treatments to manage alternate bearing - \$16,045 **\$5000 in matched funds have been obtained for this project**

This project builds on the previous work looking at mechanisms responsible for alternate bearing and tests a management plan to mitigate alternate bearing. Treatments will test a growth inhibitor (S-ABA) for ON years to reduce fruit set; 6-BA applications during ON years to increase vegetative shoot growth; and AVG applications during OFF years to increase fruit set.

Ern's Pest Control

Project Plan/ Research Grant Proposal

Project Year: 2016

Project Leader: Ernie Simpson

Mailing Address: 320 County Road 15 Orland, California 95963

Phone: 530-865-9829 Cell: 530-518-4685

E-mail: ernsimp17@sbcglobal.net

Cooperator: Dani Lightle, Orchards Advisor, UC Cooperative Extension, Orland

Commodity: Olive

Problem and its Significance:

Since the detection of Olive Fruit Fly in California in 1998, it has been a concern to olive growers in commercial orchards; preventative sprays are necessary. Trapping to monitor the Olive Fruit Fly populations in individual orchards is recommended. This will allow growers and PCA's to follow trends to their orchards and help evaluate spray program efficacy. Having an idea of area-wide population trends will help growers and PCA's interpret the results from their orchards.

Objectives:

- 1: Provide timely information to area growers regarding area-wide olive fruit fly population trends.
- 2: Continue to develop a historical perspective of olive fruit fly populations for the area.

Plans and Procedures:

Starting in early April plastic McPhail traps using Torula yeast tablets dissolved in water as the bait will be placed in one tree at 12 sites (6 in Glenn County and 6 in Tehama County). The same sites that have been used in previous years will be monitored again to allow for comparison of current years trap catches to previous years. Earlier work in Glenn and Butte Counties has shown that the plastic McPhail traps catch more flies than the commonly used yellow panel trap. Traps will be checked and flies counted weekly. The results and field observations will be posted on the Glenn County UC website ([Http://ceglenn.ucdavis.edu](http://ceglenn.ucdavis.edu)) and reported via email to the COC for further distribution. Trapping results will be reported as male and female flies for individual traps and combined and averaged by site for a graphic presentation of the data. Trapping and reporting will be continued through December or until trap catches decline for the year.

Budget Request

Budget Year: 2016

Funding Source: California Olive Committee

Salaries _____ \$4735

Supplies and Expenses: Trapping Supplies _____ \$ 300

Travel 2545 mi. @ \$.575/mi. _____ \$1465

This may vary due to fuel prices

Total _____ \$6500

Originator's Signature _____

Ernie Simpson

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project year: 2016

Anticipated Duration of the project: April –November 2016

Project Leader: Jim Stewart

Location: Tulare County

Mailing Address: PO Box 1095, Exeter CA 93221

Phone: (559) 730-6243

FAX: (559) 592-4105 E-mail: jsagipmc@verizon.net

Project Title: Southern San Joaquin Valley Olive Fruit Fly Monitoring Project

Cooperating personnel: Bert Quezada, Doug Bigham

Keywords: Olive Fruit Fly, Monitoring, Traps,
Commodity: Olive

PROBLEM AND ITS SIGNIFICANCE:

The monitoring of Olive Fruit Fly (OLFF) in commercial olive groves in the Southern San Joaquin Valley started in 2001. OLFF is potentially the most significant insect pest in commercial Olive.

OBJECTIVES:

The objective of this project would be to continue the monitoring program of adult OLFF in commercial olive groves in the Southern San Joaquin Valley. Detection and seasonal monitoring of OLFF and the accurate timing of control measures, primarily bait sprays, would be the goal of this project. Correlation of fly collections with fruit susceptibility to infestation would indicate to growers when initial bait treatments should be applied. In addition, monitoring would continue to give growers information on the general OLFF population. This information would be specific for only the groves being monitored and would be available to growers to aid in making OLFF management decisions in their respective groves in the area being trapped.

PLANS AND PROCEDURES:

The same nine sites used in the years 2013 to 2015 in commercial olive groves will be set up with traps in April of 2015. The locations will be Ivanhoe, Woodlake, Exeter, South Exeter, Tonyville, West Lindsay, Strathmore, Porterville and Terra Bella. In addition, a site in the city of Visalia would also be monitored. All of these sites are in Tulare County where a high percentage of the commercial olives are located in the Southern San Joaquin Valley. Many of the sites have been monitored starting in 2001. All traps will be in place by the first week of April and the program will end the last week of November. Two yellow panel traps with ammonium carbonate bait and male pheromone will be used per site. Traps will be serviced and OLFF counted weekly. Reports detailing the number of OLFF found at each location will be submitted to the California Olive Committee and interested parties within 24 hours on a weekly basis.

BUDGET REQUEST

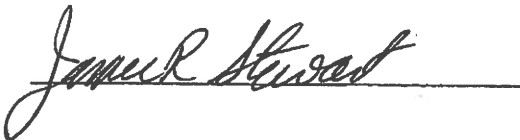
Budget year: April 1, 2016-December 1, 2016

Funding Source: California Olive Committee
Leffingwell Ag Sales Co., Inc.
Ag IPM Consultants, Inc.

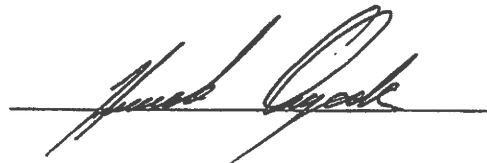
Salaries and benefits:	<u>\$15,400.00</u>
Supplies:	
Traps, bait and pheromone	<u>1200.00</u>
Travel:	
Mileage to trap sites	<u>2,400.00</u>
Equipment:	<u>0.00</u>
	TOTAL <u>\$19,000.00</u>

Funding would be split equally between the above listed funding sources.

Total funding from the California Olive Committee would be: \$6,333.33



James R. Stewart
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Olive Workgroup / Department
of Botany and Plant Sciences,
UCR

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2016

Anticipated Duration of Project: New 2-year proposal to determine the efficacy of the PGR and pruning treatments to manage alternate bearing; this requires yield data for 2 consecutive years.

Project Leaders: Carol Lovatt and Elizabeth Fichtner

Project Leaders' Contact Information:

CL-Professor of Plant Physiology, Botany and Plant Sciences-072, UC-Riverside, CA 92521-0124 Phone: 951-827-4663; Fax: 951-827-4437; Email: carol.lovatt@ucr.edu

EF-Farm Advisor, Orchard Systems, Cooperative Extension, 4437 S. Laspina St., Tulare, CA 93274, Phone: 559-684-3310; Fax: 559-685-3319; Email: ejfichtner@ucdavis.edu

Location: Lindcove Research and Education Center, Exeter

Project Title: Managing Alternate Bearing in Olive with PGRs and Pruning

Cooperating Personnel:

Lindcove Research and Education Center, Exeter

Keywords: Alternate bearing, ON-crop trees/year, OFF-crop trees/year, vegetative shoot growth, spring bud break, bud abscission, inhibition of floral development, plant growth regulators, flower and fruit thinning/removal

Commodity(s): Table Olive

Relevant AES/CE Project No.: 4556H

Problem and its Significance: Alternate bearing (AB), production of a heavy "on-crop" (high yield, ON trees) followed by a light "off-crop" (low yield, OFF trees), occurs in perennial fruit and nut crops and in forest species (where it is called "masting"). AB is a serious problem of significant economic consequence to table olive growers, and probably oil olive growers (Sibbett 2000). Industry-wide, yield can vary from 46,300 tons one year to 195,000 tons the next (USDA NASS 2011 CA Olive Probability Survey Report). In ON years, trees produce a large number of small size fruit with reduced commercial value. In OFF years, trees produce large fruit but too few to provide a good income to the grower. For olive, the ON-crop takes longer to mature, attain size and accumulate oil. The delayed harvest further exacerbates AB.

In tree crops and forest species, there are four known mechanisms by which the ON-crop of fruit reduces floral intensity and yield in the year following the ON-crop. Unfortunately, for California olive growers, all four mechanisms are operating in the 'Manzanillo' olive. Our results provided clear evidence that the ON-crop of fruit (i) reduces summer vegetative shoot growth, starting in July and thus, the number of node pairs that can bear inflorescences the next spring (olive has the potential to produce 2-4 inflorescences per node pair), (ii) causes the abscission of floral buds, with greatest period of floral bud abscission between September and October [consistent with the report of Dag et al., (2010) that olive fruit must be removed before

September to increase return bloom], (iii) inhibits the expression of a key gene required for normal floral development, and (iv) inhibits spring bud break.

There is a recurring need to mitigate the problem of alternate bearing. Alternate bearing is initiated by external factors (freeze; lack of chilling; low or high temperatures at bloom affecting not only the crop trees, but also the pollinizer trees) that cause poor flowering or pollination or excessive flower and fruit drop, resulting in an OFF-crop that is typically followed by an ON-crop, depending on how long it takes the trees to recover from the stress causing the loss of yield. Conversely, optimal conditions during bloom and fruit set such that normal crop thinning fails to take place result in an ON-crop, which is followed by an OFF-crop. Since climate is a factor initiating AB, the need for a corrective strategy reoccurs. The best solution is a flexible management strategy that is easily adapted to ON- or OFF-crop trees and utilized annually.

Results of our attempt to mitigate alternate bearing in 'Manzanillo' olive with foliar-applied PGRs. The best PGRs identified in our branch injection research for increasing summer vegetative shoot growth and spring bud break of ON-crop 'Manzanillo' olive trees and the PGR + urea treatment successful in preventing floral bud abscission in pistachio (the only other crop in which the abscission of floral buds during the ON-crop year is known to perpetuate alternate bearing) were applied to ON-crop trees to test their capacity to increase yield the following year. The experiment included 15 individual tree replications per treatment: (1) ON-crop control trees; (2) ON-crop trees receiving foliar-applied 6-BA + low-biuret urea in June and July and 6-BA only the following spring; (3) ON-crop trees receiving foliar-applied cytokinin X (a proprietary natural product) + low-biuret urea in June and July and cytokinin X only the following spring; (4) OFF-crop trees receiving foliar-applied cytokinin X in June and July; and (5) OFF-crop control trees. The PGRs were applied at 0.9 g/tree and low-biuret urea at 0.18 kg N per tree with a 400-psi handgun sprayer at 4 gallons per tree for good coverage. No applications were made after the spring applications for Year 2.

For Year 1, ON-crop trees treated with 6-BA plus low-biuret urea in June and July 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 ($P < 0.0001$) (Table 1). The June and/or July application times clearly increased fruit set. Increasing yield with 6-BA and urea in the ON-crop year followed with a spring application of 6-BA resulted in trees that were not "more OFF" the following year; all Year 1 ON-crop trees had the same OFF-crop yields in Year 2 (Table 2). Thus, trees receiving foliar-applied 6-BA and urea in the ON-crop year and 6-BA in the spring had better 2-year average yields and 2-year cumulative yields, but not significantly better than most other treatments ($P = 0.1045$) (Table 3). Year 1 treatments applied to ON-crop trees had no effect on Year 2 yield (Table 2), which indicates that applications of 6-BA or cytokinin X in spring were without effect. (Note: the optimal application time is February, but due to adverse weather the applications could not be made until mid-March, which our prior data showed to be less effective).

Year 1 ON-crop trees produced more fruit of sizes tiny, small and medium than OFF-crop trees. OFF-crop trees produced more extra-large and jumbo size fruit (Table 1). OFF-crop trees treated with cytokinin X in June and July produced significantly more colossal-size fruit in the OFF-crop year compared to all ON-crop trees regardless of treatment ($P = 0.0614$). In Year 2, the OFF-crop trees (now ON-crop trees) produced more fruit of sizes sub-petite, petite, small, medium and large than the now OFF-crop trees (Table 2). In Year 2, all trees produced the same amount of extra-large fruit; Year 1 ON-crop trees treated with cytokinin X in March following the ON-crop year produced more jumbo size fruit than trees in all other treatments in Year 2, except Year 1 ON-crop trees treated with 6-BA in March following the ON-crop year; 6-BA

treated trees produced more jumbo size fruit than the Year 1 OFF-crop trees (now ON-crop trees).

Whereas the PGR treatments had some positive effects on fruit size, we failed to reduce the severity of alternate bearing and increase yield in the year following the ON-crop. This may be 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 'Manzanillo' olive trees is from September to October. We applied the treatment to reduce olive floral bud abscission in June and July, which was likely too early to be effective.

Proposed management strategy. Given the fact that the ON-crop causes 70% to 76% of buds on bearing shoots to abscise and for those buds remaining inhibits floral development and since all four mechanisms operating during the ON-crop year in olive have a less severe effect on flowering and yield for nonbearing shoots compared to bearing shoots, it is clear that it is essential to increase the number of nonbearing shoots during the ON-crop year to successfully mitigate alternate bearing. An increase in the number of nonbearing shoots would also reduce the negative whole tree (crop load) effect on return bloom and our data show that nonbearing shoots respond better to PGRs, which would further increase return bloom and yield the year following the ON-crop. Thus, it is also important to use PGRs in the spring prior to the OFF-crop year to increase bud break and also during bloom to increase fruit set.

Inflorescence pruning or chemical inflorescence thinning during the ON-bloom would provide an effective cultural practice to help evening out the yield of ON/OFF cycles in alternate bearing 'Manzanillo' olive orchards. Most growers understandably are reluctant to thin in the ON-crop year prior to fruit set, but because inflorescences are easy to see, the degree of thinning can be adjusted to accommodate differences in bloom intensity and potential set from year to year and among trees within an orchard in a single year. Fruit removal by pruning, hand thinning or chemical thinning must be done before mid-July to reduce the inhibition of summer shoot extension growth and increase return bloom. It is difficult to see differences in set at this time. It should also be noted that the negative effects of the ON-crop of fruit are cumulative – the longer the ON-crop of developing fruit is on the tree, the more severe the impact on return bloom and yield. Thus, the goal of thinning reproductive structures in olive should be to increase the number of nonbearing shoots on ON-crop trees. Mechanical pruning does not lend itself well to creating nonbearing shoots, it tends to simply shift crop from one area of the tree to another in any given year. A chemical thinner (e.g., NAA or S-abscisic acid, which is less sensitive to temperature fluctuations than NAA) can be applied to reduce the number of fruit per shoot and the number of shoots with no fruit more uniformly around the tree.

Proposal goal, objective and research plan. Using our discovery that all four known mechanisms perpetuating alternate bearing in trees function in olive, as well as what we have learned about the timing and efficacy of PGR treatments that we have tested as branch injections and whole tree sprays, the goal of our proposal is to develop a flexible management practice that can be adapted to ON- and OFF-bloom trees to even out alternate bearing in 'Manzanillo' olive orchards, so that growers do not experience the dismally low yields an OFF-crop year. Using 'Manzanillo' olive trees, which have been subjected to light hand-pruning to maintain space and sunlight within rows and between rows, our objective is to test the following treatments: (1) untreated ON-crop (ON bloom) control trees; (2) ON-crop (ON bloom) trees sprayed with the growth inhibitor S-abscisic acid (S-ABA) (500 mg/L) at bloom to reduce fruit set, the treatment is applied as a 2-ft wide strip as the sprayer drives down the row on both sides of the tree to achieve a 30% to 40% reduction in yield; (3) ON-crop (ON bloom) trees sprayed with the growth inhibitor S-abscisic acid (S-ABA) (1000 mg/L) at bloom to reduce fruit set, the treatment is applied as a 2-ft wide strip as the sprayer drives down the row on both sides of the tree to achieve a 30% to 40% reduction in yield; (4) ON-crop (ON bloom) trees sprayed with the growth inhibitor S-abscisic acid

(S-ABA) (500 mg/L) at bloom to reduce fruit set, the treatment is applied as a 2-ft wide strip as the sprayer drives down the row on both sides of the tree to achieve a 30% to 40% reduction in yield, trees are also sprayed with 6-BA in mid-July to increase summer vegetative shoot growth, at the beginning of September to increase floral bud retention, and the following February to increase spring bud break; (5) OFF-crop (OFF bloom) control trees; and (6) OFF-crop (OFF bloom) trees sprayed with the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) at 10% bloom to increase fruit set and yield in the OFF-crop year.

Selection of these PGRs was based on their known abilities: (i) 6-BA to increase summer vegetative shoot growth during the ON-crop year (results of our research with olive and avocado) and to increase spring bud break (results of our research with olive; also avocado); (ii) S-ABA to reduce flowering and fruit set, increase fruit size during the ON-crop year and increase yield the following year (avocado); and (iii) AVG to increase fruit set (walnut and cherry). The PGRs proposed for use, 6-BA as MaxCel, S-ABA as ProTone and AVG as ReTain are all products of Valent BioSciences™, which will contribute financially to the research.

Literature Cited:

Dag, A., A Bustan, A. Avni, I. Tzipori, S. Lavee and J. Riov. 2010. Timing of fruit removal affects concurrent vegetative growth and subsequent return bloom and yield of olive (*Olea europaea* L.). *Scientia Hort.* 123:469-472.
 Sibbett, S. 2000. Alternate bearing in olive trees. *California Olive Oil News*. Vol. 3, Issue 12.

BUDGET REQUEST – Lovatt and Fichtner
Budget Year: 1 January 2016 – 31 December 2016

Funding Source: California Olive Committee

Salaries and Benefits:

Postdocs/RA's

Toan Khuong- Assistant Specialist @ \$ 4,362/mo. variable time equivalent to 25% x 1 mo. (Under my supervision, assists in laying out the experiment in the orchard, making maps, treatment applications, harvest, fruit size determination, data management, data sheets, data entry, and statistical analyses of the data.)	<u>1,091</u>
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SRA's and Lab/Field Assistance

Lab Assistant I @ \$ 15.07/hr x 100 hours. (To assist with laying out the experiments in the orchards, treatment applications, harvest, fruit size determination.)	<u>1,507</u>
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Subtotal	Sub2	<u>2,598</u>
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Employee benefits:

TK = \$1,091 x 76.11%	<u>830</u>	
TBA Lab Asst I = \$1,507 x 2.76%	<u>42</u>	
	Sub6	<u>872</u>
	TOTAL	<u>3,470</u>

Supplies and Expenses	Sub3	<u>5,638</u>
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Recharge to the Lindcove REC: use of olive grove, water, fertilizer,
Weeding, pest management, pruning, and harvest = \$5,638

Equipment

Sub4 _____ 0

Travel

Sub5 _____ 2,937

5 roundtrips to Exeter

(520 mi x 5 = 2,600 mi x \$0.6014 = \$1,564;

UCR vehicle rental 10 days x \$47.268/day = \$473

\$90/day per diem (Lindcove Trailer plus meals) x 2 people x 5 trips (1.5 days each) = \$900

SUBCONTRACT

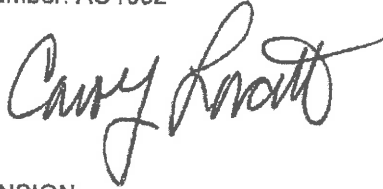
Sub7 _____ 4,000

Elizabeth Fichtner, Farm Advisor, Orchard Systems, Cooperative Extension,
4437 S. Laspina St., Tulare, CA 93274

Department account number: AO1082

TOTAL _____ 16,045

Originator's Signature



Date _____ 10/19/2015

COOPERATIVE EXTENSION

County Director: Allison Ferrybee (acting CD)

Date 10/20/2015

Program Director: _____

Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: Michael L. Rosen

Date 10/20/2015

UC COC LIAISON OFFICER: _____

Date _____

BUDGET SUBCONTRACT

Budget Year: 1 January 2016 – 31 December 2016

Elizabeth Fichtner
Farm Advisor, Orchard Systems, Cooperative Extension,
4437 S. Laspina St., Tulare, CA 93274

Funding Source:

Salaries and Benefits:

Postdocs/RA's/SRA's and Lab/Field Assistance

215 hours of labor for Student Assistant II/Lab Assistant I _____ 3,203

Subtotal

Sub2

Employee benefits:

Sub6 _____ 0

TOTAL _____ 3203

Supplies and Expenses

Sub3 _____ 0

Field supplies: bags, ribbons, cooler box

Equipment

Sub4 _____ 0

Travel

Sub5 _____ 400

SUBTOTAL _____ 3603

UC ANR Overhead @11%

Sub6 _____ 396

\$3603 x 11% =

SUBCONTRACT TOTAL _____ \$4,000

Elizabeth Fichtner, Farm Advisor, Orchard Systems, Cooperative Extension,
4437 S. Laspina St., Tulare, CA 93274

Elizabeth Fichtner

Originator's Signature

Date 10/20/2015

COOPERATIVE EXTENSION

County Director: Allison Ferry-Abbe (acting CD)

Date 10/20/2015

Program Director: _____

Date _____

AGRICULTURAL EXPERIMENT STATION

Department Chair: _____

Date _____

UC COC LIAISON OFFICER: _____

Elizabeth Fichtner

Date 10/20/2015

Table 1. Effect of a proprietary natural cytokinin (CKX) applied to OFF-crop trees and CKX and 6-benzyladenine (6-BA) plus low-biuret urea applied to ON-crop trees in June and July, with CKX and 6-BA also applied to the ON-crop trees the following spring on the Year 1 yield and fruit size distribution of 'Manzanillo' olive trees located at the Lindcove REC, Exeter, CA.

Year 1 tree status	Year 1 treatment	Total yield (kg/tree)	Fruit size distribution (pack out) based on fruit diameter in mm (No./100 fruit/tree)										
			Tiny (<16)	Small (16 to <17)	Medium (17 to <19)	Large (19 to <20)	X-large (20 to <22)	Jumbo (22 to <24)	Colossal (24 to <26)	Super Colossal (>26)			
OFF	Control	44.4 ^c	1.2 b	0.8 b	13.1 b	17.4 a	53.9 a	13.3 a	0.3 ab	0.0 a			
OFF	CKX	48.5 c	0.9 b	1.2 b	10.7 b	15.5 ab	52.8 a	18.4 a	0.5 a	0.0 a			
ON	Control	155.3 b	19.4 a	24.8 a	42.8 a	9.7 bc	3.2 b	0.1 b	0.0 b	0.0 a			
ON	CKX+urea	157.9 b	25.7 a	23.6 a	37.6 a	9.6 bc	3.2 b	0.1 b	0.0 b	0.0 a			
ON	6-BA+urea	170.4 a	27.6 a	26.8 a	39.5 a	4.6 c	1.4 b	0.0 b	0.1 b	0.0 a			
P-value		<0.0001	<0.0001	<0.0001	<0.0001	0.0043	<0.0001	<0.0001	0.0614	--			

^c Values in a vertical column followed by different letters are significantly different at the P-value specified by Fisher's Protected LSD Test.

Table 2. Effect of the Year 1 applications of a proprietary natural cytokinin (CKX) applied to OFF-crop trees and CKX and 6-benzyladenine (6-BA) plus low-biuret urea applied to ON-crop trees in June and July, with CKX and 6-BA also applied to the ON-crop trees the following spring on the Year 2 yield and fruit size distribution of 'Manzanillo' olive trees located at the Lindcove REC, Exeter, CA.

Year 1 tree status	Year 1 treatment	Total yield (kg/tree)	Fruit size distribution on (pack out) based on fruit weight (g) (kg/tree)										
			Sub-petite (2.21-2.66)	Petite (2.67-3.08)	Small (3.09-3.58)	Medium (3.59-4.22)	Large (4.23-5.07)	X-large (5.08-7.27)	Jumbo (7.28-9.76)	Colossal (9.77-13.97)	Super Colossal (≥13.98)		
OFF	Control	149.4 ^a	8.7 a	20.1 a	32.3 a	43.1 a	30.4 a	14.8 a	0.1 c	0.0 a			
OFF	CKX	130.2 a	5.8 a	15.9 a	26.0 a	35.7 a	26.2 a	19.7 a	0.8 c	0.0 a			
ON	Control	42.1 b	0.5 b	0.9 b	2.3 b	5.8 b	12.8 b	18.3 a	1.5 bc	0.0 a			
ON	CKX+urea	47.9 b	0.2 b	0.3 b	1.8 b	6.1 b	10.7 b	25.0 a	3.7 a	0.0 a			
ON	6-BA+urea	45.0 b	0.2 b	0.4 b	0.8 b	4.0 b	10.3 b	26.5 a	2.8 ab	0.0 a			
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.1794	0.0002	0.4160	--		

^a Values in a vertical column followed by different letters are significantly different at the P-value specified by Fisher's Protected LSD Test.

Table 3. Effect of the Year 1 applications of a proprietary natural cytokinin (CKX) applied to OFF-crop trees and CKX and 6-benzyladenine (6-BA) plus low-biuret urea applied to ON-crop trees in June and July, with CKX and 6-BA also applied to the ON-crop trees the following spring on the 2 year average yield and 2-year cumulative yield of 'Manzanillo' olive trees located at the Lindcove REC, Exeter, CA.

Year 1 tree status	Year 1 Treatment	2-year average (kg/tree)	2-year cumulative (kg/tree)
Off	Control	96.9 ab ²	193.8 ab
Off	CKX ^y	89.3 b	178.7 b
On	Control	98.7 ab	197.4 ab
On	CKX + N	102.9 ab	205.8 ab
On	6-BA + N	107.8 a	215.7 a
Year			
1		115.3 a	--
2		83.0 b	--
<i>P</i> -value			
Treatment (T)		0.1045	0.1045
Year (Y)		<0.0001	--
T x Y		<0.0001	--

² Values in a vertical column followed by different letters are significantly different at the *P*-value specified by Fisher's Protected LSD Test.

CALIFORNIA OLIVE COMMITTEE RESEARCH PROPOSAL

Project Year: 2016/17

Anticipated Duration of Project: 2 years

Project Leader: Ron Haff

Location: USDA, ARS, WRRRC, Albany CA

Mailing Address: 800 Buchanan St, Albany CA 94710

Phone: 510-559-5868

FAX: 510-559-5684

E-mail: ron.haff@ars.usda.gov

Cooperating Personnel: Pei Liang, USDA ARS; Eric Jackson, USDA ARS

Project Title: Real-time removal of insect damaged olives from the processing stream

PROJECT SUMMARY

Problem and its Significance:

The olive fruit fly occurs in all olive growing areas of California and poses a severe economic threat for the state's commercial olive growers. Most research has focused on control strategies, including the breeding of sterile flies, trapping and monitoring, ecological management systems, and importation of natural enemies. However, a rapid non-destructive method for detection and removal of damaged olives would aid with post-harvest management and would benefit the industry, as well as consumers.

Recently published research has indicated that fruit fly damage in olives can be detected based on absorption of light within specific wavebands in the visible/Near-infrared (NIR) region (Tables 1 and 2) (Moscetti et al., 2015). Given such specific wavebands of interest, economical, high speed sorting devices have been previously developed in our lab (Haff and Jackson, 2008). Thus, the development of such a device to removed infested olives would involve the application of already developed procedures and practices over predetermined wavebands, reducing the problem to one of mainly optics, electronics, and material handling. The development of such a device is the logical conclusion to a great deal of previous research in NIR and sorting technologies.

Trial #	Dataset				Selected Features (nm)						Error rate (%)			AUC	Wilks' λ	Pr (> F)
	Scatt. C.	Norm.	SG*	Deriv.	1	2	3	4	5	6	fp	fn	total			
01	MSC	Yes	13	-	1178	1226	1246	1566	2150	2246	18.75	0.00	9.38	0.9531	0.3525	< 0.001
02	SNV	Yes	9	1st	1168	1420	1602	1712	-	-	12.50	6.25	9.38	0.9531	0.4590	< 0.001
03	MSC	Yes	13	-	1108	1232	1416	1486	2148	-	6.25	18.75	12.50	0.9570	0.3586	< 0.001
04	MSC	Yes	9	-	1232	1764	2248	-	-	-	12.50	18.75	15.63	0.9141	0.4658	< 0.001
05	SNV	-	5	1st	1420	1710	-	-	-	-	31.25	12.50	21.88	0.8984	0.5328	< 0.001

Table 1. Results based on pretreatments, GA and LDA performed on the spectra acquired from olive fruit. The selected features corresponded to the wavelengths yielding the lowest error rates of classification per each dataset.

Trial #	Dataset				Selected Features (nm)						Error rate (%)			AUC	Wilks' λ	Pr (> F)
	Scatt. C.	Norm.	SG*	Deriv.	1	2	3	4	5	6	fp	fn	total			
01	MSC	Yes	13	-	1108	1232	1416	1486	2148	-	12.50	0.00	6.25	0.9766	0.3586	< 0.001
02	-	-	13	1st	1108	1168	1418	1466	1710	1984	6.25	12.50	9.38	0.9922	0.3758	< 0.001
03	-	Yes	13	1st	1138	1418	1464	-	-	-	12.50	18.75	15.63	0.9297	0.4588	< 0.001
04	SNV	-	9	1st	1168	1420	1602	1712	-	-	25.00	6.25	15.63	0.9531	0.4590	< 0.001
05	MSC	Yes	5	1st	1422	1712	-	-	-	-	31.25	12.50	21.88	0.8984	0.5874	< 0.001

Table 2. Results based on pretreatments, GA and QDA performed on the spectra acquired from olive fruit. The selected features corresponded to the wavelengths yielding the lowest error rates of classification per each dataset.

Objective:

The objective of this project is the development of a real-time, non-destructive sorting device that could detect and remove insect-damaged olives from the processing stream at a speed that would not hinder the normal processing of the fruit and a cost that could reasonably be absorbed by producers.

Plans and Procedures:

The approach to building a sorting device will be based on the design of commercially available NIR/vis modular sorters (Figure 1) which employ bandpass filters and splitting mirrors to isolate wavebands of interest for measurement with photodiode detectors. During the first year, the results presented in Tables 1 and 2 will be analyzed in terms of matching the most effective wavebands in terms of sorting accuracy with commercially available bandpass filters which generally have a width of about 40 nm, more or less. The availability and affordability of splitting mirrors at the required wavelengths will be determined. A "benchtop" scale prototype will be constructed and tested.

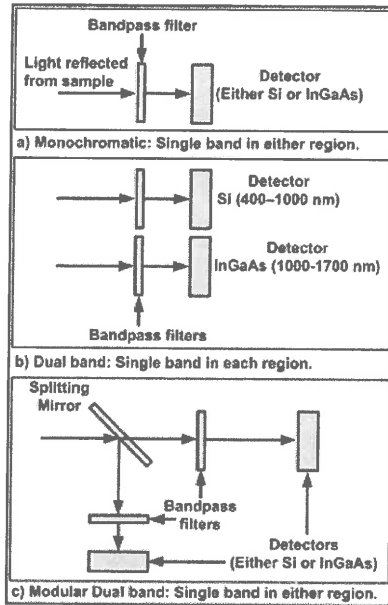


Figure 1. The design of the modular dual band sorter allows easy changing of the mirror, filters, and detectors and is therefore able to detect any bands between 400 nm and 1700 nm at either detector.

If the results of the first year indicate the building of a full scale sorter for commercial use is justified, a proposal will be submitted for the second year.

References

- Haff, R.P. and Pearson, T.C. Spectral band selection for optical sorting of pistachio nut defects. *Transactions of the ASABE*. 49(4): 1105-1113. 2006.
- Haff, R.P. and Jackson, E.S. Low cost real-time sorting of in shell pistachio nuts from kernels. *Applied Engineering in Agriculture*. 24(4):487-490. 2008.
- Moscetti, R., Haff, R.P., Stella, E., Contini, M., Monarca, D., Cecchini, M., and Massantini, R. Feasibility of NIR Spectroscopy to detect olive fruit infested by *Bactrocera oleae*. *Postharvest Biology and Technology*. 99: 58-62. 2015.

BUDGET REQUEST

Budget Year: 2016/17

Indirect Research Costs (IRC)	<u>\$3,500</u>
Salaries and Benefits (Peishish Liang, Postdoc, 25%)	<u>\$25,000</u>
Equipment	<u>\$4,000</u>
Travel	<u>\$2,500</u>
Total	<u>\$35,000</u>



Originator's Signature

Date 9/23/2015

University of California
Division of Agricultural Sciences
PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2016 Anticipated Duration of Project: 3rd year of 3 years

Principal Investigators: J. E. Adaskaveg

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

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

Keywords: Bactericides, biological controls, and systemic acquired resistance (SAR) compounds

JUSTIFICATION/ BACKGROUND

Olive knot caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (Psv) occurs throughout olive (*Olea europaea*) growing regions of the world including California (Young, 2004). The pathogen enters through wounds causing hyperplastic outgrowths (knots, tumors, galls, etc.) on branches and occasionally on leaves and fruit. Olive knot is one of the most economically important diseases of olives as infection may lead to tree defoliation, dieback, and reduced tree vigor, which ultimately lowers fruit yield and quality (Schroth, 1973). Psv can be found as both an endophyte and epiphyte of the olive phyllosphere, but the main source of inoculum are Psv-induced olive knots. Inoculum production of the pathogen is promoted during wet periods. It is exuded from knots and disseminated by rain, wind, insects, birds, as well as human activity. We demonstrated that inoculum is produced very rapidly after wetting olive knots. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission, frost, and hail damage, as well as pruning and harvesting practices. These latter orchard practices lead to direct mechanical damage of the knots and exposure of inoculum. After entering its woody host, the pathogen actively induces knot formation by production of indoleacetic acid (IAA) and cytokinins. In California, infections occur mostly during the rainy season (late fall, winter, and spring) but knots do not develop until new growth starts in the spring. Infections can occur at fairly low temperatures (5-10° C) and thus, wetness is the main limiting factor for the disease. Historically, the most susceptible olive cultivars were Manzanillo, Sevillano, Ascolano, and Mission, and none of the newer cultivars is resistant to the pathogen.

Formation of olive knots on wounded, inoculated branches depends on inoculum concentration as well as cultivar. We are focusing our studies on cv. Manzanillo but will include cv. Arbequina in selected trials, both of which are highly susceptible to the disease. Knot induction is usually localized to the initial entry point of the bacterium. Systemic movement of the pathogen has rarely been observed (Wilson and Magie, 1964). In spring 2014 evaluations of our fall 2013 trials in commercial and experimental olive plots we noticed apparent systemic movement of Psv which we never observed in any of our previous trials. Infections caused bark blistering and cracking as well as development of knots in proximity to and away from the initial point of inoculation, even on neighboring branches. In more severe cases, inoculated branches died. Potential causes of systemic movement have not been well characterized. Thus, one of our objectives was to determine environmental or other factors leading to these symptoms and whether the pathogen is migrating internally or externally on the host. These studies were initiated in 2015.

In 2016, we plan to continue these low-temperature studies in addition to implementing chemical treatments during periods of frost to determine if treatments are able to control Psv movement and knot development under these conditions. After repeated applications, the antibiotic oxytetracycline has been reported to move systemically to young citrus tissue. This strategy may also provide improved control of

olive knot in these situations and therefore will be evaluated along with other treatments. Additionally, soil applications of kasugamycin will be evaluated to determine if systemic uptake can be achieved.

Sanitation and prevention are the most successful strategies for management of olive knot. Any horticultural practice that promotes tree health, minimizes tree stress, and results in less leaf drop will reduce infections. Removal of knots during dry periods (i.e., summer to early fall) reduces inoculum and 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 and impractical. 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. Additional applications may be needed during winter rains or spring/summer hail storms. New copper formulations have been developed to reduce the metallic copper equivalent while maintaining the efficacy of the treatment. Our evaluations of copper sensitivity in populations of the olive knot pathogen indicated a reduced sensitivity of all strains with several strains showing an increased tolerance to copper. These results demonstrate a potential risk towards resistance development of Psv to copper with its continual and often exclusive use. Although the combination of copper and mancozeb is highly toxic to strains of Psv that are less sensitive to copper, the EPA will not allow additional crops to be added to the mancozeb label. In 2015 trials, we tested several copper hydroxide formulations at the highest labeled rates which resulted in exceptional disease control. High rates of copper were effective even when challenged with a copper-tolerant Psv strain. In order to maintain copper efficacy and reduce resistance development, we are planning to evaluate high rates of copper in mixed treatments with antibiotics and other chemicals. Trials performed in 2015 with selected copper enhancing compounds did improve copper performance as compared to copper alone (using low rates of copper) and will be further evaluated. We will no longer pursue systemic acquired resistance (SAR) compounds for the control of olive knot as past trials have resulted in highly inconsistent disease control. Additionally, in more recent trials where we inoculated SAR-treated plants with reduced Psv concentrations showed very poor to no control when compared to copper treatments.

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. We plan to continue its evaluation, especially in mixtures with copper. 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 in Sept. 2014. In Sept. 2015, we proposed and IR-4 accepted an "A" priority for oxytetracycline based on the need to develop several active ingredient that along with copper can be used in rotation or in mixtures. These antibiotics proposed uses were considered low risk because they were requested as after harvest, dormant and leaf drop treatments prior to the development of the crop in the current season. Thus, we will continue to evaluate kasugamycin, oxytetracycline, and other antibiotics as well as adjuvants that may optimize performance. Having several years of data will provide the best use strategies for growers.

In our research on sanitation treatments 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. The quaternary ammonia sanitizers are volatile compounds that leave near zero residues and are not corrosive to equipment. We extensively tested Deccosan 321 (Maquat) for its effectiveness against Psv and obtained federal registration of it as a sanitizer of field equipment for use on olives in 2015. We initiated several small field trials in the spring of 2015 and plan to continue these trials in 2016.

RESEARCH OBJECTIVES

1) Continuation of quaternary ammonium compound (QAC) trials.

- a. Evaluate the performance of the quaternary ammonium compound Deccosan 321 as an equipment sanitizer under field conditions in comparison to chlorine by itself and in conjunction with additional foliar treatments (copper and kasugamycin).

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

PLANS AND PROCEDURES

1) *Continuation of quaternary ammonium compound (QAC) trials.*

a. We initiated several small field trials with Deccosan 321 in 2015 and are planning to expand these trials in 2016. Trials will be performed in olive orchards at UC Riverside (cv. Arbequina), UC Davis (cvs. Arbequina and Manzanillo), and at a commercial orchard in Yuba county (cv. Arbequina). Trials will involve the contamination of field pruning equipment with a suspension of Psv, sanitation with Deccosan 321, and then making pruning cuts and injuries to disease-free olive branches and twigs. Specifically, a handheld gas-powered hedger will be used to simulate larger commercial pruning equipment. The hedger pruning teeth will be contaminated by spraying with a suspension of Psv, sanitized using Deccosan 321 at labeled rates and exposure durations, and the hedger will then be used to make pruning wounds that will include lateral cuts along larger limbs (i. e., scraping against branches) and terminal stub cuts of smaller branches. Pruning of olive branches with a disinfected hedger will be used as a negative control, and pruning with a contaminated non-sanitized hedger as a positive control. Wounds created by a Psv-contaminated and QAC-sanitized hedger will also be spray-treated with copper hydroxide or a copper hydroxide-kasugamycin mixture in additional treatments. The experiment will be laid out using a randomized complete block design.

b. Deccosan 321 efficacy under different pH conditions will be investigated using an in vitro direct contact assay. For this, a biological buffer solution will be chosen that can accommodate a pH range of 5 to 9. Psv will be mixed with a fixed concentration of Deccosan 321 prepared in buffer at pH 5, 6, 7, 8, or 9. After selected time periods, suspensions will be diluted 1000-fold with sterile water, and then spiral-plated onto culture media for enumeration of viable bacteria to determine Deccosan 321 efficacy. For controls, Psv suspensions at the various pH values will be diluted and plated without exposure to Deccosan 321. This information will be useful to find out of efficacy of Deccosan 321 can be improved by changing the acidity of the solution.

2) **Efficacy of new bactericides.**

a. Optimize the efficacy of antibiotic treatments (kasugamycin, oxytetracycline, streptomycin) against Psv in greenhouse and field trials using various formulations (technical and commercial grades), application timings, and additives (UV blockers, buffering agents, etc.). We will be exploring strategies that can potentially optimize the effectiveness of the antibiotics kasugamycin, oxytetracycline, and streptomycin. In greenhouse trials, we will compare technical and commercial formulations of the antibiotics using cvs. Manzanillo and Arbequina. In the field, application timings will be adjusted so that the antibiotics are applied during the period in the day when there is reduced sunlight to protect treatments from heat and UV degradation (our past treatments have typically been applied midday with intense sunlight). Addition of

adjuvants such as UV blockers and buffering agents to antibiotics will also be evaluated for improved disease control.

b. We will continue to evaluate copper-activity enhancing materials in field trials as a strategy to reduce copper resistance development. Previous trials have resulted in improved control when mixing these compounds with copper, especially against a copper-tolerant strain. Modifying rates of copper or CAE compounds in combination may increase control.

c. Persistence of copper-antibiotic mixed treatment after a period of simulated rain using overhead irrigation. Plants will be wounded and treated followed by a period of rain before wounds are Psv inoculated. We will evaluate selected agricultural stickers and oils compared to hydrated lime mixed with antibiotics and copper.

d. Field trials using high rates of copper formulations (7 lbs/acre) mixed with streptomycin (Firewall), oxytetracycline (Fireline), and kasugamycin (Kasumin).

For the above trials, we will design the trials with 4 or more replications of each treatment and evaluate the data using analysis of variance and mean separation procedures using SAS version 9.4.

3) Efficacy of bactericides under different environmental conditions.

a. Continue to conduct growth chamber studies to reproduce systemic infections of Psv. Plants will be subjected to low temperatures (-5°C) for selected periods of time (4 to 12 h) after wounding and inoculation. Plants will then be transferred to the greenhouse and observed for disease development. Scanning electron microscopy (SEM) of frost-damaged olive twig sections will be performed to visualize bark injuries. To track the movement of Psv and to determine if migration is systemically inside plant tissues or epiphytically, we will use selective re-isolation techniques and electron microscopy. Secondary wounds will be made at different distances from the initial inoculated wound. Development of knots and re-isolation of Psv from these wounds and from inside tissues distal to the original inoculation site will suggest systemic movement. Tissue samples will be taken after different incubation periods at different distances from the initial wound and examined using SEM. Inoculations may also be done with a copper-resistant strain to allow for discrimination of the inoculated isolate from potentially naturally present Psv strains (the majority of field strains are copper-sensitive).

b. Determine if protective treatments can reduce infection of olives under low-temperature conditions using different rates and application timings. Greenhouse grown cv. Manzanillo olives will be subjected to low temperature conditions (-5°C) in growth chambers. We will focus on several combinations of conditions to evaluate the best time to apply treatments (high copper rates and antibiotics) when freezing events are predicted to occur which will include:

1. Wounding healthy olive twigs and treating wounds with select compounds. Inoculating wounds with a Psv suspension. Placing trees in growth chambers for a duration of 12 hours at -5°C. Removing trees to greenhouse for growth until evaluations are made.
2. Wounding healthy olive twigs and treating wounds with select compounds. Placing trees in growth chambers for a duration of 12 hours at -5°C. Removing trees and inoculating wounds before moving trees to greenhouse until evaluations.
3. Wounding healthy olive twigs and placing plants in growth chamber. Removing plants, treating with compounds and inoculating with a Psv suspension before placing trees in greenhouse until evaluations.

c. Greenhouse trials using antibiotics applied as a soil drench will be conducted on potted olive plants. Different rates, application intervals, and number of applications will be evaluated to determine greatest disease control. Plants will be wounded and inoculated in between applications intervals or after the final application is made.

For the above trials, we will design the trials with 4 replications of each treatment and evaluate the data using analysis of variance and mean separation procedures using SAS version 9.4.

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Budget Request:

Budget Year: 2016	Funding Source: Olive Board of California	
Salaries and Benefits:	Post-Docs/RAs	22,000
	Lab/Field Ass't	2,000
	Subtotal	24,000
	Employee's Benefits*	14,000
	Subtotal	38,000
Supplies and Expenses		0
Equipment and University Land and Orchard charges		1,000
Operating Expenses/Equipment Travel (Davis Campus only)		0
Travel		3,000
Department Account No. _____	Total	\$42,000

*-Note: Benefits for UCR employees have increased dramatically over last years.

J. E. Adaskaveg Date: Sept. 30, 2015

Originator's Signature (PI)
Katherine Burkovic
 Dept. Chair _____ Date: Oct. 2, 2015
 (Riverside Campus)

Liaison Officer _____ Date: _____

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2016

Anticipated Duration of Project: one year

Project Leader: C. H. Pickett

Location: UC Berkeley quarantine

Mailing Address: CDFA, 3288 Meadowview Rd., Sacramento, CA 95832

Phone: 916.262-2053 FAX:916.262-2059

E-mail: cpickett@cdfa.ca.gov

Project Title: Renewal: Biological Control of Olive Psyllid Parasitoid, *Psyllaephagus euphyllurae* (Hymenoptera: Encyrtidae)

Cooperating Personnel: Dr. Kent Daane

Keywords: olive psyllid, biological control

Commodity(s): olive fruit

Relevant AES/CE Project No.:

Problem and its Significance:

The olive psyllid, *Euphyllura olivina* (Hemiptera: Psyllidae), was first reported in California in 2007 infesting olive trees in San Diego and Orange counties. It has now spread to Riverside and Los Angeles counties and has been found on olive trees at one private residence in Monterey County. So although we don't know for certain that it will become a serious pest in commercial production regions of central and northern California, we do know that it is spreading. This pest naturally occurs throughout the Mediterranean Basin, both coastally and inland, and exclusively attacks the flower blossoms and growing tissue of olive (Tzanakakis 2006, unpubl. data). The olive psyllid is reproductively active during spring months when nymphal populations can cause significant reductions to the olive fruit set. Spring infestations have been reported reducing fruit yields by up to 60% in some parts of the Mediterranean (Jardak, T, 1984, Tzanakakis, M. E. 2006).

The principal parasitoid attacking this pest in the western Mediterranean Basin, *Psyllaephagus euphyllurae* (Garcia-Mercet 1921, Aversenq 2005) was initially discovered and collected from Spain as part of other foreign exploration activities. Through funding from the Olive Commission in 2011 and funding from the Federal Specialty Crops Block Grant Program, 2012-2015, we have made a great deal of progress in demonstrating, under quarantine conditions, the host specificity of this parasitoid: that it attacks only olive psyllid (Table 1). This information is required to obtain a field release permit in California. It also shows that *P. euphyllurae* is highly host specific, a trait shared with other parasitoids released over past decades in California and that have successfully suppressed agricultural pests. However, it is possible we may be asked for additional testing, delaying release for another season. Also our Block Grant ended in June 2015 and may not be renewed. If the Block grant is not funded, we will solicit the COC for another year of funding beyond 2015.

Table 1. Non-target psyllid species, selection criteria, and preliminary results in host specificity testing.

Psyllid species	Selection criteria	Non-target attacks
Asian citrus psyllid, <i>Diaphorina citri</i>	Relatedness	none
<i>Ceanothia ceanothi</i>	Natural habitat near olive production	none
Fremontia psyllid, <i>Dichlidophebia fremontiae</i>	Relatedness, and habitat proximity	Not completed
Potato psyllid, <i>Bactericera cockerelli</i>	Native pest psyllid	none
French broom psyllid, <i>Arytinnis</i>	Beneficial insect attacking a	none

Our long term goal is to establish permanent populations of this parasitoid in southern California, before the olive psyllid spreads farther north into commercial production areas. By doing so, it may be possible to greatly delay, or even prevent its spread north and contain the population within southern California. Establishment of *P. euphyllurae* will also permanently reduce olive psyllid populations throughout its current and future distribution in California. Two hyperparasitoids (parasitoids that kill other parasitoids) have been found while collecting in Spain; these do not occur in California allowing the candidate parasitoid to exert far more impact on olive psyllid in its new home. A second parasitoid was discovered attacking olive psyllid in southeastern Spain during a recent collecting trip in support of the above project in a region that matches climatically with the central valley of California. Both *Psyllaephagus euphyllurae* and *Psyllaephagus pulchellus* co-occur in the more inland, and southern regions of Spain. With continued funding, we can insure permitting and release of the one parasitoid recently tested and most commonly associated with olive psyllid in Spain, *P. euphyllurae*.

Now is the time to be pro-active with this pest. Without an effective parasitoid in California, populations of olive psyllid will be more costly to control. We have in culture what is likely the most important natural enemy attacking the olive psyllid in the western Mediterranean Basin, the native home for both of these insects. We also have in place trained personnel who can rapidly complete studies and move on to field releases, hopefully before this pest establishes permanent populations in key olive growing regions.

Objectives:

1. Complete host specificity testing of *P. euphyllurae*, if required.
2. Release for permanent establishment *P. euphyllurae* into olive psyllid infested trees.

Plans and Procedures:

The goal of host range testing is to show that the candidate parasitoid for introduction attacks only the target pest insect, e.g. the olive psyllid, or has only a very narrow range of species that it can develop on. At this time, testing has been done on four species of psyllids. These represent native and non-native species, of which one is being considered for release as a biocontrol agent for an invasive weed. However, additional replication is needed for some species, and we hope to complete testing on a fifth non-target psyllid.

Non-Target Psyllids for host testing. Several native and exotic psyllids are in culture at quarantine laboratories located at UC Riverside, UC Berkeley, and CDFA in Sacramento, and USDA ARS in Albany. These psyllids were selected and tested for several reasons, including relatedness to the olive psyllid, occurrence in habitat similar to and near where commercial olives are grown, and their availability. California has a rich diversity of native psyllids associated with its native vegetation (Percey et al. 2011). However, only a small subset can be chosen for testing since there are over 165 representative species. We have also tested against the Asian citrus psyllid, which is related to the

olive psyllid. If *P. euphyllurae* is unable to attack and develop on a related psyllid, then it is unlikely to develop on the native, more distantly related psyllids. Several native psyllids are associated with native plants common to foothill regions of southern and central California, i.e. *Ceanothus* spp., *Fremontodendron californicum*, and *Rhus ovata* (Table 1). Associated psyllids are *Ceanothia* sp., *Diclidophlebia fremontiae*, and *Calophya triozmima*. The biological control agent, *Arytinnis hakani*, imported for control of French broom *Cytisus scoparius*, has also undergone testing.

Host plants. Insects will be reared on respective host plants (Table 2). Psyllids require new plant growth for feeding and reproduction, therefore plants will routinely be clipped to maintain a constant supply of new flush growth. Most of these psyllids are host specific and require rearing on a single species of plant. Host plants will be purchased from nearby nurseries. Plants lacking availability will be grown from cuttings at the CDFA in Sacramento, California.

Table 2. List of host plants and non-target psyllids.

Psyllid species	Host plant	Botanical name
Asian citrus psyllid, <i>Diaphorina citri</i>	Citrus	<i>Citrus</i> spp.
<i>Ceanothia ceanothi</i>	Ceanothus	<i>Ceanothus integerrimus</i>
Fremontia psyllid, <i>Diclidophlebia fremontiae</i>	Flannel bush	<i>Fremontodendron californicum</i>
Potato psyllid, <i>Bactericera cockerelli</i>	Egg plant	<i>Solanum melongena</i>
French broom psyllid, <i>Arytinnis hakani</i>	French broom	<i>Genista monospessulana</i>

Rearing olive psyllid, its parasitoid, and non-target psyllids. The olive psyllid, olive tree saplings, and non-target host plants are currently in culture at CDFA’s quarantine and greenhouse in Sacramento. Psyllids and plants are periodically shipped or hand-carried to Riverside for host specificity testing on an as-needed basis. Additional olive psyllids are collected in the field in southern California as well. Prior to testing, saplings are transferred to Ray Leach ‘Cone-tainers’ which have done well in host range tests for the Asian citrus psyllid parasitoid, *Tamarixia radiata* (Hoddle and Pandey 2014). Host plants will be placed in Bugdorms (Megaview Science, Taiwan) and maintained in a laboratory until needed for testing in Quarantine.

Host Testing.

The remaining choice and no-choice tests will be conducted in a laboratory inside of the UC Berkeley Quarantine facility using sequential no-choice, and choice exposure testing procedures. Rooted seedlings with 10 to 20 nymph test insects will be caged with the candidate parasitoid, *P. euphyllurae* alone or together with the olive psyllid, also placed on rooted seedlings. The three experimental treatments will be as follows:

Sequential no-choice tests: *P. euphyllura* is exposed to olive psyllid first, then to a non-target psyllid; or *P. euphyllura* is exposed to the non-target then to olive psyllid. Exposure times will be ca. 4 hrs, and temperatures will be set at 25 to 27 °C, with a 40% RH and 14:10 L:D photophase, following Hoddle and Pandey (2014).

Choice tests: *P. euphyllura* is exposed to olive psyllid and a second non-target host simultaneously, with each foraging on respective host plants, for 4 hrs. under the same environmental conditions as above.

Data will be recorded on the number of psyllids successfully developing to adults, psyllid nymph mortality, and number of parasitoids emerging from host psyllids. Observations will also be recorded once each hour, on the number of attempts at parasitism, and number of contacts between hosts and parasitoids.

Releases.

Once a field release permit is obtained, parasitoids will be released during spring months onto trees infested with olive psyllid in southern California. Past surveys of infested trees in southern California have found no native parasitoids or predators feeding on these psyllids. Release trees will be monitored for establishment and impact of released parasitoids for as long as funding is available. Travel to Spain to field collect additional parasitoids may be required if we are unable to mass rear adequate numbers. This trip would be made during May when olive psyllids are at their highest numbers both in Spain and southern California. Collections would be made at locations known to harbor high numbers of parasitoids.

References

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BUDGET REQUEST

Budget Year: 2016

Funding Source:

Salaries and Benefits: _____

Postdocs/RA's _____

SRA's

Lab/Field Assistance (9 months @\$2400/mo., 75%) \$16,200

Subtotal Sub2 \$16,200

Employee benefits (@40%) Sub6 \$6,480

TOTAL \$22,680

Supplies and Expenses Sub3 _____ 0

Equipment Sub4 _____ 0

Travel

In state, releases and monitoring \$2,000

Overseas, collecting \$7,000

Sub5 \$9,000

Grand Total \$31,680

Department account number:

Originator's Signature

Date _____

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2016

Anticipated Duration of Project: 10 years

Project Title:

Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard

Project Leaders:

Dr. John Preece: Research Leader, USDA-ARS National Clonal Germplasm Repository, UC Davis, 1 Shields Ave., Davis CA 95616. John.Preece@ars.usda.gov, (530)-752-7009

Dr. 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], L.Ferguson@ucdavis.edu

Mr. Dan Flynn: University of California Olive Center, Davis CA
JDFlynn@UCDavis.edu; (530)-752-5170

Mr. James M. Jackson: Principal Superintendent, Plant Sciences Field Facility, UC Davis CA
JMJackson@ucdavis.edu; (530)-753-2173 and (530)-681-2279

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2013 Current Funding Request: 15,096.00

Problems and Significance:

To facilitate mechanical harvesting the newest table olive orchards are planted in hedgerows and require regular mechanical pruning to keep the trees small. Our 12 X 18' foot research planting established at Nickels Soils Laboratory in 2002 has demonstrated to us this will be difficult with the 'Manzanillo' olive cultivar. Such hedgerow 'Manzanillo' orchards designed for mechanical harvesting would be easier to maintain if they could be grafted on dwarfing rootstocks. Among those olives with promise for use as a dwarfing rootstocks are:

Nikitskaya,

Olea cuspidate

Verticillium Resistant Oblonga

Dwarf D

Little Ollie (2015 addition)

In 2013 we proposed propagating these rootstocks and testing with grafted and non-grafted own rooted 'Manzanillo' controls for their dwarfing potential with 'Manzanillo' to produce a tree that is more amenable to mechanical harvesting. The own rooted 'Manzanillos' and 'Manzanillo' grafted to 'Manzanillo' in this orchard could also serve as the next generation hedgerow trained mechanically pruned orchard for mechanical harvesting with trunk and canopy contact shakers.

In 2013 year we were awarded funding to propagate the desired rootstocks and locate a suitable orchard site for establishment of the propagated trees. Both objectives have been achieved but due to difficulty of propagation with some cultivars and difficulty in locating a site with proper infrastructure planting was in spring 2014.

Progress through 9/30/2015:

This application for initial funding was for two purposes:

- I. Propagation and grafting of the rootstocks with ‘Manzanillo’ scions.**
 - a. Dr. John Preece supervised the development of specific propagation techniques for 112 each of the following olive cultivars to be used as dwarfing rootstocks; Nikitskaya, *Olea cuspidate*, Verticillium Resistant Oblonga and Dwarf D. Dwarf D proved very difficult to root as cuttings and this means that there were sufficient trees only for the closer spacing. At the wider spacing, Little Ollie, which roots easily is being tested, which adds another potential rootstock and expands the scope of the study in a logical way.
- II. Establishing the next generation olive hedgerow orchard for evaluation of mechanical harvesters.**
 - a. Field 3556, a four acre block located in Plant Sciences Field Facility located on the UC Davis Campus and maintained by UC Davis Plant Sciences field personnel was chosen as the planting site. This site has the added advantage of being located adjacent to oil orchards being developed by the UC Olive Center. The trees were planted in 2014. Attachment I: Field Map: 3556.
- III. Experimental Field Design:**
 - a. Split plot design with the north half of the field at spaced at 10 X 16’ and the south at 10 X 8’.
 - b. There are 4 Randomized Complete Blocks
 - c. Four different dwarfing rootstocks grafted with ‘Manzanillo’
 - d. Own rooted ‘Manzanillo’ and ‘Manzanillo’ grafted to a ‘Manzanillo’ grafting controls.
 - e. Sevillano pollinizers were planted as border rows around the perimeter of the orchard and in the middle, as a row between the wide and narrow spacing.

Progress Summary:

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 were planted into the orchard and ‘Little Ollie’ replaced the originally planned ‘Dwarf D’ at the wider spacing. This completes the planting and 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 was replaced. Sierra Gold Nursery and staff of the National Clonal Germplasm Repository grafted the trees from September 28 – Oct. 1, 2015. This will give a cooler time of the year for the grafts to heal and take. Following grafting, the orchard was sprayed with Kocide to control olive knot.

2016 Objectives:

- I. **Finish grafting all rootstocks, once the 2015 plants are established: Attachment I: Field 3556 Plot Map**
- II. **Collect data to study the any growth differences among the scions on the different rootstocks compared to the controls**

Experimental Procedures:

Complete grafting. Based on experience gained on grafting, the final trees planted in 2015 will be sufficiently large for grafting late summer, 2016. This will be completed and will add Little Ollie as an experimental rootstock at the wider spacing.

Two scions were bark or whip grafted onto each rootstock. During 2016, the weaker of the two grafts will be pruned off to a single scion per rootstock.

The goal is to be able to dwarf the olive trees by using one or more of these rootstocks. Therefore, data will focus on measurements of vegetative vigor, including branch numbers and lengths, tree height, tree caliper of both the rootstock and scion. During 2015, there were fruit on the Manzanillos, and although it is early in the study yield data will be collected.

Data will be analyzed using ANOVA with an LSD means separation.

Desired Result:

At maturity the rootstocks will maintain tree size at 10 feet or less, and the trees can be harvested with trunk shakers or canopy contact harvesters. The experimental design will also allow a determination of ‘Manzanillo’ tree yields at 10 X 16’ and 8 X 16’ feet spacings.

BUDGET REQUEST: 2016

BUDGET REQUEST

Budget Year: 2016

Funding Source: COC

<u>Orchard Maintenance Costs:</u>	<u>3,600.00</u>
UC Davis Plant Sciences Field Facility: 4 acres @ 650.00/acre per year = \$2600	
Supplies, such as herbicides \$1000.00	
<u>Sub 1</u>	<u>3,600.00</u>
<u>Data Collection:</u>	<u>10,000.00</u>

Student workers to collect the tree growth and fruiting data.

Sub 2	<u>13,600.00</u>
UC Overhead @ 11%:	1,496.00
Sub 3	<u>15,096.00</u>
TOTAL BUDGET REQUEST	<u>15,096.00</u>

UNIVERSITY OF CALIFORNIA



Originator's Signature

10/02/2015
Date

Agricultural Experiment
Station

Department Chair

Date

Liaison Officer

Date

Scope of Work

Drs. John Preece and Louise Ferguson and Mr. Dan Flynn:
Responsible for overall coordination of the project and orchard management.

Mr. James M. Jackson:
Responsible for orchard management implementation.

***** INFORMATION *****

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: NO-COST EXTENSIONS

BACKGROUND: Each year, researchers will request no-cost extensions and the Subcommittee is asked to grant the Chairman the authority to approve the requests. The Chairman will be provided requests complete with end dates. Once the Chairman approves the requests, staff will issue letters of approval or rejection to the researcher.